

Morphometry and cell volumes of diatoms from a tropical estuary of India

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Cell volumes and per cell carbon contents of different diatoms (10 centric, 14 pennate and 1 dinoflagellate) collected from Dona Paula Bay in the central west coast of India have been analyzed. Morphometric information on the phytoplankton types recovered through many replicate samples for calculating the cell volumes and to estimate per cell carbon content has been presented. The data on the above aspects are of great significance for instance for comparison of data from different geographic locations. Cell dimensions of all identified plankton species comprising diatoms (90% of total cell counts), dinoflagellates (1%) and others (9% of total cell counts) were recorded for calculating per cell carbon content of the observed species. The carbon content, while correlating positively with increasing cell size of phytoplankton species recorded, was found to be directly dependant on cell volume. The present study elucidates that the use of a standardized species list with fixed size-classes and biovolume will be necessary for a realistic measurement of carbon contents. The present study also suggests that morphometric measurements are necessary for obtaining dependable estimations of cell volumes and carbon contents.

[Keywords: Cell volume, Phytoplankton, Biomass, Dinoflagellate, Diatom]

Introduction

Autotrophic single celled phytoplankton is the key components in aquatic ecosystems and forms the base of the pyramid of biological productivity. Thus, the knowledge of their species composition, productivity and biomass are essential for an understanding of the aquatic ecosystems. An important step for improvement of the phytoplankton composition analysis has been the development of standard counting and calculation procedures. In this regard, reliability of biovolume, derived from the morphometric measurements, in providing a more accurate picture on phytoplankton biomass was demonstrated several decades ago by Paasche¹. Cell volumes can be calculated from cell-size and shape by use of appropriate geometric formulae²⁻⁴. As it is impossible to measure and calculate every individual cell in routine counting, the same shape and a mean size was originally assumed for different species⁵. Such simplification is reported to introduce a hardly quantifiable error into the biovolume calculations⁶. The use of a standardized species list with fixed size-classes and biovolumes⁷ is shown to be very practical and highly useful to measure the phytoplankton biomass. Since many species do show a wide range in size, the calculation can be improved by including

several replicate measurements from many individuals of a given species/genus collected from the environmental samples.

This study delineates the cell volumes of phytoplankton genera collected during the pre-monsoon month of March 2007 from the Dona Paula Bay, a typical tropical estuarine region. It experiences mostly the marine conditions both during low tide and high tide periods during the non-monsoon months of February to May. There are many studies on the annual variations in the phytoplankton compositions of this Bay⁸⁻¹⁰. Whereas the Cell volume considerations have not been reported for phytoplankton assemblages in this highly productive tropical estuarine region. The present study consists morphometric information on the phytoplankton types recovered through many replicate samples for calculating the cell volumes and to estimate per cell carbon content.

Materials and Methods

Five surface water samples (0.5m just below surface) from Dona Paula Bay, Goa were collected during low tide times in the premonsoon month of March 2007. The chlorophyll *a* concentration was measured from two 1 L replicates for each of these five samples. For this, volumes of 1 litre water samples were filtered onto Whatman 25 mm GF/F

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glass fiber filters (0.7 μm particle retention), and, chlorophyll was extracted with 90% (v/v) acetone and analyzed fluorometrically using a Fluorometer (Turner Designs, Fla, USA, Model 10 AU) essentially following Strickland and Parsons¹¹.

Samples were fixed with Lugol's iodine solution (1% w/v) and 3% formaldehyde for estimating phytoplankton cell count. It is stored in dark until taken up for analyses usually within 24-48 hrs after collecting the samples. The settling and siphoning procedure¹² was followed to concentrate the samples from 5000 ml to 50 ml to achieve 100 \times concentrate. Phytoplankton cells were identified following Tomas¹³, by transferring three 1-ml concentrates of each sample in to Sedgwick-rafter plankton counting chamber. All the 1000 squares on the chamber were screened and phytoplankton identified at least up to generic level whenever noticed. Simultaneously, the length (max dimension), breadth and/or, diameter of individual cell were also recorded under 400 \times magnification. The morphometric details of the phytoplankton had utilized to identify the species/genera. The cell volumes of each individual cell were calculated using a method similar to that previously described by Sun and Liu⁴. The cell volumes of all the mixed populations of phytoplankton were then converted to cell carbon (0.225 pg μm^{-3}) as suggested by Reimann *et al.*².

The length and breadth of individual cells of all the phytoplankton genera from all the five samples were measured with the help of an oculometer to estimate the surface area and cell volumes of individual cells. The cell dimensions namely the maximum length and maximum width/breadth/depth was recorded for each individual cell of each species or genus. The phytoplankton assemblage was mostly comprised of diatoms in the Dona Paula Bay during March 2007. In spite of the fact that the centric diatoms were mostly spherical with different diameters and height/depth variations, for calculating their surface area, maximum and minimum cell dimensions were recorded. The averages of these dimensions from individual species/genus were used for calculating their cell surface area. The cell volume calculations were accomplished by subjecting all the genera /species identified during this study to the method of Sun and Liu⁴. Further, simple length \times breadth \times depth formula was used to calculate the cell volume especially for centric diatoms. The third measurement, depth, was quite similar to that of

breadth. This was done in order to see how these volumes derived using cell dimensions recorded during this study compare to the ones calculated using the formulae of Sun and Liu⁴ based on geometric shape codes/formulae.

Monocultures of the predominant diatoms during the March 2007 namely *Melosira*, *Amphora* and *Navicula* sp were obtained by repeated sub-culturing in f/2 medium²³ for measuring the cell sizes. Once the cultures attained the highest chl a of $\geq 0.2 \mu\text{g ml}^{-1}$ in the culture medium, the cell dimensions from at least 150 individual cells were measured as described above.

Results and Discussion

During the study period, the average chlorophyll concentration analyzed in the five samples was $2.353 \pm 0.26 \mu\text{g l}^{-1}$. Its concentration ranged from 1.913 to 2.874 $\mu\text{g l}^{-1}$. Phytoplankton cell counts ranged from 1.296 to 2.020 ($\times 10^4$) cells l^{-1} with an overall average of $1.623 \pm 0.12 (\times 10^4) \text{l}^{-1}$ from the five samples analyzed.

During this sampling, 26 different species/genera of phytoplankton were identified. A large majority of them belonged to diatoms. There were others two dinoflagellate, two each of blue green algae and unidentified algae. Based on the number of cells of each species/genus, diatoms contributed $\sim 90\%$, dinoflagellates, 1% and other species 9% of the total cell counts during March 2007. The higher dominance index during this time at Dona Paula Bay was due to greater contribution of *Coscinodiscus*, *Melosira*, *Navicula*, *Amphora*, *Thalassionema*, *Pleurosigma* and *Chaetoceros*. The common species of diatoms recorded were: *Biddulphia aurita*, *Biddulphia* sp., *Chaetoceros* sp., *Coscinodiscus perforatus*, *Coscinodiscus radiatus*, *Coscinodiscus* sp., *Cyclotella* sp., *Melosira* sp., *Planktoniella sol*, *Rhizosolenia* sp., *Amphora* sp., *Asterionella formosa*, *Navicula membranacea*, *Navicula* sp., *Nitzschia longissima*, *Nitzschia* sp., *Pleurosigma angulatum*, *Pleurosigma elongatum*, *Pleurosigma* sp., *Synedra* sp., *Thalassionema frauenfeldii*, *Thalassionema nitzschioides* and *Thalassiothrix longissima*. The two species of dinoflagellate, *Triceratium* sp., *Gymnodium* sp., was a minor component.

The mean length, breadth and the estimated cell surface area of phytoplankton genera/species observed during March 2007 are presented in Table 1. Cell dimensions of all cells viewed from three

Table 1—Morphometric details, estimated surface areas, shape codes and estimated cell-volumes and, mean per cell carbon of different species of phytoplankton recorded from Dona Paula Bay in March 2007.

Phytoplankton species	n	Length (µm) Min – Max (Mean ± SD)	Breadth (µm) Min – Max (Mean ± SD)	Area Min – Max (Mean ± SD)	A* Min – Max (Mean ± SD)	B® Min – Max (Mean ± SD)	Shape code	per cell carbon (ng)	
								A*	B®
Centric diatoms <i>Odontella</i> (<i>Biddulphia</i>) <i>aurita</i>	6	37.5 – 42.5 (40 ± 3.1)	25 – 37.5 (29.5 ± 5.7)	937.5 – 1575 (1188.5 ± 270.4)	23437.5 – 52734.3 36330.7 ± 14743.4	18407.7 – 43295 28534.0 ± 11579.4	29	1.839	1.494
<i>Biddulphia</i> sp.	5	37.5 – 45 40.5 ± 3.21	25 – 37.5 30.5 ± 5.7	937.5 – 1406.2 1238.7 ± 269.3	23437.5 – 52734.3 38909.3 ± 14894.3	18407.7 – 43295 30559.3 ± 11697.9	29	1.969	1.647
<i>Chaetoceros</i> sp.	5	17.5 – 22.5 20 ± 1.76	10 – 12.5 10.5 ± 1.11	175 – 250 210 ± 28.5	1750 – 3125 2225 ± 533.2	1374.4 – 2454.3 1747.5 ± 418.8	29	0.112	0.098
<i>Coscinodiscus perforatus</i>	4	27.5 – 37.5 32.5 ± 4.56	25 – 42.5 33.7 ± 7.77	687.5 – 1593.7 1123.4 ± 406.9	17187.5 – 67734.3 40285.1 ± 22680.8	14848.9 – 46939.8 29768.4 ± 14498.1	4	2.039	1.705
<i>C. radiatus</i>	4	42.5 – 60 48.7 ± 7.7	42.5 – 60 50 ± 7.3	1806.2 – 2256.2 2478.1 ± 777.1	107171.8 – 216000 128109.3 ± 60671.1	60291.5 – 169646 98407.9 ± 48604.0	4	6.085	4.981
<i>Coscinodiscus</i> sp.	19	12.5 – 42.5 30.7 ± 10.4	12.5 – 42.5 30.5 ± 10.10	306.2 – 1806.2 1039.4 ± 644.0	8000 – 125000 3784.7 ± 32887.0	6283.1 – 98174.7 30051.8 ± 26099.7	4	1.915	1.521
<i>Cyclotella</i> sp.	4	32.5 – 37.5 35 ± 2.0	35 – 36 35.2 ± 0.5	1137.5 – 1312 1233.7 ± 73.5	39812.5 – 45937.5 43496.2 ± 2792.2	29035.1 – 38656.9 33759.8 ± 3929.0	4	2.201	1.709
<i>Melosira</i> sp.	28	5 – 20 9.55 ± 5.31	2.5 – 12.5 6.87 ± 4.98	12.5 – 262.5 89.0 ± 103.8	31.25 – 4500 1095.4 ± 1619.2	49 – 3534.2 900.6 ± 1253.9	4	0.055	0.045
<i>Planktoniella sol</i>	4	41 – 43 41.6 ± 0.06	43 – 44.5 44 ± 0.39	4178 – 5654.8 4965.6 ± 116.7	345662.8 – 381796.5 360195.6 ± 3510.0	344977.8 – 366698.2 355127.0 ± 2988.7	4	21.44	14.11
<i>Rhizosolenia</i> sp.	3	67.5 – 7.0 69.1 ± 1.4	5 – 7.5 5.83 ± 1.44	337.5 – 525 404.1 ± 104.8	1687.5 – 3937.5 2458.3 ± 1281.3	1325.3 – 3092.5 1930.7 ± 1006.3	28	0.124	0.107
Pennate diatoms <i>Amphora</i> sp.	50	12.5 – 50 20.3 ± 8.43	7.5 – 17.5 12.17 ± 2.86	112 – 875 254.6 ± 152.7	843.5 – 15312.5 3352.5 ± 2744.2	245.4 – 5345 1214.3 ± 993.2	17	0.169	0.136
<i>Asterionella formosa</i>	8	375 – 165 122.5 ± 42.8	2.5 – 10 4.68 ± 2.47	187.5 – 1650 1188.5 ± 270.4	1875 – 8000 2681.6 ± 2391.5	1875 – 8000 2681.6 ± 2391.5	22	0.135	0.135
<i>Meuniera</i> (<i>Navicula</i>) <i>membranacea</i>	10	25 – 40 36 ± 7.28	25 – 37.5 31.2 ± 6.1	625 – 1575 1151.8 ± 400.6	15625 – 56250 38060.9 ± 19159.4	12271.8 – 44178.6 29892.9 ± 15047.8	11	1.926	1.613

Contd—

Table 1—Morphometric details, estimated surface areas, shape codes and estimated cell-volumes and, mean per cell carbon of different species of phytoplankton recorded from Dona Paula Bay in March 2007—*Contd.*

Phytoplankton species	n	Length (µm) Min – Max (Mean ± SD)	Breadth (µm) Min – Max (Mean ± SD)	Area Min – Max (Mean ± SD)	A* Min – Max (Mean ± SD)	B® Min – Max (Mean ± SD)	Shape code	per cell carbon (ng)	
								A*	B®
<i>Meuniera</i> (<i>Navicula</i>) sp.	31	7.5 – 60 17.9 ± 11.6	2.5 – 20 9.19 ± 4.10	18.75 – 1200 200.6 ± 250.7	46.8 – 24000 268.0.9 ± 5132.7	36.8 – 18849.5 2105.6 ± 4031.2	11	0.135	0.106
<i>Nitzschia longissima</i>	13	262 – 285 269.4 ± 11.0	10 – 12.5 12.11 ± 1.72	2600 – 4275 3270.1 ± 525.8	25750 – 44140.6 40430.2 ± 12108.3	12875 – 32062.5 20215.1 ± 6054.1	13	2.046	1.992
<i>Nitzschia</i> sp.	18	20 – 222.5 102.6 ± 78.1	25 – 50 13.05 ± 14.05	75 – 5000 1550.3 ± 1725.3	187.5 – 250000 40202.2 ± 78538.6	93.7 – 125000 20101.1 ± 39269.3	13	2.035	1.717
<i>Pleurosigma angulatum</i>	5	165 – 172.5 169.5 ± 3.25	30 – 32.5 32.5 ± 1.76	5100 – 5862.5 5507.5 ± 288.3	153000 – 205187.5 179375 ± 18734.1	76500 – 91101.5 89687.5 ± 9367.0	13	9.080	7.540
<i>P. elongatum</i>	9	150 – 217.5 166.6 ± 22.0	25 – 40 35.5 ± 6.22	4375 – 9787.5 5980.5 ± 1678.1	135000 – 440437.5 220791.6 ± 99572.6	54687.5 – 220218.7 110395.8 ± 49786.3	13	11.177	5.588
<i>Pleurosigma</i> sp.	23	85 – 217.5 121.5 ± 44.1	5 – 40 19.0 ± 12.8	425 – 9787.5 2770.9 ± 2676.0	2125 – 44043.7 84122.9 ± 113575.9	1125 – 333046.8 56434.7 ± 82379.3	13	4.258	3.857
<i>Synedra</i> sp.	14	75 – 100 84.8 ± 6.7	5 – 7.5 6.07 ± 1.28	375 – 656.25 516.0 ± 119.9	1875 – 4921.8 3270.0 ± 1413.3	1875 – 4921.8 3270.0 ± 1413.3	10	0.165	0.166
<i>Thalassionema frauenfeldii</i>	7	25 – 55 35.3 ± 12.9	5 – 12.5 8.21 ± 31.13	125 – 550 305.35 ± 178.5	500 – 5000 2892.8 ± 2204.6	500 – 5000 2892.8 ± 2204.6	10	0.143	0.146
<i>T. nitzschioides</i>	3	20 – 30 23.4 ± 3.8	5 – 10 5.90 ± 3.63	50 – 300 144.3 ± 98.9	125 – 9000 1190.3 ± 1833.4	125 – 9000 1190.3 ± 1833.4	10	0.060	0.060
<i>Thalassiothrix longissima</i>	3	75 – 85 79.1 ± 5.20	5 – 7.5 5.83 ± 1.44	375 – 637.5 466.6 ± 148.0	1875 – 4781.2 2864.5 ± 1660.1	1875 – 4781.2 2864.5 ± 1660.1	10	0.145	0.145
<i>Triceratium</i> sp.	3	30 – 32.5 31.6 ± 1.4	12.5 – 15 13.3 ± 1.44	375 – 406.2 422.9 ± 58.0	4687.5 – 7312.5 5692.7 ± 1416.3	4871.3 – 6860.5 5816.3 ± 998.2	18	0.423	0.399
Dinoflagellate <i>Gymnodium</i> sp.	3	30 – 37.5 35 ± 4.33	25 – 27.5 26.6 ± 1.44	825 – 1031 931.2 ± 103.2	22687.5 – 28359.3 24828.1 ± 3081.0	11879.1 – 14848.9 12999.9 ± 1613.2	3	3.224	1.882

*Per the length × breadth × depth dimensions measured during this study; ® per formulae provided Sun and Liu⁴.

replicates of 1 ml concentrates were measured. Morphometrics of individual species/genus whose total were less than 10 are not included for calculating cell-surface area and cell-volume (Table 1). The data presented here is for a total of 10 centric, 14 pennate

diatoms and, 1 dinoflagellate. This was done in order to provide a realistic representation. The highest surface area calculated was for the species, *Planktoniella sol*, *Coscinodiscus radiatus*, *Coscinodiscus* sp., among the centric diatoms. It was

followed by *C. perforatus*, *Odontella (Biddulphia) aurita*, *Odontella (Biddulphia) sp.*, *Cyclotella sp.*, *Rhizosolenia sp.*, *Triceratium sp.*, *Melosira sp.* and *Chaetoceros sp.* The differences in cell surface area were larger among the individuals of *Pleurosigma elongatum*, *Pleurosigma sp.*, *Pleurosigma angulatum*, *Nitzschia sp.*, *Nitzschia longissima*, *Asterionella formosa*, *Meuniera (Navicula) membranacea*, *Meuniera (Navicula) sp.*, *Amphora sp.*, *Synedra sp.*, *Thalassiothrix longissima*, *Thalassionema frauenfeldii* and *T. nitzschioides*. Among pennate diatoms, the estimated surface area was the least for *Chaetoceros sp.* and *T. nitzschioides*; the greatest for *P. elongatum* in the phytoplankton assemblage observed.

The size frequency for three most abundant species of phytoplankton in the bay was also calculated and the number of cells falling in the different size ranges is depicted in Fig. 1. The per cell carbon content has been calculated using the biovolume measurements obtained during this study and by using the empirical formulae given by Sun and Liu⁴

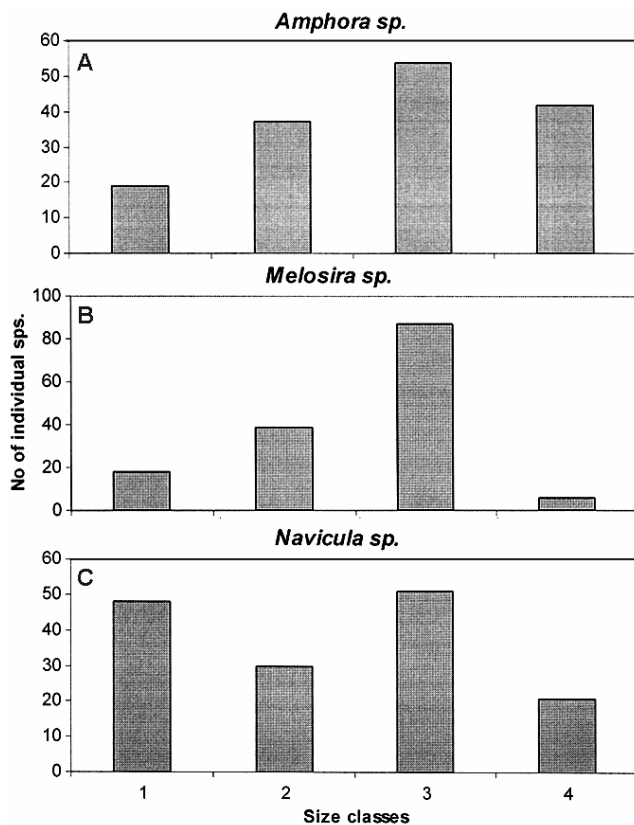


Fig. 1—Frequency of occurrence of different size classes (in terms of length) of (A): *Amphora sp.* (B): *Melosira sp.* and, (C): *Navicula sp.* cells in laboratory grown in monocultures.

(Table 1). Commensurate with biovolume, the per cell carbon was the highest in *C. gigas* and *P. sol*, followed by *C. radiatus*, *Coscinodiscus sp.*, *C. perforatus*, *Odontella (Biddulphia) aurita*, *Odontella (Biddulphia) sp.*, *Cyclotella sp.*, *Rhizosolenia sp.*, *Triceratium sp.*, *Melosira sp.*, and *Chaetoceros sp.* among the centric diatoms. Among pennate diatoms, the receding order of carbon content was: *P. elongatum*, *Pleurosigma sp.*, *P. angulatum*, *Nitzschia sp.*, *N. longissima*, *A. formosa*, *Meuniera membranacea*, *Meuniera sp.*, *Amphora sp.*, *Synedra sp.*, *T. longissima*, *Thalassionema frauenfeldii* and *T. nitzschioides* found during this study. On the whole the per cell carbon is lower in pennate diatoms.

The carbon content of phytoplankton cells is shown to vary marginally between most species². It was thus suggested that the cell carbon and cell volume relationships demonstrated for phytoplankton cells may be adopted for bacteria and protozoa¹⁴. Though correlating positively with increasing cell size, the carbon content per cell (CCPC) correlated inversely with cell volume though the CCPC has been reported to vary directly with cell size¹⁵. An identical relationship between cell carbon and cell volume has been reported for phytoplankton cells, although several problems, such as cell shrinkage due to fixation are pointed out^{14,16-19}. Besides starvation and nutrient supply¹⁵, we suggest that growth phases might also affect the carbon (and nitrogen) contents of the cell. Notwithstanding such variations, the mean per cell carbon values of phytoplankton suggested in literature^{2,15} are useful for a realistic calculation of carbon values for phytoplankton assemblages from a given location.

The size measurements of many individual cells within a species have been useful to suggest that to reduce high variance in the results of phytoplankton carbon biomass analyses, standardization of as many steps as possible of the procedures is necessary. Such efforts help to improve the quality of size-classing phytoplankton, counting methods and the comparability of the results. The biovolume derived through the size measurements agree to a great extent with the ones derived using the empirical formulae of Sun and Liu⁴. Notwithstanding some differences in the cell volumes calculated using their formula and ours, results from this study suggest that morphometric measurements are necessary. Such efforts would prove useful in obtaining dependable estimations of cell volumes and carbon contents. The

per cell carbon content in the phytoplankton genera/species identified during this study will be useful for calculation of phytoplankton carbon biomass. Since biovolume of individual cells of phytoplankton genera/species did not vary too widely (Table 1), unlike those from the experimental set-up, it is suggested that these measurements would prove useful in providing information on carbon content per cell.

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