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Research
Araştırma Makalesi

Determination of *tdh* and *trh* Positive *Vibrio alginolyticus* Isolates from Black Mussel (*Choromytilus meridionalis*) in Aegean Sea coast of Turkey

Türkiye'nin Ege Denizi kıyısındaki Kara Midye (*Choromytilus meridionalis*)'lerden *tdh* ve *trh* Pozitif *Vibrio alginolyticus* İzolatlarının Belirlenmesi

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Abstract: *Vibrio alginolyticus* is one of the important pathogens, especially found in bivalve mollusks and food poisoning in humans. The severity of food poisoning is directly proportional to the virulence genes of *V. alginolyticus*. Tdh-Related Hemolysin (*trh*) and Thermostable Direct Hemolysin (*tdh*) genes have an important place among the virulence genes found in *V. alginolyticus*. In this research, 17 *V. alginolyticus* were isolated from 17 orders (80.95%) of 21 sets of black mussels (*Choromytilus Meridionalis*) samples purchased from local divers in İzmir and Balıkesir regions. While *trh* gene was detected in 7 (42.17%) of 17 isolates, *tdh* gene was found in 6 (35.29%) and both *trh* and *tdh* genes were found in 2 (11.76%) isolates; no *trh* or *tdh* gene was found in 2 isolates (11.76%). The results of the study are also important in terms of public health. Black mussel is a product that is mainly consumed in coastal areas in Turkey and is mostly sold uncontrolled by mussel sellers. Vibrios with virulence genes can cause food poisoning, especially in summer. In addition, *V. alginolyticus* may be a *tdh-trh* reservoir for other vibrio species. To clarify this, more detailed research should be done with other vibrio species and other bivalve species.

Keywords
- *Vibrio alginolyticus*
- Black mussel
- *trh*
- *tdh*
- Turkey

Özet: *Vibrio alginolyticus*, özellikle çift kabuklu yumuşakçalarda bulunan ve insanlarda gıda zehirlenmesine neden olan önemli patojenlerden biridir. Gıda zehirlenmesinin şiddeti, *V. alginolyticus*'un virülans genleri ile doğru orantılıdır. *V. alginolyticus*' ta tespit edilmiş virulans genler içinde Tdh-İlişkili Hemolizin (*trh*) ve Termostabil Direkt Hemolizin (*tdh*) genleri önemli bir yer tutar. Bu çalışmada, İzmir ve Balıkesir yörelerindeki yerel dalgıçlardan satın alınan 21 takım (Her takımda yüz civarında midye olmak üzere) kara midye (*Choromytilus Meridionalis*) numunesinin 17 takımından (%80,95) 17 adet *V. alginolyticus* izole edilmiştir. On yedi izolatın 7'sinde (%42,17) *trh* geni, 6'sında (%35,29) *tdh* geni, 2'sinde (%11,76) hem *trh* hem *tdh* geni bulunurken; 2 izolatta (%11,76) *trh* ya da *tdh* geni tespit edilmemiştir. Çalışma sonuçları halk sağlığı açısından da önemlidir. Kara midye, Türkiye'de ağırlıklı olarak kıyı bölgelerde tüketilen ve çoğunlukla kontrolsüz olarak seyyar midyeciler tarafından satılan bir üründür. Virulans genlere sahip vibriolar özellikle yaz aylarında gıda zehirlenmeleri meydana getirebilir. Ek olarak, *V. alginolyticus*, diğer vibrio türleri için *tdh-trh* rezervuarı olabilir. Bunu netleştirmek için diğer vibrio türleri ve diğer çift kabuklu türleri ile daha detaylı araştırmalar yapılmalıdır.

Anahtar kelimeler
- *Vibrio alginolyticus*
- Kara midye
- *trh*
- *tdh*
- Türkiye

1. INTRODUCTION

Vibrio alginolyticus is one of the most widespread *Vibrio* spp. to be found in seawater, bottom of the sea, fish, bivalve and can keep its virulence under adverse conditions (Benkahla et al., 2007; Covazzi et al., 2008; Karim et al., 2018). This microorganism causes conjunctivitis, wound infections, and gastroenteritis in humans (Osorio and Klose, 2000; Türk et al., 2011; Weils and Wendy, 2012). Pathogenicity is closely related to virulence genes (Hentschel et al., 2000; Zhangx and Austin, 2005; Chewdhury and Chewdhury, 2015). *tdh*-related hemolysin (*trh*) and thermostable direct hemolysin (*tdh*) genes are the notable virulence features in *V. alginolyticus* (Reina et al., 1995). After rupture of the erythrocyte membranes, hemolysis occurs and hemoglobin is released (Lida et al., 1997; Bej et al., 1999). Haemolysins are maked by many different microorganisms (*Vibrio* spp., *Pseudomonas* spp.) (Lida and Honda, 1997). Numerous studies claim that hemolysins are involved in disease pathogenesis (Osorio et al., 2000; Stalin and Srinivasan, 2016).

Vibrio alginolyticus is an important pathogen in marine aquaculture in South Asia and Europe (Lopes et al., 1993; Balebona et al., 1998). The detection of *V. alginolyticus* with pathogenic genes from bivalve is also notable for human health. Because bivalves are often eaten raw, salted, and undercooked (Elliott et al., 1992).

Although cases of food poisoning due to *V. alginolyticus* have been reported worldwide, there is limited information on virulence genes with these isolates. *tdh* and *trh* genes mostly were detected in *V. cholerae* and *V. parahaemolyticus* (Tada et al., 1992; Xie et al., 2005, González-Escalona et al., 2006; Gutierrez West et.al. 2013). And there are only a few studies on *tdh* (Gargouti et al., 2015) and *trh* (Gonzales-Escolana et al., 2006, Avsever, 2016) genes in *V. alginolyticus* available. Avsever (2016) found that *trh* positive *V. alginolitycus* isolates in bivalve molluscs in the Balıkesir and Ayvalık regions between 2007 and 2010. However, there is no report for *tdh* positive *V. alginolitycus* from bivalve mollusks in Turkey.

This study aims to fix the *trh*, *tdh* genes of *V. alginolyticus* isolates obtained from black mussel located in Turkey, to take attention the potential virulence gene transfer between other Vibrio species and *V. alginolyticus*, and to note that *V. alginolyticus* is an important foodborne bacteria.

2. MATERIAL and METHODS

In this study, 21 black mussels (*Choroytilus meridionalis*) sampling were performed by local divers in İzmir (n=15) and Balıkesir (n=6) Provinces. 100 mussel (relatively small) samples were taken in each sampling. Sample collection took place from 1 May to 31 August 2018, when shellfish collection was not prohibited. The mean water temperature during the sampling seasons was 21ºC ±2. The samples were delivered to the laboratory by a special car, in a cold chain, and quickly.

2.1. Bacterial isolation

In this study, isolation of *V. alginolitycus* was done in accordance with TS/TS ISO 8914 standard (1998). Each group of mussels was accepted as a sample. The mussel groups were crushed in separate sterile mortars and homogenized. 25 g of sample for each group from the homogenate was weighed and used in the bacteriological study. For pre-enrichment, 25 g of the homogenate from mussels in each sampling were placed in peptonized water containing 225 ml of 3% NaCl and incubated at 37 ° C for 18-24 hours. In line with the pre-enrichment medium, the line was plated with TCBS (Thiosulfate Citrate Bile Sucrose) agar. After incubation at 37 ° C for one day, DNA extract was obtained from the four yellow-colored colonies (2-3 mm in diameter), with flagellar moving, oxidase-positive, Gram-negative rod.

2.2. DNA isolation

DNA extraction was performed from the isolates (ATCC 17749, *V. alginolyticus*; ATCC 19264, *Vibrio anguillarum; V. parahaemolyticus* isolates positive for *trh* and *tdh* and 17 *V. alginolyticus*

suspicious isolates with a commercial DNA isolation kit (High pure, Germany) in accordance with the user manual.

2.3. Confirmation of *V. alginolyticus* isolates with PCR

In the confirmation of the isolates, the target gene was selected as gyrB and used the PCR method specified by Luo et al. (2008). ATCC 17749, *V. alginolyticus* was performed as the positive bacteria. *V. anguillarum* ATCC 19264 (568 bp.) was performed as the negative control. The primer sequences were presented in Table 1. The PCR formation was used in a 25 µl quantity containing 10x PCR buffer (2.50 µl), *Taq* DNA polymerase enzyme (5 U/µl) (0.40 µl) (MBI, Fermantas), 5 µl bacteria DNA, 50 mM $MgCl_2$ (1.25 µl), 0.4 µM Alg F1 (2 µl), 0.4 µM Alg R1 (2 µl), 10 mM dNTPs (dATP, dCTP, dGTP, dTTP) (0.63 µl) and 11.22 µl non-nuclease water. The amplification procedure occurred of an initial denaturation at 94°C for 4 min, 32 cycles of denaturation at 94°C for 30 s, annealing at 64°C for 30 s, extension at 72°C for 1 min, and a final extension at 72°C for 8 min. After PCR amplification, 4 µl of each product was added into 1.0% agarose gel, and electrophoresis was performed. Bands were visualized with designated equipment as 568 bp. As DNA size markers, 100 DNA Ladder (MBI, Fermentas) was performed.

Table 1. *gyrB* primers.

gyrB primers (Luo et al. (2008)	5'-CATCGTCGCCTGAAGTCG CTGT -3' (AlgR1), 5'-TCAGAGAAAGTTGAGCTAACGATT-3' (AlgF1).

2.4. Detection of *tdh* and *trh* genes using PCR

DNA samples from 17 *V. alginolyticus* isolates were researched separately (PCR 1 and 2) for *tdh* and *trh* genes with PCR. PCR studies were used with the primer pairs (in Table 2) according to the method reported by Cohen et al., 2007. *V. parahaemolyticus* isolates previously found positive for *trh* and *tdh* by Terzi et al., 2009 were performed as positive bacteria. Distilled water was performed as a negative control. Master-mix occurred 5 µl genomic DNA, 10x PCR buffer (2.50 µl), 50 mM $MgCl_2$ (1.25 µl), 10 mM dNTPs (dATP, dCTP, dGTP, dTTP) (0.63µl), 5 µM *TRH*-L primer (2.0 µl), 5 µM *TRH*-R primer (2.0 µl), *Taq* DNA polymerase (5 units/µl) (0.40 µl) (MBI, Fermantas), 11.22 µl non-nuclease water for PCR 1 (*trh*); 5 µl genomic DNA, 10x PCR buffer (2.50 µl), 50 mM $MgCl_2$ (1.25 µl),10 mM dNTPs (dATP, dCTP, dGTP, dTTP)(0.63 µl), 5 µM *TDH*-L primer(1 µl), 5µ M *TDH*-R primer (1 µl), *Taq* DNA polymerase (5 U/µl)(0.40 µl) (MBI, Fermantas), 13.22 µl non-nuclease water for PCR 2 (*tdh*). The reactions (PCR 1, 2) performed with an automated thermocycler were as follows: Initial denaturation at 94°C for 5min., followed by 40 cycles of denaturation at 94°C for 30 s., primer annealing at 58°C for 45 s. and primer extension at 68°C for 75 s. A final extension was performed at 68°C for 7min. PCR end-products were separated by electrophoresis on 2% (w⁄v) agarose gel (1 hour, 75 volts) was performed. As a DNA size marker, 100 DNA Ladder (MBI-Fermentas) was used. Bands were observed with suitable equipment as 250 and 373 bp.

Table 2. *trh, tdh* primers.

trh primers (Cohen et al., 2007)	5'-GGC TCA AAA TGG TTA AGCG-3' and 5'-CAT TTC CGC TCT CAT ATGC-3'
tdh primers (Cohen et al., 2007)	5'-CCA TCT GTC CCT TTT CCT GC-3' and 5'-CCA AAT ACA TTT TAC TTGG-3'

3. RESULTS

Vibrio alginolyticus was isolated from 17 of (80.95 %) sampling of black mussels. Six isolates (35.29 %) for *tdh* gene, 7 isolates (42.17 %) for *trh* gene, two isolates (11.76 %) for *trh* and *tdh* genes

were found positive. Two isolates (11.76 %) were negative for *trh* and *tdh* genes. Results are shown in Scale 3 and Figure 1,2,3.

Table 3. Results of the *gyrB, tdh and trh positive V. alginolyticus* isolates.

Black mussel groups	***gyrB* positive *V. alginolyticus* (80.95 %)**	***trh* positive (only) *V. alginolyticus* (42.17 %)**	***tdh* positive (only) *V. alginolyticus* (35.29 %)**	***tdh-trh* positive *V.alginolyticus* (11.76 %)**	***tdh-trh* negative *V. alginolyticus* (11.76 %)**
21	17	6	7	2	2

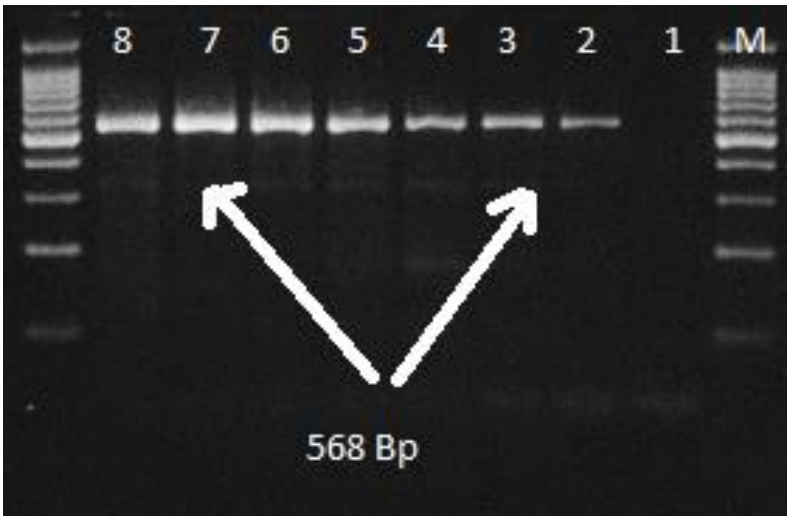

Figure 1. Confirmation of *gyrB* gene-positive *V. alginolyticus* isolates. Line 1: Negative control ATCC 19264, *Vibrio anguillarum*. Line 2: ATCC 17749, *V. alginolyticus*, Positive control,568 bp. Line 3-8: Isolates, 568 bp. M: Marker, 100 bp.

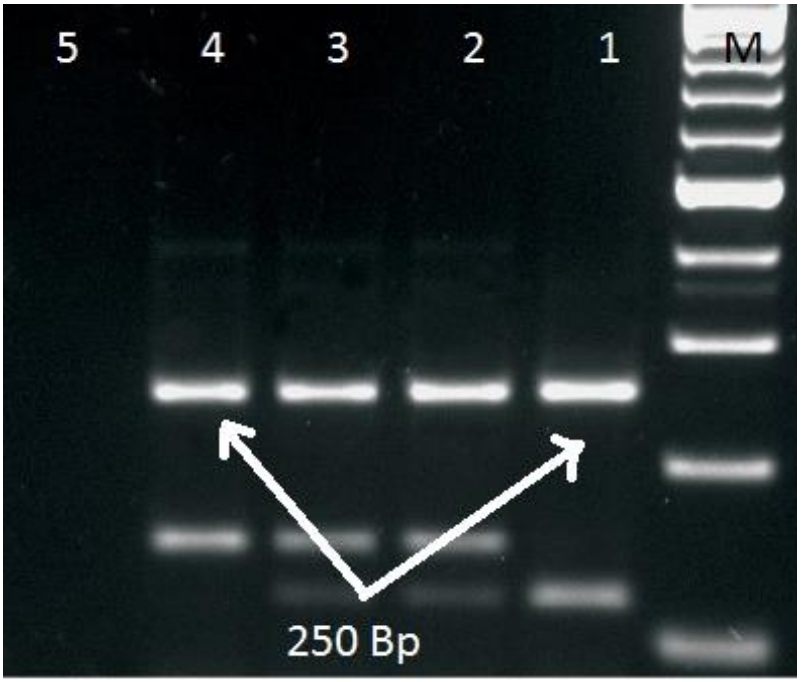

Figure 2. Detection of *trh* gene in *V. alginolyticus* isolates. Line 1: *trh* positive control (Terzi and Büyüktanır, 2009), 250 bp. Line 2, 3, 4: *trh* positive isolates, 250 bp. Line 5: Negative control distilled water. M: Marker 100 bp.

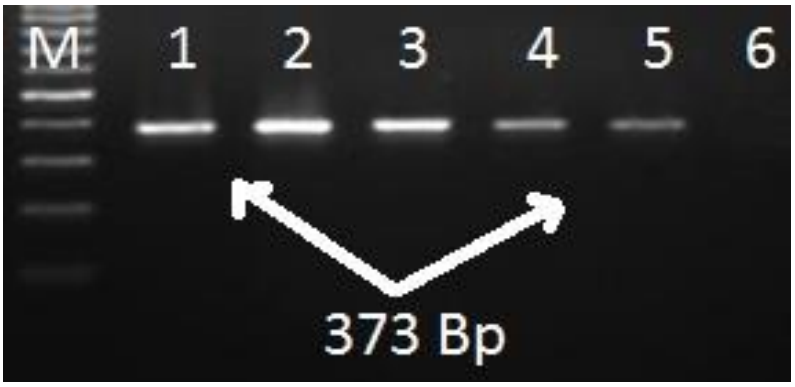

Figure 3: Detection of *tdh* gene in *V. alginolyticus* isolates. Line 1: *tdh* positive control (Terzi, Büyüktanır and Yurdusev, 2009), 373 bp. Line 2-5: *tdh* positive isolates. Line 6: Negative control distilled water. M: Marker 100 bp.

4. DISCUSSION

In this study, *Vibrio alginolyticus* was isolated from 17 of (80.95 %) sampling of black mussel. Seven isolates (42.17 %) for *trh* gene, six isolates (35.29 %) for *tdh* gene, and two isolates (11.76 %) for *trh* and *tdh* genes were found positive.

In the literature, there are studies on *Vibrio parahaemolyticus*, which has *trh* - *tdh* genes isolated from mussels but there are few studies (González-Escalona et al. 2006; Avsever, 2016) on investigating *trh* - *tdh* genes in *V. alginolyticus* bacteria isolated from mussels. So it does not seem possible to make a healthy comparison of isolation rates. Gargouti et al. (2015) isolated *V. alginolyticus* from two (20 %) of five Mantis Shrimp (*Oratosquilla oratoria*) samples collected from markets and found positive for the *tdh*, *trh* and *tox-R* genes from one (50%) of two isolates. In our study, while the isolation rate of *V. alginolyticus* was higher (80.95 %); *trh* (42.17 %) and *tdh* (35.29 %) rates were found to be relatively similar. The high isolation rate in Gargouti et. al (2015) may be due to the small number of samples used. On the other hand, Mustapha et al. (2012) isolated *V. alginolyticus* at a rate of (70 %) from shellfish, which is consistent with our study (80.95 %).

Avsever (2016) found *trh* positive *V. alginolyticus* (13.04 %) in the same region (Agean sea) between 2007-2010 from different bivalve mollusk species in Turkey. But *tdh* positive *V. alginolyticus* isolates were not reported. In this study, the *trh* positivity rate in the same region was found to be 42.17 %. This may be because the *trh* gene frequency is on the rise among Vibrio species. On the other hand, while *tdh* was not found in the study (Avsever, 2016), *tdh* positivity was found at a rate of 35.29 % in this study. This may suggest the transfer of *tdh* from other vibrios (especially *V. parahaemolyticus*) to *V. alginolyticus* (González-Escalona et al. 2006). However, further studies are needed before these can be said. Except for Avsever (2016), in Turkey, Terzi et al. (2009) noted *tdh-trh* positive *V. parahaemolyticus* in mussels from the Black Sea coast of Turkey.

Some studies have reported *V. alginolyticus* isolate carries virulence genes derived from other *Vibrio* species (Boyde et al., 2000). In America, during a *V. parahaemolyticus* outbreak, isolated *V. alginolyticus* which possessed and expressed a *trh* gene with 98% homology to the *trh2* gene of *V. parahaemolyticus* (Tada et al., 1992). *V. alginolyticus* and *V. parahaemolyticus* are highly homogeneous, with 99Æ8 and 61Æ7% similar nucleotides respectively (Osorio et al., 2000), especially because these two bacteria can exchange more often genetic material and can make each other *trh-tdh* genes in terms of the positive.

However, although there are common virulence genes between *V. alginolyticus* and *V. parahaemolyticus* and and *V. alginolyticus* might have a different virulence gene system and different pathogenic mechanism compared with *V. parahaemolyticus* although adhesion and hydrolytic

activities are also essential parameters for the infection and disease symptoms because of *V. alginolyticus* (Balebona et al., 1995).

Black mussels are eaten mainly in the Mediterranean and Aegean coastal regions of Turkey. This product, which is mostly sold uncontrolled by mobile mussels, poses a great danger, especially in the summer months. For this reason, the results of the study are also important in terms of public health. In addition, *V. alginolyticus* can be the tdh-trh reservoir for other Vibrio species. More detailed research with other vibrio species and other bivalve species should be done to clarify these.

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CONFLICT of INTEREST

The authors declare that there are no financial interests or personal relationships that could affect this work.

AUTHOR CONTRIBUTIONS

MLA; Methodology: MLA; Performing the experiment: MLA; Data analysis: MLA; Article writing: MLA, Supervision: MLA. All authors approved the final draft.

ETHICAL STATEMENTS

Local Ethics Committee Approval was not obtained. Because mussels are invertebrates and ethics committee permission is not required.

DATA AVAILABILITY STATEMENT

The data used in this study are available on the Figshare platform with the DOI address https://doi.org/10.6084/m9.figshare.11815566.v1

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Research **Araştırma Makalesi**

Histopathological Evaluation of Muscle Tissue of Horse Mackerel (*Trachurus trachurus*, Linnaeus, 1758) in Çanakkale Strait

Çanakkale Boğazındaki İstavritin (*Trachurus trachurus*, Linnaeus, 1758) Kas Dokusunun Histopatolojik Değerlendirilmesi

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Abstract: It is supported by studies that heavy metals and other water pollutants can reach humans through the food chain because of accumulation in organs of digestion, respiration, and muscles of fish, and may cause toxic effects depending on the amount of pollution and accumulation. This study was carried out by obtaining Horse mackerel samples from the Çanakkale Strait, which are fish species with high economic and nutritional value, as well as frequently consumed, seasonally (spring, summer, autumn, and winter). In the study, the tissues dissected from the dorsolateral muscles of the fresh fish were taken into Bouin's fixative, and sections were taken after routine histological follow-up. The findings revealed degenerative and inflammatory findings in the muscle tissues of fish caught especially in the autumn season. In addition, a statistically significant difference was found in autumn samples in terms of immunoreactivity ($p<0.05$).

Keywords
- Horse mackerel
- Çanakkale Strait
- Muscle tissue
- Histopathology

Özet: Ağır metaller ve diğer su kirleticilerin balıkların kas, sindirim, solunum sistemi organlarında birikmesi sonucu besin zinciri yoluyla insanlara ulaşabileceği, kirlilik ve birikim miktarına bağlı olarak toksik etkilere neden olabileceği çalışmalarla desteklenmektedir. Bu çalışma, Çanakkale Boğazı'ndan mevsimsel (ilkbahar, yaz, sonbahar ve kış) olarak avlanan, ekonomik ve besin değeri yüksek, ayrıca sık tüketilen bir balık türü olan istavrit örnekleri kullanılarak gerçekleştirilmiştir. Taze balığın dorsa-lateral kaslarından diseke edilen dokular fiksasyon için Bouin's fiksatifine konuldu ve rutin histolojik takipten sonra kesitler alındı. Bulgular, özellikle sonbahar mevsiminde yakalanan balıkların kas dokularında dejeneratif ve inflamatuar bulguları ortaya çıkardı. Ayrıca sonbahar örneklerinde immunoreaktivite açısından istatistiksel olarak anlamlı fark bulundu ($p<0.05$).

Anahtar kelimeler
- *Trachurus trachurus*
- Çanakkale Boğazı
- Kas dokusu
- Histopatoloji

1. INTRODUCTION

As a result of rapidly developing technological developments and industrialization, aquatic systems take their share from environmental pollution. Contamination of aquatic ecosystems by heavy metals and other pollutants has now become a global problem (Yılmaz, 2009). Prevention of marine pollution is one of the important goals of humanity, especially due to the negative impact of industrial developments. Despite the success in maintaining a healthy environment, the pollution problem is far

from being resolved (Moore et al., 2006). Accumulation of heavy metals such as mercury, cadmium, and lead in the bodies of aquatic organisms, especially fish, can cause serious problems (Duruibe et al., 2007). Heavy metal toxicity can damage the lungs, kidneys, liver, and other vital organs, especially the nervous system (Tchounwou et al., 2002). Long-term exposure can affect the muscular and neurological process of targeted tissue damage (Thomas and Mohaideen, 2014). The aquatic ecosystem is very sensitive to pollutants such as heavy metals and the gradual increase in the levels of such metals in aquatic environments has become a primary concern. Especially fish are among the creatures that can be highly affected by heavy metals (Ayas et al., 2007).

Fish are indicator species for monitoring metal toxicity in water. Because heavy metal ions can accumulate in such creatures more easily compared to other foodstuffs (Igwilo et al., 2006). In addition, morphological, cytological, and histopathological changes occur in different organs of the body in response to water pollution (Deore and Wagh, 2012; Atli et al., 2015; Strzyzewska et al., 2016; Kaur et al., 2018). Heavy metals are directly associated with increased incidence of cancer, neuromuscular damage, reproductive defects, and hypersensitivity to various deadly diseases (Singla, 2015). This study was realized to show the horse mackerel (*Trachurus trachurus*) specimens caught seasonally from the Çanakkale Strait which is more affected by pollution and to what extent the damage encountered organs. In the findings obtained, results have been revealed on the possibility of long-term consumption of horse mackerel, which is hunted from the Çanakkale Strait and frequently consumed by humans, to cause health problems on humans through the food chain.

2. MATERIAL and METHODS

2.1. Histological methods

In the early hours of the morning, the fish that have just died from the pier where the fishing boats docked in the Çanakkale Strait were collected seasonally. A total of 40 fish, 10 for each season, were followed up for histological examination. The tissues dissected from the dorsa-lateral muscles of the fresh fish were taken into Bouin's fixative and paraffin blocks were made after routine histological follow-up. Tissues were then fixed in Bouin's fixative for 24 hours. It was purified from water by passing through alcohol series and finally passed through xylene for transparency. For routine histopathological staining of tissues embedded in paraffin blocks, 4μ thick sections were taken in the microtome and routine Hematoxylin-Eosin (H&E) staining was performed (Çakına et al., 2021).

2.2. Immunohistochemical staining

Tissue samples were cut in a microtome with a thickness of 4 microns and taken into a water bath, and the tissue samples opened here were placed on special slides covered with Poly L-Lysine (Thermo Scientific) and adhered on a heating plate (Leica) at 40°C. All tissue samples were kept in an oven at 60°C for 1 hour, after dewaxing, they were passed through xylene twice and the paraffin was completely removed from the tissues, graded alcohols (absolute alcohol, 96% alcohol, 80% alcohol, 70% alcohol, 50% alcohol, 30% alcohol) tissue samples were both cleared of xylene and dewatered (dehydration). Tumor necrosis factor α (TNF-α), for inducible nitric oxide(iNOS) staining, is placed in heat-resistant plastic chalets containing citrate buffer (pH:6, dilution, 1:9) solution, 40 min. It was kept in a water bath (core) at 95°C for a period. Thus, the formaldehyde and paraffin used for the fixation of the tissues were completely cleaned from the tissue samples. Afterward, serum blocking solution (LAB-SA Detection System, Histostain-Plus Bulk Kit; solution A, Invitrogen) was added and left for 30 min. kept for a period. TNF-α (Ab1793) and iNOS (Ab15323) primary antibodies were administered. This procedure was done separately for each antibody. Tissue samples on which primary antibodies were added were incubated for 1 hour in a 37˚C oven. Then Seconder antibody solution (LAB-SA Detection System, Histostain-Plus Bulk Kit; A solution, Invitrogen) was added and 30 min. kept for a period. Then, enzyme conjugate solution (LAB-SA Detection System, Histostain-Plus Bulk Kit; A solution, Invitrogen) was added and 40 min. has been pending. 3,3′-Diaminobenzidene

tetrahydrochloride (DAB, Invitrogen Corporation) solution as a chromogen for 5 minutes. After it was kept in the dark, it was kept in Mayer's Hematoxylin for 5 minutes for counter-staining and tap water for 10 minutes. has been washed. Finally, it was covered with a coverslip using entellan (Bio Mount, Bio-Optica) (Numata et al., 2013; Öztürk et al., 2019).

2.3. TUNEL assay

Terminal Transferase dUTP Nick End Labeling (TUNEL, ApopTag® Plus Peroxidase In Situ Apoptosis Kit) method, which allows staining apoptotic cells, was used to determine cell death. Sections were taken after deparaffinization were washed first in distilled water and then with PBS solution for 3x5 minutes. Then 20-µg/ml Proteinase-K diluted 1/500 with PBS solution for 15 minutes at room temperature. has been applied. 5 min after washing with PBS. After being treated with 3% H_2O_2, 3x5 min. washed with PBS. Samples were incubated with Equilibration buffer for 5 minutes at room temperature. After keeping it in a humid environment with TdT-enzyme at 37˚C for 1 hour, it is kept with Stop Wash Buffer for 10 minutes. and then 30 min with Antidioxygenin Peroxidase Conjugate. treated samples 3x5 min. washed with PBS. Afterward, dyeing was done with DAB and background staining was done with Mayer's Hematoxylin. TUNEL positive cells were detected by the blind method and the averages were evaluated statistically (Öztürk et al., 2019).

2.4. Evaluation of tissue samples and statistics

Five of the sections taken from the blocks containing the dorsal muscle tissues of all fish were stained. All stained tissue samples were evaluated under Zeiss AXIO Scope 1 brand research microscope and photographed with a digital camera (AxioCam ICc 3). TNF-α and iNOS immunoreactive cells were detected using the Leica LAS V3.8 image analysis system. Staining rate semiquantitative; 0 if less than 1% of cells stain; 1+ if 1-10% of cells have staining; 2+ if 11-50% of cells have staining; 3+ if 51-80% of cells have staining; It was evaluated as 4+ if more than 80% of the cells had staining. Also, staining intensity 0=no staining; 1=pale; 2=moderate; 3=intensively determined by the blind method. Then, the total score was calculated with the formula "(1+staining intensity/3) x staining rate" (Numata et al., 2013). The resulting data were compared with the One Way-ANOVA Tukey statistical test, and $p<0.05$ results were considered statistically significant.

3. RESULTS

Degenerative and inflammatory findings were observed in horse mackerel muscle tissues stained with H&E, especially in the autumn season. Polymorphonuclear leukocyte infiltration and necrotic muscle fibrils were determined to be common among the muscle fascicles of horse mackerel specimens caught in the autumn season. In other seasons, the histopathological picture is mild, and the damage size is the lowest in the winter season (Figure 1).

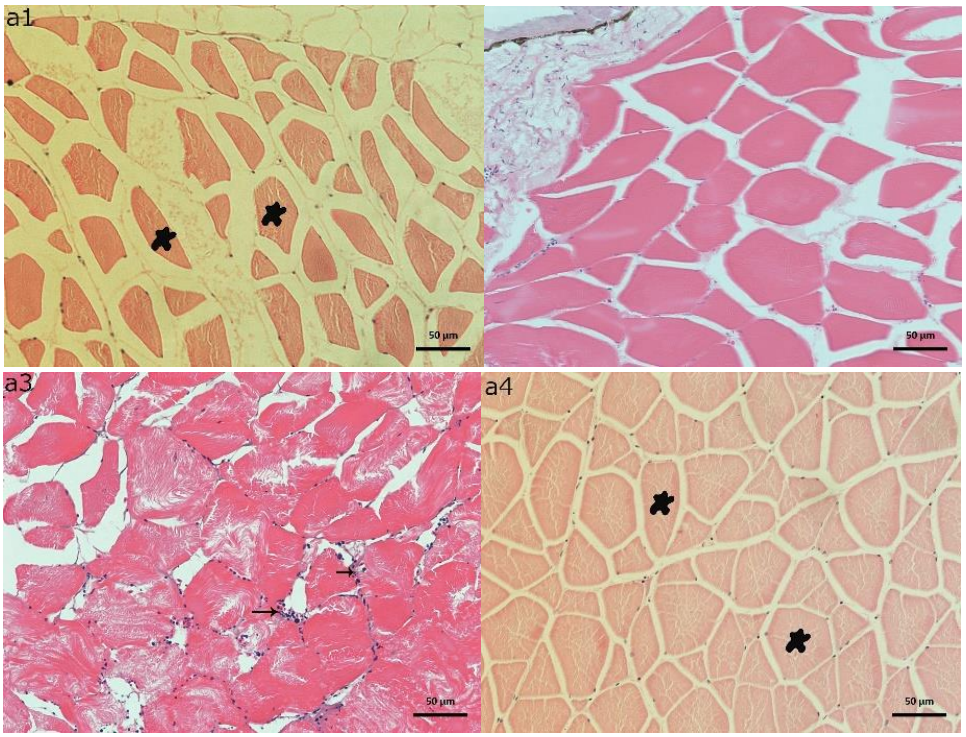

Figure 1. a1- Spring sample muscle tissue, transverse section, **a2-** Summer sample muscle tissue, longitudinal section, **a3-** Autumn sample muscle tissue, transverse section, **a4-** Winter sample muscle tissue, transverse section, H&E staining (arrow: polymorphonuclear leukocyte infiltration, star: muscle fascicle), 50 µm.

3.1. Immunohistochemical Findings

NO is a free radical with a very short half-life, which has been recognized in recent years and has proven to play an important role in many biological events such as smooth muscle relaxation, platelet aggregation, and neuronal impulse transmission. Determination of oxidative stress due to heavy metal accumulation in fish muscle tissue and its expression in muscle tissue damage were determined. It was determined that iNOS immunoreactivity was quite severe in the muscle tissue of horse mackerel in the autumn season. The severity of iNOS reactivity in the muscle tissue of the samples in other seasons was determined to be mild. There was a significant seasonal difference in tissue immunoreactivity ($p<0.05$) (Figure 2, Figure 4).

TNF-α immunoreactivity was found to be quite severe in the muscle tissue of horse mackerel specimens in the autumn season. The severity of TNF-α reactivity in the muscle tissue of the samples in other seasons was determined to be mild. There was a significant seasonal difference in tissue immunoreactivity ($p<0.05$) (Figure 3, Figure 4).

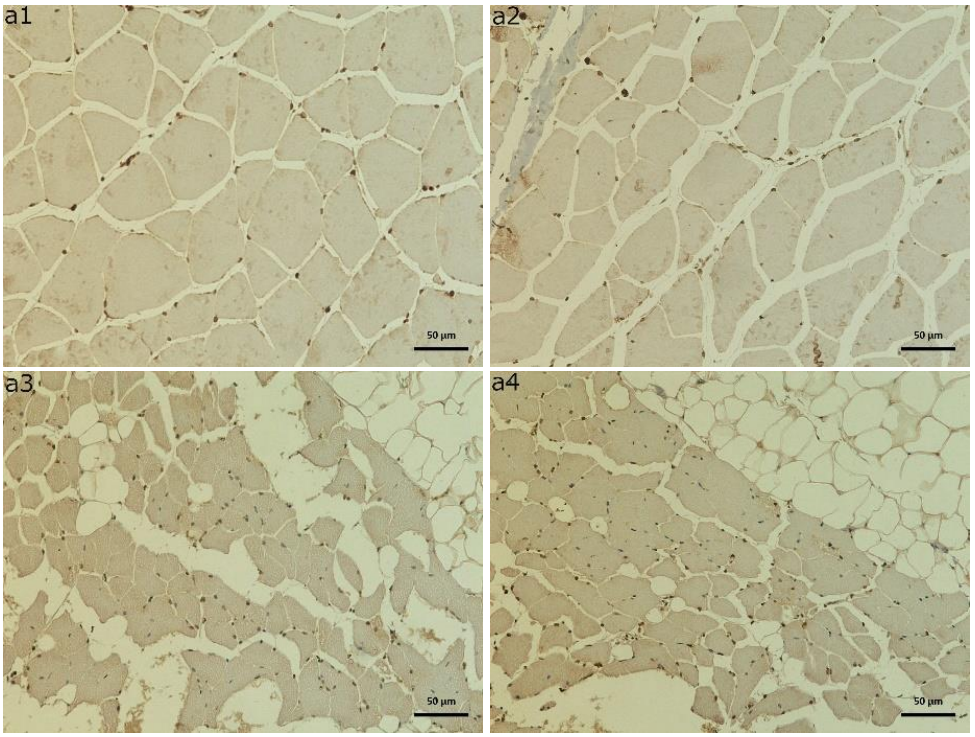

Figure 2. a1- Spring sample muscle tissue, **a2-** Summer sample muscle tissue, **a3-** Autumn sample muscle tissue, **a4-** Winter sample muscle tissue, **iNOS** reactivity, transverse section, 50 µm.

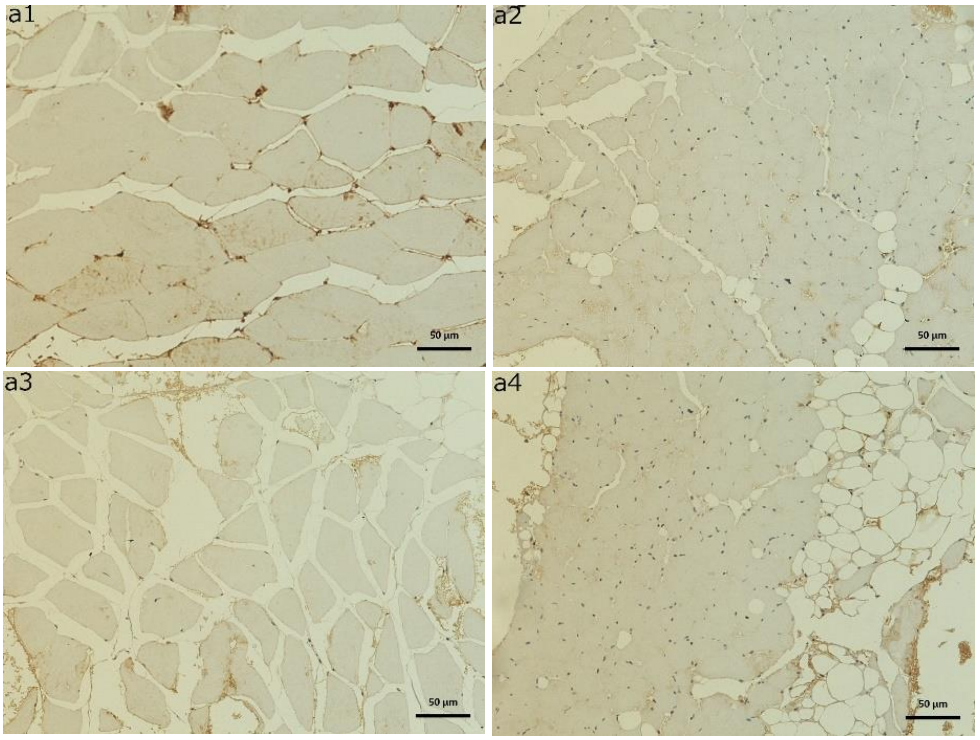

Figure 3. a1- Spring sample muscle tissue, **a2-** Summer sample muscle tissue, **a3-** Autumn sample muscle tissue, **a4-** Winter sample muscle tissue, **TNF- α** reactivity, transverse section, 50 µm.

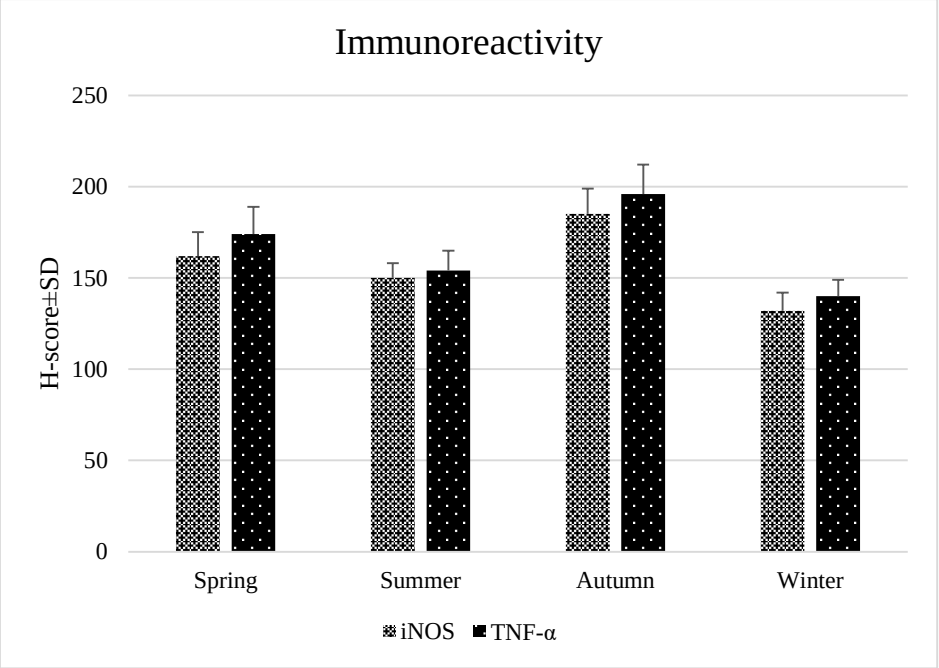

Figure 4. Distribution of iNOS and TNF-α in muscle tissue (n=8 for every sample seasonally).

3.2. TUNEL Findings

While the mechanism of programmed cell death usually occurs in embryonic tissues, disruption of oxidant-antioxidant balance and degeneration in the adult tissue are important processes that trigger apoptosis. Horse mackerel TUNEL reactivity was found to be more severe in autumn, while reactivity was mild in other seasons (Figure 5, Figure 6).

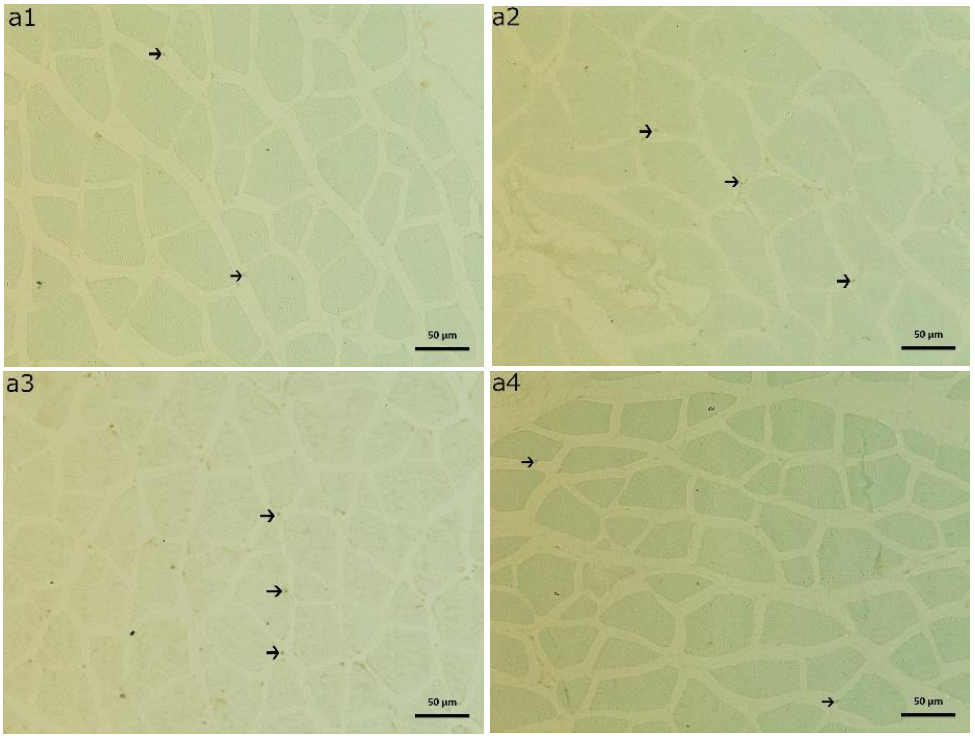

Figure 5. a1- Spring sample muscle tissue, **a2-** Summer sample muscle tissue, **a3-** Autumn sample muscle tissue, **a4-** Winter sample muscle tissue, **TUNEL** reactivity, transverse section, (arrow: apoptotic cells), 50 µm.

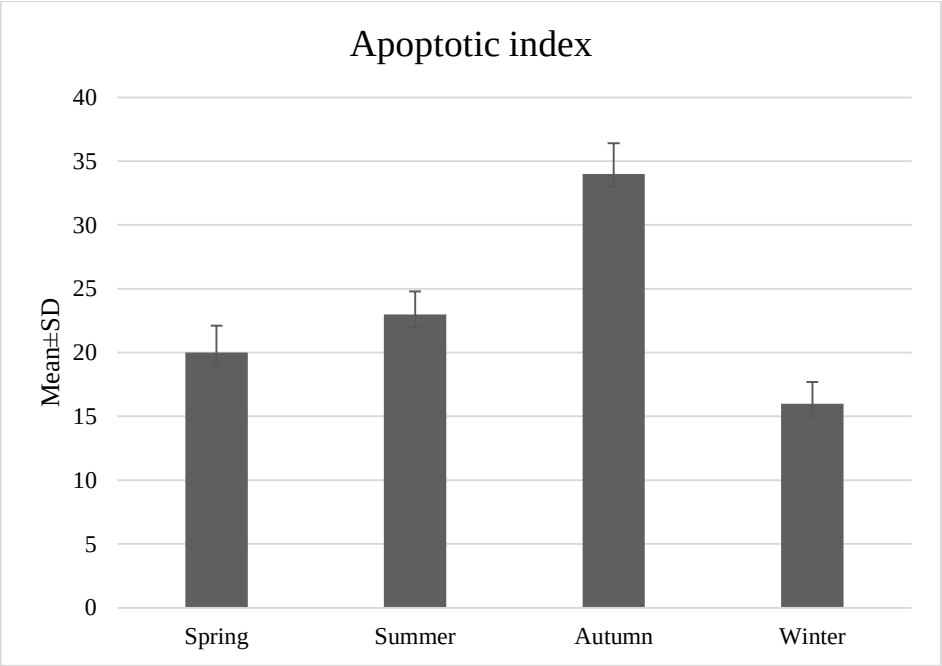

Figure 6. Apoptotic index values of muscle tissue (n=8 for every sample seasonally).

4. DISCUSSION

Today, there are almost no adequate medical and epidemiological studies on the negative effects of marine pollution on human health (Allen, 2011). Existing studies mostly focus on the formation of cancerous tissues because of the excessive accumulation of elements such as lead, cadmium, copper, nickel, and zinc (Yaman et al., 2007). This study was carried out by planning on the extent to which horse mackerel, which is one of the fish with high nutritional and economic value, is seasonally affected by pollution factors and whether it poses a threat to human health.

In studies, it has been stated that heavy metal accumulations in the seas disrupt the ecological system, this accumulation in seafood, which is a part of the food chain, cannot be ignored in terms of human health, and it may be more dangerous especially for children compared to adults (Yi et al., 2011). In a study conducted on the seawater of the Dardanelles Umurbey coast and some mollusks growing in this region (Gezen et al., 2011), because of examining the internal organs of clams, oysters, and sea snails. Zn and Mn in scallops, Zn in oysters, and Al, Zn, Fe, Cu, and Mn in sea snails were found above acceptable values. In research, the presence of many heavy metals has been detected in single and bivalve seafood grown in the Dardanelles Strait (Demir and Akkuş, 2011). In our country, the annual pollution rate is quite high compared to the regions due to the Çanakkale and Istanbul Strait transit ship passages. It is known that crustaceans accumulate pollution factors such as heavy metals with their advanced filter system. However, it is not known to what extent fish are affected by marine pollution and which fish are more affected. Support was provided with histopathological findings within the framework of the study plan on seafood, which is one of the ways to contribute to the literature and to threaten human health through the food chain. Our findings were directly on fish muscle tissue used by humans as a food source.

Histopathological biomarkers are used as a good indicator tool to reveal the effect and size of pollutants in fish. These markers have been successfully used to evaluate the effect on the vital organs of fish that respond well to toxic substances and stress (Abalaka, 2017; Dane and Şişman, 2017; Dane and Şişman, 2020). When the oxidative stress resulting from heavy metal exposure and the associated tissue damage were examined on muscle tissue, degeneration in the tissues of fish caught in the autumn season occurred more than in other seasons. In iNOS and TNF-alpha immunohistochemical

staining, the immunoreactivity of muscle fascicles was more severe. These findings are like the results of other heavy metal studies (Jabeen and Chaudhry, 2010). Al-Khayat et al. (2018), Reddy and Rawat (2013) confirmed that histopathology is invaluable biomarker for genotoxic assessments. They also drew attention to the importance of histopathological biomarkers to determine the presence of pollutants in the aquatic ecosystem (Peebua et al., 2008; Jabeen and Chaudhry, 2010; Reddy and Rawat, 2013; Viana et al., 2013). This study revealed that the histopathological changes in the muscle tissue of the horse mackerel caught in the Dardanelles vary according to the seasons and that these pathological changes are also in the muscles, albeit to a lesser extent. These histological changes may be a direct or indirect indicator of the effects of genotoxic substances, heavy metals, pesticides, salts, industrial and domestic wastes discharged into the seas. In some studies, these histopathological changes in the muscles occur as a result of exposure to various toxic substances (Mansour and Sidky, 2003; Abbas and Ali, 2007; Kaur et al. 2018; Chang et al., 2019), and in some studies, Zn reports that similar effects occur in the presence of elements such as Cu and Pb (Padrilah et al., 2018; Reddy and Rawat, 2013; Drishya et al., 2016; Abalaka, 2017; El-Khayat et al., 2018). In our previous study findings for *Sardina pilchardus* (Irkin and Öztürk, 2021), elements such as Zn, Cu, Cd, and Pb were detected, albeit in low amounts, and it was observed that histopathological changes occurred more in the samples with high detection in the autumn season. It was determined by TUNEL staining that these heavy metals, which have genotoxic effects, also increased the apoptotic index. Although it shows that heavy metal accumulation is high in metabolic organs such as organs and the liver, it is consumed in large amounts by people in muscle tissue. accumulation has also been reported. We are of the opinion that it is necessary to evaluate the results in terms of health and to focus on more comprehensive studies.

CONCLUSION

The study is a pioneering study in terms of evaluating the muscle tissue of fish caught in the Çanakkale Strait and associating it with heavy metals. Other fish species should be evaluated with similar studies and the disadvantages of their consumption should be revealed. It should be determined whether fish, which is an important food, especially in coastal cities, are exposed to pollution at a level that threatens human health. In this regard, this study results show that there is no harm in consuming horse mackerel in the autumn season,

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AUTHORS CONTRIBUTIONS

Author LCİ and ŞÖ designed the study, LCİ and RA wrote the first draft of the manuscript, LCİ, ŞÖ performed and managed statistical analyses.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICAL APPROVAL

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

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Research **Araştırma Makalesi**

Current Status, Management, and Future Prospects of Whiting (*Merlangius merlangus*) in the Sea of Marmara

Marmara Denizi'ndeki Mezgit Balığının (*Merlangius merlangus*) Mevcut Durumu, Yönetimi ve Geleceğe Yönelik Çıkarımlar

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Abstract: The current status of whiting (*Merlangius merlangus* Linnaeus, 1758) populations in the Sea of Marmara (Turkey) was evaluated by estimating growth and mortality rates in this study. The overall sex ratio (*M*:*F*) was calculated as 1:1.36. *b* value of *LWR* computed as 2.8904 for both sex groups, and *M. merlangus* showed a negative allometric growth type by Pauly's t-test result. The age of individuals in the population ranged from I to VI. The growth parameters were estimated as L_∞ = 35.74 cm, k = 0.124 yr^{-1}, and t_0 = -1.338 years for all individuals. Total mortality (*Z*), natural mortality (*M*), and fishing mortality (*F*) rates were calculated as 1.35 yr^{-1}, 0.34 $yr^{-1,}$ and 1.01 yr^{-1}, respectively. The Phi-prime growth index (φ') and exploitation rate (*E*) of the population were calculated as 2.20 and 0.75 yr^{-1}. According to the results, it is obvious that the whiting stocks in the Marmara Sea are currently used at a high capacity (E = 0.75 yr^{-1}). The impact of over-fishing can have increasingly detrimental effects on the overall population size of this population. Fisheries management practices in the Marmara Sea should be regulated by taking into account the ecosystem change, fishing fleet, and unreported catch data. In addition, temporal or spatial fishing bans can be applied by increasing the selectivity of fishing gear.

Keywords
- Age
- Growth
- Exploitation rate
- Marmara Sea
- LWR

Özet: Bu çalışmada, Marmara Denizi'ndeki (Türkiye) mezgit (*Merlangius merlangus* Linnaeus, 1758) popülasyonunun büyüme ve ölüm oranları tahmin edilerek mevcut durumu değerlendirilmektedir. Cinsiyet oranı (*E*:*D*) 1:1,36 olarak hesaplanmıştır. LWR *b* değeri her iki cinsiyet için 2,8904 olarak hesaplandı ve Pauly's t-testi sonuçlarına göre mezgit popülasyonu negatif allometrik büyüme gösterdi. Popülasyondaki bireylerin yaşları I ile VI arasında değişim göstermiştir. Büyüme parametreleri, tüm bireyler için L_∞ = 35,74 cm, k = 0,124 $yıl^{-1}$, and t_0 = -1,338 yıl olarak tahmin edilmiştir. Toplam ölüm (*Z*), doğal ölüm (*M*) ve balıkçılık ölüm (*F*) oranları sırasıyla 1,35 $yıl^{-1}$, 0,34 $yıl^{-1}$ and 1,01 $yıl^{-1}$ olarak hesaplanmıştır. Popülasyonun Phi-prime büyüme indeksi (φ') ve sömürü oranı (*E*) 2,20 ve 0,75 $yıl^{-1}$ olarak hesaplanmıştır. Sonuçlara göre Marmara Denizi'ndeki mezgit stoklarının şu anda yüksek kapasitede kullanıldığı aşikardır (E = 0,75). Aşırı avlanmanın etkisi, genel popülasyon büyüklüğü üzerinde giderek daha fazla zararlı etkiye sebep olabilir. Marmara Denizi'ndeki ekosistem değişimi, balıkçı filosu ve rapor edilmemiş av verileri dikkate alınarak balıkçılık yönetimi uygulamaları düzenlenmelidir. Av araçlarının seçiciliği artırılarak zamansal veya mekânsal avlanma yasakları uygulanabilir.

Anahtar kelimeler
- Yaş
- Büyüme
- Sömürülme oranı
- Marmara Denizi
- LWR

1. INTRODUCTION

The whiting (*Merlangius merlangus* Linnaeus, 1758) (Gadiformes, Gadidae) is bento-pelagic fish and widespread in the Black Sea, Adriatic Sea, Mediterranean Sea, and Aegean Sea (Akşıray, 1987). The adults of the whiting adapted to live in 5-16 °C water (Özdemir et al., 2018) and it is found in waters at a depth of 50 meters to 100 meters and on muddy, sandy bottoms (Frattini and Casali, 1998). The species reaches a maximum of 70 cm, and the average length is 23.5 cm (Nedreaas et al., 2014). Whiting fisheries mostly occurred by bottom trawls, gill nets, line fishing, and deep water cast nets in Turkey (Zengin, 2019; Karadurmuş et al., 2021). 93% of the whiting amount, which was 9363 tons in 2020, was obtained from the Black Sea (TÜİK, 2020). It is caught with gillnets in the Sea of Marmara and is among the most caught species as bycatch in shrimp fisheries by beam trawl. Previous studies on whiting were mostly concentrated in the Black Sea (Düzgüneş and Karaçam, 1990; Erkoyuncu et al., 1994; Samsun, 1995; Şahin and Akbulut, 1997; Samsun and Erkoyuncu, 1998; Kalaycı et al., 2007; Bilgin et al., 2012; Kasapoğlu and Düzgüneş, 2014; Mazlum and Bilgin, 2014; Samsun and Akyol, 2017; Taylan et al., 2018; Türker and Bal, 2018; Balık and Öztaş, 2019; Yıldız and Karakulak, 2019; Aksu, 2020).

Whiting is one of the important commercial demersal fisheries products in the Sea of Marmara. Annual catch statistics for whiting show a continuous decrease (about 90%) pattern in the Marmara Sea since the 2000s, especially since 2009 (TÜİK, 2020). Anthropogenic activities, environmental and biotic factors affect the dynamics of fish species in the Mediterranean and the Sea of Marmara (Brosset et al., 2015). High fishing pressure, poor fisheries management, and limited fishing regulations are responsible for the decline in stocks (Zengin and Akyol, 2009). In addition, the mucilage event seen in November 2020 seems to affect the entire ecosystem, especially fish species (Karadurmuş and Sarı, 2022). The previous main study (Atasoy et al., 2006) was carried out on the whiting growth and mortality in the Marmara Sea. The other studies (Bok et al., 2011; Demirel and Dalkara, 2012; Daban et al., 2020) only examined the length-weight relationship (*LWR*) of whiting in the Marmara Sea. Finally, Bal (2021) examined the limited population parameters of whiting in the Sea of Marmara. Despite its economic and ecological importance, the data on the growth, mortality, and exploitation rates of *M. merlangus* for the Sea of Marmara is not available in recent years. The study aimed to reveal the status of whiting stocks in the Sea of Marmara and examine them in fisheries management. National catch statistics indicate that whiting stocks in the Marmara Sea have decreased significantly. Data on the current status of populations (sex ratio, size, and age distribution), growth parameters, mortality, and exploitation rates will be used to provide essential input to demographic models that will be used to estimate the species recovery.

2. MATERIAL and METHODS

The field studies were conducted in GFCM (General Fisheries Commission for the Mediterranean) Geographical Sub-Area 28 (the Sea of Marmara) (Figure 1). Sampling studies were carried out intensively around the Kapıdağ Peninsula. A total of 33 beam trawls and 17-gill net operations were carried out in different points of the study area. The whiting specimens (n = 2522) were captured at depths ranging from 25 to 195 m by using a twin commercial shrimp trawl (10 m beam width and a 32 mm mesh size), gill nets (18-20-22-24 mm mesh size), and line fishing at monthly intervals between August 2020 and July 2021. These nominal mesh sizes were measured as the stretch measure (from knot to knot). This method allowed the sampling of the smallest individuals in the population. The MEDITS (Mediterranean International Trawl Survey) protocol was followed at all stages of the trawl survey (sampling gear characteristics, the design of the survey, the sampling methodology, and the processing of samples). Total length (*TL*) was measured using an ichthyometer with 0.01 cm precision, while body weight (*W*) was weighed using a scale with 0.01 g precision. Sex distinction was made according to the shape and color of gonads. All biological and morphometric studies were carried out

in the laboratory. The overall sex ratio (*M*:*F*) was calculated as the proportion of males to females and the difference from a balanced ratio (1:1) (Conover and Van Voorhees, 1990; Vazzoler, 1996) was analyzed using the Chi-squared (χ^2) test (Düzgüneş et al., 1983). Changing sex ratios according to size group (cm) and age (year) were examined. The *LWR* was estimated according to Froese (2006), power equation as $W = a \times TL^b$. Where *W* is the body weight (g), *TL* is the total length measurement (cm), *a* and *b* are the regression parameters. This equation was used to transform its logarithmic form as In (*W*) = In (*a*) + *b* In (*TL*). The confidence limits (*Cl*) of regression parameters and the coefficient of determination (r^2) were used to evaluate the correlation between *W* and *TL*. Pauly's t-test was used to determine if coefficient *b* was significantly different from 3 (Zar, 1999). In the determination of growth rates of *TL* and *W*, the following formula *TL* increment (%) = $[(TL_n - TL_{n-1})/TL_{n-1}] \times 100$, and *W* increment (%) = $[(W_n - W_{n-1})/W_{n-1}] \times 100$ were used, where n is age-class (Ricker, 1975).

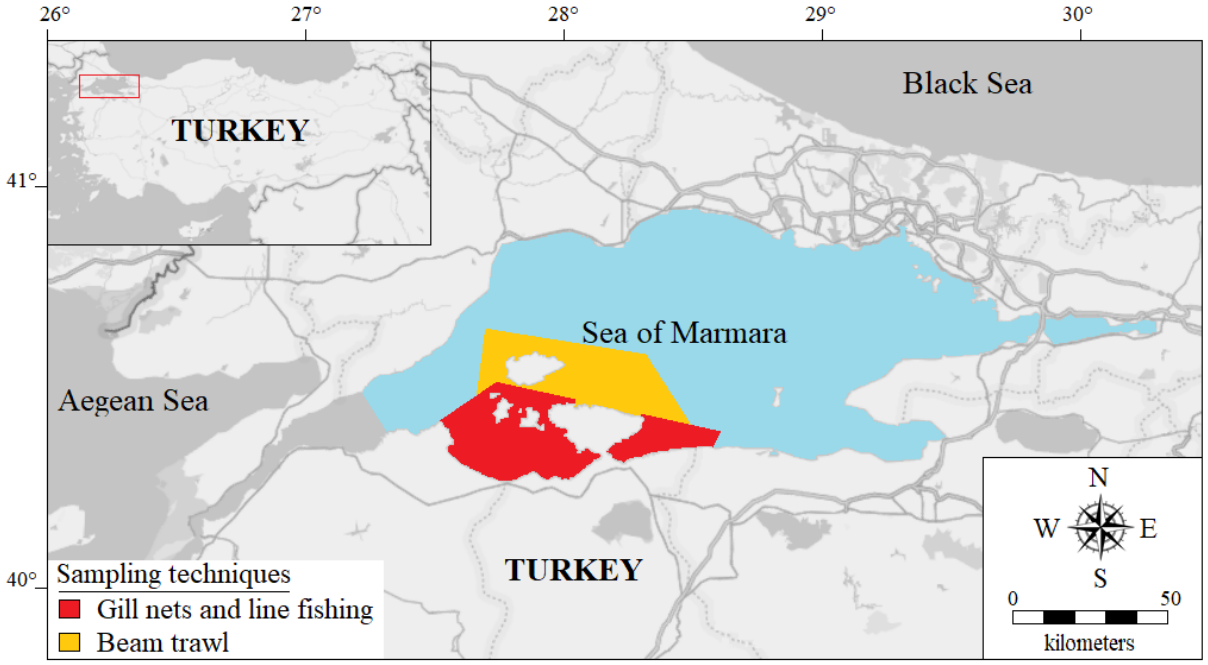

Figure 1. Map of the study area. The red area represents the sampling region made with gillnet and line fishing, and the orange area represents the region where trawl hauls were made.

All samples were grouped into length classes of 1 cm, and sagittal otoliths were removed for each size class. The otoliths were cleaned and stored for further processing and readings. Otoliths were ground manually with various abrasive papers to clarify the first annulus. These otoliths were immersed in glycerin in a petri dish and viewed under the light in a stereomicroscope. The age rings were counted according to Ross and Hüssy (2013) by using a monitoring system (Leica EZ4E (Leica Microsystems, Wetzlar, Germany) camera system). The association of one opaque zone and one translucent zone was regarded as an annulus (Campana, 2001; Ross and Hüssy, 2013). Age estimates were obtained from 618 individuals. Independent two readers undertook all readings for each otolith without prior information on length and sex. Growth curves were fitted using the least-squares method using the von Bertalanffy (1938) growth equation (VBGF):

$$L_t = L_\infty (1 - e^{-k(t - t_0)})$$

where L_∞ is asymptotic length (cm), *k* is the growth rate (yr^{-1}), *t* is age (year), and t_0 is the hypothetical age at zero-length (year). These parameters are commonly used to evaluate the current status of the fish populations by associating the values of their mortality coefficients. Phi-prime growth index (φ') was calculated using the formula Munro and Pauly (1983):

$$\varphi' = \log k + 2 \log L_\infty$$

The growth index makes it possible to compare the growth of different populations. The total mortality rate (Z) was calculated using age-based catch curve analysis (Chapman and Robson, 1960). According to the Pauly (1980) model, the natural mortality (M) rate was calculated using the following equations:

$$\log M = -0.0066 - 0.279\ (\log L_{\infty}) + 0.6543\ (\log k) + 0.4634\ (\log T)$$

The fishing mortality was calculated according to the formula: $F = (Z - M)$, and the rate of exploitation was calculated according to the formula: $E = (F / Z)$. The sea surface temperature was measured each month with a YSI® ProDss (Xylem, Rye Brook, NY) multimeter in all stations, the annual mean value (16.4 °C) was used to calculate the natural mortality rate (M). Significance levels for all statistical tests were established at $P = 0.05$ a prior with SPSS v0.26 (IBM Corp., Armonk, NY). The normality of the data was checked using the Kolmogorov-Smirnov test and the homogeneity was analyzed using the ANOVA (Analysis of Variance) test. Therefore, non-parametric test Mann-Whitney U and Kruskal Wallis H were used to analyze the statistical differences in data according to size class, age, and gender. Monthly and combined variables were analyzed using Pearson correlation and regression analysis to search for relationships among morphological characters (Sokal and Rohlf, 1969).

3. RESULTS

3.1. Population status

Sex, size, and age data were gathered from a total of 2522 *M. merlangus* samples, of which 1454 (57.65%) were females, 1068 (42.35%) were males. The overall sex ratio (M:F) was 1:1.36 which is highly significantly different from the balanced ratio of 1:1 ($\chi^2 = 59.079$; $df = 1$; $P < 0.001$). Males were dominant during the early ages, but after the age of 2 sex ratio changed in favor of females. Males dominated in the length intervals between 5 − 7 cm, and females those beyond 12 cm significantly. The sex ratio is balanced (1:1) in 2 age classes and 12 cm size classes (Table 1).

Table 1. Sex-ratios (*M*:*F*) of *Merlangius merlangus* according to size classes (*df*: 1, χ^2: Chi-square value, –: not calculated, *ns*: not significant, **: $P < 0.01$, ***: $P < 0.001$)

Size classes (*TL*, cm)	Female	Male	Sex ratio	χ^2	*Sig.*
5	0	3	-	-	-
6	3	10	1:0.30	3.77	*ns*
7	8	18	1:0.44	3.85	*ns*
8	26	32	1:0.81	0.62	*ns*
9	28	32	1:0.88	0.27	*ns*
10	42	58	1:0.72	2.56	*ns*
11	60	68	1:0.88	0.50	*ns*
12	106	108	1:0.98	0.02	*ns*
13	142	114	1:1.25	3.06	*ns*
14	266	162	1:1.64	25.27	***
15	254	176	1:1.44	14.15	***
16	196	140	1:1.40	9.33	**
17	150	72	1:2.08	27.41	***
18	76	38	1:2.00	12.67	***
19	54	21	1:2.57	14.52	***
20	19	6	1:3.17	6.76	**
21	9	6	1:1.50	0.60	ns
22	11	4	1:2.75	3.27	ns
23	3	0	-	-	-
24	1	0	-	-	-
Total	1454	1068	1:1.36	59.079	***

TL and *W* of the whiting specimens used in the analysis ranged between 5.35 − 24.2 cm and 2.40 – 98.35 g. Details on the length and weight of the whiting samples are given in Table 2. Significant differences occurred in the *TL* (*U*: $z = -7.464$; $P < 0.05$) and *W* (*U*: $z = -7.263$; $P < 0.05$) between females and males. Most fish (79.3%) were within the 12 − 18 cm length groups, and 76.14% of all samples with a mean of 14.67 cm *TL* were over minimum landing size (*MLS* > 13 cm) according to national fishery regulations (BSGM, 2020) (Figure 2). The statistical data relevant for the evaluating of the *LWR* of *M. merlangus* is included in Table 2, showing the estimated regression parameters along with their 95% confidence interval, growth type of populations, and the coefficient of correlation. The value of *a* varied between 0.0083 and 0.0122. According to the *b* value obtained from the LWR equations, Pauly's t-test result showed that *M. merlangus* ($t_{combined} = 1.961$, $P < 0.05$) exhibit negative allometric growth ($b < 3$) for males, females, and combined sexes. The high values of coefficient of determination ($r^2 > 0.95$) were calculated for whiting.

Table 2. Descriptive statistics and total length (cm) and weight (g) relationships for *Merlangius merlangus* by sex.

Sex	***n***	**Mean *TL* ± *SE* (Min-max)**	**Mean *W* ± *SE* (Min-max)**	**Regression parameters**			**Confidence intervals (95%)**		***Sig.***	**Growth**
				a	***b***	r^2	***Cla***	***Clb***		
Female	1454	15.05 ± 0.07 (6.10 – 24.20)	28.00 ± 0.38 (2.50 – 98.35)	0.0090	2.9312	0.9569	0.0083 – 0.0098	2.8992 – 2.9632	< 0.05	A^-
Male	1068	14.14 ± 0.09 (5.35 – 22.80)	23.84 ± 0.41 (2.40 – 89.40)	0.0112	2.8509	0.9602	0.0102 – 0.0122	2.8160 – 2.8858	< 0.05	A^-
Combined	2522	14.67 ± 0.06 (5.35 – 24.20)	26.24 ± 0.28 (2.40 – 98.35)	0.0101	2.8904	0.9594	0.0095 – 0.0107	2.8671 – 2.9136	< 0.05	A^-

* *n*, sample size; ±*SE*, standard error; min, minimum; max, maximum; *a*, regression intercept; *b*, regression slope; *CI*, confidence interval; r^2, coefficient determination; A^-, negative allometric growth

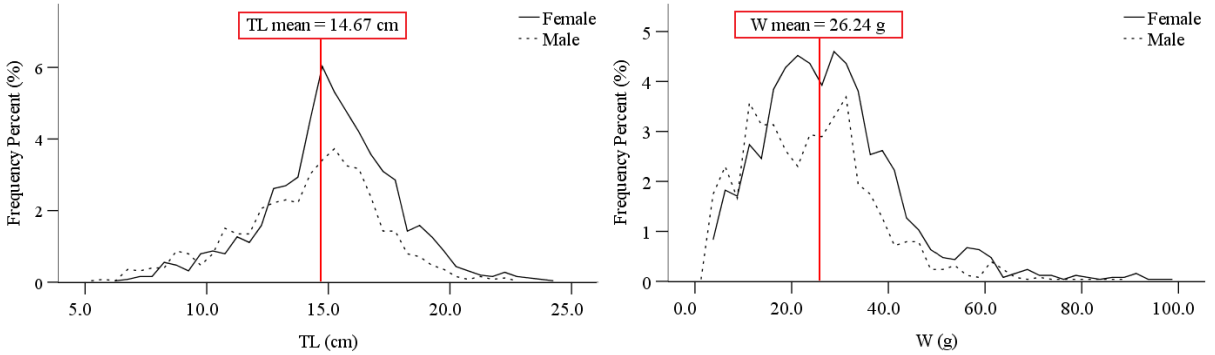

Figure 2. Sex-specific length and weight frequency distribution of *Merlangius merlangus*. Red lines indicate mean values for combined sexes (n = 2522).

The results of the otolith reading are given in Table 3. Six age classes (I − VI) were found for both sexes. Most of the samples (87.22%) consisted of younger fish below age V. Female fish were more abundant in the older age classes. Both females and males were dominant in age groups III and IV, and males more than IV years old were rare (3.39%) in the population (Figure 3). Descriptive statistics of *TL* and *W* of *M. merlangus* were given by age and sex in Table 4. The mean *TL* revealed significant differences between age groups both females (H = 583.9; df = 5; $P < 0.05$) and males (H = 460.5; df = 5; $P < 0.05$). Our results contained enough samples of the youngest fishes to accurately estimate growth at the earliest ages (below two years).

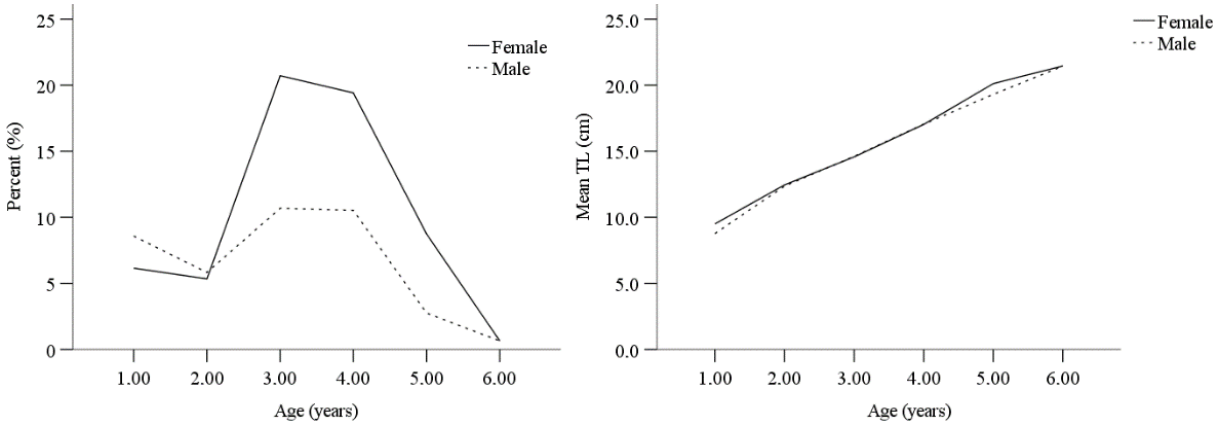

Figure 3. The proportion of specimens and length-frequency distributions by age groups.

Table 3. Age–length key of *Merlangius merlangus* from the Marmara Sea.

Total length (cm)	Age groups						
	1	**2**	**3**	**4**	**5**	**6**	**Total**
5	3						3
6	7						7
7	10						10
8	22						22
9	20						20
10	18	3					21
11	11	17					28
12		34	9				43
13		11	40				51
14		2	82	6			90
15		2	45	37	1		85
16			9	55	3		67
17			9	48	8		65
18				25	4		29
19				5	22	2	29
20				4	12	1	17
21				3	9	2	14
22				2	9	2	13
23					3		3
24						1	1
Total	**91**	**69**	**194**	**185**	**71**	**8**	**618**

3.2. Growth parameters, mortality, and exploitation rate

Age at length data was used to calculate the *VBGF* parameters (Table 5) and growth curves (Figure 4). No significant differences occurred between observed and predicted *TL* of different ages of specimens (U = 68.000; z = -0.231; $P > 0.05$). The population grew fairly slowly, achieving a mean observed size at age VI. The growth increments had no significant difference between sexes (F = 0.264; df = 2; $P > 0.05$); while the growth increments had significant differences between age groups (F = 36.310; df = 5; $P < 0.05$). Female fishes reach a significantly larger asymptotic length (L_∞ in cm) compared to males. The t_0 value was close to zero for both sexes, indicating a good growth for the smallest fish. However, estimates of the growth coefficient ($k \cdot yr^{-1}$) for both sexes (close to 1 per year) indicated slow growth in females and males. Growth parameters suggested that males (φ' = 2.20) grew relatively faster than females (φ' = 2.21). While the growth index (φ') of *M. merlangus* was estimated as 2.20 the exploitation rate (E) was calculated as 0.75. The mortality and exploitation rate of whiting are given in Figure 5.

Table 4. Variations in biometric measurements (Total length-*TL* in cm, Body weight-*W* in g) by age groups.

Sex	Summary	Age I		Age II		Age III		Age IV		Age V		Age VI	
		TL	***W***	***TL***	***W***	***TL***	***W***	***TL***	***W***	***TL***	***W***	***TL***	***W***
Female	*n*	38		33		128		120		54		4	
	Mean	9.51	7.10	12.45	14.15	14.57	23.74	17.03	37.59	20.11	62.01	21.45	75.76
	±*SE*	0.22	0.39	0.18	0.60	0.10	0.60	0.13	0.95	0.25	2.28	1.24	11.00
	Min	6.10	3.05	10.20	7.90	12.00	10.20	14.10	19.50	15.80	30.20	19.20	54.90
	Max	11.80	11.60	15.00	24.80	17.80	47.20	22.90	93.20	23.30	92.40	24.20	98.35
Male	*n*	53		36		66		65		17		4	
	Mean	8.77	5.97	12.36	14.23	14.61	24.59	17.05	38.01	19.30	55.04	21.44	67.15
	±*SE*	0.21	0.36	0.15	0.57	0.12	0.78	0.20	1.53	0.40	3.86	0.36	3.42
	Min	5.35	2.40	10.60	10.10	12.50	14.30	15.00	19.90	17.00	32.00	20.70	60.40
	Max	11.90	14.20	15.00	27.00	17.30	44.00	22.20	89.40	22.80	86.40	22.25	74.60
Combined	*n*	91		69		194		185		71		8	
	Mean	9.08	6.44	12.41	14.19	14.58	24.03	17.04	37.74	19.92	60.34	21.44	71.46
	±*SE*	0.16	0.27	0.12	0.41	0.08	0.47	0.11	0.81	0.22	1.98	0.60	5.58
	Min	5.35	2.40	10.20	7.90	12.00	10.20	14.10	19.50	15.80	30.20	19.20	54.90
	Max	11.90	14.20	15.00	27.00	17.80	47.20	22.90	93.20	23.30	92.40	24.20	98.35

n, sample size; ±*SE*, standard error; min, minimum; max, maximum

Table 5. Estimates of von Bertalanffy growth parameters by sexes.

Growth Parameters	Female	Male	Combined
L_∞ (cm)	39.61	35.36	35.74
W_∞ (g)	434.24	290.99	311.57
t_0 (year)	-1.622	-1.257	-1.338
$k \cdot yr^{-1}$	0.101	0.129	0.124
φ'	2.20	2.21	2.20

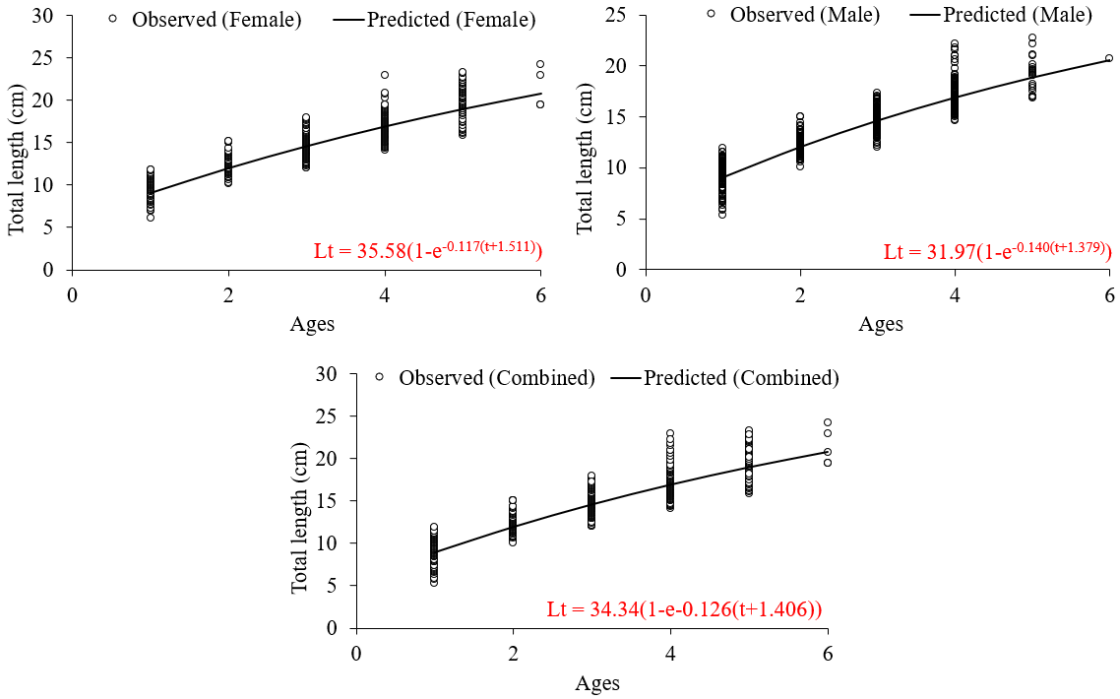

Figure 4. Length-at-age data of whiting used in the present study. Lines show estimated von Bertalanffy growth curves

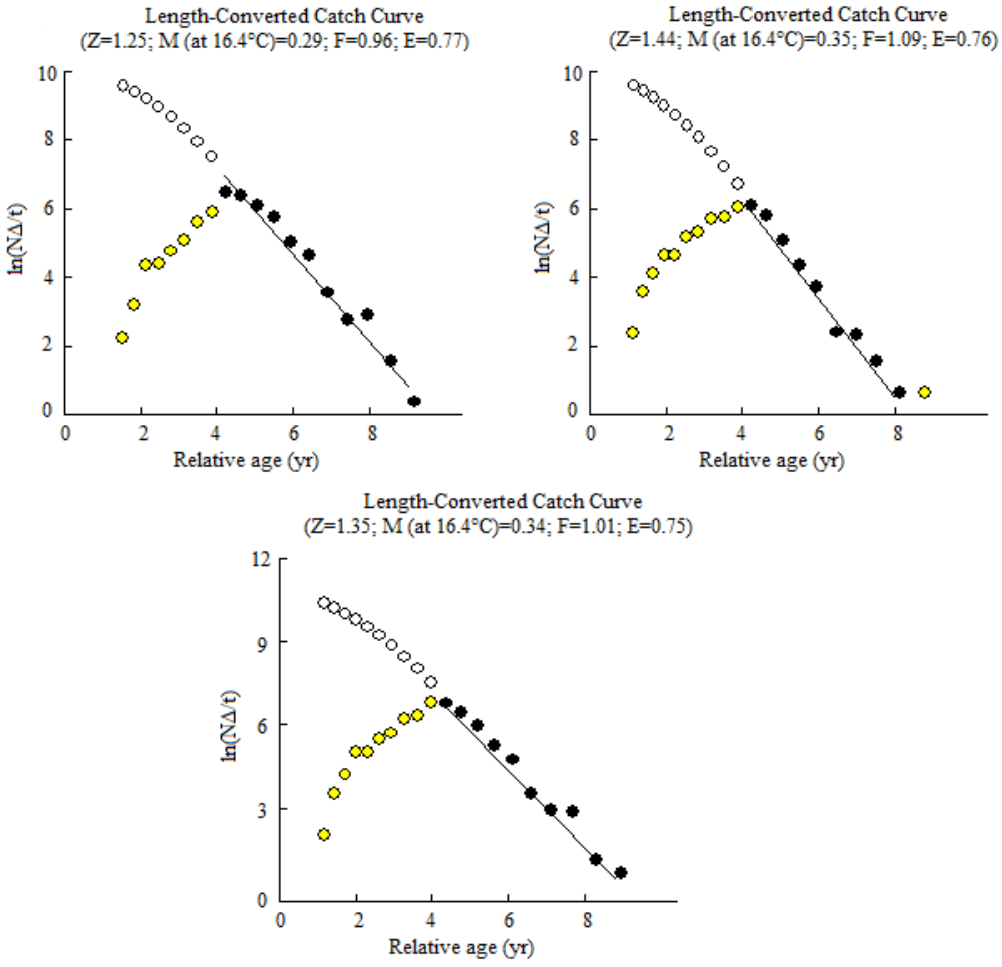

Figure 5. Estimation of mortalities using length converted catch curve analysis for whiting (○= not used in the analysis; ● = used in the analysis).

4. DISCUSSION

The number of females was found higher than males with a ratio of 1:1.36 (*M*:*F*). Unlike this study, Atasoy et al. (2006) and Bal (2021) reported that males were more dominant in the Sea of Marmara as 1:0.78 (*M*:*F*) and 1:0.64 (*M*:*F*), respectively. Many fish species tend to invest equally in the production of females and males. In natural populations, a sex ratio of 1:1 is expected which generally occurs in more stable environments that do not suffer from frequent oscillations (Fisher, 1930). Several factors, such as sampling methods, predation, variations in environmental conditions, changes in recruitment or mortality of individuals of a particular sex, may also promote changes in the sex ratio in fish (Garcia et al., 2004). The sex ratio of whiting, in our opinion, is affected not only by the location surveyed, but also by the type of fishing gear utilized. Thus, commercial fishing gear is more selective in its size composition and can mislead the results by catching larger fish.

Whiting populations may differ in terms of density, distribution, or growth rates, even in small spatial scales. These differences could be attributed to local environmental factors such as hydro geography, depth, sediment particle size, and temperature. Some previous studies reported individuals with longer than 24 cm in the Sea of Marmara (Göksungur, 2004; Demirel and Dalkara, 2012; Bal, 2021). The expected range for *a* was reported between 0.001 and 0.05 for the natural fish populations by Froese (2006) and the obtained value for all species was in accordance with the expected range. The value of *a* may differ between environments, daily or seasonally (Bagenal and Tesch, 1978). The results show that *M. merlangus* invest more in length than in weight ($b < 3$), as has been observed for previous studies (Demirel and Dalkara, 2012; Çalık and Erdoğan Sağlam, 2017; Samsun and Akyol, 2017; Aksu, 2020). The *LWR* parameters vary too between locations, depending on the competitors, abundance of food and reproductive activity, and over time (Yankova, 2016). Contrary to our study, it has been reported that *M. merlangus* shows positive allometric growth ($b > 3$) in previous research conducted in the Sea of Marmara (Bok et al., 2011; Bal, 2021). Compared to the earlier studies (Demirel and Dalkara, 2012; Kasapoğlu and Düzgüneş, 2014; Yıldız and Karakulak, 2019; Bal, 2021), some slight differences in r^2 values in the present study were regular which may be based on many factors such as season, length range, fish physiology, sampling size and habitat (Froese, 2006).

Similar age groups (Özdamar and Samsun, 1995) and older (Samsun et al., 1994; Şahin and Akbulut, 1997; Çiloğlu et al., 2001) were reported for specimens from the Black Sea. However, several authors reported a shorter life cycle (below age VI) in the Sea of Marmara (Atasoy et al., 2006) and Black Sea (Düzgüneş and Karaçam, 1990; Yıldız and Karakulak, 2019), which may be attributed to the selectivity factor of fishing gear or sampling methods (Froese, 2006). However, Çiloğlu et al. (2001) found the oldest fish among 9 years old individuals in the literature. Because the Sea of Marmara is a closed basin, populations are extremely sensitive, and excessive fishing pressure destroys stocks without growth. Whiting is one of the most caught species as bycatch in shrimp beam trawl fishery (Zengin and Akyol, 2009; Aslan İhsanoğlu and İşmen, 2020), the whiting stocks in the Sea of Marmara cannot resist fishing pressure and are overexploited. Another probable reason may be the number of samples, sampling season, or the variations of sampling methods. In the Black Sea, especially individuals in the 5-7 cm length group corresponded to 0 years of age (Polat and Gümüş, 1996; Özdemir et al., 2006; Erdoğan Sağlam and Sağlam, 2012; Mazlum and Bilgin, 2014), but no individuals aged 0 years were found in this size group in the Sea of Marmara (Atasoy et al., 2006; this study). It should be noted that different stocks of the same species may display variables due to different feeding conditions (Erkoyuncu, 1995).

Growth parameters have been the main indicator to identify fishing pressure levels and growth on fish stocks. The theoretical length of the individuals in this study (L_∞ = 35.74 cm *TL*) was lower than values estimated for the Sea of Marmara (L_∞ = 38.5 cm *TL in* Atasoy et al., 2006). Although the possibility exists that the maximum length of *M. merlangus* is longer than 45.36 cm (Şahin and Akbulut, 1997), no evidence was to support this in literature. Due to variable results of growth

parameters (k, L_∞), the growth index (φ') is commonly preferred for the estimated growth performance of fishes. Most studies (Erdoğan Sağlam and Sağlam, 2012; Yıldız and Karakulak, 2019) estimated growth index of whiting above 0.2 yr^{-1} in the Black Sea, as demonstrated in this study from the Sea of Marmara ($\varphi' = 2.2$ yr^{-1}). Differences in growth index between regions may be due to environmental conditions (such as temperature and food availability) between sampled areas (Tuck et al., 1997) or fishing pressure (Campana, 2001). Although geographical differences may affect growth parameters, further studies are needed to determine what factors account for these differences. Older populations up to 9 years of age have been reported in the Black Sea (İşmen, 1995; Süer, 2016). No individuals older than 6 years of age were found in the Sea of Marmara (Atasoy et al., 2006; this study). In addition, individuals corresponding to age 0 could not be sampled in these studies. The differences in growth parameters in the Black Sea and the Marmara Sea may be due to the smaller individuals in the populations in the Marmara Sea and the differences in the sampling method in which individuals aged 0 are neglected. It is worth noting that the higher growth rates (above 2.3 yr^{-1}) reported in the eastern Black Sea (Düzgüneş and Karaçam, 1990; Çiloğlu et al., 2001) were based on the low fishing pressure. Although geographical differences may affect growth parameters, it is unclear whether these differences are due to environmental factors or fishing pressure (Campana, 2001).

No studies have focused on the exploitation rate of the whiting fishery by estimating mortality in the Marmara Sea. In this study, the mortality parameters are mostly higher than the previous studies performed for the Black Sea (Samsun and Erkoyuncu, 1998; Yıldız and Karakulak, 2019). The total mortality rate ($Z = 1.35$ yr^{-1}) was similar to the one found by Erdoğan Sağlam and Sağlam (2012), in the southeastern Black Sea. They estimated the total mortality (Z) rate of 1.68 yr^{-1} and an exploration (E) rate of 0.84. Growth and mortality rates are important to understand the dynamics of populations and to evaluate possible sustainable harvests (Campana and Thorrold, 2001). The natural effects on mortality in the fished populations or the impact of fishing can be confused (Pauly, 1980). If the exploitation rate is higher than 0.5 may be a sign of a heavily fished population (Patterson, 1992). This observed high mortality in this study ($E = 0.75$) might be caused by food limitations, diseases, predators, or commonly illegal fishing activities. More studies are needed to determine which of these factors is responsible for the high mortality rate. As with all marine populations, the whiting will not show excessive resistance to overfishing exploitation, so management and conservation measures should be taken to minimize the impact of fishing gear on stocks (Ridgway et al., 2006).

4.1. Implications for whiting fishery management

The Marmara Sea is under the influence of significant anthropogenic activities that adversely affect the well-being of its ecosystem (Akoğlu, 2021). Fish species are also influenced by the adverse impacts of anthropogenic activities (Chassot et al., 2007). Environmental and biotic factors, such as sea surface temperatures, phytoplankton biomass, and primary productivity, play crucial roles in the dynamics of fish species in the Mediterranean (Brosset et al., 2015). As a result of these effects, the first mucilage event was reported in 2007 in the Sea of Marmara and this phenomenon reappeared in November 2020 (Savun-Hekimoğlu and Gazioğlu, 2021). As a result of causing several problems, including fisheries, ecological, social, and economic losses are inevitable (Karadurmuş and Sarı, 2022). Apart from this, it is necessary to approach this decrease in terms of fisheries management and examine the issue from an expert perspective. The catch (tons) of *M. merlangus* decreased overall in the Marmara Sea in the 2000s, and the catch amount exhibited relative stability between 2006 and 2010, followed by a sharp decline (Figure 6). No growth and mortality data recorded in this region since 2003. The high exploitation rate we have found can be attributed to overfishing, and this study determined high fishing mortality rate ($F = 1.01$ yr^{-1}) confirms this estimate. Unlike the eastern Black Sea, the Marmara Sea population of whiting is not protected by a trawl ban and is open for commercial shrimp trawl fishing seasonally. However, commercial fishers' use of this species has been low, as fishers have instead focused their attention on shrimp fishery. This situation should be put under

consideration by the stakeholders since it could pose a potential threat to the sustainability of the stocks. However, it is important to acknowledge that compliance by fishers is integral to the success of fishery management. Therefore, more lenient but well-managed alternative management techniques might be required. There is no regulation regarding the mesh size of nets such as whiting and red mullet in commercial fisheries in Turkey, except for turbot gillnets. The mesh size of the whiting nets should be regulated referred to the first maturity length and the current status of populations. Such a regulation would allow most individuals to attain sexual maturity and spawn at least once before being exposed to catch. Such regulations will also protect larger and older individuals with high reproductive capacity.

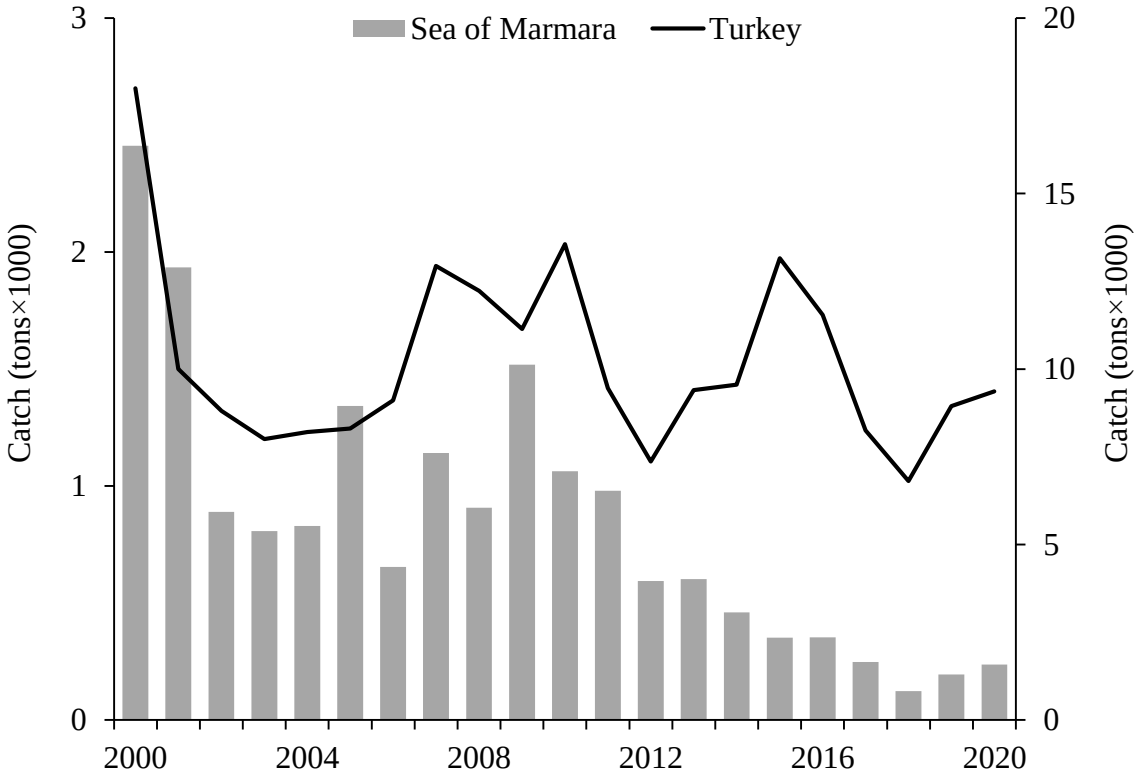

Figure 6. The catch amount (tons) of whiting in the Marmara Sea according to the data of the TÜİK (2020).

The Marmara Sea is also an important fishing ground with many commercially important fish species. Demersal fishes, especially whiting, remain an economically vital resource to the Marmara Sea fishing community and are also under the influence of the adverse impacts of anthropogenic activities. All evidence indicates that the whiting stock in the Sea of Marmara is currently being overexploited and used at a high capacity. Yet, although coupled assessment of fishing effort and catches is necessary, fisheries management practices should consider environmental aspects of the ecosystem in addition to conventional fisheries regulations. So, the risk of destruction of demersal fishes in the Sea of Marmara will remain an ongoing concern requiring long-term vigilance. Hence, effort should be directed towards the better management of the whiting stocks.

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CONFLICTS OF INTEREST

The author declares that for this article they have no actual, potential, or perceived conflict of interests.

ETHICAL STATEMENTS

Local Ethics Committee Approval was not obtained because experimental animals were not used in this study. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors. The necessary permission for the trawl survey was obtained from the Republic of Turkey Ministry of Agriculture and Forestry (Date: 19.10.2020; No: E-67852565-140.03.03-2924781).

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Research **Araştırma Makalesi**

Structure and Spatial Distribution of the Rotifera Assemblages in Kırklareli Reservoir (Kırklareli/Turkey)

Kırklareli Baraj Gölü'ndeki (Kırklareli/Türkiye) Rotifera Faunası'nın Yapısı ve Mevsimsel Dağılımı

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Abstract: This study was carried out to determine the diversity, abundance, and seasonal distribution of Rotifera in Kırklareli Reservoir. Rotifera samples were collected from May 2018 to April 2019 at three stations in the reservoir and some water quality parameters were measured. The qualitative evaluation of the samples revealed the presence of 39 species in the reservoir. The quantitative evaluation of the samples showed that 24727 ind/m³ Rotifera on average was found in the reservoir. The maximum organism number was found in the summer season (45690 ind/m^3). The average 27.3 % of the total annual Rotifera abundance was composed of *Kellicottia longispina* in Kırklareli Reservoir followed by *Polyarthra dolichoptera* (24.6 %), *Lecane luna* (6.8 %), *Asplanchna priodonta* (6.4%), *Synchaeta oblonga* (5.3%) and *Synchaeta pectinate* (4.9 %). *Asplanchna priodonta, Synchaeta oblonga, Synchaeta pectinata, Keratella cochlearis, Keratella quadrata, Polyarthra dolichoptera, Polyarthra vulgaris* and *Mytilina mucronata* were found the most common species in the reservoir. The water quality parameters measured in the reservoir were detected among the acceptable values to support aquatic life, especially the Rotifera community. The Rotifera densities and abundance showed a positive correlation with water temperature and Chlorophyll-*a*. According to these results, we conclude that Kırklareli Reservoir has an oligomesotrophic character in terms of the Rotifera fauna and the physicochemical parameters.

Keywords
- Rotifera
- Diversity
- Seasonal distribution
- Water quality
- Reservoir

Özet: Bu çalışma, Kırklareli Baraj Gölü'nün Rotifera çeşitliliğini ve mevsimsel dağılımını belirlemek amacıyla yapılmıştır. Mayıs 2018 ile Nisan 2019' tarihleri arasında gölde belirlenen üç istasyonda Rotifera örnekleri toplanmış ve bazı çevresel parametreler ölçülmüştür. Rotifera örneklerin kalitatif değerlendirmesinde 39 Rotifera türü belirlenirken kantitatif değerlendirmeler sonucunda baraj gölünde yıllık ortalama 24727 ind/m³ Rotifera tespit edilmiştir. Kırklareli Baraj Gölünde en fazla bolluğa sahip olan tür *Kellicottia longispina* (%27,3) olurken bunu *Polyarthra dolichoptera* (% 24,6), *Lecane luna* (% 6,8), *Asplanchna priodonta* (% 6,4), *Synchaeta oblonga* (% 5,3) ve *Synchaeta pectinata* (% 4,9) takip etmiştir. *Asplanchna priodonta, Synchaeta oblonga, Synchaeta pectinata, Keratella cochlearis, Keratella quadrata, Polyarthra dolichoptera, Polyarthra vulgaris* ve *Mytilina mucronata* rezervuarda en yaygın türler olarak bulunmuştur. Rezervuarda ölçülen çevresel parametreler, başta Rotifera faunası olmak üzere sucul yaşamı desteklemek için kabul edilebilir değerler arasında tespit edilmiştir. Rotifera yoğunluğu ve bolluğu, su sıcaklığı ve Klorofil-*a* ile pozitif korelasyon göstermiştir. Bu sonuçlara göre Kırklareli Rezervuarının Rotifera faunası ve fizikokimyasal parametreler açısından oligomezotrofik bir karaktere sahip olduğu sonucuna varılmıştır.

Anahtar kelimeler
- Rotifera
- Fauna
- Mevsimsel dağılım
- Su kalitesi
- Baraj gölü

1. INTRODUCTION

The rapid population growth, development of industry, pollution, and global climate change cause to decrease in clean water resources all around the world. For this reason, reservoirs built for many reasons including flood control, drinking water supply, agricultural watering, energy production, and fisheries also contain many zooplanktonic organisms.

The zooplanktonic organisms are an important biological component in aquatic ecosystems which play a vital role in the food chain, which the main function is to act as primary and secondary connections and aquatic ecosystems of the energy transfer (Altaff, 2004). Zooplankton can also be used as a biological indicator for water pollution studies because their formation, viability, and responses change under adverse environmental conditions (Oliver, 1996). Typical zooplankton assemblage of reservoirs is commonly constituted by Protozoa, Rotifera, Copepoda, and Cladocera (Rocha et al., 1999).

Rotifers are one of the most important components in the zooplankton community. They are frequently abundant in eutrophic freshwater ecosystems and are more abundant than other zooplankton groups, because of their short generation time and high reproductive rate (Herzig, 1987). They play a crucial role in the interlinking food chain in the aquatic ecosystem. They are considered to be one of the most sensitive indicators of water quality (Sladecek, 1983; Pontin and Langley, 1993). It is of the opinion of many researchers that the rotifer species composition and their abundance can be used as indicators of trophic status (Berzins and Pejler 1987; Matveeva, 1991). The distribution, abundance, and diversity of zooplankton in aquatic ecosystems depend mainly on the physicochemical properties of water and biological parameters. (Barnett and Beisner, 2007). Also, the temporal variations in the Rotifera community may depend on changes in the availability of edible phytoplankton which often vary depending on the physical processes and nutrient availability in the water bodies (Sarmento et al., 2008). Hence Rotifera association, abundance, seasonal variation, richness, and diversity can be used for the assessment of water pollution and lake management applications. Therefore, studies on seasonal variations of Rotifera in aquatic ecosystems are very important.

A number of studies have been carried out to examine the distribution and diversity of Rotifera in Turkey reservoirs (Buyurgan et al., 2010; Yıldız, 2012; Saler and Alış, 2014; Tuna and Ustaoğlu, 2016; Saler et al., 2017; Güher and Çolak, 2015; Gökçe and Turhan, 2014, Dorak et al., 2019; Dorak, 2019). But there are still reservoirs in Turkey that its zooplanktonic organisms have not been studied yet. This study aims to determine the Rotifera fauna, abundance, seasonal distribution of Kırklareli Reservoir, and some environmental parameters.

2. MATERIAL and METHODS

2.1. Study Area

Kırklareli Reservoir was built between the years 1985-and 1995 for irrigation and flood control on Şeytandere Stream. The reservoir provides drinking and using freshwater supplies to the province of Kırklareli. The reservoir is located 7 km to the northeast of Kırklareli city center (41°44'08.6"N and 27°16'59.0"E) the coordinates. The volume of the reservoir is about 112 hm^3 and the surface area is 6 km^2. The depth of the reservoir varies depending on the months and seasons, but when fully filled it is about 67 m. Although the reservoir is fed mainly by the Ana stream and Büyük stream, it is also fed by other creeks in the basin and by rainfall (Figure 1). The reservoir is surrounded by forests and partially agricultural areas. The reservoir is subjected to temporal fluctuations in water volume with high water volume in the rainy season and less water in the dry season due to high evaporation, agricultural irrigation, and drinking water supply (Anonymous, 2019).

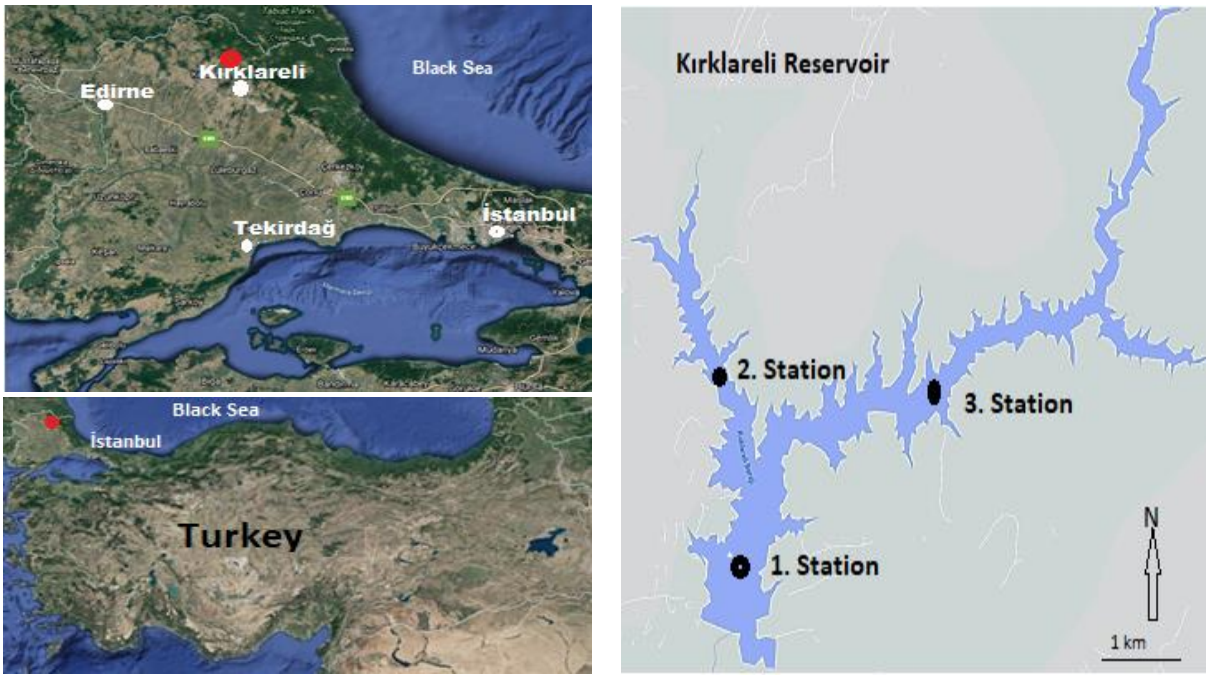

Figure 1. Location of Kırklareli Reservoir and the sampling stations.

2.2. Sampling

The Rotifera and water samples were collected at monthly intervals from May 2018 to April 2019 at three stations representing the lake's ecological characters (Table 1, Figure1). But, due to bad weather conditions, no sampling could be performed in March 2019.

Table 1. Sampling stations and coordinates in the Kırklareli Reservoir.

Sampling stations	Explanations	Geographic coordinates
1st station	This station is the middle part of the reservoir. The water in the reservoir is discharged from this place for irrigation and drinking water supply.	41°44'53,8" N 27°17'02,6" E
2nd station	This station is located on the western part of the reservoir and is where the Ana stream feeds the lake is located.	41°45'54,9" N 27°16'41,6" E
3rd station	This station is located on the eastern branch of the reservoir and is where the Büyük stream feeds the reservoir.	41°45'41,9" N 27°18'30,3" E

The Rotifera samples were collected with a Hensen type plankton net (mesh size 55 µm, mouth diameter 15 cm, length 75 cm) vertically up to the surface from the bottom point (10 m deeply) and horizontally. The samples were brought to the laboratory in 250 ml plastic bottles containing 4% formaldehyde. In the laboratory, samples were identified to species level according to Kolisko (1974); Koste (1978); Herzig (1987); De Manuel Barrabin (2000); Nogrady and Segers (2002); Ejsmont-Karabin et al., (2004) and Segers (2008). The counting of the samples was made according to Edmondson (1959) using an Olympus inverted microscope and was calculated using the following formula of Lackey (1938). Densities are presented as the number of individuals per cubic meter (ind/m^3).

$N = n \times v / V$

Where,

N = Total number of organisms/m^3 of water filtered,
n = Number of zooplankton counted in 5 ml plankton sample,
v = Volume of concentrate plankton sample (ml),
V= Volume of total water filtered through (m^3)

Some physicochemical parameters, such as water temperature (WT), conductivity (EC), pH and dissolved oxygen (DO) were measured on-site simultaneously by using Orion Star S/N 610541. Secchi disk depth (SD) of the reservoir was measured using a Secchi disk. To determine other physicochemical and biological variables of the water, sampling was made by a Ruttner water sampler. Nitrate nitrogen (NO_3-N), Nitrite nitrogen (NO_2-N) Phosphate (PO_4-P), Sulphate (SO_4^{2-}), Calcium (Ca_2^+), Magnesium (Mg_2^+), and Chlorophyll-a (Chl-*a*) were measured of the Trakya University Technology Research Development Application and Research Centre. The analysis of the ions was performed by Metrohm Ion Chromatography System using EPA 300.1 method. Metal analyzes were read on the Agilent Technologies 7700 ICP-MS System using EPA 200.7 and EPA 200.8 methods (EPA, 1994).

Simpson's diversity index was used to determine the species diversity and the species richness of Rotifera in the reservoir. The Bray-Curtis similarity index was used to examine the similarities of the sampling of the months and the seasons according to the diversity and abundance of Rotifera species (Jaccard, 1912). Spearman's correlation was used to determine the relationship of Rotifera with each other and with environmental parameters (Krebs, 1999).

3. RESULTS

3.1. Physicochemical variables

The measured in the Kırklareli Reservoir of physicochemical parameters and their minimum, maximum and average values are given in Table 2. Variations of these physicochemical parameters according to the sampling stations and months are given in Figure 2. When the mean values of each physicochemical parameter measured in the reservoir were evaluated according to Water Pollution Control Regulations (Anonymous, 2015), it has been found to vary within normal ranges.

Table 2. The measured physicochemical parameters and their minimum, maximum and average values (*below the limit of detection).

	Abbreviation	**Min.**	**Max.**	**Average**
Water temperature (^{0}C)	WT	6.00	27.00	16.50 ± 7.66
Dissolved oxygen (mg/L)	DO	7.43	13.75	9.71 ± 1.83
Secchi disk depth (cm)	SD	66.67	336.67	198.33 ± 73.53
pH	pH	8.15	9.45	8.64 ± 0.49
Conductivity (µS cm/L)	EC	213.33	322.37	248.17 ± 30.10
Nitrite nitrogen (mg/L)	NO_2-N	*	0.05	0.02 ± 0.02
Nitrate nitrogen (mg/L)	NO_3-N	0.04	2.13	0.73 ± 0.71
Ortho-phosphate (mg/L)	PO_4-P	*	0.78	0.11 ± 0.23
Sulphate (mg/L)	SO_4^{2-}	9.71	10.57	10.12 ± 0.25
Calcium (mg/L)	Ca_2^+	3.04	22.31	13.66 ± 6.60
Magnesium (mg/L)	Mg_2^+	1.90	12.30	8.19 ± 3.87
Chlorophyll-*a* (µg/L)	Chl-*a*	2.31	13.09	5.96 ± 3.49

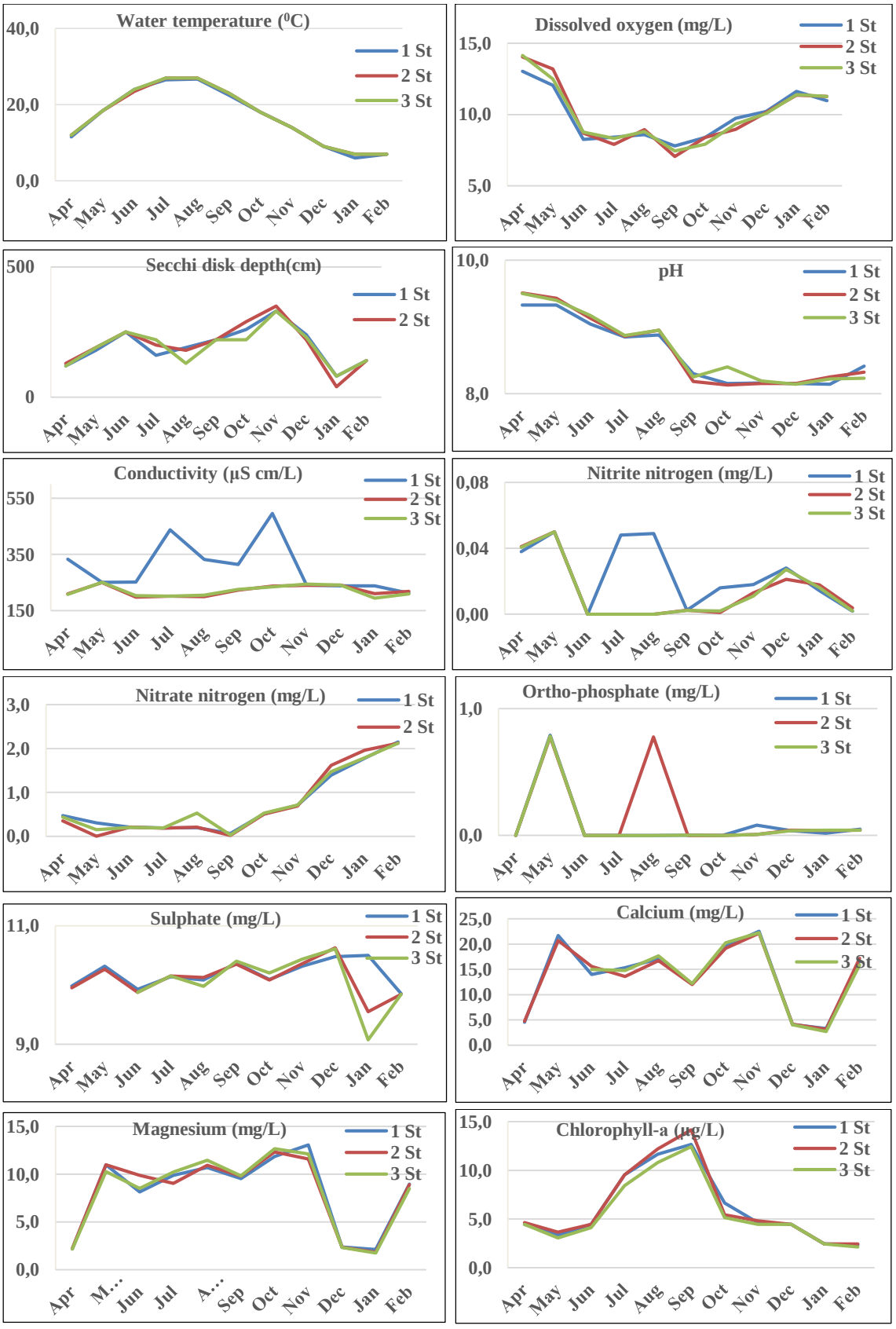

Figure 2. Variations of the physicochemical parameters according to the sampling stations and months.

3.2. Rotifer species composition and abundance

A result of the qualitative evaluation of the samples in Kırklareli Reservoir revealed the presence of 39 species belonging to Rotifera (Table 3).

When Rotifera species were evaluated in terms of the seasonal species richness, it was listed from the highest to lowest as 34 species in the summer season, 19 species in the autumn season, 14 species in the spring season, and 12 in the winter season.

According to the stations, the highest species number was found in 31 species in the 1st station, followed by the 2nd (30 species) and the 3rd stations (29 species). The maximum species diversity was recorded as 23 species in August, followed by June (16 species) and July (15 species) while the least diversity was found as 3 species in December and 2 species in January.

Table 3. The Rotifera species in Kırklareli Reservoir and the average values of their annual numbers per m^3.

ROTIFERA	**Annual average (ind/m^3)**	**%**
Anuraeopsis fissa Gosse, 1851	153 ± 483	0.6
Anuraeopsis navicula Rousselet, 1911	217 ± 442	0.9
Ascomorpha ecuadis Petry, 1850	844 ± 2558	3.4
Ascomorpha ovalis (Bengendahl, 1892)	193 ± 451	0.8
Ascomorpha saltans Bartsch, 1870	402 ± 933	1.6
Asplanchna priodonta Gosse, 1850	1592 ± 2452	6.4
Brachionus angularis Gosse, 1851	290 ± 667	1.2
Brachionus bidentatus Anderson, 1889	8 ± 25	0.02
Brachionus calyciflorus Pallas, 1766	185 ± 531	0.7
Brachionus falcatus Zacharias, 1898	8 ± 25	0.02
Brachionus plicatilis Müller, 1786	16 ± 51	0.1
Brachionus urceolaris Müller, 1773	225 ± 685	0.9
Colurella uncinata (Müller, 1773)	16 ± 51	0.1
Epiphanes macroura (Barrois & Daday, 1894)	8 ± 25	0.03
Euchlanis lyra Hudson, 1886	16 ± 51	0.1
Filinia longiseta (Ehrenberg, 1834)	161 ± 331	0.7
Gastropus minor (Rousselet, 1892)	113 ± 329	0.5
Hexarthra mira (Hudson, 1871)	32 ± 68	0.1
Kellicottia longispina (Kellicott, 1879)	6747 ± 20864	27.3
Keratella cochlearis (Gosse, 1851)	460 ± 587	1.9
Keratella quadrata (Müller, 1786)	499 ±1174	2.0
Keratella tecta (Gosse, 1851)	80 ± 254	0.3
Lecane bulla (Gosse, 1886)	80 ± 162	0.3
Lecane luna (Müller, 1776)	1685 ± 5065	6.8
Mytilina mucronata (Müller, 1773)	193 ± 284	0.8
Notommata glyphura Wulfert, 1935	8 ± 25	0.03
Polyarthra dolichoptera Idelson, 1925	6072 ± 18146	24.6
Polyarthra euryptera Wierzejski, 1891	16 ± 51	0.1
Polyarthra remata Skorikov,1896	724 ± 2233	2.9
Polyarthra vulgaris Carlin, 1943	796 ± 1820	3.2
Synchaeta oblonga Ehrenberg, 1832	1303 ± 1810	5.3
Synchaeta pectinata Ehrenberg, 1832	1206 ± 1437	4.9
Testudinella patina (Hermann, 1783)	24 ± 76	0.1
Trichocerca bicristata (Gosse, 1887)	24 ± 76	0.1
Trichocerca capucina (Wierjeski & Zacharias, 1893)	56 ± 132	0.2
Trichocerca cylindrica (Imhof, 1891)	80 ± 124	0.3
Trichocerca elongata (Gosse, 1886)	56 ± 132	0.2
Trichocerca iernis (Gosse, 1887)	24 ± 76	0.1
Trichocerca longiseta (Schrank, 1802)	113 ± 283	0.4
TOTAL	**24727 ± 35506**	**100**

The most common species in the reservoir was *A. priodonta* and was found for nine months. *S. oblonga* and *S. pectinata* were sampled for seven months and *K. cochlearis, K. quadrata, P. dolichoptera, P. vulgaris* and *M. mucronata* were sampled for five months. *A. fissa, B. bidentatus, B. falcatus, B. plicatilis, K. tecta, C. uncinata, P. euryptera, E. macroura, E. lyra, T. bicristata, T. iernis, N. glyphura,* and *T. patina*, were sampled only in one month during the study (Table 3). According to the Simpsons diversity index, while the maximum species diversity was recorded as, D=7.873 in August, followed by the June (D=6.272), May (D=6.013), February (D=4.183), and September (D=3.621), it's were found in the lowest value in November (D=1.44) and July (D=1.688).

The quantitative evaluation of the samples revealed an average value of 24727 ± 35506 ind/m^3 in the Kırklareli Reservoir. When the sampling months were evaluated based on average individual values per m^3, the maximum number of Rotifera was found in April (100584 ind/m^3) followed by July (97310 ind/m^3) and August (22754 ind/m^3), and the minimum was found in January (176 ind/m^3) followed by December (796 ind/m^3) and October (2035 ind/m^3) (Figure 3).

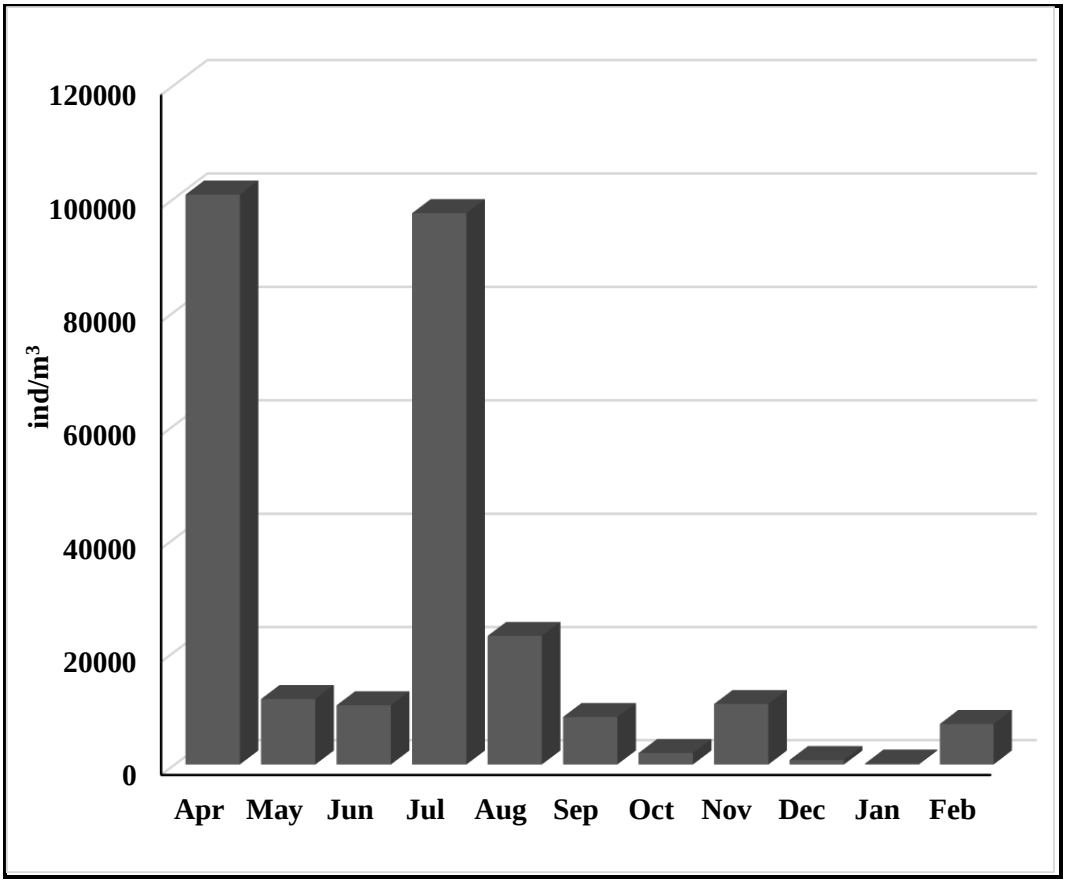

Figure 3. The abundance of Rotifera in Kırklareli Reservoir according to the sampling months.

According to the results of cluster analysis, the similarity between the month's ranges from 7 % to 60 %. The maximum organism number was found in the summer season (45690 ind/m^3), followed by the spring season (41924 ind/m^3) and autumn season (8239 ind/m^3), and the minimum was found in winter (3055 ind/m^3). The results of the cluster analysis showed that in autumn with winter (30 % similarity) and autumn with summer (18 % similarity) no obvious seasonal similarity has been identified (Figure 4).

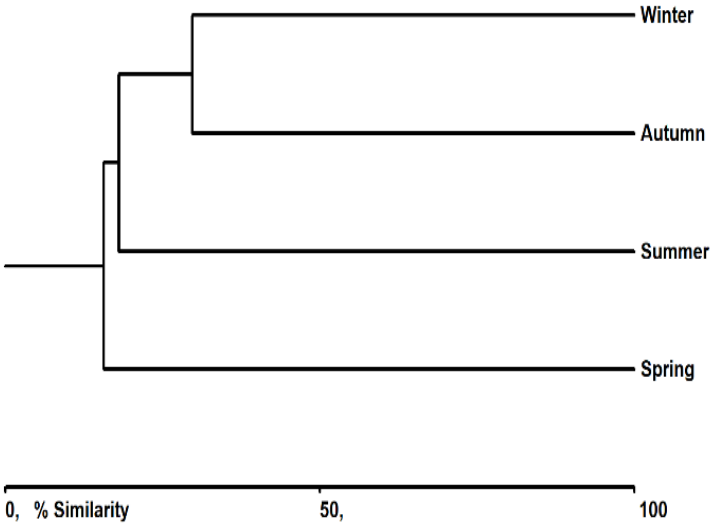

Figure 4. Cluster analysis showing the similarity index of Rotifera according to the seasonal.

The maximum number of Rotifera in Kırklareli Reservoir were recorded in the 1st station (38913 ind/m^3). This is followed by the 3rd and 2nd stations with 13806 ind/m^3 and 21462 ind/m^3, respectively. The Spearman's correlation was used to determine the relationship of Rotifera with environmental parameters. There was a positive correlation between Rotifera with pH (r=0.736) ($P< 0.01$), WT with Chl-*a* (r=0.673) ($P< 0.05$), DO with NO_2-N (r=0.651) ($P< 0.05$), Mg_2^+ with SD (r=0.645) ($P< 0.05$), EC with Chl-*a* (r=0.718) ($P< 0.05$), Ca_2^+ with Mg_2^+ (r=0.855) ($P< 0.01$) while there was negative correlation WT with DO (r=0.655) ($P< 0.05$) and NO_3-N (r=0.818) ($P< 0.01$), DO with Chl-*a* (r=0.709) ($P< 0.05$), EC with NO_3-N (r=0.664) ($P< 0.05$) (Table 4).

Table 4: According to Spearman's correlation analysis, the relationship between Rotifera and environmental parameters in Kırklareli Reservoir.

	Rotifera	WT	**DO**	**SD**	**pH**	**EC**	**NO_2N**	**NO_3N**	**PO_4**	**SO_4**	**Ca**	**Mg**	**Chl-*a***
Rotifera	1.000												
WT	**.600**	1.000											
DO	.045	**-.655***	1.000										
SD	-.118	.327	-.582	1.000									
pH	**.736****	.436	.236	-.391	1.000								
EC	.400	.582	-.464	.309	.191	1.000							
NO_2N	.321	-.165	**.651***	-.413	.202	.156	1.000						
NO_3N	-.555	**-.818****	.436	-.182	-.536	**-.664***	.000	1.000					
PO_4	-.114	-.248	.515	-.334	-.029	-.410	.433	.267	1.000				
SO_4	.064	.264	-.336	.591	-.345	.491	.183	-.391	-.010	1.000			
Ca	.291	.336	-.118	.482	.136	.255	-.128	-.173	.257	.173	1.000		
Mg	.200	.536	-.500	**.645***	-.055	.573	-.220	-.364	.019	.418	**.855****	1.000	
Chl-*a*	.382	**.673***	**-.709***	.364	-.027	**.718***	-.138	-.564	-.420	.518	.118	.545	1.000

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

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

4. DISCUSSION

As a result of the qualitative evaluation of the samples, 39 Rotifera species were found in Kırklareli Reservoir during the study period. All the species determined are recorded for the first time in Kırklareli Reservoir. According to Segers (2008); Ustaoğlu et al., (2012); Ustaoğlu (2015), and Güher (2014) all the species recorded in the present study show the widespread distribution in Turkey as well as all around the world. In this study, *A. priodonta, S. oblonga, S. pectinate, K. cochlearis, K. quadrata, P. dolichoptera, P. vulgaris* and *M. mucronata* were found the most common species in the

reservoir. The average 27.3 % of the total annual Rotifera abundance was composed of *K. longispina* in Kırklareli Reservoir followed by *P. dolichoptera* (24.6 %), *L. luna* (6.8 %), *A. priodonta* (6.4 %), *S. oblonga*, (5.3 %) and *S. pectinata* (4.9 %). The abundance of the rest of the identified species was less than 4 % individually and 24.2 % in total. In the studies carried out in Süleoğlu, Kadıköy and Kayalıköy reservoirs located in the same geographic area, 40, 32 and 33 Rotifera species were identified, respectively (Güher, 2015; Güher, 2019; Güher and Öterler, 2020). Similar results were found in this study.

In this study, the annual total number of the Rotifera was found as 24727 ± 35506 ind/m^3. The maximum Rotifera abundance was found in the summer season (45690 ind/m^3), followed by the spring season (41924 ind/m^3) and autumn season (8239 ind/m^3) and the minimum was found in winter (3055 ind/m^3). Considering the geographical region where Turkey is located, zooplankton organisms are expected to increase twice in spring and autumn during the year. But, in Kırklareli Reservoir, while Rotifera only reaches its maximum in the summer seasons, it decreases to a minimum in the winter season. In this study, the water temperature was recorded in the lowest value in winter and the highest in summer seasons. The Rotifera growth and abundance in the reservoir showed a positive correlation with WT and pH, because WT is the most important factor affecting the amount of nutrients and life in freshwater (Geller and Müller, 1981). Also, the Rotifera has a very short life cycle under suitable temperature, nutrient amount, and photoperiod conditions. Since rotifers have short breeding periods, their abundance increases rapidly under suitable environmental conditions.

To determine the trophic index of the lake, *Brachionus*:*Trichocerca* (QB/T) equality was used (Sladecek, 1983). According to this if the QB/T ratio = 1 the reservoir is considered as oligotrophic if the ratio is in the range of 1-2 the reservoir is mesotrophic and if the ratio is > 2 the reservoir is considered as eutrophic. In this study, Kırklareli Reservoir was determined (6 species of *Brachionus* and 6 species of *Trichocerca*) QB/T = 1. According to this, the reservoir showed oligotrophic property. In addition, *S. pectinata*, *P. vulgaris P. dolichoptera*, *K. cochlearis* and *A. priodonta* have been identified as the dominant species for oligotrophic conditions (Kolisko, 1974). These species were found to be common in this study. According to Sladecek (1983), *Brancionus* species indicate eutrophic habitat. They also suggested the Brachionidae family and *Brachionus* species as indicators of a highly trophic habitat. In Kırklareli Reservoir 10 species from Brachionidae were identified. For this reason, it can be said that the dam lake is closer to the eutrophic feature. However, the densities of *Brancionus* species were found to be very low in this study (Table 3).

pH is one of the important factors affecting the living life in water. In this study, the average pH value was found to be 8,64 ± 0,49 and the reservoir water was graded as alkaline water (Table 2). For the continuation of biological life in aquatic ecosystems, mean dissolved oxygen concentrations above 5 mg/L (Karpowicz and Ejsmont-Karabin, 2017) and the electrical conductivity values 250-500 µS/cm were reported to be the acceptable (Yücel, 1990). Accordingly, the values recorded in the reservoir were among the acceptable values to support aquatic life, especially the Rotifera community. Also, When the mean values of each physiochemical factor measured in the reservoir were evaluated according to Water Pollution Control Regulations (Anonymous, 2015), it was determined that the water quality of Kırklareli reservoir was generally compatible with the first-class water quality.

5. CONCLUSION

The Rotifera species in the Kırklareli Reservoir were evaluated both qualitatively and quantitatively. A total of 39 Rotifera species were determined in the qualitative evaluation of plankton samples. The maximum species diversity was recorded as 23 species in August, followed by June (16 species) and July (15 species) while the least diversity was found as 3 species in December and 2 species in January. The most common species in the reservoir were found *A. priodonta, S. oblonga, S. pectinata, K. cochlearis, K.quadrata, P. dolichoptera, P. vulgaris* and *M. mucronata.* The quantitative

evaluation of the samples revealed an average value of 24727 ind/m³ in the reservoir. While the maximum organism was found summer season (45690 ind/m^3) at 1st station (38913 ind/m^3) and in April (100584 ind/m^3), the lowest value was found winter season (3055 ind/m^3) in 3rd station (13806 ind/m^3) and in January (176 ind/m^3). When we evaluate the species identified in the reservoir, the distribution of the individuals that make up the Rotifera fauna, and physical-chemical parameters as a whole, it has been concluded that Kırklareli Reservoir is in oligomesotrophic character.

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CONFLICT OF INTEREST

There is no conflict of interest in this study.

AUTHOR CONTRIBUTIONS

No other contributors to this work.

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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Research **Araştırma Makalesi**

Heavy Metal Content of Water in Ikwu River (Umuahia, Nigeria): Pollution Indices and Health Risk Assessment Approach

Ikwu Nehri (Umuahia, Nijerya) Suyundaki Ağır Metal İçeriği: Kirlilik Endeksleri ve Sağlık Riski Değerlendirmesi

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Abstract: The heavy metal content of a local drinking water source in Southeast Nigeria was studied between January 2021 and June 2021 in 3 stations. Pollution indices (heavy metal pollution index and contamination index) and health risk assessment for non-carcinogenic were used to check the water's suitability for human consumption. Eight heavy metals were assessed with standard methods and compared with The Nigerian Drinking Water Quality Standard. Some metals (Mn, Pb, Fe, Cd, and Cr) exceeded acceptable limits. The heavy metal pollution index exceeded the threshold value (100), ranging between 503.56 and 746.80. The contamination index ranged between 10.74 and 17.12 indicating high contamination potential and all the hazard indices exceeded unity (1). The heavy metal content, pollution indices, and health risk assessment has shown that the water from the Ikwu River was not fit for human consumption. The main metals that influenced the results were Mn, Pb, Fe, Cd, and Cr, because they exceeded limits while Cd and Cr were responsible for the observed adverse health risk. The children were more vulnerable. The geogenic influence was a major factor exacerbated by season and anthropogenic activities in the river.

Keywords
- Limits
- Heavy metal
- Water quality
- Indices
- Drinking water

Özet: Güneydoğu Nijerya'da yerel bir içme suyu kaynağının ağır metal içeriği Ocak 2021 ile Haziran 2021 arasında 3 istasyonda incelenmiştir. Suyun insan tüketimine uygunluğunu kontrol etmek için kirlilik indeksleri (ağır metal kirlilik indeksi ve bulaşma indeksi) ve kanserojen olmayanlar için sağlık risk değerlendirmesi kullanılmıştır. Sekiz ağır metal, standart yöntemlerle değerlendirilmiş ve Nijerya İçme Suyu Kalite Standardı ile karşılaştırılmıştır. Bazı metaller (Mn, Pb, Fe, Cd ve Cr) kabul edilebilir sınırları aşmıştır. Ağır metal kirlilik indeksi eşik değerini (100) aştı; 503,56 ile 746,80 arasında değişmektedir. Kirlilik indeksi 10.74 ile 17.12 arasında değişmekte olup, yüksek kontaminasyon potansiyeline işaret etmekte ve tüm tehlike indeksleri birden (1) aşmaktadır. Çocuklar daha savunmasızdı. Jeojenik etki, nehirdeki mevsim ve antropojenik faaliyetlerle şiddetlenen önemli bir faktör olarak gözlenmiştir.

Anahtar kelimeler
- Limitler
- Ağır metal
- Su kalitesi
- İndeksler
- İçme suyu

1. INTRODUCTION

The future of life on earth and sustainable development can only be guaranteed by the availability of good quality water in adequate quantity (Ertaş et al., 2021). Accessibility to potable water is the ease with which a greater majority of people get good quality and quantity of water for their basic needs (Lukman et al., 2016). Safe drinking water has also been described as a basic human right (Gebrekidan and Samuel, 2011, Li and Wu. 2019). Water quality degradation reduces its uses for different purposes coupled with the challenges of water scarcity (Ertaş et al., 2021).

Water pollutants majorly include heavy metals, fertilizers, other toxic inorganic, and organic compounds, etc. (Al-Jumaily, 2016). Considering the wide range of pollutants militating against safe drinking water supplies, heavy metals deserve the highest level of attention because they are toxic even at relatively low concentrations (Marcovecchio et al., 2007; Rehman et al., 2018). Heavy metals occur naturally on earth but can be influenced by human activities (Singh, 2007). In recent times, the quantity of heavy metals has increased tremendously in the environment as a result of human activities (Al-thahaibawi, 2021). Heavy metal concentrations in the environment and exposures worldwide have increased due to industrialization, urbanization, and agriculture, thereby increasing the deleterious human health effects associated with such exposures (Rusyniak et al., 2010). The consequences of such continuous exposure include an internal imbalance in the body and the accumulation and substitution of essential elements. Heavy metals also affect the activity of various hormones and essential enzyme functions (Mukke and Chinte, 2012).

Heavy metal pollution index (HPI) and contamination index are quality indices used in rating the composite influence of dissolved heavy metals in rivers (Addey et al., 2018; Anyanwu and Umeham, 2020b; Anyanwu et al., 2020; Hamidu et al., 2021). It is calculated from the viewpoint of the suitability of water for human consumption concerning metals contamination (Majhi and Biswal, 2016). Risk assessment for non-carcinogenic effects has also been used to evaluate the potential risk of heavy metal pollution in rivers (Muhammad et al., 2011; Wongsasuluk et al., 2013; Anyanwu et al., 2020; Anyanwu and Nwachukwu, 2020; Zakir et al., 2020). Heavy metal was not included in previous studies on the river (Anyanwu and Emeka, 2019; Anyanwu et al., 2022). Hence, this study aims to assess the heavy metal content in relation to drinking water suitability using pollution indices and health risk assessment**.**

2. MATERIAL and METHODS

2.1. Study Area

The study was carried out in Ikwu River, which is located in Umuire Community along Umuahia – Uzoakoli Road, Umuahia, South-east Nigeria within 53411988 – 53448000N and 72844400 – 72852764E (Figure 1).

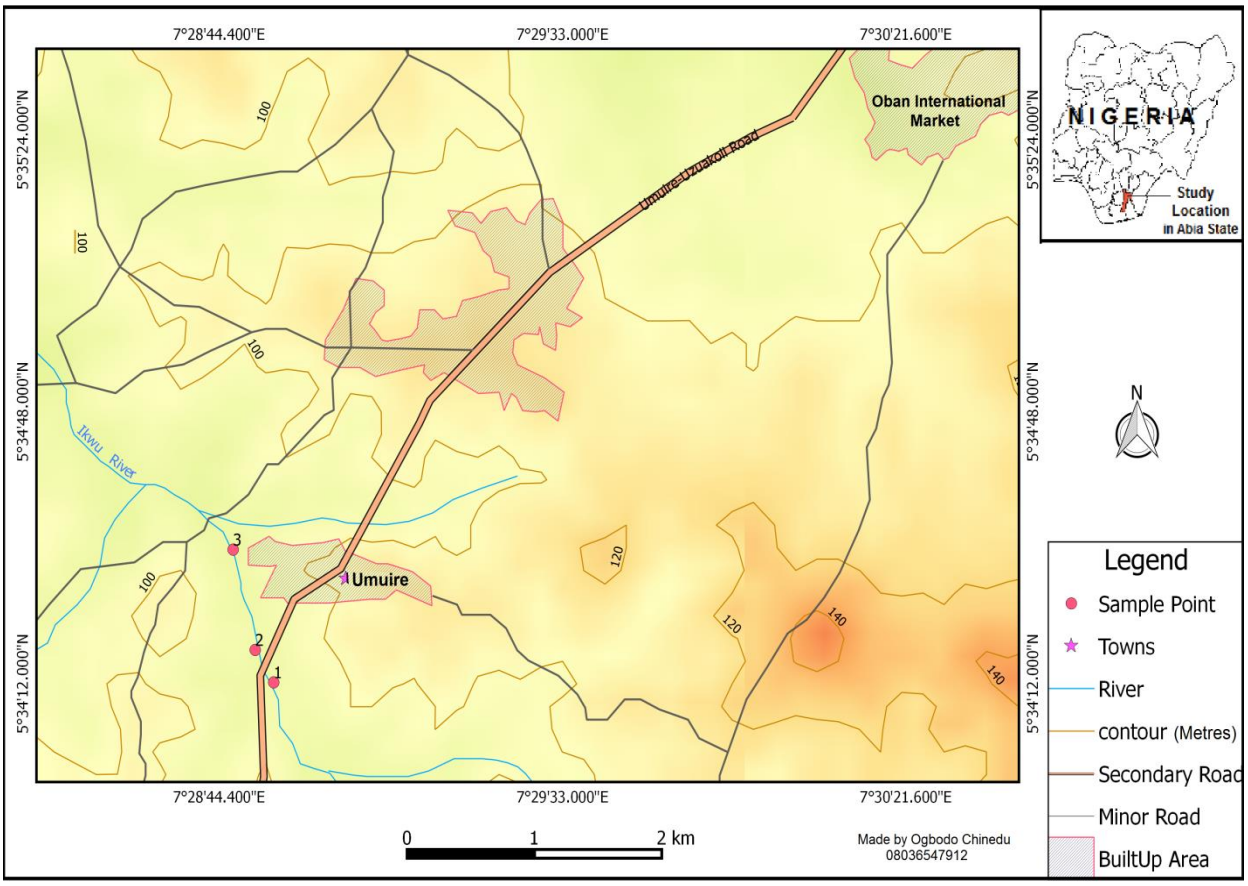

Figure 1: Map of Umuahia, Abia State, Nigeria showing the sampling stations of Ikwu River.

The popular Ubani market is in the watershed of the river. Ikwu River flows through Umuire and Umuegwu Okpula communities and discharges into the Imo River Basin. The three stations were selected based on accessibility and observed anthropogenic activities. Station 1 was the reference site, located upstream on the right along Umuahia – Uzoakoli Road. No activities were observed during the study except periodic signs of cattle watering. Previously, extraction of water for horticulture, agricultural, and drinking purposes was reported (Anyanwu and Emeka, 2019). Station 2 was located by the left side of Umuahia – Uzoakoli Road, 350 meters downstream of Station 1. Many activities were observed some distances upstream of Station 2 such as bathing, washing of cars, motorcycles, and tricycles, children swimming, abstraction of drinking water, and sand mining as the rains increased. Station 3, located within Umuire community is a major source of water for most domestic activities. It is about 430 meters downstream of Station 2. Observed human activities were abstraction of drinking water, washing of clothes, bathing, swimming, and sand mining as the rains increased. Stormwater from Umuire community is also discharging into this station after rainfall events.

2.2. Samples Collection and Analyses

Water samples were collected monthly with a one-liter water sampler from Ikwu River between January and June 2021 and transferred into a clean 250 ml plastic bottle. The samples were acidified to pH 2 with nitric acid (HNO_3) according to Sharma and Tyagi (2013). The digestion was with concentrated Analar nitric acid according to Zhang (2007) while the determination of heavy metals was carried out with UNICAM Solaar 969 atomic absorption spectrometer (AAS) which used acetylene-air flame. The data were summarized with Microsoft Excel while two-way ANOVA was used to test for significant differences in stations and months.

2.3. Pollution Assessment Indices

2.3.1. Heavy metal pollution index

The heavy metal pollution index (HPI), based on the weighted arithmetic mean method was developed by Prasad and Bose (2001). HPI indicates the total quality of water with respect to heavy metals (Horton, 1965; Mohan et al., 1996). HPI has been applied extensively (Addey et al., 2018; Anyanwu and Umeham, 2020b; Anyanwu et al., 2020; Hamidu et al., 2021). To compute HPI, unit weightage (W_i) was considered as a value inversely proportional to the recommended standard (S_i) for the relevant parameters (Prasad and Bose, 2001).

The formula for HPI was described by Mohan et al. (1996) and presented as:

$$HPI = \frac{\sum q_i \, x \, W_i}{\sum W_i} \quad (1)$$

Where *qi* is the sub-index of ith parameter. *Wi* is the unit weightage of ith parameter and n is the number of parameters considered.

W*i* = 1/Standard (S)

The sub-index (*qi*) of each parameter is defined by:

$$qi = 100 \, x \frac{C_i}{S_i} \quad (2)$$

where *Ci* is the measured value of ith parameter while *Si* is the recommended standard value of ith parameter. The critical value of HPI for drinking purposes as proposed by Prasad and Bose (2001) is 100. Eight (8) heavy metals (Mn, Cu, Pb, Fe, Zn, Cd, Cr, and Ni) were evaluated and the weightage (*Wi*) was taken as the inverse of standard permissible limits by Nigerian Standard for Drinking Water Quality (SON, 2015).

2.3.2. Contamination index

The contamination index was developed by Backman et al. (1998) and it calculates the relative contamination of different metals separately and manifests the sum of generated components as a representative. The contamination index was calculated with the equation:

$$C_d = \sum_{i=0}^{n} C_{fi}$$

$$\text{Where } Cf_i = \left(\frac{CA_i}{CN_i}\right) - 1 \quad (3)$$

Cf_i = contamination factor for *i*-th component,

CA_i = analytical value for *i*-th component and

CN_i = upper permissible concentration of *i*-th component. (N denotes the 'normative value'). The low, medium, and high contamination levels are referred to C_d values of less than 1, between 1 and 3, and greater than 3, respectively. CN_i is considered the standard permissible value (S_i) used in the calculation of HPI. This method has been widely used by various researchers (Biswas et al., 2017; Dibofori-Orji et al., 2019; Anyanwu et al., 2020; Anyanwu and Umeham, 2020b).

2.4. Health Risk Assessment

Health risk assessment was carried out for the metals that exceeded acceptable limits (Mn, Pb, Fe, Cd, and Cr). The non-carcinogenic method as described by Muhammad et al. (2011) was used for the human health risk assessment. The chronic daily intake (CDI) of heavy metals in Ikwu River water was evaluated by the equation (4):

$$CDI = \frac{C_W xIRxEFxED}{B_W xAT} \quad (4)$$

Where, CDI is the daily dose of heavy metals to which consumers might be exposed. C_W (mg/l) is the concentration of heavy metals in the river water, *IR* is the ingestion rate, *EF* is the exposure frequency, *ED* is the exposure duration, *BW* is the body weight, *AT* is the averaging time. The input parameters used in evaluating CDI values are presented in Table 1.

Table 1. Input parameters used in evaluating CDI values

Factor/parameter	Symbol	Units	Adult	Children
Exposure Duration	ED	Years	30	6
Exposure Frequency	EF	Days/year	350	350
Averaging Time	AT (ED x 365)	Days	10950	2190
Body Weight	BW	Kg	70.0	15.0
Ingestion Rate	IR	L/day	2.0	1.0

Source: (USEPA, 2004, 2006).

2.4.1. Hazard quotient

The hazard quotient (HQ) for non-carcinogenic risk was calculated using the equation by USEPA (1999):

$$HQ = \frac{CDI}{RFD} \quad (5)$$

Where, *CDI* is the daily dose of heavy metals to which consumers might be exposed and *RfD* is the reference dose (mg/kg/day) which is the daily dosage that enables the individual to sustain this level of exposure over a long period of time without experiencing any harmful effects.

If, HQ> 1, it represents adverse non-carcinogenic effects of concern while HQ< 1 represents an acceptable level (no concern) (Maigari et al., 2016).

2.4.2. Hazard index

For the risk assessment of a mixture of pollutants, the individual HQs are combined to form the hazard index (HI) (Wongsasuluk et al., 2013).

$$HI = \sum_{i=1}^{n}(HQ)i \quad (6)$$

Where, HI, is the hazard index for the overall toxic risk and n is the total number of metals under consideration. When HI is <1.0, non-carcinogenic adverse effect through ingestion is negligible (Zakir et al., 2020).

2.5. Statistical Analysis

The data were summarized using the Descriptive Statistic Package of Microsoft Excel while Two-way ANOVA without replicate was used to determine significant spatial and temporal variations.

3. RESULTS

3.1. Spatial and Temporal Variations

The summary of the heavy metal values is presented in Table 2. Iron, lead, and cadmium exceeded limits throughout the study and significantly higher values were recorded during the dry months (January - March 2021) while lower values were recorded during the onset of the wet season (April - June 2021). Iron values ranged between 0.43 and 3.11 mg/L. The lowest value was recorded in Station 1 (June 2021). The highest value was recorded in Station 2 (January 2021). Fe was significantly different ($p < 0.05$) in both stations and months. All the values were above the acceptable limit. Station 1 was significantly ($p < 0.05$) lower than Stations 2 and 3 while January was significantly ($p < 0.05$) higher than the rest of the months.

Table 2. Summary of heavy metals measured at Ikwu River (with a range in Parenthesis)

Parameter	Station 1 X±S.E.M.	Station 2 X±S.E.M	Station 3 X±S.E.M	Station *P* – Value	Month *P* – Value	SON 2015**
Mn (mg/L) *	0.22±0.06[a] (0.11 – 0.53)	0.30±0.09[b] (0.17 – 0.75)	0.30±0.08[b] (0.14 – 0.66)	$p < 0.05$	$p < 0.05$	0.2
Cu (mg/L)	0.12±0.02 (0.07 – 0.22)	0.16±0.05 (0.08 – 0.38)	0.14±0.03 (0.09 – 0.25)	$p > 0.05$	$p < 0.05$	1.0
Pb (mg/L) *	0.03±0.001[a] (0.01 – 0.06)	0.04±0.001[b] (0.02 – 0.07)	0.04±0.01[b] (0.01 – 0.09)	$p < 0.05$	$p < 0.05$	0.01
Fe (mg/L) *	0.83±0.31[a] (0.43 – 2.40)	1.08±0.41[b] (0.55 – 3.11)	1.02±0.37[b] (0.48 – 2.84)	$p < 0.05$	$p < 0.05$	0.3
Zn (mg/L)	0.54±0.24 (0.21 – 1.73)	0.66±0.30 (0.27 – 2.17)	0.61±0.24 (0.22 – 1.80)	$p > 0.05$	$p < 0.05$	3
Cd (mg/L) *	0.02±0.01 (0.01 – 0.04)	0.03±0.01 (0.01 – 0.05)	0.03±0.01 (0.01 – 0.06)	$p > 0.05$	$p > 0.05$	0.003
Cr (mg/L) *	0.05±0.01[a] (0.02 – 0.09)	0.07±0.01[b] (0.03 – 0.11)	0.06±0.06[b] (0.02 – 0.11)	$p < 0.05$	$p < 0.05$	0.05
Ni (mg/L)	0.01±0.00 (0.01 – 0.03)	0.02±0.00 (0.01 – 0.02)	0.01±0.00 (0.01 – 0.02)	$p > 0.05$	$p > 0.05$	0.02
HPI	503.56	746.80	738.46			
C_d	10.74	17.12	16.26			

*Mean Values exceeded acceptable limits; **Nigerian Standard for Drinking Water Quality (NSDWQ) (2015); SEM= Standard Error of Mean.

Lead values ranged between 0.01 and 0.09 mg/L. The lowest value was recorded in Stations 3 (April 2021) and 1 (June 2021) while the highest value was recorded in Station 3 (March 2021). All the values were above the acceptable limit. Station 1 was significantly ($p < 0.05$) different from Stations 2 and 3 while January to March 2021 was significantly ($p < 0.05$) higher than April to June 2021 values.

Cadmium values ranged between 0.01 and 0.06 mg/L. The lowest value was recorded in Station 1 (January and June 2021); Station 2 (June 2021) and Station 3 (April 2021). The highest value was recorded in Station 3 (March 2021). All the values were above the acceptable limit. Cadmium values were not significantly different ($p > 0.05$) in months and stations.

Manganese, chromium, and nickel had values that exceeded limits only during the dry months. The Manganese values ranged between 0.11 and 0.75 mg/L. The lowest and highest values were recorded in June and January 2021 in Stations 1 and 2, respectively. The values recorded from January to March 2021 were higher than the standard limits set by SON (2015) while April to June 2021 values were within the acceptable limits. Station 1 was significantly ($p < 0.05$) lower than Stations 2 and 3 while January to March 2021 was significantly ($p < 0.05$) higher than April to June 2021.

Chromium values ranged between 0.02 and 0.11 mg/L. The lowest value was recorded in Station 1 (June 2021) and Station 3 (April 2021). The highest value was recorded in Station 2 (March 2021) and Station 3 (March 2021). Chromium values were significantly different ($p < 0.05$) in the months and stations. Station 1 was significantly ($p < 0.05$) lower than Stations 2 and 3. Values from January to March 2021 were above the acceptable limit while March 2021 value was significantly ($p < 0.05$) higher than the rest of the months.

Nickel values ranged between 0.01 and 0.03 mg/L. The lowest value was recorded in Station 1 in all the months except in March 2021. The highest value was recorded in Station 1 (March 2021). The value obtained in Station 1 (March 2021) exceeded the acceptable limit. Nickel values were not significantly different ($p > 0.05$) in the months and stations.

Zinc and copper were all within their limits though higher values were recorded during the dry months. Zn values ranged between 0.21 and 2.17 mg/L. All the values were below the acceptable limit (3 mg/L). The lowest value was recorded in Station 1 (June 2021). The highest value was recorded in Station 2 (January 2021). Zn was highly significantly different ($p < 0.05$) in months but not significant ($p > 0.05$) in stations. January was significantly ($p < 0.05$) higher than the rest of the months. The copper values ranged between 0.07 and 0.38 mg/L. The lowest value was recorded in June 2021 in Station 1 and the highest value was recorded in January 2021 in Station 2. All the values were within the acceptable limit and there was no significant difference ($p > 0.05$) among the stations while January 2021 value was significantly ($p < 0.05$) higher than February to June 2021 values.

3.2. Pollution Indices

The heavy metal pollution index and contamination index showed the possible geogenic and anthropogenic impacts in the river. The HPI and C_d values are also presented in Table 2. The HPI values ranged from 503.56 (Station 1) to 746.80 (Stations 2) which exceeded the threshold value of 100. The high HPI was contributed by the high values recorded for manganese, lead, iron, cadmium, and nickel in all the stations. Stations 2 and 3 had higher HPI values.

The C_d ranged between 10.74 (Station 1) and 17.12 (Station 2) and all are greater than 3, indicating high pollution potential risk. Stations 2 and 3 also recorded the higher values.

3.3. Health Risk Assessment

3.3.1. Chronic daily intake

The chronic daily intake (CDI) of the heavy metals that exceeded limits and respective oral toxicity reference doses (RfD) values are presented in Table 3. The CDI values for Mn were 0.006 mg/kg/day (adult) and 0.007 mg/kg/day (children) in Station 1 and 0.0082 mg/kg/day (adult) and 0.019 mg/kg/day (children) in both Stations 2 and 3. CDI values for Mn recorded for adults and children in all the stations were lower than the RfD (0.14 mg/kg/day).

The CDI values for Pb were 0.0008 mg/kg/day (adult) and 0.0019 mg/kg/day (children) in Station 1 while values of 0.0011 mg/kg/day (adult) and 0.003 mg/kg/day (children) were recorded in Stations 2 and 3. Pb CDI values recorded in all the stations for adult and children were lower the RfD (0.0035 mg/kg/day).

Table 3. Chronic daily intakes of the heavy metals

Metal	Station 1		Station 2		Station 3		RfD*
	Adult	Children	Adult	Children	Adult	Children	(mg/kg/day)
Mn	0.006	0.007	0.0082	0.019	0.0082	0.019	0.14
Pb	0.0008	0.0019	0.0011	0.003	0.0011	0.003	0.0035
Fe	0.227	0.053	0.296	0.069	0.279	0.065	0.7
Cd	0.0005	0.001	0.0008	0.0019	0.0008	0.0019	0.0005
Cr	0.0014	0.003	0.0019	0.005	0.0016	0.004	0.003

*(USEPA IRIS, 2011)

The CDI values for Fe were 0.22 mg/kg/day (adult) and 0.53 mg/kg/day (children) in Station 1, 0.296 mg/kg/day (adult) and 0.069 mg/kg/day (children) in Station 2 and 0.279 mg/kg/day (adult) and 0.065 mg/kg/day (children) in Station 3. CDI values for Fe recorded in all the stations for adult and children were lower than the RfD limit value (0.7 mg/kg/day).

The CDI values for Cd were 0.0005 mg/kg/day (adult) and 0.001 mg/kg/day (children) in Station 1 while the values of 0.0008 mg/kg/day (adult) and 0.019 mg/kg/day (children) were recorded in Stations 2 and 3. Cd CDI values recorded in all the stations (adult and children) exceeded the RfD (0.0005 mg/kg/day).

The Cr CDI values recorded for children in Stations 2 and 3 exceeded the RfD (0.003 mg/kg/day).

3.3.2. Hazard quotient

The Hazard Quotients (HQs) of the heavy metals that exceeded limits is presented in Table 4. All the HQs for Mn, Pb, and Fe were less than 1 for adults and children in all the stations. However, HQs for Cd were all greater than 1 in all the stations for both adults and children except for adults (station 1) while HQs for Cr were greater than 1 for only children in stations 2 and 3.

Table 4. Hazard Quotients and Total Hazard Index of the Heavy Metals

Metals	Station 1		Station 2		Station 3	
	Adult HQ	Children HQ	Adult HQ	Children HQ	Adult HQ	Children HQ
Mn	0.043	0.050	0.059	0.14	0.059	0.14
Pb	0.23	0.54	0.31	0.86	0.31	0.86
Fe	0.32	0.076	0.42	0.099	0.40	0.093
Cd	1.00	2.00	1.60	3.84	2.60	3.80
Cr	0.47	1.00	0.63	1.67	0.53	1.33
HI	2.063	3.666	3.019	6.609	3.899	6.223

3.3.3. Hazard index

Hazard indices (HI) recorded for both adult (2.06 – 3.90) and children (3.67 – 6.61) in all the stations were greater than threshold value (1).

4. DISCUSSION

Iron, lead, and cadmium exceeded limits throughout the study. Significantly higher values were recorded in the dry months while lower values were recorded from the onset of the wet season in April 2021. This observed trend could be attributed to geogenic sources influenced by season and anthropogenic activities. Grützmacher et al. (2013) and CGWB (2014) defined geogenic sources as levels that exceeded permissible limits without any direct or indirect link to anthropogenic activities and could have negative health effects. Little or no precipitation, low flow rate, higher air temperatures, and higher evaporation during the dry months contribute to the concentration and higher values of the metals (Etesin et al., 2013; Houssou et al., 2017; Haque et al., 2019). The dry periods or seasons are also associated with increased human visitations and activities because rivers and streams are major sources of water for drinking and most domestic activities in the region (Onyele and Anyanwu, 2018; Anyanwu and Umeham, 2020a, b). However, sand mining activities started and increased with the rains in the river as observed by Anyanwu et al. (2020) and Anyanwu and Umeham (2020a, b). These activities tend to impact negatively on the water quality as observed in Stations 2 and 3 (Anyanwu and Umeham, 2020a, b). On the other hand, the lower values recorded from the onset of the rains (April 2021) could be as a result of dilution (Griffin, 2017). More water is released into the river channel during the wet season. Ezekiel and Dikam (2020) also observed that iron, lead, cadmium and manganese exceeded limits in River Dilimi, Jos North, Plateau State, Nigeria and attributed it to anthropogenic impacts.

Manganese, chromium, and nickel had values that exceeded limits only during the dry months. This observed trend could also be attributed to season and anthropogenic influences as observed in iron, lead, and cadmium (Etesin et al., 2013; Houssou et al., 2017; Haque et al., 2019).

Zinc and copper were all within acceptable limits though higher values were recorded in the dry months. This could also be attributed to season and anthropogenic influences as observed in the other metals. Ezekiel and Dikam (2020) also observed that zinc and copper were within limits in River Dilimi, Jos North, Plateau State, Nigeria despite anthropogenic impacts.

All the HPI exceeded the threshold value (100) in all the stations. Stations 2 and 3 had higher HPI values attributable to geology, season, and human activities, especially sand mining activities. The

contribution of sand mining to heavy metal contamination has been variously reported (Pillay et al., 2014; Anyanwu and Umeham, 2020b; Ijaola and Simon, 2021). The HPI values recorded in this study were lower than 1408.33 recorded in River Povpov, Itakpe, Kogi State, Nigeria (Ameh and Akpah, 2011) but higher than 619.8 recorded in Eme River, Umuahia (Anyanwu and Umeham (2020b) and 512.4 recorded in Iyiakwu River, Elemaga (Anyanwu et al., 2020). Both rivers were subjected to more intense sand mining activities.

The C_d values were all greater than 3, indicating high pollution potential risk. Stations 2 and 3 also recorded the higher C_d values; attributed to the factors influencing the HPI. The high C_d was also influenced by the high values recorded for manganese, lead, iron, cadmium, and nickel in all the stations. Herojeet et al. (2015) suggested that Fe and Cd were among the metals that contributed to the high C_d values recorded in the Sirsa River, Himachal Pradesh, India. The C_d values were lower than 18.87 recorded in Eme River, Umuahia (Anyanwu and Umeham, 2020b) and 3.32 recorded in Iyiakwu River, Elemaga (Anyanwu et al., 2020).

The health risk assessment showed the CDI was varied among the metals. CDI values for Mn recorded in all the stations for adults and children were lower than the reference dosage and therefore do not pose any health risk to people drinking water from the stations. The CDI values were slightly lower than the values recorded by Anyanwu et al. (2020) and the same as the only CDI recorded for Mn in Station 1 of Ossah River, Umuahia (Anyanwu and Nwachukwu, 2020). Health effects from Mn are not critical except at concentrations exceeding 5 mg/L (Dimirkou and Doula, 2008).

The CDI values of Pb recorded for adults and children in all the stations were lower than the reference dosage. Thus, lead does not pose any health risk for those exposed to drinking the water. The CDI values recorded by Anyanwu et al. (2020) were slightly lower.

The CDI values for Fe recorded in all the stations for adults and children were lower than the reference dosage. Consequently, Fe does not pose a health risk for those exposed to drinking the water. Though the CDI values were lower than the reference dosage, they could have been influenced by the high Fe content of the river. Related studies recorded high CDI values for Fe (Ekere et al., 2014; Maigari et al., 2016; Onyele and Anyanwu, 2018; Anyanwu et al., 2020; Anyanwu and Nwachukwu, 2020). Naturally, iron has high concentrations on earth and is more abundant in the Nigerian freshwater environment (Adefemi et al., 2004; Aiyesanmi, 2006; Kumar et al., 2010; Iwuoha et al., 2012). Iron in high concentrations is associated with higher risks for cancer, heart disease, and other ailments (arthritis, endocrine problems, diabetes, and liver disease (Elci et al., 2008).

The CDI values for Cd recorded in all the stations (adult and children) exceeded the reference dosage. As a result, Cd poses a health risk for those exposed to drinking the water. The high CDI values of Cd could be as a result of the high Cd content in the river. Cadmium CDI values were lower in Ekere et al. (2014) and higher in Anyanwu et al. (2020). Generally, Cadmium was considered as toxic trace element (Mandour, 2012). Cadmium toxicity is through ingestion and chronic exposure in humans affect the kidney as the critical target organ (Johri et al., 2010; Unisa et al., 2011).

The Cr CDI values recorded for children in Stations 2 and 3 exceeded the reference dosage. Thus pose a serious health risk for children exposed to drinking the water in the stations. The values were within the ranges recorded by Anyanwu et al. (2020) and Anyanwu and Nwachukwu (2020). Chromium is considered carcinogenic and genotoxic at higher concentrations (Paustenbach et al. 2003; Moffat et al., 2018).

Some HQ values for cadmium (adults and children) and chromium (children) exceeded 1 and were attributed to high CDI values. The high HQ values make exposed individuals vulnerable. Therefore, the metals pose long term health risks to the water users. Hazard indices (HI) for both adult and children in all the stations were higher than the threshold value (1). The long-term health risk is therefore high, and the non-carcinogenic adverse effect cannot be ignored.

5. CONCLUSION

The heavy metal content, pollution indices, and health risk assessment has shown that the water from Ikwu River was not fit for human consumption. The main metals that influenced the results were manganese, lead, iron, cadmium, and chromium, because they exceeded limits while cadmium and chromium were responsible for the observed adverse health risk. The children were more vulnerable. Geogenic influence was a major factor exacerbated by season and anthropogenic activities in the river.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

EDA designed the research. EDA, OGA and OBN conducted the field research, analyzed the data, and interpreted the results. All the authors contributed to writing the manuscript, reading and approving the final manuscript.

ETHICAL APPROVAL STATEMENTS

Not applicable

DATA AVAILABILITY STATEMENT

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.

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Research **Araştırma Makalesi**

Little Known Aspects of Aquatic Insects: Myiasis

Sucul Böceklerin Az Bilinen Yönleri: Miyazis

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Abstract: Among invertebrates, Diptera, an aquatic insect, has the largest group of species. Aquatic Diptera larvae live in a highly distinctive environment in contact with vertebrates, humans, contaminated water, and depositing eggs in the host organism due to their life cycle. This study aims to describe various aspects of *Clogmia albipunctata* one of myiasis insects causing a disease that affects both living and dead vertebrates as well as humans and whose symptoms are often overlooked. Furthermore, the study is remarkable since it is the first report of *C. albipunctata* (Psychodidae) in an indoor drainage system, except for humans, vertebrates, and natural ecosystems. SEM images gave a detailed description of the larvae and confirmed the species identification. When their life cycles were investigated, it was determined that in addition to myiasis, *C. albipunctata* larvae (drain fly or moth fly) played a vital role in the movement of bacteria from drains to indoor places, such as toilets, bathrooms, showers, and kitchens. Multi-drug resistant bacteria populate *C. albipunctata*, which possesses synanthropic behavior, and may play a major role in its transmission. This study focused on accidental myiasis.

Keywords
- Aquatic Diptera
- *Clogmia albipunctata*
- Myiasis
- Psychodidae
- Turkey

Özet: Omurgasızlar arasında Diptera, en fazla sucul türe sahip olan böcek grubudur. Sucul Diptera'nın yaşam döngüsünde omurgalılar ve insanlarla karşılaşması, konakçı organizmaya yumurtlaması veya yumurta bırakılan kontamine su ile olan temas, sucul Diptera larvalarının çok farklı bir çevrede yaşamasına sebep olur. Bu çalışmanın amacı, insanlar kadar canlı ve ölü omurgalıları etkileyen ve semptomları sıklıkla gözden kaçan miyaz böceklerinden biri olan *Clogmia albipunctata*' yı çeşitli yönleriyle tanımlamaktır. Ayrıca çalışma, *C. albipunctata*' nın (Psychodidae) insan, omurgalılar ve doğal ekosistemler dışında bir kapalı gider sistemindeki ilk kayıt olması nedeniyle dikkat çekicidir. SEM görüntüleri, larvanın detaylı tanımlamasına izin vermiş ve tür tanımlamasını doğrulamıştır. Yaşam döngüleri incelendiğinde miyazise ek olarak *C. albipunctata* larvalarının (gider sineği ya da güve sineği), bakterilerin giderlerden tuvalet, banyo, duş, mutfak gibi iç mekanlara taşınmasında kritik bir rol oynadığı görülmüştür. Ayrıca, çoklu ilaca dirençli bakteriler, sinantropik davranışa sahip olan ve bulaşıda önemli bir rol oynayabilen *C. albipunctata*' da yerleşir. Bu çalışma, sucul böceklerin tesadüfi miyazisi üzerine odaklanmıştır.

Anahtar kelimeler
- Sucul Diptera
- *Clogmia albipunctata*
- Miyazis
- Psychodidae
- Türkiye

1. INTRODUCTION

The population dynamics of freshwater benthic macroinvertebrates alter over time, depending on water quality and ecosystem productivity. Benthic macroinvertebrate community composition and ecological tolerance values of those invertebrate species based on environmental resistance provide important information for aquatic biomonitoring. Therefore, invertebrates have a crucial position in

aquatic ecology studies. Diptera, one of the aquatic insects, has the largest group of species among invertebrates. In its life cycle, aquatic Diptera encounters vertebrates and humans and contacts with contaminated water or laying eggs in the host organism, which leads Diptera larvae to live in a very different habitat and to be identified under different bio-ecological conditions: myiasis.

The term myiasis was first used by Hope in 1840 (El-Dib et al., 2020). Myiasis is defined as the infestation of human and vertebrate animals with insect larvae that feed on the host's dying (necrotic) or alive tissue, liquid body substances, or swallowed food for at least a period of time (El-Badry, 2014; El-Dib et al., 2020; Gökçe, 2020). As insect larvae, Diptera, Lepidoptera, Hymenoptera, and Coleoptera larvae cause myiasis (Cordeiro and Wagner, 2018). Myiasis is classified in two ways: anatomically, according to the location of the infestation on the host, and parasitically, according to the parasite's level of dependence on the host (Boumans et al., 2009; Hovius et al., 2011; Amro et al., 2018).

Myiasis is a condition in which invertebrate (especially Diptera) larvae infest the tissue and organ cavities of people and vertebrates, and lesions occur since the larvae feed with living or dead tissues, body fluids, or undigested food (Gökçe, 2020). Especially Calliphoridae, Sarcophagidae, and Destridae are groups that cause mostly myiasis in Diptera. Also, Fanniidae, Muscidae, Phoridae, Syrphidae, Psychodidae (Diptera) are crucial families that are responsible for myiasis worldwide (Ježek and van Harten, 2009; Gökçe, 2020).

Obligatory, facultative, and accidental myiasis are the three types of myiasis (Zittra et al., 2020; Mokhtar et al., 2016). There are two causes of accidental myiasis. The first is ingesting food contaminated with larvae. The second is when flies lay their eggs in either the host's anus or their urogenital area, thus causing the larvae to enter the rectum or urogenital tract. Nevertheless, the majority of the digested larvae are unable to complete their life cycle in the digestive or urogenital systems of their hosts. Cutaneous, subcutaneous, or cavitary groups are seen in myiasis according to the habitation of the attached larvae (Mohammed and Smith, 1976; Hjaija et al., 2018; Sarkar et al., 2018; El-Dib et al., 2020). Human myiasis is most commonly found in open wounds that have not been cared for properly. Furthermore, it can also affect body orifices including the oral cavity, eyes, ears, anus, and urogenital tract. Urogenital myiasis is a condition in which fly larvae infest the urinary canal and genital organs like the vaginal or penile orifices (Rasti et al., 2016; Hjaija et al., 2018; Pijáček M, Kudělková, 2018).

Mature flies are seen between the late prevernal and serotinal seasons and they lay ova. On the other hand, some myiasis agents are larvae inhabiting in aquatic habitats. The prevalence and frequency of myiasis are determined by fly and susceptible animal populations, as well as climate and environmental conditions (Kvifte and Wagner, 2017).

This study aims to describe different aspects of myiasis disease which affects both live and deceased vertebrates and human beings, but whose symptoms are frequently disregarded. Furthermore, the study is important because it is the first record about *Clogmia albipunctata* (drain fly or moth fly) as the habitat in Turkey, except for humans, vertebrates, and natural aquatic ecosystems.

2. MATERIAL and METHODS

2.1. Sampling and identification

In this study, larvae samples were collected around the sink and the drain filter in the building on the university campus in Malatya. Organic matter residue on the body of larvae prevents microscopic examination and clear SEM images. For the preparation of the specimens, a 10% KOH solution was utilized. The specimens were kept in 10% KOH solution at room temperature for 4 hours for cleaning from organic matter residue on the body of the larvae. Larvae were not left in the solution for a longer period of time to avoid degeneration of the soft portions of the body and the integrity of the body. After that, specimens were washed with distilled water and were preserved in 80% ethyl alcohol and

glycerol solution. The identification process was performed according to Kvifte and Wagner (2017), Cordeiro and Wagner (2018) by using a stereomicroscope (Leica MZ7.5). The samples were photographed, and their morphological measurement was performed using Leica camera DFC295 (Leica Application Suite, LAS version 4.5LAS). Scanning electron microscope images (SEM; LEO EVO-40xVP) were taken by Laboratory (İnönü University Scientific and Technology Research Centre).

3. RESULTS

Larvae were collected around the indoor drain filter of a building in the university campus. A total of 42 specimens were identified as aquatic larvae, *Clogmia albipunctata* Williston, 1893 (Diptera: Psychodidae) in the area with wet and partially organic materials.

All of the specimens were at the 4th instar stage. The body lengths ranged from 5.120 to 6.10 mm (Figure 1.). The body has 26 pseudo segments (annuli), is covered with well-sclerotized light brown color tergal plates; and one of the remarkable characteristics is the bristly body.

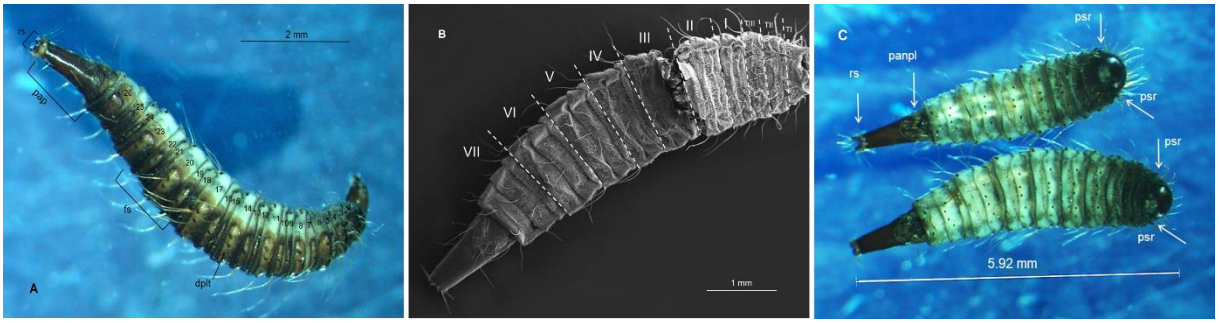

Figure 1. (A) *C. albipunctata* has totally 7 segments that are secondarily divided into 26 pseudosegments. (B) bristly 7 abdominal and 3 thoracic segments have filiform setae dorsolaterally view (65 X). (C) spiraculum and respiratory siphon are clearly recognizable in the whole body ventral view (*dplt:* dorsal plate; *fs:* filiform seta; *panpl:* preanal plate; *pap:* post abdominal process; *psr:* prothoracic spiracle; *rs:* respiratory siphon).

The head capsule is sub-oval and sclerotized. The hypostome has three teeth (Figures 2. and 3). The thorax is covered with tooth-like scales spination (Figure 3.).

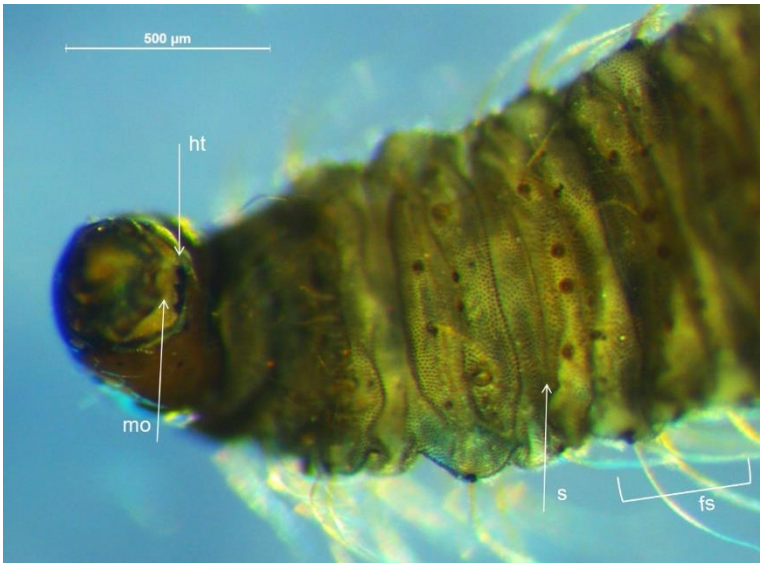

Figure 2. *C. albipunctata* larva has well development and sclerotized head capsule. Hypostomal three sharp teeth are prominent (*fs:* filiform seta; *ht:* hypostomal teeth; *mo:* mouth opening; *s:* spines).

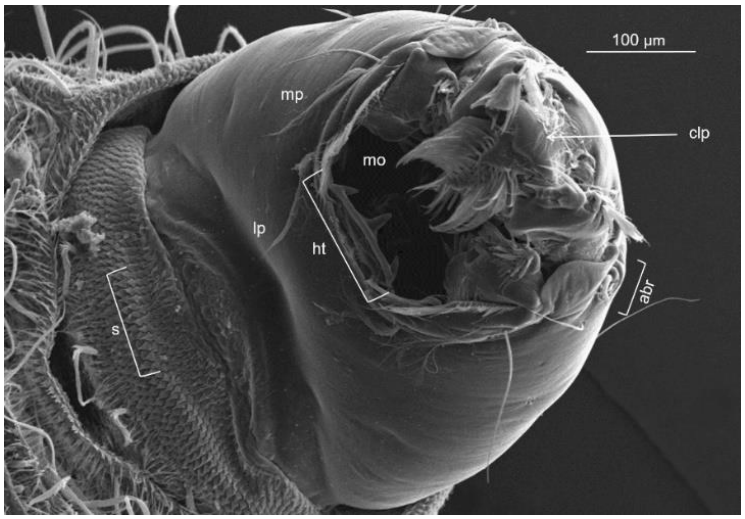

Figure 3. Head with the left antenna from a ventral view. Hypostomal teeth are clearly visible, median tooth slightly longer than corner teeth (550 X) (*abr:* antennal basal ring; *clp:* clypeus; *ht:* hypostomal teeth; *lp:* labial palpus; *mo:* mouth opening; *mp:* maxillary palpus; *s:* spines).

Prothoracic spiracles are present (Figure 4.). At the terminal end of the body, the respiratory siphon plate is prominent.

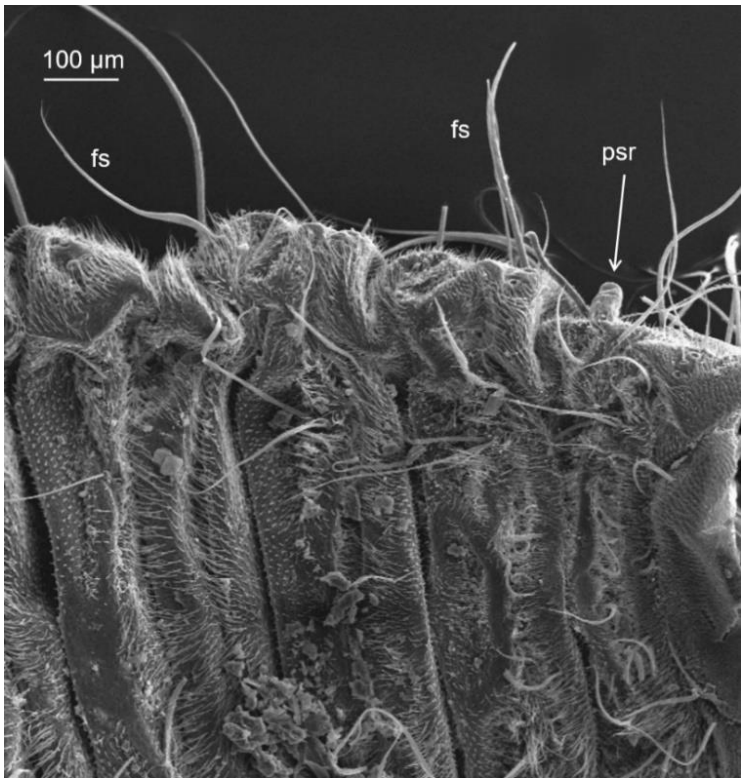

Figure 4. Prothorax has two finger-like spiracles in lateral positions, ventral view (250 X) **(***fs:* filiform seta; *psr:* prothoracic spiracle).

The preanal plate of *C. albipunctata* has a distinctive form (Figure 5.). SEM images were used to provide a detailed larval description and validation of the identification. After the filter which had found the larvae around it, was cleaned and covered, the larvae were not observed again. Also, no adult specimens were recorded.

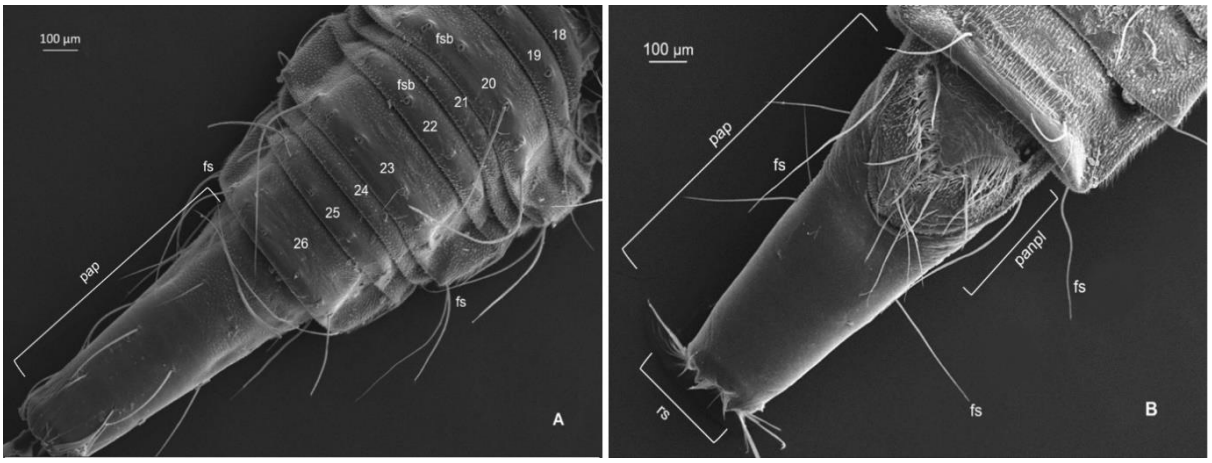

Figure 5. (A) *C. albipunctata* larval post abdominal part from dorsal view (210 X); (B) respiratory siphon plate from ventral view (230 X) (*fs:* filiform seta; *fsb:* filiform seta base; *panpl:* preanal plate; *pap:* post abdominal process; *rs:* respiratory siphon).

As in family characteristics, *C. albipunctata* has a four-stage life cycle as a holometabolous fly: egg, four larval instars, pupa, and adult. *C. albipunctata* is a synanthropic and cosmopolite aquatic Dipteran species (Wagner, 2011). The female ones lay their eggs on the surface of the water. Since they accumulate in higher numbers in indoor and outdoor wastewater pipe systems, they are commonly seen in wet bathrooms, hospitals, and drains (Ledwoch et al., 2018). They feed on the biofilm layer (protozoa, bacteria, algae) in pipes and drains (Ledwoch et al., 2018; Faulde and Spiesberger, 2013). Larval development depends on the amount of food and temperature. Adults and larvae are harmless. However, due to synanthropic life, larvae cause myiasis in vertebrates and humans.

The subfamily Psychodinae has previously been recorded in the Nearctic, Neotropic, Oriental, Afro-tropic, and Australian zoogeographic regions (Wagner and Andersen, 2007). As a cosmopolitan species, *C. albipunctata* is common in most of the zoogeographic areas. Table 1 shows myiasis and zoogeographic location records of the species as larvae and adults. It can be regarded as an invasive species according to Oboňa and Ježek (2012).

Table 1. As a cosmopolitan species, *C. albipunctata* is widespread in most of the zoogeographic areas. Myiasis and zoogeographic field records of the larvae and adults are presented.

Life stage	Location	Country	References
Larvae	Intestinal myiasis	Japan	Tokunaga, 1953
Adult	Geographical location	Italy	Sarà and Salamanna, 1968
Larvae	Nasopharyngeal myiasis	Nigeria	Mohammed and Smith, 1976
Larvae	Urinary and intestinal myiasis	Malaya	Smith and Thomas, 1979
Adult	Geographical location	Senegambia	Wagner, 1983
Adult	Geographical location	Colombia	Wagner and Joost, 1994
Adult	Geographical location	Nicaragua	Maes and Killick-Kendrick, 1994
Adult	Geographical location	Germany, Central Europa	Werner, 1997
Adult	Geographical location	Tanzania,	Wagner and Andersen, 2007
Larvae	Intestinal myiasis	Taiwan	Tu et al., 2007
Adult	Geographical location	Mexico	Ibáñez-Bernal, 2008
Adult	Drain, hospital operation room	Belgium	Boumans et al., 2009
Adult	Geographical location	Arabian Peninsula and UAE	Ježek and Harten, 2009
Adult	Geographical location	Czech Republic	Wagner, 2011
Larvae	Urogenital myiasis	Germany	Hovius et al., 2011
Adult	Geographical location	Slovakia	Oboňa and Ježek, 2012
Larvae	Urinary myiasis	Egypt	El-Badry et al., 2014
Adult	Geographical location	Honduran	Bravo et al., 2014
Adult	Geographical location	Spain	Kvifte et al., 2016
Adult	Geographical location	Uruguay	Martinez et al., 2016
Larvae	Intestinal myiasis	Malaysia	Mokhtar et al., 2016
Adult	Geographical location	Thailand	Kvifte and Andersen, 2016
Larvae	Urinary myiasis	Iran	Rasti et al., 2016
Adult	Geographical location	Netherlands	Ciliberti et al., 2017
Larvae	Urinary myiasis	Israel	Sarkar et al., 2018
Larvae	Urinary myiasis	Palestine	Hjaija et al., 2018
Larvae	Drain	Venezuela	Cazorla-Perfetti, 2019
Larvae	Urogenital myiasis	Libya	Amro et al., 2019
Adult	Geographical location	Finland	Salmela et al., 2019
Adult	Geographical location	Austria	Zittra et al., 2020
Larvae	Intestinal myiasis	Egypt	El-Dib et al., 2020
Larvae	Urinary myiasis	Czech Republic	Pijáček and Kudělková, 2020
Larvae	Urogenital and gastrointestinal myiasis	Turkey	Gökçe, 2020
Adults	Geographical location	Ukraine	Oboňa et al., 2021
Larvae	Human residual root myiasis.	China	Liu et al., 2021
Larvae	Drain, Department flat, Academic facility	Turkey	Present study

4. DISCUSSION

Considering their life cycles, *C. albipunctata* larvae as well as myiasis play an important role in transporting bacteria since they move into indoor spaces through drains such as toilets, bathrooms, showers, and kitchen drains. It was noted by Faulde and Spiesberger (2012) that 45 bacterial species were isolated from the larvae of *C. albipunctata* collected in a hospital. Since *C. albipunctata*, which has a synanthropic behavior, is colonized by multi-drug resistant bacteria, it may play a crucial role in the transmission and contamination of multidrug-resistant bacteria that cause serious nosocomial infections. This relationship between bacteria and larvae often occurs in the environments such as hospitals and schools. The eggs and larvae pose a dangerous threat because they live in the biofilm contaminated with the patient's bacterial flora. The biofilm develops and spreads rapidly and can span distances of many kilometers. During the third and fourth larval stages, the larvae living in the biofilm may begin to move and thus can come out of damp areas such as showers, bathtubs, toilets, and kitchens. At this point, it can carry drug-resistant bacteria from the microbial flora of the biofilm to the environment (Rupprecht et al., 2020).

The emergence of *C. albipunctata*, on the other hand, primarily indicates inadequate water and pest

management and sanitation in hospitals and other facilities (Faulde and Spiesberger, 2012). *Bacillus thuringiensis* is often regarded as the most effective larvicidal agent. It is frequently utilized as a microbiological agent against the world's most common insect pests. *B. thuringiensis* is known for producing a wide range of insecticidal proteins. According to Houston et al. (1989), the application of *Bacillus thuringiensis* serotype *israelensis* can reduce the incidence of drain flies by 79%.

Myiasis cases in Turkey were found to be caused by Diptera. Species belonging to the family Calliphoridae (Şenel et al., 2016), Sarcophagidae (Yücesan et al., 2021), Oestridae (Erenler et al., 2019), Psychodidae (Şahin et al., 2018; Gökçe, 2020; Şen and Polat, 2021), and Simuliidae (Akarsu et al., 2003) were recorded as the causative agent of myiasis in Turkey. These species are mostly aquatic Diptera larvae (Psychodidae and Simuliidae). Myiasis has become more common in rural regions due to sociocultural patterns and poor sanitation. This study focused on a different aspect of aquatic insects and described the 4th instar *C. albipunctata*, the first record in the drain in Turkey, in detail, and presented it to attract attention to myiasis which is usually overlooked.

5. CONCLUSION

This study revealed that all of the myiasis cases in Turkey is caused by synanthropic Dipterous larvae. Ecological factors such as temperature, nutrients, and moist conditions influence larval growth. Climatic change is a serious point as much as personal hygiene, and the spread and prevalence of accidental myiasis affect environmental health.

Today, two problems (low water quality and water scarcity), affect water consumption all over the world. At this point, an increase in the number of myiasis agents can be seen in aquatic insects due to low sanitation. In addition, there is an increase in the development of Dipterous larvae in the biofilm layer in drains and wastewater channels in indoor and outdoor environments. Along with its effect, myiasis creates serious health concerns by transmitting resistant pathogenic bacteria. The more eggs that get laid on the biofilm layer due to an increase in temperature exacerbate the insect invasion. For environmental health, disinfection processes that will leave minimum residue and ensure that other natural populations are minimally affected should be carried out. It is advised to provide regular drain cleaning to prevent hospital infections.

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CONFLICT OF INTEREST

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

AUTHOR CONTRIBUTIONS

DG is the corresponding author for the present study in all processes of manuscript preparation and final draft.

ETHICAL APPROVAL STATEMENTS

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

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 **Araştırma Makalesi**

Keten ve Çiya Tohumu ile Zenginleştirilmiş Yayın Balığı (*Siluris glanis*) Köftelerinin Bazı Kalite Kriterlerinin Araştırılması

Investigation of Some Quality Criteria of Catfish (*Siluris glanis*) Balls Enriched with Chia and Flaxseed

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Özet: Bu çalışmada, farklı oranlarda (%4 ve %8) keten ve çiya tohumu ile zenginleştirilmiş yayın balığı (*Silurus glanis*) köftelerinin bazı kalite kriterlerinin incelenmesi amaçlanmıştır. Balık köfteleri depolamanın belirli günlerinde (1, 7 ve 14. gün) toplam aerobik mezofilik bakteri (TAMB), psikrotrofik bakteri, maya-küf, tiyobarbitürik asit (TBARS), toplam uçucu bazik azotu (TVB-N), pH ve duyusal kalite parametreleri yönünden araştırılmıştır. Mikrobiyolojik analiz sonuçlarına göre keten ve çiya tohumu ile zenginleştirilmiş köfte örneklerinde bakteri sayısı, kontrol grubu örneklere göre daha düşük bulunmuş ve tüm gruplarda depolama süresine paralel olarak artış ($p<0,05$) tespit edilmiştir. TVB-N ve pH değerleri kontrol grubunda daha yüksek bulunurken, TBARS değeri keten ve çiya tohumu ilaveli balık köftelerinde daha yüksek saptanmıştır. Duyusal analiz sonuçları incelendiğinde ise depolama boyunca doku hariç tüm gruplar arasından en çok beğenilen grup kontrol grubu olmuştur. Çalışmamızda keten ve çiya tohumu ilavesinin genel olarak kalite kriterleri üzerine olumlu etki ettiği görülmüştür.

Keywords
- Keten tohumu
- Çiya
- Balık köftesi
- Yayın balığı
- Kalite

Abstract: This study aimed to examine some quality criteria of catfish (*Silurus glanis*) fishballs enriched with flax and chia seeds at different rates (4% and 8%). Total aerobic mesophilic bacteria (TAMB), psychrotrophic bacteria, yeast-mold, thiobarbituric acid (TBARS), total volatile basic nitrogen (TVB-N), pH and sensory levels on certain days of storage (1, 7, and 14 days) of fishballs investigated in terms of quality parameters. According to the results of microbiological analysis, the number of bacteria in the ball samples enriched with flax and chia seeds was lower than in the control group samples, and an increase was detected in all groups in parallel with the storage period ($p<0.05$). While TVB-N and pH values were higher in the control group, TBARS value was higher in the fishballs supplemented with flax and chia seeds. When the sensory analysis results were examined, the control group was the most liked group among all groups except tissue during storage. In our study, it was observed that the addition of flax and chia seeds had a positive effect on the quality criteria in general.

Anahtar kelimeler
- Flaxseed
- Chia
- Fishballs
- Catfish
- Quality

1. GİRİŞ

Son yıllarda hızlı kentleşme ve çalışan nüfusundaki artış nedeniyle tüketiciler hazır gıda tüketimine yönelmiştir. Hazır yemek teknolojisi ile lezzetli, pratik, güvenilir, kaliteli ürünler tüketime hazır hale getirilip, sunulmaktadır. Özellikle toplu tüketime sahip alanlarda (catering firmaları, lokantalar, okullar, oteller, hastaneler vb.) sunduğu alternatifler sayesinde tercih edilebilir hale gelmiştir. Ayrıca

sağlıklı besinleri tercih eden ve günlük yaşantısında yoğun iş temposuna sahip yemek yapmaya vakit bulamayan insanlar için de alternatif bir seçenektir (Kaba vd., 2013; Kara, 2017). Günümüzde sağlıklı ve dengeli beslenme önem kazanmış ve bu doğrultuda tüketici beslenme alışkanlıklarında da değişiklikler olmuştur (Taşkaya vd., 2003; Demircioğlu, 2018; Arslan 2020). Su ürünleri bu bağlamda protein değeri yüksek, vitamin ve mineraller içeriği zengin, doymamış yağ asitlerini ve esansiyel amino asitlerini yüksek oranda bünyesinde bulunduran değerli bir besin maddesidir (Anonim, 2021). Gelişen teknolojilere paralel olarak diğer gıda maddeleri gibi su ürünleri de farklı şekillerde işlenip, paketlenerek tüketime hazır bir hale getirilmektedir. Hazır yemek teknolojisinde su ürünlerinin önemi de tartışılmazdır. Su ürünleri çeşitli şekillerde işlenerek sofralarımızdaki yerini almıştır. İşlenmiş su ürünleri gerek lezzetleri gerekse sunmuş oldukları alternatifler sayesinde aranılan ürünler arasında yerini almıştır. Bu ürünlerden birisi de balık köftesidir. Temizlenerek kıyma haline getirilen balık etinin içerisine çeşitli baharat ve katkı maddeleri ilave edilerek köfte haline getirilmekte ve ambalajlanmaktadır (Yanar ve Fenercioğlu, 1999; Çapkın, 2020; Kaba vd., 2013). Et ve et ürünleri ile ilgili yapılan araştırmalarda bitkisel kökenli maddelerin kullanımının tüketiciler açısından pek çok avantaja sahip alternatif ürünleri ortaya çıkardığı belirtilmiştir. Koruyucular, kıvam artırıcılar, renklendiriciler, besin değerini düzenleyiciler ya da bunların birçoğunu yapısında bulundurabilen farklı tahıl unlarının kullanımı bunlara örnek olarak sayılabilir. Özellikle de unların kullanımı ile ilgili yapılan araştırmalarda, bileşenlerin sağladığı fonksiyonel özellikler sayesinde ürünün kalitesinin arttırılabileceği ile ilgili bilgiler vardır (Kurt ve Kılınççeker, 2012; Kılınççeker, 2015). Çeşitli tahıllar veya baklagil tohumları gibi fonksiyonel özelliğe sahip tarımsal ürünler un haline getirilerek köfte gibi gıdalarda kullanımı ile dağılma, büzüşme, fire kaybı, renk değişimi gibi birçok problemi azaltarak ürünün raf ömrünü uzatmaktadır (Kılınççeker, 2015). Gerek dünya nüfusundaki artış gerekse pandemi süreci beslenme kavramının değişmesine ve beraberinde fonksiyonel gıdalara olan ilginin artmasına neden olmuştur. Bitkisel kaynaklı ürünler gıdalara ilave edilmeye başlanmıştır (Ertugay vd. 2020).

Bitkisel kaynaklara birer örnek de keten ve çiya tohumudur. Keten (*Linum usitatissimum*) 30-100 cm boyunda, mavi çiçekli, tek yıllık bir kültür bitkisidir. Tohumları yumurta şeklinde, yassı, parlak ve lezzetlidir. Keten tohumunun genellikle ticari olarak kahverengi ve sarı türlerinin üretimi yapılmaktadır. İki türe ait besin içeriği benzer olmasına rağmen sarı keten tohumu daha çok tercih edilmektedir. (Ergene ve Bingöl, 2019; Kılınççeker ve Kırpık, 2019). Keten tohumu %35-45 oranında yağ içerir. Keten tohumu yağı bitkisel kaynaklar içinde %59 α-linoleik asit (ALA) içeriğince en zengin kaynak olması nedeniyle önemli bir endüstriyel üründür. Keten tohumunun antifungal özelliğe sahip olduğundan, bazı gıdalarda küf gelişimini inhibe etmek amacıyla kullanım alanına sahiptir. Keten tohumu temel besin ögelerinin yanında polifenoller, tokoferoller ve lignin gibi yarar sağlayan bileşenleri de içermesinden dolayı, diyete ilave edilerek kardiyovasküler hastalıklar, diyabet, obezite, bağırsak ve prostat karsinomlarına karşı koruyucu etki göstermektedir. (İşleroğlu vd., 2005; Ergene ve Bingöl, 2019).

Çiya (*Salvia hispanica* L.), Lamiaceae ailesine mensup, anavatanı güney Meksika'dan kuzey Guatemala'ya kadar uzanan tek yıllık otsu bitkidir Çiya İspanyolca yağlı anlamına gelen Çiyan/chien kelimesinden türetilmiştir. Besleyici ve fonksiyonel özelliği nedeniyle çiya tohumunun önemi dünya çapında gittikçe artırmış ve besin, kozmetik, ilaç üretimi gibi birçok alanda da kullanım imkanı bulmuştur. Çiya tohumu %17-24 protein, %18-30 diyet lif ve %25-40 oranında yağ ihtiva etmektedir. Yağ içeriğinin %80'i de α-linolenik asit (omega-3; n3) ve linoleik asitten (omega-6; n-6) oluşmaktadır Ayrıca kalsiyum, fosfor, potasyum, demir ve magnezyum, niasin ve A vitamini bakımından da zengindir (Erdoğdu ve Geçgel, 2019). Yaz aylarında çiçeklenen bitkinin boyu yaklaşık 1 m'yi bulabilmektedir. Çiya tohumu yaklaşık 2 mm boyunda, oval, gri, siyah, kahverengi ya da beyaz renkli olup üzerinde koyu renkli noktalar mevcuttur. Kardiyovasküler hastalıklara karşı koruyucu özelliğe sahip olmakla birlikte antioksidan özelliği sayesinde kanser riskini azaltabilmektedir (Arnak, 2020). Tüketicilerin çiya tohumuna olan ilgilerinin artmasındaki en önemli etken kan basıncı kontrolü ve kan

şekeri seviyesi düzenlemedeki rolü ile reflü ve mide yanması gibi rahatsızlıkların iyileştirilmesindeki etkisinden kaynaklanmaktadır (Erdoğdu, 2019; Ergene ve Bingöl, 2019; Ergür ve Emir Çoban, 2020).

Yayın Balığı, Siluridae familyasına mensup olup tatlı su balıklarının en büyüğüdür. 3-5 m boy ve 250-300 kg ağırlığında olanlara rastlanmıştır. Ülkemizde birçok göl ve akarsuda bulunan yayın balığı (*Silurus glanis*) fazla rağbet gören bir türdür. Sportif balıkçılıkta da oldukça popülerdir. Eti oldukça lezzetli, kılçığı fazla olmayan, yüksek protein içeriğine ve düşük yağ oranına sahip, ekonomik değeri yüksek insanlar tarafından tüketilen bir tatlı su balığıdır (Kamarı, 2007; Saylar, 2009; Uysal vd., 2009; Çağıltay, 2011). Yayın balığı ve aynı familyaya mensup türlerin etleri kullanılarak yapılan balık köftesi, burger vb. ürünlerle ilgili yapılan çalışmalar Türkiye'de ve dünyada sınırlıdır. 2017 yılı yayın balığı yetiştiricilik üretimi yaklaşık 765 tondur. Ülkemizde iç sulardan avcılık yoluyla elde edilen yayın balığı miktarları 2018 yılında 362 tondur. 2018 yılında avcılık yoluyla elde edilen yayın balığı miktarı avcılıktan elde edilen toplam iç su ürünleri içerisinde %1,2'lik paya sahiptir (Yeşilçiçek, 2019).

Bu çalışma ile yayın balığı etinin köfte olarak değerlendirerek hem sağlık hem de ürün kalitesine olumlu etkileri olan bitkisel kökenli maddelerden keten ve çiya tohumu unları ilavesi ile tüketicilere farklı alternatif bir ürün sunulması amaçlanmıştır.

2. MATERYAL VE METOT

2.1. Materyal

Araştırmanın materyalini oluşturan yayın balıkları Ardahan İli'ndeki yerel bir satıcıdan (yeni doğadan avlanmış) tedarik edilmiştir. Keten ve çiya tohumları ise piyasadan temin edilerek öğütülmüştür. Köfte üretiminde kullanılan baharatlar da piyasadan temin edilmiştir.

Ortalama 108,33±7,63 cm boy ve 14,66±1,52 kg ağırlığındaki 9 adet yayın balıkları (her biri 3 balıktan oluşan toplam 3 grup) strafor kutularda soğuk zincir kurallarına uygun olarak Atatürk Üniversitesi Su Ürünleri Fakültesi Laboratuvarına getirilmiştir. Balıkların önce derileri yüzülerek, başları kesilmiş, iç organları temizlenmiş ve filetoları çıkarılmıştır. Daha sonra kıyma makinesinde (Arçelik K1768) kıyma haline getirilmiştir.

2.2. Köftelerin hazırlanması

Geleneksel köfte hamuru üretimi piyasa baz alınarak Can (2012)'e göre %88,5 kıyılmış balık eti, %2 tuz, %5 soğan, %3 sarımsak, %0,5 kimyon, %0,5 karabiber ve %0,5 kırmızıbiber eklenerek hazırlanmıştır. İlave edilen maddelerin oranları balık eti üzerinden hesaplanmıştır. Piyasadaki ürünler baz alınarak yaklaşık olarak 20 g ağırlığında, 32 mm çapında yuvarlak köfteler üretilmiştir. Hazırlanan köfteler beş gruba ayrılmıştır. Bitkisel unlarla zenginleştirilmemiş grup kontrol (K), %4 keten tohumu ilaveli grup (K4), %4 çiya tohumu ilaveli grup (Ç4), %8 keten tohumu ilaveli grup (K8) ve %8 çiya tohumu ilaveli grup (Ç8) olarak hazırlanmıştır. Köfteler ön çalışmalarla belirlenen sıcaklık ve sürede (175°C'lik fırında 5 dk.) pişirilerek, kilitli buzdolabı poşetlerine yerleştirilmiş ve buzdolabı koşullarında (4±1ºC) muhafaza edilmiştir.

2.3. Mikrobiyolojik analizler

Mikrobiyolojik analizler için, 25 g balık örneği steril stomacher poşetine alınmış ve üzerine 225 ml steril serum fizyolojik ilave edilerek stomacher cihazında (Lab Stomacher Blender 400-BA 7021 Sewardmedical, England) homojenize edilmiştir. Toplam aerobik mezofilik ve psikrotrofik bakteri analizleri için Plate Count Agar (PCA, Condalab) kullanılmış ve besiyerleri sırasıyla 30°C'de 2 gün ve 4°C'de 10 gün süreyle inkübasyona bırakılmıştırlar. Maya-küf sayımı için ise Potato Dextrose Agar (PDA, Condalab) besiyeri kullanılmış, 25ºC'de 5 gün inkübe edilmiştir (Gökalp vd.,2001).

2.4. Kimyasal analizler

pH analizi için 10 g örneğe distile su ile ilave edilmiş ve 1 dakika boyunca homojenize edildikten sonra belirlenmiştir (AOAC, 1990).

TBARS değeri Lemon (1975) ve Kılıç ve Richards (2003)'ün kullandığı yöntem modifiye edilerek

yapılmıştır. 100 g örneğe %7,5'luk triklorasetik asit (TCA, Isolab) eklenmiş ve homojenize edilerek filtre kağıdından süzülmüştür. Filtrata tiyobarbütirik asit (TBA, Isolab) ayracı eklenerek su banyosunda (Memmert) 100ºC'de yaklaşık 40 dakika bekletilmiştir. Daha sonra su banyosundan alınarak soğumaya bırakılmış ve spektrofotometrede (Shimadzu) 532 nm köre karşı okuma yapılmıştır Elde edilen veriler ile TBARS değeri aşağıdaki formüle göre hesaplanmıştır:

TBARS= ((Abs/ k (0,006) x 2/ 1000 x 6,8) x 1000/örnek ağırlığı

TVB-N değerini belirlemek için Malle ve Poumeyrol (1989) tarafından önerilen yöntem kullanılmıştır. 100 gram örneğe %7,5'lik (v/v) TCA eklenmiş ve homojenize edilerek santrifüj işlemine tabi tutulmuş ve filtre kağıdından süzülmüştür. Elde edilen filtrata %10'luk NaOH (Tekkim) (w/v) eklenmiş içinde %4'lük borik asit (Tekkim) içeren erlene son hacim yaklaşık 50 ml olana kadar köpük önleyici ve kaynama taşı da eklenerek distilasyon işlemi gerçekleştirilmiştir. Elde edilen distilat 0,05 M HCl (Tekkim) çözelitisi ile titre edilerek 100 gramdaki TVB-N değeri hesaplanmıştır.

2.5. Duyusal analizler

Duyusal analizlerde balık köfteleri görünüş, koku, lezzet, 10 puan üzerinden değerlendirilmiştir. Köfte örnekleri Atatürk Üniversitesi Su Ürünleri Fakültesi öğrencileri ve öğretim elemanlarından oluşan 10 kişilik panelist grubu tarafından yaklaşık 5 dakika tavada ısıtıldıktan sonra 10 puan üzerinden değerlendirilmiştir. Duyusal değerlendirme işlemi yaklaşık 1 saatte tamamlanmıştır. Puanlamada 10 puan çok iyi, 5 puan önemsiz, 4 puan ve aşağısı bozulmuş olarak kabul edilmiştir (Haq vd., 2013).

2.5. İstatistiksel analizler

Üç tekerrürlü ve 2 paralelli olarak gerçekleştirilen deneylerin sonucunda uygulamalar arasındaki fark varyans analizi (ANOVA) ve Duncan çoklu karşılaştırma testine tabi tutulmuş ve % 95 güven aralığında belirlenmiştir. İstatistiksel analiz, SPSS (Statistical Package for Social Science software) (Inc. Version 17.0, ABD) programı kullanılarak gerçekleştirilmiştir.

3. BULGULAR VE TARTIŞMA

3.1. Mikrobiyolojik sonuçlar

Farklı oranlarda (%4 ve %8) keten ve çiya tohumu ile zenginleştirilmiş yayın balığı köftelerinin soğukta muhafazası (4±1ºC) sırasındaki mikrobiyolojik analiz sonuçları Tablo 1'de verilmiştir.

Tablo 1. Farklı oranlarda (%4 ve %8) keten ve çiya tohumu ile zenginleştirilmiş yayın balığı *(Siluris glanis*) köftelerinin mikrobiyolojik analiz sonuçları (log kob/g) (Ort. ± SD)

Analizler	Depolama Süresi (gün)	Köfte Örnekleri				
		K	K4	Ç4	K8	Ç8
Toplam aerobic Mezofilik bakteri sayısı	1	3,23±0,22^{a}	2,86±0,10^{a}	2,97±0,08^{a}	2,60±0,33^{a}	2,62±0,22^{a}
	7	5,92±0,33^{b}	2,57±0,17^{a}	3,53±0,51^{a}	3,63±0,22^{b}	2,62±0,22^{a}
	14	8,56±0,41^{c}	6,85±0,24^{b}	7,00±0,12^{b}	6,27±0,34^{c}	6,65±0,44^{c}
Psikrotrofik bakteri sayısı	1	3,11±0,11^{a}	2,65±0,08^{a}	2,69±0,36^{a}	2,29±0,50^{a}	2,18±0,32^{a}
	7	5,23±1,22^{b}	2,54±0,29^{b}	3,33±0,59^{a}	3,61±0,35^{b}	3,33 ±0,33^{b}
	14	8,11±0,32^{c}	6,51±0,34^{b}	6,40±0,31^{b}	5,96±0,29^{c}	5,89±0,30^{c}
Maya-küf sayısı	1	2,00±0,00^{a}	2,00±0,00^{a}	2,00±0,00^{a}	2,00±0,00^{a}	2,00±0,00^{a}
	7	3,83±0,79^{b}	2,47±0,33^{a}	2,78±0,50^{b}	2,16±0,00^{a}	2,42±0,19^{a}
	14	5,19±0,24^{c}	4,54±0,30^{b}	4,82±0,31^{a}	4,08±0,11^{b}	4,68±0,36^{b}

K: Kontrol, K4: %4 keten tohumu ilaveli örnek, Ç4: %4 çiya tohumu ilaveli örnek, K8: %8 keten tohumu ilaveli örnek, Ç8: %8 çiya tohumu ilaveli örnek. Farklı harflerle gösterilen ortalamalar istatistiksel olarak birbirinden farklıdır ($p<0,05$).

Toplam aerobik mezofilik bakteri sayısı, depolama süresi ve sıcaklığa göre değişiklik göstermekte ve ürünün mikrobiyolojik durumu hakkında bilgi vermektedir (Bostan vd., 2011). Taze balıklarda

toplam aerobik bakteri için kabul edilebilir limit değeri 6 log kob/g olarak bildirilmiştir (Anonim 2022).

Keten ve çiya tohumu ile zenginleştirilmiş köfte örneklerinde depolamanın ilk gününde toplam mezofilik aerobik bakteri sayısı kontrol grubu örneklere göre daha düşük bulunurken, tüm gruplarda depolama süresine paralel olarak artış ($p<0,05$) tespit edilmiştir. Toplam aerobik mezofilik bakteri sayısı en düşük K8 grubu (2,60±0,33) köfte örneklerinde belirlenmiştir. TMAB sonuçlarına göre en düşük bakteri sayısı K8 grubu (2,60±0,33) köfte örneklerinde tespit edilmiştir. Toplam bakteri sayısının tüm gruplarda 14 günlük depolama periyodu sonunda kabul edilebilir limit değerini aştığı belirlenmiştir. Uçak (2020) alabalık burgerlerine ait toplam mezofilik bakteri sayısını depolamanın 15. gününde kontrol grubunda 7,29, %0,5 nar çekirdeği ekstraktı ile zenginleştirilmiş grupta 6,97 ve %1 nar çekirdeği ekstraktı ile zenginleştirilmiş grupta 6,79 log kob/g olarak bildirmişlerdir. Bu sonuçların çalışmamız sonuçlarına benzer olduğu görülmüştür. Da Silva vd. (2021) hindistan cevizi unu ile kapladıkları balık nuggetların toplam mezofilik bakteri sayılarının depolama süresi boyunca kabul edilebilir limit değerini (6 log kob/g) aşmadığı vurgulanmıştır. Kılıççeker (2014) adaçayı ve ısırgan otu ekstraktları ile kapladıkları balık köftelerin toplam mikroorganizma sayısı açıdan muhafaza süresinin tüm gruplar üzerinde etkisinin istatistiksel açıdan anlamlı olduğu belirlenmiştir ($p<0,05$). Yapılan bir başka çalışmada gümüş balığından hazırlanan köftelerin toplam canlı bakteri sayısında muhafaza süresince artış olduğu gözlemlenmiştir (Duman ve Peksezer 2016). Kaba vd. (2012), dumanlanmış zargana balığı kullanarak hazırladıkları köftelerin derin dondurucuda (-18°C) 6 aylık depolama süresi boyunca mikrobiyolojik kalite kriterleri yönünden tüketilebilirlik sınır değer olan 6 log kob/g değerini aşmadığını rapor etmişlerdir.

Soğukta muhafaza edilen su ürünlerinin kalitesinde meydana değişimlerin ve raf ömrünün belirlenmesinde psikrotrofik bakterilerin mezofil bakterilere kıyasla daha etkili olduğu bilinmekte olup; psikrotrofik bakteriler için kabul edilebilirlik sınır değer 6 log kob/g olarak verilmiştir (Mol vd., 2007). Keten ve çiya tohumu ile zenginleştirilmiş köfte örneklerinde depolamanın ilk gününde psikrotrofik bakteri sayısı kontrol grubu örneklere göre daha düşük bulunurken, tüm gruplarda depolama süresine paralel olarak artış ($p<0,05$) tespit edilmiştir. En düşük bakteri sayısı ise Ç8 grubu (2,18±0,32) köfte örneklerinde belirlenmiştir. Depolama süresince psikrotrofik bakteri sayısında görülen artışların istatistiksel açıdan önemli olduğu saptanmıştır ($p<0,01$). Psikrotrofik bakteri sayısı, K8 ve Ç8 gruplarında depolama süresi boyunca kabul edilebilir limit değerini aşmadığı, diğer gruplarda ise aştığı belirlenmiştir. Sur ve Karabıyıklı Çiçek (2021), çiya tohum yağının sahip olduğu esansiyel yağların çiya ve eklendiği ürüne antimikrobiyal etki kazandırdığını ve bu antimikrobiyal etkinin de genel olarak Gram pozitif ve Gram negatif bakteriler gibi mikroorganizmalar üzerinde inhibitif ve bakteriostatik etki gösterdiğini vurgulamışlardır. Bu bulgu çalışma bulgularımızı desteklemektedir. Keten tohumunun da içerdiği fenolik asitlerden dolayı; antioksidan, antimikrobiyal ve anti-kanser etki gösterdiği bildirilmiştir (Özgöçmen 2020). Kaya (2019) yaban mersini ilaveli aynalı sazan etinden yapılan balık köftelerinin psikrotrofik aerob bakteri sayısı açısından gruplar arasında önemli farklılığın olduğunu tespit etmişlerdir ($p<0,05$). Çapkın (2020), aynalı sazan balığından hazırladığı köftelerle ilgili çalışmasında toplam psikrotrofik aerobik bakteri sayılarında muhafaza süresince artışlar tespit etmiştir. Çalışma verileri çalışmamızla uyum göstermektedir. Syahrul et al. (2022), kedi balığı atıklarından üretilen balık köftelerinin mikrobiyal açıdan gruplar arasında önemli farklılığın olmadığını rapor etmişlerdir.

Maya ve sayısı tüm gruplarda 2,00 log kob/g olarak saptanırken depolama sonuna kadar artış göstermiştir. En yüksek maya ve küf sayısı depolamanın 7. gününde kontrol grubu (5,19±0,24) örneklerde bulunmuştur. Depolama süresi ve uygulama işlemleri maya ve küf sayıları üzerine önemli derecede etkili olduğu tespit edilmiştir. Çorapçı (2018), et ve et ürünlerinde maya ve küfler için kabul edilebilir bir limit değerin olmadığını vurgulamıştır. Maya ve küf sayısı tüm gruplarda depolama başlangıcında 2,00 log kob/g olarak saptanırken, en yüksek maya-küf sayısı depolamanın 7. gününde

kontrol grubu (5,19±0,24) örneklerde bulunmuştur. Elshafie vd. (2018)'nin yaptığı çalışmada çiya tohumun esansiyel yağlarının gıda kaynaklı patojen küflere karşı doğal fungistatik ve fungisidal etkili bileşiklere sahip olduğunu bildirmiştir. Çalışmadan elde ettiğimiz bulgular bu çalışma bulguları ile uyuşmaktadır. Maya-küf sayısının tüm köfte gruplarında depolama süresi boyunca arttığı gözlenmiştir. Maya ve küf sayıları üzerine grupların ve depolama süresinin etkisinin de önemli olduğu bulunmuştur ($p<0,05$). Çapkın vd. (2020) kadife balığı köftelerinin maya ve küf sayılarının depolama süresine bağlı olarak arttığını bildirmişlerdir. Can ve Emir Çoban (2012) aynalı sazan balığı ile hazırlanan köftelere %1 oranında timol sürülmesinin diğer gruplara kıyasla maya-küf sayısını düşürdüğü tespit edilmiştir. Çalışmamızda keten ve çiya tohumu ilavesinin mikrobiyolojik kalite üzerine olumlu etki ettiği görülmüştür.

3.2. Kimyasal sonuçlar

Farklı oranlarda (%4 ve %8) keten ve çiya tohumu ile zenginleştirilmiş yayın balığı (*Siluris glanis*) köftelerinin soğukta muhafazası (4±1ºC) sırasındaki kimyasal analiz sonuçları Tablo 2'de verilmiştir

Tablo 2. Farklı oranlarda (%4 ve %8) keten ve çiya tohumu ile zenginleştirilmiş yayın balığı *(Siluris glanis*) köftelerinin kimyasal analiz sonuçları (Ort. ± SD)

Analizler	Depolama Süresi (gün)	Köfte Örnekleri				
		K	K4	Ç4	K8	Ç8
TVB-N	1	7,37±0,95^{a}	7,17±0,20^{a}	7,02±0,16^{a}	7,07±0,12^{a}	6,37±0,53^{a}
	7	17,40±0,87^{b}	14,81±0,66^{b}	15,08±0,99^{b}	14,37±0,66^{b}	15,30±0,60^{b}
	14	27,32±2,63^{c}	23,21±1,99^{c}	22,41±1,12^{c}	20,69±0,43^{c}	21,18±1,22^{c}
TBARS	1	0,87±0,10^{a}	1,22±0,10^{a}	1,09±0,04^{a}	1,54±0,12^{a}	1,24±0,05^{a}
	7	2,70±0,45^{b}	4,62±1,07^{b}	3,62±0,27^{b}	4,26±0,92^{b}	3,05±0,21^{b}
	14	5,40±0,60^{c}	6,88±0,29^{c}	6,57±0,42^{c}	6,33±0,33^{c}	6,09±0,11^{c}
pH	1	6,40±0,20^{a}	6,53±0,00^{b}	6,46±0,02^{b}	6,51±0,03^{b}	6,42±0,02^{b}
	7	6,81±0,04^{b}	6,76±0,09^{c}	6,73±0,03^{c}	6,72±0,04^{c}	6,64±0,05^{c}
	14	6,42±0,02^{a}	6,38±0,05^{a}	6,22±0,10^{a}	6,33±0,11^{a}	6,24±0,07^{a}

K: Kontrol, K4: %4 keten tohumu ilaveli örnek, Ç4: %4 çiya tohumu ilaveli örnek, K8: %8 keten tohumu ilaveli örnek, Ç8: %8 çiya tohumu ilaveli örnek. Farklı harflerle gösterilen ortalamalar istatistiksel olarak birbirinden farklıdır ($p<0,05$).

Enzim ve mikroorganizma faaliyetleri sonucunda ürünlerin pH seviyeler yükselmekte ve kalite açısından ürünlerde farklılıklar meydana gelmektedir. Genel olarak taze balığın pH değeri 6,0-6,5 arası, tüketilebilirlik sınır değeri de 6,8-7 arası bildirilmiştir (Çapkın 2020). Köfte örneklerinin pH değerlerinde muhafaza süresince dalgalanmalar görülmüş ve gruplar arasında istatiksel açıdan önemli farklılıklar ($p<0,05$) tespit edilmiştir. En düşük pH değeri 14. gün Ç4 (6,22) grubu örneklerde bulunurken, en yüksek 9. gün K (6,81) grubu örneklerde tespit edilmiştir. Santillán-Álvarez vd. (2017) çiya tohumu ile zenginleştirilen yeniden yapılandırılmış sazan balığında pH değerini ortalama 6,21 olarak belirlemiş ve tazelik için önerilen tüketilebilir sınır değerini (6,49) aşmadığını bildirmiştir. Smaldone vd. (2017) uskumru ve gökkuşağı alabalığı kullanarak hazırladıkları köftelerin pH değerlerini depolamanın ilk gününde sırasıyla 6,12 ve 6,14 olarak bulurken, 22 günlük depolama sonunda 4,94 ve 4,97 değerlerine düştüğünü tespit etmişlerdir. Özpolat ve Emir Çoban (2012) karabalık ve sarıbalığın köfte olarak değerlendirildiği çalışmalarında iki grup köfte örneği arasında depolama süresince önemli bir farkın olmadığını saptamışlarıdır. Kullanılan balık türü, köfte katkı maddeleri, paketleme ve depolama koşullarının farklı olmasından dolayı çalışmamız diğer çalışmalarla benzerlik ve farklılıklar arz etmektedir. Kesemen (2018) tavuk köftelerinde çiya unu kullanımının kontrol grubuna göre pH değerini düşürdüğünü rapor etmiştir. Yapılan bir başka çalışma da ise kaju lifi ilavesinin pH değerini düşürdüğü bildirilmiştir (Guedes-Oliveira et al. 2016). Mahmoudzadeh et al. (2010), pisi balığı ve kertenkele balığı kullanarak hazırladıkları köftelerin derin dondurucuda (-18°C) 5 aylık depolama süresi boyunca pH değerlerinde artış olduğunu vurgulamışlardır. Zaki (2018) deve

etinden hazırladığı burger formülasonuna %1, %3 ve %5 oranlarında çiya ilavesininin pH değerlerinde depolama süresince artış olduğunu saptanmıştır.

Tarım ve Orman Bakanlığının yayınladığı kriterlere göre taze balık etinde TVB-N için <20 uygun, 20-28 arası kabul edilebilir, >28 değerler kabul edilemez olarak bildirilmiştir (Anonim 2002; Çapkın 2020). Kimyasal kalitenin belirlenmesinde önemli bir parametre olan TVB-N değeri, depolama boyunca tüm gruplarda tüketilebilirlik sınır değeri olarak kabul edilen 32-36 mg/100 g değerinin altında saptanmış olup, tüketilebilir ürün sınıfında yer almıştır (Varlık vd., 2004). TVB-N değerleri üzerine grupların ve depolama süresinin etkisi önemli bulunmuştur ($p<0,005$). Depolamaya paralel olarak tüm gruplarda TVB-N değerlerinde artış belirlenmiştir. En fazla artış kontrol grubu (K) örneklerde görülmüştür. En düşük TVB-N değeri de ise depolamanın 0. gününde Ç8 grubu örneklerde gözlemlenmiştir. TVB-N değeri depolama süresi boyunca 6,37-27,32 mg/100 g arasında gözlemlenmiştir. Depolama süresi ile keten tohumu ve çiya ilavesinin örneklerin TVB-N değerleri üzerine önemli derecede ($p<0,05$) etkili olduğu saptanmıştır. Yapılan bir çalışmada farklı bitkisel unlarla (buğday, arpa, yulaf, çavdar ve biber) zenginleştirmiş sazan balığı köftelerinin TVB-N değerlerinde depolamaya bağlı olarak artış olduğu ve depolama süresi boyunca tüketilebilir sınır değerini aşmadığı bildirilmiştir (Kılınççeker 2015). Çalışmadan elde edilen sonuçlar çalışmamızla paralellik göstermektedir. Cadun vd (2015) farklı lif (buğday ve elma) türlerinin balık köftelerinin kalitesine etkisini araştırdıkları çalışmalarında TVB-N değerlerinin depolama süresi buyunca tüm gruplarda önemli bir şekilde arttığını vurgulamışlardır. Ali et al. (2019), kabak püresi veya patates püresi ile formüle edilmiş tilapia balık burgerlerinde toplam uçucu nitrojen (9.45–11.20 mg N/100 g), değerlerini kontrol grubuna göre daha düşük tespit etmişlerdir. Özdemir (2019) tütsülenmiş alabalık eti kırıntıları ile hazırlanan burger tipi köftelerin TVB-N değerlerinin tüm örnek gruplarında depolama başlangıcından sonuna kadar artış gösterdiğini saptamışlardır. Ali et al. (2017) tatlı su çipurasından üretilen etlere farklı oranlarda kabak ve patates püresi ilave ederek hazırladıkları balık köftelerinde TVB-N değeri kontrol grubuna kıyasla daha düşük bulunmuştur. Ünlüsayın vd. (2002), sudak ve kadife balığı fileto atıklarından üretilen balık köftelerinin TVB-N değerlerinin depolama periyodu boyunca artış gösterdiğini bildirmişlerdir.

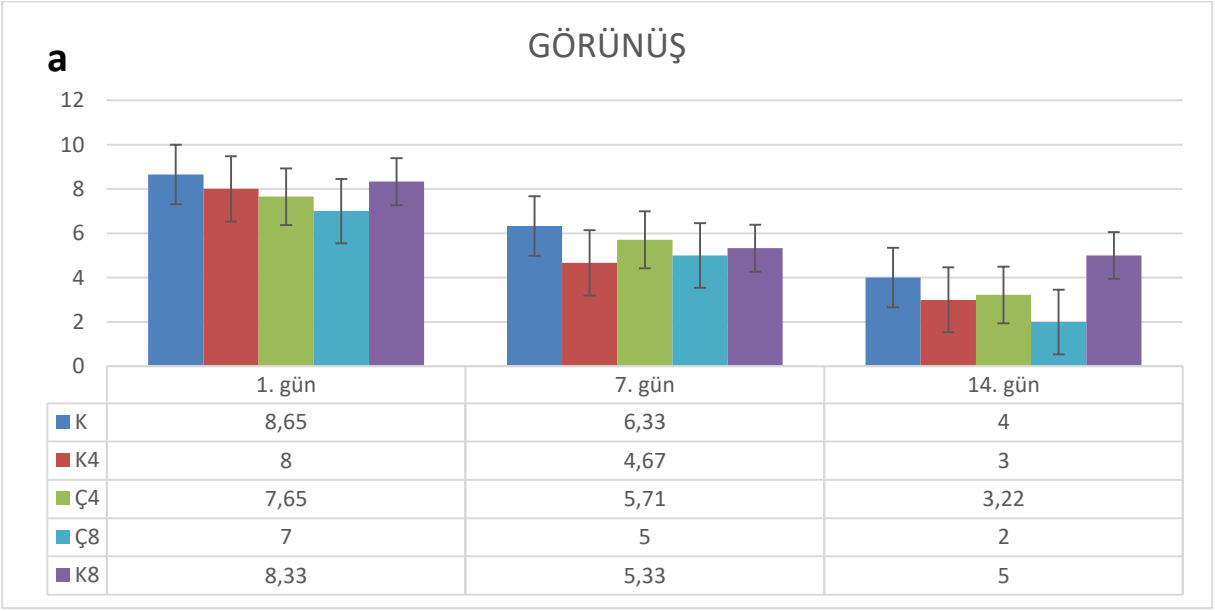
Balık etinde bozulma göstergesi olan TBARS değeri yağların acılaşma derecesini belirlemeye yarayan bir kimyasal kalite metodudur. TBA tüketilebilirlik sınır değeri balık etinde 7-8 mg MA/kg'dan arasındadır (Varlık vd. 2004). TBARS değeri araştırma sonuçlarımıza göre depolama süresi boyunca tüm gruplarda tüketile bilirlik sınır değerinin altında belirlenmiştir. TBARS değerlerinde de depolamaya paralel olarak artışlar belirlenmiştir. En düşük TBARS değeri K grubu (0. gün 0,87 µmol malonaldehit (MA)/kg) örneklerinde saptanırken, en yüksek K4 grubu örneklerde (14. gün 6,88 µmol malonaldehit (MA)/kg) örneklerinde bulunmuştur. Balık köftelerinin TBARS değerleri depolamaya bağlı olarak artış göstermiştir. İstatistiki analiz verilerine göre kontrol grubu ile bütün gruplar arasındaki fark önemli bulunmuştur ($p<0,05$). TBARS değeri kontrol grubuna kıyasla keten ve çiya tohumu ilaveli balık köftelerinde daha yüksek bulunmuştur. TBARS değerindeki artışın, keten ve çiya tohumlarının omega-3 içeriklerinin yüksek olmasından dolayı olduğu düşünülmektedir. Yapılan bir çalışmada keten tohumlu atıştırmalıkların en yüksek TBARS değerine sahip olduğunu rapor etmişlerdir (Vadukapuram vd., 2014). Can ve Emir Çoban (2012) aynalı sazan balığı köftelerinin muhafaza süresince tüketile bilirlik sınırlarının değerinin altında olduğunu rapor etmişlerdir. Riernersman vd. (2016), balık etinden üretilen burgerlerde çiya tohumu eklenmeyen grupta TBARS değeri çiya tohumu unu ilave edilen gruba kıyasla daha yüksek bulunmuştur. Heck vd. (2017) burger üretiminde hayvansal yağ yerine çiya veya keten tohumu yağı kullanımının çiya yağı kullanılan burgerlerde diğer gruplara kıyasla daha yüksek lipit oksidasyonuna sahip olduğunu bildirmişlerdir. Pintado et al. (2016) tarafından yapılan bir başka çalışmada da çiya unu ilave edilmiş frankfurterlerde TBARS değerleri daha yüksek saptanmıştır.

Sağlık üzerine pek çok faydalı etkileri bulunan antioksidan özelliklere sahip bitkisel tohumların

(keten ve çiya) köftelerin kimyasal özellikleri üzerine olumlu etkileri görülmüştür.

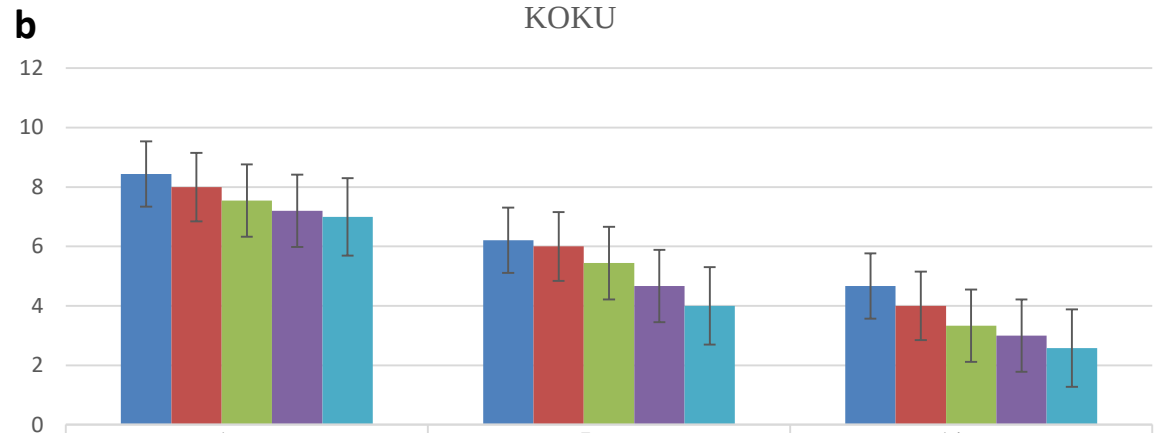
3.3. Duyusal sonuçlar

Farklı oranlarda (%4 ve %8) keten ve çiya tohumu ile zenginleştirilmiş yayın balığı köftelerinin soğukta muhafazası (4±1ºC) sırasındaki duyusal analiz sonuçları Şekil 1'de verilmiştir. Duyusal analiz sonuçlarına göre tüm örnek gruplarında depolamaya paralel olarak bir azalma görülmüş ve gruplar arasındaki fark istatistiksel olarak önemli bulunmuştur ($p<0,05$). Duyusal parametreler açısından doku hariç en fazla beğenilen grup kontrol grubu olmuştur. En az beğenilen grup ise %8 çiya tohumu ilave edilen grup (Ç8) olmuştur, bu durum çiya tohumunun alışılmadık bir lezzet vermesinden kaynaklandığı düşünülmektedir. Heck vd. (2017) çiya yağı kullanılan burgerlerin diğer gruplara kıyasla daha yüksek daha düşük duyusal puanlar aldığını rapor etmişlerdir. Çalışma sonuçları çalışmamızla benzerlik göstermektedir. Santillán-Álvarez vd. (2017) sazan balığı etine farklı oranlarda (%1, 4 ve 8) çiya tohumu unu ilavesinin duyusal açıdan çiya tohumu unuyla hazırlanan grupların kontrol grubuna yakın sonuçlar verdiğini bildirilmiştir. Yapılan bir başka çalışmada ise balık köftelerine %0, 3, 6 ve 9 seviyelerinde bambu lifi ilavesinin duyusal açıdan düşük oranda (% 3) lif ilavesinin örneklerin duyusal kalitesini artırmada faydalı olabileceğini vurgulamışlardır (Kılınççeker ve Karahan, 2019). Zeng et al. (2016) bambu filizi diyet lifi kullanılarak panelenmiş balık köftelerinin derin yağda kızartılması sırasında hamura %6 bambu filizi diyet lifi eklenmesinin, kızarmış köftelerin duyusal kalitesini iyileştirdiğini rapor etmişlerdir. Barros et al. (2018) %10 çiya unu içeren tavuk nuggetların duyusal açıdan panelistler tarafından kabul edilebilir olduğunu bildirmiştir. Kılınççeker (2020) nohut unu ilaveli tavuk köftelerinin duyusal açıdan tat üzerinde olumlu etkisinin olduğunu belirtmiştir. Bilgin vd. (2001) farklı işleme teknikleri (sıcak dumanlama, haşlama, kızartma) uyguladıkları *Clarias gariepinus*'un duyusal parametreler açısından panelistlerce daha çok beğenildiğini bildirmişlerdir. Akter et al. (2013) kedi balığı köftelerinin depolama süresi sonunda duyusal kalitesinin (doku, lezzet ve renk) azaldığını saptamışlardır. Hashim et al. (2019) 3 farklı sebze (domates, ıspanak, brokoli) ilavesi ile hazırladıkları kedi balığı köftelerinin duyusal parametreler açısından beğenildiğini saptamışlardır. Kedi balığı ile yapılan başka bir çalışmada da benzer bulgulara rastlanılmıştır (Sukkaseam et al. 2017).



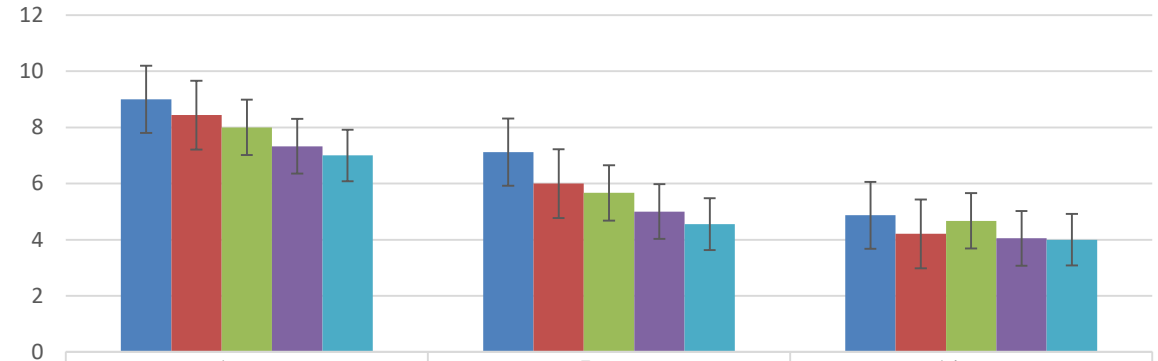
	1. gün	7. gün	14. gün
K	8,65	6,33	4
K4	8	4,67	3
Ç4	7,65	5,71	3,22
Ç8	7	5	2
K8	8,33	5,33	5

b

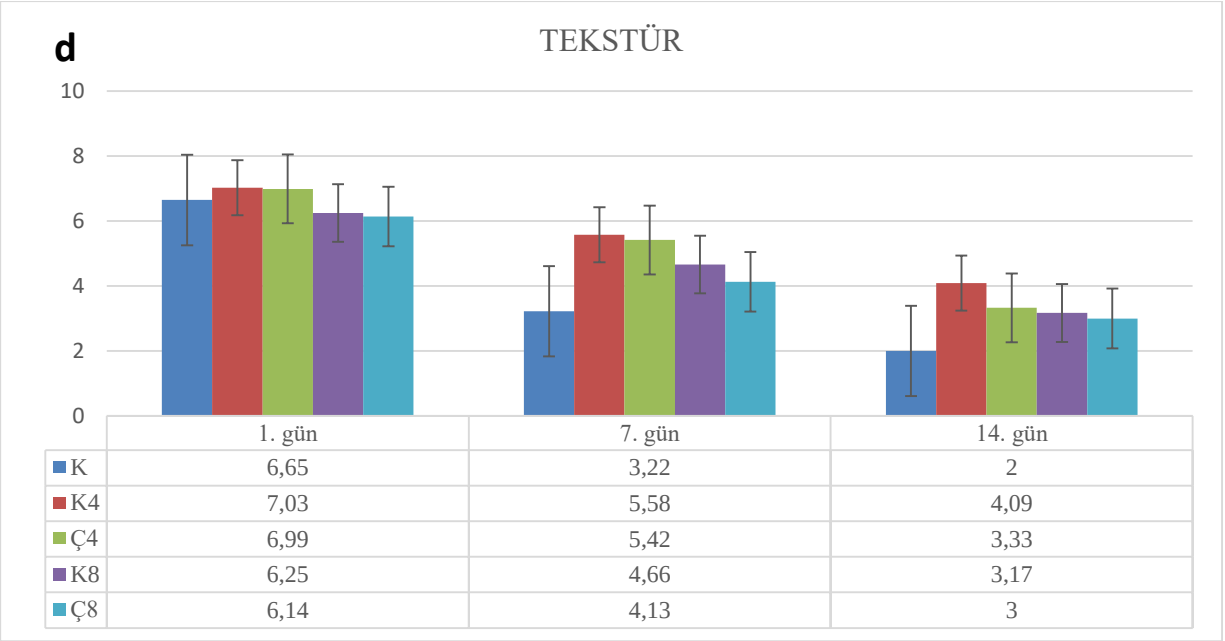


	1. gün	7. gün	14.gün
K	8,44	6,21	4,67
K4	8	6	4
Ç4	7,55	5,44	3,33
K8	7,2	4,67	3
Ç8	7	4	2,58

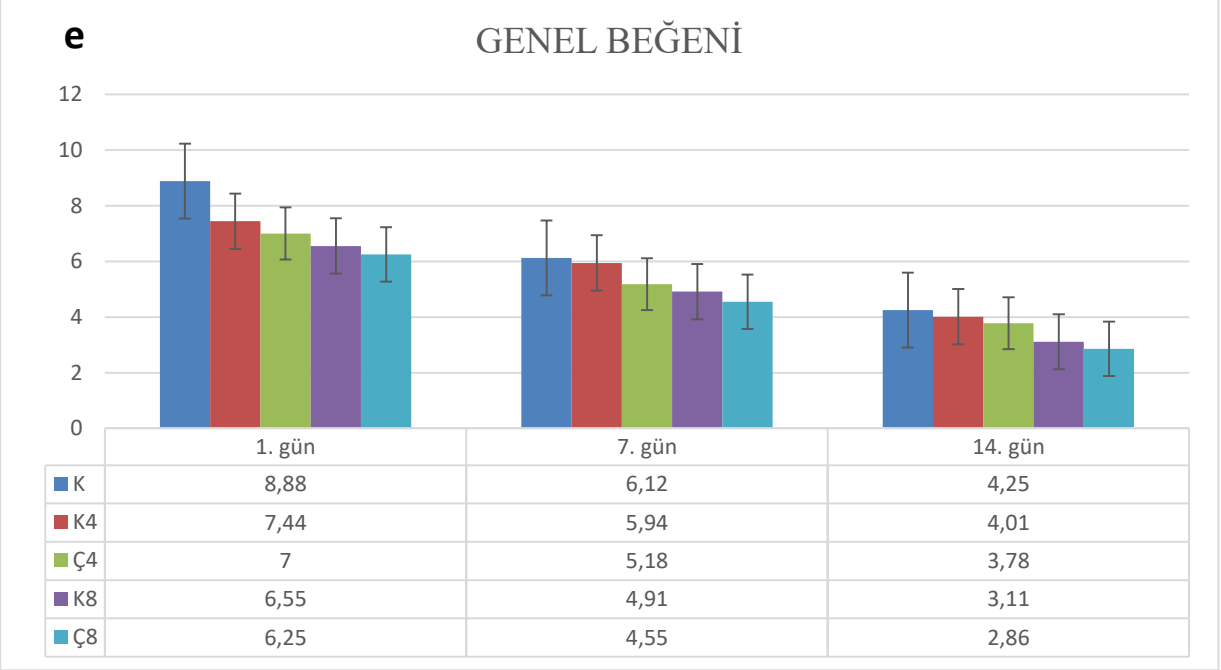
c



	1. gün	7. gün	14. gün
K	9	7,12	4,87
K4	8,44	6	4,21
Ç4	8	5,67	4,67
K8	7,33	5	4,05
Ç8	7	4,55	4



	1. gün	7. gün	14. gün
K	6,65	3,22	2
K4	7,03	5,58	4,09
Ç4	6,99	5,42	3,33
K8	6,25	4,66	3,17
Ç8	6,14	4,13	3



	1. gün	7. gün	14. gün
K	8,88	6,12	4,25
K4	7,44	5,94	4,01
Ç4	7	5,18	3,78
K8	6,55	4,91	3,11
Ç8	6,25	4,55	2,86

Şekil 1. Farklı oranlarda (%4 ve %8) keten ve çiya tohumu ile zenginleştirilmiş yayın balığı *(Siluris glanis)* köftelerinin duyusal analiz sonuçları. K: Kontrol, K4: %4 keten tohumu ilaveli örnek, Ç4: %4 çiya tohumu ilaveli örnek, K8: %8 keten tohumu ilaveli örnek, Ç8: %8 çiya tohumu ilaveli örnek.

4. SONUÇ

Bu araştırma sonucunda balık köftelerinin kalite kriterleri üzerine keten ve çiya tohumunun olumlu etkileri belirlenmiş, sağlık açısından önemli fonksiyonel özelliklere sahip keten ve çiya tohumu gibi bitkisel unlarla zenginleştirilen balık köftelerinin sunulabileceği görülmüştür. Günümüzde çalışan kadın ve yalnız yaşayan insan sayısını artışına paralel olarak önem kazanan hazır yemek (catering) teknolojisi ile hazırlanabilecek ürünler için de bu çalışmanın olanaklar sağlayacağı açıktır. yrıca su ürünlerinde keten ve çiya tohumunun kullanımıyla ile ilgili çalışmaların sınırlı olduğu görülmüş ve balık etinin bu şekilde değerlendirilerek hem raf ömrünün uzatılması hem de hazır gıda tüketiminin yaygınlaştığı günümüzde tüketiciye alternatif olarak sunulması ile ekonomik katkı sağlaması açısından

da önemli olduğu görüşündeyiz.

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Research **Araştırma Makalesi**

Determination of Letal Concentrations (LC_{50}) of Cyfluthrın, Dimethoate Insecticides on *Gammarus pulex* (L., 1758)

Cyfluthrin, Dimethoate Böcek İlaçlarının *Gammarus pulex* (L., 1758) Üzerindeki Letal Konsantrasyonlarının (LC_{50}) Belirlenmesi

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Abstract: In this study, *Gammarus pulex* (L., 1758) individuals obtained from Tunceli Munzur Stream were exposed to different concentrations of two insecticides containing cyfluthrin and dimethoate active ingredients, and LC_{50} values were determined from acute toxicity tests. During the study, parameters such as water temperature, pH, and dissolved oxygen were constantly controlled to ensure that they did not change. In this study, experiments were carried out by placing 0.5 liters of water in 1-liter glass aquariums. 10 *G. pulex* individuals were used for each concentration group. To determine the LC_{50} value, the mobility of the living things was observed and recorded in 24-hour periods. *G. pulex*, which lost its motility, was removed from the aquarium and excluded from the study. The study was carried out in 3 replications and the LC_{50} value for dimethoate was determined as 170.51 ± 8.15 µg/l, while the LC_{50} value for cyfluthrin was determined as 0.800 ± 0.12 µg/l.

Keywords
- *Gammarus pulex*
- Cyfluthrin
- Dimethoate
- Pesticide
- Acute toxicity

Özet: Bu çalışmada Tunceli Munzur Akarsuyundan elde edilen *Gammarus pulex* (L., 1758) bireyleri cyfluthrin ve dimethoate etken maddelerini içeren iki insektisitin farklı konsantrasyonlarına maruz bırakılarak, akut toksisite testlerinden LC_{50} değerleri belirlenmiştir. Çalışma boyunca su sıcaklığı, pH, çözünmüş oksijen gibi parametreleri sürekli kontrol edilerek değişmemesi sağlanmıştır. Yapılan bu çalışmada deneyler 1 litrelik cam akvaryumlarda 0,5 litre su konularak gerçekleştirilmiştir. Her bir konsantrasyon grubu için 10 adet *G. pulex* bireyi kullanılmıştır. LC_{50} değerinin belirlenmesi için 24 saatlik periyotlarla canlıların hareketlilik durumları gözlemlenerek kaydedilmiştir. Hareketliliğini kaybetmiş *G. pulex*'ler akvaryum içerisinden alınıp çalışma dışı bırakılmıştır. Çalışma 3 tekrarlı yürütülmüş olup, dimethoate için LC_{50} değeri 170,51 ± 8,15 µg/l tespit edilirken, cyfluthrin için LC_{50} değeri 0,800 ± 0,12 ng/l olarak olarak belirlenmiştir.

Anahtar kelimeler
- *Gammarus pulex*
- Cyfluthrin
- Dimethoate
- Pestisit
- Akut toksisite

1. INTRODUCTION

All kinds of damage done by people to the environment they live in by unnatural means create environmental pollution. This environmental pollution, on the other hand, directly or indirectly affects all living things, especially humans, at least once in different stages of their lives from birth to death. Although it is in the hands of people to reduce or increase these effects, they are constantly increasing due to economic concerns. Pesticides used to establish large industrial facilities, to release waste into

the environment, to mix with water, and to get more efficiency in agriculture are just a few examples of the factors that cause environmental pollution.

The effect of pollutants on the organism may differ according to abiotic factors (temperature, oxygen, pH, light, etc.) and factors such as height, weight, and sex of the vivid.

The transmission routes of pesticides to the aquatic environment are generally by mixing with wind rainwater, drainage waters, surface flows, and irrigation waters, spraying against aquatic organisms or plants living in water channels, mixing with sewage and sewage waters in residential areas, and the discharge of pesticide manufacturing residues. In addition, as a result of direct applications to water (for example, in mosquito control), pesticides are retained by aquatic plants or bottom mud (Atamanalp and Yanık 2001).

Pesticide residues in water accumulate in dissolved form or the form of transformation products, sediments, benthic invertebrates, aquatic plants, plankton, aquatic organisms, and fish (Sarıgül, 2007).

As a result of unconscious and misuse of pesticides, negative effects on nature and human life occur. Unconscious and overused pesticides affect non-target organisms by being carried into rivers, lakes, and seas by winds, rainwater, and groundwater.

These negativities seen in aquatic creatures do not have the same effect on every living thing; It affects different events of living things such as nutrition, circulation, and reproduction and creates a stress effect on living things.

The organisms most affected by the pollution of the aquatic environment are the organisms living in that aquatic environment. These organisms living in polluted environments will either move away from this environment, adapt to this environment, or perish by dying. For this reason, living things choose the most suitable habitats for themselves. Such organisms are an indicator of their habitat, an indicator, or a biomarker. *Gammarus*, which is a clean water indicator, is one of the creatures that have both economic and aquatic indicator features (Demirsoy, 1998).

Many scientific studies have been conducted to examine the effects of pesticides on various aquatic organisms. In one of these studies, Felten et al (2007) investigated the effect of cadmium on physiological and behavioral responses of *Gammarus pulex*. Adam et al. (2009) applied propiconazole, tebuconazoline, 3-iodo-2-propynyl butyl carbamate (IPBC, fungicide), and cypermethrin to *G. pulex* as a single or a mixture to determine the toxicity of insecticides and fungicides used as wood preservatives. Vellinger et al (2013) studied the single and combined effects of cadmium and arsenate in *Gammarus.* Uğurlu et al. (2015) investigated the toxicological effects of thiamethoxane on *Gammarus kischineffensis*. Demirci (2018) evaluated the acute toxic effects of imidacloprid and acetamiprit on *G. kischineffensis.* In this study, it was aimed to determine the acute toxicity of dimethoate and cyfluthrin pesticides on *G. pulex*, which is a clean water indicator.

2. MATERIAL and METHOD

2.1. Material

2.1. 1. Collection of *G. pulex*

The *G. pulex* individuals used in the study were collected from the side branches of Munzur Stream in Tunceli province with the help of a bottom scoop, the air was reinforced, and they were brought to the Munzur University Fisheries Faculty research laboratory with tanks (Figure 1).

G. pulex individuals were placed in 40x20x20 cm aquariums and adapted to laboratory conditions for 4 weeks. Airflow was provided with air motors for the oxygen requirement of the aquariums.

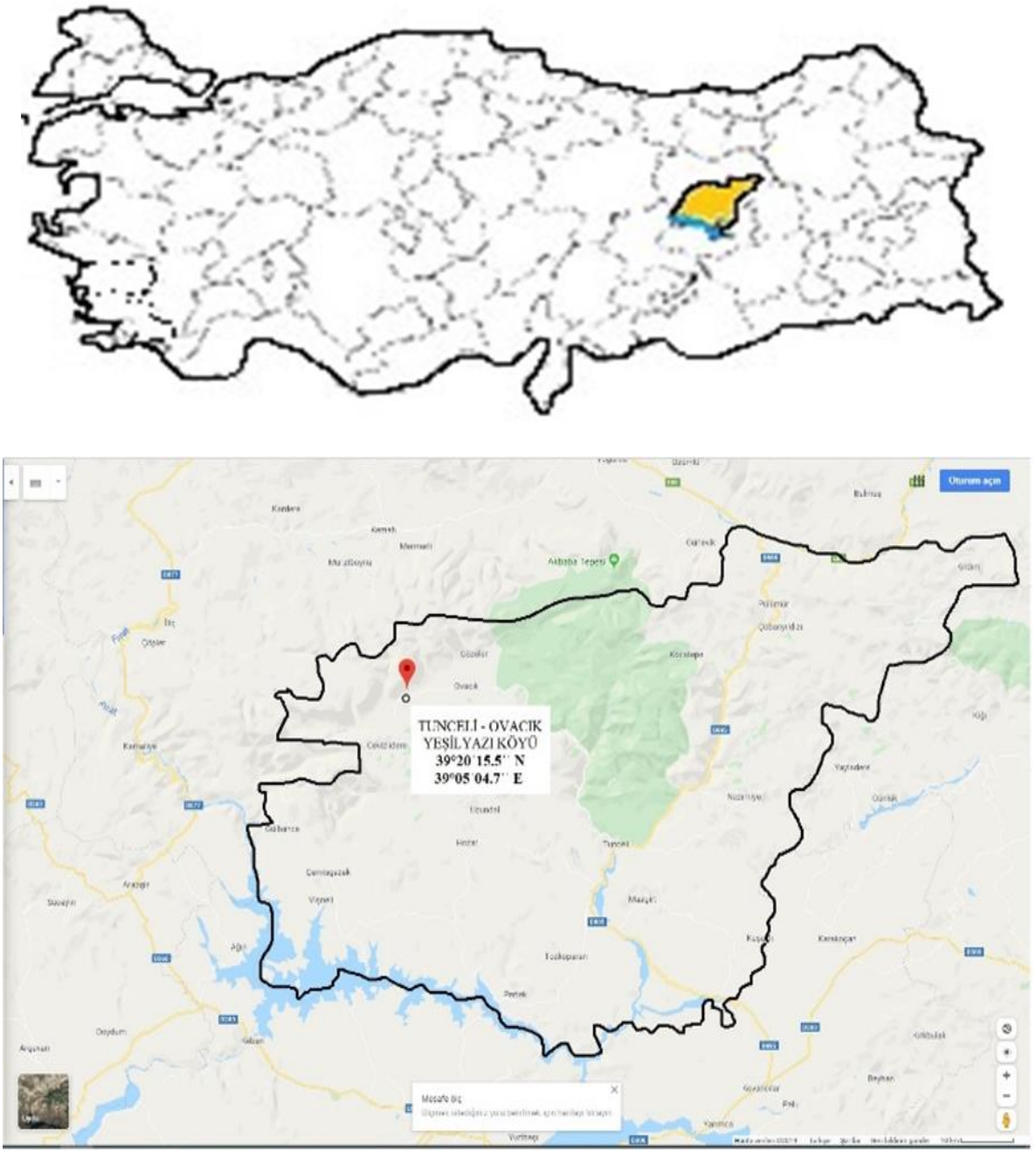

Figure 1. The area where the experimental organism (*G.pulex*) was collected

2.1.2 Adaptation of *G. pulex* to the Laboratory

For the adaptation of *G. pulex* to laboratory conditions, suitable environments for natural habitats have been prepared. For this purpose, sediment taken from the natural habitat of *G. pulex* was washed with pure water and placed in stock aquariums. Again, water brought from the natural environment of *G. pulex* was added to them. The stock aquariums were supplemented with oxygen using an air motor. In ambient lighting, a photoperiod of 12 hours dark and 12 hours light was used. With the thermostatic air conditioner, the ambient temperature where the aquariums are left is fixed at 18 ^{0}C. After the adaptation medium was prepared, *G. pulexs* collected from the Munzur Stream were placed in stock aquariums. *G. pulex* is left to adapt to laboratory conditions. 70% of the water in the stock aquariums was renewed once a week. For the feeding of *G. pulex*, shrub willow tree leaves were collected and left to rot.

2.1.3. Range Experiment

Before the study, a range determination study was performed to determine dimethoate and cyfluthrin concentrations. In the spacing tests, 10 *G. pulex* individuals were placed in each of the aquariums.

Range determination experiments were performed for each pesticide. After the range determination experiments, the concentration ranges of dimethoate and cyfluthrin pesticides were determined to be applied in the LC_{50} experiments.

During the first application, abnormal movements such as fast and reverse swimming were observed in living things. On the 3rd and 4th days of the application, limitation of movement was observed.

2.1.4. Experiment Design

Glass aquariums with a volume of 1 liter were used for the experiments in the study and 10 *G. pulex* were placed in each aquarium. According to the results of range determination experiments, the trial design for dimethoate pesticide (K (0,0), D1 (25µg/l), D2 (50 µg/l), D3 (100µg/l), D4 (200 µg/l) and D5 (400 µg/l)) concentrations were determined (Table 1).

For Cyfluthrin pesticide, the trial design (K (0.0), C1 (0.2 µg/l), C2 (0.4 µg/l), C3 (0.8 µg/l) and C4 (1.6 ng) /l) concentrations were determined (Table 2).

LC_{50} experiments were carried out statically over a period of 96 hours. In each experiment, dead individuals were counted and removed from the aquarium in 24-hour periods. No feeding was done to the animals during the experiment. In the experimental application, water taken from the environment where *G. pulex* was collected was used. All experimental studies were applied in 3 replications.

Table 1. Experiment design and concentrations determined for dimethoate

Recurrences	Groups (µg/l dimethoate)					
	K	**D1**	**D2**	**D3**	**D4**	**D5**
I	0,0	25,0	50,0	100,0	200,0	400,0
II	0,0	25,0	50,0	100,0	200,0	400,0
III	0,0	25,0	50,0	100,0	200,0	400,0

Table 2. Experiment design and concentrations determined for Cyfluthrin

Recurrences	Groups (µg/l cyfluthrin)				
	K	**C1**	**C2**	**C3**	**C4**
I	0,0	0,2	0,4	0,8	1,6
II	0,0	0,2	0,4	0,8	1,6
III	0,0	0,2	0,4	0,8	1,6

2.5. Determining the LC_{50} Value

To determine the LC_{50} value, experimental groups in which different dimethoate and cyfluthrin concentrations were applied separately were formed together with the control group. For each group, 10 live were used. 96 hours after administration of dimethoate and cyfluthrin, viable and deceased individuals were counted. LC_{50} value was determined by using SPSS 24.0 statistical package program Probit Analysis.

In all experimental stages of the study, 0.5 liters of dechlorinated water taken from the natural environment of the creatures were used in 1-liter glass aquariums.

For each concentration, 10 *G. pulex* were placed in these aquariums. To determine the LC_{50} value, the mobility of the living things was observed and recorded in 24-hour periods.

G. pulex, which lost its mobility, was removed from the aquarium and excluded from the study.

3. RESULTS

3.1. Acute Toxicity (LC_{50}) Values

3.1.1. LC_{50} Value of Dimethoate Insecticide

In the study, the LC_{50} value of dimethoate insecticide on *G. pulex* was determined as 3 repetitions and the average values are given in Table 3. The mean LC_{50} value of the dimethoate pesticide was found to be 170.51±8.15 µg/l, the lower band level average value was 119.89±7.9 µg/l, and the upper band level average value was 228.53±9.0 µg/l. (Table 3).

Table 3. LC_{50} values of *G. pulex* exposed to dimethoate insecticide

	LC_{50} Value		
	LC_{50} (µg/l)	**Lower Level (µg/l)**	**High level (µg/l)**
I. Recurrence	178.69	127.80	237.59
II. Recurrence	162.37	111.96	219.51
III. Recurrence	170.50	119.90	228.48
Average	170.51 ± 8.15	119.89 ± 7.9	228.53 ± 9.0

Table 4. Mortality rates after 96 hours in *G. pulex* exposed to dimethoate insecticide

Dimethoate concentrations applied to the experimental groups (µg/l)		**Number of *G. pulex* used in trial**	**Number of *G. pulex* died during 96 Hours**	**% Death**
I. Recurrence	0 (K)	10	0	0
	25 (D1)	10	1	10
	50 (D2)	10	2	20
	100 (D3)	10	4	40
	200 (D4)	10	6	60
	400 (D5)	10	9	90
II. Recurrence	0 (K)	10	0	0
	25 (D1)	10	1	10
	50 (D2)	10	2	20
	100 (D3)	10	4	40
	200 (D4)	10	8	80
	400 (D5)	10	9	90
III. Recurrence	0 (K)	10	0	0
	25 (D1)	10	1	10
	50 (D2)	10	2	20
	100 (D3)	10	4	40
	200 (D4)	10	7	70
	400 (D5)	10	9	90

In the study, mortality rates of all groups (K, D1, D2, D3, D4 and D5) were determined in *G. pulex* individuals exposed to dimethoate insecticide within 96 hours. (Table 4).

G. pulex individuals have a 10% death rate in all 3 repetitions in the D1 group, a 20% mortality rate in each relapse for the D2 group, a 40% mortality rate in each replication for the D3 group, and 60% in the I. Replica for the D4 group. 80% in recurrence and III. It was determined that the highest mortality rate was 70% in the recurrence and 90% in the D5 group (Table 4).

3.1.2. LC_{50} Value of Cyfluthrin Insecticide

In this study, the LC_{50} value of cyfluthrin on *G. pulex* was determined in 3 repetitions and the average values are given in Table 5. The mean LC_{50} value of the Cyfluthrin insecticide was found to be

0.800 ± 0.12 µg/l, the lower band level average value was 0.570 ± 0.12 µg/l, and the upper band level average value was 1.059 ± 0.13 µg/l (Table 5).

Table 5. LC_{50} values of *G. pulex* exposed to Cyfluthrin insecticide

	LC_{50} Value		
	LC_{50} (µg/l)	**Lower Level (µg/l)**	**High Level (µg/l)**
I. Recurrence	0,714	0,486	0,965
II. Recurrence	0,752	0,525	1,005
III. Recurrence	0,935	0,700	1,207
Average	0,800±0,12	0,570±0,12	1,059±0,13

In the study, mortality rates of all groups (K, C1, C2, C3 and C4) were determined within 96 hours of *G. pulex* individuals exposed to cyfluthrin insecticide. (Table 6). *G. pulex* individuals C1 group death at a rate of 10% in each 3 replication, C2 group 30% in I. Replica, II. 40% in recurrence, III. 20 mortality rate in recurrence, 80% in I. recurrence in C3 group, II. 60% in recurrence and III. 50% mortality rate in recurrence, I., and II. for the C4 group. It was determined that the mortality rate was 90% in recurrences and 80% in III recurrences (Table 6).

Table 6. Mortality rates after 96 hours in *G. pulex* exposed to Cyfluthrin insecticide

Cyfluthrin concentrations (µg/l) applied to the experimental groups		**Number of *G. pulex* used in trial**	**Number of *G. pulex* died during 96 Hours**	**% Death**
I. Recurrence	0 (K)	10	0	0
	0,2 (C1)	10	1	10
	0,4 (C2)	10	3	30
	0,8 (C3)	10	8	80
	1,6 (C4)	10	9	90
II. Recurrence	0 (K)	10	0	0
	0,2 (C1)	10	1	10
	0,4 (C2)	10	4	40
	0,8 (C3)	10	6	60
	1,6 (C4)	10	9	90
III. Recurrence	0 (K)	10	0	0
	0,2 (C1)	10	1	10
	0,4 (C2)	10	2	20
	0,8 (C3)	10	5	50
	1,6 (C4)	10	8	80

4. DISCUSSION

In recent years, it has been revealed in scientific studies that pesticides or insecticides used to increase productivity in agriculture and animal husbandry harm both terrestrial and aquatic organisms, which are out of their intended use and are not targeted, even in the smallest amounts. For this purpose, the acute toxicity of dimethoate and cyfluthrin insecticides used as pesticides in agriculture *on G. pulex* was investigated.

As a result of the research, for dimethoate; The mean LC_{50} value was 170.51±8.15 µg/l, the lower band level mean value was 119.89±7.9 µg/l, and the upper band level average value was 228.53±9.0 µg/l, while cyfluthrin For LC_{50} mean value 0.800 ± 0.12 µg/l, lower band level mean value 0.570 ± 0.12 µg/l, upper band level mean value 1.059 ± 0.13 µg/l.

As can be seen from the determined values, it was observed that both insecticides were effective on *G. pulex*. It was determined that even very low concentrations of Cyfluthrin insecticide were effective on *G. pulex*.

Many researchers have examined the effects of pesticides on other non-target aquatic organisms. Among these researchers, Köprücü and Aydın (2004) determined that deltamethrin pesticide; Aydın and Köprücü (2005), diazinon pesticide; Aydin et al. (2005) determined the acute toxicity of cypermethrin pesticide on the embryo and larvae of *Cyprinus carpio*. In another study, Ural and Şimşek (2006) investigated the acute toxicity of dichlorvos pesticide on Silurus glanis offspring. Serdar (2021) calculated the LC_{50} value of Cyfluthrin pesticide in zebra mussels as 553.22 ± 27.3 µg/L in his study. In a different study, Yüksel et al. (2020) calculated the LC*50* value of the *G. pulex* they exposed to malathion pesticide as 1.03 ± 0.07 mg/L in their study. It has been determined that the findings obtained as a result of these studies on various fish species and the findings of this study show a complete similarity in terms of dying or adversely affecting the life of the living things even at low concentrations.

Güner (2020), acute toxicity of cyhalofop butyl (LC_{50}) study on *Gambusia holbrooki.* The acute toxicity (LC_{50}) of this herb (Chillinger 200 EC 200, cyhalofob butyl), which is used extensively in the Thrace region, including Cyhalofob butyl, has been investigated. The acute toxicity (LC_{50} value) of this herbicide was investigated in common mosquito fish (*Gambusia holbrooki*) in the Thrace Region. The Lethal Dose 50 experiment was performed in 3 replicates in static test runs (water temperature 27.70 ±0.56 □C, water pH 8.88± 0.37, and conductivity 718.25±21.113 µhos). The experimental results obtained for the Chillinger 200 ec during the experiments were evaluated with the Trimmed Spearman-Karber method.

Serdar et al (2019), evaluation of the acute toxic effect of cadmium on *Gammarus pulex* (freshwater amphipoda) at different temperatures. As a result of the study, it was aimed to determine the change of LC_{50} values of Cd in *G. pulex* at 10, 14, and 18° C. 96 hours were determined at different temperatures of 10, 14, and 18 °C. LC 50 values were obtained by probit analysis; 51.79 ± 1.2 µg L -1 for 10°C, 47.67 ± 0.6 µg L -1 for 14°C, and 33.93 ± 0.6 µg L -1 for 18°C. It was determined that the values decreased depending on the temperature increase of LC*50*.

Serdar (2019), investigated the effect of dimethoate pesticides on some biochemical biomarkers in *Gammarus pulex.* The acute toxicity value (LC*50*) of dimethoate pesticide in *G. pulex* was determined. Superoxide dismutase (SOD), glutathione S-transferase (GST), glutathione peroxidase (GPx), and catalase (CAT) activities and malondialdehyde (MDA), glutathione (GSH) levels of *G. pulex* organism exposed to sublethal concentrations were investigated. Analyzed by ELISA for 24 and 96 hours. In conclusion, this study demonstrated the abilities of dimethoate pesticides to induce oxidative stress. The results showed MDA, GSH levels, SOD, CAT, GPx, and GST activities. *G.pulex* has stated that it can be used as an effective biomarker.

Cold and Forbes (2004) investigated the effects of short-term pyrethroid pesticide applications on the survival and proliferation of *G. pulex*. As a result, they determined that the exposure concentrations of the widely used pesticide esfenvalerate significantly affect the survival and reproduction of *G. pulex.*

Lukancic et al (2009), Physiological responses of two freshwater shellfish, *Asellus aquaticus L*. and *G. fossarum*, after exposure to two pesticides were measured. Both species responded to short-term exposure with elevated Respiratory (R) levels or lower levels of Electron Transfer System (ETS) activity. In both test types, it showed an effect for 1 hour at a concentration of 10 mg/L. Laboratory tests of both test types prove that *G. fossil* is more sensitive to short-term pesticide exposure than *A. aquaticus*. In this study, *G. pulex*'ler individuals were affected as a result of short-term exposure to pesticides. Studies show similarity in this aspect.

5. CONCLUSION

Pesticides used in agriculture contaminate the waters, which are vital for life, by mixing with the waters in various ways. The pesticides that fish and other aquatic organisms take into their bodies affect the natural balance by making a negative impact on human health and the food pyramid as a result of the consumption of fish by humans, birds, and other creatures that consume fish.

To minimize these damages, pesticide use should be controlled and farmers should be educated by authorized persons. Less toxic pesticides should be preferred, access to water sources should be prevented while spraying, pesticide containers used should not be washed in water sources, and used tools and containers should be destroyed and not released into the environment. Samples should be taken frequently from water sources and evaluations should be made.

More detailed research and scientific studies should be carried out on the damage caused by pesticides to nature and humans. Considering the importance of water for humans and other living things, it is necessary to investigate the effects and harms in these areas and to take necessary precautions.

In addition, it is obvious that new studies are needed to investigate the biological and environmentalist alternative removal methods of the determined harmful effect, to reduce the toxic effect on living things, and according to the conditions of the developing world.

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CONFLICT OF INTEREST

I declare that there are no financial interests or personal relationships that could affect this work

AUTHOR CONTRIBUTION

A.A; performing the experiment, article writing, R.A; inspection, O.S; data analysis

ETHICAL STATEMENTS

Ethical approval is not required as the creatures used in this study are invertebrates.

DATA AVAILABILITY STATEMENT

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

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Research **Araştırma Makalesi**

Protective Effects of Different Egg Yolk Sources on Cryopreservation of Scaly Carp (*Cyprinus carpio*) Sperm

Farklı Yumurta Sarısı Kaynaklarının Pullu Sazan (*Cyprinus carpio*) Spermasının Kriyoprezervasyonu Üzerine Koruyucu Etkileri

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Abstract: Egg yolk is one of the most widely used cryoprotective components of extenders, especially for the cryopreservation of mammalian species' sperm cells. However, there is a lack of information regarding their efficacy in cryopreservation of fish sperm cells. Thus, the objective of this experiment was to compare the effectiveness of egg yolk from different avian species (duck, goose, and chicken) on post-thaw quality and fertilization ability of scaly carp (*Cyprinus carpio*) semen following cryopreservation. Sperm samples diluted with the sucrose-based extender at the ratio of 1:10 were supplemented with 10, 15, and 20% ratios of different avian egg yolks. In the control group, sperm samples were diluted with the sucrose-based extender, without egg yolk. Following dilution, sperm samples were equilibrated at +4ºC for 10 min and aspirated into 0.25-ml straws. Then, sperm samples were frozen 3 cm above the liquid nitrogen (LN_2) surface and plunged directly into the LN_2. The frozen sperm cells were thawed in a water bath at 35 ºC for 30 s and fertilization was carried out using a $1x10^5$ spermatozoa/egg ratio. Based on the results, supplementation of sperm cells with 20 % duck egg yolk in a sucrose-based extender exhibited the best post-thaw progressive motility (67.8 ±1.24%), progressive motility duration (32.6 ±1.45 s), viability (82.4 ±1.36%), and fertility (92.6 ±1.28%) compared to the control group ($P<0.05$). The results of the experiment showed that duck egg yolk could be used as an alternative instead of chicken egg yolk in a sucrose-based extender for the cryopreservation of scaly carp sperm.

Keywords
- Cryopreservation
- Egg yolk
- Fertility
- Extender
- Sperm

Özet: Yumurta sarısı, özellikle memeli türlerine ait sperm hücrelerinin dondurularak saklanmasında kullanılan sulandırıcıların en yaygın kriyoprotektif bileşenlerinden biridir. Dolayısıyla, balık sperm hücrelerinin dondurularak saklanmasındaki etkinlikleri hakkında bilgi eksikliği bulunmaktadır. Bu nedenle bu çalışmanın amacı, farklı kanatlı türlerine (ördek, kaz ve tavuk) ait yumurta sarılarının, kriyoprezervasyonu takiben pullu sazan (*Cyprinus carpio*) spermasının çözüm sonu kalite ve fertilizasyon yeteneği üzerindeki etkinliklerinin karşılaştırılmasıydı. Sukroz bazlı sulandırıcı ile 1:10 oranında dilüe edilen sperm örneklerine %10, 15 ve %20 oranlarında farklı kanatlı yumurtası sarıları ilave edildi. Kontrol grubunda ise sperm örnekleri, yumurta sarısı içermeyen sukroz bazlı sulandırıcı ile dilüe edildi. Dilüsyon işlemini takiben, sperm örnekleri +4ºC'de 10 dakika ekilibere edilerek 0,25 ml'lik payetlere çekildi. Daha sonra sperm hücreleri sıvı nitrojen (LN_2) yüzeyinin 3 cm üzerinde dondurularak doğrudan sıvı azota (LN_2) aktarıldı.

Anahtar kelimeler
- Kriyoprezervasyon
- Yumurta sarısı
- Fertilite
- Sulandırıcı
- Sperm

Dondurulan sperm hücreleri 35 ºC su banyosunda 30 sn süre ile çözdürüldü ve 1x10^5 spermatozoa/yumurta oranı kullanılarak fertilizasyon işlemi gerçekleştirildi. Sonuçlara göre, sperm hücreleri sukroz bazlı sulandırıcıda %20 ördek yumurtası sarısı ile takviye edildiğinde, kontrol grubu ile karşılaştırıldığında en iyi çözüm sonu progresif motilite (%67,8 ±1,24), progresif motilite süresi (32,6 ±1,45 s), canlılık (%82,4 ±1,36) ve fertilite (%92,6 ±1,28) değerleri elde edilmiştir (P<0.05). Çalışmanın sonuçları, pullu sazan sperminin dondurularak saklanmasında sukroz bazlı sulandırıcı kullanıldığında, tavuk yumurtası sarısı yerine ördek yumurtası sarısının alternatif olarak kullanılabileceğini göstermiştir.

1. INTRODUCTION

The cryopreservation technique, which is an important biotechnological tool for the conservation of aquatic genetic resources, has been successfully utilized for the long-term storage of sperm cells in the aquaculture industry. The benefits of cryogenic preservation of sperm cells in the aquaculture industry can be summarized as follows: year-round supplying of sperm, artificial hybridization between species, transportation of sperm cells among fish farms, reduction in the nursing cost of male broodstock, and establishment of cryobanks (Bozkurt, 2019; Yavaş et al., 2014).

Although cryopreservation of sperm cells offers many advantages mentioned above, it is also a complex process leading to several forms of cellular damage (Purdy, 2006). The main cryodamage of the cryopreservation process on sperm cells is associated with cold shock and intercellular ice crystal formation, which may lead to a decrease in motility and fertilizing ability of sperm following thawing (Matsuoka et al., 2006; Bozkurt et al., 2019).

Even though cryoprotectants can inhibit cryodamages during the cryopreservation process, they can become toxic to the sperm cells at high levels (Tekin et al., 2007). Therefore, egg yolks from different avian species in an extender have been used to protect sperm cells against cold shock damages during cryopreservation in domestic animals recently (Aboagla and Terada, 2004). The useful effect of avian egg yolks in the cryopreservation process can be ascribed to a resistance factor, which is necessary to protect sperm cells from the cold shock and to maintain viability as well (Webb et al., 2011).

Chicken egg yolk traditionally has been used as a complementary for the cryopreservation of sperm cells due to its easy availability (Bathgate et al., 2006). It ensures protection to sperm membranes against the cryodamages, which occur due to the significant temperature variations during cryopreservation (Andrabi, 2009). However, it has been reported that extenders including egg yolks from different avian species other than that of chicken significantly improved post-thaw quality parameters of bovine (Su et al., 2008), equine (Trimeche et al., 1997; Webb et al., 2011; Burris and Webb, 2009), and ovine (Ali et al., 2013) sperm. It is supposed that the post-thaw quality improvement in sperm cells is based on the variations in the biochemical composition of different avian egg yolks (Bathgate et al., 2006).

As far as we know, there is limited knowledge in terms of the protective roles of avian egg yolk sources on cryopreservation of fish sperm. Thus, the present study was performed to explore the protective effect of egg yolks of different avian species (duck, goose, and chicken) on post-thaw quality and fertilization ability of cryopreserved scaly carp sperm.

2. MATERIAL and METHOD

2.1. Broodstock

Mature male (2478.3±3.2 g, n=13) and female (3628.4±2.7 g, n=3) scaly carp broodstock (2- to 3 years old) were provided by a state aquaculture production station located in Şanlıurfa (Turkey) in June 2021. The broodstock was held in wintering ponds under a natural photoperiod regime. For gamete collection, male and female broodstock were transferred into the hatchery and were held separately in shadowed tanks supplied with continuously (4.0 l/min) well-aerated water at 22°C.

2.2. Collection of gametes

Each brood fish was taken out from the water, and its abdomen was dried. Before injections and stripping, individuals were anesthetized separately in a 50-L tank with 0.7 ml/l diethyl ether (Sigma-Aldrich, Germany) for a few minutes. The urogenital papillae of all broodstock were dried to avoid contamination of gametes with water, urine, or feces.

Carp pituitary extract (CPE), which was suspended in 0.65% NaCl solution, was injected intramuscularly into the brood fishes. Adult males were injected with 1 mg/kg of body weight of CPE 12 h before stripping. Females were injected at 4 mg/kg body weight of the same hormone in two doses, of which 10% of the total dose was administered 24 h before stripping while the remaining 90% was injected 12 h later.

Sperm was stripped by gentle abdominal massage directly into 10-ml glass tubes, which were covered with a parafilm and stored in a styrofoam box holding crushed ice (4±2°C). The sperm quality parameters were evaluated following stripping in 10 minutes at the laboratory. Eggs were also collected by gentle abdominal massage in a dry metal bowl. The eggs were checked visually and only transparent, and well-rounded eggs were used for the fertilization experiments.

2.3. Evaluation of sperm quality

The motility of selected sperm samples was evaluated with the aid of an activation solution (AS) (45mM NaCl, 5mM KCl, and 30mM Tris-HCl, pH 8.2). For this aim, each 1 µl of sperm sample was placed on a glass slide and activated by adding a 10 µl activation solution (AS). Sperm motility was determined using a phase-contrast microscope at 100x magnification (BX43; Olympus, Tokyo, Japan). The percentages (%) and duration (s) of motility were evaluated nine times for each sample. Samples showing below 80% motility were discarded. Sperm motility (%) was evaluated as the percentage of cells exhibiting progressive forward movement, whereas the duration of motility (s) was evaluated until forward movement stopped.

For the purpose of spermatozoa density evaluation, sperm samples were diluted at a ratio of 1:1000 with Hayem solution (35.2 mM Na_2SO_4, 17.1 mM NaCl, 1.8 mM $HgCl_2$, 200-ml bicine). In this way, spermatozoa density was evaluated using a 100 µm deep Thoma hemocytometer (TH-100; Hecht-Assistent, Sondheim, Germany) at 400x magnification with an Olympus BX50 phase contrast microscope (Olympus) and expressed as spermatozoa $x10^9$/ml (three replicates). While indicator papers (Merck, 5.5–9) were used to measure sperm pH, whereas semen colour was evaluated visually within 30 minutes following sperm collection.

Sperm viability was evaluated according to Bjorndahl et al. (2003) using eosin-nigrosin stain (0.67 g eosin Y, 0.9 g of sodium chloride, and 10 g nigrosin dissolved in 100 ml of distilled water). For this aim, a mixture of 5 µl of sperm with 5 µl of the stain was spread on a clean slide and remained to air dry in a dust-free environment. The percentage of live sperm cells was calculated from a total of 300 sperm cells examined under ×100 oil immersion with a phase-contrast microscope (Olympus). In this way, unstained sperm cells were considered alive, while stained sperm cells were considered as dead (Bozkurt and Yavaş, 2021).

2.4. Sperm cryopreservation

Sperm samples (n=13) exhibiting high progressive motility (>80%) and having approximately 12×10^9 spermatozoa/ml sperm density were used in this study. Sperm samples individually were split into four subsamples, and each sample was diluted at a ratio of 1:10 (v:v) with the base extender, which was composed of 3.4314 g sucrose, 0.3427 g NaCl, 21 µl NaOH, 0.5 ml antibiotic (10,000 Unit/ml penicillin and 10,000 µg/ml streptomycin), 100 ml distilled water, pH: 7.7, 325 mmol/kg Osm (Irawan et al. 2010) containing 0 (control), 10, 15 and 20% egg yolk from each of the three avian species such as duck, goose, and domestic chicken. Diluted sperm samples were drawn into 0.25-ml straws by sealing with polyvinyl alcohol (PVA) and were equilibrated in a cool chamber at +4°C for 15 min to obtain isothermal conditions before freezing. Sperm samples were frozen 3 cm

above the liquid nitrogen (LN_2) surface inside a polystyrene box for 10 min. Then, in each experiment, the frozen samples were plunged into the LN_2 for 1 min and finally, nine straws per sperm sample were frozen. Subsequently, the straws were plunged into the LN_2 (-196°C) storage tank. For thawing, the straws were removed from the LN_2 tank and immersed in a 35°C water bath for 30 s, meanwhile the straws were kindly agitated. Thawed sperm samples were activated using an activation solution (AS) and examined under a phase-contrast microscope (Olympus) for the post-thaw sperm characteristics.

2.5. Fertilization experiments

For fertilization, pooled eggs from mature four females were used. The fertilization process was performed at spermatozoa to egg ratio of $1x10^5$ in dry Petri dishes (containing about 500 eggs) using fresh or thawed sperm. Thawed sperm was added over the eggs and kindly mixed before activation with 20 ml of fertilization solution (3 g urea and 4 g NaCl in 1-L distilled water). Following fertilization, the eggs were stirred for 30 min and then, the eggs were washed with the tannic acid solution (0.5 g/l) to eliminate adhesiveness for 10 min. Following, the eggs were rinsed with hatchery water and kindly transferred to Zuger glass incubators with running water (22°C) and kept until eyeing (14-16 h) and hatching (3-4 d). Dead eggs were removed from each incubator during incubation. Fertilization ratios were evaluated in the 4-cell stage under a stereo-microscope at 20-fold magnification. Fertilizing experiments were replicated three times.

2.6. Statistical analysis

Mean values (±SD) regarding freezing and fertilizing experiments were used for statistical analysis. Motility values were normalized through arcsine transformation and differences among the parameters were analyzed using one-way ANOVA. Duncan's post-hoc test was implemented for all comparisons among the treatments at a level of $P<0.05$. All statistical analyses were performed using SPSS 17 for Windows statistical software package.

3. RESULTS

3.1. Sperm quality parameters

In fresh sperm, the mean percentage (%) and duration (s) of motile spermatozoa were 87.30±7.80% and 75.07±14.25 s, respectively. Mean spermatological properties of fresh sperm are given in Table 1.

Table 1. Mean spermatological properties of fresh scaly carp (*Cyprinus carpio*) sperm(n=13).

Volume (ml)	Motility (%)	Motility Duration (s)	Density ($x10^9$/mL)	Total Density ($x10^9$)	pH	Colour
2.96 ±0.48	87.30 ±7.80	75.07 ±14.25	12.11 ±2.50	35.84 ±2.91	7.65 ±0.46	Milky white

3.2. Chemical composition of avian egg yolks

The protein, total fat, dry matter, and raw ash contents of duck, goose, and chicken egg yolks are summarized in Table 2. Duck egg yolk contained more protein than the other two types of egg yolk ($P<0.05$), and goose egg yolk contained more total fat, dry matter, and raw ash than the others ($P<0.05$).

Table 2. Content of egg yolks from different avian species.

Egg Origin	Protein	Total Fat	Dry Matter	Raw Ash
Duck	18.4^{b}	27.0^{a}	53.8^{ab}	2.1^{ab}
Goose	15.6^{a}	34.7^{b}	56.4^{b}	2.4^{b}
Chicken	16.8^{ab}	29.6^{a}	51.0^{a}	1.6^{a}

Different superscripts indicate significant differences within columns ($P<0.05$).

The fatty acid and cholesterol contents of duck, goose, and chicken egg yolks are summarized in Table 3. There are some variations among the avian species in terms of fatty acid levels (P<0.05). Chicken egg yolk contains more cholesterol than duck and gooses egg yolks (P<0.05).

Table 3. Fatty acid (% w/w of total lipids) and cholesterol (mg/g of yolks) content of different avian egg yolk types.

Component	**Avian Egg Yolk Types**			**Reference**
Fatty acid	**Duck**	**Goose**	**Chicken**	**-**
14 : 0	0.5 ± 0.1^{a}	0.7 ± 0.2^{a}	0.4 ± 0.1^{a}	Surai et. al. 1999
16 : 0	26.4 ± 1.1^{a}	31.2 ± 0.9^{b}	25.8 ± 0.8^{a}	Surai et. al. 1999
16 : 1n7	2.7 ± 0.2^{a}	3.8 ± 0.1^{a}	2.1 ± 0.2^{a}	Surai et. al. 1999
18 : 0	6.4 ± 0.2^{a}	7.0 ± 0.5^{a}	8.6 ± 0.3^{a}	Surai et. al. 1999
18 : 1n-9	47 ± 1.2^{b}	41.9 ± 1.3^{a}	40.5 ± 1.1^{a}	Surai et. al. 1999
18 : 1n-7	1.9 ± 0.1^{a}	2.0 ± 0.2^{a}	1.6 ± 0.2^{a}	Surai et. al. 1999
18 : 2n-6	5.6 ± 0.3^{a}	9.3 ± 0.4^{ab}	14.7 ± 0.5^{b}	Surai et. al. 1999
18 : 3n-3	0.3 ± 0.0^{a}	0.4 ± 0.1^{a}	0.4 ± 0.0^{a}	Surai et. al. 1999
20 : 1n-9	0.5 ± 0.1^{a}	0.4 ± 0.1^{a}	0.3 ± 0.1^{a}	Surai et. al. 1999
20 : 4n-6	4.0 ± 0.1^{b}	2.3 ± 0.1^{ab}	1.7 ± 0.0^{a}	Surai et. al. 1999
20 : 6n-3	0.6 ± 0.1^{a}	0.3 ± 0.2^{a}	1.6 ± 0.2^{a}	Surai et. al. 1999
Cholesterol	Duck	Goose	Chicken	Reference
	10.6 ± 0.01^{a}	-	22.9 ± 0.02^{b}	Surai et. al. 1999
		15.81 ± 0.1^{ab}		Golzar Adabi et al. 2013

Different superscripts indicate significant differences within columns (P<0.05).

3.2. Post-thaw quality parameters

The effect of supplementation of egg yolks of different avian species at different concentrations to the sucrose-based extender on post-thaw progressive motility, motility duration, and viability parameters of frozen–thawed scaly carp sperm are presented in Figures 1-3.

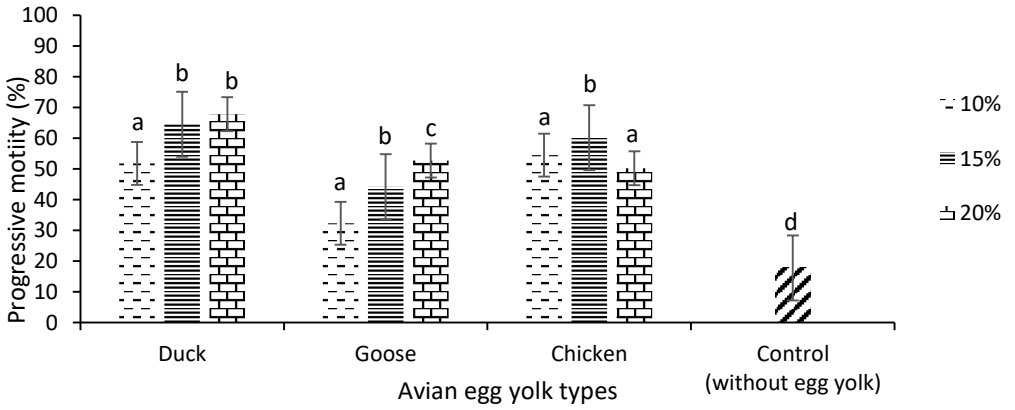

Figure 1. The mean post-thaw progressive motility (%) of frozen-thawed scaly carp (*Cyprinus carpio*) sperm. Different letters indicate differences among treatments (ANOVA, P<0.05, n=9).

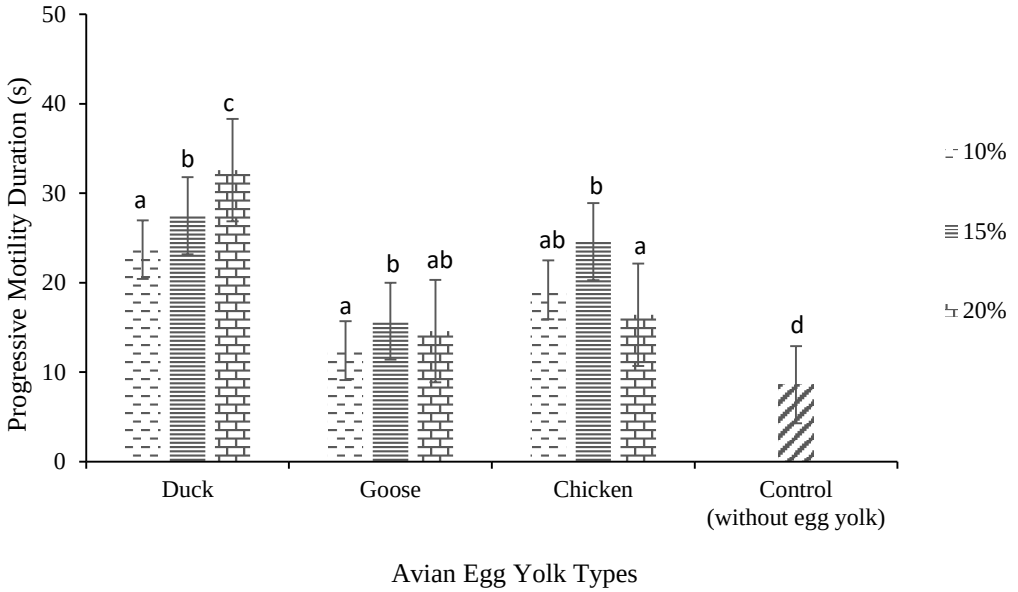

Figure 2. The mean post-thaw progressive motility duration (s) of frozen-thawed scaly carp (*Cyprinus carpio*) sperm. Different letters indicate differences among treatments (ANOVA, P<0.05, n=9).

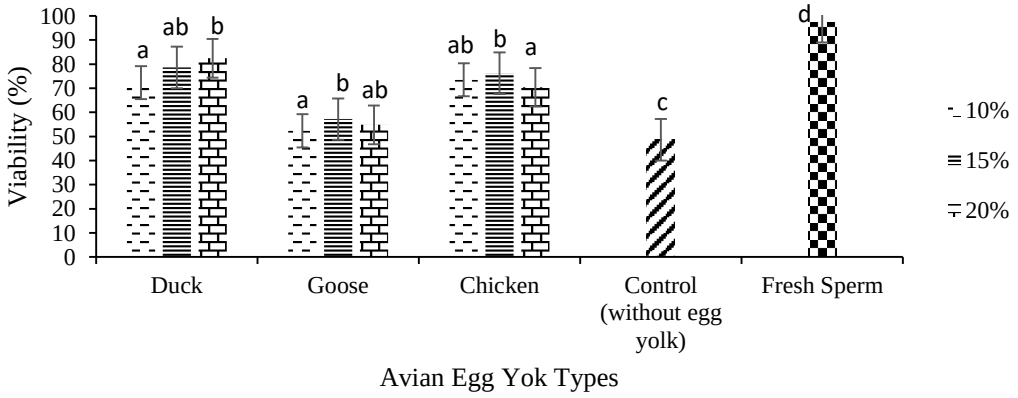

Figure 3. The mean post-thaw viability (%) of frozen-thawed scaly carp (*Cyprinus carpio*) sperm. Different letters indicate differences among treatments (ANOVA, P<0.05, n=9).

According to the results, duck egg yolk had the best cryoprotective effect in terms of the highest progressive sperm motility, and motility duration (67.8% and 32.6 s respectively) as compared to the other avian egg yolks (P<0.05) analyzed. Additionally, sperm cryopreserved in duck egg yolk recorded the highest viability rate (82.4%) than sperm cryopreserved in goose and chicken egg yolk containing extenders (P<0.05). Sperm diluted in goose egg yolk-based extender showed lower percentages in terms of progressive sperm motility, motility duration, and viability (P<0.05). Supplementation of all types of avian egg yolks in extenders caused an increase in all post-thaw quality parameters in comparison to those that did not contain egg yolks (control group) (P<0.05).

3.3. Fertilization

Supplementation of the sucrose-based extender with different avian egg yolk types caused an increase in post-thaw fertility in comparison to those that did not contain avian egg yolk (P<0.05). Fertilization rates were determined higher than 50.0% in all avian egg yolk-containing extenders. Cryopreserved sperm with an extender containing 20% duck egg yolk provided the highest fertilization result (94 %) when compared to the other tested groups (P<0.05). Sperm extended in

goose egg yolk containing extender caused lower fertility and there were no significant differences in the concentration of its (P>0.05, Figure 4).

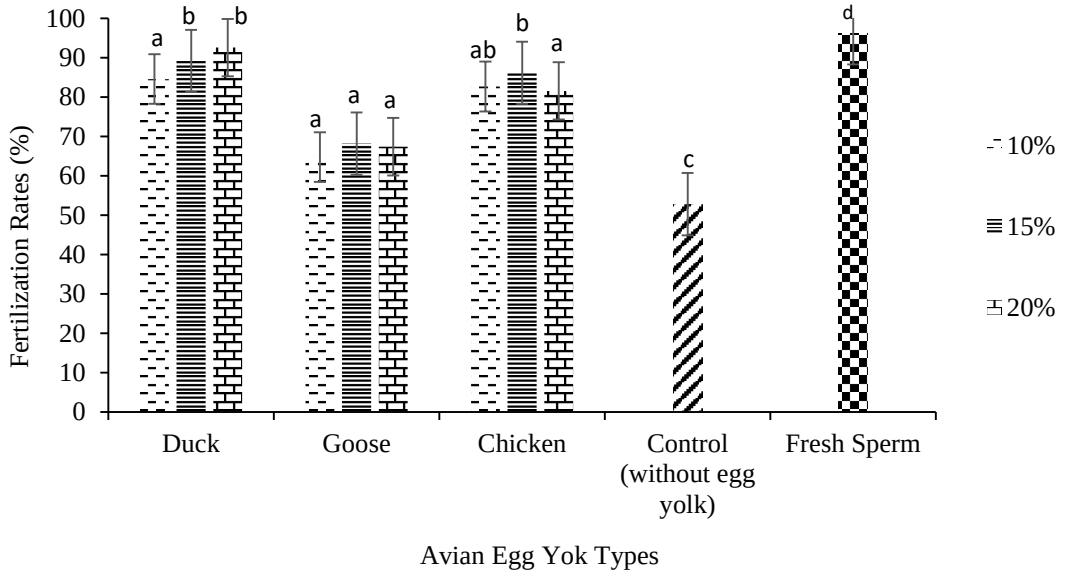

Figure 4. The mean post-thaw fertility (%) of frozen-thawed scaly carp (*Cyprinus carpio*) sperm. Different letters indicate differences among treatments (ANOVA, P<0.05, n=3).

4. DISCUSSION

Much experimental-based research has revealed the cryoprotective effect of avian egg yolks to improve post-thaw sperm quality and fertility following cryopreservation mainly in mammalian species (Aboagla and Terada, 2004; Clulow et al., 2007; Moreno et al., 2008; Akhter et al., 2017).

On the other hand, limited data are available regarding fish sperm cryopreservation using different avian egg yolk sources as a component of extenders. Additionally, most cryopreservation linking studies in aquaculture to date have not tested the role of egg yolk compositions in extender formulations.

According to previous studies regarding mammalian species, it should be also noted that the post-thaw quality of sperm may be attributed to the variations in biochemical composition of egg yolks in different avian species, especially in terms of fatty acids and cholesterol (Bathgate et al., 2006).

From this point of view, fatty acid and cholesterol contents of egg yolks belonging to different avian species are summarized in Table 3. According to Table 3, it seems that there are variations among the avian species in terms of fatty acid levels (P<0.05). On the other hand, chicken egg yolk contains more cholesterol than duck and goose egg yolks (P<0.05).

Many researchers stated that the variations in the chemical composition of the egg yolks in avian species affect their protection ability during cryopreservation (Bathgate et al., 2006; Moreno et al., 2008; Surai et al., 1999). The most important finding of this study is that sperm frozen in duck egg yolk containing extender exhibited higher post-thaw quality and fertility than sperm frozen in other avian egg yolks. The difference may be ascribed to the higher levels of protein and monounsaturated fatty acids, and lower levels of lipid and cholesterol in the duck egg yolk. The components of protein and fatty acid have been demonstrated to be effective in the protection of sperm during cryopreservation (Prasard et al., 1988; Maurice et al., 1994). From this point of view, it is clear that the levels of these components in duck egg yolk may provide better protection to the sperm resulting in higher progressive sperm motility, and fertility after thawing.

It should be noted that the results of this study are in agreement with that of other researchers. For instance, Humes and Webb (2006) reported that chucker egg yolk improved the motility of stallion sperm rather than chicken egg yolk following cryopreservation. This result may be associated with higher levels of protein present in chucker egg yolk. Additionally, the results of this study match with the findings of previous studies proving extenders containing egg yolks from the avian species other than chickens resulted in significantly high post-thawing evaluation parameters in sperm of some mammalian sperm such as boar (Bathgate et al. 2006), buffalo (Akhter et al., 2017; Waheed et al., 2012), stallion (Webb et al., 2011; Burris and Webb, 2009; Clulow et al., 2007), bulls (Su et al., 2008), and rams (Ali et al., 2013; Gholami et al., 2012).

On the other hand, Bozkurt et al. (2014) reported that common carp sperm cryopreserved in a glucose-based extender containing turkey and quail egg yolks provided high sperm quality like the sperm samples cryopreserved in the chicken egg yolk. Even though there was no report concerning the effect of sucrose-based extenders on fertilization results in fish sperm cryopreservation, the beneficial effects of egg yolk supplementations to extenders seem to be species-specific.

In conclusion, duck egg yolk improved post-thaw quality, as well as fertility in scaly carp spermatozoa. Consequently, duck egg yolk may be a promising alternative for replacing chicken egg yolk in extenders for scaly carp sperm cryopreservation.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

The authors declare that all authors contributed equally to the article.

ETHICAL STATEMENTS

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

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 **Araştırma Makalesi**

Juvenile *Parasagitta setosa* (J. Müller, 1847) (Chaetognatha) from Shallow Waters of the Southern Black Sea: Temporal Size Structure, Gonad Maturity, and Gut Content

Güney Karadeniz'in Sığ Sularında Juvenil *Parasagitta setosa* (J. Müller, 1847) (Chaetognatha): Zamansal Boyut Yapısı, Gonad Gelişimi ve Mide İçeriği

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The present study aimed to assess the abundance, body length, maturity stage, and gut content of *Parasagitta setosa* in the southern Black Sea, Turkey. The study was conducted twice a month from January 2008 to December 2008. Vertical hauls with a 112 µm mesh size plankton net were used from a depth of 50 m to the surface of the Sinop coast. During the study period, the abundance of this species was generally low, varying between 10 and 980 ind.m^{-2}. In particular, the abundance of *P. setosa* was low from December to July but increased from August. Small size individuals were predominated, with both 1 – 1.99 mm and 2 – 2.99 mm size classes accounting for 62% of the total *P. setosa* sample. Four developmental stages were determined based on ovary and seminal vesicle development. Stage I (immature) was the predominant developmental stage in the *P. setosa* population. A total of 1580 individuals were dissected; however, only 53 individuals had food items in their gut (3.4%), with copepods (54.73%) accounting for the predominant group the food content. It was determined that the abundance values and feeding ratios of *P. setosa* were low in the coastal area of Sinop and new individuals join in the population during the summer-autumn period.

Keywords
- Population structure
- Developmental stages
- Feeding
- Turkey

Özet: Mevcut çalışma, Türkiye'nin Güney Karadeniz bölgesinde *Parasagitta setosa* bolluğunu, vücut uzunluğunu, olgunluk evresini ve bağırsak içeriğini değerlendirmeyi amaçlamıştır. Çalışma, Ocak 2008 – Aralık 2008 tarihleri arasında ayda iki kez gerçekleştirildi. Sinop kıyısında, 50 m derinlikten yüzeye dikey çekimlerle 112 µm göz açıklığına sahip plankton kepçesi kullanıldı. Çalışma süresi boyunca, türün bolluk değerleri genellikle düşük olup, 10 ile 980 birey.m^{-2} arasında değişmiştir. Özellikle, *P. setosa* bolluğu Aralık'tan Temmuz ayına kadar düşüktü ancak Ağustos ayından itibaren artmıştır. *P. setosa* popülasyonuna küçük boyutlu bireylerin baskın olduğu belirlenmiştir. Toplam *P. setosa* örneğinin %62'sini oluşturan 1 – 1.99 mm ve 2 – 2.99 mm boy sınıflarına ile küçük boyutlu bireyler baskındı. Ovaryum ve seminal vezikül gelişimi kullanılarak dört gelişim evresi belirlenmiştir. *P. setosa* popülasyonunda evre I (olgunlaşmamış evre) baskın olmuştur. Toplam 1580 birey disekte edilmiştir. Bununla birlikte, sadece 53 bireyin (%3,4) bağırsaklarında besin maddeleri bulunmuştur. Besin içeriğinin başlıca grubunu kopepodlar (%54,73) oluşturmuştur. Sinop kıyısal alanında *P. setosa* türünün bolluk değerlerinin ve beslenme oranlarının düşük olduğu ve yeni bireylerin yaz - sonbahar döneminde populasyona katıldığı belirlenmiştir.

Anahtar kelimeler
- Populasyon yapısı
- Gelişimsel evreler
- Beslenme
- Türkiye

1. INTRODUCTION

Chaetognatha is a small marine animal that consists of mainly pelagic species, except for the benthic genus *Spadella*. Chaetognatha has a wide distribution in marine waters, ranging from coastal waters to deep waters and from the surface to the bottom of the water. They are usually abundant in plankton and constitute a major component of the total zooplankton biomass (Alvarino, 1983; Bone et al., 1991). Chaetognatha are both primary and secondary consumers in marine ecosystems, playing a crucial role in the marine food web and contributing to the matter and energy cycles of the marine ecosystem. Chaetognatha feed on fish larvae (Johnson et al., 2006; Vdodovich et al., 2018) and other micro- and mesozooplankton, mainly copepods (Pearre, 1981; Kehayias et al., 1996; Fulmer and Bollens, 2005; Terbıyık Kurt, 2018; Wang et al., 2020), also chaetognaths (Pearre, 1982), whereas they serve as food for many large carnivorous organisms, including seabirds (Mehlum and Gabrielsen, 1993), amphipods (Marion et al., 2008), decapods, mysids (Hopkins et al., 1994), fish and fish larvae (Young and Davis, 1990; Johnson et al., 2008).

Chaetognatha are protandric hermaphrodite animals, and the seminal vesicule in these species mature earlier than their ovaries. Female gonads (in the trunk region) and male gonads (in the tail region) occur in different parts of the body (Alvarino, 1992; Kehayias et al., 1999). Fertilized eggs are released into the water, where they swim near the surface for a few days and then hatch as 'larvae'. The development of chaetognath larvae into adult form is direct without any metamorphosis process (Alvarino, 1990).

Parasagitta (Syn: *Sagitta*) *setosa* is a Chaetognatha species commonly found in the Black Sea (Moldoveanu and Timofte, 2004; Arashkevich et al., 2014; Lebedeva et al., 2015; Stefanova, 2015; Yıldız and Feyzioglu, 2016; Üstün et al., 2018; Üstün et al., 2019). Although their distribution and daily migration model (Vinogradov et al., 1985; Vinogradov et al., 1986; Besiktepe and Unsal, 2000; Erkan et al., 2000; Mutlu, 2006; Marinova and Stefanova, 2009) has been well studied in the Black Sea, studies on sexual development and morphological characteristics (Feyzioğlu et al., 1998; Feyzioğlu et al., 2010), gut content (Dirts and Utkina, 1988; Vdodovich et al., 2018), genetic characteristics (Peijnenburg et al., 2004; Peijnenburg et al., 2006) and fatty acid composition (Şen Özdemir et al., 2020) are still limited.

Coastal shallow waters with ecological and economic significance provide a variety of ecosystem services, including nutrient supply, nutrient conversion, protection from predators, and spawning (Hughes et al., 2014). Maybe, the most referred function among all is that it serves as a nursery where the offspring of numerous vertebrate and invertebrate species can grow and mature before migrating elsewhere during maturity (Lefcheck et al., 2019). The objective of the present study is to determine the population structure, gonad development, and stomach content of the juvenile stage of *P. setosa* which is one of the key species of the Black Sea living in the shallow waters of Sinop.

2. MATERIAL and METHODS

Samples were collected from a single station located in the coastal water of Sinop, Turkey (42°00′21″N, 35°09′32″E, and a depth of 50 m), twice a month from January 2008 to December 2008. A detailed description of the study area and a part of the abundance data were presented in Üstün et al. (2018). A plankton net with a 50 cm diameter and 112 μm mesh size was vertically towed from the bottom to the surface of the water column during the daytime. After sampling, the collected material was transferred into a bottle and preserved in a solution of borax-buffered 4% formaldehyde in seawater. In the laboratory, all *P. setosa* specimens were separated from the whole sample under a stereomicroscope Novex RZ 65500. Systematic classification and nomenclature of this species were performed according to the World Register of Marine Species (WoRMS 2022). The body length of *P. setosa* individuals was measured by metric ocular stereomicroscope. Body length was measured from the tip of the head to the end of the tail, excluding the tail fin. Size classes were arbitrarily set at 1 mm

intervals (the 2 mm size class includes individuals from 2.00 to 2.99 mm, etc.) (Zo, 1973). Abundance values were calculated as individuals per square meter (ind.m^{-2}).

The maturity stages were classified according to Kehayias et al. (1999), based on the development of the ovaries and seminal vesicles: Stage I — young without visible ovaries; Stage II — immature with visible ovaries but not visible seminal vesicles; Stage III — both ovaries and seminal vesicles visible; and Stage IV — seminal vesicles filled with sperm, large ova in ovaries.

Individuals containing food items in their gut were dissected, and food organisms in the gut were classified by their species or genus whenever microscopic examination was possible. Food items in the first third section of the gut were not taken into account while counting the amount of food items as they may have been captured in the collector of the mesh (Øresland, 1987; Kehayias et al., 2005). The feeding ratio was indicated as the food-containing ratio (FCR; percentage of chaetognaths containing food in their gut) and the number of prey per chaetognath (NPC) (Batistić et al., 2003).

3. RESULTS

The annual mean abundance of *P. setosa* was calculated as 329.2 individuals (ind.)m^{-2} in the study area in 2008. The low abundance of *P. setosa* was observed from January 2008 to the end of May 2008. Then, the increase in abundance that started at the end of May 2008 continued until the end of December 2008 (Figure 1). The minimum abundance value was recorded on 26 February 2008, 16 April 2008, and 8 May 2008 (10 ind.m^{-2}), while the maximum values were determined on 23 September 2008 and 21 November 2008 (980 ind.m^{-2} and 975 ind.m^{-2}, respectively). Specimen of *P. setosa* was not found on 30 April 2008 in samples (Figure 1).

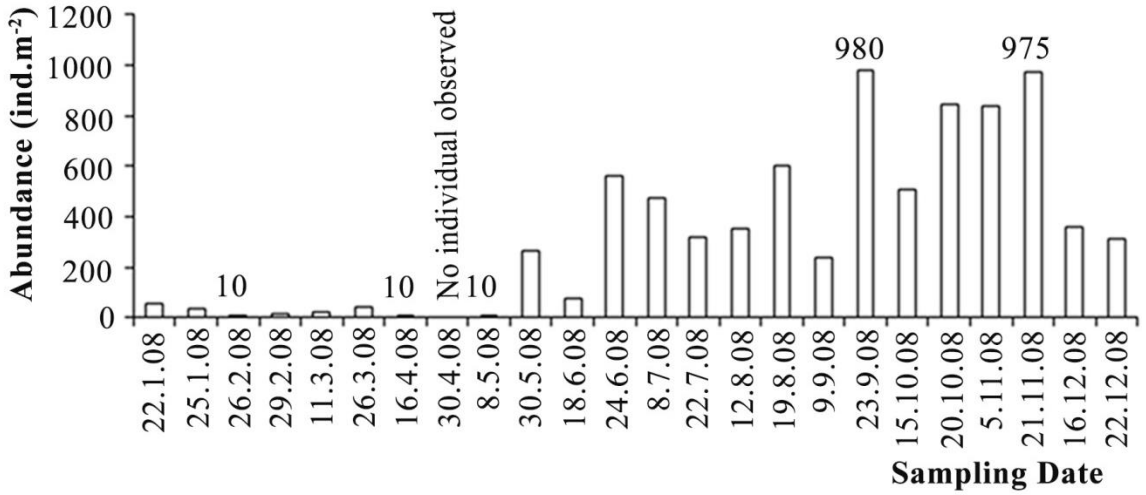

Figure 1. Abundance (ind.m^{-2}) values of *P. setosa* in Sinop, southern Black Sea.

Table 1. Length group (mm) and size frequency (%) of *P. setosa* in Sinop, southern Black Sea.

Length group (mm)	Frequency (%)
0.5 – 0.99	2.97
1 – 1.99	32.53
2 – 2.99	29.18
3 – 3.99	15.70
4 – 4.99	6.77
5 – 5.99	4.05
6 – 6.99	1.90
7 – 7.99	2.22
8 – 8.99	1.39
9 – 9.99	1.46
10 – 10.99	1.08
11 – 11.99	0.63
12 – 12.99	0.13

In this study, the body lengths of 1580 individuals were measured and varied between 0.53 mm (24 June 2008) and 12.80 mm (25 January 2008). The small-sized individuals of *P. setosa* (1 – 1.99 mm and 2 – 2.99 mm) dominated the population and comprised 32.53% and 29.18%, respectively, of the whole population in Sinop. The mean body length of *P. setosa* was 2.98 mm, ranging between 0.81 mm and 6.27 mm (Table 1, Figure 2). A high fraction of individuals of *P. setosa* were between 1 mm and 3 mm long, which was recorded between late May 2008 and December 2008.

In total, 93% of the population was in developmental stage 1, with the proportion decreasing towards higher developmental stages (3.5% in stage 2, 3.2% in stage 3, and 0.32% in stage 4). Individuals in stage 1 were present year-round and dominated the population between the end of May 2008 and the end of November 2008. The maximum number of individuals in stage 1 was determined on 23 September 2008 (186 ind.) and 21 November 2008 (190 ind.). Individuals in stage 2 and stage 3 were detected from the end of June and to be beginning of December 2008. The highest number of individuals in stage 2 was detected on 5 November 2008 (22 individuals, 13%). The highest number of individuals in stage 3 was detected on 8 July 2008 (16 individuals, 17%) followed by 5 November 2008 (15 individuals, 9%). Individuals in stage 4 were found on 8 July 2008 (1 individual), 23 September 2008 (1 individual), and 5 November 2008 (3 individuals, 2%). Individuals in stages 2, 3, and 4 were not observed at the end of December 2008 or in the middle of June 2008. Stage 2 was represented by 1 individual on 29 February 2008. Individuals in stage 1 were also found in a very number on 29 February 2008. The size of individuals in stage 1 ranged between 0.53 mm and 12.8 mm (mean: 2.7 mm); in stage 2, between 2.5 mm and 12 mm (mean: 6.65 mm); in stage 3, between 3.25 mm and 11.3 mm (mean: 6.6 mm) and stage 4, between 5.9 mm and 11.8 mm (mean: 9.4 mm) (Figure 2).

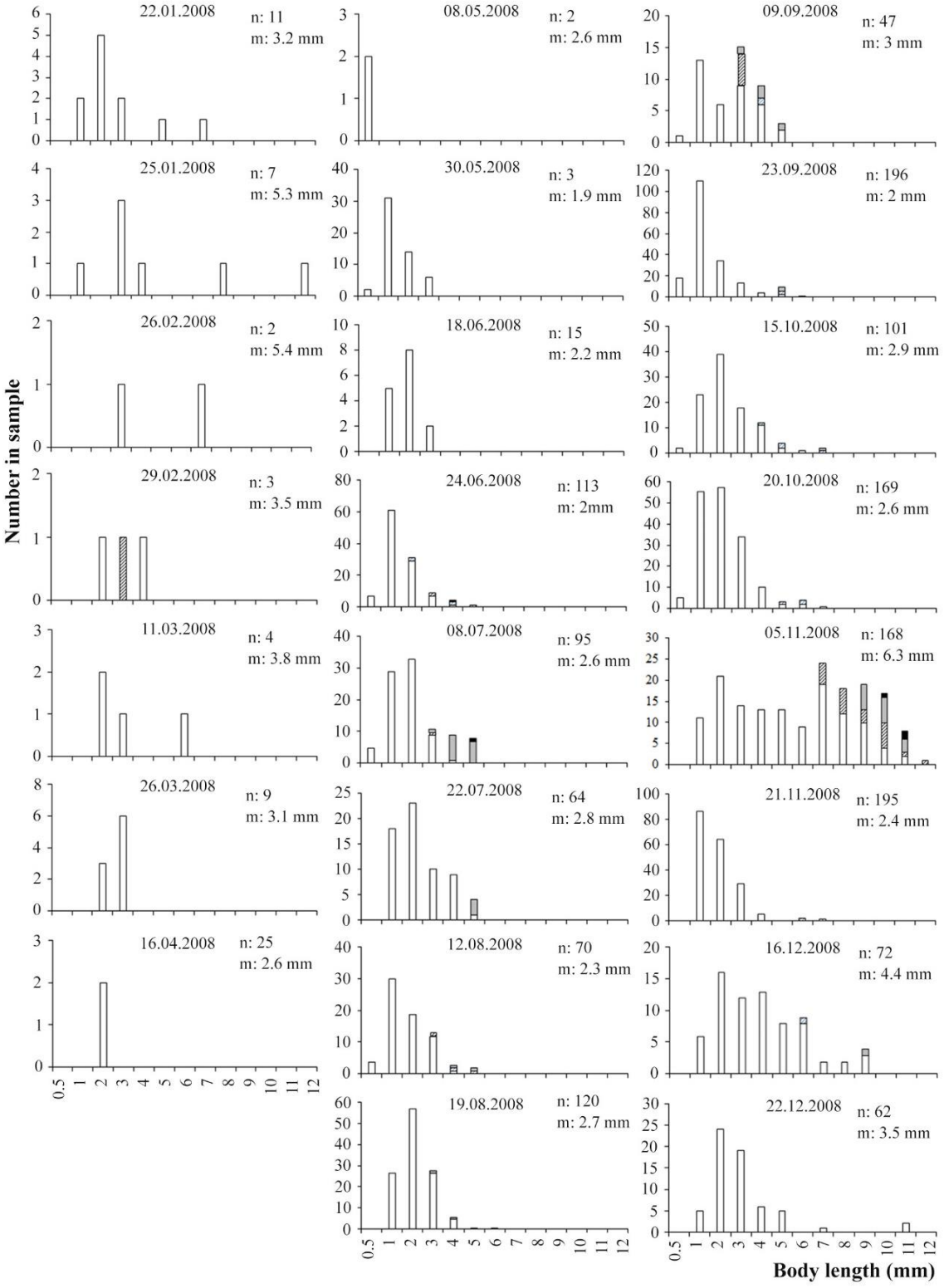

Figure 2. Distribution of different maturity stages of *P. setosa* in Sinop, southern Black Sea. Values are represented in body length (mm) versus the number in the sample. N: number of sampled specimens, m: mean body length, white bar: stage 1, crisscross bar: stage 2, grey bar: stage 3, black bar: stage 4.

Table 2. The number of food items in gut content, FCR (the food containing ratio), and NPC (the number of prey items per chaetognath) of *P. setosa* in the sampling period in Sinop, southern Black Sea.

Date	DUF	UC	Cn	Ac	Pp	FCR	NPC
30/5/08	1					1.9	0.019
18/6/08	1					6.7	0.067
24/6/08	4			1		4.4	0.044
8/7/08	2	2				4.2	0.042
22/7/08	4	1				7.8	0.078
12/8/08		2				2.9	0.029
19/8/08	4					3.3	0.033
9/9/08	2	2			1	11	0.11
23/9/08			2			1	0.01
15/10/08		5	1	1		7	0.07
20/10/08		5	1			3.6	0.036
5/11/08	2	2		1		3	0.03
21/11/08	1			1		1	0.01
22/12/08	3	1				6.5	0.065

* DUF: Digested unidentified food; UC: Unidentified Copepoda; Cn: Copepoda nauplii; Ac: *Acartia clausi;* Pp: *Paracalanus parvus*

Table 3. Food composition of the gut content of *P. setosa* in Sinop, southern Black Sea

Gut contents	(%)	Stage 1 (%)	Stage 2 (%)	Stage 3 (%)
Digested unidentified food	45.28	37.74	5.66	1.89
Unidentified Copepoda	37.74	28.30	7.55	1.89
Copepoda nauplii	7.55	5.66	1.89	
Acartia clausi	7.55	3.77	3.77	
Paracalanus parvus	1.89		1.89	

In the present study, a total of 1580 individuals were investigated, and food items were found in the gut contents of only 53 individuals. Individuals containing food items in their gut were observed from the end of May 2008 to the end of December 2008. The FCR varied between 1% and 11%, and the NPC was between 0.01 and 0.11, according to the month. In total, the FCR and the NPC of *P. setosa* were calculated as 3.4% and 0.034, respectively. As a result of the food content analysis, it was determined that the majority of individuals had an empty gut (96.6%). Most of the prey items in specimens who had prey items in their guts were digested. However, most food items could not be identified due to digestion. The ratio of identifiable food organisms within digested and undigested foods was approximately 54.7%. Copepods dominated the diet of *P. setosa*. Cannibalism was not observed. The highest feeding intensity was determined in stage 1 of *P. setosa*. Stage 4 individuals had no food in their gut (Table 2, Table 3).

4. DISCUSSION

The annual mean abundance of *P. setosa* in the current study (329 ind.m^{-2}) was quite low compared to previous studies conducted at the same station in 1999 (1748 ind.m^{-2}) and 2002 – 2006 (680 ind.m^{-2} in 2002, 455 ind.m^{-2} in 2003, 736 ind.m^{-2} in 2004, 435 ind.m^{-2} in 2005, 541 ind.m^{-2} in 2006) in Sinop coastal waters, whereas these values were higher than data obtained in 2007 (116 ind.m^{-2}) in the same area. The highest abundance values were always observed in autumn period both in current (980 ind.m^{-2} in September 2008) and previous studies (13300 ind.m^{-2} September 1999, 2050 ind.m^{-2} in September 2002, 1700 ind.m^{-2} November 2003, 3585 ind.m^{-2} October 2005, 2600 ind.m^{-2} September

2006, 295 ind.m^{-2} September 2007) in this region. Only, in 2004, the maximum value was observed in August (3900 ind.m^{-2}) (Ünal, 2002; Üstün et al., 2016; Üstün et al., 2018). The maximum abundance values obtained from other studies conducted in the coastal regions of the eastern Black Sea (Trabzon) and the western Black Sea were higher than those obtained in this study. In addition, the peak values in the Trabzon region were determined during summer months (Beşiktepe, 1998; Feyzioğlu et al., 2010; Yıldız and Feyzioğlu, 2014). Food and temperature are the principal factors affecting the growth of *P. setosa* in the Black Sea (Besiktepe and Unsal, 2000). A high abundance of *P. setosa* was detected in the summer and autumn months when copepod abundance and temperature are high (Besiktepe and Unsal, 2000; Ünal, 2002). Coastal regions are highly sensitive and variable systems against environmental factors (such as precipitation, and terrestrial inputs). Therefore, the abundance values of species may vary in coastal areas which have different topographical and hydrographic structures (Calbet et al., 2001; Terbıyık Kurt and Polat, 2013).

The peak *P. setosa* abundance in the Black Sea was reached in July/August and September when most of the smaller individuals (juvenile) settle in the upper strata. Spawning begins in July, and the number of adult individuals decreases rapidly and is replaced by young individuals in July/August (Niermann et al., 1998; Besiktepe and Unsal, 2000). In the summer and early autumn, populations distributed in the upper strata were dominated by *P. setosa* juveniles. Adult *P. setosa* carry out diel vertical migration from the oxygen minimum zone to the surface layers (Niermann et al., 1998; Besiktepe and Unsal, 2000; Mutlu 2006).

In the present study, based on the number of immature individuals (stage 1) in the Sinop coastal area, it was determined that the breeding period continued from June to December. Microscopic examinations revealed that the number of individuals with eggs (stages 2, 3, and 4) was high in early July, early September, and early November. After these months, the number of immature individuals increased, whereas the number of individuals with eggs decreased. Qresland (1983) suggested that *P. setosa* died after breeding.

The body length of *P. setosa* ranged from 1.4 to 20.6 mm in the eastern Black Sea (Feyzioğlu et al., 1998), 1 to 19 mm in the western Black Sea (Beşiktepe, 1998) and 0.5 – 1mm to > 20mm in Sinop (Ünal, 2002). The body lengths recorded in the present study were shorter than those recorded in the previous studies. However small-sized individuals were found to be dominant in all these studies. These differences may be due to the use of different sampling mesh and larger mesh sizes, as well as the fact that other studies have been carried out in deeper regions. Ünal (2002) mentioned that large/adult individuals showed a higher distribution density in deep waters, whereas small/young individuals were more abundant in coastal waters.

The very low number of adults and large-sized individuals in the present study could be attributed to the possibility that these were present in deep waters and were thus unnoticed during sampling or were dead after breeding. Therefore, the abundance and body length of *P. setosa* recorded in the present study were compared with those recorded in studies conducted in the coastal area.

The feeding rates (values of FCR and NPC) determined in this study were lower than those recorded by studies carried out on other seas (Table 4). Drits and Utkina (1988) examined the nocturnal feeding of *P. setosa* in the deep waters of the Black Sea during April-May 1984. They found that copepodite V and *Calanus* and *Pseudocalanus* females formed the stomach contents of members with lengths ranging from 16-21 mm. In the current study, *A. clausi* and *P. parvus*, which are characteristic of coastal areas, were detected in the stomach contents of *P. setosa*, while Drits and Utkina (1988) detected the presence of deep-water copepod species. In the present study, copepods (54.7%) provided the main food source of *P. setosa*, which aligns with the well-documented fact that copepods are the preferred prey of chaetognaths (Duro and Saiz, 2000; Batistić et al., 2003; Kehayias and Ntakou, 2008).

Table 4. Reported FCR and NPC values for *P. setosa* in other regions.

	Duró and Saiz (2000) Catalan Sea Mediterranean Sea	Batistić¤ et al. (2003) South Adriatic	Tönnesson and Tiselius (2005) West Sweden	Kehayias et al. (2005) North Agean Sea	Kehayias and Ntakou (2008) East Agean Sea
FCR	8.2 – 10.7% (in day)	0 – 6% (5.9% in total)	52.5% (in total)	8.3% (in total)	39.3% (in total)
NPC	0.08 – 0.11 (in day)	0 – 0.04	0.28 – 0.56 (in day)		

Significant amounts of unidentified food items were detected in the gut contents of *P. setosa*. Similarly, a high percentage of unidentified food items was noted in the diet of this species; it consisted of a high population of stage 1 individuals in the eastern Aegean Sea (Kehayias and Ntakou, 2008). Smaller prey selected by small-sized individuals can be digested relatively quickly; therefore, the identification of food in the gut contents is a difficult task (Pearre, 1974). Thus, the high proportion of unidentified food items in the present study can be attributed to the high prevalence of small-sized individuals.

In conclusion, the abundance and length composition values obtained in this study are lower than the results obtained in other studies conducted in the Black Sea. Feeding ratio values that are low suggest that it has a quite limited effect on the creatures that constitute its food. Conducting studies also on the deep water column and determining the relationship between the results found and the environmental parameters (such as temperature, and salinity) will help to better understand the place and importance of the species in the pelagic ecosystem of the Black Sea to explain the population structure and feeding regime of *P. setosa* in a better way.

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CONFLICT OF INTEREST

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

AUTHOR CONTRIBUTIONS

Funda Üstün is only author in the paper.

ETHICAL STATEMENTS

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

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 **Araştırma Makalesi**

Türkiye'de Çift Kabuklu Yumuşakçalarda Betanodavirus Varlığının Araştırılması

Investigation of Betanodavirus Presence in Bivalve Mollusks in Türkiye

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Özet: Viral nervöz nekrozis (VNN), özellikle larva ve yavru deniz balıklarında, bazen de yetişkinlerde görülen önemli viral bir hastalıktır. Akdeniz'de artık levreklerde endemik olarak kabul edilen ve sık sık salgınlara neden olan betanodavirusların RGNNV genotipinin yanısıra, son birkaç yılda çipuralarda da salgınlar daha sık bildirilmeye başlanmış ve RGNNV/SJNNV genotipi izole edilmiştir. Bu çalışmanın amacı resmi otoriteden onaylı doğal yataklarda yetiştirilen akivades (*Ruditapes decussatus*) ve kara midye (*Mytilus galloprovincialis*) istasyonlarında VNN etkeni betanodavirus varlığının araştırılmasıdır. Çalışmada 2016-2020 yılları arasında beş adet akivades, sekiz adet kara midye istasyonundan toplam 50 örnekleme yapılmıştır. Akivades örneklemesi Ağustos aylarında, kara midye örneklemesi ise Eylül aylarında gerçekleştirilmiştir. Her örneklemede her istasyondan 30 adet örnek alınmıştır ve her biri beş örnekten oluşmak üzere toplam 300 adet havuz oluşturulmuştur. Çift kabuklu yumuşakçaların hepatopankreaslarından hazırlanan örnekler, Real-Time Reverse Transcriptase Polimerase Chain Reaction (RT-qPCR) testi ile betanodavirus yönünden araştırılmıştır. RT-qPCR testleri sonucunda hem akivades örneklerinde hem kara midye örneklerinde betanodavirus nükleik asidi tespit edilmemiştir. Türkiye'de çift kabuklu yumuşakçalarda betanodavirus varlığı ilk defa bu çalışma ile araştırılmıştır. Sonuç olarak bu çalışma ile sadece doğal yataklarda bulunan midye ve akivadeslerde araştırma yapılmıştır, ancak, virüsün daha çok endemik olduğu Güney Ege ve Akdeniz bölgelerinde resmi onaylı akivades veya kara midye istasyonu bulunmadığından bu bölgelerde örnekleme yapılmamıştır. Kabuklularda betanodavirus epidemiyolojisini daha iyi anlamak için bu bölgeleri de içeren daha ileri ve genişletilmiş çalışmalara ihtiyaç vardır.

Anahtar kelimeler
- Akivades
- Betanodavirus
- Kara midye
- RT-qPCR

Abstract: Viral nervous necrosis (VNN) is an important viral disease that is seen especially in larval and juvenile, occasionally in adult marine fish. VNN is now accepted as endemic in the Mediterranean basin and outbreaks in sea bream caused by RGNNV/SJNNV genotype have been isolated as well as the RGNNV genotype of betanodaviridae which causes frequent outbreaks. The aim of this study was to investigate the presence of the betanodavirus in carpet shell (*Ruditapes decussatus*) and black mussel (*Mytilus galloprovencialis*) stations approved by the official authorities in Turkey. A total of 50 samplings were carried out from five carpet shells and eight black mussel stations between 2016-2020. Sampling of carpet shells were conducted in August and black mussels in September by every year. Thirty samples were collected from each station, and a total of three hundred pools consisting of five samples each were produced. Samples prepared from the hepatopancreas of the bivalve mollusks were tested by Real-Time Reverse Transcriptase Polymerase Chain Reaction (RT-qPCR). Betanodavirus nucleic acid was not detected in any of carpet shell and black mussel samples. The

Keywords
- Black mussel
- Betanodavirus
- Carpet shell
- RT-qPCR

presence of betanodavirus in bivalve mollusks in Turkey was investigated for the first time in this study. In conclusion, this study was conducted in only officially approved carpet shell and black mussel stations, however, since there were no officially approved stations in the Southern Aegean and Mediterranean regions, where the virus is more endemic, sampling was not conducted. Further and expanded studies are necessary including these regions to better understand the betanodavirus epidemiology in shellfish.

1. GİRİŞ

Viral Nervöz Nekrozis (VNN) hastalığı, *Nodaviridae* ailesi içinde yer alan betanodaviruslar tarafından meydana getirilen önemli viral bir hastalıktır. VNN, su ürünleri yetiştiriciliğinin sürdürülebilirliği ve gelişimi anlamında en büyük zorluktur ve üretime yönelik önemli bir risk oluşturduğu vurgulanmaktadır (Costa ve Thompson, 2016; Thiery vd., 2011; Toffan vd., 2017). En çok levrek, grouper ve yassı balıklarda görülmekle birlikte, başlıca deniz balıklarını etkilemektedir, bununla birlikte çipuralarda da VNN salgınları bildirilmiştir (Bitchava vd., 2007; Comps ve Raymond 1996; Munday vd., 2002; Sano vd., 2011; Toffan vd., 2017; Volpe vd., 2020).

Betanodaviruslar yaklaşık 25 nm çapında, ikosahedral yapıda, zarsız ve yuvarlak morfolojiye sahiptir (Breuil vd., 1991). Genomları, tek iplikçikli, pozitif polariteli ve iki segmentlidir (Johnson vd., 2003). RNA1 segmenti (3,1 kb), RNA bağımlı RNA polimerazın (RdRp) sentezinden sorumludur (Gallagher ve Rueckert, 1988; Nagai ve Nishizawa, 1999). RdRp, virus tarafından kodlanan tek enzimdir ve virusun sıcaklığa adaptasyonunda görev alır (Ahlquist vd., 2003; Hata vd., 2010; Panzarin vd., 2014). RNA1'in replikasyonu sırasında, RdRp tarafından subgenomik RNA3 transkripti üretimi yönlendirilir (Johnson vd., 2003). RNA2 segmenti, kapsid proteinini kodlar ve farklı genotiplerin konakçı tropizmi ve immunoreaktivisinden sorumludur (Ito vd., 2008; Iwamoto vd., 2004; Johnson vd., 2003; Nishizawa vd., 1995; Panzarin vd., 2016). RNA3 olarak bilinen üçüncü transkript, viral replikasyon sürecinde RNA1 terminusundan ayrılır ve antinekrotik ölüm faktörü B1 (Cai vd., 2010; Fenner vd., 2006; Huang vd., 2001; Iwamoto vd., 2005) ve hücre RNA sessizleşmesinin inhibitörü B2 yapısal olmayan proteini kodlar (Fenner vd., 2006; Iwamoto vd., 2005; Nagai ve Nishizawa, 1999). RNA3, ayrıca virus RNA'sının konakçı hücrede toplanmasında görev alır.

Betanodaviruslar, RNA2 segmentinin T4 değişken bölgesinin filogenetik analizine göre resmi olarak *Striped jack nervous necrosis virus* (SJNNV), *Tiger puffer nervous necrosis virus* (TPNNV), *Barfin flounder nervous necrosis virus* (BFNNV) ve *Redspotted grouper nervous necrosis virus* (RGNNV) olarak dört major genotipte gruplandırılırlar (ICTV, 2021). Johansen vd. (2004), bir kalkandan (*Scophtalmus maximus*) izole ettikleri ve Turbot nodavirus (TNV) adını verdikleri yeni bir genotip önermişler, ancak henüz resmi olarak onaylanmamıştır. Genotipler türlere özgü olmaktan ziyade, optimum olarak geliştikleri bir su sıcaklığına ihtiyaç duyarlar (Costa ve Thompson, 2016). Virusun segmentli olmasından dolayı, reassortant oluşumu şimdiye kadar SJNNV ve RGNNV genotipleri arasında tespit edilmiştir ve levrek, çipura, dil balıklarında RGNNV/SJNNV veya SJNNV/RGNNV reassortantları pek çok kez rapor edilmiştir (Bithcava vd., 2019; He ve Teng, 2015; Toffan vd., 2017; Volpe vd., 2020).

Betanodaviruslar genel olaral Nervöz nekrozis virus (NNV) olarak ta anılmaktadırlar. NNV'ye en az 173 farklı kültürü yapılan veya yabani balık ve omurgasız türünün duyarlı olduğu ve bu türlerin en az 62'sinde salgın meydana getirdiği bildirilmektedir (Bandin ve Souto, 2020). VNN, genel olarak çiftlik balıklarında salgınlar meydana getirmektedir, ancak, başta orfoz olmak üzere yabani balıklarda da şiddetli salgınlar rapor edilmiştir (Gomez vd., 2009; Vendramin vd., 2013). Yabani balıklarda asemptomatik NNV enfeksiyonları da tespit edilmiştir (Baeck vd., 2007; Barker vd., 2002; Ciulli vd., 2007; Gomez vd., 2004; Gomez vd., 2008a; Liu vd., 2015; Panzarin vd., 2012). Yumuşakçalarda NNV varlığı Akdeniz, Güney Kore, Çin ve Japonya'da tespit edilmiştir (Ciulli vd., 2010; Fichi vd., 2015; Gomez vd., 2008b; Gomez vd., 2010; Gomez vd., 2006; Panzarin vd., 2012).

Çift kabuklu yumuşakçalar, diğer kabuklular gibi filtrasyon ile beslenen canlılardır ve çevrelerindeki sularda bulunan patojenleri yoğunlaştırabilirler (Metcalf vd., 1979; Rippey, 1994). Bu nedenle, çift kabuklu yumuşakçaların çeşitli patojenik virusların yayılmasında rol oynadıkları bildirilmiştir (Gomez vd., 2008b; Kim vd., 2016). Bu çalışmanın amacı, Kuzey Ege ve Marmara Deniz'lerinde yer alan ve resmi otoriteden onaylı doğal yataklarda yetiştirilen akivades ve kara midyelerde betanodavirus varlığının araştırılmasıdır.

2. MATERYAL VE METOT

2.1. Örnekleme

Kuzey Ege ve Marmara Deniz'lerinde yer alan ve resmi otoriteden onaylı doğal yataklarda bulunan akivades ve kara midye istasyonları, 2016-2020 yılları arasında örneklenmiştir. Örneklemeler Ağustos ve Eylül aylarında gerçekleştirilmiş olup, her bir istasyondan 30 adet akivades ve/veya kara midye alınmıştır (Tablo 2).

2.2. Örneklerin hazırlanması

Soğuk zincirde vakit geçirilmeden laboratuvara ulaştırılan kara midye ve akivades örneklerinden hepatopankreas alındıktan sonra 5 örnekten 1 havuz oluşturuldu. Steril havanda pens ve makas yardımıyla parçalara ayrıldıktan sonra steril kum (Sea sand, Merck, Germany, CAS-No: 14808-60-7) ile ezilerek, %2 FBS, %2 antibiyotik içeren EMEM (Eagle's Minimum Essential Medium) (Sigma-Aldrich, United Kingdom, Product No: M4655) vasatı ile 1/5 oranında homojenize edildi. Homojenizatlar 15 ml hacimdeki steril santrifüj tüplerine (Sarstedt, Germany, Ref: 62.554.502) aktarıldı, +4 °C ve 4000 g'de 15 dk santrifüj (ThermoFisher SL 16R, Germany) edildi ve elde edilen süpernatantlar 0,45 µm'lik membran filtreden (Sartorius, USA) geçirilerek inokulumlar hazırlandı ve vakit geçirilmeden moleküler testlere geçildi.

2.3. RNA ekstraksyionu

İnokulumlardan 200'er µl alınarak 32 gözlü ekstraksiyon pleytlerine konuldu. RNA ekstraksiyonu ticari kitin protokülüne (MagNA Pure LC Total Nucleic Acid Isolation Kit, Roche, Germany, Product No. 03038505001) uygun olarak otomatik ekstraksiyon cihazında (MagNA Pure LC System, Roche, Germany) yapıldı. Ekstraksiyon işleminin doğrulanması ve olası çapraz kontaminasyonun tespit edilmesi amacıyla, ekstraksiyon işleminde pozitif kontrol ve negatif kontrol kullanıldı. Pozitif kontrol olarak referans betanodavirus, negatif kontrol olarak ise EMEM vasatı kullanıldı.

2.4. Real Time RT-PCR

Çalışmada, betanodavirusların RNA2 segmentinin T4 değişken bölgesine göre dizayn edilen Panzarin vd. (2010) tarafından geliştirilen primer ve problar kullanıldı (Tablo 1).

Tablo 1. Real Time RT-PCR testinde kullanılan primer ve prob dizilimleri

Primer	Hedef Bölge	Nt Pozisyonları	5'→ 3' Dizilim	Amplikon Büyüklüğü (bç)	Referans Yayın
RNA2 Forward	RNA2	392–410	CAA-CTG-ACA-RCG-AHC-ACA-C	69	Panzarin vd., 2010
RNA2 Reverse		445–460	CCC-ACC-AYT-TGG-CVA-C		
RNA2 Prob		422–442	TYC-ARG-CRA-CTC-GTG-GTG-CVG*		

*Reporter; FAM, quencher; BHQ1

Real Time RT-PCR (RT-qPCR) testinde ticari kit (Real Time Ready Virus Master, Roche, Germany, Cat. No. 05 992 877 01) ve Real Time PCR cihazı (Roche LightCycler® 480 Multiwell Plate 96) kullanıldı. Mastermiks, her bir örnek için 7,6 µl H_2O, 1 µl F primer (10 µM), 1 µl R primer (10

µM), 1 µl Prob (10 µM), 4 µl 5x buffer, 0,4 µl enzim ile hazırlandı. Mastermiksten 96 gözlü Real Time PCR pleytlerine (Roche LightCycler® 480 Multiwell Plate 96, White, Germany, Ref: 04 729 692 001) 15'er µl konulduktan sonra üzerlerine 5'er µl örnek RNA'sı, her pleyte pozitif ve negatif kontrol eklendi. Pleyt üzeri şeffaf bant ile kapatıldıktan sonra 1500g'de +4 ºC'de 2 dk santrifüj edildi ve PCR cihazına yerleştirildi. Reaksiyon ve ısı koşulları ise reverse transkripsiyon için 53ºC 4 dakika ve 50ºC 6 dakika, reverse transkriptazın inhibisyonu için 95ºC 65 saniye, ön denatürasyon 95ºC 10 saniye, bağlanma ve sentez 54ºC 30 saniye ve uzama 72ºC 1 saniye 45 döngü olarak ayarlandı.

Çalışmada kullanılan RT-qPCR testi, saha örnekleri çalışılmadan önce optimize edildi. RT-qPCR testinin verimliliği: %98, duyarlılık: %100, özgüllük: %100, Slope değeri: -3.373, R^2: 0.98 ve minimum tespit limiti 2.82×10^1 kopya/mL olarak hesaplandı. Pozitif kontrol olarak, RT-qPCR testi optimizasyonunda da kullanılan ve kopya sayısı bilinen plazmid DNA kullanıldı (Kaplan ve Karaoğlu, 2021).

3. BULGULAR

Çalışmaya ilk olarak 2016 yılında başlanmış ve tüm akivades ve kara midye örnekleri NNV yönünden negatif bulunmuştur. İlk örnekleme yılını takiben 2017, 2018, 2019 ve 2020 yıllarında da örneklemeye devam edilmiş ve yine tüm örnekler NNV yönünden negatif bulunmuştur (Tablo 2).

Tablo 2. Örnekleme yapılan lokasyonlar ve yıllara göre test sonuçları

Kodu	Tür	Lokasyon	Örnekleme yılları ve test sonuçları				
			2016	2017	2018	2019	2020
A1	Akivades	İnciraltı - İzmir	Negatif	Negatif	Negatif	Negatif	Negatif
A2	Akivades	Foça - İzmir	-*	Negatif	Negatif	-	-
A3	Akivades	Kalınburun - Erdek- Balıkesir	Negatif	Negatif	Negatif	Negatif	Negatif
A4	Akivades	Hakimin Koyu - Erdek - Balıkesir	Negatif	Negatif	Negatif	Negatif	Negatif
A5	Akivades	Bandırma - Balıkesir	Negatif	Negatif	Negatif	Negatif	-
M1	Kara Midye	Koyun Adası - Erdek - Balıkesir	Negatif	Negatif	Negatif	Negatif	-
M2	Kara Midye	Urla - İzmir	Negatif	Negatif	Negatif	Negatif	-
M3	Kara Midye	Aliağa - İzmir	Negatif	Negatif	-	Negatif	Negatif
M4	Kara Midye	İstanbul Boğazı	Negatif	Negatif	Negatif	Negatif	Negatif
M5	Kara Midye	Balıkçı Adası-İstanbul	Negatif	Negatif	-	-	Negatif
M6	Kara Midye	Gelibolu - Çanakkale	-	Negatif	Negatif	Negatif	Negatif
M7	Kara Midye	Yenice - Bandırma - Balıkesir	-	-	Negatif	Negatif	-
M8	Kara Midye	Sarı Burun – Erdek - Balıkesir	-	-	Negatif	Negatif	Negatif

*Üretim olmadığından örnekleme yapılmamıştır.

4. TARTIŞMA

VNN, Türkiye'de ilk defa 2011 yılında Akdeniz'de bir deniz işletmesinde klinik enfekte levreklerde tespit edilmiştir (Özkan Özyer vd., 2014). 2012 ve 2014 yıllarında ise kuluçkahane tarama çalışmaları sırasında asemptomatik yavru levrek ve çipuralardan NNV tespit edilmiştir. 2014 yılında ayrıca %5 ve %10 mortalite görülen bir kuluçkahane ve deniz işletmesinde fingerling levreklerde yine klinik olarak tespit edilmiştir (Kalaycı vd., 2016). NNV, ayrıca subklinik enfekte fingerling levreklerde 2016 ve 2017 yıllarında tespit edilmiştir, ilk tespiti takiben yapılan izleme çalışmalarında 2018 yılında yine aynı işletmede fingerling levreklerde tespit edilmiş, ancak fry ve daha küçük balıklarda tespit edilmemiştir. Fingerling boylarda tespit edilen virusun deniz suyu kaynaklı olabileceği değerlendirilmiştir (Kaplan ve Karaoğlu, 2021). Benzer şekilde 2016 yılında yapılan bir tarama programında, Karadeniz'de subklinik enfekte levreklerde tespit edilmiştir, ancak ilk tespiti takiben yapılan izleme çalışmalarında virus tekrar tespit edilmemiştir (Kaplan vd., 2021). NNV, Türkiye'de son olarak 2019 yılında, 90-100 g ağırlığındaki çipuralarda subklinik olarak tespit

edilmiştir. Aynı çalışmada virus tespit edilen çiftlik ile ilişkili kuluçkahanelerde yapılan araştırmalarda, virus tekrar tespit edilmiş ve enfeksiyon kaynağı hem epidemiyolojik hem moleküler düzeyde ortaya konulmuştur (Kaplan vd., 2022). Hastalık ilk çıktığı 1985'ten bu yana, Güney Amerika hariç dünyanın pek çok bölgesinde bildirilmiştir (Costa ve Thompson, 2016). SJNNV genotipi, 2007 yılında İber Yarımadasında izole edilene kadar coğrafi olarak Japonya suları ile sınırlıyken (Cutrín vd., 2007), aynı yıl içerisinde Adriyatik Denizi'nde (Hırvatistan ve İtalya) Avrupa deniz levreğinden izole edilen reassortant betanodavirus (SJNNV/RGNNV) izolatları ilk defa tanımlanmıştır (Toffolo vd., 2007). Bunun çapraz reassortantı olan RGNNV/SJNNV genotipi ise günümüzde Güney Avrupa ve İber Yarımadasında çipura ve dil balıklarında yaygın olarak bulunmaktadır (Olveira vd., 2009; Panzarin vd., 2012). VNN, Akdeniz havzasında endemiktir ve hastalığın ortaya çıkışı hem çiftlik hem de vahşi balıklarda çok kez ortaya konulmuştur ve bölgede endemik olarak seyretmektedir (Berzak vd., 2019; Costa ve Thompson, 2016; Munday vd., 2002; OIE, 2017; Toffan vd., 2017). RGNNV, bu bölgede en çok tespit edilen virus türüdür (Costa ve Thompson, 2016), ancak SJNNV türü de 2009 yılında İber Yarımadasında bildirilmiştir (Panzarin vd., 2012). Reassortant RGNNV/SJNNV ve SJNNV/RGNNV suşları sadece Akdeniz'de bildirilmiştir (He ve Teng, 2015). Türkiye'de levreklerde hem klinik hem subklinik olarak NNV tespit edilmesine rağmen çipuralarda şimdiye kadar sadece subklinik olarak tespit edilmiştir.

Yumuşakçaların akuatik virusların potansiyel bir rezervuarı olabileceği bildirilmiştir (Gomez vd., 2010; Panzarin vd., 2012). Yumuşakçalar betanodavirusların ayrıca doğal rezervuar ve muhtemel taşıyıcıları olarak işlev görürler. Betanodaviruslar, sağlıklı görünüşte yabani ve çiftlik omurgasızlarından çift kabuklular (midye; *Mytilus galloprovincialis* ve istiridye; *Ruditapes philippinarum*), kabuklu (karides; *Pandalus hypsinotus*, yengeç; *Charybdis bimaculata,* dikenli ıstakoz; *Pamulirus versicolor* veya karındanbacaklı; *Opistobranchia*) gibi yumuşakçalarda tespit edilmiştir (Gomez vd., 2008b; Gomez vd., 2008c).

Bu çalışmada, 2016, 2017, 2018, 2019 ve 2020 yıllarında kayıtlı istasyonlardan örneklenen, hem kara midyelerinde hem akivadeslerde NNV tespit edilmemiştir. NNV, Türkiye'nin Akdeniz ve Güney Ege kıyılarında levreklerde hem klinik hem subklinik olarak, çipuralarda ise subklinik olarak tespit edilmiştir (Kalaycı vd., 2016; Kaplan ve Karaoğlu, 2021; Kaplan vd., 2022; Özkan Özyer vd., 2014). Karadeniz'de tespit edilen NNV ise su kaynaklı olmayıp, daha önce enfekte olan, ancak virusu persiste olarak taşıyan ve virus saçılımının olmadığı düşünülen levreklerde tespit edilmiştir (Kaplan vd., 2021). 5 yıllık tarama sonuçları dikkate alındığında, örneklenen bölgelerde virus sirkülasyonunun olmayabileceği sonucu çıkarılabilir.

NNV, şimdiye kadar 12 familya ve dokuz takıma ait 21 deniz omurgasız türünde tespit edilmiştir (Bandin ve Souto, 2020). Bu tespitlerin çoğu, filtre besleme aktiviteleri nedeniyle çevredeki sudan, viruslar da dahil olmak üzere farklı partiküller biriktirebilen çift kabuklu yumuşakçalarda gerçekleştirilmiştir (Ciulli vd., 2010; Gomez vd., 2008c, Panzarin vd., 2012). Akdeniz ülkeleri dışında Kore, Japonya ve Çin kıyılarında bulunan farklı kabuklu türlerinde de NNV tespit edilmiştir (Gomez vd., 2006; Gomez vd., 2008b; Kim vd., 2018). Berzak vd. (2019), tarafından Akdeniz'de İsrail kıyılarında toplanan 33 adet kum yengecinin 2'sinde NNV tespit edilmiştir. Ayrıca, Fransa'da pasifik midyesinde (*Cassostrea gigas*), İtalya'da istiridyede (*Ruditapes philipinarum*) (Gomez vd., 2008c) ve ahtapotta (*Octopus vulgaris*) (Fichi vd., 2015), Yunanistan'da Akdeniz midyesinde (*Mytilus galloprovincialis*), Avrupa istiridyesinde (*Ostrea edulis*) ve başka bir istiridye türünde (*Venus verrucosa*), ayrıca kırmızı ağızlı kabukluda (*Stramonita haemastoma*) (Bitchava vd., 2019) NNV tespit edilmiştir. Ayrıca, İtalya'da bir deniz kaplumbağasından (Caretta caretta) balık dışındaki bir deniz omurgalısından ilk betanodavirus izolasyonu rapor edilmiş ve virusa karşı duyarlı konakçıların yelpazesinin genişlediği bildirilmiştir (Fichi vd., 2016). Virus tespit edilen bu kabukluların hiçbirinde NNV'ye ilişkin klinik bir bulguya rastlanmamıştır (Bandin ve Souto, 2020). Omurgasızlar arasında en sık tespit edilen RGNNV genotipidir, ancak BFNNV genotipleri de Asya sularında tekli veya RGNNV

genotipleri ile beraber tespit edilmiştir (Bandin ve souto, 2020; Kim vd., 2018). Akdeniz'de tespit edilen virusların tamamı Akdeniz'de endemik olan RGNNV genotipidir. Bu da virusun endemik olduğu bölgelerde, çift kabukluların virusu filtrasyon sırasında alarak, horizontal olarak enfekte olduklarını ve virus replikasyonu olmasa bile rezervuar olduklarını göstermektedir.

5. SONUÇ

Sonuç olarak, bu çalışmada Ege ve Marmara Denizi'nde lokalize olan kayıtlı midye ve akivades istasyonlarında beş yıl boyunca NNV araştırması yapılmış ve tüm örnekler negatif bulunmuştur. İstasyonların konumları göz önüne alındığında, Akdeniz'de endemik olan ve Türkiye'de de daha önce tespit edilen RGNNV genotipi betanodavirusun optimum gelişme ısısından düşük ısıların bulunduğu ve daha önce virus tespit edilmeyen bölgelerde oldukları görülmektedir. Bu çalışma, sadece kayıtlı istasyonlarda gerçekleştirilmiştir ve özgünlük olarak değerlendirildiğinde, Türkiye'de çift kabuklu yumuşakçalarda NNV varlığı ilk kez araştırılmıştır. NNV'nin Türkiye kıyılarındaki kabuklulardaki varlığının daha iyi anlaşılması için, virusun optimum ısısına daha yakın olan ve virusun daha önce de tespit edildiği Güney Ege ve Akdeniz kıyılarını içeren, kayıtlı istasyon olmasa bile doğal hayatı kapsayan daha geniş tarama çalışmalarının yapılması gerektiği düşünülmektedir.

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Review **Derleme**

Climate Change's Impact on Aquaculture and Consequences for Sustainability

İklim Değişikliğinin Su Ürünleri Yetiştiriciliği Üzerindeki Etkisi ve Sürdürülebilirlik için Sonuçları

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Abstract: Aquaculture is the fastest-growing sector of food production, with catch fisheries currently accounting for more fish biomass. Unfortunately, the sustainability of aquaculture is jeopardized due to the projected repercussions of climate change, which are not only a future but also a present reality. We examine the probable impacts of climate change on aquaculture productivity and the consequences for the sector's long-term viability in this review. Various aspects of a changing climate have been considered, including rising temperatures, sea-level rise, illnesses, toxic algal blooms, changes in rainfall patterns, the unpredictable supply of external inputs, changes in sea surface salinity, and catastrophic climatic events. Climate change's impacts will be long-lasting and almost certainly permanent, wreaking havoc on the economy of people who work in the industry. As a result, the fisheries authorities must put in greater effort to comprehend the scope of climate change's influence on aquaculture and plan for its potential implications, as well as identify the sorts of consequences and design an adequate reaction to manage them.

Keywords
- Climate Change
- Aquaculture
- Sustainability
- Fisheries

Özet: Su ürünleri yetiştiriciliği, mevcutta daha fazla balık biokütlesi oluşturan av balıkçılığı ile gıda üretiminin en hızlı büyüyen sektörüdür. Ne yazık ki, su ürünleri yetiştiriciliğinin sürdürülebilirliği, iklim değişikliğinin sadece gelecek değil, aynı zamanda bugünün bir gerçeği ve öngörülen etkileri nedeniyle tehlikeye atılmaktadır. Bu derlemede iklim değişikliğinin su ürünleri verimliliği üzerindeki olası etkilerini ve sektörün uzun vadeli uygulanabilirliği üzerindeki sonuçları incelenmektedir. Yükselen sıcaklıklar, deniz seviyesinin yükselmesi, hastalıklar ve zehirli alg patlamaları, yağış düzenlerindeki değişiklikler, öngörülemeyen dış girdi arzı, deniz yüzeyi tuzluluğundaki değişiklikler ve zararlı iklim olayları dahil olmak üzere değişen bir iklimin çeşitli yönleri göz önünde bulundurulmuştur. İklim değişikliğinin etkileri uzun süreli ve kalıcı olacağı için sektörde çalışan insanların ekonomisine zarar verecektir. Sonuç olarak, balıkçılık yetkilileri, iklim değişikliğinin su ürünleri yetiştiriciliği üzerindeki etkisinin kapsamını kavramak ve olası etkilerini planlamak, ayrıca sonuçların çeşitlerini belirlemek ve bunları yönetmek için yeterli bir önlem almak için daha fazla çaba sarf etmelidir.

Anahtar kelimeler
- İklim değişikliği
- Su ürünleri
- Sürdürülebilirlik
- Balıkçılık

1. INTRODUCTION

Aquaculture, or fish, shellfish, and aquatic plant cultivation, is the world's fastest-growing food production sector. Between 1990 and 2016, global aquaculture production expanded fold, with an average annual growth rate of 5.8% from 2000 to 2016. (FAO, 2018). Aquaculture's contribution to world fish output has continued to climb, reaching 82.1 million tons (46%) of the predicted 179 million tons of global production, according to FAO (2020). Furthermore, aquaculture's proportion of

world fish output is predicted to increase from 46 percent now to 53 percent in 2030. (FAO, 2020). The most pressing question, however, is whether the industry can develop sustainably and quickly enough to satisfy future predicted demand, which is being worsened by a rapidly rising human population and a changing environment. Climate change is now considered a severe threat to the world's food supply, both in terms of quality and quantity ((Beach and Viator, 2008; Hamdan et al., 2015; Myers et al., 2017). Climate change will influence small-scale food producers' lives and revenue, as well as poor net food consumers' livelihoods, limiting access to food through food price rises and instability (Maulu et al., 2021; Elsheikh, 2021a).

Climate change is defined as changes in the statistical distribution of weather over long periods, often decades to millions of years. These changes can occur in the average weather or just in the distribution of weather events around an average, and they can occur in a single place or all over the world (Yazdi and Shakouri, 2010). Also, Climate change is gradual changes in temperature, precipitation, atmospheric moisture, wind intensity, as well as sea level, all of these changes happen at a breakneck speed (Sesana et al., 2021)

The bulk of contemporary research in aquaculture indicates that some climatic changes, such as rising temperatures, altering precipitation patterns, and increased frequency of some extreme events, have already had an impact on water supplies, while others are still emerging. Because of the sector's substantial contribution to global food security, nutrition, and livelihoods, climate change implications on aquaculture sustainability have recently attracted a lot of attention (Fleming et al., 2014; Blanchard et al., 2017; Troell et al., 2017; Zolnikov, 2019; FAO, 2020).

The repercussions also will be long-lasting and certainly irreversible, wreaking havoc on the economy of individuals who work in the industry (Barange et al., 2018; Dabbadie et al., 2018). At both the regional and global levels, the impacts of climate change on aquaculture have been thoroughly researched and evaluated (De Silva and Soto, 2009; Yazdi and Shakouri, 2010; Clements and Chopin, 2016; Bueno and Soto, 2017; Chung et al., 2017; Ellis et al., 2017; Froehlich et al., 2017; Handisyde et al., 2017; Harvey et al., 2017; Klinger et al., 2017; Beveridge et al., 2018; Dabbadie et al., 2018; Elsheikh, 2021b). However, in the bulk of this research, there has been a trend toward focusing on the negative implications of predicted climate change on aquaculture while ignoring the beneficial effects, which are crucial for adaptation measures. This review examines the consequences of climate change on aquaculture output and the implications for sustainability, focusing on how each factor of climate change will influence the sector.

Direct effects of climate change on aquaculture production include affecting the physical and physiology of finfish and shellfish stocks in production systems, while indirect effects include affecting product prices, fishmeal and fish oil costs, and other goods and services required by fishers and aquaculture producers (Handisyde et al., 2006; De Silva and Soto, 2009; Freeman, 2017; Adhikari et al., 2018). Aquaculture has recently seen considerable technical advancements, allowing the industry to extend its present output to fulfill the growing demand for aquatic goods. Climate change, on the other hand, is gradually becoming one of the key concerns challenging the sustainability of food production systems, including aquaculture (Lim-Camacho et al., 2014; IPCC, 2018; FAO, 2020). The following are some of the projected effects of climate change on aquaculture productivity and sustainability: rising temperature, ocean acidification, diseases and harmful algal blooms changes in rainfall/precipitation patterns, sea-level rise, the uncertainty of external input supplies, changes in sea surface salinity, and severe climatic events (Handisyde et al., 2006; Brander, 2007; Ficke et al., 2007; Barange et al., 2018).

The significance of temperature in aquatic animal growth and development is crucial, because fish are poikilothermic, and they may be particularly vulnerable to temperature changes caused by climate change (Ngoan, 2018; Sae-Lim et al., 2017; Adhikari et al., 2018). Most fish, especially cold-water species such as Atlantic halibut, Salmon, and Cod, as well as intertidal shellfish, are expected to die

more as a result of the 1.5°C rises in average global temperature forecast for this century. As a result, prolonged temperature stress can have a range of effects on aquaculture productivity, the most common of which is a decrease in output (Hamdan et al., 2012; Gubbins et al., 2013).

Ocean acidification occurs when the pH of ocean water drops over time (typically decades) as a result of CO_2 accumulation from the atmosphere (Richards et al., 2015; Bahri et al., 2018). The seas are thought to store 50 times the amount of CO_2 that the atmosphere does. (Seggel et al., 2016). At 1.5°C or higher global warming, the expected increase in CO_2 absorption by seas would have negative consequences for the growth, development, calcification, survival, and abundance of various aquatic species (IPCC, 2018). Increased CO_2 levels in water might lead to a drop in pH, endangering the environmental sustainability of aquaculture production systems by causing water quality to deteriorate, resulting in low output. Furthermore, when ocean acidity rises, the supply of carbonate essential for the formation of coral skeletons (Calcification) in shell-forming animals including shrimp, mussels, oysters, and corals decreases, posing a danger to marine aquaculture output (Yazdi and Shakouri, 2010; Kroeker et al., 2014; Rodrigues et al., 2015).

Aquaculture diseases are predicted to be affected by a changing temperature regime, such as bacterial, parasitic, viral, and fungal infections, but in an unpredictable way. What is known, however, is that when cultured species are subjected to heat stress, they become more sensitive to illness and that rising temperatures may lead to the spread of exotic diseases (Collins et al., 2020). The sensitivity of finfish and shellfish to pathogens is a primary factor of illness, and both direct and indirect temperature stresses are likely to have an impact. As a result, warm water disease outbreaks are expected to become more common, with the possibility of new ones emerging as a result of climate change (Chiaramonte et al., 2016). The replication rate, pathogenicity, life cycle duration, and transmission of infections among numerous finfish and shellfish species are predicted to increase when the temperature rises (Sae-Lim et al., 2017). Furthermore, rising temperatures may hasten the introduction of epizootic illnesses in aquaculture, posing significant economic concerns. Epizootic disease outbreaks are already one of the most significant challenges limiting the success of aquaculture production systems in many places across the world. (Marcogliese, 2008; Maulu et al., 2019). In Chilean aquaculture, an exceptional loss of fish has been documented owing to the spread of *Pseudochattonella cf. verruculosa* and *Alexandrium catenella* species, whose outbreaks were linked to climate-induced changes in water column stratification. (Trainer et al., 2019). Furthermore, diseases such as inflammation, atrophy, and necrosis have been documented in numerous organs of bivalve mollusks as a result of hazardous algal blooms in several investigations (Haberkorn et al., 2010; Basti et al., 2011; Hégaret et al., 2012).

Changes in rainfall patterns will have two distinct effects on aquaculture productivity and sustainability by increased flooding and periods of low or no rainfall (Drought), Drought risks are anticipated to be greater at 2°C of global warming in a particular location than at 1.5°C, according to the IPCC (2018), whereas flooding occurrence patterns are impossible to predict with certainty. Increased rainfall, especially if it comes in the form of heavier storms, will exacerbate the production risks in lowland regions. (Bell et al., 2010). These dangers include the loss of fish in ponds due to flooding, the invasion of ponds by undesired species, and pond damage caused by infilling and the washing away of walls (Rutkayova et al., 2017). Mixing pond water and fish with wild fish might have a detrimental impact on aquaculture production's environmental sustainability, primarily through the introduction of invasive fish species and worsening of water quality. Furthermore, pond fish losses endanger the social and economic aspects of aquaculture sustainability by reducing producers' profits and causing poverty in communities. (Maulu et al., 2021). Droughts can cause water stress, such as shortages and degradation in quality, which can have a severe impact on aquaculture productivity (Hambal et al., 1994). Water shortages projected as a result of climate change may exacerbate competition for water among many user groups, including aquaculture, agriculture, residential

consumption, and industry (Handisyde et al., 2006; Barange et al., 2018). This will have an impact on all aspects of aquaculture sustainability (Maulu et al., 2021).

According to IPCC (2018) forecasts, sea level rise will be roughly 0.1 meters lower under 1.5°C global warming compared to 2°C by 2100. This trend, however, is predicted to continue after 2100, with the degree and pace of increase likely to be determined by future GHG routes (IPCC, 2018). Coastal habitats such as mangroves and salt marshes, which are critical for maintaining wild fish supplies and producing seeds for aquaculture production, may be destroyed by rising sea levels (Kibria et al., 2017). This will have a detrimental impact on aquaculture breeding initiatives as well as the sector's economic viability. Higher sea levels are expected to have an impact on aquaculture production facilities such as ponds, cages, tanks, and pens, particularly in lowland areas, due to saline water intrusion (Kibria et al., 2017). Aquaculture, freshwater fisheries, and agricultural productivity are all thought to be harmed by groundwater salinization. As a result, salinization makes aquaculture unfit for production, resulting in greater production costs and reduced profits. Changes in species composition, organism abundance and distribution, ecosystem productivity, and phenological shifts are all predicted to occur as sea levels rise, posing a danger to inland and marine aquaculture output (Doney et al., 2012).

Variations in sea salinity are likely to have a detrimental impact on the economic benefits of some aquaculture species, thus jeopardizing the social and economic viability of aquaculture production. The increased salinity effect, on the other hand, has been closely linked to aquaculture production systems in coastal areas downstream (Nguyen et al., 2018). In general, changes in water salinity will result in greater mortalities for a variety of species, posing a threat to the sector's economic and social viability through increasing species losses and higher management costs (Maulu et al., 2021).

The appropriateness of habitat and the geographic dispersion of marine fishes are largely influenced by oxygen availability (Zambonino-Infante et al., 2013). Reduced oxygen concentrations are expected to occur more frequently, and for longer periods in the future as a result of climate change. It is important to recognize the difficulty posed by the ocean's growing low-oxygen zones. One of the biggest imminent risks to future fisheries resources and marine ecosystems, according to the United Nations Environment Program's 2003 yearbook, is oxygen depletion (Townhill et al., 2017). Regions of the ocean with low oxygen concentrations are expected to develop more frequently and last longer in the future as a result of long-term climate change. When oxygen levels are low, marine species must work harder to fulfill their metabolic needs, which can have an impact on their ability to grow, feed, and reproduce. The analysis carried out has shown that there is already a sizable body of information about the physiological and behavioral reactions of fishes and shellfish to oxygen, particularly hypoxia. However, there is still a significant information vacuum about how these changes may appear as implications for fisheries and ecosystems. The only approach to keep the sector's output going might be to adjust to the anticipated changes in the short term while pursuing mitigating measures in the long run. However, the ability of producers in various parts of the world to adapt will determine if adaptation is effective. By advocating changes in fishing practices, changes in governance, and the deployment of efficient management plans and strategies, aquaculture producers may also adapt to climate change by assuring a steady supply of fish from captured fisheries (Frusher et al., 2014). There is a need to incorporate climate variability and change in the modeling of aquaculture undertakings to reduce the impacts of climate change in fisheries-based livelihoods. According to FAO (2020), global production from capture fisheries has stagnated or declined in some years over the past few decades. Inputs from capture fisheries, such as fishmeal, fish oils, brood stocks, and wild seeds, are significantly reliant on aquaculture output at the moment (Malcorps et al., 2019). Therefore, effective management of resources from fisheries may contribute to a sustainable supply of aquaculture inputs.

At a time when a rising global population needs to be fed and catch fisheries are at their peak and may eventually decrease, the availability of fishmeal and fish oils is already seen as a barrier to the development of aquaculture. The usage of plant-based aquaculture feeds in place of fishmeal currently depends on a few key crops including soy, maize, and wheat, all of which might be consumed directly by humans and are all negatively impacted by climate change. The processing ability, the presence of anti-nutritional components, storage stability, and application to the appropriate fish species in aquaculture are all factors that need to be investigated for aquaculture feed (Hall, 2015). Nevertheless, aside from climate change hazards, the success of the aquaculture insurance industry may rely on how effective and low-risk aquaculture develops. Since aquaculture is a relatively new field, research is needed to explain its advantages and effects on farmers' financial circumstances, particularly in the most disadvantaged regions.

Recirculating Aquaculture Systems (RAS) is one of the possible adaption options when taking environmental sustainability and sensitivity to the impacts of climate change on fish output into consideration. RAS are extremely productive, intensive farming techniques for a wide range of seafood products (Ahmed & Turchini, 2021). They may be used all year long, in a variety of locations, including near to important seafood markets, and are not impacted by seasonality or environmental factors. However, RAS are costly, intricate, and highly constructed systems that need for significant capital expenditure, which is why they mostly function in highly industrialized nations. Moreover, one of the major limitations of RAS is its high energy consumption.

2. CONCLUSION

The possible consequences of climate change on aquaculture productivity were emphasized in this review, which covered significant areas of climate change and aquaculture production. Climate change, which is both a current and future reality, is posing an increasing threat to the aquaculture business. These effects on aquaculture are expected to be both positive and negative, with the negative effects outnumbering the positive ones. Furthermore, while climate change is a global problem for food production, the risks connected with aquaculture are expected to differ by geographical or climatic zones, national economy, water environment, production techniques, production scale, and the farmed species of aquaculture producers. As a result, aquaculture producers must adapt to the options available and minimize the repercussions by making necessary changes to their production operations to develop resilience and maintain output in a changing environment. As the aquaculture industry grows, so does the danger of climate change, necessitating the development of research and field studies to mitigate the risks associated with climate change and its influence on aquaculture.

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Author declares that there is no conflict of interest.

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

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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
ORCID's of the authors should be identified. Please visit https://orcid.org to register an ORCID.

MANUSCRIPT FORMAT
Manuscripts in original articles, short communication, case report and reviews should be prepared in accordance with the format below*.

<table>
<tr><th>Original Article</th><th>Short Communication</th><th>Case Report</th><th>Review Article</th></tr>
<tr><td colspan="4">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</td></tr>
<tr><td>2. Material and Methods
3. Results
4. Discussion
5. Conclusion</td><td>2. FREE CONTENT</td><td>2. Case Report</td><td>2. FREE CONTENT</td></tr>
<tr><td colspan="4">3. Discussion
4. Conclusion
Acknowledgement
Funding
Conflict of Interest
Author Contributions
Ethical Statements
Data Availability Statement
References</td></tr>
</table>

* **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
Kısa başlık
Yazarlar
Kurum bilgileri
Sorumlu yazar e-posta adresi
ORCID bilgileri

Başlık
Özet
Anahtar kelimeler

Title
Abstract
Keywords

1. Giriş</td></tr>
<tr><td>2. Materyal ve Metot
3. Bulgular
4. Tartışma
5. Sonuç</td><td>2. SERBEST İÇEREİK</td><td>2. Olgu Sunumu</td><td>2. SERBEST İÇEREİK</td></tr>
<tr><td colspan="4">3. Tartışma
4. Sonuç
Teşekkür
Finans
Çıkar Çatışması Beyanı
Yazar Katkıları
Etik Onay Beyanı
Veri Kullanılabilirlik Beyanı
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
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NOT: Dergi yazım kurallarına uygun olarak hazırlanmayan makaleler değerlendirmeye alınmamaktadır.