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Estimation of Diatom and Dinoflagellate cell volumes from surface waters of the Northern Indian Ocean.

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Abstract

Phytoplankton samples collected from the Northern Indian Ocean (Bay of Bengal, Northern Arabian Sea, and Dona Paula Bay Goa, west coast of India), were utilized to quantify changes in cell size, cell volume and carbon per cell of the diatoms and dinoflagellates. The Dataset from the Bay of Bengal also provides inter- and intra-annual variations (April 2008 to March 2010). The variations in cell size and volume were large in regions influenced by the riverine influx or terrigenous inputs. An interregional comparison of commonly available forms (8 species) points out that cell volumes are larger in the North Atlantic and lowest in the Mediterranean. The information provided will be useful in estimation of carbon biomass and biogeochemical studies.

Keywords Diatoms; Dinoflagellates; Cell Volume; Dona Paula Bay; Bay of Bengal

1. Introduction

Trait-based characteristics are increasingly used to predict the phytoplankton community distribution along the environmental gradient (Margalef, 1978; Reynolds, 1988). They are not necessarily taxonomy related but determined based on size and the physiological processes such as growth (light and nutrient assimilation) and loss (sinking and grazing) (Morabito et al., 2007). The cell size is referred as a master trait which places important constraints on many key organismal characteristics and biotic interactions (Bartho et al., 2013 and references therein). Smaller organisms have several advantages over large ones for e.g a lower sinking rate, which is proportional to cell radius squared (Stokes law) (Smyada, 1970). Higher surface to volume ratio that helps efficient acquisition of limiting nutrients (Ploug et al., 1999; Sherwood et al., 1975) and higher maximum growth rates (Banse, 1976). In contrast, the large size organisms carry the advantage of motility, access nutrient resources unavailable to other organisms; avoid grazing and higher possibility of survival (Reynolds, 2006). The trade-off between these traits represents an ecological strategy to exploit better the available resources (Litchman et al., 2010). Since micro-phytoplankton exhibit, a wide range in their size (20 – 200) and shape, quantification of cell numbers only will not provide accurate information on Carbon biomass. Hence, there is a need to convert cell count to cell volume since a large number of small cells are equivalent to few larger cells in terms of carbon biomass (Harrison et al., 2015). Cell size and its carbon content evaluations from cell volume can provide useful inputs to ecosystem applications, modeling and biogeochemistry studies. Phytoplankton cell volume and its associated parameters have been reported from Chinese sea, Baltic Sea, Mediterranean Sea, Beagle Channel and North of Atlantic, (Almandoz et al., 2011; Barton et al., 2013; Olenina et al., 2006; Sarno et al., 1993; Stanca et al., 2013; Sun et al., 2000). However, a similar kind of work from the waters surrounding the Indian subcontinent is lacking. Although Harrison (Harrison et al., 2015) has cited some of the references in this context, published literature is meager. In the Indian waters, the Phytoplankton cell volume is measured in a few cases from the mangrove habitat and near coastal sites (Biswas et al., 2010; Mitra et al., 2012; Munir et al., 2015). This study provides information on cell volume and carbon per cell of diatom and dinoflagellate from coastal and open ocean stations. The dataset is further compared for inter bioregional variations.

Materials and Methods

2.1. Study area

The surface water samples from the Bay of Bengal hereafter referred as “BoB” (XBT program using ships of opportunity) were collected from April 2008 to March 2010 on seven occasions along the Chennai - Port Blair; 81°00' E/ 13°00' N to 92°00' E/ 11°23' N, and on six occasions (April 2008 to March 2010) along Port Blair to Kolkata; 12°00' N / 93°14'E to 21° 00'N / 88° 23' E at 22 different stations. The stations are categorized into C-P open ocean (CPOS), Andaman Region (AR), P-K Open Ocean (PKOS) and River Mouth (RM) regions as shown in Figure 1. From the Northern Arabian Sea (NAS) the surface water samples were collected while on a cruise SSK60 from 25th January 2014 to 1st February 2014 (40 stations covering 6 transects; 20°13' E / 68°90' N to 18°50' E / 69°99' N) and one coastal station located off Goa, Dona Paula Bay (15°27'N, 73°48'E), weekly twice from 1st September to 24th December 2015 with a total 34 samples.

1.2. Hydrological parameters

From the BoB vertical temperature profile of the water column was recorded by launching XBT- MK21 - T7 Probes (Sippican Inc.) at one-degree intervals. From the Northern Arabian Sea, the temperature was recorded using CTD. In the Dona Paula Bay, surface water temperature was measured *insitu*. The conductivity of surface seawater from the Bay of Bengal and Dona Paula Bay was measured using Autosal and later converted into salinity (Guildline autosal 8400B). From the Northern Arabian Sea, the conductivity was measured using dual conductivity (SBE4) sensor fitted to CTD.

In all regions, for nutrients, 10 ml of seawater samples were collected into 10 ml cryovials, immediately frozen in liquid nitrogen and then analyzed using Skylar, (San++ segmented flow analyzer) following the method of Grasshoff et al. (1983).

2.3 Estimation of micro-phytoplankton cell volume

From the BoB, three liters of surface water samples were collected separately and preserved with different preservatives. (0.40% of Lugol's iodine, 0.60% buffered formaldehyde and 0.20 % Glutaraldehyde). The samples were allowed to settle in the laboratory for quantification of diatoms and dinoflagellates through a microscope. From the Northern Arabian Sea, only one liter of surface water samples was collected and fixed with 0.40% Lugols iodine for the estimation of diatom cell volume and a similar procedure was followed as that of BoB. For the estimation of dinoflagellates, thirty-five liters of surface water

samples were collected and concentrated to 50.0 ml, using 20 μ nylon mesh. The samples were immediately fixed with 0.40% Lugol's iodine. At the end of the cruise, the samples were brought to the laboratory and concentrated to 35.0 ml and 5.00 ml of this concentrated sample was analyzed for dinoflagellates. For the coastal station of Dona Paula Bay, one liter of surface water was concentrated to 20.0 ml, of which 2.00 ml of sample was dispensed on a 3.80 cm Petri dish and measured for both diatoms and dinoflagellates.

The cell dimensions of diatoms and dinoflagellates from the BoB were measured using an Ocular micrometer, calibrated with a stage micrometer. From the Northern Arabian Sea and Dona Paula Bay, the cells were measured using image analysis software (Q-Capture Pro 7, Olympus Inc). In all the three sites cells were observed using an inverted microscope (Olympus IX71) at 100 and 200 times magnification. The measured dimension for each taxon was calculated for its cell volume using assigned geometric shape (Hildebrand, 1999; Sun and Liu, 2003). The range of cell size and cell volume, its classification according to size classes, the median value of cell volume and the number of cells measured (N) from three different regions are provided in Appendix (1A and 1B). A comparative analysis of the cell volume, 10 species of diatoms and dinoflagellates (which has a minimum number of 8 measurements is presented in Fig.2a-g). The rest of the species with cell volume are provided in Appendix 1A.

The carbon per cell was calculated using the equation provided by Menden-Deuer and Lessard (2000). The median volume was converted to carbon per cell using the equation $C = aV^b$ where a and b are 0.288 and 0.811 for diatoms, 0.216 and 0.939 for other protists, and 0.003 and 1 for *Noctiluca scintillans* (Macartney) Kofoid & Swezy, 1921. We also measured cell volume of live and fixed cells. The data is provided in (Appendix 1A and 1B). Studies on phytoplankton cell volume have emphasized that at least a minimum of 10 to 50 randomly selected cells for each species should be measured. Although we have measured most of the cells up to 25 or more, it was not possible to measure all the taxa since some of them were rare forms and they are measured as they occurred in the samples. The dataset from three different sites of northern Indian Ocean is compared with the published literature from different bioregions to evaluate the variations in the cell size (Appendix 2).

Results and Discussion

3.1 Hydrological parameters

The BoB, (CPOS and PKOS) comprised of stations that are away from the riverine influence, whereas the AR and RM are closer to the Irrawaddy and Hooghly - Ganga river basins. The variations in Sea Surface Temperature (SST), Sea Surface Salinity (SSS) and nutrients during the observation period are provided in details in another publication (Chitari et al in press). In brief, the SST was low during monsoon (NEM and SWM; 26.1 to 29.9°C) and relatively higher during the intermonsoon (SIM and FIM; 28.2 to 31.0°C). The SSS was relatively high in CPOS (29.2 to 34.4) when compared to P-K (25.7 to 34.4psu). Low SSS, was observed during the SWM, especially in the RM and was relatively high during the SIM and FIM.

Nutrient concentrations in the surface waters of the BoB were below detectable range for the most part of the year, especially during the SIM. In the CPOS, maximum concentrations of DIN, and DIP were observed on some occasions during the monsoon and was upto 3.02 and 2.88 $\mu\text{mol L}^{-1}$. In the PKOS it was on par with CPOS. However, in the AR and RM, it was noticed that the concentration was upto 4.23 $\mu\text{mol L}^{-1}$ for DIN and 3.08 $\mu\text{mol L}^{-1}$ for DIP. The relatively higher nutrient concentration can be attributed to freshwater discharge.

The temperature in the Northern Arabian Sea was observed to be low compared to BoB and Dona Paula Bay. The nutrients were higher (Nitrate $>2.00\ \mu\text{mol}$) compared to BoB and both are attributed to winter convective mixing. In the Dona Paula Bay high nitrate (0.40 to 8.00 μmol) and phosphate (0.01 to 0.68 μmol) concentration was also observed. The details of hydrological parameters of the northern Arabian Sea (Roy et al., 2015, Sarma et al., 2015) and Dona Paula Bay (Patil and Anil 2011; 2015) are available in the published literature.

3.2 Microphytoplankton cell volume

A total of 219 micro-phytoplankton species, 90 diatoms, and 129 dinoflagellates were measured during the study period from three different sites of Indian Ocean (BoB, Northern Arabian Sea, and Dona Paula Bay) (Appendix 1A and 1B). Regarding species composition, amongst the diatoms, *Chaetoceros* spp. followed by *Rhizosolenia* spp. were the dominant forms, whereas amongst the dinoflagellate, genus *Tripos* spp. was dominant and this was followed by *Protoperdinium* spp. The higher number of size classes was observed in diatoms especially in the Dona Paula Bay and River Mouth (Hooghly Estuary) when compared to dinoflagellates except for *Pyrocystis pseudonociluca* Wyville-Thompson, 1876 in the open ocean. The higher number of size classes observed in diatoms belonged to *Bacteriastrum*

furcatum Shadbolt, 1854, *Ditylum brightwellii* (T. West) Grunow, 1885, *Guinardia striata* (Stolterfoth) Hasle, 1996, *Guinardia delicatula* (Cleve) Hasle, 1997, *Leptocylindrus danicus* Cleve, 1889, *Proboscia indica* (H.Peragallo) Hernández-Becerril, 1995, *Rhizosolenia hylina* Ostefeld, 1901, *Rhizosolenia hebetata* f. *semispina* (Hensen) Gran, 1908, *Rhizosolenia setigera* Brightwell, 1858, *Proboscia alata* (Brightwell) Sundström, 1986 and *Pseudosolenia calcar-avis* (Schultze) B. G. Sundström, 1986. Such a size variation in the Dona Paula Bay and the River Mouth can be attributed to the nutrients and variation in salinity. Finenko (Finenko et al., 2003) observed diatoms possess a greater degree of plasticity and are dependent on the growth conditions (mainly nutrients and irradiance). Patil and Anil (2015) also observed blooms of these forms in the Dona Paula Bay and are driven mainly by variation in salinity (14 to 30 psu) and nutrients by freshwater discharge. Similarly, their variations in the Andaman Region can also be attributed to terrigenous inputs and rainfall.

The Cumulative variance in the cell volume between similar taxa measured by ocular micrometer and image analysis software showed maximum variations in most complex shapes. In the simplest forms having minimum line parameters, the CV was within a range of 2-3%. However, a maximum variation of 21% was observed in more complex shapes having multiple line parameters such as *Climacodium frauenfeldianum* Grunow, 1868 and then followed by *Chaetoceros* spp. Ehrenberg, 1844 and *Thalassionema frauenfeldii* Tempère & Peragallo, 1910. (Appendix 3).

3.3 Seasonal and Spatial variations in micro-phytoplankton cell volume in the Bay of Bengal.

Seasonal variations in cell volume among the diatoms along the BoB was maximum during the SWM (July 2008, Sept 2008 and July 2009), and minimum during Intermonsoon (April 2008, March 2009 and March 2010). Among the diatoms, variations were observed in *L. danicus*, *G. striata* *Thalassionema nitzschoides* (Grunow) Mereschkowsky, 1902, *Proboscia alata*, *R. hebetata* f. *semispina*, *Rhizosolenia castracanii* H.Peragallo, 1888 and *Rhizosolenia bergonii* H.Peragallo, 1892 (Figure 2a-g).

In some of the dinoflagellates, maximum variation was observed during the monsoon and minimum during Intermonsoon (*P. pseudonoculica*, *Triplos furca* (Ehrenberg) F.Gómez, 2013 and *Triplos fusus* (Ehrenberg) F.Gómez, 2013) and can be attributed due to wind-driven mixing (Figure 2a-g). Irrespective to the seasons, the Andaman Region and River Mouth showed maximum variations in cell volume when compared to the open ocean sectors of C-P

and P-K (figure 2a-g). Dinoflagellates are known to be a poor competitor for nitrates and half of them are heterotrophic. Vertical migration in the water column allows them to persist with non-competitive parameters for nitrogen uptake and growth (Eppley et al., 1969; Smyada, 1997). The utilization of energy for mobility could be one of the reasons for minimum variation in cell volume.

3.4 Comparison of cell volumes from the Indian ocean with different regions of the world

The cell volume data from this study is compared with the information available, from Atlantic (Barton et al., 2013, Olenina et al., 2006), Pacific (Sun et al., 2000), and the Mediterranean Sea (ki Tai Kim, 1995) and is summarized in Figure 3. Out of 219 species measured for cell volume from this study, we could compare only 10 species for which the reference data in all the regions were available. (Figure 3, Appendix 2). The maximum cell volume was observed from the waters of North Atlantic and the minimum was observed from the Mediterranean Sea. Larger cell size observed in the Northern Atlantic, compared to the Mediterranean could be due to variation in temperature. Smith and Reynolds (2003) observed annual mean SST within a range of 0 to 25.0°C. In the Mediterranean waters, several authors (Sarno et al., 1993 and Stanca et al., 2013) observed temperature variation from 3.00 to 30.0°C. The temperature variations in the two different regions could be the factor for the variations in the cell volume.

Till date, only 8.00% of the studies have estimated cell volume in the waters surrounding Indian subcontinent (Leblanc et al., 2012). In the Atlantic, Pacific and Arctic region several organized groups such as HELCOM (Helsinki Commission), PEG (Phytoplankton Expert Group), ECS (European Committee for Standardization) have set up standard protocols, to estimate biovolumes using recommended shapes of Hildebrand (Hildebrand et al., 1999), and Sun and Liu (Sun and Liu, 2003) for various phytoplankton species (Harrison et al., 2015; Olenina et al., 2006.). In the Indian waters, although few datasets are available there is a need to follow the most simple and common protocol to facilitate inter bioregional comparison.

According to Harrison (Harrison et al., 2015), the diatom cell volumes and carbon estimates are a single largest source of uncertainty. Since larger diatoms are 20,000, times more in its cell volume than the small diatoms. Volumes of big dinoflagellates are 1500 times larger than small dinoflagellates. The ranges in diatom cell volumes are 10 times greater than across

dinoflagellates (i.e.>20,000 vs. 1500 times). The Information from the Indian Ocean Region provided in this paper adds a number of species from the open ocean and provide their size ranges.

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Figures

Figure 1: Locations of sample collection from the Northern Indian Ocean (Bay of Bengal, Northern Arabian Sea, and Dona Paula Bay). In the Bay of Bengal, samples were collected from four different tracks (Chennai to Port Blair open ocean – CPOS; Andaman Region – AR; Port Blair to Kolkata open ocean – PKOS; and River Mouth- RM). From the Northern Arabian Sea samples were collected from 40 stations and in the Dona Paula Bay from one station.

Figure 2a-g: Intra- and Inter-annual variations in the cell volume (log transformed values) of 10 diatoms and dinoflagellates species from the Bay of Bengal, which had minimum numbers of 8 measurements. The cells measured were from April 2008 to March 2010 (**a**; April 2008, **b**; July 2008 **c**; Sept 2008, **d**; March 2009, **e**; July 2009, **f**; Sept 2009, **g**; March 2010) along the 4 different tracks (Chennai to Port Blair open ocean – CPOS; Andaman Region – AR; Port Blair to Kolkata open ocean – PKOS; and River Mouth – RM). The regions are denoted in different shades. Species of Diatoms are indicated in bold and Dinoflagellates are indicated in regular font.

Figure 3: Comparison of cell volume from 4 different geographical regions. These includes present dataset, Mediterranean Sea (ki Tai Kim 1995), Pacific Ocean (Sun et al. 2000) and North Atlantic (Olenina et al. 2006, Barton et al. 2013). The ten species which are found to be common in all the 4 regions were clustered using the Bray–Curtis similarity coefficient and group average method (log transformed). The species used for clustering are marked by (*) and is provided in Appendix 2.

Appendix 1. Supplementary Data

Appendix 1A: List of Diatoms and Dinoflagellates cell sizes, cell volume and Carbon per cell from the Bay of Bengal and Northern Arabian Sea. The columns from left to right (**A** to **L**) denote **A** - Serial number; **B**– Species; **C** - length range of the cell (μm); **D** - width range of the cell (μm); **E** and **I** - number of cells; **F** and **J** - range of cell volume from minimum-maximum (μm^3); **G** and **K**- median value (μm^3); **H** and **L**; carbon per cell (pg C cell^{-1}) measured for Bay of Bengal and Northern Arabian Sea respectively.

Appendix 1B: List of Diatoms and Dinoflagellates cell sizes, cell volume and Carbon per cell from the Dona Paula Bay. The columns from left to right (**A** to **L**) denote, **A** - Serial number; **B** – species; **C** - length range of the cell (μm); **D** - width range of the cell (μm); **E** and **I** - number of cells measured; **F** and **J** - range of cell volume from minimum – maximum (μm^3); **G** and **K** - median value (μm^3); **H** and **L** - carbon per cell (pg C cell^{-1}) measured for live and fixed cells respectively.

Appendix 2. Supplementary Data

Appendix 2: Comparison of average cell volume ($\mu\text{m}^3 \times 10^4$) of the present study (Northern Indian Ocean) with different geographical regions. The species denoted with (*) were common to all the 4 regions. The columns (**A**-**E**) denote, **A** - serial number; **B** – Species; **C** - Present study (Northern Indian Ocean); **D** - Olenina et al. 2006 (Northern Atlantic), **E**- ki Tai Kim 1995 (Mediterranean); **F** - Sun et al. 2000 (Pacific); **G** - Barton et al. 2013 (Northern Atlantic).

Appendix 3. Supplementary Data

Appendix 3. Comparison of cell volume measured using Image analysis software (Q-Capture Pro7) and Ocular micrometer. Columns left to right (**A to J**) denote, **A** -Serial number; **B** – Species; **C** and **E** are the average values of cell volume; **D** and **F** are the number of cells (**N**) measured using Image Analysis and Ocular micrometer; **G** - represents Coefficient of Variation (CV%) of cell volume. **H** and **I** represent Cell carbon ($\text{pg carbon cell}^{-1}$) obtained from the average values of cell volume measured by Image Analysis (Q-Capture Pro 7, Olympus Inc) and Ocular micrometer respectively. The **J** column denotes coefficient of variation (CV %) of Cell carbon.

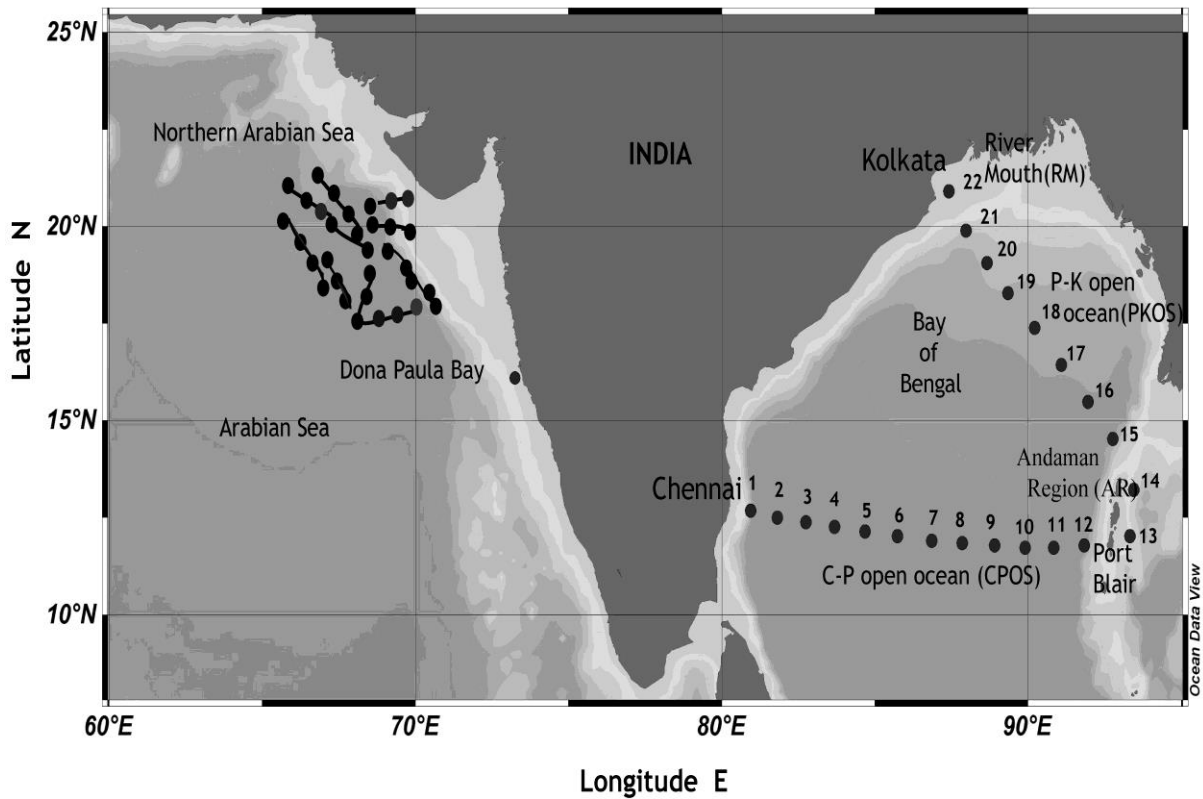


Figure 1

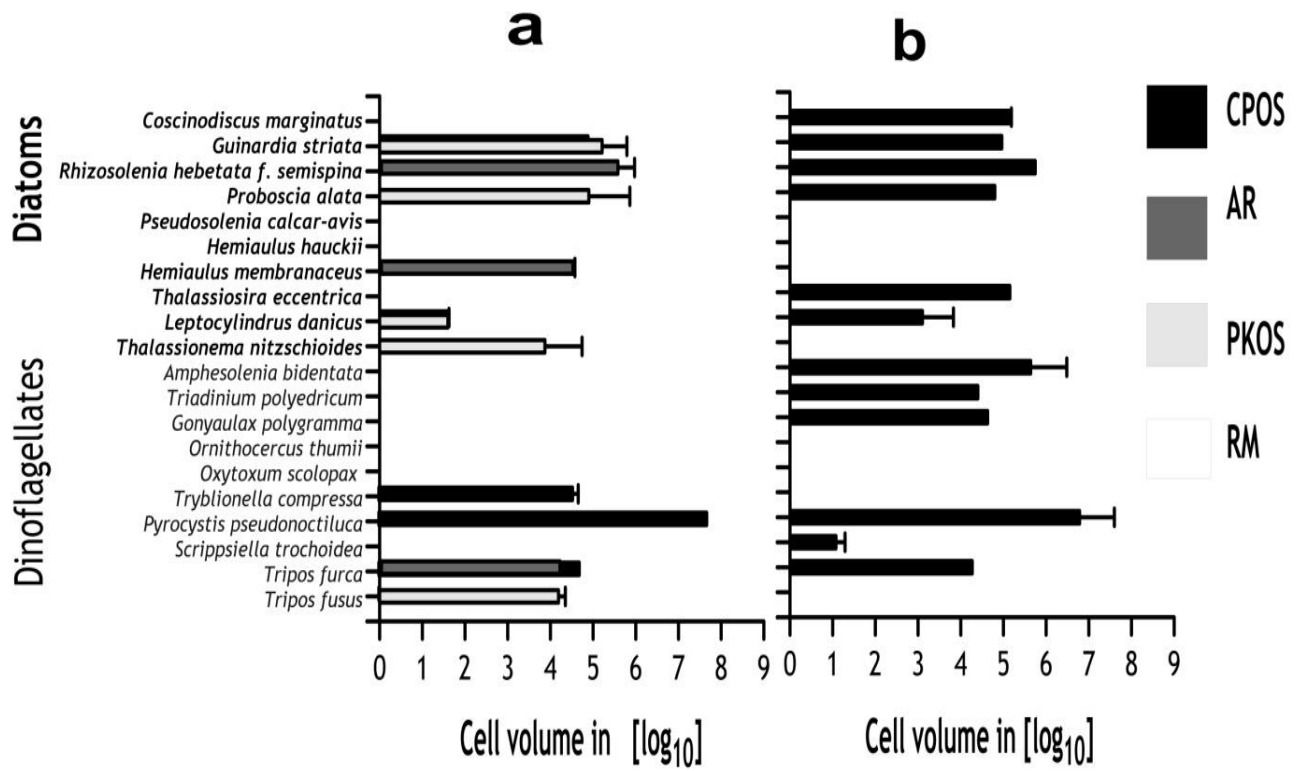


Figure 2a and b

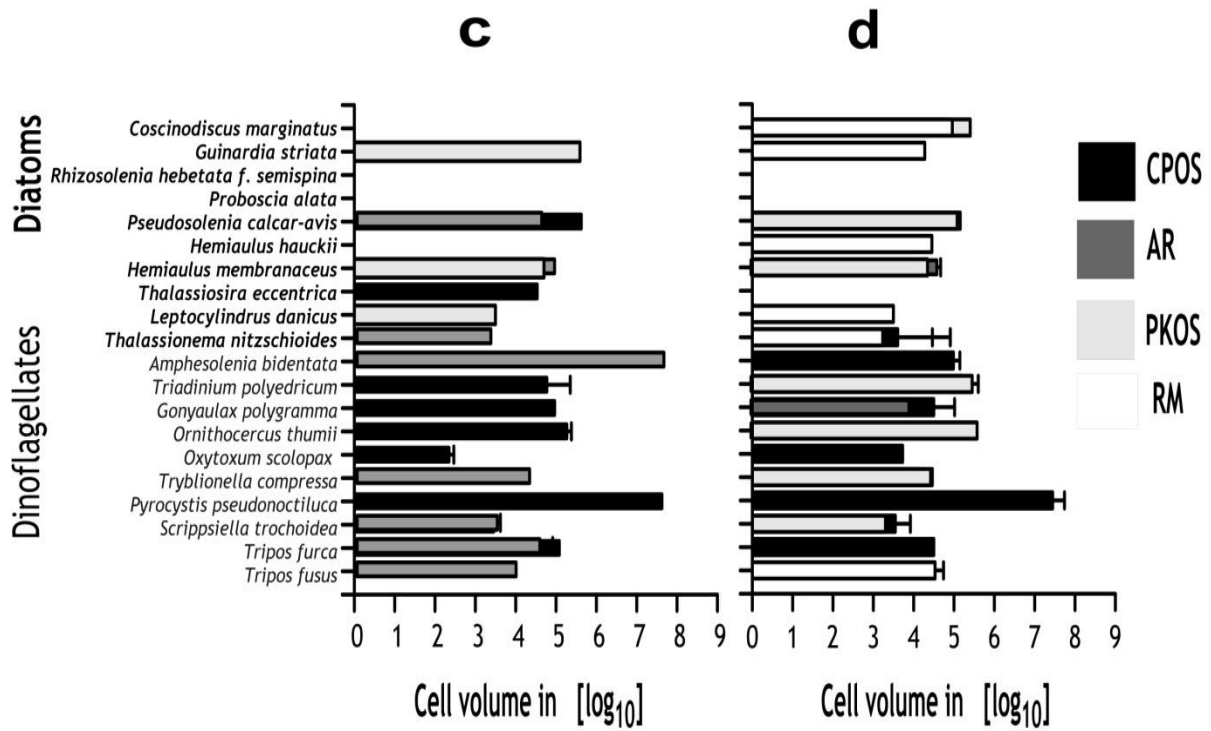


Figure 2c and d

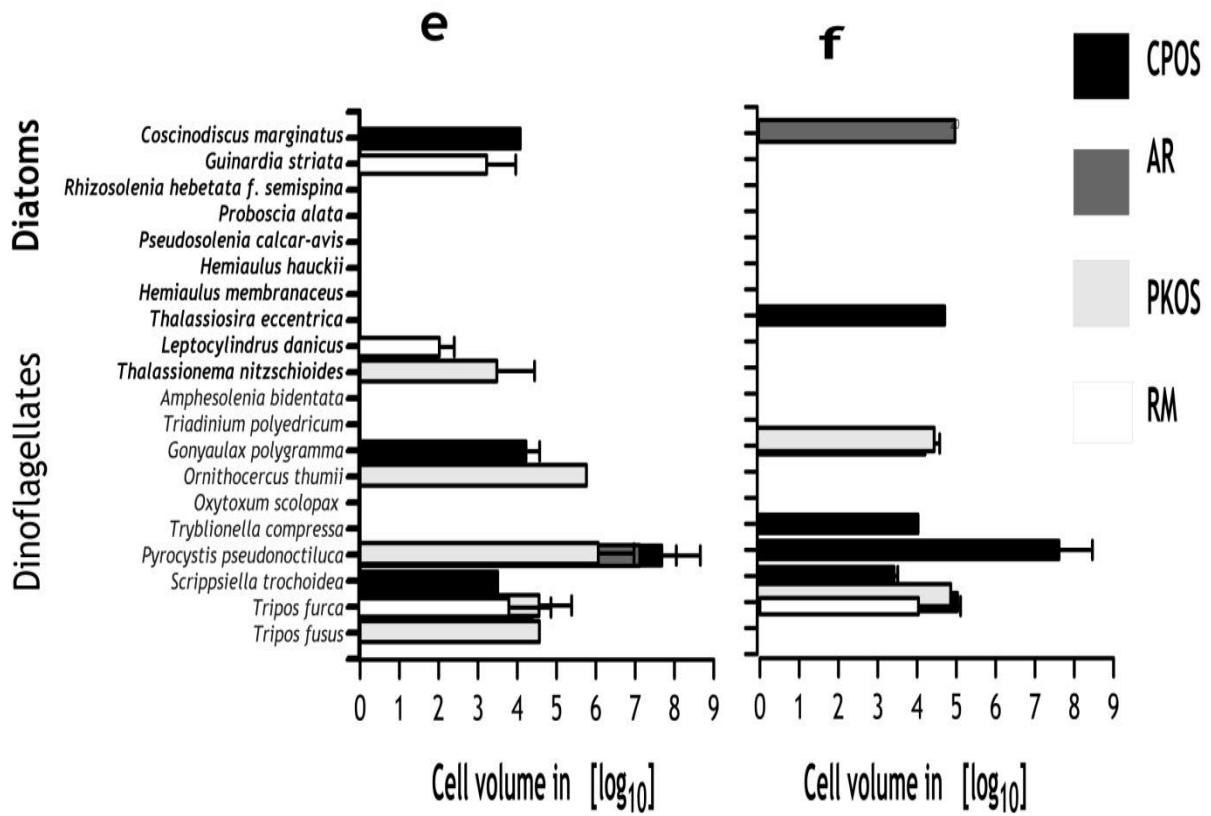


Figure 2e and f

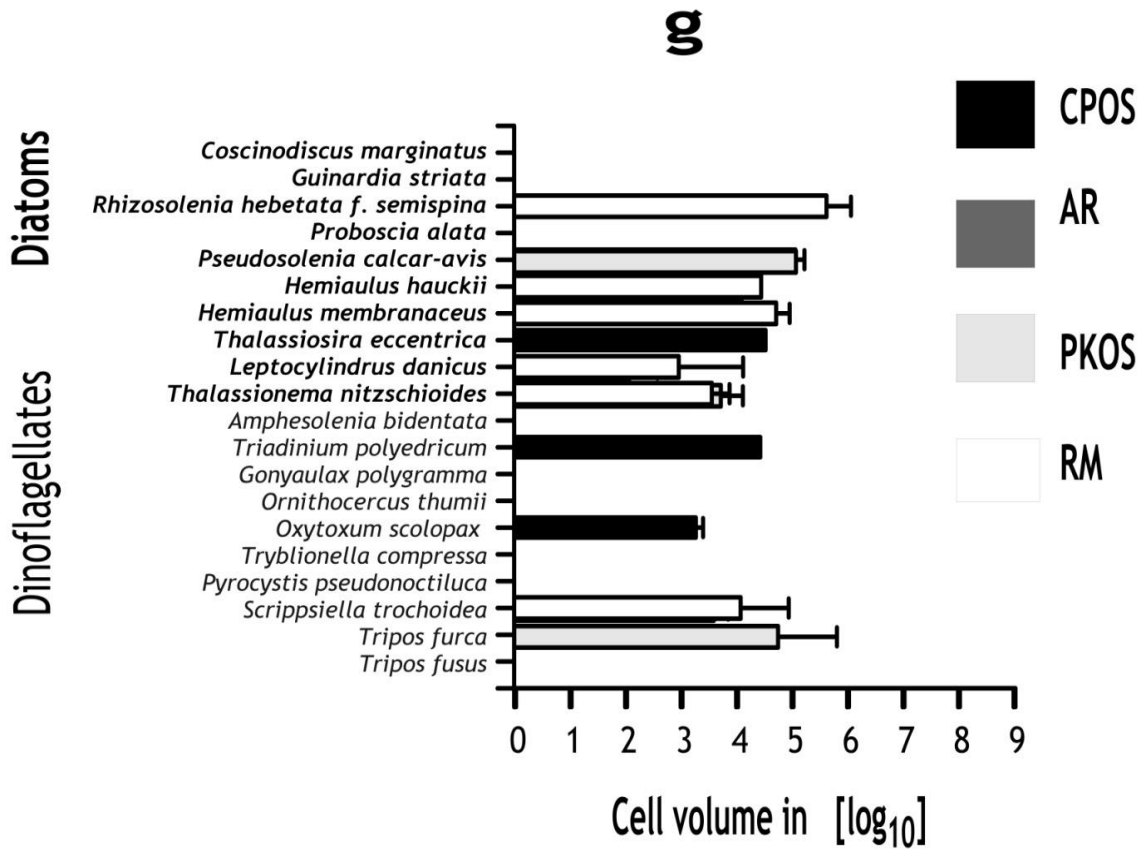


Figure 2g

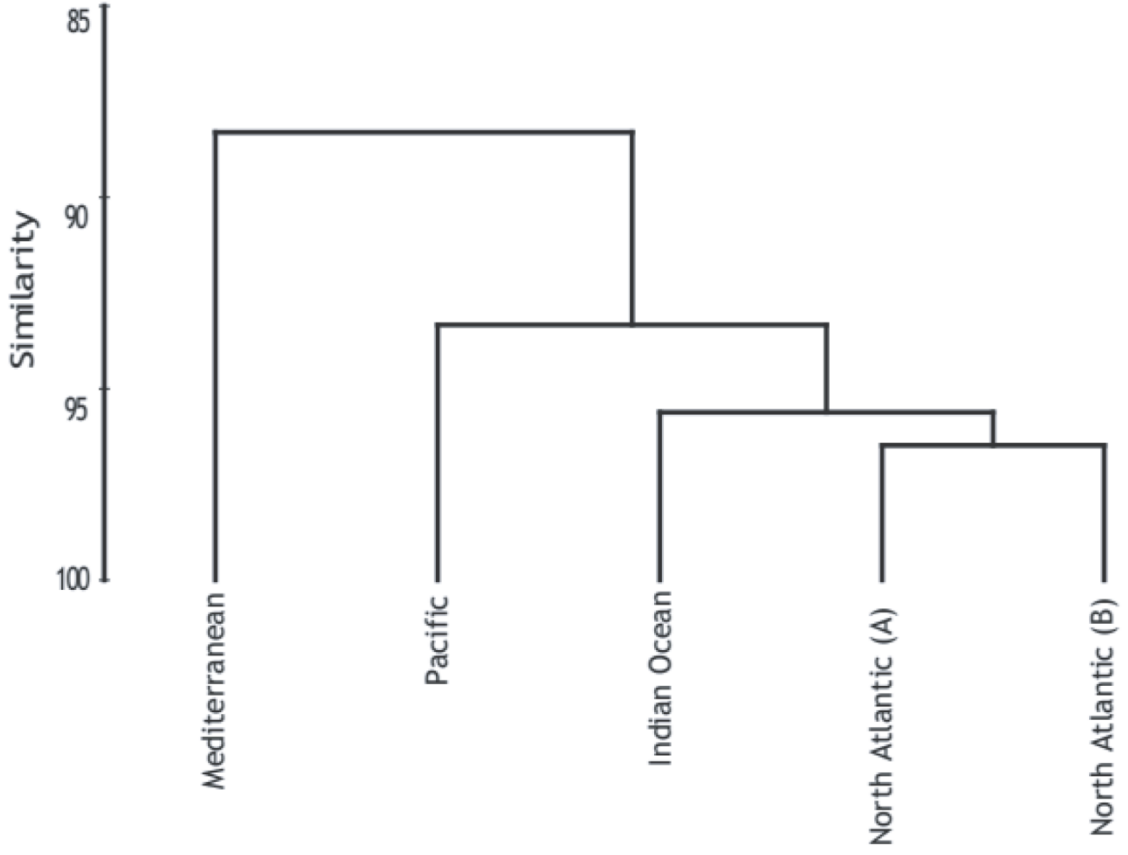


Figure 3