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EFFECT OF pH IN DIATOM CULTURE AND SPECIES COMPETITION

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ABSTRACT

The axenic cultures of two marine species of diatoms *S. costatum* and *T. fluviatilis* were grown in the laboratory to study the pH tolerance in individual batch cultures and species competition in mixed cultures. The experiments were performed at varying pH levels in the range 7.0 to 10.0. Of the two species *T. fluviatilis* was hardly affected by varying pH up to its maximum tolerable level of 9.5, whereas the growth of *S. costatum* was adversely affected by increasing pH and ceased when the pH exceeded 9.0. However the growth rate was more or less same for both the species in the optimum pH. The optimum range of pH for *S. costatum* and *T. fluviatilis* is 7.5 to 8.2 and 8.0 to 8.5 respectively.

In the mixed culture, in all the pH ranges *T. fluviatilis* dominated over *S. costatum*. The growth rate of *S. costatum* was reduced very much and ceased when the pH exceeded 8.5 where as in *T. fluviatilis* the growth rate was maintained as in individual batch culture. This dominance results because of *T. fluviatilis* unique ability to tolerate high alkaline conditions and allelopathic interaction with the other species.

INTRODUCTION

THE LARGE scale cultivation of unicellular marine algae in controlled conditions is a prerequisite to meet the growing demand in aquaculture systems as supplimentary food. Attempts to maximize phytoplankton biomas yields *via* the supply of excess nutrients while still maintaining desired species in culture have met with varying degrees of success. The growth and metabolic activity of planktonic algae have been influenced by varying environmental conditions. Among the various environmental factors, pH is an important factor which can influence the growth of organisms either directly or by altering the nutritional status of the medium. The effect of pH on micro algal cultures has been studied by many authors. Kylin (1917) studied a wide variety of marine algae in sea water at pH adjusted to 3.6 to 10.0 and observed that the majority survived for at least a few days in pH 6.8 to 10.0. Bachrach and Lucciardi (1932) reported a narrow pH range for the diatoms *Navicula* sp. and *Nitzschia* sp. cultivated in enriched natural sea water. The best growth of *Isochrysis galbana* was obtained at pH 8.0 and inhibition occurred over 8.25 (Kain and Fogg, 1958 b). Humphrey (1975) reported that *Phaeodactylum tricornutum* was among a small group of marine algae that could tolerate pH value > 10.0. Among a group of marine unicellular algal species possessing similar growth characteristics in intensive culture, *P. tricornutum* is often the successful competitor, because it is able to withstand even high pH (Goldman, 1976). The allelopathic nature and high pH tolerance of *P. tricornutum* was also studied by Goldman and Ryther (1976). The studies of Goldman *et al.* (1982 a, b) showed that the marine diatom *P. tricornutum* tolerates alkaline pH values upto $\approx$ 10.3. The present study deals with the effect of pH on the growth of diatoms *Thalassiosira fluviatilis* and *Skeletonema costatum* in batch cultures in the laboratory.

Material and Methods

The experiment was conducted with the two species of diatoms *Thalassiosira fluviatilis* Hust. and *Skeletonema costatum* (Grev.) Cl. grown in constant conditions of salinity (28‰), temperature (28° to 30°C), illumination (4000 lux) and the culture medium composition. Both the sepecies came from clones isolated from Vellar Estuary (11°29'N ; 79°46'E) and maintained axenically in the laboratory. The f/2 medium (Guillard and Ryther, 1962) was conveniantly modified to suit the cultures and the medium was made up in aged and filtered sea water with the salinity adjusted to 28‰ and sterilized by autoclaving at 15 lbs pressure for 15 minutes. Continuous illumination of 4000 lux was provided for 14 hrs by 40 W 'Cool white' fluorescent lamps and the temperature ranged from 28° to 30°C. Both species grew well under these conditions. The cultures were shaken periodically to keep the cells in suspension. All experiments were done in triplicate. Cell counts were made with a Haemocytometer (Neubauer, Fein optic). The results presented are the means.

Medium composition

Potassium nitrate	: 225 mg
Potassium hydrogen ortho phosphate	: 26.00 ,,
Ferric chloride hexa hydrate	: 2.95 ,,
Manganous chloride	: .30 ,,
Sodium meta silicate	: 34.00 ,,
Sodium salt of EDTA	: 15.00 ,,
Vitamin B_{12}	: 10.00 μg
Soil extract	: 25.00 ml
Aged sea water (salinity 28‰)	: 1 lit

Culture system and pH used

The test organisms were cultured in various pH ranging from 7.0 to 10.0. The pH used were 7.0, 7.5, 8.0 8.2, 8.5, 8.7, 9.0, 9.5 and 10.0.

The batch cultures of *S. costatum*, *T. fluviatilis* and bialgal (mixed) cultures were grown separately at all pH levels. Inacula of same age and size were used to develop the cultures. For mixed cultures, half the size of the inacula of individual cultures of both species were used to avoid crowding of cells. During the exponential growth phase, the cell numbers were determined (upto stationary phase) and the specific growth rate (k') was calculated by using the formula given by Fogg (1966).

Relative growth constant (k') = (log N_2— log N_1)/t

Where N_1 and N_2 are the initial and final cell concentrations after an intervel of time 't'.

Generation time 'G' in days = 0.301/k'

Results

Effect of pH in monoalgal cultures

Skeletonema costatum

The effect of pH in monoalgal culture of *S. costatum* is presented in the Table 1. Maximum growth rate was noticed at pH 8.0. Beyond pH 8.5 the growth was very much arrested and no growth was observed at pH 9.5 and 10.0. In these pH level the inaculated cells remained inactive and later became non-viable. Relatively high growth rate was noticed in the pH range of 7.5 to 8.5. Maximum cell number was observed at pH 8.0 with the specific growth rate of 0.54 (1.8 divisions/day). The yield was reduced to a magnitude of 25 times at pH 8.7 when compared with the maximum yield at pH 8.0. After the growth period, the pH of the culture medium was found to be slightly increased (from 8.0 to 8.5).

Thalassiosira fluviatilis

The optimum growth of *T. fluviatilis* was observed at pH 8.2. In this pH the mean

TABLE 1. *Growth pattern of* S. costatum *in various pH levels.* Skeletonema costatum (*mono-culture*)

pH used	Growth pattern (cells/ml). No. of days after inoculation						
	0	1	2	3	4	5	6
7.0	715	1,400	3,500	6,250	15,950	29,500	53,500
7.5	715	2,270	7,250	24,000	73,500	2,32,500	5,72,800
8.0	715	2,115	8,600	31,524	98,750	6,18,500	12,83,150
8.2	715	2,075	7,500	24.750	80,750	2,63,250	6,22,725
8.5	715	1,750	4,250	12,650	32,450	88,550	1,18,660
8.7	715	1,470	3,055	8,250	15,875	32,500	51,800
9.0	715	1,015	2,650	4,500	7,810	12,800	17,150
9.5	715	No growth					
10.0	715	No growth					

TABLE 2. *Growth pattern of* T. fluviatilis *in various pH levels.* Thalassiosira fluviatilis (*mono-culture*)

pH used	Growth pattern (cells/ml). No. of days after inoculation						
	0	1	2	3	4	5	6
7.0	950	1,270	2,600	4,350	10,500	18,500	30,615
7.5	950	2,200	5,620	15,782	32,480	72,250	1,37,300
8.0	950	2,500	8,450	20,230	59,180	1,82,950	3,22,950
8.2	950	3,350	12,700	45,200	1,52,600	5,18,750	15,07,900
8.5	950	3,150	11.070	26,020	75,910	2,44,875	4,97,450
8 7	950	2,300	6,500	18,270	48,860	1,03,100	1,81,000
9.0	950	1,800	4,500	6,750	15,910	29,750	54,140
9.5	950	980	1,270	2,750	4,100	5,500	6,750
10.0	950	No growth.					

TABLE 3. *Specific growth rate and generation time of* S. costatum *and* T. fluviatilis *in various pH levels*

pH used	*S. costatum*			*T. fluviatilis*		
	Specific growth rate (K)	Generation time (G) hrs	Divisions/ day	Specific growth rate (K)	Generation time (G) hrs	Division/ day
7.0	0.31	23.3	1.03	0.25	28.9	0.83
7.5	0.48	15.1	1.59	0.36	20.1	1.19
8.0	0.54	13.4	1.80	0.42	17.2	1.40
8.2	0.49	14.7	1.63	0.53	13.6	1.76
8.5	0.37	19.5	1.23	0.45	16.1	1.49
8.7	0.31	23.3	1.03	0.38	19.0	1.26
9.0	0.23	31.4	0.76	0.29	24.9	0.96
9.5	No growth		—	0.14	51.6	0.47

TABLE 4. *Growth pattern of* S. costatum *and* T. fluviatilis *in various pH levels of bi-algal cultures*

pH used	Growth pattern (cells/ml) No. of days after inoculation							
	0	1	2	3	4	5	6	7
Skeletonema costatum								
7.0	360	695	1,450	4,510	11,500	24,150	41,900	58,650
7.5	360	940	2,070	4,967	11,750	26,685	58,483	96,770
8.0	360	1,015	2,580	6,280	16,180	39,880	1,00,500	1,73,900
8.2	360	485	700	1,560	2,455	4,301	7,362	9,870
8.5	360	410	550	1,090	1,387	2,180	3,890	5,150
8.7	360	No growth						
9.0	360	Do.						
9.5	360	Do.						
10.0	360	Do.						
Thalassiosira fluviatilis								
7.0	425	560	940	1,750	3,240	5,500	8,450	10,670
7.5	425	1,250	3,890	6,270	12,285	25,940	65,030	1,01,900
8.0	425	1,490	4,110	10,760	26,150	78,615	2,04,100	3,70,150
8.2	425	1,570	5,490	17,230	56,620	1,95,365	6,32,825	15,72,500
8.5	425	1,540	4,450	11,965	31,990	92,290	2,40,161	5,10,960
8.7	425	1,015	3,560	6,910	11,475	19,390	33,550	73,850
9.0	425	780	1,725	3,545	6,390	11,635	15,825	20,350
9.5	425	620	1,050	1,560	1,810	2,100	2,410	2,940
10.0	425	No growth	—	—	—	—	—	—

TABLE 5. *Specific growth rate and generation time of* S. costatum *and* T. fluviatilis *at various pH in bi-algal cultures*

pH used	*S. costatum*			*T. fluviatilis*		
	Specific growth rate (K)	Generation time (G) hrs	Divisions/ day	Specific growth rate (K)	Generation time (G) hrs	Division/ day
7.0	0.32	22.6	1.06	0.20	36.1	0.66
7.5	0.35	20.6	1.17	0.34	21.2	1.13
8.0	0.38	19.0	1.26	0.42	17.2	1.40
8.2	0.21	34.4	0.70	0.51	14.2	1.69
8.5	0.17	42.5	0.56	0.44	16.4	1.46
8.7	No growth		—	0.32	22.6	1.06
9.0	Do.		—	0.24	30.1	0.80
9.5	Do.		—	0.12	60.2	0.40
10.0	Do.		—	No growth	—	—

divisions per day was 1.76. The growth rate decreased below and above the optimum pH (Table 2), and was completely arrested at pH 10.0. The maximum cell numbers obtained at pH 8.2 was 15.08×10^6/ml. It is about 28 times higher than that at pH 9.0. Relatively high growth was observed in the pH range 7.5 to 8.7 and the cells were healthy and viable. But at extreme pH levels, the cells were not so healthy as in optimal pH. During the growth period the optimum pH (8.2) of the medium raised and went upto 9.0 at the end of the growth period, since the medium was not buffered.

Species competition in various pH levels

The growth pattern of *S. costatum* was adversely affected in the mixed cultures (Table 4). The specific growth rate of 1.26 divisions/day was recorded at pH 8.0. The growth was absolutely nil in pH 8.7 and above. At pH 8.5, the cells remained viable for one week only and little growth was noticed. But the growth of *T. fluviatilis* was not at all affected in the mixed cultures when compared with mono-algal culture. The optimum growth rate was observed at pH 8.2 with 1.69 divisions/day. The cells of *T. fluviatilis* was healthy and viable as in monoalgal culture, even after two weeks, whereas the cells of *S. costatum* was unhealthy and moribund within 10 days of growth period. The growth of *T. fluviatilis* was normal in all pH levels as in monoalgal cultures, and the other was suppressed at all pH levels. The exponential growth of *T. fluviatilis* continued upto a cell density of 15.72×10^5 cells/ml and attained the stationary phase.

Discussion

Although data on pH responses by marine algae are limited, numerous marine species appear to be unable to tolerate pH values much above 9.5 (Humphrey, 1975 ; Goldman, 1976) and typically grow optimally in narrow pH range bracketing the pH of sea water which is ≈ 8.1 to 8.3 (Kain and Fogg, 1958a, b, 1960 ; Hayward, 1968 ; Humphrey, 1975). These findings closely agrees with the pH optima of *T. fluviatilis* and *S. costatum*. Hayward (1968), Humphrey (1975) and

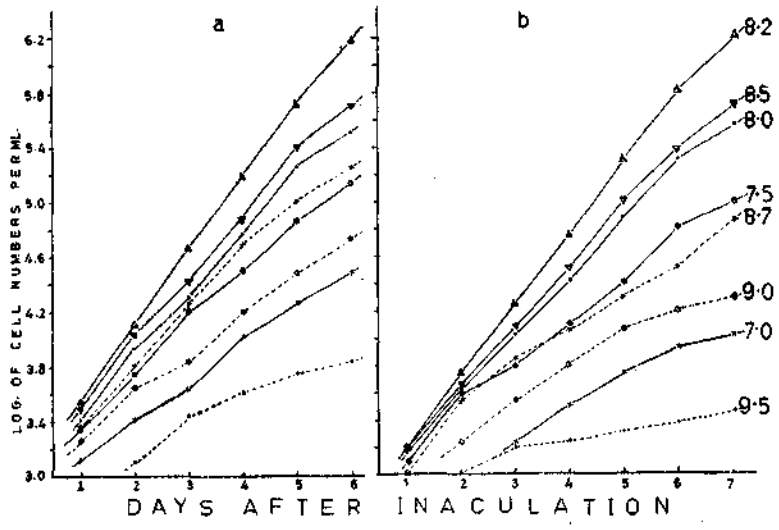

Fig. 1. Growth pattern of *T. fluviatilis* as a function of pH : a. In mono-algal culture and b. In bi-algal (mixed) culture.

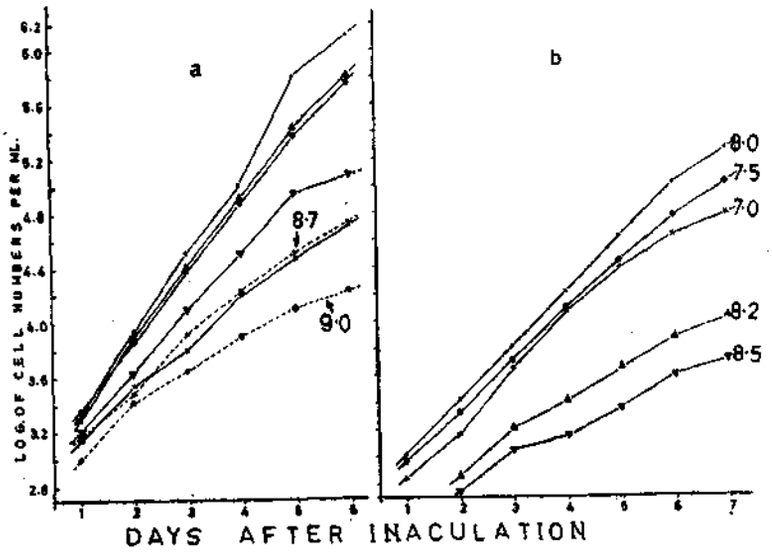

Fig. 2. Growth pattern of *S. costatum* as a function of pH : a. In mono-algal culture and b. In bi-algal culture.

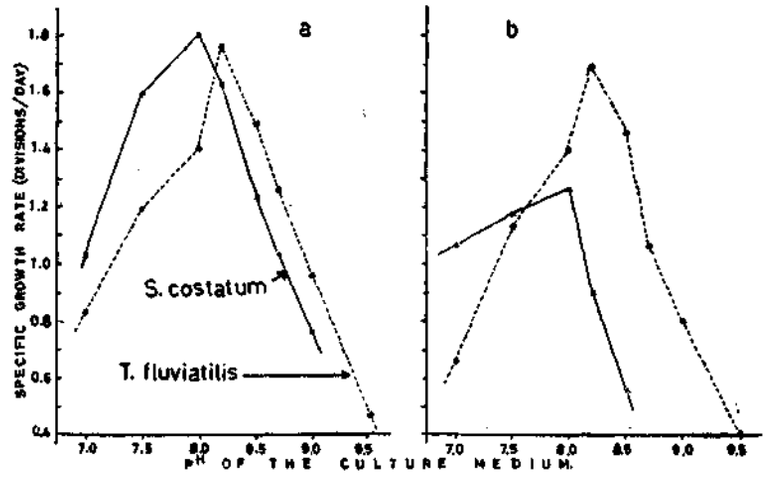

Fig. 3. Species competition in mixed cultures as a function of pH : a. Unialgal culture and b. bi-algal culture.

Goldman (1976) observed that the marine diatom *Phaeodactylum tricornutum* was capable of growing at pH levels upto 10.0 and above, eventhough its pH optimum was closer to 8.0. The present study reveals that the diatom *T. fluviatilis* tolerates higher range of pH than *S. costatum*. Above pH 9.0, the growth of *S. costatum* was completely arrested in mono-algal culture (Fig. 2). In mixed cultures *S. costatum* was not able to tolerate pH values above 8.5, whereas *T. fluviatilis* has overcome the growth of *S. costatum* in all the pH levels

upto 9.5. The failure of *S. costatum* to grow in high pH (above 9.0) may be due to the non-availability of some essential nutrients in high pH. Goldman *et al.* (1982) pointed out that the ability of *Phaeodactylum tricornutum* to tolerate pH values > 9.3 simply reflect a reduced requirement for an essential nutrient that become less available with increasing pH. This may be the reason for the high tolerance capacity of *T. fluviatilis* in high range of pH. Among a group of marine unicellular algal species possessing similar growth characteristics in intesive culture, *P. tricornutum* was often the successful competitor, because of its ability to withstand even high pH (Goldman, 1976).

On the basis of the above results (Fig. 3) it is intuitively obvious that *T. fluviatilis* is the successful competitor in the mixed culture with *S. costatum*. Eventhough their pH optima are closer to 8.0 in monocultures, *S. costatum* was highly suppressed in mixed culture. It suggests that in mixed cultures, due to competition for nutrients, *S. costatum* was not able to grow in the presence of *T. fluviatilis*. That's why the growth of *S. costatum* was highly decreased even in pH 8.0. Maestrini and Kossut (1981) reported that *S. costatum* did not grow at all in dialysis sacks grown in situ due to the depletion of nutrients. But *Thalassiosira pseudonana* has grown well in the same dialysis sacks even in nutrients limited conditions. Hence, it is evident that the nutrients uptake by the diatom *S. costatum* is very low when compared with *T. fluviatilis*. Further the interaction among marine phytoplankton species through the excretion of growth inhibiting substances (allelopathy) has been suggested to play an important role in aquatic environments (Pratt and Fong, 1940; Margalef, 1958; Hellebust, 1974). McLachlan and Craigie (1964) reported that the phenolic substances produced by *Fucus* was toxic to seven species of unicellular algae including *S. costatum*. Such chemical alterations in the growth media may also have been a major determinant of the growth of *S. costatum* in high pH. Even the autotoxins secreted by *S. costatum* itself check their own growth in high pH (Levring, 1945). The allelopathic nature by secreting toxic compounds (Sharp *et al.*, 1979) and high pH tolerance of *P. tricornutum* was the cause of its success in intensive cultures (Goldman and Ryther, 1976).

In waste water treatment or energy production applications, pH control probably is not required. But for those mass culture applications that require the maintenance of specific species, such as aquaculture or the production of chemical derivatives pH control is necessary. The present study shows that *T. fluviatilis* is the successful competitor in mixed culture with *S. costatum*, because it can tolerate high pH values and grows well even in nutrients limited medium due to increase of pH.

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