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THE EFFECT OF pH IN INTENSIVE MICROALGAL CULTURES.
II. SPECIES COMPETITION[1]

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Abstract: The results of a series of competition experiments between the chlorophyte *Dunaliella tertiolecta* (Dun) Butcher and the diatom *Phaeodactylum tricornutum* (TFX-1) Bohlin demonstrate conclusively that *Phaeodactylum tricornutum* dominates in intensive marine cultures when the pH rises above ≈ 10. This dominance results because of the diatom's unique ability among marine species to tolerate alkaline conditions. When the pH is regulated both freshwater and marine algae, once firmly established in culture at their respective pH optima, can resist invasion from competing species. Hence, pH control may be a method for maintaining species other than *P. tricornutum* in mass culture. When *Dunaliella tertiolecta*, however, is grown under even slight pH stress it becomes susceptible to invasion by *Phaeodactylum tricornutum*. This susceptibility to takeover by *P. tricornutum* increases with increasing pH. In contrast, the freshwater chlorophyte *Chlorella vulgaris* Beij., which also is sensitive to increasing pH, is capable of remaining dominant at any pH within its tolerance range when invaded by the pH-insensitive chlorophyte, *Scenedesmus obliquus* (Turp.) Kutz. Although allelopathic interactions may be responsible for the success of *Chlorella vulgaris* under seemingly stressful conditions, the success of *Phaeodactylum tricornutum* at increasingly higher pH seems to be related primarily to the alga's pH tolerance characteristics and not to any chemical interactions with competing species.

INTRODUCTION

In Part I of this study (Goldman *et al.*, 1982), we demonstrated that the marine diatom *Phaeodactylum tricornutum* could tolerate alkaline pH values up to ≈ 10.3, whereas the chlorophyte *Dunaliella tertiolecta* was unable to grow when the pH exceeded ≈ 9.3. Although data on pH effects on marine algae are limited, there is some evidence indicating that many marine species have a similar sensitivity to extreme alkaline conditions as does *D. tertiolecta*. Thus, we hypothesized that in intensive cultures that are poorly buffered *Phaeodactylum tricornutum* enjoys a competitive advantage that is related primarily to pH. This hypothesis is consistent with the repeated observation that in large-scale outdoor cultures in which pH is not regulated and is allowed to rise above 10, *P. tricornutum* often dominates (Goldman & Ryther, 1976; D'Elia *et al.*, 1977; Goldman & Mann, 1980).

Other hypotheses to explain the success of *P. tricornutum* in large-scale cultures have been offered including allelopathy (Sharp *et al.*, 1979), light limitation (Nelson *et al.*, 1979), temperature (Goldman & Ryther, 1976), and silicon limitation (D'Elia *et al.*, 1979; Goldman & Mann, 1980). Both temperature and silicon limitation

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play important rôles in establishing some of the conditions favoring *P. tricornutum*. For example, when silicon is in excess, *P. tricornutum*, which does not have a silicon requirement, competes most favorably in the temperature range 10–20 °C (Goldman & Ryther, 1976); but when silicon is limiting the temperature range favoring dominance by this species is extended down to <5 °C as other cold-water and silicon-requiring diatoms are eliminated from competition (Goldman & Mann, 1980). These observations, together with the pH data from Part I of our study (Goldman *et al.*, 1982) lead us to suggest that *P. tricornutum* always will be favored when the pH is $\geqslant 10$ and the temperature is in the range 10–20 °C.

Because *P. tricornutum* is an undesired species for many mass culture applications (Goldman & Stanley, 1974), a major goal in mass culturing marine microalgae has been to find ways to prevent its incursion into and takeover of large-scale cultures. In the present study we explore further the rôle of pH regulation in this process by carrying out a series of competition experiments between *P. tricornutum* and *Dunaliella tertiolecta* and between two freshwater microalgae at varying pH levels.

MATERIALS AND METHODS

CONTINUOUS CULTURES

The test algae, nutrient media, culture system, and modes of pH control were described in detail in Part I (Goldman *et al.*, 1982). In summary, two marine species, *Phaeodactylum tricornutum* (TFX-1) Bohlin and *Dunaliella tertiolecta* (Dun) Butcher and two freshwater species, *Scenedesmus obliquus* (Turp.) Kutz. and *Chlorella vulgaris* Beij. were grown in nutrient-enriched continuous cultures at a nominal dilution rate (D) of $0.5 \cdot \text{day}^{-1}$. The pH in six cultures was regulated by a pH-stat system that controlled the flow of 1% CO_2 gas into the cultures to keep the pH from rising above the designated value. In the seventh culture continuous bubbling of laboratory air was provided so that the pH rose to a level controlled primarily by the photosynthetic demand for inorganic carbon. Two mM HCO_3^- was supplied in the liquid medium to allow for the initial rise in pH to the designated value before bubbled CO_2 became the major source of inorganic carbon. In this way inorganic carbon never was limiting in the pH-controlled cultures.

SPECIES COMPETITION

We cultured each species to steady state at varying pH in an overall range between 7.6 and 10.6. As described in Part I (Goldman *et al.*, 1982), both freshwater species grew in the pH range 7.9 to 10.6, whereas the pH ranges were 7.6 to 10.3 for *Phaeodactylum tricornutum* and 7.6 to 9.3 for *Dunaliella tertiolecta*. Once steady state was attained, we added the respective contaminant species ($<1\%$ of total culture biomass) to each freshwater or marine culture and observed the outcome of competition over a 2- to 3-wk period. Cell counts of the individual species in each culture were taken daily using a Spencer Bright-line hemacytometer. We calculated specific growth rates (μ) of individual species according to the following equation: $\mu = D + t^{-1} \ln (X_t \cdot X_0^{-1})$, where t is the time interval in which a change in cell number from X_0 to X_t is observed.

RESULTS

When *Chlorella vulgaris* was established as the dominant species, it successfully resisted competition from *Scenedesmus obliquus* over the entire range of pH tested (7.9 to 10.6). Similar results were obtained when *S. obliquus* was dominant initially and *Chlorella vulgaris* was the contaminant. In all cases the contaminant species rapidly disappeared from the culture after being introduced. *Phaeodactylum tricornutum*, when dominant initially, similarly was able to resist competition from *Dunaliella tertiolecta* at all pH values in the range 7.6 to 10.3, and the latter species quickly disappeared from the culture. When, however, *D. tertiolecta* was dominant initially, the outcome of competition with *Phaeodactylum tricornutum* was a complex function of pH. Only at the lowest pH (7.60) was it impossible for *P. tricornutum* to grow, leaving the steady state population of *Dunaliella tertiolecta* unperturbed (Fig. 1A). Then, when we increased the controller setting to pH 10, the culture pH rose only to 9.60 so that there was no further addition of CO_2-enriched air; growth of the chlorophyte stopped ($\mu = 0.05 \cdot \text{day}^{-1}$) and cell washout ensued (Fig. 1A).

At pH values of 8.05 (Fig. 1B), 8.70 (Fig. 2A), and 9.10 (Fig. 3A) it still was possible to maintain steady state populations of *D. tertiolecta* although there was about a two-fold decrease in steady-state cell numbers with increasing pH. Yet, in all three cultures, *Phaeodactylum tricornutum* increased exponentially after being introduced – either after a 9-day lag at pH 8.05, or immediately at pH 8.70 and 9.10. Moreover, initial growth rates of the contaminant increased with increasing pH from $0.77 \cdot \text{day}^{-1}$ at pH 8.05 to $1.18 \cdot \text{day}^{-1}$ at pH 9.10 (Table I). Correspondingly,

TABLE I

Initial growth rates of *Phaeodactylum tricornutum* when introduced as contaminant in steady-state cultures of *Dunaliella tertiolecta*.

Culture pH	CO_2 (%)	Dilution rate (day^{-1})	Initial growth rate (day^{-1})
7.60	1	0.44	0
8.05	1	0.59	0.77[a]
8.70	1	0.59	0.90
9.10	1	0.60	1.18
9.50	0.036	0.55	1.37
10.05	None	0.48	0.93

[a] After 9-day lag.

when we increased the pH from 8.70 to 9.18 (Fig. 2A) and from 9.10 to 9.40 (Fig. 3A), the growth rate of *Dunaliella tertiolecta* declined below the dilution rate and cell washout ensued. In both cases *Phaeodactylum tricornutum* was equal in cell number to *Dunaliella tertiolecta* when the experiments were terminated. Unfortunately, most of the experiments had to be terminated before the outcome of competition was complete due to difficulties in counting individual species resulting from cell clumping.

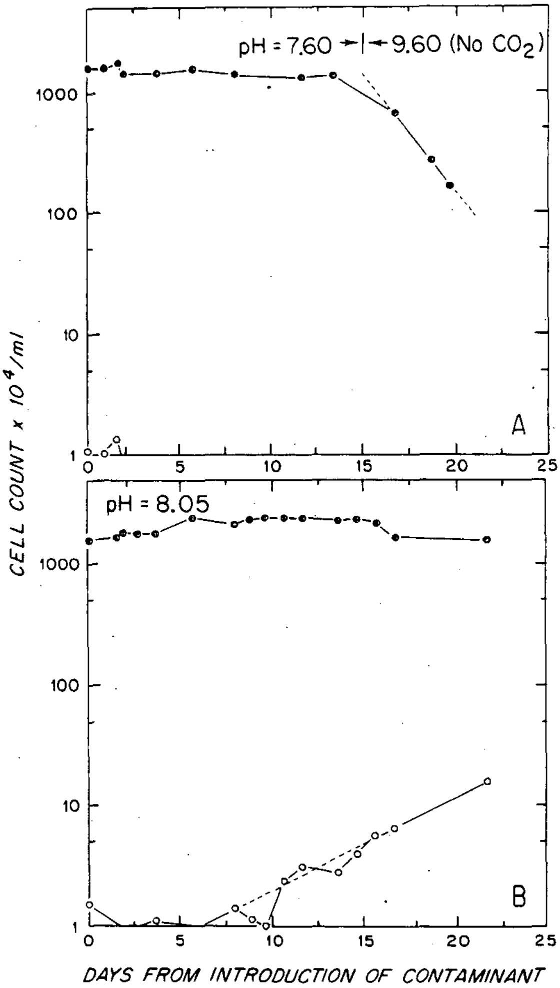

Fig. 1. Competition between *Dunaliella tertiolecta* and *Phaeodactylum tricornutum* in continuous culture maintained at a nominal dilution rate of 0.5 · day^{-1} and at varying pH: except where noted, pH control was with 1% CO_2, regulated with a pH-stat system; *Dunaliella tertiolecta* (●) initially was at steady state and *Phaeodactylum tricornutum* (○) was introduced as the contaminant; symbols and legend are the same for all figures; dashed lines were drawn by eye to estimate growth rates of *P. tricornutum* and washout rates of *Dunaliella tertiolecta*; A, pH 7.60 and 9.60; B, pH 8.05.

The most rapid initial growth rate of *Phaeodactylum tricornutum* (1.37 · day^{-1}) was measured in the air-grown culture of *Dunaliella tertiolecta* which initially was

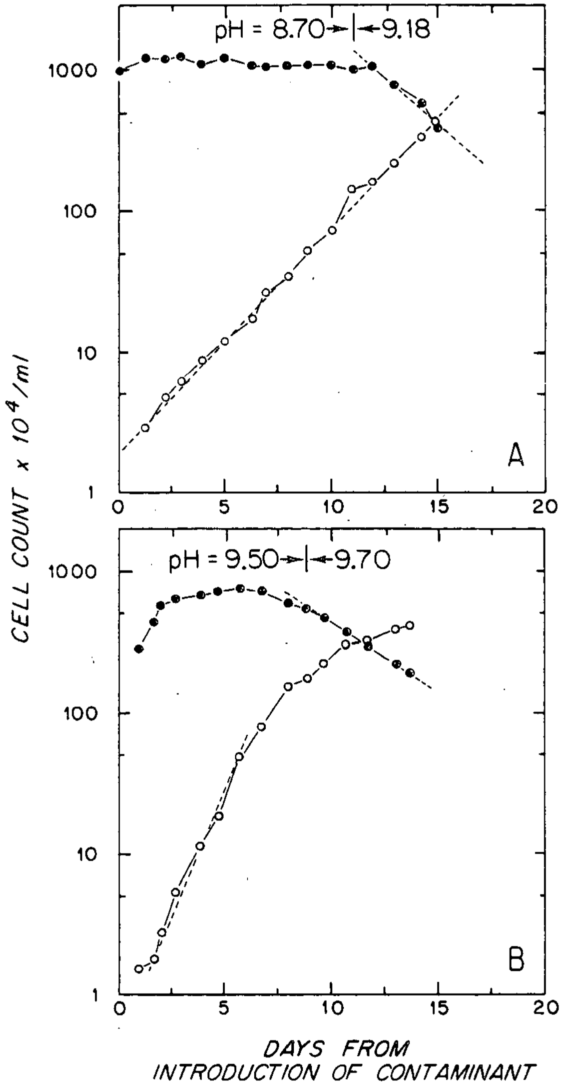

Fig. 2. Competition between *Dunaliella tertiolecta* and *Phaeodactylum tricornutum* in continuous culture maintained at a nominal dilution rate of 0.5 · day^{-1} and at varying pH: A, pH 8.70 and 9.18; B, pH 9.50 and 9.70 (0.036% CO_2 in air supplied continuously).

at pH 9.3 (Fig. 2B). As the former species increased in number, the pH rose leading to a decline in the population of the latter until it was surpassed by the diatom at the end of the experiment. Finally, when the controller was set at pH 10

(Fig. 3B) the pH initially increased only to ≈ 9.4, thereby preventing any CO_2 from entering the culture. As *Phaeodactylum tricornutum* rapidly grew at 0.93 · day^{-1},

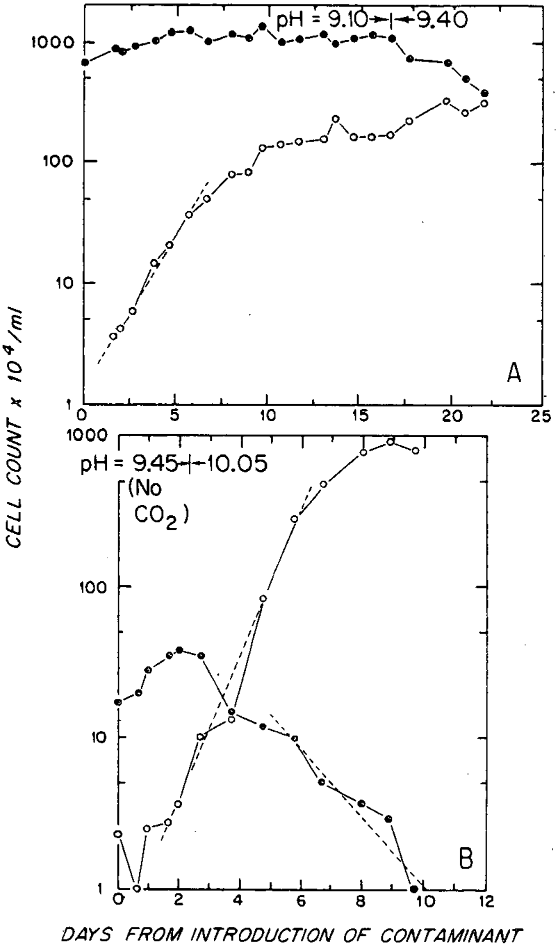

Fig. 3. Competition between *Dunaliella tertiolecta* and *Phaeodactylum tricornutum* in continuous culture maintained at a nominal dilution rate of 0.5 · day^{-1} and at varying pH: A, pH 9.10 and 9.40; B, pH 9.45 and 10.05.

however, the pH finally rose to 10 and CO_2 was supplied to the culture. Under these conditions the net growth rate of *Dunaliella tertiolecta* was reduced to zero and within ten days the chlorophyte was eliminated from the culture.

DISCUSSION

COMPETITION AT OPTIMAL pH

Based on continuous culture theory (Spicer, 1955), for any dilution rate (D) below the maximum specific growth rate ($\hat{\mu}$), a single species culture will attain steady state. Accordingly, the impact of pH on continuous culture growth of algae (including species competition) can be described in a manner similar to the way in which the effects of other abiotic factors such as light intensity and temperature have been described. Such regulatory factors can be best quantified in terms of their modifying effects on $\hat{\mu}$ (Goldman & Carpenter, 1974). For example, each species has an optimal pH (or pH range) for growth; above and below this optimal pH growth rates are adversely affected. Thus, when D is fixed, as in the current experiments, adverse effects of pH on $\hat{\mu}$ can lead to situations where $\hat{\mu}$ approaches D, with concomitant reductions in steady-state biomass levels; or, in more severe cases in which $\hat{\mu}$ is diminished below D, cell washout can result.

In the present studies the experimental D of $0.5 \cdot \text{day}^{-1}$ was considerably below each species' $\hat{\mu}$, at least when the algae were grown at their respective optimal pH (Goldman & Peavey, 1979; Goldman & Graham, 1981). Under these conditions the hardy weed species, which not only tolerate a broad range of pH extending into the alkaline region, but also are hardly affected by pH within that range (i.e. *Scenedesmus obliquus* and *Phaeodactylum tricornutum*, cf. Goldman *et al.*, 1982), always remained dominant when exposed to a small contaminant population (initially $< 1\%$ of the total biomass). Thus, pH had little effect on $\hat{\mu}$ of the dominant species so that the relationship between $\hat{\mu}$ and D was never altered (i.e. $\hat{\mu} \gg D$). In an ecological sense, a key determinant of this competition is the actual ratio of dominant to contaminant species at the onset of competition. When the contaminant population is exceedingly small compared with that of the well-established species the opportunities for species displacement are reduced considerably because the niche is already occupied (Whittaker *et al.*, 1973). The same argument applies for explaining the success of both *Dunaliella tertiolecta* and *Chlorella vulgaris* in avoiding displacement by their respective contaminants when each of these species was firmly established in culture under conditions of optimal pH. For each species $\hat{\mu}$ was much greater than D and the niche was completely occupied before the contaminant was introduced.

We thus conclude that, in principal, pH control may be a simple way to maintain for sustained periods desired marine species other than *Phaeodactylum tricornutum* in large-scale outdoor cultures. The success of this technique, however, hinges on satisfying the requirements that the desired species be established in sufficient numbers before contamination occurs, that the optimal pH for the desired species be well identified, and that rigid pH control be sustained throughout the culture. Moreover, the opportunities for species displacement to result from even small changes in environmental factors other than pH (e.g., percentage CO_2 in bubbled gas, light intensity, dilution rate, nutrient flux and composition, and temperature) are well documented (Golueke, 1960; Goldman & Ryther, 1976; Goldman & Mann, 1980; Azov *et al.*, 1980). Hence, although pH control is mandatory for preventing the displacement of a desired marine mass culture by *P. tricornutum*, the regulatory effects of pH control may be overshadowed by changes in other environmental conditions. Such changes could easily alter the balance in favor of *P. tricornutum* by lowering $\hat{\mu}$ of the desired species. Then the weed diatom could compete for the niche even though the desired species initially was well established and growing at near optimal pH.

COMPETITION AT EXTREME pH

In sharp contrast to the above types of subtle pH-related interactions that occur under near optimal pH conditions, is the obvious result of competition at extreme pH between species with distinctly different pH maxima. The results of the competition experiments between the marine algae at pH values >9.3 demonstrate this point vividly. In this case, the inability of *Dunaliella tertiolecta* to maintain a steady state at a dilution rate of $0.5 \cdot \text{day}^{-1}$ ($\hat{\mu} < D$) when the pH exceeded 9.3 (Fig. 1A and Goldman *et al.*, 1982) is consistent with the outcome of the competition experiments between this alga and *Phaeodactylum tricornutum* (Fig. 3B). These findings confirm our earlier hypothesis (Goldman *et al.*, 1982) that, because most marine algae respond to high pH as does *Dunaliella tertiolecta*, *Phaeodactylum tricornutum* can grow without competition in unbuffered cultures when the pH rises to 10 and above. Hence, the success of *P. tricornutum* in previous large-scale outdoor experiments (cf. Goldman & Ryther, 1976; D'Elia *et al.*, 1977; Goldman & Mann, 1980) more than likely was caused by alkaline conditions that resulted from the lack of pH control.

COMPETITION UNDER pH STRESS

Intuitively it would appear that only under optimal pH could pH-sensitive species, such as *Dunaliella tertiolecta* compete favorably with species like *Phaeodactylum tricornutum*. Yet, the results of the competition experiments performed under non-optimal pH conditions (Figs. 1–3) are not so easily interpreted.

On the one hand, the increasing success of *P. tricornutum* in invading stable cultures of *Dunaliella tertiolecta* when the pH was raised from 8.05 to 9.10 appears coupled to the pH-dependent decrease in steady state biomass of *D. tertiolecta* (Fig. 1 in Goldman *et al.*, 1982). Under such conditions it is most probable that $\hat{\mu}$ of the chlorophyte decreased dramatically with increasing pH (reflecting the pH stress), thereby easing the pressure on *Phaeodactylum tricornutum* to compete for the available resources. This argument is consistent with the observation that during the initial periods when *P. tricornutum* successfully encroached the cultures, steady state of *Dunaliella tertiolecta* was sustained for periods up to three weeks (Figs. 1B, 2A, and 3A).

In contrast, *Chlorella vulgaris*, although displaying the same sensitivity to high pH as the marine chlorophyte, was able to resist competition from the pH-insensitive *Scenedesmus obliquus* at any pH between 7.9 and 10.3 as long as it was dominant initially. Such a response is suggestive of an allelopathic interaction between the two species. In fact, *Chlorella vulgaris*, along with many other algal species, can excrete compounds toxic to other algae under certain conditions, one of which is high pH (Pratt & Fong, 1940; Proctor, 1957; Kroes, 1971). Proctor (1957) identified these products as long-chain fatty acids which become more soluble (and possibly more toxic) with increasing pH. Thus, the ability of *C. vulgaris* to persist at all pH values when dominant initially may reflect, on the one hand, an ability to occupy a niche through excellent growth at optimal pH and, on the other hand, an ability to survive under pH stress by eliminating competitors via allelopathic interactions. Once again, the relative ratio of initially dominant to contaminant populations probably is a major determinant of the outcome of this form of competition.

Sharp *et al.* (1979) suggested that the success of *Phaeodactylum tricornutum* in competition with the diatom *Thalassiosira pseudonana* (3H) resulted from allelopathy. D'Elia *et al.* (1979), however, could find no evidence for allelopathy in competition experiments involving these diatoms. The latter authors, upon finding that *Phaeodactylum tricornutum* grows faster than *Thalassiosira pseudonana* (3H) at very low light intensity, postulated that light reduction via self-shading among cells in mass culture could provide the former species with a competitive advantage (Nelson *et al.*, 1979).

Our results do not support either of the above explanations for the success of *Phaeodactylum tricornutum*. First, if low average light intensity be a prerequisite for dominance by *P. tricornutum*, then invasion by the diatom should have been most pronounced at low pH where the biomass of *Dunaliella tertiolecta* (and concomitantly, shelf-shading) was maximum, and least effective at high pH (≈ 9.10) where opposite conditions prevailed. Although the increase in the initial growth rates of *Phaeodactylum tricornutum* (Table I) may have been due to more available light as the steady state biomass of *Dunaliella tertiolecta* decreased with increasing pH, the results shown in Figs. 1–3 are not consistent with an enhancement effect favoring *Phaeodactylum tricornutum* at low culture light intensities. Secondly, it is hard to reconcile the ability of *P. tricornutum* to invade and establish a firm foothold in the *Dunaliella tertiolecta* cultures to an allelopathic interaction when there was no measurable deviation from steady-state growth of the chlorophyte during the early stages of succesful invasion by the diatom.

It may be possible to maintain other more desired species in intensive culture with pH control; but the attainment of such conditions may be difficult because *Phaeodactylum tricornutum* grows well over a wide pH range and seems to be capable of exploiting rapidly any competitive situation in which even slight stress is put on the already established and desired species. Such questions will, however, only be answered definitively when long-term outdoor experiments involving pH manipulation are performed.

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