

## Temporal and Spatial Variations of Zooplankton Community and Biochemical Response due to Water Quality in a Deep Dam Lake (Turkey)

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### Abstract

In this study, the zooplankton community and its relationship with environmental factors were investigated in the Karakaya Dam Lake (KDL). The physico-chemical characterization showed that there were obvious changes in the water quality and zooplankton population structure, which was mainly due to the organic matter source. Reactive phosphate (SRP) and nitrogen values were found in low concentrations. A total of 22 zooplankton taxa were determined, including 14 taxa of Rotifera, followed by 6 taxa of Cladocera and 2 taxa of Copepoda. The most abundant species were *Synchaeta oblonga* (Rotifera), *Bosminia longirostris* (Cladocera), and *Cyclops scutifer* (Copepoda). The abundance of zooplankton was highest in spring. Canonical correspondence analysis (CCA) was used to examine relationships between measured environmental variables and zooplankton composition. According to CCA, dissolved oxygen, and SRP values, there was a significant relation to zooplankton abundant with these factors. The triplots diagram demonstrated variations of the structure of zooplankton population composition which can be explained by the environmental variables. Oxidative stress (catalase, glutathione S-transferase, glutathione reductase) and neurotoxicity (acetyl cholinesterase) biomarkers were analyzed in copepod species. Glutathione reductase and acetyl cholinesterase activities were significantly inhibited in the summer. Catalase activity was induced in the spring. The seasonal changes of biomarker indicated that the KDL may be at risk of pollution that originated from agricultural and industrial activities.

**Keywords:** Community structure, zooplankton, water quality, oxidative stress, Karakaya Dam Lake

## Derin Bir Baraj Gölü'nde (Türkiye) Zooplankton Topluluğunun Zamansal ve Mekansal Değişimleri ve Su Kalitesine Bağlı Biyokimyasal Cevabı

Bu çalışmada, Karakaya Baraj Gölü'nde (KDL) zooplankton topluluğu ve çevresel faktörlerle olan ilişkileri araştırıldı. Fiziko-kimyasal karakterizasyon, su kalitesi ve zooplankton populasyon yapısında, esas olarak organik madde kaynağından kaynaklanan değişiklikler olduğunu gösterdi. Reaktif fosfat (SRP) ve azot değerleri düşük konsantrasyonlarda bulundu. Rotifera'nın 14 taksonu, Cladocera'nın 6 taksonu ve Copepoda'nın 2 taksonu olmak üzere toplam 22 zooplankton taksonu tespit edildi. En bol bulunan türler *Synchaeta oblonga* (Rotifera), *Bosminia longirostris* (Cladocera) ve *Cyclops scutifer* (Copepoda) olarak belirlendi. Zooplankton bolluğu, ilkbaharda en yüksek seviyede idi. Analiz edilen çevresel değişkenler ile zooplankton bileşimi arasındaki ilişkileri incelemek için kanonik uyum analizi (CCA) kullanıldı. CCA verilerine göre, çözünmüş oksijen ve SRP değerleri ile zooplankton arasında anlamlı bir ilişki gözlemlendi. Triplot diyagramı, çevresel değişkenlerle açıklanabilen zooplankton populasyon kompozisyonunun yapısının varyasyonlarını gösterdi. Oksidatif stres (katalaz, glutatyon S-transferaz, glutatyon redüktaz) ve nörotoksisite (asetil kolinesteraz) biyobelirteçleri copepod türlerinde analiz edildi. Glutatyon redüktaz ve asetil kolinesteraz aktivitelerinin yaz aylarında önemli ölçüde inhibe olduğu gözlemlendi. Katalaz aktivitesi ilkbaharda indüklendi. Biyobelirteçlerin mevsimsel değişiklikleri, KDL'nin tarımsal ve endüstriyel faaliyetlerden kaynaklanan kirlilik riski altında olabileceğini gösterdi.

**Anahtar Kelimeler:** Kominite yapısı, zooplankton, su kalitesi, oksidatif stres, Karakaya Baraj Gölü

## INTRODUCTION

The watercourse ecosystems are habitats for many aquatic organisms which play an important role in continuing the food web (Sarker et al., 2018). Due to the continuous increase of xenobiotic compounds and various industrial and domestic wastes, pollution of aquatic ecosystems has been a major concern in recent years (Park et al., 2020). Therefore, it is important to control water pollution, monitor water quality, and comment on the changes in water quality (Khadse et al., 2019; Jia et al., 2019). Water pollution can affect organisms at the molecular and biochemical levels including neurotoxicity, genetic toxicity, oxidative stress, reproductive properties and population structure (Demailly et al., 2019; Park et al., 2020; Hussain et al., 2020). Antioxidant enzyme responses of these organisms are essential for determining mechanisms of toxicity. When the antioxidant defense system is active, reactive oxygen species (ROS) homeostatic balance is protected. This means, organisms can be protected from the toxic effects of (ROS) (Klumpenet al., 2017; Souza et al., 2019; Akbulut and Turhan, 2021). Exposure to pollutants may results in an increase in ROS production and antioxidant activity. Catalase (CAT), glutathione S-transferase (GST), and glutathione reductase (GR) are the beneficial antioxidant enzymes that aquatic organisms produce to reduce the harmful effects of ROS (Liu et al., 2018; Park et al., 2020). In addition, acetyl cholinesterase (AChE) activity acts as a biomarker when exposed to organophosphate pesticides (Tiwari et al., 2019).

Consequently, the description and monitoring of pollutants in the ecosystem has to be based on a temporal (seasonal) scale and from a multi-biomarker approach. On the other hand, biomonitoring studies should be performed with suitable indicator species (Barka et al., 2020). Zooplankton, one of the indicator species, play an important role in the transfer of energy and nutrients from producers to secondary consumers and are very susceptible to environmental changes (Gorokhova et al., 2016; Gökçe et al., 2018; Kadiene et al., 2019; Gökçe et al., 2020).

Copepods are dominant members of zooplankton communities and are frequently used as a model organism in ecotoxicological studies (Hussain et al., 2020). Di-Marzio et al (2013) enounced that the influences of pollutants on copepods in freshwater environments have been poorly studied even though they are considered a suitable candidate for toxicity assays. In the present study, using the copepods, *Cyclops scutifer* and *Microcyclops varicans*, as model organisms, we investigated their biochemical response to the effects of pollutants in KDL evaluations of biochemical parameters (CAT, GST, GR and AChE). In this study, the spatial and temporal variation of water quality and pollutants on the zooplankton community in KDL in the upper Euphrates River were investigated. The aims of this study were to 1) reveal the ecosystem properties of KDL, located on the upper Euphrates River Basin by examining water quality values, 2) investigate spatial and temporal zooplankton community dynamics, and 3) evaluate the effects of physical-chemical variables of ecosystem quality on the Copepods using a range of biological markers. Thus, assessment and monitoring of Karakaya Dam ecosystem health status were performed and reliable data were obtained for future monitoring assessment.

## MATERIAL AND METHODS

### Field sampling and laboratory analyses

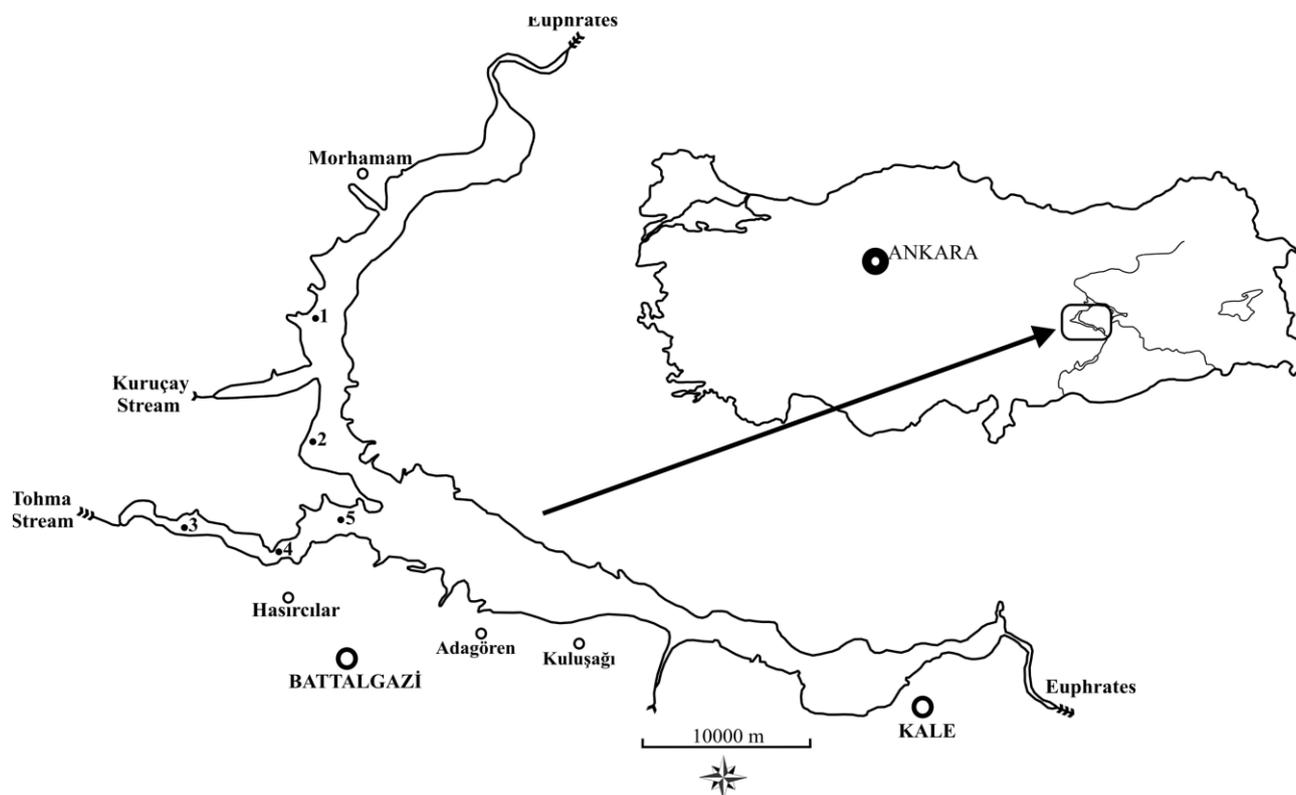
The Euphrates, one of the world's most important transboundary rivers, originates in the east of Anatolia and empties into the Persian Gulf (Uçkun and Gökçe, 2015). Karakaya Dam, which is an important water reservoir on the Euphrates River Basin, is one of the most important water resources in terms of both irrigation and fishing.

The sampling points in Karakaya Dam Lake were chosen to determine the limnological structure of the lake, as well as the effect of the residential areas on the lake and 5 sampling points were determined to show ecosystem quality and pollutants entering the system (Figure 1). St.3, St.4 and St.5 sampling points are the regions where the untreated/partially treated wastes from the

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Organized Industrial Zone are mixed with the lake ecosystem and this area is a narrow bay. St.1 and st.2 sampling points are the wider part of the dam morphometry. St.2 had a very shallow depth of about 5 m. St 1 had the depth of about 35 m and

St.3, St.4 and St.5, each had a depth of nearly 16 m. The water and zooplankton samples were collected per month between October 2010 and November 2011.



**Figure 1.** Map of Karakaya Dam Lake. Sampling stations surveyed in this study are indicated.

Water samples were taken at 5 meter intervals with a Ruttner water sampler (Hydro-Bios, 2 L). Temperature, dissolved oxygen (YSI-55), conductivity (EC25; YSI-30), and pH (YSI-60) were measured in the field. Dissolved oxygen (DO; YSI 55), temperature, pH (YSI-60), and conductivity (EC25; YSI-30) were determined in the field. Ammonium (NH<sub>4</sub>-N; DIN38 406-E5-1 standard method), nitrite (NO<sub>2</sub>-N; DIN38 405-D10 standard method), nitrate (NO<sub>3</sub>-N; DIN38 405-D9-2 standard method) and soluble reactive phosphate (SRP; DIN38 405-D11-1 standard method) were determined from filtered water samples in the laboratory (Gökçe, 2014).

Zooplankton samples were obtained by filtration of 100 L of water using 55- $\mu$ m pore-sized plankton net (Hydro-Bios) and were stored in 4% formaldehyde. 80 individuals of copepod were kept

for enzymatic activity analysis at -40 °C by washing twice in 10 mL centrifuge tubes. An inverted microscope (Leica DM), and identify the zooplankton taxa (Armengol et al., 1998; Ustaoglu 2004).

#### Antioxidant enzymes analyses

##### Sample preparation

Preliminary studies were done to optimize the methodology including whole-body copepod use in the biochemical analyses. As a result of the examined samples under the microscope, it was determined that the dominant copepod in the water was *C. scutifer* with rate of 92.4%. Since it is not possible to separate organisms while performing biochemical analyzes, organisms were used in the analysis without separating them. Copepod samples (80 individuals) were sonicated in K-phosphate buffer, at pH 7.4 and then the samples were

centrifuged at 10000 rpm for 10 min at 4°C. The supernatants were used as enzyme sources for CAT,

GR, GST and AChE activities.

### Biochemical assays

Enzyme activities were determined using a spectrophotometer. Assays were run in triplicate. The total protein content was determined by the Bradford (1976) method using BSA as the standard.

CAT activity assay was performed according to Luck (1963). The decomposition of the substrate  $H_2O_2$  was monitored spectrophotometrically.

GR activity was measured using the method Cribb et al., (1989). The solution includes NADPH, and GSSG in potassium phosphate buffer containing EDTA. The reaction was started by the addition of the GSSG and the decrease in absorbance. The enzyme activity was indicated as  $nmol\ min^{-1}\ mg\ protein^{-1}$ .

GST assay was performed according to Habig et al. (1974) using 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate. The assay mixture contained CDNB, sample, 0.01 M Tris buffer and GSH. GSH was used as the cofactor in the reaction and absorbance was recorded.

AChE activity analysis was measured from the method presented in Ellman et al. (1961). Acetylthiocholine iodide (ATCh) was used with 5,5-dithiobis-2-nitrobenzoate (DTNB) as the thiol indicator. 0.1 M phosphate buffer (pH 8.0), 0,01 M DTNB, and 0.015 M of acetyl choline iodide was added. The absorbance was measured at 5 min and 12 min at 412 nm. All measured enzyme activity values were indicated as  $nmol\ min^{-1}\ mg\ protein^{-1}$ .

### Data analyses

The Shannon-Weaver diversity index ( $H'$ ) (Shannon and Weaver, 1963; Derevenskaia et al., 2021) and species abundance ( $ind\ m^{-3}$ ) were calculated using the zooplankton taxa community (Djurhuus et al, 2018). CANOCO (version 4.5) was used to perform a canonical correspondence analysis (CCA) to examine the variation in zooplankton composition due to temporal and spatial distributions of environmental variables (Jongman et al., 1995; Lepš and Šmilauer, 2003). Before the CCA, the species data was transformed by  $\log_{10}(x+1)$ . The CCA findings were shown in a triplots graphic that combined response factors (zooplankton species and biomarkers) with

environmental variables. Biomarker data were analyzed using GraphPad Prism (Version 5.0; Graph Pad Software Inc., USA). Data were compared using the Kruskal-Wallis test followed by the unpaired t test (for pairwise comparisons) (Yamamuro et al., 2019).

## RESULTS AND DISCUSSION

### Physico-chemical properties of KDL

Determining the physico-chemical properties of the KDL water and the seasonal and spatial biomarker levels of copepod living in this reservoir are important in terms of evaluating the health of the reservoir. Temperature is the vital factor that affects the metabolic activities of aquatic organisms (Akther et al., 2018; Bulut et al., 2021). The water temperature of KDL varied between 8-30.4 °C (Table 1). According to the temperature values, thermal stratification was observed in October 2010, April, May, June, July, August, September and October 2011 (Figure 2). The area between the surface layer of the lake and the depth of five meters is defined as the epilimnion layer at all stations of KDL. On the other hand, the DO value, which varies according to temperature, photosynthesis rate of organisms and water regime of lakes, is an important factor for the health of aquatic organisms (Guo et al., 2021). According to the correlation analysis, DO and SRP values were found to be significantly related to other water quality variables ( $r= 0.944$  and  $r= 0.885$ ,  $p<0.005$ ; respectively). DO concentrations had varied from 1.11-13.13  $mg\ L^{-1}$  and were observed at very low values in the summer. These results may be due to the increase in temperature during the summer months. Güher and Öterler (2020) reported that the water DO concentration changes inversely with the temperature change. DO values in spring were higher than other seasons in all sampling stations (Table 1). This situation may be due to the circulation of the lake water and the increase in phytoplankton during the sampling period (Gökçe, 2014). Khan and Bari (2019) reported a positive relationship between DO concentration and total phytoplankton density in the aquatic ecosystem.

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**Table 1.** Physicochemical parameters (Mean ±SD values) according to stations and seasons in KDL between October 2010 and November 2011

Season	Stations	Secchi	DO (mg L <sup>-1</sup> )	T (°C)	pH	EC (µS cm <sup>-1</sup> )	NO <sub>3</sub> -N (mg L <sup>-1</sup> )	NO <sub>2</sub> -N (µg L <sup>-1</sup> )	NH <sub>4</sub> -N (mg L <sup>-1</sup> )	SPR (µg L <sup>-1</sup> )
Autumn	1	3.4 ± 0.3	6.4 ± 0.5	15.9 ± 2.9	8.4 ± 0.3	390.9 ± 39.1	0.008 ± 0.001	0.9 ± 1.1	0.01 ± 0.01	0.03 ± 0.04
	2	2.9 ± 0.2	7.4 ± 0.7	19.1 ± 4.6	8.8 ± 0.2	406.5 ± 43.9	0.007 ± 0.002	0.6 ± 0.9	0.02 ± 0.06	0.07 ± 0.09
	3	2.6 ± 0.1	4.7 ± 0.6	18.2 ± 3.7	8.4 ± 0.3	486.2 ± 12.3	0.004 ± 0.001	1.1 ± 0.9	0.03 ± 0.08	0.03 ± 0.04
	4	2.7 ± 0.2	4.9 ± 0.8	17.4 ± 3.7	8.5 ± 0.5	485.5 ± 46.3	0.007 ± 0.001	1.2 ± 1.2	0.03 ± 0.08	0.04 ± 0.06
	5	2.7 ± 0.2	5.0 ± 1.0	17.3 ± 3.9	8.5 ± 0.5	446.9 ± 26.8	0.007 ± 0.001	1.4 ± 1.2	0.02 ± 0.06	0.04 ± 0.04
Winter	1	3.9 ± 0.5	7.5 ± 0.8	10.2 ± 2.4	8.4 ± 0.2	412.5 ± 26.6	0.001 ± 0.001	0.3 ± 0.6	0.00 ± 0.00	0.01 ± 0.03
	2	3.6 ± 0.3	7.6 ± 0.8	10.6 ± 2.4	8.3 ± 0.5	403.5 ± 8.0	0.003 ± 0.002	0.6 ± 0.6	0.00 ± 0.00	0.01 ± 0.01
	3	2.9 ± 0.3	7.4 ± 0.9	10.7 ± 2.3	8.6 ± 8.6	441.4 ± 23.7	0.001 ± 0.001	0.3 ± 0.5	0.01 ± 0.01	0.02 ± 0.02
	4	3.0 ± 0.3	7.5 ± 0.7	10.6 ± 1.8	8.6 ± 0.1	437.5 ± 24.1	0.002 ± 0.001	1.1 ± 0.9	0.01 ± 0.01	0.04 ± 0.03
	5	2.8 ± 0.2	7.4 ± 0.9	10.3 ± 1.9	8.6 ± 0.1	421.9 ± 13.4	0.003 ± 0.001	0.8 ± 0.6	0.01 ± 0.01	0.04 ± 0.02
Spring	1	2.9 ± 0.1	8.8 ± 0.5	13.3 ± 3.5	8.7 ± 0.4	412.5 ± 26.5	0.002 ± 0.002	0.3 ± 0.7	0.00 ± 0.00	0.32 ± 0.70
	2	2.3 ± 0.4	9.4 ± 0.6	16.8 ± 5.0	8.9 ± 0.1	404.9 ± 6.4	0.006 ± 0.013	1.0 ± 1.9	0.00 ± 0.00	2.70 ± 6.60
	3	1.4 ± 0.5	8.6 ± 1.2	16.7 ± 3.9	8.9 ± 0.5	405.7 ± 9.3	0.019 ± 0.042	0.3 ± 0.6	0.01 ± 0.01	1.30 ± 2.21
	4	1.5 ± 0.4	8.8 ± 1.3	15.7 ± 4.6	8.9 ± 0.5	493.4 ± 38.3	0.017 ± 0.018	1.3 ± 2.0	0.01 ± 0.01	1.40 ± 1.97
	5	1.6 ± 0.7	9.4 ± 0.6	15.5 ± 4.8	9.0 ± 0.3	470.1 ± 28.4	0.016 ± 0.010	0.9 ± 2.0	0.01 ± 0.01	2.90 ± 4.30
Summer	1	2.2 ± 0.2	6.4 ± 0.6	20.7 ± 1.5	8.5 ± 0.4	449.7 ± 21.7	0.000 ± 0.000	0.9 ± 0.7	0.00 ± 0.00	0.07 ± 0.06
	2	1.9 ± 0.2	6.8 ± 0.7	27.0 ± 2.0	8.5 ± 0.3	383.0 ± 7.2	0.000 ± 0.000	0.6 ± 0.8	0.00 ± 0.00	0.06 ± 0.04
	3	1.8 ± 0.1	5.1 ± 0.5	24.0 ± 1.6	8.7 ± 0.4	469.4 ± 18.2	0.003 ± 0.001	1.8 ± 1.7	0.01 ± 0.01	0.16 ± 0.10
	4	1.8 ± 0.1	5.0 ± 1.0	23.4 ± 1.6	8.6 ± 0.3	447.1 ± 19.7	0.003 ± 0.001	1.7 ± 1.6	0.00 ± 0.00	0.14 ± 0.09
	5	1.7 ± 0.1	4.7 ± 0.2	22.6 ± 1.8	8.6 ± 0.4	435.2 ± 24.7	0.004 ± 0.001	1.8 ± 1.4	0.00 ± 0.00	1.10 ± 0.06

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However, as can be seen in Figure 2, DO was found in low concentrations at the bottom depths of St.3 and St.4 in contrast to the surface water in autumn, spring and summer. This may have resulted

from the oxidation events that took place in the bottom layers of the decomposition of organic materials coming from the organized industrial zone.

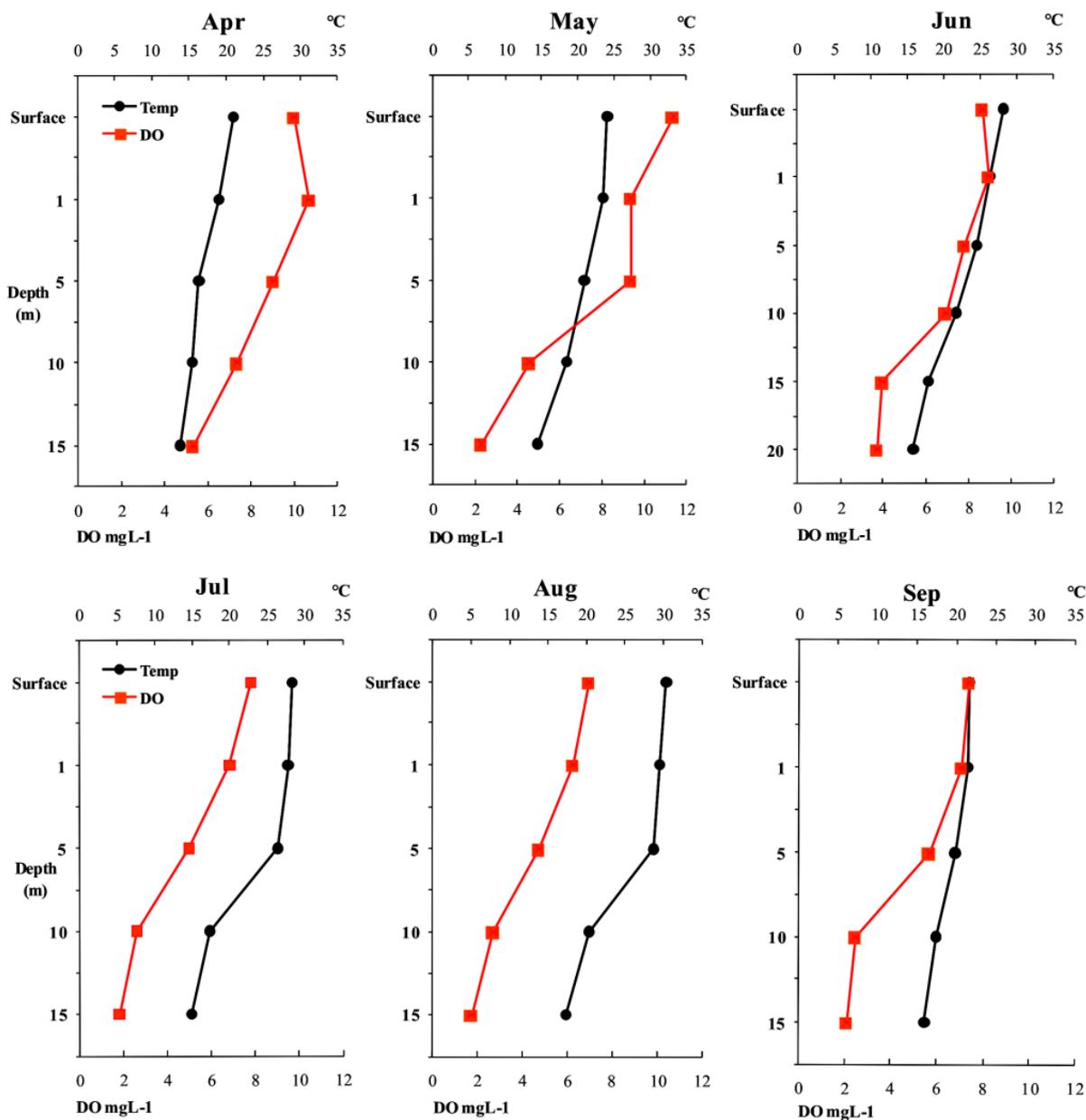


Figure 2. Vertical profiles (every 5 m of depth) of temperature (°C) and dissolved oxygen (mg L<sup>-1</sup>) in KDL.

It was revealed that the temperature was related to pH ( $r= 0.769$ ) and SRP ( $r= 0.680$ ) during the sampling period. Since most of the metabolic reactions of aquatic organisms are pH- dependent, the health of these organisms is affected by pH change (Güher and Öterler, 2020). The pH values generally were in alkaline values (7.2-9.9, January, St.2- April, St.3) in KDL. It was determined by correlation analysis that pH values were related to nitrogen salts ( $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$ ;  $r= 0.656$  and  $r= 0.694$ ,  $p < 0.005$ ; respectively) and SRP ( $r= 0.863$ ).

Electrical conductivity (EC) is closely related to ion concentration and temperature of the water (Akther et al., 2018). This situation was also found in the CCA. The EC value in KDL varies between 289.1 and 680  $\mu\text{S}/\text{cm}^{-1}$  (October 2011, St.1 and October 2010, St.3).

Nitrogens and phosphorous are the major limiting factors for aquatic organisms (Deepika et al., 2019; Dorche et al., 2018). In this study,  $\text{NO}_3\text{-N}$ ,  $\text{NO}_2\text{-N}$ ,  $\text{NH}_4\text{-N}$  and SRP, which are sources of nitrogen and phosphorus, were generally found at low concentrations in KDL (Table 1). The data is compatible with a previous study on the physico-chemical properties of KDL water (Gökçe and Turhan, 2014). Among the studied parameters,  $\text{NO}_3\text{-N}$  concentration was determined higher in the spring season than in other seasons (Table 1). It is thought that this situation may be caused by the entry of agricultural and domestic pollutants into the lake water through precipitation. In a previous study, Arab et al. (2019) reported that the increase in  $\text{NO}_3\text{-N}$  concentration in the aquatic ecosystem is due to the conversion of  $\text{NH}_4\text{-N}$  into  $\text{NO}_3\text{-N}$ , which is discharged into the environment from domestic and agricultural activities.  $\text{NO}_2\text{-N}$ , another nitrogenous compound, is an intermediate oxidation product between  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  (Kükrer and Mutlu, 2019). This close interaction was also seen in the correlation analysis ( $r= 0.943$ ).  $\text{NO}_2\text{-N}$

concentrations were determined at relatively low levels ( $\leq 0.002$  mg/L) in all sampling seasons and increased in summer when compared to other seasons (Table 1).

While the concentrations of  $\text{NH}_4\text{-N}$ , another nitrogenous compound, were generally determined as  $\leq 0.03$  mg  $\text{L}^{-1}$  during the study periods, the highest concentration (0.373 mg  $\text{L}^{-1}$ ) was determined at St.4 in October 2010. The low  $\text{NO}_3\text{-N}$  and  $\text{NO}_2\text{-N}$  concentrations in this period may indicate that the nutrient salts are in the  $\text{NH}_4\text{-N}$  form (Table 1). The highest SRP was determined as 20.33  $\mu\text{g}$   $\text{L}^{-1}$  in March 2011 in St.2. It has been shown in previous studies that there is an increase in the concentration of SRP in the waters of dam lakes in Turkey such as Uzunçayır Dam Lake, Keban Dam Lake and Kayalıköy Reservoir in spring season (Gökçe and Turhan, 2014; Kutlu et al., 2020; Varol, 2020; Güher and Öterler 2020). This increase may have resulted from the agricultural activities carried out around the lake. Hamil et al. (2018) reported that the use of fertilizers containing potassium nitrate may cause increased SRP levels in the aquatic ecosystem.

### Zooplankton composition

During the sampling period, 14 taxa belonging to Rotifera, 6 taxa belonging to Cladocera, 2 taxa belonging to Copepoda equated to a total of 22 zooplankton taxa that were identified (Table 2). The most abundant group was Rotifera (75%), followed by Cladocera (12%) and Copepoda (13%). *S. oblonga* (26560 ind  $\text{m}^{-3}$ ) was the most abundant species of Rotifera, while *B. longirostris* (5040 ind  $\text{m}^{-3}$ ) was the most abundant species of Cladocera. *Notholca squamula* (880 ind  $\text{m}^{-3}$ ) for the rotifer species and *Daphnia longispina* (640 ind  $\text{m}^{-3}$ ) for the cladocera species were detected in very low abundance. *C. scutifer* abundance was about 10720 ind  $\text{m}^{-3}$ , *M. varicans* abundance was 880 ind  $\text{m}^{-3}$ .

**Table 2.** Frequency of zooplankton species in the sampling stations during the study period in KDL

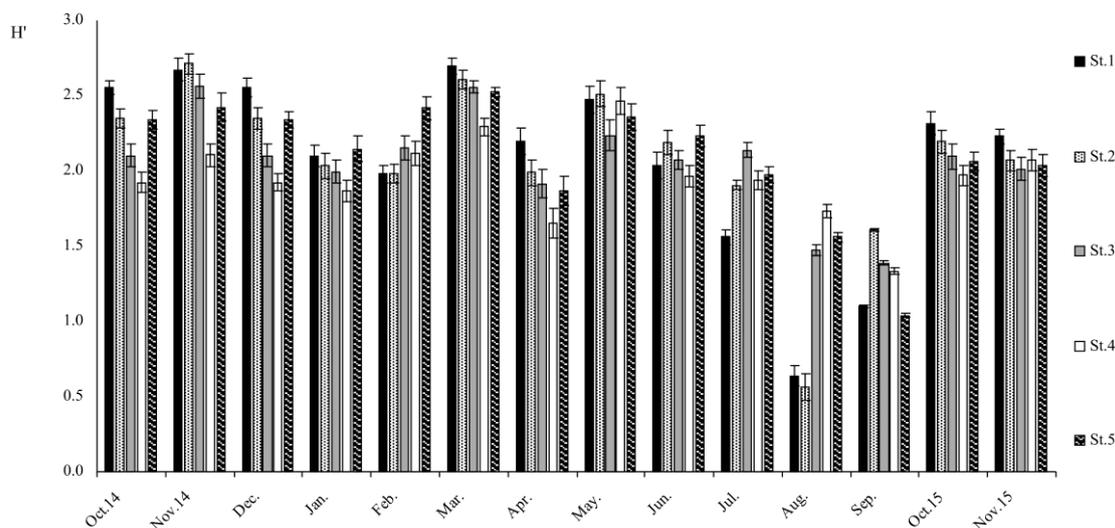
Taxa	St1	St2	St3	St4	St5
<b>Rotifera</b>					
<i>Ascomorpha saltans</i>	****	****	*****	*****	*****
<i>Asplanchna priodonta</i>	*****	*****	*****	*****	*****
<i>Brachionus calyciflorus</i>	*	**	**	**	**
<i>Filinia longiseta</i>	**	**	**	**	**
<i>Keratella cochlearis</i>	*****	*****	*****	*****	*****
<i>K. quadrata</i>	**	**	**	**	**
<i>K. tecta</i>	**	**	**	**	**
<i>Kellicottia longispina</i>	**	**	**	**	**
<i>Lecane luna</i>	*	*	*	*	*
<i>Lepadella patella</i>	**	**	*		
<i>Notholca squamula</i>	*		*		*
<i>Polyarthra dolicoptera</i>	*****	*****	*****	*****	*****
<i>Synchaeta oblonga</i>	*****	*****	*****	*****	*****
<i>Trichocerca similis</i>	**	**		*	**
<b>Cladocera</b>					
<i>Alonarectangula</i>	**	**	**	**	**
<i>Bosminialongirostris</i>	**	**	**	**	**
<i>Ceriodaphniareticulata</i>	**	**	**	**	**
<i>Chydorussphaericus</i>	**	**	**	**	**
<i>Daphnia longispina</i>	*	*	*	*	*
<i>D. cucullata</i>	**	**	**	**	**
<b>Rotifera</b>					
<i>Cyclops scutifer</i>	*****	*****	*****	*****	*****
<i>Microcyclops varicans</i>	*	**	*	*	*
Nauplius	*****	*****	*****	*****	*****

**Table 3.** Density (number of individuals /m<sup>3</sup>, ±SD values) zooplankton species in the sampling stations in KDL

		Density									
Year	Months	St.1		St.2		St.3		St.4		St.5	
2010	October	1520	± 61	1920	± 90	1920	± 98	1280	± 76	2000	± 90
	November	2480	± 121	1920	± 88	2400	± 126	1520	± 100	248	± 141
	December	880	± 67	1440	± 61	1600	± 70	1840	± 67	1680	± 67
2011	January	1840	± 108	1520	± 95	1280	± 78	1120	± 76	1760	± 100
	February	960	± 56	1280	± 74	2720	± 144	2160	± 118	2800	± 130
	March	3120	± 115	2640	± 107	1360	± 55	1680	± 81	1200	± 48
	April	4240	± 254	4160	± 305	5200	± 387	4640	± 408	5360	± 415
	May	5440	± 260	8180	± 383	7120	± 396	10080	± 488	6000	± 320
	June	4080	± 296	4000	± 265	1920	± 109	1680	± 100	1600	± 84
	July	640	± 46	640	± 38	960	± 50	960	± 59	880	± 52
	August	240	± 31	320	± 46	560	± 45	640	± 42	480	± 35
	September	240	± 22	400	± 27	320	± 28	400	± 33	320	± 31
	October	2320	± 111	1840	± 100	1600	± 91	1120	± 66	1200	± 69
	November	1840	± 91	1360	± 76	1600	± 95	1680	± 93	1600	± 93

The abundance of zooplankton was highest in April (23600 ind m<sup>-3</sup>) and May (36820 ind m<sup>-3</sup>), and lowest in July (4080 ind m<sup>-3</sup>), August (2240 ind m<sup>-3</sup>), and September (1680 ind m<sup>-3</sup>) (Table 3). This was due to the increase of phytoplankton in parallel with the increase of temperature and photoperiod in spring. The Shannon–Weaver analysis (H') is an indicator for diversity for water systems. Datta et al. (2010) reported that communities were affected as the stress increases, and accordingly, species diversity decreases with deteriorating criteria of

water quality such as physicochemical properties. Our data demonstrated that KDL is placed in a light state of pollution in fall, winter, and spring, a moderate state of pollution in summer (Table 1). St.5 had the highest diversity (H'=2.09), while St.4 had the lowest diversity of species (H'=1.95). The highest mean diversity was observed in March, (H'=2.53), May (H'=2.40), and the lowest mean diversity was observed in August (H'=1.19) (Figure 3). High levels of nitrate in spring support these results (Table 1).



**Figure 3.** Diversity of zooplankton diversity due to spatial and temporal variation of KDL according to months and sampling stations

### Biochemical assays

In this study, seasonal and regional changes in biomarkers such as CAT, GST, GR, and AChE activities of copepods in KDL were studied. Biomarkers are used to assess the impact of pollutants in aquatic organisms as the first signals of pollution exposure. Numerous studies on aquatic pollution have reported that environmental pollutants cause oxidative stress in aquatic organisms (Barka et al., 2020) (Table 4). Besides pollution, seasonal changes are also indicated as an important factor affecting biomarker responses (Barda et al., 2014). In our study, significant changes were detected in copepod CAT, GR, GST, and AChE activities associated with season (Table 4). On the other hand, interrelated changes were detected between some physico-chemical parameters and some enzyme activities depending on stations and seasons. Similarly, it has been shown in previous studies that interrelated changes were detected between some physico-chemical parameters and biomarkers (Barda et al., 2014; Amira et al., 2018; Pastorino et al., 2020; Djebbi et al., 2021).

In this study, CAT activities of copepods in KDL were higher in spring (Table 4). This increase in CAT activity may be due to changes in energy levels and metabolic activities with the increase in temperature, nutrient amount and reproductive activity in spring (Nahrang et al., 2013). Chainy et al. (2016) reported that seasonal changes in antioxidant defense may be associated with age, reproductive cycle, and food availability. Moreover, many studies reported that higher CAT activities were determined in several aquatic organisms living in polluted fields (Barda et al., 2014; Uluturhan et al., 2019). On the other hand, the fact that the SPR value is relatively high in the spring compared to other seasons (Table 1) may cause an increase in the CAT activity value.

While GR activity did not determine seasonal variation, only St.1 and St.5 determined significant inhibition in the spring (Table 4). Our results showed that AChE activity ranged from 3.36 to 5.26  $\text{nmol min}^{-1} \text{mg protein}^{-1}$ . Similarly, Barka et al. (2020) reported that the AChE activity of copepod collected from the Mcherga reservoir was between 4 and 7  $\text{nmol min}^{-1} \text{mg protein}^{-1}$ .

**Table 4.** Differences in biomarker levels in copepods from KDL according to seasons and sampling stations

Season	Stations	n	GST <sup>a</sup>	GR <sup>a</sup>	CAT <sup>b</sup>	AChE <sup>a</sup>
Autumn	1	3	10.0 ± 1.00	17.7 ± 1.41 <sup>h</sup>	5.14 ± 0.50	4.50 ± 0.16 <sup>u</sup>
	2	3	9.6 ± 0.78 <sup>a</sup>	17.3 ± 1.40	4.89 ± 0.04 <sup>l</sup>	5.07 ± 0.41
	3	3	11.0 ± 1.12 <sup>b</sup>	17.9 ± 0.60	5.65 ± 0.37 <sup>m</sup>	4.75 ± 0.16
	4	3	10.5 ± 2.00	18.2 ± 2.47	5.72 ± 0.52	4.62 ± 0.32 <sup>v</sup>
	5	3	10.1 ± 0.91 <sup>c</sup>	18.1 ± 1.74	5.51 ± 0.45	4.60 ± 0.24 <sup>w</sup>
Winter	1	3	8.0 ± 0.95	17.2 ± 1.31 <sup>j</sup>	5.13 ± 0.43	5.25 ± 0.58 <sup>x</sup>
	2	3	8.1 ± 0.96	15.1 ± 1.15 <sup>1,2</sup>	4.48 ± 0.22 <sup>n,4,5</sup>	4.28 ± 0.44
	3	3	9.5 ± 1.04	19.4 ± 1.26	5.59 ± 0.33 <sup>o,4</sup>	3.87 ± 0.40
	4	3	9.0 ± 0.88	20.4 ± 1.32 <sup>2</sup>	6.00 ± 0.31 <sup>5</sup>	4.48 ± 0.26 <sup>y</sup>
	5	3	8.6 ± 1.02	20.7 ± 0.80 <sup>k,1</sup>	5.38 ± 0.35 <sup>p</sup>	3.76 ± 0.32
Spring	1	3	10.4 ± 0.47 <sup>d</sup>	12.6 ± 1.00 <sup>h,j,3</sup>	5.91 ± 0.25 <sup>6</sup>	4.70 ± 0.37 <sup>z</sup>
	2	3	9.9 ± 0.96 <sup>e</sup>	14.6 ± 0.99	6.52 ± 0.18 <sup>l,n,r</sup>	3.72 ± 0.92
	3	3	12.2 ± 0.73 <sup>f</sup>	16.9 ± 1.18 <sup>3</sup>	7.04 ± 0.32 <sup>m,o,s,6</sup>	3.33 ± 0.65
	4	3	11.7 ± 0.98	15.9 ± 1.15	6.81 ± 0.29	3.42 ± 0.90
	5	3	11.9 ± 0.80 <sup>g</sup>	16.0 ± 1.18 <sup>k</sup>	6.86 ± 0.30 <sup>p</sup>	3.01 ± 0.14
Summer	1	3	6.0 ± 0.60 <sup>d</sup>	17.4 ± 3.00	5.60 ± 0.27	3.58 ± 0.15 <sup>u,x,z</sup>
	2	3	5.8 ± 0.73 <sup>a,e</sup>	17.2 ± 3.09	5.09 ± 0.16 <sup>r</sup>	4.62 ± 1.00
	3	3	7.0 ± 0.50 <sup>b,f</sup>	18.9 ± 4.20	5.95 ± 0.22 <sup>s</sup>	3.36 ± 0.25
	4	3	7.6 ± 1.00	18.3 ± 3.00	6.07 ± 0.41	3.59 ± 0.18 <sup>v,y</sup>
	5	3	6.9 ± 0.20 <sup>c,g</sup>	17.5 ± 2.10	5.73 ± 0.30	3.62 ± 0.15 <sup>w</sup>

<sup>a</sup>: The enzyme activity was indicated as nmol min<sup>-1</sup> mg protein<sup>-1</sup> ± standard error.

<sup>b</sup>: The enzyme activity was indicated as μmol min<sup>-1</sup> mg protein<sup>-1</sup> ± standard error

The same letters indicate the difference when comparing different seasons for the same station ( $p \leq 0.05$ )

The same numbers indicate the difference when comparing different stations for the same season ( $p \leq 0.05$ )

Additionally, it had been observed that AChE activities of copepods in KDL were significantly inhibited in summer. Intense pesticides and fertilizers are used in the summer for gardening around KDL (Varol and Sünbül, 2019). Various neonicotinoid pesticides, including organophosphates known to cause AChE inhibition, are used in apricot orchards around the reservoir (Uluturhan et al., 2019; Djebbi et al., 2021). Results of previous studies showed that the water and sediment of KDL were contaminated with metals such as Fe, Zn, and Cu (Özmen et al., 2006; Gökçe and Özhan, 2011; Ural et al., 2012). Rodriguez et al. (2018) reported that AChE activities in *Paracartia latisetosa* in Lake Faro were at lower levels during the summer. The authors attribute this low enzyme

activity to intensive agricultural activities around the water source.

GST activity in copepods showed the same profile as AChE, with significant inhibition during the summer (Table 4). While the activity of this enzyme increases to reduce the negative effects of pollutants (Amira et al., 2018; Bouzahouane et al., 2018; Vrankovic et al. 2018) it is also known that various pollutants cause inhibition of this enzyme activity in aquatic organisms (Vrankovic et al., 2018). In our study, the inhibition of GST activity in almost all stations in summer may indicate that the pollution is seasonal or that the decrease in activity is due to seasonal physiological changes in sampled organisms (Santos et al., 2021).

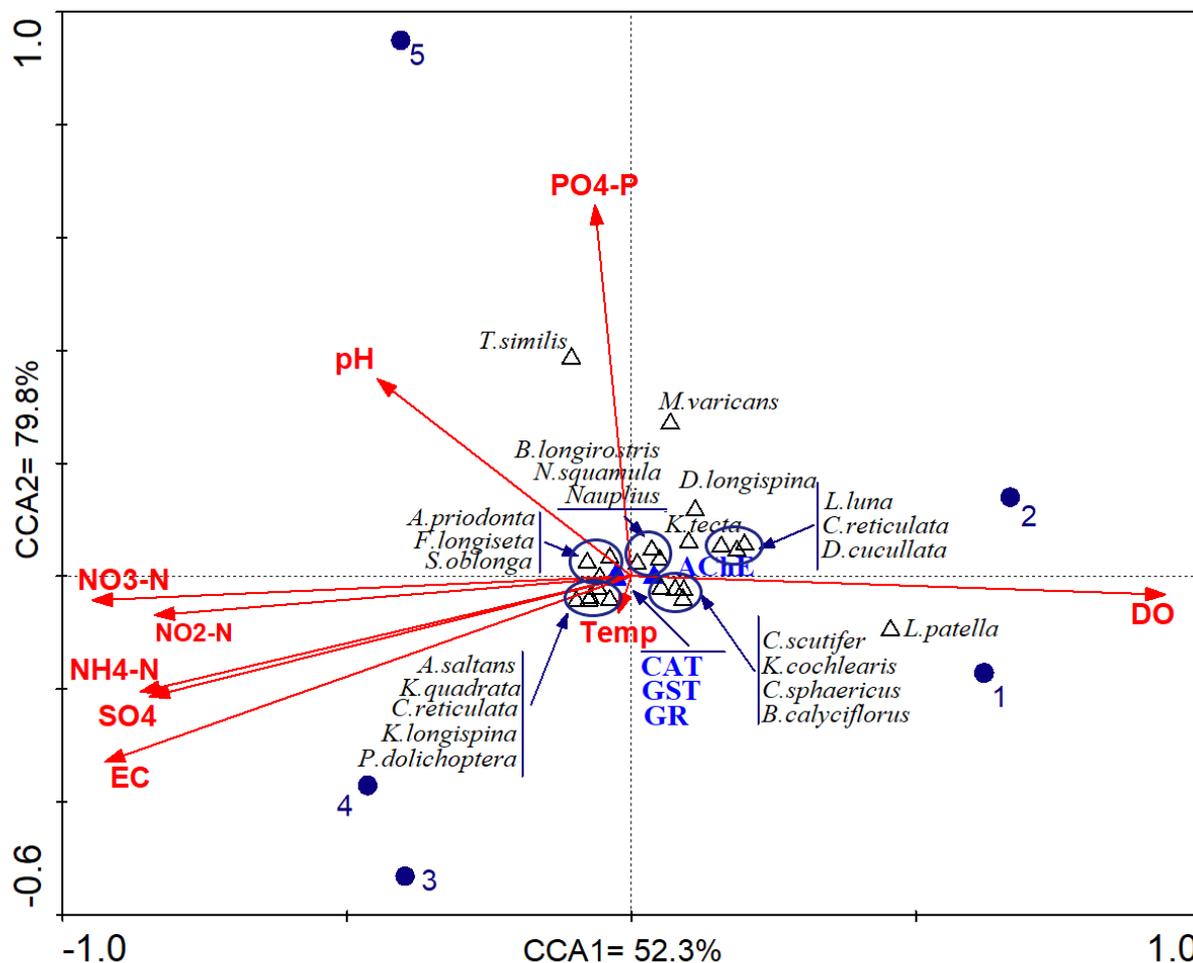
On the other hand, no significant differences were observed between sampling points in GST and AChE activity values (Table 4).

In addition, the decreasing GST and AChE activity in the summer and the increasing CAT activity in the spring may be due to the increase in temperature. Pastorino et al., (2020) reported that temperature may affect biomarkers in aquatic organisms. In addition, increase in water temperature, decrease in DO concentration, and level of lake water in summer may cause a decrease in enzyme activity values in copepod. Moreover, the low diversity of zooplankton in KDL in summer supports that these organisms may be in adverse conditions. Furthermore, the fact that the nitrite concentration is higher in summer than in other

seasons may be another reason for the decrease in enzyme activities. NO<sub>2</sub>-N is known to be toxic to animals and humans (Kükrer and Mutlu, 2019).

**Physico-chemical properties, biomarker, and zooplankton relationships**

Relationships between determined environmental factors, and zooplankton population and copepod enzyme activities of the sample station were evaluated by using CCA. Figure 4 demonstrated the CCA ordination diagram with only the two most important ordination axes. According to correlation analysis, DO and SRP values were found to be significantly related to zooplankton species abundance ( $r= 0.944$  and  $r= 0.887$ ; respectively).



**Figure 4.** CCA triplots for zooplankton abundance and environmental variables (variables are represented by arrows. Species are depicted by points; the numbers indicate sampling stations).

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This triplots diagram demonstrated variations of the structure of zooplankton population composition which can be explained by the eight environmental variables. In particular, while *Trichocerca similis* was closely affected by the amount of SPR, *L. patella*, *C. scutifer*, *K. cohlearis*, *C. sphaericus* and *Brachionus calyciflorus* were affected by the amount of DO. The EC values, SO<sub>4</sub> (r= 0.946), NH<sub>4</sub>-N (r= 0.925) and NO<sub>3</sub>-N (r= 0.890) were seen to be significantly correlated in Figure 4. *Polyarthra dolicoptera*, *Ascomorpha saltans*, *Ceriodaphnia reticulata*, *Kellicottia longispina* and *Keratella quadrata* were found to be closely these variables.

Similar to the evaluations described above, it was observed that the enzyme activities were closely affected by environmental variables in the CCA triplots (Figure 4). The amount of AChE was found to be correlated with DO concentration. CAT, GST and GR enzymes, which are affected by physical-chemical changes in the ecosystem, were found to be related to temperature, pH, NO<sub>3</sub>-N, NH<sub>4</sub>-N, SO<sub>4</sub>, and EC amounts. In this study, the effect of water quality was determined by biochemical activity and diversity of zooplankton in the KDL. Relationships between determined environmental factors, zooplankton population and copepod enzyme activities of the sample stations were displayed by a CCA. Figure 4 showed the CCA ordination diagram with only the two first and most important ordination axes. This diagram shows the patterns of variation in the composition of the zooplankton, which can be explained by the eight environmental variables.

## CONCLUSION

This research provides insight into the implementation of biomarkers with copepod to disclose early signs of possible deterioration to the health of the KDL ecosystem. Some biochemical and physico-chemical parameters which are standard methods were measured in this study. This study indicated that AChE and GST were affected by the environmental parameters. DO and temperature caused inhibition of these biomarker activities in copepod. Consequently, despite the absence of a significant pollution problem as a result of the ecological assessment of the KDL, it is under threat due to the residential areas, agricultural lands around the KDL and decrease a in the water level in summer. Therefore to follow the changes in the

ecosystem the water features and biota in the lake should be monitored continuously. Moreover, this study is the first research to detected biomarker levels of zooplankton in KDL, which would possibly be a reference for the future investigations on the dam lake.

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## CONFLICT OF INTEREST STATEMENT

The author declares that there is no conflict of interest in this study.

## RESEARCH AND PUBLICATION ETHICS STATEMENT

The author declares that the research and publication ethics are complied with in the study.

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