

BIOVOLUME AND BIOMASS OF COMMON DIATOM SPECIES FROM THE COASTAL WATERS OF KARACHI, PAKISTAN

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Abstract

The biovolumes of two groups of diatoms pennate and centric including thirty three common species were calculated for the estimation of carbon biomass at two stations of Manora Channel, coastal waters of Karachi, Pakistan (northern Arabian Sea). Measurements were taken during the routine cell identification by Light microscopy. Total biovolume ranged from $558\mu\text{m}^3$ to $392500\mu\text{m}^3$ and biomass from 1.1pgCcell^{-1} to 3.9pgCcell^{-1} . Total carbon biomass was higher for centric diatoms compared to pennates. Cell biovolume showed the same pattern at both stations and a positive relationship with cell carbon biomass. This is the first detailed study based on geometrical shapes of various species and their biovolume and carbon biomass calculations from northern Arabian Sea. The obtained data will help to assess the diatom community analysis and their size related contribution of carbon biomass within the study area.

Introduction

The diatoms are basic unit of primary production in the ocean (Kopczynska *et al.*, 1986). The primary production based on carbon fixation by diatoms is estimated as 25.8×10^{15} g C yr⁻¹ which is about 43% of the world ocean primary production. Diatoms have great variety of shapes and sizes in various species, which are important for their biovolume and biomass (Smayda, 1978; Snoeijs, 1994; Kumar *et al.*, 2009;). The variations in cell sizes are related with environmental factors. These include temperature, light, salinity and most important the nutrients, these all factors can affect the biovolume and biomass (Mullin *et al.*, 1966).

Diatom species of large size respond to the environmental variables differently than smaller one. For example large species give strong response to the salinity as compared to small species. In ecological studies the larger species are always in less abundance and can not be estimated properly and on the other hand if considering the biomass of smaller species which have usually low values the total biomass can be underestimated (Snoeijs *et al.*, 2002). According to Husted (1957) large diatom species should be studied separately. It can be concluded that species respond differently to environment and therefore both of them in diatom community should be considered for taking complete ecological information.

The total carbon biomass of diatoms is an important and basic parameter to evaluate their role in the silicon budget of ocean (Hasle, 1969). The commonly used procedure for measuring the phytoplankton biomass is *In situ* measurement of chlorophyll or fluorometric chlorophyll measurement from chlorophyll extraction. These are the methods which can not give sufficient knowledge about the carbon biomass of diatom community especially on species levels (Garibotti *et al.*, 2003). The method of cell counting can not provide complete information related with the biomass of diatoms therefore measurement of different dimensions of cell is a helpful method for calculating the biovolume and then converting it to biomass (Havskum, 2004). According to Sun & Liu (2003) the calculation of cell dimensions and converting it from biovolume to biomass is very essential to determine their role in biogeochemical cycle of the ocean. Biovolume can be estimated by several methods and used in regular analysis. This research is based on the work carried previously by Hillebrand *et al.*, (1999) and Sun & Liu (2003) for calculating biovolume of diatom species analyzed with

the Utermohl (1958) method. The objective of this research is to estimate the biovolume and biomass of common diatom species from coastal waters of Karachi and it would be a suggestion that measurements of different size classes of diatoms will be helpful in community biomass analysis which can decrease the chances of variability in the results.

Materials and Methods

Samples were collected from two sites of Manora Channel, station A ($24^{\circ}49.77'N$ $66^{\circ}57.85'E$) a polluted area with impact from Layari River and station B ($24^{\circ}47.93'N$ $66^{\circ}58.87'E$) outside Manora Channel in the open water, a non-polluted station with more oceanic ecosystem influence. Samples were taken with 1.7L Niskin bottle at the depth of 2m, preserved in 1% Lugol's solution and stored in dark-coloured bottles at 4°C.

A set of geometric shapes were selected for determining biovolume of diatoms as proposed previously by Hillebrand *et al.*, (1999) and Sun & Liu (2003). The linear dimensions were measured manually using a micrometer during the identification of the diatom cells. The cell volume of each species was computed by applying average dimensions for each species from each sampling station to the geometrical shape that most closely resembled the species form e.g., cylinder, rectangular box, prism on elliptic base etc. According to Smayda (1978) at least 25 randomly selected cells for each species should be measured but this can not be applied for rare species so they were measured as they occurred. The mean biovolume was calculated from mean value of individual cell biovolumes. The species with maximum linear dimensions of less than 20 μm (Verity *et al.*, 1992) all cells can be calculated with prolate spheroid forms in which depth equals width.

Carbon estimates were derived from measured cell dimensions, calculated cell volumes using the geometric formulae (Hillebrand *et al.*, 1999; Sun & Liu, 2003) and then by applying literature-derived carbon to volume ratios for different taxonomic groups of diatoms given by Menden-Deuer & Lessard (2000) as $\text{Log pg C cell}^{-1} = \text{log } -0.541 + 0.811 \times \text{log V } (\mu\text{m}^3)$.

Result

The cell biovolume and carbon biomass of 34 common species were calculated from two stations A, B of Manora Channel in which eighteen were represented pennate genera and sixteen were represented centric diatoms. Diatom categories are presented in Table 1.

Table 1. Values of Biovolume and carbon biomass of major diatom species from stations A, B of Manora Channel.

S. No.	Morphotype	Shape code	Station A		Station B		References
			Biovolume (μm^3)	Carbon biomass C($\mu\text{g cell}^{-1}$)	Biovolume (μm^3)	Carbon biomass C($\mu\text{g cell}^{-1}$)	
Pennate diatoms							
1.	<i>Asterionellopsis glacialis</i>	B	500	1.6	500	1.6	
2.	<i>Amphora</i> spp.	CY	8086	2.62	1938	2.12	
3.	<i>Asterionella formosa</i>	B	5402	2.48	3613	2.34	Sun & Lui, 2003
4.	<i>Cylindrotheca closterium</i>	PS			5233	2.47	
5.	<i>Gyrosigma</i> spp.	EP	2951	2.1	1688	2.07	Hillebrand <i>et al.</i> , 1999
6.	<i>Licmophora paradoxa</i>	SC	16354	2.87			Sun & Lui, 2003
7.	<i>Nitzschia longissima</i>	EP	1143	1.94	558	1.68	
8.	<i>Navicula directa</i>	EP	68524	3.38	4300	2.4	
9.	<i>Navicula transitans</i>	EP	2607	2.23	1053	1.91	
10.	<i>Navicula</i> spp.	EP	2242	2.17	17990	2.91	
11.	<i>Pseudo-nitzschia</i> spp.	EP	12304	2.77	1084	1.92	
12.	<i>Pleurosigma</i> sp. 1	EP	38944	3.18	13289	2.8	
13.	<i>Pleurosigma</i> sp. 2	EP	9771	2.69	7724	2.61	
14.	<i>Pleurosigma directum</i>	EP	45000	3.23	84780	3.45	
15.	<i>Pleurosigma normani</i>	EP	10313	2.71	27377	3.05	
16.	<i>Pleurosigma macrum</i>	EP	2944	2.27	2944	2.27	
17.	<i>Synedra</i> spp.	B	1359	2	1817	2.1	
18.	<i>Thalassionema nitzschoides</i>	B	1359	2	740	1.78	
Centric diatoms							
1.	<i>Coscinodiscus radiatus</i>	C	363063	3.96	305208	3.9	Sun & Lui, 2003
2.	<i>Corethron criophilum</i>	C2H	39266	3.18	6924	2.57	
3.	<i>Chaetoceros danicus</i>	EP	-	-	118	1.14	
4.	<i>Chaetoceros decipiens</i>	EP	16485	2.87	18396	2.91	
5.	<i>Chaetoceros affinis</i>	EP	589	1.7	240	1.39	
6.	<i>Ditylum brightwellii</i>	TP	54125	3.29	43841	3.22	
7.	<i>Guinardia flaccida</i>	C	330060	3.93	309622	3.91	
8.	<i>Eucampia zodiacus</i>	EP	14570	2.83	22811	2.99	
9.	<i>Odontella sinensis</i>	EP	7440	2.59	9279	2.67	
10.	<i>Odontella aurita</i>	EP	7948	2.62	7729	2.61	
11.	<i>Odontella mobileinsis</i>	EP	46969	3.24	5854	2.51	
12.	<i>Planktoniella sol</i>	C	142281	3.63	392500	3.99	
13.	<i>Rhizosolenia setigera</i>	C	82052	3.44	16441	2.87	
14.	<i>Rhizosolenia imbricata</i>	C	31477	3.1	12051	2.76	
15.	<i>Rhizosolenia styliformis</i>	C	2337	2.19	294375	3.89	
16.	<i>Thalassiosira</i> spp.	C	153219	3.66	95555	3.49	

Shapes used to estimate biovolume from linear dimensions B=rectangular box, C=cylinder, EP=prism on elliptic base, PP=parallelogram base, TP=prism on triangular base, SC=sickle shape cylinder, CY=cymbelloid shape, C2H=cylinder 2 half sphere, PS=prolate spheroid.

Total carbon biomass decreased from station A (inside) to station B (outside) for pennate taxa but in contrast increased for centric taxa as shown in Fig. 1. Cell biovolume also showed the same pattern (Table 2).

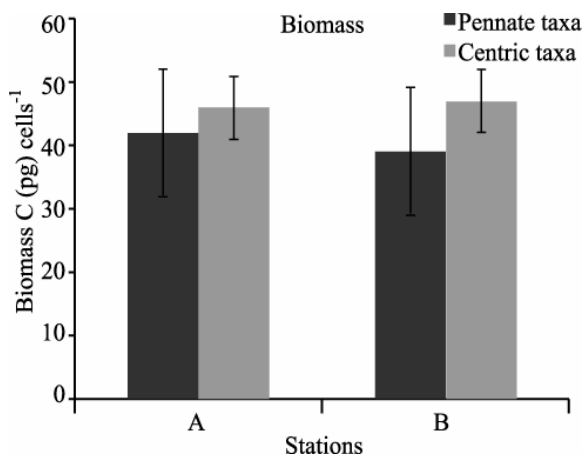


Fig. 1. Total biomass C (pg) cells⁻¹ of pennate and centric diatoms at stations A, B.

The largest diatom observed in the present study was *Thalassiosira* spp., from station A waters with transapical axis (200 μm) and *Coscinodiscus radiatus* from station B with transapical axis (180 μm). The longest diatom was *Pleurosigma directum* with 400 μm apical axis from station A. Chain forming diatoms like *Pseudo-nitzschia* spp., and *Eucampia zodiacus* were also observed in the samples with long apical axis. The smallest diatom observed was *Chaetoceros affinis* (15 μm) apical axis (10 μm) transapical axis and *Navicula transitans* (20 μm) apical axis and (5 μm) transapical axis.

Table 2. Maximum and minimum ranges of values for biovolume (μm^3) and biomass ($\mu\text{g cell}^{-1}$) of diatoms from two stations A and B from Manora Channel.

Diatoms	Stations	Biovolume (μm^3)	Biomass C($\mu\text{g cell}^{-1}$)
Pennate	A	500-68524	1.6-3.3
Centric		589-330060	1.7-3.9
Pennate	B	500-84780	1.6-3.9
Centric		118-392500	1.1-3.9

Biovolume and biomass calculations for each major species or pennate and centric taxa are presented in Table 1. From station A the obtained cell biovolume for pennate taxa ranged from 500 to 68524 μm^3 and biomass was ranged from 1.6 to 3.3 pg C cell^{-1} . For centric taxa obtained cell biovolume ranged from 589 to 330060 μm^3 and biomass ranged from 1.6 to 3.9 pg C cell^{-1} . From station B obtained cell biovolume for pennate taxa ranged from 500 to 84780 μm^3 and biomass ranged from 1.6 to 3.9 pg C cell^{-1} . Centric taxa cell biovolume ranged from 118 to 392500 μm^3 and biomass ranged from 1.1 to 3.9 pg C cell^{-1} (Table 2).

Minimum carbon biomass estimates were obtained from the pennate diatoms *Asterionellopsis glacialis*, 1.6 pg C cell^{-1} from both stations A, B. Maximum carbon values were observed in the centric diatom *Coscinodiscus*

radiatus, and *Planktoneilla sol* was 3.9 pg C cell^{-1} , whereas the centric diatom *Guinardia flaccida* also attained higher carbon values of 3.93 pg C cell^{-1} . Next to *Coscinodiscus radiatus* and *Planktoneilla sol*, *Rhizosolenia styliformis*, *Pleurosigma directum*, *Thalassiosira* spp were the most important contributors to diatom biomass from both stations (Table 1).

Statistical analysis: Regression analysis was applied to determine relationship between biovolume and biomass at both stations A and B for total pennate and centric species biovolume and biomass. At station A, the relationship between biovolume and biomass was $R^2=0.55$ and at station B, $R^2=0.52$ for pennate species. At station A the relationship between biovolume and biomass was $R^2=0.63$ and at station B, $R^2=0.55$ for centric species (Fig. 2).

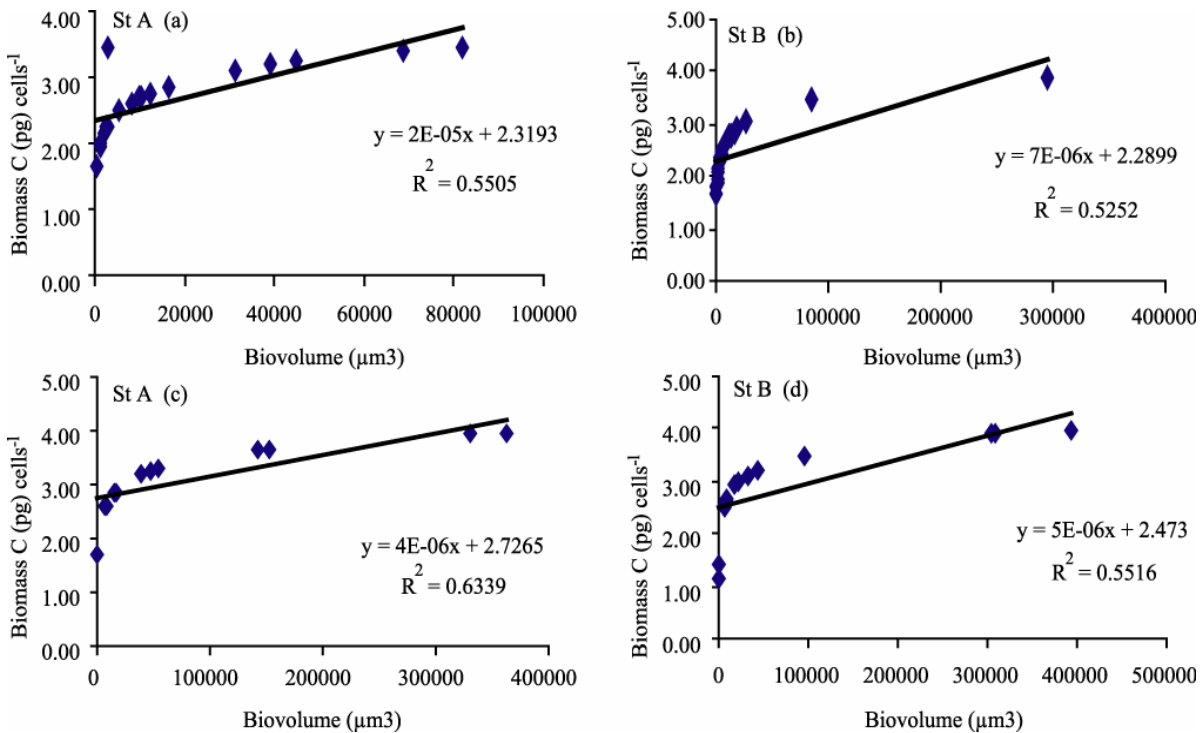


Fig. 2. Regression relationship between biovolume and biomass of pennate and centric species from two stations A, B (a, b) pennate (c, d) centric.

Discussion

The functioning of an ecosystem is dependent upon the size structure of microbial communities. Generally larger phytoplankton cells dominate the biomass in variable eutrophic environment like coastal areas and small phytoplankton cells dominate in stable environment such as open ocean (Li, 2002; Pennington *et al.*, 2006; Tabassum & Saifullah, 2010; Tabassum & Saifullah, 2011; Naz *et al.*, 2012). The observed biovolume and biomass in our studies has shown the same results that both were found in high values and larger cell size at station A which is near shore station influenced by the pollution and river inputs as compared to station B which is near to open waters with more oceanic influence. Similar observation was reported by Brown *et al.*, (2002) from Coast of Oman, Central Arabian Sea.

The effect of cell size in the plankton communities creates a complex competition among different species that influences the species diversity and evolution process. The size measurements for calculating the biovolume of different species of diatoms was the first step during the process of routine cell counting by Utermohl's method in this study. This method is used successfully for calculating biovolume of the cells and converting it to biomass (Rott *et al.*, 2007) but it can also bias the results because the light halos surrounds the cell and the real dimension of the cell can not be seen (Hillebrand *et al.*, 1999). The geometrical models were selected which resembles the most with the particular species because there is a great variation in size and shapes among different species. These geometrical shapes used in this study were previously proposed by Hillebrand *et al.*, (1999) and Sun & Liu (2003).

The studies from Kerguelen Plateau, Southern Ocean (Indian sector) reports that the largest species were centric diatoms viz., *Coscinodiscus* spp., *Thalassiosira* spp., and *Rhizosolenia setigera*. These results are similar with the observations of Cornet-Barthaux & Armand (2007). *Pseudo-nitzschia* species were reported with smallest biovolume from Ross Sea by Moro *et al.*, (2000) but other species with large biovolumes. This difference may be because of difference in temperature and light in two different regions. Among pennate species *Thalassionema nitzschoides* and *Synedra* spp., and among centric species *Chaetoceros affinis* the estimated biovolume was lowest at both station **A** and **B** but relatively high values were found at station **A** than station **B** (Table 1). Similar results were reported from Indian Ocean Dona Poul Bay by Kumar *et al.*, (2009). The highest biovolume values were reported for *Coscinodiscus radiatus* and *Planktonella sol* which was the same observation. On the whole per cell carbon estimated was highest for centric diatoms at station **A**. In contrast *Rhizosolenia styliformis* has high cell carbon per cell at station **B** than station **A**. In pennate diatoms per cell carbon estimated was high at station **A** than station **B**. As a whole per cell carbon is high in centric and lower in pennate types.

The data collected from both stations showed almost similar diatom species but variations in size distribution were found. The phenomenon of upwelling occurs seasonally in the region due to the Asian monsoon system which plays an important role for determining the size distribution of diatoms because it brings the nutrient to the euphotic zone. Due to the upwelling, phytoplankton especially diatoms store nutrients in their vacuoles. The stored nutrients increase the cell size and as a result cell volume also increases. Tada *et al.*, (2000) has reported that cell biovolume vary with the cell size. Our results demonstrate a positive relationship of biovolume with carbon biomass at both stations A and B which is similar reported by Montagne *et al.*, (1994). The problems of cell shrinking, supply of nutrients and the growth phase of different species are the major factors which determine the cell size of species. Further investigation would be helpful in the determination of diatom community biomass related with environmental parameters at the coastal waters of Karachi, Pakistan.

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References

- Brown, S.L., M.R. Landry, S. Christensen, D. Garrison, M.M. Gowing, R.R. Bidigare and L. Campbell. 2002. Microbial community dynamics and taxon-specific phytoplankton production in the Arabian Sea during the 1995 monsoon seasons. *Deep. Sea. Res. II*, 49: 2345-2376.
- Cornet-Barthaux, V.L. and B. Armand. 2007. Biovolume and biomass estimates of key diatoms in the Southern Ocean. *Aquat. Microb. Ecol.*, 48: 295-308.
- Garibotti, I.A., M. Vernet, W. A. Kozlowski and M.E. Ferrario. 2003. Composition and biomass of phytoplankton assemblages in coastal Antarctic waters: a comparison of chemotaxonomic and microscopic analyses. *Mar. Ecol. Prog. Ser.*, 247: 27-42.
- Naz, T., Z. Burhan, S.Munir, P.J.A Siddiqui. 2011. Taxonomy and seasonal distribution of *Pseudo-nitzschia* species (Bacillariophyceae) from the coastal waters of Pakistan. *Pak. J. Bot.*, 44(4): 1467-1473.
- Pennington, J.T., K.L. Mahoney, V.S. Kuwahara, D.D. Kolber, R. Calienes and F.P. Chavez. 2006. Primary production in the eastern tropical Pacific. *Progr. Oceanogr.*, 69: 285-317.
- Husted, F. 1957. Di Diatomeen flora des Flußsystems der Weser im Gebiet der Hansestadt Bremen. *Abh. Nature.*, Ver. Bremen., 34: 181-440.
- Hasle, G.R. 1969. An analysis of the Phytoplankton of the Pacific Southern Ocean: abundance, composition and distribution during the Bratigg expedition, 1947-1948. *Hvalrad Skr Sci Results. Mar. Biol. Res.*, 52: 1-18.
- Havskum, H., L. Schlüter, R. Scharek, E. Berdalet and S. Zohquet. 2004. Routine quantification of Phytoplankton groups— microscopy or pigment analyses? *Mar. Ecol. Prog. Ser.*, 273: 31-42.
- Hillebrand, H., C.D. Durselen, D. Krischtel, D. Pollinger and T. Zohary. 1999. Biovolume calculation for pelagic and benthic microalgae. *J. Phycol.*, 35: 403-424.
- Kumar, M.S.R., N. Ramaiah and D.L. Tang. 2009. Morphometry and cell volumes of diatoms from a tropical estuary of India. *Ind. J. Mar. Sci.*, 38: 160-165.
- Kopczynska, E.E., L.H. Weber and S.Z. El Sayed. 1986. Phytoplankton species composition and abundance in the Indian Sector of the Antarctic Ocean. *Polar. Biol.*, 6:161-169.
- Li, W.K.W. 2002. Macroecological patterns of phytoplankton in the northwestern North Atlantic Ocean. *Nature*, 419: 154-157.
- Mullin, M.M., P.R. Sloan and R.W. Eppley. 1966. Relationship between carbon content, cell volume and area in phytoplankton. *Limnol. Oceanogr.*, 11: 307-311.
- Moro, I., R. Paccagnella, C. Barbante and C. Andreoli. 2000. Microalgal communities of the sea ice, ice-covered and ice-free waters of Wood Bay (Ross Sea, Antarctica) during the austral summer 1993-94. *Mar. Ecol.*, 21: 3-4.
- Montagnes, D.J.S., J.A. Berges, P.J. Harrison and F.J.R. Taylor. 1994. Estimating carbon, nitrogen, protein, and chlorophyll *a* from volume in marine phytoplankton. *Limnol. Oceanogr.*, 39: 1044-1060.
- Menden-Deuer, S. and E.J. Lessard. 2000. Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. *Limnol. Oceanogr.*, 45: 569-579.
- Rott, E., N. Salmaso and E. Hoehn. 2007. Quality control of Utermöhl-based Phytoplankton counting and biovolume estimates—an easy task or a Gordian knot? *Hydrobiologia.*, 578: 141-146.
- Snoeijs, P., S. Busse and M. Potapova. 2002. The importance of diatom cell size in community analysis. *J. Phycol.*, 38: 265-272.
- Snoeijs, P.S. 1994. Distribution of epiphytic diatoms species composition diversity and biomass of different microalgae host along seasonal salinity gradients in the Baltic Sea. *Diatom. Res.*, 9: 189-211.
- Sun, J. and D. Liu. 2003. Geometric models for calculating cell biovolume and surface area for Phytoplankton. *J. Plank. Res.*, 25: 1331-1346.
- Smayda, T.J. 1978. The size of cell. *Phytoplankton Manual. UNESCO.*, 225-229, 273-279.
- Tada, K., S. Pithakpole, R. Yano and M. Montani. 2000. Carbon and nitrogen content of *Noctiluca scintillans* in the Soto Island Japan. *J. Plank. Res.*, 22: 1203-1211.
- Tabassum, A and S.M Saifullah. 2010. The planktonic diatom of the genus *Chaetoceros* Ehrenberg from northwestern Arabian Sea bordering Pakistan. *Pak. J. Bot.*, 42(2): 1137-1151.
- Tabassum, A and S.M. Saifullah. 2011. Marine centric diatom *Rhizosolenia* Brightwell: Its occurrence and distribution in neritic waters of Pakistan. *Pak. J. Bot.*, 43(4): 2187-2193.
- Utermöhl, H. 1958. Zur Vervollkommung der quantitativen Phytoplankton Method. *Mitt. Ver. Theor. Angew. Limnol.*, 9: 1-38.
- Verity, P.G., C.Y. Robertson, C.R. Tronzo, M.G. Andrews, J.R. Nelson and M.E. Sieracki. 1992. Relationships between cell volume and the carbon and nitrogen content of marine photosynthetic nanoplankton. *Limnol. Oceanogr.*, 37: 1434-1446.