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A report on salinity-governance of auxospore size in euryhaline diatoms of a well mixed estuary on North-Eastern coastal Bay of Bengal

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Abstract. A three yearlong study was performed on the well mixed waters of the Hooghly Estuary on the North-Eastern coast of Bay of Bengal that revealed the significant effect of salinity towards the induction and governance of auxospores and their sizes in euryhaline centric and raphid pennate diatoms. The diatom species were chosen randomly (also due to their ubiquitous nature in the study area) in order to observe the effect of salinity in an unrestrictive manner [viz. *Coscinodiscus radiatus* Ehrenberg, *Coscinodiscus concinnus* W. Smith, *Coscinodiscus lineatus* Ehrenberg, *Coscinodiscus excentricus* Ehrenberg, *Chaetoceros danicus* Cleve, *Chaetoceros lorenzianus* Grunow, *Chaetoceros decipiens* Cleve, *Chaetoceros minimus* (Levander) D. Marino, G. Giuffre, M. Montresor & A. Zingone, *Pleurosigma formosum* W. Smith and *Nitzschia sigmoidea* (Nitzsch)]. Correlation (r-values at 5% level of significance) values between salinity and auxospore size revealed that lower salinity was conducive for larger auxospore (-0.7391 to -0.9282) production in case of all the species (total ten) studied upon. Nutrient parameters were also found to impart significant influences on auxospore size but not as prominent as salinity. Large auxospore was found to be the prerequisite to greater biovolume and vice versa, pointing to lower salinity regimes of a well mixed estuary favorable for sexual reproduction in euryhaline diatoms.

Keywords: Diatoms, Auxospore, Salinity, Biovolume, Hooghly Estuary.

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Introduction

Auxospores are sexual spores of diatoms and also their rejuvenescent spore since during asexual reproduction, the two frustules of a diatom cell separate and act as two different templates for the next generation. The larger epitheca gives rise to a cell line identical to the parental cell in size but the hypotheca of the parent cell forms a cell line which is smaller and epitheca guided cells in future generations continue be smaller to a point when the cells lose their metabolic viability (Crawford, 1974). That is where the auxospore is the evolutionary modification since is germinates to give rise to similar sized cells and restore the cell line. Evolutionary significance of auxospore has widely been acknowledged and accepted as Round and Crawford (1981) first initiated the hypothesis of monophyletic origin of diatoms and their phylogenetic connections to scale bearing progenitors.

A number of parameters influence the induction of auxospore formation in nature but they are very poorly understood and much work needs to be done to properly understand the dynamics of auxospore induction in diatoms. Since phytoplanktons have an innate connection with their surrounding environment, the slightest shifts in it generate responses in phytoplanktons. “In particular, diatoms are the most widely used indicators for palaeosalinity reconstructions (Fritz, 1990; Fritz et al., 1991; Juggins, 1992; Cumming and Smol, 1992; Ryves et al., 2004; Saunders, 2010)”. Salinity has often been considered by (Round, 1960; Underwood, 1994; Sullivan and Currin, 2000; Trobajo et al., 2004), to be an important factor determining diatom distribution in estuaries, and in fact, tolerance to changes in salinity (eventuated, for example, by tides, changing freshwater inputs and rainfall) has been inferred, from field studies, to be a prerequisite for most diatoms living in estuaries and coastal wetlands). However, culture-based studies on the growth of these species under wide salinity regimes are required to better interpret the role of the salinity in determining their natural distributions.

The salinity exerts one of the most significant stresses one any aquatic organism, more so in case of the phytoplanktons which are in incessant contact with their fluid ambience. Since auxospore generation and stress or adverse conditions go hand in hand (since origin of sexual reproduction in algae has been hypothesized and proven to have been associated with adverse nutrient deficient or highly stressful environment), the investigation on the possible effect of increase or decrease in salinity on the governance of auxospores in diatoms (decrease in salinity might trigger larger auxospore induction as a consequence to larger cell volume in centric and pennate diatoms of a well mixed estuary and vice versa) has always been of utmost significance only intensifying in case of a well mixed tropical estuary such as the Hooghly Estuary, since data regarding the auxospore and its dynamics with the physicochemical environment in a well mixed estuary are highly deficient.

The three year study was performed to understand the triggering factors of auxospore induction as well as the parameters that govern their sizes in euryhaline diatoms of a well mixed tropical estuary. The study was performed taking into account three stations that are varying in chemical and biological parameters, most notably salinity. As auxospore is one of the most important features of a diatom cell, parameters that influence their size are of equal importance from ecological point of view. Studies on auxospore are very rare since these propagules are rarely observed and an account such as the present one is always of significance to ecologists and provide platform to future studies as well.

Material and methods

Study area

The primary samplings were performed at Kachuberia (21° 51’ 39” N, 88° 8’ 37” E), Chemaguri (21° 40’ 43” N, 88° 7’ 28” E) and Gangasagar (21° 38’ 0” N, 88° 5’ 0” E) located in the Hooghly Estuary (Figure 1). The selection of these locales were mainly based on their clearly discernible salinity differences ranging from region with high riverine influence (Kachuberia) as, to brackish water regions (Chemaguri) and marine dominated region (Gangasagar). The Hooghly Estuary is primarily fed by the River Ganges. The River Hooghly is the chief distributary of the River Ganges and is transformed into an irregular coastal marshy habitat to form the largest delta in the world, with severe fluvial deposits. The Hooghly Estuary exchanges flow with the Bay of Bengal near the Sagar Island and its tidal domain for nearly 250 km. the mouth of the estuary has a funnel shape and the predominant tidal regime is semidiurnal in nature. The vertical tide range at the mouth varies from 5.2 m during the spring tide to 1.8 m during the neap period. This is a well mixed estuary due to the intense tidal and wave actions with a meso-macrotidal setting (2.5-7 m amplitude). Mean current velocities are between 108 and 117 cm s^{-1} during high and low tides respectively (Biswas et al., 2004). The climate of this

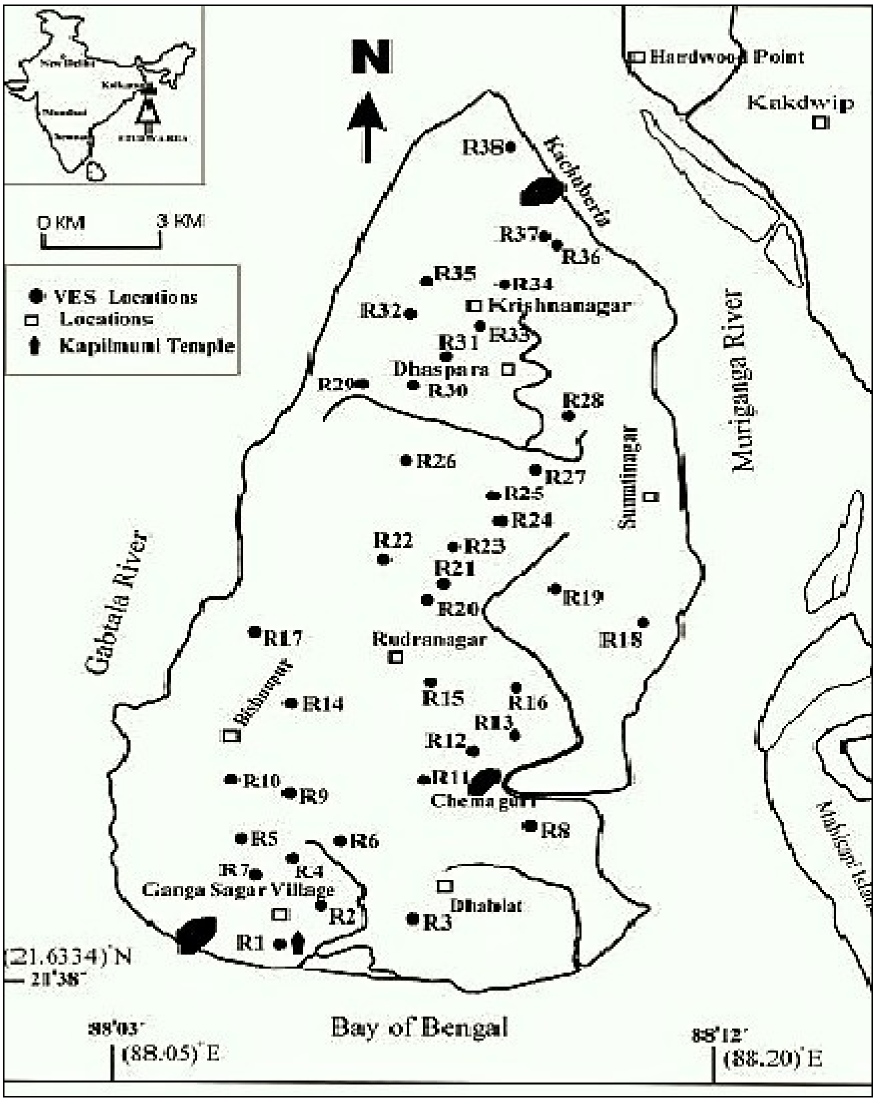

Figure 1. Map showing study sites with sampling stations in black blotches (Mukherjee et al., 2015).

region is characterized by the Southwestern monsoon (July-October), Northeast monsoon or post monsoon (November-February) and premonsoon (March-June); at least 70-80% of the rainfall occurs during the summer monsoon (SW monsoon), resulting in the high river discharge (2,952-11,897 $m^3 s^{-1}$), which gradually diminishes to 900-1,500 $m^3 s^{-1}$ during non-monsoonal months (Mukhopadhyay et al., 2006).

Sample collection and analyses

Monthly data of surface water salinity, air temperature, water temperature, wind speed, dissolved oxygen, primary productivity and essential nutrients (nitrate, phosphate and silicate) were recorded and monthly phytoplankton sampling was performed from study sites for a total period of two years, 2011 and 2012. The essential meteorological back ground data of the days of the collections were procured from the usage of Shimadzu Weather Station. Salinity of the water was first measured with the aid of refractometer and then estimated using argentometric method and the factor corrected values were put in.

Water samples for the estimation purposes were collected using the Niskin water sampler. For the estimation of phosphate, nitrate and silicate, the standard methods were followed (Grasshoff et al., 1983). Gross primary production and community respiration were measured *in situ* using the light and dark bottle method (Strickland and Parsons, 1972). The phytoplankton (diatom) sampling was performed using a handheld net [phytoplankton net (bolting silk no. 30, mesh size 20 µm) equipped with flow meter] from different stations in the Hooghly Estuary. The mesh size was fixed for the microphytoplanktons and not the nanoplanktons to maintain a certain degree of separation during the study and also due to the reason that increased turbidity rendered the usage of smaller mesh size impossible due to large quantities of silt clogging. The chief gear used in this purpose was country boats to ensure the least disturbance of the prevalent population of phytoplanktons. After the collection, the phytoplankton concentrates were transferred into 25 mL Tarson polythene containers and 4% formalin along with Lugol's iodine were used as preservatives depending on the need of clarification and specific preferences. The formalin is a very good agent for clearing cells off organic debris but it doesn't stain cells hence it is hard to discriminate between living, viable cells from dead, resuspended ones. The samples were then analyzed under light microscopes, bright field microscopes and phase contrast microscopes for their accurate and proper identification, using proper literature (Desikachary 1986-1989; Hasle and Syvertsen, 1997; Al-Kandari et al., 2009). All measurements were performed using ocular micrometer and standardized stage micrometer under high power objectives (x45).

Ten species were selected depending on the presence of auxospore during the study tenure viz. *Coscinodiscus radiatus* Ehrenberg, *Coscinodiscus concinnus* W. Smith, *Coscinodiscus excentricus* Ehrenberg, *Coscinodiscus lineatus* Ehrenberg, *Chaetoceros danicus* Cleve, *Chaetoceros decipiens* Cleve, *Chaetoceros lorenzianus* Grunow, *Chaetoceros minimus* (Levander) D. Marino, G. Giuffre, M. Montresor & A. Zingone, *Nitzschia sigmoidea* (Nitzsch) W.Smith and *Pleurosigma formosum* W. Smith (Figure 2), comprising both centric and pennate diatoms. A total of 100 cells of each species collected at each season on average were checked for the presence of auxospore and the mean size along with the standard deviation were plotted in the tables. Same process was followed in case of calculating biovolumes of the cells (length x breadth x height for cells not spherical and π x radius^2 x height for spherical cells). These species are also euryhaline species and distributed pan tropically, hence observations made on them should reflect similar scenario elsewhere also, making it a study with global implication.

Data compilation and statistical analyses

All data statistical calculations were performed using the MS-Excel software but for statistical analyses, the 'add-in' of Excel, the Xlstat software was used and the principal component analysis tool of the data analysis package of the Xlstat 12 was employed to deduce the Pearson correlation was performed at the 5% significance level. All the figures were converted to encapsulated postscript files from MS Office artworks by using relevant softwares. The tables were produced using the table function and not the spreadsheets.

Results

The study to delineate the most important governing factor for the induction of auxospores in diatoms in a well mixed tropical estuary was carried out in the waters of the Hooghly Estuary on the North-Eastern coast of India at the confluence of Bay of Bengal. Detecting a particular parameter in well mixed tropical estuaries is hard and equally important because of the obvious reason of it being well mixed and all the physicochemical parameters apparently contribute to some extent. For the current study few very important parameters were selected viz. dissolved nitrate-nitrogen, dissolved phosphate-phosphorus, dissolved silicate, surface water salinity, dissolved oxygen and

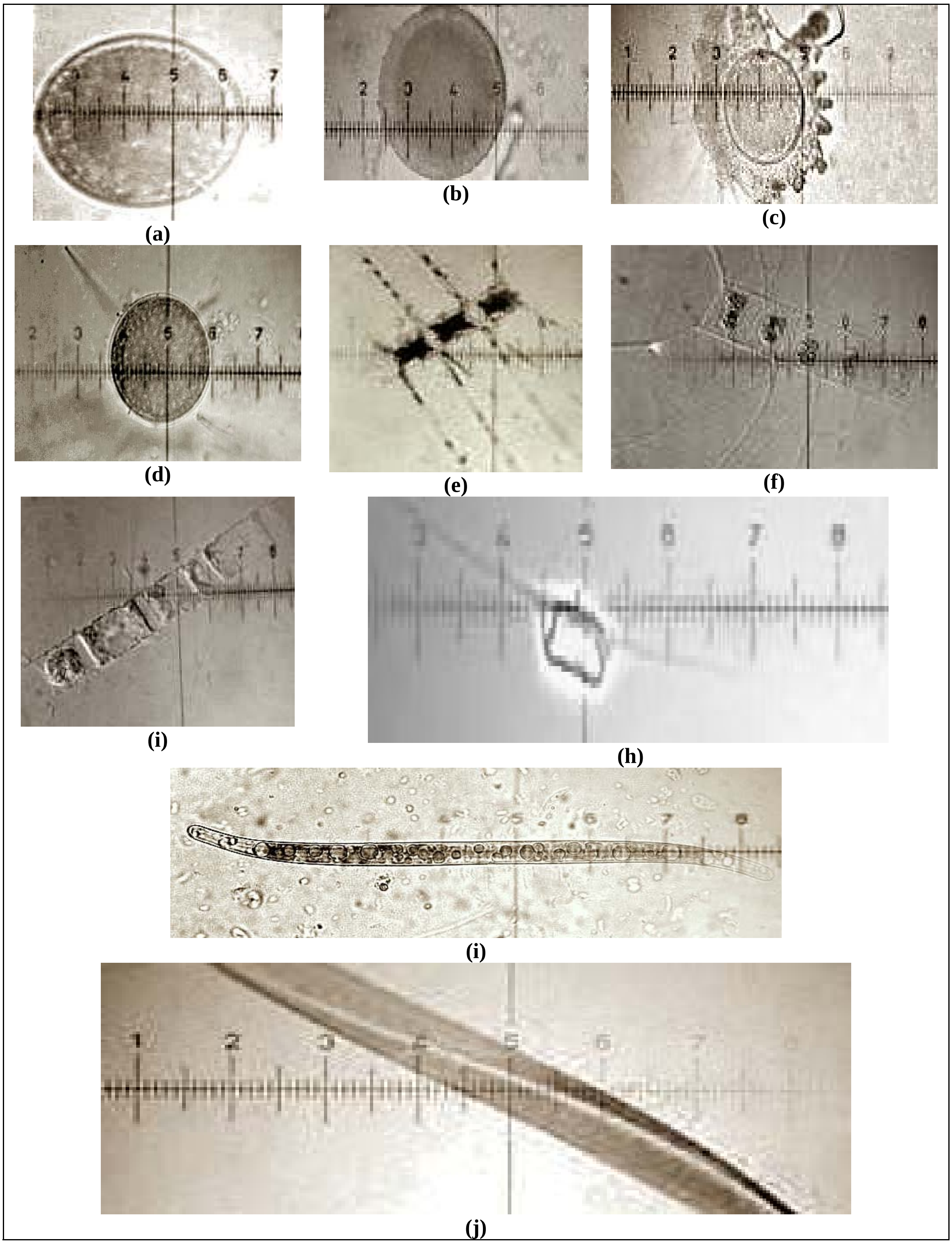

Figure 2. Micrography of selected species. (a) *Coscinodiscus radiatus* under high power objective (x40) with auxospore initial cell [one standardized ocular small division = 3.32 µm]; (b) *Coscinodiscus concinnus* under high power objective (x40); (c) *Coscinodiscus excentricus* under high power objective (x40) [soon after release from the mother cell, note the mucilaginous outgrowths]; (d) *Coscinodiscus lineatus* under high power objective (x40); (e) *Chaetoceros danicus* under high power objective (x45); (f) *Chaetoceros decipiens* under high power objective (x40); (g) *Chaetoceros lorenzianus* under high power objective (x40); (h) *Chaetoceros minimus* under high power objective (x45); (i) *Nitzschia sigmoidea* under high power objective (x40); (j) *Pleurosigma formosum* under high power objective (x40).

primary productivity, thereby encompassing the major chemical and biological parameters. The study continued for three years (Table 1) and seasonal variations in the parameters were quite prominent.

The general trend was the increase in chief nutrients such as nitrate and phosphate during the monsoon months, although silicate always registered the highest values during the post monsoon periods. Salinity was found to be lowest during the monsoon period, and highest during premonsoon followed by the post monsoon. Primary productivity was found to be highest in the post monsoon, followed by premonsoon and lowest in monsoon. The dissolved oxygen followed the trend of primary productivity closely.

Table 1. Spatio-temporal variation in selected parameters (nutrients, dissolved oxygen, primary productivity, salinity) governing the growth and development of phytoplanktons in well mixed waters of the Hooghly Estuary. The study encompassed three years, from 2010 to 2013, which were again subdivided into seasons viz. premonsoon (March to June), monsoon (July to October) and post monsoon (November to February) respectively.

Parameter	Year	Season	Kachuberia	Chemaguri	Gangasagar
NO_3-N (μM)		Pre'2010	14.02±3.14	14.22±2.24	16.57±2.66
	2010-2011	Mon'2010	18.77±2.31	19.75±1.56	20.89±1.37
		Post'2010	12.95±3.50	13.55±2.95	13.89±3.12
		Pre'2011	15.94±1.57	16.05±1.89	16.71±2.84
	2011-2012	Mon'2011	20.82±2.03	20.55±1.63	21.16±1.44
		Post'2011	11.84±1.68	12.30±2.84	12.72±2.10
		Pre'2012	12.35±2.88	12.26±3.25	14.75±3.89
	2012-2013	Mon'2012	20.41±2.65	20.85±2.14	21.67±2.62
		Post'2012	14.59±3.03	15.21±2.12	15.43±1.99
PO_4-P (μM)		Pre'2010	1.57±0.45	2.04±0.36	1.96±0.45
	2010-2011	Mon'2010	1.78±0.40	2.25±0.44	2.31±0.94
		Post'2010	1.10±0.26	1.24±0.12	1.21±0.12
		Pre'2011	1.80±0.55	2.24±0.54	2.15±0.55
	2011-2012	Mon'2011	1.66±0.48	1.84±0.49	1.88±0.52
		Post'2011	0.82±0.12	1.24±0.25	1.15±0.16
		Pre'2012	1.83±0.24	2.22±0.55	2.15±0.50
	2012-2013	Mon'2012	1.98±0.46	2.45±0.54	2.60±0.48
		Post'2012	1.24±0.27	1.60±0.22	1.65±0.66
SiO_4 (μM)		Pre'2010	58.22±16.62	60.32±14.52	65.24±17.86
	2010-2011	Mon'2010	66.51±10.55	71.39±15.67	74.98±30.06
		Post'2010	72.36±15.36	77.65±22.64	84.75±23.47
		Pre'2011	50.88±13.52	52.05±20.48	54.32±21.96
	2011-2012	Mon'2011	75.62±15.24	83.26±21.47	88.86±23.19
		Post'2011	81.25±12.66	82.87±18.54	86.96±16.67
		Pre'2012	49.58±11.47	51.66±18.45	54.59±22.93
	2012-2013	Mon'2012	83.25±17.84	88.23±15.28	91.06±08.17
		Post'2012	73.15±09.45	75.66±13.82	79.89±13.32
PP ($mgCm^{-3}h^{-1}$)		Pre'2010	25.17±3.09	30.29±2.72	30.16±2.59
	2010-2011	Mon'2010	18.83±2.72	22.47±3.15	23.15±2.54
		Post'2010	31.29±5.08	32.11±6.71	32.99±5.37
		Pre'2011	26.31±3.78	29.78±3.56	30.69±5.49
	2011-2012	Mon'2011	21.21±1.97	23.49±1.21	23.48±2.00
		Post'2011	31.27±5.11	32.49±5.23	33.51±5.15
		Pre'2012	25.55±0.83	28.53±2.65	30.05±3.70
	2012-2013	Mon'2012	23.21±1.01	24.33±3.14	24.98±3.67
		Post'2012	30.89±3.86	33.32±3.66	35.42±3.93

Table 1. Continued.

Parameter	Year	Season	Kachuberia	Chemaguri	Gangasagar
D.O. (mgL^{-1})		Pre’2010	5.20±0.62	4.80±0.67	5.80±0.74
	2010-2011	Mon’2010	3.85±0.84	3.52±0.47	4.27±0.77
		Post’2010	6.95±0.23	5.94±0.62	7.05±0.95
		Pre’2011	5.30±0.38	4.50±0.74	5.52±0.64
	2011-2012	Mon’2011	4.22±0.41	3.98±0.22	4.47±0.32
		Post’2011	6.28±0.82	6.20±0.93	6.86±0.86
		Pre’2012	4.98±0.80	4.82±1.02	5.10±0.98
	2012-2013	Mon’2012	4.13±0.44	4.22±0.53	4.47±0.32
		Post’2012	6.35±0.74	6.37±0.69	6.70±0.87
Salinity (psu)		Pre’2010	16.30±3.52	18.25±2.52	19.10±3.63
	2010-2011	Mon’2010	07.63±4.62	09.52±2.12	11.32±1.36
		Post’2010	12.30±2.84	15.24±3.32	16.96±6.94
		Pre’2011	16.77±2.31	19.30±2.55	21.30±3.30
	2011-2012	Mon’2011	05.95±2.36	09.54±3.55	11.85±3.85
		Post’2011	13.82±2.84	15.35±5.23	17.22±6.69
		Pre’2012	18.77±4.14	20.45±2.05	22.81±1.25
	2012-2013	Mon’2012	06.64±2.47	12.35±5.58	16.28±4.94
		Post’2012	15.33±2.40	18.70±2.05	20.03±1.63

Observation of auxospore in nature is quite rare but in well mixed estuarine habitats auxospore induction is readily observed in few euryhaline species among which the following were the few selected due to their year round availability at all the sampling sites during high viz. *Coscinodiscus radiatus*, *Coscinodiscus concinnus*, *Coscinodiscus lineatus*, *Coscinodiscus excentricus*, *Chaetoceros danicus*, *Chaetoceros lorenzianus*, *Chaetoceros decipiens*, *Chaetoceros minimus*, *Pleurosigma formosum* and *Nitzschia sigmoidea*. The mean auxospore size was selected due to the fact that during a single collection (one month), a selected species might not have harbored an auxospore but did so in other months in a season. Hence seasonal mean was scientifically and statistically more accurate and valid choice.

From Table 2 it can be easily observed that the auxospore sizes in all the selected study species of diatoms varied greatly from season to season and the largest auxospores in terms of size was observed during monsoon. The centric diatoms in general displayed auxospores of larger sizes due to the advantage over their pennate counterparts in possessing greater surface area to volume ratio. Premonsoon and post monsoon both recorded drastically reduced auxospore sizes.

The auxospore size fluctuation pattern was reflected in the trend of variation in biovolume of the selected diatom species (Table 3), which might have been reflections upon the fact that larger cell volume was the prerequisite to auxospore formation or auxospore generation increased the cellular dimensions. Both ways, dependence on salinity for the size determinacy of auxospores and the relationship between auxospore size and cell volume of the diatoms can be better highlighted using the Table 4, where the r-values clearly suggest that salinity, among the selected parameters is the most significant factor in governance of auxospore size in euryhaline diatoms of a well mixed estuary, since other parameters such as the nutrients all have relatively less significant positive r-values (dissolved nitrate being the most significant among the nutrients) but none as important as salinity.

Discussion

The reason behind the rise in nutrients during monsoon in general was due to the immense riverine run off deposited in the estuarine basin which

Table 2. Seasonal variation in average surface area values or the sizes of the auxospores observed in the selected species (the only ones in which prominent auxospores were observed) during the tenure of the study. There is considerable fluctuation in the size of the auxospores formed in the species mentioned and only the average value is given (sizes were measured from specimens collected from the three study sites) to make the table concise and to provide an idea of the seasonal variations that took place in the Hooghly estuarine waters on the whole.

Year	Auxospore Area (μm²)	*C. radiatus*	*C. concinnus*	*C. lineatus*	*C. eccentricus*	*C. danicus*	*C. lorenzianus*	*C. decipiens*	*C. minimus*	*P. formosa*	*N. sigmoidea*
2010-2011	Pre'2010	1,050.72	1,150.52	112.60	2,135.87	132.20	680.60	135.20	58.50	70.62	1,040.60
	Mon'2010	2,190.5	2,320.66	225.91	4,553.68	280.60	1,152.00	286.40	132.00	120.50	2,192.00
	Post'2010	1,040.74	1,220.75	116.86	2,243.48	155.30	790.44	167.50	72.70	70.74	1,140.44
2011-2012	Pre'2011	946.95	1,173.60	107.81	2,229.15	145.40	779.40	146.80	53.25	76.95	1,139.40
	Mon'2011	1,899.60	2,348.51	244.52	4,659.41	272.00	2,145.60	378.75	126.58	129.60	2,105.20
	Post'2011	1,051.65	1,153.43	127.22	2,433.27	158.40	988.36	142.83	55.45	81.65	1,248.36
2012-2013	Pre'2012	1,348.72	1,146.13	91.51	2,228.79	141.60	685.40	35.74	55.60	68.72	1,045.40
	Mon'2012	2,488.25	2,323.68	232.22	4,855.40	392.40	1,968.80	90.65	135.20	128.25	4,108.30
	Post'2012	1,441.58	1,217.50	137.88	2,740.52	204.40	889.53	41.22	60.44	81.58	1,149.32

**C. radiatus= Coscinodiscus radiatus, C. concinnus= Coscinodiscus concinnus, C. lineatus= Coscinodiscus lineatus, C. eccentricus= Coscinodiscus eccentricus, C. danicus= Chaetoceros danicus, C. lorenzianus= Chaetoceros lorenzianus, C decipiens= Chaetceros decipiens .C. curvisetus= Chaetoceros curvisetus, C. quarctatus= Chaetoceros quarctatus, P. formosa= Pleurosigma formosa and N. sigmoidea= Nitzschia sigmoidea*.

Table 3. Seasonal variation in average cell biovolumes observed in the selected species (the only ones in which prominent auxospores were observed) during the tenure of the study. There is considerable fluctuation in the size of the auxospores formed in the species mentioned and only the average value is given (sizes were measured from specimens collected from the three study sites) to make the table concise and to provide an idea of the seasonal variations that took place in the Hooghly estuarine waters on the whole.

Year	Cell Biovolume (μm³)	*C. radiatus*	*C. concinnus*	*C. lineatus*	*C. eccentricus*	*C. danicus*	*C. lorenzianus*	*C. decipiens*	*C. minimus*	*P. formosa*	*N. sigmoidea*
	Pre'2010	19,233	18,755	8,910	24,869	2,051	2,204	1,295	68	1,245	18,720
2010-2011	Mon'2010	116,088	112,082	21,120	77,175	6,093	10,023	6,589	260	4,286	55,672
	Post'2010	23,729	43,125	16,740	29,586	2,678	2,482	1,657	71	2,634	29,310
	Pre'2011	20,864	17,568	10,164	25,672	2,572	2,162	1,167	70	1,210	20,423
2011-2012	Mon'2011	175,840	138,840	23,680	86,130	8,360	8,356	4,220	235	5,034	54,428
	Post'2011	47,689	49,627	17,176	32,308	2,807	2,378	2,384	72	1,869	31,680
	Pre'2012	18,767	21,360	12,127	22,126	2,248	2,162	1,362	63	1,482	28,984
2012-2013	Mon'2012	146,322	161,754	26,648	78,916	7,632	11,562	8,034	216	4,327	76,628
	Post'2012	38,763	46,559	14,162	30,889	2,556	2,385	2,286	78	1,662	41,896

**C. radiatus= Coscinodiscus radiatus, C. concinnus= Coscinodiscus concinnus, C. lineatus= Coscinodiscus lineatus, C. eccentricus= Coscinodiscus eccentricus, C. danicus= Chaetoceros danicus, C. lorenzianus= Chaetoceros lorenzianus, C decipiens= Chaetoceros decipiens .C. curvisetus= Chaetoceros curvisetus, C. quarctatus= Chaetoceros quarctatus, P. formosa = Pleurosigma formosa and N. sigmoidea= Nitzschia sigmoidea*.

Table 4. R-values at 5% significance level, of the dependence of auxospore size with salinity of the ambient surface water as well as the inclination of biovolume towards auxospore size. The size dependency of the auxospores was also correlated with the nutrient parameters. The following species were the only ones in which prominent auxospores were observed during the course of the study.

	Auxospore area : nitrate	Auxospore area : phosphate	Auxospore area : silicate	Auxospore diameter : Salinity	Auxospore Diameter : Cell Biovolume
C. radiatus	+0.8558	+0.5844	+0.4790	-0.7391	+0.8624
C. concinnus	+0.9379	+0.5040	+0.5042	-0.9034	+0.9546
C. lineatus	+0.9191	+0.3812	+0.6249	-0.9282	+0.8970
C. eccentricus	+0.9274	+0.4828	+0.5649	-0.8820	+0.9880
C. danicus	+0.8637	+0.4982	+0.6207	-0.7553	+0.8908
C. lorenzianus	+0.8299	+0.3129	+0.6635	-0.7933	+0.8270
C. decipiens	+0.5729	+0.0175	+0.2888	-0.7865	+0.9032
C. minimus	+0.9116	+0.4734	+0.544	-0.9142	+0.9848
P. formosa	+0.9299	+0.4492	+0.5791	-0.8926	+0.9378
N. sigmoidea	+0.7960	+0.5483	+0.5613	-0.6617	+0.9187

**C. radiatus= Coscinodiscus radiatus, C. concinnus= Coscinodiscus concinnus, C. lineatus= Coscinodiscus lineatus, C. eccentricus= Coscinodiscus eccentricus, C. danicus= Chaetoceros danicus, C. lorenzianus= Chaetoceros lorenzianus, C decipiens= Chaetoceros decipiens .C. curvisetus= Chaetoceros curvisetus, C. quarctatus= Chaetoceros quarctatus, P. formosa= Pleurosigma formosa and N. sigmoidea= Nitzschia sigmoidea*.

includes flood induced washed out agricultural fields also, thereby injecting copious amount of natural and anthropogenic nutrients into the estuarine sink. At the end of the monsoon months, when excess runoffs cease to empty in the estuary, the dissolved silicate values start to rise and which was reflected in the comparatively higher values exhibited during post monsoon.

The estuarine region is populated by the both euryhaline and stenohaline phytoplanktonic species which have certain level of tolerance towards changes in the water salinity and since the estuary under observation was a well mixed estuary, the general and significant drop in salinity affected all the species and only those with extremely polarized tolerances, meaning able to withstand very large salinity fluctuations were able to thrive. This directly affected the primary productivity and consequent production of oxygen. The nutrient load which gets deposited in the estuary during monsoon provides the impetus for the growth of the phytoplanktons during the post monsoon, leading to greater primary productivity and dissolved oxygen which was clearly evident from the study (Table 1).

From the Tables 2, 3 and 4, a picture emerges where it is clear that dilution of surface water salinity actually was conducive for the generation of larger auxospore and cells with greater biovolume as well. Salinity dilution only happened during the monsoon period which recorded the largest auxospore in all the species (both centric and pennate) while in premonsoon and post monsoon, surface water salinity was higher and auxospores were reduced in size. Sexual reproduction in diatoms involves formation of auxospore but in most species this is a relatively rarely observed phenomenon.

Auxospores are rejuvenation tactics deployed by the diatoms to counter their gradual decrease in size through asexual reproduction (where smaller hypotheca of one generation becomes the epitheca of the next generation leading to size loss) to retain the size of the parent cell. At the same time sexual reproduction in diatoms is indicative of stressful situations and generally phytoplanktons employ asexual means of propagation in favorable conditions (Crawford, 1974; Round and Crawford, 1981), which should have been the case in monsoon season due to the immense nutrient load being added into the estuarine basin. But from the study it is clear that auxospores are largest during monsoon which could be pointing to the salinity stress introduced by the dilution of the salt content by massive freshwater load.

All the parameters were taken into consideration during the plotting of a principal component biplot curve (Figure 3) as part a multivariate analysis and it clearly depicted that among all the parameters considered for the study, salinity influenced the auxospore size most significantly and lower salinity was proven to be prerequisite to larger auxospores in both centric and pennate diatoms. Nutrients such as nitrate, phosphate etc also have positive influences in the generation of auxospore, but not as significant as that of salinity. The evolutionary significance of auxospore formation and their structure has already been accepted (Round and Crawford, 1981; von Stosch, 1982; Kaczmarska et al., 2000, 2001) and the study with results showing auxospore formation and increase in biovolume may be linked should provide more impetus for future studies.

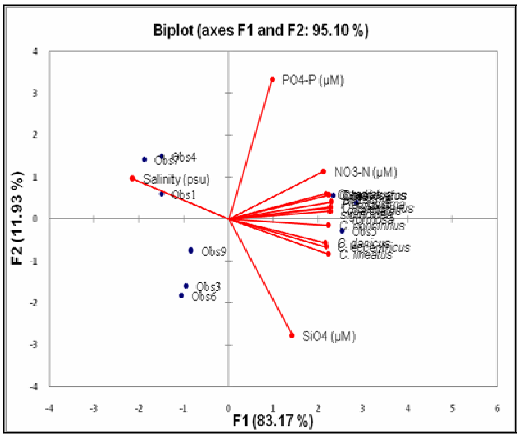

Figure 3. Figure representing the Principal Component Biplot Curve to show the influence of selected physicochemical parameters on Auxospore size during the study period.

Conclusion

Auxospores are one of the most important evolutionary significant features of the diatoms and knowledge regarding their morphogeny has only started to brush the surface and that too mainly on cultured specimens. In nature auxospores are quite rare and well mixed tropical estuaries with their large amount of nutrient load often prove to be the places to find them in diatoms. The study reported here is of immense significance from the point of view of induction of auxospore in centric and raphid pennate euryhaline diatoms. Among many feasible parameters salinity proved to be the most important in size governance of auxospore and it was also found that larger cell volume might have been the prerequisite to larger auxospore or vice versa. The lower saline regions of a well mixed tropical estuary such as the Hooghly Estuary was observed to be the zones with largest auxospores therefore germinating into healthier cells.

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