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GROWTH CHARACTERISTICS OF SOME NANNOPLANKTERS

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ABSTRACT

In this account the growth characteristics or growth kinetics of ten species of nannoplankters, mostly phytoflagellates were presented. The growth constant and mean generation time under controlled conditions of temperature and light showed variations in different species. It is believed that the growth constant and generation time will depend upon the environmental conditions of the algal cells and the Class and genus of the algae. The growth kinetics of other micro-algae studied elsewhere also is briefly discussed.

INTRODUCTION

ALTHOUGH the basic knowledge on the role of planktonic micro-algae in the economy of the sea is well recognised, information on the growth characteristics of the nannoplankters, especially in a culture system is still meagre. The lack of knowledge on these organisms is due largely to the difficulty of making comparative observations and raising them on mass scale in a culture system. Realising the importance of these organisms as the essential food of almost all the larval forms, the isolation, maintenance and mass culture of these micro-algae is a pre-requisite in the hatchery systems throughout the world. They are likely to be of great importance as the chief food of the molluscan larvae, particularly in the initial stages. Oyster larvae can ingest nothing larger than 10 microns and appear to rely for food on minute phytoflagellates excluding the diatoms.

Apart from the dinoflagellates and Coccolithophores, the most commonly occuring marine flagellates are those forming part of the nannoplankton. In this category, termed as the phytoflagellates, with characteristic pigments belong to the algal Classes, Haptophyceae, Chrysophyceae (including Silicoflagellates) and Prasinophyceae (Chlorophyceae) are probably the most common, although a limited number of flagellates of the Classes Cryptophyceae, Euglenophyceae and Chlamydomonadineae are usually present.

Previous work on the growth of marine micro-algae has been summarised by Harvey (1955) and growth rate alone has been studied by Braarud (1937) under controlled conditions of light and temperature. In a subsequent paper Braarud (1945) has recorded the relative growth constant of many species grown in culture system under controlled conditions. Kain and Fogg (1958 a, b; 1960) have studied the growth constants of three species of marine phytoplankton under controlled conditions of light, temperature, salinity and nutrients. Recently Joseph and Nair (1975) have studied the growth kinetics of three species of estuarine phytoplankters in a culture system. Nair (1974) has reviewed the growth kinetics of several species of phytoplankters from the natural marine environment and Ammini Joseph and Nair (1984) have studied the growth kinetics of few selected species of nannoplankters in a culture system.

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Material and Methods

Ten species of micro-algae which are nannoplankters were grown in the algal culture room for the purpose of feeding the larvae of oysters. These nannoplankters were isolated by the serial dilution culture method. In this method, four dilution steps (the inocula corresponding to 0.001, 0.01, 0.1 and 1 ml of sample already filtered through 10 μ seive) have been employed and in each step ten culture tubes (25 ml) were used which were filled with sterilized seawater (salinity 32-34‰ and pH 7.8-8.0) and Miquel's media added. These tubes were incubated in the algal culture room where the temperature is 25° C and have controlled conditions of light (1 to 2 k lux) for 12 hours. After 2-4 weeks, the cultures were examined under the microscope and the presence of each species in the tubes were noted. Subsampling the inocula from these tubes, a pure culture of the species could be raised gradually by increasing the volume from 25 ml to 250 ml, 500 ml and 1 litre conical flasks. Finally the stock cultures of these newly isolated ones were maintained in Hauffkin's culture flasks (3 or 4 litre) providing sufficient light (1-2 k lux) without aeration. These algae inoculated in a limited volume of the medium, proliferate in a characteristic pattern consisting of a lag, exponential, declining and death phases.

The species studied were *Isochrysis galbana Pavlova lutheri, Dicrateria inornata, Chromulina freiburgensis* (all flagellates belonging to Haptophyceae), *Tetraselmis chuii, T. gracilis, T. tetrahele* (flagellates of Chlorophyceae), *Chlorella salina, Nannochloris* sp. (Chlorophyceae) and *Synechocystis salina* (Cyanophyceae). The stock cultures were maintained in the algal culture room using Conway or Walne's medium. After determining the cell concentration of the stock of all the ten species, 5-10 ml of the same were inoculated to 1 litre conical flasks having the enriched medium. After inoculation, the flasks were placed in front of 2 tube lights (1 k lux). Next day onwards, the growth rate were estimated by noting the cell concentration by using a haemocytometer. The increase in growth rate were observed for 15 days.

Results

The growth characteristics of ten species of nannaplankters represented in Figs. 1-3 indicate different growth pattern in each species. In Fig. 1, the growth pattern of 4 species of Haptophyceae, *Isochrysis galbana, Pavlova lutheri, Dicrateria inornata* and *Chromulina freiburgensis* are presented. From an intial inoculum of 3. 2 million cells/ml *Isochrysis galbana* reached its optimum growth rate during 9th to 12th days, the maximum being on 11th day and gradually entered the declining phase on 15th day. The initial inoculum of

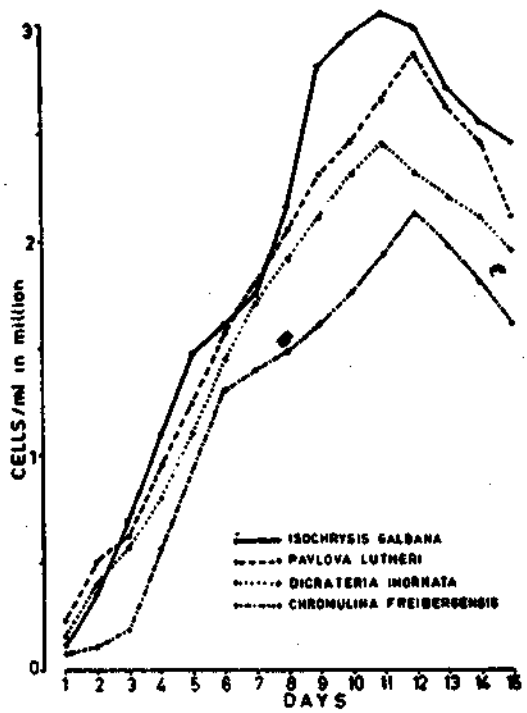

Fig. 1. Growth characteristics of four members of Haptophyceae.

Pavlova was 1.8 million cells/ml and it reached its maximum growth on 12th day and showed sign of decline on 14th day. Similarly, *Dicrateria*, from an initial inoculum of 2.2 million cells/ml, reached its maximum growth on the 11th day as in *Isochrysis* and slowly entered the declining phase in subsequent days. While in *Chromulina*, from an initial inoculum of 2.5 million cells/ml, the maximum growth reached on 12th day as in the case of *Pavlova* and its growth retarded in subsequent days.

In *Tetraselmis chuii*, the inoculum had a concentration of 1.2 million cells/ml at the beginning and the maximum concentration was attained on 11th day and the density started to decline on subsequent days (Fig. 2).

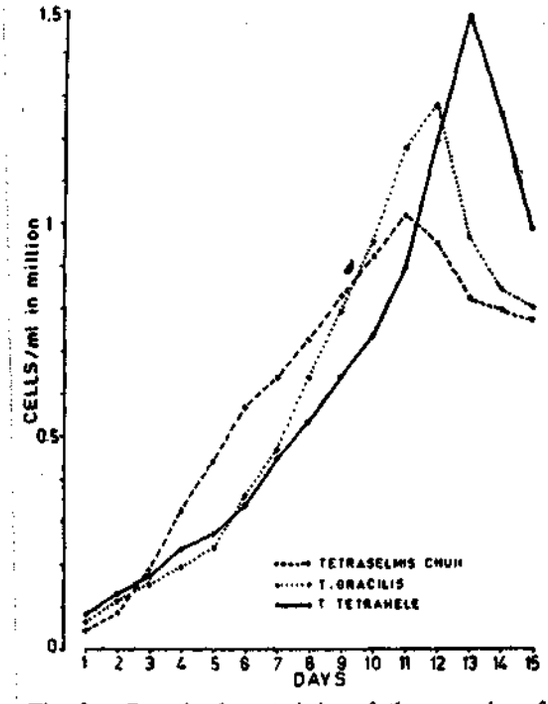

Fig. 2. Growth characteristics of three species of *Tetraselmis*.

Similarly in *T. gracilis* from an initial concentration of 1.4 million cells/ml the maximum was observed on 12th day and immediately entered the declining phase. In the case of *T. tetrahele*, the maximum concentration noted was on 13th day attaining 1.5 million cells/ml and sharp decline was evident on subsequent days.

From an initial inoculum of 12.8 million cells/ml of *Chlorella*, it attained its maximum growth in 12 days and gradually entered the declining phase in subsequent days (Fig. 3).

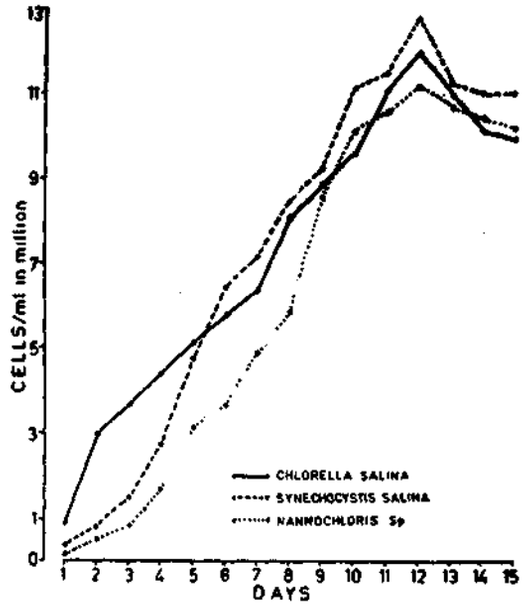

Fig. 3. Growth characteristics of species of *Chlorella*, *Synechocystis* and *Nannochloris*.

Similarly in *Nannochloris* sp. from an initial inoculum of 11.6 million cells/ml reached its maximum growth on 12th day and started to decline in subsequent days. In the case of *Synechocystis salina*, the inoculum had a density of 13.4 million cells/ml and the maximum growth of cells was noted on 12th day as in the other two species earlier mentioned. On the subsequent days, the cultures entered the declining and stationary phases.

Growth characteristics

Growth was estimated solely by cell counts, from 2nd day onwards upto 15th day. The mean generation time and the relative growth constant of the ten species of nannoplankters are presented in Table - 1. It was found that

TABLE 1. *Relative growth constant and mean generation time of ten species of nannoplankters*

Species	'k' values	t_g(hrs)
Isochrysis galbana	0.050	14
Pavlova lutheri	0.043	16
Dicrateria inornata	0.031	22
Chromulina freiburgensis	0.047	15
Tetraselmis chuii	0.033	21
T. gracilis	0.035	20
T. tetrahele	0.028	25
Chlorella salina	0.035	20
Nannochloris sp.	0.024	29
Synechocystis salina	0.043	16

all the Haptophycean forms have a mean generation time, *i.e.* the doubling time for a single cell, was less than 24 hours; for *Isochrysis*, 14 hours; for *Pavlova* 16 hours and for *Chromulina*, it was 15 hours. In the case of *Dicrateria*, the mean generation time was 22 hours. While all the species of *Tetraselmis* showed more than 20 hours for the cells to multiply. However, in *Chlorella*, the generation time was 20 hours and in *Nannochloris*, it was 29 hours. The Cyanophycean member, *Synechocystis* showed a mean generation time of 16 hours for its cells to multiply. The growth constant 'k' also showed varying values with respect to these organism's mean generation time. Among all the forms, *Isochrysis galbana* indicated a high 'k' values when compared to other nannoplankters.

DISCUSSION

The mean generation time and growth constant have been studied elsewhere for a number of phytoplankters especially diatoms in culture (Harvey, 1934; Braarud, 1945; Kain and Fogg, 1958 a, b; Ketchum, 1939; Spencer, 1954). Since the nannoplankters multiply by cell division, the individual cells have a variable generation time when they double themselves. These vary according to the environmental conditions and the Class and genus of the organisms. The mean value for a division time is expressed by the equation:

$$\ln.nt = \ln.n_o + kt$$

where n_o is the initial number of cells, nt is the number of cells at the end of time t expressed in hours and 'k' a constant that depends on the organisms and the environment. If t_g is the mean generation time for cell in hours, then

$$t_g = \frac{0.7}{k} \text{ or } k = \frac{0.7}{t_g} \text{ (Strickland, 1960)}$$

So by taking the natural logarithm of (ln) cell numbers at the initial and final phase of the experiment, the growth constant (k) and mean generation time (t_g) can be calculated.

According to Strickland (1960), k values for various phytoplankters are generally near the optimum and the results are usually within the range of 0.02 to 0.15 units (hours). However, Braarud (1945) reported about 0.15 for small diatoms decreasing to 0.07 to 0.05 units for larger species. The values given by Smyda (1957) are near to 0.05 and Harvey (1934) reported a value of 0.12 for *Chaetoceros* sp. The generation time for the dinoflagellate *Gymnodinium* sp. is 12 hours indicating a 'k' value of around 0.06 as reported by Ragotskie and Pomeroy (1957). Harvey *et al.* (1935) estimated

'k' as about 0.035 for total phytoplankton population.

In the case of *Asterionella japonica*, the growth constant has been found to be between 0.7 and 1.2 units per day (Kain and Fogg, 1958a) and for the flagellate *Isochrysis galbana*, a value of 0.55 units per day under optimum conditions was observed by Kain and Fogg (1958b) as in the present investigation. However, the growth constant was lower being only 0.3 units per day observed in the case of *Phaeodactylum tricornutum* (Ketchum, 1939; Spencer, 1954), the values being 1.7 units per day; *Dunaliella bioculata* has 1.8 units per day (Eddy, 1956) and *Chlorella pyrenoidosa* showed 2.0 units per day (Thacker and Babcock, 1957). The shortest generation time recorded was 5 hours for *Thalassionema nitzschioides*, representing a 'k' value of 0.14 and for the mixed population, the shortest doubling time was 9 hours, 'k' being 0.08 units (Nair, 1974). *Skeletonema costatum* has generation time of 13 hours, indicating a 'k' of 0.055 (Parsons *et al.*, 1961). Joseph and Nair (1975) observed that the growth constant 'k' in the case of *Tetraselmis gracilis* as 0.07 (t_g being 10 hours) and *Thalassiosira subtilis*, 'k' was 0.056 (t_g being 13 hours) while in *Synechocystis salina*, 'k' was 0.048 and generation time 14.6 hours in natural conditions.

Thus it is clear that the growth constant and generation time will depend on the environment of the algal cells and the Class and genus of the algae. However, under the diverse conditions found in the natural environment, variation in 'k' are bound to be considerable, depending on the species, light conditions, temperature and the nutrient properties of the water. Recent studies of Subba Rao (1981) showed that enrichment of algae with different levels of trace metals had a varied effect on the division rates of the cells. Besides inhibition or enhancement of growth of phytoplankton, other reported effects of trace metals are extended lag phase, morphological changes such as giant cell formation and decreased density of final population.

It is believed that 'k' values for warm tropical waters may be several times as great as the values given above for temperate and sub arctic regions (Wood, 1958). Also it is difficult to know how different the 'k' values found for cultures under continuous illuminations. According to Sweeny and Hastings (1958) that this can have an effect on the time of day during which cell division occurs but the effect on generation time may not be very great. Recent studies of Ammini Joseph and Nair (1984) showed that the growth measurements of nannoplankters exhibited the peak growth and activity from 2-6 days of inoculum and then the growth rate declined gradually. Further it was stated by these authors that the nutrient utilization capacity of the Haptophycean members especially nitrates and phosphates were higher than other phytoflagellates.

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