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PROGRESSIVE CELL SIZE DECREMENT AND AUXOSPORE FORMATION IN CULTURES OF DIATOMS

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ABSTRACT

Achnanthes brevipes v. *intermedia* (Kütz.) Cleve, *Amphiprora alata* (Ehr.) Kütz., *Amphiprora ornata* Bailey, *Amphiprora paludosa* Wm. Sm., *Amphiprora paludosa* v. *punctulata* Grun., *Melosira dubia* Kütz., *Synedra tabulata* (Agardh) Kütz., *Thalassiosira fluviatilis* Hustedt and *Triceratium dubium* Brightwell isolated from marshes, estuaries or coastal regions were made axenic and used in studies on progressive size diminution and auxospore formation.

Rates of progressive size diminution were studied in cells grown in different salinities for 36 subcultures and frequency curves, mean length of apical, transapical and pervalvar axes were obtained. Reduction in dimension of transapical and pervalvar axes was negligible wher compared to reduction in apical axis of pennate diatoms. In *A. paludosa* and *S. tabulata*, where it was possible to study the rate of size diminution, apical axis decrement was greater in high saline media and in large cells. Concomitant with decrease in cell diameter there was a corresponding increase in pervalvar axis in centric diatoms and this feature was more obvious in *T. fluviatilis*.

Three types of behaviour were noted in these diatoms as regards auxospore formation. In *A. brevipes* v. *intermedia*, *M. dubia* and *T. dubium* auxospores were frequently observed in all salinity media. On the other hand, *T. fluviatilis* auxosporulated only as a response to changes in salinity of the media. *A. paludosa* and *S. tabulata* showed progressive size diminution with no induction of auxospores even with periodic changes in salinity. Occasional large cells appeared though in cultures of these and other diatoms and the cause of their occurrence was not quite clear.

INTRODUCTION

REPEATED cell division in diatom population result in progenies with smaller and smaller individuals. Assuming that all cells of a population divide with equal frequency, distribution of cells with different sizes will follow a simple binomial rule (Pfitzer, 1869; Macdonald, 1869). The theoretical size reduction, derivable on the basis of Macdonald-Pfitzer rule, does not, however, occur in a number of diatoms (Rao and Desikachary, 1970). The rate of size decrement is also different under different environmental conditions. Size decrement in larger cells appears to be more than in smaller cells and the rate of decrement is greater in hypersaline media (Rao, 1978, 1980). Cells after attaining a particular size range during progressive reduction, produce auxospores which restore the original size. Size decrement unaccompanied by auxospore formation results in death of cells (Geitler, 1932). Information on progressive cell size diminution and rate of size decrement in many diatoms is unknown. The aim of the present study is to obtain data on some estuarine diatoms.

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Material and Methods

Diatoms used

Achnanthes brevipes v. *intermedia* (Kütz.) Cleve (A 1078) + *Amphiprora alata* (Ehr.) Kütz. (A 1119), *Amphiprora ornata* Bailey (A 1200), *Amphiprora paludosa* Wm. Sm. (A 1208), *Amphiprora paludosa* v. *punctulata* Grun (A 1209), *Melosira dubia* Kütz. (A 1118), *Synedra tabulata* (Agardh) Kütz. (A 1104), *Thalassiosira fluviatilis* Hustedt (A 1205) and *Triceratium dubium* Brightwell (A 1101) isolated from these collections were used in the present study. These diatoms were made axenic by antibiotic treatment (Droop, 1967) and maintained in Guillard's F/2 medium (Guillard and Ryther, 1962).

The diatoms were grown in different salinities ranging from 0.05% to 60% for 30 subcultures to find out the rate of progressive size diminution and auxospore formation.

Results

Progressive cell size diminution of diatoms in different salinity regimes

To assess rate of progressive cell size diminution in different salinity regimes, periodic cell transfers to diatoms were made at the end of 15 days in series 1. 0.05‰, 1‰, 5‰, 15‰ and 30‰ (control) and series 2. F/2 medium (control) (30‰), F/2* + 15 g/l NaCl (45‰) and F/2 + 30 g/l NaCl (60‰) medium and the experiment run for 36 subcultures. The two series were run one after the other and therefore comparison of rate of cell size diminution in these media with two separate controls became inevitable. At the end of each transfer, dimensions of at least 100 cells, selected at random in each sample were measured. From these measurements, percentage frequency curves and mean length of apical, transapical and pervalvar axes were obtained. There was hardly any perceptible reduction in dimensions of transapical and pervalvar axes when compared with the amount of reduction in apical axis in pennate diatoms and therefore these were omitted from the present consideration. Concomitant with decrease in frustule diameter or valve angle, there was increase in length of pervalvar axis in centric diatoms and therefore this aspect was studied.

Although the diatoms grew in many salinities on the first transfer, it was not possible to subculture them in the respective media in subsequent transfers. Except *T. fluviatilis* which grew in all salinities ranging from the fresh water 0.05‰ to the highest, 60‰ others failed to grow in the different salinity media from the second or subsequent transfer. *A. brevipes* v. *intermedia*, *A. paludosa* and *M. dubia* could not be maintained in 0.05‰—1.0‰ and *A. alata*, *A. ornata*, *A. paludosa* v. *punctulata* in 0.05 - 5‰. *S. tabulata* could be maintained only in a narrow range of 15‰ -30‰ and *T. dubium* in 15 - 45‰. Frequency curves of cells with different dimensions and mean apical axis/diameter of these diatoms were obtained in salinities where it was possible to maintain them. Though frequency curves were obtained in all subcultures, besides the inoculum, those obtained at the end of 6, 12, 18, 24, 30 and 36 transfers were only plotted.

Size diminution in *Achnanthes brevipes* v. *intermedia (Kütz.) Cleve*

Irrespective of the salinity of the medium there was periodic increase and decrease in mean length of apical axis during several

* Accession number of the culture maintained in the culture collection centre, Centre of Advanced Study in Botany, University of Madras, Madras 600 005.

transfers within the respective media (Fig. 1). A reading of frequency curves showed that in all media, cells with increased length of apical axis periodically occurred and this could be possible only due to auxospore formation taking place within the respective media. While progressive size diminution shifted the frequency towards smaller individuals, regular occurrence of large individuals followed by their division contributed more number of cells with large dimensions. Two or sometimes even three distinct populations could be seen in any one subculture. Populations with large cells were derived from populations of small cells by auxopore formation. Progressive apical axis reduction could be seen in all these distinct populations. As cells of one population reached a particular size limit, there was a decrease in their number and a simultaneous increase of another auxospore population (Fig. 2).

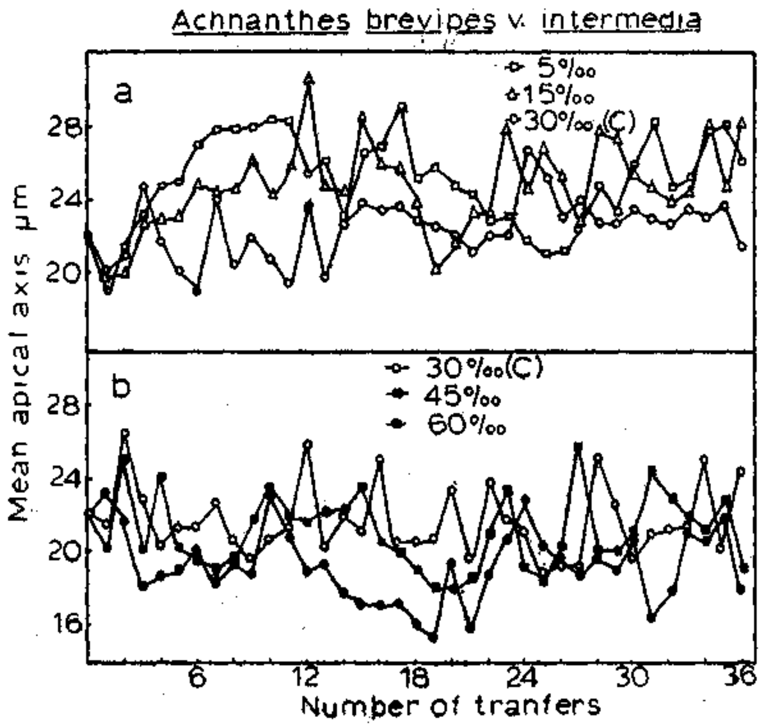

Fig. 1. Mean length of apical axis of *Achnanthes brevipes* v. *intermedia* in different salinities for 36 transfers: a. < 30‰ salinity and b. > 30‰ salinity.

Amphiprora alata (Ehr.) Kütz., *Amphiprora ornata* Bailey and *Amphiprora paludosa* v. *punctulata* Grun.

Cells of *A. alata* had a dimension range of 42.0-75.0 μm with the maximum number of cells having apical axis length between 45.0 and 60.0 μm. In *A. ornata* cells ranged from 22.5-42.0 μm with the majority having apical axis in the range of 27.0-30.0 μm. Cells of *A. paludosa* v. *punctulata* had the smallest range of dimension and the apical axis ranged between 19.0-34.8 μm. Length of apical axis curves of these cultures over the study period were wavy indicating that the mean size of the population frequently shifted from large to

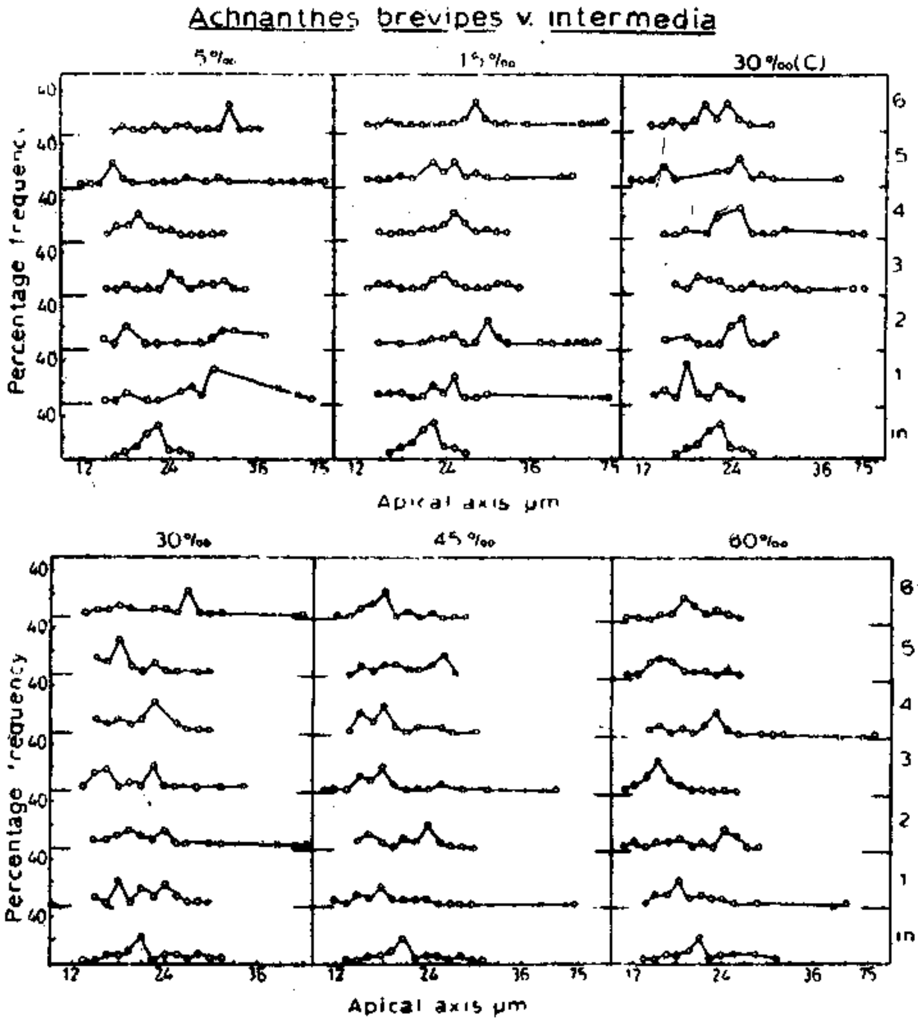

Fig. 2. Percentage frequency graph showing apical axis of *Achnanthes brevipes* v. *intermedia* in different salinities. Frequency curves obtained at the end of 6, 12, 18, 24, 30 and 36th transfer only are represented.

small cells and vice versa (Fig. 3, 5, 7). Perhaps due to periodic auxospore formation a certain percentage of large cells was always produced in any population which accounted for the way nature of the mean length of apical axis. Frequency curves indicated that the population which remained usually unimodal tended to become bimodal with a population derived by the activity of large individuals (Figs. 4, 6, 8). These compensated the otherwise steady rate of reduction and cells maintained a fluctuating mean apical axis.

Amphiprora paludosa Wm. Sm.

A. paludosa was studied first in high salinity media. There was progressive reduction in average length of apical axis and the rate of reduction differed in different salinities. Size reduction was very rapid and mean length of apical axis reduced from 90.0 to 40.0 μm. Reduction was more in high saline media than control. When grown in low salinity media rate of reduction was not rapid and during the study period average length of apical axis showed reduction from 40.0-30.0 μm less so in small cells maintained a minimum size range (Fig. 11). Frequency curves reflected this feature more clearly (Fig. 12).

Melosira dubia Kütz.

Auxospores were frequently observed in all the salinity regimes. As a result, frequency

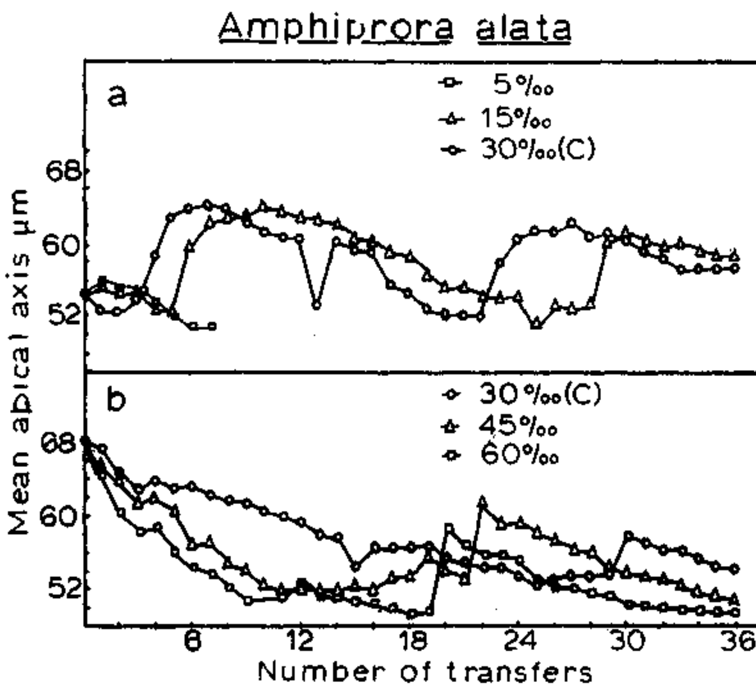

Fig. 3. Mean length of apical axis of *Amphiprora alata* in different salinities for 36 transfers : a. < 30‰ salinity and b. > 30‰ salinity.

and the rate of reduction in different media were more or less similar (Fig. 9). The population remained unimodal throughout and the rapid rate of reduction in apical axis in large and a comparatively small rate in small individuals was quite evident from the frequency curves (Fig. 10).

Synedra tabulata (Agardh) Kütz.

Cells of *S. tabulata* after a period of length reduction which was steep in large cells, but

curves showed a sizable percentage of large cells and the diameter ranged from 10.5-45.0 μm (Fig. 14). The mean cells diameter during the entire period fluctuated between 15.0-30.0 μm (Fig. 13). Length of pervalvar axis of the double cell (two cells each with a compliment of two valves and one girdle band) ranged from 30.0 - 49.5 μm with the mean around 38.0 μm. These appeared to increase concomitant with a decrease in diameter of cells.

Triceratium dubium Brightwell

T. dubium could be maintained only between salinities 15‰ and 45‰. In these cultures there was periodic auxospore formation during 15, 16). Progressive size decrement in this diatom was accompanied by increase in pervalvar axis.

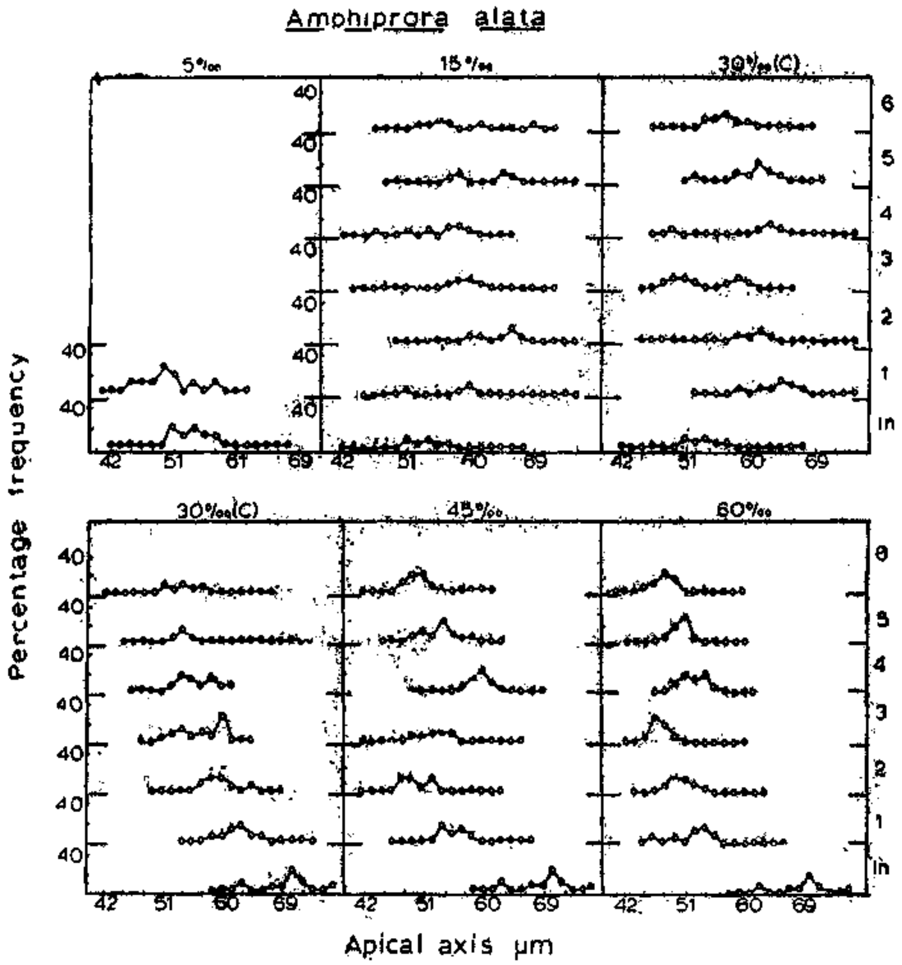

Fig. 4. Percentage frequency graph showing apical axis of *Amphiprora alata* in different salinities. Frequency curves obtained at the end of 6, 12, 18, 24, 30 and 36th transfer only are represented.

regular transfers within the solution. Rate of reduction in 15‰ salinity medium was slightly lower than 30‰ medium and was maximum in NaCl amended medium (Figs.

Thalassiosira fluviatilis Hustedt

T. fluviatilis with a diameter range of 9.0-16.5 μm with an average of 12.1 μm when inoculated into different media showed reduction

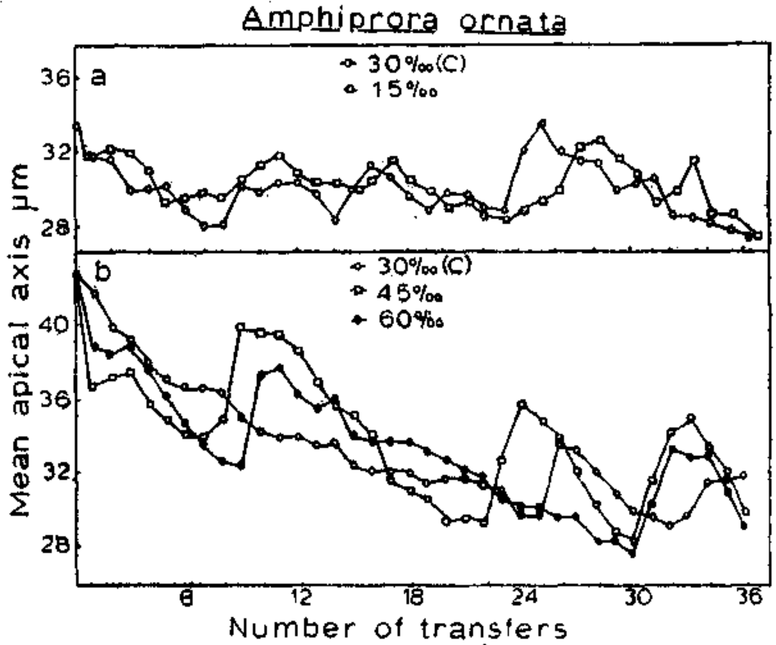

Fig. 5. Mean length of apical axis of *Amphiprora ornata* in different salinities for 36 transfers: a. < 30‰ salinity and b. > 30‰ salinity.

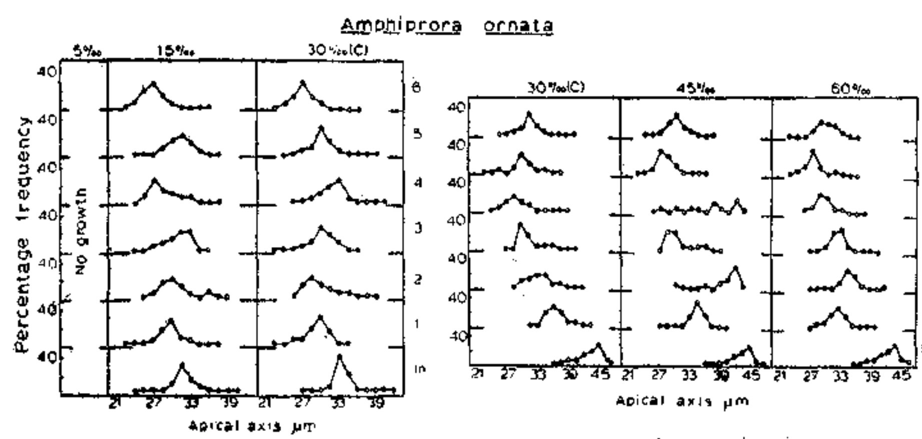

Fig. 6. Percentage frequency graph showing apical axis of *Amphiprora ornata* in different salinities. Frequency curves obtained at the end of 6, 12, 18, 24, 30 and 36th transfer only are represented.

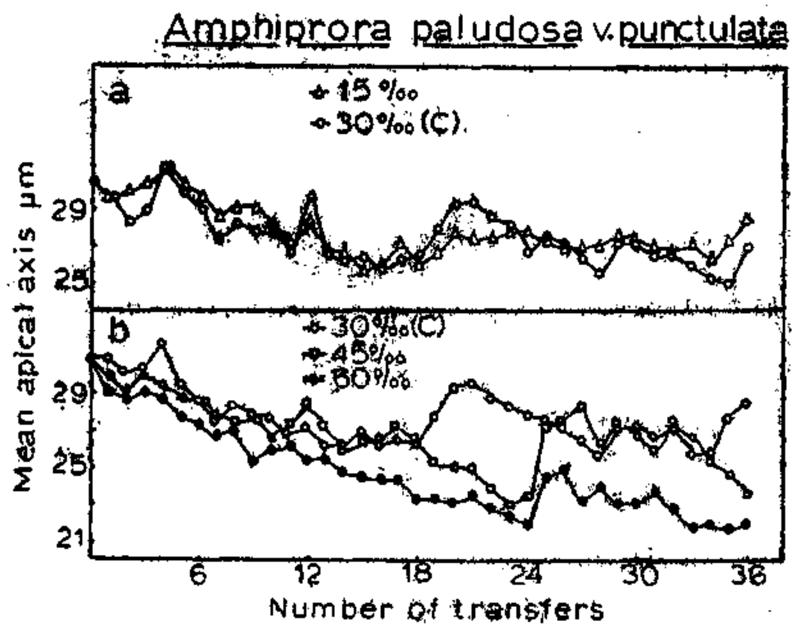

Fig. 7. Mean length of apical axis of *Amphiprora paludosa* v. *punctulata* in different salinities for 36 transfers : a. < 30‰ salinity and b. > 30‰ salinity.

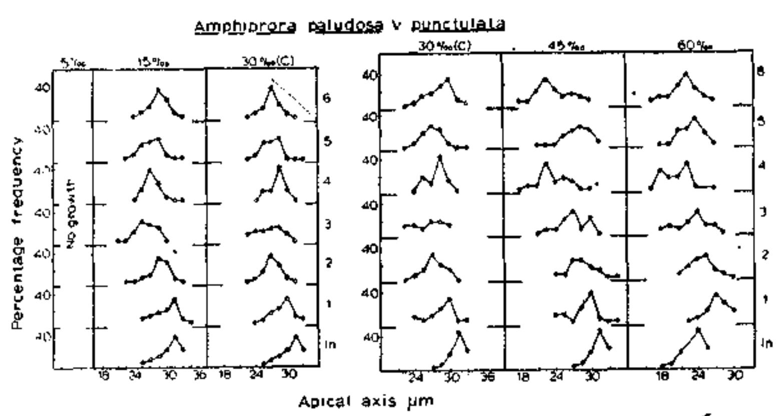

Fig. 8. Percentage frequency graph showing apical axis of *Amphiprora paludosa* v. *punctulata* in different salinities. Frequency curves obtained at the end of 6, 12, 18, 24, 30 and 36th transfer only are represented.

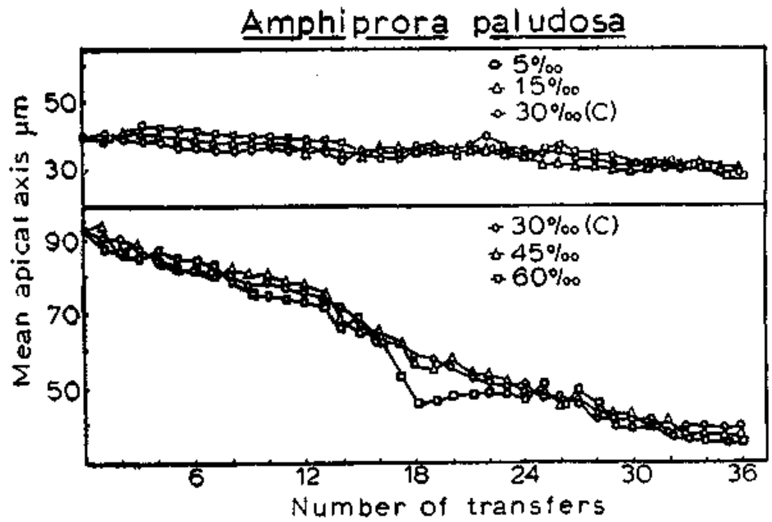

Fig. 9. Mean length of apical axis of *Amphiprora paludosa* in different salinities for 36 transfers : a. < 30‰ salinity and b. > 30‰ salinity.

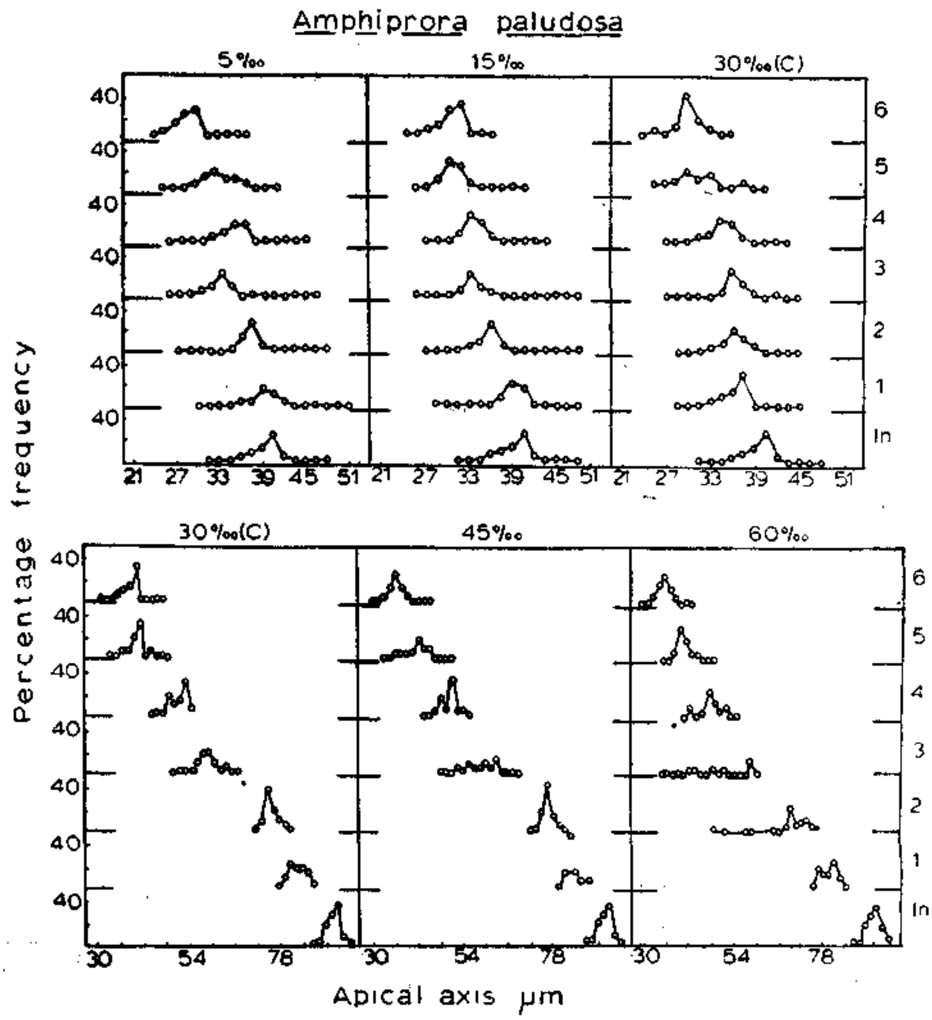

Fig. 10. Percentage frequency graph showing apical axis of *Amphiprora paludosa* in different salinities. Frequency curves obtained at the end of 6, 12, 18, 24, 30 and 36th transfer only are represented.

upto 8.0 μm in the high saline media (Fig. 17) and the diatom appeared to maintain this diameter. Reduction in mean cell diameter was more steep in large cells and gradually levelled of as the diameter of cells decreased. The diatom showed further decrease in size in 5‰, 1‰ and 0.05‰ salinities and the minimum mean cell diameter ranged from 6.0 - 4.0 μm, 0.05‰ salinity registering the smallest range of cell dimension (Fig. 18). When such cells were transferred into media of high salinity there was auxospore formation resulting in the occurrence of large cells which had a diameter range of 13.0 - 16.5 μm. As in the other two centric diatoms small cells showed increase in pervalvar axis.

Salinity and length of pervalvar axis in centric diatoms

Fig. 19, 20 and 21, show relationship of diameter/length of angle to pervalvar axis in *Melosira dubia*, *Triceratium dubium* and *Thalassiosira fluviatilis* respectively. For every diameter/length of angle, length of pervalvar axis in at least 25 cells were measured and the mean calculated. Mean pervalvar axis/diameter was plotted against each diameter or angle. In these diatoms mean length of pervalvar axis was more or less the same in cells of large dimensions while small cells were distinctly elongated. The extent of this elongation depended on salinity of the medium. In *M. dubia* and *T. dubium* this elongation was more or less uniform in all salinity media while in *T. fluviatilis* pervalvar elongation in low salinity media was very great compared to high saline media, the pervalvar axis of cells from 0.05‰ salinity being the longest.

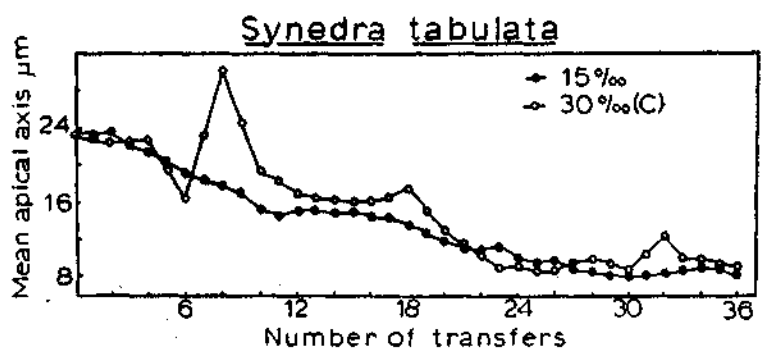

Fig. 11. Mean length of apical axis of *Synedra tabulata* for 36 transfers in ≤ 30‰ salinity.

Discussion

Progressive cell size decrement and auxospore formation

An unusual feature of a diatom cell is that during its division one of the resultant cells is smaller than the parent by approximately twice the thickness of its cingulum. Thus, any population will consist of cells possessing a range of cell sizes and in the course of several divisions the minimal size range for the particular diatom will be reached. This progressive reduction in diatom cell size was first explained independently by Pfitzer (1869, 1871) and Macdonald (1869). Size reduction in

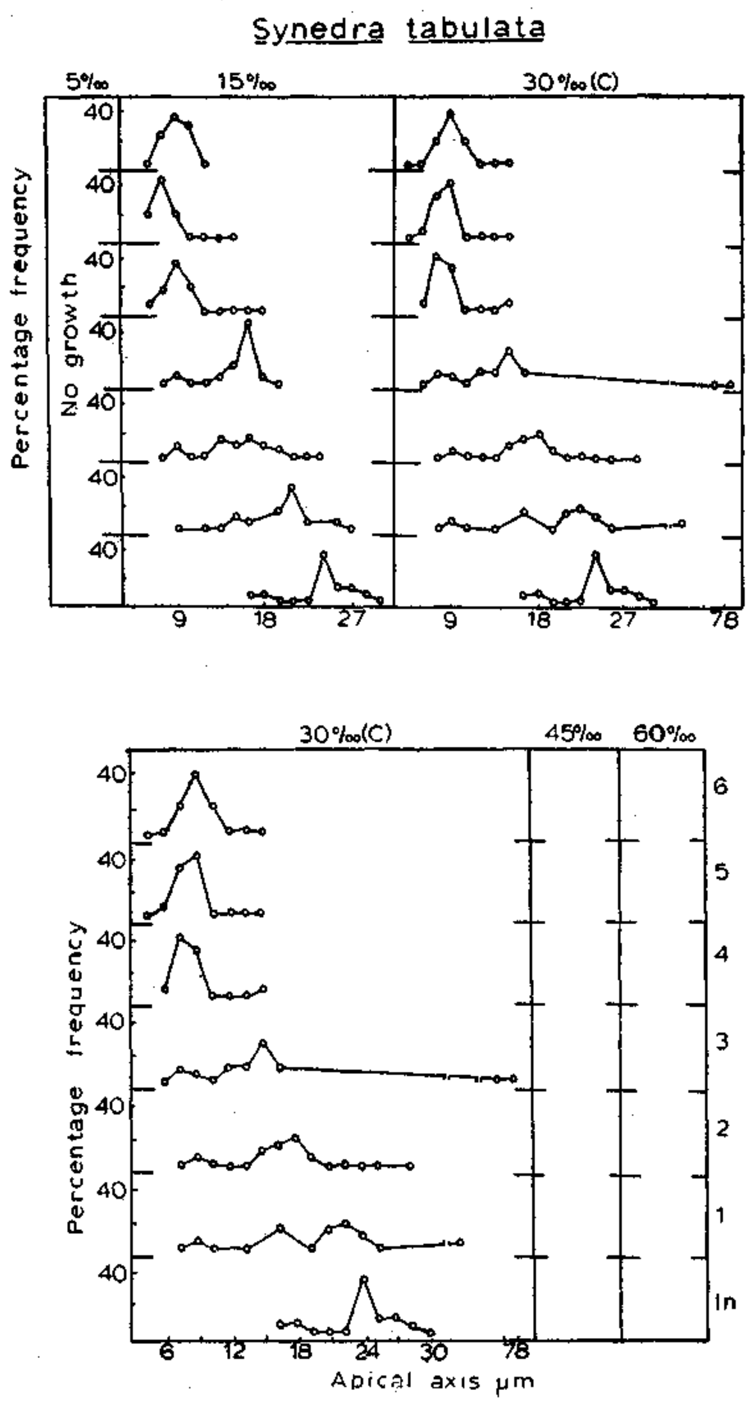

Fig. 12. Percentage frequency graph showing apical axis of *Synedra tabulata* in different salinities. Frequency curves obtained at the end of 6, 12, 18, 20, 24, 30 and 36th transfer only are represented.

most diatoms whose life-cycle has been studied, is eventually compensated by auxospore formation which restores the original size. Size decrement unaccompanied by auxospore formation may result in death of cells (Geitler, 1932).

number of diatoms. Rao (1980) recognized three patterns of size reduction in culture. 1. Diatom cells which after progressive diminution in size and reaching lower dimension range maintain at the same level without further reduction. 2. Diatom cells that

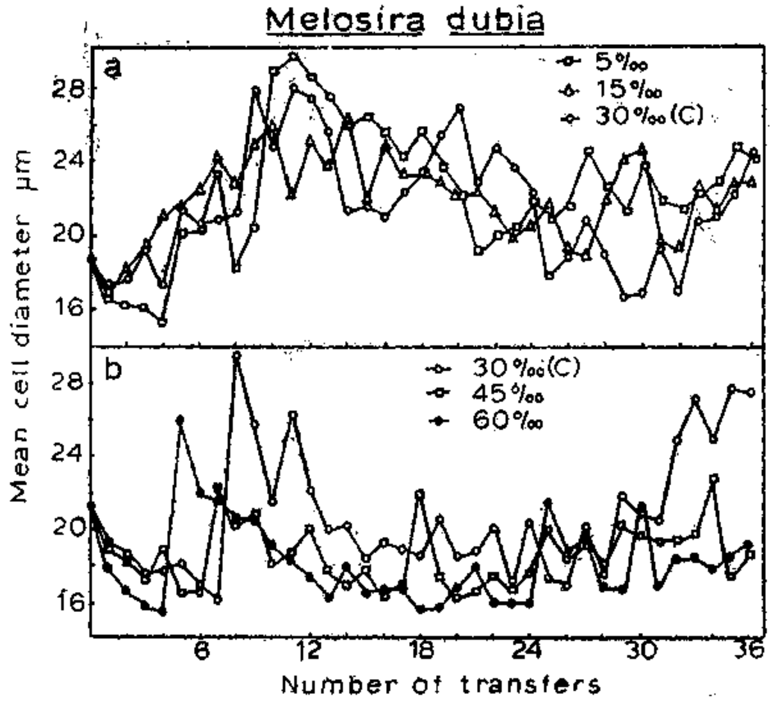

Fig. 13. Mean diameter of *Melosira dubia* in different salinities for 36 transfers: a. < 30‰ salinity and b. > 30‰ salinity.

Studies made on diatom population during the past several years (Geitler, 1932; Wiedling, 1943; 1948; Denffer, 1949; Locker, 1950; Erben, 1959; Hustedt, 1967; Geissler, 1970; Rao and Desikachary, 1970; Hostetter and Hoshaw, 1972; Desikachary and Rao, 1973; Hostetter, 1980), revealed that the pattern of size reduction accompanying cell division may vary and the theoretical size reduction of Macdonald-Pfitzer's rule does not occur in a

maintain the same dimensions throughout without any reduction. 3 a. Diatom cells which show progressive size diminution and also auxospore formation at intervals showing a fluctuating range of dimensions and 3 b. Diatom cells that form repeated auxospores so that a small percentage of large cells is always present in a population at any given time, the diatom already reaching the minimal size range (Fig. 22).

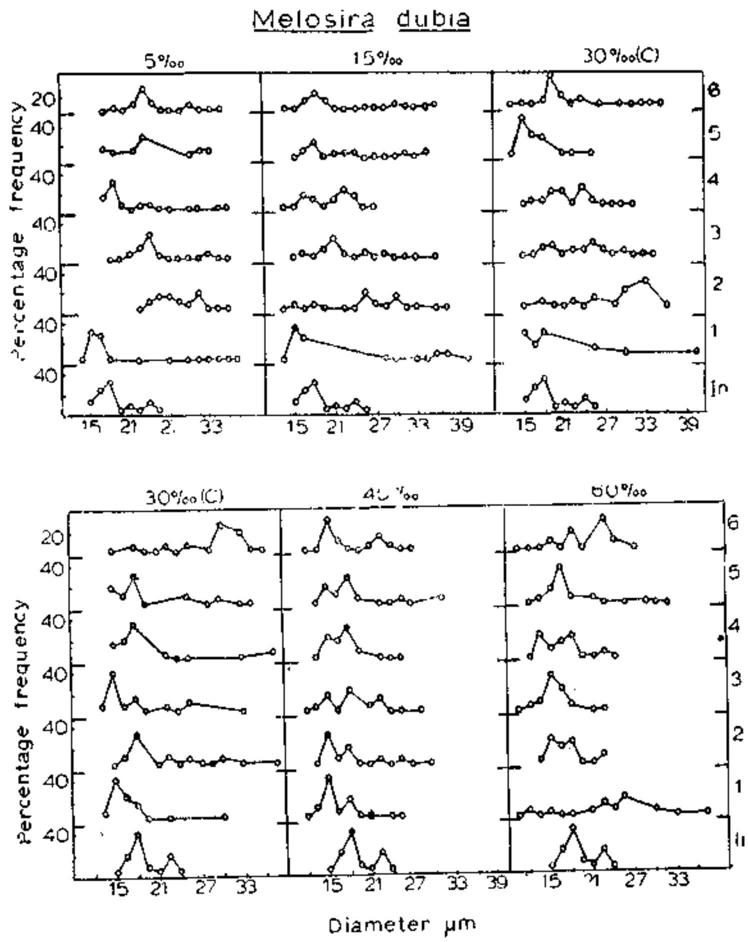

Fig. 14. Percentage frequency graph showing diameter of *Melosira dubia* in different salinities. Frequency curves obtained at the end of 6, 12, 18, 24, 30 and 36th transfer only are represented.

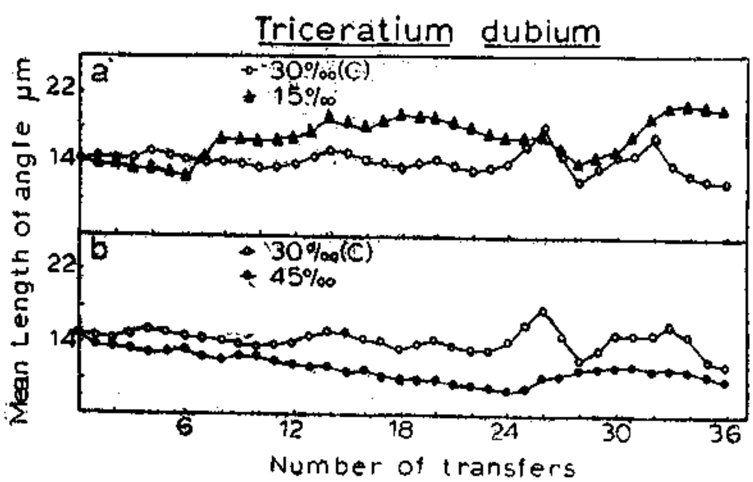

Fig. 15. Mean length of the angle of *Triceratium dubium* in different salinities for 36 transfers: a. < 30‰ salinity and b. > 30‰ salinity.

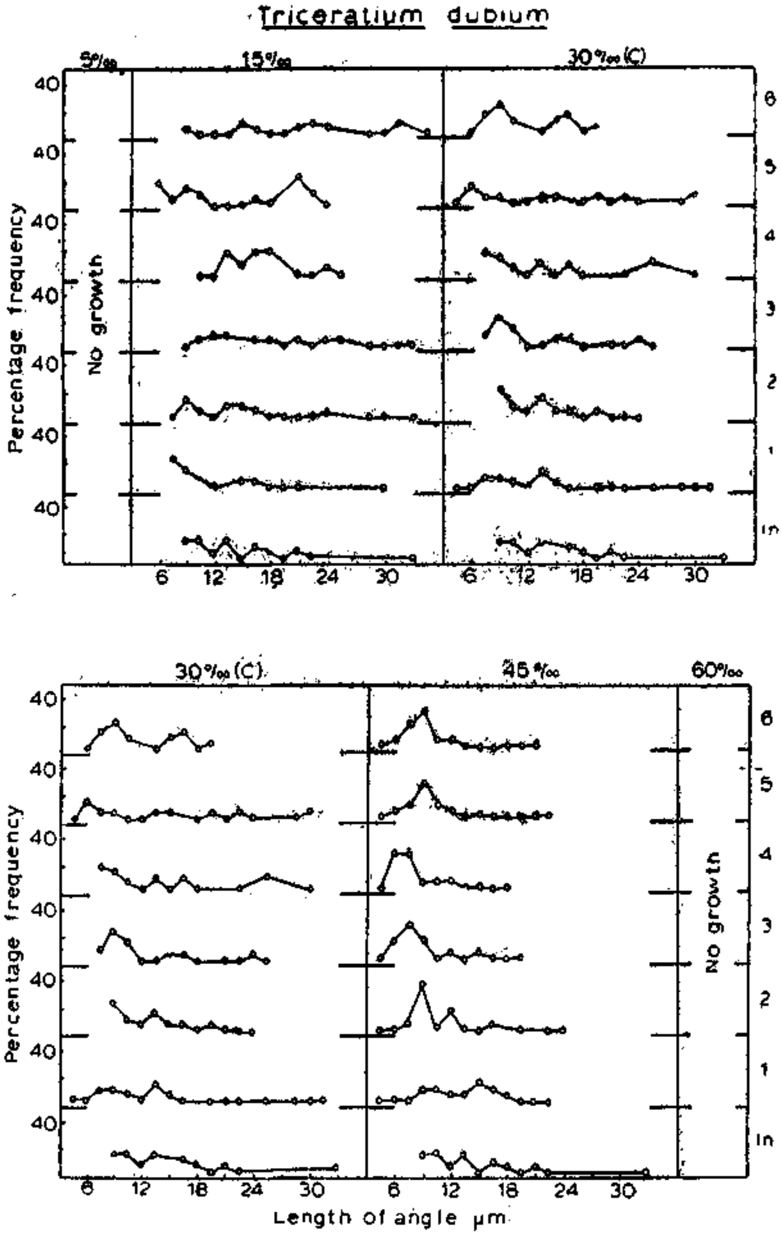

Fig. 16. Percentage frequency graph showing length of the angle of *Triceratium dubium* in different salinities. Frequency curves obtained at the end of 6, 12, 18, 24, 30 and 36th transfer only are represented.

In the present work, the behaviour of *Achnanthes brevipes* v. *intermedia*, *Melosira dubia* and *Triceratium dubium* is similar to the behaviour of diatoms of the type 3 a reported by Rao (1980). There was a regular occurrence of large individuals contributing a number of distinct populations and progressive apical axis/diameter reduction taking place mation, a small percentage of large cells was always present in any population. As sexual process of auxospores, or, for that matter, any other type of auxospore formations has not been reported in species of *Amphiprora*, it is not very clear how a small percentage of large cells is present at any one time during the ourse of subculturing.

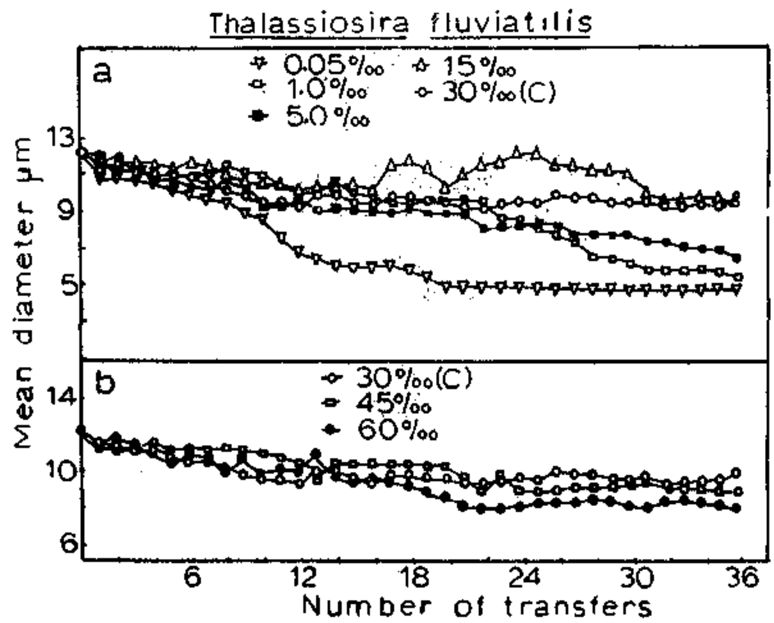

Fig. 17. Mean diameter of *Thalassiosira fluviatilis* in different salinities for 36 transfers : a. < 30‰ salinity and b. > 30‰ salinity.

in each of them and this happened in all saline media. That these diatoms produce auxospores very frequently was also confirmed by microscopic observations. *Amphiprora paludosa* v. *punctulata* is considered as belonging to type 3 b. In this diatom, the length of apical axis fluctuated between 19.5-34.5 μm and obviously the diatom had reached the minimal size range. Perhaps due to auxospore for- Behaviour of *Amphiprora alata* and *A. ornata* is slightly different in that these cultures had larger cells, progressive cell size diminution was still taking place and large cells were frequently occurring in the several treatments. *Amphiprora paludosa* and *Synedra tabulata* are examples of the type in which there was a progressive reduction in average length of apical axis which was steep in larger, but less so in smaller and almost flat in cells near about the minimum size range. Auxospores were not observed in these cultures*.

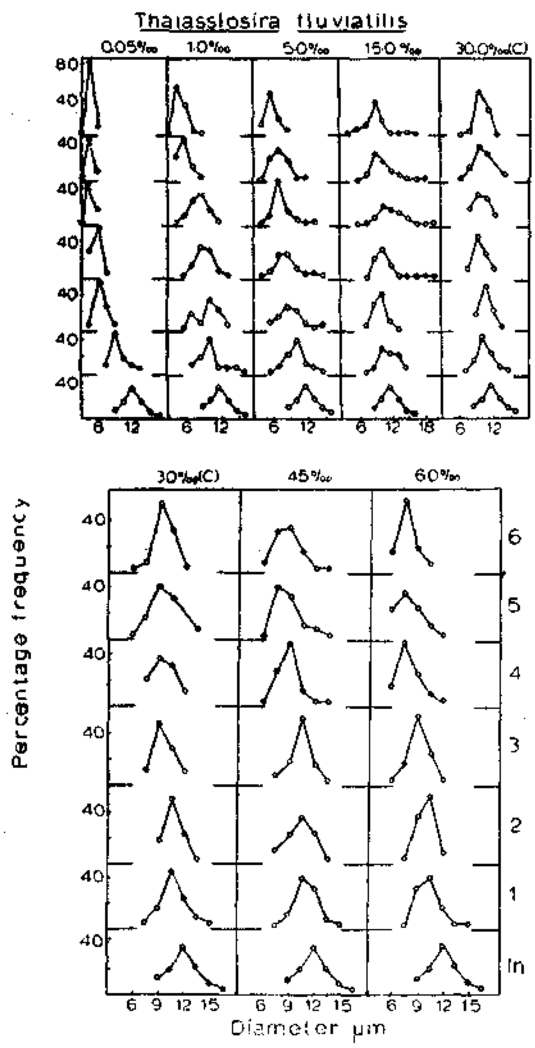

Fig. 18. Percentage frequency graph showing diameter of *Thalassiosira fluviatilis* in different salinities. Frequency curves obtained at the end of 6, 12, 18, 24, 30 and 36th transfer only are represented.

* Nuclear change accompanying actual process of auxospore formation have not been studied. It is not known whether this involves sexual, asexual or vegetative increase in size. Occurrence of distinctly large cells among a population of small cells is considered as auxospore formation.

Only in *A. paludosa* and *S. tabulated* it was possible to study the rate of size diminution as frequent occurrence of large cells in treatments in the other diatoms interfered with the rate of size decrement. Rate of size reduction in these two diatoms was variable and it depended on both size of the population and salinity of the media in which they grew. Greater the cell dimension greater was the reduction in any treatment. Similarly reduction was greater in high salinity media. *S. tabulata* was studied only through a small range and even here this trend was quite apparent.

Thalassiosira fluviatilis is the only diatom that grew in all salinity media. The pattern of progressive diameter reduction in the high (15-60‰) and the low (0.05-5‰), saline media varied. In the former the diatom appeared to maintain a mean cell diameter of 8.0 µm while in the latter it maintained at a mean cell diameter of 4.0-5.0 µm, cells with the smallest dimensions occurring in 0.05‰ salinity. These small cells when transferred into media of different salinities produced auxospores and in this behaviour they were similar to *Cyclotella meneghiniana* studied earlier in this laboratory (Rao, 1971, 1978). While large cells with 16.5 µm appeared regularly, possibly as a result of auxospore formation in 15‰ and 30‰ (control), in low salinity media (0.5-5‰) and NaCl amended media they occurred only when a change in salinity of the medium was effected.

That the minimum size range of particular diatom is environmentally influenced has a great bearing on the occurrence and distribution of the same species at different geographical locations and in different waters. The same species could exist in several waters with a different dimension range. It is not possible to explain why under the same condition in some cultures there is auxospore for-

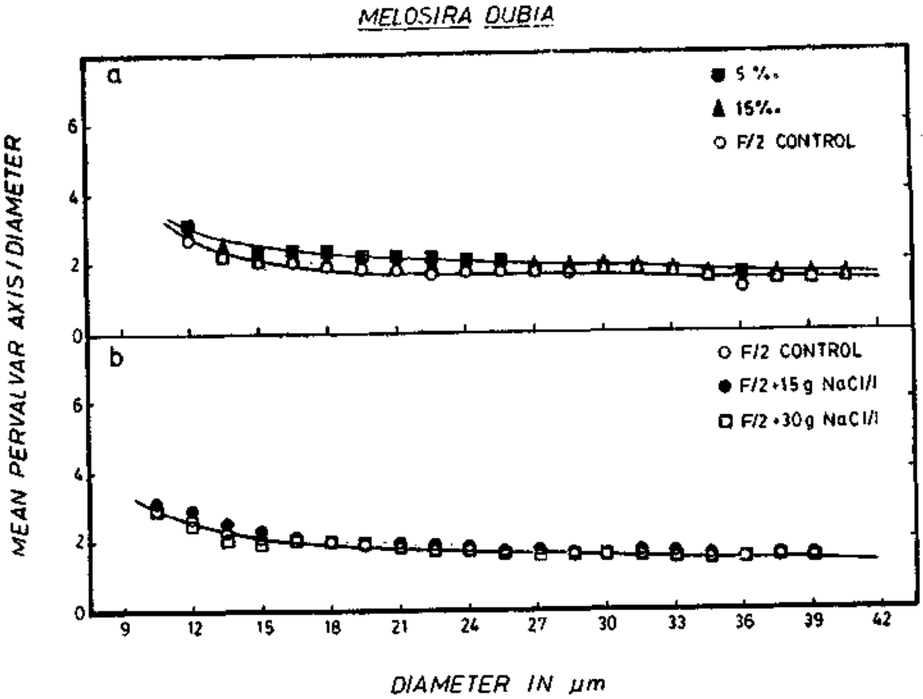

Fig. 19. Relationship of cell diameter to pervalvar axis in *Melosira dubia* : a. < 30‰ salinity and b. > 30‰ salinity.

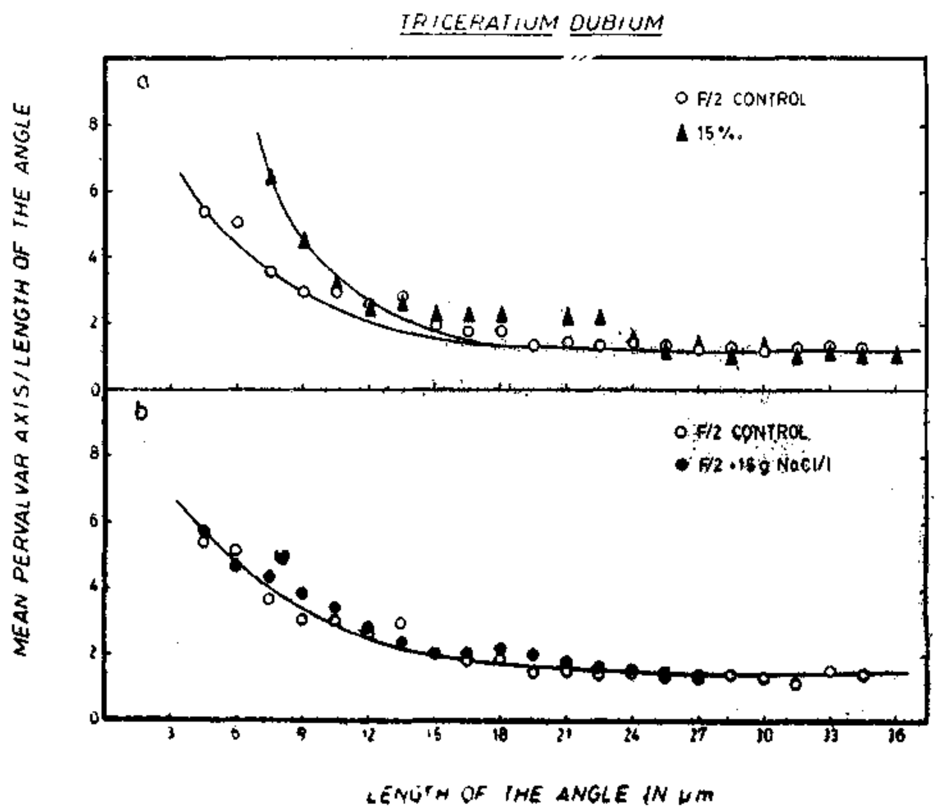

Fig. 20. Relationship of angle of cell to pervalvar axis in *Triceratium dubium* : a. < 30‰ salinity and b. > 30‰ salinity.

mation while in others only vegetative mutiplication. Rao (1980) reported that *Cyclotella meneghiniana* behaved as type 1 and 3 depending on salinity of the medium, other conditions remaining same. A similar behaviour was noticed in *T. fluviatilis* also.

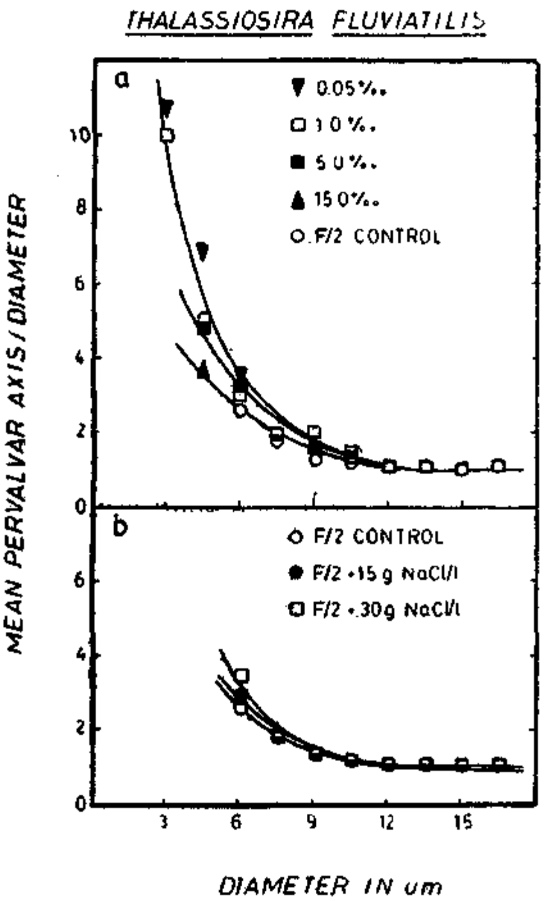

Fig. 21. Relationship of cell diameter to pervalvar axis in *Thalassiosira fluviatilis*: a. < 30‰ salinity and b. > 30 % salinity.

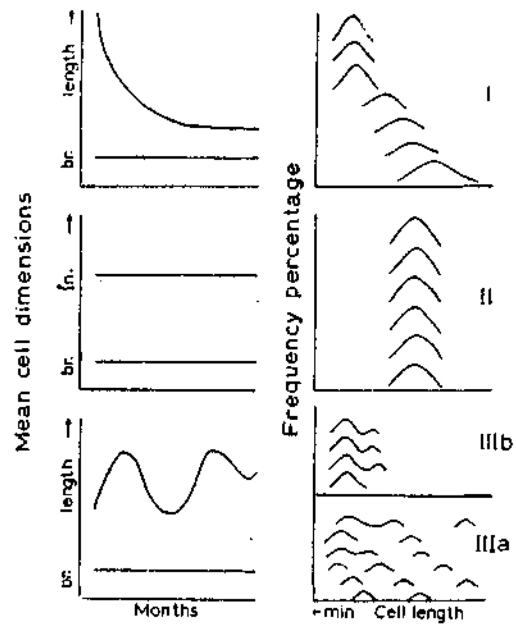

Fig. 22. Mean cell dimensions and length frequency curves of the 3 types of behaviour of diatoms in culture (after V.N.R. Rao, 1980).

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