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External morphological variations and temporal distribution of the dinoflagellate *Ceratium hirundinella* in two dam reservoirs in the Tigris River basin (Turkey)

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Abstract: Seasonal distribution, abundance, and external morphological changes of *Ceratium hirundinella* in the Kralkızı and Dicle dam reservoirs on the Tigris River were investigated during 1 year (February 2008 to January 2009). This species was present in both dam reservoirs during all 4 seasons. The highest mean density of *C. hirundinella* in Kralkızı Dam Reservoir was recorded in December, while in Dicle Dam Reservoir it was highest in June. *C. hirundinella* cells had a fourth horn in both dam reservoirs during the study period, and morphologically they showed the highest relative standard deviation in the fourth horn. In addition, a new fifth horn occurred on some cells, always between the antapical and postequatorial horns. Total length, length of antapical horn, and length of the fourth horn of *C. hirundinella* displayed significant seasonal variations ($P < 0.05$). The total cell length showed significant correlations with the length of the apical horn and the length of the antapical horn.

Key words: *Ceratium hirundinella*, external morphological changes, seasonal distribution, abundance

1. Introduction

The species *Ceratium hirundinella* (O.F.Müller) Dujardin is a widespread freshwater planktonic dinoflagellate in lakes and reservoirs in temperate regions (Wetzel, 1983; Reynolds, 1984; Heaney et al., 1988; Padisak et al., 2003). *C. hirundinella* populations often appear in the spring in these systems, reach maximum abundance during the summer, and decrease in the autumn during the mixing period (Padisak, 1985; Perez-Martinez and Sanchez-Castillo, 2002). However, *C. hirundinella* is also a very common species throughout the winter period, and it is present in the phytoplankton community during all seasons in some Mediterranean Spanish reservoirs (Perez-Martinez and Sanchez-Castillo, 2001) and Croatian lakes (Gligora et al., 2003). *C. hirundinella* is one of the few phytoplankton species that shows external morphological changes (Lindström, 1992; Gligora et al., 2003; Parodi et al., 2007). The changes include variations in cell size, length of the various horns, and occurrence of a fourth horn (Huber-Pestalozzi, 1950; Hutchinson, 1967).

The aim of the present study is to describe the abundance, seasonal distribution, variations in total cell length, width of cingulum and length of the different horns, and presence or absence of the fourth horn of *Ceratium hirundinella* in the Kralkızı and Dicle dam reservoirs on the Tigris River (Turkey).

2. Materials and methods

2.1. Study area

The Kralkızı Dam Reservoir (KDR) and Dicle Dam Reservoir (DDR) are located within the boundaries of Diyarbakır Province in the Tigris River basin (Turkey) (Figure 1). The KDR (38°19′59″N to 38°25′20″N, 39°47′16″E to 40°01′37″E; 710 m a.s.l.) has a surface area of 57.5 km^2 and a volume of 1919 hm^3. The Kralkızı Dam was constructed in 1997 for hydroelectric power generation. The Dicle Dam Reservoir (8°13′49″N to 38°25′09″N, 40°01′00″E to 40°14′16″E, 710 m a.s.l.) has a surface area of 24 km^2 and a volume of 595 hm^3. The Dicle Dam was also constructed in 1997 for hydroelectric power generation, irrigation, and drinking water (Varol et al., 2012).

The continental climate of the Tigris River basin is a subtropical plateau climate and is similar to those of the Mediterranean region. According to meteorological measurements between 1970 and 2010, the average annual temperature in Diyarbakır was approximately 15.7 °C, with a maximum of 38.5 °C in summer and a minimum of –2.5 °C in winter. The average annual precipitation was 463 mm, 89% of which fell between October and April (Varol, 2013).

2.2. Sampling and analysis

In this study, 7 sampling sites were selected on the dam reservoirs. Four sites (K-1, K-2, K-3, and K-4) were located

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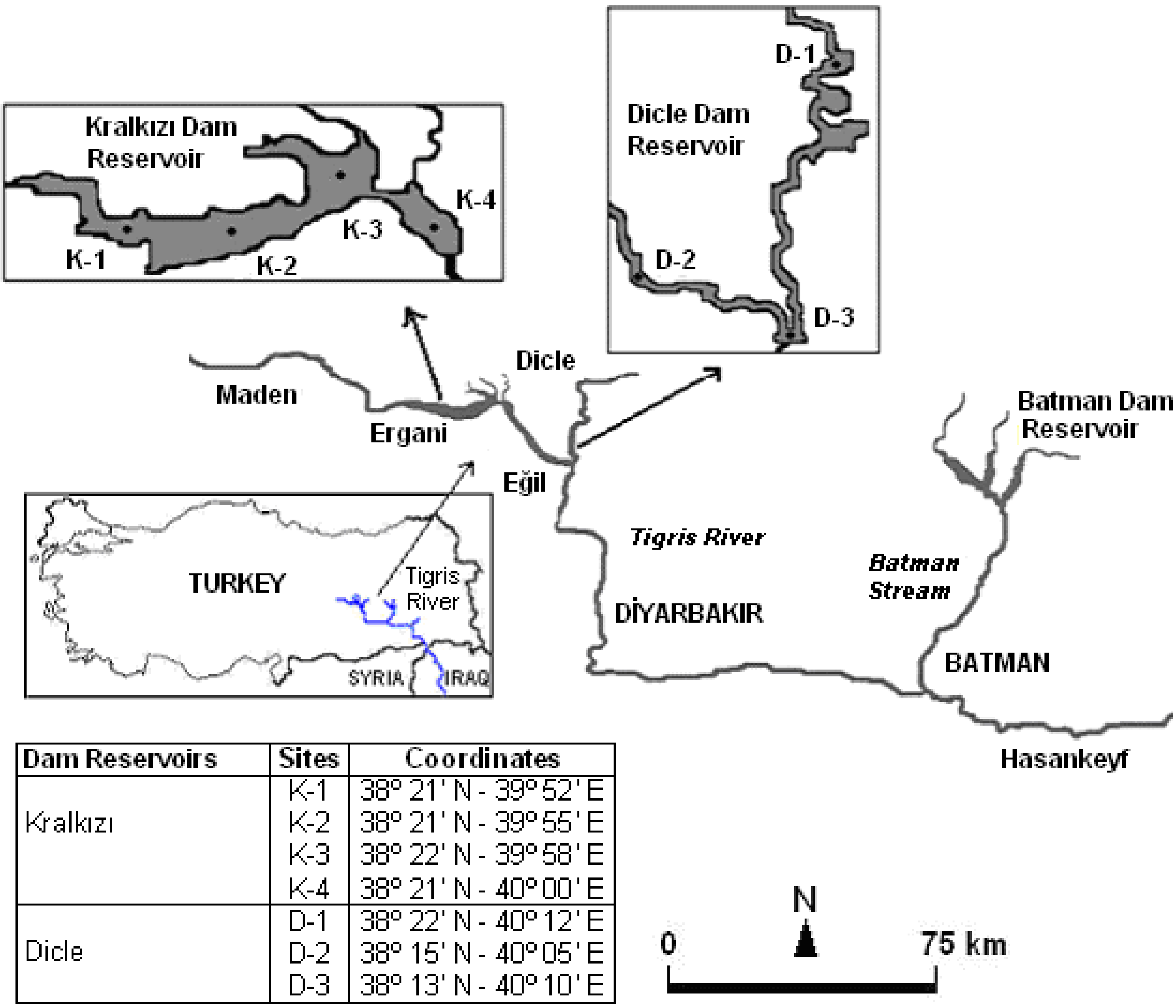

Dam Reservoirs	Sites	Coordinates
Kralkızı	K-1	38° 21' N - 39° 52' E
	K-2	38° 21' N - 39° 55' E
	K-3	38° 22' N - 39° 58' E
	K-4	38° 21' N - 40° 00' E
Dicle	D-1	38° 22' N - 40° 12' E
	D-2	38° 15' N - 40° 05' E
	D-3	38° 13' N - 40° 10' E

Figure 1. Map showing the sampling sites on the Kralkızı and Dicle dam reservoirs in the Tigris River basin (Turkey). Reproduced from Varol (2013).

in the KDR and 3 sites (D-1, D-2, and D-3) were located in the DDR (Figure 1). Phytoplankton samples were taken monthly between February 2008 and January 2009.

Samples for qualitative analysis of phytoplankton were collected using a plankton net with 55-µm mesh size, while quantitative samples were taken with a 1-L Ruttner water sampler. All samples were preserved with formaldehyde in the field. For quantitative phytoplankton analysis, phytoplankton cells were counted with an inverted microscope (Olympus CKX-41), using Utermöhl's sedimentation method (Utermöhl, 1958). For qualitative analysis, the samples were examined with an Olympus BX51 microscope equipped with an Olympus DP71 digital camera. Identification of *Ceratium hirundinella* was carried out according to Huber-Pestalozzi (1950).

Water samples were also taken simultaneously with the phytoplankton samples. The samples were analyzed for 9 parameters including water temperature, pH, dissolved oxygen, electrical conductivity, turbidity, nitrate nitrogen, orthophosphate phosphorus, calcium, and magnesium. Water temperature, pH, dissolved oxygen, and electrical conductivity were measured in the field with a portable multimeter. All other parameters were determined in the laboratory following standard protocols (APHA, 1999).

Five individuals of *Ceratium hirundinella* cells per sample were analyzed for the biometric studies. Total length and cingulum width and the lengths of apical horn, antapical horn, postequatorial horn, and fourth horn of all examined individuals were determined. Lengths of horns were measured from the base of the horn to the end point (Figure 2). All measurements were performed using

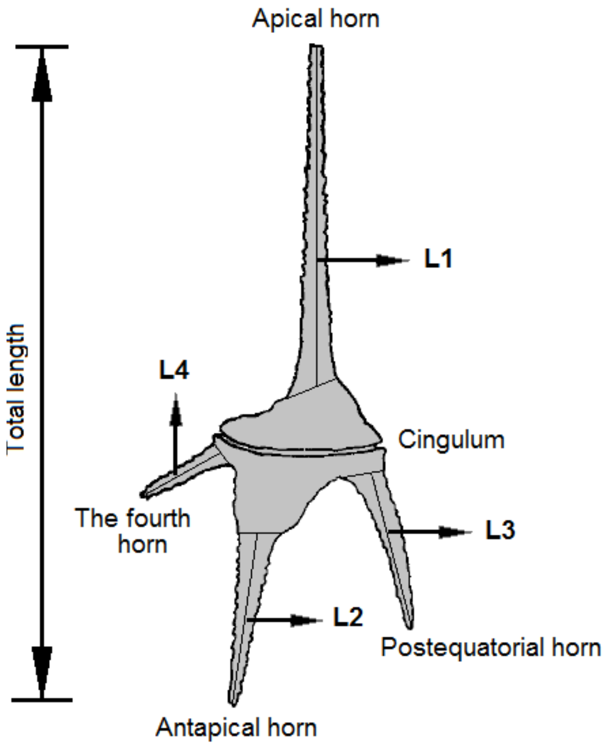

Figure 2. Scheme of dorsal view of *Ceratium hirundinella* cell with some morphological terms (L1: length of apical horn; L2: length of antapical horn; L3: length of postequatorial horn; L4: length of the fourth horn).

Image Analysis Pro 5.0 software (Olympus Soft Imaging Solutions GmbH).

The relationships among the dimensions of the different horns and the cingulum, and water quality parameters and *Ceratium hirundinella* density, were tested by using correlation analysis with statistical significance set at P < 0.05. Analysis of variance (ANOVA) and the Kruskal–Wallis test were performed to analyze the significant spatial and temporal differences (P < 0.05).

3. Results

Minimum, maximum, and mean values of the water quality parameters measured in Kralkızı and Dicle dam reservoirs are presented in Table 1.

C. hirundinella was present in the Kralkızı Dam Reservoir during all 4 seasons; it showed the highest densities in the autumn and winter seasons (ANOVA, P < 0.05). Population density of *C. hirundinella* decreased from February to June and reached its minimum in July. Abundance of the species increased from August to December, in which the highest mean density was recorded (14,000 cells/L) (Figure 3). In addition, the number of *C. hirundinella* cells did not show significant spatial variations among the 4 sampling sites (ANOVA, P > 0.05). Water quality parameters did not show significant correlation with *C. hirundinella* density in the KDR. Cysts of *C. hirundinella* were not observed in the KDR during the study period.

C. hirundinella was present in all seasons during the study period in the DDR. Although the mean numbers of cells were higher in summer and autumn, there were no statistically significant differences among the seasons (ANOVA, P > 0.05). The cells of *C. hirundinella* remained

Table 1. Mean, minimum, and maximum values of water quality parameters in Kralkızı and Dicle dam reservoirs.

	Kralkızı Dam Reservoir			Dicle Dam Reservoir		
	Mean	Min	Max	Mean	Min	Max
WT (°C)	17.34	4.40	27.20	17.89	4.00	26.60
pH	8.48	8.16	8.70	8.48	7.88	8.94
DO (mg/L)	8.81	6.84	11.40	9.54	8.18	13.25
EC (µS/cm)	278	252	308	293	230	353
Turb (NTU)	1.4	0.3	3.6	1.3	0.2	5.9
NO_3-N (mg/L)	0.141	0.002	0.483	0.206	0.005	0.886
PO_4-P (mg/L)	0.037	0.017	0.099	0.038	0.010	0.104
Ca (mg/L)	38.06	30.12	57.85	41.05	28.97	76.88
Mg (mg/L)	9.87	8.46	11.32	9.03	6.88	11.31

WT (water temperature), DO (dissolved oxygen), EC (electrical conductivity), Turb (turbidity), NO_3-N (nitrate nitrogen), PO_4-P (orthophosphate phosphorus), Ca (calcium), and Mg (magnesium).

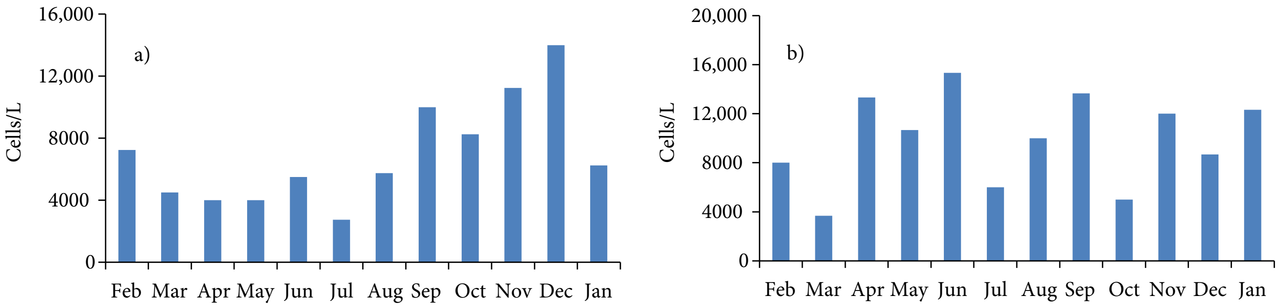

Figure 3. Seasonal distribution of the mean density of *Ceratium hirundinella* cells in Kralkızı (**a**) and Dicle (**b**) dam reservoirs.

at the lowest mean density in March. The highest mean densities were recorded in June (15,330 cells/L) and September (13,670 cells/L) (Figure 3). In the DDR, *C. hirundinella* density did not show significant spatial variations (ANOVA, P > 0.05). Physical and chemical variables were also not significantly correlated with *C. hirundinella* density in the DDR (P > 0.05). Cysts of *C. hirundinella* were observed rarely in November and December samples (Figure 4).

In the KDR, total length, length of antapical horn, and length of fourth horn of *C. hirundinella* displayed significant seasonal variations (Kruskal–Wallis, P < 0.05) among 12 months. The total cell length ranged between 220.0 and 331.4 µm (Table 2). The greatest length was observed in June, and the shortest length was recorded in August. High positive correlations (P < 0.05, r^2 = 0.74 to 0.79) were observed between total length, length of the apical horn, and length of the antapical horn (Figure 5; Table 3). The length of the apical horn was measured to be 124.2 ± 13.9 µm. It was shortest in August, September, and January (102.9 µm), while it was at a maximum in June (162.9 µm). The length of the antapical horn ranged between 54.3 and 105.7 µm, while the postequatorial horn was between 28.6 and 74.3 µm. The longest antapical and postequatorial horns were recorded in June. All cells of *C. hirundinella* had a fourth horn during the investigation period. However, the length of the fourth horn changed throughout the study period (2.9–42.9 µm), and the longest fourth horn was observed in October. In February, March, and January, the fourth horn was very short (mean length of 2.9 µm). Width of the cingulum was 80.3 ± 8.5 µm, and it was widest in November. In September, some individuals with 5 horns were observed (Figure 4). The new horn occurred between the antapical and postequatorial horns.

The Kruskal–Wallis test indicated that there were significant seasonal differences in the medians of total length, length of apical horn, length of antapical horn, length of postequatorial horn, length of fourth horn, and width of cingulum of *C. hirundinella* in the DDR. The total cell length ranged from 231.4 to 362.9 µm with an average of 284.56 µm (Table 3). The greatest length was recorded in May. The total cell length showed significant correlations with all measurements (Table 3), but it was strongly correlated with length of the apical horn (P < 0.05, r^2 = 0.90) and length of the antapical horn (P < 0.05, r^2 = 0.83) (Figure 5). The apical horn and antapical horn showed similar seasonal changes (P < 0.05, r^2 = 0.69). The length of the apical horn ranged between 91.4 and 185.7 µm, while the length of the antapical horn ranged between 48.6 and 102.9 µm. The longest apical and antapical horns were observed in June. The postequatorial horn was 57.96 ± 11.3 µm long. *C. hirundinella* had a fourth horn during all months in the DDR, and it reached 60 µm long in May. Width of the cingulum was 86.9 ± 8.4 µm; it was smaller in the summer season. In June and November, some individuals had 5 horns (Figure 4). The new horn was observed between the antapical and postequatorial horns.

4. Discussion

Ceratium hirundinella is generally found during the summer in warm waterbodies in temperate regions (Padisak, 1985; Heaney et al., 1988; Rengefors et al., 1998). However, it was present during all seasons in the Kralkızı and Dicle dam reservoirs. Similarly, Perez-Martinez and Sanchez-Castillo (2001) found that *C. hirundinella* was present during all seasons in 70% of 100 Spanish reservoirs examined. For the development of *C. hirundinella*, water temperature values must range between 4 and 23 °C, with optimum development at 12–23 °C (Heaney et al., 1983, 1988; Lindström, 1992; Rengefors et al., 1998; MacDonagh et al., 2005). Because water temperature values ranged from 4.4 to 27.2 °C in Kralkızı and 4 to 26.6 °C in Dicle, water temperature in the Kralkızı and Dicle dam reservoirs would allow for *C. hirundinella* development throughout the year. In the Bermejales Reservoir (Spain), this species was present during all seasons, since the temperature values were between 7.8 and 24 °C (Perez-Martinez and

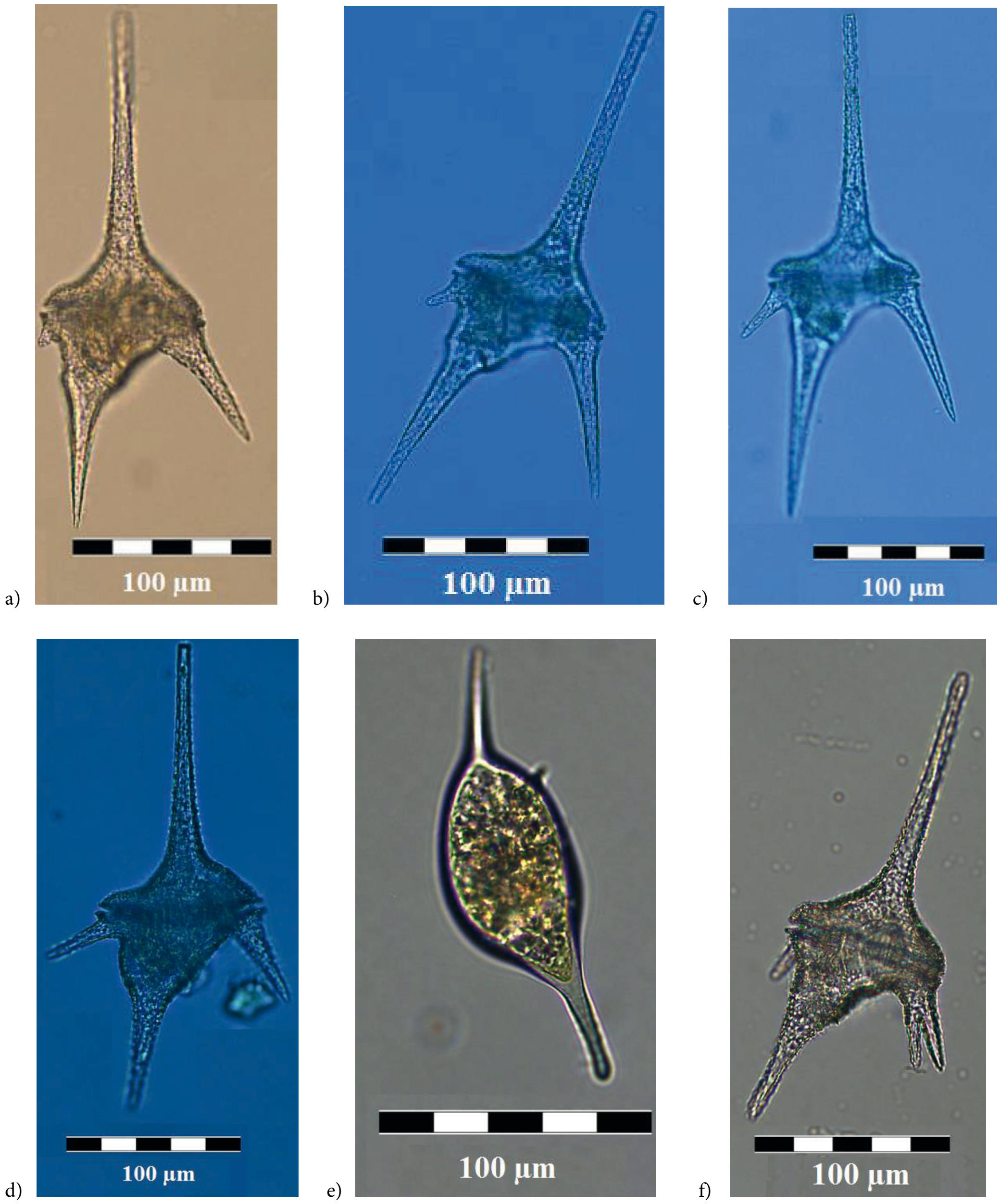

Figure 4. Some photomicrographic figures of *Ceratium hirundinella* cells in both dam reservoirs: **a–d**) cells with 4 horns; **e**) cyst; **f**) cell with 5 horns.

Sanchez-Castillo, 2002). In addition, it was reported that the species was also common in Ponikve and Njivice lakes (Croatia) throughout the year (Gligora et al., 2003).

The length and number of horns and the cell size of *C. hirundinella* could change seasonally. Such morphological variations have been attributed to a buoyancy adaptation of the cells to seasonal changes in water density and viscosity because of temperature changes (Hutchinson, 1957). Dottne-Lindgren and Ekbohm (1975) reported that the variation in length of *C. hirundinella* was due to changes in the metabolism of the cells. However, some studies (Lindström, 1992; Bertolo, 2010) suggested that external morphological changes in *C. hirundinella* improve protection against predators, such as zooplankton grazers and planktivorous fish. The morphological variability of *C. hirundinella* is likely to be related to predation or changes in environmental factors such as water temperature and concentration of nutrients in Kralkızı and Dicle dam reservoirs, too.

Table 2. Total length, length of various horns, and width of cingulum (in µm) of *C. hirundinella* cells in Kralkızı and Dicle dam reservoirs.

	Total length	Apical horn	Antapical horn	Postequatorial horn	Fourth horn	Cingulum
Kralkızı						
Mean	272.6	124.2	74.1	56.9	10.9	80.3
Median	271.4	125.7	74.3	57.1	8.6	80.0
Minimum	220.0	102.9	54.3	28.6	2.9	65.7
Maximum	331.4	162.9	105.7	74.3	42.9	102.9
Std. dev.	24.4	13.9	12.4	11.4	10.4	8.5
Relative std. dev.	9.0	11.2	16.8	20.1	96.0	10.5
Dicle						
Mean	284.6	130.1	75.9	58.0	23.4	86.9
Median	282.9	128.6	72.9	58.6	24.3	85.7
Minimum	231.4	91.4	48.6	28.6	2.9	71.4
Maximum	362.9	185.7	102.9	77.1	60.0	105.7
Std. dev.	30.6	19.7	14.2	11.3	16.4	8.4
Relative std. dev.	10.8	15.2	18.7	19.5	70.1	9.6

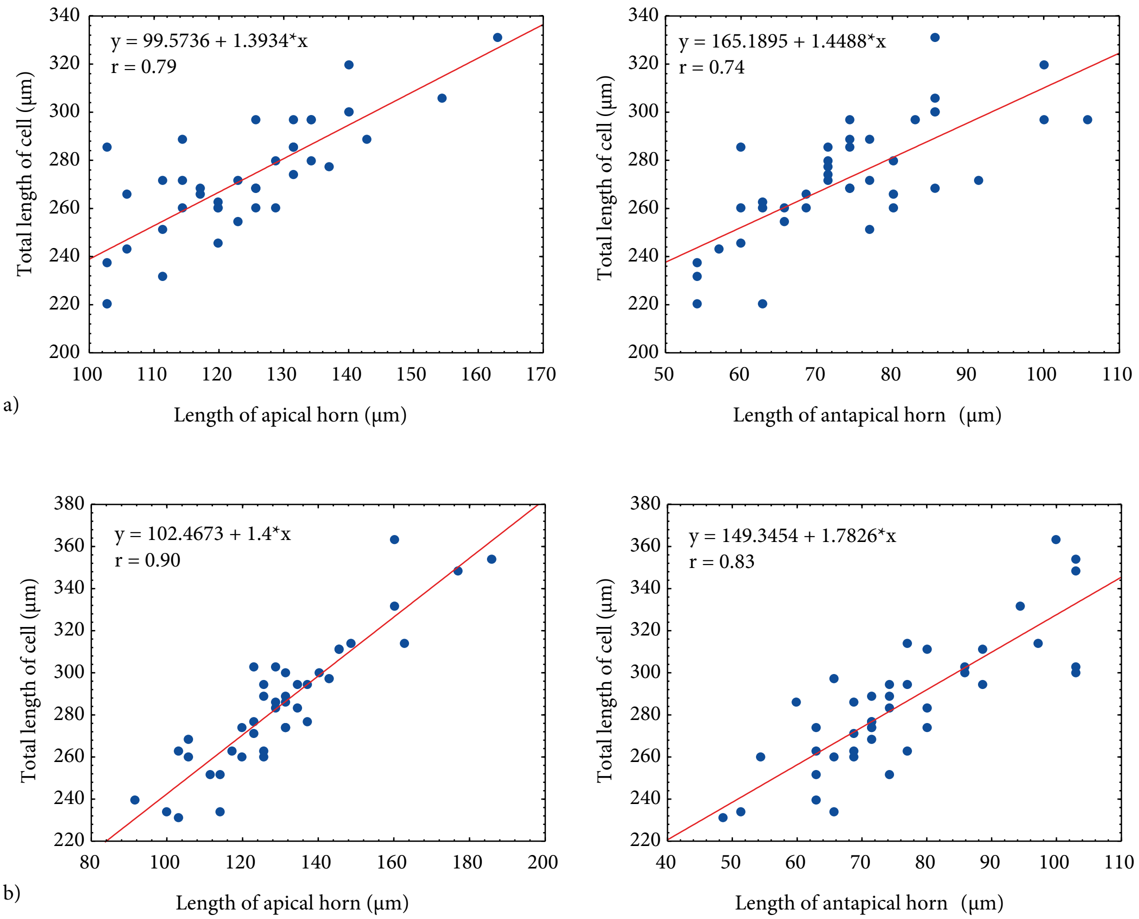

Figure 5. Correlations between total length, length of the apical horn, and length of antapical horn of *C. hirundinella* cells in Kralkızı (**a**) and Dicle (**b**) dam reservoirs.

Table 3. Correlation matrix among total length (TL), length of apical horn (L1), antapical horn (L2), postequatorial horn (L3), and fourth horn (L4), and width of cingulum (W) of individuals in both dam reservoirs.

	TL	L1	L2	L3	L4	W
Kralkızı						
TL	1.00					
L1	**0.79**	1.00				
L2	**0.74**	**0.58**	1.00			
L3	**0.40**	**0.42**	**0.63**	1.00		
L4	–0.10	–0.05	0.16	**0.38**	1.00	
W	**0.41**	0.22	0.21	–0.18	–0.18	1.00
Dicle						
TL	1.00					
L1	**0.90**	1.00				
L2	**0.83**	**0.69**	1.00			
L3	**0.55**	**0.37**	**0.64**	1.00		
L4	**0.55**	**0.45**	**0.46**	**0.59**	1.00	
W	**0.50**	**0.33**	0.29	0.25	**0.41**	1.00

Bold values indicate significant correlations ($P < 0.05$).

The smallest cells of *C. hirundinella* in the Kralkızı and Dicle dam reservoirs were observed in September. In Lake Erken (Sweden) (Lindström, 1992) and Ponikve and Njivice lakes (Gligora et al., 2003), the smallest cells were also observed in September. The largest cells appeared in June in the KDR and in May in the DDR. Correspondingly, Lindström (1992) reported that *C. hirundinella* cells were the largest in spring in Lake Erken. During the study period, *C. hirundinella* cells showed the highest relative standard deviation in the fourth horn in both dam reservoirs. A similar situation was also observed by Parodi et al. (2007) in Paso de las Piedras Reservoir (Argentina). Some individuals had 5 horns in the Kralkızı and Dicle dam reservoirs. Similarly, Gligora et al. (2003) reported that cells with 5 horns occurred in Ponikve Lake; the new horn was always between the antapical and postequatorial horn and was completely developed and separated.

In temperate systems, the lowest water temperature for *C. hirundinella* excystment and appearance has been reported to be 4 °C (Heaney et al., 1983; Rengefors et al., 1998). Because the temperature values in both dam reservoirs were higher than 4 °C, this species could be present throughout the year in the Kralkızı and Dicle dam reservoirs. *C. hirundinella* cells can form cysts in order to survive periods of unfavorable environmental conditions. The cysts may sink to the bottoms of lakes and remain there until environmental conditions become favorable (Harland, 1988; Rengefors and Anderson, 1998). In the KDR, no cysts of *C. hirundinella* were observed. A similar result was reported by Perez-Martinez and Sanchez-Castillo (2002) in the Bermejales Reservoir. On the other hand, cysts of *C. hirundinella* were rarely observed in November and December in the DDR. The appearance of cysts in the DDR occurred 10 months later than the first sampling month (February).

In conclusion, *C. hirundinella* was commonly present in the Kralkızı and Dicle dam reservoirs in the winter months despite lower water temperatures. *C. hirundinella* also showed external morphological changes in both dam reservoirs. All cells of *C. hirundinella* had a fourth horn during the research period. Some individuals had 5 horns; the new horn was between the antapical and postequatorial horns. Cysts of *C. hirundinella* were observed only in the DDR. Further laboratory and field studies are required to fully understand the factors that affect the seasonal distribution and morphological changes of *C. hirundinella*.

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