

BODY SIZE VARIATIONS OF *DAPHNIA CUCULLATA* SARS IN A RESERVOIR (ELAZIG-TURKIYE)

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Abstract. *Daphnia cucullata* is one of the most abundant zooplankton species, found in 85 percent of freshwater and as a model aquatic organism it was chosen from this study. That is why this zooplankton species was chosen. The plankton samples were collected from Keban Reservoir from 2020-2023 with a standard plankton net (55- μ m mesh size) at the designated station, Plankton samples were stored in 4% formaldehyde solution. Some water quality parameters were determined by making instant physical measurements with a YSI (EXO 2) model device. Water temperature, pH, electrical conductivity, dissolved oxygen, turbidity (Secchi Disk Depth) and Chlorophyll a were measured. In this study, the four-year body changes of *D. cucullata* in August from 2020 to 2023 were monitored. During four years of progressive eutrophication in Keban Reservoir, we found that among the five morphological parameters analyzed in *D. cucullata* length of head, carapace length, length of tail spine, body length, width of carapace had a tendency to decrease.

Keywords: *Daphnia cucullata, reservoir, morphology, water quality, Türkiye*

Introduction

Increasing pressure exists on water resources and on the cultural over-fertilization of freshwater bodies (Schindler, 2012). Anthropogenic eutrophication is the result of increased discharges of nutrients (mainly nitrogen and phosphorus) directly into lakes, reservoirs, rivers or river basins. Increased nutrient inputs, mainly due to industrial activities and high human population growth, and increased fertilizer use in agriculture, combined with the effects of climate change, may lead to degradation of inland waters (Moss, 2011).

The zooplankton plays a major role in transferring energy between primary producers and higher consumers in the trophic web, and is therefore a major contributor in the recycling of nutrients (Lampert and Sommer, 1997). Physicochemical parameters like temperature, dissolved oxygen, pH, conductivity and turbidity can also determine the composition of the water column (Devetter, 1998; Špoljar et al., 2018). Therefore, zooplankton has the characteristics to be an indicator of environmental conditions and trophic status (Kuczyńska-Kippen et al., 2020) as a number of studies in the past have pointed to this (Gulati, 1983; Sládeček, 1983; Berziņš and Pejler, 1989). In recent years authors have demonstrated the usefulness of zooplankton as indicators of water quality and the trophic state of water bodies by using only one group of zooplankton, that is rotifers (May and O'Hare, 2005; Galir et al., 2018) or microcrustaceans (Pinto-Coelho et al., 2005; Haberman et al., 2007; Cheng et al., 2010; Ejsmont-Karabin and Karabin, 2013), authors have demonstrated the usefulness of zooplankton as indicators of water quality and trophic status of water bodies. Some other studies have considered both zooplankton groups (Brito et al., 2011; Obertegger and Manca, 2014; Haberman and Haldna, 2014; Kehayias and Doulka, 2014; Tasevska, 2017; Pociecha, 2018; Stamou,

2019). Cladocerans are a primarily freshwater monophyletic group. They are a major component of the microcrustacean zooplankton. They inhabit most continental freshwater and saltwater habitats, being more abundant in temporary and permanent reservoirs. Cladocerans are important components of freshwater fauna; they are particularly significant in stagnant water food webs (Forro et al., 2008). Cladocerans are recognized for their trait elasticity with respect to body size in response to both biotic and abiotic factors (Korosi et al., 2008; Havens and Beaver, 2011).

In spite of its wide ecological range, *D. cucullata* shows a considerable variation in body size, with adult females varying in length from 1 mm to 2 mm (Błędzki and Rybak, 2016). However, it is one of the smallest *Daphnia* species. It is characterized by a head that, as a result of cyclomorphosis, forms an exceptionally strong protrusion that ends in a sharp point (helmet). The elongated head, rather than fish pressure, is thought to protect *D. cucullata* from invertebrate predation (Pijanowska, 1990), together with the long tail spine.

Body size is among the most important determinants of organismal function and ecological role (LaBarbera, 1986; Peters, 1983). Biologists have expended considerable effort in documenting patterns of size distribution and evolutionary size change among organisms (McKinney, 1990; Stanley, 1973), as well as in analyzing the consequences of size and size change for various biological attributes. If *Daphnia cucullata* can change morphology in different aquatic ecosystems depending on trophic level, such changes can be expected in a single water body during rapid eutrophication (Bonner, 1988; Calder, 1984; McMahon, 1983; Schmidt-Nielsen, 1984).

This study aimed to qualify *Daphnia cucullata* morphological variation during four years of progressive eutrophication in the Keban Reservoir. This study aimed to determine how morphological patterns such as head length, shell length, tail spine length, body length and shell width of *D. cucullata* changed over the years.

Materials and methods

Study sites

Keban reservoir has been built between 1965 and 1975 for producing electrical energy on the Euphrates River, in the Keban district of Elazig province. The reservoir volume at normal water elevation is 31,000.00 hm³ and the reservoir area is 675.00 km². It is one of the few largest reservoirs in the world and in Turkey. The reservoir is located in the Upper Euphrates Basin of Eastern Anatolia, 40 km northwest of Elazığ, 65 km northeast of Malatya, and 10 km below where the Karasu and Murat Rivers meet, in one of the narrowest straits where the river flows. Keban Reservoir is the largest artificial lake in Turkey after Ataturk Dam Lake with these characteristics. Since electricity is produced in the dam, the minimum water code must remain at a certain level. This situation causes the water code to remain at a certain level. The water level in the reservoir has not changed over the years (Ozgen, 2000).

Sampling methods

The plankton samples were collected from Keban Reservoir in August of 2020-2021-2022 and 2023 with a standard plankton net (55- μ m mesh size) from the designated stations (Fig. 1). Plankton samples were preserved in 4% formaldehyde solution. Some water quality parameters were determined with a YSI (EXO 2) model device.

Temperature, pH, electrical conductivity, dissolved oxygen and turbidity (Secchi Disk Depth) were measured instantaneously in situ. Chlorophyll a, was determined according to standard methods. Total phosphorus (ascorbic acid method 12 after acidification) and total nitrogen (by method of 2.6-dimethylphenol after persulfate separation) were determined by the Hach-Lange DR6000 apparatus (Gales, 1966).



Figure 1. The view of Keban Reservoir that the samples were taken

Morphological analysis

In order to avoid genetic variability in a given year due to parthenogenetic reproduction, the summer season (august) was chosen. In the summer season the proportion of adult females in the whole population of *D. cucullata* is the largest (Wiktor, 1961) and cyclomorphosis changes are the most pronounced (Lampert and Wolf, 1986).

We analyzed morphological variability in 20 populations of *D. cucullata* using under an inverted microscope (GMBH D-6330 diavert inverted microscope, Earnst Leitz Ltd., Canada) and identified under a compound microscope (Nikon Eclipse E 100, Nikon Instruments Inc., Japan). The measured features were length of head, B- carapace length, C-length of tail spine, D-body length, E-width of carapace (*Fig. 2*). The measurements of morphological parameters were done three times for each specimen and then the mean values were used for analyses. *D. cucullata* is characterized by a head which forms an extraordinarily strong protrusion that ends in a sharp, somewhat dorsally curved point (*Fig. 2*).

Results

During four years of progressive eutrophication in Keban Reservoir, the biomass of Daphniidae, mostly represented by *D. cucullata*, clearly increased. Additionally, Daphniidae abundance remained remarkably unchanged (*Fig. 3*). In the 2020 years of

reservoir, *Daphnia cucullata* (G.O. Sars), *Daphnia longispina* (O.F. Muller), *D. magna* (Straus), *Bosmina longirostris* (O.F. Muller) and *Chydorus sphaericus* (O.F. Muller), were the most frequently encountered species among cladocerans. In 2023, *D. longispina* almost disappeared from the reservoir.

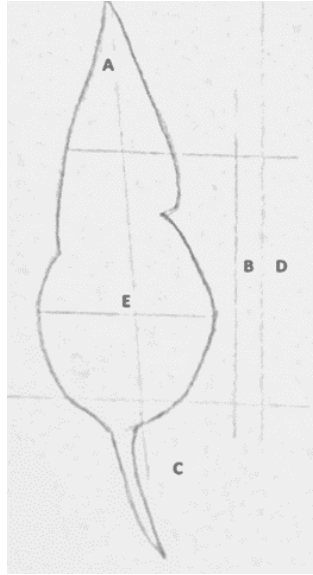


Figure 2. *Daphnia cucullata* morphometric measurements: (A) length of head, (B) carapace length, (C) length of tail spine, (D) body length, (E) width of carapace (original)

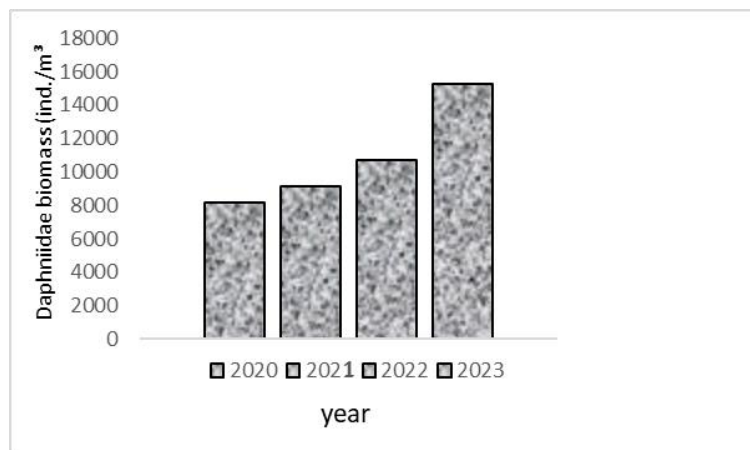


Figure 3. Annual changes of Daphniidae biomass (ind./m³)

The minimum water temperature recorded in Keban reservoir was 22°C in 2020, while the maximum was 25°C in 2023. In 2020, the lowest pH value observed was 6.9, rising to a high of 7.9 in 2023. The lowest value of dissolved oxygen was 6.3(mg/L) in 2021, while the highest was 6.9(mg/L) in 2022. In 2020, the minimum recorded electrical conductivity value was 376(μS cm⁻¹), increasing to a high of 429 (μS cm⁻¹). Turbidity measurements ranged from a minimum of 2.9 (m) in 2023 to a maximum of 5.4 (m) in 2020. While chlorophyll-a value was 2.85 (μg/L) in 2020, the peak value was 3.55(μg/L) in 2023. Total phosphorus and total nitrogen values increased over the years (Table 1).

Table 1. Annual changes of some water quality parameters of Keban Reservoir

	2020	2021	2022	2023
Water temperature (°C)	22	23	24	25
pH	6.9	7.1	7.4	7.9
Dis. Oxygen (mg/L)	6.7	6.3	6.9	6.5
Electrical conductivity ($\mu\text{S cm}^{-1}$)	376	399	415	429
Secchi disk (m)	5.4	4.8	3.4	2.9
Chl-a $\mu\text{g/L}$	2.85	3.1	3.45	3.55
Total phosphorus (mgP/L)	0.020	0.025	0.030	0.035
Total nitrogen (mgN/L)	0.50	0.55	0.60	0.66

Changes in chlorophyll concentrations have been detected in the last 4 years. Trophic Status Index according to Carlson calculated based on summer chlorophyll has clearly increased. The summer Secchi depth gradually decreased from 5.4 m in 2020 and fell below 2.9 m in 2023. Five morphological trait values tended to decrease over time: length of head, carapace length, length of tail spine, body length and width of carapace a decreasing *Daphnia cucullata* as an indicator of the eutrophic status of lakes.

Statistical analysis

The data obtained from the research were analyzed using SPSS (Statistical Package for Social Sciences) 24.0 program. Descriptive statistics, t-test and ANOVA were used to evaluate the data. In the analysis and interpretation of the data, normality distributions were first made according to the skewness and kurtosis values of the research results. Since the standard deviation values (standard error) were between $-1.96 < X > 1.96$, it was accepted that the data were normally distributed (Tabachnick and Fidell, 2013; George and Mallery 2010). Accordingly, the one-way ANOVA test and frequency analysis, which are parametric tests, are also parametric tests; Cohen's was used for the measurement of impact values. Therefore, using the criteria suggested by Cohen (1988), these criteria are; 0.01 was considered a small effect, 0.06 was considered a medium effect, and 0.14 was considered a large effect (Table 2).

Table 2. Skewness and Kurtosis values of morphological measurements of *D. cucullata*

	Skewness		Kurtosis	
	Value	SE	Value	SE
Length of head	0.991	0.269	-0.009	0.532
Carapace length	0.599	0.269	0.106	0.532
Length of tail spine	0.990	0.269	1.413	0.532
Body length	0.888	0.269	0.808	0.532
Width of carapace	0.838	0.269	-0.193	0.532

ANOVA results revealed that there were significant changes among the parameters. Five morphological traits of *D. cucullata* were found to differ over time (2020-2023) and there was a significant difference between years. According to the ANOVA results, length of head (18.965; $p < 0.01$), carapace length ($F = 18.965$; $p < 0.01$), Carapace

length ($F = 30.240$; $p < 0.01$), Length of tail spine ($F = 13.904$; $p < 0.01$), Body length ($F = 15.919$; $p < 0.01$), Width of carapace ($F = 18.964$; $p < 0.01$) showed that these morphological characteristics decreased over the years and there was a significant difference between these characteristics and the year variable (*Table 3*).

When *Table 4* is examined, it is seen that the values of five morphological traits of *D. cucullata* tended to decrease over time. Length of head ($r = -0.653$, $p < 0.01$), length of carapace ($r = -0.737$, $p < 0.01$), length of tail spine ($r = -0.588$, $p < 0.01$), body length ($r = -0.621$, $p < 0.01$), width of carapace ($r = -0.648$, $p < 0.01$) are negatively and highly correlated.

Table 3. One-way ANOVA testing differences between morphological measurements on *Daphnia cucullata* in years 2020–2023

	Year	N	\bar{x}	ss		Sum of squares	df	Mean square	F	P
Length of head	2020	20	33.90	9.83	Between groups	2628.938	3	876.313	18.965	0.000
	2021	20	28.35	7.75	Within groups	3511.750	76	46.207		
	2022	20	22.80	4.31	Total	6140.688	79			
	2023	20	18.70	3.06		8760.050	3			
Carapace length	2020	20	50.95	12.54	Between groups	7338.700	76	2920.017	30.240	0.000
	2021	20	40.10	9.70	Within groups	16098.750	79	96.562		
	2022	20	32.95	9.62	Total	2285.437	3			
	2023	20	22.50	6.49		4164.050	76			
Length of tail spine	2020	20	33.65	7.72	Between groups	6449.487	79	761.812	13.904	0.008
	2021	20	26.75	8.60	Within groups	13114.700	3	54.790		
	2022	20	23.60	7.40	Total	20871.100	76			
	2023	20	18.95	5.52		33985.800	79			
Body length	2020	20	79.75	22.78	Between groups	6814.138	3	4371.567	15.919	0.000
	2021	20	66.80	15.84	Within groups	9102.850	76	274.620		
	2022	20	56.05	11.50	Total	15916.988	79			
	2023	20	45.20	14.00		2628.938	3			
Width of carapace	2020	20	44.95	12.81	Between groups	3511.750	76	2271.379	18.964	0.000
	2021	20	38.55	14.03	Within groups	6140.688	79	119.774		
	2022	20	27.00	9.85	Total	8760.050	3			
	2023	20	21.55	4.55						

Table 4. Pearson correlation analysis results on the relationship between *D. cucullata*'s five morphological feature values and years

Correlations						
	Year	Length of head	Carapace length	Length of tail spine	Body length	Width of carapace
Year	1					
Length of head	-0.653**	1				
Carapace length	-0.737**	0.630**	1			
Length of tail spine	-0.588**	0.497**	0.689**	1		
Body length	-0.621**	0.662**	0.906**	0.654**	1	
Width of carapace	-0.648**	0.635**	0.841**	0.665**	0.801**	1

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

Discussion

Zooplankton is widely accepted as an important indicator of trophic status in freshwaters. However, zooplankton was not considered as biological indicators in the EU Water Framework Directive (Ejsmont-Karabin and Karabin, 2013). Nevertheless, indicators of trophic status based on the community of rotifers and crustaceans are commonly used for the assessment of the trophic status of lakes (Liang et al., 2020).

We observed changes in the morphological structure of *Daphnia cucullata* in Keban reservoir. Reduction in body size was the most obvious change. This change was manifested not only in the length of the head, but also in a decrease in the values of all measured morphological features. Comparison of annual changes in *D. cucullata* biomass also confirmed this (Fig. 2). Some studies have shown that as the trophic level increases, the body size of *D. cucullata* decreases (Karpowicz et al., 2020).

It is thought that the main reason for the changes in the body parameters of *D. cucullata* is the increase in total some water quality parameters. The increase in chlorophyll a, total phosphorus and total nitrogen depending on the years may have caused to decrease the morphological characteristics of *D. cucullata*. As eutrophication increases, small-bodied species will become increasingly numerous and dominate over larger-bodied cladocerans (Hillbricht-Ilkowska, 1977). Secchi disk visibility (SDV) is a key visual indicator of water clarity and is commonly used as a key indicator of eutrophication (Sługocki and Czerniawski, 2018).

Nürnberg (1996) reported the Secchi disk depth as > 4 m in oligotrophic lakes, 2-4 m in mesotrophic lakes and 1-1.9 m in eutrophic lakes. OECD (1982) stated that the Secchi disk depth of oligotrophic lakes is 5.4-28.3 m (average 9.9 m), the Secchi disk depth of mesotrophic lakes is 1.5-8.1 m (average 4.2 m) and the secchi disk depth of eutrophic lakes is 5.4-28.3 m (average 9.9 m). disc depth 0.8-7.0 m (average 2.45 m) noted that it varies between.

Wetzel et al. (1975) stated that ultraoligotrophic lakes have total phosphorus contents ranging from < 1-5 µg/L, oligomesotrophic lakes from 5-10 µg/L, and mesoeutrophic lakes from 10-30 µg/L.

Whittaker (1970) found that oligotrophic lakes have total phosphorus concentrations < 1-5 µg/L, mesotrophic lakes have total phosphorus concentrations 5-10 µg/L, and eutrophic lakes have total phosphorus concentrations 10-30 µg/L; Taylor et al. (1970) found that oligotrophic lakes contain <10 µg/L total phosphorus, mesotrophic lakes contain 10-30 µg/L total phosphorus, and eutrophic lakes contain >30 µg/L total phosphorus. Based on this, it can be said that this reservoir is eutrophic regarding total phosphorus content.

Hakanson and Jansson (1983) found oligotrophic lakes have total nitrogen contractions to be <0.35 mg N/l and mesotrophic lakes to be 0.35-0.50 mgN/L; Nürnberg (1996) found oligotrophic lakes to be < 0.35 mg N/L and mesotrophic lakes to be 0.35-0.65 mg N/L. Based on this, it can be said that Keban Reservoir is mesotrophic in terms of total nitrogen content. The increase in these parameters over the years may have caused the body change of *D. cucullata*.

The larger species, *D. magna*, disappeared in reservoir and in the following period *D. longispina* was found only occasionally. The morphology of *Daphnia cucullata* in the Keban Reservoir (2020-2023) rapidly changing water could be influenced by factors such as food availability, fish predation and exclusive presence of *D. cucullata* among Daphniidae.

D. cucullata has a wide range of ecological tolerance. It occurs in oligotrophic (Karpowicz et al., 2020) as well as hypertrophic lakes (DeMott et al., 2001; Górnaiak and Karpowicz, 2014). A decrease in body length of *D. cucullata* under more eutrophic conditions has been observed previously (Patalas and Patalas, 1966; Smakulska and Górnaiak, 2004). This was confirmed by our results. Dense populations of planktivorous fish may have contributed to the reduction in biomass and body size of *D. cucullata*. Hrbacek and Hrbackova-Esova (1959, 1960), detected the presence of ‘dwarf’ *D. cucullata* in lakes with dense fish populations. investigations into the dynamics of aquatic ecology will be greatly enriched by an increased understanding of water quality controls on zooplankton taxonomic composition and size spectra.

Conclusions

D. cucullata's wide distribution and size variability under different trophic conditions make it a good biological indicator of inland waters ecological status. Another advantage of *D. cucullata* as a lake trophic state indicator is that it is easy to sample and does not require special taxonomic knowledge, which other biological indicators (i.e. phytoplankton, phytobenthos, macroinvertebrates) require. This study showed that the body size of *D. cucullata* decreased as the trophic level of the waters increased.

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