Available online at www.ijpab.com

ISSN: 2320 – 7051
Int. J. Pure App. Biosci. **3 (3):** 94-104 (2015)

INTERNATIONAL JOURNAL OF PURE & APPLIED BIOSCIENCE

Research Article

OPEN ACCESS

Laboratory experiment reveals some key factors behind auxospore induction in two ubiquitous centric diatoms of Hooghly Estuary, Bay of Bengal, India

Abhishek Mukherjee, Subhajit Das, Sabyasachi Chakraborty and Tarun Kumar De*

Department of Marine Science; Calcutta University; 35, B.C. Road, Kolkata-700 019

*Corresponding Author E-mail: tarunde@yahoo.co.in

ABSTRACT

An experiment was performed taking into account four species each of Coscinodiscus Ehrenberg and Chaetoceros Ehrenberg to delineate the potential triggering factors inducing auxospores. Auxospores are sexual spores of diatoms and are normally formed as response to adverse environmental conditions to curtail the size reduction of diatoms. The parameters considered to be most important for this purpose are salinity, turbidity, pH and plant nutrients. Two experimental set ups were established, each of which contained a gradient of all the parameters resulting out of simulated dilution of the stock media solution to mimic possible conditions experienced by the subject species in nature. Unfiltered, turbid and filtered, clear natural seawater were employed as the basic culture media for the two groups of the sets. The various experimental results as well as statistical data pointed towards salinity and turbidity rather than the more widely accepted parameters such as nutrients and pH to serve as potential triggering factor. When only salinity was considered, the auxospore induction appeared to be conservative and 15-22 psu was the range for optimal generation. The range broadened when turbid water was used and the species developed auxospores in 8-30 psu of salinity. The potential turbidity range was found to be 80 – 270 NTU depending upon the presence of auxospores. This experiment shed important lights in the generation of auxospores and in well mixed estuaries, it might be induced among species near the confluence in drier periods and can extend to off shore as well during monsoon based on the results obtained.

Key words: *Auxospore; Diatom; Hooghly estuary; Salinity; Turbidity*

INTRODUCTION

The environment in which phytoplanktons grow is highly dynamic. This is more applicable to aquatic diatoms of a well mixed tropical estuary where the biogeochemical cycles are intricately linked as spatio-temporal changes in mixed layer depth[25], coupled with tidal mixing[18] and wind mixing[22] cause periodic fluctuations in stability and temperature of the ocean surface, changing irradiance, nutrient availability (and thus maximum growth rate), on a wide variety of time scales.

Diatoms are one of the most significant marine primary producers and play out the most important role in the oceanic carbon and silica cycles[20]. Although the intricate life histories of several diatoms have been described[7,8,4], field observations of sexual stages in marine planktonic species are extremely scant [14,11, 24,5], though a few studies on the centric diatoms have been dealt with using molecular techniques and those still are not nearly enough[13].

The auxospore is unique to the diatoms and in general the cell results from allogamous fusion. The major function of auxospores in the life of a diatom is to restore the size which progressively reduces with each generation due to the unique morphology of the diatom valves.

The larger epitheca gives rise to cells of equal diameter but the smaller hypotheca start to produce smaller cells and ultimately the cell line perishes due to the size of the cell which is no longer viable enough to perform any sort of physiological activities. "Typically the auxospore cell wall consists of two biogenic constituents: organic matter and siliceous ingredients, although the two differ from those in vegetative cells", according to[19].

The current study dealt with the delineation of the ecological factors that might serve to be the key agents in the induction of the auxospores in the centric diatoms in a well mixed tropical estuary such as the Hooghly in the north east coast of Bay of Bengal. The species selected as the subjects of the experiment were *Coscinodiscus radiatus* Ehrenberg, *Coscinodiscus concinnus* Wm. Smith, *Coscinodiscus linetus* Ehrenberg, *Coscinodiscus eccentricus* (Ehrenberg) Cleve, *Chaetoceros danicus* Cleve, *Chaetoceros lorenzianus* Grunow, *Chaetoceros curvisetus* Cleve *and Chaetoceros coarctatus* Lauder since these are the more ubiquitous species encountered along the stretches of the Hooghly estuary and any observations made out of them can be hoped to reflect in similar species around the well mixed estuaries of the tropical world.

The primary objective of the experiment was to narrow down the most potential triggering factors behind the induction of the auxospores in the dominant centric diatoms in the well mixed waters of the Hooghly estuary. Since auxosporulation is the size-reduction-curtailing mechanism employed by the diatoms; knowledge on the physicochemical parameters which govern the process is of utmost importance. Nutrient dynamics in open oceans, lakes or stratified estuaries follow a rather straightforward path compared to the well mixed estuaries of the tropics which are generally formed by the large rivers emptying into the bay or the sea and because the study took place in such a site, ecological implications of the outcome gains even more weightage. This is the first report of any experiment on auxospores in centric diatoms of Hooghly estuary and the factors taken into considerations were salinity, pH, turbidity and chief plant nutrients which are mostly considered to be important behind the generation of any propagules in phytoplanktons. The study and the results eventuating out of the experiments are of immense ecological significance and the knowledge regarding auxospores always play the pivotal role in our understandings of the centric diatoms.

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 (Fig. 1). The selection of these locales were mainly based on their clearly discernible salinity differences and mangrove vegetation covers, ranging from region with high riverine influence (Kachuberia) as mangrove impoverished zone, to brackish water regions and mangrove vegetation (Chemaguri) and marine dominated region (Gangasagar) with sparse mangrove cover. 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 5.2m during the spring tide to 1.8m during the neap period. This is a well mixed estuary due to the intense tidal and wave actions with a meso-macrotidal setting (2.5-7m amplitude). Mean current velocities are between 108 and 117 cm s^{-1} during high and low tides respectively. The climate of this 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 (2952-11897 m^3s^{-1}), which gradually diminishes to 900-1500 m^3s^{-1} during non-monsoonal months"[3,16].

Sample collection and analyses

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[9].

Salinity of the stock was estimated by following the argentometric titration method[21].The pH was measured using a Hach portable digital pH meter calibrated at pH 7 using buffer solutions from Merck. The water turbidity was monitored using a Systronics turbidity meter and recorded in nephelometric turbidity units.

The phytoplankton (diatom) sampling was performed using a handheld 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. 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 Olympus light microscopes, bright field microscopes and Magnus inverted microscope for their accurate and proper identification, using proper literature[10,6,1]. All measurements were performed using standardized ocular micrometer and stage micrometer under high power objectives (x40).

Selection of species

Eight species were selected depending on the presence of auxospore during the study tenure viz. *Coscinodiscus radiatus* Ehrenberg, *Coscinodiscus concinnus* W.Smith, *Coscinodiscus lineatus* Ehrenberg, *Coscinodiscus eccentricus* Ehrenberg, *Chaetoceros danicus* Cleve, *Chaetoceros lorenzianus* Grunow, *Chaetoceros curvisetus* Cleve, *Chaetoceros coarctatus* Lauder comprising both groups of dominant centric diatoms. The selection was purely based on the abundance and dominance of these species in the study sites as well as any other tropical estuaries making them the perfect study subject and can be assumed that observation on them and inferences drawn from them will be reflected in species in other estuaries as well, under similar circumstances.

Preparation of the experimental set ups

The aim of the experiment was to narrow down the potential physico-chemical parameters responsible for the induction of auxospores in centric diatoms found in a well mixed tropical estuary. The stock water sample collected had mean salinity value of 30.21 psu, pH at 8.38, turbidity at 270 NTU, dissolved nitrate-nitrogen at 22.14 μML^{-1}, dissolved phosphate-phosphorus 3.05 μML^{-1} and 103.82 μML^{-1} of dissolved silicate-silica. From that a salinity gradient was prepared by diluting the stock with Milli-Q water so that it formed the gradient as mentioned in the table 1. For each salinity stage, ten replicate sets were prepared. The salinity gradient automatically formed gradients of all the other parameters selected. The basis of the preparation of the various salinity concentrations was to simulate the salinity zone encountered at the study sites from near coast to offshore. The salinity levels were checked by using a refractometer at every stage and other parameters were checked by following standard method mentioned earlier.

Data compilation and statistical analyses

All data statistical calculations were performed using the MS-EXCEL software but for the 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 software packages. The tables were produced using the table function and not the spreadsheets.

RESULTS

An experiment was performed in laboratory conditions under controlled environment to monitor the effect of varying concentrations of salinity (6 to 30 psu) on the induction of auxospores in eight of the most dominant centric diatom species found in estuarine and coastal upwelling zones.

The subject species for the experiment were *Coscinodiscus radiatus, Coscinodiscus concinnus, Coscinodiscus lineatus, Coscinodiscus eccentricus, Chaetoceros danicus, Chaetoceros lorenzianus, Chaetoceros curvisetus* and *Chaetoceros quarctatus*. The reason behind the selection of the species was due to their ubiquitous nature as flagship diatom species throughout the worldwide well mixed tropical or subtropical estuaries with similar nutrient budget patterns. In other words, any observations made from the experiment on them or inferences drawn should reflect analogous patterns in other parts of the tropical to subtropical world as well.

The table 1 is indicative of the selected parameters considered to be paramount such as the chief plant nutrient like nitrate-nitrogen, phosphate-phosphorus and silicate-silica; as well as governing physicochemical variables like pH and salinity. From table 2 and Fig. 2 , it is clearly visible that the auxospore induction among the selected subject species took place most prominently between 15-22 psu salinity range when clear filtered natural seawater from the same collection site as that the diatoms were used. This phenomenon broadened in spectrum considerably when the seawater was used as it is, in its nature turbid condition, as evidenced from Fig.3 and table 3.

This is clearly observed in table 4 where, the appearance of auxospore has been depicted in the light of increase in turbidity. The values considered here are the integer mean values of each of the experimental set up created by dilution of the unfiltered natural seawater while attempting to prepare a salinity gradient. By taking into account all the four tables and two figures mentioned above, it can be summarised that the phenomenon of the induction of auxospores in the selected subject species of centric diatoms occurred in a more conservative manner during the usage of filtered seawater or indirectly saying when all other background parameters were taken into account excluding turbidity. And when turbidity was considered, the auxospore formation appeared to be more wide spread at even higher salinities.

Another study was performed to understand the effect of salinity on the size of the auxospores induced in the artificial laboratory condition where it was observed that in all the species the higher the salinity was the smaller was the size of the auxospore induced which has a direct pertaining to the effect of salinity on the size of the cell itself. The observation from table 5 shows that auxospores induced in filtered natural seawater expressed significantly high negative correlation values among all the selected diatom species (r-values ranging from -0.60 to -0.99) while this was not the case when turbidity along with all the other parameters came under consideration as unfiltered seawater was employed. The size-salinity relationship changed prominently in this case and the r-values were not statistically significant always. This experiment could point towards the effect of salinity and turbidity rather than all the other preconceived parameters to be important for the induction or generation of auxospores in natural conditions as well.

DISCUSSION

The experiment was focused on the conditions under which centric diatoms readily induce auxospores. The environment in which phytoplankton (both aquatic and oceanic) grow is highly variable in time and space. Diurnal and seasonal changes in mixed layer depth[25], coupled with tidal mixing[18] and wind mixing[22] cause periodic fluctuations in stability and temperature of the ocean surface, changing irradiance, nutrient availability (and thus maximum growth rate), on a wide variety of time scales. Diatom cells are enclosed in two siliceous thecae and during mitotic division each daughter cell retains one maternal theca and synthesizes a new one internally. It follows that the two daughter cells differ slightly in size, which causes a progressive reduction of the average cell size in a growing population[12,17]. This continual size reduction can be countered by the emergence of the sexual cycle and the generation of the auxospore. Within the auxospore, which is not surrounded by rigid siliceous thecae, a large sized initial cell is formed[2].

Origin of sex in phytoplanktons was postulated to be a response to adverse conditions when nutrient availability or any other growth promoting and/or supporting parameters were absent or not enough to sustain asexual reproduction. Since auxospores are the size rejuvenescent spores of the diatoms, it can also be surmised that auxospores only form in nature as responses to adverse conditions neither when sustenance of the usual asexual reproduction by the diatoms no longer remains the viable nor the feasible option due to higher expenditure and investment of resources.

The experiment was primarily focused on the effect of varying concentration of salinity, along with other chief variables, on the dominant diatoms of a well mixed estuary such as the Hooghly estuary. Natural sea water collected from the sites where the subject species were collected was used as the primary culture media. The results shown in the table 1 are the values of each of the parameters that were obtained by diluting the original stock in order to get the preferred salinity while the other parameters changed along with and automatically developed a gradient. Any artificial sequential modification (gradients at equal interval) during the preparation of the gradients was avoided strongly so that the results could also be applicable to natural conditions and furthermore the conditions created during the culture reflected mostly the environments the species would normally experience in their natural habitats.

The limited range in appearance of auxospores in the cells when filtered seawater was used within the salinity range of 15-22 psu (Fig. 2 and table 2) and the same phenomenon being wide spectrum when turbid seawater was employed (Fig. 3 and table 3) could point to a scenario where the former might have indicated the preferred salinity range for the auxospores to be induced in the selected centric diatoms, since the subject species comprise both euryhaline and marine species, an increase in salinity from the most preferred level for the euryhaline species or reduction in salinity level for the marine species could pose some osmotic adversity in the metabolism of these species and can prompt them to resort to sexual mode of reproduction instead. This range of salinity is readily encountered by these species in the nature during the post and premonsoon periods of the year in their natural habitat at the site of the collection, when the due to the excessive phytoplankton bloom reduces the dissolved nutrient concentrations in the water ad also very little to no rain fall causes no large scale river runoff ensuring clearer water in the basin and further clear water off shore free from any suspended colloid particle load.

The other set up that sparked the onset of sexual reproduction among the subject species was more analogous to environmental conditions encountered during monsoon periods which are characterised by highly turbid weathers with increased nutrient concentrations. A very surprising outcome of this experiment was the presence of auxospores among the species along a wider range of salinity gradients, and salt concentrations which were devoid of sexual reproduction now bore auxospores. The reason behind this could be that although the salt content provided osmotic shock, the suspended colloids provided them with sources to obtain nutrients which in itself are contrary to the conditions that trigger sexual reproduction.

Since the experimental set up was prepared in such a way that the gradients be formed from the highest concentration of all the parameters to the lowest by diluting the stock water with double distilled Milli-Q water, the higher the salinity, the higher were all the others including the nutrient in the first set up without any turbidity and there auxospores appeared within 15-22 psu range. In the second set up, that was not the case and auxospores among the species were detected almost throughout the gradient (10-30 psu) and the only parameter that was changed in this set was the turbidity as unfiltered seawater was used. The only possible inference that can be drawn from here is that since the cultures were grown in artificial optimal conditions and provided 20 Klux of light energy, the turbid condition greatly impeded the light penetration and ultimately rendering photosynthesis low or hindered greatly. This created a condition where sexual reproduction could have been the preferred choice by the diatoms (each concentration had ten set ups for the entire range and auxospores were detected in the majority of the ten sets, for every concentration of salinity in the second set up described in Fig. 3 and table 3).

From the correlation values shown in table 5, it can be clearly said that a very precise and significant correlation exists between the auxospore sizes and the salinity gradient (r = -0.60 to -0.99) when the clear filtered seawater was used, whereas the same cannot be said for the auxospores sizes recorded (r = -0.04 to -0.75) form the experimental set up containing turbid seawater as the media. The auxospore initial cells mimic the size of the parent cell and indirectly point to the effect of salinity on the size governance in diatom cells[15]. Again, the absence of any such pattern (Fig. 3) of cell size or auxospore size increase or decrease evidenced from the r-values in the set ups containing turbid water pointed towards the hindrance in the pattern due to the photosynthetic malfunction imposed by the increased scattering of incident light by the suspended particulate matters.

Table 1: The table is highlighting the selected significant parameters considered as influential in auxospore induction. The analyses were made on both the unfiltered and filtered seawaters used for the experiment and the gradient preparation was focused mainly on the salinity while the other parameters automatically changed as dilution increased.

*Sal (psu) fil	Turb (NTU)	Sal (psu) unfilt	pH	T Nit-N (μML^{-1})	D Nit-N (μML^{-1})	T Phos-P (μML^{-1})	D Phos-P (μML^{-1})	T Sil-Si (μML^{-1})	D Sil-Si (μML^{-1})
30.21	270.40	30.32	8.38	177.12	22.14	8.54	3.05	373.75	103.82
±0.61	±12.47	±0.20	±0.13	±21.66	±1.73	±2.19	±0.23	±54.06	±27.39
26.07	248.37	26.24	8.27	173.28	21.66	8.00	2.86	350.42	97.34
±0.78	±4.78	±0.33	±0.05	±29.05	±1.57	±1.92	±0.82	±69.38	±15.63
24.20	230.44	24.28	8.14	161.20	20.15	6.94	2.48	337.68	93.80
±0.33	±9.55	±0.09	±0.19	±30.44	±2.65	±1.25	±1.05	±41.60	±21.28
22.04	185.94	22.12	8.03	154.80	19.35	6.30	2.25	325.58	90.44
±0.93	±5.69	±0.15	±0.12	±22.87	±1.37	±1.84	±0.93	±35.76	±8.47
20.11	152.8	20.20	7.90	152.96	19.12	5.88	2.10	306.00	85.00
±0.80	±6.33	±0.36	±0.11	±14.70	±2.31	±1.43	±0.66	±54.29	±12.66
18.09	135.78	18.15	7.79	151.68	18.96	5.48	1.96	279.28	77.58
±0.73	±8.20	±0.59	±0.26	±5.65	±2.14	±1.65	±0.73	±39.94	±15.36
15.18	110.65	15.26	7.52	135.84	16.98	4.87	1.74	256.39	71.22
±0.47	±5.28	±0.21	±0.29	±12.86	±2.66	±1.35	±0.84	±42.76	±9.24
12.23	89.72	12.53	7.36	130.24	16.28	4.28	1.53	236.52	65.70
±0.82	±2.44	±0.24	±0.19	±30.72	±2.84	±1.15	±0.22	±33.54	±14.52
10.05	80.25	10.09	7.13	118.88	14.86	3.41	1.22	213.62	59.34
±0.91	±3.05	±0.56	±0.06	±11.92	±3.03	±0.73	±0.51	±41.90	±12.08
8.10	70.55	8.16	6.97	100.00	12.50	2.88	1.03	189.18	52.55
±0.43	±1.86	±0.46	±0.17	±6.38	±2.10	±1.06	±0.39	±23.03	±13.52
6.03	50.77	6.10	6.82	97.20	11.15	2.38	0.85	146.95	40.82
±0.97	±2.94	±0.23	±0.31	±14.77	±1.68	±0.64	±0.26	±20.77	±9.45

*the values in the table are presented as mean ± standard deviation among ten experimental set ups; fil = filtered, unfil = unfiltered, T = Total, D = Dissolved, Sal = Salinity, Turb = Turbidity, Nit-N – Nitrate-Nitrogen, Phos-P = Phosphate-Phosphorus, Sil-Si = Silicate-Silicate.

Table 2: The table is depicting the presence and absence of the auxospores in the experimental laboratory conditions under varying salinity in eight of the most dominant centric diatom species belonging to two genera *Coscinodiscus* and *Chaetoceros* respectively.

Salinity (psu) Gradient→	6	8	10	12	15	18	20	22	24	26	30
Coscinodiscus radiatus	X	X	X	√	√	√	√	√	X	X	X
Coscinodiscus concinnus	X	X	X	X	√	√	√	√	X	X	X
Coscinodiscus lineatus	X	X	X	X	√	√	√	X	X	X	X
Coscinodiscus eccentricus	X	X	X	√	√	√	X	X	X	X	X
Chaetoceros danicus	X	X	X	X	√	√	√	X	X	X	X
Chaetoceros lorenzianus	X	X	X	X	X	√	√	√	X	X	X
Chaetoceros curvisetus	X	X	X	X	√	√	√	X	X	X	X
Chaetoceros coarctatus	X	X	X	X	√	√	√	√	X	X	X

The source medium for the experiment used was filtered natural seawater of 30 psu. The salinity values considered here are mean integer values only.

Table 3: The table is depicting the presence and absence of the auxospores in the experimental laboratory conditions under varying salinity in eight of the most dominant centric diatom species belonging to two genera *Coscinodiscus* and *Chaetoceros* respectively.

Salinity (psu) Gradient→	**6**	**8**	**10**	**12**	**15**	**18**	**20**	**22**	**24**	**26**	**30**
Coscinodiscus radiatus	X	X	√	√	√	√	√	√	√	√	√
Coscinodiscus concinnus	X	X	X	X	√	√	√	√	√	√	X
Coscinodiscus lineatus	X	X	X	X	√	√	√	√	√	√	√
Coscinodiscus eccentricus	X	X	√	√	√	√	√	√	√	√	X
Chaetoceros danicus	X	X	X	√	√	√	√	√	√	√	√
Chaetoceros lorenzianus	X	X	X	X	√	√	√	√	√	√	√
Chaetoceros curvisetus	X	X	X	√	√	√	√	√	√	√	√
Chaetoceros coarctatus	X	X	X	√	√	√	√	√	√	√	√

The source medium for the experiment used was unfiltered natural turbid coastal seawater of 30 psu. The salinity values considered here are mean integer values only.

Table 4: The following table is showing the appearance of auxospores in the diatom cells as increasing range of turbidity under laboratory controlled environment. The turbidity was recorded as it is in the unfiltered natural seawater collected from the coast to offshore (from very high to lower turbidity) ranging from 270 to 50 NTU. The turbidity reading considered here are the mean integer values only.

Turbidity (NTU) Gradient→	**50**	**70**	**80**	**90**	**110**	**135**	**152**	**185**	**230**	**248**	**270**
Coscinodiscus radiatus	X	X	√	√	√	√	√	√	√	√	√
Coscinodiscus concinnus	X	X	X	X	√	√	√	√	√	√	X
Coscinodiscus lineatus	X	X	X	X	√	√	√	√	√	√	√
Coscinodiscus eccentricus	X	X	√	√	√	√	√	√	√	√	X
Chaetoceros danicus	X	X	X	√	√	√	√	√	√	√	√
Chaetoceros lorenzianus	X	X	X	X	√	√	√	√	√	√	√
Chaetoceros curvisetus	X	X	X	√	√	√	√	√	√	√	√
Chaetoceros coarctatus	X	X	X	√	√	√	√	√	√	√	√

Table 5: The table below is a comparative account of the range of correlation values determined between the auxospore diameter recorded in both the filtered and unfiltered natural seawater of varying salinity, made by diluting a stock of known salt concentration.

Species↓	**r-value of auxospore diameter: salinity of filtered natural seawater**	**r-value of auxospore diameter: salinity of unfiltered natural seawater**
Coscinodiscus radiatus	-0.8005	-0.2003
Coscinodiscus concinnus	-0.6766	-0.5476
Coscinodiscus lineatus	-0.8885	-0.0423
Coscinodiscus eccentricus	-0.6798	-0.0253
Chaetoceros danicus	-0.9332	-0.5846
Chaetoceros lorenzianus	-0.9998	-0.7698
Chaetoceros curvisetus	-0.6023	-0.3341
Chaetoceros coarctatus	-0.9741	-0.7503

Fig. 1 The above figure is depicting the map of the collection sites for the study. The black patches are pointing the locations of the stations

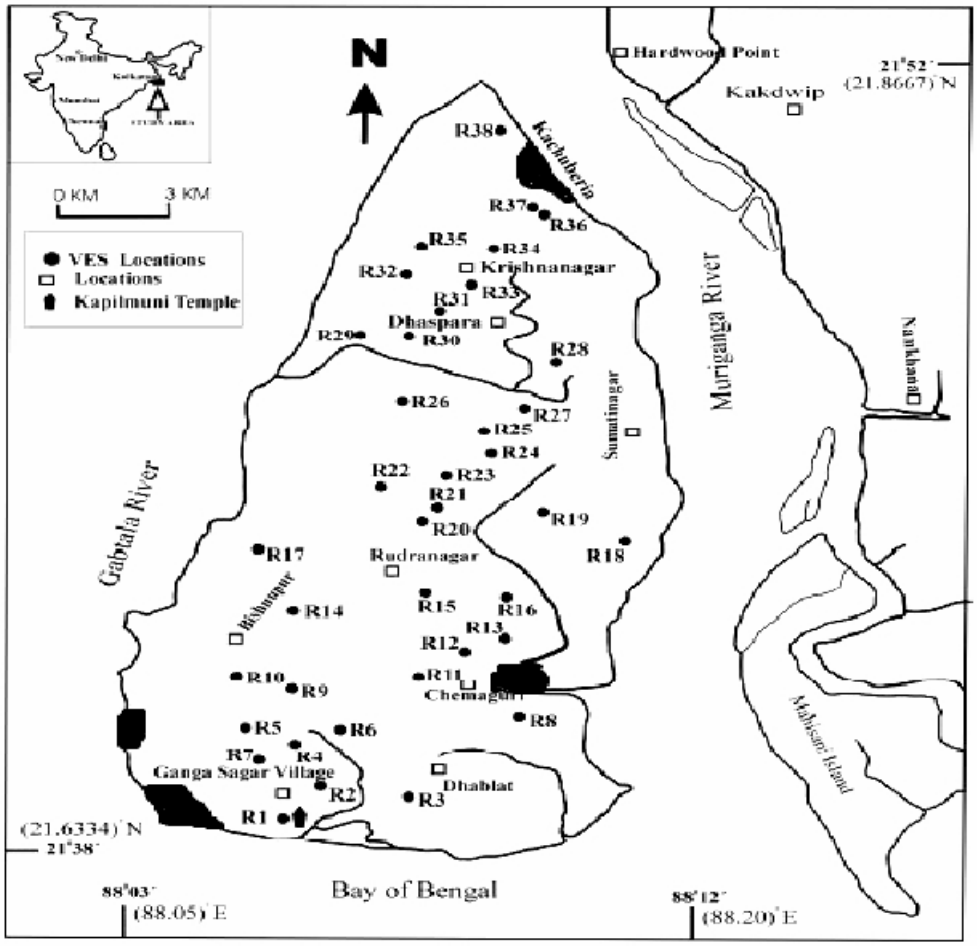


Fig. 2 Size dependence of auxospores (expressed in terms of diameter) in varying salinity of natural filtered seawater under laboratory conditions showing a conservative behaviour

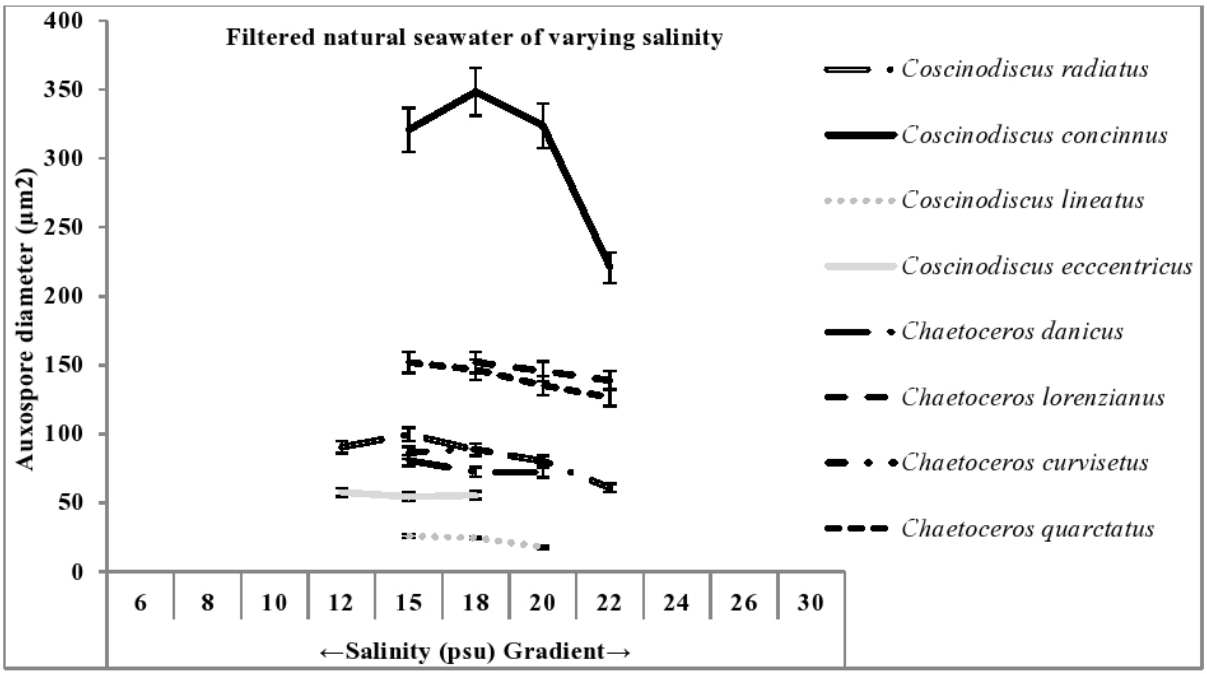


Fig. 3 Auxospore size not following a set pattern along with the variation in salinity of the unfiltered natural turbid seawater and not showing any reservation to a particular set of salinity range

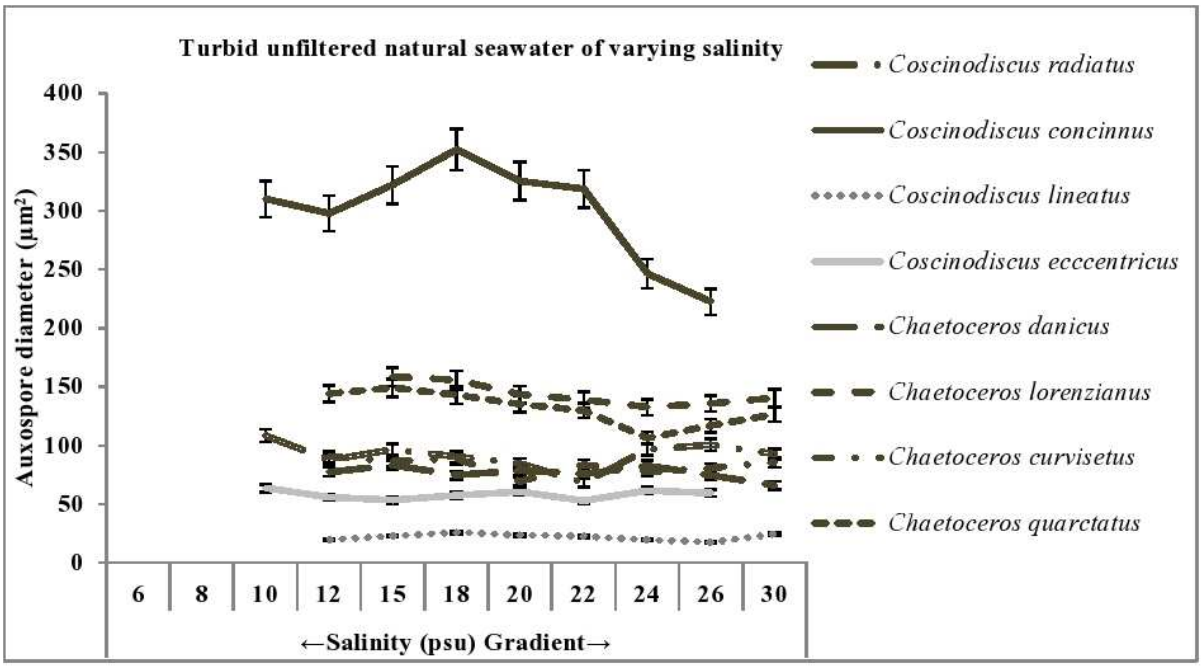
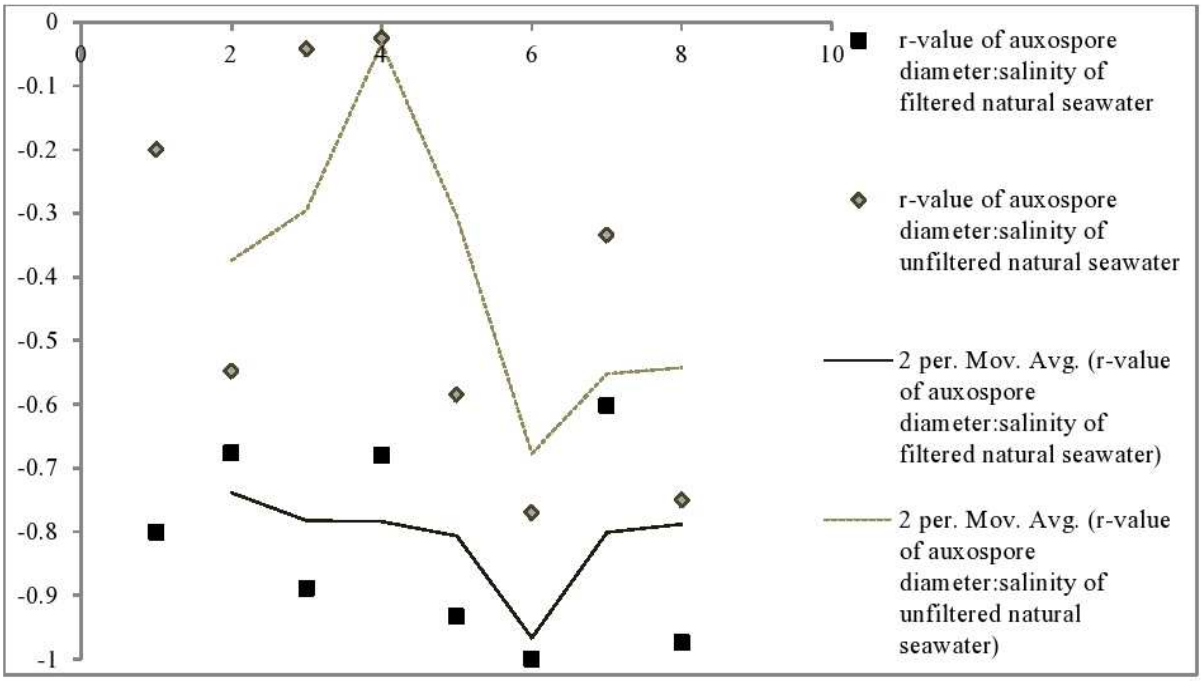


Fig. 4 The scatter plot diagram above is depicting the "two period movement average" trend lines of the correlation values performed between the auxospore diameter and the salinity variation while in filtered and unfiltered natural seawater respectively, at 95% significance level



CONCLUSION

The experiment was of immense significance since it dealt with the conditions that might trigger auxospore formation in the dominant centric diatoms such as *Coscinodiscus* and *Chaetoceros* which in itself is very important since induction of sexual reproduction in diatoms and ontogeny of auxospores in diatoms have yet to be clearly understood. Auxospores aid in the recycling of the nutrients at nutrient poor environment during adverse situations and silica sinking has also a very important role in the induction of auxospores in diatoms.

Any studies that reveal some insights regarding the functioning of auxospores is rare and that in well mixed estuaries are even rarer and can only help us broaden our knowledge base regarding the physiology of diatoms. This study is the first of its kind performed on diatoms collected from the Hooghly estuary and requires further observations to increase our understanding of the sexual cycle of diatoms.

Acknowledgement

The financial aids from University Grants Commission (UGC) and Department of Science and Technology (DST-PURSE) New Delhi, India are gratefully acknowledged. It is always hard to work in the remote and partly inaccessible islands of the Sundarbans by road and the financial support is immensely appreciated for the hassle free completion of the study. We also owe our sincere gratitude to the department of forestry of West Bengal, India for providing us with the opportunity to work in the Sundarban deltaic region.

REFERENCES

1. Al-Kandari, M. Al-Yamani, F.Y. and Al-Rifaie, K. Marine Phytoplankton Atlas of Kuwait's Waters. Kuwait Institute for Scientific Research, Kuwait, (2009)
2. Assmy, P. Henjes, J. Smetacek, V. and Montresor, M. Auxospore formation in the silica-sinking oceanic diatom *Fragilariopsis kerguelensis* (Bacillariophyceae). *J. Phycol.*, **42:** 1002–1006 (2006)
3. Biswas, H. Mukhopadhyay, S.K. De, T.K. Sen, S. and Jana, T.K. Biogenic controls on the air-water carbon dioxide exchange in the Sundarban mangrove environment, northeast coast of Bay of Bengal, India. *Limnol. Oceanogr*., **49:** 95–101(2004)
4. Chepurnov, V. A. Mann, D. G. Sabbe, K. and Vyverman, W. Experimental studies on sexual reproduction in diatoms. *Int. Rev. Cyt.*, **237:** 91–154 (2004)
5. Crawford, R. M. The role of sex in the sedimentation of a marine diatom bloom. *Limnol. Oceanogr*., **40:** 200–204 (1995)
6. Desikachary, T.V. "Atlas of diatoms 1-6." Madras Science Foundation, Madras, 1986-89.
7. Drebes, G. Sexuality. *In* Werner, D., (Ed.) The Biology of Diatoms. Blackwell Scientific Publications, Oxford, 250–283 (1977)
8. Edlund, M. B. and Stoermer, E. F. Ecological, evolutionary, and systematic significance of diatom life histories. Journal of Phycology, **33:** 897–918 (1997)
9. Grasshoff, K., Erhardt, M. and Kremling, K. Methods of Seawater Analysis. Verlag Chemie, Weinmeim, (1983)
10. Hasle, G.R. and Syvertsen, E.R. Marine diatoms. *In* Tomas, C.R. (Ed), Identifying Marine Phytoplankton. Academic Press, London, 1997, 5–385.
11. Jewson, D. H. Life cycle of a *Stephanodiscus* sp. (Bacillariophyta). *J. Phycol*., 28:856–866 (1992).
12. Kaczmarska, I. Bates, S. S., Ehrman, J. M. and Leger, C. Fine structure of the gamete, auxospore and initial cell in the pennate diatom *Pseudonitzschia* multiseries (Bacillariophyta). *Nova Hedwig.,* **71:** 337–357 (2000)
13. MacDonald, J. D. On the structure of the diatomaceous frustule, and its genetic cycle. Ann. Mag. Nat. Hist., **(4)3:** 1-8 (1869)
14. Mann, D.G. Why didn't Lund see sex in *Asterionella*? A discussion of the diatom life cycle in nature. *In* Round, F.E. (Ed), *Algae and the aquatic environment*, Biopress, Bristol, 1988, 383–412.
15. Mukherjee, A. Chakraborty, S. Das, S. and De, T. K. Salinity might be the most influential governing factor of cell surface size of *Coscinodiscus* in well mixed tropical estuarine waters. *Int. J. Life Sci. Edu. Res*., **1(2):** 81 – 90 (2013)
16. Mukhopadhyay, S.K. Biswas, H. De, T.K. and Jana, T.K. Fluxes of nutrients from the tropical River Hoogly at the land-ocean boundary of Sundarban, NE coast of Bay of Bengal. *Ind. Jour. Mar. Sys*., **62:** 9-21(2006)
17. Pfitzer, E. U¨ber den Bau und Zellteilung der Diatomeen. *Bot. Zeit*. [Ed. Hanstein], **27:** 774–776 (1869)

18. Pingree, R. D. Holligan, P. M. and Mardell, G. T. The effect of vertical stability on phytoplankton distribution. *Deep Sea Res*., **25:** 1011- 1028 (1978)
19. Round, F.E. Crawford, R.M. and Mann, D.G. The Diatoms: Biology and morphology of the genera. Cambridge University Press, Cambridge, 1990, 747.
20. Smetacek, V. Diatoms and the ocean carbon cycle. *Protist*., **150:** 25–32 (1999)
21. Strickland, J.D.H. and Parsons, T.R. A practical handbook of seawater analysis, 2nd Edn. *Fish. Res. Board*, Canada, 1972.
22. Therriault, J.C. and Platt, T. Environmental control of phytoplankton patchiness. *Can. J. Fish. Aqua. Sci.*, **38:** 638-641, (1981)
23. Von Stosch, H.A. On auxospore envelopes in diatoms. *Bacillaria* **5:** 127–156, (1982)
24. Waite, A. and Harrison, P. J. Role of sinking and ascent during sexual reproduction in the marine diatom *Ditylum brightwellii*. *Mar. Eco. Prog. Ser*., **87:** 113–22, (1992)
25. Woods, J. D. and Barkmann, W. The response of the upper ocean to solar heating. I. The mixed layer. *Quat. J. Royal Met. Soc*., **112:** 1-27, (1986)