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Impact of Sodium Bicarbonate-Enriched Diet on Muscle pH and Quality of Rainbow Trout (*Oncorhynchus mykiss*) During Cold Storage

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ABSTRACT

The effect of sodium bicarbonate (SB) supplementation to fish feed on the quality characteristics of rainbow trout (*Oncorhyncus mykiss*) under post-harvest cold storage conditions was investigated in this study. Rainbow trout fed with 0, 1M, 2M and 3M SB supplemented diets for 21 days before harvesting were stored at 4ºC. The highest post-harvest pH level was observed in the group fed with 2M and 3M SB. TVB-N value was found between 14.88-15.68 mg/100g in fish fed with 1M, 2M and 3M SB supplemented diets, while it was determined as 20.14 mg/100g for the control group. At the beginning of storage, similar TBARs values were observed in all groups; however the increase in TBARs in the groups fed with SB supplemented feeds was slower than in the control. Sensory scores of trout fed with SB supplemented diets at the beginning of storage were higher than those of the control group. However, no difference was determined between the groups regarding shelf life (13 days). The best texture scores were observed in the 3M group throughout the storage period. Therefore, it can be concluded that SB addition to pre-harvest rainbow trout feeds can be used as a feed supplements to improve fish flesh quality.

Keywords: Sodium bicarbonate, rainbow trout, pH, cold storage, post-harvest quality

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Sodyum Bikarbonatla Zenginleştirilmiş Diyetin Soğukta Depolama Sırasında Gökkuşağı Alabalığının (*Oncorhyncus mykiss*) Kas pH'ı ve Kalitesi Üzerine Etkisi

Öz: Bu araştırmada balık yemlerine sodyum bikarbonat (SB) ilavesinin gökkuşağı alabalığının (*Oncorhyncus mykiss*) hasat sonrası soğuk depolama koşullarında kimyasal ve duyusal özelliklerine etkisi araştırılmıştır. Hasattan önce 21 gün boyunca 0, 1M, 2M ve 3M SB katkılı yemlerle beslenen gökkuşağı alabalığı 4ºC'de depolanmış, raf ömrü sonuna kadar kimyasal ve duyusal değerlendirmeler yapılmıştır. Hasat sonrası en yüksek pH düzeyi 2M ve 3M SB katkılı yemlerle beslenen grupta gözlenmiştir. TVB-N değeri 1M, 2M ve 3M SB katkılı yemlerle beslenen gökkuşağı alabalığında 14,88-15,68 mg/100g arasında bulunurken, kontrol grubunda 20,14 mg/100g olarak belirlenmiştir. Depolamanın başlangıcında tüm gruplarda benzer TBARs değerleri gözlenmiş; ancak SB katkılı yemlerle beslenen gruplarda TBARs artışının kontrole göre daha yavaş olduğu görülmüştür. Depolama başlangıcında SB katkılı yemlerle beslenen alabalıkların görünüm, koku, tekstür, renk ve genel kabul edilebilirlik puanları kontrol grubuna göre daha yüksek bulunmuştur. Ancak raf ömrü (13 gün) açısından gruplar arasında fark gözlenmemiştir. Tüm depolama süresi boyunca en iyi doku skorları 3M grubunda gözlemlenmiştir. Bu nedenle, hasat öncesi gökkuşağı alabalığı yemlerine SB ilavesinin et kalitesini artırmak amacıyla yem katkı maddesi olarak kullanılabileceği sonucuna varılabilir.

Anahtar kelimeler: Sodyum bikarbonat, gökkuşağı alabalığı, pH, soğuk depolama, hasat sonrası kalite

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Introduction

The accumulation of lactic acid due to glycolysis in the post-mortem stage decreases muscle pH in fish. Low pH in the muscle may cause the formation and increase of gaps between muscle clusters (gaping), decreased water holding capacity, denaturation of muscle and sarcoplasmic proteins and lipid oxidation observed in fish fillets. Liu et al., (2020) stated that fish with high muscle pH have higher water content than those with low pH. Consequently, fish tissues with high pH can be described as softer and watery, while others can be described as harder and dry (Chan et al. 2022). Significant correlations were observed between muscle tissue and muscle degradation parameters due to increased cathepsin B+L activity with decreased muscle pH. Bahuaud et al. (2010)

reported that cathepsin B activity is associated with muscle deterioration, and cathepsin L gene expression is associated with muscle deterioration and tissue. However, it was found that there was more metmyoglobin and lipid oxidation in Asian sea bass (*Lates calcarifer*) minced meat at pH 6 than at pH 7 (Thiansilakul et al. 2011). Post-mortem pH value in fish muscle can be affected by many factors such as species, season, size, fishing method, killing method, and starvation status (Özyurt et al. 2007; Li et al. 2017).

Water is an important component of food, and meat products are often lightly salted to increase water holding capacity and improve sensory properties. The functional properties of proteins after processing (e.g. solubility and water binding) are important as they affect the ability of proteins to interact with other components and, therefore, affect the overall product quality. Salting meat products with a combination of sodium chloride (NaCl) and sodium bicarbonate (NaHCO3) has been associated with better texture, aroma, flavor, odor, color and higher water holding capacity. Sodium ions cause swelling of meat proteins. As sodium ions form an ion cloud around the filaments and osmotic pressure increases within the myofibrils, the filament lattice swells and water holding capacity increases (Siró et al. 2009). While water retention in muscle is lowest when the pH of the muscle is close to the isoelectric point (pI) of myofibrillar proteins, displacing the pH away from the isoelectric point improves water retention. Sodium bicarbonate (SB) is an amphoteric compound with good buffering capacity; aqueous solutions become slightly alkaline due to the formation of hydroxide ions (OH-) and carbonic acid (H2CO3), predominantly in the form of bicarbonate (HCO3-) between pH 6.4 and 10.3. It is known that when SB is used in meat products, muscle pH increases and therefore the protein configuration changes (Åsli and Mørkøre 2012; Åsli and Mørkøre 2013; Åsli et al. 2016).

Sodium bicarbonate has been used for many years as a food ingredient, in nutrition and in industrial processes (Balestra and Petracci 2019). It is among the foods generally recognized as safe (GRAS) by the FDA, and the use of sodium bicarbonate as a food additive has been approved by the European Union (EFSA 2011). Adding SB to the water during rearing and post-harvest transportation of fish helps to balance the pH of the water as well as the salt balance of the fish (Martins et al. 2017). There is evidence to support the use of SB in small ruminant production (Jallow and Hsia 2014; Akter 2021; Hassan et al. 2022; Vicente et al. 2023), but the use of SB in aquaculture and changes in post-harvest meat quality have not been focused.

Rainbow trout (*Oncorhynchus mykiss*) is the most common and economical fish species among trout species. The most preferred storage method for fresh trout in retail and industrial applications is cold (2-4 ^{o}C) and ice storage. There are many factors that affect the storage quality of fish during cold storage. Factors such as fish nutrition, pre-harvest handling and ambient conditions after harvest, packaging material used during storage, and processing techniques can be considered among the important factors affecting the quality of cold-stored trout (Aksun and Tokur 2014). This study aimed to investigate the effects of feeding rainbow trout (*Oncorhynchus mykiss*) with different concentrations of sodium bicarbonate added feeds before harvesting on chemical and sensory quality during cold storage. Thus, providing a high pH value to post-mortem fish will improve the meat quality of rainbow trout.

Materials and Methods

Material

In the study, sodium bicarbonate was purchased from a local market. Commercial trout feed was obtained from a private feed company (Abalıoğlu, Denizli, Turkey). A total of 240 rainbow trout (*Oncorhynchus mykiss*) with an average live weight of 250±10 g was used. The content of 6 mm diameter trout feed was 41% crude protein, 23% crude fat, 12% crude ash, 10% moisture, 3% crude cellulose, 1.5% phosphorus and energy content was 4375 kcal/kg. Analytical-grade chemicals and reagents were acquired from Merck (Darmstadt, Germany) or Sigma (St. Louis, MO, USA).

Preparation of feeds containing sodium bicarbonate, and feeding

The addition of sodium bicarbonate (SB) to the feeds was applied by absorbing method. Solutions containing 1, 2 and 3 M sodium bicarbonate were sprayed on the feeds at a rate of 10% of the feed and mixed at regular intervals until the feeds absorbed the solution. The feeds that absorbed the SB solution well were kept at room temperature and allowed to dry. As a control group, the feeds were treated with pure water without SBC. The prepared feeds were kept in a dry environment at room temperature until the feeding study was carried out.

In the feeding phase of the study, the facilities of a local trout farming enterprise in the region were used. After the fish were adapted to the rectangular concrete ponds for one-week, experimental feeding was carried out twice in the morning and evening at 3% of the fish weight. Fish were fed with control and experimental group feeds for 21 days. Then, no feeding was done for one day, and harvesting was applied on the 23rd. All the experiments were conducted in accordance with the protocols approved by Çukurova University Animal Experiments Local

Ethics Committee (Approval date: 12.12.2023, Decision number:10).

Cold Storage of Rainbow Trout and Quality Control Analyses

The harvested fish were placed in foam boxes on ice and brought to the laboratory. The washed fish were placed on foam plates, covered with stretch film and placed in the refrigerator (2-4 ºC). At least three parallel pH measurements, total volatile basic nitrogen (TVB-N), thiobarbituric acid reactive substances (TBARs) analysis and sensory analyses were carried out for each research group on 0^{th}, 3^{nd}, 6^{th}, 9^{th}, 11^{th} and 13^{th} days of the cold storage.

A sample of 5 g of fish meat was stirred in 50 mL of distilled water for 5 min using Ultra-turax. The pH of the fish meat was then measured using a digital pH meter (WTW 315i pH Meter; Weilheim, Germany) (Lima dos Santos et al. 1981). The method of Antonocopoulos et al. (1973) was used to measure rainbow trout's total volatile base-nitrogen (TVB-N) concentration following steam distillation. Based on titration using 0.1 N HCl and a boric acid solution, TVB-N analysis was performed. The results of the analysis were given as mg TVB-N per 100 g of material.

The method of Tarladgis et al. (1960) was used to measure thiobarbituric acid reactive compounds (TBARs) to assess the level of lipid oxidation in the samples. After distillation of 10 g rainbow trout muscle, 5 mL distillate and 5 mL thiobarbituric reagent (0.02 M TBA in 90% glacial acetic acid) were combined and the mixture was incubated in boiling water for 35 min. Absorbance against the blank containing distilled water instead of the sample was measured using a spectrophotometer (Perkin Elmer, Lambda 25) at 538 nm, and the results were presented in mg of malonaldehyde (MA) per kilogram of the sample.

For sensory evaluation, raw fish was evaluated for color, odor, texture, appearance and overall acceptability by 6 panellists using a score of 1-9 (1; disliked at all-9; liked very much) (Paulus et al. 1979). Evaluation continued until the fish became inedible. The study was considered concluded when the panellists acquired the inedibility ratings (4 and below).

Statistical analysis

One-way analysis of variance (ANOVA) was used to evaluate the data, and the Duncan multiple comparison test was used to examine any differences between the groups during storage. Significance level was $p<0.05$.

Results And Discussion

Chemical Changes of Rainbow Trout Fed with SB Supplemented Feed during Cold Storage Period

Muscle pH in live fish is generally around 7, and post-mortem pH is known to vary between 6.0 and 7.0 (Abbas et al. 2008). In the post-mortem period, glycolysis occurs in muscle tissue under anaerobic conditions and lactic acid is formed. Lactic acid formation is known to have a reducing effect on pH. In this study, the pH level of rainbow trout fed with different concentrations of SB supplemented diets is shown in Table 1. At the beginning of storage, the highest pH level was observed in the group fed with 2M and 3M SB supplemented feeds, while the lowest muscle pH was observed in the 1M group ($p<0.05$). Moreover, the highest muscle pH was observed in 2M and 3M groups until the 11th day of cold storage ($p<0.05$). However, on the 13th day, the last day of storage, pH levels were similar in the control group, 1M and 3M groups ($p>0.05$), while the lowest muscle pH was found in trout fed with 1M SB supplemented feeds. Bodas et al. (2007) found that, although the initial pH of sheep fed with sodium bicarbonate supplemented feeds was low, it decreased more slowly in the first 45 minutes after death compared to the control group. However, they determined that the pH decrease rates in muscle after 24 hours were similar in all groups. Ogunwole et al. (2014) stated that the addition of sodium bicarbonate to drinking water had no effect on the muscle pH of broiler chickens. The pH increase observed during storage in this study is an expected result due to the release of alkaline compounds due to endogenous and/or microbial enzymes. Similarly, many researchers reported that the pH value in the muscle increased as the storage process progressed (Abbas et al. 2008; Cao et al. 2020; Lan et al. 2023). Although it is generally stated that the limit value for pH consumption in fish is 6.80-7.00 (Stansby, 1982; Oehlensclager 1992), this value can be affected by factors such as processing and storage conditions and species.

The initial changes that may occur in fish muscle at the post-mortem stage in fish are due to endogenous enzymes that provide proteolysis of muscle proteins and connective tissue. Therefore, it is stated that the chemical changes occurring at the beginning of the post-mortem stage are caused by autolysis rather than microorganism activities (Delbarre-Ladrat et al. 2006). Although there are different views on how the process occurs, many studies suggest that cathepsin, proteasome and

especially calpain enzyme systems play a role in postmortem proteolysis and meat softening (Kaur et al. 2021). Proteolytic degradation leading to softening of myofibrils occurs in a case-specific manner, and the activity of enzymes such as calpains is influenced by factors such as pH, temperature and different processing methods (Kaur et al., 2021; Ma and Kim. 2020). Wilhelm et al (2010) reported that low pH values in muscles are consistent with Ca2+ release and increased Ca2+ release leads to increased calpain activity. Generally, the breakdown of proteins and other nitrogenous compounds in fish muscle is measured by an increase in total volatile basic nitrogen (TVB-N). In this study, as shown in Table 2, the TVB-N value determined as 20.14 mg/100g at the beginning of storage in the control group was significantly higher (14.88-15.68 mg/100g) than the rainbow trout fed with 1M, 2M and 3M SB supplemented feeds (p<0.05). The lowest TVB-N levels were observed in rainbow trout fed 2M and 3M SB supplemented diets until the 3rd day of storage. On the other hand, the lowest TVB-N content during the whole storage period was found in the group fed with feeds containing the highest level of SB (3M group) (p<0.05). The experimental groups (1M, 2M, and 3M) exhibited high muscle pH levels at the start of storage when autolysis was more efficient and microbial activity had not yet increased. The low pH value in the control group might have led to increased enzymatic activity, which would explain why the TVBN value in the control group was higher than in the other groups. Therefore, it can be said that the addition of SB to the feed may cause better initial meat quality. Consistent with the results of this study, there are many studies indicating that the low TVBN value at the beginning of storage increases during storage due to bacterial and internal enzyme activities and reduction of protein and non-protein nitrogenous components (Shokri et al. 2020; Xu et al. 2022; Javadifard et al. 2023). Lang (1983) suggested that the general quality classification of fish and fish products according to TVBN values should be as follows: up to 25 mg/100 g 'high quality'; up to 30 mg/100 g 'good quality'; up to 35 mg/100 g 'limit of acceptability'; and above 35 mg/100 g 'spoiled'. This classification does not always correspond with other quality parameters, but in this study, it was observed to be consistent with the sensory results.

Seafood products are extremely sensitive to oxidation due to their polyunsaturated fatty acid profile. Secondary oxidation products released from lipid oxidation both cause bitter taste in meat and act as precursors for protein oxidation (Hematyar et al. 2019). In addition, it was stated that lipid oxidation products may contribute significantly to the increased risk of cardiovascular and neurological diseases as well as non-infectious chronic diseases such as cancer (Grootveld et al. 2020). TBARs (thiobarbutyric acid reactive substances) analysis is one of the most common methods used to determine the formation of secondary lipid oxidation products in seafood. In this study, TBARs values of rainbow trout fed with different ratios of SB supplemented diets during storage period are shown in Table 3. At the beginning of storage, TBARs values of 2M, 3M groups and control group were similar, while the lowest value was found in 1M group. However, the increase in TBARs in the groups fed with SB-supplemented feeds increased more slowly than in the control group and in general, TBARs values of all groups were lower than the control group during storage. While TBAR values increased with storage time for all samples, they remained below the acceptability limit of 2 mg MA kg-1, as noted by Connell et al. (1990). Prior to this investigation, studies investigating the effect of adding sodium bicarbonate on meat quality generally focused on parameters like meat firmness and water holding capacity rather than lipid quality (Jallow and Hsia 2014; Bodas et al. 2007; Ogunwole et al. 2014; Bodas et al. 2009). Therefore, a comparison could not be made for the results of this study.

Sensory Changes in Rainbow Trout Fed with SB Supplemented Feed during Cold Storage Period

The sensory scores (appearance, color, odor, texture and overall acceptability) of rainbow trout during cold storage are shown in Fig 1. In this study, it was determined that the appearance, color, odor, texture and general acceptability scores in all groups reached the unconsumable limit (4 and below) on the 13th day from the initial good quality values (8-9). Therefore, there was no effect on shelf-life extension of rainbow trout fed with SB supplemented feeds. However, the initial scores of the control group were lower than those of the trout fed diets supplemented with SB in all parameters. Furthermore, it was noted that during the entire storage period, the group fed with feeds supplemented with 3M SB scored higher in texture evaluations than the control group. However, the same trend was not observed for color assessment. In general, the 3M SB supplemented group scored higher in appearance and odor evaluations. Therefore, it can be said that the addition of SB improves the sensory properties of rainbow trout in general, although it has no effect on shelf life extension. Bodas et al (2007) found that although Hue values and yellowness values of sheep fed with SB supplemented feeds were different, whiteness and redness colors were similar to the control. On the other hand, Jallow and Hsia (2014) reported that meat color and texture firmness of sheep fed with SB

supplemented feeds were higher than the control group. In this study, similarly, the addition of SB improved texture values but had no significant effect on color values.

Conclusion

It was observed that the muscle pH of rainbow trout fed different levels of SB for 21 days before harvesting was at a higher pH level. However, TVBN and TBARs values, which are indicators of spoilage, were lower and sensory scores were higher in rainbow trout fed with sodium bicarbonate supplemented feeds during cold storage. However, no difference was observed in terms of shelf life. Considering that the addition of SB to pre-harvest feed enriches meat quality, it can be recommended as a potential feed additive in fish feeding.

Storage Days	Control	1M	2M	3M
0	6.70±0.02^{b1}	6.43±0.13 ^{a1}	6.78±0.02 ^{c1}	6.81±0.04^{c1}
3	6.79±0.12^{a12}	6.78±0.06 ^{a2}	6.83±0.02^{b1}	6.85±0.34^{b1}
6	6.87±0.02^{a2}	6.93±0.13^{b2}	7.02±0.12^{c2}	7.09±0.10^{c2}
9	7.12±0.14^{a3}	7.21±0.05 ^{b4}	7.20±0.36^{b3}	7.24±0.12 ^{c23}
11	7.08±0.10^{a3}	7.09±0.06 ^{a3}	7.14±0.02^{b2}	7.16±0.10 ^{b2}
13	7.38±0.02^{b4}	7.36±0.10^{b5}	7.09±0.03^{a2}	7.36±0.09^{b3}

Table 1. Effect of sodium bicarbonate addition to the diet on the pH level of rainbow trout during post-harvest cold storage.

n = 3, ± SD. Letters (a–d) in the same line indicate difference between groups ($p < 0.05$)
Numbers (1-5) in the same column indicate difference according to storage days ($p < 0.05$)

Table 2. Effect of sodium bicarbonate addition to the diet on the TVB-N (mg/100g) level of rainbow trout during post-harvest cold storage.

Storage Days	Control	1M	2M	3M
0	20.14±1.26^{c1}	15.68±1.02^{b1}	14.88±0.42^{a1}	15.26±1.29^{b1}
3	23.47±0.81^{c2}	22.10±0.37^{c2}	19.27±0.38^{b2}	18.34±0.57^{a2}
6	22.56±0.45^{b1}	22.36±0.66^{b2}	26.92±0.44^{c3}	19.75±1.07^{a2}
9	29.30±0.63^{d3}	23.69±0.70^{b2}	25.38±0.40 ^{c3}	21.76±0.78^{a3}
11	34.28±0.47 ^{c4}	32.07±1.37^{c3}	28.64±1.17^{b4}	25.18±0.72^{a4}
13	37.21±0.17^{b5}	35.07±0.37^{b4}	34.94±0.17^{b5}	29.10±0.72^{a5}

n = 3, ± SD. Letters (a–d) in the same line indicate difference between groups ($p < 0.05$)
Numbers (1-5) in the same column indicate difference according to storage days ($p < 0.05$)

Table 3. Effect of sodium bicarbonate addition to the diet on the TBARs (mg MA/kg) level of rainbow trout during post-harvest cold storage.

Storage Days	Control	1M	2M	3M
0	0.44±0.05 ^{b1}	0.40±0.03 ^{a1}	0.46±0.04^{b1}	0.44±0.01^{b1}
3	0.66±0.04^{c12}	0.55±0.00 ^{b2}	0.52±0.01^{b1}	0.48±0.01^{a1}
6	0.84±0.02^{c2}	0.62±0.03^{b2}	0.60±0.02^{b2}	0.54±0.00 ^{a12}
9	0.85±0.04 ^{c2}	0.76±0.01^{b2}	0.62±0.01^{a2}	0.56±0.01^{a12}
11	1.05±0.07 ^{b3}	0.98±0.01 ^{b3}	0.91±0.01 ^{a3}	0.85±0.00 ^{a2}
13	1.65±0.04^{c4}	1.58±0.04^{b4}	1.41±0.01^{b4}	1.05±0.00^{a3}

n = 3, ± SD. Letters (a–d) in the same line indicate difference between groups ($p < 0.05$)
Numbers (1-5) in the same column indicate difference according to storage days ($p < 0.05$)

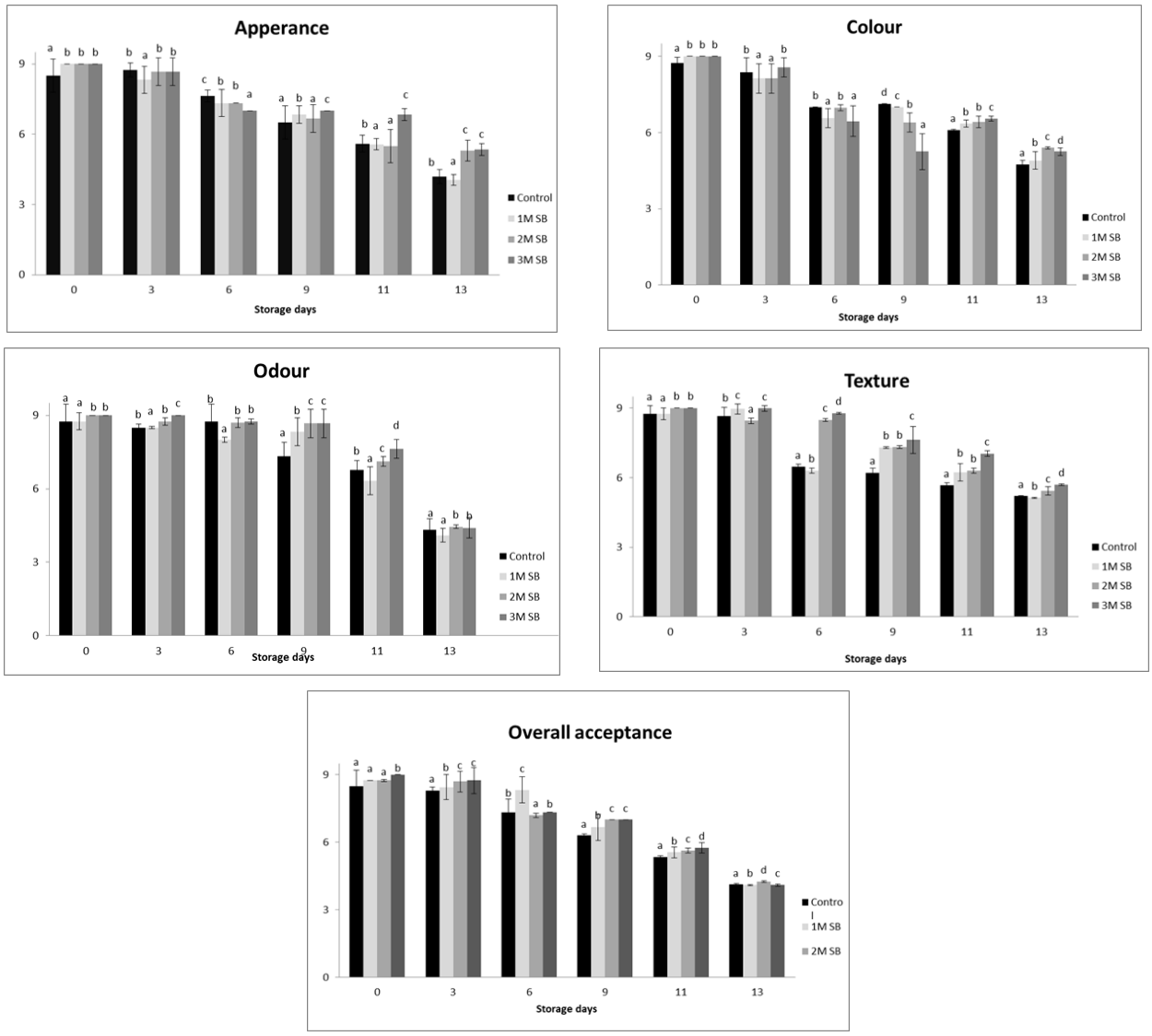

Figure 1. Effect of sodium bicarbonate addition to the diet on the sensory scores of rainbow trout during post-harvest cold storage. Bars with the different letters (a-d) indicate significant differences ($p < 0.05$).

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Sır Baraj Gölü'nün (Kahramanmaraş) Zooplankton Faunası ve Mevsimsel Değişimi

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ÖZ

Bu çalışmada; Kahramanmaraş ili Ceyhan Nehri üzerinde yer alan Sır Baraj Gölü'nün zooplankton tür kompozisyonu ve çeşitliliğinin mevsimsel dağılışı, 2021 yılı boyunca 6 istasyonda araştırılmıştır. Çalışma süresince; Rotifera'dan 39, Cladocera'dan 12, Copepoda'dan 8 ve Amphipoda'dan 1 tür olmak üzere toplam 60 takson tespit edilmiştir. Bunlar arasında rotifer *Lecane sympoda*, Türkiye zooplankton faunası için yeni kayıttır. Sır Baraj Gölü'nde zooplanktonun en yüksek tür sayısı sonbahar mevsiminde (39 tür), en düşük sayı ise 24 tür ile kış aylarında tespit edilmiştir. İlk üç örnekleme istasyonunun tür zenginliği, 4., 5. ve 6. istasyonların zenginliğinden daha yüksek bulunmuştur.

Anahtar kelimeler: Zooplankton, Sır Baraj Gölü, fauna, tür kompozisyonu, Kahramanmaraş

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Zooplankton Fauna of Sır Dam Lake (Kahramanmaraş)

Abstract: In this study, the seasonal distribution and composition of zooplankton species of the Sır Dam Lake, located on the Ceyhan River in Kahramanmaraş province, were investigated at 6 stations during 2021. A total of 60 taxa were identified: 39 rotifers, 12 cladocerans, 8 copepods and 1 amphipod. Among the rotifers, *Lecane sympoda* is new record for zooplankton fauna of Türkiye. The highest number of zooplankton species in Sır Dam Lake was detected in autumn (39 species), and the lowest number was detected in winter with 24 species. The species richness of stations 1, 2 and 3 was found to be higher than stations 4, 5 and 6.

Keywords: Zooplankton, Sır Dam Lake, fauna, species composition, Kahramanmaraş

Alıntılama
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Giriş

Zoocoğrafik açıdan birçok türün yayılışında kıtalar arası bir köprü niteliği taşıyan Türkiye, sadece akarsuları ile değil aynı zamanda doğal ve yapay göl ve gölcükleri ile de yüksek sayıda sucul yaşama ev sahipliği yapmaktadır. Son yıllarda iklim değişiklerinin doğal bir sonucu olarak ortaya çıkan kuraklık veya sel taşkınları, tatlı su kaynaklarını ve içeriğindeki biyoçeşitliliği tehdit eder hale gelmiştir (Sorf vd. 2015). Antropojenik kaynaklı kirlilik ve egzotik türlerde görülen artışlar ise tehdidin diğer bir boyutunu oluşturmaktadır (Lespinas vd. 2014). Bu yüzden ekosistemlerin mevcut durumlarının izlenmesi ve koruma tedbirlerinin alınabilmesi için limnolojik çalışmaların yapılması artık bir zorunluluk halini almıştır (Crul 1995).

Tatlı su ekosistemlerinin temel biyolojik bileşenlerinden biri olan planktonik hayvanlar, birincil ve küçük ikincil üretimi tüketerek ve daha yüksek trofik tüketicilere yiyecek sağlayarak besin ağlarında kritik bir aracı konuma sahiplerdir. Besin zincirinin bir halkasından diğerine madde ve enerji akışını sağlayan zooplankton grupları, içinde yaşadıkları ekosistemin işleyiş mekanizmasında ve dinamizminde önemli bir rol oynarlar (Capriulo vd. 2002; Lampert ve Sommer 1997; Turner 2004; Çelik ve Saler 2016). Ancak bu gruplar, kirletici maddelere ve sıcaklığa karşı oldukça duyarlıdırlar ve bu da

onların topluluk yapısını ve çeşitliliğini büyük ölçüde etkiler (Gannon ve Stemberger 1978; Zhao vd. 2018). Bir ekosistemin zooplankton çeşitliliği ve miktarı, o sistemin su kalitesini ve gölün trofik durumunu da yansıtacağı için limnolojik çalışmalarda faydalı bir gösterge olarak kabul edilir (Bonecker vd. 2013; Dodson vd. 2005).

Sır Baraj Gölü (Kahramanmaraş), Akdeniz Bölgesi'nin en büyük akarsularından biri olan Ceyhan Nehri üzerinde bulunan 6 rezervuardan biridir. Küçükyılmaz vd. (2023), 380 ton kapasiteli 4 adet su ürünleri yetiştiricilik tesisi bulunan Sır Baraj Gölü'nde özellikle Aksu Deresi ile göle karışan fabrika ve şehir kanalizasyon atıklarından kaynaklı kirlilik sorunu yaşandığını bildirmişlerdir. Baraj gölünde şimdiye kadar detaylı bir yıllık zooplanktonik araştırma yapılmamıştır. Dorak vd. (2019), 2015 yılının sadece yaz aylarında yaptıkları örneklemelerde Copepoda'dan 4, Cladocera'dan 4 ve Rotifera'dan 13 olmak üzere toplam 21 tür tanımlamışlardır.

Bu çalışmada, 2021 yılı boyunca Sır Baraj Gölü'nün zooplankton tür kompozisyonu ile türlerin yer ve zamana bağlı dağılışlarının belirlenmesi amaçlanmıştır.

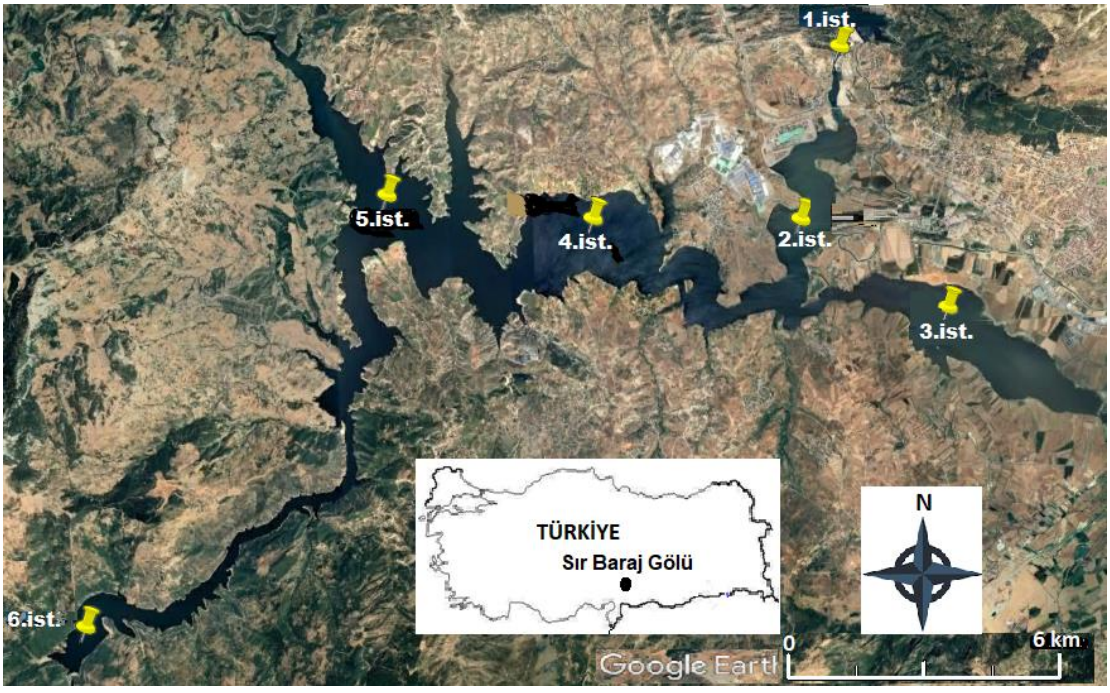
Materyal ve Metot

Araştırma alanının özellikleri

Sır Baraj Gölü (Kahramanmaraş), Akdeniz Bölgesi'nin en büyük akarsularından biri olan Ceyhan Nehri üzerinde bulunur (Şekil 1). 1987-1991 yılları arasında hidroelektrik enerji elde etmek için inşa edilen baraj, 37°35'40.37"K ve 36°45'29.06"D koordinatlarında yer alır. Baraj gölünün rakımı 1281 m, hacmi 1.120 hm^3 ve maksimum derinliği 30 m civarında olup mezotrofik karakterde olduğu bildirilmiştir (Dorak vd. 2019). Normal su kotunda gölalanı 48 km^2'dir (Küçükyılmaz vd. 2023).

Örneklerin toplanması ve tespiti

Zooplakton örnekleri, 2021 yılının Ocak ve Aralık ayları arasında baraj gölünde belirlenen 6 istasyondan mevsimsel periyotlarla toplanmıştır. Materyal, göz açıklığı 55 µm olan Hensen plankton kepçesi ile toplanmış ve % 4'lük formaldehit içinde tespit edilmiştir. Tür teşhislerinde, örnekler Olympus BX51 marka mikroskop altında incelenmiş olup türlerin total ve taksonomik önem taşıyan bölümlerinin fotoğrafları, Olympus BX51-DP71 kameralı mikroskopta çekilmiş ve tüm görüntüler kayıt altına alınmıştır. Türlerin teşhisinde farklı araştırmacıların kaynaklarından faydalanılmıştır (De Smet 1996; De Smet ve Pourriot 1997; Einsle 1996; Flössner 1972; Harding ve Smith 1974; Kiefer 1978; Voigt ve Koste 1978; Negrea 1983; Nogrady vd. 1995; Reddy 1994; Rylov 1963; Scourfield ve Harding 1966; Segers 1995). Bu çalışmada tür zenginliği, zooplankton türlerinin toplam sayısını ifade etmektedir

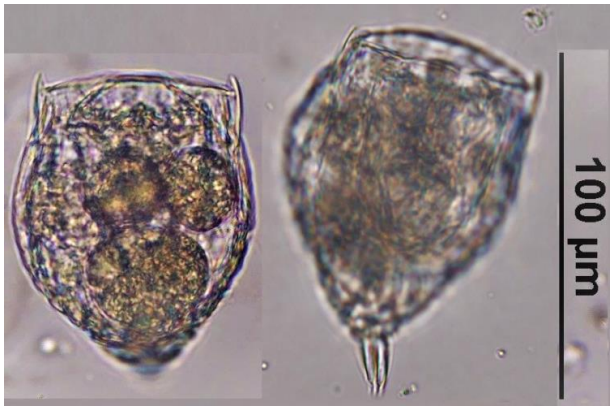


Şekil 1. Sır Baraj Gölü ve örnekleme istasyonları

Figure 1. Sır Dam Lake and sampling stations

Bulgular

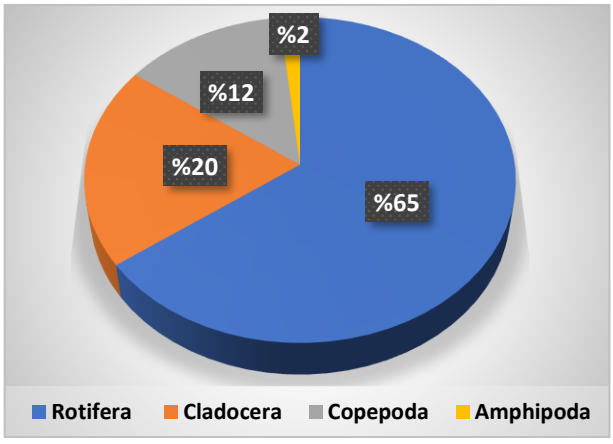
2021 yılı boyunca Sır Baraj Gölü'nün 6 istasyonunda; Rotifera grubundan 39, Cladocera grubundan 12, Copepoda grubundan 8 ve Amphipoda grubundan 1 takson olmak üzere toplam 60 takson tanımlanmıştır. Rotifera'nın Lecanidae familyasının bir üyesi olan *Lecane sympoda* Türkiye zooplankton faunası için yeni kayıttır (Şekil 2). Ayrıca kladoserlerden *Bosmina coregoni*, Türkiye'nin Akdeniz Bölgesi için Hatay bildiriminden (Bozkurt ve Aktaş 2016) sonra ikinci kayıttır.



Şekil 2. *Lecane sympoda*, dorsal ve ventral

Figure 2. *Lecane sympoda*, dorsal and ventral

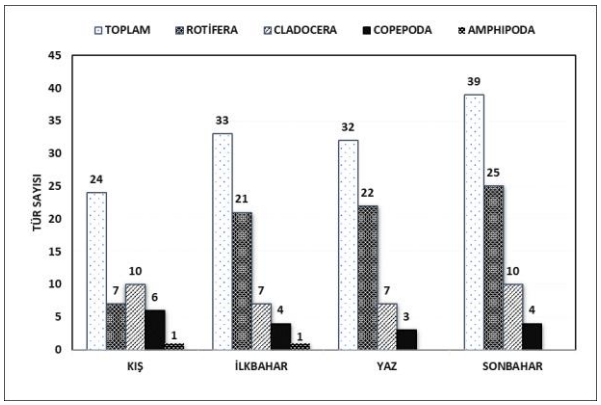
Sır Baraj Gölü'nde tespit edilen zooplankton türleri, üç grubun (Rotifera, Cladocera ve Copepoda) temsilcilerinden ibarettir. Ayrıca bir amfipod takson, iki mevsimde de kepçe materyalinde bulunduğu için faunanın bir üyesi olarak eklenmiştir. Zooplankton tür zenginliği (tür sayısı) açısından Rotifera grubu en baskın grubu (%65) oluştururken, onu Cladocera (%20) ve Copepoda (%12) grupları takip etmiştir (Şekil 3).



Şekil 3. Sır Baraj Gölü'nde zooplankton gruplarının yüzde dağılımları

Figure 3. Percent distribution of zooplankton groups in Sır Dam Lake

Sır Baraj Gölü'nde mevsimsel tür zenginliği açısından; en yüksek sayı 39 tür ile sonbahar mevsiminde, en düşük sayı ise 24 takson ile kış mevsiminde tespit edilmiştir. İlkbahar ve yaz aylarında benzer sayıda (33 ve 32) tür tespiti yapılmıştır (Şekil 4). Toplam zooplankton zenginliği genel olarak sonbaharda yüksek olmasına rağmen, zooplankton gruplarının zenginliklerinde mevsimsel farklılıklar görülmüştür. Rotifera, kış mevsimi dışında genel olarak zooplanktonun en baskın grubunu oluşturmuştur. Rotiferlerin en yüksek takson sayıları sonbahar mevsiminde 25 tür olarak kaydedilmiş olmasına karşın kış mevsiminde tüm istasyonlarda en düşük sayılar görülmüştür Cladocera, kış mevsiminde zooplanktonun en baskın grubu olmuştur. Kladoserler, kış ve sonbahar mevsimlerinde her biri 10 tür ile temsil edilirken, ilkbahar ve yaz aylarında çeşitli sebeplere bağlı olarak tür sayılarında düşüşler olduğu görülmüştür. Copepoda grubunda ise kış mevsiminde en yüksek sayı olan 6 tür, yaz mevsiminde ise sadece 3 tür tanımlanmıştır. Sır Baraj Gölü'nde kopepodlar, Cyclopoida, Calanoida ve Harpacticoida olmak üzere 3 grubun üyeleri ile temsil edilmiştir. Sır Baraj Gölü'nün tür kompozisyonu ve türlerin yer ve zamana bağlı dağılışları ise Tablo 1'de gösterilmiştir.



Şekil 4. Zooplankton gruplarının tür zenginliğindeki mevsimsel değişimler

Figure 4. Seasonal changes in species richness of zooplankton groups

Türlerin mevsimsel dağılımına bakıldığında, bazı türler yıl boyunca varlıklarını sürdürmelerine karşın bazıları ise sadece bir mevsimde görülmüştür. *Keratella cochlearis, K. quadrata, Rotaria neptunia, Polyarthra dolichoptera, P. vulgaris, Bosmina longirostris, Ceriodaphnia dubia, Ceriodaphnia pulchella, Chydorus sphaericus, Daphnia longispina* ve *Acanthocyclops robustus* yıl boyunca her mevsim görülmüştür. Buna karşın *Euchlanis incisa, Ilyocryptus sordidus, Eucyclops macrurus, Macrocyclops albidus* sadece kış mevsiminde; *Brachionus urceolaris, Cephalodella catellina,*

Colurella adriatica, Epiphanes senta, Daphnia magna, Thermocyclops dybowskii sadece ilkbahar mevsiminde; *Asplanchna girodi, Conochilus dossuarius, Dicranophorus epicharis* sadece yaz mevsiminde; *Ascomorpha ecaudis, Brachionus budapestinensis, Cephalodella megalocephala, Colurella obtusa, Lecane bulla, L. sympoda, Lepadella rhomboides, Synchaeta pectinata* ve *Simocephalus vetulus* türleri sadece sonbahar mevsiminde kaydedilmiştir.

Tablo 1. Sır Baraj Gölü'nün altı istasyonunda zooplankton tür kompozisyonu ve türlerin mevsimsel dağılışları

Table 1. Seasonal distribution and composition of zooplankton species at six stations of Sır Dam Lake

	KIŞ-2021						İLKBAHAR						YAZ						SONBAHAR					
Taksonlar / İstasyonlar	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**
Rotifera																								
Ascomorpha ecaudis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+
Asplanchna girodi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-
Asplanchna priodonta	-	-	-	-	-	-	+	+	+	+	-	-	+	-	-	-	-	-	+	-	-	+	+	+
Brachionus budapestinensis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Brachionus calyciflorus	-	-	-	-	-	-	-	+	+	+	-	-	-	-	+	+	-	+	-	-	-	-	-	-
Brachionus urceolaris	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cephalodella catellina	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cephalodella intuta	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-
Cephalodella megalocephala	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Colurella adriatica	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Colurella obtusa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Conochilus dossuarius	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Dicranophorus epicharis	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Epiphanes senta	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Euchlanis dilatata	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Euchlanis incisa	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Keratella cochlearis	-	+	-	-	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	-	+	+	+
Keratella quadrata	-	-	-	-	+	+	+	-	+	+	-	-	-	-	+	-	+	+	+	+	-	+	+	-
Keratella tecta	-	-	-	-	+	-	-	-	-	+	+	-	-	+	+	+	+	+	-	-	-	-	-	-
Keratella tropica	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	+	+	+	-	-	-	+	+	-
Lecane bulla	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Lecane closterocerca	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-
Lecane sympoda	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Lecane lunaris	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Lepadella patella	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-
Lepadella rhomboides	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Lindia torulosa	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Notholca acuminata	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Pleurotrocha petromyzon	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-
Polyarthra dolichoptera	+	+	-	-	-	-	-	+	+	+	-	-	+	+	+	+	+	+	+	+	-	+	+	+
Polyarthra vulgaris	-	+	-	-	-	-	-	+	+	+	-	-	+	+	+	+	+	+	+	+	-	+	+	+
Pompholyx complanata	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	+	+	+
Pompholyx sulcata	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+	+	+	-	-	-	-	-	-
Rotaria neptunia	-	+	+	-	+	+	+	+	+	-	-	-	+	-	-	-	-	-	+	+	-	+	-	-
Synchaeta oblonga	-	-	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-
Synchaeta pectinata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Synchaeta stylata	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-
Trichocerca weberi	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-
Trichotria pocillum	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	+	-	-	-	-	-
Cladocera																								
Bosmina longirostris	+	-	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-	+	+	+
Bosmina coregoni	-	-	-	-	+	+	-	-	-	-	-	-	-	+	+	-	-	-	+	-	-	+	+	-
Ceriodaphnia dubia	-	-	-	-	+	+	-	-	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+
Ceriodaphnia pulchella	-	+	+	-	+	+	-	-	-	+	+	-	+	-	-	+	-	+	+	+	+	-	+	-
Chydorus sphaericus	-	-	+	+	+	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-
Daphnia longispina	-	-	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
Daphnia magna	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Tablo 1 devamı																								
Daphnia pulicaria	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+	-	+	-	-	-	+	+	+
Diaphanosoma birgei	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
Eurycercus lamellatus	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-
Ilyocryptus sordidus	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Simocephalus vetulus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-
Copepoda-Cyclopoida																								
Acanthocyclops robustus	-	-	+	-	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-	+	+	+
Cyclops vicinus	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Eucyclops macrurus	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Macrocyclops albidus	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mesocyclops leuckarti	-	-	-	-	+	+	-	-	-	-	-	-	-	+	+	+	-	+	-	-	-	+	+	+
Thermocyclops dybowskii	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Copepoda-Calanoida																								
Acanthodiaptomus denticornis	-	+	-	-	+	+		-	-	-	-	-	+	-	-	-	-	-	+	+	+	+	+	+
Copepoda- Harpacticoida																								
Nitokra hibernica	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Amphipoda																								
Gammarus sp.	-	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Türlerin istasyonlara göre dağılımı karşılaştırıldığında, 2. İstasyonun en zengin tür çeşitliliğine (37 tür) ev sahipliği yaptığı görüldü. Onu, 3. (34 tür) ve 1. (32 tür) istasyonlar takip etmiştir. Yıl boyunca 4., 5. ve 6. istasyonlarda ise tür sayıları 25, 24 ve 23 olarak tespit edilmiştir.

Tartışma ve Sonuç

Bu çalışma, 2023 yılında gerçekleşen Kahramanmaraş depremleri öncesinde alınmış materyalden yapılmıştır. Bu yüzden deprem öncesi Sır Baraj Gölü'nün kısmi biyoçeşitliliğini ortaya koyması açısından büyük önem taşımaktadır. Baraj gölünün 2021 yılı zooplankton zenginliği, 6 istasyonda incelenmiş ve Rotifera grubundan 39, Cladocera grubundan 12, Copepoda grubundan 8 ve Amphipoda grubundan 1 takson olmak üzere toplam 60 takson tanımlanmıştır. Rotifera'dan *L. sympoda* Türkiye zooplankton faunası için yeni kayıttır. Segers (2007), bu türün global dağılış alanının Palearktik, Oriental ve Afrotropikal bölgeler olduğunu açıklamıştır. Bu çalışma ile *L. sympoda*'nın Palearktik bölgedeki dağılış alanına Kahramanmaraş ilindeki bu ekosistem de dahil olmuştur. Bu tür, Türkiye'ye en yakın İran'ın batı bölgesinden kaydedilmiştir (Löffler 1961). Ustaoğlu (2015), güncel Türkiye içsu zooplankton kontrol listesinin 417 rotifer, 103 kladoser ve 141 kopepod olmak üzere toplam 661 taksondan oluşmuş olduğunu bildirmiştir. Bu çalışma ile toplam sayı 662 taksona çıkmıştır.

Rotifera grubu, kış mevsimi dışında zooplanktonun en baskın (%65) grubu olmuştur. Rotiferler gerek yüksek üreme potansiyeline sahip olmaları ve gerekse ekstrem koşullara bile toleransları sayesinde mevsimsel süksesyonunu devam ettirebilmektedirler (Gannon ve Stemberger 1978).

Dorak vd. (2019), 2015 yılı yaz aylarında Sır Baraj Gölü'nün zooplankton faunasını incelemişler ve Copepoda'dan 4, Cladocera'dan 4 ve Rotifera'dan 14 olmak üzere toplam 22 tür tespit etmişlerdir. Ayrıca hesapladıkları TSI (Trofik durum indeksi) değerine göre; gölü, mezotrofik olarak tanımlamışlardır. Bu çalışmada rapor ettikleri türlerden *Acanthocyclops viridis, Ceriodaphnia quadrangula, Diaphanosoma brachyurum, Ascomorpha saltans, Conochilus unicornis, Keratella valga, Trichocerca capucina* ve *Trichocerca similis* türleri bizim çalışmamızda görülememiştir. Üstelik bizim çalışmamızda yaz mevsiminde 32 tür tanımlanmıştır.

Sır Baraj Gölü'nü kuzeyden besleyen Kılavuzlu Baraj Gölü'nde yapılan bir çalışmada da 33'ü Rotifera'ya, 14'ü Cladocera'ya ve 10'u Copepoda'ya ait olmak üzere toplam 57 zooplankton türü tespit edilmiştir (Bozkurt 2016). Bu türlerin bir bölümü bizim çalışmamızda da tespit edilmiştir.

Yapılan bazı araştırmalar Sır Baraj Gölü'nün son yıllarda hem evsel hem de endüstriyel atıklar nedeniyle kirlilik baskısı altında olduğunu ortaya koymaktadır. Canpolat ve Uzun (2019), Kahramanmaraş Organize Sanayi Bölgesi atık sularının deşarj olduğu Aksu Çayı ve çayın Sır Barajı'na karıştığı bölgeden aldıkları su örneklerinde demir (Fe), çinko (Zn), bakır (Cu), krom (Cr), nikel (Ni) ve kadmiyum (Cd) gibi ağır metallerin en yüksek konsantrasyon değerlerinin sonbahar

mevsimin de ölçüldüğünü ve buna göre de bölgede ciddi bir ağır metal kirliliğinin yaşandığını bildirmişlerdir.

Küçükyılmaz vd. (2023), trofik durum indeksi TSI (TP, TN, SD, CHL) verilerini kullanarak yaptıkları değerlendirmede Sır Baraj Gölü'nün yüzey suyunda ötrofik, orta derinlikte ve dip suyunda hipertrofik ve genel olarak bakıldığında ötrofik karakterde bir göl olduğunu belirlemişlerdir. Ayrıca bu çalışmada, Aksu Deresi'nin şehir kanalizasyonu ve çeşitli fabrika atıklarını taşıması nedeniyle Sır Baraj Gölü'ne boşaldığı bölgenin en sorunlu bölge olduğu rapor edilmiştir. Araştırmacılar, bizim çalışmamızın da 3. istasyonu olan bu bölgenin suyunun, amonyum azotu ve toplam azot yönünden II. sınıf, toplam fosfor ve çözünmüş oksijen yönünden III. sınıf su kalitesine sahip olduğunu tespit etmişlerdir.

Zooplankton, su ortamındaki değişikliklere karşı oldukça duyarlı olup içinde yaşadıkları suların kalitesinin ve trofik durumunun belirlenmesinde kullanılabilecek iyi biyolojik göstergeler olduğu öne sürülmüştür (Gannon ve Stemberger 1978). *Asplachna girodi, Brachionus calyciflorus, B. angularis, Keratella cochlearis, K. quadrata, K. tecta, Euchlanis dilatata, Lecane luna, Pompholyx sulcata, Daphnia cucullata* gibi daha pek çok türün ötrofik suların indikatörleri oldukları bildirilmektedir (Rylov 1963; Munoz-Colmenares vd. 2021). *Acanthocyclops* spp. ve *Cyclops* cinsleri dünya çapında mezo-ötrofik sularda bulunabilir (Ma vd. 2019; Haberman ve Haldna 2014). Bu çalışmada örnekleme yaptığımız 3. istasyon yıl boyunca en düşük çözünmüş oksijen değerlerine (Küçükyılmaz vd. 2023) sahip olmasına karşın 34 zooplankton türü içermektedir. Baraj gölünde yıl boyunca kaydedilen türlerin de genel olarak ötrofik ya da mezotrofik suların indikatörleri olduğu görülmektedir.

Son yıllarda küresel iklim değişimlerinden kaynaklanan seller ve kuraklık, tatlısu ekosistemleri üzerinde artan bir strese neden olmaktadır. Kirletici maddelere ve sıcaklığa karşı oldukça hassas olan zoplanktonik organizmalar, ekolojik etkileşimleri ve besin döngüsündeki önemleri nedeniyle ekosistem sağlığının korunmasında ve işleyişinde hayati rol oynarlar. Bu yüzden Akdeniz Bölgesi'nin önemli ekosistemlerinden biri olan Sır Baraj Gölü, gerek yukarıda bahsedilen kirletici faktörler, gerek iklim değişimi ve gerekse depremin etkileri sonrası yaşanan gelişmeler nedeniyle ekolojik açıdan düzenli aralıklarla saha çalışmaları yapılarak izlenmeyi hak etmektedir.

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Zooplankton of The Mingachevir Reservoir (Azerbaijan)

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ABSTRACT

The article provides information on the biodiversity of multicellular zooplankton communities (metazooplankton) distribution by biotopes, development dynamics, abundance and biomass by seasons in the Mingachevir Reservoir in the current period (2021-2023). As a result of the study 36 species of zooplankton were found belonging to 3 taxonomic groups (13 species of rotifers, 12 species of cladocerans, 11 species of copepods). Cosmopolitan and phytophiles-littoral species are more common in the reservoir. The total abundance of zooplankton varied between 4136-46410 ind./m^3, biomass 986.46-10013.98 mg/m^3.

Keywords: species composition, zooplankton, abundance, biomass.

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Introduction

Zooplankton is a very important and functional component of the ecosystem in every water basins. Whether migration, feeding traits, or as a consumer of primary fertility, zooplankton provides the direction and intensity of matter and energy flow in stagnant water basins, self-cleaning process. Its species composition and development dynamics make it possible to carry out a diagnostic assessment of the changes occurring in the water basin ecosystem (Andronikova 1996, Basharova 1995). Reservoirs are ecosystems that exists for a long time, and have their own stable level of planktonocenosis, which is the main indicator of the hydrobiological regime. From this point of view, the study of zooplankton of the Cascade Reservoirs (Varvara, Mingachevir, Yenikiand, Shamkir) of the Kura River, which has a great role in all areas of water management in our republic, caused interest. It is known that in the part of the Kura River flowing through our republic, 4 reservoirs forming a cascade - Shamkir, Yenikend, Mingachevir and Varvara Reservoirs are in operation. Among these reservoirs, according to their size and chronological order, the largest and oldest is Mingachevir (commissioned in 1953), and the smallest and youngest is Yenikend (commissioned in 2000) (Aliev and Bagirova 2009). The aim of this study is compare the species composition, abundance and biomass of zooplankton in the the Cascade Reservoirs of the Kura River in the current period.

Materials and Methods

Study Area

The Mingachevir Reservoir is situated on the territory of the Azerbaijan Republic - 40°55'30.1"N. and 46°47'44.3"E. It is located in the Samukh depression in the middle flowing of the Kura River. It is surrounded by the mountains: Gochashen (or Akar-Bakar) from the north and east, Bozdag from the south and southwest, and Palandoken Mountains from the northwest. The length of the reservoir is 75 km, the maximum width is 20 km, the maximum depth is 75 m (in the part near the dam), the average depth is 27 m, the area is 62000 ha. The length of the reservoir dam is 1550 m, the height is 80 m. A hydroelectric power station (HPS) consisting of 6

hydrounits with a total capacity of 371000 KW was built in this dam. Two large irrigation canals which commissioned in 1958 - the Karabakh (172 km long) and Shirvan (128 km long) canals take their source from the Mingachevir Reservoir (Ahmedzade and Gashimov 2016, Khalilov 2003, Tarverdiyev 1974). The main water sources of the Mingachevir Reservoir are Kura, Ganikh, Gabirri, and partially Ganjachay Rivers. According to its hydrological characteristics, the reservoir is conditionally divided into 4 parts: upper - river part (109 km^2), middle-semi-lake part (158 km^2), lower - lake part (316 km^2) and Khanabad Gulf (42 km^2). The river part covers the valleys of the rivers that flow into the reservoir and respectively are called Kura, Ganikh and Ghabirri Gulfs. The semi-lake part - located between the gulfs of the rivers and the lake part. Its area is 158 km^2, its depth is up to 30 meters, its length is 11 km. Its maximum depth is 56m. The lake part - area is 316 km^2, its length is 35 km, its maximum width is 11 km, and its average width is 10.8 km. This part is the deepest part of the reservoir (especially the right bank 55-75 m), it extends to the dam. Khanabad Gulf - covers the eastern end of the reservoir. Its area is 42 km^2. The northern and eastern shores are sloping, and the southern shore is steep. Here, the depth varies between 5-40 m (Tarverdiyev 1974).

Sampling and analysis

Zooplankton samples in the Mingachevir Reservoir were collected seasonally in 2021-2023. To collect samples from 9-12 stations in the central parts of the reservoir, as a means of transport were used motor boats and from the sublittoral zone 0-5 m, 5-10 m, 10-15 m, 15-20 m, etc. from the depths were used rowboats (Figure 1). For this purpose were used Apstein net (No. 77) to collect quantitative samples and scoop-nets of different sizes to collect qualitative samples from different areas of the reservoir (stone, sand, black sludge).

Quantitative samples were taken through in the vertical direction hauls from depths selected from relatively deep parts of the reservoir (30-50 m), but in the horizontal direction hauls, the Apstein net was inserted into the water at a depth of 0.5-1.0 m and drawn along the direction of movement of the boat at a distance of 5 meters. At each station, air and water temperatures were measured with a thermometer (Tetra TH Digital and Testo 610). The collected samples were fixed in 4% formalin and 70% alcohol solution depending on the density of organisms. Both live and fixed zooplankton samples were analyzed under OLYMPUS CX 41 RF and NICON SMZ 1270 microscopes and their identification was based on taxonomic keys (key-books and international taxonomic data systems). Bogorov chamber and stamped (marked) pipette for quantitative analysis were used (Abakumova 1983, Alekseev and Tsalolokhin 2010, Andronikova 1996, Borutsky et al. 1991, Vinberg and Lavrenteva 1982, Kutikova 1970, Manuylova 1964, Plotnikov et al. 2017, Rylov 1948, Smirnov 1971, Tsalolikhin 1995, Witty 2004). The biomass of species with large morphometric measurements was determined under a microscope using an ocular micrometer and an objective micrometer according to the methods of Ruttner-Kolisko (1977), Balushkina and Vinberg (1979). When it was impossible to determine the mass of small organisms, information about their mass was obtained from scientific literature. At the same time, the sizes of individuals of the species, male, female (with eggs, eggless), juveniles and nauplii stages were taken into account (Ulomsky 1947, 1951, 1952, 1955, 1958, Plotnikov et al. 2017).

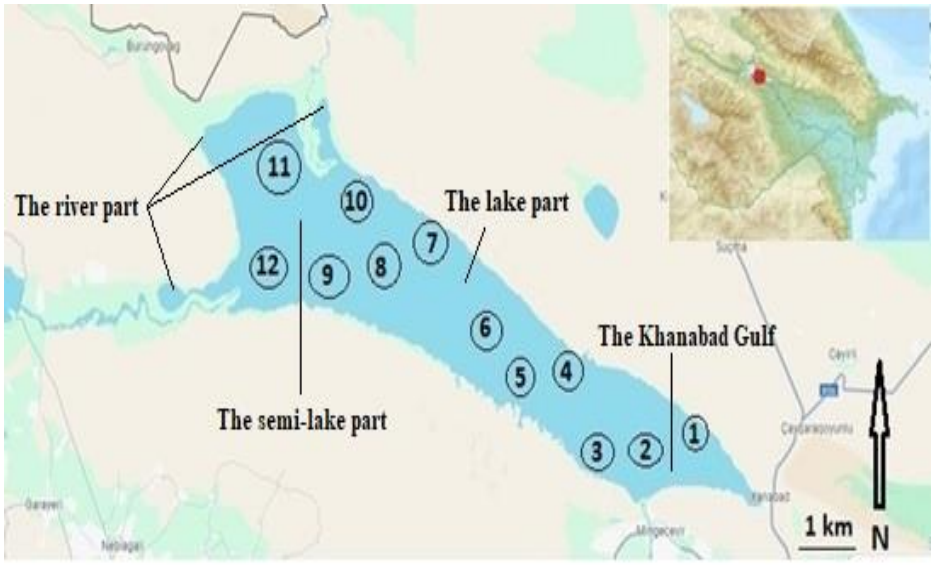

Figure 1. Map-scheme showing the location of biological stations in the Mingachevir Reservoir

In order to determine the frequency of occurrence, the percentage of the number of samples in which individuals of the species were found among all the samples taken from the reservoir was determined and was performed based on the following formula:

$$pF = \frac{n}{N} \cdot 100\%$$

where pF is the frequency of occurrence

n – the number of samples in which the species was recorded

N – number of all samples (Kojova 1970)

The frequency of occurrence of individuals of the species in the sample was considered if pF > 75% - constant, pF<75% >50% - regular, pF=25-50% - irregular and pF < 25% -random.

In order to reveal the similarities and differences in the distribution of zooplankton species found in the plankton of both studied reservoirs, using the similarity formula proposed by Sorensen (1948) the similarity coefficient of zooplankton species in the studied reservoirs was calculated based on the following formula:

$$K = \frac{2C}{A+B} \cdot 100\%$$

where, K- the similarity coefficient,

A and B – the general number of species found and identified in each of the compared reservoirs,

C-the number of common species to both reservoirs being compared.

The result are expressed in % the calculations and are based on the presence of species identified in both reservoirs, the fauna of which are compared. In the calculation, if C=0, then K=0, which means that there is no commonality or similarity between the 2 reservoirs being compared, and if A=B=C, then K=100%, which means the identification of the species in the reservoirs is compared.

Results

During the present study 2021-2023, 36 species were recorded belonging to 3 taxonomic groups of zooplankton in the Mingachevir reservoir. In terms of the number of species, rotifers are richest group with 13 species (36.1%), followed by cladocerans with 12 species (33.3%), copepods with 11 species (30.6%). Brachionidae family with 6 species belonging to 2 genera, Daphnidae family with 6 species belonging to 4 genera, Chydoridae family with 3 species belonging to 2 genera, Cyclopoidae family with 9 species belonging to 6 genera showed species richness.

Polyarthra vulgaris Garlin, 1943, *Asplanchna priodonta* Gosse, 1850, *Daphnia longispina* Müller, 1785, *D. hyalina* (Leyding, 1860), *Chydorus sphaericus* (Müller, 1785), *Bosmina longirostris* (Müller, 1785), *Arctodioptomus acutulobatus* Sars, 1903, *Termocyclops dybowskii* (Lande, 1890) were the most frequent species in all seasons of the year (Table 1).

The species recorded during the study are typical for the inland water basins of Azerbaijan. They are recorded in both mixohaline (saltish) and freshwater basins. Oligo-beta-alpha-mesosaprobe (o-β-α) species, saprobic index varied between 1.0-2.8. According to their origin they belong to the Boreal, Ponto-Caspian and endemic to Azerbaijan genetic groups (Kasymov 1972, Plotnikov et al. 2017).

Zoogeographically the main part of zooplankton species is cosmopolitan (58.3%). Holarctic and polyarctic species respectively account for 16.7% and 14.0% of the total species. According to the habitat character phytophilic-littoral species (41.7%) predominated in the samples collected from the reservoir, eurybionty species made up 30.5% of the total number of species, and true plankton species 27.8%. Littoral species *L. luna, C. reticulata, S. vetulus, M. brachiata, M. albidus, M. gracilis* and benthic species of the genus *Alona* sp. were recorded more in the shallow coastal waters (Table 1).

In winter (January-February) at a temperature range of +3.4-9.5°C were recorded 19 species in the zooplankton complex of the reservoir: *A. priodonta, B. calyciflorus, B. falcatus, K. cochlearis, P. vulgaris, T. tetractis, D. longispina, D. hyalina, M. brachiata, B. longirostris, Ch. sphaericus, A. affinis, A. acutulobatus, M. fuscus, M. albidus, C. strenuus, M. leuckartii, A. gigas, T. dybowskii. Among these species D. hyalina, B. longirostris, Ch. sphaericus, A. acutulobatus, T. dybowskii* were the leading species. These species are found in the zooplanktonocenosis of the reservoir throughout the year. Almost all of the 36 recorded species participate to one degree or another in the formation of the zooplankton complex in the reservoir in spring and summer (Table 1). In the spring season, under temperature conditions of +4.2-10.5°C *B. longirostris, Ch. sphaericus, A. acutulobatus were dominant, B. calyciflorus, K. cochlearis, K. quadrata, A. priodonta, S. pectinata, D. longispina, D. hyalina, M. brachiata, M. albidus, M. leuckarti* were subdominant species. In summer (June-August) in the shallow areas of the water reservoir at temperature range +18.2-25.5°C mass development of *A. acutilobatus* was recorded.

Table 1. Zooplankton species composition, zoogeography, habitat character and seasonal development dynamics in the Mingachevir reservoir in 2021-2023

№	Species	Zoogeography	Habitat	Winter	Spring	Summer	Autumn
	Rotifera						
1	*Synchaeta pectinata* Ehrenberg, 1832	K	Eut	-	+++	++++	++++
2	*Polyarthra vulgaris* Garlin, 1943	H, A	Eut	++	+++	++++	++++
3	*Asplanchna priodonta* Gosse, 1850	K	Eut	++	+++	++++	++++
4	*Lecane luna* (Müller, 1776)	K	L,Ph	-	+	++	++++
5	*Trichotria tetractis* (Ehrenberg, 1830)	K	L	+	++	++++	+++
6	*Lepadella ovalis* (Müller,1786)	K	Ph	-	++	++	+
7	*Brachionus leydigi* Cohn, 1862	K	L	-	+	++	+
8	*B.bennini* Leislsing, 1924	K	Pl	-	+	++	+
9	*B. falcatus* Zacharias, 1898	E, N	Ph	+	+++	+++	-
10	*B.calyciflorus* Pallas, 1766	K	Pl	++	++	++++	++++
11	*Keratella cochlearis* (Gosse, 1851)	K	Eut	++	+++	++++	++++
12	*K.quadrata* (Müller, 1786)	K	Eut	-	++	++++	++
13	*Filinia longiseta* (Ehrenberg, 1834)	K	Eut	-	+	++	-
	Cladocera						
14	*Diaphanosoma brachyurum* (Lievin, 1848)	H P	Pl	-	+	++++	++++
15	*Daphnia pulex* Leydig, 1860	H	Pl	-	+	+	+
16	*D. longispina* (Müller, 1785)	H	Pl	+++	+++	++++	++++
17	*D.hyalina* Leyding, 1860	P	Pl	+++	+++	++++	++++
18	*Simocephalus vetulus* Müller, 1776	K	Ph, L	-	++	++++	++
19	*Moina brachiata* Jurine, 1820	K	Ph, L	+	+	++++	++
20	*Ceriodaphnia reticulata* (Jurine, 1820)	K	Ph, L	-	+	++++	++
21	*Chydorus sphaericus* (Müller, 1785)	K	Eut	+++	++++	++++	++++
22	*Alona affinis* (Leydig, 1860)	K	Ph, L, Bt	+	+	++	++
23	*Coronatella rectangula* Sars, 1862	K	Ph, L,Bt	-	+	++	++
24	*Bosmina longirostris* (Müller, 1785)	K	Eut	+++	++++	++++	++++
25	*Leptodora kindtii* (Focke, 1844)	H	Pl	-	+	++	+
	Copepoda						
26	*Arctodiaptomus acutulobatus* Sars, 1903	P	Pl	++++	++++	++++	++++
27	*Macrocylops fuscus* (Jurine, 1820)	H	Ph	+	+++	++++	++++
28	*M.albidus* (Jurine, 1820)	K	Ph,L	+	+++	++++	++++
29	*Cyclops strenuus* Fisher, 1851	H	Pl	+	++	++	++
30	*C.vicinus* Uljanin, 1875	H, P	Eut	-	++	+++	++
31	*Megacyclops gigas* (Claus, 1857)	P	Pl	++	++	+++	+++
32	*M. viridis* (Jurine, 1820)	K	Eut	-	++	++	++
33	*Metacyclops gracilis* (Lilljeborg, 1858)	P	Ph, L	-	++	+++	+
34	*Mesocyclops leuckarti* (Claus, 1857)	K	Eut	+	++	+++	++
35	*Termocyclops dybowskii* (Lande, 1890)	P	Ph	+	++	+++	++++
36	*Attheyella crassa* (Sars, 1863)	H	L, Ph	-	+	++	-
	Total			19	36	36	36

Note: Species distribution or zoogeographic character (Segers 2007; 2008, Boxshall and Defaye 2008; Forro et al. 2008): A – Australia, P – Palaearctic, K – cosmopolitans, H – Holarctic, E – Ethiopia, N – Neotropics. Habitat character (Kutikova 1970; Manuylova 1964; Dumont and Negrea 2002; Dussart and Defaye 2002; 2006): Pl – planktonic, L – littoral, Ph – phytophilic, Eut – eurytopic. Bt - benthic. Frequency of occurrence of species (Kojova, 1970): pF < 25% (+), pF=25-50% (++), pF<75% >50% (+++), pF > 75% (++++), was not recorded (-).

In June 2021 the amount of *A. acutulobatus* at a depth of 0-5 meters was 8.600 ind./m3, and biomass was 4,644.05 mg./m^3 in Khanabad Gulf. In the autumn (September-November) at a temperature range of +17.8-23.5°C *Ch. sphaericus*, *B. longirostris*, *A. acutulobatus* were the leading species in both shallow and deep parts of the reservoir.

The total amount of zooplankton in the Mingachevir Reservoir ranged between 4136 and 46410 ind./m^3, biomass from 986.46 to 10013.98 mg/m^3. During the study years the highest values were recorded in June 2021 (35250 ind./m^3 and 9517.61 mg/m^3), in July 2022 (46410 ind./m^3.-1013.98 mg/m^3,), in June 2023 (10306 ind./m^3-2302.84 mg/m^3) (Table 2).

Table 2. Reletive density and biomass of zooplankton species in the Mingachevir Reservoir by seasons 2021- 2023

	Date of sampling			**2021**		
		february	march	june	august	september
	Number of species	11	22	26	26	26
Total: - N, ind/m^3		14475	32667	35250	9750	17039
N%	Rotifera	9	19,6	22,3	23,6	29,0
	Cladocera	34.4	34.6	27.1	35.4	36.0
	Copepoda	56.6	45.8	50.6	41.0	35.0
Total: - B, mq/m^3		4605.96	5928.43	9517.61	1791.80	3094.65
B%	Rotifera	0.2	0.6	0.5	0.7	0.7
	Cladocera	20.8	25.1	22.0	30.6	37.8
	Copepoda	79.0	74.3	77.5	68.7	61.5
	Date of sampling			**2022**		
		january	march	june	july	october
	Number of species	17	27	32	33	30
Total: - N, ind/m^3		4136	9419	17005	46410	45610
N%	Rotifera	13.1	23.4	32.3	23.1	22.6
	Cladocera	35.1	28.6	29.2	32.1	36.6
	Copepoda	51.8	48.0	38.5	44.8	40.8
Total: - B, mq/m^3		1150.92	2216.51	3691.71	10013.98	9369.15
B%	Rotifera	0.5	0.4	3.0	0.4	0.5
	Cladocera	17.0	16.1	25.0	20.8	21.1
	Copepoda	82.5	83.5	72.0	78.8	78.4
	Date of sampling			**2023**		
		january	march	april-may	june	october-november
	Number of species	19	36	36	36	36
Total: - N, ind/m^3		7279	9104	5000	10306	6623
N%	Rotifera	24.5	28.7	26.3	22.7	30.7
	Cladocera	23.1	27.7	31.1	28.0	32.0
	Copepoda	52.4	43.6	42.6	49.3	37.3
Total: - B, mq/m^3		1731.55	1872.91	986.46	2302.84	1145.25
B%	Rotifera	0.5	0.4	0.5	0.3	0.6
	Cladocera	14.6	19.0	25.2	17.7	20.4
	Copepoda	85.0	80.6	74.3	82.0	79.0

Analysis of the developmental dynamics of zooplankton by taxonomic groups showed that cladocerans and copepods have the ability to create high abundance and biomass in the zooplanktocenosis of the reservoir. In 2021, the highest abundance and biomass of cladocerans were recorded in March in the lake part of the reservoir (7300 ind./m^3) and in Khanabad Gulf (1103.81mg/m^3), the highest quantitative parameters of copepods were recorded in June (10800 ind./m^3-5021.08 mg/m^3) in Khanabad Gulf. In 2022, the highest amount of cladocerans were recorded in July in the semi-lake part of the reservoir (8620 ind./m3), biomass (1211.99 mg/m^3) in the Khanabad Gulf. In the same month, copepoda species made up 50.4% of the total amount of zooplankton and 79.6% of its biomass in the Khanabad Gulf. In 2023, the highest amount of cladocerans (respectively 24.3%-13.8% of the total amount and biomass) and copepods (53.7% of the total amount, 85.8% of the total biomass) were recorded in June in Sovkhoz kosa area (the southern part of the reservoir).

In all seasons of the research years (2021-2023), A. acutulobatus which dominated both in terms of amount and ability to create biomass, accounted for 10-35% of the total amount of zooplankton and 30-65% of its biomass. In this regard, *D. longispina, D. hyalina, Ch. sphaericus, T. dybowskii* were in second place, making up 2-15% of the total amount and biomass. Although *B. longirostris* is in the second place in terms of the ability to create amount, it accounted for only 0.5-1.0% of the total biomass of zooplankton.

Discussion

The analysis of existing literature sources about the zooplankton of reservoirs of Azerbaijan showed that the basis of zooplankton are ciliates, rotifers, cladocerans and copepods in reservoirs. Although ciliates and rotifers predominate in biodiversity and amount, cladocerans and copepods have always been at the forefront in terms biomass production (Aliev and Bagirova 2009, Kasymov 1972).

In the 50s and 80s of the previous century, much scientific research was carried out by Likhodeyeva (1963), Kasymov (1965), Ahmedov (1971), Alekperov (1987) and others in the Cascade Reservoirs of the Kura River. Only the hydrofauna of Yenikend Reservoir was studied in 2000-2005 (Aliev and Bagirova 2009, Kasymov 1972, Alekperov and Tapdiqova 2022).

Metazooplankton in the Mingachevir Reservoir was first studied by Likhodeyeva (1963) who found 21 zooplankton species. Then Ahmedov (1971) recorded 38 zooplankton species. Both researchers showed that species *A. priodonta, P. vulgaris, D. brachyurum, D. longispina, D. hyalina, B. longirostris, L. kindtii, S. sarsi, A. acutilobatus, M. fuscus, T. dybowskii* are the most common and permanent species in Mingachevir Reservoir (Ahmedov 1971, Likhodeyeva 1963). Protozooplankton (fauna of cilates) of Mingachevir Reservoirs was studied in the 1970s by Alekperov (1987) and who found 50 species of free-living ciliates in the Mingachevir Reservoir (Alekperov 1987, Alekperov and Tapdiqova 2022). The latest studies in the Cascade Reservoirs of the Kura River were carried out in 2001-2005 after the Yenikend Reservoir was put into operation, and the results of the research showed that the species composition of metazooplankton consisted of 26-31 species (Ahmedov 2003, 2006). As a result of the analysis of available literature materials, the study of the state of zooplankton in the Mingachevir Reservoir under modern conditions caused interest.

As a result of the comparative analysis of zooplankton data obtained in the Mingachevir Reservoir in 2021-2023 with the results of research in the 60s of the previous century, it was found that 16 species *(F. longiseta, B. calyciflorus, K. cochlearis, K. quadrata, A.priodonta, S. pectinata, D. brachyrum, D. pulex, D. hyalina, C. reticulata, M. brachiata, B. longirostris, Ch. sphaericus, L. kindtii, M. fuscus, T. dybowskii)* out of 21 species recorded by Likhodeyeva (1963), 25 species *(S. pectinata, A. priodonta, T. tetractis, B. leydigi, B. calyciflorus, K. cochlearis, K. quadrata, L. ovalis, P. vulgaris, F. longiseta, D. longispina, S. vetilus, C. reticulata, M. brachiata, B. longirostris, Ch. sphaericus, A. rectangula, L .kindtii, A. acutulobatus, M. fuscus, M. albidus, C. visinus, A. gigas, M. grasilis, T. dybowskii)* out of 38 species recorded by Ahmedov (1971) have preserved their existence for 55-60 years. The species registered in 2021-2023 – *L. luna, B. bennini, B. falcatus, A. affinis, C. strennus, M. viridis, M. leucarti, A. crassa* are new species for reservoirs. Of the species presented by both researchers as permanent species, 1 species - *T. dybowskii* - retains its dominance for many years, *B. longirostris, Ch. sphaericus, A. acutulobatus* dominated in the zooplankton complex of the reservoir in all seasons of 2021-2023.

Starting from the Central Aran zone of Azerbaijan and moving towards the Western zone, Varvara is the first, Shamkir is the last reservoirs of the Cascade Reservoirs of the Kura River. For the purpose of comparative study of the zooplankton of the Cascade Reservoirs, zooplankton of the Varvara Reservoir was studied in 2019-2021 and 41 metazooplankton species were recorded. 19 of them belong to rotifera (*S. pectinata, P. vulgaris, A. priodonta, L. luna, L. quadridentata, L. lunaris, T. tetractis, B. quadridentatus, L. ovalis, B. bennini, B. falcatus, B. diversicornis, B. calyciflorus, B. angularis, P. patulus, K. cochlearis, K. quadrata F. longiseta, H. mira*), 12 of them belong to cladocera (*D. longispina, D. hyalina, S. vetulus M. brachiata, C. reticulata, S. mucronata, M. hirsuticornis, G. testudinaria, Ch. sphaericus P. aduncus, A. affinis, B. longirostris*), 10 of them belong to copepoda (*A. acutulobatus M. fuscus, M. albidus, E. serrulatus, E. macruroides, P. fimbriatus, C. vicinus, A. gigas, M. gracilis, T. dybowskii*) (Alekperov and Tapdiqova 2021). When comparing the similarity coefficient according to Sorensen (1948) in Mingachevir and Varvara Reservoirs, it was found that: 1 species of rotifers *(Brachionus leydigi),* 4 species of cladocerans *(Diaphanosoma brachyrum, Daphnia pulex, Alona rectangula, Leptodora kindtii),* 4 species of copepods *(Cyclops strenuus, Megacyclops viridis, Mesocyclops leuckarti, Atheyella crassa)* were recorded in the Mingachevir Reservoir, but these species were not found in the Varvara Reservoir, 7 species of rotifers *(Lecane quadridentata, L.lunaris, Brachionus quadridentatus, B.diversicornis, B.angularis, Platyas patulus, Hexarthra mira),* 4 species of cladocerans *(Scapholeberis mucronata, Macrothrix hirsuticornis, Graptoleberis testudinaria, Pleuroxus aduncus),* 3 species of copepods *(Eucyclops serrulatus, E.macruroides Paracyclops fimbriatus)* were recorded in the Varvara Reservoir, these species were not found in the Mingachevir Reservoir.

The similarity coefficient of Rotifera between the Mingachevir Reservoir (13 species) and the Varvara Reservoir (19 species) are 12 species *(S. pectinata, P. vulgaris, A. priodonta, L. luna, T. tetractis, L.*

ovalis, B. benini, B. falcatus, B. calyciflorus, K. cochlearis, K. quadrta, F. longiseta) or K=75,0%.

The similarity coefficient of Cladocera between the Mingachevir Reservoir (12 species) and the Varvara Reservoir (12 species) are 8 species *(D. longispina, D. hyalina, S. vetulus, M. brachiata, C. reticulata, Ch. sphaericus, A. affinis, B. longirostris)* or K=66.7%.

The similarity coefficient of Copepoda between the Mingachevir Reservoir (11 species) and the Varvara Reservoir (10 species) are 7 *species (A. acutulobatus, M. fuscus, M. albidus, C. vicinus, A. gigas, M. gracilis, T. dybowskii)* or K=66.7%.

Between the Mingachevir and Varvara Reservoirs, a high similarity coefficient (75,0%) belongs to Rotifers, similarity coefficient is the same between Cladocerans and Copepods (66.7%).

Conclusion

As a result of research conducted after an almost 60-year break, information was obtained on the species composition, quantity parametrs, distribution by biotopes and the dynamics of development by season of multicellular zooplankton communities (metazooplankton) in the Mingachevir Reservoir under current conditions. It was determined that the metozooplankton of the reservoir consists of 36 species belonging to 3 taxonomic groups. In terms of biodiversity rotifers (36.1%) dominated. *P. vulgaris, A. priodonta, D. longispina, D. hyalina, Ch. sphaericus, B. longirostris, A. acutulobatus, T. dybowskii* were the most frequent species in all seasons of the year.

Acknowledgements

All zooplankton samples were collected by the author herself and analyzed in laboratory conditions.

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A New Water Mite Species (Acariformes, Hydrachnidia) for the Fauna of Türkiye

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ABSTRACT

This study provides the first record and morphological description of the freshwater mite genus *Tartarothyas* Viets, 1934 and *Tartarothyas micrommata* Viets, 1934, from Aksu Stream in Isparta Province, Türkiye. The results of this study are expected to contribute significantly to a better understanding of the water tick fauna in Turkey. The findings emphasize the importance of comprehensive morphological reassessments and DNA analyses to better understand the species' evolutionary relationships.

Keywords: New record, Hydrachnidia, Acari, Tartarothyas, Türkiye

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Türkiye Faunası İçin Yeni Bir Su Akarı Türü (Acariformes, Hydrachnidia)

Öz: Bu çalışma, Türkiye'nin Isparta İli Aksu Deresi'nden *Tartarothyas* Viets, 1934 cinsi ve *Tartarothyas micrommata* Viets, 1934 türüne ait ilk kaydı ve morfolojik tanımını sunmaktadır. Bu çalışmanın sonuçlarının, Türkiye'deki su kenesi faunasının daha iyi anlaşılmasına önemli ölçüde katkı sağlayacağı düşünülmektedir. Bulgular, türün evrimsel ilişkilerini daha iyi anlamak için kapsamlı morfolojik yeniden değerlendirmelerin ve DNA analizlerinin önemini vurgulamaktadır.

Anahtar kelimeler: Yeni kayıt, Hydrachnidia, Acari, Tartarothyas, Türkiye

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Introduction

In freshwater habitats, the most notable group of Arachnida is the Hydrachnidia, also known as water mites. Globally, more than 6,000 species representing 57 families, 81 subfamilies, and more than 400 genera have been described (Cimpean and Battes 2018). Water mites, such as *Tartarothyas*, play a critical role in freshwater biodiversity and serve as valuable indicators for environmental quality assessment (Di Sabatino et al. 2008). With the highest number of reported species (1,642 species), the Palearctic region is among the most well-studied regions (Cimpean and Battes 2018) followed by Neotropical region with 1491 species (Goldschmidt et al. 2021).

The first studies on water mites in Türkiye were conducted by Thon (1905) on specimens collected in the vicinity of Mount Erciyes, where two new species were described. To date, 348 species belonging to 63 genera and 25 families have been recorded from Türkiye. With the addition of the present study; Türkiye presently comprises a total of 349 species and 64 genera (Erman et al. 2019, Esen 2022, Kijevcanin 2024).

Since the genus *Tartarothyas* Viets, 1934 was reported from Southeastern Europe (*T. micrommata* Viets, 1934), only a few findings have been reported from the Western Palearctic region. Currently, two species belonging to this genus, *T. micrommata* Viets, 1934 and *T. romanica* Husiatinschi, 1937, are recognized in the European fauna. (Gerecke 1996, Di Sabatino et al. 2010, Goldschmidt et al. 2021) The genus is mostly crenobiont, with almost all the finds from Europe, North America and the Indo-Australian

regions relating to springs, particularly helocrenes (Di Sabatino et al. 2008, 2010).

The aim of this study is to contribute to a better understanding of the ecosystem by incorporating a species that has not yet been recorded from Türkiye's freshwater systems into the fauna list.

Materials and Methods

The samples were collected from the Aksu stream near Pazarköy in Isparta province, at, 37° -44′ -31.97″ N, 30°-01′-32.96″ E (Fig 1).

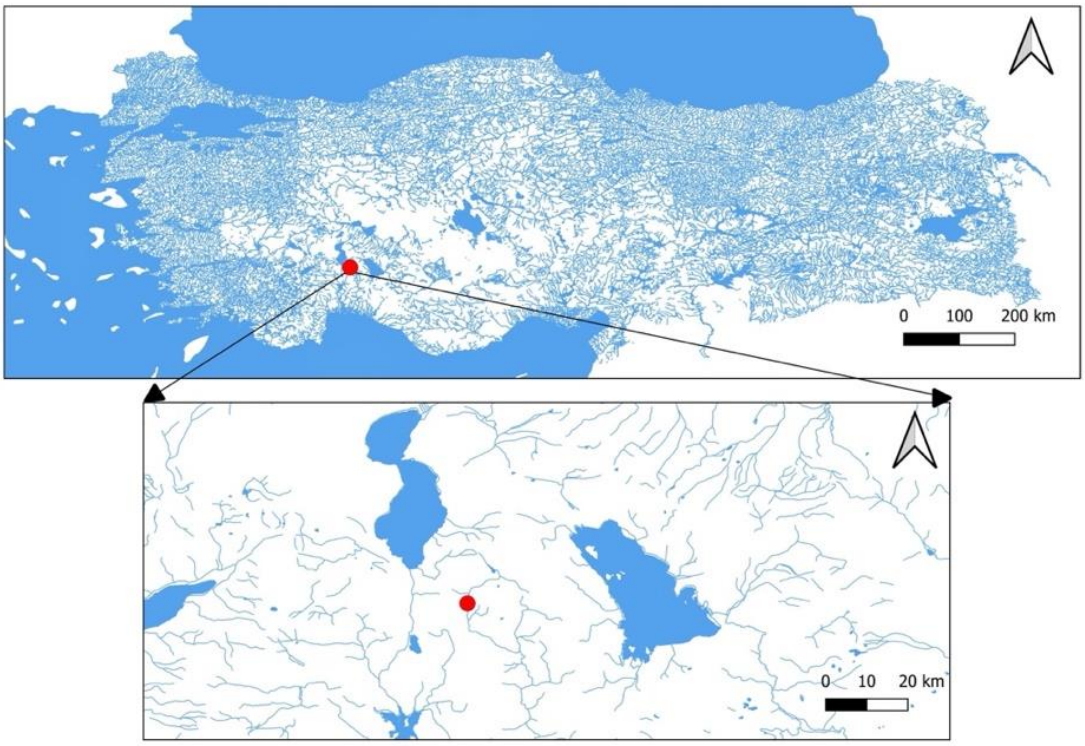

Figure 1. Map of the study area

Water mites were collected from habitats with vegetation growth along the smaller tributaries of the Aksu River using with hand nets and were fixed in Koenike's solution. The specimen were dissected and exhibited in a laboratory. All measurements are given in micrometers. The following abbreviations are used: Ac: acetabula, Cx-I: the first coxae, P-1: palp segment 1, and IV-L-5: fourth leg, fifth segment.

Result

Family: Hydryphantidae
Subfamily: Tartarothyadinae
Genus: *Tartarothyas* Viets, 1934

Lateral eyes reduced in size, not encapsulated; Ac oval, in the membranous area between the gonopore and genital flaps; suture line separating Cx-I/II nearly parallel to the longitudinal idiosomal axis; chelicerae with very strong claws (Harvey 1987).

Tartarothyas micrommata Viets, 1934
Synonimes: *Tartarothyas fonticola Motas & Tanasachi, 1957*
Tartarothyas vietsi Motas & Tanasachi, 1962

Diagnosis; (Adult-Female) legs without swimming setae; dorsal plates absent; glandularia platelets absent; lateral eyes reduced; body not elongated (Harvey 1987).

Description: Adults. Integument smooth. Genital flaps with 2-3 pairs of setae and 3 pairs of acetabula; the first pair was longest, and the third pair was ovoid (Fig. 2A). Female: body 835/545; Capitulum with two seate on anterior margin, capitulum L 213, P-1 with a thickened, sub-distal seta on the medial surface and with distal extension; P-5, with two slightly processes (Fig. 2.B). P-1-5: 30-62-57-100-42. Cx I-II with 1-2 stout setae on distal ends (Fig. 2.A). Legs without swimming setae; claws completely smooth; leg IV: 95-120-110-175-212-190 (Fig.3). Anus without associated sclerite (Fig 2A). genital field L/W 112/125. Palp (Fig 4A-B): Inside the palp, thickened, sub-distal seta is observed on the medial surface and with distal extension. Chelicerae (Fig 4C).

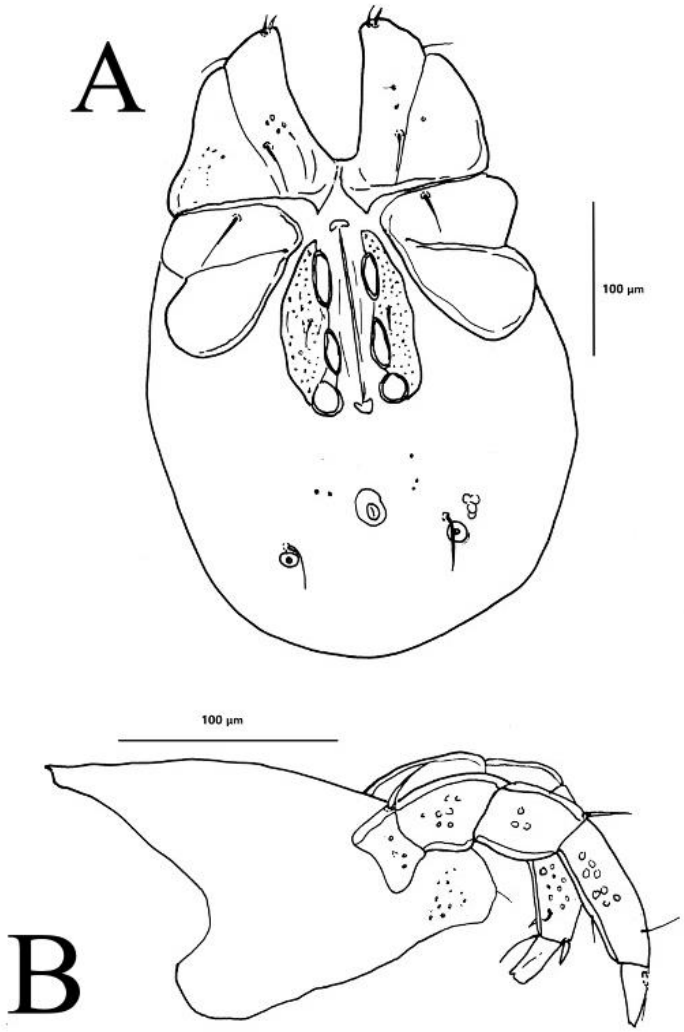

Figure 2. *Tartarothyas micrommata* Female ; A) Ventral view B) Capitulum, palps.

Figure 3. *Tartarothyas micrommata* IV. Leg

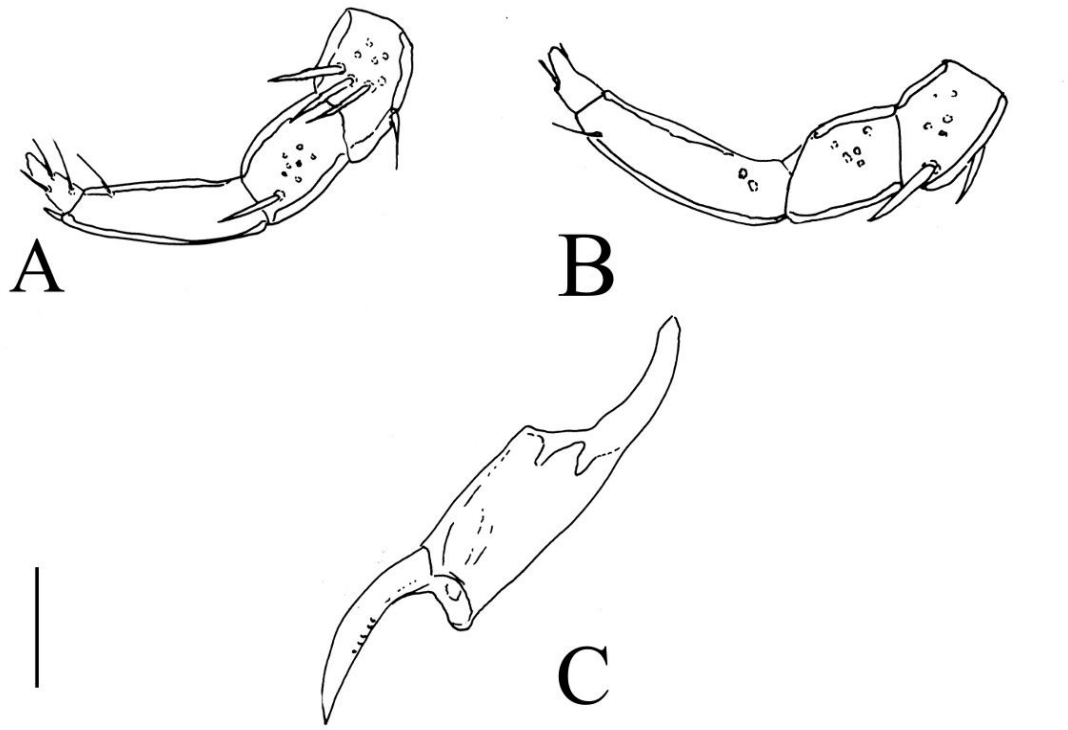

Figure 4. A) Palp inside, B) Palp outside C) Chelicerae (bar represents 100µm)

Discussion

It is noteworthy that, among the few previously described species of *Tartarothyas*, nine are currently known across four different continents, all of which exhibit remarkable morphological similarity. Moreover, the morphological differences used for species separation in Europe have shown considerable variability within larger populations, leading to the synonymization of three out of five temporarily described European species (*T. fonticola* (Motas and Tanasachi 1957), *T. raetica* Bader, 1989 and *T. suecica* Bader, 1989) (Gerecke 1996; Di Sabatino et al. 2008). In addition to size differences, the species primarily vary in palpal setation. However, some setae are challenging to recognize, making the available explanations frequently incomplete and the illustrations unreliable. Additional differences pertain to the size and shape of the acetabula and cheliceral claws. Published measurements of genital field length are difficult to compare, as previous species descriptions did not specify the distances used.

Thus, a comprehensive reassessment of the variability in morphological characteristics across all currently recognized taxa would be beneficial. Furthermore, molecular data would be invaluable in elucidating the evolutionary relationships within this fascinating genus. According to Goldschmidt and Ramirez (2020), water mites play a crucial role in the diversity of freshwater systems and are excellent indicators for assessing water quality and environmental integrity. Due to the limited number of available specimens, the evaluation and study of these species will be difficult.

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Effects of Dietary Mannan Oligosaccharide and Fructo Oligosaccharide Combinations on the Culture Performance of Red Swamp Crayfish

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ABSTRACT

This research explored the impact of dietary prebiotics on the growth performance of red swamp crayfish over two distinct 90-day trials (each with 3 replicates). In the first trial (initial weight: 0.085 g, 7 experimental groups), mannan-oligosaccharide (M0, M1, M2, M3) and fructo-oligosaccharide (F0, F1, F2, F3) were added at concentrations of 0, 1, 2, and 3 g kg^{-1}. The highest weight gain (WG) and specific growth rates (SGR) were recorded in the M3 group (WG: 8.05 g, SGR: 5.07) and F3 group (WG: 8.00 g, SGR: 5.06). Similarly, the M3 and F3 groups showed the most favorable feed conversion ratios (FCR) and survival rates (SR). In the second trial (initial weight: 0.087 g, 10 experimental groups), the combined use of MOS and FOS (M3+F3) delivered the best performance (WG: 8.82 g, SGR: 5.12, FCR: 1.29, SR: 93%), compared to the M1+F1 group (WG: 6.94 g, SGR: 4.86, FCR: 1.64, SR: 82%). While hepatopancreas tissues remained normal in all groups, the probiotic-supplemented groups exhibited significantly higher crude protein and lower fat content, total hemocyte counts, and intestinal bacteria counts compared to the control group ($p<0.05$). A combination of 3 g kg^{-1} MOS and FOS is recommended to enhance crayfish farming productivity.

Keywords: Growth, Crayfish, *Procambarus clarkii*, prebiotics, proximate analysis.

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Mannan-Oligosakkarit ve Frukto-Oligosakkarit Kombinasyonlarının Kırmızı Bataklık Kereviti Yetiştiriciliği Performansı Üzerine Etkileri

Öz: : Bu araştırma, iki farklı deneme boyunca (her biri 3 tekrarlı ve 90 günlük) kırmızı bataklık kerevitlerinin büyüme performansına yemle verilen prebiyotiklerin etkileri incelemiştir. Birinci denemede (başlangıç ağırlığı: 0,085 g, 7 deney grubu), mannan-oligosakkarit (M0, M1, M2, M3) ve frukto-oligosakkarit (F0, F1, F2, F3) 0, 1, 2 ve 3 g kg^{-1} düzeylerinde eklenmiştir. En yüksek ağırlık artışı (WG) ve spesifik büyüme oranları (SGR) M3 grubunda (WG: 8,05 g, SGR: 5,07) ve F3 grubunda (WG: 8,00 g, SGR: 5,06) kaydedilmiştir. Aynı şekilde, M3 ve F3 grupları en iyi yem değerlendirme oranlarına (FCR) ve hayatta kalma oranlarına (SR) sahip olmuştur. İkinci denemede (başlangıç ağırlığı: 0,087 g, 10 deney grubu), MOS ve FOS'un (M3+F3) birlikte kullanımı, M1+F1 grubuna kıyasla en iyi sonuçları vermiştir (WG: 8,82 g, SGR: 5,12, FCR: 1,29, SR: %93; M1+F1 WG: 6,94 g, SGR: 4,86, FCR: 1,64, SR: %82). Tüm gruplarda hepatopankreas dokuları normal kalırken, probiyotik takviyeli gruplarda ham protein ve yağ seviyeleri anlamlı derecede daha yüksek, toplam hemosit sayısı ve bağırsak bakterileri sayısı ise kontrol grubuna kıyasla daha düşük bulundu ($p<0.05$). *Procambarus clarkii* yetiştiriciliğinin verimini artırmak için 3 g kg^{-1} MOS ve FOS kombinasyonunun kullanılması önerilmektedir.

Anahtar kelimeler: Büyüme, kerevit, *Procambarus clarkii*, prebiyotik, besin bileşen analizi.

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Introduction

The rapid growth of the global population is increasing the demand for quality food sources. Challenges like accessing affordable animal protein and climate change scenarios are among the most discussed topics. Especially during and after the COVID-19 pandemic, disruptions in the supply chain made us realize the importance of local agricultural production and the difficulties of external dependency. Among the self-sufficiency areas of

countries, food production is the most crucial. Aquatic animal products have significant nutritional and economic value. Therefore, aquaculture has the potential to meet human nutritional needs in terms of quantity, quality, and diversity (Genc et al. 2020). However, the aquaculture sector often resorts to using chemicals and antibiotics to reduce disease risks in intensive production. In 2006, the European Union banned the excessive and unnecessary use of antibiotics in animal production (except under veterinary supervision) (Wall et al. 2016). Consequently, restrictions on antibiotic use in aquaculture have also been introduced based on the recommendations of the European Union and FAO (Boix et al. 2014). The ban on chemical agents like antibiotics, which leave residues, has led to increased research into alternative feed additives that can promote healthy growth in animals. In this context, the effectiveness of organic acids, enzymes, probiotics, prebiotics, and synbiotics is being tested (Barug et al. 2006; Akhter et al. 2015; Guerreiro et al. 2018; Reverter et al. 2021; Hoseinifar et al. 2024; Genc et al. 2024a; Genc et al. 2024b). It has been suggested that adding prebiotics to feed can have positive effects on animal health, thereby enhancing efficiency and sustainability in aquaculture (Wee et al. 2022).

Following finfish, decapod crustaceans represent a substantial market demand in the aquaculture industry. Among decapod crustaceans, the red swamp crayfish (*Procambarus clarkii*) ranks as the second most cultivated species, following the Pacific white shrimp (*Penaeus vannamei*). This crayfish species has become a common product that can be cultured in all regions except Antarctica and Oceania (Hobbs Jr 1988; Hobbs Jr et al. 1989; Gherardi 2006; Lodge et al. 2012; Souty-Grosset et al. 2016). The red swamp crayfish is predominantly produced in China and the United States (FAO 2022). Secondary immune system development in decapods is not as advanced as it is in vertebrates, thus requiring constant immune stimulation for healthy culture. Prebiotics are easily accessible and easy-to-apply components that serve as immune stimulants (Cerezuela et al. 2011; Ringø et al. 2010; Dinçer 2022). Prebiotics are fermentable food components resistant to gut enzymes, playing a role in the proliferation and activity of beneficial bacteria in the intestines of terrestrial and aquatic animals. Prebiotics can increase farming efficiency by enhancing nutrient utilization (Gibson and Roberfroid 1995; Manning and Gibson 2004; Guerreiro et al 2014; Ringø et al. 2010, 2014; Akhter et al. 2015).

Among the prebiotics used in aquaculture are β-glucan, inulin, arabinoxylan oligosaccharide (AXOS) (Li et al. 2021), mannan oligosaccharide (MOS) (Mazlum et al. 2011; Genc et al. 2007), galacto-oligosaccharide (GOS), fructo-oligosaccharide (FOS), galacto-glucomannan (GGM), isomalto-oligosaccharide (IMO), and xylo-oligosaccharide (XOS) (Wee et al. 2022). MOS and FOS, in particular, have been reported to improve gut health, boost immune systems, and promote growth (Gibson et al. 2017; Akhter et al. 2015; Assan et al. 2022). MOS is a component derived from the cell wall of the yeast *Saccharomyces cerevisiae*. It is stable at both high and low temperatures, does not react with feed ingredients, and has the ability to suppress mold (Ringø et al. 2010, 2014; Torrecillas et al. 2014; Song et al. 2014; Genc and Kumtepe 2024). Dietary MOS can enhance immune response and prevent pathogen proliferation in the digestive system (Santin et al. 2001; Patterson and Burkholder 2003; Song et al. 2014). FOS, derived from sucrose molecules, is an indigestible compound that has been reported to enhance immune response by increasing the gut bacterial diversity of the host (Dong and Wang 2013). Moreover, there are studies indicating that FOS enhances feed utilization (Ringø et al. 2010; Zhang et al. 2010; Ringø et al. 2014; Song et al. 2014; Guerreiro et al. 2014, 2016).

The cambarid family of freshwater crayfish (*Procambarus* spp.) is native to the Americas, while the astacid family (*Astacus* spp., *Pontastacus* spp.) is found in Europe and Asia, and the parastacid family (*Cherax* spp.) is distributed in Australia and its surroundings (Kumlu 2001; González et al. 2009; Kumlu 2010). Compared to the European crayfish (*Astacus astacus*) and the Eastern European/Turkish crayfish (*Pontastacus leptodactylus*), the red swamp crayfish has higher environmental tolerance and disease resistance. This species is preferred in aquaculture due to its short incubation period (20-25 days) and ability to reproduce multiple times throughout the year (Arslan 2024). The red swamp crayfish is appreciated for its taste in the food industry and its appearance in the aquarium industry (FAO 2009; Holdich 2002). It has been suggested that culturing this species under controlled conditions (in recirculating systems) could reduce the risk of invasion and benefit the food and aquarium sectors (Arslan 2024). However, heterogeneous growth and cannibalism are issues in the culture of *P. clarkii* (Dinçer 2022; Byeon and Lee 2024).

To address these problems, it has been found beneficial to use shelter to prevent cannibalism under controlled conditions (Karplus et al. 1995; Ramalho et al. 2008; González et al. 2011; Huang et al. 2011; Mazlum et al. 2017; Su et al. 2020; Yu et al. 2020). A hypothesis has been developed prioritizing the use of prebiotics to enhance culture efficiency. There are only a limited number of studies addressing the supplementation of MOS and FOS in red swamp

crayfish (*P. clarkii*) culture, with none focusing specifically on the potential effects of their combined use. Therefore, this study aims to determine the effects of different doses of MOS and FOS prebiotics, as well as their combinations, added to species-specific experimental diets on the growth parameters and nutrient composition of red swamp crayfish under controlled conditions (indoor tanks/laboratory scale).

Materials and Methods

Location, broodstock maintenance, and juvenile production

The feeding trials were conducted at the Department of Fisheries and Aquaculture Engineering, Faculty of Agriculture, Ankara University, Ankara, Türkiye. Juvenile red swamp crayfish (*P. clarkii*) were produced by breeding four female and four male broodstock at the research unit. Mating behaviors were observed over a two-week period, during which crayfish were provided a diet of trout pellet feed (45% crude protein), spinach leaves (blanched), and fresh peas. The matured females and males were stocked for mating in an environment equipped with 5-6 cm diameter PVC pipes serving as hiding areas, under clear water conditions with aeration, filtration, and circulation providing a total water volume of 200 L. Successful mating was confirmed by the observation of pleopodal eggs after approximately one month. Crayfish carrying pleopodal eggs were transferred to an incubation tank maintained at 24ºC, where fry hatching occurred after about 22 days. When juvenile crayfish started to detach from the pleopods and move independently on the tank floor, approximately 7 days after hatching, the females were removed from the tank. The juveniles were fed for 10 days with an experimental diet prepared in ground form, in accordance with the recommended formulation for crustaceans (described in the following section: *Experimental Diets and Feeding*). After this period, the juveniles were weighed and distributed into experimental tanks. Their acclimation to the experimental tanks was monitored for three days before the trials commenced. Details of the experimental plans (Trial I and Trial II) are presented below.

Experimental diets and feeding

An experimental diet formulation was prepared following the recommendations for crustaceans by NRC (2011). The raw materials were ground, and MOS and FOS prebiotics were added at 0, 1, 2, and 3 g kg^{-1}. The mixture was then pelletized (using a cold pelleting machine, Pasfil, Istanbul) into pellets 1.2 mm in diameter and 3-4 mm in length. The pellets were conditioned with steam under 1 ATM pressure for approximately 20 minutes to achieve gelatinization. After drying in an oven at 40ºC for 24 hours, the pellets were ground and broken (in suitable sizes for crayfish juveniles), labeled and stored at -18ºC. The basal diet had a nutritional composition of 90.50% dry matter, 6.05% ash, 38.14% protein, and 9.13% lipid (Table 1, Table 2). During the trials, feeding was conducted three times daily according to a graduated schedule. Specifically, the feeding rates were: 8% of live weight for days 0-15, 7.2% for days 15-30, 6.6% for days 30-45, 5.8% for days 45-60, 5% for days 60-75, and 4.2% from day 75 to day 90. Feed amounts were determined based on bi-weekly live weight measurements (Croll and Watts 2004; Dinçer 2022).

Table 1. The composition of experimental diets for Trial I (per 100 g)

	K	1M	2M	3M	1F	2F	3F
Fish meal[a]	30	29.8	29.8	29.8	29.8	29.8	29.8
Soy pulp meal[b]	12	11.8	11.8	11.8	11.8	11.8	11.8
Corn gluten[c]	13	12.8	12.8	12.8	12.8	12.8	12.8
Wheat flour[d]	36.3	35.9	34.9	33.9	35.9	34.9	33.9
Fish oil[e]	2.6	2.6	2.6	2.6	2.6	2.6	2.6
Soy lecithin[f]	2	2	2	2	2	2	2
Vitamin premix[g]	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Mineral premix[g]	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin C[g]	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Guar gum + Cholestrol[h]	3	3	3	3	3	3	3
MOS	**0**	**0.1**	**0.2**	**0.3**	**0**	**0**	**0**
FOS	**0**	**0**	**0**	**0**	**0.1**	**0.2**	**0.3**

table continues

Proximate analysis (%)							
Dry matter	90.50	90.59	90.59	90.64	90.59	90.59	90.64
Crude ash	6.05	6.06	6.08	6.08	6.06	6.06	6.08
Crude protein	38.14	38.07	38.03	38.01	38.07	38.03	38.01
Crude lipid	9.13	9.06	9.06	9.06	9.06	9.06	9.06

[a]Anchovy meal, Sürsan Aquculture Ltd. Samsun, Turkiye, [b]Kırcı Soy Producs, Balıkesir, Türkiye, [c]Cargill, İstanbul, Türkiye, [d]İpek Wheat Fab., Nevsehir, Türkiye, [e]Anchovy oil, Sürsan Aquculture Ltd. Samsun, Türkiye, [f]Sigma Aldrich Chemicals, St. Louis, MO, ABD, [g]DSM Food producs, Türkiye, [h]Guar gum, Kartal Chemical Products Ltd, Istanbul, Turkiye, Cholesterol, Sigma, Germany.

Table 2. The composition of experimental diets for Trial II (per 100 g)

	K	**1M1F**	**1M2F**	**1M3F**	**2M1F**	**2M2F**	**2M3F**	**3M1F**	**3M2F**	**3M3F**
Fish meal[a]	30	29.8	29.8	29.8	29.8	29.8	29.8	29.8	29.8	29.8
Soy pulp meal[b]	12	11.8	11.8	11.8	11.8	11.8	11.8	11.8	11.8	11.8
Corn gluten[c]	13	12.8	12.8	12.8	12.8	12.8	12.8	12.8	12.8	12.8
Wheat flour[d]	36.3	34.9	33.9	32.9	33.9	32.9	31.9	32.9	31.9	30.9
Fish oil[e]	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6
Soy lecithin[f]	2	2	2	2	2	2	2	2	2	2
Vitamin premix[g]	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Mineral premix[g]	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin C[g]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Guar gum + Cholestrol[h]	3	3	3	3	3	3	3	3	3	3
MOS	**0**	**0.1**	**0.2**	**0.3**	**0**	**0**	**0**	**0**	**0.1**	**0.2**
FOS	**0**	**0**	**0**	**0**	**0.1**	**0.2**	**0.3**	**0**	**0**	**0**
Proximate analysis (%)										
Dry matter	90.50	91.15	91.15	91.15	91.37	91.37	91.37	91.47	91.47	91.59
Crude ash	6.05	6.08	6.08	6.08	6.17	6.11	6.11	6.19	6.19	6.19
Crude protein	38.14	37.71	37.71	37.71	37.62	37.62	37.62	37.55	37.55	37.55
Crude lipid	9.13	9.06	9.06	9.06	9.06	9.06	9.06	9.06	9.06	9.04

[a]Anchovy meal, Sürsan Aquculture Ltd. Samsun, Turkiye, [b]Kırcı Soy Producs, Balıkesir, Türkiye, [c]Cargill, İstanbul, Türkiye, [d]İpek Wheat Fab., Nevsehir, Türkiye, [e]Anchovy oil, Sürsan Aquculture Ltd. Samsun, Türkiye, [f]Sigma Aldrich Chemicals, St. Louis, MO, ABD, [g]DSM Food producs, Türkiye, [h]Guar gum, Kartal Chemical Products Ltd, Istanbul, Turkiye, Cholesterol, Sigma, Germany.

Trial I: Effects of different levels of dietary MOS and FOS on the culture performance of red swamp crayfish

The feeding trial was conducted over 90 days using feeds supplemented with different levels of MOS and FOS prebiotics. The experiment was carried out under controlled conditions with a daily water exchange rate of 3%, aeration, and a photoperiod of 12 hours light and 12 hours dark in tanks with a water volume of 40 L. Each tank housed 15 crayfish, corresponding to a density of 15 crayfish per 0.24 m^2. A total of seven groups, including a control group, were set up with three replicates each. Juvenile crayfish with an initial weight ranging from 0.084 to 0.085 g were randomly selected and distributed according to a completely randomized design. To simulate natural conditions, complex nets and plastic pipes were placed in the tanks as hiding areas. During the experimental period, feeding was performed three times daily following the schedule outlined in the feeding plan (starting at 8% of live weight for the first 15 days and gradually decreasing to 4.2% for the final 15-day period). Water quality parameters, including dissolved oxygen, pH, water temperature, and ORP (oxidation-reduction potential), were measured daily, while nitrite, nitrate, total ammonia nitrogen, and total phosphorus levels were measured weekly. At the end of Trial I, the effectiveness of prebiotic feed additives was evaluated based on growth parameters and nutrient composition analysis.

Trial II: Effects of dietary MOS and FOS combinations on the culture performance of red swamp crayfish

In Trial II, the prebiotic doses used in Trial I were applied in combination to investigate their effects. Nine different prebiotic combinations were formed by using one dose of MOS and one dose of FOS, as applied in the first trial. The second trial comprised ten groups, including a control group, with juvenile red swamp crayfish distributed into 30 tanks following a completely randomized design, with three replicates per group (40 L tanks, 15 crayfish per 0.24 m^2). Juvenile crayfish with an initial weight of 0.087-0.089 g were used at the beginning of Trial II. Feeding was conducted three times a day, starting with 8% of the body weight for the first 15-day period and decreasing to 4.2% for the last 15 days, under the same conditions as Trial I (3% daily water exchange, aeration, and a 12-hour light/12-hour dark photoperiod). Water quality parameters, including dissolved oxygen, pH, water temperature, and ORP, were measured daily, while nitrogenous compounds and total phosphorus were measured weekly. At the end of Trial II, the prebiotic combinations that showed the best culture performance in crayfish were determined.

Water quality parameters

Water quality parameters such as temperature, dissolved oxygen ad pH were daily monitored (YSI Pro20 and YSI EcoSense) in tanks. Additionally, nitrite, nitrate, total ammonia and phosphate were weekly determined using the photometric kit method (Hanna, HI801-01 iris Visible Spectrophotometer, USA) according to the protocol. Moreover, Oxidation Reduction Potential (ORP) measurement (Pinpoint ORP Monitor, American Marine Inc.) was conducted as an additional parameter indicating the suitability of the aquaculture environment for living organisms.

Determination of growth parameters

Body weight measurements were taken at the beginning and 15-day intervals until the end of the experiment'. The formulas of variables including growth and nutrient utilization performance (survival rate (SR), body weight gain (BWG), specific growth rate (SGR), and feed conversion ratio (FCR)) were provided below.

SR (%) = (Day 0 number of crayfish– Final day number of crayfish x 100) / Day 0 number of crayfish

BWG (g) = Final day body weight – Day 0 body weight

SGR (% day^{-1}) = 100 x (ln Final day body weight – ln Day 0 body weight) / Trial period (days)

FCR = Total feed consumption during the trial / Live weight gain

Proximate analysis

After the trials, crayfish samples underwent nutrient analysis following the AOAC protocol (Horwitz 2000). Initially, crayfish samples were dried in an oven at 105.0 ± 0.5ºC until their weights stabilized. Subsequently, protein content was determined using the Kjeldahl method on whole-body dry matter. The crude lipid level was calculated using the Soxhlet extraction protocol. Lastly, ash content was determined using the incinerator protocol (8 hours, 525ºC). The following formulas were employed for the calculations:

Humidity (%) = (dry sample weight - wet sample weight) / (wet sample weight) x 100

Crude Protein (%) = [(titration amount - blank sample x 0,1 x 14,007 x 6,25) / sample amount] x 100

Raw Oil (%) = (the amount of oil in the soxhlet jug/sample amount) x 100

Crude Ash (%) = [(weight of first porcelain cup - weight of last porcelain cup) / sample amount] x 100

Hepatopancreas histomorphology

Two crayfish from each experimental group were sedated in ice water to take a hepatopancreas sample. Subsequently, 0.5 g of hepatopancreas tissue was extracted from the posterior-lateral region of the carapace. The extracted tissue was fixed in a 10 mL buffered formaldehyde solution, placed in histological follow-up cassettes, and labeled. Following a two-day fixation process, the hepatopancreas tissue samples underwent hydration (distilled water), dehydration (increasing ethyl alcohol series), clearing (xylene cold-hot), and paraffinization (warm liquid paraffin series) stages for histomorphological examination using a standard manual protocol. The samples were then transferred to paraffin blocks in tissue embedding containers and sectioned at a 5-6 µm thickness using a rotary microtome (Shandon). The tissue sections were spread into a container with water at 45ºC, transferred to labelled slides, and stained with hematoxylin and eosin following deparaffinization, dehydration, and hydration stages. The stained preparations were examined under a light microscope (CM40 Leica) with a camera, and microphotographs were recorded (Vogt et al. 1985; Genc et al. 2007; Kaya et al. 2019).

Total bacterial counts

Approximately 0.3 g of intestinal contents were collected under aseptic conditions from two crayfish in each tank to determine the total bacterial count in the digestive tract. The samples were transferred into sterile tubes with slight dorsoventral pressure from the anal pore. Dilutions were prepared by placing the samples in tubes containing sterile physiological saline (NaCl 0.85%: 9 mL for ~1 g sample) and serially diluted in the range of 10-1 to 10-7. Inoculation was performed using the patch method on agar plates (Difco^{TM}) in triplicate, representing each group. Petri dishes were incubated at 36 ± 1ºC for 48 hours to calculate the total aerobic bacterial count (TBC) (Okpala et al. 2014). Bacterial count results (average of three plates) are expressed as colony-forming units (CFU g^{-1}).

Total hemocyte count (THC)

Hemolymph samples from red swamp crayfish were collected from the sinus using an anticoagulant syringe (3 mL, BDMicroFine, anticoagulant: 26 mM $C_6H_8O_7$, 100 mM glucose, 450 mM NaCl, 30 mM $C_6H_5Na_3O_7$, 10 mM $C_{10}H_{16}N_2O_8$, pH 4.6). The total hemocyte count was determined using a counting chamber (Neubauer slide) under the microscope (Söderhäll and Smith 1983).

Statistical analysis

Data obtained from the trials were evaluated using a one-way analysis of variance (ANOVA) and Duncan's comparison test (mean ± standard deviation) after checking the normality and homogeneity of variance. The alpha significance level was set at 0.05. Analyses were conducted using the SPSS program (SPSS 17.0, Chicago, IL, USA).

Results

Water quality assessments

During Trial I, the dissolved oxygen level ranged from 6.46 to 6.53 mg/L, pH varied between 6.59 and 6.74, water temperature ranged from 20.91 to 20.97ºC, and ORP fluctuated between 117 and 153 mV. Weekly assessments revealed nitrite levels between 0.16 and 0.21 mg L-1, nitrate levels between 0.95 and 1.00 mg L-1, total ammonia levels between 0.49 and 0.69 mg L-1 and total phosphate levels between 1.17 and 1.20 mg L-1. Statistical analysis indicated no significant difference in most measured water quality parameters ($p>0.05$), except for ORP, and total ammonia values, which exhibited a statistical difference ($p<0.05$), (Table 3).

In Trial II, dissolved oxygen levels were observed between 5.94 and 6.10 mg L-1, pH levels ranged from 6.25 to 6.32, water temperature fluctuated between 20.9 and 21.0ºC, and ORP ranged from 159 to 165 mV. Weekly measurements indicated nitrite levels between 0.14 and 0.16 mg L-1, nitrate levels between 1.27 and 1.52 mg L-1, total ammonia levels between 0.85 and 0.97 mg L-1, and phosphate levels between 1.17 and 1.46 mg L-1. Statistical analysis did not reveal any significant differences between the groupsin terms of most water quality parameters ($p>0.05$). However, significant differences wereobserved in ORP and phosphate values ($p<0.05$). While no statistical difference was foundbetween the control group and the 2M3F group regarding water phosphate levels ($p>0.05$), a significant difference was noted when compared with the 1M3F, 2M1F, 3M2F, and 3M3F groups ($p<0.05$), (Table 3).

Table 3. Water quality parameters measured during the trials

	Groups	pH*	DO (mg/L)	T (°C)	$NO2^-$ (mg/L)	$NO3^-$ (mg/L)	TAN (mg/L)	T-P (mg/L)	ORP (mV)
Trial I	**Control$_1$**	6,74±0,21^a	6,51±0,05^a	20,95±0,02^a	0,21±0,03^a	1,00±0,12^a	0,57±0,07^{ab}	1,17±0,04^a	123±10^{ab}
	1M	6,65±0,19^a	6,51±0,05^a	20,91±0,03^a	0,19±0,04^a	0,98±0,05^a	0,58±0,09^{ab}	1,18±0,07^a	139±13^{bc}
	2M	6,60±0,25^a	6,46±0,04^a	20,96±0,08^a	0,16±0,05^a	0,95±0,10^a	0,49±0,04^a	1,19±0,08^a	146±19^c
	3M	6,59±0,27^a	6,54±0,04^a	20,93±0,06^a	0,20±0,03^a	0,98±0,18^a	0,69±0,13^b	1,18±0,06^a	117±9,0^a
	1F	6,63±0,15^a	6,53±0,02^a	20,92±0,09^a	0,16±0,04^a	0,98±0,08^a	0,54±0,09^{ab}	1,19±0,05^a	147±9,0^c
	2F	6,66±0,28^a	6,49±0,08^a	20,97±0,01^a	0,20±0,03^a	0,95±0,14^a	0,56±0,07^{ab}	1,19±0,08^a	146±5,0^c
	3F	6,60±0,19^a	6,50±0,09^a	20,93±0,06^a	0,16±0,04^a	0,99±0,12^a	0,61±0,04^{ab}	1,18±0,06^a	153±13^c
Trial II	**Control$_2$**	6,28±0,01^a	6,05±0,52^a	21,0±0,05^a	0,16±0,05^a	1,33±0,21^a	0,93±0,06^a	1,18±0,11^a	162±2,6^{abc}
	1M1F	6,32±0,07^a	6,08±0,49^a	20,9±0,03^a	0,15±0,03^a	1,32±0,24^a	0,85±0,08^a	1,29±0,10^{abc}	159±1,00^a
	1M2F	6,28±0,06^a	6,05±0,55^a	21,0±0,08^a	0,15±0,03^a	1,46±0,18^a	0,86±0,09^a	1,32±0,10^{abc}	165±1,5^c
	1M3F	6,31±0,05^a	5,94±0,50^a	21,0±0,11^a	0,15±0,03^a	1,47±0,15^a	0,91±0,08^a	1,37±0,04^{bc}	163±2,1^{abc}
	2M1F	6,27±0,03^a	6,01±0,49^a	21,0±0,15^a	0,15±0,04^a	1,30±0,40^a	0,86±0,07^a	1,38±0,07^{bc}	162±1,7^{abc}
	2M2F	6,26±0,02^a	6,10±0,50^a	21,0±0,02^a	0,14±0,04^a	1,52±0,14^a	0,92±0,02^a	1,33±0,03^{abc}	163±2,1^{bc}
	2M3F	6,26±0,05^a	5,99±0,52^a	20,9±0,06^a	0,15±0,04^a	1,49±0,08^a	0,90±0,06^a	1,17±0,10^a	164±1,5^{bc}
	3M1F	6,29±0,07^a	6,09±0,52^a	20,9±0,17^a	0,16±0,04^a	1,27±0,36^a	0,91±0,06^a	1,26±0,14^{ab}	160±1,0^{ab}
	3M2F	6,32±0,05^a	6,04±0,49^a	21,0±0,10^a	0,16±0,04^a	1,35±0,22^a	0,92±0,03^a	1,46±0,05^c	164±2,5^{bc}
	3M3F	6,25±0,06^a	6,02±0,47^a	20,9±0,13^a	0,16±0,04^a	1,45±0,22^a	0,94±0,03^a	1,39±0,07^{bc}	165±3,1^c

*For Trial I and Trial II, different superscript letters within the columns for each parameter indicate statistically significant differences ($p<0.05$). M: MOS, F: FOS, DO: Dissolved oxygen, T: Water temperature, Nitrite: NO_2^-, Nitrate: NO_3^-, TAN: Total ammonia nitrogen ($NH_3 + NH_4^+$), T-P: Total phosphorus, ORUP: Oxidative reduction potential (mV). Data are presented as mean ± standard deviation.

Growth

In Trial I, MOS and FOS prebiotics were added separately to the feeds as feed additives and fed and given to 7 groups, including the control group, for 90 days. In Trial II, co-administrations of MOS and FOS prebiotics were tested 10 dietary groups for 90 days. The SGR in Trial I reveals a clear trend of improvement with increasing levels of MOS and FOS, reaching the highest in the 3M group (5.07 ± 0.04% day^{-1}). The weight gain in Trial I also followed a similar pattern, with the 3M group exhibiting the highest value (8.05 ± 0.22 g). The FCR results in Trial I support the growth metrics, with the 3M group displaying the best ratio (1.28 ± 0.04 g). Lower FCR values indicate more efficient feed utilization, emphasizing the positive effect of higher MOS levels (Table 4).

In Trial II, the SGR values verified the improvement with 3M3F group showing the highest SGR (5.12 ± 0.03% day^{-1}). This suggests that the combination of MOS and FOS in Trial II synergizes with crayfish growth. The weight gain results in Trial II support the SGR findings, with the best value in 3M3F group achieving the highest value (8.82 ± 0.27 g). The FCR results in Trial II align with the growth metrics, and the 3M3F group exhibits the lowest FCR (1.29 ± 0.04). This underscores the efficiency of feed conversion when using combined prebiotics (Table 4).

Proximate analysis

In Trial I, the comprehensive assessment of whole-body nutrient components resulted (in dry matter basis) from feeding red swamp crayfish with prebiotic-added feeds at varying rates. Notably, the 3M group exhibited the highest crude protein level at 40.07 ± 0.25%. Conversely, the control group displayed the lowest crude protein level, registering at 37.73 ± 0.15% ($p<0.05$). No statistical differences were observed among the Trial I groups ($p>0.05$). Findings from the whole-body nutrientcomponent analysis in Trial II revealed that, when utilizing prebiotic combinations as feedadditives, the 3M2F group exhibited the highest protein content, recording 41.23 ± 0.74%. In contrast, the control group presented the lowestvalue at 38.33 ± 1.02% ($p<0.05$). Regarding ash content, the control group recorded thehighest rate (31.23% ± 1.06%), whereas the 3M2F group maintained the lowest level at 28.53 ± 0.87% ($p < 0.05$). All experimental groups had a similar dry matter value ($p>0.05$). The highest crude lipid content was in the control group (7.50%), and the lowest was in the 3M group (6.30%), (Table 5).

In Trial II, the 3M2F group had the highest crude protein content (41.23%), while the control group had the lowest (38.33%). The control group had the highest crude lipid content (7.37%), and the 3M2F group had the lowest (5.97%).

Table 4. Effects of different dietary MOS, FOS and combinations on growth of red swamp crayfish (90 days)

	Groups	IW (g)*	FW (g)	WG (g)	SGR	FCR	SR
Trial I	**Control$_1$**	0.084 ± 0.001^{a}	7.128 ± 0.505^{a}	7.04 ± 0.51^{a}	4.93 ± 0.08^{a}	1.47 ± 0.10^{b}	84.45 ± 3.85^{ab}
	1M	0.085 ± 0.001^{a}	7.147 ± 0.196^{a}	7.06 ± 0.20^{a}	4.93 ± 0.03^{a}	1.45 ± 0.04^{b}	82.22 ± 3.85^{a}
	2M	0.085 ± 0.001^{a}	7.287 ± 0.446^{a}	7.20 ± 0.45^{a}	4.95 ± 0.07^{ab}	1.43 ± 0.09^{b}	88.89 ± 3.85^{abc}
	3M	0.085 ± 0.001^{a}	8.139 ± 0.219^{b}	8.05 ± 0.22^{b}	5.07 ± 0.04^{b}	1.28 ± 0.04^{a}	93.33 ± 0.00^{c}
	1F	0.085 ± 0.000^{a}	7.157 ± 0.505^{a}	7.07 ± 0.51^{a}	4.92 ± 0.08^{a}	1.46 ± 0.10^{b}	86.67 ± 6.67^{abc}
	2F	0.085 ± 0.000^{a}	7.243 ± 0.241^{a}	7.16 ± 0.24^{a}	4.94 ± 0.04^{a}	1.44 ± 0.05^{b}	86.67 ± 0.00^{abc}
	3F	0.085 ± 0.001^{a}	8.084 ± 0.612^{b}	8.00 ± 0.61^{b}	5.06 ± 0.09^{b}	1.29 ± 0.10^{a}	91.11 ± 3.85^{b}
Trial II	**Control$_2$**	0.087 ± 0.001^{ab}	7.039 ± 0.098^{a}	6.95 ± 0.98^{a}	4.87 ± 0.02^{a}	1.64 ± 0.02^{b}	86.67 ± 0.00^{ab}
	1M1F	0.088 ± 0.001^{ab}	7.031 ± 0.114^{a}	6.94 ± 0.11^{a}	4.86 ± 0.03^{a}	1.64 ± 0.03^{b}	82.22 ± 3.85^{a}
	1M2F	0.087 ± 0.001^{a}	7.123 ± 0.146^{a}	7.04 ± 0.15^{a}	4.89 ± 0.03^{a}	1.62 ± 0.04^{b}	88.89 ± 3.85^{ab}
	1M3F	0.088 ± 0.001^{ab}	7.111 ± 0.438^{a}	7.02 ± 0.44^{a}	4.88 ± 0.06^{a}	1.62 ± 0.09^{b}	91.11 ± 3.85^{ab}
	2M1F	0.088 ± 0.001^{ab}	7.194 ± 0.379^{a}	7.11 ± 0.38^{a}	4.89 ± 0.05^{a}	1.60 ± 0.09^{b}	86.67 ± 6.67^{ab}
	2M2F	0.087 ± 0.000^{a}	7.200 ± 0.043^{a}	7.11 ± 0.04^{a}	4.91 ± 0.01^{a}	1.60 ± 0.01^{b}	91.11 ± 7.70^{ab}
	2M3F	0.088 ± 0.002^{ab}	8.343 ± 0.263^{b}	8.25 ± 0.26^{b}	5.06 ± 0.03^{b}	1.38 ± 0.05^{a}	84.45 ± 3.85^{ab}
	3M1F	0.088 ± 0.001^{ab}	8.548 ± 0.300^{bc}	8.46 ± 0.30^{bc}	5.08 ± 0.03^{bc}	1.35 ± 0.05^{a}	91.11 ± 3.85^{ab}
	3M2F	0.087 ± 0.002^{a}	8.809 ± 0.339^{bc}	8.72 ± 0.34^{bc}	5.13 ± 0.02^{c}	1.31 ± 0.05^{a}	91.11 ± 7.70^{ab}
	3M3F	0.089 ± 0.001^{b}	8.905 ± 0.267^{c}	8.82 ± 0.27^{c}	5.12 ± 0.03^{c}	1.29 ± 0.04^{a}	93.33 ± 0.00^{b}

*For Trial I and Trial II, different superscript letters within the columns for each parameter indicate statistically significant differences ($p<0.05$). M: MOS, F: FOS, IW (g): Initial weight, FW (g): Final live weight, BWG (g): Weight gain, SGR (%day^{-1}): specific growth rate, FCR: Feed conversion ratio, SR: Survival rate. Data are presented as mean ± standard deviation.

The control group had the highest crude ash content (31.23%), and the 3M2F group had the lowest (28.53%). There was no significant difference in dry matter content among the groups (Table 5).

Table 5. Effects of different dietary MOS, FOS and combinations on proximate analysis (dry matter basis) of red swamp crayfish (90 days)

	Groups	Crude protein	Crude lipid	Crude ash	Dry matter
Trial I	**Control$_1$**	37.73 ± 0.21^{a}	7.50 ± 0.20^{c}	32.77 ± 0.83^{a}	22.43 ± 0.90^{a}
	1M	38.50 ± 0.52^{ab}	6.9 ± 0.3^{abc}	32.03 ± 1.02^{a}	22.93 ± 0.47^{a}
	2M	39.10 ± 0.62^{bc}	6.8 ± 0.1^{ab}	31.60 ± 1.20^{a}	23.23 ± 0.50^{a}
	3M	40.07 ± 0.25^{c}	6.3 ± 0.4^{a}	31.03 ± 0.38^{a}	23.10 ± 0.85^{a}
	1F	38.07 ± 0.65^{ab}	7.3 ± 0.5^{bc}	31.93 ± 1.02^{a}	23.27 ± 0.55^{a}
	2F	38.33 ± 0.71^{ab}	7.4 ± 0.5^{bc}	32.17 ± 1.35^{a}	22.83 ± 0.95^{a}
	3F	38.03 ± 0.81^{ab}	7.1 ± 0.4^{bc}	32.00 ± 1.23^{a}	23.27 ± 0.60^{a}
Trial II	**Control$_2$**	38.33 ± 1.02^{a}	7.37 ± 0.12^{c}	31.23 ± 1.06^{c}	23.53 ± 1.21^{a}
	1M1F	39.10 ± 1.37^{a}	7.10 ± 0.20^{c}	30.20 ± 0.26^{bc}	23.93 ± 0.67^{a}
	1M2F	39.30 ± 1.04^{ab}	7.13 ± 0.49^{c}	30.03 ± 0.67^{bc}	24.27 ± 1.42^{a}
	1M3F	38.73 ± 1.53^{a}	6.87 ± 0.06^{bc}	30.33 ± 0.64^{bc}	24.63 ± 0.96^{a}
	2M1F	38.87 ± 0.06^{a}	7.30 ± 0.46^{c}	30.27 ± 0.40^{bc}	24.07 ± 0.72^{a}
	2M2F	40.30 ± 0.95^{ab}	6.17 ± 0.32^{ab}	29.63 ± 0.74^{ab}	24.43 ± 1.18^{a}
	2M3F	39.27 ± 0.40^{ab}	6.73 ± 0.60^{bc}	30.20 ± 0.98^{bc}	24.23 ± 0.74^{a}
	3M1F	39.43 ± 1.46^{ab}	6.83 ± 0.12^{bc}	29.87 ± 0.38^{b}	24.30 ± 0.66^{a}
	3M2F	41.23 ± 0.74^{b}	5.97 ± 0.81^{a}	28.53 ± 0.87^{a}	24.77 ± 1.01^{a}
	(table continues…)				
	3M3F	39.03 ± 1.31^{a}	6.87 ± 0.40^{bc}	29.80 ± 0.26^{b}	24.87 ± 0.95^{a}

*For Trial I and Trial II, different superscript letters within the columns for each parameter indicate statistically significant differences ($p<0.05$). M: MOS, F: FOS. Data are presented as mean ± standard deviation.

Hepatopancreas histomorphology

In trial I, there were no significant differences in hepatopancreatic tissues among the groups, indicating that the prebiotic supplements did not adversely affect tissue morphology. The star-shaped tubular structure and cell types within appeared normal, affirming the health of the hepatopancreatic tissues. The tubular epithelial morphology confirmed that the prebiotics did not harm the crayfish. In trial II, applying different prebiotic combinations at varying doses did not alter the hepatopancreatic tissue morphology compared to the control group. In summary, Trial II demonstrated that the crayfish from all treatment groups exhibited normal hepatopancreatic tubular tissues, epithelial vacuoles, and tubular digestive and absorption functions without deviation from the expected normal.

Total bacterial counts

Trial I, the 2F group showed the highest bacterial count in the intestinal content ($4.60 \pm 0.26 \times 10^5$ CFU g^{-1}), while the control group had the lowest count ($3.88 \pm 0.13 \times 10^5$ CFU g^{-1}) ($p<0.05$). The 2F group was similar to the 2M ($4.55 \pm 0.40 \times 10^5$ CFU g^{-1}) and 3M groups ($4.43 \pm 0.29 \times 10^5$ CFU g^{-1}) ($p>0.05$). In trial II, the 3M2F group exhibited the highest bacterial count in intestinal content ($5.03 \pm 0.51 \times 10^5$ CFU g^{-1}), whereas the control group had the lowest count ($4.05 \pm 0.21 \times 10^5$ CFU g^{-1}), (Table 6).

Hemolymph parameters

In trial I, the 3M group exhibited the highest total hemocyte count ($81.67 \pm 2.75 \times 10^6$ cell mL^{-1}), while the control group had the lowest count ($38.17 \pm 2.75 \times 10^6$ cell mL^{-1}) ($p < 0.05$). The results indicated a significant increase in the number of hemocytes containing immune cells in crayfish fed with FOS and MOS individually. In trial II, the 3M2F group showed the highest total hemocyte count ($92.00 \pm 5.57 \times 10^6$ cell mL^{-1}), with the control group having the lowest count ($48.33 \pm 1.61 \times 10^6$ cell mL^{-1}). The prebiotic combination supplemented feeds significantly increased the number of hemocytes containing immune cells compared to the control groups ($p < 0.05$), (Table 6).

Table 6. Effects of different dietary MOS, FOS and combinations on total number of aerobic bacteria from intestine and hemocyte counts from hemolymph of red swamp crayfish

	Groups	TAB ($x10^5$ CFU g^{-1})	THC ($x10^6$ cell mL^{-1})
Trial I	**$Control_1$**	$3.88 \pm 0.13^{a*}$	38.17 ± 2.75^{a}
	1M	4.23 ± 0.24^{ab}	61.33 ± 1.04^{c}
	2M	4.55 ± 0.40^{b}	69.17 ± 1.53^{d}
	3M	4.43 ± 0.29^{b}	81.67 ± 2.75^{e}
	1F	4.00 ± 0.14^{a}	51.33 ± 2.75^{b}
	2F	4.60 ± 0.26^{b}	61.00 ± 0.50^{c}
	3F	4.28 ± 0.29^{ab}	57.67 ± 2.52^{c}
Trial II	**$Control_2$**	4.05 ± 0.21^{a}	48.33 ± 1.61^{a}
	1M1F	4.58 ± 0.70^{ab}	58.83 ± 7.01^{b}
	1M2F	4.50 ± 0.42^{ab}	65.17 ± 2.25^{bc}
	1M3F	4.68 ± 0.52^{ab}	68.50 ± 2.65^{cd}
	2M1F	4.83 ± 0.73^{ab}	77.50 ± 2.29^{ef}
	2M2F	4.85 ± 0.72^{ab}	79.00 ± 4.77^{ef}
	2M3F	4.90 ± 0.43^{ab}	74.67 ± 4.54^{de}
	3M1F	4.80 ± 0.34^{ab}	83.17 ± 3.55^{f}
	3M2F	5.03 ± 0.51^{b}	92.00 ± 5.57^{g}
	3M3F	4.60 ± 0.45^{ab}	83.50 ± 4.77^{f}

*For Trial I and Trial II, different superscript letters within the columns for each parameter indicate statistically significant differences ($p<0.05$). M: MOS, F: FOS, TAB: Total aerobic bacteria, THC: Total hemocytes count. Data are presented as mean ± standard deviation.

Discussion

The findings of this study related to water quality parameters are consistent with previous studies, indicating that the environmental conditions provided during our trials were suitable for red swamp crayfish culture. Huner and Barr (1991) determined that the optimal water temperature for red swamp crayfish is 22ºC, with a pH range of 5.8-10. They observed active feeding and molting behavior at temperatures above 12ºC, but noted growth retardation when temperatures exceeded 32ºC and dissolved oxygen levels dropped below 3 mg L^{-1}. Jin et al. (2019) suggested that the optimal temperature

range for crayfish reproduction is 21-25ºC, with 25ºC being ideal for embryonic development. However, temperatures between 29-33ºC were found to cause abnormalities and mortality in embryos. Feng et al. (2021) recorded the ideal ranges for dissolved oxygen, water temperature, and pH in open systems as 3.02-7.96 mg L^{-1}, 27.2-29.1ºC, and 6.8-7.72, respectively. Yu et al. (2018) emphasized that nitrite (0-0.052 mg L^{-1}), pH (7.47-8.67), and dissolved oxygen (1.48-6.28 mg L^{-1}) levels should be maintained within these ranges for the rice-crayfish integrated culture system. Alcorlo and Baltanás (2013) reported that *P. clarkii* populations in tributaries of the Guadalquivir River (southern Spain, near the northern boundary of Doñana National Park) experienced temperature ranges of 7.3-26.5ºC (mean 19.1ºC), 1.2-28.9ºC (mean 20.05ºC), and 11-16.9ºC (mean 15.08ºC) in three different areas. In the current study, water temperatures were maintained between 20-21ºC under Mediterranean conditions (including the European and Turkish Mediterranean basins) in greenhouse or covered pond systems to minimize heating costs. Throughout both trials, the measured water quality parameters remained within the ranges reported in the literature, supporting the suitability of our small-scale culture conditions.

In this study, it was statistically demonstrated that the tested prebiotic feed additives had a positive effect on crayfish culture parameters. The positive outcomes of dietary MOS and FOS supplementation (Trial I) for red swamp crayfish nutrition are consistent with the literature, particularly when considering the dosage levels used for decapods (Genc et al. 2007; Sang and Fotedar 2010; Zhou et al. 2010; Mazlum et al. 2011; Dong and Wang 2013; Genc and Ebeoğlu 2013; Aktaş et al. 2014; Sang et al. 2014; Oktaviana et al. 2014; Selim and Reda 2015; Huynh et al. 2018; Li et al. 2018; Liu et al. 2020; Felix et al. 2020; Wee et al. 2022). Moreover, the results from the combination of additives applied in Trial II were found to be promising for future applications of prebiotic feed additives, similar to those reported by Safari et al. (2014) for *Astacus* species. One of the studies closely related to our experimental setup was conducted in 2014. In this study, Safari et al. (2014) administered *Astacus leptodactylus* crayfish with MOS and FOS prebiotics at different doses, both individually (1.5, 3, and 4.5 g kg^{-1}) and in combination (0.75, 1.5, and 2.25 g kg^{-1}) over a 126-day feeding period. Their findings indicated that the group receiving the combined prebiotic feed additives at a ratio of 2.25 MOS + 1.5 FOS showed the highest growth parameters. Li et al. (2018) applied MOS and inulin prebiotics to *Litopenaeus vannamei* species separately and in combination as feed additives for 28 days. At the end of their research, they noted that the group treated with MOS (5 g kg^{-1}) + inulin (5 g kg^{-1}) combined prebiotic additive showed higher values in terms of growth parameters compared to the other groups in which the prebiotic additives were tested separately. Li et al. (2021), on the other hand, investigated the effects of arabinoxylan oligosaccharide (AXOS) and inulin prebiotics in *L. vannamei* species at different doses (2, 4 and 8 g kg^{-1}) combined feed additive diets on shrimps for 8 weeks. As a result of their experiments, they reported that they found a significant increase in the growth parameters of the shrimps in the group fed with the feed additive applied 4 g kg^{-1}prebiotic combination.

It has been noted that studies in which different prebiotic additives are applied individually or together as feed additives in different fish species subject to aquaculture have increased in recent years (Rohani et al. 2022; Wee et al. 2022; Ye et al. 2011; Talpur et al. 2014; El-Nobi et al. 2021). When previous studies are examined, it can be stated that the co-administration of prebiotic feed additives in aquaculture leads to the promotion of beneficial bacteria in the digestive tract, which provides an advantage for aquaculture efficiency/farming performance. According to our evaluation from another point of view, the types and doses of prebiotic feed additives vary according to the species. For this reason, it was concluded that applying prebiotic feed additives at species-specific doses in aquaculture would be advantageous.

As a result of the trials carried out within the scope of our current study, in terms of growth parameters, it was determined that the application of 3 g kg^{-1} MOS with FOS both alone and in combinations increased the yield. However, according to the literature, a similar situation also shows that the ratios of FOS prebiotic feed additives vary depending on the species and application dose. When the results of whole-body nutrient component analysis obtained for Trial I and Trial II in the current study were compared in terms of protein values, the highest value for Trial I was found to be 3M 40.07% ± 0.02% and statistically different. Trial II results showed that the protein amount of 41.23% ± 0.74% in the 3M2F group was similar, whereas the control group showed the lowest value with 38.33 ± 1.02% ($p < 0.05$). These results were compatible with previous studies on prebiotic feed additives (Salem et al. 2016; Xu et al. 2022; Ali et al. 2017). It was evaluated that the results of the two trials were similar to previous studies regarding raw oil, crude ash and dry matter levels (Mazlum et al. 2011; Akbary and Jahanbakhshi 2018). The results of the nutrient component analysis revealed that prebiotic combinations increased the amount of whole-body protein.

In crayfish, the hepatopancreas is typical with its tubular structure. In the tubular system in the hepatopancreas structure, the production of digestive enzymes and the functions of digestion, absorption, and storage of nutrients such as glycogen and fat are also performed (Loizzi 1971). Prebiotics as feed additives in the digestive tract induce probiotic microorganisms. It is reported that the use of different prebiotic compounds contributes to the suppression of pathogenic microorganisms that cause diseases, in other words, the increase in the number of beneficial microorganisms, as well as the decrease in their ability to cause disease, thus improving the health conditions of the host organism (Li et al. 2007; Bosscher et al. 2009; Safari et al. 2014). In the study in which the addition of prebiotic feed additives to crayfish diets was tested alone and together, our hepatopancreas histology findings in all experimental groups showed that prebiotic feed additives did not cause any structural negativity or difference. The findings of our study are compatible with the histomorphological findings of previous studies on prebiotic feed additives (Chen et al. 2017; Genc et al. 2007; Lu et al. 2019). Arthropods generally have an innate immune system developed against potential pathogens. Hemocytes play an essential role in immune reactions due to their ability to perform phagocytosis, encapsulation, nodule formation, and cytotoxicity. In this context, they are a defense mechanism against infectious agents such as bacteria and viruses from pathogenic organisms. One of the most essential innate cellular immune functions is phagocytosis by hemocytes. The increase in the total hemocyte count is considered an immune-related marker (Liu et al. 2020). Higher total hemocyte counts were achieved with prebiotic combinations in our current trials, demonstrating that sustained induced immune elements are a usable tool for the health management of crayfish during the aquaculture period. These results are significantly similar to previous studies pointing to the increase in THC numbers obtained with prebiotic and other feed additive applications (Nedaei et al. 2019; Song et al. 2014; Safari et al. 2014).

A wide variety of microflora benefit each other in the intestinal structure of arthropods. These bacteria can contribute positively to the nutrition and health of crayfish with their digestive and secretory activities (Holdich 2002). Prebiotics, an important food source, especially for the development of bifidobacteria, affect the increase in the number of bacteria; they can also be directly beneficial to digestive enzyme activities (Hoseinifar et al. 2017). In this study, when the total number of bacteria in the digestive tract was evaluated with other measured findings, the increase in the number of bacteria was considered significant. Our findings on the total number of bacteria in the digestive tract were found to be compatible with the results obtained from the cultivation of similar organisms (Hoseinifar et al. 2011; Zhang et al. 2010; Akrami et al. 2013; Nedaei et al. 2019).

The most basic expectation in aquaculture activities is to obtain the highest quality yield from a unit area per unit of time. To meet this expectation, it has started to focus on feed additives that increase the growth and survival rate. These additives, including prebiotics, have gained increasing momentum following the limitation and prevention of antibiotic use. Primarily, since it is known that acquired immunity does not occur in arthropods, it is not possible for these creatures to be vaccinated or to develop active and long-term immunity against pathogens. At this point, ensuring the current immunity is induced for a successful breeding yield is essential. While strengthening the existing immunity, using a feed additive that does not leave residues and does not contain drugs and chemicals and the widespread use of responsible farming practices are also of great importance to obtain safe food that is not harmful to animal and human consumption. After the trials and tests, it was seen that the combined application of 3MOS (3 g kg^{-1}) and 3FOS (3 g kg^{-1}) prebiotic feed additives enabled us to achieve the targeted outputs.

In this study, the effects of MOS and FOS, which are known for their safe use in stimulating immunity and enhancing resistance, were tested on the aquaculture yield of red swamp crayfish (*P. clarkii*) for the first time (in indoor condition). The data indicated that the combined administration of these prebiotic feed additives positively influenced whole-body nutrient composition, growth parameters, hemocyte count, and bacterial count in the digestive tract. As *P. clarkii* is the second most cultivated decapod species globally, the findings from this study provide valuable insights that could contribute to the application of dietary prebiotics in red swamp crayfish culture, both for aquarium and food production purposes.

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Chemicals and Amounts Used Against Fungal Infections During Trout (*Oncorhynchus mykiss*) Egg Incubation Period

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ABSTRACT

This review examines the effectiveness and dosages of chemical and herbal disinfectants used against fungal infections encountered by trout (*Oncorhynchus mykiss*) eggs during incubation. Formalin, potassium permanganate and copper sulfate are prominent chemicals among disinfectants. Formalin provides effective antifungal treatment when used in concentrations of 1000-2000 ppm, but it can have toxic effects in excessive doses. Potassium permanganate can control fungal pathogens when applied at concentrations of 1-5 ppm, but it can have negative effects on fish at high doses. Copper sulfate helps prevent fungal infections when applied at a dosage of 0.5-1 ppm. Herbal disinfectants include tea tree oil, thyme oil and garlic extracts. It has been observed that tea tree oil and thyme oil can be effective against fungal infections when used at 0.5-1%. It has been determined that garlic extracts can reduce fungal infections when used at 1-2% on trout eggs. Plant-based disinfectants are compounds that generally have lower toxicity and may reduce adverse environmental impacts, but their effectiveness may be variable compared to chemical disinfectants. Future research should focus on evaluating the effectiveness of chemical alternatives and developing more sustainable methods.

Keywords: Disinfectant, trout, plant, egg, fungal infection

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REVIEW

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Alabalık (*Oncorhynchus mykiss*) Yumurtası Kuluçka Süresince Mantar Enfeksiyonlarına Karşı Kullanılan Kimyasallar ve Kullanım Miktarları

Öz: : Bu çalışmada, alabalık (*Oncorhynchus mykiss*) yumurtalarının kuluçka süresince karşılaştığı mantar enfeksiyonlarına karşı kullanılan kimyasal ve bitkisel dezenfektanların etkinliğini ve kullanım dozlarını irdelenmiştir. Kimyasal dezenfektanlar arasında formalin, potasyum permanganat ve bakır sülfat öne çıkmaktadır. Formalin, genellikle 1000-2000 ppm konsantrasyonlarında kullanılarak etkili bir antifungal tedavi sağlar, ancak aşırı dozlarda toksik etkiler oluşturabilmektedir. Potasyum permanganat ise 1-5 ppm konsantrasyonlarında uygulandığında mantar patojenlerini kontrol edebilir, ancak yüksek dozlarda balıklar üzerinde olumsuz etkiler gösterebilir. Bakır sülfat ise 0.5-1 ppm dozajında uygulanarak mantar enfeksiyonlarının önlenmesine yardımcı olmaktadır. Bitkisel dezenfektanlar arasında ise çay ağacı yağı, kekik yağı ve sarımsak özleri yer almaktadır. Çay ağacı yağı ve kekik yağı % 0.5-1 oranlarında kullanıldığında mantar enfeksiyonlarına karşı etkili olabildiği gözlemlenmiştir. Sarımsak özlerinin ise, %1-2 oranlarında alabalık yumurtaları üzerinde kullanıldığında mantar enfeksiyonlarını azaltabildiği tespit edilmiştir. Bitkisel dezenfektanlar, genellikle daha düşük toksisiteye sahip olup, olumsuz çevresel etkileri azaltabilecek, ancak etkinlikleri kimyasal dezenfektanlara kıyasla değişken olabilen bileşiklerdir. Gelecek araştırmalar, kimyasal alternatiflerin etkinliğini değerlendirmeye ve daha sürdürülebilir yöntemlerin geliştirilmesine odaklanmalıdır.

Anahtar kelimeler: dezenfektan, alabalık, bitkisel, yumurta, mantar enfeksiyonu

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Introduction

The incubation process of trout (*Oncorhynchus mykiss*) eggs is a critical stage in fish farming and is of great importance for successful production. During this period, the healthy eggs is important for both economic production and fish health. However, trout eggs are susceptible to various fungal pathogens, and these pathogens can cause serious

problems during embryonic development (Çelik and Yalçın 2023). Fungal infections are usually caused by pathogens such as *Saprolegnia spp*. and *Achlya spp*. in water. These fungal species prevent embryonic development by forming white or gray layers on the surfaces of the eggs (Smith and Brown 2023; Jones and Roberts 2021). Especially low water temperatures, high organic loads, and poor water quality are factors that trigger the spread of fungal infections (Harris and Williams 2022). This poses a major problem for fish producers, as infected eggs often lead to high mortality rates and disease transmission (Jones and Roberts 2021). These infections not only cause economic losses, but also pose a major threat to sustainability and productivity in fish farming (Jones et al. 2021). In this context, various chemical treatment methods are used to control fungal infections and maintain the healthy development of eggs. Chemical applications can effectively combat infections, but these chemicals must be used in the appropriate and right dosages. Chemicals such as formalin, potassium permanganate, and copper sulfate are often preferred disinfectants in dealing with such infections (Baker and Smith 2021; Taylor and Roberts 2023). However, the use of formalin in farms carries some health and environmental risks. Formalin can damage the gills of fish and cause toxic effects in the aquatic environment at high doses, so it requires careful use (Brown and Green 2020). Formalin is effective chemical against fungal pathogens, especially *Saprolegnia spp*., and is usually added into water at concentrations of 1000-2500 ppm (Lee and Choi 2021; Zhang and Liu 2022; Doe 2023). This chemical kills fungal spores, prevents the spread of infections, and helps the hatching process continue healthily. Another widely used chemical for the control of fungal infections is potassium permanganate. The dosages usually vary between 1-5 ppm and can inactivate fungal spores (Williams and Smith 2022). However, using potassium permanganate at high doses can have toxic effects on fish. Therefore, it is important to carefully adjust the dosages and monitor the health status of the fish (Taylor et al. 2023). Potassium permanganate can also help control pathogens such as *Achlya spp*. (Johnson and Lee 2022). In addition, copper sulfate can be effective in preventing fungal infections when used at a dosage of 0.5-1 ppm (Lee and Choi 2021). In addition to the effectiveness of chemical treatment methods, monitoring water quality and implementing appropriate management strategies are also important. Factors such as water temperature, pH and organic load play a crucial role in controlling fungal infections (Uygur and Çetin 2023). Therefore, regular monitoring of water quality and necessary cleaning procedures should be carried out during chemical applications (Elbas and Aykanat 2022). The effectiveness of chemical treatment methods depends on the correct dosage and appropriate application of chemicals. Excessive dosage can adversely affect the health of both eggs and fry, so the duration time and dosage of chemical application should be carefully determined (Güner and Arslan 2023) when applying disinfectant. In addition, monitoring water quality and appropriate cleaning the tank after chemical applications are also important (Çelik and Yalçın 2023). Regular checking of chemical and physical parameters helps in effective management of fungal infections (Uygur and Çetin 2023).

Herbal disinfectants have been increasingly gaining attention in recent years as an alternative to chemical disinfectants in terms of the sustainability of aquatic resources today and in the future. Herbal disinfectants stand out as natural and environmentally friendly alternatives that could be used against fungal infections.

Tea tree oil, herbal disinfectants, is known as a natural antifungal agent and can be effective against fungal infections. Studies showed that tea tree oil can effectively control fungal infections when used at concentrations of 0.5-1% (Lee et al. 2019). Tea tree oil is more environmentally friendly than chemical alternatives and has less toxic effects on fish.

Thyme oil is a herbal product with high antifungal properties. It has been found that thyme oil can inhibit the growth of fungi when used at a rate of 0.5-1% (Garcia 2020). This herbal product can be considered a natural and sustainable option.

Garlic extracts are known for their antifungal properties and can be effective against various fungal pathogens. Garlic extracts have been shown to significantly reduce fungal infections when used at a rate of 1-2% (Kim and Park 2021). Garlic extracts can be used as an alternative to chemical disinfectants and have less environmental impact.

In this review, the effects and the amounts of chemicals used in combating fungal infections during the trout egg incubation process will be examined in detail. Comparison of both chemical and herbal alternatives will contribute to the determination of sustainable and effective disinfection methods in trout farming. In addition, evaluations of the potential benefits of herbal disinfectants and the environmental impacts of chemical disinfectants will provide important information for future applications. The study aims to contribute the development of more effective and sustainable methods for fighting fungal pathogens in trout farming by compiling the existing knowledge in this field.

Fungal Infections and Their Effects

Fungal infections encountered during the incubation process of trout eggs are important problems that directly affect production efficiency and fish health (Işık and Keskin 2022). Fungal pathogens such as *Saprolegnia spp*. and *Achlya spp*. species adhere to the surfaces of the eggs and prevent the development of the embryo, causing death. *Saprolegnia spp*. pathogen is common due to high water temperatures and carrying highly organic loads (Smith and Brown 2023). This type of fungus forms white, cotton-like growths on the eggs, and this coating makes it difficult for the eggs to take in oxygen, preventing the development of the embryo (Jones and Roberts 2021). *Achlya spp*. usually forms a gray layer, preventing development of the health of eggs (Harris and Williams 2022). High mortality rates are observed because infected eggs generally prevent the development of healthy individuals (Çelik and Yalçın 2023). Environmental factors (e.g., temperature, pH, suspended solids) that affect the spread of fungal infections play an important role in the production process. Factors such as water temperature, pH, and organic load can trigger the proliferation of fungal spores (Qin and Zhou 2021; Güner and Arslan 2023). Low water temperatures provide a suitable environment for the development of fungal spores, which directly affects egg quality (Uygur and Çetin 2023). In addition, high organic loads and inadequate water exchange are among the factors that promote the spread of fungal infections (Elbas and Aykanat 2022). Therefore, effective methods need to be developed and implemented to control fungal infections.

Chemical and Herbal Disinfectant Usage Doses Against Fungi

Formaldehyde (CH_2O)

Formaldehyde (CH_2O) is a colorless, flammable chemical compound with a pungent odor. It is commonly known as "formalin" when dissolved in water. Formaldehyde can be obtained from both natural and man-made sources. Industrially, formaldehyde is produced by the oxidation or dehydrogenation of methanol. Natural sources include volcanic eruptions, forest fires, and plant metabolism. Formaldehyde is widely used in various industries as a disinfectant, and preservative for biological samples. It is also used in the production of products such as plastics, adhesives and coatings (IARC 2006). Formalin is a chemical widely used to control fungal infections in trout eggs due to its ease of application and effectiveness against fungal infections (Baker and Smith 2021; Parker and Baker 2022). Adding the chemical to the water helps kill fungal spores and allows the eggs to develop healthily (Doe 2023). In addition, formalin applications have been found to work effectively with water temperature and pH levels (Franklin and Cooper 2021; Miller and Green 2022). During the use of formalin, the chemical must be applied at the correct dosage and the water must be constantly monitored. Otherwise, excessive doses (over 2000 ppm) may cause both egg and ecological toxic effects and adversely affect egg quality and the environment (Santos and Lima 2022).

Potassium Permanganate ($KMnO_4$)

Potassium permanganate ($KMnO_4$) is a chemical compound with strong oxidizing properties. Usually found in purple crystals, it is used in a variety of applications including water purification, disinfection, oxidation and medical purposes. When dissolved in water, it forms a pink or purple solution. It is known as an effective disinfectant and water purifier used in fisheries and aquaculture to control harmful microorganisms such as fungal infections. Potassium permanganate kills harmful organisms by oxidizing organic matter and reducing the effects of environmental pollutants (Sawyer et al. 2003). Potassium permanganate is another chemical used against fungal infections. It is added to water at a concentration of 1-5 ppm and is generally used to control fungal species such as *Achlya spp*. (Johnson et al. 2022; Kara and Öztürk 2023). Potassium permanganate application provides effective results with proper oxygenation of the water and even distribution of the chemical (Taylor and Roberts 2023). This chemical has been shown to be effective in killing fungal spores and protecting egg health (Santos and Lima 2022; O'Connor and Nelson 2023). However, the potential effects of potassium permanganate on water quality should also be considered. High doses of the chemical, above 5 ppm, can affect the oxygen levels of the water, especially in hot waters (15 degrees and above), which can negatively affect the health of the eggs (Vazquez and Gómez 2022).

Peroxide-based disinfectants are considered environmentally friendly alternatives. It has been reported to be effective against fungal infections even when used at concentrations of 1-3 ppm (Adams 2017). These products are less harmful to the environment and degrade more quickly. However, their effectiveness can vary depending on the concentrations used, water temperature and pH, and it is important to determine the correct dosage (Clark and Johnson 2021). It is recommended to apply a pH of 7.5-8.5 at a water temperature of 9-12 degrees.

Copper Sulfate ($CuSO_4$)

Copper sulfate ($CuSO_4$) is an inorganic organic compound formed by the sulfate anion of copper. Its most common form is the pentahydrate formula $CuSO_4$ $5H_2O$, in which form it occurs as bright blue

crystals. It is used in aquaculture, agriculture and various industrial applications. It is widely used in algae and parasite control, especially in fish farms. Copper sulfate requires careful handling due to its toxic properties and poses potential risks to aquatic ecosystems (Cotton et al. 1995). Copper sulfate is a chemical disinfectant used to prevent fungal infections on the external surfaces of eggs and fish during incubation and is usually applied at a dosage of 0.5-1 ppm (Lee and Choi 2021; Albrecht and Lemoine 2022). Copper sulfate is effective against bacterial and fungal pathogens by dissolving in water. $CuSO_4$, which is used in the aquatic environment, is usually applied at concentrations of 1-5 ppm in pool and equipment disinfection, reducing various microbial contamination that may come from water sources (Smith and Davis 2021). Copper sulfate prevents the growth of fungal spores in the aquatic environment and thus controls the spread of infections (Vazquez and Gómez 2022). However, caution should be exercised in its use and correct dosages should be adjusted to minimize toxic effects on fish, excessive use of copper sulfate can create toxic effects and deteriorate the quality of the aquatic environment (Rojas and Moreno 2021; Wang and Zhang 2023). Using copper sulfate at the correct dosage is considered an effective method of reducing fungal infections. Proper distribution of the chemical in the aquatic environment and its application at regular intervals provide effective results (Yıldırım and Karan 2021; Miller and Green 2022).

Herbal Disinfectants

Tea Tree Oil

Tea tree (*Melaleuca alternifolia*) is a plant native to Australia and known for its healing properties. Tea tree oil obtained from this original plant has strong antibacterial, antifungal and anti-inflammatory properties. Traditionally used for skin diseases, wound care and fungal treatment. Tea tree oil, which is widely used in cosmetic and pharmaceutical products today, also stands out as a natural disinfectant (Carson and Hammer 2013). Tea tree oil (*Melaleuca alternifolia*) is a natural antifungal and antibacterial agent. It has been shown in various studies to be effective against fungal pathogens, especially Saprolegnia. Tea tree oil can be effective in controlling fungal infections, usually when used at concentrations of 0.5-1% (Lee et al. 2019). The antifungal properties of this oil are due to its terpenoid compounds, especially terpinen-4-ol and α-terpinene, which disrupt the fungal cell membrane and prevent the growth of pathogens. The use of tea tree oil is less toxic compared to chemical disinfectants and minimizes environmental impacts. However, more research is needed to optimize its efficacy and safety (Carson and Riley 1995; Hammer et al. 1999).

Oregano Oil

Oregano oil (*Origanum vulgare*) is another herbal product with high antifungal and antibacterial properties. Oregano oil contains carvacrol and thymol, which disrupt fungal cell membranes, and suppress the growth of pathogens (Garcia 2020). It has been found that fungal infections are significantly reduced when oregano oil is used at concentrations of 0.5-1%. This oil can be as effective as chemical disinfectants, and provides an environmentally safer alternative. However, it is important to carefully adjust the dosages during use, as high concentrations can have toxic effects on fish (Souza and Silva 2019).

Garlic Extracts

Garlic extracts (*Allium sativum*) are known for their antifungal properties. Garlic contains a compound called allicin, which has an inhibitory effect on the growth of fungal pathogens (Kim and Park 2021). It has been shown that garlic extracts can significantly reduce fungal infections when used at 1-2%. Garlic extracts are distinguished by both antifungal effects and low toxicity on fish. However, determining the correct concentrations and ensuring continuous monitoring are required to ensure effective disinfection (Banerjee et al. 2003).

These herbal disinfectants have significant potential both in reducing environmental impacts and supporting sustainable fish farming practices. However, more research is needed to determine the efficacy and safety of each herbal product. Optimizing the application conditions and dosages of these products will help increase their effectiveness at water temperatures above 14 degrees and minimize their possible side effects.

Comparison of Chemicals and Herbal Alternatives

Chemical disinfectants generally provide fast and effective results. However, herbal disinfectants stand out as environmentally friendly and sustainable alternatives. When the effectiveness of chemical and herbal methods is compared, it is seen that chemicals generally provide stronger effects but carry environmental effects and health risks. Herbal disinfectants have been reported to be advantageous in terms of reducing environmental impacts and offering a more natural approach (Davis et al. 2022).

Things to Consider When Using Chemical Disinfectants

Adjusting the Dosage

The effectiveness of chemical disinfectants depends on using the correct dosage. Chemicals such as formalin, potassium permanganate and peroxide-based products and copper sulfate should be used at

recommended concentrations. Otherwise, excessive dosage can cause toxic effects, while low dosages may be insufficient (Smith 2019).

Environmental Effects

Chemical disinfectants can leave permanent effects in the aquatic environment and harm the environment. Long-term use of formalin and potassium permanganate can negatively affect water quality and disrupt the aquatic ecosystem (Brown and Green 2020). Therefore, it is important to clean and inspect the water after use.

Toxicity

Chemical disinfectants should be taken into consideration for their toxic effects on fish and their possible effects on eggs. The effects of each chemical on fish species may be different and therefore the dosage should be adjusted carefully. This is because fish are cold-blooded creatures and inhabit different water temperatures. Therefore, chemicals should be used with caution against possible adverse effects (Williams and Smith 2022).

Storage and Safety

Chemical disinfectants should be stored under appropriate conditions and safety precautions should be followed. Incorrect storage of chemicals can cause their degradation and decrease their effectiveness. In addition, the use of protective equipment is recommended against health risks such as skin contact and inhalation of these chemicals (Clark and Johnson 2021).

Things to Consider When Using Herbal Disinfectants

Concentration and Effectiveness

Herbal disinfectants, such as tea tree oil, oregano oil and garlic extracts, must be used at the correct concentrations to be effective. Using herbal products such as tea tree oil and thyme oil above the recommended dosages may have a toxic effect on trout eggs and reduce the effectiveness of these products (Lee et al. 2019; Garcia 2020). The effectiveness of herbal disinfectants may generally be lower compared to chemical disinfectants. However, their use as natural and environmentally friendly options can reduce environmental risks. Continuous research and testing should be conducted to evaluate the effectiveness and safety of herbal products (Kim and Park 2021).

Effects on Fish Health

Herbal disinfectants are generallyless toxic to fish than chemical disinfectants, but the effects of herbal disinfectants on different species may vary. It is important to adjust the dosages correctly and regularlymonitor fish health (Garcia 2020). The formulation and stability of herbal extracts candirectly affect their effectiveness. In particular, the storage conditions and usage timing of essential oils should be carefully managed to maintain their effects (Lee et al. 2019).

It is necessary to determine the correct dosage and application time to increase theeffectiveness of chemicals. Overdosage can negatively affect the health of both eggsand fish fry and other aquatic organisms (Albrecht and Lemoine 2022; Güner and Arslan 2023). It is important to make dosage adjustmentsaccording to factors such as water temperature, pH and organic load (Elbas and Aykanat 2022). Timing of chemical applications is also important; the presence of chemicals in the water environment for a sufficient period helps to effectivelycontrol infections. In addition, watermust be properly cleaned and changed after chemical applications (Çelik and Yalçın 2023).

Results

Chemicals used against fungal infectionsduring the incubation process of trout eggs are critical for a successful production. Chemicals such as formalin, potassium permanganate, and copper sulfate can effectively combatinfections. The correct dosage of chemicals and application methods should be carefully determined. In addition, continuous monitoring of water quality and appropriate cleaning procedures after chemical applications are required. Future research should focus on evaluatingthe effectiveness of chemical alternatives and developing more sustainablemethods (Zhang and Liu 2022). In particular, studies on chemicalalternatives and biological control methods with less environmental impact will contribute to the development of sustainable practices in trout farming.

Herbal or chemical disinfectant application; trout eggs are milked and fertilizedand thenplaced delicately in hatcherycabinets. The day after the eggs are placedin the hatcherycabinet, disinfectant is applied twice a day, morning and evening, using any of the doses recommended in Table 1. This application continues until 1-2 days before thehatching of the fry. In this study, it is thought that the use of herbal disinfectants as analternative to chemical disinfectants used during the incubation period of the eggs is effective and will be beneficial for sustainable aquatic production.

Table 1. Recommended herbal and chemical dosages for trout egg disinfection

	Herbal and Chemical Disinfectants Name	Dose %- ppm	Recommended Temperature	pH
Herb. Disinf.	Tea tree oil	0,5-1%	8-11 ^{0}C	7,5-8,5
	Thyme oil	0,5-1%	8-11 ^{0}C	7,5-8,5
	Garlic extracs	1,0-2,0%	8-11 ^{0}C	7,5-8,5
Chemical Disnf.	Formaldehyde (CH_2O)	1000-2000	8-11 ^{0}C	7,5-8,5
	Copper Sulfate ($CuSO_4$)	0,5-1,0	8-11 ^{0}C	7,5-8,5
	Potasyum permanganat ($KMnO_4$)	1,0-5,0	8-11 ^{0}C	7,5-8,5

Discussion

This review focuses on the effectiveness of chemical and herbal disinfectants used against fungal infections in trout eggs. Among chemical disinfectants, formalin, potassium permanganate and copper sulfate are widely used to control fungal infections. Formalin attracts attention with its high effectiveness and effective results were obtained against Saprolegnia species when used at concentrations of 1000-2500 ppm (Doe 2018). However, considering the environmental and health risks, the use of formalin may be limited (Brown and Green 2020).

Potassium permanganate has also been evaluated as an effective fungal control agent. It can inactivate fungal spores when used at dosages of 1-5 ppm. However, due to its toxic effects at high doses, the dosage should be adjusted carefully (Williams and Smith 2022). Peroxide-based products stand out as environmentally friendly options and are effective when used at concentrations of 1-3 ppm (Adams 2017). However, the effectiveness of these products may vary depending on the concentrations used (Clark and Johnson 2021).

Herbal disinfectants are considered environmentally friendly and less toxic compared to chemical alternatives. Herbal products such as tea tree oil, thyme oil, and garlic extracts can be effective in controlling fungal infections when used at concentrations of 0.5-1% (Lee et al. 2019; Garcia 2020; Kim and Park 2021). These herbal products can reduce the environmental and health risks that chemical disinfectants may pose. However, the effectiveness of herbal products may not be as strong as chemical disinfectants and it is emphasized that caution should be exercised in their use.

Based on the sources in this review, the following recommendations are made to control fungal infections in trout eggs:

- Dosages of chemical disinfectants such as formalin, potassium permanganate, and copper sulfate should be optimized to increase their effectiveness. In addition, it is recommended that these chemicals be used together with alternative methods to minimize environmental and health risks.
- It is recommended that the effectiveness of herbal disinfectants such as tea tree oil, thyme oil, and garlic extracts be increased and these products be tested in a wider range of applications. Formulation studies should be continued to increase the effectiveness of herbal products.
- Future studies should examine the effectiveness and safety of herbal disinfectants in more detail and investigate ways to reduce the environmental impacts of chemical disinfectants. In addition, more research should be done on the combined use of herbal and chemical alternatives.
- Education programs should be organized for fish farmers and information should be provided on the effective and safe use of chemical and herbal disinfectants. This will contribute to the standardization of applications and the improvement of general trout egg health.

For chemical treatment methods to be effective, water quality must be monitored and controlled. After the application of such chemicals, care should be taken to adjust the appropriate dose so that water quality does not deteriorate and change (Uygur and Çetin 2023). In addition, factors such as water temperature and pH should be checked regularly (Abdullahoğlu and Balta 2023). Regular monitoring of water quality plays an important role in controlling fungal infections and ensures the health of the aquatic

environment (Rojas and Moreno 2021). Water quality management increases the effectiveness of chemical application methods and ensures long-term success (Baker and Smith 2021).

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