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Section A: Environmental Science

Review Article

Inter-Relationship between Artificially Triggered Phytoplankton Bloom and Nutrient Levels in Brackish Water Ponds of Indian Sundarbans

Subhasmita Sinha[1] *, Kakoli Banerjee[2], Sufia Zaman[3], and Abhijit Mitra[3]

[1]Department of Marine Science, University of Calcutta, 35 B.C. Road, Kolkata 700 019, West Bengal, India

[2]School of Biodiversity and Conservation of Natural Resources, Central University of Orissa, Landiguda, Koraput, Odisha 764020, India.

[3]Department of Oceanography, Techno India University, Salt Lake Campus, Kolkata 700 091, India

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Abstract: We analyze in this paper the oscillation of nitrate, phosphate and silicate due to enhancement of phytoplankton standing stock through iron fertilization. Observations of selected variables exhibit the depletion of nutrients with the increase of phytoplankton volume. We also observe significant positive correlation between phytoplankton cell volume and cell carbon content. This observation holds good for all the six major *Coscinodiscus* species available in the brackish water ponds of Indian Sundarbans. The maximum values of phytoplankton volume and carbon content in 1.5 ppm iron fertilized pond speaks in favour of phytoplankton bloom due to optimum iron level in aquatic phase.

Key words: *Coscinodiscus spp.*, iron fertilization, cell volume, cell carbon, nutrient level

INTRODUCTION

Fertilizing a pond with organic or inorganic fertilizer is often done to initiate an algal bloom. The natural and artificial fertilizers provide principal chemical nutrients necessary for algal growth and reproduction. The principal nutrients are nitrogen (N), phosphorous (P), and potassium (K). These traditional aquaculture pond fertilizers can be supplemented with about 0.05% iron to improve their effectiveness in promoting phytoplankton growth in brackishwater or seawater[1]. Mineral sources of iron such as ferrous sulphate and ferrous oxide are less expensive sources of iron for fertilizers, but chelated iron compounds should be used to enhance iron solubility and fertilizer effectiveness. Another important advantage of iron fertilization is that ferrous iron in pond bottom sediment precipitates hydrogen sulphide gas produced by microbial activity. Two important indicators were considered in the present experiment (i) volume of dominant phytoplankton species and (ii) carbon content in the phytoplankton species. Apart from these nutrients [like nitrate (NO_3), phosphate (PO_4) and silicate (SiO_3)] were also monitored for eight subsequent months during 2012.

MATERIALS AND METHODS

Study sites: Sundarbans delta is one of the dynamic mangrove dominated estuarine deltas of the world[2], which is situated at the apex of Bay of Bengal. A major portion of this delta (62%) lies in Bangladesh and the remaining 38% is within the Indian sub-continent. In the Indian Sundarbans, approximately 2069 sq. km of area is occupied by the tidal river system or estuaries, which finally end up in the Bay of Bengal[3]. These estuaries feed several brackishwater ponds in the area. We selected four ponds [control and three experimental with different (0.5 ppm,1.5 ppm and 2.5 ppm) iron sulphate doses] in the Kakdwip area of Indian Sundarbans ($21^0$52'35.7"N & $88^0$11'55.0″E) and treated them differently to observe the effect of iron addition on cell volume and cell carbon of six major *Coscinodiscus* species (*Coscinodiscus eccentricus, Coscinodiscus jonesianus, Coscinodiscus radiatus, Coscinodiscus gigas* and *Coscinodiscus oculusiridis*) available in the present study area.

Salinity: The surface water salinity in the selected ponds was recorded by means of an optical refractometer (Atago, Japan) and cross-checked in laboratory using Mohr-Knudsen method. The correction factor was found out by titrating silver nitrate solution against standard seawater (IAPO standard seawater service Charlottenlund, Slot Denmark, chlorinity = 19.376 psu).

Dissolved iron: Surface water samples were collected from the three ponds using 10-l Teflon-lined Go-Flo bottles fitted with Teflon taps and deployed on a rosette or on Kevlar line, with additional surface sampling carried out by hand. Shortly after collection, samples were filtered through Nuclepore filters (0.4 µm pore diameter) and aliquots of the filters were acidified with sub-boiling distilled nitric acid to a pH of about 2 and stored in cleaned low-density polyethylene bottles. Dissolved Fe was separated and pre-concentrated from the brackishwater using dithiocarbamate complexation and subsequent extraction into Freon TF, followed by back extraction into HNO_3[4]. Extract was analysed for dissolved Fe by Atomic Absorption Spectrophotometer (Perkin Elmer: Model 3030). The accuracy of the dissolved heavy metal determinations is indicated by good agreement between our values and reported for certified reference seawater materials (CASS 2) (**Table 1**).

Table 1: Analysis of reference material for near shore seawater (CASS 2)

Element	Certified value ($\mu g\ l^{-1}$)	Laboratory results ($\mu g\ l^{-1}$)
Fe	2.97 ± 0.12	2.61 ± 0.14

Nutrient analyses: Surface waters for nutrient analyses were collected in clean TARSON bottles and transported to the laboratory in ice-freezed condition. Triplicate samples were collected from the same collection site to maintain the quality of the data. The standard spectrophotometric method of[5] was adopted to determine the nutrient concentration in surface water. **Nitrate** was analysed by reducing it to nitrite by passing the sample with ammonium chloride buffer through a glass column packed with amalgamated cadmium filings and finally treating the solution with sulphanilamide. The resultant diazonium ion was coupled with N - (1-napthyl) - ethylene diamine to give an intensely pink azo dye. Determination of the **phosphate** was carried out by treatment of an aliquot of the sample with an acidic molybdate reagent containing ascorbic acid and a small proportion of potassium antimony tartarate. Dissolved **silicate** was determined by treating the sample with acidic molybdate reagent. The resultant silico-molybdic acid was reduced to molybdenum blue complex by ascorbic acid and incorporation of oxalic acid prevented formation of similar blue complex by phosphate.

Cell volume: Net samples for phytoplankton were collected around 12.00 noon with a conical nylon net bag (30 cm diameter) made of a 30 No. bolting silk from the four selected ponds and preserved in 4% neutral formaldehyde. Phytoplankton samples were observed with a ZEISS research microscope coupled with an image analyzing system. Phytoplankton cell identifications were based on standard taxonomic keys[6,7]. Linear dimensions of the phytoplankton species were measured on the basis of taxonomic information and shape code. For each species of *Coscinodiscus* the best fitting geometric shape (cylindrical) and corresponding equation was used to calculate the cell volume[8].

Cell carbon: The six *Coscinodiscus sp*. are under diatom group. The cell volume of diatoms was converted into cell carbon as per the expression **cell carbon (pg) = 0.288 [live cell volume (μm^3)]** $^{0.811}$, which is the standard expression for transforming cell volume into cell carbon[9].

Statistical analysis: To explore the relationships between phytoplankton cell volume and cell carbon, scatterplots and allometric equations were computed. To assess whether cell volume, carbon content and environmental variables varied significantly between the ponds, two-way ANOVA was performed. All statistical calculations were performed with SPSS 9.0 for Windows.

RESULTS AND DISCUSSION

Hydrological parameters: The average nitrate concentrations ranged as per the order control pond (23.78 µgat/l) > 0.5 ppm $FeSO_4$ treated pond (23.67 µgat/l) > 2.5 ppm $FeSO_4$ treated pond (15.33 µgat/l) > 1.5 ppm $FeSO_4$ treated pond (12.46 µgat/l). Mean phosphate and silicate exhibited similar trends with highest values in the control pond (5.63 µgat/l and 67.69 µgat/l respectively), followed by 0.5 ppm $FeSO_4$ treated pond (5.08 µgat/l and 61.25 µgat/l respectively), 2.5 ppm $FeSO_4$ treated pond (4.02 µgat/l and 54.17 µgat/l respectively) and 1.5 ppm $FeSO_4$ treated pond (2.27 µgat/l and 51.01 µgat/l) (**Figures 1, 2 and 3**).

All the hydrological variables showed a distinctive response to iron after the seven days experimental period. The hydrological parameters exhibited considerable response to Fe fertilization. The nitrate level in control pond, 0.5 ppm treated pond showed an increment of 77.04% and 58.79% respectively. However in 1.5 ppm and 2.5 ppm treated pond water, the nitrate level decreased by 32.71% and 15.55% respectively. A similar trend is observed for the phosphate level. Maximum percentage decrease of 81.36% of phosphate level is observed in the pond treated with 1.5 ppm ferrous sulphate followed by the 2.5 ppm treated pond with a decrease in percentage of 38.46%. Whereas the control and the pond treated with 0.5 ppm dose there is a percentage increase of phosphate level of 43.22% and 21.59% respectively. The silicate level also exhibited a similar pattern of uptake in the four ponds.

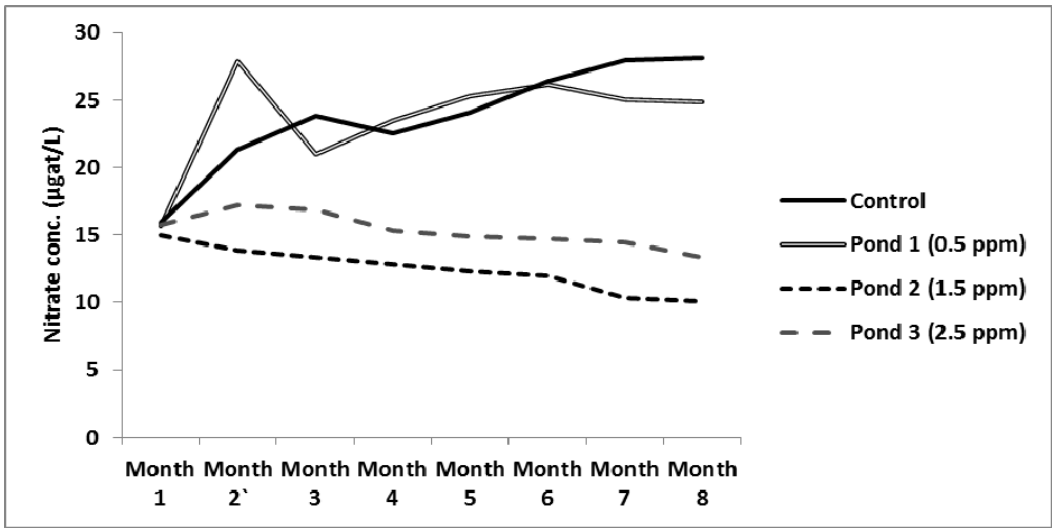

Figure 1: Monthly variations of NO_3 (µg at/L)) in the four selected ponds.

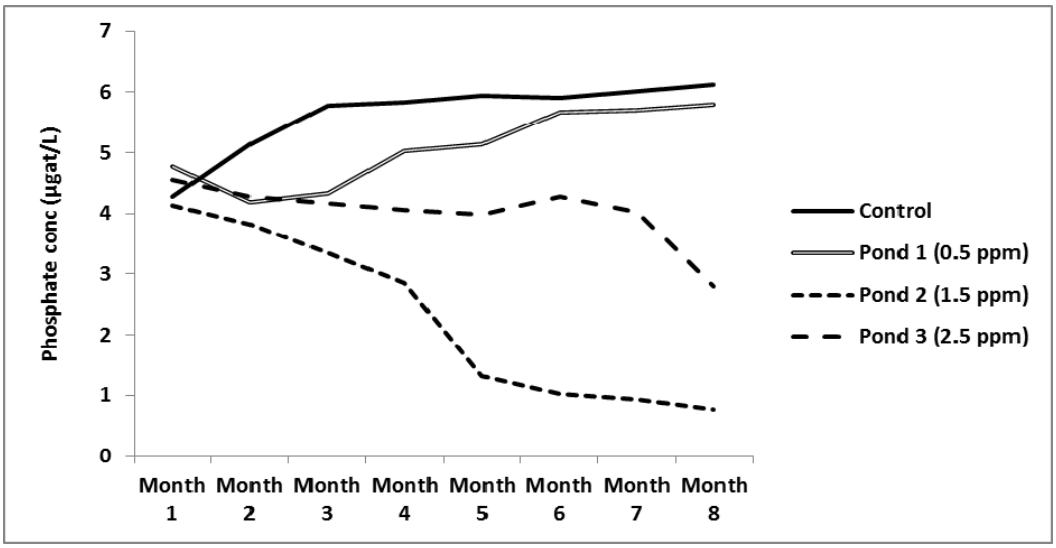

Figure 2: Monthly variations of PO_4 (µg at/L) in the four selected ponds.

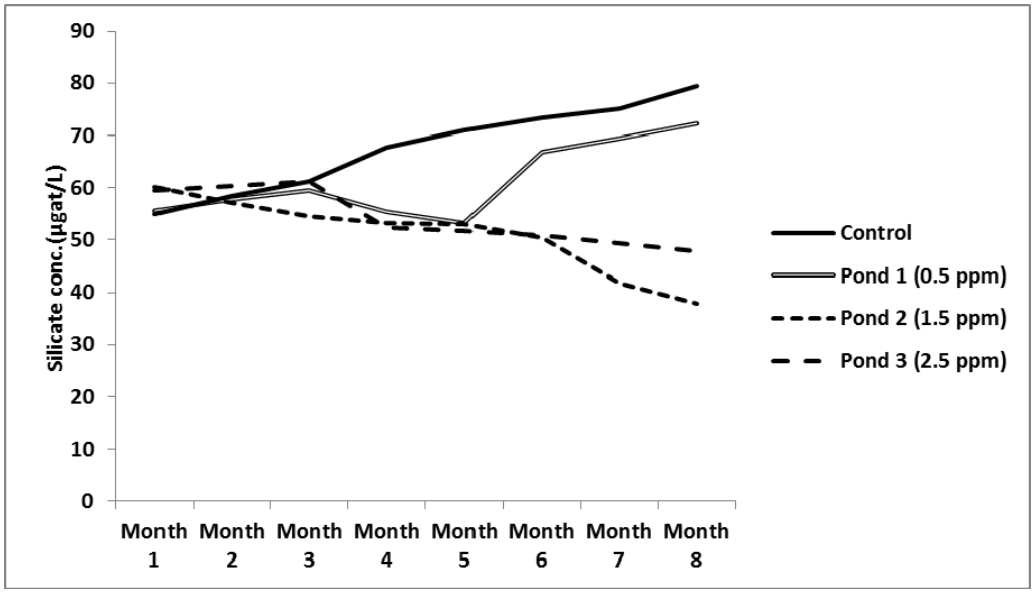

Figure 3: Monthly variations of SiO_3 (µg at/L) in the four selected ponds.

The control and 0.5 ppm treated pond showed increase in silicate level with a percentage of 44.40% and 29.82%. On the other hand the silicate level showed a decreased percentage of 37.13% and 19.70% in the 1.5 ppm dose and 2.5 ppm dose treated ponds (**Table 2**)

Table 2: Comparative study of nutrient level (µg at/L) between ponds before and after the experiment

	Control	Pond 1 (0.5 ppm)	Pond 2 (1.5 ppm)	Pond 3 (2.5 ppm)
Nitrate (µg at/L)				
Initial (Before)	15.90	15.65	14.95	15.76
Final (After)	28.15	24.85	10.06	13.31
Increase/Decrease (%)	+ 77.04	+ 58.79	- 32.71	- 15.55
Phosphate (µg at/L)				
Initial (Before)	4.28	4.77	4.13	4.55
Final (After)	6.13	5.80	0.77	2.80
Increase/Decrease (%)	+ 43.22	+ 21.59	- 81.36	- 38.46
Silicate (µg at/L)				
Initial (Before)	54.98	55.63	60.20	59.55
Final (After)	79.39	72.22	37.85	47.82
Increase/Decrease (%)	+ 44.40	+ 29.82	- 37.13	- 19.70

'+' indicates increase (%) '-' indicates decrease (%)

Cell volume: The average cell volume of phytoplankton ranged from (3642.74 µm^3 in *Coscindiscus oculusiridis* in control pond) to (90921.09 µm^3 in *Coscindiscus radiatus* in 1.5 ppm $FeSO_4$ treated pond). Phytoplankton cell volume was maximum in 1.5 ppm $FeSO_4$ treated pond (average value 32734.01 µm^3) followed by 2.5 ppm $FeSO_4$ treated pond (average value 16172.50 µm^3) followed by 0.5 ppm $FeSO_4$

treated pond (average value 14496.87 μm^3) and control pond (average value 11744.95 μm^3) (**Figure. 4**). Here the average values of all six species were taken into consideration.

Cell carbon: The average cell carbon of phytoplankton ranged from (222.70 picogram in *Coscindiscus oculusiridis* in control pond) to (3026.00 picogram in *Coscindiscus radiatus* in 1.5 ppm $FeSO_4$ treated pond). Phytoplankton cell carbon content was maximum in 1.5 ppm $FeSO_4$ treated pond (average value 1249.31 picogram) followed by 2.5 ppm $FeSO_4$ treated pond (average value 711.13 picogram), followed by 0.5 ppm $FeSO_4$ treated pond (average value 643.17 picogram) and control pond (average value 531.67 picogram) (**Figure 5**). Here also the average values of all six species were taken into consideration.

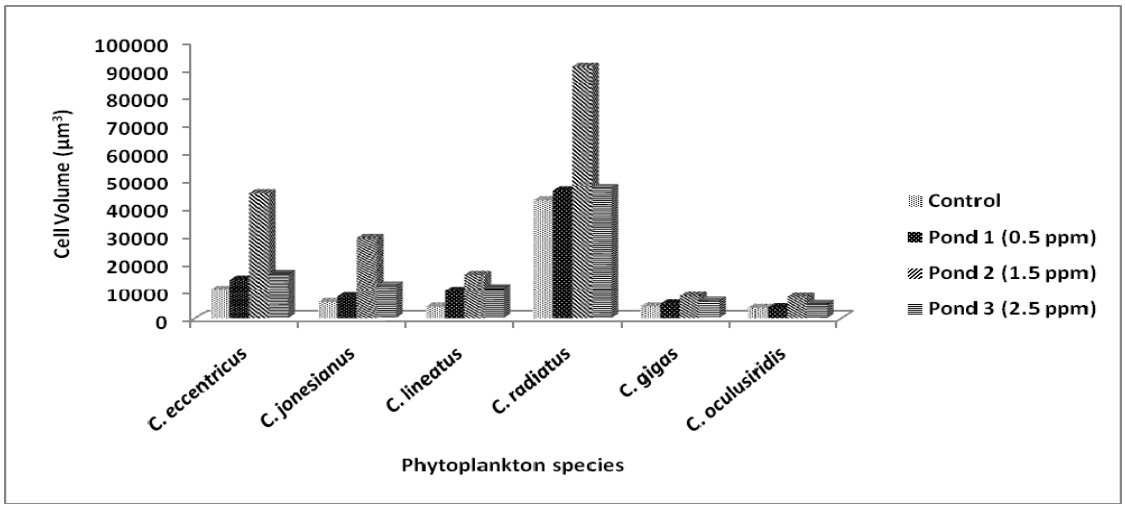

Figure 4: Variations of average cell volume (μm^3) of six *Coscinodiscus sp.* in four ponds

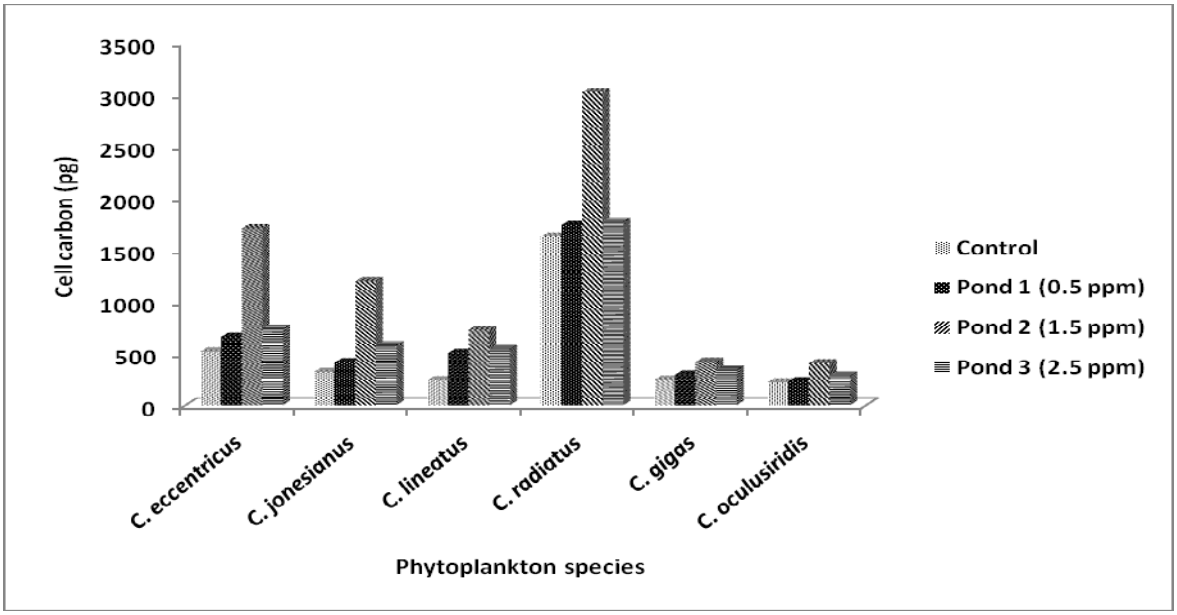

Figure 5: Variations of average cell carbon (pg) of six *Coscinodiscus sp.* in four ponds

Statistical interpretation: To explore the relationships between phytoplankton cell volume and cell carbon, scatterplots and allometric equations were computed. Significant positive correlations were found between phytoplankton cell volume and cell carbon content for all six species. It is noteworthy that phytoplankton carbon content is accounted solely due to phytoplankton volume (**Figures 6-11**). Two way ANOVA results confirm significant variation of cell volume, cell carbon, and nutrient level between the ponds (**Table 3**).

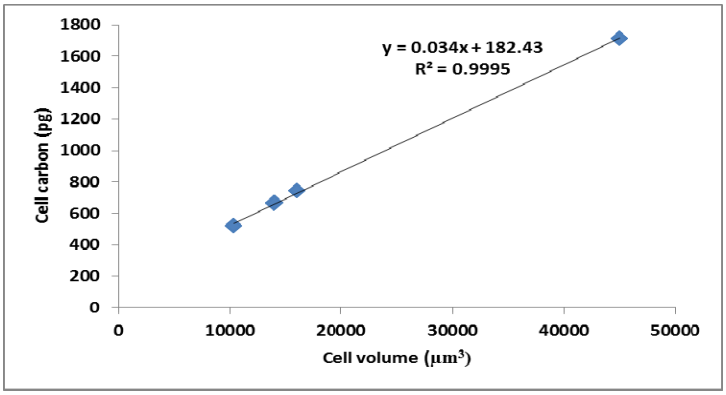

Figure 6: Allometric equation for *C. eccentricus* in all four ponds

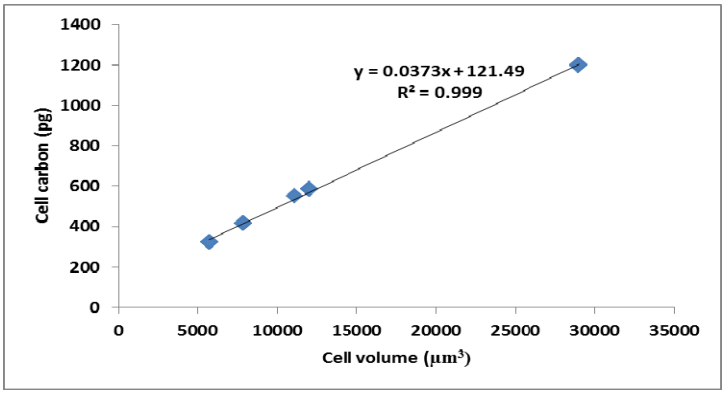

Figure 7: Allometric equation for *C. jonesianus* in all four ponds

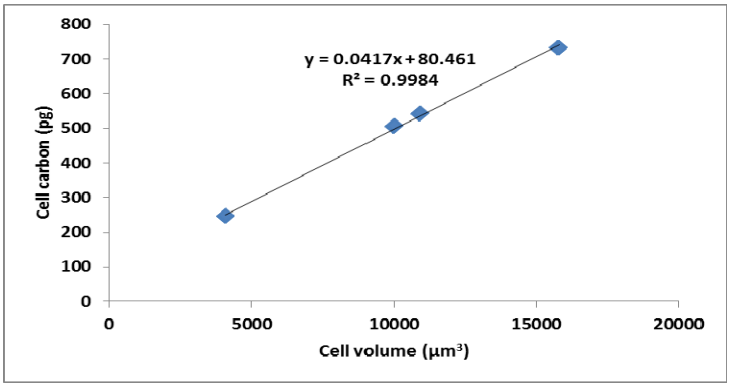

Figure 8: Allometric equation for *C. lineatus* in all four ponds

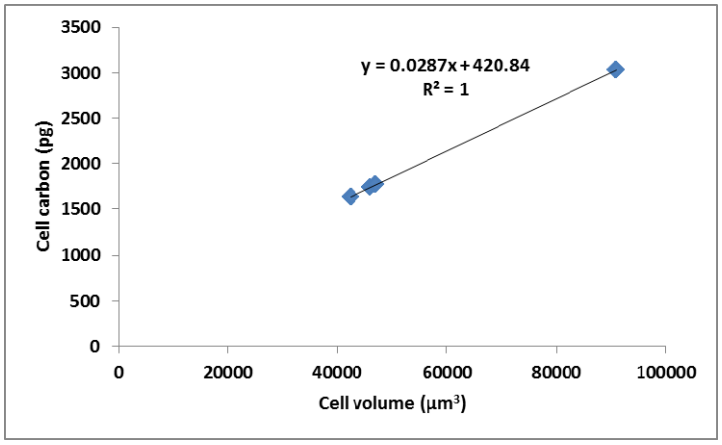

Figure 9: Allometric equation for *C. radiatus* in all four ponds

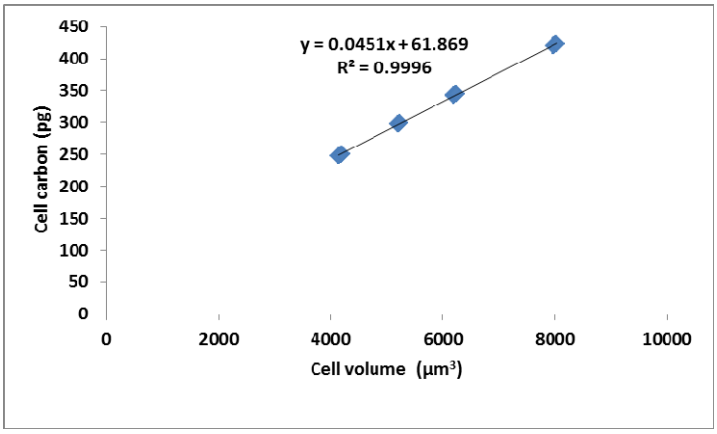

Figure 10: Allometric equation for *C. gigas* in all four ponds

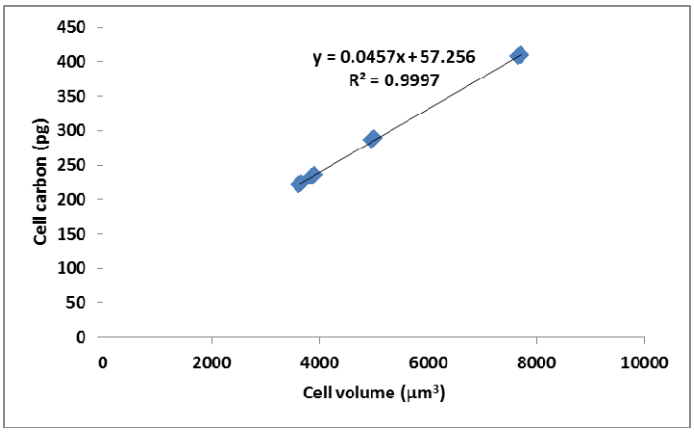

Figure 11: Allometric equation for *C. oculusiridis* in all four ponds

Table 3: ANOVA results showing cell volume, cell carbon and nutrient level variations between ponds during 2012

Variable	F_{cal}	F_{crit}
Cell Volume (µm^3)		
C. eccentricus	57881518.24	3.07
C. jonesianus	34778.78	3.07
C. lineatus	16491497.16	3.07
C. radiatus	210694146.20	3.07
C. gigas	1042251.42	3.07
C. oculusiridis	4165024.18	3.07
Cell Carbon (pg)		
C. eccentricus	49107732.49	3.07
C. jonesianus	30459.25	3.07
C. lineatus	35205336.21	3.07
C. radiatus	133944769.1	3.07
C. gigas	940636.28	3.07
C. oculusiridis	5749601.24	3.07
Nutrient Level (µg at/L)		
Nitrate	29.81	3.07
Phosphate	18.25	3.07
Silicate	6.42	3.07

The persistence of High Nitrate Low Chorophyll (HNLC) conditions in the surface waters of several large regions of the world's oceans comprises a familiar enigma in oceanography[10]. The factors that prevent the utilization of nitrate also regulate the rate at which carbon dioxide is taken up by phytoplankton and, ultimately, the amount of carbon exported from the surface waters. The oceans are both a major source and sink for atmospheric carbon dioxide, and processes that control the balance of these fluxes are thought to have a major effect on global climate[11]. Understanding the factors that limit the uptake of

excess plant nutrients is, therefore, a key to understanding climate change. Grazing pressure exerted on phytoplankton by rapidly reproducing microzooplankton and micronutrient (iron) deficiency may function jointly in these HNLC waters[12]; yet the relative importance of each of these factors in controlling the biomass and rates of phytoplankton production has remained contentious[13]. The experimental tools available to the oceanographer have, until recently, been inadequate to resolve the relative importance of these processes. *In vitro* enrichment experiments[14-17] where iron is added at nanomolar levels to samples of seawater invariably do not represent the *in situ* phytoplankton grazer community. The present study exhibits considerable growth of phytoplankton volume and phytoplankton carbon in iron sulphate treated ponds along with significant lowering of nutrients (NO_3, PO_4 and SiO_3). The increase in phytoplankton carbon content in $FeSO_4$ treated pond also confirms the role of iron in accelerating carbon dioxide uptake from the ambient waters. On contrary in the control pond, all species of *Coscinodiscus* showed lowest cell volume and carbon that speak in favour of the role of iron fertilization in enhancing the bloom condition of phytoplankton and utilization of nutrients from ambient water. Significant variations of different nutrient levels are observed between four ponds and cell volumes of six different *Coscinodiscus sp*. are also observed to vary significantly The results of nutrient variations show that the decrease is maximum in 1.5 ppm treated pond, which indicates that the process of uptake of nutrients are greatly favoured in this particular dose of ferrous sulphate. Most of the dissolved Fe in seawater is complexed with organic ligands[18-20]. In the open ocean, organic compounds with extremely high selectivities and affinities for ferric iron (siderophores and unidentified compounds with similar properties) bind >99% of the organically complexed iron[18-22]. Recently, 2 main types of Fe-binding organic ligands have been identified and most ligands were in the colloidal fractions (0.03~0.4 μm)[23]. Humic substances (humic and fulvic acids) contribute to iron complexation, keeping iron in a soluble form at both high pH and high concentrations of anions and cations in estuarine systems[24]. Humic substances are also able to stimulate the growth of coastal phytoplankton in laboratory cultures[25-27] and contribute to the phytoplankton bloom in coastal waters[28]. Fe bound with siderophore desferrioxamine B (DFB) can be utilized by marine diatoms and natural phytoplankton communities[29-31], but some studies have indicated that siderophore bound Fe cannot be taken up by marine diatoms[32-35], and the addition of DFB can reduce the biological uptake of Fe and the growth rate of phytoplankton[30, 36, 37]. Our synchronizes with the works observation of[38] who observed increased export of carbon to sub-Antarctic sediments during the Last Glacial Maximum at times of higher iron flux.

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Corresponding Author: Subhasmita Sinha;
Department of Marine Science, University of Calcutta, 35 B.C. Road, Kolkata 700 019, West Bengal, India