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# Spatial and Temporal Variations in Water Quality Along the Bua River, Malawi

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## ABSTRACT

Water quality in freshwaters is declining worldwide due to increases in human populations, expansion of agricultural activities, and climate change. There are quite a number of regions of the world, Africa inclusive, that are understudied, and little to no baseline information exists related to water quality. This study was focused on the Bua River in Malawi, which supports sustenance fishing and basic needs for local communities. A portion of the river has elevated levels of protection because it is found within the Nkhotakota Wildlife Reserve. The focus of this study was to understand the spatial-temporal variations of water quality at five sites in the Bua River from May 2018 to June 2020 capturing the three main seasons (warm wet, cool dry, and hot dry). Although other water quality parameters did not vary spatially. Spatially, the Bua River mouth registered the highest values of Soluble reactive phosphorus and the Bua River upstream had the lowest. However, there were greater temporal differences across seasons for water temperature, water pH, and chlorophyll a. For instance, chlorophyll-a was higher during the hot dry season (3.28 µgL-1) compared to the cool dry season (2.10 µgL-1) and warm wet season (1.91 µgL-1). Water transparency, as measured by secchi depth was lowest during the warm wet season, which coincides with higher concentrations in SRP. All measurements of salt content, Electrical Conductivity (EC), Total Dissolved Solids (TDS), and salinity) were higher during the hot dry season and correlated negatively with SRP. Similarly, bicarbonate and alkalinity were also higher during the hot dry season. Principle Component Analysis indicated that the parameters responsible for variations of Bua River water quality are mainly related to soluble minerals, water temperature, and surface runoff associated with agricultural activities and domestic waste accounting for 78.49 % of the total variance in the data set.

**Keywords:** Water quality, freshwater habitats, principal-components analysis, seasonality, Southern Africa

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## Introduction

Rivers support various ecosystem services worldwide and provide important societal services, including food, water for domestic use (washing clothes, drinking water, cooking uses, and watering livestock), agriculture, hydroelectric power generation, navigation, and industrial production. These sustained pressures on rivers lead to fragmentation, habitat degradation, pollution, loss of connectivity, and a decline in water quality affecting many fundamental processes and functions. Sometimes, balancing these competing ecosystem services that rivers provide among the different resource users become a main concern worldwide (Islam et al. 2017).

Surface water quality of rivers is influenced by anthropogenic influences (urban, industrial, agricultural, exploitation of water resources) and natural processes (changes in precipitation, erosion, weathering) degrade surface water and impact their ecosystem services.

The aquatic ecosystems have been continuously modified by agriculture, disposals from urban, mining and industrial wastes, and engineering modifications to the environment and inappropriate resource management along the catchments globally (Allan 2004). More industries would mean increased discharge of effluent and other wastes into Rivers, whereas fertilizers from agricultural runoff are the main source of nutrients in aquatic ecosystems. The increased nutrients may lead to increased primary productivity, a phenomenon commonly called cultural eutrophication. The effects of eutrophication include oxygen depletion in the water column (Aure and Stigebrandt 1990), increased phytoplankton production and algal blooms (Dillion and Rigler 1974), presence of cyanobacteria associated with organic enriched nutrients (Laws 2000) and impacts on macro invertebrates and other benthic communities. Thus, addition of large amounts of exogenous nutrients, whether from agricultural runoffs or other point or non-point sources will not only increase phytoplankton biomass, but will also favour nitrogen fixing bacteria and the presence of potentially toxic phytoplankton species to fish and humans (Guildford et al. 1999). Overall, degradation of River water quality will have detrimental negative impact to fish and other aquatic organisms.

The pollution of rivers and aquifers is a growing threat to freshwaters in southern Africa (Dan-Hassan et al. 2012; Amadi 2010). River pollution results in changes in water quality and quantity, as well as in loading of silt and other materials, which directly affect a river's form (Limuwa et al. 2013). More generally, the majority of pollution into rivers originates from industrial and domestic wastewater as well as agricultural drainage (Carpenter et al. 1998; Jarvie et al. 1998). Seasonal variations in both anthropogenic activities and natural processes such as temperature and precipitations, affect the quality of river water and lead to different attributes between seasons (Vega et al. 1998).

Inappropriate use of the water resources has an immediate impact on the livelihoods of some of the world's most vulnerable human communities such as fishers, as they rely on the water resource for basic needs. Current data indicate that the Bua River fishery continues to dwindle due to overfishing, but little has been done to elucidate the effect of water quality and habitat alterations on *Opsaridium microlepis* and other Riverine fish species. As noted by Limuwa et al. 2013 that *O. microlepis* is facing serious problems not only as a result of fishing pressure, but also from environmental degradation, such observations call for measures to monitor the River ecosystem to come up with good management practices.

Our objective was to understand the temporal-spatial variations of water quality throughout the Bua River, Malawi in order to understand its ability to fully support competing ecosystem services that it currently provides. Large gaps remain in our understanding of water quality in many key rivers throughout the world, but particularly in understudied areas in Africa. Lake Malawi/Nyasa support a unique biodiversity of fishes and its rivers, such as the Bua River, provide an important connection between the Lake and its surrounding landscape. Therefore, it was evaluated on a monthly basis, the water quality at five sites in the Bua River from May 2018 through June 2020 capturing the three main seasons (warm wet, cool dry, and hot dry) to a provide baseline understanding of its water quality both longitudinally and over time and to identify possible pollution sources. Ultimately, our results aimed at informing the local communities about the water quality of the Bua River, and identifying some of the key factors that managers and policymakers can target for monitoring/research programs.

## Materials and Methods

### Study Area

The Bua River is one of the major rivers that drains into the western coast of Lake Malawi/Nyasa, Malawi flowing through the Nkhotakota Wildlife Reserve where it has elevated levels of protection. It has a catchment area of 10,658 km$^2$, it is 186 km long, and its width varies from 16 to 87 km (Kelly et al. 2019). The climate of the Bua River catchment is classified as sub-tropical (Government of Malawi 2017) with three seasons, including warm wet (1st November - 30th April); cool dry (1st May - 31st August); and hot dry (1st September - 31st October; Government of Malawi 2017). The average annual rainfall is estimated as 897 mm (range 800-1000 mm; Government of Malawi 2011). Our sampling sites are concentrated in the lower river from Tongole Pool in the upper extent of the Nkhotakota Wildlife Reserve, excluding administrative districts from Mchinji to Kasungu (Figure 1, Table 1). As the Bua River leaves the Nkhotakota Wildlife Reserve, it passes through agricultural lands dominated with rice fields and sugarcane plantations. Where the river meets the lakeshore floodplain, the catchment drops rapidly through a series of steep slopes leading to high levels of sedimentation across the lakeshore floodplain as the gradient flattens out (Kelly et al. 2019). This lower part of Bua River supports potamodromous fish species that migrate from the lake to spawning grounds in the mainstream and tributaries that have gravel and sandy-bottomed shallows, including Mpasa *O. microlepis*, which is an important fish for economic and cultural purposes (Tweddle 1983).

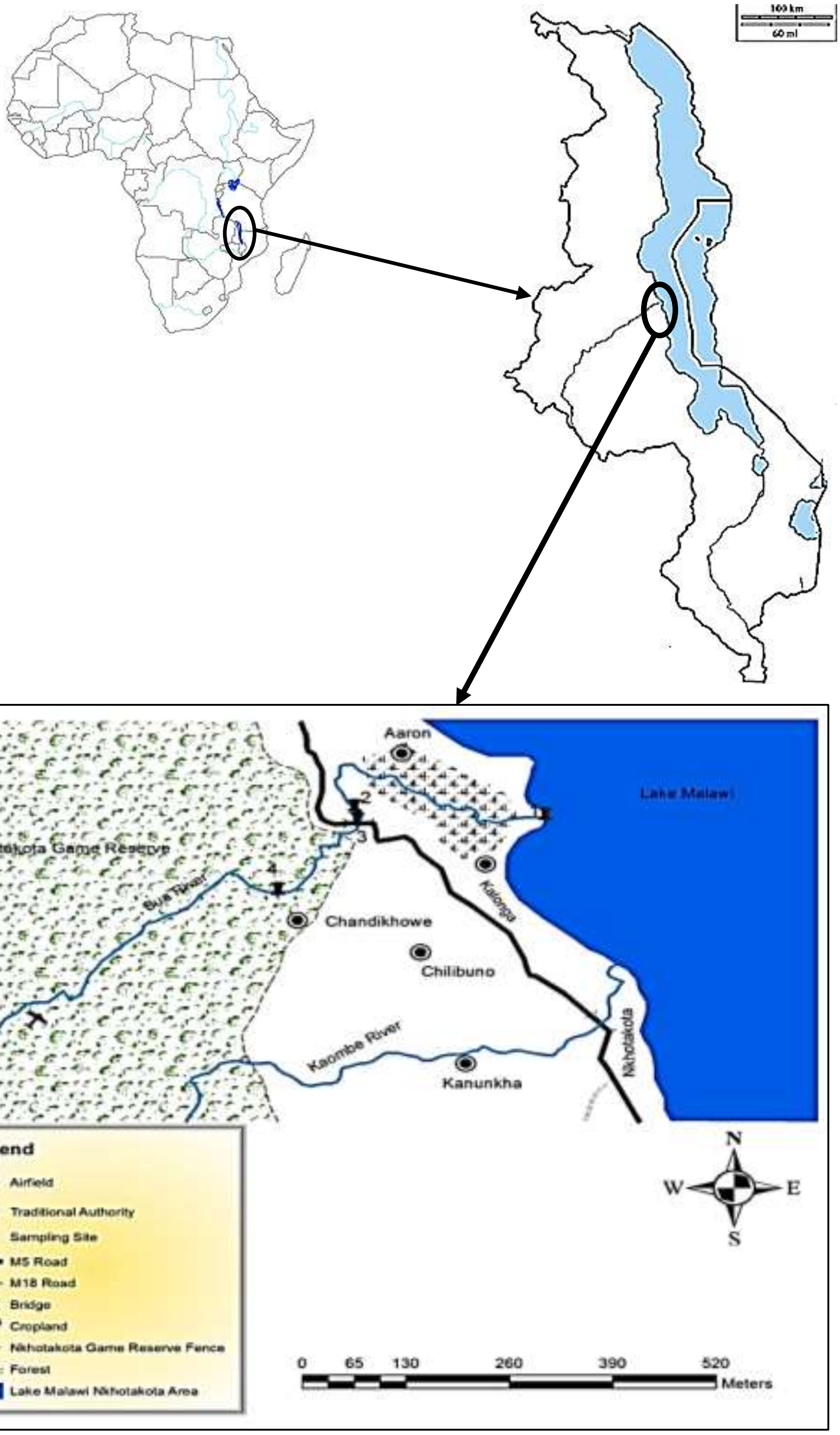


**Figure 1**. The Bua River, Malawi showing five sampling sites

**Table 1.** Description and coordinates of five sampling sites along the Bua River, Malawi from May 2018 to June 2020

| ID | Site Name | Description | GPS Coordinates |
|---|---|---|---|
| **1** | Bua Mouth | 10m upstream of confluence with Lake Malawi/Nyasa | -12°47`19.59``S 34°16`30.92``E |
| **2** | Bua Weir | Irrigation intake | -12°47`05.75``S 34°11`41.81``E |
| **3** | Bua Bridge | At road crossing (M5) | -12°47`15.86``S 34°11`46.72``E |
| **4** | Nandinga Pool | Inside the Nkhotakota Wildlife Reserve | -12°49`54.57``S 34°09`32.23``E |
| **5** | Tongole Pool | Upstream of Nandinga pool, but inside Nkhotakota Wildlife Reserve | -12°54`42.47``S 34°02`56.53``E |

**Water Sampling and Analysis**

The water samples were collected from five sampling sites across three seasons (warm wet, cool dry and hot dry). The sample collection was done monthly and targeted the first week of each month between May 2018 to June 2020, except for July 2018 and May 2020. The sample sites start where the Bua River meets Lake Malawi/Nyasa and move upstream pass a diversion dam into the Nkhotakota Wildlife Reserve. The grab samples were taken from 20 cm below the surface with 1L polyethylene bottles. The samples were stored on wet ice in a cooler before analyses for chlorophyll-a and phosphorus within 24 hours.

Water temperature (oC), pH, electrical conductivity (µS/cm), total dissolved salts (mg/L), and salinity (psu) were measured by using a multiparameter water quality monitoring instrument *in situ* (Orion ThermoScientific, 8107UWMMD/013005MD). Calibration of sensors was performed before every survey. Water transparency was also determined *in situ* using a secchi disk that is 30 cm in diameter. It was measured alkalinity and bicarbonates with acidimetric titration using the Gran function plot method (Wetzel and Likens 2000).

The water samples were stored in a freezer before analysing for SRP. After thawing, 1 L water sample was filtered immediately through 47 mm diameter GF/F Whatman filter paper. Following the protocol from Stainton et al. (1977) and Wetzel and Likens (2000), the soluble reactive phosphorus was measured. Then, the filters were wrapped in aluminium foil and kept them frozen for subsequent chlorophyll analysis. Chlorophyll-a were extracted in 90 % acetone for 24 hours and fluorescence readings were made using a Turner 10-000 R fluorometer after the addition of 2 drops of 2 M HCl.

All water quality parameters were evaluated across seasons and sites using both Spearman's Rank Correlation and Kruskal-Wallis and Mann-Whitney's tests. Spearman's rank correlation coefficient allowed us to measure the correlation coefficient between all parameters because it is a non-parametric measure of association between the variables of non-normally distributed datasets. Kruskal-Wallis and Mann-Whitney non-parametric tests were performed to analyse the significant spatial and temporal differences for each parameter in this study ($\alpha = 0.05$). IBM SPSS Statistics 20.0 were used to analyse the data.

# Results

**Correlation of Water Quality Parameters**

In the Bua River, Chlorophyll-a had strong positive significant correlation with EC (r= 0.550, p= 0.000), salinity (r= 0.549, p= 0.000) and TDS (r= 0.564, p= 0.000) suggesting that with increase or decrease in the concentration of Chlorophyll-a that electrical conductivity, salinity and total dissolved salts would also increase or decrease. Similarly, water clarity correlated positively with EC (r= 0.573, p= 0.000), salinity (r= 0.569, p= 0.000), TDS (r= 0.560, p= 0.000), bicarbonate (r= 0.619, p= 0.000), alkalinity (r= 0.562, p= 0.000) and showed a negative correlation with SRP (r= -0.631, p= 0.000; Table 2) suggesting that water clarity (secchi depth) increased with decreasing soluble reactive phosphorus concentration.

All measurements of salt content correlated positively with each other and negatively with SRP, EC, and salinity (Table 2; r= 0.976, p= 0.000), EC and TDS (r= 0.958, p= 0.000), EC and bicarbonate (r= 0.691, p= 0.000), EC and Alkalinity (r= 0.678, p= 0.000), salinity and TDS (r= 0.952, p= 0.000), salinity and bicarbonate (r= 0.550, p= 0.000), salinity and alkalinity (r= 0.537, p= 0.000), TDS and bicarbonate (r= 0.525, p= 0.000), TDS and alkalinity (r= 0.516, p= 0.000), bicarbonate and alkalinity (r= 0.974, p= 0.000; Table 2). SRP correlated negatively with EC (r= -0.795, p= 0.000), salinity (r= -0.773, p= 0.000), TDS (r= -0.783, p= 0.000), bicarbonate (r= -0.626, p= 0.000) and alkalinity (r= -0.463, p= 0.010).

**Table 2.** Correlation of water quality parameters for all the five sites in the Bua River, Malawi from May 2018 to June 2020

| | SD(m) | Chl-a | WT | pH | EC | Salinity | TDS | SRP | Bicarb | Alka |
|---|---|---|---|---|---|---|---|---|---|---|
| **Secchi Depth(m)** | 1 | | | | | | | | | |
| **Chlorophyll 'a'** | 0.237* | 1 | | | | | | | | |
| **Water Temp** | -0.243** | 0.276** | 1 | | | | | | | |
| **Water pH** | 0.242** | -0.075 | -0.336** | 1 | | | | | | |
| **EC** | 0.573** | 0.550** | 0.234* | -0.061 | 1 | | | | | |
| **Salinity (psu)** | 0.569** | 0.549** | 0.267** | -0.074 | 0.976** | 1 | | | | |
| **TDS(mg/L)** | 0.560** | 0.564** | 0.203* | -0.017 | 0.958** | 0.952** | 1 | | | |
| **SRP** | -0.631** | -0.355 | 0.205 | 0.094 | -0.795** | -0.773** | -0.783** | 1 | | |
| **Bicarbonate** | 0.619** | 0.341* | -0.094 | 0.130 | 0.691** | 0.550** | 0.525** | -0.626** | 1 | |
| **Alkalinity(mg/L)** | 0.562** | 0.355* | 0.027 | 0.172 | 0.678** | 0.537** | 0.516** | -0.463* | 0.974** | 1 |

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

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

Chlorophyll-a varied temporally in the Bua River with the highest mean monthly concentration recorded in December followed by November and the lowest mean monthly concentration was recorded in March. The long-term variation of chlorophyll-a varies throughout the year with the warm wet season registering both the highest and lowest values (Figure 2). The highest phytoplankton biomass of 14.32 µgL-1 was recorded at Bua mouth in November of 2018 and the lowest phytoplankton biomass was recorded at Bua mouth in January of 2020. Kruskal Wallis test revealed significant differences ($p<0.01$) across the categories of sampling months with an overall mean chlorophyll-a concentration of 2.45 µgL-1.

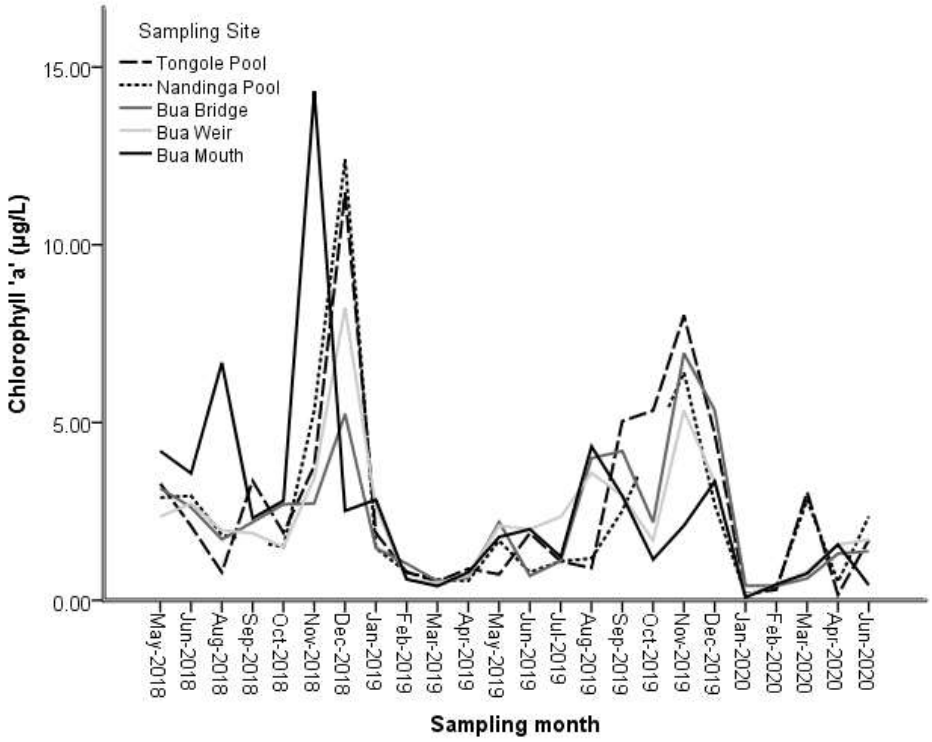


**Figure 2.** Monthly Chlorophyll-a (µg/L) variations by station, (May, 2018 to June, 2020)

Phytoplankton biomass estimated as Chlorophyll-a ranged from 0.08 µgL-1 to 14.32 µgL-1, with higher mean values ($p<0.05$) during the hot dry season (3.28 µgL-1) as compared with cool dry season (2.10 µgL-1) and wet season (1.91 µgL-1). The Mann-Whitney post hoc test revealed significant differences ($p<0.05$) between cool dry season and hot dry season and also between hot dry season and warm wet season (Figure 3).

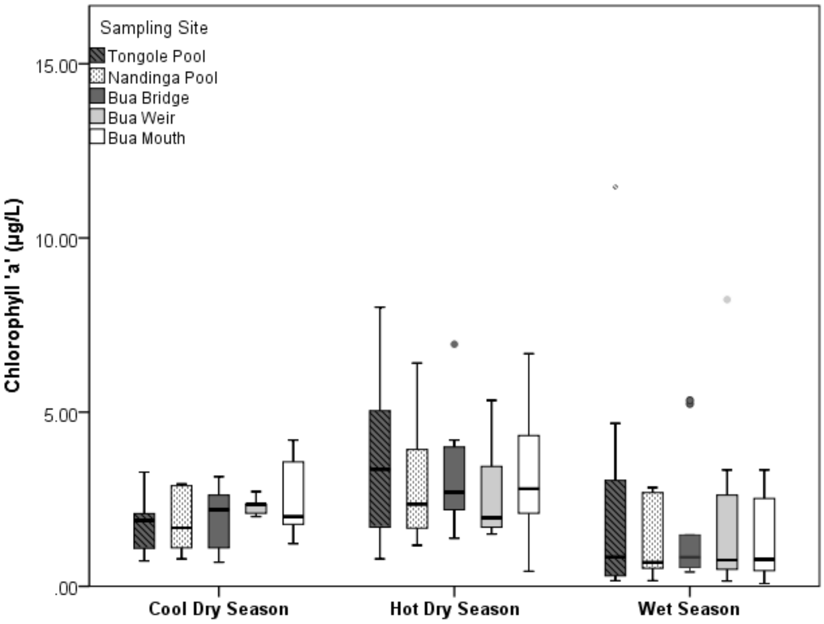


**Figure 3.** Seasonal Chlorophyll a variation in Bua River, Malawi by site

Mean monthly secchi depth measurements varied monthly ($p=0.000<0.05$). It decreased appreciably from September to March, and the followed an increasing trend from April to August revealing the significant aspect of seasonality (Figure 4, Figure 5).

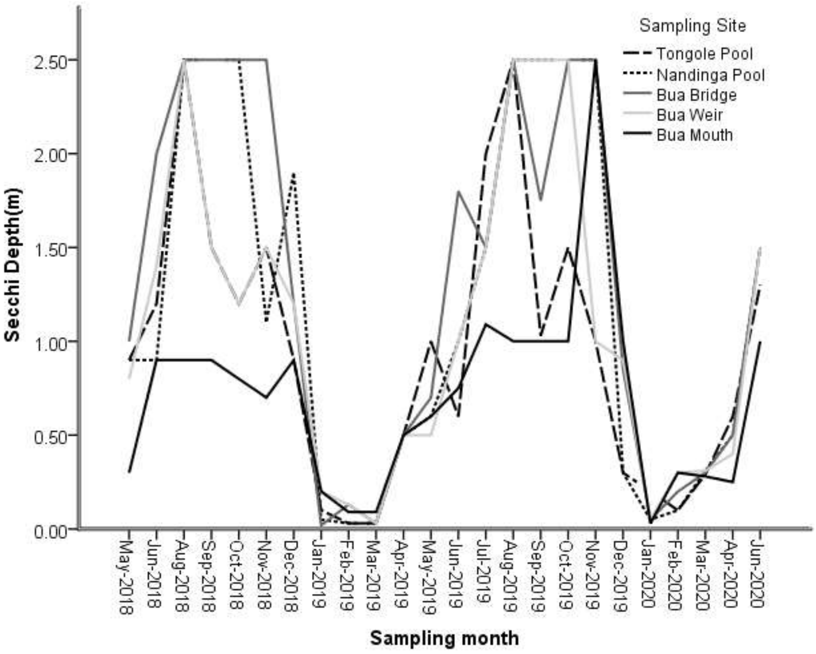


**Figure 4.** Monthly Secchi depth variations by site in the Bua River, Malawi from May, 2018 to June, 2020

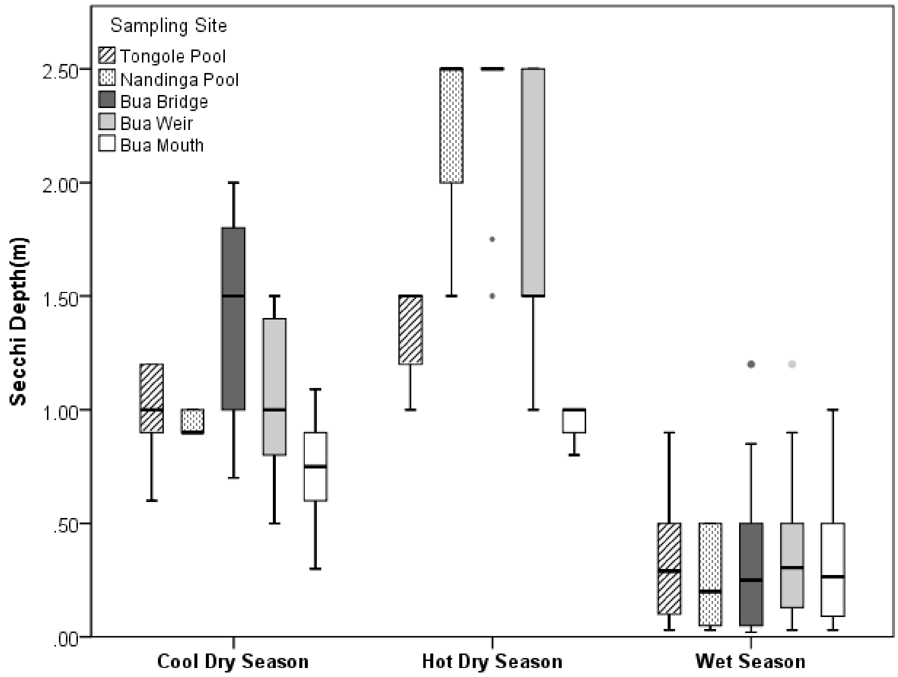


**Figure 5.** Seasonal Secchi depth (m) variations in the Bua River, Malawi from May, 2018 to June, 2020 by site

Water temperature varied monthly and responded to seasonal changes as expected. The highest monthly water temperature of 34°C was recorded at Bua Weir and Bua Bridge in December 2018 and also at Bua Nandinga pool in March 2019. In contrast, the lowest monthly water temperature of 19.70°C was recorded at Nandinga pool in June 2020. Generally, the long-term variation of temperature varied consistently with regards to seasonal changes, with the lower values being recorded in the cooler months and vice versa (Figure 6). Kruskal Wallis test revealed significant differences ($p<0.01$) across the categories of sampling months with an overall mean water temperature of 26.35°C.

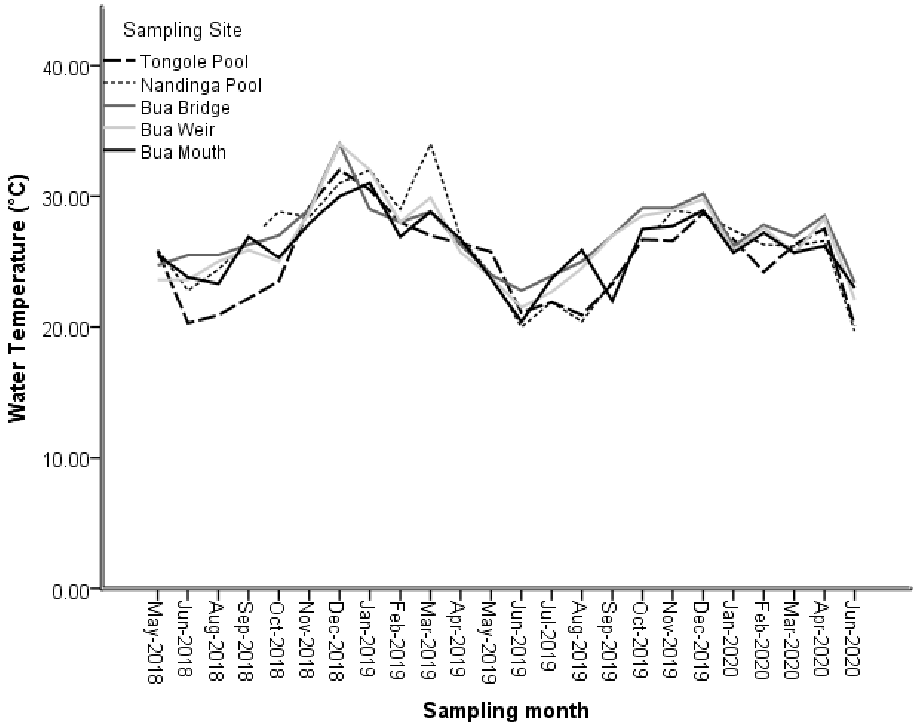


**Figure 6.** Monthly Temperature (°C) variations by site in the Bua River, Malawi from May, 2018 to June, 2020

Water temperature varied significantly across the seasons ($p<0.05$) with higher mean temperature observed during the wet season (28.30ºC) followed by hot dry season (25.44ºC) and lastly cool dry season (23.31ºC). Spatially, during the cool dry season Bua Bridge registered the highest mean water temperature of 24.18ºC and Nandinga pool registered the lowest mean water temperature of 22.92ºC while during the hot dry season, Bua Bridge registered the highest mean water temperature of 26.82ºC and Tongole pool registered the lowest mean water temperature of 23.71ºC. On the other hand, during the wet season Nandinga pool registered the highest mean water temperature of 28.79ºC and Bua mouth registered the lowest mean water temperature of 27.71ºC (Figure 7). Overall, a well-defined spatial variation was observed with Bua Bridge registering the highest mean water temperature of 27ºC and Tongole pool registering the lowest mean water temperature of 25.25ºC.

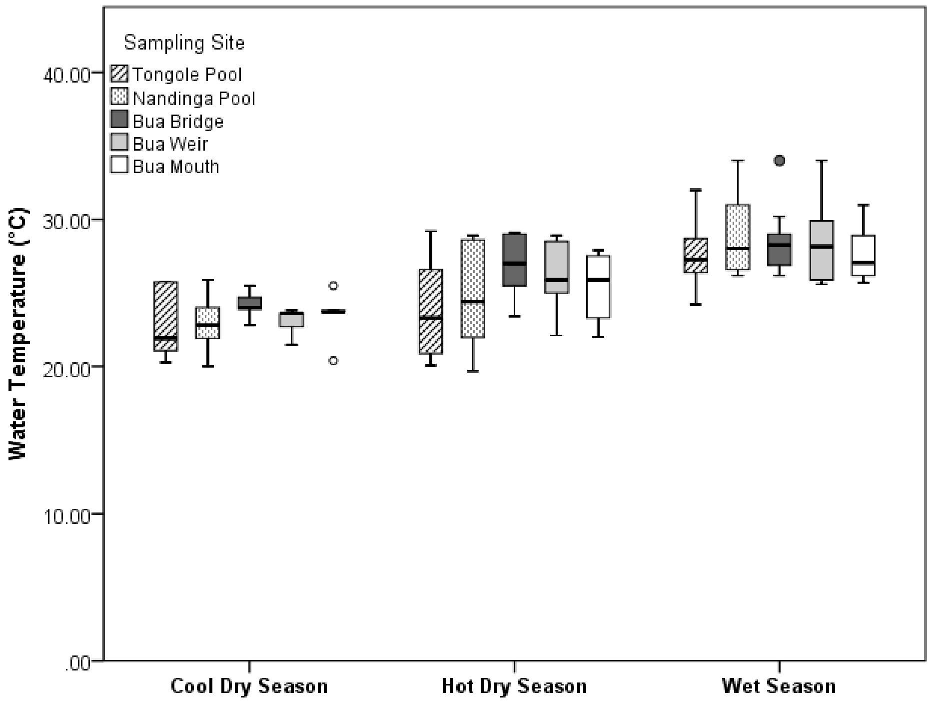


**Figure 7.** Seasonal water temperature variations in Bua River, Malawi from May, 2018 to June, 2020

Soluble reactive phosphorus (SRP) greatly varied throughout the study period with a minimum concentration of 0.98 µg/L (Bua weir, December 2018) and a maximum concentration of 30.2 µg/L, (Nandinga pool, January 2019; Table 3; Figure 8). The highest mean monthly phosphorus concentration of 22.49µg/L was recorded in the month of January whereas the lowest mean monthly phosphorus concentration of 2.25 µg/L was recorded in the month of October (Figure 8). Kruska-Wallis test revealed significant differences ($p<0.05$) across the categories of sampling months with an overall mean phosphorus concentration of 9.37 µg/L (Table 3).

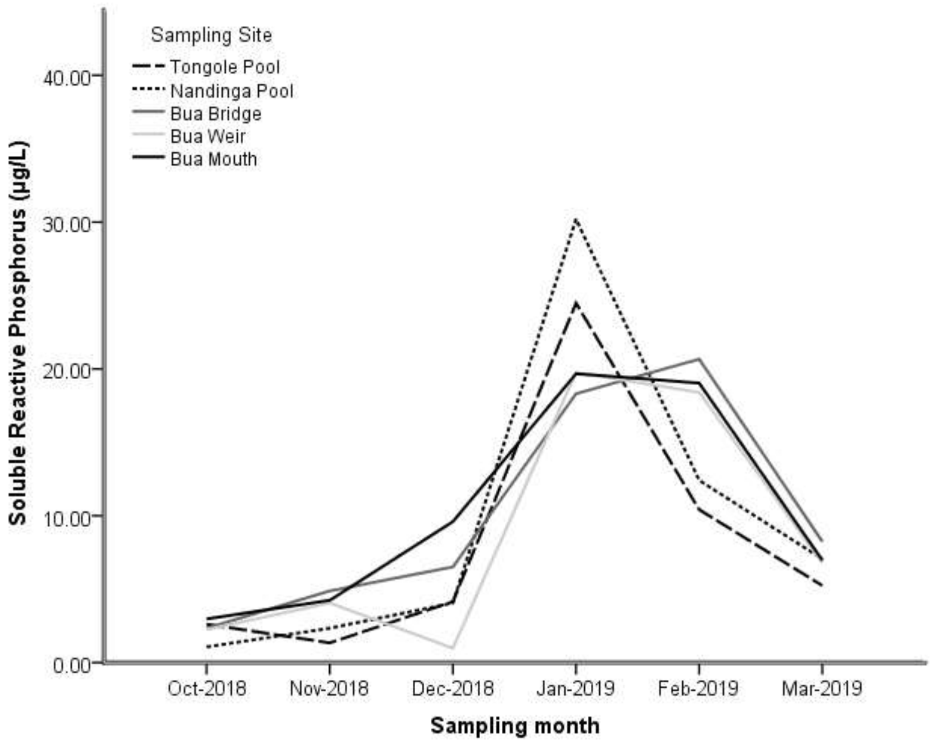


**Figure 8.** Monthly Soluble Reactive phosphorus (µg$L^{-1}$) measurements in across sites in the Bua River, Malawi

**Table 3.** Descriptive statistics for various water quality parameters across five sites in the Bua River, Malawi from May 2018 to June 2020

| Parameter | Range | Minimum | Maximum | Mean | Std. Deviation | Variance |
|---|---|---|---|---|---|---|
| **Secchi Depth (m)** | 2.48 | 0.02 | 2.50 | 1.03 | 0.82 | 0.67 |
| **Chlorophyll a (µg/L)** | 14.24 | 0.08 | 14.32 | 2.45 | 2.39 | 5.73 |
| **Temperature (°C)** | 14.30 | 19.70 | 34.00 | 26.20 | 3.12 | 9.71 |
| **Water pH** | 3.76 | 6.22 | 9.30 | 7.95 | 0.67 | 0.45 |
| **EC (µS$cm^{-1}$)** | 304.78 | 78.82 | 383.60 | 242.19 | 73.27 | 5369.21 |
| **Salinity (psu)** | 0.18 | 0.06 | 0.24 | 0.17 | 0.04 | 0.00 |
| **TDS (mg/L)** | 126.00 | 51.90 | 177.90 | 118.64 | 31.74 | 1007.21 |
| **Alkalinity (mg/L)** | 144.60 | 56.70 | 201.30 | 129.44 | 44.21 | 1954.56 |
| **SRP (µg/L)** | 29.22 | 0.98 | 30.20 | 9.37 | 8.06 | 64.96 |
| **Bicarbonate (mg/L)** | 184.10 | 58.50 | 242.60 | 151.14 | 53.95 | 2910.43 |

The distribution of soluble reactive phosphorus was not uniform across temporal categories with a significant difference ($p < 0.01$) between the wet season and hot dry season. The wet season had recorded higher SRP mean values (12.65 µg/L) compared with the hot dry season which had the lowest (2.81 µg/L). Spatially, Bua Bridge and Bua mouth registered and recorded the highest mean soluble reactive phosphorus concentration of 3.61 µg/L and Nandinga pool registered the lowest mean soluble reactive phosphorus concentration of 1.70 µg/L during the hot dry season, while, Bua mouth registered the highest mean soluble reactive phosphorus concentration of 13.82 µg/L and Tongole pool registered the lowest mean soluble reactive phosphorus concentration of 11.07 µg/L during the wet season (Figure 9).

Spatially, the overall distribution of soluble reactive phosphorus was not uniform across the sampling stations. Bua mouth recorded the highest mean soluble reactive phosphorus of 10.41 µg/L and Tongole pool recorded the lowest mean soluble reactive phosphorus concentration of 8.04 µg/L

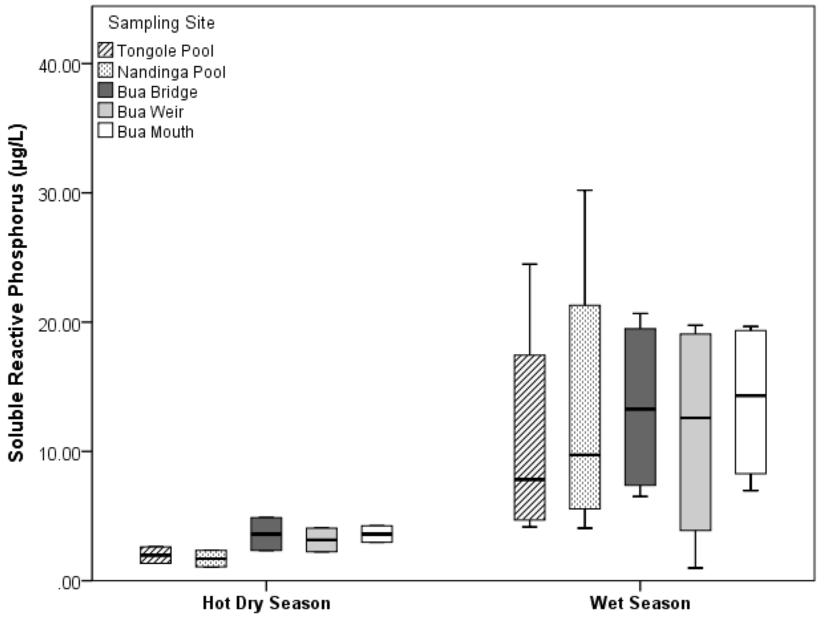


**Figure 9.** Seasonal Soluble Reactive Phosphorus variations (µgL$^{-1}$) in Bua River, Malawi across sites from May, 2018 to June, 2020

The highest monthly surface water pH value of 9.3 was recorded at Bua Bridge in July 2019 whereas the lowest monthly pH value of 6.22 was recorded at Tongole pool in March 2019 (Figure 10). The months of January, February and March of 2019 were associated with low mean water pH values of 7.29, 7.22 and 6.83 respectively. The highest mean monthly water pH value of 8.55 was recorded in July and the lowest mean monthly pH value of 7.46 was recorded in March. Kruskal-Wallis test revealed significant differences ($p< 0.05$) across months with an overall mean water pH value of 7.95 (Table 3). The cool dry season (mean pH= 8.32) was associated with consistently higher mean values above 8.00 than the other seasons (Figure 11). Significant differences in mean values were found across the seasons ($p < 0.05$) with higher mean value recorded during the cool dry season (8.32), followed by the hot dry season (7.92) and lower mean value recorded during the wet season (7.78). Mann-Whitney test showed that no significant differences ($p = 0.06>0.05$) existed between hot dry season and warm wet season.

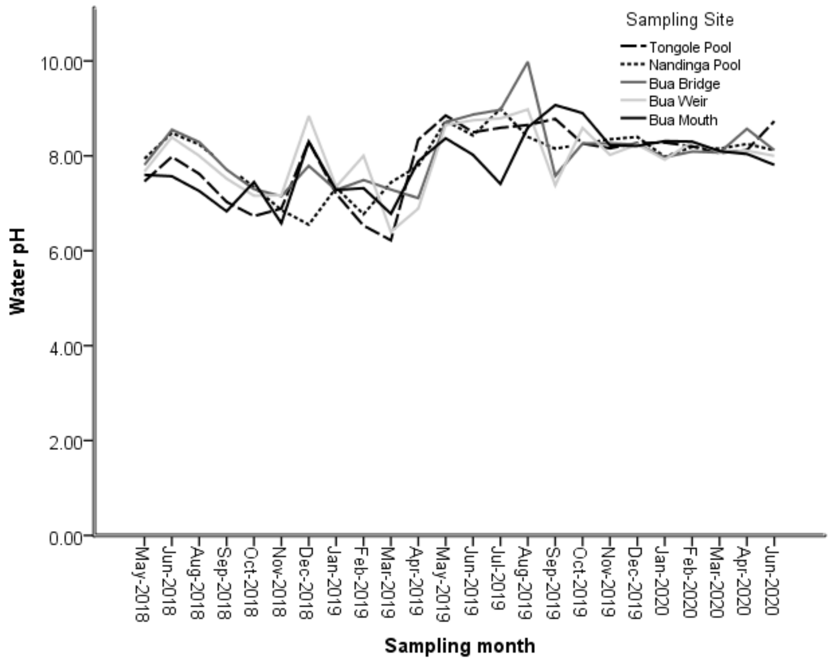


**Figure 10.** Monthly pH variations in the Bua River, Malawi from May, 2018 to June, 2020

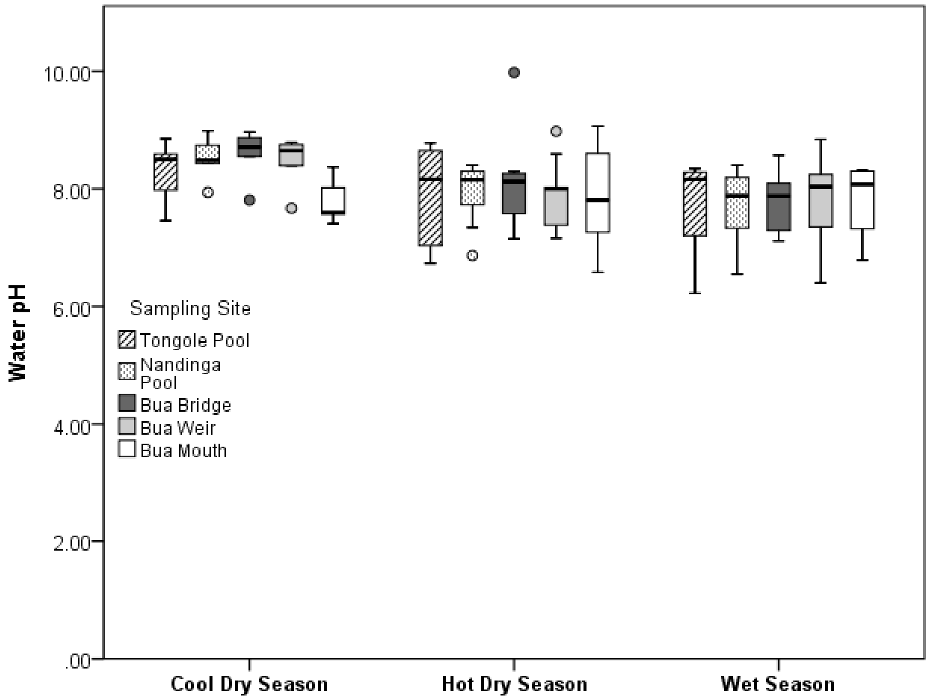


**Figure 11.** Seasonal pH variations in the Bua River, Malawi across sites from May, 2018 to June, 2020

Most pH values were well above 7.00, indicating that the River is relatively alkaline in nature. Throughout the study period, it was noted that acid buffering capacity for Bua River alternated between 56.70 mg/L and 201.30 mg/L, with the highest mean monthly alkalinity (192.96 mg/L) recorded in November 2018 and lowest mean monthly alkalinity (86.74 mg/L) recorded in March 2019. Significant differences ($p<0.05$), were found between seasons with higher mean values during the hot dry season (161.32 mg/L) and cool dry season (130.96 mg/L) with the lowest mean value of 115.99 mg/L observed during the warm wet season. Spatially, acid buffering capacity alternated between 124.30 mg/L and 135.14 mg/L across the sampling stations, with the highest mean alkalinity recorded at Bua Mouth and lowest mean alkalinity at Nandinga Pool.

Bicarbonate measurements ranged from a minimum of 58.50 mg/L to a maximum of 242.60 mg/L with an overall mean of 151.14 mg/L (Table 3). Bicarbonate had the highest mean values during the hot dry season (195.63 mg/L) followed by cool dry season (155.95 mg/L) and then the warm wet season (127.18 mg/L). The distribution of Bicarbonate was not uniform across sampling stations, with the highest mean value (157.40 mg/L) recorded at Bua Mouth and lowest mean Salinity measurements ranged from a minimum of 0.06 psu to a maximum of 0.24psu with an overall mean value of 0.17 psu (Table 3). The maximum salinity measurement was recorded at Bua Bridge in the month of October 2019 and the minimum salinity measurement was recorded at Bua mouth in the month of February 2019 (Figure 12). The highest mean monthly salinity measurement of 0.23 psu was registered in November whereas the lowest mean monthly salinity measurement of 0.13 psu was registered in April (Figure 12). Kruska-Wallis test revealed significant differences ($p<0.05$) across the categories of sampling months with an overall mean salinity measurement of 0.17 psu (Table 3). value (145.49 mg/L).

Salinity measurements were consistently higher during the hot dry season (median=0.1940 psu) than the other seasons. Both Levene's test for equality of variance and Kruskal-Wallis non-parametric test indicated significant differences ($p<0.05$) among seasons with higher mean value during the hot dry season (0.2 psu) followed by the cool dry season (0.16) and the warm wet season (0.15 psu). On spatial scale, all stations recorded an average salinity value of 0.17 psu and no significant differences were found across the sampled stations. (Figure 13).

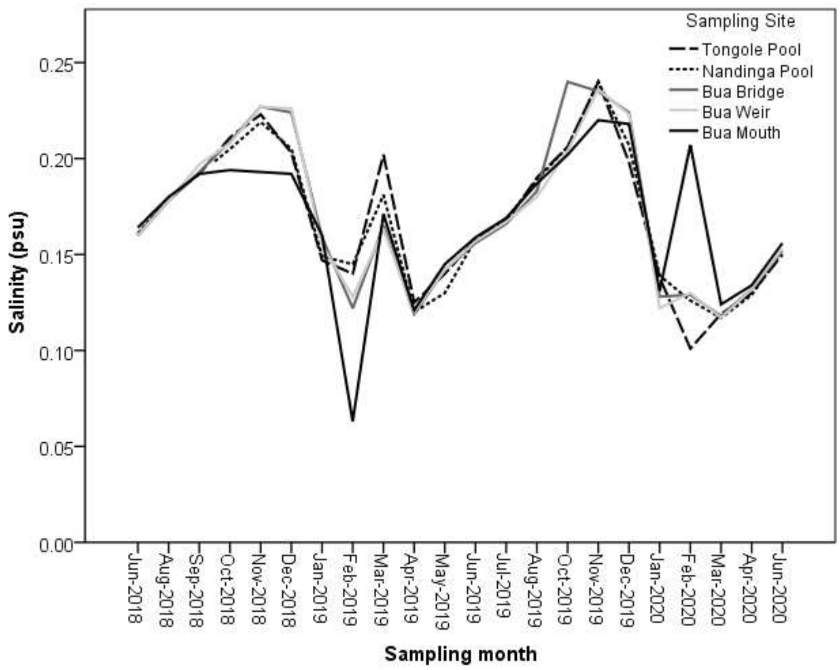


**Figure 12.** Monthly salinity (psu) variations in the Bua River, Malawi across sites from May, 2018 to June, 2020

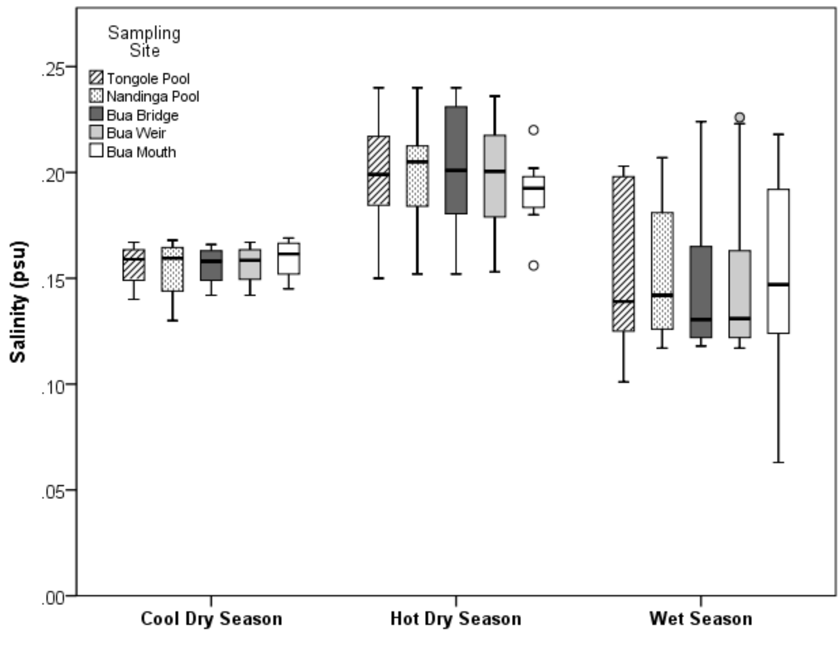


**Figure 13.** Seasonal salinity variations in Bua River, Malawi from May, 2018 to June, 2020

Electrical conductivity ranged from a minimum value of 78.82 µScm$^{-}$1 to a maximum value of 383.6 µScm$^{-}$1 with an overall mean of 242.19 µScm-1 (Table 3). Spatially, electrical conductivity did not differ between sampling stations ($p>0.05$) with average range between 232.96 µScm$^{-}$1 recorded at Nandinga pool and 247.20 µScm-1 recorded at Bua mouth. Significant differences ($p<0.05$) were found between cool and hot seasons, wet and hot season and no significant differences ($p>0.05$) were observed between cool dry season and wet season, with higher mean values in hot dry season (300.23 µScm$^{-}$1) and cool dry season (212.92 µScm$^{-}$1) and lower mean value in wet season (206.90 µScm$^{-}$1). The highest mean monthly conductivity of 364.44 µScm$^{-}$1 was recorded in November and the lowest mean monthly conductivity of 153.25 µScm$^{-}$1 was recorded in April.

Mean Total dissolved salts were higher during the hot dry season (140.58 mg/L) followed by the cool dry season (110.63 mg/L) and the warm wet season registered 102.53 mg/L. Significant differences ($p<0.05$) were observed between cool dry season and hot dry season, warm wet season and hot dry season and no significant difference ($p>0.05$) between cool dry season and warm wet season. The highest mean monthly TDS of 160.51 mg/L was recorded in December and the lowest mean monthly TDS of 74.66 mg/L was recorded in April.

**Principle Component Analysis**

Based on Principal Component Analysis (PCA), 78.49 % of the total variance in the data set could be explained from two main components. Component 1 explains 63.38 % and is attributed to EC, Temperature, Secchi depth, Chlorophyll a, Salinity, TDS, Alkalinity and Bicarbonate. Component 2 explains 15.11 % and is attributed to pH and SRP (Table 4). Factors in component 1 are associated with high eigenvalue (6.34) and loading values as compared to factors in component 2 which has eigenvalue of 1.51 and lower loading values. SRP had a negative loading factor with factor 1 and its negative relationship with salinity, electrical conductivity, TDS, alkalinity, and transparency (Figure 14, Figure 15).

**Table 4.** Factor loading matrix and variance explained

| Variable | Component/Factor | |
|---|---|---|
| | 1 | 2 |
| **Secchi Depth** | **0.861** | 0.097 |
| **Chlorophyll 'a'** | **0.794** | 0.094 |
| **Water Temperature** | **0.681** | 0.154 |
| **Water pH** | 0.495 | **0.697** |
| **Electrical Conductivity** | **0.926** | -0.318 |
| **Salinity** | **0.871** | -0.354 |
| **TDS** | **0.933** | -0.301 |
| **Alkalinity** | **0.809** | 0.474 |
| **SRP** | **-0.648** | **0.582** |
| **Bicarbonate** | **0.835** | 0.322 |
| ***Eigenvalue*** | *6.340* | *1.580* |
| ***% Total variance*** | *63.380* | *15.100* |
| ***Cumulative %*** | *63.380* | *78.490* |

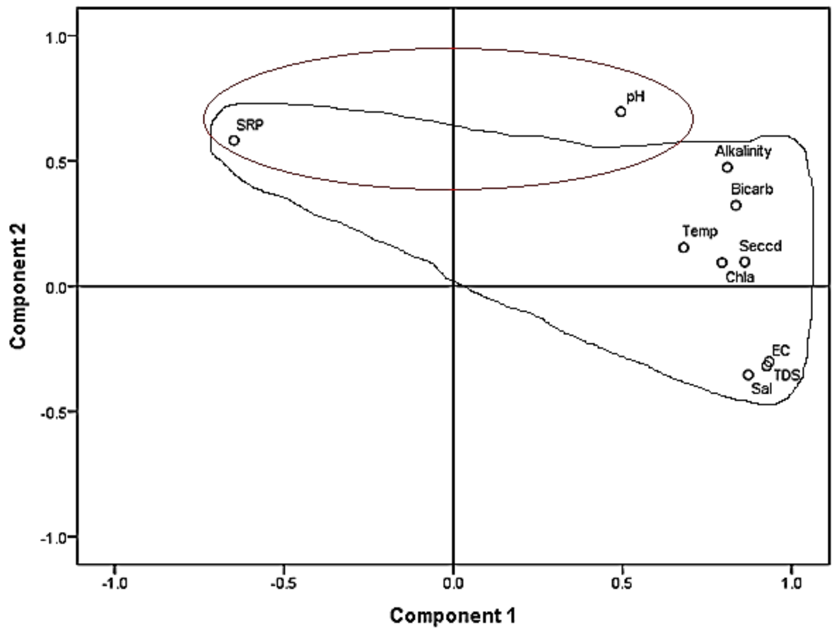


**Figure 14.** The loading plot of factors for component 1 and component 2

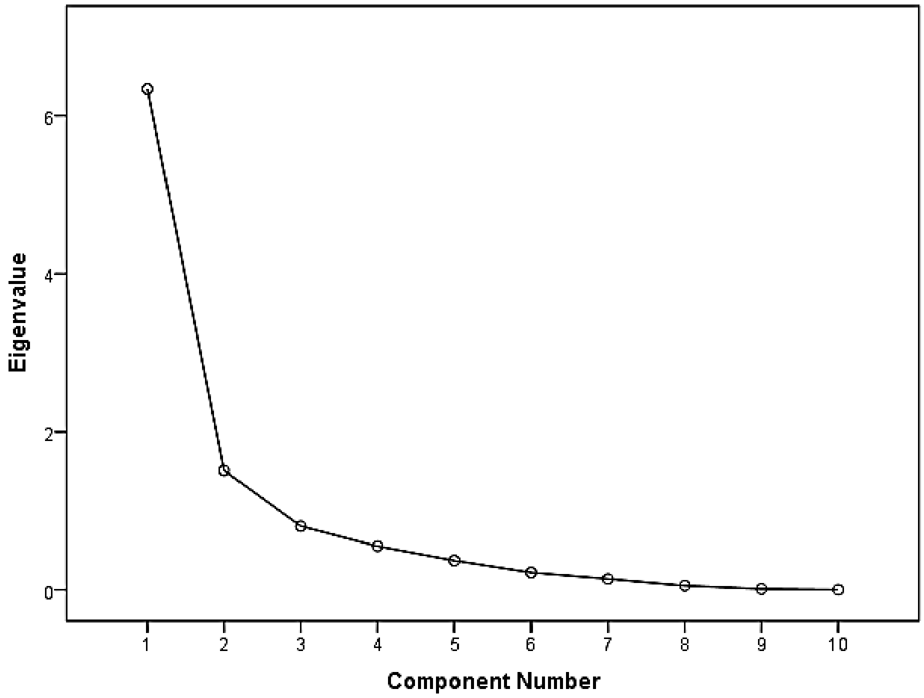


**Figure 15.** Scree plot of Eigenvalue against components

## Discussion

The absence of significant spatial variation in all the parameters assessed, except SRP, is an indication that the water quality conditions are somehow similar along the different sections of the Bua River. However, most of the water quality parameters varied temporally.

The recorded low mean secchi depth value indicated that the mainstream Bua River is associated with high turbidity especially during the warm wet season probably due to high silt loading during rain events and to a lesser extent from the phytoplankton biomass, indicating that the suspended particulate matter contributed more to decreasing water transparency (Figure 5; Panigrahi et al. 2007). Rainfall increased flow and water turbidity due to silt and suspended particulate matter. High turbidity in water prevents light from reaching phytoplankton thereby reducing their capacity for photosynthesis resulting in reduced growth and also increased flow reducing the residence time of phytoplankton in the water column, as such less time is available for nutrient uptake. The limited phytoplankton growth observed in the wet season (Figures 2 and Figure 3) is more likely to have occurred in the Bua River because of lack of light from low water transparency or short residence times that do not allow phytoplankton to reach the maximum concentration permitted by the available nutrients (Araújo et al. 2011). Since the primary producers form the base of the food chain, any deleterious impacts will probably also be manifested in the invertebrate and fish communities (Wood and Armitage 1997).

On the other hand, the hot dry season was associated with high mean secchi depth and high mean phytoplankton biomass. This was mainly attributed to decreased water level and settling of suspended particulate matter which allowed maximum light penetration for photosynthesis and also decreased water flow allowing more nutrient uptake for phytoplankton growth. As an important index representing the phytoplankton biomass, chlorophyll-a did not exhibit a significant correlation with soluble reactive phosphorous indicating that phytoplankton were generally affected by other limiting environmental factors rather than soluble reactive phosphorus, which calls for further study.

Variations in the concentration of soluble reactive phosphorus were highly tied to seasonal changes with higher mean concentration in the wet season than the hot dry season, with Nandinga pool reaching as high as 30 $\mu gL^{-1}$. The high recorded level of soluble reactive phosphorus suggests being from fertilizer inputs from agricultural fields as runoff along the Bua River during periods of high rainfall. Ravindra and Kaushik (2003), indicated that the increased concentrations of phosphorus in the River water might be due to agricultural runoff containing phosphate fertilizers and detergents, which has the potential for pollution of the surface water and cause eutrophication. As noted during field observation visits, Bua River is associated with periods of low flow during the hot dry season where the velocity decreases creating conditions for sedimentation. Thus under oxic conditions, orthophosphate may combine with particles such as iron, aluminium and calcium forming stable products that can accumulate in the bed sediments (Addiscott et al. 2000) and be released into the River system when the sediment gets disturbed by factors such as rainfall (Webster et al. 2001). Phosphorus is essential for primary production i.e the growth of algae and other aquatic plants, but excess phosphorus may lead to eutrophication. In the present study soluble reactive phosphorus concentration changed by over 70 % from that of the oligotrophic nature observed in hot dry season, and since this change is much greater than 15 % prescribed by South African Water Quality Guidelines (DWAF 1996) it shows that the River is greatly impacted by phosphorus. The presence of relatively high amounts of soluble reactive phosphorus pollution indicates the impact of agricultural diffuse water and domestic discharges (Wu 2005).

Bua River ecosystem, as a running water system exhibit daily and seasonal temperature patterns which might expose aquatic organisms to potentially lethal or sub-lethal conditions. Anthropogenic causes of temperature changes in River systems include those resulting from stream regulation and changes in riparian vegetation (Wellborn and Robinson 1996; Ward and Stanford 1982; Quinn et al. 1997). Duffus (1980) postulate that an increase in water temperature decreases oxygen solubility and might also increase the toxicity of certain chemicals, both which result in increased stress in the associated organisms. It must be pointed out here that many life cycles of aquatic organisms such as migration, breeding and emergence are cued into temperature. As such, false temperature cues caused by modified temperature regimes may affect the timing of life history and thus interfering with normal development (Dallas and Day 2004). Fish, insects, phytoplankton, zooplankton and other aquatic species all have chosen temperature ranges such that deviations from the optimum range affect aquatic life as it determines which organisms will thrive and which will diminish in numbers and size. As observed by Jain et al. (2013), sudden changes in water temperature are believed to be deleterious to fish with abrupt changes of ±5°C or greater likely to be harmful. Nevertheless, the observed temperature in Bua River still falls within the optimum range (18°C to 33°C) for the survival of tropical fish (Bone and Moore 2008) and the mean monthly temperature change of 1.74°C is less than 5°C.

It must be emphasized here that during the entire period of study water pH values were well within the optimum range for the survival of aquatic life (WHO 2006; UNECE-ECS 1992; DWAF 1996). The observed fluctuations in River pH can be caused by external factors such as agricultural runoff, acidic mine drainage (AWD), and fossil fuel emissions such as carbon dioxide, which creates a weak acid when dissolved in River water. Internally, the water pH is influenced by the metabolism of aquatic organisms and may oscillate due to metabolic processes associated with photosynthetic activity that capture $CO_2$ from the water (Araújo et al. 2011). The substantial low mean alkalinity and Bicarbonate observed during the wet season in the River system was mainly attributed to the influx of acid-forming sulphates from fertilizers. But the fact that seasonal variation was less than 1pH unit value and the minimum alkalinity ranged from 56.70 mg/L to 201.30 mg/L suggests that the River has relatively strong buffering capacity. In poorly buffered waters, pH can change rapidly, which in turn may have severe effects on the aquatic biota (DWAF 1996) thereby predisposing fish and other organisms to opportunistic infections such as Epizootic ulcerative syndrome (EUS). According to Wood and Rogano (1986), the direct effect of a change in pH is an alteration in the water, ionic and osmotic balance of individual whole organisms. This can, in turn, have sub-lethal effects such as slow growth and reduced fecundity (Berrill et al. 1991).

The correlation matrices showed that EC had strong positive significant correlations ($p<0.01$) with salinity ($r = 0.976$), TDS ($r = 0.879$), Bicarbonate ($r = 0.691$) and Alkalinity ($r = 0.678$). And on the other hand all the measurements of dissolved salt content had strong negative significant relationship ($p<0.01$) with SRP. These associations indicate that the parameters are affected by same factors such as low water levels in hot dry season and the dilution/ amplification of the water by rainfall in wet season. Similar observations were made by Marthe et al. (2015) who noted that nutrients have strong negative correlations with measured physicochemical parameters which indicate that these nutrients are usually brought to the water during rain events.

Factor analysis of the data sets outputs two factors with a total variance of 78.49 %. Factor one is the most important with strong significant loading of alkalinity, electrical conductivity, bicarbonate, total dissolved salts, secchi depth, temperature and salinity. Within the first factor, observed demarcations are that alkalinity, EC and bicarbonates indicate dissolution from natural formations, soluble reactive phosphorus indicates inputs from agricultural runoff whereas TDS, transparency and salinity explains pollution through runoff from catchment area. And lastly in the same category, temperature indicates seasonal influence in water quality. On the other hand, factor two which comprises of pH and soluble reactive phosphorus are attributed to anthropogenic activities and domestic wastes. Moreover, as Al-Badaii et al. (2013) observes, pollution can be accompanied with cultivation of the surrounding regions where phosphate, nitrogen and sulphate fertilizers are utilized. This was highly anticipated because the catchment is associated with maize farming, rice farming and extensive sugarcane plantations. To sum up, factors from principle component analysis indicated that the parameters responsible for variations of Bua River water quality are mainly associated with soluble minerals and temperature as natural sources as well as agricultural activities, surface runoff and domestic waste as anthropogenic activities.

Generally, it can be concluded from the study that the Bua River water resource at a large scale is moderately polluted. There are some smaller scale environmental incidents observed along the different sections of the river that have resulted in the deterioration in the physicochemical quality and a general rise in the nutrient level. This is mainly due to anthropogenic activities. The general public, therefore, has to be civic educated and be made aware of the consequences of the pollution. Periodic monitoring and preventative measures need to be emphasised to save the aquatic system from eutrophication. Additional work is also needed to determine the dynamics of the watershed's response to runoffs and land management practices under varying climatic conditions to better understand the complex physical and chemical processes causing the degradation observed in the present study, and also to ascertain the role of River discharge in nutrient dynamics within the River. Furthermore, absence of significant spatial variation in most of the parameters assessed is an indication that the water quality conditions are equally impacted along the different sections of the River. As such management measures must consider the whole stretch of the River from upper (i.e Mchinji area) to lower sections (Bua mouth).

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# An Investigation on Some Population Parameters of Tigris Trout (*Salmo tigridis* Turan, Kottelat & Bektaş, 2011) for Çatak Stream, Upper Tigris Basin/ Türkiye

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**A B S T R A C T**

The present study examined length-weight relationship and some growth parameters of Tigris Trout (*Salmo tigridis* Turan, Kottelat & Bektaş, 2011) caught in Çatak Stream. By using electroshock and casting nets, 162 Tigris trout were caught between January 2008 and January 2009. The fork lengths and total weights were measured, and sexes of the caught fish were identified. Furthermore, their age was also identified in line with their otoliths. The age range was found out as II–VII age. The mean of fork length was found to be 19.69 ± 3.80 cm for males and 19.89 ± 4.63 cm for females. While the mean of total weight was 97.19 ± 5.98 g for males and 102.18 ± 6.10 g for females. The length weight relationship was found as $W = 0.187\ L^{2.834}$. Length and weight relationship according to von Bertalanffy growth equations, they were estimated as $L_t$=36.467 $\left(1 - e^{-0.188(t+1.180)}\right)$, $W_t$ = 495.428 $\left(1 - e^{-0.188(t+1.180)}\right)^{2.834}$ respectively. Growth type was found to be negative allometric in males and females individuals. In line with the samples examined, it was revealed that the population was mainly composed of II and III elderly individuals. The male/female ratio was found out as 1:2 among the samples.

**Keywords:** Çatak Stream, *Salmo tigridis,* Tigris trout, growth parameters

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**Yukarı Dicle Havzası, Çatak Çayı Dicle Alabalığı (*Salmo tigridis* Turan, Kottelat & Bektaş, 2011)'nın Bazı Populasyon Parametrelerinin İncelenmesi**

**Öz:** Bu çalışmada Çatak Çayı'ndan yakalanan Dicle alabalıklarının (*Salmo tigridis* Turan, Kottelat & Bektaş, 2011) boy-ağırlık ilişkisi ve büyüme özellikleri incelenmiştir. Ocak 2008-Ocak 2009 tarihleri arasında elektroşok ve serpme ağ kullanılarak toplam 162 adet Dicle Alabalığı yakalanmıştır. Yakalanan balıkların çatal boyları, total ağırlıkları ölçülmüş, cinsiyetleri belirlenerek otolitlerinden yaş tayinleri yapılmıştır. Çalışmada alabalıkların yaş aralığı, II-VII olarak tespit edilmiştir. Ortalama çatal boy, erkeklerde 19,69±3,80 cm, dişilerde 19,89±4,63 cm, ortalama total ağırlık erkeklerde 97,19±5,98 g, dişilerde 102,18±6,10 g olduğu belirlenmiştir. Boy ağırlık ilişki $W$=0,187 $L^{2,834}$, boyca ve ağırlıkça Von Bertalanffy büyüme eşitlikleri sırasıyla $L_t$=36,467 (1-$e^{-0,188*(t+1,180)}$), $W_t$=495,428 (1-$e^{-0,188*(t+1,180)}$)$^{2,834}$ olarak hesaplanmıştır. Dişi ve erkek bireylerde büyüme tipi negatif allometri olarak bulunmuştur. İncelenen örneklerde populasyonun ağırlıklı olarak II ve III yaşlı bireylerden oluştuğu belirlenmiştir. Örneklerde erkek/dişi oranı 1:2 olarak belirlenmiştir.

**Anahtar kelimeler:** Çatak Çayı, *Salmo tigridis,* Dicle alabalığı, büyüme parametreleri

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## Introduction

Natural trout, which show a wide distribution in the waters of our country, are known as mountain trout, Black Sea trout, red spotted, Tigris and Anatolian trout. Natural trout is distributed in streams between 100 and 2300 m in altitude (Kocabas et al. 2013). In these regions, called trout zones, the flow of water is fast, and the amount of oxygen is high. Generally, the body structures of trout are shuttle-shaped, and the sides are flat. On the lateral lines, there are 10–12 large red spots with small red spots (Aras et al. 1986; Geldiay and Balık 1996). Recently, deterioration in physical habitat structures of rivers, deterioration of water quality, a decrease of flow rates due to global climate change, dams and obstacles to prevent passing fish in rivers have negatively affected the fish stocks in these places. In addition to these negativities, excessive catching pressure, which is economically valuable and believed to cure diseases, has brought natural trout stocks to extinction. More studies are required on trout populations in Turkey; however, the studies are limited because of the geographic location of the habitat and the difficulty of obtaining samples. It can be said that most of the existing literature is based on taxonomic studies (Alp and Kara 2004). A number of studies have examined natural trout stocks in Turkey. Among the studies conducted on natural trout, Aras (1976) and Aras et al. (1986) deal with bio-ecological status. Çetinkaya (1996) and Alp and Kara (2004) tried to figure length-weight relationship and condition factors. Kocaman et al. (2004) and Yüksel et al. (2020a), examined population parameters, while Tanır and Fakıoğlu (2017) conducted biomass and density studies. Yüksel et al. (2020b) determined morphometric features. Duman et al. (2011) investigated the nutritional content of their meat. Çatak Stream natural trout species was named as *Salmo trutta macrostigma* (non Dumeril 1858) until 2011 (Tortonese 1954; Geldiay and Balık 1996). However, Turan et al. (2011), in their study supported by molecular methods, it was determined that natural trout living in Çatak Stream belonged to a different group of family was renamed as *Salmo tigridis* (Tigris trout). There are only two studies related to *S. tigridis* (Çetinkaya 1996; Akkuş and Sarı 2017) at Çatak Stream, which is located in the triangle of Hakkari, Van and Siirt provinces (east-south Turkey). Despite the number of natural trout populations in many regions of Turkey, the information about their growth and reproductive characteristics is insufficient (Yüksel et al. 2020a). The lack of an up-to-date study on the population parameters of the *S. tigridis* causes uncertainty about this species which is rapidly declining due to overfishing in the region. The aim of this study is to find out the population characteristics of the *S. tigridis* as well as shedding light onto the measures to be taken to protect the decreasing species of *S. tigridis*.

## Materials and Methods

The study was conducted on the Çatak Stream, one of the main tributaries of the Tigris River. Çatak Stream, which is located in the Upper Tigris Basin, arises from the mountainous parts of the village of Sıcaksu at an altitude of 2338 m in Van-Gürpinar district. Çatak Stream is located in an area which is difficult to reach due to the steep terrain conditions (Figure 1).

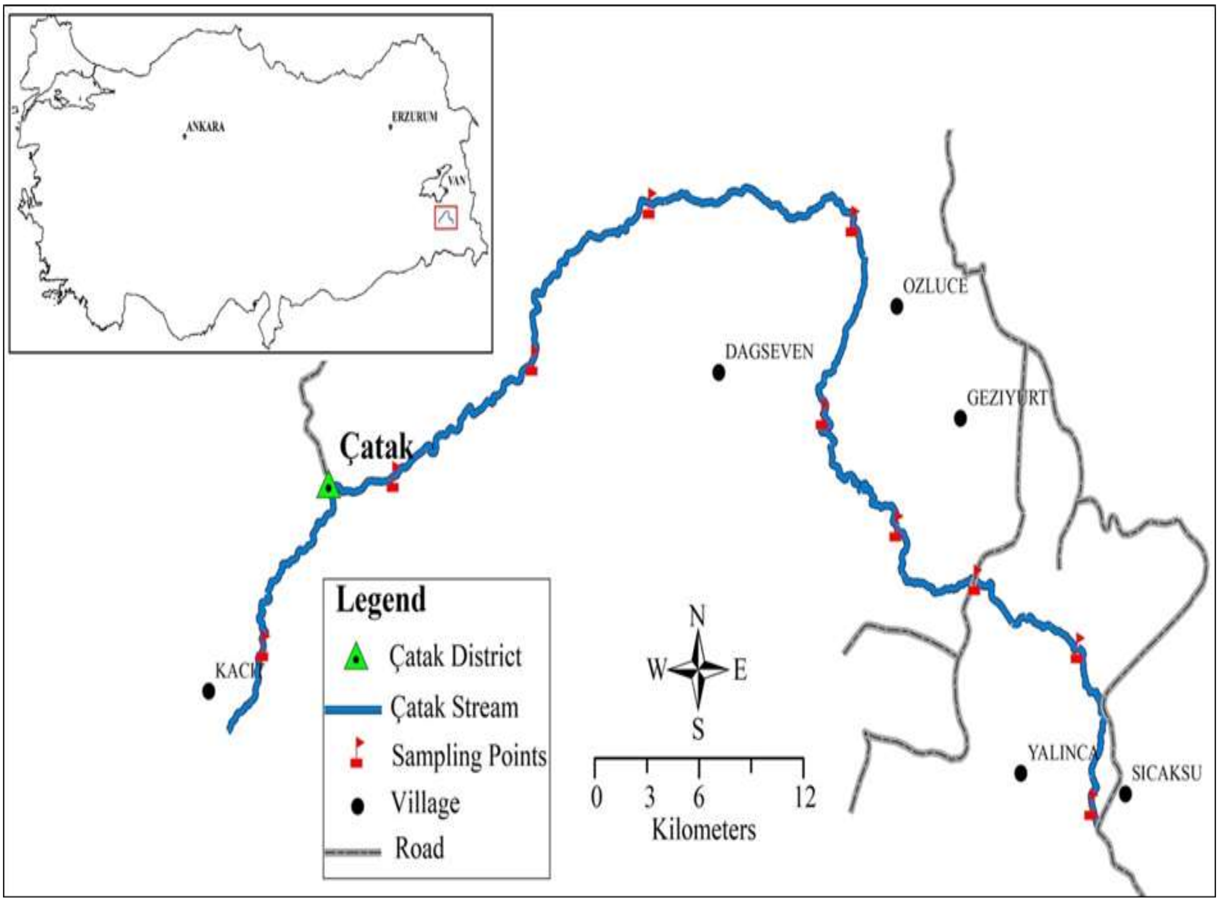


**Figure 1.** Çatak Stream and sampling points

The sex of the fish was determined by visual or microscopic examination of the gonads. Seasonal fish sampling was carried out with a 12 Volt DC and 7–14 amp battery, portable electroshock device and fish scoop with 2–4 mm mesh sizes. In the deep areas, where the electroshock device is not effective, the scattering net with 5 mm mesh size is used in the sampling. Sampling studies were carried out at 10 different points with a length of 200 meters (Gelwick 1990), chosen by taking the terrain conditions and the habitat structure of the river into account. The total weights of the caught fish were made on a digital scale with ± 0.1 g sensitivity, and fork lengths were determined on a measuring board with a precision of ± 1 mm. The age of the fish was determined by reading the age rings of the otolith from each fish under the light microscope. Furthermore, $W = a L^b$ equation was used to calculate the length -weight relationship (Froese 2006). Where, *W* and *L* indicates as total weight (g) and fork length (cm) respectively. The a and b values of LWRs are calculated from the regression analysis. The t-test ($p<0.05$) was applied to determine whether the b value is different from 3.

Von Bertalanffy (1957) growth constants have also been used $L_t = L_\infty (1 - e^{-K (t - t0)})$, $W_t = W_\infty (1 - e^{-K (t - t0)})^b$ for equations. $L_\infty$ represents the maximum theoretical length that a Tigris trout can reach, and $W_\infty$ represents the maximum theoretical weight that it can reach. In order to compare the growth parameters calculated in the study with the previous studies in the literature, fi ($\acute{Ø}$) value developed by Pauly and Munro (1984) was calculated. $\acute{Ø} = \ln K + 2 \ln L_\infty$ was used to calculate $\acute{Ø}$.

## Results

In line with the findings of the present study, it was found that examined fork length values of 162 individuals ranged between 10.8 and 31 cm, and their weights ranged between 15 and 308 g. The maximum and minimum fork length values are 13.3–31cm in males and 10.8–29.5cm in females, respectively. Total weight values were between 31.1–308 g in males and 15–234.8 g in females. Average weight and length values of age groups between II and VII are given in Table 1.

**Table 1.** Fork length and weight values (min-max)

| Age | Number | | Male | | Female | |
|---|---|---|---|---|---|---|
| | Male | Female | Fork L. (cm) (min-max) | Weight (g) (min-max) | Fork L. (cm) (min-max) | Weight (g) (min-max) |
| 2 | 35 | 13 | 19.9-13.8 | 91.8-31.1 | 19.8-10.8 | 91.8-15 |
| 3 | 45 | 22 | 20.1-15 | 102.9-41.2 | 18.3-14.9 | 73.5-36 |
| 4 | 14 | 7 | 24.1-21 | 148-97 | 24-20.5 | 146.4-108.6 |
| 5 | 6 | 4 | 25.5-24.5 | 181.1-158.1 | 24.5-24 | 160-126.1 |
| 6 | 4 | 6 | 29-25.9 | 312.2-224.8 | 27.2-26 | 226.8-189.7 |
| 7 | 4 | 2 | 31-26.5 | 318.2-203.2 | 29.5-27 | 234.8-234.1 |

During the process of reading ages, it was found out that the rate of VII-old fish was the lowest among the examined individuals, and it was 3.70%. The proportion of the other age groups was 6.17% among the elderly individuals in VI, 6.17% among the in V age group, 12.96%, among the elderly in IV, 41.35%; among the elderly in III, 29.62% among the elderly in II. Individuals belonging to age group III are the most dominant age group with a rate of 41.35%. The population was predominantly composed of II and III (70.98%) elderly individuals (Figure 2).

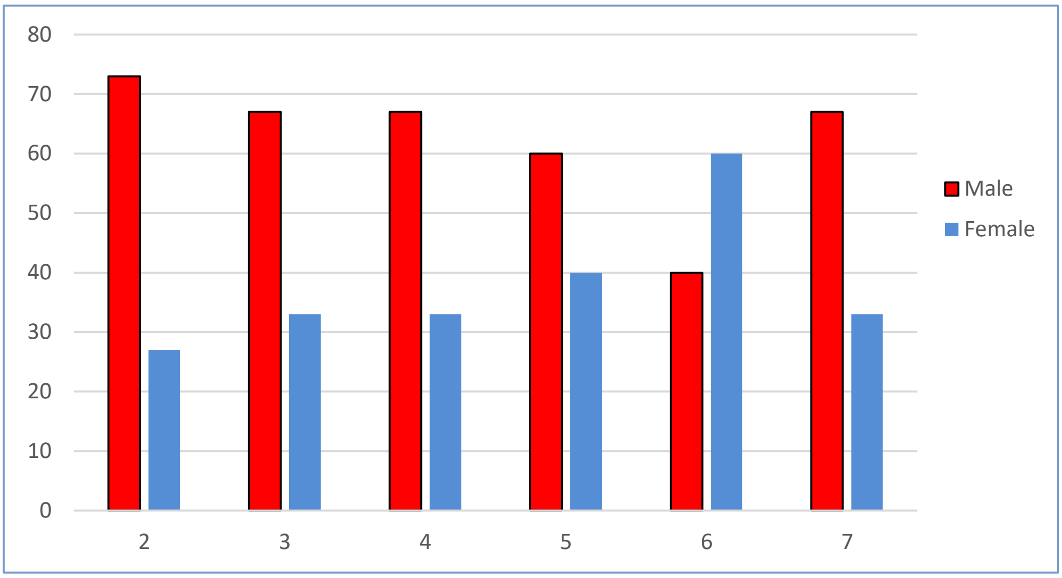


**Figure 2.** Proportional (%) distribution of male and female individuals in age groups

Length -weight relationships of the individuals sampled in the study were calculated for male and female individuals (Figure 3).

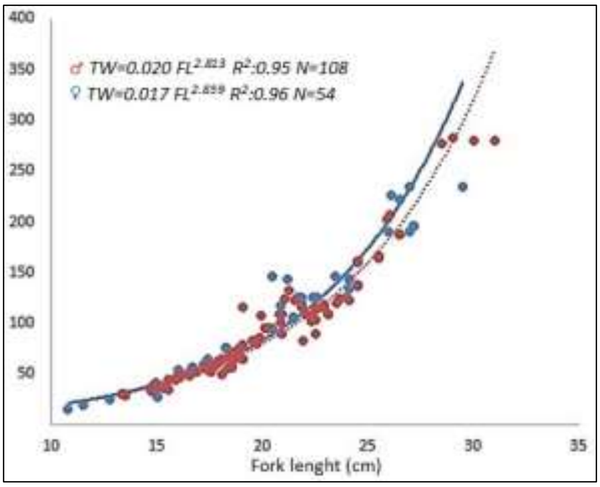


**Figure 3.** Relationship for male and female fish

As a result of the regression analysis, the regression coefficient value (b) was calculated as 2.834 (negative allometric growth; t-test $p<0.05$) in all individuals, 2.813 (negative allometric growth; $p<0.05$) in male subjects, and 2.859 (negative allometric growth,; t-test $p<0.05$) in female subjects (t-test, $p<0.05$). $R^2$ value calculated as a result of regression analysis, and it was calculated as $R^2 = 0.961$ in all individuals. Furthermore, $R^2 = 0.966$ was in females, and $R^2 = 0.957$ was in males (Table 3). In the study, the (b) value, which is calculated for females, males and all individuals, was calculated as (2.89) among males as the lowest level. Von Bertalanffy growth parameters of Çatak Stream *Salmo tigridis* population were calculated for all individuals (Table 3). Von Bertalanffy growth parameters for male individuals**,** the maximum theoretical length was $L_\infty = 37.235$, and maximum theoretical weight was $W_\infty = 524.982$. Brody growth coefficient was revealed as $K = 0.175$, theoretical age was $t_0$= -2.201, and $\acute{Ø}$ value was calculated as 5.49. In female individuals**,** the maximum theoretical length was $L_\infty = 35.297$, and maximum theoretical weight was $W_\infty = 454.989$. Brody growth coefficient was revealed as $K = 0.248$, theoretical age was $t_0$= -1.330, and $\acute{Ø}$ value was calculated as 5.73.

**Table 2.** Regression analysis parameters

| Sex | Number of individuals (n) | Regression parameters a | b | $r^2$ | t- test | Growht type |
|---|---|---|---|---|---|---|
| All indv. | 162 | 0.018 | 2.834 | p<0.05 | 0.961 | Negative allometric |
| Male | 108 | 0.017 | 2.813 | p<0.05 | 0.966 | Negative allometric |
| Female | 54 | 0.020 | 2.859 | p<0.05 | 0.957 | Negative allometric |

**Table 3**. Von Bertalanffy growth parameters calculated for all individuals

| Von Bertalanffy growth parameters (male) | | | | | Von Bertalanffy growth equation (male) |
|---|---|---|---|---|---|
| *$L_\infty$ (cm)* | *$W_\infty$ (g)* | *K* | *$t_0$* | *Ǿ* | $L_t = 37.235 \cdot (1 - e^{-0.175 \cdot (t+2.201)})$ |
| 37.235 | 524.982 | 0.175 | -2.201 | 5.49 | $W_t = 524.982 \cdot (1 - e^{-0.175 \cdot (t+2.201)})^{2.813}$ |
| Von Bertalanffy growth parameters (female) | | | | | Von Bertalanffy growth equation (female) |
| $L_\infty$ (cm) | $W_\infty$ (g) | K | $t_0$ | Ǿ | $L_t = 36.467 \cdot (1 - e^{-0.248 \cdot (t+1.33)})$ |
| 35.297 | 454.989 | 0.248 | -1.330 | 5.73 | $W_t = 454.989 \cdot (1 - e^{-0.248 \cdot (t+1.133)})^{2.859}$ |

## Discussion

In a nutshell, in the present study conducted with *Salmo tigridis* individuals in Çatak Stream, the maximum fork length was 31 cm, and the oldest individuals were in VII age group. In the study conducted by Çetinkaya (1996) related to *S. tigridis* individuals in Çatak Stream, the maximum fork length value was 38 cm, and the oldest individual was reported as VIII. In the study we conducted on the same population, it was obvious that there were no VIII elderly individuals in the area, and the fork length value decreased to 31 cm. This situation is thought to have been caused by the catching large individuals aged VIII along with overfishing, habitat destruction and pollution in the past 14 years. It was reported by Alp and Kara (2004) that the populations other than *Salmo platycephalus* in the Zamantı Stream were exploited at a high degree, and those individuals belonging to the high age group were extinct. With the withdrawal of older individuals from VIII, the length value decreased from 38 cm to 31 cm. The decrease in the size of the fish in fish populations is an indicator of overfishing (Avşar 2005). When the studies conducted by other researchers in different regions are examined, it can be claimed that the maximum fork length in the *Salmo trutta macrostigma* population of the Fırnız Stream in Seyhan Basin is 48.5 cm, and the largest age detected is X (Alp and Kara 2004). It was observed that the length and age values obtained from these studies are larger than those obtained from our study. As it is widely known, the growth in fish varies depending on many parameters such as water temperature, nutrient abundance and habitat structure (Çelikkale 1994; Avşar 2005). Therefore, it is expected that these values will come out differently in the studies carried out in streams with different ecological characteristics. In addition, the conservation activities carried out in different regions, which are not of the same sensitivity, are considered as other reasons for the emergence of this situation. When the ratio of age groups in the population is examined, it is seen that the population is composed of mainly II and III age groups with a rate of 70.98%. The proportion of individuals between the ages of IV–VII in the population is 29.02%. This is thought to be due to the intense catching pressure of individuals aged IV–VII, who are larger in length and weight than those of the younger II and III. Korkmaz (2005), in the study on mountain trout in Kadıncık Creek in Tarsus, asserted that 10.94% of the age composition of individuals were older than 3 years due to an intense catching pressure on adult fish. By Alp and Kara (2004), it is stated that there is an illegal and excessive prey pressure on the *S. trutta* population which is one of the most economical fishes among inland water fishes. Female / male ratio was calculated as 1:2 Çetinkaya (1996), in the study of Çatak Stream *S. tigridis* population, reported this ratio as 1:2. This value is consistent with the value calculated in our study. When other studies were examined in terms of female/male ratio, Alp et al. (2005) found 1:0.66 in the Fırnız Stream. In the study of Kocabas et al. (2012) the ratio was 1:1.08 in Uzungöl Stream. On the other hand, Yüksel et al. (2020a) reported the ratio of Munzur Stream as 1:0.96. By the time the other studies are taken into consideration, it is seen that the ratio of male individuals is higher than female individuals similar to the rate in our study. The number of male individuals in populations performing migration movements from trout species is expected to rise more than female individuals (Maitland and Campbell 1992). In general, males come to the reproductive area before females and leave the area later than females (Nikolsky 1963). In freshwater fish populations, males are expected to be more common in the first age groups (Alp et al. 2005). The value of (b) obtained from the regression analysis to determine the length-weight relationship reveals the type of growth in fish (Erkoyuncu 1995). The b values obtained in our study ranged between 2.89 and 2.81, and negative allometric growth was observed for all individuals, female and male individuals. Other studies on natural trout in Turkey (b) value varied a lot. Çetinkaya (1996) found out Çatak Stream *S. tigridis* individuals as 3.07. Yüksel (1997) claimed for Erzurum Teke Stream *S. t.*

*macrostigma* individuals as 2.59, while Arslan et al. (2004) pointed out 2.89 for *Salmo trutta labrax* individuals living in Çoruh Basin. On the other hand, Alp and Kara (2004) found out Seyhan, Ceyhan and Euphrates in the *S. t. macrostigma* individuals in the range of 3.027–2.878. The same range was 2.89–3.04 for Çoruh Basin Blood Brook Salmo trutta individuals (Arslan et al. 2004). Yüksel et al (2020a) conducted a similar study in Munzur River *S. t. macrostigma* and found the range as 2.9854–2.7251. In a similar vein, Tanır and Fakıoğlu (2017), who studied Çoruh Basin *S. trutta* individuals, reported the range of 3.0672–3.3158. It was observed that the value of (b) obtained in our study was lower than the values obtained from other studies except for the value of 2.59 reported by Yüksel (1997). This situation is thought to be due to the severe winter conditions prevailing for eight months in the Eastern Anatolia Region where the Çatak Stream, which rises at an altitude of 2338 m, is located. During the long winter months, the water temperature in Çatak Stream is nearly zero degrees. Trout stops the feed intake when the water temperature falls below 2 ºC (Çelikkale 1994). In fish, malnutrition that occurs in cold seasons causes a decrease in the length-weight relationship (b) (Arslan et al. 2004). Çetinkaya (1996), (b) value was reported as 3.07 for the Çatak Stream *S. tigridis* individuals. It is seen that this value is higher than the value we calculated (2.89–2.81). This difference is due to the deterioration of the river habitat in the last 14 years due to environmental conditions, the decrease in the amount of food and over-catching due to the withdrawal of large individuals from the population. In addition, as shown in the above mentioned literature (Arslan et al. 2004; Tanır and Fakıoğlu 2017), it is seen that there are differences between the values of (b) in studies conducted at different times in the same habitats. Depending on water temperature, nutrient abundance and reproductive activities, temporal and spatial differences occur in the length-weight relationship of fish populations (Wootton 1991). Populations of the same fish species in different streams may vary in length and weight values depending on environmental factors such as nutrient abundance, water temperature and habitat characteristics (Nikolsky 1963). Considering this situation, it is expected that there will be a difference between (b) value calculated by Çetinkaya (1996) in the same habitat and (b) value calculated in our study in 2019. The value (b) obtained in the study shows that the growth type of all individuals and sexes examined was negative allometry. Aras et al. (1986) claimed that *S. t. macrostigma* growth is usually isometric (b = 3) although (b) values vary between 2.3 and 4.0, he said. The negative allometric growth type detected in this study is consistent with this study. The value of (a) calculated in the regression analysis is used as an indicator to determine the degree of fattening (Condition Factor, K) of the fish (Avşar 2005). In our study, (a) value was calculated as 0.0187. When (a) values obtained from other studies are examined, it can be said that Kara et al. (2011) found 0.016 in the *S. platycephalus* population in the Zamantı Stream of the Seyhan River. For the same value, Yüksel et al (2020a) found 0.0187 in Munzur Stream among *S. t. macrostigma* individuals. Gülle et al. (2007) also reported that the trout in the Western Mediterranean basin is in the range of 0.011–0.015. These values are similar to the values obtained in our study. Since not all qualitative testable growth constants can be evaluated together, there is a high probability of error. In order to solve this problem, Φ 'value which reflects the total growth performance is calculated. In the study, Φ' value was calculated as 5.52 for *S. tigridis*. Considering the values obtained from other studies on natural trout are taken into consideration, the values were 5.81 in the Zamantı Stream (Kara et al. 2011), it was as 5.62 in the Munzur Stream (Yüksel et al. 2020a). It is thought that the differences between Φ 'values reported in other studies with the value of 5.52 in our study are caused by ecological differences that have direct effects on growth performance. *S. tidridis* (Tigris trout), which lives in Çatak Stream, is one of the important biodiversity richness of Turkey. In this study, it was observed that there were no VIII elderly individuals seen in the past years in the population. Of the 162 individuals examined, only 6 VII elderly individuals were found, and II–III elderly individuals were dominant in the whole sample, indicating excessive and illegal fishing on the population. In the Statement No: 5/1 on Regulation of Commercial Fisheries, natural trout fishing in rivers and tributaries of Van province is completely prohibited. In the Statement on the Regulation of Amateur Fisheries Hunting No. 5/2, Çatak Stream (80% of it), including Elmacı Stream starting from Taşliyazı Stream of Gürpınar district, is closed to fishing throughout the year. Although the whole of the stream is closed to commercial fishing and amateur fishing throughout the year, illegal fishing in the region continues extensively on the Tigris trout which shows that the measures are not applied. The continued illegal fishing in the region in the near future may cause the Tigris trout to disappear completely in the Çatak Stream. For this reason, the area declared as fishing prohibition should be expanded to cover the whole Çatak Stream, and the measures should be implemented strictly. In some studies, it has been observed that stream rehabilitation and road construction works have greatly changed the natural flow regime and habitat structure of the river. Rivers are living ecosystems with flow regimes, basements and coastal structures. It is evident that the natural trout species that adapt to this structure will affect the natural trout species living in the high parts of the stream, such as trout negatively. Preventing interferences that disrupt the natural structures of The Tigris living in the Çatak Stream is of high importance for the continuity of the trout.

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# Stock Assessment Indicators for Sustainable Exploitation of *Chrysichthys walkeri* in Lake Volta, Ghana, West Africa

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**ABSTRACT**

The biological indicators of *Chrysichthys walkeri* (Claroteid catfishes) in lake Volta were investigated for their sustainable management. A total of 846 samples were collected from the lake from March to December 2020. The recorded total length of the fish samples was analyzed using *TropFishR*. The von Bertalanffy parameters including asymptotic length ($L_\infty$), growth rate ($K$), and growth performance index ($\Phi$) were estimated as 28.88 cm, 0.42 per year, and 2.54 respectively. Mortality parameters were calculated as total mortality rate ($Z$) = 2.56 per year, natural mortality rate ($M$) = 0.91 per year and fishing mortality rate ($F$) = 1.65 per year. The exploitation rate ($E$) was 0.64. From the study, it was concluded that the population of the species *C. walkeri* from the Lake Volta is overexploited. There is the need to ensure continuous monitoring of fishing effort to ensure the sustainable exploitation and management of *C. walkeri.*

**Keywords:** *Chrysichthys walkeri*, growth, mortality, *TropFishR*, Lake Volta, Ghana

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## Introduction

Lake Volta occupies about 4% of the area of Ghana. It was recorded as the largest man- made Lake, which is the largest of its kind and has provided enormous biological and socio-economic benefit like generation of electricity (1060 MW) at Akosombo and Kpong dam (Fobil and Attuquayefio 2003). The Lake Volta is a highly significant water body richly surrounded by Togo, Cote d´Ivoire, Mali, Benin and Burkina Faso distributing the largest drains in the Lake Basin (Bene 2007). It is drained by several major rivers: the Mouhoun (Black Volta), the Nakambe (White Volta) with the Nazinon (Red Volta) as its tributary, the Oti River and the Lower Volta (Bene 2007). It is estimated that a total of 300.000 people depends on the lake for their livelihood of which 80.000 are fishers and 20.000 fish processors and traders (Braimah 1995). The fishery is solely artisanal with about 17.500 canoes actively fishing in the Lake operating from about 2.000 fishing villages. More than 90% of the inland fresh water fish are produced from the Volta Lake (Béné 2007). Study by Dankwa et al. (1999) and Briamah (2001) revealed the existence of 121 and 140 fish species for the whole Lake Volta respectively. Some of the dominant species recorded from the Lake include *Chrysichthys* sp., *Sierathrissa* sp., *Tilapia* sp., and others (Ofori-Danson 1999).

Belonging to the family Claroteidae, *Chrysichthys walkeri* is one of the five species belonging to the genus Chrysichthys in freshwaters of Ghana (Okyere and Boahemaa-Kobil 2020). It is an endangered fish species said to be endemic to the Pra Basin in Ghana (Lalèyè et al 2021; Froese and Pauly 2020). However, other studies on the species suggest its presence in Nigeria as well (Taiwo and Aransiola 2003; Ikusemiju and Olaniyan 1977; Nwafili1 et al. 2012). According to Nwafili1 et al. (2012), *C. walkeri* is almost indistinguishable from its counterpart *Chrysichthys nigrodigitatus* and is considered as an important commercial value fish, desirable for human consumption (Olopade et al. 2015). This species is of food and commercial importance with high culture potential (Dankwa et al. 1999).

There are few studies on the species and these include studies on its food and feeding habits (Ikusemiju and Olaniyan 1977), its length-weight relationship, condition factors and fecundity (Taiwo and Aransiola 2003) and its proximate composition consumption (Olopade et al. 2015). In Ghana, there are even fewer references on *C. walkeri,* with such reference including the study on changes in the fish community of the Kpong Headpond, lower Volta River (Quarcoopome 2011) and life history of Chrysichthys catfish in Volta Lake, Ghana (Vanderpuye 1979). It is therefore evident that, there is a paucity of information on population indicators for the sustainable management of *C. walkeri*.

The sustainable management of fisheries is a multi-dimensional and multi-level activity, that deals with a wide range of considerations including survival of the fish stocks and the fisheries (FAO 1999). It is an activity requiring reliable and invaluable information such as biological indicators. According to Hoggarth et al. (2005), biological indicators monitor the current status of a fishery and determines if fishery objectives are being achieved. Encompassing the categories of catch of fish, size of fish stock and amount of fishing, biological indicators serve as important tools of stock assessment (Hoggarth et al. 2005). They are therefore invaluable, to ensuring the sustainable management of important fisheries resources such as the stocks of *C. walkeri*.

This study was undertaken to address the knowledge gap concerning information on some population parameters such as growth rates, mortality rate and exploitation rates for sustainable management of *C. walkeri*. It focuses on the indicators such as growth parameters, mortality parameters and exploitation rate.

## Materials and Methods

### Study Area

Four landing communities within the Stratum VII of the Lake Volta, which lies between longitude 0°10’ and 1°05W and latitude 8°8’ and 8°20’N, and extends 60 km south and 50 km north of Yeji, were selected. These communities were Tonka, Vutideke, Brekente and Fante Akura which are all landing sites within the Stratum VII of Lake Volta (Figure 1). Yeji is the capital of Pru District in the Brong-Ahafo region with a population of 28.515 (GSS 2014). Selection of these sampling inland fishing communities was based on two-stage stratified sampling criteria, which were geographical isolation and the level of fishing activities based on the number of fishing boats.

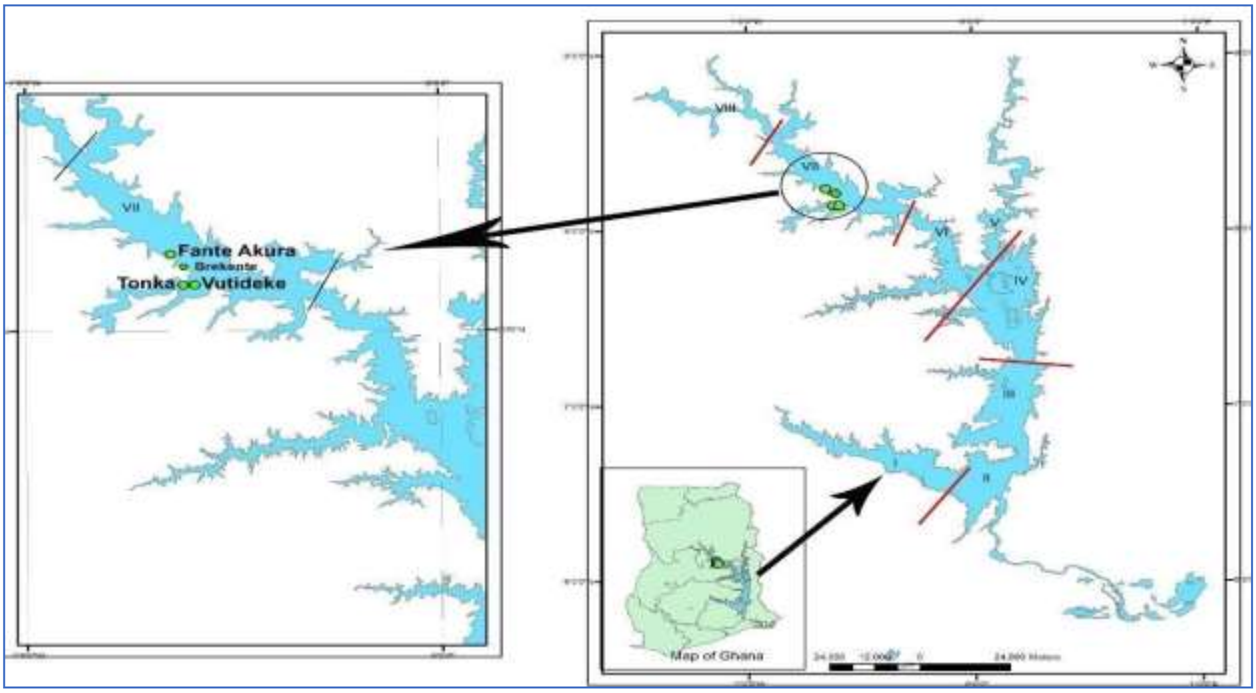


**Figure 1.** Map showing the study areas within Stratum VII of the Volta Lake, Ghana

### Data Collection

Samples of *C. walkeri* from the Volta Lake, Ghana were obtained from randomly selected fishermen who apply multifilament fishing gears in their fishing activities. Samples were obtained over a ten (10) month period (i.e., March 2020 to December, 2020) and were identified at the sampling sites using the taxonomic identification keys by Lowe-McConnell and Wuddah (1972). Measurements of length and weight were performed using a 100 cm graduated wooden measuring board and to the nearest gram 0.1 g using the digital balance.

**Estimation of Parameters**

***Growth parameters***

Growth parameters which follow the Von Bertalanffy Growth Function (*VBGF*) including growth rate (*K*) and asymptotic length ($L_\infty$) were estimated using the ELEFAN_ SA. Estimation of longevity ($t_{max}$) of the species was done using the method:

$t_{max} = 3/K$ (Anato 1999)

The growth performance index was calculated using the formula:

$\Phi' = 2logL_\infty + log\ K$ (Pauly and Munro 1984)

The theoretical age at length zero ($t_o$) followed the equation:

$Log_{10}(-t_0) = -0.3922 - 0.2752\ log_{10}\ L_\infty - 1.038log_{10}\ K$ (Pauly 1979)

**Mortality Parameters**

The total mortality (*Z*) was computed using linearized length-converted catch curve (Pauly and David 1981; Sparre and Venema 1992)

The natural mortality rate (*M*) was calculated using:

$M = 4.118K^{0.73}L\infty^{-0.333}$ (Then et al. 2015)

Fishing mortality (*F*) was calculated as:

$F = Z - M$ (Qamar et al. 2016)

The exploitation rate (*E*) was computed using:

$E = F/Z$ (Georgiev and Kolarov 1962)

**Length at First Capture ($Lc_{50}$)**

Using a backwards extrapolation of the descending limb of the length-converted catch curve, the probability of capture was estimated. A selectivity curve was generated using linear regression fitted to the ascending data points from a plot of the probability of capture against length, which was used to derive values of the lengths at capture at probabilities of 25%, 50% and 75% (Pauly 1987).

**Virtual Population Analysis (*VPA*)**

*VPA* is a method that allows for the reconstruction of the population from total catch data by age or length. The initial step was to estimate the terminal population (*Nt*), followed by the successive estimation of F values and finally, the population sizes are computed for each length class (midpoint). These procedures were estimated using the method developed by Pope (Pope 1972).

**Yield Per Recruit**

The relative yield-per-recruit (*Y/R*) was estimated using the knife-edge method (Beverton and Holt 1957).

## Results

**Length Distribution**

Table 1 shows the length distribution and catch composition of *C. walkeri* obtained during the study period. The length interval ranged from 5.5 cm - 27.5 with the highest number of specimens obtained during periods of August and September and the least during Mar - Apr, 2020 (Table 1). In all, a total of 846 specimen of *C. walkeri* were sampled for the study.

**Table 1.** Catch composition and length distribution of *C. walkeri* obtained from March - December, 2020

| lengthClass | 2020 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
| **5.5** | 4 | | | | | | | | | |
| **6.5** | 4 | | | | | 1 | 2 | | | |
| **7.5** | 2 | | | | | 3 | 4 | 2 | 1 | 1 |
| **8.5** | 5 | | 1 | 1 | | 7 | 12 | 9 | 4 | 2 |
| **9.5** | 4 | 3 | | 1 | | 6 | 44 | 15 | 9 | 9 |
| **10.5** | 6 | 1 | 4 | 3 | 5 | 24 | 50 | 11 | 3 | 4 |
| **11.5** | | 6 | 9 | 3 | 11 | 17 | 54 | 11 | 9 | 14 |
| **12.5** | | 6 | 8 | 1 | 9 | 12 | 16 | 6 | 3 | 8 |
| **13.5** | 2 | 4 | 5 | 2 | 14 | 11 | 6 | 5 | 3 | 4 |
| **14.5** | 1 | 7 | 12 | 6 | 15 | 18 | 6 | 5 | 4 | 2 |
| **15.5** | 2 | 6 | 12 | 8 | 14 | 5 | 3 | 5 | 5 | 6 |
| **16.5** | 4 | 6 | 4 | 8 | 16 | 7 | 4 | 11 | 5 | 3 |
| **17.5** | 3 | 3 | 4 | 6 | 10 | 3 | 2 | 6 | 1 | 2 |
| **18.5** | 2 | 2 | 1 | 8 | | 4 | | 3 | | |
| **19.5** | 1 | | | 10 | | 3 | | | 2 | 2 |
| **20.5** | 1 | 1 | 1 | 1 | 1 | 1 | | | 1 | |
| **21.5** | 1 | 1 | 3 | 1 | | | | | 2 | |
| **22.5** | | | | 2 | | 1 | | | | |
| **23.5** | | | | 2 | | 1 | | | | |
| **24.5** | 1 | 1 | | 1 | | | | 1 | | |
| **25.5** | | 1 | | 1 | 1 | | | | | |
| **26.5** | | | | 1 | | | | | | |
| **27.5** | 1 | 1 | | 1 | | | | | | |
| **Total** | 44 | 49 | 64 | 67 | 96 | 124 | 203 | 90 | 52 | 57 |

Reconstructed length frequency of *C. walkeri* with superimposed growth curves is shown in Figure 2. The asymptotic length ($L_\infty$) was 28.88 cm with a growth rate ($K$) of 0.42 per year. Growth performance index ($\Phi'$) was 2.54. The longevity ($t_{max}$) was approximately seven (7) years. The age at zero length ($t_o$) was estimated as - 0.40 years.

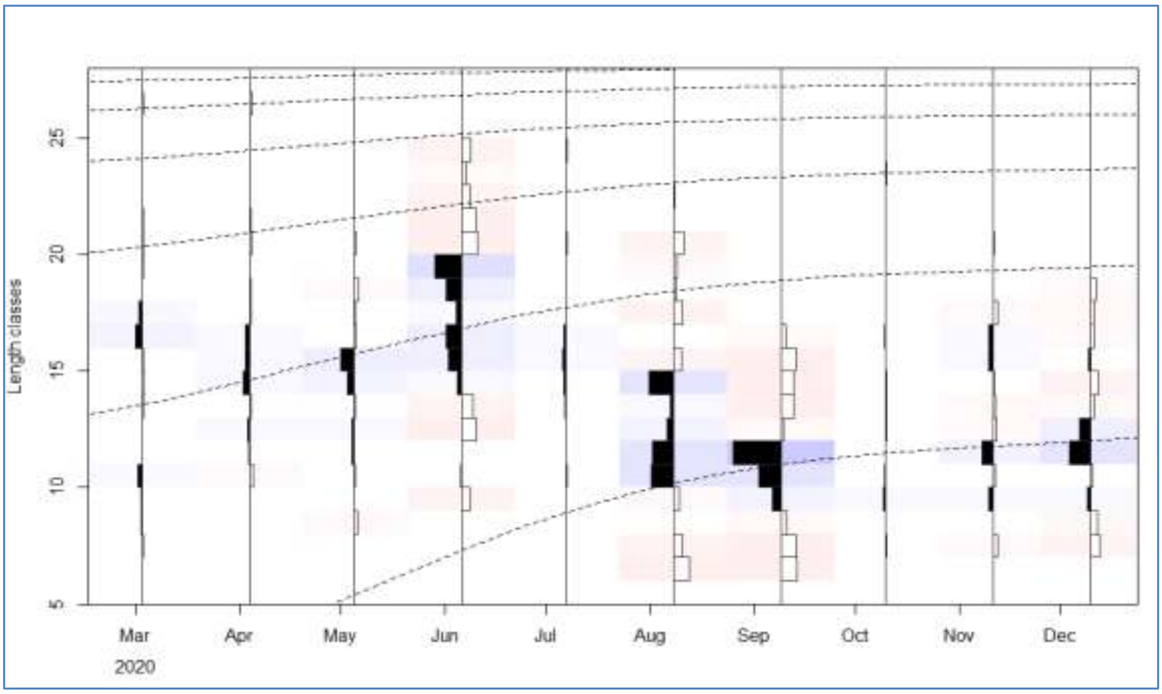


**Figure 2.** Reconstructed length-frequency distribution with growth curves.

**Mortality Parameters**

The linearized length-converted catch curve was used for the estimation of instantaneous total mortality ($Z$) as shown in Figure 3. The total mortality rate ($Z$) was calculated as 2.56 per year. The natural and fishing mortality rates were estimated at $M$ = 0.91 per year and $F$ = 1.65 per year respectively. The current exploitation rate ($E$) was obtained at 0.64.

**Length at First Capture**

The corresponding lengths at capture ($Lc$) for *C. walkeri* were estimated as $Lc_{75}$ = 3.27 cm, $Lc_{50}$ = 4.80 cm and $Lc_{75}$ = 6.33 cm (Figure 3).

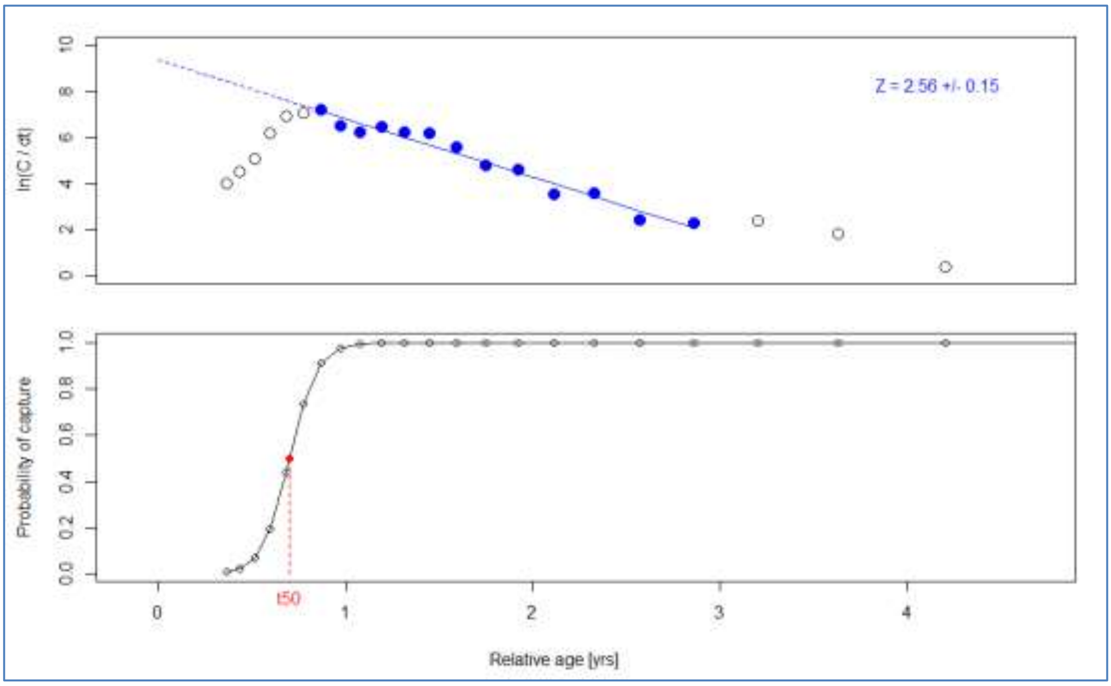


**Figure 3**. Linearized length-converted catch curve.

**Virtual Population Analysis**

The virtual population analysis of *C. walkeri* is shown in Figure 4. Individuals within the range of 11 - 12 cm experienced the highest level of exploitation (catch = 13400 per year). Natural losses were highest among individuals within the length range of 6 cm – 8 cm. Surviving individuals in the stock exhibited a declining trend with increased rate of fishing pressure. The highest number of survivors (692421) in the stock was observed in the length range of 6 – 7 cm whereas the lowest number of survivors (1078.38) was observed for individuals at a length range of 27 – 28 cm. Fishing effort was highest (*F* = 0.32 per year) on individuals within the length range of 11 – 12 cm and lowest (*F* = 0.006 per year) on individuals at length range of 6 – 7 cm.

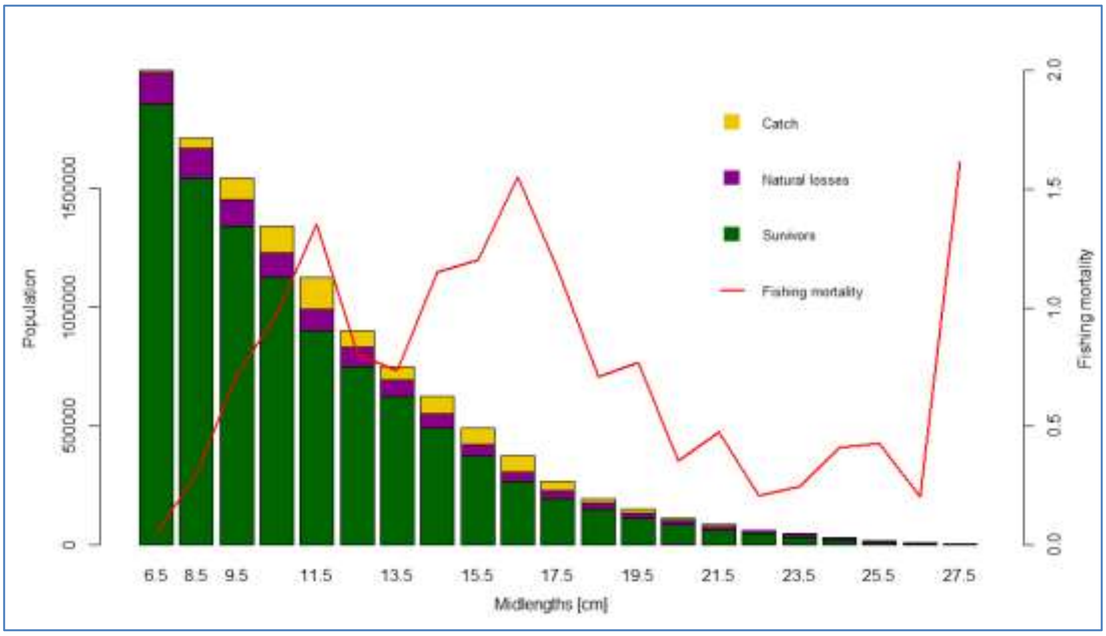


**Figure 4.** Length structured virtual population analysis of *C. walker* in Lake Volta

**Relative Yield Per Recruit**

The plot of relative yield per recruit against fishing mortality showed that the indices for sustainable yield were 0.8 for $F_{0.5}$ and 2.5 for $F_{msy}$ as indicated in Figure 5.

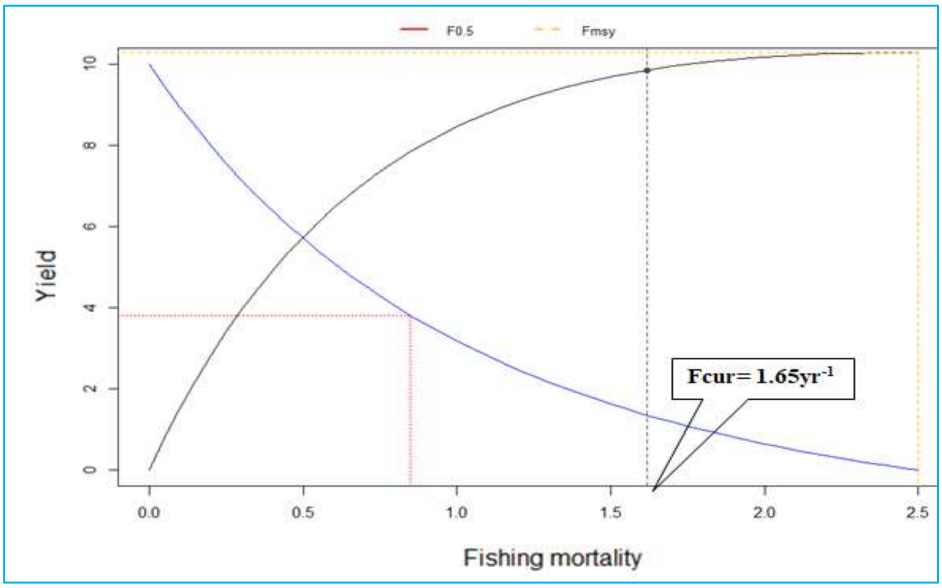


**Figure 5.** Yield-per-recruit plot of *C. walkeri* in the Lake Volta. The black dot represents yield under the current fishing pressure. The yellow and red lines represent maximum allowable fishing mortality and fishing mortality with a 50% reduction related to the virgin biomass.

## Discussion

There is little information on the population dynamics of *C. walkeri* and hence, the present study compares the information obtained on *C. walkeri* with its almost indistinguishable counterpart *C. nigrodigitatus* (Olopade et al. 2015). The present study will provide baseline information for the sustainable management of *C. walkeri* in the Lake Volta.

The growth rate for *C. walkeri* estimated for the study was 0.420 per year. This was lower than the estimates for *C. nigrodigitatus* from Ghana (0.65 per year) (Ofori-Danson et al. 2002), Nigeria's Nun River (0.538 per year) (Abowei and Hart 2007) and Nigeria's lower cross river (1.50) (Udoh et al. 2015). Variations in growth rate of this study compared to others may be is a result of influences of gonad maturity, sex, growth phase, habitat, fish adaptive life pattern, location, food abundance or number of sampled specimen (Froese 2006; Qamar et al. 2016). The growth rate from the present study (0.420 per year) signified that *C. walkeri* in Lake Volta is a slow-growing species, characterized by its longevity period ($t_{max}$) of seven (7) years. The asymptotic length ($L_\infty$) for the present study was 28.88 cm which is relatively lower when compared to the asymptotic length ($L_\infty$) of the *C. nigrodigitatus.* The differences in asymptotic length may be are results of the differences in environmental factors, productivity, length of the largest species, the age analysis method utilized and fishing pressure (Park et al. 2008; Sequeira et al. 2009; Wehye et al. 2017).

Growth performance index is a function of the asymptotic length and it is used in comparing the growth curves between populations of the same species and/or different species that belong to the same family (Park et al. 2013). It can be affected by determinants of the growth potential of species such as genetic make-up, overfishing and the diet of the fish species (Sambo and Haruna 2012). The growth performance index for *C. walkeri* for the present study (2.54) was lower than estimates by Ofori-Danson et al. (2002) (3.12), Abowei and Hart (2007) (2.63) and Udoh et al. (2015) (4.31) for the related species, *C. nigrodigitatus.* Differences in growth performance indexes may be due to the differences in environmental factors, such as temperature, salinity of study water and variability in the lifespan between study areas (Park et al. 2013).

The total mortality rate estimated from the current study was 2.56 per year. This was found to be lower than estimates by Ofori-Danson et al. (2002) (3.77 per year) and Udoh et al. (2015) (4.31 per year) but higher than estimate by Abowei and Hart (2007) (1.68 per year). The variation in total mortality estimates in relation to the current study could be a result of differences in environmental parameters and the level of fishing effort. The natural mortality rate estimated from the present study was 0.91 per year which was lower than estimates of mortality rate by Ofori-Danson et al. (2002) (1.24 per year) and Udoh et al. (2015) (1.38 per year) for *C. nigrodigitatus* but higher than the estimate of natural mortality rate by Abowei and Hart (2007) (0.70 per year) for *C. nigrodigitatus*. From the present study, the fishing mortality rate ($F$ = 1.65 per year) was greater than the natural mortality rate ($M$ = 0.91 per year). This estimate suggests that for *C. walkeri* species in Lake Volta, fishing mortality is the most important form of mortality confronting this species, as the impact of fishing activities on the decline of *C. walkeri* is superior to natural mortality induced conditions (de Costa et al. 2018). The fishing mortality rate for *C. walkeri* was however, lower than the fishing mortality rate required at the maximum sustainable yield ($F_{msy}$). The exploitation rate from the present study was 0.64, which is lower than the value provided by Ofori-Danson et al. (2002) (0.65) but higher than provided by Abowei and Hart (2007) (0.58) and Udoh et al. (2015) (0.62) for *C. nigrodigitatus*. The exploitation rate of *C. walkeri* in Lake Volta was above the optimum of 0.5, and this suggests that the species is overexploited (Gulland 1971).

The estimated length at first capture from the current study for *C. walkeri* was 4.80 cm. This estimate was less than the value recorded by Udoh et al. (2015) from Nigeria for *C. nigrodigitatus* (i.e., 36.3 cm). The potential reason for this variation in estimates could be linked to the type and mesh size of fishing gear, time, duration of sampling, fish landing site sampled (Ofori-Danson et al. 2018).

Length-based VPA provides a medium for estimating fishing pressure on various length groups using fish landings from fishing operations (Neethiselvan and Venkataramani 2002). The high fishing mortality on a large-sized individual was experienced by individuals within the interval of 11 cm - 12 cm. Also, the number of individuals of the species subjected to natural losses as well as the number of survivors declined as they matured.

In conclusion, *C. walkeri* from Lake Volta is a slow-growing species with a growth rate of 0.42 per year. The exploitation rate ($E$ = 0.64) was above the optimum level of 0.5 indicating that this species is overexploited in Ghana's Lake Volta. There is therefore the need to reduce the fishing effort through the implementation of closed fishing season, alternative livelihoods for fisher folks and the application of sustainable fishing practices. In addition, the continuous monitoring of the above stated fishing management practices for sustainable exploitation of *C. walkeri* is essential for the achievement of *SDGs* 1, 2 and 14.

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# The Accumulation of Some Heavy Metals in Northern Pike *(Esox lucius* L., 1758*)* Inhabiting Sıdıklı Küçükboğaz Dam Lake (KIRŞEHİR)

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## ABSTRACT

Pike (*Esox lucius*) samples were obtained from the designated locations of Sıdıklı Küçükboğaz Dam Lake between March 2012 and February 2013. Some heavy metal concentrations (Cu, Fe, Mn, Zn, Cr and Al) in the muscle, liver, skin, intestine and gills of this fish were measured by Flame Atomic Absorption Spectrometry (AAS) device and it was observed that heavy metals accumulated at different levels in different tissues of pike. Heavy metal concentration in different fish tissues varied as dry weight Cu: 0.1123-0.3764, Fe: 4.3135-9.505, Mn: 0.5442-0.1684, Zn: 3.3065-2.475, Cr: 0.1889-2.517, Al: 2.17-1.045 µg $g^{-1}$. When the heavy metal levels in the tissues were compared with the national and international permissible limits, it was seen that the values of all metals in the muscles of the fish were below the determined limit values according to the analysis results. Seasonal changes in metal (Cu, Fe, Mn, Zn, Cr and Al) concentrations were observed in pike tissues, but it was concluded that these differences would not negatively affect the consumption recommendations based on the levels permitted by FAO/WHO (2020). According to the analysis, it was concluded that the fish collected from this region can be safely consumed by humans and the lake water can be used for irrigation purposes in agriculture.

**Keywords:** *Esox lucius,* heavy metal, Sıdıklı Küçükboğaz Dam Lake, Kırşehir

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### Sıdıklı Küçükboğaz Baraj Gölü (Kırşehir)'nde Yaşayan Turna Balığı (*Esox lucius* L., 1758)'nda Bazı Ağır Metallerin Birikimi

**Öz:** Turna (*Esox lucius*) numuneleri, Mart 2012 ile Şubat 2013 tarihleri arasında Sıdıklı Küçükboğaz Baraj Gölü'nün belirlenen yerlerinden temin edilmiştir. Turna'nın kas, karaciğer, deri, bağırsak ve solungaçlarındaki bazı ağır metal konsantrasyonları (Cu, Fe, Mn, Zn, Cr ve Al) Alev Atomik Absorpsiyon Spektrometresi (AAS) cihazı ile ölçülmüş ve farklı dokularında farklı seviyelerde ağır metallerin biriktiği gözlemlenmiştir. Farklı balık dokularındaki ağır metal konsantrasyonu, kuru ağırlık olarak; Cu: 0.1123-0.3764, Fe: 4.3135-9.505, Mn: 0.5442-0.1684, Zn: 3.3065-2.475, Cr: 0.1889-2.517, Al: 2.17-1.045 mg/kg şeklinde değişiklik göstermiştir. Dokulardaki ağır metal seviyeleri ulusal ve uluslararası izin verilen sınırlarla karşılaştırıldığında, balıkların kaslarındaki tüm metal değerlerinin analiz sonuçlarına göre belirlenen sınır değerlerinin altında olduğu belirlenmiştir. Turna dokularında metal (Cu, Fe, Mn, Zn, Cr ve Al) konsantrasyonlarında mevsimsel değişiklikler gözlemlenmiş, ancak bu farklılıkların FAO/WHO (2020) tarafından izin verilen seviyelere göre tüketim önerilerini olumsuz etkilemeyeceği sonucuna varılmıştır. Yapılan analize göre, bu bölgeden toplanan balıkların insanlar tarafından güvenle tüketilebileceği ve göl suyunun tarımda sulama amaçlı olarak kullanılabileceği sonucuna varılmıştır.

**Anahtar kelimeler:** *: Esox lucius*, ağır metal, Sıdıklı Küçükboğaz Baraj Gölü, Kırşehir

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## Introduction

It is known that heavy metals are among the leading toxic substances that pollute the natural environment. Pollution when combined with water has become a serious health problem in recent years as it creates a widespread threat by increasingly accumulating through the food chain in all organisms in the ecosystem (Yilmaz et al. 2016; Polat and Akkan 2016; Uncumusaoglu and Akkan 2017; Akkan et al. 2018; Mutlu et al. 2018). Therefore, they are easily soluble in water resulting that they can be absorbed very easily by aquatic organisms and their binding to the proteins of living things is quite strong (Kalay et al. 2004; Muşlu 2008). It eventually causes structural dysfunctions at cellular and molecular levels in aquatic organisms and increases in the frequency of DNA breaks (Kalay and Karataş 1999; Levesque et al. 2002; Akkan et al. 2013; Giordano et al. 1989).

When low ambient concentrations are examined, heavy metals seem to accumulate in metabolically active tissues such as liver, kidney and spleen at high levels and this accumulation is associated with metal detoxification and disfunction of metabolic events (Sharma 1983; Cicik 2003).

The aquatic environment is highly susceptible to the harmful effects of heavy metal contamination because aquatic organisms are in close and prolonged contact with soluble metals. Increasing heavy metal accumulation in fish constituting a ring of the biological cycle and consumed as an important protein source, causes both toxic effects on fish and negative effects on human health (Dural et al. 2007; Canbek et al. 2007; Sipahi et al. 2013). Therefore, it can be considered as one of the most important indicators of the impact of metal pollution in water ecosystems. The release of industrial and agricultural wastes has recently increased heavy metal concentrations in lake ecosystems, which leads aquatic organisms to be exposed to these metals at high levels (Kalay and Canlı 2000; Sankar et al. 2006; Said et al. 2014). When heavy metals released into the aquatic environment with different types of wastes exceed the legal limits, especially when they are used as drinking water or irrigation water, they reach more dangerous dimensions and cause many problems in living organisms, community-environmental health and agriculture (Segar and Pellenbarg 1973). Therefore, an accurate water pollution assessment and monitoring is of great importance because of its direct effects on aquatic life and human health (Saha et al. 2017). Lead (Pb), cadmium (Cd) (Kumar et al. 2013), nickel (Ni) (Kaaber et al. 1978, 1979), chromium (Cr) (U.S.EPA 1999), mercury (Hg) and arsenic (As) (Sankhla et al. 2016) are among the most important heavy metals that cause water pollution and pose a great threat due to their toxic effects.

The aim of this study is to determine the heavy metal pollution level of this lake by analyzing some heavy metal accumulation levels in the muscle, liver, skin, intestine and gills of Northern Pike collected from different locations of Sıdıklı Küçükboğaz Dam Lake.

## Materials and Methods

### Study Area and Sampling

Sıdıklı Küçükboğaz Dam Lake is located in the Central Basin of Kızılırmak River, in the Southwest of Kırşehir Province, on the Körpeli Boğaz Stream, in the north of Hirfanlı Dam Lake. It is connected to a small river known as Kepez Özü. The reservoir volume of the lake is 28,500 $m^3$, the reservoir area is 1.65 $km^2$ and the area to be irrigated is 4,945 hectares. It is 30 km away from Kırşehir city center (Figure 1). In the dam lake, there are *Cyprinus carpio* (European carp), *Silurus glanis* (Wels catfish), *Tinca tinca* (Tench), *Capoeta tinca* (Anatolian khramulya), *Squalius cephalus* (chub), and *Esox lucius* (Northern Pike).

**Figure 1**. Satellite Map Image of Sıdıklı Küçükboğaz Dam Lake (Taken from Google Earth.)

**Field and Laboratory Studies**

Study materials consist of 15 pike samples (*E. lucius*) collected by sampling every month in a one-year period from different parts of the Sıdıklı Küçükboğaz Dam Lake between March 2012 and February 2013.The samples were caught using fanned nets and fishing lines with 12x12, 16x16, 18x18 and 22x22 mm mesh sizes and brought to the laboratory in suitable sized bags. While water samples were taken into brown bottles of 500 ml, sediment samples were taken 3 m from the beach and the lake floor with a plastic shovel placed in plastic bags and brought to the laboratory.

**Preparation of Tissues and Organs of Northern Pike (*E. lucius*) for Analysis**

Approximately 0.5 g of tissue samples (muscle, gill, liver, skin, intestine) from each fish were excised, washed with distilled water, weighed, packed in polyethylene bags and stored at -20°C until metal analysis. All tissue samples were transferred to 100 ml teflon tubes and samples (0.5 g) using the microwave solubilization program (pressure 200 psi, ramp time 15 mins. Temperature 210°C, maximum power 450 W, retention time 10 mins.) in a microwave oven (CEM MARS -5 Closed Cup Microwave Digestion System) was dissolved with 5 mL $HNO_3$ (65% (m/m)). Teflon tubes were sealed with a watch glass and heated at 50-100°C on a hot plate for 4 hours until the solution slowly evaporated to almost dryness. 2 ml of $HNO_3$ (65% (m/m)) and $H_2O_2$ (30% (m/m)) were added to the residue and the solution was evaporated again on the hotplate. After cooling, 2.5 ml of 1N $HNO_3$ was added to the digested residue and transferred to 10 ml graduated flasks, then diluted to level with deionized water (Ciftci et al. 2011). Samples were filtered through a 0.45 μm syringe filter (Sartorius) prior to analysis.

**Preparation of Sediment for Analysis**

Sediment samples were taken from the shore of Sıdıklı Küçükboğaz Dam Lake with a plastic scoop, placed in 500 ml colored bottles and brought to the laboratory for analysis. It was placed in a petri dish, dried in an oven at 60ºC and pounded in a mortar until it was pulverized. 0.1 g was weighed and put into a teflon tube and 5 ml of concentrated $HNO_3$ and then 1 ml of concentrated $HClO_4$ were added and left for one hour. Solubilization was carried out for one hour and allowed to cool at room conditions. Excess acids in the environment were evaporated at 50-100 ºC. It was completed to 10 ml with deionized water, and kept in a cool place without light until it was analyzed in glass tubes by filtering.

Sediment solubilization was done in a microwave oven. Microwave solubilization method provides great advantages in terms of short time, less acid consumption and preventing possible metal loss (Karadede et al. 2004).

**Preparation of Water Samples for Analysis**

The pH of the water samples taken from the Sıdıklı Küçükboğaz Dam Lake was first measured during the field, taken in 500 ml brown bottles, brought to the laboratory and the pH was measured and recorded again. Then it was filtered and taken into another bottle of the same size, 1 ml of concentrated $HNO_3$ was added (to prevent bacteria that may occur) and stored in a cool and dark environment until the analysis.

**Analysis Method**

In this study the accumulation of heavy metals copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), chromium (Cr) and aluminum (Al) were analyzed. Analyzes were carried out with a Flame Atomic Absorption Spectrometer (AAS) (Analytik Jena AG, Jena, Germany) equipped with a 50 mm burner head and an injection module (SFS-6). All absorption lines of an element in the 185-900 nm spectral range were analyzed analytically using a Xe short arc lamp as a continuous lamp source. The spectral background of the sample in AAS is always corrected simultaneously, independently and directly on the analysis line. All measurements were made under optimum conditions in triplicate using an injection module SFS-6, which provides computer-controlled aspiration of cavities, analytical solutions and samples (Ciftci et al. 2011; Ciftci and Er 2013). All solutions were prepared using ultrapure water (specific resistance 18 M/cm) from a MilliQ purification system (Millipore Corporation, Massachusetts, USA). Standard analysis solutions were prepared from 1000 mg L-1 stock solutions (Merck). Results are expressed in $\mu g\ g^{-1}$. One-way ANOVA and Tukey tests were used to test the differences in metal levels between samples (significance level $p < 0.05$). All statistical calculations were made through SPSS 16.0 for Windows.

## Results

This study was carried out on the Northern Pike found in Sıdıklı Küçükboğaz Dam Lake and provides information on the metal accumulation levels in the studied tissues. According to the results obtained in this study, it was determined that the heavy metals that accumulated the most in the liver were Cu and Fe. It was observed that Mn, Cr and Al accumulated in the gills at the highest rates. Compared to other tissues, Zn accumulated mostly in the intestines. Cu is least found in muscle tissue and the order of change in other tissues is gill > skin > intestine. In the analysis for Fe, the least accumulation was observed in the skin. Accordingly, the relationship between other tissues was in the form of intestine > gill > muscle tissue. Mn is mostly found in the skin and intestines after the gills, and its level is very low in the muscle tissue. Metal accumulation rates for Zn are gill > liver > skin > muscle tissue. Looking at the Cr levels, the least accumulation was observed in the skin, and the order in other tissues was liver> muscle tissue> intestine. Finally, in the results obtained for the Al analysis, no significant accumulation was observed in the muscle tissue, while the Zn ratio in the intestines was found to be higher than in the liver.

Mean concentrations of Cu, Fe, Mn, Zn, Cr and Al metals accumulating in different tissues of Northern Pike (*E. lucius*) are given in Table 1. During the study, research was carried out on 15 fish materials collected in monthly periods. The results of the analysis show that heavy metals are mostly accumulated in the liver and gills. While Fe, Zn and Al values were determined to be the most abundant metals in the liver with concentrations of 10.551 µg/kg, 2.6935mg/kg and 0.801 mg/kg, respectively, the second highest metal concentration was found to be the most abundant in the gills with Zn, Fe and Al values of 4.686 mg/kg, 2.804 µg/kg and 1.548 mg/kg respectively. Metal concentrations accumulated in the muscles, on the other hand, showed the lowest concentration level throughout the study. Except for Mn and Al metals, all metal concentrations accumulating in the liver were measured at higher levels than others. Considering the different tissues of pike, the lowest Cr metal level was recorded as 0.048 µg/kg in the skin, and the highest value was measured with 0.689 mg/kg in the liver.

**Table 1.** Concentrations of heavy metals in various tissues of Northern Pike (gill, intestine, skin, muscle tissue and liver) (one year period) (mg/kg)

| | Mean.±SE (Min.-Max.) | | | | |
|---|---|---|---|---|---|
| **Metal (mg/kg)** | **Gill** X± SE Min.–Max | **Intestine** X± SE Min.–Max | **Skin** X± SE Min.–Max | **Muscle** X± SE Min.–Max | **Liver** X± SE Min.–Max |
| **Cu** | 0.187±0.0045$^{a,x}$ (0.0588-0.3898) | 0.205±0.0083$^{a,x}$ (0.1178-0.2536) | 0.165±0.0036$^{a,x}$ (0.0788-0.3575) | 0.2726±0.0231$^{a,xy}$ (0.0553-0.7616) | 0.572±0.0305$^{b,z}$ (0.3764-1.0527) |
| **Fe** | 2.804±0.2560$^{b,y}$ (1.7021-4.3135) | 3.227±0.3347$^{a,y}$ (1.9015-4.879) | 1.063±0.0698$^{a,y}$ (0.588-1.888) | 1.231±0.0589$^{a,yz}$ (0.7413-1.7027) | 10.551±0.1694$^{c,w}$ (9.505-11.65) |
| **Mn** | 0.7005±0.0032$^{b,x}$ (0.5442-0.8164) | 0.222±0.0039$^{a,x}$ (0.1501-0.4247) | 0.3316±0.0070$^{a,xy}$ (0.1771-0.5946) | 0.1166±0.0055$^{a,x}$ (0.0208-0.3575) | 0.1096±0.0407$^{a,x}$ (0.0502-0.1684) |
| **Zn** | 4.686±0.2546$^{a,x}$ (3.3065-5.7035) | 9.762±0.2430$^{a,y}$ (8.175-10.39) | 2.8385±0.0320$^{a,z}$ (2.305-3.2105) | 1.6521±0.0440$^{a,z}$ (1.054-2.05) | 2.6935±0.0905$^{a,yz}$ (1.913-3.4295) |
| **Cr** | 0.5976±0.2608$^{a,x}$ (nd-2.2015) | 0.1527±0.0037$^{a,x}$ (0.047-0.3384) | 0.048±0.0020$^{a,xy}$ (nd-0.1259) | 0.058±0.0038$^{a,xy}$ (nd-0.17) | 0.689±0.3252$^{a,xy}$ (nd-2.517) |
| **Al** | 1.548±0.0766$^{a,x}$ (0.7318-2.17) | 0.862±0.0625$^{a,x}$ (0.325-1.5185) | 0.804±0.0224$^{a,xy}$ (0.555-1.287) | 0.712±0.0143$^{a,xy}$ (0.56-1.099) | 0.801±0.0070$^{a,xy}$ (0.5419-1.045) |

Horizontally, the letters a, b and c show statistically significant differences ($p < 0.05$).
The letters x, y, z and w vertically show statistically significant differences ($p < 0.05$).
nd: not detected.
X ± SE: Mean ± standard error. Number of samples taken annually n:15

According to the results of the analysis, while Fe has the highest accumulation, Cr lowest and the highest accumulation rate of Fe and Cu metals is in the liver. In addition, it was determined that the relationship between heavy metal concentrations was listed as Fe> Zn> Al> Cu> Mn> Cr. Similarly, in the study of Tekin-Özan and Kır (2007), while it is known that the pike (*E. lucius*) living in Lake Işıklı detected Cu, Mn and Cr in the liver below the analysis limit, they also determined the Fe and Zn values.

Since fish is a strong source of protein, the metal concentrations accumulated in the muscle tissues are very important. The result shows that the heavy metal concentrations accumulated in the muscle tissue of Northern Pike were found to be lower than the maximum values recommended and allowed by FAO (2005) and WHO (1995), which is very important. Accordingly, the mean heavy metal concentrations in the muscles, Cu; 0.2726 mg/kg, Fe; 1.231 mg/kg, Mn; 0.1166 mg/kg, Zn; 1.6521 mg/kg, Cr; 0.058 mg/kg, and Al; 0.712 mg/kg calculated as. In the studies (Türkmen et al. 2009; Company et al. 2010), the researchers similarly found that the muscle was not an active tissue in accumulating heavy metals. In addition to analyzes made in the muscle due to its importance in nutrition, analyzes were also performed on the gills and skin, especially the liver, due to its high capacity in accumulating heavy metals.

According to the results of the metal analyzes in the water of the Sıdıklı Küçükboğaz Dam Lake, the Cu (0.0120 mg/L) and Cr (0.583 mg/L) accumulation levels were measured below the analysis limit. It was determined that the Zn (0.769 mg/L) accumulation level was insignificant, while the Fe (0.489 mg/L) and Mn (0.491 mg/L) accumulation levels were significant. According to the results of the metal analyzes made in the sediment of the Sıdıklı Küçükboğaz Dam Lake, it was seen that the Cu (0.1651 mg/kg), Fe (21.7495 mg/kg), Zn (0.8997 mg/kg) and Cr (0.1701 mg/kg) accumulation levels were insignificant, while the Mn (5.9197 mg/kg) accumulation levels were significant.

Heavy metal deposition levels of the study were evaluated statistically by One Way ANOVA and Tukey tests. Cu and Fe values in liver and other tissues; Zn and Al values in the gill, intestine and other tissues were determined to be statistically significant ($p < 0.05$). Considering the differences between all tissues, the Cr value was not considered statistically significant ($p < 0.05$). In the liver, the differences in Cu and Fe accumulations were found to be statistically significant, while the values obtained in terms of other metals were insignificant ($p > 0.05$). While Mn value in gill and skin, values in Zn and Al accumulation in intestines and gills were statistically significant, changes in accumulation of other metals were statistically insignificant ($p > 0.05$).

## Discussion

In aquatic ecosystems, sediment is constantly addressed in monitoring studies, as it acts as a natural reservoir of pollutants that tend to precipitate and is the natural memory of the aquatic ecosystems in which it is located (Mutlu et al. 2020). In the heavy metal analysis carried out in the sediment of Sıdıklı Küçükboğaz Dam Lake, Cr was measured below the analysis limit except for the months of May-June and July. Cu, Fe, Mn, Zn and Al were detected every month. It has been determined that the metal that accumulates the most in the sediment is Al in the Spring-Summer-Autumn and Winter seasons. According to the study carried out in the study area covering a 28 km coastline along the Black Sea, it was determined that the heavy metals that accumulated most in the sediment were Fe and Al (Fikirdeşici Ergen et al. 2018).

According to the analysis made in Kapulukaya Dam Lake (Kırıkkkale), it has been shown that Al is present in the sediment to a significant extent (Binici and Pulatsü 2018). The presence of heavy metals in ionic form in water can cause an increase in toxicity effects. The presence of heavy metals in the form of toxic ions in waters can directly harm organisms and their consumers. Some of the heavy metals found in this way are taken directly by living things after they enter the water, while others are carried to the sediment (Kaptan 2014). For this reason, the analyzes to be made are of great importance. As a result of heavy metal analysis in the water of Sıdıklı Küçükboğaz Dam Lake, Cr (0.583 mg/L) was measured below the analysis limit in all seasons. Cu (0.0120 mg/L) has only been detected in August. Fe (0.489 mg/L), Mn (0.491 mg/L) and Zn (0.769 mg/L) were detected between June and September. Al has been detected between May and September. Al (1.001 mg/L) was detected at the highest rate in Spring, Al, Zn and Cr in Summer. It has been determined that the most common metals in water are Al and Zn. According to the results of heavy metal analysis carried out in Ulubat Lake Water, it was determined that the highest level of accumulation was Zn (6.13mg/L) metal in Spring (Berber et al. 2021). Studies have shown that Al (35.28 mg/L) in the Kovada lake in the Antalya basin, Al (27.59 mg/L) in the Karakuyu Marshes, and Al (9.57 mg/L) and Zn (309.86 mg/L) in the Burdur Basin Lakes show the highest level of accumulation (Oruçoğlu and Beyhan 2019). As a result of heavy metal analysis in some tissues and organs of Northern Pike living in Sıdıklı Küçükboğaz Dam Lake; Fe and Cu were detected in the liver, and Zn in the intestine and gill in the samples collected in the spring. In the samples collected during the summer months, Cu and Fe were detected in the liver, Mn in the gill and skin, and Zn and Al in the intestine Cu and Fe were detected in the liver in the samples collected in autumn, Zn in the intestine and gills, and Mn in the gills in the samples collected in the winter months. Cu, Fe, Zn, Cr, Al do not differ between tissues. In the study conducted in Ladik Lake (Samsun), Al, Cr, Mn and Zn levels in the tissues of fish were measured at the level of gill>liver>muscle in all seasons. Cu and Fe levels were found in the order of liver>gill>muscle (Erdoğan et al. 2021). Zn, Cu, Pb and Cd levels were investigated in the liver, gill and muscle tissues of economically important species (*Caranx rhoncus, Scomber japonicus, Pegusa lascar*) in Mersin Bay and it was determined that Cu accumulates at the highest level in the liver (Karayakar et al. 2017). These results showed parallelism with our study. At the end of the study, it was determined that heavy metals accumulated mostly in the liver and gills. The liver is an important organ where metals are stored, both because it plays an active role in metabolism and because it is the organ where metalloproteins, which are metal-binding proteins, are produced (Al-Yousuf et al. 2000; Liu et al. 2012). Metals taken up with respiratory water adhere to the mucus in the gills, and the metals remain between the lamellae as respiratory water passes through the gill lamellae. Thus, the metal concentration in the gills can reach high levels (Heath 1995). The low level of accumulation of metals in the muscle tissue can be explained by the fact that the muscle is not a metabolically active organ (Karadede et al. 2004) however, researches are carried out with the concern that the metals accumulated in this tissue can reach the human by joining the food chain (Wang et al. 2010).

This study was carried out to determine the heavy metal levels accumulated in the muscles, liver, skin, intestines and gills of the Northern Pike living in the Sıdıklı Küçükboğaz Dam Lake. A natural advantage of this study is it enables us to obtain information about heavy metal pollution of the reservoir. Although the metal levels determined in Sıdıklı Küçükboğaz Dam Lake are not excessively high in general, a metal pollution tending to increase has been detected. According to the results, significant differences were found in the heavy metal accumulation levels in the tissues and organs of Northern Pike. According to these results, it was concluded that the heavy metal levels accumulated in the muscle tissue of Northern Pike in this region were not significantly found, which poses no danger in consuming them as food. In addition, the results underline the importance of controlling the commercial activity on the Sıdıklı Küçükboğaz Dam Lake and the streams feeding it, in addition to preventing the leakage of pesticides and chemical fertilizers used in agricultural activities into the lake. Considering that the water of the dam lake is used for agriculture and irrigation purposes, it is very important to take precautions against all kinds of dangers thought to increase when fish consumption is taken into account in terms of protein content. In addition, metal pollution of the lake water and regular inspections are of great importance. In addition to these, tourism activities should be arranged by considering the pollution capacity of the lake. It should not be forgotten that it is an inevitable fact that wastes left to nature consciously or unconsciously will return to humans.

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# Zooplankton Fauna and Seasonal Changes of Two Karstic Sinkhole Lakes: Meyil and Kızören (Konya/Türkiye)

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**ABSTRACT**

Meyil and Kızören sinkholes are large karstic formations located in the Konya plain, Türkiye. Seasonal distribution of planktonic community of these two karstic lakes have yet to be studied. In this study, samples were collected from pelagic station in different seasonal periods (March-July-October 2018) and their physico-chemical parameters were determined and evaluated with seasonal species composition. As a result, fourty zooplankton species were identified in the two lakes, amongst which are thirty-four rotifers, five cladocerans and one copepod. Rotifers were founded as the dominant group for both lakes. All species identified as a new record for Meyil and Kızören sinkhole lakes. Furthermore, this study contributes to the literature by explaining the first detailed data for zooplankton fauna of sinkhole lakes in Türkiye as of the sampling date.

**Keywords:** Zooplankton fauna, karstic lake, Kızören Sinkhole, Meyil Sinkhole, Türkiye

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**İki Karstik Obruk Gölünün Zooplankton Faunası ve Mevsimsel Değişimleri: Meyil ve Kızören (Konya/Türkiye)**

**Öz:** Meyil ve Kızören obrukları, Türkiye’de Konya ovasında yer alan büyük karstik oluşumlardır. Bu iki karstik gölün planktonik topluluğunun mevsimsel dağılımı henüz çalışılmamıştır. Bu çalışmada, farklı mevsimsel dönemlerde (Mart-Temmuz-Ekim 2018) pelajik istasyondan örnekler toplanmış ve bunların fiziko-kimyasal parametreleri belirlenmiş ve mevsimsel tür kompozisyonu ile değerlendirilmiştir. Sonuç olarak, iki gölde otuz dört rotifer, beş kladoser ve bir kopepod olmak üzere kırk zooplankton türü tespit edilmiştir. Her iki gölde de baskın grup olarak Rotifera belirlenmiştir. Tüm türler Meyil ve Kızören obruk gölleri için yeni kayıt olarak belirlenmiştir. Ayrıca bu çalışma, örnekleme tarihi itibariyle Türkiye'deki obruk göllerinin zooplankton faunasına ilişkin ilk detaylı verileri ortaya koyarak literatüre katkı sağlamaktadır.

**Anahtar kelimeler:** Zooplankton faunası, karstik göl, Kızören Obruğu, Meyil Obruğu, Türkiye

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## Introduction

The name in Turkish “Obruk” which is the most critical karstic formation consisting of wide and deep cylindrical well-shaped sinkholes in Konya plain in Türkiye (Canik and Çörekçioğlu 1985; Ertek 2009; Günay et al. 2011). Sinkholes are observed in areas where underground caves are formed due to the erosion of limestone on the surface and groundwater filling the void created by the collapse of these caves (Palmer 1991). The sinkholes in Türkiye are mostly located in Konya Province, and to date, the number of sinkholes within the borders of this province is around 332 (Orhan et al. 2020). Community research, which is the subject of lake studies, provides researchers with information about climate change or the ecosystem's structure (Olden et al. 2006; Gürbüzer et al. 2019). Zooplanktonic organisms are both crucial components of the food chain and one of the biological study communities in freshwater ecosystems that are used as an indicator for environmental changes (Attayde and Hansson 2001; Jeppesen et al. 2001; Yağci 2016; Montes-Ortiz and Elias-Gutierrez 2018).

Many previous freshwater zooplankton researches have been conducted in Türkiye, and these are mainly focused on lakes and rivers. Such as those by Daday (1903), Geliday (1949), Tokat (1972), Dumont (1981), Ustaoğlu and Balık (1987), Emir (1991), Gündüz (1991), Segers et al. (1992), Altındağ (1999), Altındağ and Yiğit (2002), Ustaoğlu (2004), Kaya et al. (2008), Altındağ et al. (2009), Buyurgan et al. (2010), Gürbüzer et al. (2017) and Gürbüzer et al. (2019). Bozkurt and Bozça (2019), Bozkurt (2019) and Apaydın Yağcı et al. (2021).

However, concerning the sinkhole lakes of Türkiye, there were no detailed studies on the zooplankton fauna (Rotifera, Clodocera and Copepoda) in the literature. We aimed this study to explain the zooplankton fauna of the Meyil and Kızören sinkholes and discuss species diversity in three different seasons. Seasonal species composition was evaluated along with some physical and chemical parameters. At the same time, we aimed to contribute to the literature by explaining the first detailed data for zooplankton fauna of the sinkhole lakes in Türkiye as of the sampling date.

## Materials and Methods

### Studied Sites

The Meyil Sinkhole is located in a formation called uvala, and it spreads over a large area reaching 650 meters in diameter with an asymmetrical slope. The northern slopes of the sinkhole are significantly steeper than the southern slopes. Meyil Sinkhole contains Neogene rocks such as; limestone, marl, and sandstone. The total depth of the sinkhole is 104 meters; the depth of the lake inside is 40 meters. Accordingly, the height of the slopes between the lake level and the plateau surface is 64 meters (Biricik 1992). It is located near Meyil Plateau in the northern part of Karapınar District in Konya Province and located at 37°59'17.83"N - 33°21'12.98"E coordinates (Figure 1).

The Kızören Sinkhole is located at the 75th kilometer of the Konya-Aksaray highway, near Kızören Town. The steepness of the sinkhole slopes is varied-the gradients within the section consisting of crystalline limestone forming a steeper profile. The sinkhole slopes are largely covered with travertine sediments showing water level changes. The total depth of the sinkhole is 171 meters; the depth of the lake inside is 145 meters. The sinkhole diameter is 300 meters; The diameter of the lake inside is about 240 meters (Biricik 1992). Kızören Sinkhole is located in the Karatay District of Konya Province and located at 38°10'29.61"N - 33°11'8.89"E coordinates (Figure 1).

The formation of the sinkhole is directly related to the groundwater level. Climate is an essential factor for groundwater. There is a direct relationship between precipitation and groundwater (Yılmaz 2010). Around the Konya Plain, determined that drought and excessive water use, which occurred due to global climate change, caused the groundwater level to decrease (Bozyiğit and Tapur 2009). For this reason, a direct relationship is not expected between the annual average rainfall and the water levels in the Meyil and Kızören Sinkholes.

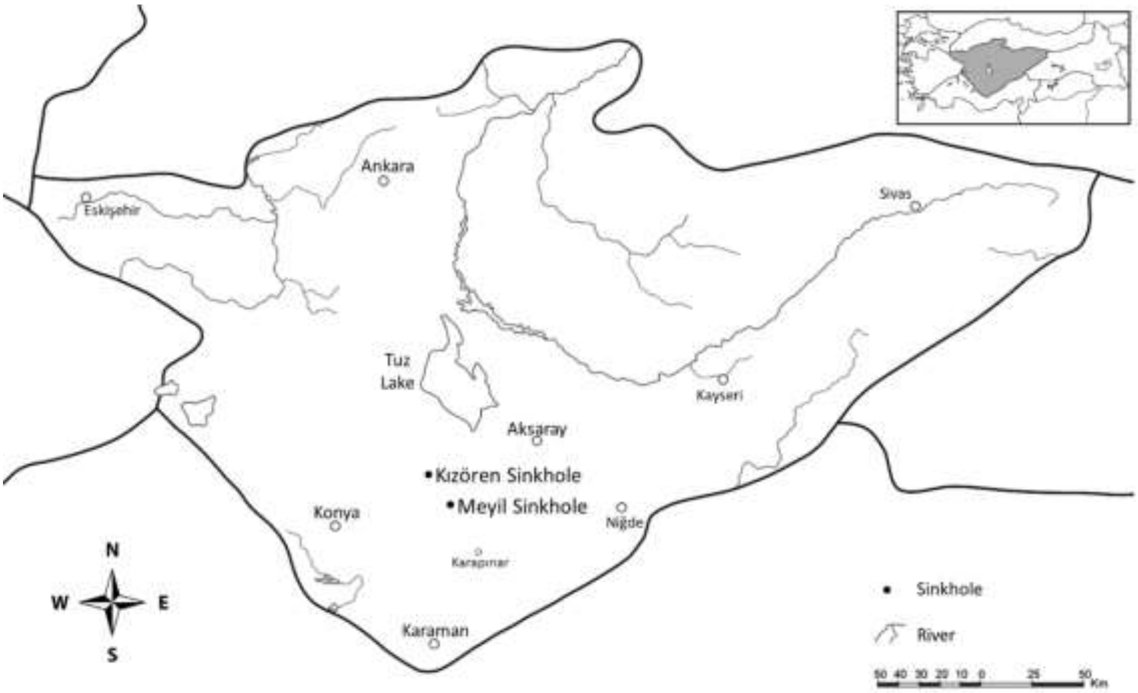


**Figure 1.** Map of study areas (Central Anatolia Region, Türkiye)

### Sample Collecting

Zooplankton samples were collected with a plankton net (Hydro-Bios, mesh size 55 µm and 25 cm in diameter) in seasonal periods (March-July-October 2018). After collection, samples were immediately fixed with 4% formaldehyde in 100 ml plastic bottles. Zooplankton sampling was carried out at one station from each lake due to safety concerns on the shores. The distance between the plateau and the water surface is approximately 30 meters at Kızören Sinkhole and is surrounded by steep slopes. Therefore, zooplankton and water samples were collected only from an old pier. As for Meyil Sinkhole, the coastal zone, which consists of loose material, had almost become swampy with the drop in the water levels. Therefore, zooplankton and water samples were collected from only one spot with firm soil. In the winter season, sampling was not possible due to rugged terrain and weather conditions for both lakes (Figure 2).

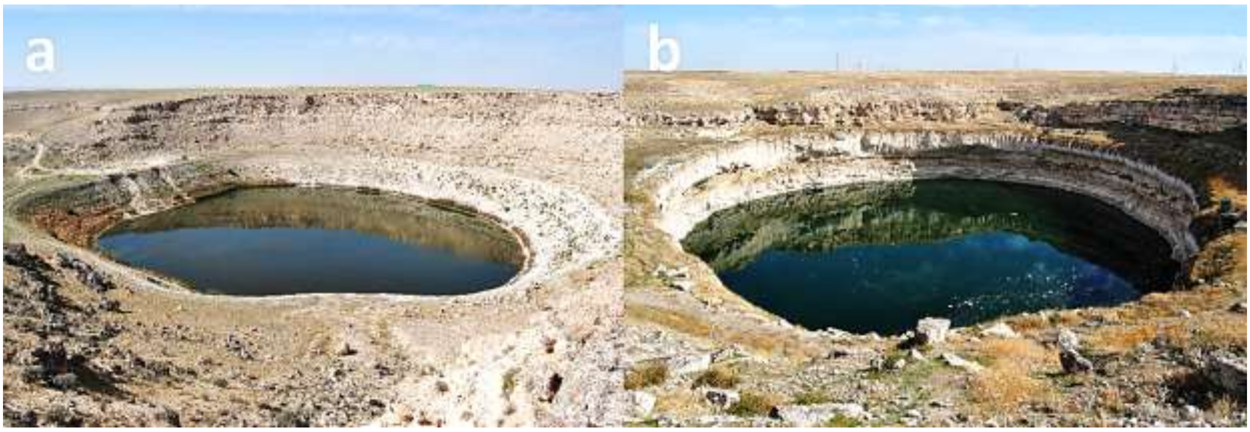


**Figure 2.** Study sites; a) Meyil Sinkhole and b) Kızören Sinkhole (Photographs taken by Oğuzhan Durmaz, 2015)

In addition, for both lakes, some physical and chemical parameters (pH, temperature, dissolved oxygen and electrical conductivity) were measured during the sampling periods. These measurements were made with Hanna HI 9812-5 (Romania) Multiparameter and Ohaus Starter 300D (USA) oxygen meter devices.

**Identification of Zooplankton Species**

The zooplankton species in this study were described down to species level based on monographs published by previous researchers (Ward and Whipple 1945; Donner 1965; Kolisko 1974; Koste 1978; Edmonson 1959; De Smet 1996; Smirnov 1996; Harding and Smith 1974; Nogrady et al. 1995). A microscope (Leica DMIL inverted, Leica DMSL stereo) and digital camera (DFC320, Leica Suite Application, Germany) were used for species identification of the collected zooplankton samples.

## Results

The zooplankton fauna of Meyil and Kızören Sinkhole Lakes were researched in different seasonal periods (March-July-October 2018). Some zooplankton species identified were determined only in one season in the Meyil and Kızören sinkhole lakes (Table 1). *Filinia longiseta* (Rotifera) has been recorded at Kızören sinkhole lake during spring whereas, at Meyil, four species from Rotifera and only *Arctodiaptomus* sp. from Copepoda have been recorded. It was not identified at the species level because no adult samples were found. Ten species belonging to Rotifera and one belonging to Copepoda were recorded in Meyil Sinkhole, and a total of 30 species from Rotifera and five from Cladocera were recorded in Kızören Sinkhole (Table 1) overall

**Table 1.** Seasonal list of zooplankton taxa recorded from Meyil and Kızören sinkhole lakes

| Species | Meyil Sinkhole | | | Kızören Sinkhole | | |
|---|---|---|---|---|---|---|
| Rotifera | Spring | Summer | Autumn | Spring | Summer | Autumn |
| *Asplanchna priodonta* Gosse, 1850 | - | - | - | - | - | + |
| *Asplanchna girodi* de Guerre, 1888 | - | - | - | - | - | + |
| *Brachionus angularis* Gosse, 1851 | + | - | - | - | - | - |
| *Brachionus calyciflorus* Pallas, 1766 | - | - | - | - | + | - |
| *Brachionus plicatilis* Müller, 1786 | - | + | - | - | - | - |
| *Brachionus quadridentatus* Hermann, 1783 | + | - | + | - | - | + |
| *Brachionus urceolaris* Müller, 1773 | - | - | + | - | - | + |
| *Cephalodella forficula* (Ehrenberg, 1830) | - | - | - | - | - | + |
| *Cephalodella* sp. (Bory de St.Vincent, 1826) | - | - | + | - | - | - |
| *Colurella adriatica* Ehrenberg, 1831 | - | - | - | - | - | + |
| *Colurella colurus* (Ehrenberg, 1830) | - | - | - | - | - | + |
| *Colurella obtusa* (Gosse, 1886) | - | - | - | - | - | + |
| *Dicranophorus epicharis* Harring & Myers, 1928 | - | - | - | - | - | + |
| *Euchlanis dilatata* Ehrenberg, 1832 | - | - | - | - | - | + |
| *Filinia longiseta* (Ehrenberg, 1834) | + | - | - | + | + | + |
| *Hexarthra polyodonta* (Hauer, 1957) | + | + | - | - | + | + |
| *Keratella quadrata* (Müller, 1786) | - | + | - | - | + | + |
| *Keratella tropica* (Apstein, 1907) | - | - | - | - | - | + |
| *Lecane bulla* (Gosse, 1851) | - | - | - | - | + | + |
| *Lecane clostrocerca* (Schmarda, 1859) | - | - | - | - | + | + |
| *Lecane grandis* (Murray, 1913) | - | - | - | - | - | + |
| *Lecane hamata* (Stokes, 1896) | - | - | - | - | + | + |
| *Lecane lamellata* (Daday, 1893) | - | + | - | - | - | - |
| *Lecane luna* (Müller, 1776) | - | - | - | - | - | + |
| *Lecane lunaris* (Ehrenberg, 1832) | - | - | - | - | - | + |
| *Lecane nana* (Murray, 1913) | - | - | - | - | - | + |
| *Lecane ohiensis* (Herrick, 1885) | - | - | - | - | - | + |
| *Lepadella patella* (Müller, 1773) | - | - | - | - | + | + |
| *Philodina megalotrocha* Ehrenberg, 1832 | - | - | - | - | + | + |
| *Polyarthra vulgaris* Carlin, 1943 | - | + | - | - | + | - |
| *Synchaeta pectinate* Ehrenberg, 1832 | - | - | - | - | - | + |
| *Trichocerca pusilla* (Jennings, 1903) | - | - | - | - | - | + |
| *Trichotria pocillum* (Müller, 1776) | - | - | - | - | + | + |
| *Trichotria tetractis* (Ehrenberg, 1830) | - | - | - | - | - | + |
| Cladocera | | | | | | |
| *Alona guttata* Sars, 1862 | - | - | - | - | + | + |
| *Chydorus ovalis* Kurz, 1875 | - | - | - | - | - | + |
| *Chydorus sphaericus* (O.F.Müller, 1776) | - | - | - | - | - | + |
| *Coronatella rectangula* Sars, 1862 | - | - | - | - | - | + |
| *Oxyurella tenuicaudis* (Sars, 1862) | - | - | - | - | - | + |
| Copepoda | | | | | | |
| *Arctodiaptomus* sp. (Kiefer, 1932) | + | - | - | - | - | - |
| Total number of taxa | 5 | 5 | 3 | 1 | 12 | 33 |

The dominant zooplankton group was formed by the Rotifera phylum for both lakes. According to Segers (2007), most of the rotifer species found are cosmopolitan. In addition, some water parameters (pH, dissolved oxygen, electrical conductivity and water temperature) were measured and evaluated with seasonal species composition for both lakes (Table 2).

**Table 2.** Seasonal measured water parameters during sampling for both lakes

| Water Parameters | Meyil Sinkhole Lake | | | Kızören Sinkhole Lake | | |
|---|---|---|---|---|---|---|
| | Spring | Summer | Autumn | Spring | Summer | Autumn |
| pH | 9.3 | 9.2 | 9,0 | 8.2 | 8.0 | 8.3 |
| DO | 10.48 | 3.30 | 5.71 | 7.60 | 7.48 | 4.25 |
| EC | 920 | 930 | 1000 | 980 | 860 | 669 |
| T | 16 | 26 | 21,5 | 15 | 24 | 20,5 |

DO: Dissolved oxygen (mg/L), EC: Electrical (µS/cm), T: temperature (°C)

## Discussion

The results showed that different seasonal conditions and water parameters might be structurally affected by zooplankton species composition. These data are essential in terms of ecological evaluation (Table 2). In lakes located in calcareous regions, the dissolved carbonate can increase the pH to around 9. In lakes that do not have an external flow, there is an accumulation of alkaline substances with evaporation, and the pH can rise to 12 (Tanyolaç 2011). In freshwaters, pH is in relationship with many chemical parameters. This relationship also dramatically affects the zooplankton species distribution. It is known that the alkaline limit in terms of zooplankton abundance is 8.5 pH (Bērziņš and Pejler 1987). There is an inverse relationship between oxygen and pH. High pH and low oxygen values have a lethal effect on living things (Tanyolaç 2011). It is known that both sinkholes consist of limestone rocks. Thus, both sinkhole lakes were classified as alkaline according to mean pH values. Significant differences were not observed between seasonal pH values (Table 2). Accordingly, it's seen that pH is not a solely dominant limiting factor in species richness between seasons.

However, for Meyil Sinkhole, pH values of 9 or more may be associated with low numbers of species in all seasons (Table 2). In Meyil Sinkhole Lake, EC values did not yield essential differences during seasonal measurements. In Kızören Sinkhole Lake, an inverse proportion was observed between seasonal EC values and a seasonal number of species (Table2). Considering that the maximum acceptable EC value for fisheries is 500 µs / cm (McKee and Wolf 1963), the average EC values for both lakes are high. Most rotifer species are tolerant of a wide range of conductivity values (Neschuk et al. 2002; Malekzadeh Viayeh and Špoljar 2012). However, in this study, the highest number of Rotifer species was identified in the Kızören Sinkhole in autumn, with the lowest electrical conductivity values. Electrical conductivity values may affect species diversity and richness. Akdemir (2008) investigated ostracod samples collected from two separate maar lakes (Acı and Meke) and a sinkhole lake (Meyil) in Konya, Türkiye, in another study he conducted in Meyil Sinkhole Lake. As a result of his research, he suggested that the most significant number of species is found only in Lake Meyil, which may be related to low salinity and increased oxygen levels.

Temperature is a limiting factor for the abundance and distribution of zooplankton (Mikschi 1989). Our study observed an inverse proportion between seasonal water surface temperature and dissolved oxygen in Meyil Sinkhole (Table 2). The highest temperature in Kızören Sinkhole is observed in summer. However, the dissolved oxygen value was lower in autumn than in summer. It was thought that the low dissolved oxygen values measured in the summer season were caused by the decrease in the water level for both sinkholes and the reduction in the groundwater level due to the evaporation effect. However, the low dissolved oxygen value in the Kızören Sinkhole in autumn can be explained by the decrease in the water level as well as the increase in zooplankton species diversity (Table 1). Unlike other karst lakes, the coastal zone of the sinkhole lakes is generally in the form of a circle, and there are no indentations (Figure 2). This situation may affect zooplankton species diversity. There are studies on zooplankton in karstic sinkholes with similar structures in the literature. Cervantes-Martinez and Gutiérrez-Aguirre (2015) identified the zooplankton species from two karstic sinkholes in Mexico. Those are *Lecane bulla*, *Lecane lunaris* and *Lepadella patella* from Rotifera and we identified these species at the Kızören sinkhole. The rotifer species (*Lecane bulla*, *Lecane lunaris* and *Lepadella patella*) identified in this study are similar to those at Kızören sinkhole lake. Another detailed research was conducted by Montes-Ortiz and Elias-Gutierrez (2018); they identified the zooplankton species in different seasonal periods in Cenote Azul Sinkhole in Mexico. *Coronatella rectangula* from Cladocera, one of these identified species, was also recognised at the Kızören Sinkhole. However, Cladocera was the most abundant zooplanktonic group in Cenote Azul Sinkhole (Montes-Ortiz and Elias-Gutierrez 2018).

It is known that biological studies on sinkholes are quite limited in Türkiye. This situation reveals that more studies are needed to determine the biological diversity in the sinkholes. It may be recommended to carry out further studies on the diversity and abundance of organisms living in both sinkholes with applications where the sampling frequency and the number of stations will be increased. As a result, this study will contribute to future research on the fauna of sinkhole lakes with a valuable and primary data set.

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# A Study on Planktonic and Epipelic Algae Occurring in Karakaya Dam Lake (Elazığ, Türkiye)

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**A B S T R A C T**

This study aimed to determine the species composition of two algal associations in Karakaya Dam Lake. For this purpose, monthly variations in species composition of phytoplankton and seasonally variations in epipelic algae occurring in the dam lake were studied between June 2010 and May 2011. Five stations were selected in Karakaya Dam Lake. A total of 38 algal taxa belonging to Bacillariophyta (27 taxa), Charophyta (2 taxa), Chlorophyta (3 taxa), Cyanobacteria (3 taxa), Miozoa (2 taxa) and Ochrophyta (1 taxon) were identified in the phytoplankton. The diatom genera *Cocconeis, Cymbella, Fragilaria, Nitzschia, Stauroneis* and *Surirella* were represented with two taxa in the phytoplankton. *Ceratium hirundinella* was noticed with its continuous occurrence in the phytoplankton throughout the year. A total of 25 algal taxa belonging to Bacillariophyta (21 taxa), Charophyta (1 taxon), Chlorophyta (2 taxa) and Cyanobacteria (1 taxon) were identified in the epipelon. Seasonal variations of algal species with respect to occurrence frequency and abundance were compared and discussed in relation to physical and chemical properties of the dam lake.

**Keywords:** Phytoplankton, epipelon, Karakaya Dam Lake, Elazığ, Türkiye

**A R T I C L E  I N F O**

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**Karakaya Baraj Gölü (Elazığ, Türkiye) Planktonik ve Epipelik Algleri Üzerine Bir Araştırma**

**Öz:** Bu çalışma, Karakaya Baraj Gölü'ndeki iki alg topluluğunun tür kompozisyonunu belirlemeyi amaçlamıştır. Bu amaç doğrultusunda; Haziran 2010 ile Mayıs 2011 arasında gölde fitoplanktonik alg türlerinin ortaya çıkış ve bolluklarındaki değişimler aylık olarak incelenirken epipelik alglerdeki değişimler ise mevsimsel olarak incelenmiştir. Bu araştırma için Karakaya Baraj Gölü'nde beş istasyon seçilmiştir. Baraj gölü fitoplanktonunda Bacillariophyta (27 takson), Charophyta (2 takson), Chlorophyta (3 takson), Cyanobacteria (3 takson), Miozoa (2 takson) ve Ochrophyta'ya (1 takson) ait toplam 38 alg taksonu tespit edilmiştir. *Cocconeis, Cymbella, Fragilaria, Nitzschia, Stauroneis* ve *Surirella* cinsleri fitoplanktonda iki taksonla temsil edilmiştir. Tüm algler arasında *Ceratium hirundinella*, yıl boyunca fitoplanktonda sürekli bulunmasıyla dikkat çekici olmuştur. Karakaya Baraj Gölü epipelik alg topluluğunda Bacillariophyta (21 takson), Charophyta (1 takson), Chlorophyta (2 takson) ve Cyanobacteria'ya (1 takson) ait toplam 25 alg taksonu kaydedilmiştir. İstasyonlarda diyatomelerin ve diğer alg türlerinin ortaya çıkışlarındaki mevsimsel değişimler ve bollukları karşılaştırılmış ve baraj gölünün fiziksel ve kimyasal özellikleri ile ilişkili olarak tartışılmıştır.

**Anahtar kelimeler:** Fitoplankton, epipelon, Karakaya Baraj Gölü, Elazığ, Türkiye

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## Introduction

The presence, absence or abundance of planktonic and benthic algal species in an aquatic ecosystem are based on certain ecological conditions. In fact, many external and internal factors affect the seasonal changes in species composition and biomass of algal communities in dam lakes. Seasonal/periodical fillings, discharge of wastewater and fluctuations in lake water level are considered to be major external factors changing physicochemical and biological characters of dam lakes (Geraldes and Boavida 1999) whilst water temperature, pH and concentrations of nutrients are commonly accepted to be the most significant internal parameters (Philips et al. 2000). Thus it is significant to study phytoplankton and other algal communities to determine the changes in aquatic ecosystems since algae react to the physical and chemical changes most quickly occurring due to external factors in the structure of a lake ecosystem (Habib et al. 1997). Such reactive ability of algal species makes them? be used as one of the major indicators of environmental variables and trophic levels of lakes (Reynolds et al. 2002).

Studies on algae occurring in dam lakes and reservoirs have particularly received attention after 80's in Türkiye (Aykulu and Obalı 1981; Gönülol and Aykulu 1984; Yıldız 1985; Gönülol 1985a, 1985b; Altuner and Gürbüz 1996; Yazıcı and Gönülol 1994; Şipal et al. 1996; Gönülol and Obalı 1998; Çetin and Şen 1997,1998) Particularly floristic and ecological studies carried out on the phytoplankton of lakes have rapidly increased in numbers (Çetin and Yıldırım 2000; Gürbüz and Kıvrak 2003; Atıcı 2002; Albay and Akçaalan 2003; Çetin and Şen 2004; Baykal et al. 2004; Baykal and Açıkgöz 2004; Çetin 2004; Kıvrak and Gürbüz 2005; Sömek et al. 2005; Çetin and Şen 2006; Çelik and Ongun 2006; Aykulu et al. 2006; Pala 2007; Taş and Gönülol 2007; Ongun Sevindik 2010; Sönmez 2011; Atıcı and Alaş 2012; Öterler 2013; Tokatlı and Atıcı 2014; Atıcı et al. 2016; Sönmez et al. 2017; Atıcı et al. 2018; Tokatlı et al. 2020a, 2020b).

Karakaya is one of the largest dam lakes in Türkiye. The dam lake (or reservoir) was constructed in 1976-1987 and water retention was started in 1987. The reservoir has a surface area of 268 km$^2$ with a maximum (high from river bed) depth of 173 m. The total lake volume is 9580 hm$^3$ (DSİ 2019). The mean depth of the lake is determined as 35.7 m. The lake is mainly supported by Fırat River. The dam lake has a great significance for the region as it is commonly used for irrigation, energy production and fisheries purposes. The dam lake provides 102 hm$^3$ of potable water capacity per year. The cage fish culture has been performed intensively in Karakaya Dam Lake. There are 38 fish farms with the capacity of approximately 6.000 tonnes/year in the region. Through searching the literature, it was come across no detailed study engaged in seasonal variations of planktonic and benthic algae occurring in Karakaya Dam Lake. Therefore, the present research aimed to study the planktonic and epipelic algae occurring in Karakaya Dam Lake in relation to physico-chemical factors for a period of a year (Figure 1).

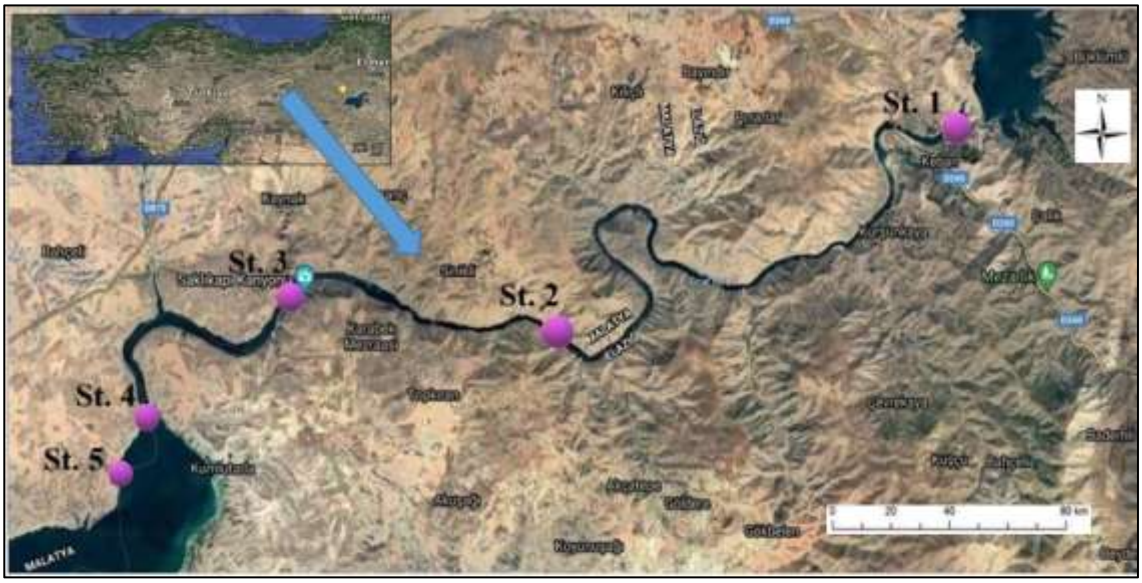


**Figure 1.** Location of sampling station and Karakaya Dam Lake

## Materials and Methods

Karakaya Dam Lake, which is one of the most important rervoir of Türkiye, sample stations are located in the East Anatolia Region between the locality of 38.48127 – 38.43147 N and 38.43833 – 38.28149 E. Five stations were selected in Karakaya Dam Lake (Figure 1). Physical-chemical measurements and analyses were performed at different depths of stations (respectively mean depth 0-5-10 m). These depths are given in parenthesis after the number of each station. pH level was measured directly by using a pH meter (HACH HQ40 d), whilst a Secchi disk was used to measure water transparency. Chlorophyll-*a* was measured by fluorometric method in which the water sample was filtered through Whatman GF/C filter that was then treated with acetone (APHA 1995). The spectrophotometric method (Hach-Lange DR 6000 spectrophotometer) was employed for the analysis of sulfate, total phosphorus and total nitrogen by using the suitable kits (Merck).

Phytoplankton sampling was carried out by using plankton net of the brand Hydro-Bios with pore of 55 µm. For epipelic algae, muddy sediment samples were collected and placed into plastic containers for laboratory observation.

To prepare permanent diatom slides, subsamples were taken in which acid solution was added to digest organic materials. The samples were boiled on a hot plate for 15 minute to expedite the digestion process. They were subsequently left to cool. Samples were neutralized by rinsing with distilled water and dried on coverslips that were mounted on glass slides with Entellan. For other algae temporary slides were prepared. Olympus CX21FS1 research microscope and Olympus CKX41 inverted microscope were employed for identification of species and counting algal cells respectively. The relative abundance method was applied for individual numbers of diatoms and results were expressed as "% organism" (Sladeckova 1962). At least 100-200 individuals based on the abundance of diatoms were observed and counted in each slide. Algal species were identified according to Krammer and Lange-Bertalot (1986, 1988, 1991a, 1991b) and John et al. (2003). Names of species are updated in accordance with Guiry and Guiry (2022).

## Results

Changes in values of physicochemical parameters were given in Figures 2, 3, 4, 5, 6, 7 and 8.

The mean values of surface water temperature measured at stations were found to vary in the range of 8.4-30.3 ºC through the year (Figure 2).

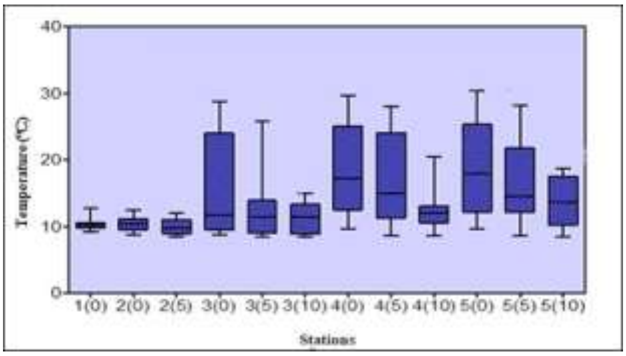


**Figure 2.** Variations of temperature values at stations

Values of pH at stations varied in the range of 8.1-8.73 (Figure 3). This simply shows that the dam lake has neutral and slightly alkaline water characteristic. pH values were slightly higher at station 4 than those recorded at other stations.

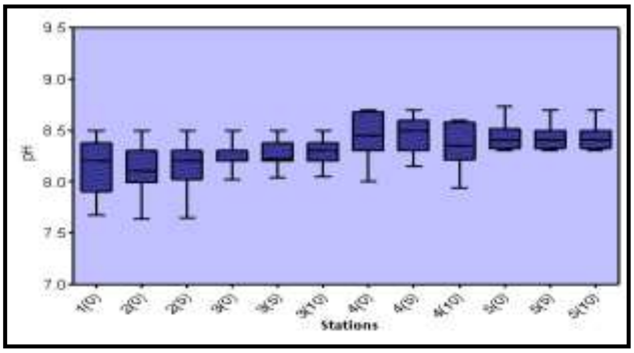


**Figure 3.** Variations of pH values at stations

Secchi disk depth at stations varied in the range of 2 m and 10.5 m (Figure 4). The figure shows that variations in Secchi disk depth between seasons and stations were considerable. The highest visibility (10.5 m) was recorded at Station 2 in July whilst the lowest visibility (2 m) was reached at stations 4 and 5 (Figure 4).

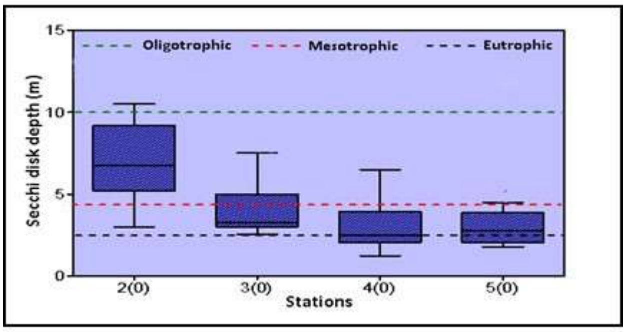


**Figure 4.** Secchi disk depth values (m) at stations

Chlorophyll-*a* was measured between 0.1-5.59 µg/L (Figure 5) The lowest concentration was determined at station 5 whilst the highest concentration was analysed at 5m depth at station 2.

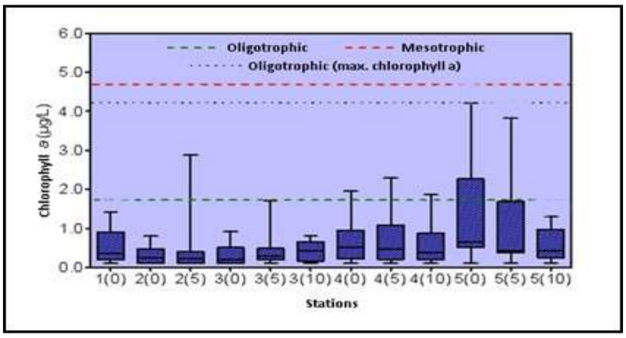


**Figure 5.** Variations of chlorophyll-*a* concentrations (µg/L) at stations

Variations in concentrations of plant nutrients were as follows; variations for sulfate ($SO_4^{-2}$) concentrations varied as 20.08-56-98 mg/L. Total phosphorus (TP) and total nitrogen (TN) concentrations varied between 0.01-0.08 mg/L and 0.67-0.91 mg/L respectively (Figure 6, 7 and 8).

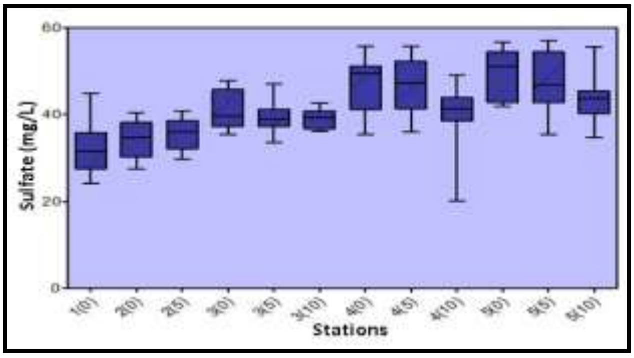


**Figure 6.** Variations of sulfate concentrations (mg/L) at stations

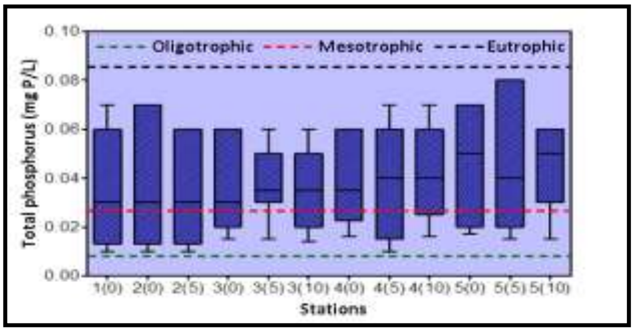


**Figure 7.** Variations of total phosphorus concentrations (mg P/L) at stations

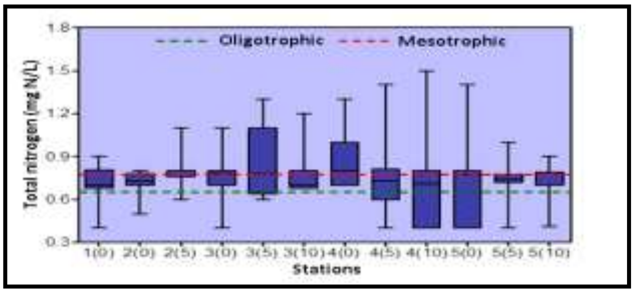


**Figure 7.** Variations of total nitrogen concentrations (mg N/L) at stations

**Phytoplankton Community**

A total of 38 algal taxa belonging to Bacillariophyta (27 taxa), Charophyta (2 taxa), Chlorophyta (3 taxa), Cyanobacteria (3 taxa), Miozoa (2 taxa) and Ochrophyta (1 taxon) were identified in the phytoplankton of Karakaya Dam Lake (Table 1). The diatom genera *Cocconeis, Cymbella, Fragilaria, Nitzschia, Stauroneis* and *Surirella* were represented with two taxa in the phytoplankton. Dinoflagellate *Ceratium hirundinella* was noticeable with its continuous occurrence in the phytoplankton throughout the year. *Asterionella formosa, Cocconeis pediculus, Cymbella affinis, Diatoma vulgaris, Fragilaria crotonensis, F. rumpens, Gyrosigma acuminatum, Melosira varians, Navicula cryptocephala, Nitzschia sigmoidea, Rhoicosphenia abbreviata* and *Ulnaria ulna* from Bacillariophyta and *Dolichospermum planctonicum* and *Oscillatoria limosa* from Cyanobacteria were also noticeable diatoms with respect to frequency of occurrence (Table 1, Figure 8). Contrary, *Cocconeis placentula* (April), *Craticula cuspidata* (March), *Cymbella cistula* (March), *Stauroneis anceps* (March), *S. construens* (April) and *Surirella ovalis* (September) were considered as rarely occurring algae as they were recorded in only one sample throughout the study. It is worth to mention that *Dinobryon divergens* was the only representative of Ochrophyta (Table 1).

The percentage participation of algal group is shown in Figure 9. As seen, Bacillariophyta constituted the main part of the phytoplankton in the lake. The percentage participation of other algal groups was low remaining under 8 %

**Table 1.** The list of algae recorded in the phytoplankton of Karakaya Dam Lake

| Taxa | Jun. | Jul. | Aug. | Sept. | Oct. | Nov. | Dec. | Jan. | Feb. | Mar. | Apr. | May. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **BACILLARIOPHYTA** | | | | | | | | | | | | |
| *Achnanthidium minutissimum* (Kützing) Czarnecki | - | - | - | + | - | - | - | + | - | - | - | - |
| *Asterionella formosa* Hassall | - | - | - | + | - | + | + | + | + | + | + | - |
| *Aulacoseira granulata* (Ehrenberg) Simonsen | - | - | - | - | - | - | - | - | - | + | + | - |
| *Cyclotella meneghiniana* Kützing | + | - | - | + | - | - | - | - | - | + | - | + |
| *Cocconeis pediculus* Ehrenberg | + | + | - | + | + | - | - | + | + | + | - | - |
| *Cocconeis placentula* Ehrenberg | - | - | - | - | - | - | - | - | - | - | + | - |
| *Craticula cuspidata* (Kutzıng) D.G.Mann | - | - | - | - | - | - | - | - | - | + | - | - |
| *Ctenophora pulchella* (Ralfs ex Kützing) D.M.Williams & Round | - | - | - | + | - | - | - | - | - | + | - | + |

**Table 1.** Continued.

| Taxa | Jun. | Jul. | Aug. | Sept. | Oct. | Nov. | Dec. | Jan. | Feb. | Mar. | Apr. | May. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **BACILLARIOPHYTA** | | | | | | | | | | | | |
| *Cymbella affinis* Kützing | + | + | - | + | + | + | + | + | + | + | + | + |
| *Cymbella cistula* (Ehrenberg) O.Kirchner | - | - | - | - | - | - | - | - | - | + | - | - |
| *Diatoma vulgaris* Bory | + | + | + | + | + | + | + | - | + | + | + | + |
| *Epithemia gibba (*Ehrenberg) Kützing | - | - | - | + | - | - | - | - | - | + | + | - |
| *Fragilaria crotonensis* Kitton | + | + | - | - | - | + | + | + | + | + | + | + |
| *Fragilaria rumpens* (Kützing) G.W.F.Carlson | + | + | + | - | - | - | - | - | + | + | - | + |
| *Gomphonema truncatum* Ehrenberg | + | - | - | - | + | - | - | + | - | + | - | - |
| *Gyrosigma acuminatum* (Kützing) Rabenhorst | - | - | - | + | + | + | + | + | + | + | + | - |
| *Halamphora veneta* (Kützing) Levkov | - | - | - | + | - | - | - | + | - | + | - | + |
| *Melosira varians* C.Agardh | - | - | + | + | + | + | + | + | - | - | - | - |
| *Navicula cryptocephala* Kützing | - | + | + | - | + | + | - | + | + | + | + | + |
| *Nitzschia intermedia* Hantzsch | - | - | - | + | + | - | - | - | - | + | + | + |
| *Nitzschia sigmoidea* (Nitzsch) W.Smith | - | - | - | + | + | + | + | + | - | + | + | + |
| *Rhoicosphenia abbreviata* (C.Agardh) Lange-Bertalot | + | + | + | + | + | - | - | + | + | - | + | + |
| *Stauroneis anceps* Ehrenberg | - | - | - | - | - | - | - | - | - | + | - | - |
| *Staurosira construens* Ehrenberg | - | - | - | - | - | - | - | | - | - | + | - |
| *Surirella librile* (Ehrenberg) Ehrenberg | - | - | - | - | - | - | - | - | + | + | - | - |
| *Surirella ovalis* Brébisson | - | - | - | + | - | - | - | - | - | - | - | - |
| *Ulnaria ulna* (Nitzsch) Compère | - | - | - | + | + | + | + | + | - | + | + | - |
| **CHAROPHYTA** | | | | | | | | | | | | |
| *Closterium aciculare* T.West | + | + | - | - | - | - | - | - | + | - | - | - |
| *Spirogyra gracilis* Kützing | - | + | + | - | - | + | - | - | - | - | - | + |
| **CHLOROPHYTA** | | | | | | | | | | | | |
| *Microspora tumidula* Hazen | + | + | - | - | - | - | - | - | + | - | - | - |
| *Monactinus simplex* (Meyen) Corda | + | + | - | - | - | + | + | - | - | - | - | + |
| *Stigeoclonium tenue* (C.Agardh) Kützing | - | + | - | - | + | + | - | - | - | - | - | - |
| **CYANOBACTERIA** | | | | | | | | | | | | |
| *Aphanizomenon flos-aquae* Ralfs ex Bornet & Flahault | - | - | + | - | + | - | - | - | - | - | - | - |
| *Dolichospermum planctonicum* (Brunnthaler) Wacklin, L.Hoffmann & Komárek | - | + | - | + | + | + | + | - | - | + | + | + |
| *Oscillatoria limosa* C.Agardh ex Gomont | + | + | + | - | + | + | - | + | - | + | + | + |
| **MIOZOA** | | | | | | | | | | | | |
| *Ceratium hirundinella* (O.F.Müller) Dujardin | + | + | + | + | + | + | + | + | + | + | + | + |
| *Peridinium bipes* F.Stein | - | - | - | + | - | - | - | - | - | - | + | - |
| **OCHROPHYTA** | | | | | | | | | | | | |
| *Dinobryon divergens* O.E.Imhof | + | - | - | - | - | - | - | - | - | + | - | - |

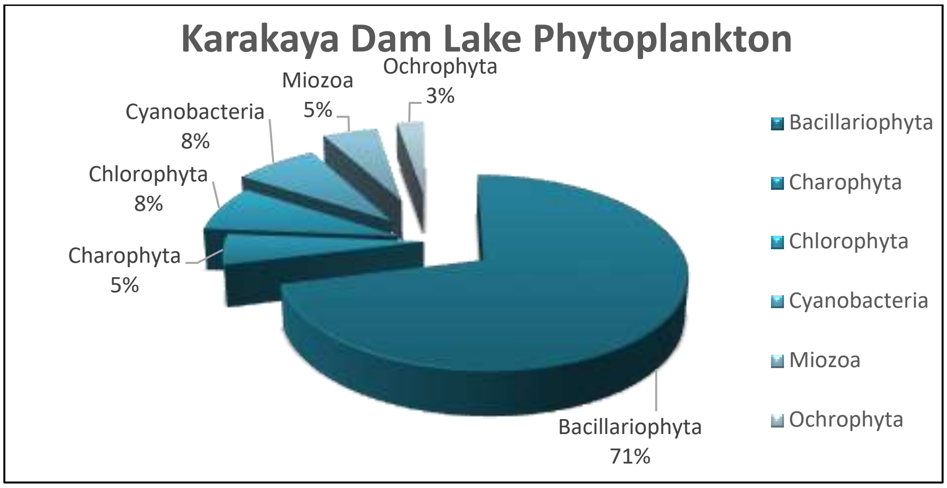

Figure 9. Percentage (%) distribution of phytoplanktonic algal groups in Karakaya Dam Lake

**Epipelon Community**

A total of 25 algal taxa belonging to Bacillariophyta (21 taxa), Charophyta (1 taxon), Chlorophyta (2 taxa) and Cyanobacteria (1 taxon) were identified in the epipelic association of Karakaya Dam Lake (Table 2). *C. pediculus*, *C. affinis*, *D. vulgaris*, *Gomphonema truncatum*, *N. cryptocephala* and *R. abbreviata* were observed in all seasons. Contrary, *C. cistula* was observed only in one season (spring) in the epipelon during the study. *Spirogyra gracilis* and *O. limosa* were the only representatives of Charophyta and Cyanobacteria respectively (Table 2).

**Table 2.** The list of algae recorded in the epipelon of Karakaya Dam Lake

| Taxa | Summer | Autumn | Winter | Spring |
|---|---|---|---|---|
| **BACILLARIOPHYTA** | | | | |
| *Achnanthidium minutissimum* (Kützing) Czarnecki | - | + | + | - |
| *Asterionella formosa* Hassall | - | + | + | + |
| *Cocconeis pediculus* Ehrenberg | + | + | + | + |
| *Ctenophora pulchella* (Ralfs ex Kützing) D.M.Williams & Round | - | + | - | + |
| *Cyclotella meneghiniana* Kützing | + | + | - | + |
| *Cymbella affinis* Kützing | + | + | + | + |
| *Cymbella cistula* (Ehrenberg) O.Kirchner | - | - | - | + |
| *Diatoma vulgaris* Bory | + | + | + | + |
| *Epithemia gibba (*Ehrenberg) Kützing | - | + | - | + |
| *Fragilaria crotonensis* Kitton | + | - | + | + |
| *Fragilaria rumpens* (Kützing) G.W.F.Carlson | + | - | + | + |
| *Gomphonema truncatum* Ehrenberg | + | + | + | + |
| *Gyrosigma acuminatum* (Kützing) Rabenhorst | - | + | + | + |
| *Halamphora veneta* (Kützing) Levkov | - | + | + | + |
| *Melosira varians* C.Agardh | + | + | + | - |
| *Navicula cryptocephala* Kützing | + | + | + | + |
| *Nitzschia intermedia* Hantzsch | - | + | - | + |
| *Nitzschia sigmoidea* (Nitzsch) W.Smith | - | + | + | + |
| *Rhoicosphenia abbreviata* (C.Agardh) Lange-Bertalot | + | + | + | + |
| *Surirella librile* (Ehrenberg) Ehrenberg | - | - | + | + |
| *Ulnaria ulna* (Nitzsch) Compère | - | + | + | + |
| **CHAROPHYTA** | | | | |
| *Spirogyra gracilis* Kützing | + | - | - | + |
| **CHLOROPHYTA** | | | | |
| *Microspora tumidula* Hazen | + | - | + | - |
| *Stigeoclonium tenue* (C.Agardh) Kützing | + | + | - | - |
| **CYANOBACTERIA** | | | | |
| *Oscillatoria limosa* C.Agardh ex Gomont | - | + | - | + |

The trophic level of the lakes reveals the basic ecological character of the ecosystem in question; It is also the most basic criterion in determining aquaculture strategies. In the trophic classification process, the annual average total phosphorus concentration and Secchi disk visibility findings of the Karakaya Dam Lake were used. Although there is no data covering all trophic levels given in Table 3, considering the terrace depths and phosphorus values of the lake, it can be said that the second station is oligotrophic and the other stations are mesotrophic according to the categories of OECD (1982) and Hakanson and Jansson (1983) possible.

Redfield (1934), in his studies, determined that the carbon: nitrogen: phosphorus (C: N: P) ratios of water and the carbon: nitrogen: phosphorus ratios in the phytoplankton are very similar to each other and these ratios (C: N: P) are 106: He gave it as 16:1. Based on this ratio and in accordance with Liebig's Law of Minimum, it was determined that phosphorus is the limiting element in the development of phytoplankton in Karakaya Dam Lake (Table 3).

**Table 3.** Characteristic properties of lakes in different trohic levels (OECD1982; Hakanson and Jansson1983).

| Trophic Level | Secchi Disk Dept (m) | Clorophyll-a (μg/L) | Total-Phosphorus (mg/L) | Total-Nitrogen (mg/L) |
|---|---|---|---|---|
| Oligotrophic | >5 | <2.5 | <0.01 | <0.35 |
| Mesotrophic | 3-6 | 2-8 | 0.008-0.025 | 0.3-0.5 |
| Eutrophic | 1-4 | 6-35 | 0.02-0.1 | 0.35-0.6 |
| Hipertrophic | 0-2 | 30-400 | >0.08 | >0.6 |
| Karakaya Dam Lake | 1.25–10.5 | 0.10-5.59 | 0.00-0.08 | 0.00-1.5 |

When it was looked at the fish species in the categories of OECD (1982) and Hakanson and Jansson (1983), the fact that the trout is not naturally found in the lake, but the vaccinated and escaped trout in the lake find a habitat in the lake, and the predominance of whitefish in the lake shows that the lake has a predominantly mesotrophic character.

In terms of maximum and mean chlorophyll a concentration, all sampling points were located in the oligotrophic border. It has tended to increase with the areas affected by fish farming facilities.

The difference between the low concentrations of the station representing the Keban outlet river area and the high concentrations of the station representing the Karakaya surface water was found to be statistically significant. However, other sampling points could not be distinguished as significant.

## Discussion

Karakaya is one of the largest dam lakes in Türkiye. Phytoplankton and epipelic algae of the lake were studied for a period of a year in order to determine seasonal changes of species composition and abundance in both algal associations in relation to physical and chemical properties of the lake. Trophic status of the lake was also evaluated in terms of algal species and its physical-chemical characteristics.

Physicochemical data and recorded algae indicated that Karakaya Dam Lake shows both oligotrophic and mesotrophic lake characteristics. The lake could be included oligotrophic and mesotrophic lake category with respect to Secchi disk depth measured at stations (Nürnberg 1996; Vollenweider and Kerekes 1982). Maximum and mean chlorophyll-*a* concentrations also supported oligotrophic statue of Karakaya Dam Lake (Håkanson 1993). According to Thomann and Mueller (1987) if the total phosphorus concentration in the lake is <10 μg/L the lake should be considered as an oligotroph. If concentrations are 10-20 μg/L and >20 μg/L it should be placed in mesotrophic and eutrophic lake category respectively. Total phosphorus concentrations analyzed in dam lake water (0.01-0.08 mg P/L) clearly indicated that the studied part of Karakaya Dam Lake has oligotrophic lake characteristic. However, concentrations of total nitrogen (0.67-0.91 mg N/L) showed that the lake is on the border of oligotrophy-mesotrophy.

*C. hirundinella* was noticeable alga with its continuous occurrence in the phytoplankton throughout the study. *A. formosa, C. pediculus, C. affinis, D. vulgaris, F. crotonensis, G. acuminatum, N. cryptocephala, N. sigmoidea* and *R. abbreviata* (Bacillariophyta); *D. planctonicum* and *O. limosa* (Cyanobacteria) were also noticeable algae with their frequency of occurrence. Many studies have shown that these species are characteristic algae of mesotrophic lakes (Rawson 1956; Hutchinson 1967; Fogg et al. 1973; Round 1973; Wetzel 1983; Reynolds et al. 1993; Bellinger and Siegee 2010; Laplace-Treyture and Feret 2016). Thus, occurrence of these algae may support the view that the lake also shows mesotrophic characteristic in terms of algal evidence.

A total of 38 algal taxa belonging to Bacillariophyta (27 taxa), Charophyta (2 taxa), Chlorophyta (3 taxa), Cyanobacteria (3 taxa), Miozoa (2 taxa) and Ochrophyta (1 taxon) were identified in the phytoplankton. The largest share of the phytoplankton belonged to Bacillariophyta (27 taxa) in Karakaya Dam Lake. The highest number of taxa (25) was found at in March, while the lowest number (9) was recorded in August. The diatom genera *Cocconeis, Cymbella, Fragilaria, Nitzschia, Stauroneis* and *Surirella* were represented with two taxa in the phytoplankton. *C. hirundinella* was noticed with its continuous occurrence in the phytoplankton throughout the year. *D. planctonicum* and *O. limosa* were observed in belonging to Cyanobactria in the phytoplankton. The species composition of the phytoplankton and epipelon of Karakaya Dam Lake showed similarities to those of many lakes and reservoirs in Türkiye (Çetin and Yıldırım 2000; Atıcı 2002; Çetin and Şen 2004; Kıvrak and Gürbüz 2005; Aykulu et al. 2006; Pala 2007; Taş and Gönülol 2007; Ongun Sevindik 2010; Sönmez 2011; Atıcı and Alaş 2012; Öterler 2013; Sönmez et al. 2017).

A total of 25 algal taxa belonging to Bacillariophyta (21 taxa), Charophyta (1 taxon), Chlorophyta (2 taxa) and Cyanobacteria (1 taxon) were identified in the epipelon. The largest share of the epipelon belonged to Bacillariophyta (25 taxa) in Karakaya Dam Lake. The highest number of taxa (21) was found at in spring, while the lowest number (13) was recorded in summer. The species composition and seasonal succession of the phytoplankton were almost similar at three stations during the study. The reasons for the similarities might be attributed to the similar environmental conditions at the stations. Seasonal values of almost all physicochemical parameters measured and analyzed showed similarities at stations. This may explain that in different parts of the same ecosystem, phytoplankton display similar growth characteristics under similar environmental conditions.

The increase both in species composition and algal individual numbers phytoplankton during the spring and autumn months in Karakaya Dam Lake were noticeable in the period of increasing water temperature. This finding may suggest that water temperature is one of the main factors affecting the species composition and abundance of planktonic algae in the dam lake. A strong relationship between phytoplankton composition and water temperature was also reported in some studies (Jaworska et al. 2014, Rakocevic 2012). However, it is difficult to mention any relations between phytoplankton growth and pH since high and low individual numbers were observed at similar pH levels in the present study. However, there was a noticeable correlation between the growth of phytoplankton and transparency in Karakaya Dam Lake since the larger populations of algae occurred during summer when transparency was high. In contrast low individual numbers coincided with low transparency in winter.

It is also hard to establish a relation between plant nutrients (TN and TP) and seasonal occurrence of phytoplankton species at stations. Although TN and TP concentrations were similar at stations seasonal occurrence of algal species varied from one station to another. Some species occurred at only one station and absent at others. Contrary, some species occurred at all stations. These findings suggest that occurrence of algal species in the Karakaya Dam Lake might be affected not only by the nutrients, but also by other factors. However, it was noticeable to observe that high chlorophyll a concentrations coincided with high amount of nitrogen and phosphorus in lake water.

The species diversity of Bacillariophyta was the highest in spring and autumn, and the lowest in winter and summer. *C. affinis* and *D. vulgaris*, which were reported to be found in eutrophic waters by Reynolds et al. 1994, were identified in Lake Karakaya in almost all seasons. According to these findings, it may be possible to express that *F. crotonensis, N. cryptocephala* and *R. abbreviata* may occur in mesotrophic as well as eutrophic lakes depending on the conditions.

*G. acuminatum and N. sigmoidea* is accepted by various authors as the characteristic indicator of both oligotrophic and mesotrophic lakes (Rawson 1956; Bellinger and Siegee 2010 and Laplace-Treyture and Feret 2016). It was also reported that *G. acuminatum and N. sigmoidea* strongly related to the trophic status of the lakes and announced to be the specific alga of oligotrophic lakes (Hutchinson 1967). In contrast, Wetzel (1983) reported that *G. acuminatum and N. sigmoidea* may be dominant in intermediate-sized eutrophic lakes. However, the present study is partly in harmony with these studies. Considering insistent occurrence of this diatom at three stations in the Karakaya Dam Lake, one may suggest that *G. acuminatum and N. sigmoidea* may incline to occur in lakes with various trophic status.

Occurrence of Ochrophyta species in Karakaya Dam Lake generally coincided with increasing temperature. However, occurrence of *D. divergens* with highly numbers was observed only in March when water temperature was already high. Studies (Reynolds et al. 1993 and Reynolds et al. 1994) revealed that phosphate and nitrate have an influence on the development of this species. Thus, *D. divergens* could have developed in Karakaya Dam Lake with the support of nitrate and phosphate that were always in sufficient concentrations to support the growth of plankton species.

*D. planctonicum* and *O. limosa* were only representatives of Cyanobacteria in Karakaya Dam Lake. Cyanobacteria species were observed mostly in summer and autumn. They were encountered with much fewer individual numbers in spring. Excessive Cyanobacteria development in summer may be explained by sufficient amount of nutrients and high water temperature (Round 1973; Reynolds et al. 1993 and Reynolds et al. 1994). Moreover, in cases where water movements are stable and lake is rich in nitrate, these species are known to reproduce rapidly (Fogg et al. 1973) Karakaya Dam Lake. *D. planctonicum and O. limosa* were recorded at all stations in all seasons.

It is important to determine the characteristics of the reservoirs, which have an important potential in terms of aquaculture and various study subjects, in terms of maximizing the evaluation of these water bodies that constitute the inland water wealth of our country. Such studies also provide the basis for the creation of databases related to our lakes.

Although fish farming in its current state does not seem to affect the water quality of the 10th Region of Karakaya Dam Lake in terms of nutrient salt forms; The increase in chlorophyll a determined at the Dam Lake station may represent the effect of fish farming on possible trophic level progression.

In order for a water source to be used in accordance with its purposes, it must be periodically monitored continuously. A managed monitoring program that fully evaluates the data will provide very useful information for environmental management.

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# Spatial and Temporal Changes of Planktonic Microcrustaceans (Cladocera, Copepoda) and their Relationship with Physicochemical Parameters in Kırklareli Reservoir (Kırklareli-Türkiye)

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## ABSTRACT

The present study focused on monthly densities and abundances of planktonic microcrustaceans (Cladocera and Copepeda) in Kırklareli reservoir, as well as their relationship with some physicochemical parameters at three stations for the period from May 2018 to April 2019. In the present study, a total of 26 planktonic microcrustaceans species, 14 from Cladocera and 12 from Copepoda were identified. The mean annual microcrustacean abundance recorded during the study was 24076 ind/m$^3$, 67% of which was Cladocera (16054 ± 12414 ind/m$^3$) and 33% was Copepoda (8022 ± 5564 ind/m$^3$). The maximum abundance of planktonic microcrustaceans were found in September (51521 ind/m$^3$) and the minimum was found in January (2919 ind/m$^3$). The most common species in the reservoir were *Bosmina longirostris, Daphnia pulex, Daphnia cucullata, Daphnia galeata, Daphnia longispina* and *Diaphanosoma brachyurum* from Cladocera and *Cyclops abyssorum, Cyclops vicinus, Eudiaptomus vulgaris* and *Arctodiaptomus wierzejskii* from Copepoda. The environmental parameter values measured in the reservoir were acceptable to support aquatic life, especially the zooplankton community. The total density and abundance of zooplanktonic microcrustaceans showed positive correlation with the water temperature and Chlorophyll-a. According to these results, we concluded that Kırklareli reservoir has a mesotrophic character in terms of the microcrustacean fauna and the physicochemical parameters.

**Keywords:** Cladocera, Copepoda, diversity, water quality, reservoir

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## Kırklareli Baraj Gölü'nde (Kırklareli-Türkiye) Planktonik Mikrocrustacea'ın (Cladocera, Copepoda) Mevsimsel değişimi ve Fizikokimyasal Parametrelerle İlişkisi

**Öz:** Bu çalışma, Kırklareli baraj Gölü'nde planktonik mikrocrustacea (Cladocera, Copepoda) faunasının mevsimsel değişimi ve bazı fizikokimyasal parametrelerle ilişkilerini belirlemek amacıyla Mayıs 2018-Nisan 2019 tarihleri arasında üç farklı istasyonda yapılmıştır. Cladocera'dan 14 ve Copepoda'dan 12 olmak üzere 26 tür tespit edilmiştir. Çalışma sırasında yıllık ortalama 24076 birey/m$^3$ mikrocrustacea bulunurken, bunun % 67 Cladocera (16054 ± 12414 birey/m$^3$) ve % 33 Copepoda (8022 ± 5564 birey/m$^3$) bireylerinden oluştuğu tespit edilmiştir. Planktonik mikrocrustacea maksimum bolluğu Eylül (51521 birey/m$^3$) ve minimum (2919 birey/m$^3$) Ocak ayında bulunmuştur. Rezervuardaki en yaygın türler Cladocera'dan *Bosmina longirostris, Daphnia pulex, Daphnia cucullata, Daphnia galeata, Daphnia longispina, Diaphanosoma brachyurum* ile Copepoda'dan *Cyclops abyssorum, Cyclops vicinus, Eudiaptomus vulgaris, Arctodiaptomus wierzejskii*'dir. Rezervuarda ölçülen çevresel parametreler başta zooplankton topluluğu olmak üzere sucul yaşamı desteklemek için kabul edilebilir değerler arasında tespit edilmiştir. Toplam mikrocrustacea yoğunluğu, su sıcaklığı ve Chlorophyll-*a* ile pozitif korelasyon göstermiştir. Bu sonuçlara göre Kırklareli rezervuarının mikrocrustacea faunası ve fizikokimyasal parametreler açısından mezotrofik bir karaktere sahip olduğu sonucuna varılmıştır.

**Anahtar kelimeler:** ***:*** Cladocera, Copepoda, çeşitlilik, su kalitesi, baraj gölü

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## Introduction

The zooplanktonic organisms, which play a role in the transformation of plant based foods into animal proteins in freshwater ecosystems, are the food source of many living things and are among the important energy dynamics of aquatic ecosystems. These organisms occupy an intermediate position in the food web and are among the important zooplankton groups that mediate the transition of energy from low to high trophic levels (Sharma et al. 2010). The freshwater zooplankton comprises Protozoa, Rotifera, Cladocera, Copepoda and Ostracoda. Cladocera and Copepoda are planktonic microcrustaceans that are widely distributed in freshwaters. They are crucial groups among zooplankton and form the most useful and nutritive group of crustaceans for higher members in the food chain such as fishes. Cladocera are known as filter feeders as they filter the water to trap the organisms in it. Cladocera are highly sensitive against even low concentrations of pollutants (Murugan et al. 1998). Copepoda provide an important food source for fish and are influenced by negative environmental factors in water bodies. In addition, these organisms play a role in preventing pollution by controlling the phytoplankton population due to grazing pressure on phytoplankton (Trivedi et al. 2003).

The variability in the distribution of zooplankton in freshwater ecosystems is affected by abiotic and biotic parameters or by a combination of both (Beyst et al. 2001; Escribano and Hidalgo 2000). Although zooplankton exists under a wide range of environmental conditions, yet many species are limited by temperature, dissolved oxygen, salinity and other physicochemical factors. To conserve freshwater ecosystems from further deterioration there is a need for regular monitoring. A comprehensive biomonitoring process involves both physicochemical and biological approaches and gives the exact status of the aquatic ecosystem. Biomonitoring of water bodies also helps to understand the composition of biota and its dynamics. Zooplankton has been recently used as an indicator to observe and understand changes in the ecosystem (Li et al. 2000). For this reason, studies on Cladocera and Copepoda, which are an important component of the aquatic ecosystem, are extremely important.

A number of studies have been carried out to examine the distribution and diversity of microcrustaceans (Cladocera and Copepoda) in the inland waters of Türkiye (Ustaoğlu 2015; Güher 2014). However, studies on reservoirs are limited (Saler and Alış 2014; Ulgu and Bozkurt 2015; Güher and Çolak 2015; Güher 2019; Dorak et al. 2019; Dorak 2019; Bozkurt and Kara 2020; and Güher and Öterler 2021). Also, the distribution and diversity of planktonic microcrustaceans (Cladocera and Copepoda) of Kırklareli Reservoir have not been studied so far. Therefore, the aim of this paper is to investigate the influence of physicochemical parameters of water of Kırklareli Reservoir on species composition, relative abundance and seasonal dynamics of the planktonic microcrustaceans (Cladocera and Copepoda).

## Materials and Methods

### Study Area

Kırklareli Reservoir was built between the years 1985-1995 for irrigation and flood control on Şeytandere stream. The reservoir which supplies freshwater to the province of Kırklareli for drinking and domestic usages is located between 41°44'08.6"N and 27°16'59.0"E coordinates. The volume of the reservoir is about 112 $hm^3$ and the surface area is 6 $km^2$. Although the reservoir is fed mainly by the Ana stream and Büyük stream, it is also fed by other creeks in the basin and by rainfall (Figure 1). The reservoir is subjected to temporal fluctuations in water volume with high water volume in the rainy season and less water in the dry season due to high evaporation. The microcrustaceans and water samples were collected monthly from May 2018 to April 2019 at three stations representing the lake's ecological characters. The first sampling station is located in the middle of the reservoir (41°44'53,8"N 27°17'02,6"E), the second sampling station is located in the western part of the reservoir where Ana stream feeds the reservoir (41°45'54,9"N 27°16'41,6"E) and the third sampling station is located in the eastern branch of the reservoir where Büyük stream feeds the reservoir (41°45'41,9"N 27°18'30,3"E) (Figure 1).

### Sampling

The planktonic microcrustaceans and water samples were taken at monthly intervals from May 2018 to April 2019 in three stations in the reservoir (Figure 1). The planktonic microcrustaceans and water samples were carried out at monthly intervals from May 2018 to April 2019 in three stations in the reservoir (Figure 1). Due to unfavourable weather conditions, no sampling could be performed in March 2019. Planktonic microcrustacean samples were collected with a Hensen type plankton net (mesh size 55 μ, mouth diameter 15 cm, length 75 cm) vertically up to the surface from the bottom point (10 m deeply) and horizontally. Plankton samples taken from the reservoir were immediately preserved in 4 % formaldehyde in the field and then brought to the laboratory for further analyses. In the laboratory, samples were identified at the species level according to Dussart (1967, 1969), Flössner (1972), Smirnov (1974), Kiefer (1978), Margaritora (1983), Korinek (1987), Apostolov and Marinov (1988), Dussart and Defaye (2002, 2006) and Bledzki and Raybok (2016). The counting of the samples was made according to Edmondson (1959) using an Olympus inverted microscope. Densities were presented as the number of individuals per cubic meter (ind/m$^3$).

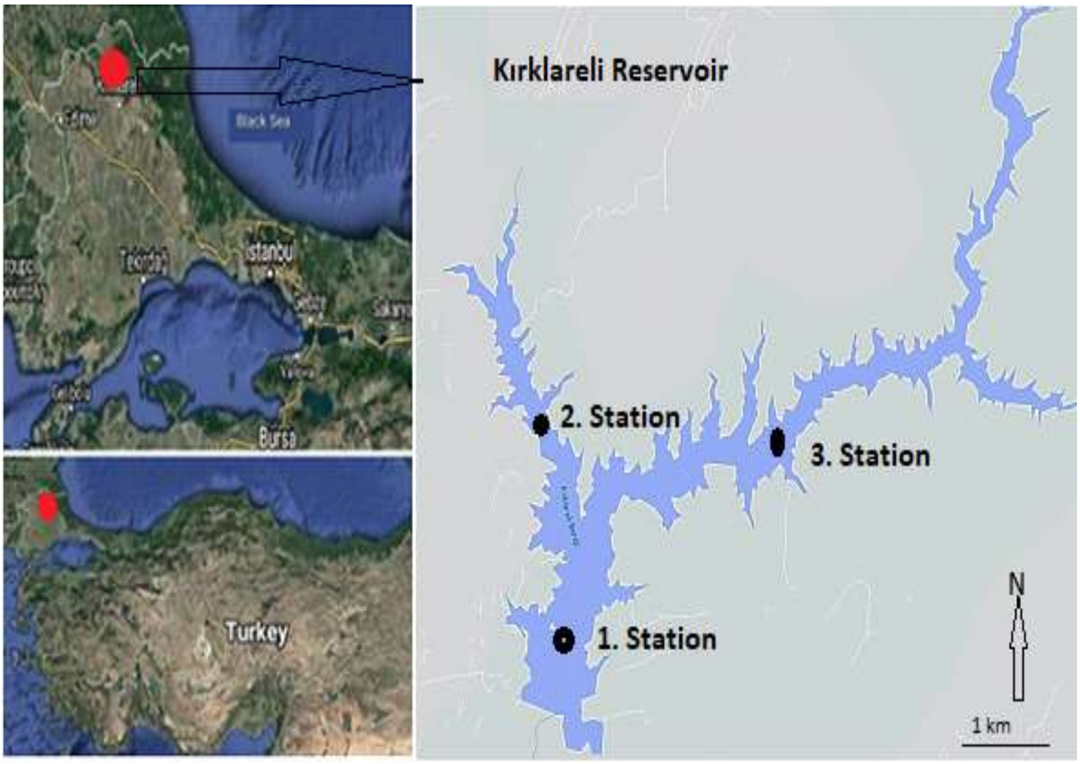


**Figure 1.** Location of Kırklareli Reservoir and the sampling stations

The water samples were taken with Ruttner water sampling bottles from about 15 to 20 cm below the water surface. Some physicochemical parameters, such as Water temperature (WT), Dissolved oxygen (DO), pH and Conductivity (EC) were measured on site simultaneously by using Orion Star S/N 610541 water analyser. The light permeability (LP) of the reservoir was measured using a Secchi disk. Nitrate nitrogen ($NO_3$-N), Nitrite nitrogen ($NO_2$-N) Phosphate ($PO_4^-$P), Sulphate ($SO_4^{2-}$), Calcium ($Ca_2^+$) and Magnesium ($Mg_2^+$) were measured at the Trakya University Technology Research Development Application and Research Centre. The analyses were performed by Metrohm Ion Chromatography System and the Agilent Technologies 7700 ICP-MS System (EPA 2001), immediately after the sample collection.

**Statistical Analyses**

Shannon-Weaver index, Margalef diversity and Simpson's diversity indexes were used to determine the species diversity and the species richness of planktonic microcrustaceans in the reservoir (Shannon and Weaver 1949, Margalef 1958). Bray-Curtis similarity index was used to examine the similarities of the sampling based on the months and the seasons according to diversity and abundance of Cladocera and Copepoda species (Jaccard 1912). Spearman's correlation was used to determine the relationship of Cladocera and Copepoda groups with each other and with environmental parameters (Krebs 1999). In addition, the statistical association between Cladocera and Copepoda assemblage structure and environmental variables were quantified with the canonical correspondence analysis (CCA) by the Past Version3.14 (Hammer et al. 2001).

## Results

**Physicochemical Variables**

The measured environmental parameters and their minimum, maximum and average values are given in Table 1. Variations in these environmental parameters according to the sampling stations and months are given in Figure 2. The WT ranged between the lowest value of 6.00 ℃ obtained during the winter season in January and the highest of 27.00 ℃ obtained during the summer season in July (average 16.50 ± 7.66 ℃). The summer season temperature was significantly higher ($p< 0.05$) than the winter season temperature. DO fluctuated between the lowest monthly mean of 7.43 mg/L obtained in September in winter season and the highest monthly mean of 13.75 mg/L recorded in April in the spring season. The monthly average of the DO values measured in the reservoir was 9.71 ± 1.83 mg/L. Light permeability was highest at 336.67 cm obtained in November in the autumn season and lowest at 66.67 cm in January in the winter season (average 198.33 ± 73.5 cm). Significantly higher transparency ($p<0.05$) was obtained in the autumn season. The pH value of the reservoir was moderately alkaline varying from 8.15 to 9.45. The lowest pH was obtained in December, while the highest was obtained in April (average 8.64 ± 0.49). The monthly mean variations in conductivity were similar. The monthly average of EC values measured in the reservoir was 248.17 ± 30.10 µScm/L. The $NO_2$-N concentration in the reservoir was found below the limit of detection in June, while it was highest in May with 0.05 mg/L (average 0.02 ± 0.02 mg/L). The highest concentration of $NO_3$-N recorded was 2.13 mg/L in February in the winter seasons, while the lowest was obtained as 0.04 mg/L in September in the autumn seasons. The monthly average of $NO_3$-N values measured in the reservoir was 0.73 ± 0.71 mg/L. The $PO_4$-P concentration in the reservoir was found below the limit of detection in April June, July, September and October, while it was highest in May, respectively 0.78 mg/L (average 0.11 ± 0.23 mg/L). The maximum value of sulphate was recorded in December and the minimum in January. The mean value of $SO_4^{2-}$ in the reservoir was 10.12 ± 0.25 mg/L. The maximum $Mg_2^+$ value was detected as 12.30 mg/L in October in the autumn season and the minimum as 1.90 mg/L in January in the winter season (average 8.19 ± 3.87 mg/L). The maximum $Ca^{2+}$ concentration was measured as 22.31mg/L in November and the minimum as 3.04 mg/L in January (average 13.66 ± 6.60 mg/L). The highest and the lowest chlorophyll-*a* values were recorded 13.09 µg/L in September in the summer and the autumn season and 2.31µg/L in February in the winter season, respectively. The mean chlorophyll-*a* in the reservoir was 5.96 ± 3.49 µg/L (Figure 2 and Table 1).

**Table 1.** The measured physicochemical parameters in the reservoir and their minimum, maximum and average ± stdev values (*below limit of detection)

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

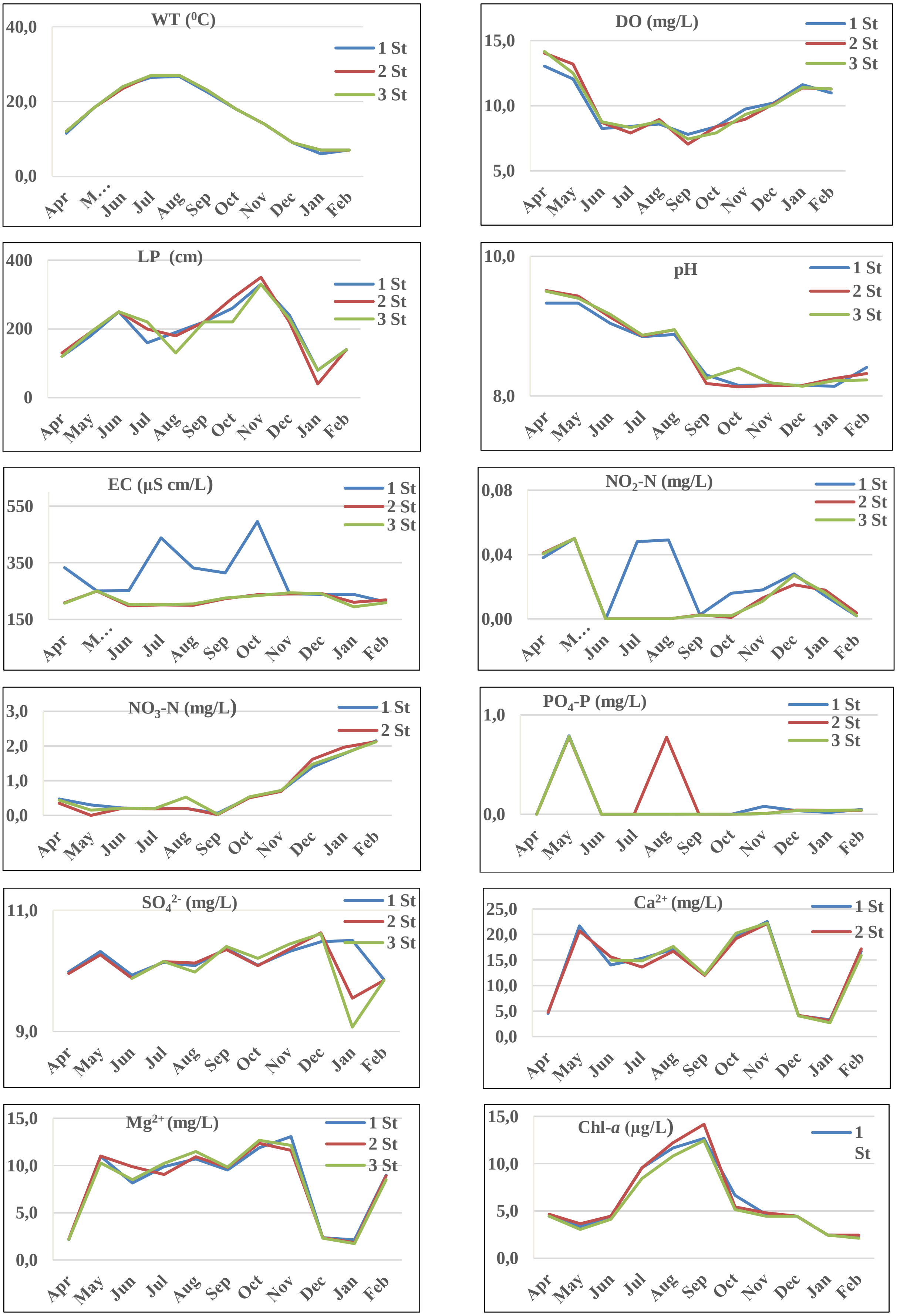


**Figure 2.** Variations of the physicochemical parameters according to the sampling stations and months in the reservoir

The results of the cluster analysis showed that the stations were very similar in terms of environmental parameters (91.0 % to 98.5 % similarity) (Figure 3).

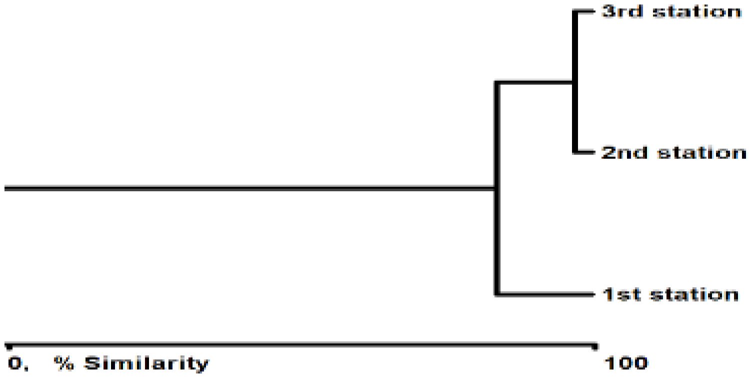


**Figure 3.** Cluster analysis showing the similarity index of the physicochemical parameters according to the sampling stations

**Community Structure Of Planktonic Microcrustaceans**

A total of 26 planktonic microcrustaceans species, 14 from Cladocera and 12 from Copepoda were identified, from 3 stations in Kırklareli Reservoir. Cyclopoid copepodites, Calanoid copepodites, and Nauplius were also observed (Table 2).

**Table 2.** Cladocera and Copepoda species in Kırklareli Reservoir and the average values of their annual numbers per m$^3$

| **CLADOCERA** | **Average ± stdev** | **%** |
|---|---|---|
| *Diaphanosoma brachyurum* (Liévin,1848) | 2477 ± 3907 | 15.4 |
| *Daphnia pulex* Leydig, 1860 | 281 ± 308 | 1.8 |
| *Daphnia cucullata* Sars, 1862 | 1062 ± 1299 | 6.6 |
| *Daphnia galeata* Sars, 1863 | 850 ± 2283 | 5.3 |
| *Daphnia hyalina* Leydig 1860 | 1070 ± 2817 | 6.7 |
| *Daphnia longispina* (O.F.Müller, 1876) | 1471 ±1917 | 9.2 |
| *Ceriodaphnia quadrangula* (O.F.Müller, 1785) | 1102 ± 2096 | 6.9 |
| *Bosmina longirostris* (O.F.Müller, 1785) | 6796 ±6375 | 42.3 |
| *Pleuroxus aduncus* (Jurine, 1820) | 8 ± 25 | 0.1 |
| *Chydorus ovalis* Kurz, 1875 | 40 ± 87 | 0.3 |
| *Chydorus sphaericus* (O.F.Müller, 1776) | 712 ± 1502 | 4.4 |
| *Alona guttata* Sars, 1862 | 8 ± 25 | 0.1 |
| *Leptodora kindtii* (Focke, 1844) | 88 ±119 | 0.6 |
| *Cercopagis pengoi* (Ostroumov, 1892) | 88 ± 280 | 0.6 |
| Total | 16054 ± 12414 | 100 |
| **COPEPODA** | | |
| *Eudiaptomus vulgaris* (Schmeil, 1898) | 97 ± 122 | 1.2 |
| *Arctodiaptomus wierzejskii* (Richard, 1888) | 48 ± 69 | 0.6 |
| *Macrocyclops albidus* (Jurine, 1820) | 8 ± 25 | 0.1 |
| *Cyclops abyssorum* G.O.Sars, 1863 | 249 ± 328 | 3.1 |
| *Cyclops strenuus* Fischer, 1851 | 16 ± 51 | 0.2 |
| *Cyclops vicinus* Uljanin, 1875 | 185 ± 265 | 2.3 |
| *Acanthocyclops robustus* (G.O.Sars, 1863) | 88 ± 203 | 1.1 |
| *Mesocyclops leuckarti* (Claus, 1857) | 64 ± 126 | 0.8 |
| *Megacyclops viridis* (Jurine, 1820 | 88 ± 156 | 1.1 |
| *Thermocyclops crassus* (Fischer, 1853) | 56 ± 94 | 0.7 |
| *Canthocamptus microstaphylinus* Wolf, 1905 | 40 ± 127 | 0.5 |
| *Canthocamptus staphylinus* (Jurine, 1820) | 24 ± 55 | 0.3 |
| Nauplius | 3788 ± 2532 | 47.2 |
| Cyclopoid copepodit | 2252 ± 2317 | 28.1 |
| Calanoid copepodit | 1017 ± 845 | 12.7 |
| Total | 8022 ± 5564 | 100 |

The seasonal species richness of planktonic microcrustaceans was listed from the highest to the lowest as; summer (11 Cladocera, 10 Copepoda species), autumn (13 Cladocera, 8 Copepoda species), spring (9 Cladocera, 4 Copepoda species) and winter (4 Cladocera, 4 Copepoda species). According to the stations, the highest species number was found in the 1st station (13 Cladocera, 11 Copepoda species) followed by the 2nd (13 Cladocera, 9 Copepoda species) and the 3rd stations (11 Cladocera, 10 Copepoda species). The maximum species diversity was recorded as, 10 species from Cladocera in August and in October and 8 species from Copepoda in July, while the least diversity was found as 1 species from Cladocera in February and 1 species from Copepoda in December and February.

The most common species in the reservoir was *Bosmina longirostris* from Cladocera and was found in all sampling months. *Daphnia pulex*, *D. cucullata D. galeata* and *D. longispina* were sampled for seven months and *Diaphanosoma brachyurum* was sampled for six months and *Chydorus sphaericus*, *Daphnia hyalina* and *Leptodora kindtii* were sampled for five months. The most common species from Copepoda were *Cyclops abyssorum* and *C. vicinus* found during six months and *Eudiaptomus vulgaris* and *Arctodiaptomus wierzejskii* found during five months. Also, Cyclopoid copepodites, Calanoid copepodites and Nauplius from Copepoda were found in all sampling months. *Pleuroxus aduncus, Alona guttata* and *Cercopagis pengoi* from Cladocera and *Macrocyclops albidus, Cyclops strenuus* and *Canthocamptus microstaphylinus* from Copepoda were sampled only in one month during the study.

According to the Shannon Diversity Index, while species richness was at its maximum (0.82) in September it was found in the lowest value (0.555) in June. According to the Simpsons Diversity Index, while species richness was at its maximum in August (6.969), its lowest value (2.362) was found in April. According to the Margalef Index, while species richness was at its maximum in October (8.081), its lowest value (5.942) was found in September ($p>0.005$) (Table 3).

**Table 3.** Species diversity and species richness values of microcrustacean groups according to the sampling months

| Index | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Feb |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Shannon J' | 0.571 | 0.641 | **0.555** | 0.727 | 0.777 | **0.82** | 0.73 | 0.798 | 0.734 | 0.726 | 0.679 |
| Simpsons Diversity (1/D) | **2.362** | 4.283 | 2.686 | 5.969 | **6.969** | 6.209 | 6.466 | 5.352 | 3.238 | 3.269 | 2.609 |
| Margaleff M Base 10, | 6.829 | 6.595 | 6.119 | 6.057 | 6.261 | **5.942** | **8.081** | 6.796 | 7.966 | 5.999 | 7.178 |

As a result of the quantitative evaluation of plankton samples, the annual average value of planktonic microcrustaceans in the reservoir was found as 24076 ind/m$^3$. The annual average values according to the groups were 16054 ± 12414 ind/m$^3$ for Cladocera and 8022 ± 5564 ind/m$^3$ for Copepoda. In other words, the planktonic microcrustaceans in the Kırklareli Reservoir consists of 67 % Cladocera and 33 % Copepoda. However, a large part of Copepoda consists of larval individuals such as Nauplius or copepodite stage (12 % adult individuals, 88 % larval individuals) (Table 2). When the results were evaluated in terms of sampling months, the maximum abundance of planktonic microcrustaceans were found in September (51521 ind/m$^3$) followed by October (46502 ind/m$^3$) and July (41976 ind/m$^3$) and the minimum was found in January (2919 ind/m$^3$) followed by February (7962 ind/m$^3$) (Figure 4). According to the results of cluster analysis, January-December (80 %), February-April-May (69 %) and July-August (63 %) were the most similar to each other while the least similar months were June-July (17 %), July-November and July-December-January (7 %) (Figure 5). In other words, spring was very similar to the summer season (60 % similarity) and autumn was very similar to the summer season (72 % similarity) (Figure 6).

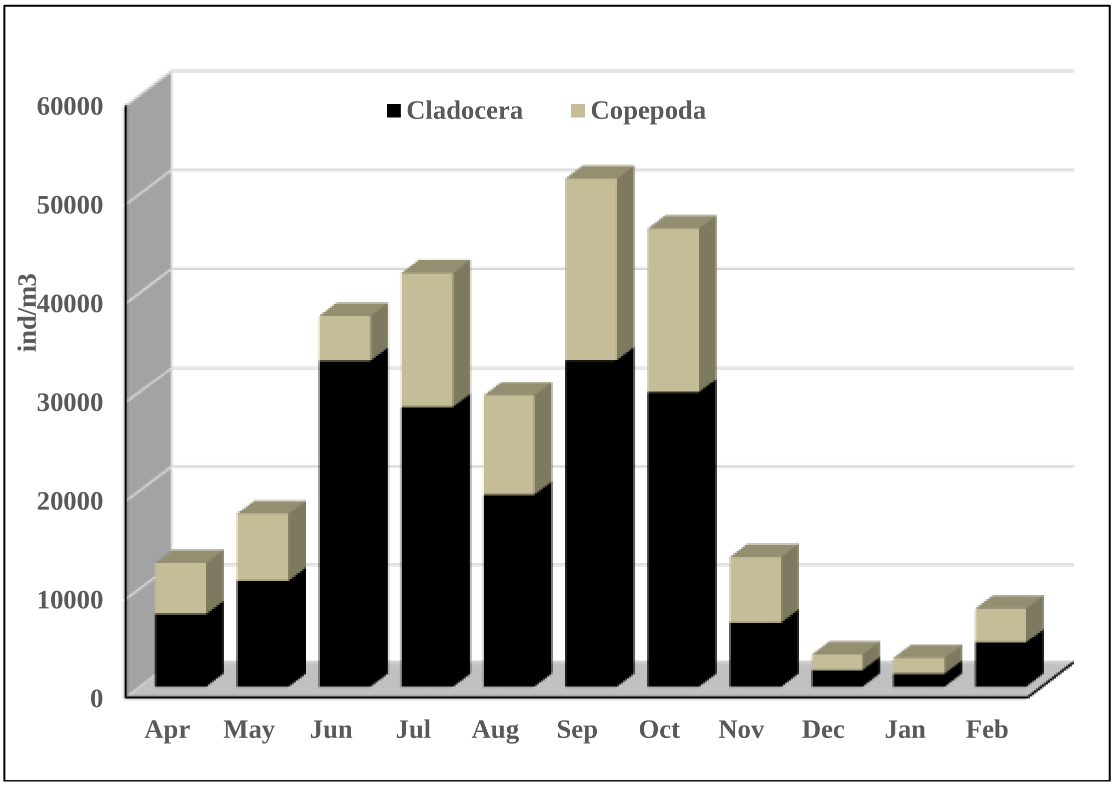


**Figure 4.** The abundance of planktonic microcrustacean in Kırklareli Reservoir according to the sampling months

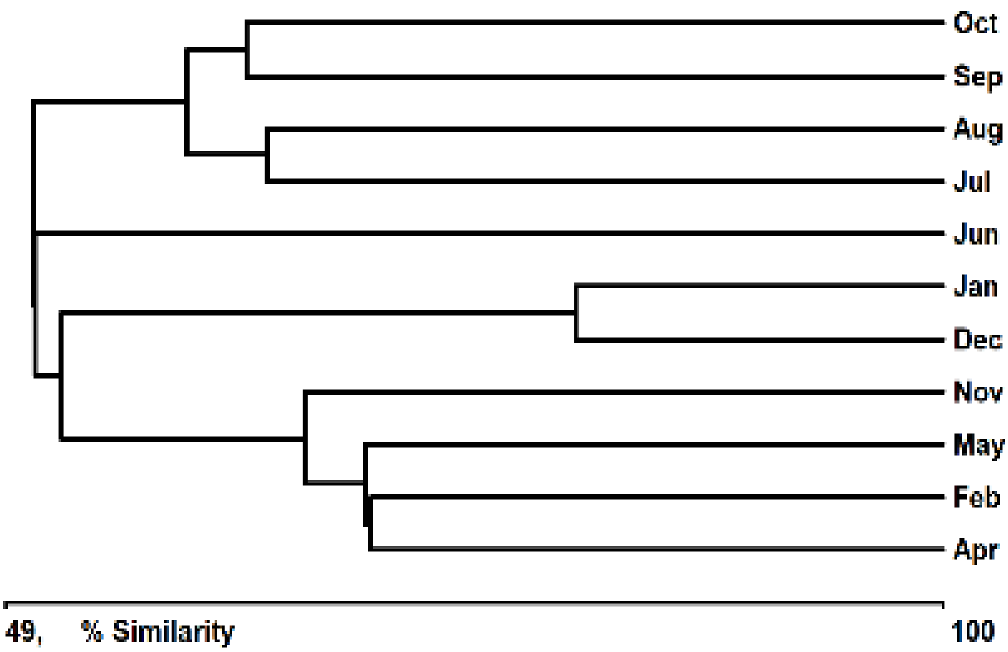


**Figure 5.** Cluster analysis showing the similarity index of planktonic microcrustaceans according to the sampling months

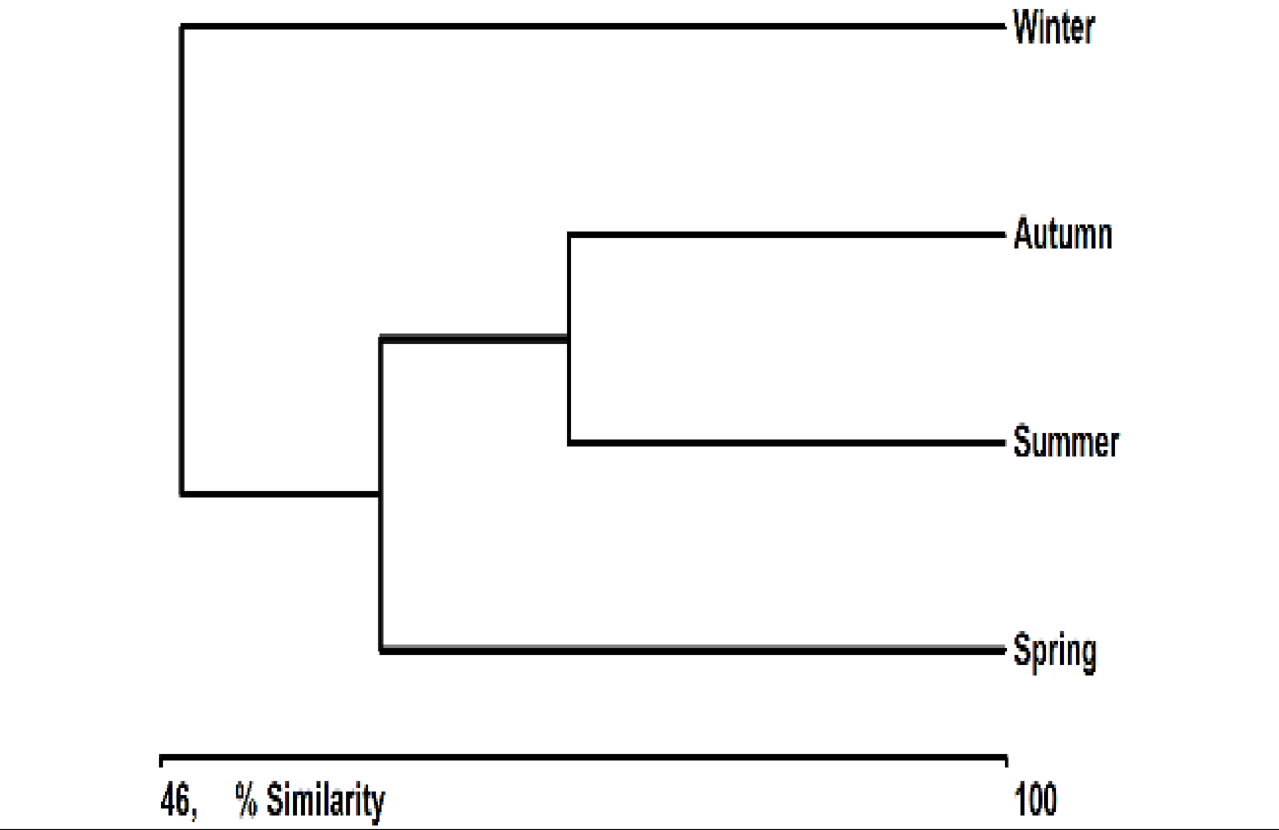


**Figure 6.** Cluster analysis showing the similarity index of planktonic microcrustaceans according to the sampling seasons

The Spearman's correlation was used to determine the relationship of Cladocera and Copepoda groups with environmental parameters. There was a positive correlation between Cladocera with WT (r= 0.809) ($p< 0.01$), EC (r=0.636) ($p< 0.05$) and Chl-*a* (r=0.636) ($p< 0.05$), while there was a negative correlation with DO (r=0.736) ($p< 0.01$) and $NO_3$-N (r=0.800) ($p< 0.01$). Also, there was a positive correlation between Copepoda with WT (r=0.711) ($p< 0.05$), EC (r=0.875), $Mg_2^+$ (r=0.738) and Chl-*a* (r=0.806) and Cladocera (r=0.797) ($p< 0.01$), while there was a negative correlation with DO (r=0.656) and $NO_3$-N (r=0.715) ($p< 0.05$). There was a positive correlation between WT with Chl-*a*, $NO_3$-N with DO, $Mg^{2+}$ with LP, Chl-*a* with EC and $Ca^{2+}$ with $Mg^{2+}$, while there is a negative correlation between WT with DO and $NO_3$-N (Table 4).

**Table 4.** The relationship between microcrustacean groups and environmental parameters in Kırklareli Reservoir as revealed by the Spearman's correlation analysis (Cop: Copepoda, Clad: Cladocera).

| | **WT** | **DO** | **LP** | **pH** | **EC** | **$NO_2N$** | **$NO_3N$** | **$PO_4^-P$** | **Ca** | **Mg** | **Chl-*a*** | **Clad** | **Cop** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **WT** | 1 | | | | | | | | | | | | |
| **DO** | **-.655*** | 1 | | | | | | | | | | | |
| **LP** | .327 | -.582 | 1 | | | | | | | | | | |
| **pH** | .436 | .236 | -.391 | 1 | | | | | | | | | |
| **EC** | .582 | -.464 | .309 | .191 | 1 | | | | | | | | |
| **$NO_2N$** | -.165 | **.651*** | -.413 | .202 | .156 | 1 | | | | | | | |
| **$NO_3N$** | **-.818**** | .436 | -.182 | -.536 | **-.664*** | .000 | 1 | | | | | | |
| **$PO_4^-P$** | -.248 | .515 | -.334 | -.029 | -.410 | .433 | .267 | 1 | | | | | |
| **Ca** | .336 | -.118 | .482 | .136 | .255 | -.128 | -.173 | .257 | 1 | | | | |
| **Mg** | .536 | -.500 | **.645*** | -.055 | .573 | -.220 | -.364 | .019 | **.855**** | 1 | | | |
| **Chl-*a*** | **.673*** | **-.709*** | .364 | -.027 | **.718*** | -.138 | -.564 | -.420 | .118 | .545 | 1 | | |
| **Clad** | **.809**** | **-736**** | .427 | .391 | **.636*** | -.486 | **-.800**** | -.543 | .273 | .527 | **.636*** | 1 | |
| **Cop** | **.711*** | **-.656*** | .333 | .210 | **.875**** | -.193 | **-.715*** | -.349 | .433 | **.738**** | **.806**** | **.797**** | 1 |

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

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

The Canonical Correspondence Analysis (CCA) was used to determine the relationship of Cladocera and Copepoda species with environmental parameters. The eigenvalues of the first two axes were calculated as 0.052 and 0.026, respectively. In the analysis, the two axes explain 100% of the variance of the species, 66.11% (Axis 1) and 33.89% (Axis 2). The distributions of *Canthocamptus staphylinus*, *Thermocyclops crassus*, *Macrocyclops albidus*, *Acanthocyclops robustus*, and *Mesocyclops leuckarti* from Copepoda and *Daphnia hyalina* from Cladocera were affected by DO and $NO_3$-N. Environmental parameters did not affect the distributions of *Cyclops vicinus* and *Arctodiaptomus wierzejiskii* from Copepoda and *Daphnia pulex, Cercopagis pengoi* and *Pleuroxus aduncus* from Cladocera. These species were found to have the highest ecological tolerance. While WT, $SO_4^{2-}$, $Ca_2^+$, $Mg_2^+$, LP, $PO_4$-P and pH are effective in the distribution of *Daphnia cucullata, Bosmina longirostris,* and *Chydorus ovalis* from Cladocera and *Megacyclops viridis* and *Eudiaptomus vulgaris* from Copepoda. EC and $NO_2$-N parameters are effective in the distribution of *Daphnia galeata* and *Chydorus sphaericus* from Cladocera and *Cyclops strenuous, Canthocamptus microstaphylinus* and Calanoid copepodit from Copepoda. (Figure 7).

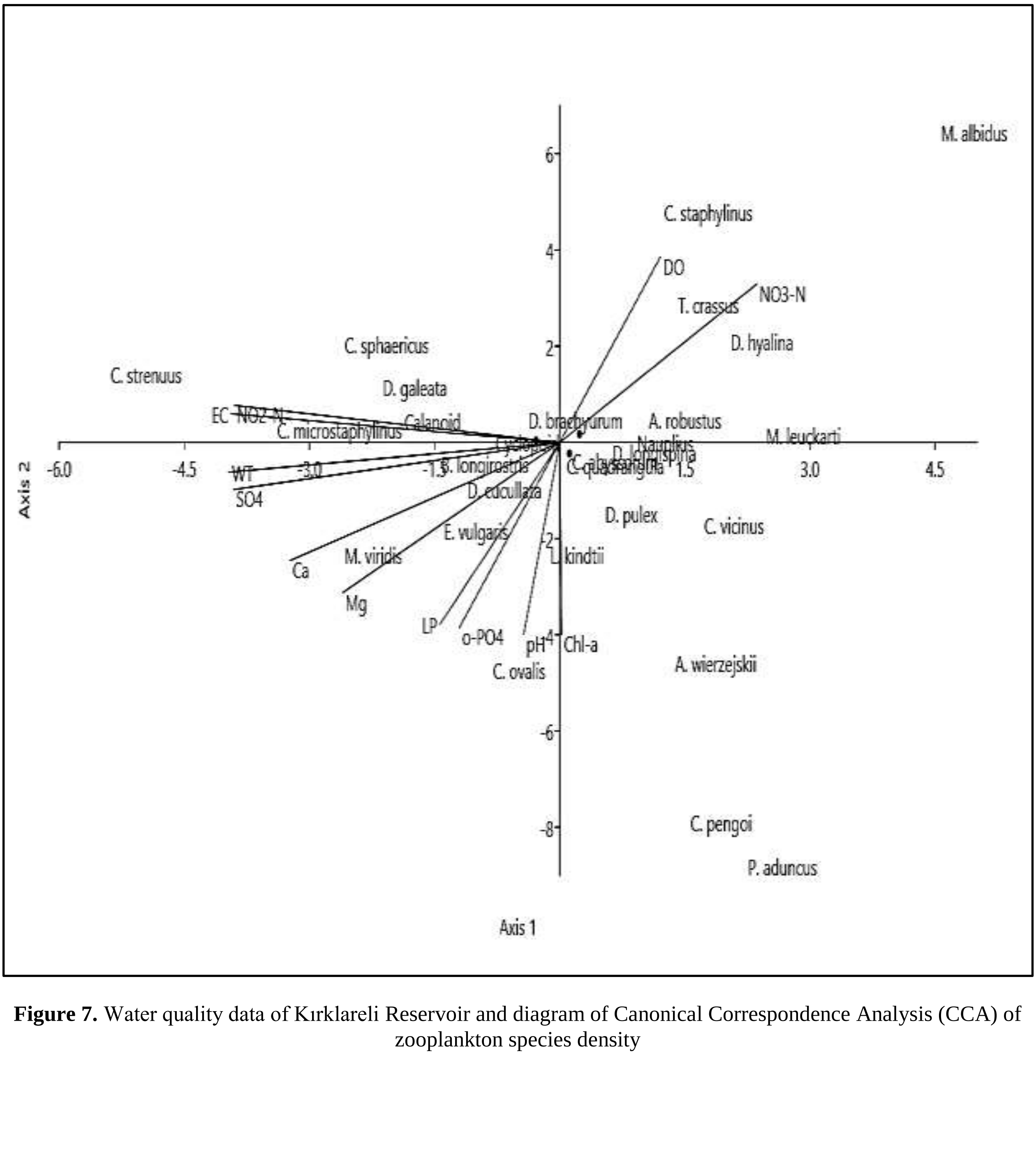


**Figure 7.** Water quality data of Kırklareli Reservoir and diagram of Canonical Correspondence Analysis (CCA) of zooplankton species density

## Discussion

In the present study, which was performed for one-year period in Kırklareli reservoir, a total of 26 species have been identified as 14 species belonging to Cladocera and 12 species to Copepoda. The most common species in the reservoir were *D. brachyurum, B. longirostris, D. pulex, D. cucullata D. galeata* and *D. longispina* from Cladocera and *C. abyssorum, C. vicinus, E. vulgaris* and *A. wierzejskii* from Copepoda. Most of the zooplankton species found in the present study are reported to be widespread in freshwater ecosystems of all sizes in different geographic regions (Güher 2014, Ustaoğlu 2015). This is due to the fact that these species are able to adapt to very different ecological conditions and have a cosmopolitan distribution. The annual mean zooplankton abundance recorded during the study were 24076 ind/$m^3$, and 67% of it was Cladocera (16054 ± 12414 ind/$m^3$) and 33% was Copepoda (8022 ± 5564 ind/$m^3$) (Table 2). Considering the geographical region where Türkiye is located, zooplankton organisms are expected to increase twice in spring and autumn during the year. But, in Kırklareli reservoir, while Cladocera reaches its maximum in the summer and Copepoda in the autumn, Cladocera and Copepoda become minimum in the winter season. As one can see from the results of Bray-Curtis similarity index, according to the diversity and abundance of zooplankton species, similarities of months are associated with seasons. When the present results are compared with the studies carried out in the reservoirs in the same area, the abundances of Cladocera and Copepoda are relatively low (Güher and Çolak 2015, Güher 2019, Güher and Öterler 2021). The relatively low abundances of Cladocera and Copepoda were as a result of the hydrodynamics of the reservoir such as the low water volume, short residence time, relative old age of the reservoir and its morphometry.

Simpsons diversity index results showed that species richness of zooplankton is higher during the summer months. The increase in the temperature and the increase in aquatic macrophytes forming very specific habitats within the water body support species richness. The Shannon diversity index revealed no significant differences in species diversity of zooplankton by months. Species diversity and species richness increase and decrease in the same months and are affected by similar conditions (Table 4).

The correlations of Cladocera and Copepoda with nitrate and phosphate may not necessarily be a direct consequence of the zooplankton utilizing the nutrients, but could be attributed to the dependence of the phytoplankton (which serves as food for the zooplankton) on these nutrients. The positive correlation of zooplankton with transparency was as a result of the transparency of the water which supports zooplankton growth and abundance. The correlation with Chl-*a* depends on the use of phytoplankton as food by Cladocera and Copepoda.

The water temperature was recorded in its lowest value in winter and in the highest in the summer seasons. Similarly, the abundance of planktonic microcrustaceans was recorded in its lowest in the winter and in its highest in late summer and early autumn seasons. The zooplankton growth and abundance in the reservoir showed a positive correlation with WT, because WT is the most important factor affecting the amount of nutrients and life in freshwater (Geller and Müller 1981). pH is one of the important factors affecting the living life in water. Many species of fish and aquatic organisms develop well in waters with a pH range of 6.5 - 8.5 (Arrignon 1976; Dauba 1981). In this study, the average pH value was found to be 8,64 ± 0,49 and the reservoir water was graded as alkaline water (Table 1). Mean dissolved oxygen concentrations above 5 mg/L (Karpowicz and Ejsmont-Karabin 2017) and the electrical conductivity values around 250-500 μS/cm were reported to be the acceptable (Yücel 1990). Accordingly, the values recorded in the reservoir were among the acceptable values to support aquatic life, especially the zooplankton community.

According to the results of CCA analysis, high correlations (for CCA axis 1: 0.052, for CCA axis 2: 0.026) between Cladocera and Copepoda species and the environmental variables shows that, WT, $SO_4^{2-}$, $Ca_2^+$, $Mg_2^+$, LP, $PO_4$-P and pH are the significant factors determining the distribution of zooplankton organisms. CCA analysis showed that *D. hyalina, D. cucullata, B. longirostris, C. ovalis* from Cladocera and *C. staphylinus, T. crassus, M. albidus, A. robustus, M. leuckarti, M. viridis and E. vulgaris* from Copepoda are affected by similar environmental conditions.

Zooplanktonic organisms play an important role as indicators in determining water quality, eutrophication and water pollution level. Cladocera and Cyclopoid Copepods are particularly well adapted to eutrophic conditions (Gannon and Stremberger 1978). *C. sphaericus* (712 ± 1502 ind/$m^3$; 4.4 %), *B. longirostris* (6796 ±6375 ind/$m^3$; 42.3%) and *C. vicinus* (185 ± 265 ind/$m^3$; 2.3%) in Kırklareli reservoir are known as typical indicators of eutrophic lakes (Ryding and Rast 1989, Makarewicz 1993). Also, Yağcı (2016) reported that *C. quadrangula, B. longirostris, C. sphaericus, D. longispina* and *Cyclops strenuus paternonis* are mesotrophic-eutrophic indicators. In the present study, although *D. brachyurum, B. longirostris, D.*

*pulex, D. cucullata, D. galeata* and *D. longispina* from Cladocera and *C. vicinus, E. vulgaris* and *A. wierzejski* from Copepoda are the most common species in Kırklareli reservoir, the abundances of these species were very low. This shows that the reservoir has mesotrophic characteristics.

In conclusion, a total of 26 species from planktonic microcrustaceans (14 Cladocera and 12 Copepoda) were found in Kırklareli reservoir. As a result of quantitative evaluation of the samples, 67 % of the total microcrustaceans were Cladocera (16054 ± 12414 ind/m$^3$) and 33 % were Copepoda (8022 ± 5564 ind/m$^3$). When the mean values of each environmental factor measured in the reservoir were evaluated according to Water Pollution Control Regulations (Anonymous 2015), it can be concluded that they are within the normal ranges. When we examined the species identified in the reservoir, the distribution of the individuals that make up the microcrustaceans fauna and physicochemical parameters as a whole, it has been concluded that Kırklareli reservoir posesses mesotrophic characteristics in terms of zooplankton.

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# An Investigation on *Argulus foliaceus* (Crustacea) Infestation of Fishes in the Kunduzlar Dam Lake

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## ABSTRACT

In this study, crustacean parasite species, *Argulus foliaceus* was investigated on the seven fish species (*Alburnus escherichii*, *Barbus plebejus*, *Capoeta tinca*, *Carassius gibelio*, *Chondrostoma nasus*, *Cyprinus carpio*, *Squalius cephalus*) from the Kunduzlar Dam Lake in Kırka-Eskişehir, Türkiye. The parasite was found on the gills, fins, and skin of all of the host fish species except *Chondrostoma nasus*. Its prevalence and mean intensity of the parasitic infection value for the infected six fish species are as follows: 3 out of 38 *Alburnus escherichii* (prevalence 7.9%, mean intensity 4.3±3.1), 9 out of 16 *Barbus plebejus* (56.2%, 47.9±28.5), 12 out of 47 *Capoeta tinca* (25.5%, 27.5±18.9), 7 out of 17 *Carassius gibelio* (41.1%, 6.9±5.3), 4 out of 4 *Cyprinus carpio* (100%, 4.3±4.2) and 6 out of 14 *Squalius cephalus* (42.8%, 2.3±1.8). *Argulus foliaceus* was recorded for *A. escherichii* among the six host fish species for the first time within the study area from Türkiye. Thus, a new host fish species and a new locality have been added to the geographical infestation zone of the *A. foliaceus* species from Türkiye, which is located in the southeast region of Eurasia. Parasitological findings were evaluated concerning seasonal, age, and gender groups of the host fishes. In conclusion, *A. foliaceus* infestation was observed higher in summer due to the increased in water temperature, which is significantly different from winter, spring, and autumn. The infestation of *A. foliaceus* was more common in larger fish than in smaller fish. Moreover, the similar rates of infestation were recorded between the male and female fish.

**Keywords:** *Argulus*, crustacea, freshwater fish species, Kunduzlar Dam Lake, Türkiye

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## Kunduzlar Baraj Gölü Balıklarında *Argulus foliaceus* (Crustacea) Enfestasyonu Üzerine Bir Araştırma

**Öz:** Bu çalışmada, crustacean parazit türü olan *Argulus foliaceus* Türkiye'de Kunduzlar Baraj Gölü'nden (Kırka-Eskişehir) yedi balık türü (*Alburnus escherichii*, *Barbus plebejus*, *Capoeta tinca*, *Carassius gibelio*, *Chondrostoma nasus*, *Cyprinus carpio*, *Squalius cephalus*) üzerinde araştırılmıştır. Parazit *Chondrostoma nasus* hariç tüm konakçı balık türlerinin solungaçlarında, yüzgeçlerinde ve derisinde bulunmuştur. Enfekte altı balık türü için parazitin enfeksiyon oranı, ve ortalama yoğunluğu şu şekildedir: 38 *Alburnus escherichii*'den 3'ü (enfeksiyon oranı %7.9, ortalama yoğunluk 4.3±3.1), 16 *Barbus plebejus*'dan 9'u (%56.2, 47.9±28.5), 47 *Capoeta tinca*'dan 12'si (%25.5, 27.5±18.9), 17 *Carassius gibelio*'dan 7'si (%41.1, 6.9±5.3), 4 *Cyprinus carpio*'dan 4'ü (%100, 4.3±4.2) ve 14 *Squalius cephalus*'tan 6'sı (%42.8, 2.3±1.8). *Argulus foliaceus*, Türkiye'den çalışma alanında altı konak balık türü arasındaki *A. escherichii*'den ilk kez kaydedilmiştir. Böylece Avrasya'nın güneydoğu bölgesinde yer alan Türkiye'den, *A. foliaceus* türünün coğrafi dağılışına yeni bir konak balık türü ve yeni bir yerleşim yeri eklenmiştir. Parazitolojik bulgular mevsimler ile konak balıkların yaş ve cinsiyet grupları açısından değerlendirilmiştir. Sonuç olarak *A. foliaceus* türü kış, ilkbahar ve sonbahar dönemlerine göre su sıcaklığındaki artış nedeniyle yaz aylarında daha fazla gözlenmiştir. *Argulus foliaceus* enfestasyonu büyük balıklarda küçük olanlara gore daha yaygındı. Ayrıca, erkek ve dişi balık örnekleri arasında benzer oranlarda enfeksiyon kaydedilmiştir.

**Anahtar kelimeler:** *Argulus*, krustacea, tatlısu balık türleri, Kunduz Baraj Gölü, Türkiye

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## Introduction

As a crustacean parasite species, *Argulus foliaceus* is a common invasive ectoparasite species for freshwater fishes. It's a low host specificity that lives on the gills, mouth cavity, skin, and fins of host fishes. The parasite species is causing infestations of host fish species in natural environments and culture fishing ponds. Moreover, it can cause bacterial, fungal, and viral secondary parasitic diseases in relevant tissues and organs and thus can cause economic losses (Walker et al. 2008).

The aim of this present study is to reveal the occurrence of *Argulus foliaceus* in fish species living in the Kunduzlar Lake Dam from Türkiye, and to compare the infection of prevalence and the mean intensity of *A. foliaceus* between fish species living in the same locality. Moreover, the aim is to determine between *A. foliaceus* infestation and the each fish species for size and gender groups, and seasonal changes.

## Materials and Methods

### Study Area

The Kunduzlar Dam Lake Kırka-Eskişehir-Türkiye, is located at 39º20'30" Parallel North and 30º32'25" Meridian East. The minimum water elevation of the Dam Lake is 1011.50 m, and the maximum water elevation is 1027.10 m. The lake area at maximum water level is 4.40 $km^2$ (Özbek and Öztürk 2010).

### Sampling Methods And Laboratory Examination

*Argulus foliaceus* specimens were examined at the skin, fin, gill, and mouth cavity of the fish via with Olympus x30 stereo microscope. Parasites found on these organs were taken to a physiological water environment with the help of a scalpel, were washed with tap water, and their mucus was cleaned. They were placed in warm AFA (Alcohol-Formaldehyde-Acetic acid) fixative, and they were left for 12-24 hours. Then, the dehydration process was applied to parasites with the help of the ethyl alcohol series (35%, 50%, 70%). Following this, some of the parasites were preserved in 70% ethyl alcohol. Another part of them was made into preparation in glycerine gel.

### Statistics

For the species definition of the parasite, Bykhovskaya-Pavlovskaya et al. (1962) were used. Infestation prevalence, parasite intensity, and minimum-maximum numbers of parasites were calculated according to Bush et al. (1997). Parasitological data obtained were analyzed using SPSS 11.5 software package. Accordingly, relations between infection data of the parasite species, size and older groups of host fish, and seasonal changes were evaluated through the chi-square test.

## Results

### *Argulus foliaceus* infestation on the fish species

Within the study, a total number of 175 fish specimens were examined for *A. foliaceus* infestation on the fish species in the Kunduzlar Dam Lake from Türkiye. Seven fish species were examined, and all of the six fish species, *Alburnus escherichii*, *Barbus plebejus*, *Capoeta tinca*, *Carassius gibelio*, *Cyprinus carpio*, and *Squalius cephalus*, were infected with *A. foliaceus*, but it was not found on *Chondrostoma nasus*. *Argulus foliaceus* was recorded on gills, fins and skin of the infected host fish species: 3 out of 38 *A. escherichii* (prevalence 7.9%, mean intensity 4.3±3.1), 9 out of 16 *B. plebejus* (56.2%, 47.9±28.5), 12 out of 47 *C. tinca* (25.5%, 27.5±18.9), 7 out of 17 *C. gibelio* (41.1%, 6.9±5.3), 4 out of 4 *C. carpio* (100%, 4.3±4.2), and 6 out of 14 *S. cephalus* (42.8%, 2.3±1.8). Among these fish species, the highest prevalence of infestation was found in *C. carpio* and the highest parasite intensity was observed in *B. plebejus*. The lowest prevalence of infestation was found in *A. esherihii* (Table 1).

### *Argulus foliaceus* infestation on the fish species with related to seasonal changes

*Argulus foliaceus* infestation on fish species was evaluated over four seasons, i.e., spring, summer, autumn, and winter. Accordingly, *A. foliaceus* infestation was encountered on *A. esherishii* in winter and *B. plebejus* in spring. However, in summer, the infestation was generally at high values, and these values are significantly different from the values in other seasons ($P<0.05$). The highest prevalence of infestation in summer was found in *B. plabejus*, *C. carpio*, and *C. tinca*. The highest mean intensity of parasite was recorded on *B. plebejus* and *C. tinca* (Table 2).

**Table 1.** Occurence of *Argulus foliaceus* infestation of the host fish species from Lake Dam Kunduzlar.

| Infection Parameters | Fish species | | | | | |
|---|---|---|---|---|---|---|
| | *Alburnus escherichii* | *Barbus lebejus* | *Capoeta tinca* | *Carassius gibelio* | *Cyprinus carpio* | *Squalius cephalus* |
| Exm/Inf | 38/ 3 | 16/ 9 | 47/ 12 | 17/ 7 | 4/ 4 | 14/ 6 |
| (%) | (7.9) | (56.2) | (25.5) | (41.1) | (100) | (42.8) |
| M&SD | 4.3±3.1 | 47.9±28.5 | 27.5±18.9 | 6.9±5.3 | 4.3±4.2 | 2.3±1.8 |
| M-MPN | 1-7 | 1-86 | 1-62 | 1-14 | 1-12 | 1-8 |
| TPN | 13 | 431 | 330 | 48 | 17 | 14 |

Exm/Inf: Examined/Infected fish number, (%) Prevalence, M&SD: Mean parasite number and standard deviation, M-MPN: Minimum-Maximum parasite number, TPN: Totalparasite number

**Table 2.** Distribution of *Argulus foliaceus* infestation from Lake Dam Kunduzlar according to the seasons

| Seasons | Infection Parameters | Fish species | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Alburnus escherichii* | *Barbus plebejus* | *Capoeta tinca* | *Carassius gibelio* | *Cyprinus carpio* | *Squalius cephalus* |
| Spring | Exm/Inf | - | 4/ 0 | - | - | - | - |
| | (%) | | (0.0) | | | | |
| | M&SD | | 0±0 | | | | |
| | M-MPN | | 0 | | | | |
| | TPN | | 0 | | | | |
| Summer | Exm/Inf | 8/ 3 | 8/ 8 | 12/11 | 10/ 7 | 4/ 4 | 4/ 3 |
| | (%) | (37.5) | (100) | (91.6) | (70.0) | (100) | (75.0) |
| | M&SD | 4.3±3.1 | 53.7±24.0 | 29.8±18.1 | 6.9±5.3 | 4.3±4.2 | 3.7±3.4 |
| | M-MPN | 1-7 | 19-86 | 1-62 | 1-14 | 1-12 | 1-8 |
| | TPN | 13 | 430 | 328 | 48 | 17 | 11 |
| Autumn | Exm/Inf | 19/ 0 | 4/ 1 | 13/ 1 | 7/ 0 | - | 6/ 3 |
| | (%) | (0.0) | (25.0) | (7.7) | (0.0) | | (50.0) |
| | M&SD | 0±0 | 1.0±0.0 | 2.0±0.0 | 0±0 | | 1.0±0.0 |
| | M-MPN | 0 | 1 | 2 | 0 | | 1 |
| | TPN | 0 | 1 | 2 | 0 | | 3 |
| Winter | Exm/Inf | 11/ 0 | - | - | - | - | - |
| | (%) | (0.0) | | | | | |
| | M&SD | 0±0 | | | | | |
| | M-MPN | 0 | | | | | |
| | TPN | 0 | | | | | |

***Argulus foliaceus* infestation with related to size groups of the fish species**

The distribution of *A. foliaceus* infestation for size groups of fish species is shown in detail in Table 3. The first and second size groups of *B. plebejus* were higher values of both infection prevalence and mean intensity than *C. tinca*. Even though it is statistically insignificant (P>0.05), as a noteworthy difference, the value of infestation vanished within the third size group of *B. plebejus* and *C. tinca*. It was a remarkable finding that *A. foliaceus* infestation was seen only in the largest third-size group of the other four fish species.

**Table 3.** Distribution of *Argulus foliaceus* infestation from Lake Dam Kunduzlar according to the host fish size and groups

| Infection Parameters | Size groups | *Alburnus escherichii* and size | | *Barbus plebejus* and size | | *Capoeta tinca* and size | | *Carassius gibelio* and size | | *Cyprinus carpio* and size | | *Squalius cephalus* and size | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Exm/Inf | I. group | - | - | 15.8-16.2 cm | 4/ 2 | 14.0-15.6 cm | 7/ 1 | - | - | - | - | - | - |
| (%) | | | | | (50.0) | | (14.3) | | | | | | |
| M&SD | | | | | 78.5±10 | | 10±0 | | | | | | |
| M-MPN | | | | | 71-86 | | 10 | | | | | | |
| TPN | | | | | 157 | | 10 | | | | | | |
| Exm/Inf | II. group | - | - | 18.0-23.8 cm | 10/ 7 | 16.2-24.3 cm | 37/ 11 | - | - | - | - | - | - |
| (%) | | | | | (70) | | (29.7) | | | | | | |
| M&SD | | | | | 39.1±25 | | 28.9±19 | | | | | | |
| M-MPN | | | | | 1-74 | | 1-62 | | | | | | |
| TPN | | | | | 274 | | 320 | | | | | | |
| Exm/Inf | III. group | 11.3-14.5 cm | 38/ 3 (7.9) | 33.0-34.5 cm | 2/ 0 | 27.8-31.6 cm | 2/ 0 | 18.0-23.2 cm | 17/ 7 | 20.4-29.5 cm | 4/ 4 | 12.0-15.7 cm | 14/ 6 |
| (%) | | | 4.3±3.1 | | (0) | | (0) | | (41.1) | | (100) | | (42.8) |
| M&SD | | | 1-7 | | 0±0 | | 0±0 | | 6.9±5.3 | | 4.3±4.2 | | 2.3±1.8 |
| M-MPN | | | 13 | | 0 | | 0 | | 1-14 | | 1-12 | | 1-8 |
| TPN | | | | | 0 | | 0 | | 48 | | 17 | | 14 |

***Argulus foliaceus* infestation with related to gender groups of the fish species**

The distribution of *A. foliaceus* infestation concerning gender groups of fish species is shown in Table 4. Both male and female groups of fish species were examined, except for *C. carpio*. Infestation prevalence of the parasite was higher in female than male groups of *B. plebejus*, *C. gibelio*, and *S. cephalus*. On the other hand, mean infestation intensity was higher in male than female groups of the same host fish species; *B. plebejus*, *C. gibelio*, and *S. cephalus*. Moreover in *C. tinca* specimens, both infestation prevalence and mean intensity were higher in male than female fish specimens. However, the difference in infection between the groups of fish is not statistically significant (P>0.05).

**Table 4.** Distribution of *Argulus foliaceus* infestation from Lake Dam Kunduzlar according to host fish gender groups

| Infection Parameters | Gender | Fish species | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Alburnus escherichii* | *Barbus plebejus* | *Capoeta tinca* | *Carassius gibelio* | *Cyprinus carpio* | *Squalius cephalus* |
| Exm/Inf | Male | 20/ 2 | 8/ 4 | 26/ 10 | 8/ 3 | 4/ 4 | 12/ 5 |
| (%) | | (10) | (50.0) | (38.4) | (37.5) | (100) | (41.6) |
| M&SD | | 3.0±2.8 | 57.5±28.8 | 31.8±17.5 | 8.0±5.6 | 4.3±4.2 | 2.6±2.1 |
| M-MPN | | 1-5 | 19-86 | 2-62 | 3-14 | 1-12 | 1-8 |
| TPN | | 6 | 230 | 319 | 24 | 17 | 13 |
| Exm/Inf | Female | 18/ 1 | 8/ 5 | 21/ 2 | 9/ 4 | | 2/ 1 |
| (%) | | (5.5) | (62.5) | (9.5) | (44.4) | | (50.0) |
| M&SD | | 7.0±0.0 | 40.2±28.9 | 5.5±6.3 | 6.0±5.8 | - | 1.0±0 |
| M-MPN | | 7 | 1-74 | 1-10 | 1-12 | | 1 |
| TPN | | 7 | 201 | 11 | 48 | | 1 |

## Discussion

*Argulus foliaceus* is a crustacean ectoparasite species that live in different habitats and is common on various fish species (Lamarre and Cochran 1992; Székely and Molnár 1997; Yıldız and Kumantaş 2002; Öktener 2003; Kır et al. 2004; Tekin-Özan and Kır 2005; Poulin 2007; Walker et al. 2008; Öztürk 2010; Açıkel and Öztürk 2013). It is emphasized that this parasite does not make an evident host preference amongst fish species that are living in the same environment (Lamarre and Cochran 1992; Mikheev et al. 1998; Taylor et al. 2009). In parallel with this view, *A. foliaceus* infestation was found in 6 fish species in the study. This result supports the view that *A. foliaceus* lives in many various fish species as a parasite.

*Argulus foliaceus* mostly prefers to invade fish species such as *Cyprinus carpio*, *Abramis brama*, *Tinca tinca*, *Carassius auratus* and *Scardinius erythrophtalmus* (Pojmanska 1993; Kır et al. 2004; Walker et al. 2008; Pekmezci et al. 2011). The parasite was a higher value of infestation in *C. tinca* and *B. plebejus* species in comparison with other host fish species in this study. This may indicate that the parasite prefers certain fish species. In this respect, an interesting result was that *A. foliaceus* infestation was not found in *Chondrostoma nasus*. Thus, it can be said that *A. foliaceus* may adapt to live in many fish species, yet it prefers physiologically more appropriate fish species (Taylor et al. 2009).

It is a known fact that one of the abiotic factors that are most effective in the prevalence and intensity of *A. foliaceus* infection in fish is water temperature (Harrison 2006). Öztürk et al. (2000) indicate that the infestation prevalence of *A. foliaceus* reaches its highest value in summer. In another study, this parasite was encountered in the summer and autumn (Öztürk 2002). Açıkel and Öztürk (2013) observed *A. foliaceus* on *Squalius cephalus* only in the summer months. Similar results were recorded in this study as well. The parasite infestation generally reached high values in the host fish specimens examined in summer, and there was a significant difference between the values of the summer and other seasons. These results are in parallel with the view that *A. foliaceus* is a species that is prone to hot weathers (Molnar and Szekely 1995; Özer and Erdem 1999; Yıldız and Kumantaş 2002; Kır et al. 2004; Tekin-Özan and Kır 2005; Açıkel and Öztürk 2013).

Grutter (1994) indicates a relationship between *A. foliaceus* infestation and the size or age groups of fish. According to Poulin (2007), the prevalence and intensity of *A. foliaceus* infection change in direct proportion to the size of the host fish. In another study, it is stated that larger fish have more parasites as they are exposed to longer periods of the parasite (Lamarre and Cochran 1992). In parallel with these data, Walker et al. (2008) did not encounter any *A. foliaceus* infestation in young host fish specimens. Öztürk (2002) indicates that the prevalence and intensity of *A. foliaceus* infestation found in second and third-size groups of fish are more dominant than in the first group. The data in the present study support the data obtained by the above-mentioned researchers, and the value of infestation of *A. foliaceus* was more common in larger host fish than in smaller ones.

It is a known fact that there is not an evident interaction between gender groups of fish and *A. foliaceus* infection (Açıkel and Öztürk 2013; Öztürk 2002). In the present study, the value of infestation of *A. foliaceus* was not significantly different between male and female older groups of the host fish.

It is a known fact that *A. foliaceus*, which is nourished by the mucus and blood of its host, is effective, especially on gills and skin (Székely and Molnár 1997). It is also known that the parasite species causes a toxic effect on the parts, and through the wounds emanated, it causes secondary bacterial and fungal infections (Walker et al. 2008). Accordingly, the present research field is a natural environment, and the parasitic occurrence was found at a low intensity, the above-mentioned pathological symptoms were not encountered in the host fish. However, in the case of preference of Lake Dam Kunduzlar, which is an appropriate ecological environment for cyprinid species, to become a culture fish area in the future, then one should keep in mind that *A. foliaceus* is a potential pathogenic parasitic species for the host fish, especially in the summer period.

In conclusion, the infestation of *A. foliaceus* on the six fish species from Kunduzlar Dam Lake were evaluated for the first time, related to some environmental factors, such as seasonal change, size, and gender groups of the fishes. Moreover, *A. foliaceus* is a new parasite record for *A. escherichii* from Türkiye. So, *A. escherichii* is a new host record for *A. foliaceus* from Türkiye. Thus, a new host fish species and a new locality has been added to the geographical spread zone of the parasite species from Asia Minor, Türkiye, which is located in the southeast region of Eurasia.

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# Sentetik Apo-ester ve Kadife Çiçeğinin (*Tagetes erecta*) Sarı Prenses Balığının (*Labidochromis caeruleus*) Pigmentasyonunda Karotenoyit Kaynağı Olarak Kullanılması

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## Ö Z

Bu çalışma, %4 kadife çiçeği (*Tagetes erecta*), 0 ve 100 mg/kg sentetik apo-ester içeren diyetlerle 60 gün beslenen sarı prenses balığının (*Labidochromis caeruleus*) deri rengi ve yetiştiricilik parametreleri üzerindeki etkilerini belirlemek amacıyla gerçekleştirilmiştir. Deri rengi, kolorimetrik yöntemle bir yansıtma spektroskopisi kullanılarak ölçülmüştür. Kadife çiçeği ve sentetik apo-esterin balıkların renk parametreleri (L*,a*,b*,Ch, $H°_{ab}$) üzerindeki etkilerinin benzer olduğu belirlenmiştir ($p>0,05$). Her iki karotenoyit kaynağı, karotenoyit içermeyen diyete kıyasla balıklarda daha yüksek bir sarılık (+b*) değeri sağlamış ($p<0,05$), ancak açıklık (+L*) ve kırmızılık (+a*) değerlerini etkilememiştir ($p>0,05$). Diğer yandan, karotenoyit kaynaklarının balıkların büyüme performansı, yem değerlendirme ve hayatta kalma oranı üzerinde bir etkisi olmamıştır ($p>0,05$). Bu çalışma, kadife çiçeğinin sarı prenses balığı diyetlerinde sentetik apo estere alternatif bir doğal karotenoyit kaynağı olarak başarılı bir şekilde kullanılabileceğini göstermiştir.

**Anahtar kelimeler:** *Labidochromis caeruleus,* kadife çiçeği, apo-ester, pigmentasyon, karotenoyit

## M A K A L E  B İ L G İ S İ

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## The Use of Apo-ester and Marigold (*Tagetes erecta*) as Carotenoid Source in Pigmentation of Electric Yellow Cichlid (*Labidochromis caeruleus*)

**Abstract:** This study was carried out to determine the effects on skin pigmentation and rearing parameters of electric yellow cichlid (*Labidochromis caeruleus*) fed diets containing 4% marigold (*Tagetes erecta*), 0 and 100 mg/kg synthetic apo-ester for 60 days. Skin colour was measured by colorimetric method using reflectance spectroscopy. Effects of marigold and synthetic apo-ester on colour parameters (L*,a*,b*,Ch, $H°_{ab}$) of fish were similar ($P>0.05$). Both carotenoid sources provided higher a yellowness (+b*) value in fish ($P<0.05$) but did not affect lightness (+L*) and redness (+a*) value, compared to diets without carotenoids ($P>0.05$). On the other hand, carotenoid sources had no effect on growth performance, feed utilization and survival rate of fish ($P<0.05$). This study demonstrated that marigold can be successfully used as an alternative natural carotenoid source to the synthetic apo-ester in electric yellow cichlid diets.

**Keywords:** *Labidochromis caeruleus,* marigold, apo-ester, pigmentation, carotenoid

**Alıntılama**

Yanar M, Terzi D, Evliyaoğlu E. 2022. Sentetik Apo-ester ve Kadife Çiçeğinin (*Tagetes erecta*) Sarı Prenses Balığının (*Labidochromis caeruleus*) Pigmentasyonunda Karotenoyit Kaynağı Olarak Kullanılması LimnoFish. 8(3): 288-296. doi: 10.17216/LimnoFish. 1018012

## Giriş

Dünyada olduğu gibi ülkemizde de akvaryum balıklarına olan ilgi ve talep giderek artmakta ve bu gelişmeye paralel olarak akvaryum balıkçılığı sektörü büyümektedir. Sarı prenses balığı (*Labidochromis caeruleus,* Cichlidae) sürümü fazla olan popüler bir akvaryum balığı türüdür. Karakteristik rengi sarı olup, pazar fiyatının oluşmasında önemlidir. Balıklarda sarıdan kırmızıya kadar değişen renkleri sağlayan karotenoyit grubu pigmentler olup, diğer hayvanlar gibi balıklar da bu maddeleri sentezleyemezler. Ancak bunları yedikleri bitkilerden ve/veya dolaylı olarak hayvanlardan sağlarlar (Goodwin 1984; Torrissen vd. 1989). Ötrofik havuzlarda balıklar, karotenoyitce zengin alg ve zooplaktonik organizmalardan beslendikleri için renkleri parlak ve albenili olmaktadır (Hata ve Hata 1973). Entansif balık yetiştiriciliğinde bu doğal yem kaynaklarından yararlanılamadığı ve dolayısıyla yeterli düzeyde renk kalitesi oluşmadığı için, balıkların diyetlerine doğal veya sentetik karotenoyit kaynaklarının ilave edilmesi bir gereklilik olarak ortaya çıkmaktadır.

Kadife çiçeği (*Tagetes erecta*), yurdumuzda ilkbahar başlangıcından sonbahar sonuna kadar çiçek açan, park ve bahçelerde bol bulunan, sarı ve turuncu renklerde olan bir peyzaj bitkisidir. Oldukça yoğun miktarda karotenoyit (1320-5700 mg/kg) içerir (Piccaglia vd. 1998). İçerdikleri karotenoyitlerin önemli kısmını ksantofiller oluşturur. Bu ksantofillerin %80'ine yakını lüteindir (Sivel vd. 2014). Önceleri kanatlı hayvanların yumurtalarının renklenmesinde kullanılan kadife çiçeğinin (Santos-Bocanegra vd. 2004), daha sonra Japon balığı (Şeker 2004), yeşil kaplan karidesi (Göçer vd. 2006) ve gökkuşağı alabalığında (Büyükçapar vd. 2007) kullanılabileceği gösterilmiştir. Sentetik bir karotenoyit olan apo-ester (β-apo-8′karotenoik asit etil ester) ise endüstride kanatlı hayvanların yumurtasının renklendirilmesinde yaygın olarak kullanılmaktadır. Apo-esterin Japon balığı dışında (Yanar vd. 2008), başka bir balık türünde kullanıldığına rastlanmamıştır.

Karotenoyitlerin renklenme sağlamasının yanı sıra, bazı balık türlerinde yaşama oranını ve büyüme performansını artırdığı ileri sürülmüştür (Torrissen 1984a; Storebakken vd. 1987; Choubert ve Storebakken 1989; Boonyaratpalin ve Unprasert 1989; Goswami 1993; Liu vd. 2019). Bunun olası nedenleri; karotenoyitlerin A vitamini sentezinin öncüsü olması, A vitamini gibi iş görmesi (Schiedt vd. 1985; Guillou vd. 1992; Christiasen vd. 1994; White vd. 2003), güçlü bir antioksidan olması (Bjerkeng ve Johnsen 1995; Shimidzu vd. 1996; Nakano vd. 1999; Bell vd. 2000) ve bağışıklık sistemini güçlendirmesi (Nakano vd. 1995; Amar vd. 2004) ile ilişkilendirilmiştir. Ancak karotenoyitlerin balıkların büyüme ve yaşama oranı üzerindeki etkileri henüz tartışmalı olup, sarı prenses balığı özelindeki etkileri, bir çalışmanın dışında (Yeşilayer vd. 2020) bilinmemektedir.

Bu çalışmada, doğal bir karotenoyit kaynağı olan kadife çiçeğinin sarı prenses balığının renklenmesi üzerindeki etkileri, sentetik bir karotenoyit olan "apo-ester" ile karşılaştırılmalı olarak test edilmiştir. Ayrıca bu karotenoyit kaynaklarının balığın büyüme performansı, yem değerlendirme ve yaşama oranı üzerindeki etkileri de belirlenmiştir.

## Materyal ve Metot

Yerel akvaryumculardan sağlanan sarı prenses balıkları (*Labidochromis aeruleus*) 3 hafta karantinada tutulduktan sonra 2 hafta deney koşullarına uyumları sağlanmıştır. Bu süreçte balıklar bazal diyetle beslenmişlerdir. Balıklar 110 L hacmindeki akvaryumlara 15 adet stoklanmıştır. 3 diyet grubu hazırlanmış ve deneme 3 tekerrürlü olarak yürütülmüştür. Deneme süresi 60 gün tutulmuş ve bu süre içinde balıklar serbest yemleme yöntemiyle 3 öğün beslenmişlerdir. Balıkların renk, boy ve ağırlık ölçümleri 20 gün aralıklarla yapılmıştır. Ölçümler esnasında balıklar 0,25 mL/L 2-fenoksietanol ile anestezi edilmiştir. Akvaryumlar merkezi bir hava motoruyla sürekli havalandırmıştır. Akvaryumların suları günde %70-80 oranında değiştirilmiş, yem artıkları ve dışkılar sifonlanarak ortamdan uzaklaştırmıştır. Suların amonyum ($NH_4^+$-N), nitrit ($NO_2^-$-N) ve nitrat ($NO_3^-$-N) içerikleri haftada bir defa spektrofotometrik (Spectroguant® NOVA 60 Merck) olarak, sertlik ($CaCO_3$) ise titrimetrik (APHA, 1998) olarak ölçülmüştür. Akvaryumlara 12 saat aydınlık 12 saat karanlık periyodu uygulanmıştır. Sıcaklık, oksijen (Oxyguard Handy Polaris) ve pH (Testo 206) ile her gün ölçülmüştür. Yetiştiricilik süresince su sıcaklığı 25-26 °C, pH 7,7-7,8; oksijen 6,5-7,5; amonyum <0,075; nitrit <0.042; nitrat <20 ve sertlik ($CaCO_3$ olarak) 285 mg/L olarak belirlenmiştir.

Balıklar için eşit protein ve eşit enerji içeriğine sahip 3 farklı diyet hazırlanmıştır:

1) Bazal diyet veya kontrol grubu (karotenoyit katkısı olmayan yem)
2) %4 kadife çiçeği içeren diyet (100 mg total karotenoyit/kg yem)
3) %1 Carophyll Yellow içeren diyet (100 mg sentetik apo-ester/kg yem).

Yem bileşenleri önce değirmende öğütüldü, sonra içine su ilave edilerek hamur kıvamına getirildi. %10 apo-ester (β-apo-8′karotenoik asit etil ester) içeren ve ticari ismi Carophyll Yellow (F. Hoffman La Roche & Co., Ltd., Basle, İsviçre) olan sentetik renklendirici, 60°C suda çözüldükten sonra yemin içine ilave edildi. Karanfil çiçeği ise fırında 50°C sıcaklıkta 2-3 gün kurutulup un haline getirildikten sonra yeme ilave edildi. Hamur haline getirilen deney yemleri karıştırılıp homojenize edildikten sonra 1,5 mm göz açıklığına sahip yem makinasından geçirilerek spagetti formuna getirildi ve 1,5 mm uzunluklarda kesilerek pelet formuna dönüştürüldü. Peletler daha sonra plastik poşetlere konarak kullanılana kadar -20°C'de saklandı. Diyetlerin formülasyonu ve besin kompozisyonları Tablo 1'de ayrıntılı olarak gösterilmiştir.

Kadife çiçeğinin total karotenoyit analizi spektrofometrik olarak belirlenmiştir. Total karotenoyit analizinde Torrissen ve Naevdal (1984)'ın yöntemi takip edilmiş ve çözücü olarak aseton kullanılmıştır. Çözeltinin ekstinşın katsayısı (extinction coefficient, $E_{1\%,\ 1cm}$) 450 nm'de 2500 alınmıştır (Schidet ve Liaaen-Jensen 1995). Kadife çiçeğinin total karotenoyit içeriği kuru maddede 2500 mg/kg olarak belirlemiş ve diyetlere katılmasında bu miktar esas alınmıştır. Balıkların deri rengi, kolorimetrik yöntemle bir yansıtma spektroskopisi kullanılarak (HunterLab 2,3), L* (+açıklık; -koyuluk), a* (+kırmızılık; -yeşilik) ve b* (+ sarılık; -mavilik) tristimulus değerleri baz alınıp, bunların renk parametrelerine dönüştürülmesi ile ölçülmüştür.

Diğer yandan, rengin yoğunluğu ve açıklığını/berraklığını ifade eden Chroma (Ch) ile kırmızı ve sarı renkler arasındaki ilişkiyi ifade eden H°$_{ab}$ değeri, a* ve b* değerlerinden yararlanılarak hesaplanmıştır. Ch, $(a^{*2} + b^{*2})^{1/2}$ denkleminden; H°$_{ab}$ a*>0 olması durumunda; $\tan^{-1}$ (b*/a*), 0<a olması durumunda ise 180+ $\tan^{-1}$ (b*/a*) denkleminden hesaplanmıştır (Hunt, 1977). Renk ölçümlerinde balıkların kuyruk kaide kısmından baş bölgesine kadarki bölgede sırta yakın her iki yanal taraf kullanılmıştır.

**Tablo 1.** Diyetlerin formülasyonu ve kimyasal kompozisyonları
**Table 1.** Formulation and chemical composition of diets

| | Diyet grupları | | |
|---|---|---|---|
| **Besin bileşenleri** | **Bazal diyet** | **%4 Kadife çiçeği** | **100 mg/kg Apo-ester** |
| **Balık unu** | 350 | 349 | 350 |
| **Soya unu** | 350 | 355 | 350 |
| **Mısır unu** | 224 | 180 | 223 |
| **Ay çiçeği yağı** | 20 | 20 | 20 |
| **Balık yağı** | 20 | 20 | 20 |
| **di-kalsiyum fosfat (DCP)** | 5 | 5 | 5 |
| **Limostane (kalker)** | 5 | 5 | 5 |
| **Kavilamicine[R,a]** | 2 | 2 | 2 |
| **Tuz** | 5 | 5 | 5 |
| **Metiyonin** | 2 | 2 | 2 |
| **Lisin** | 2 | 2 | 2 |
| **Vitamin+minaral premix[b]** | 15 | 15 | 15 |
| **Kadife çiçeği** | 0 | 40 | 0 |
| **CARPHYLL Yellov[R] %10** | 0 | 0 | 1 |
| **Total** | 1000 | 1000 | 1000 |
| **Kimyasal kompozisyon** | | | |
| **Kuru madde (%)** | 82,46 | 82,32 | 82,46 |
| **Ham protein (%)** | 42,02 | 42,04 | 42,02 |
| **Ham yağ (%)** | 9,10 | 9,06 | 9,10 |
| **Ham selüloz (%)** | 4,067 | 3,984 | 4,067 |
| **Gross enerji (kJ/g DW)** | 18,74 | 18,71 | 18,74 |
| **Total karotenoyit (mg/kg)** | <1 | 100 | 100 |

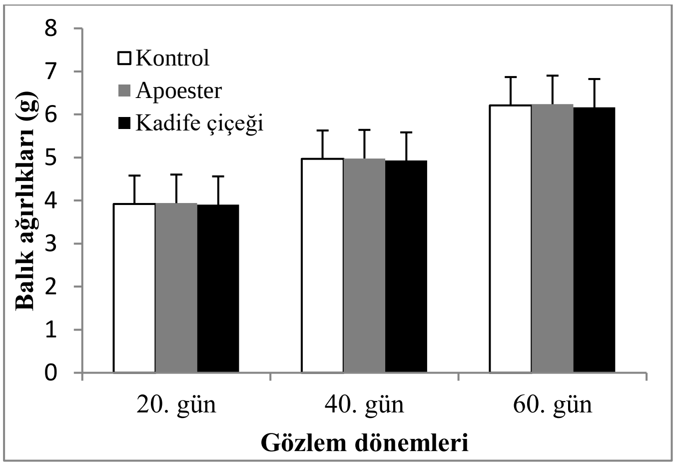
[a]: 10 g/kg avilamycin içerir.
[b]: 16,000 IU retinol, 1500 IU vit D3, 50 IU α-tocoferol, 70 mg ascorbic acid, 10 mg thiamin, 15mg riboflavin, 10 mg pantotenoic acid, 5 mg pyridoxine, 0.02 mg $B_{12}$, 7 mg K, 100 mg niacin, 0,25 mg biotin, 2 mg folic acid, 100 mg inositol, 1mg choline, 30 mg Zn, 25 mg Mn, 25 mg Mg, 2 mg Fe, 0,7 mg I, 1 mg Cu ve 0.2 mg Co içerir.
Diyetlerin kimyasal bileşenleri hesaplama yoluyla elde edilmiştir.

Veriler tek yönlü varyans analizi (one-way ANOVA) ile test edilmiş ve gruplar arasında fark var ise, bunlar Duncan çoklu karşılaştırma testinde %5 önem seviyesine göre değerlendirilmiştir. Söz konusu istatistiksel analizler SPSS paket programında (versiyon 20,0) yapılmıştır. Veriler ortalama±standart hata olarak ifade edilmiştir. Bu çalışma, bir yüksek lisans tez projesi olup, Çukurova Üniversitesi Hayvan Deneyleri Yerel Etik Kurulunun 12.11.2020 tarihli 8 nolu toplantısında araştırma etiği yönünden değerlendirilerek onaylanmış ve deneyler Avrupa konseyinin deneylerde veya diğer bilimsel amaçlarda kullanılan hayvanların korunmasına yönelik 86/609/EEC sayılı kararına göre gerçekleştirilmiştir.

## Bulgular

### Yetiştiricilik Parametreleri

Balıklar, kadife çiçeği ve apo-ester katkılı diyetleri kontrol grubuyla benzer şekilde iştahla tüketmişlerdir. Başlangıç ağırlığı 3,04 g olan diyet grupları 60 gün süren yetiştiricilik periyodu sonunda 6,17-6,24 g ağırlığa ulaşmışlardır (Şekil 1). Deneme sonunda balıkların canlı ağırlık kazancı %102-105, spesifik büyüme oranı 1,15-1,16, yem çevrim oranı 1,53-1,55 ve yaşama oranı %98-99 arasında gerçekleşmiş olup, bu parametreler bakımından diyet grupları arasında önemli düzeyde bir fark çıkmamıştır (p>0,05) (Tablo 2).




**Şekil 1.** Farklı diyetlerle beslenen sarı prenses balıklarının gözlem dönemlerindeki büyüme performansı

**Fig 1.** Growth performance of electric yellow cichlid fed different diets in observation periods

**Tablo 2.** Farklı diyetlerle beslenen sarı prenses balığının yetiştiricilik parametreleri
**Table 2.** Rearing parameters of electric yellow cichlid fed different diets

| | **Deneme grupları** | | |
|---|---|---|---|
| **Yetiştiricilik parametreleri** | **Kontrol** | **%4 kadife çiçeği** | **100 mg/kg apo-ester** |
| **Başlangıç ağırlık (g)** | 3,04 ± 0,06 | 3,04 ± 0,06 | 3,04 ± 0,06 |
| **Son ağırlık (g)** | 6,21 ± 0,33 | 6,17 ± 0,30 | 6,24 ± 0,21 |
| **Canlı ağırlık kazancı (%)** | 104,05±11,64 | 102,80 ± 10,49 | 105,04 ± 10,09 |
| **Spesifik büyüme** | 1,15 ± 0,09 | 1,15 ± 0,09 | 1,16 ± 0,08 |
| **Yem çevrim oranı** | 1,53 ± 0,18 | 1,55 ± 0,20 | 1,53 ± 0,21 |
| **Yaşama oranı (%)** | 99,26 ± 0,74 | 99,26 ± 0,74 | 98,52 ± 0,74 |

## Renklenme

Balıkların deneme başında 60,04 olan deri parlaklığı değeri (L), deneme sonunda gruplar arasında 55,94-58,02¸ deneme başında 3,41 olan kırmızı renk değeri (a) ise deneme sonunda gruplar arasında 3,14-3,15 arasında gerçekleşmiş olup, diyete ilave kadife çiçeği veya sentetik apo-esterin balıkların parlaklık ve kırmız renk değeri üzerinde bir etkisi olmamıştır (p>0,05). Diğer yandan, deneme başında 26,13 olan sarı renk değeri (b), deneme sonunda kontrol grubunda 25,47 iken, kadife çiçeği ve apo-ester katkılı diyetlerle beslenen gruplarda sırasıyla 35,91 ve 37,67 olarak gerçekleşmiş olup, her iki karotenoyit katkılı diyet grubu, kontrol grubundan daha yoğun bir sarı renklenme göstermiştir (p<0,05). Ancak her iki karotenoyit kaynağının sarı renklenme üzerindeki etkileri benzer bulunmuştur (p>0,05) (Tablo 3). Diğer yandan, karotenoyit kaynaklarının balıkların $H°_{ab}$ ve Ch değerleri üzerindeki etkileri, b* değeri üzerindeki etkilerine benzer bir etki göstermiştir. Deneme başında 82,43 olan $H°_{ab}$ değeri, deneme sonunda gruplar arasında 82,52-85,12 olarak gerçekleşmiş olup, apoesterdeki bu değer kontrol grubundan daha yüksek çıkmıştır (P<0.05). Deneme başında 26,37 olan Ch değeri, deneme sonunda gruplar arasında 25,79-37,82 olarak gerçekleşmiş olup, her iki karotenoyit katkılı diyet grubundaki bu değerler, kontrol grubundan daha yüksek çıkmıştır (p<0,05), Ancak her iki karotenoyit kaynağının Ch değerleri üzerindeki etkileri benzer bulunmuştur (p>0,05)

**Tablo 3.** Farklı diyetlerle beslenen sarı prenses balıklarının gözlem dönemlerinde deri renk değerleri
**Table 3.** Skin colour values of electric yellow cichlid fed different diets in observation periods

| | Balıkların gözlem dönemlerinde deri renk değerleri | | | | |
|---|---|---|---|---|---|
| **Deneme grupları** | **L** | **a** | **b** | **H°$_{ab}$** | **Ch** |
| **0. gün** | | | | | |
| **Kontrol** | 60,04 ± 1,88 | 3,41 ± 0,30 | 26,13 ± 1,33 | 82,43±0,67 | 26,37±1,33 |
| **Kadife çiçeği** | 60,04 ± 1,88 | 3,41 ± 0,30 | 26,13 ± 1,33 | 82,43±0,67 | 26,37±1,33 |
| **Apoester** | 60,04 ± 1,88 | 3,41 ± 0,30 | 26,13 ± 1,33 | 82,43±0,67 | 26,37±1,33 |
| **20. gün** | | | | | |
| **Kontrol** | 60,11 ± 2,15 | 3,42 ± 0,33 | 26,37 ± 1,69$^{b}$ | 82,24±0,86 | 26,63±1,68$^{b}$ |
| **Kadife çiçeği** | 57,96 ± 2,03 | 3,39 ± 0,33 | 30,28 ± 1,80$^{ab}$ | 83,40±0,63 | 30,49±1,80$^{ab}$ |
| **Apoester** | 59,45 ± 2,09 | 3,38 ± 0,30 | 32,43 ± 1,85$^{a}$ | 83,41±0,94 | 32,66±1,81$^{a}$ |
| **40. gün** | | | | | |
| **Kontrol** | 58,14 ± 2,48 | 3,44 ± 0,35 | 25,81 ± 2,04$^{b}$ | 81,85±1,08$^{b}$ | 26,09±2,02$^{b}$ |
| **Kadife çiçeği** | 57,81 ± 2,13 | 3,35 ± 0,35 | 33,88 ± 2,34$^{a}$ | 83,82±0,80$^{ab}$ | 34,09±2,32$^{a}$ |
| **Apoester** | 57,63 ± 2,09 | 3,33 ± 0,31 | 36,52 ± 2,18$^{a}$ | 84,60±0,53$^{a}$ | 36,70±2,18$^{a}$ |
| **60. gün** | | | | | |
| **Kontrol** | 58,02 ± 2,67 | 3,35 ± 0,41 | 25,47 ± 2,30$^{b}$ | 82,52±1,05$^{b}$ | 25,79±2,26$^{b}$ |
| **Kadife çiçeği** | 56,57 ± 2,73 | 3,18 ± 0,41 | 35,91 ± 2,65$^{a}$ | 84,64±0,75$^{ab}$ | 36,09±2,65$^{a}$ |
| **Apo-ester** | 55,94 ± 2,91 | 3,14 ± 0,36 | 37,67 ± 2,23$^{a}$ | 85,12±0,54$^{a}$ | 37,82±2,23$^{a}$ |

Değerler ortalama ± standart hata olarak belirtilmiştir. Her sütunda farklı harflerle gösterilen ortalamalar istatistiki olarak farklıdır ($p<0,05$).

## Tartışma ve Sonuç

Çalışmada yeme kadife çiçeği veya apo-ester ilave edilmesi, sarı prenses balığının büyüme performansı, yem değerlendirme ve yaşama oranı üzerinde bir etkisi olmamıştır ($p>0,05$). Ancak bu konu tartışmalı olup, karotenoyitlerin balıkların yetiştiricilik parametreleri üzerindeki etkilerine yönelik farklı sonuçlar ileri sürülmüştür. Sentetik karotenoyitlerin Atlantik salmonu (Torrissen 1984; Christiansen vd. 1994; Christiansen ve Torrissen 1996), kırmızı tilapia (*Oreochromis niloticus*) (Boonyaratpalin ve Unprasert 1989), sarı kedi balığı (*Pelteobagrus fulvidraco*) (Liu ve ark. 2019), kuruma karidesi (*Penaeus japonicus*) (Chien ve Jeng 1992) ve hint sazanında (*Catla catla*) (Goswami 1993) büyüme ve yaşama oranını artırdığı ileri sürülmüştür. Buna karşın, sentetik karotenoyitlerin *Penaeus japonicus* (Negre-Sandargues vd. 1993) ve sarı levrekte (*Perce flavescens*) (Abd El-Gawad vd. 2019), daha yüksek bir yaşama oranı sağlamasına rağmen, büyüme performansı üzerinde bir etkisinin olmadığı bildirilmiştir. Karotenoyitlerin balıkların yetiştiricilik parametreleri üzerindeki etkileri kadife çiçeği ve apo-ester özelinde tartışılırsa; çalışmamızdaki bulgular, literatürlerdeki sonuçlarla paralellik göstermektedir: Alma vd. (2013), Japon balığı diyetine, kadife çiçeği katkısının balığın yaşama oranı, büyüme performansı ve yem değerlendirmeye bir etkisinin olmadığını ileri sürmüştür. Jagadeesh vd. (2014) ise bir akvaryum balığı olan *Etroplus maculatus* (Cichlidae) diyetinde kadife çiçeği katkısının balığın ağırlık kazancını olumlu etkilerken, diğer yetiştiricilik parametrelerini etkilemediğini belirtmiştir. Yine aynı araştırmacılar başka bir çalışmada (Jagadeesh vd. 2015), bir akvaryum balığı olan konkinyus (*Pethia conchonius*) diyetine ilave edilen kadife çiçeğinin balıkların yaşama oranını olumlu etkilediği, ancak diğer yetiştiricilik parametreleri üzerinde bir etki yapmadığını rapor etmiştir. Yeşilayer vd. (2020) ise, çalışmamızda olduğu gibi, sarı prenses yavrusu diyetlerine kadife çiçeği ekstratı katkısının balığın yetiştiricilik parametreleri üzerine bir etkisinin olmadığını bildirmiştir. Diğer yandan, sentetik apo-ester, Japon balığı diyetinde kullanıldığında, çalışmamızdaki, sonuçlara benzer şekilde, yetiştiricilik parametreleri üzerinde bir etki göstermemiştir (Yanar vd. 2008).

Yeme katılan kadife çiçeği ve sentetik apo-ester, sarı prenses balığının derisindeki renk parametreleri (L*,a*,b*, Ch ve H°$_{ab}$) üzerinde benzer etkiler göstermiş olup, her iki karotenoyit kaynağı da kontrol grubuyla karşılaştırılırsa, balığın karakteristik sarı renk yoğunluğunu önemli düzeyde arttırmış, ancak bu kaynakların kırmız renk ve parlaklık üzerinde bir etkisi olmamıştır. Çalışmamıza benzer bir araştırmanın kurgulandığı kanatlılarda, sentetik apo-esterin yumurta sarısının renklendirilmesinde kadife çiçeğine göre daha etkili olduğu rapor edilmiştir (Santos-Bocanegra vd. 2004). Bu farklılığın nedeni apo-esterin stabilizasyonunun daha yüksek olmasına bağlanmıştır (Pasarin ve Rovinaru 2018). Japon balığı üzerinde yapılan benzer bir çalışmada ise, apo-esterin doğal karotenoyit kaynağı olarak kullanılan yoncaya göre daha iyi bir renklenme sağladığı bildirilmiştir (Yanar vd. 2008). Ancak, çalışmamızda apo-ester, kadife çiçeğine karşı sayısal olarak daha yüksek bir renklenme sağlasa da, istatistiksel açıdan bu fark önemsiz bulunmuştur. Kadife çiçeği çalışmamızdaki balık türünden farklı olarak, tilapia (Boonyaratpalin ve Unprasert (1989), Japon balığı (Şeker 2004; Alma vd. 2013), yeşil kaplan karidesi (Göçer vd. 2006), gökkuşağı alabalığı (Büyükçapar vd. 2007), *Barilius bendelisis* (Jha vd. 2012), *Etroplus maculatus* (Jagadeesh vd. 2014) ve *Pethia conchonius (*Jagadeesh vd. 2015) diyetlerinde de kullanılmış ve etkili bir pigmentasyon sağlanmıştır. Diğer yandan, sarı prenses balığının diyetinde alg türlerinden *Sargassum baveanum*, *Gracilaria persica* ve *Entromorpha intestinali* (Pezeshk vd. 2019), hayvansal canlı yemlerden *Chirinomus* sp., *Culex* sp. *Artemia* sp. (Maleknejad vd. 2014) ve kırmızı biber (Yılmaz ve Ergün 2011) kullanılarak istenen düzeyde pigmentasyon sağlanmış olup, Lab değerleri sırasıyla 59,25-73,72; -4,41-9,55 ve 29,12-60,92 aralığında bulunmuştur. Çalışmamızda 60 günlük deneme sonunda kadife çiçeğinin balık derisinde tespit edilen L*, a*, b*, Ch ve $H°_{ab}$ değerleri (sırasıyla 56,57; 3,18; 35.91; 84,64 ve 36,09), Yeşilayer vd. (2020)'nın 0,5 gr ağırlığındaki yavru sarı prenseslerde aynı sürede ve aynı karatenoyit kaynağının kullanıldığı çalışmada elde edilen sonuçlara (sırasıyla 57,19; -2,91; 23,75; 23,97 ve 96,93) nispeten yakın bulunmuştur.

Tablo 3'de kadife çiçeği ve apo ester katkılı diyetlerle beslenen balıklarda sarı renklenmenin 20. ve 40. gözlem dönemlerinde kontrol grubuna göre yoğun bir artış gösterdiği, ancak 60. gözlem döneminde bu artış hızının göreceli olarak azaldığı dikkat çekicidir. Bu trend, özellikle apo-ester ile beslenen grupta daha belirgindir. Bunun nedeninin, karotenoyitlerin dokularda yapacağı birikimin sınırlı olduğu, belli bir miktardan sonra doyum noktasına ulaştığı ve bundan sonra dışardan bir karotenoyit girişi olsa da, dokularda artık daha fazla birikemeyeceğinden kaynaklandığı düşünülmektedir. Dolayısıyla balıklarda renklenmenin sağlanması için araştırmalar sonucunda oluşturulan protokollerde önerilen optimal karotenoyit miktarı ve uygulama süresinin aşılması, daha fazla bir renklenme sağlamayacak, aksine yem maliyet artışına neden olacaktır. Dolayısıyla sarı prenses balıklarının karakteristik sarı renklenmesinin sağlanması için 100 mg/kg apo-ester veya %4 kadife içeren diyetlerle 60 gün beslenmeleri yeterli gözükmektedir. Karotenoyit kaynağının maliyeti ve sentetik ürünlere duyulan kaygılar söz konusu olduğu durumlarda ise, kadife çiçeği, sentetik apo-estere alternatif bir doğal karotenoyit kaynağı olarak başarıyla kullanılabilir.

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# Recent Advances in Packaging Technology of Seafood Products

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## A B S T R A C T

Packaging is developing about the development of consumer demands and awareness day by day**.** Packaging technology is a technology that is constantly evolving. Monitoring seafood products at all stages from the moment they are caught to the moment they are consumed is extremely important for the quality of seafood products and prevention of diseases caused by seafood. Therefore, by using of improved packaging techniques, not only can be prevented the contamination of fishery products with microorganisms, but also the quality and suitability of fishery products for consumption can also be monitored at every stage. In this review, conducted studies regarding advanced packaging materials and products for seafood products have been mentioned and studies on recent advanced packaging applications in fresh and processed fishery products in recent years were compiled.

**Keywords:** recent advances, packaging technology, seafood products, fishery products

## A R T I C L E I N F O

**REVIEW**

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**Su Ürünleri Ambalaj Teknolojisindeki Son Gelişmeler**

**Öz:** Ambalaj teknolojisinin gelişimi ile ilgili tüketici talepleri ve farkındalığı her geçen gün artmaktadır. Paketleme teknolojisi sürekli gelişen bir teknolojidir. Su ürünlerinin yakalandıkları andan tüketiciye ulaşana kadar her aşamada izlenmesi hem ürünlerin kalitesi açısından hem de su ürünleri kaynaklı hastalıkların önlenmesi açısından son derece önemlidir. Bu nedenle, geliştirilmiş paketleme teknikleri kullanılarak, sadece su ürünlerinin mikroorganizmalarla kontaminasyonu önlenmekle kalmaz, aynı zamanda su ürünlerinin tüketime uygunluğu ve kalitesi de her aşamada izlenebilir. Bu amaçla yapılan bu derleme çalışmasında, gıda ürünleri için ileri ambalaj malzemeleri ve ürünlerinden bahsedilmiş ve son yıllarda taze ve işlenmiş su ürünlerinde ileri ambalaj uygulamalarına ilişkin çalışmalar derlenmiştir.

**Anahtar kelimeler:** son gelişmeler, ambalaj teknolojisi, su ürünleri, balıkçılık ürünleri

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## Introduction

Nowadays, food production and consumption activities improve many packaging requirements and the development of new forms of packaging (Bose et al. 2021). Advanced food packaging technologies not only ensure the safety of foods from microbial pathogens and pollutants, but also extend the shelf-life of the food products (Rangaraj et al. 2021). The improvement of synthetic packaging materials has many advantages that it leads to more attractive and safe packaging of poultry, meat and seafood products (Torosaurus 2012). However, food packagings produced from petroleum-based plastics have a hazardous effects on the environment and also human health. For this reason, packaging from biodegradable polymers is needed to be improved for the food packaging industry (Florez et al. 2022). Conventionally, food packaging is used not only for developing food quality, but also for supplying consumers with annotations of the characteristics of food products. In recent years, a new generation of smart packaging is being progressed to observe the properties of packaged food products by providing real-time information such as quality, safety and maturity (Cheng et al. 2022). Smart packaging combines both intelligent and active components. The sensing properties of metal oxide nanoparticles, antimicrobial activity, oxygen and ethylene scavenging, bio-nanocomposite films are all in the research areas of smart food packaging (Nicolic et al. 2021). Active food packaging has also been used in recent years thanks to consumer desires for preservative-free and processed foods. Additionally, in terms of improving concern and overutilization of synthetic non-biodegradable polymers, there is a developing interest in renewable biopolymer products in packaging technology (Priyadarshi et al. 2021). Active packaging not only improves the condition of food packaging but also extends the shelf life of food products. However, smart packaging not only effects the condition of packaged food products, but also effects the environment surrounding food products (Florez et al. 2022). Hence, intelligent food packaging is being produced for monitoring of the properties of foods continuously during the stages of processing, transportation and storage of food products. This kind of innovative packaging has many advantages such as improving the food quality, reducing the safety problems of foods, decreasing the food waste, which gives rises to favourable to the improvement of a safer, healthier, and also stronger food supply chain (Poyatos-Racionero, et al. 2018). Edible packaging is one of the most popular packaging methods that many studies have been carried out on this subject for extending the shelf-life of food and fishery products. However, it has yet to be effectively applied to the markets. Packaging made of plastics is widely used in the markets instead of biodegradable packaging, which have been made from various biological resources, edible films and coatings. The antioxidant capacity and antimicrobial characteristics of these edible packagings can be developed by adding phenolic compounds, essential oils and fruit extracts (Yuvaraj et al. 2021). Even though the majority of novel solutions and their applications have been considered in the food packaging processes, elaborate research could be required for their extensive implementations in the food industry (Soro et al. 2021). The growth of active and intelligent packaging films has been observed in recent years that they caused the use of proteins, polysaccharides, and anthocyanins from different plant sources (Yong and Liu 2020). Applyling natural antimicrobial agents effectively eliminates many species of pathogenic and spoilage microorganisms (Zhang et al. 2021). Additionally, intelligent pH-indicator polysaccharide-based films have been produced in recent years. However, further researches about the polysaccharide-based films in food packaging technology and their combinations with other materials could be studied in the future (Florez et al. 2022). Nano-biocomposites have been applied for developing the smart and active packaging at an accelerating pace (Tyagi et al. 2021). Recent advances have been made in the applications of graphitic carbon nitride (g-C3N4), hexagonal boron nitride (h-BN), 2DMs (including graphene, transition metal dichalcogenides TMDs), transition metal carbides and nitrides (MXenes), layered double hydroxides (LDHs)) in active packaging (such as antimicrobial activity, mechanical property, thermal stability and improving the barrier property etc.) and intelligent packaging (such as gas sensors, time-temperature indicators, pH indicators, intelligent labels, preparing leak indicators, etc.) (Yu et al. 2021). Furthermore, in the coming years growing technological advances together with the increasing market demands are leading to give the adoption of intelligent packaging in food industries (Poyatos-Racionero et al. 2018). Accordingly, the purpose of this review is not only can be outlined the recent advances about the food packaging technologies, but also can be summarized the effects of these advanced packaging technics on to the shelf-life of fishery products during transporting, marketing and storage.

**Recent Advanced Materials and Instruments for Food Packagings**

The processing, packaging and safety of foods have been effected from modern food science and technologies (Dzikunoo et al. 2021). Expanded polystyrene is widely applied in foam packaging. Thus, developing foams are required to apply biodegradable packaging for food products by using renewable materials such as starch-based foams instead of synthetic plastics (Tapia-Placido et al. 2022). But EPS-based foams lead to environmental problems in terms of little recycled and non-biodegredable. So, developing foams are needed for applying biodegradable packaging for food products by using renewable materials such as starch-based foams instead of synthetic plastics (Tapia-Blacido et al. 2022).

Active packaging comprises active compounds such as antimicrobial peptides (AMPS) that it is inclusive of them in different nanocarriers to obtain their controlled release for preserving of the food products during storage. In addition, AMPs have a good potential materials in food packaging because of eliminating the infectious bacteria and foodborne pathogens (Liu et al. 2021). Active packaging materials are improved to extend the shelf life of food products with antioxidants and antimicrobials, whereas smart packaging materials are improved to observe the maturity, quality and safety of foods in real time. Additionally, photoactivated materials can photocatalysis or photoluminescence for being used to produce these active and smart food packagings. Moreover, photocatalysis-based instruments can be used as color indicators for gas removal and to eliminate a broad spectrum of microbes, whilst photoluminescence-based sensors can observe environmental changes in food packagings such as freshness, the apperance of specific microorganisms and gas leakage (Xu et al. 2022).

Nanotechnology is also one of the most important areas of advanced packaging, which transports food products safely without spoiling the quality, nutrition and taste. Additionaly, it also prevents the microbial contamination and preserves the mechanical, chemical and physical properties of food products. Many nanomaterials have been applied in food packaging technology to provide bio-based, active, advanced and intelligent packagings (Chausali et al. 2022). Silver-based nanomaterials have been used in different applications in food packaging sector, which contain thermal resistant high performance packages, edible nanocoatings, functionalized packagings with antimicrobial and also improved physicochemical properties (Mathew and Radhakrishnan 2021).

Increased rates of the using plastic materials have hazardous effects on the environment, aquatic animals and human health (Bulat and Kılınç 2020). Therefore, in recent years, the active biofilms obtained from biopolymers, fibers and mineral clays have been considered a good alternative instead of the synthetic plastics in food packaging (Bourakadi et al. 2021). Additionally, the continuously increasing usage of plastic materials cause to improve the presence of nano and microplastics contamination in variety of food products. Therefore, their potential remaining effects on human health are uncertain in the future (Shruti et al. 2021). Because of this approach, alternative sustainable packaging material is necessary to develop for human health and also for environment instead of using plastic packaging (Haghighi et al. 2020). Instead of being used petro-based materials, innovative antimicrobial packaging materials can be improved by using antimicrobial agents. Edible packaging typically uses biodegradable and sustainable materials that is very suitable for coating or wrapping around the foods (Petkoska et al. 2021). Antimicrobial agents into the biopolymer-based edible films has been a significant improvement in active food packaging technology (Chawla et al. 2021). The commercial usage of biodegradable protein films is still very limited in the industry because of their poor barrier and mechanical performance. In contrast to this approach, processing of proteins may be able to be a good impact on the properties of biodegradable films (Romani et al. 2018). These biodegradable films are being produced not only from the animals (gelatine, myofibrillar protein, collagen), but also plants (maize, rice, wheat, barley etc.), sunflower and aquatic plants for improvement of packaging material (Assad et al. 2020). A lot of antimicrobial substances can also be combined with the materials in the packaging technology, including antimicrobial polymers, biotechnological products etc. (Singh et al. 2016). Essential oils and also their compounds can be added into the packaging that are especially very important for environmental concerns. These bio-based packagings have been mostly used in many types of foods such as fish, fruit, meat, raw and processed food with effective results. The preservation of foods occurs not only can be obtained by diffusion of the active compounds of essential oil, but also it can be incorporated into the packaging to the foods (Ribeiro-Santos et al. 2017), but also packaging films include antimicrobial agents can be extended the shelf life of foods by inhibiting the spoilage (Singh et al. 2016). Although, many studies have been done on the usage of bio-based materials for food packaging, further studies are needed to develope their functioning mechanisms, performances and also improve greener methods for the processing and production of these bio-based materials (Wang et al. 2022).

Nanoemulsions of natural oils are gaining importance in various fields containing agriculture and food sectors, in terms of efficiency, possess developed functionalities and more stability. For example; neem oil and its nanoemulsions as well as pectin, starch and chitosan based active packaging of foods containing vegetables and fruits have also been used (Kumar et al. 2022). Chitosan is a nontoxic, biodegradable, and biocompatible natural polymer. It is a good option for antimicrobial films due to its film-forming properties, ability it absorbs nutrients used by bacteria and inhibiting a variety of bacteria species with its ability to adhere to water with enzyme systems (Darmadji et al. 1994). Additionaly, it is one of the properest polysaccharides in the nature that it has a lot of properties such as antifungal, antimicrobial, antioxidant activity and non-toxicity characteristics. For these reasons, active or intelligent chitosan-based films have been developed for the usage of food packaging in recent studies (Florez et al. 2022). Biopolymer-based food packaging such as starch has been replaced in recent years because it is eco-friendly, inexpensive biopolymer and sustainable. Thermoplastic starch is one of the best materials for obtaining starch derivatives that is applied for food packaging. Although, it has some disadvantages, including inadequate water barrier characteristics, hygroscopic nature and low gas permeability (Bangar et al. 2021). Clodextrins have some benefits that they have been garnering increasing attention day by day because of being formed supramolecular structures and several complexes. So, they are not only used for food packaging but also they can be used for the applications in other fields of science (Liu et al. 2022). Plant-based food wastes can also be used as raw materials in biodegradable packaging for improvement of packaging performance (Zhang and Sablani 2021). Kefiran is an exopolysaccharide obtained from the microflora of kefir grains that it has tremendous potential in food packaging applications due to its film forming, biological properties, electrospinning abilities, biocompatibility (Carvalho and Conte-Junior 2021). Cellulose containing composites is the reinforcing material such as nanocrystal or nanofibres, which are becoming more frequently, when compared with cellulose-based packaging (Garrido-Romero et al. 2022). The clay-polymer nanocomposites containing bioactive compounds for food packaging applications can provide some advantages by affording barrier properties, enhancing the mechanical properties of the polymer matrix, stabilizing the bioactive compounds and also a vehicle for their controlled release (Cheikh et al. 2022).

Variety of food packaging technologies are applied individually or in mixture for observing effective food conservation such as active food packaging; oxygen scavengers; carbondioxide emitters; moisture regulators; antioxidant and antimicrobial packaging; intelligent packaging, including freshness indicators; retort pouch processing and edible films; time–temperature indicators and leakage indicators; coatings/biodegradable packaging (Kontominas et.al. 2021). Nanotechnology offers exciting opportunities for the integration with the packaging, the development of active components, communication, miniaturization and batteries. However, the use of nanotechnology in intelligent packaging is still limited (Ros-Lis and Benitez 2021).

Intelligent packaging integrated with $O_2$ sensors has been developed and demonstrated its ability to monitor $O_2$ content throughout the food supply chain of many different packaged foods (Cruz-Romero et al. 2019). Many commercial intelligent packaging products have been given such as integrity indicators, time temperature indicators, freshness indicators, and radio frequency identification tags (Poyatos-Racionero et al. 2018). Freshness indicators are not only efficient intelligent and simple products, but also they can be scientifically and directly monitored the freshness of foods without damaging packaging and food easily. The overall control of food safety and quality can be monitored by these intelligent technologies such as sensor, bar code etc. (Shao et al. 2021). These freshness indicators can be produced as a label, ensuring a rapid response to storage temperature or food quality that changes according to the color changes. Additionally, Electrospinning, as a sophisticated and an efficient method of preparing continuous nanofibers that it has been developed as an encouraging to produce food packaging materials. Subsequently, electrospun nanofibers have been recycled as a novel product to improve intelligent packaging (Forghani et al. 2021). Moreover, the safety and quality of packaged foods were ensured developing oxygen indicators by electrospinning process that they detected the damaged food packages during the supply chain (Yılmaz and Altan 2021). Electronic nose is one of the most important monitoring device that it is a good alternative for measuring the characteristics of flavour and aroma in relation to volatile compounds in the quality of food products. Nowadays, the improvement of the usage of e-nose applications for the food industries has been increasing because of the emergencing of gas-sensing detection; it has a new potential (Ali et al. 2020). Nanomaterials of different kinds expose a immense potential for intelligent food packaging. Nanosensors obtain selective and sensitive platforms that they cause to sense of deteriorative markers (Mohammadpour and Naghib 2021). Furthermore, two-dimensional materials can be widely used in food packaging and they have some good advantages such as high mechanical performance, unique electrocatalytic activity and large specific surface area. (Yu et al. 2021). The popularity of nanotechnology in food packaging is also increasing day by day in terms of the various bio-nanocomposites including nanohybrids, organic and inorganic nanomaterials that are very suitable for food packaging materials due to their antimicrobial, antioxidant, thermal mechanical, barrier advanced properties (Perera et al. 2021). In addition to this, nanoclays have been used with essential oils and bio-extracts that included both synthetic and bio-based polymers to manufacture nanocamposites with improved the antimicrobial, barrier and antioxidant activities (Nath et al. 2022). Although the risks and benefits of engineered nanoparticles in food-contact applications are discussed, they can be also examined whether or not and in which conditions they can be released from the food contact polymers (Enescu et al. 2019). In addition to this; proper risk assessments are developing enormous rate by using the composite films for example metallic micro-/nanoparticles in food contact materials, regarding the effective evaluation, human toxicity and and confirmation of the advantages on the use of metallic particles are necessary to declared (Videira- Qintela et al. 2021). Moreover, smart biomaterials can also be interacted with the food product or the headspace for ensuring a preferred result for bio-derived products due to enhance the sensor activities, safety and also shelf life. However, the environmental influences should be arranged by specific standards to sustain more efficient food packaging for future markets (Reshmy et al 2021). Additionaly, the contaminants in food packaging technology and the determination of the most appropriate safety assessments of these contaminants are also required to determine in this rapidly increasing field (Karmaus et al. 2018).

**Recent Advanced Packaging Technologies in Raw and Processed Seafood Products**

Fish, poultry, meat are very perishable that they should be packed using the most probable technique and they should be processed very quickly. Retorting is heated in hermetically sealed containers that it extends the shelf life and also preserves the food products. According to this method; retort-processed seafood products are ready to eat food products that they have a shelf life of more than a year in optimal temperatures (Bindu et al. 2012). The application of the packaging, temperature/pressure treatments, the use of microbial-derived compounds, natural compounds (e.g. biopolymers, plant/algal extracts, gelatin) and various combinations of these applications have been provided to extend the shelf life of fishery products (Nie et al. 2022). Proper packaging maintains the quality of seafood for a long time after processing (Nagarajarao 2016). The effects of packaging technics on the microbiological flora of the packaged seafood have been reviewed by Kılınç and Çaklı, (2001). Additionally, many packaging technologies have been used for extending the shelf life of raw and processed fishery products such as vacuum and modified atmospheric packagings (Kılınç and Çaklı 2004). Advances published on vacuum and modified atmosphere packaging of seafood products have been discussed between the years 2000 and 2010 (Fletcher 2012). Alternative renewable materials began to take place with the polystyrene materials for packaging (Hansen et al. 2012). In present years effect of vacuum impregnated grape seed extract and fish gelation on quality of seabass during storage (4°C) suggested that FGG is a good promising for sea bass preservation (Zhao et al. 2021). Many studies have been done and developed about the application of new and alternative packaging technics. Nonwoven fabrics, containing bamboo and silver have been used for packaging of somon fillets to have a longer shelf life (Kılınç et al. 2017), whereas the effects of absorbent pads containing black seed and rosemary oils on the shelflife of sardine (*Sardina pilchardus*) fillets have been carried out (Kılınç and Altaş 2016).

Edible coatings can cause to develop the quality of a variety of seafood products by retarding microbial growth, moisture loss and reducing lipid oxidation. Additionally, food additives have some special properties that are contained antioxidant and antimicrobial agents. Biodegradable edible coatings have also many advantages that they have edible and generally more environmentally (Dehghani et al. 2018). Many types of edible films have been used on the surface of seafood products for increasing the shelf life and quality. These films include many special characteristics of compounds that they have antioxidants, chemical and natural antimicrobial agents, vitamins, enzymes, minerals (Janes and Dai 2012). Bioactive films produced from marine gelatin are being improved for food packaging to do continuation good alternatives to other synthetic materials. The use of these films in food packaging for the protection of fish, meat and vegetable products will become widespread commercially (Abdelhedi et al. 2022). In recent years, the production of fish gelatin and chitosan has also been improving commercially. Moreover some bioactive components can be separated from gelatin extraction or chitosan to produce film-forming formulations. They not only cause to extend food shelf life, but also they lead to produce packaging and reduce the food losses (Caba et al. 2019). The improvement of gelatin-based films for usage in food packaging has attracted more attention because of their biodegradability, non-toxicity, renewability and availability recently. Advanced properties of gelatin film have been studied by incorporating modifiedkappa-carrageenan and zein nanoparticles for active food packaging that it showed the valuable potential for the application of the various food products (Maroufi et al. 2021). Further, the addition of plant essential oils, plant extracts and nanoparticles to starch films can develope their antioxidant, antibacterial, and barrier properties. In addition to this, adding anthocyanins into the starch films can make not only the films pH-responsive, but also can indicate the degree of spoilage in fish products and achieve the purpose of warning consumers (Cui et al. 2021).

Nanotechnology applications in food packagings and importance for seafood have been reviewed by (Sürengil and Kılınç 2011). Additionally, incorporation of natural anthocyanins and TiO2 nanoparticles developed the bacteriostatic properties, moisture, mechanical resistance and inhibited oxidative reactions of smart films. According to the fish degradation, which was well correlated with ammonia production, the films changed the color. These active/biodegradable smart films are not only able to improve quality of fish, but also can be prolonged the shelf life, inhibited the fish spoilage and reduced the fish wastes (Sani et al. 2022). Novel techniques containing biosensor, colorimetric sensor, electrochemical biosensor, enzyme biosensor, electronic nose, electronic tongue, computer vision techniques, fluorescence spectroscopy, HSI spectroscopy and Vis/NIR spectroscopy as rapid and reliable tools for evaluating fish freshness in timely manner and an effective methods have been used (Wu et al. 2016). New approaches in packaging technology are improving increasingly by using different natural functional additives and combination methods. Although they had some difficulties about consumer acceptance and sometimes incomplete development or low retail and legal restrictions, intelligent packaging systems are still being innovated and show good potential for developing the present methods for providing the safety of packed meat products (Schumann and Schmid 2018). Smart deep learning-based attitude has been used for non-desructive freshness diagnosis of common carp fish. This method is well ability of classifying and monitoring fish freshness as a precise, low-cost, non-destructive, automated and real-time techniques (Taheri-Garavand et al. 2020). Composite active/intelligent food packaging film was improved with polyvinyl alcohol and gelatin combine with amaranthus leaf extract to observe the freshness of fish. Fish packed in neat films had a shelf life of 3 days, while samples in active films spoiled after 12 days. On the other hand, the films were active as they minimised oxidative rancidity and delayed microbial growth of chilled fish (Kanatt 2020).

The development of new fish processing and packaging technologies not only give rise to produce alternative novel products, but also achieve good management, reduce food waste and improve the shelf-life of raw and processed fishery products (Tsironi et al. 2020). Intelligent packaging in food products indicates many types of packaging that includes intelligent agents such as sensors and detectors. This type of packaging is used to warnings about possible problems in food packages, convey information about food safety and quality of foods. Markers and sensors are capable for measuring not only chemical, biological or physical variables in food products but also, biosensors and color sensors are mostly applied in the food packaging industry as well as color sensors are commonly used as labels in intelligent packaging (Pirsa et al. 2022). Additionally, a non destructive method, observing the volatile compounds based on trimethylamine of packed cod fillets has been developed that this method monitors the changes in freshness quality of fillets (Heising et al. 2015).

The use of electronic sensors in the seafood packaging industry have many advantages in manufacturing processes. This not only gives rise to pack seafood products properly but also, immediately detect the pollution particle in fishery products and improve the processing of seafood industry by doing the packing process faster (Badilla and Gaynor 2018). Oxygen absorber are used for prevention of rancidity and discoloration, whereas antioxidant releaser are used for enhancement of oxidative stability for fatty fish. Furthermore, moisture absorber is applied for declining of moisture condensation within the package and shelf life extention of fresh fish, whilst carbon dioxide emitter is used for reducing in head space volume of modified atmosphere packaging and shelf life extention of fresh fish. Besides, antimicrobial releaser is practised for retardation of microbial growth and shelf life extension of fresh and smoked fish (Kontominas et al. 2021). Label with a dye in intelligent packaging that changes color in accordance with a chemical reaction, which is affected by temperature or compounds. Physical (e.g. temperature) or chemical (e.g. pH, volatile composition) factors affecting or producing a chemical reaction that changes the color of freshness indicator (Azeredo and Correa 2021). pH is used as non-destructive methods for observing the changes in the freshness situation of packed cod fish examined, whereas volatile compounds in packed fish that affect the electrode signals of the electrodes with increasing the volatile amines content of fresh cod fillets stored at 0–15 °C (Heising et al. 2014). A wireless basic volatile sensor have a good correlation with the ammonia gas concentration and microbial analysis, identify that it shows a potential instrument for monitoring of fish spoilage for both 24°C and 4°C storage conditions (Bhadra et al. et al. 2015). Intelligent food packaging (A dual-channel indicator), including a built-in sensor inside the fish bags, can be able to real-time monitoring of fish safety and quality by visibly discernible out-put signals due to the increasing production of amines according to the fish spoilage (Duan et al. 2022). Additionally, the freshness label produces a noticeable color change according to the fish spoilage with increasing the level of the total volatile basic nitrogen (TVB-N), which can indicate the spoilage of the fish. (Wang et al. 2022). The development of colorimetric and optical sensors is highly not only attractive, but also they give rise to real time operation and easy visualization (Ma et al. 2021). Aggregation-induced emission indicator is very sensitive to ammonia vapor for detecting the spoilage of the seafood. The color variation of this indicator was in agreement with the increasing tendency of the value of total volatile basic nitrogen and total viable bacteria count, which confirmed the accuracy of the this indicator (Zhu et al. 2021). A biopolymer-based pH indicator was carried out to monitor the spoilage of fish at (23°C) room temperature in terms of the color change of pH indicator from red to yellow after 1 day (Sobhan et al. 2022). The developed novel paper based pH-sensitive intelligent detector can be used for different variety of samples and also in different packaging sizes by adjusting the detector's pH. This detector is not only inexpensive, but also can detect freshness level and spoilage in the form of an on-package detector (Alamdari et al. 2021). The polyvinyl alcohol/glycerol film incorporated with curcimin and anthocyanins at a ratio of 2:8 (v/v) could provide three different colors, which were assigned to spoilage for packaged fish, medium freshness and the sign of freshness (Chen et al. 2020). The intelligent pH indicator films were made by incorporating curcumin-loaded Pickering emulsion with polyvinyl alcohol matrix and corn starch. The pH indicator films made with curcumin-loaded Pickering emulsion were prepared on examining fish freshness that they had more pronounced color change from yellow to red (Liu et al. 2021). Intelligent colorimetric indicator films with 10% purple tomato anthocyanin caused to change their color during spoilage of fish, indicating their potential usage for monitoring fish freshness and spoilage (Li et al. 2021). The intelligent packaging film based on bacterial cellulose and pelargonidin had a great color difference from red to colorless with changing of total volatile basic nitrogen and sensory scores, when operated to observe the freshness of tilapia fillets (Huimin et al. 2021). Hydroxypropyl methylcellulose/microcrystalline cellulose biocomposite film combined with butterfly pea (*Clitoria ternatea*) anthocyanin as a pH-responsive indicator was improved. In lights of aboveapproach; the spoilage level of mackerel (*Scomber scombrus*) was determined. According to the changes in the color of this indicator as follows; blue, green ocean, and colonial blue colors showed that it was spoiled, violet color showed that it was suitable for eating, deep purple and light purple colors showed that it was fresh. (Boonsiriwit et al. 2021). Gradient colorimetric indicators were prepared by using piezoelectric inkjet-printing to observe the freshness of packaged ocean perch, catfish and wild haddock fillets during storage. Based on the spoilage of fish products, the color changes of the indicator labels were determined with the increasing of trimethylamine, total volatile basic nitrojen and dimethylamine levels in the packages (Luo et al. 2021). pH-responsive chitosan-based film incorporated with alizarin, which was very sensitive to ammonia vapor with changing a pH and also the color from slightly yellow to purple, was improved (Ezati and Rhim 2020). Cellulose nanofibers and carboxymethyl cellulose intelligent pH responsive color indicator films were developed using shikonin. The monitoring of color changes of indicator films from a reddish pink to blue-violet indicated as the spoilage of fish (Ezati et al. 2021). A halochromic indicator produced from polylactic acid and anthocyanins for monitoring the visual freshness of fish roe and shrimp were improved. This developed indicator showed the levels of the spoilage of products as fresh, medium fresh, and spoiled (Ghorbani et al. 2021). For monitoring the shrimp spoilage correlated with the increasing ammonia vapors; the novel pH colorimetric film was developed from sugarcane wax on agar matrix combined with butterfly pea flower extract (Hashim et al. 2022).

**Table 1.** Intelligent Packaging for Raw and Processed Fishery Products

| Type of Packaging | Type of changes/ increasing product | Potential Benefit | References |
|---|---|---|---|
| The use of pH and conductivity electrodes | Volatile compounds | Freshness status of packed cod fish for sensing non-destructively | Heising et al. 2014 |
| A hydrogel-pH-electrode based near-field passive volatile sensor | Basic volatile spoilage compounds | Non-destructive detection of fish spoilage | Bhadra et al. 2015 |
| A chitosan-based pH-responsive film | Ammonia | Determining of the fish spoilage | Ezati and Rhim 2020 |
| Visual pH-sensitive films containing curcumin and anthocyanins | Volatile amines | Non-destructively monitoring the real time freshness of fish products | Chen et al. 2020 |
| Intelligent pH-responsive color İndicator films | pH changes | Monitoring seafood quality | Ezati et. al. 2021 |
| pH- responsive indicator | Ammonia | Sustainable, Monitoring of the freshness of mackerel | Boonsiriwitt et al. 2021 |
| Inkjet –printed gradient colorimetric indicators | Volatile amines | Monitoring the freshness of ocean perch, catfish or wild haddock fillets | Luo et al. 2021 |
| A novel paper based pH-sensitive intelligent detector | pH changes | Detecting of the freshness level and spoilage of seafood | Alamdari et al. 2021 |
| Colorimetric and optical indicators | Total volatile basic nitrogen | Non-destructively and visual monitoring of seafood spoilage | Ma et al. 2021 |
| Aggregation-induced emission indicator | Ammonia | Monitoring of seafood spoilage | Zhu et al. 2021 |
| Intelligent colorimetric indicator film | Total volatile basic nitrogen | Monitoring of the freshness of tilapia fillets during storage | Huimin et al. 2021 |
| The intelligent pH indicator film | pH changes | Monitoring of fish freshness | Liu et al. 2021 |
| Intelligent colorimetric indicator films | pH changes | Monitoring of fish spoilage or freshness | Li et al. 2021 |
| A halochromic indicator | Total volatile basic nitrogen | Monitoring of the freshness of fish roe and shrimp | Ghorbani et al. 2021 |
| A dual-channel indicator | Amines | Monitoring of fish spoilage | Duan et al. 2022 |
| Fish freshness indicator label | Total volatile basic nitrogen | Tracking of fish freshness visually and non-destructively | Wang et al.2022 |
| A biopolmer-based pH indicator | pH changes | Cost effective, simple, environmentally friendly, mitigate the impact of possible outbreaks and detecting fish spoilage | Sobhan et al. 2022 |
| A novel pH colorimetric film | Ammonia | Optical tracking of shrimp freshness | Hashim et al. 2022 |

Together with these trends; number of challenges have been appeared for fish producers in accordance with the development of new product, processing and packaging technologies that ensure the seafood products have maintained their sensory and nutritional qualities before they reach to the consumers (Hyldig et al. 2012). Nevertheless, migration of potentially toxic materials and their transformation products from packaging materials are required to be monitored continuously in terms of human health (Szczepanska et al. 2018).

## Discussion

The continuous improvement of technologies cause to the development of packaging techniques. Ensuring that fish and aquatic products are monitored at every stage until they reach consumers can only be achieved by using improved packaging techniques.

In addition to the advantages brought by the development of packaging technology, it is necessary to focus on the disadvantages and not to prefer the use of materials and methods that will harm human health in packaging. As a result, thanks to improved packaging techniques, determining that seafood products can be consumed or not consumed without the need for analysis to determine their quality will not only eliminate the analysis costs, but also will lead to give many advantages in terms of time. In addition, it is also envisaged that packaging techniques will be more advanced in the future than today and that today's unknown topics related to advanced packaging techniques will be solved.

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