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Seasonal changes of cell volumes of four diatom species in the Iskenderun Bay, North-eastern Mediterranean Sea

İskenderun Körfezi'nde (Kuzeydoğu Akdeniz) dağılım gösteren dört diatom türünün hücre hacimlerinin mevsimsel değişimleri

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Abstract

The cell volumes of four diatom species in the Iskenderun Bay were calculated and results hence evaluated on seasonal basis. The selected species were *Thalassiothrix fraunfeldii, Hemiaulus hauckii, Guinardia flaccida* and *Pseudosolenia calcar-avis* which commonly occur in the area. The statistical analyses showed that, cell volumes of all species were different among the seasons ($p<0.01$)($p<0.05$). Moreover, cell volumes of three species (*T. fraunfeldii, P. calcar-avis* and *G. flaccida*) were found different among locations. There were correlations of less importance between cells volumes and seawater temperature. The results of the study showed that, complex biological and environmental factors and their interactions affect the phytoplankton cell volumes.

Key words: Phytoplankton, diatom, cell volume, cell size, north-eastern Mediterranean.

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Introduction

Determination of microbial biomass is quite important for estimation of energy flux and tropic situation in an aquatic ecosystem. The increase in biomass of primary producers affects the nutritional level of higher producers. Since, the increase of algae biomass affects the conversion of inorganic substances to organic matter. Thus, an effective and reliable estimation of algae biomass is very important to evaluate the situation of the ecosystem. For the determination of algae biomass, many methods including very classical-ordinary ones and sophisticated equipments are developed and still used in present time. The most frequently measured biochemical parameter in oceanography is chlorophyll in determination of plant biomass and productivity (Jeffrey *et al.*, 1997). But, chlorophyll concentration can be affected by environmental conditions and doesn't give any information on species composition. One of the oldest but still useful and reliable methods is the determination of phytoplankton abundance by counting cells of each species under microscope. However, using only the cell counts is not sufficient in biomass estimation (Smayda, 1978). For a mixed sample, high numbers of small-sized species may contribute only a minor fraction of the overall biomass, whereas other larger-sized species which are much less abundant in numbers may dominate the overall biomass (Hillebrandt *et al.*, 1999). Thus, in aquatic environment, cell volume should be calculated for each species. Phytoplankton cell volume can be calculated by using some volume equations developed according to the geometric shape of cell (Edler, 1979; Hillebrandt *et al.*, 1999; Sun and Liu, 2003). Then, the cell volume values can be converted to total biomass by multiplying the cell volume of each species with its cell abundance.

Diatoms, one of the major groups of phytoplankton, dominate in nutrient rich coastal water (Miller, 2004). They need high nutrient concentrations for rapid growth. They have great ecological importance and may contribute 20 to 25% of the world net primary production (Werner, 1977). However, diatoms are known to respond to changes in environmental variables, primarily temperature (Miranda *et al.*, 2005).

Iskenderun Bay is located on the north-eastern corner of the Eastern Mediterranean Sea. It covers one of the largest continental shelf areas in the Eastern Mediterranean. The primary production in the bay is 2-4 times higher compared to open sea waters (Yılmaz *et al.*,1992). The microphytoplankton of the bay generally dominant by diatoms in terms of abundance (Polat *et al.*, 2000) as observed in other coastal areas.

The aim of this study is to analyse and compare seasonal changes in cell volumes of some diatom species in Iskenderun Bay, North-eastern Mediterranean. In this respect, four diatom species which are common in the region were selected and their cell volume changes were investigated on seasonal basis at three sampling sites.

Material and Methods

Plankton samples were collected from three sites situated in the Iskenderun Bay, North-eastern Mediterranean Sea (lat. 36° 40.5'N - 36° 46' N and long. 35° 45' E - 35° 52' E). At each site, samples were taken along one transect. The first sampling site was located near the coast (8m deep) while the second site was located ~12-13 km offshore (50-60m deep). The third

sampling site was between locality 1 and locality 2 (25-30 m deep) (Figure 1). Samples were taken monthly intervals at each location in years of 2000-2001. Sea surface temperature and salinity were measured by YSI model salinometer in each sampling period.

Surface plankton samples with 55 μm mesh size net were collected from each sampling sites. Samples were fixed with neutralized formaldehyde until it reached a concentration of 2%. Microscopic investigations were carried out by phase-contrast microscope (Olympus BX-50). Four diatom species which are commonly present in sampling sites and seasons were selected for investigation in this research.

The selected species are *Thalassiothrix fraunfeldii* Grunow, *Hemiaulus hauckii* Grunow in Van Heurck, *Guinardia flaccida* (Castracane)H. Peragallo and *Pseudosolenia* (*Rhizosolenia*) *calcar-avis* Schultze from diatoms (Bacillariophyceae).

The dimensions of about twenty cells for each species were measured using ocular-micrometer under the microscope. Cell volumes were calculated for each species using the equations for the geometric shape developed by Hillebrandt *et al.*(1999). In this respect, the volume formulas of rectangular box for *Thalassiothrix fraunfeldii*, elliptic prism for *Hemiaulus hauckii* and

cylinder for *Guinardia flaccida* and *Pseudosolenia calcar-avis* were used. Cell volumes were calculated as μm^3.

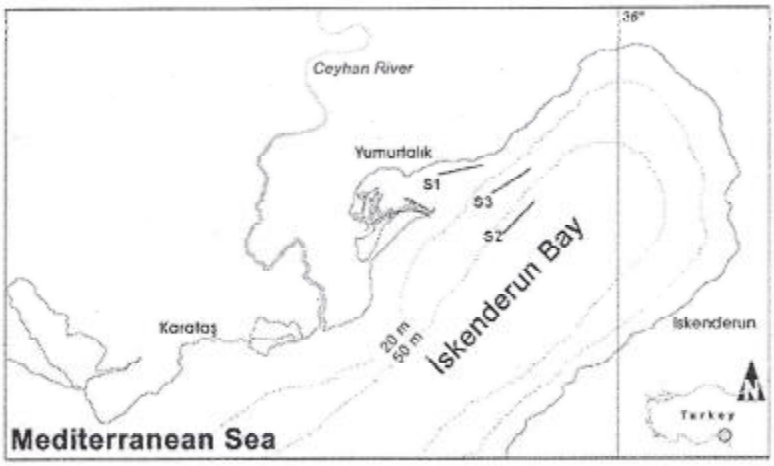

Figure 1. Location of sampling sites in the Iskenderun Bay.

The physico-chemical data and cell volume values belonging to samples taken in monthly intervals were evaluated on seasonal basis. All results were statistically analysed to find out the effect of seasons and sampling sites on cell volume for each species. Factorial experimental design analysis was performed (Hinkelmann and Kempthorne, 1994) and mathematical model of the study was as follows:

$$Yijk = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + eijk$$

In this equation,

Yijk : Cell volume value belonging to i^{th} season, j^{th} site and k^{th} species,

μ : mean of population,

α_i : the effect of i^{th} season,

β : the effect of j^{th} site,

$(\alpha\beta)_{ij}$: interaction effect of season x site,

eijk : error of the experimental design.

In this analysis, in addition to effects of seasons and locality on cell volume, the interaction effect of seasons and sites was also investigated. Then, the factors affecting cell volume were analysed with the DUNCAN multiple comparison test to find out differences. Furthermore, correlations between phsico-chemical factors (temperature and salinity) and cell volume values were computed. In these analyses however, 9.1 version of SPSS statistical package program was used.

Results

The mean sea surface temperature was highest in summer as 29.±0.39°C and lowest in winter as 17.6±0.32°C (Table 1). Salinity reached highest level in autumn (38.3±0.179) and it was lowest in spring (36.9±0.18).

Table 1. The seasonal range (in parentheses) and mean values of temperature and salinity.

	Summer	Autumn	Winter	Spring
Temperature	(28.1-30.1)	(21.0-29.2)	(16.6-18.5)	(18.0-23.7)
	29±0.39	25.02±1.08	17.6±0.32	20.9±0.79
Salinity	(37.6-38.9)	(37.3-39.0)	(37.3-38.0)	(36.3-37.6)
	38.1±0.17	38.3±0.17	37.5±0.11	36.9±0.18

The minimum, maximum and mean cell volumes for each species in different seasons are represented in Table 2. The lowest mean cell volume values of *T. fraunfeldii* and *G. flaccida* were recorded in spring, whereas the lowest mean values for *H. hauckii* and *P.calcar-avis* were recorded in autumn. The highest mean cell volume values of H.hauckii were recorded in winter whereas it was the highest in summer for *P. calcar-avis* and in

autumn for *T. fraunfeldii* and *G. flaccida* (Table 2). The lowest cell volume value for *T. fraunfeldii* (Table 2) was recorded at site 2 (the farthest site to the coast) in the winter. In summer and autumn however, *G. flaccida* and *H. hauckii* showed the lowest volume values at site 3. It was highest in summer at site 1 for *T. fraunfeldii* and site 2 for *G. flaccida*. Furthermore, it was highest at site 3 in spring for *H. hauckii* and *P. calcar-avis*.

Table 2. The seasonal range and mean cell volumes (μm^3 $x10^3$) of four diatom species.

	Summer	Autumn	Winter	Spring
Species		(range) mean±SE		
T. fraunfeldii	(1.169–4.703)	(1.500–4.631)	(1.087–3.895)	(1.574–2.763)
	2.493± 0.155	2.852 ± 0.098	2.591± 0.084	2.235± 0.042
H. hauckii	-	(5.168–32.133)	(8.033–44.10)	(6.492 – 49.303)
	-	15.971± 0.794	19.637± 1.221	19.292± 1.208
P.calcar-avis	(316.61-1948.18)	(204.76–1058.31)	-	(218.684–1850.967)
	969.282±57.819	368.27±21.24	-	794.474 ± 77.912
G. flaccida	(31.415–302.097)	(70.685–345.550)	-	(64.695–191.637)
	131.151 ± 7.397	150.455± 6.521	-	129.892± 3.915

The results of variance analyses of cell volume values for each species are given in Table 3. The differences among seasons for all species were found significant ($p<0.01$) ($p<0.05$). However, the effects of each locality on cell volumes were analysed and the difference among sampling sites in term of cell volumes were important for all species except *H. hauckii*. The interaction effect of season and locality was found significant only for *T. fraunfeldii* ($p<0.01$) (Table 3). Interaction effects were not significant for other species.

The results of the Duncan multiple comparison test were shown in Table 4. *T. fraunfeldii* reached the highest mean cell volume in autumn (Table 2) and the cell volume of *T. fraunfeldii* in autumn was found statistically different than those of other seasons (Table 4).

The mean cell volume of *H. hauckii* in autumn was found statistically different than those of spring and winter. The mean cell volume of *R. calcar-avis* in autumn was statistically different from the spring and summer. *G. flaccida*, reached its highest mean value in autumn, the mean cell volume values in the spring and summer were statistically different from the autumn.

Table 3. The variance analysis of cell volumes of four diatom species.

Thalassiothrix fraunfeldii			
S.V.	D.F.	M.S.	F
Season	3	4050176.7	10.62**
Site	2	2323734.4	6.09**
Season x Site	5	3420802.4	8.97**
Error	209	381313.7	_
Total	219		
Hemiaulus hauckii			
S.V.	D.F.	M.S.	F
Season	2	220077137.2	3.45*
Site	2	1637934.3	0.026
Season x Site	3	18236464.8	0.28
Error	152	63635570.6	_
Total	159		
Pseudosolenia calcar-avis			
S.V.	D.F.	M.S.	F
Season	2	5781877815875.7	41.58**
Site	2	664274273196	4.78**
Season x Site	3	171229574270.1	1.23
Error	152	139038748744.3	_
Total	159		
Guinardia flaccida			
S.V.	D.F.	M.S.	F
Season	2	7970434575.3	3.66*
Site	2	10740560857	4.93**
Season x Site	4	1298029260.7	0.59
Error	171	2174995877	_
Total	179		

S.V.: Source of Variation, D.F.: Degree of Freedom, M.S.:Mean Square, F: f-test statistics

*: $p<0.05$

**: $p<0.01$

The effects of locality on cell volumes were analysed and cell volumes of *T. fraunfeldii* at site 1 were found statistically different than in other sites.

Table 4. The results of Duncan multiple comparison tests

Species	Seasons	Sites
T. fraunfeldii	$spring_{(a)}$ $summer_{(b)}$ $winter_{(b)}$ $autumn_{(c)}$	$3_{(a)}$ $2_{(a)}$ $1_{(b)}$
H. hauckii	$autumn_{(a)}$ $spring_{(b)}$ $winter_{(b)}$	–
P. calcar-avis	$autumn_{(a)}$ $spring_{(b)}$ $summer_{(c)}$	$2_{(a)}$ $1_{(a)}$ $3_{(b)}$
G. flaccida	$spring_{(a)}$ $summer_{(a)}$ $autumn_{(b)}$	$3_{(a)}$ $2_{(b)}$ $1_{(b)}$

However, mean cell volume at site 3 was different than site 1 and site 2 for *G. flaccida*. The effect of localities on cell volumes of *H. hauckii* was not significant ($p>0.05$). Moreover, cell volumes of four diatom species showed no important correlations with temperature and salinity ($p>0.05$).

Discussion

Phytoplankton cells have quite different shape and dimensions and these properties are very important in the life cycle of these organisms. Cell shapes and size are not constant and can make difference due to the effect of its environment. Organisms are sensitive to a wide range of environmental perturbations (Harris, 1986) and the variation of individual cell dimensions are related to some environmental factors such as temperature and salinity (Smayda, 1978). At the same time, nutrient concentrations and physical processes can cause differences in cell dimensions. Of these, light is the important factor affecting cell dimensions. Thompson *et al.* (1991) found that cell volume decrease with decreasing light intensities, ranging from 23% to 72% for cell volume (n=9 species). In addition to environmental factors, physiological situations of the cell also affect the dimensions of the cell. This phenomenon is very clear in the vegetative reproduction stage of diatoms. Within a diatom population, mean cell size usually decreases with each cell division (Round *et al.*, 1990). In such a condition, all axis of a cell changes in different ratios and the outline of a cell can change from linear or linear lanceolata to oval or nearly circular (Round *et al.*,1990). However, in diatoms, the decrease of cell size during vegetative growth is restored by sexual reproduction (Hasle and Syvertsen, 1997; Sze, 1998).

The variations in phytoplankton cell dimensions are very important specifications in identifying the communities. On the other hand, the

variations in cell size can also cause differences in the estimation of phytoplankton biomass.

In the present study, changes of cell volumes according to season and locality were investigated for four diatom species which are common in North-eastern Mediterranean. The distribution of these species was cosmopolite and exists in almost all seasons as recorded in previous studies in the North-eastern Mediterranean coast of Turkey (Polat *et.al.*, 2000; Eker and Kıdeyş, 2000). Therefore, seasonal changes in cell volumes of these species were feasible to study.

Statistical analyses showed that cell volumes of all related species had differed among seasons ($p<0.01$). Mean cell volume of *H. hauckii* was high in winter and spring. The adaptive properties of some species may explain the high cell volumes in that period. Larger phytoplanktons use their vacuoles to store nutrients and the vacuole increase in size as the cell volume increases (Smayda, 1970). For this reason, large cells exhibit higher uptake rates and storage capacities of nutrients in their vacuoles which are used as reserves for unsuitable conditions (Malone, 1980; Harris, 1986). The high cell volumes in winter and spring are probably related with the increase of available nutrients in surface waters due to deep mixing. In winter, increased water density due to low temperature make easy floating of phytoplanktonic organisms and this situation may also favour occurrence of large size individuals. For example, *Rhizosolenia hebetata* a dimorphic diatom species, has a thicker cell wall in cold waters whereas the cell wall is thinner in warm waters (Round *et al.*, 1990). In contrast to the other species, the highest mean cell volumes of *Pseudosolenia (Rhizosolenia) calcar-avis* and *G. flaccida* were found in summer and in autumn, respectively. The reason of this can be due to delayed adaptation of these species to the changing environmental conditions. On the other hand, recent studies have revealed that large diatoms, such as members of the genus *Rhizosolenia*, are strongly positively buoyant (Moore and Villareal, 1996). These large diatom species can be decreasing their cell density to compensate cell size and weight of heavy siliceous cell wall. Therefore, the increased cell volume in summer may be related to positive buoyant property of *P. calcar-avis* in this study. Another possible reason is grazing pressure by zooplankton. In temperate waters, spring outburst of phytoplankton grazed down by zooplankton in the summer (Wimpenny, 1966). Moreover, it was suggested that at least 75% of the daily primary production is consumed by zooplankton (Steeman-Nielsen, 1972) and the size distribution of phytoplankton are associated with the

effects of grazing (Grigorszky *et al.*,1998; Frost, 1980). The size selective grazing of zooplankton suppresses small-sized phytoplankton whereas large phytoplankton cells stay unaffected (Harris, 1986). Thus, the large size can also be thought as adaptive mechanism and some phytoplankton escape from grazing by becoming too large to ingest (Guillard and Kilham, 1976). In a similar way, in present study, the effects of grazing may be less on large members of *P. calcar-avis*.

On the other hand, the mean cell volumes of *T. fraunfeldii* and *G. flaccida* were found lower in spring. This result can be attributed to decrease in cell size due to the increase of cell division rates in this season.

There were significant differences in cell volumes of *T. fraunfeldii*, *P. calcar-avis* and *G. flaccida* in regard to locality ($p<0.01$). Temperature and salinity were not significantly different among sampling sites. Thus, the differences in cell volumes in regard to locality can not be explained by only these factors. The interaction effects between seasons and locality on cell volumes of *T. fraunfeldii* was also found to be important ($p<0.01$). The highest cell volume of *T. fraunfeldii* was found at site 1 which is the closest area to the coast and under the effect of land-based inputs. Probably, cell volume of this species was affected by nutrient rich and turbulent waters in this locality. On the other hand, cell volume of *P.calcar-avis* was the highest at site 3 which is the farest site to the coast. It can be suggested that, cell volume differences among localities might resulted from species specific properties as well as complex effects of environmental factors.

The seasonal differences in cell volume values among all species in this study could be due to some mechanisms special for each species. In diatoms, the size range seems to be species dependent and the specific variation may be as large as 8 to 10 times the length of the apical axis or the diameter (Hasle and Syvertsen, 1997).

According to these results, cell volumes of these species showed seasonal differences. It is well known fact that the temperature has a great effect on phytoplankton physiology. However, this research showed that the differences in cell volumes in different seasons can not be explained with only temperature changes. In such situations, the effects of other environmental properties such as density, nutrient concentrations and grazing should not be disregarded. However, an answer to the question "which factors are more important" is quite difficult to get, since a change in

one factor may effect others and such changes may have complex impacts on cell physiology.

Finally, the calculation of cell volumes for each period and even each sampling area are very important to prevent biased estimations of phytoplankton biomass. Further investigations are necessary for other diatom species living in different climatological and environmental conditions to obtain detailed information on size variation mechanism.

Özet

Bu çalışmada, İskenderun Körfezi'nden 2000-2001 yılları arasında alınan örneklerde dört diatom türünün hücre hacimleri hesaplanmış ve hücre hacimlerinin mevsimsel değişimleri incelenmiştir. Seçilen türler bölgede yaygın bulunan *Thalassiothrix fraunfeldii, Hemiaulus hauckii, Guinardia flaccida* ve *Pseudosolenia (Rhizosolenia) calcar-avis* türleridir. İstatistiksel analizler sonucunda tüm türlerin hücre hacimleri mevsimler arasında önemli farklılıklar göstermiştir. Üç türün hücre hacimleri de (*T. fraunfeldii, P. calcar-avis* ve *G. flaccida*) lokaliteler arasında da farklılık göstermiştir. Türlerin hücre hacimleri ile sıcaklık arasında önemli bir korelasyon bulunmamıştır. Elde edilen sonuçlardan hücre hacmi değişimlerinin yalnızca sıcaklık değişimlerinden kaynaklanmadığı, kompleks biyolojik ve çevresel faktörlerin ve bunların etkileşimlerinin hücre hacimlerini etkilediği söylenebilir.

References

Edler, L. (1979). Recommendations for Marine Biological Studies in the Baltic Sea. Phytoplankton and Chlorophyll. Baltic Marine Biologists Publication, no.5, p.38.

Eker, E., Kıdeyş, A. E. (2000). Weekly variations in phytoplankton structure of a harbour in Mersin Bay (North-eastern Mediterranean). *Turk. J. Botany* 24: 13-24.

Frost, B.W. (1980). Grazing. In: The Physiological Ecology of Phytoplankton. (ed. I. Morris), Blackwell Scientific Publ., Oxford, pp. 465-491.

Grigorszky, I., Nagy, S., Tóth, A., Máthé, C., Müller, Z., Borbély, G. (1998). Effect of large-and of small-bodied zooplankton on phytoplankton in a eutrophic oxbow.*J. Plankton Res.* 20: 1989-1995.

Guillard, R.R.L., Kilham, P. (1976). The Ecology of Marine Planktonic Diatoms. In: The Biology of Diatoms. (ed.D.Werner), University of California Press, Berkeley, pp.372-460.

Harris, G.P. (1986).Phytoplankton Ecology. Chapman and Hall Ltd., London, p.384.

Hasle, G.R., Syvertsen, E. E. (1997). Marine Diatoms. In: Identifying Marine Phytoplankton. (ed. C.R. Tomas), Academic Press, New York, pp.5-385.

Hillebrandt, H., Dürselen, C.D., Kirschtel, D., Pollingher,U., Zohary, T. (1999). Biovolume Calculation for Pelagic and Benthic Microalgae. *J.Phycol.*35: 403-424.

Hinkelmann, K., Kempthorne, O. (1994). Design and Analysis of Experiments.vol 1, Wiley Series in Probability and Mathematical Statistics, p. 495.

Jeffrey, S.W., Mantoura, R.F.C. (1997). Development of pigment methods for Oceanography: SCOR-supported working groups and objectives.In: Phytoplankton Pigments in oceanography, Guidelines to Modern Methods. (eds. S.W.Jeffrey, R.F.C.Mantoura, S.W.Wright), UNESCO, pp.19-36.

Malone, T.C. (1980). Algal Size. In: The Physiological Ecology of Phytoplankton.(ed.I.Morris), Blackwell Scientific Publ., Oxford, pp. 433-463.

Miller, B. (2004). Biological Oceanography. Blackwell Publ., UK, p.402

Miranda, M.D.,Gariano, M., Serra, E. (2005). Changes in the cell size of the diatom *Cylindrotheca closterium* in a hyperhaline pond. *Chemisty and Ecology* 21: 77-81.

Moore, J.K., Villareal, T.A. (1996). Size-ascent rate relationships in positively buoyant marine diatoms. *Limnol.Oceanogr.* 41: 1514-1520.

Polat, S., Sarıhan, E., Koray, T. (2000). Seasonal Changes in the Phytoplankton of the Northeastern Mediterranean (İskenderun Bay). Turk.*J. Botany* 24:1-12.

Round, F.E., Crawford, R.M., Mann, D.G. (1990). The Diatoms, Biology and Morphology of the Genera. Cambridge Univ.Press, New-York, p.747.

Sze, P. (1998). A Biology of the Algae. WCB Mc Graw-Hill, p. 278.

Smayda, T. J. (1970). The suspension and sinking of phytoplankton in the sea. *Oceanogr. Mar. Biol. Ann. Rev.* 8: 353-414.

Smayda, T. J. (1978). From Phytoplankter to Biomass. In: Phytoplankton Manual, (ed. A. Sournia), UNESCO, Paris, pp. 273-279.

Steeman Nielsen, E. (1972). The rate of primary production and the size of the standing stock of zooplankton in the oceans. *Int. Revue der Gesamten Hydrobiologie* 57: 513-516.

Sun, J., Liu, D. (2003). Geomatric models for calculating cell biovolume and surface area for phytoplankton. *J.Plankton Res.* 25: 1331-1346.

Thompson, P.A., Harrison, P.J., Parslow, J.S. (1991). Influence of Irradiance on cell volume and carbon quota for ten species of marine phytoplankton. *J.Phycol.* 27: 351-360.

Werner, D. (1977). Introduction with a note on taxonomy. In: The Biology of Diatoms. (ed. D. Werner) Univ. California Press, pp.1-23.

Wimpenny, R.S. (1966).The Plankton of the Sea.Faber and Faber Ltd., London, p.426.

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