

SALINITY AND DIATOMS*

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ABSTRACT

Salinity has long been recognised as an important factor in the distribution of Diatoms. Studies on *Cyclotella meneghiniana* have shown that salinity can play a significant role in some aspects of the life of this diatom. It is suggested that variations in salinity in natural environments can also influence the productivity of diatom populations.

INTRODUCTION

ESTUARIES are subjected to great changes in salinity (NaCl) from a very near fresh water condition to saline conditions as near (rarely even more) to that of the sea, while a totally fresh water and marine environments do not show such a fluctuation. One may reasonably expect that planktonic diatoms living in such a biotope would be well adapted to live under such fluctuating conditions while truly fresh water and marine diatoms may not be adapted and may even be affected by such fluctuations. Laboratory studies of diatoms have largely confirmed this general pattern of behaviour. It has been shown that marine diatoms are unable to tolerate a lowering of salinity, optimal growth occurring only at salinities equivalent to or very near sea water (Ryther, 1954; Kain and Fogg, 1958; Jorgensen, 1960; McLachlan, 1961; Guillard and Ryther, 1962). It has also been noted that certain fresh water diatoms do not tolerate any increase in the total solids of the medium (Chu, 1942; Rodhe, 1948; as quoted by Lewin and Guillard, 1963). Diatoms from estuaries have the widest adaptability to any change in salinity of the external medium (Williams, 1964). There seems to be even races within a species, whose behaviour is in keeping with the environment in which they are found. While the growth rates of estuarine clones are not very much affected in media of wide salinities, clones isolated from sea do not even survive in lower salinities. The physiological differences between clones correspond to conditions of salinity that obtain in zones from which they are collected (Guillard and Ryther, 1962; Guillard, 1963). It would appear that the ability of the diatom to survive and grow in fluctuating salinities is, therefore, one of the chief characteristics that determines its occurrence in an estuary and it is again this ability that would determine whether a particular species in the estuarine area can successfully extend into fresh water situations on the one hand and into the marine environment on the other.

Cyclotella meneghiniana Kütz

Cyclotella meneghiniana is one such diatom which is known to occur in all these environments. Although this diatom is recorded from the coastal area (Subrahman-

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yan, 1946), the population densities are never so great as to show them up in counts, while fairly large populations are found in the estuarine portions of the river Cooum, which joins the sea near Madras. Iyengar and Venkataraman (1951) also found this diatom in large quantities in the estuarine portions of river Cooum. In purely fresh water areas distal to the river mouth cells were not recorded in any great quantity. It would, therefore, be interesting to study in laboratory culture the behaviour of this diatom to changes in salinity that is normally encountered in any estuary.

Three isolates of *C. meneghiniana*, 1. from the estuarine portions of river Cooum, 2. from a brackish water beach-pool and 3. from a fresh water garden pond, were studied. Reimann medium (Reimann *et al.*, 1963, p. 76; $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ —70.8 mg; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ —24.6 mg; K_2HPO_4 —8.7 mg; Na_2SiO_3 —56.8 mg; EDTA (di-Sodium salt)—5.0 mg; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ —0.278 mg; $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ —0.018 mg; soil extract—15 ml* ; distilled water 1000 ml) was used in the present study as a basal medium. NaCl was added in various concentrations such as 1 g/l, 2 g/l, 5 g/l, 10 g/l, 15 g/l, 20 g/l, 25 g/l and 30 g/l to this medium to provide media with different salinities. The pH of these media was adjusted to 7.9 before autoclaving. Stock cultures of these isolates were maintained in Reimann medium. Inoculum for growth studies was from a 7-days-old grown culture and the inoculum level was adjusted to give about 3000 cells/ml initially in the respective culture media. Artificial light of 1,500 lux units was provided in a 18/6 light dark cycle. Temperature was constant at $24 \pm 1^\circ\text{C}$. Growth was estimated by counting cells in a haemocytometer periodically and finally expressed as number of divisions per day.

The division rates of the three isolates of *C. meneghiniana* in different salinities is shown in Fig. 1a. All isolates grew well in all salinities. The isolate from fresh water pond grew to the same extent as estuarine isolates, although growth slightly declined at higher salinities.

Guillard and Ryther (1962) isolated a number of clones of *Cyclotella nana* which differed in their salinity tolerance. Estuarine clones showed the widest adaptability and grew in salinities ranging from 0.5 to 32.0‰ while clones from Sargasso sea did not survive a lowering of salinity. We did not study any isolate of *C. meneghiniana* derived from a purely marine environment. Nevertheless, the present study showed that within this species there is not much difference between the fresh water and estuarine isolates in their capacity to tolerate a wide range of salinity. Even on the first transfer all the isolates began growing in all the salinities tested and there appeared to be no need for 'training' the diatom to withstand or tolerate varying salinities (Reimann *et al.*, 1963). To understand fully the behaviour or reactions of *C. meneghiniana* to sudden or drastic changes in salinity, experiments, briefly called as cross-inoculation experiments, were also conducted. *C. meneghiniana* growing in Reimann medium and in different NaCl amendments (30 g/l NaCl, not included) were transferred from one medium into the others. As many as a hundred single transfers were made with the Cooum (estuarine) isolate. If the size of cells were such that auxospores could be formed, a marked increase or decrease in salinity induced their formation leading to production of larger cells. The percentage increase or decrease in average cell diameter on transfer followed by a month's growth in these media were calculated (Table 1). Increase in cell diameter was met with in cells on transfer into 1 g/l, 2 g/l and 5 g/l NaCl amended Reimann medium,

* Equal quantities of dried, sieved garden soil and distilled water were autoclaved at 15 lb/square inch for 15 minutes, filtered and used.

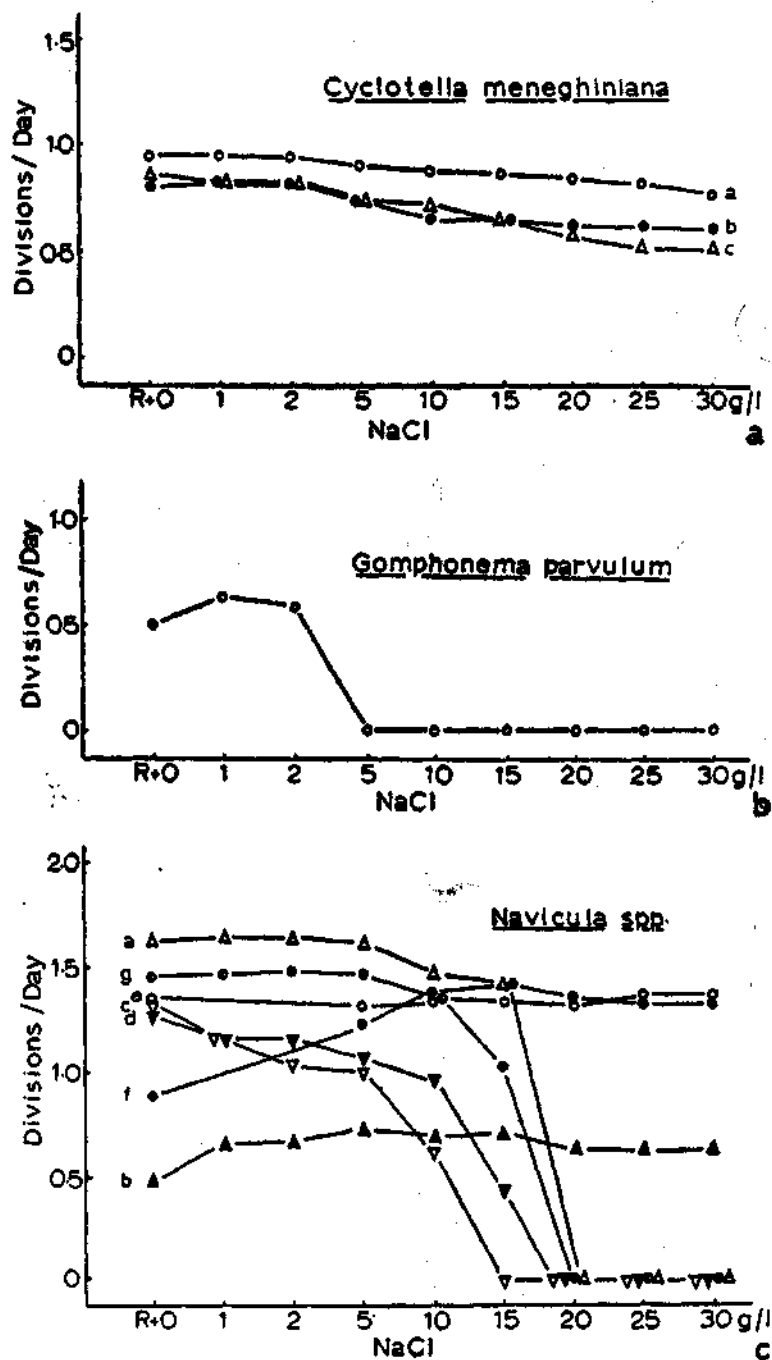


Fig. 1 (a). Division rates of three isolates of *Cyclotella meneghiniana* in media of different salinities. (a) Cooum isolate; b. brackish pool isolate; c. garden pond isolate, (b). Division rates of *Gomphonema parvulum* in media of different salinities, and (c). Division rates of species of *Navicula* in media of different salinities, a. *N. minima* (8), b. *N. minima* (9), c. *N. pelliculosa* (10) d. *N. pelliculosa* (11), e. *N. salinarum* (12), f. *N. salinarum* (13) and g. *N. vitabunda* (14).

the last one yielding the greatest percentage increases in average diameters. Reimann medium and 10 g/l NaCl amended Reimann medium were also equally good. In other words, it would appear that *C. meneghiniana*, even though it can grow in fresh water conditions or in varying salinities, grows very well and produces auxospores in lower salinity ranges used in these experiments. It can also survive in high salinity (sea) and when dilution takes place again produce auxospores.

Similar studies with the fresh water and brackish water isolates showed that their behaviour to changes in salinity were more or less similar. When transferred from Reimann medium to higher salinities or from higher to lower salinities these isolates formed auxospores in much the same way as did the Coom isolate (Rao, unpublished).

Table 1. showing percentage increase or decrease in average cell diameter in *Cyclotella meneghiniana* (Coom isolate) on transfer of cells from one medium to seven other media used

INOCULUM FROM ↓	TREATMENTS							
	1 Reimann medium	2 R+ 1g/l NaCl	3 R+ 2g/l NaCl	4 R+ 5g/l NaCl	5 R+ 10g/l NaCl	6 R+ 15g/l NaCl	7 R+ 20g/l NaCl	8 R+ 25g/l NaCl
Reimann medium	—	+38.30	+91.40	+85.26	+54.45	+10.93	+10.21	+10.10
Reimann+ 1g/l NaCl	+5.46	—	-2.40	+0.48	-9.63	-12.91	-10.93	-10.18
Reimann+ 2g/l NaCl	+5.19	+13.60	—	+7.29	+6.37	-4.00	-1.30	-1.70
Reimann+ 5g/l NaCl	+0.73	+0.91	+8.25	—	-9.52	-6.81	-10.51	-9.60
Reimann+ 10g/l NaCl	+3.18	+22.93	+29.03	+9.90	—	-2.37	-4.15	+1.18
Reimann+ 15g/l NaCl	+61.33	+13.56	+77.61	+35.18	+2.33	—	+2.45	+10.13
Reimann+ 20g/l NaCl	+13.40	+13.31	+133.48	+113.26	+2.14	+1.53	—	-3.04
Reimann+ 25g/l NaCl	+54.19	+96.40	+115.10	+151.61	+27.43	-1.51	+3.44	—

C.D. at 1% level 34.01

4 3 2 5 7 6 8

Broadly speaking, in any one year there can be two incidences of auxospore formation in an estuarine environment such as the one which we have studied, once on dilution when fresh water flows into the sea through the estuary and again on return of saline conditions by addition of sea water into the estuary with a reduction or stoppage of flow of fresh water. Auxospore formation is considered to be a process of rejuvenation and is said to be followed by a high rate of division leading to formation of blooms. Sudden blooms seem to be caused by a two way increase, especially in species which have a wide range of cell dimensions. Auxospore formation leads to a sudden increase in cell volume. For instance, in this diatom, by auxospores formation a near ten-fold increase in cell volume has been noticed. Thus, the immediate consequence of auxospore formation is an increase in total volume of cell

per cell. This coupled with the postulated high rate of cell division following auxospore formation can contribute within a short time a large population both in number and by cell volume, from a relatively small pre-existing initial population. The observation made by Iyengar and Venkataraman (1951) on the abundances of *C. meneghiniana* in the Cooum estuary seems especially significant here. The diatom reached at least two peaks of abundance. Both these blooms were preceded by heavy showers and the chloride content of the waters at these times was very low. The alga disappeared (or decreased) with increase in salinity during summer and the blooms followed dilution or fall in salinity. Estuaries must support a small population in that portion of the estuary which by its salinity is most favourable for the maintenance of the species and this population can act as the necessary seed material for such abundances. In what state or stage the species is maintained in the off season one does not know. We do not know whether the diatom perennates in a manner other than its planktonic existence (Lund, 1954, 1955 ; Braarud, 1962).

In nature, besides salinity, there may be other factors such as temperature, light, nutritional, special growth factors etc., acting separately or in conjunction that determine the occurrence of sudden planktonic blooms. Such a bloom formation is immediately followed by a quick disappearance. It is common knowledge that inhibitory forces begin to work within any large population. This coupled with naturally occurring predatory mechanisms existing in any biotope can lead to a decline of the bloom. All that is suggested here is that salinity relationships of the algae probably play a prominent role, in producing abundance especially of euryhaline species.

The ability to tolerate and grow in various salinities, efficient maintenance of a small inoculum throughout in places of various salinities of the river and the sea, effective use of changes in salinity for reproduction and auxospore formation, and in short synchronizing its life cycle to changes in hydrological conditions of the environment, are some of the main features for causation of abundance of *C. meneghiniana* in the Cooum estuary (Desikachary and Rao, 1969).

OTHER DIATOMS

Diatoms (Table 2) isolated from fresh water, brackish water and marine environments were also studied for their tolerances to salinity. Clones of diatoms isolated from fresh water areas were maintained in Reimann medium. Diatoms from marine environments were maintained in Reimann medium + 30 g/l NaCl. Those isolated from the local estuarine situations with intermediate salinities were initially maintained in Reimann medium adjusted to a salinity nearest to the habitat from which these were isolated. As the isolates grew equally well in Reimann medium without NaCl these were subsequently maintained in this medium. Only one isolate of *Amphora coffeaeformis* (isolate No. 2 ; see Table 2) which did not grow in this medium was maintained in 5 g/l NaCl amended Reimann medium. Experimental conditions were identical as those in studies with *Cyclotella meneghiniana*.

The rates of division of *Gomphonema parvulum* in different salinities is shown in Fig. 1b. The diatom grew well in Reimann medium only. It did not appear to tolerate an increase in salt concentration of the medium.

Among the *Navicula* species (Fig. 1c), *Navicula salinarum* isolated from a marine environment and *Navicula minima* from Cooum estuary grew well in all

salinities and these two species are euryhaline. *N. pelliculosa*, *N. vitabunda* and *N. minima* (fresh water isolates), did survive up to 20‰ NaCl although growth was comparatively higher in Reimann medium, gradually falling and becoming practically nil at higher NaCl concentrations.

The behaviour of *Amphora coffeaeformis* to changes in salinity varied. Clones of this species though isolated from different environments grew in all salinities studied. Isolates from particular salinity showed greater growth when grown in that salinity than in other NaCl concentrations. One particular isolate (No. 2), however, showed optimal growth in a narrow range of salinity (5-10‰ NaCl) and growth declined with increase or decrease in NaCl concentrations. *Amphora ovalis* isolated from a marine environment grew well only at high salinity, while estuarine clone grew well in all salinities. Both species of *Amphora* (Fig. 2a) are euryhaline, their degree of euryhalinity being different. There is, therefore, some difference in the behaviour of different clones of these species depending upon the place of origin of the isolates.

In *Nitzschia* (Fig. 2b) clones of *N. amphibia* (isolate 16) and *N. vitrea* from fresh water areas did not tolerate increased salinity. All the other species including *N. amphibia* (isolates 15 and 17) derived from another fresh water area and an estuary did survive in higher salinities up to 20-25‰ NaCl. The different isolates of *N. amphibia* differ in their responses to salinity and this behaviour seems to be like those of *Amphora coffeaeformis*. We did not study any *Nitzschia* species from marine environment.

A composite graph showing division rates of diatoms isolated from fresh water environments is presented in Fig. 3a. Except *Gomphonema parvulum* and *Nitzschia vitrea* which may be considered by their behaviour as typical fresh water species, all the other isolates though derived from fresh water localities tolerated NaCl concentrations to varying degrees. However, growth was much better in lower salinities and best in fresh water medium. The division rates of diatoms from the estuaries is shown in Fig. 3b. A majority of diatoms from this environment were able to tolerate wide changes in salinity. The division rates of diatoms from the marine areas are given in Fig. 4a. It is clear that isolates did survive in all salinities, although growth was much better in higher salinities. However, no diatom which can be considered as typically marine, was studied.

These results confirm that we may have representatives of both steno- and euryhaline diatoms in every situation. Some species seem to have even races, clones differing in their behaviour to changes in salinity. The occurrence and abundance of the individual species in any given environment may depend upon the conditions that are favourable for their optimum development. Among the euryhaline diatoms there are forms which exhibit optimum growth in lower, middle or higher salinity ranges. In a way we are faced with what is called valency as an expression of growth of organisms to borrow a term used in studies with other environments, for example thermal situations (Vouk, 1948). These forms can be conveniently called as micro-, meso-, and macro-valent euryhaline species, though they are all euryhaline. The ideas that are sought to be conveyed here are very nearly those of Kolbe (1927), but with one difference. The hypothetical curves given by Kolbe (p. 114) do not fully represent diatoms which may survive in all ranges of salinities. The hypothetical curves given in Fig. 4b will broadly represent our idea in the use of terms proposed here. This incorporates expression of optimal growth of euryhaline diatoms which may tolerate and grow in a very wide range of salinity. It is,

TABLE 3. List of centric and pennate diatoms and their abundance in the inoculum (plankton sample) and that obtained in media of different salinities

No.	Diatoms	FINE NET (600)						COARSE NET (200)									
		Inoculum or Plankton sample	Reimann medium	Sodium chloride amended Reimann medium						Inoculum or Plankton sample	Reimann medium	Sodium chloride amended Reimann medium					
				5 g/l	10 g/l	15 g/l	20 g/l	25 g/l	30 g/l			5 g/l	10 g/l	15 g/l	20 g/l	25 g/l	30 g/l
CENTRALES																	
1.	<i>Biddulphia mobiliensis</i> (Bailey) Grun.	R	—	—	VR	—	—	VR	R	—	—	—	VR	—	—	—	
2.	<i>Biddulphia sinensis</i> Grev.	R	—	—	VR	—	—	VR	R	—	—	—	—	—	—	—	
3.	<i>Chaetoceros affinis</i> Lauder	C	—	—	VR	VR	VR	—	C	—	—	—	VR	C	VR	—	
4.	<i>Chaetoceros decipiens</i> forma <i>singularis</i> Grun.	—	—	D	—	A	R	R	—	—	—	C	—	—	C	—	
5.	<i>Chaetoceros didymus</i> Ehr.	C	—	—	—	R	—	VR	—	—	—	—	VR	R	C	—	
6.	<i>Chaetoceros diversus</i> Cleve	R	—	—	—	—	—	—	—	—	—	—	VR	C	—	—	
7.	<i>Chaetoceros lorenzianus</i> Grun.	C	—	—	—	—	—	—	—	—	—	—	VR	C	—	—	
8.	<i>Coscinodiscus sublineatus</i> Grun.	C	—	—	—	VR	VR	—	R	—	—	—	—	—	—	—	
9.	<i>Cyclotella meneghiniana</i> Kütz.	—	A	A	D	A	C	C	R	—	A	D	A	A	A	C	
10.	<i>Ditylum brightwellii</i> (West) Grun.	C	—	C	C	R	—	—	—	—	—	VR	R	R	R	—	
11.	<i>Lithodesmium undulatum</i> Ehr.	R	—	—	—	—	—	VR	VR	—	—	—	—	—	—	—	
12.	<i>Melosira sulcata</i> (Ehr.) Kütz.	R	—	—	—	VR	—	—	—	—	—	—	—	—	—	—	
13.	<i>Rhizosolenia alata</i> Brightwell] forma <i>gracillima</i> (Cleve) Grun.	C	—	—	—	—	—	—	—	—	—	—	—	VR	—	—	
14.	<i>Skeletonema costatum</i> (Grev.) Cleve	C	—	C	A	C	VR	—	—	—	—	C	D	C	VR	VR	
15.	<i>Thalassiosira subtilis</i> (Ostenfeld) Grun.	—	—	—	—	R	R	C	C	—	—	—	—	—	C	R	

PENNALES

16.	<i>Amphiprora paludosa</i> W. Smith var <i>subsalina</i> Cleve	—	—	R	R	A	A	D	D	—	—	—	—	R	C	D	A
17.	<i>Amphora coffeaeformis</i> Agardh	—	C	R	C	VR	C	A	D	—	A	C	C	A	C	D	A
18.	<i>Asterionella japonica</i> Cleve	C	—	—	VR	VR	—	—	—	C	—	—	VR	—	—	VR	VR
19.	<i>Bacillaria paradoxa</i> Grmelin	VR	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
20.	<i>Gyrosigma balticum</i> (Ehr.) Rabenh.	—	—	—	VR	VR	—	—	—	—	—	—	VR	—	—	—	—
21.	<i>Navicula halophila</i> (Grun.) Cleve	—	C	C	C	R	A	—	—	—	C	C	R	C	—	A	—
22.	<i>Navicula minima</i> Grun.	—	C	R	—	C	—	—	—	—	—	—	—	—	—	—	—
23.	<i>Nitzschia closterium</i> (Ehr.) W. Smith	—	R	C	R	—	—	VR	VR	—	R	—	—	—	—	VR	—
24.	<i>Nitzschia obtusa</i> var. <i>scalpelliformis</i> Grun.	R	—	R	R	—	R	R	—	R	—	—	—	R	R	R	—
25.	<i>Nitzschia seriata</i> Cleve	R	—	—	—	—	VR	VR	VR	R	—	—	—	VR	VR	—	VR
26.	<i>Nitzschia vitrea</i> Norman	—	—	—	—	VR	—	—	—	—	—	—	—	—	—	—	—
27.	<i>Pleurosigma directum</i> Grun. var. <i>membranacea</i> Subrah.	—	—	—	R	—	—	R	—	—	—	—	VR	—	—	—	—
28.	<i>Pleurosigma normanni</i> Ralfs.	—	—	—	—	VR	—	—	—	—	—	—	—	—	—	—	VR
29.	<i>Synedra formosa</i> Hantzsch.	R	—	—	—	—	VR	—	VR	R	—	—	—	—	—	R	VR
30.	<i>Thalassionema nitzschioides</i> Grun.	C	—	—	VR	—	VR	VR	—	C	—	—	—	—	—	—	VR
31.	<i>Thalassiothrix frauenfeldii</i> Grun.	R	—	—	—	—	VR	VR	—	C	—	—	—	—	R	—	—

D=Dominant (above 40%); A=Abundant (20-40%); C=Common (5-20%); R=Occasional (2-5%); VR : also present (less than 2%).

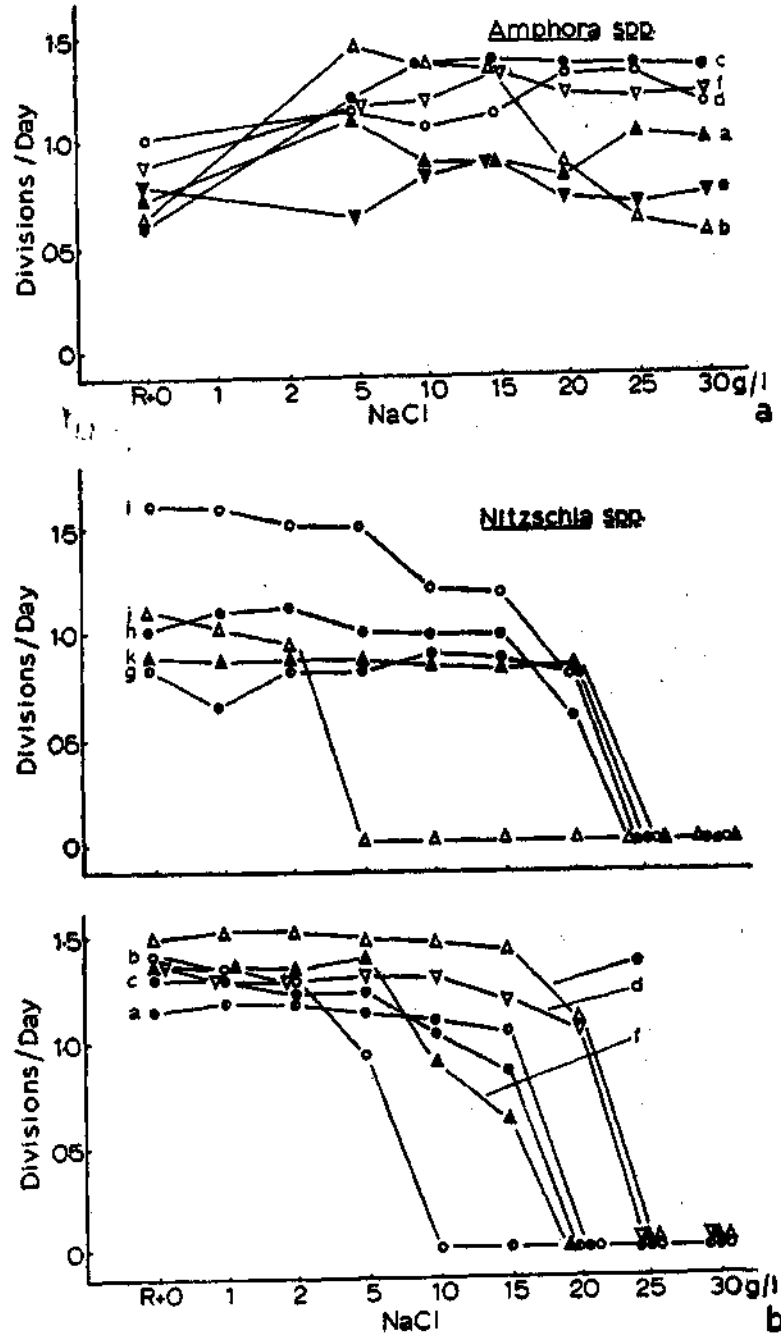


Fig. 2 (a). Division rates of species of *Amphora* in media of different salinities, a. *A. coffeaeformis* (1), b. *A. coffeaeformis* (2), c. *A. coffeaeformis* (3), d. *A. coffeaeformis* (4), e. *A. ovalis* (5); and (b). Division rates of species of *Nitzschia* in media of different salinities, Bottom figure: a. *N. amphibia* (18), b. *N. amphibia* (16), c. *N. amphibia* (17), d. *N. frustulum* (18), e. *N. frustulum* (19), f. *N. frustulum* (20); Top figure: g. *N. palea* (21), h. *N. palea* (22), i. *N. palea* (23), j. *N. vitrea* (24), and k. *Nitzschia* sp. (25).

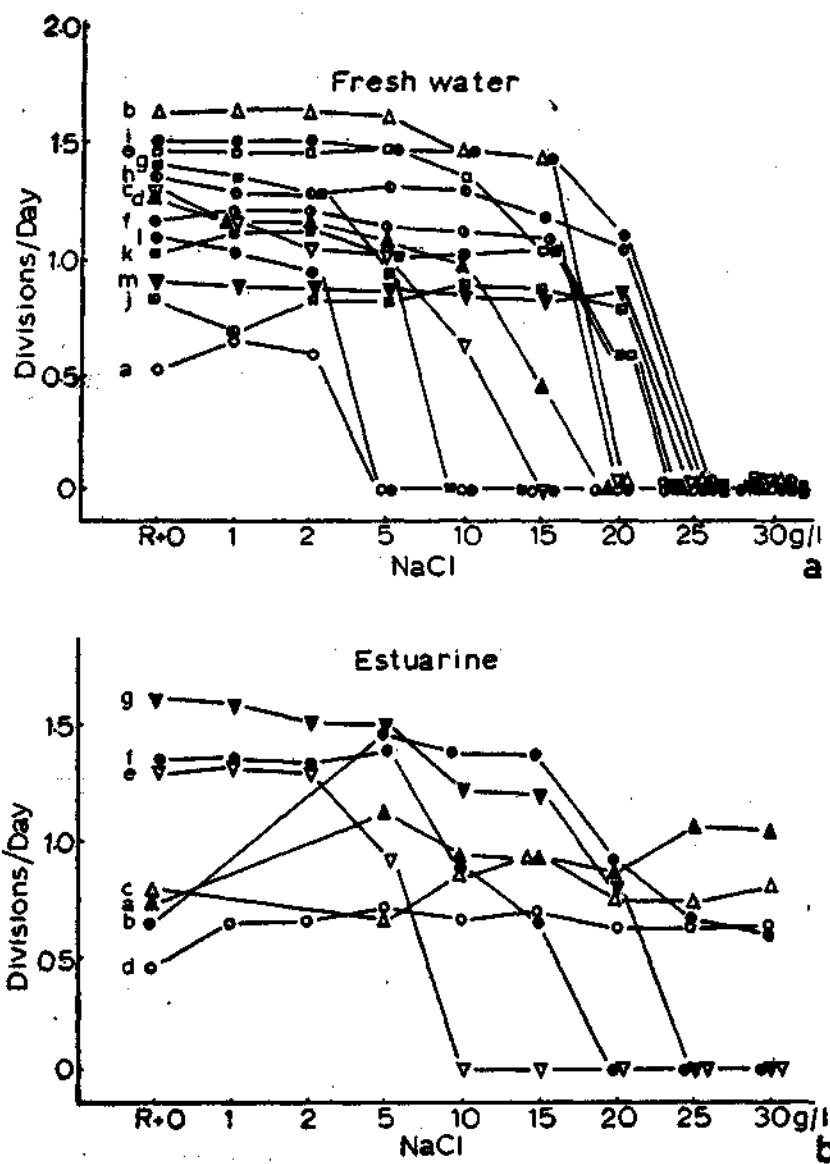


Fig. 3 (a). Composite graph showing division rates of diatoms isolated from fresh water environment in various salinities. a. *Gomphonema parvulum* (7), b. *Navicula minima* (8), c. *Navicula pelliculosa* (10) d. *Navicula pelliculosa* (11), e. *Navicula vitabunda* (14), f. *Nitzschia amphibia* (15), g. *Nitzschia amphibia* (16), h. *Nitzschia frustulum* (18), i. *Nitzschia frustulum* (19), j. *Nitzschia palea* (21), k. *Nitzschia palea* (22), l. *Nitzschia vitrea* (24), m. *Nitzschia* sp. (25); and (b). Composite graph showing division rates of diatoms isolated from Cooum and Adyar estuaries, Madras. a. *Amphora coffeaeformis* (1), b. *A. coffeaeformis* (2), c. *A. ovalis* (5), d. *Navicula minima* (9), e. *Nitzschia amphibia* (17), f. *N. frustulum* (20), and g. *Nitzschia palea* (23).

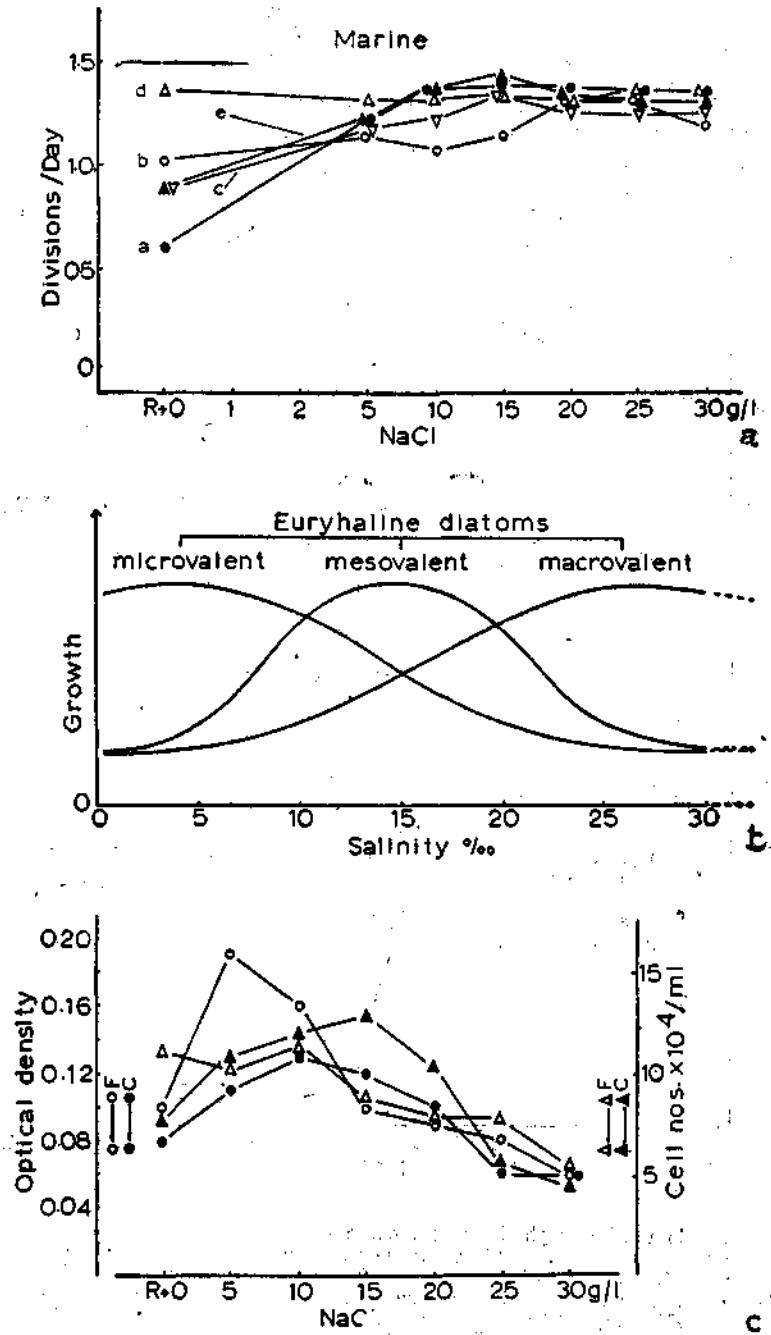


Fig. 4. (a). Composite graph showing division rates of diatom isolated from the sea. a. *Amphora coffeaeformis* (3), b. *A. coffeaeformis* (4), c. *A. ovalis* (6), d. *Navicula salinarum* (12), e. *N. salinarum* (13); (b) Hypothetical curves representing the proposed behaviour of euryhaline diatoms, and (c). Cell numbers and optical density of pigment extract of planktonine dia of different salinities. F. Plankton collected using a fine net (600 meshes/inch), C. Plankton collected using a coarse net (200 meshes/inch).

however, recognised that no set of terminologies can completely express the behaviours of every species of diatoms.

EURYHALINE COMPONENTS OF PHYTOPLANKTON

In many investigations on near shore waters, one type of phytoplankton blooms, commonly reported, coincides with a marked decrease in salinity and the individual dominant plankton elements have been traced to an estuarine origin. A large decrease in salinity naturally favours mesovalent or even microvalent euryhaline species, while a smaller decrease may favour only macrovalent species. The results obtained here on the behaviour of euryhaline diatoms occurring in various environments suggest a possibility of these taking a part in building of diatom abundance. It would be advantageous in studying phytoplankton if we can determine its euryvalent component and understand its contributions to the abundances. In coastal water, euryhaline diatoms may ordinarily exist in very low numbers although capable of optimum growth at a lower salinity. This ability of the diatom species to grow, yet differentially, in varying salinities can be used with advantage to estimate the euryhaline component of the diatom flora in any given environment. This will bring in experimental methods into phytoplankton studies. Borrowing from microbiological techniques studies were undertaken to explore this feasibility.

Phytoplankton was collected from the Madras coast and was inoculated into Reimann medium and Reimann medium amended with different NaCl concentrations such as 5 g/l, 10 g/l, 15 g/l, 20 g/l, 25 g/l and 30 g/l. Inoculum level was adjusted to give about 3,000 cells/ml initially in each media, optical density of the pigment extract (10 ml of 90% acetone was used for extraction) being 0.003. Experimental conditions were same as was obtained in the previous studies on other diatoms. Cell numbers and the relative optical density of the pigment extract at the end of 10 days' growth is given in Fig. 4c. Diatoms occurring in these media were also qualitatively and quantitatively estimated (Table 3). Fig. 4c and Table 3 shows that there was more growth as expressed by cell numbers and optical density in media with low salinity and in fact, diatoms such as *Cyclotella meneghiniana*, *Skeletonema costatum*, and *Amphora coffeaeformis* were found to occur regularly in all the salinities, in various proportions. This indicates that the phytoplankton used as inoculum contained euryhaline diatoms, though in low numbers ordinarily not recognizable or countable. Their capacity to grow in lower salinities, favoured their growth and multiplication and these became dominant elements in cultures. The extent of occurrence of a particular species in different salinities is a measure of its euryvalency. This procedure may also give an idea of the presence or absence of the dominant euryhaline diatoms in the phytoplankton in the off season when they are not recognizable by the methods now being used or are too few to be got in any counting procedures currently employed.

The absence of some species from such cultures cannot be used, of course, as a negative evidence. Firstly, there is always a possibility of the existence of such diatoms (in a vegetative or perennating conditions) in parts of the biotope other than the one from which the inoculum was derived. Our inability to understand, and to replicate requisite conditions, most favourable, for the growth of some of these species at least cannot also be forgotten (Droop, 1958; Provasoli, 1958). Secondly, it is possible that a temporary addition of one or more species (allochthonous origin), especially from areas very close to land or from near estuaries, during periods favourable for their growth and multiplication, may cause a brief, though sometimes signi-

TABLE 2. Diatoms from fresh water, brackish water and marine environments used for study of their tolerance to salinity

No.	Diatoms used in this study		Place of collection	Date	Salinity of water at the time of collection
1.	<i>Amphora coffeaeformis</i> Agardh	(FE)	River Cooum, Madras	Jan. '66	0.20‰
2.	<i>Amphora coffeaeformis</i> Agardh	(E)	River Cooum, Madras	Jan. '66	6.42‰
3.	<i>Amphora coffeaeformis</i> Agardh	(M)	Moist sand, shore collection, Kovelong, Madras	Mar. '70	Marine
4.	<i>Amphora coffeaeformis</i> Agardh	(M)	-do- -do- -do-	Mar. '70	Marine
5.	<i>Amphora ovalis</i> Kütz.	(E)	River Adyar, Madras	Mar. '70	10.82‰
6.	<i>Amphora ovalis</i> Kütz.	(M)	Moist sand, shore collection, Kovelong, Madras	Mar. '70	Marine
7.	<i>Gomphonema parvulum</i> (Kütz.) Grun.	(F)	Garden pond, Univ. Botany Lab. Madras	Jan. '66	0.05‰
8.	<i>Navicula minima</i> Grun.	(F)	-do- -do- -do-	Jan. '66	0.05‰
9.	<i>Navicula minima</i> Grun.	(E)	River Adyar, Madras	Mar. '70	10.82‰
10.	<i>Navicula pelliculosa</i> (Breb.) Hilse	(F)	Muddy water of small puddle in Triplicane, Madras	Nov. '65	0.05‰
11.	<i>Navicula pelliculosa</i> (Breb.) Hilse	(F)	-do- -do- -do-	Nov. '65	0.05‰
12.	<i>Navicula salinarum</i> Grun. Cleve and Grun.	(M)	From shore collections opposite Marina, Madras	Oct. '65	Marine
13.	<i>Navicula salinarum</i> Grun. Cleve and Grun.	(M)	-do- -do- -do-	Oct. '65	Marine
14.	<i>Navicula vitabunda</i> Hust.	(F)	Tirupathi lake, Andhra Pradesh	Jan. '66	0.05‰
15.	<i>Nitzschia amphibia</i> Grun.	(F)	Tirupathi lake, A.P.	Jan. '66	0.05‰
16.	<i>Nitzschia amphibia</i> Grun.	(F)	Vedanthangal lake, Madras	Jan. '66	0.05‰
17.	<i>Nitzschia amphibia</i> Grun.	(E)	River Cooum, Madras	Jan. '66	6.42‰
18.	<i>Nitzschia frustulum</i> (Kütz.) Gran.	(F)	Tirupathi lake, A.P.	Jan. '66	0.05‰
19.	<i>Nitzschia frustulum</i> (Kütz.) Gran.	(F)	Vedanthangal lake, Madras	Mar. '66	0.05‰
20.	<i>Nitzschia frustulum</i> (Kütz.) Gran.	(E)	River Cooum, Madras	Jan. '66	6.42‰
21.	<i>Nitzschia palea</i> (Kütz.) W. Smith	(F)	Tirupathi lake, A.P.	Jan. '66	0.05‰
22.	<i>Nitzschia palea</i> (Kütz.) W. Smith	(F)	Vedanthangal lake, Madras	Mar. '66	0.05‰
23.	<i>Nitzschia palea</i> (Kütz.) W. Smith	(E)	River Cooum, Madras	Jan. '66	6.42‰
24.	<i>Nitzschia vitrea</i> Norman	(F)	Muddy soil from Aminjikarai, Madras	Oct. '65	0.05‰
25.	<i>Nitzschia</i> sp.	(F)	Tirupathi lake, A.P.	Jan. '66	0.05‰

(F) Fresh water; (FE) Fresh water portion of Estuary; (E) Estuary; (M) Marine.

ficant, appearance in the coastal plankton. Such species will therefore not be revealed in isolated or single experiments. However, studies when carried out over a prolonged period of time may enable us to understand the periodicity and survival of euryhaline diatoms in a marine environment.

REFERENCES

- BRAARUD, T. 1962. Species distribution in marine phytoplankton. *J. Oceanogr. Soc. Japan*, Anniversary Volume, 20 : 628-649.
- CHU, S. P. 1942. The influence of mineral composition of the medium on the growth of planktonic algae I. Methods and culture media. *J. Ecol.*, 30 : 284-325.
- DESIKACHARY, T. V. and RAO, V. N. R. 1969. Salinity variations and *Cyclotella meneghiniana* Kuetz. *Proc. XI. Int. bot. Congr. Seattle, U.S.A.*, p. 46.
- DROOP, M. R. 1958. Optimum, relative and ionic concentrations for growth of some euryhaline algae. *Verh. int. Ver. Limnol.*, 13 : 722-730.
- GUILLARD, R. R. L. 1963. Organic sources of nitrogen for marine centric diatoms. In C. H. Oppenheimer, C. C. Thomas, [Ed.] *Symp. Marine Microbiology* Ch. 9 : 93-104. Springfield, Illinois, U.S.A.
- and RYTHER, J. H. 1962. Studies on marine planktonic diatoms *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran. *Can. J. Microbiol.*, 8 : 229-239.
- IYENGAR, M. O. P. and VENKATARAMAN, G. 1951. The ecology and seasonal succession of the algal flora of the river Cooum at Madras with special reference to the Diatomaceae. *J. Madras Univ.*, B, 21 : 140-192.
- JORGENSEN, E. G. 1960. The effects of salinity, temperature and light intensity on growth and chlorophyll formation of *Nitzschia ovalis*. *Carnegie Inst. Wash. Yearbook*, 59 : 348-349.
- KAIN, J. M. and FOGG, G. E. 1958. Studies on the growth of marine phytoplankton 1. *Asterionella japonica* Gran. *J. mar. biol. Ass. U.K.*, 37 : 397-413.
- KOLBE, R. W. 1927. Zur Okologie, Morphologie und Systematik der Brackwasser-Diatomeen. *Pflanzenforschung*, 7 : 1-146.
- LEWIN, J. C. and GUILLARD, R. R. L. 1963. *Diatoms*. *A. Rev. Microbiol.*, 17 : 373-414.
- LUND, J. W. G. 1954. The seasonal cycle of the plankton diatom *Melosira italica* (Ehr.) Kütz. subsp. *subarctica* O. Müll. *J. Ecol.*, 42 : 151-179.
- . 1955. Further observations on the seasonal cycle of *Melosira italica* (Ehr.) Kütz. subsp. *subarctica* O. Müll. *Ibid.*, 43 : 90-102.
- MCLACHLAN, J. 1961. The effect of salinity on growth and chlorophyll content in representative classes of unicellular marine algae. *Can. J. Microbiol.*, 7 : 399-406.
- PROVASOLI, L. 1958. Nutrition and ecology of Protozoa and Algae. *A. Rev. Microbiol.*, 12 : 279-308.
- REIMANN, B. E. F., LEWIN, J. C. and GUILLARD, R. R. L. 1963. *Cyclotella cryptica*, a new brackish water diatom species. *Phycologia*, 3 : 75-84.
- RODHE, W. 1948. Environmental requirements of freshwater plankton algae. *Symb. Bot. Upsal.*, 10 : 1-149.

- RYTHER, J. H. 1954. The ecology of phytoplankton blooms in Moriches Bay and great South Bay Long Island, New York. *Biol. Bull.*, **106** : 198-209.
- SUBRAHMANYAN, R. 1946. A systematic account of the marine plankton diatoms of the Madras Coast. *Proc. Indian Acad. Sci.*, **24B** : 85-197.
- VOUK, V. 1948. Thermal-vegetation and ecological-valences theory. *Hydrobiologia*, **1** : 90-95.
- WILLIAMS, R. B. 1964. Division rates of marsh diatoms in relation to salinity and cell size. *Ecology*, **45** : 877-880.