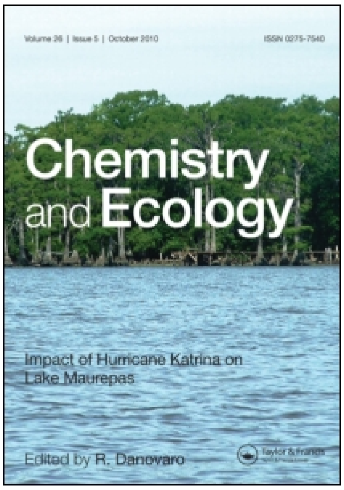

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Changes in the cell size of the diatom *Cylindrotheca closterium* in a hyperhaline pond

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Changes in the cell size of the diatom *Cylindrotheca closterium* in a hyperhaline pond

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Cell length of the diatom *Cylindrotheca closterium* (Ehr.) Reimann & Lewin was investigated on a fortnightly basis from January to April 2000 in a hyperhaline pond (Sardinia, Mediterranean Sea) and relationships, with changes in temperature, salinity, and nitrite concentrations were assessed using single and multiple regression analyses. Results of our study indicate that *C. closterium* cell size was inversely related to temperature changes, but that salinity and nitrite concentrations may have a major idiosyncratic effect on cell size variability.

Keywords: Diatoms; *Cylindrotheca closterium*; Hyperhaline ponds; Cell size; Temperature salinity

1. Introduction

Diatoms represent a major component of aquatic plankton and are therefore an important target in marine research. This component is known to respond to changes in several environmental variables, primarily temperature (*e.g.* [1]). Recent investigations have documented the inverse relationship between ectotherm size and temperature increase [2]. One of the few exceptions has been reported for a diatom species (*Phaeodactylum tricornutum*, [2]), but other studies showed that other diatoms escape from the general inverse rule [3].

Shallow water ecosystems such as coastal lagoons and ponds are generally characterised by important physical and chemical gradients that make these systems highly unstable and subject to unpredictable fluctuating conditions [4, 5]. These environments, indeed, are largely controlled by the dynamic balance between external water inputs (marine and fresh-waters) and wind and wave auxiliary energy inputs [6, 7]. Moreover, in these environments seasonal changes of temperature and salinity are known to be extremely wide [5, 8], even at temperate latitudes, so that most organisms living in these environments display a wide ecological niche [9].

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In order to provide additional information on environmental constraints influencing ectotherm cell size, in this study we investigated seasonal changes in cell size of the diatom *Cylindrotheca closterium* (Ehr.) Reimann & Lewin, in relation to seasonal variability of temperature, salinity and nitrite concentrations in a hyperhaline pond.

2. Materials and methods

2.1 *Sampling and environmental variables*

Superficial water samples for inorganic nutrient and phytoplankton counting were collected on an almost fortnightly basis from January to May 2000 in a hyperhaline pond in Southern Sardinia (Mediterranean Sea, Italy) using a modified Ruttner bottle. In the flood period, the area of the basin was about 3 ha with a maximum depth of about 50 cm. Water samples for phytoplankton counting were immediately fixed in neutralized formalin (1% final concentration [10]) and stored at room temperature in the dark until identification. Water samples for nutrient determination, kept at sampling temperature in a thermostatic bag were brought to the laboratory within 2 hours of collection. At each sampling date, water temperature, pH and salinity, were measured *in situ* using portable field instruments.

2.2 *Cell size determination*

Taxonomic identification, diatoms cell volume and diatoms cell counting were carried out following the Utermöhl method [11] using an inverted microscope equipped with phase contrast. In this work we have considered just *Cylindrotheca closterium*, being generally the dominant species. Cell size of about 50 cells for each sample was measured using a micrometer ocular ($\pm 1.0\,\mu m$).

2.3 *Nitrite concentrations*

Water samples for nitrite determinations were filtered onto Whatman GF/F glass-fibre filters and the filtrate were immediately analysed according to [12].

2.4 *Statistical analysis*

One-way analysis of variance (ANOVA) was performed to investigate temporal changes in cell size and cell volume of the dominant diatom *Cylindrotheca closterium*. Spearman-Rank correlation analysis was applied on the entire data set and, then, multiple linear regression analysis was applied to determine the relationships between cell size and the physico-chemical variables investigated.

3. Results and discussion

Mean values of *Cylindrotheca closterium* cell length, abundance, cell volume, the contribution to the whole phytoplankton abundance and of all measured environmental parameters are reported in table 1.

Together with C. *closterium*, other diatoms, *Navicula transitans* var. *derasa* f. *delicatula* Heimdal, *Skeletonema costatum* (Grev.) Cleve, *Amphora veneta* Kutz, *Hantzschia amphyoxis*

Table 1. Abundance, cell length and volume of *Cylindrotheca closterium* and physico-chemical variables during the study period in the Stani Saliu pond.

Date	Length μm	Density cell I^{-1}	Volume μm^3	Contribution to total phytoplankton abundance %	Contribution to total diatom abundance %	Nitrite $\mu g\,I^{-1}$	Temperature °C	Salinity ‰
15 January	88.8 ± 3.0	14.1×10^6	41.98 ± 18.55	67	96	27.85	7.87	39.50
31 January	83.9 ± 4.4	7.8×10^6	29.65 ± 15.17	90	90	7.6	11.47	43.87
16 February	81.0 ± 4.8	5.5×10^5	26.17 ± 14.37	39	41	1.31	11.50	50.97
6 March	77.1 ± 10.0	131.8×10^6	22.87 ± 11.00	64	72	7.6	11.47	60.70
28 March	73.0 ± 7.8	8×10^5	20.56 ± 10.51	36	36	U.d.l	14.53	74.67
13 April	73.0 ± 5.6	2.3×10^5	20.78 ± 9.56	24	24	U.d.l.	16.17	76.03
28 April	62.0 ± 15.2	84.3×10^4	36.46 ± 18.81	0.4	1.4	U.d.l.	19.57	127.90

U.d.l. = under detection limits.

(Ehr.) Grun, *Thalassionsira* sp., *Navicula* sp., and Chlorophyta nd. were present during the study period. The density of *C. closterium* decreased constantly from January (14.1×10^6 cell I^{-1}) to April (4.3×10^4 cell I^{-1}). The cell volume of *C. closterium* decreased progressively from January (41.98 ± 18.55 μm^3) to the first sampling of April (20.78 ± 9.56 μm^3) according to the cell length variation, then re-increased in the last sampling date (36.46 ± 18.81 μm^3; table 1).

C. closterium contributed up to 90% of the whole phytoplankton in January and only 0.4% in April. At the same time, it accounted for 96% of the total diatom populations in January and only ca. 1% in April, because of a bloom of *Skeletonema costatum*.

C. closterium is a cosmopolitan diatom found from freshwaters to hyperaline waters, generally displaying a length ranging from 30 to 400 μm [13]. However, in the present study, *C. closterium* length displayed remarkably lower values and clear seasonal changes, decreasing significantly from January (88 ± 3.0 μm) to April (62 ± 15.2 μm).

Results of the Spearman-rank correlation analysis indicate that during the present study significant correlations were found between mean cell diatom length and temperature, salinity and nitrite concentrations (table 2). Whilst the positive relationships between cell size and temperature well fits with most previous studies [14, 15], the negative correlation between these variables is contrasting with some literature information. The biology of diatoms, due to their reproduction type, characterized by the progressive decrease of the frustule size with increasing number of generations, brings naturally to temporal cell size variations. In addition, recent investigation has pointed out that changes in water temperature may also alter considerably their cell size spectra [3]. Nevertheless, literature information reports contrasting results about the relationships between temperature and diatom cell size [16–20]. Although the cell size response to changes in environmental variables might be better defined in terms of cell volume [21], our results, based simply on diatom cell length, would indicate that *Cylindrotheca closterium* displays a clear inverse relationship between temperature and cell size, in good

Table 2. Spearman-rank correlation analysis on length, salinity, temperature and nitrite concentrations ($p < 0.01$).

	Length	Salinity	Temperature	Nitrite
Length	–			
Salinity	−0.964	–		
Temperature	−0.929	0.964	–	
Nitrite	0.906	−0.991	−0.927	–

Table 3. Multiple linear regression analysis results on the entire data set. Reported are the regression coefficients and the confidence intervals.

	Coeff	Std Error	T	p	Confidence lower limit 99,0%	Confidence upper limit 99,0%
Constant	91,5361	1,8703	48,9414	0.000001	82,9250	100,1472
Salinity	−0.2369	0.0224	−10.5377	0.0004	−0.3405	−0.1334
Nitrite	0.2319	0.0670	3.4605	0.0258	−0.0766	0.5406

agreement with the general rule for ectotherms [2], but in contrast with the recent literature (Montagnes and Franklin 2001).

The variability of the cell size of *C. closterium* could also have been related to other environmental variables. Therefore, in order to evaluate possible idiosyncratic effects of the other measured environmental variables on the diatom cell size, we performed a multiple regression analysis (table 3). Our results identified salinity and nitrite concentrations as the possible physico-chemical parameters that could have affected the length of this diatom. Moreover, the discrimination of the most important contributors to the significant multiple regression indicates that salinity would play a major role on cell length variability.

The results of the present study suggest that size of diatoms can not be simply ascribed to temperature changes but that these changes are more likely the result of idiosyncratic effects of a wider set of environmental variables. Further investigations on other diatom species under different environmental conditions are needed to further support these findings.

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