Comparison of potential growth rates of *Ceratium hirundinella* with observed population density changes

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Abstract

Ceratium hirundinella cells in Lake Constance divided during the second half of the night. Growth rates are calculated from the fraction of cells undergoing cell division. Potential growth rates are compared with observed changes in population density. The discrepancy between both is discussed as a possible function of fungal parasitism.

Introduction

Measurements of the growth rate of natural populations of phytoplankton have been a major challenge for phytoplankton ecologists. Contrary to cultures, where growth rates can be derived directly from observed population increase, calculation of growth rates in the field is complicated by concomitant growth and loss. Indirect methods of calculating growth rates by estimates of the loss rate have been used by Reynolds *et al.* (1982), Sommer & Stabel (1983), Sommer (in prep.) for diatoms which have the advantage of a skeleton remaining countable after cell-death. A powerful tool of direct measurement of the growth rate is the estimate of cells undergoing mitosis, as it has been recently reviewed by Mc Duff & Chisholm (1982).

As their division stages are easily recognizable and cell divison is commonly synchronized, this method has been first used for dinoflagellates: marine species of *Ceratium* (Gough, 1905; Apstein, 1911; Elbrächter, 1973; Weiler & Chisholm, 1976), and the fresh water species *Ceratium hirundinella* O. F. Müller (Entz, 1931; Heller, 1973; Frempong, 1982), *Peridinum cinctum* Lef. (Pollingher & Serruya, 1976). However, only Pollingher & Serruya (1976) and Heller (1977) have compared the growth rates derived from cell division with the observed rate of increase of the populations in the lake.

The present study examines the nocturnal phasing of *Creatium hirundinella* cell divisions on three sampling occasions in Lake Constance, compares calculated growth rates with observed population density changes and discusses discrepancies between potential and realized growth as a possible function of fungal parasitism. In Lake Constance, a mesotrophic, warm monomictic lake of 500 km^2 surface area and 250 m maximum depth, *Ceratium hirundinella* is usually a dominant species in mid-August (Sommer 1981). In the year of this study (1982) *Ceratium* failed its August dominace, maximum population densities remained one order of magnitude smaller than in the previous three years.

Methods

Sampling and counting

Samples were taken at 1 m intervals from the surface down to 10 m depth with 9 l van Dorn-bottles of nearly 1 m length from the deepest point, 'Überlinger See', the north western bight of Lake Constance. By that sampling procedure the entire

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water column from 0 to 10 m was covered, in which according to previous experience more than 95% of the cell number of *Ceratium hirundinella* of the entire water column could be expected. On July 20/21 and 27/28 samples were taken hourly. Cell division only occurred during nighttime. Therefore, on August 10/11 and 17/18 sampling frequency was increased to intervals of 30 minutes during nighttime, whereas daytime sampling frequency was reduced. Unfortunately on August 3, the time of highest Ceratium densities, no nocturnal study was possible.

Lugol's iodine solution was used for preservation. Samples from the separate depths were mixed and the integrated sample was allowed to settle for more than 24 h in 100 or 50 ml Utermöhl-chambers and counted under the inverted microscope. At least 100 cells of each category were counted per sample. The following categories of cells were distinguished: (O) complete cells, with no apparent sign of division, (A) cells in the process of division, i.e. cells with an oblique fissure on the theca, (B) apical halves after division, (C) antapical halves after division. For the morphological distinction see Heller (1977). Additionally dead cells (D), i.e. empty cell-walls were counted. Most of them contained spherical particles, that stained brown with Lugd's iodine.

Calculation of growth rates

In principle there are two possibilities to estimate the fraction of cells undergoing division: Either the maximum of the curve of dividing cells or the area beneath the curve of dividing cells divided by the time of division (i.e. the time a certain stage of division is visible) is taken. As it has been shown by Mc Duff & Chisholm (1982), the first method is only applicable when cell division is so tightly synchronized that there is at least one sample in which the total number of dividing cells on a given day can be seen in division. This was not the case in our study; therefore, we had to obtain a reliable estimate for the time, during which a given division stage was visible (t_D). The dark brown colour of *Ceratium* cells after iodine fixation made nucleus staining impossible. Therefore, the only division stage, for which the time of visibility is clearly defined, is category A. It begins with the appearance of the division fissure on the theca and ends with the separation of the halves. Heller (1977) has estimated t_d from the time shift between the maxima of category A and of categories B and C. This was possible because of the pronounced maxima in his curves. In our case maxima were not clear enough (Fig. 1) to allow this method. Therefore, we chose the time shift between the gravity centers of the curves for A and B and C as an alternative approach. Theoretically the estimates of t_D derived from the A–B and from the A–C time shift should have been identical, but because of data scatter there were slight differences (Fig. 1). For the calculation of the growth rate the average between both

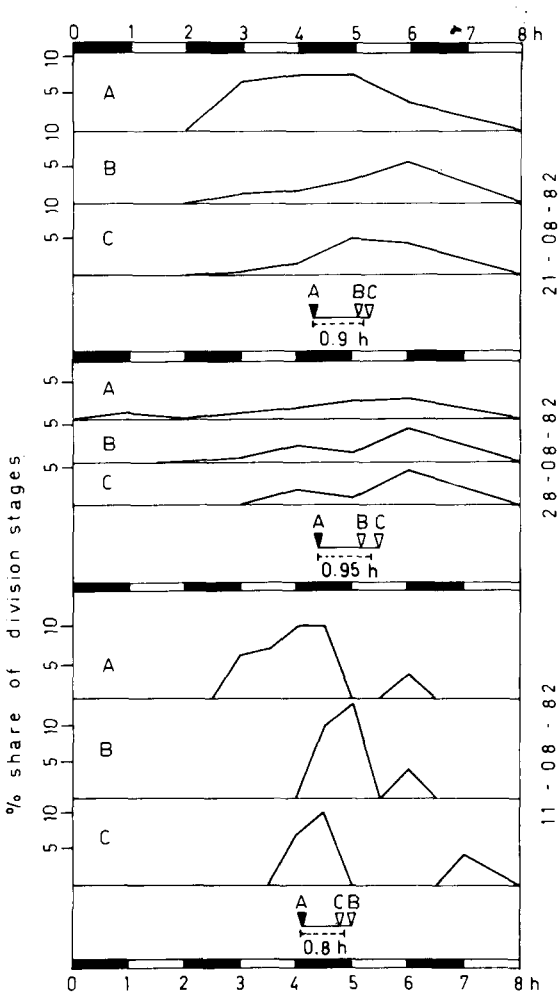

Fig. 1. Phasing of *Ceratium hirundinella* cell divisions. A: cells in division, B: apical halves, C: antapical halves (all in % of total cell number). The triangles indicate the gravity centers of the curves of the division stages. The broken line indicates the calculated division time.

estimates was taken. The following equations were used:

Total number of cells:

$$\text{equ. 1:} \quad N_{TOT} = N_O + N_A + \frac{N_B + N_C}{2}$$

The fraction of the single categories per sample were expressed as percent shares of the total cell number.

$$\text{equ. 2:} \quad p_A\,(\%) = \frac{N_A \cdot 100}{N_{TOT}}$$

Gravity center of the curve of cells in the process of division:

$$\text{equ. 3:} \quad T_A = \frac{\Sigma\, p_A \cdot t_s}{\Sigma\, p_A}$$

(t_s = sampling time)

Division time:

$$\text{equ. 4:} \quad t_D = \frac{(T_B - T_A) + (T_C - T_A)}{2}$$

Total fraction of cells undergoing mitosis during one day-night cycle:

$$\text{equ. 5:} \quad P_A = \frac{A}{t_D}$$

when A is the area covered by the curve of P_A.

The potential growth rate:

$$\text{equ. 6:} \quad \mu = \ln \frac{100 + P_A}{100}$$

The observed (net) rate of increase/decrease between two sampling occasions.

$$\text{equ. 7:} \quad k = \frac{\ln N_2 - \ln N_1}{t_2 - t_1}$$

Results

There were great differences between the counts of N_{TOT} within one sampling day. By definition N_{TOT} does not increase until cells of category B and C have grown up to complete cells. Hence, the differences within one day are certainly due to horizontal heterogeneity in the distribution of *Ceratium*. The value of N_{TOT} given together with standard deviations in Fig. 2 is the average for all sampling times on one day. As an average of 24 to 30 individual values it probably integrates out most of the short-distance heterogeneities in the lake. Until August 3 population densities were in the same range as in the corresponding time of the last three years. Contrary to our previous experience the population began to decrease already in the week from August 3 to 6. In agreement with earlier observations (Entz, 1931; Heller, 1977) cell divisions were restricted to the second half of the night (Fig. 1) although synchronisation was not as tight as reported by Heller (1977). Calculated potential growth rates were 0.27 d^{-1} for July 20/21, 0.12 day^{-1} for July 27/28 and 0.20 day^{-1} for August 10/11, which corresponds to doubling times of 2.6, 5.8, 3.5 days respectively. On August 17/18 the population density was too low to obtain reliable counts of the division stages.

Discussion

Division time and reliability of growth rate estimates

As it has been emphasized by Mc Duff & Chisholm (1982) the estimate of the division time is the Achilles' heel of the calculation of μ. The time shifts between the gravity centers of the curve of the dividing cells and the curves of the two different types of post-divisional semicells give two independent estimates of t_D for each night; the agreement between them can be taken as an indication for the reliability of the estimate. On July 20/21 the estimates for t_D were 0.8 and 1.0 h, on July 20/21 0.8 and 1.1 h and on August 10/11 0.7 and 0.9 h. On July 27/28, when the difference was biggest, substituting a division time of 0.8 h into the calculation would have given a growth rate of 0.14 day^{-1}, substituting t_D = 1.1 h would have given 0.10 day^{-1}, instead of 0.12 day^{-1}

which was obtained by using the average division time. We consider the smallness of these differences as encouraging with respect to the reliability of our method.

Furthermore we found surprising agreement between the division times of the three sampling occasions. This shall not be taken as an indication for constancy of t_D, because the whole period of study fell into a phase of fairly stable weather with only minor changes in water temperature. (Surface temperature varied between 18.7 and 21.4 °C and temperatures of 10 m depth from 13.7 to 17.4 °C.) It needs observations over a far wider range of environmental conditions to decide whether the division time is constant or variable. In Esthwaite Water Heller (1978) found a t_D of 3 h in summer and Heaney *et al.* (1953) found 8 h in winter.

The discrepancy between μ and k

Only on one occasion population did development follow the prediction of growth derived from cell divisions (July 27 to August 3, Fig. 2). It was the only sampling interval during which an increase in population density could be observed. Surprisingly enough, this increase followed the lowest rate of cell divisions observed. It is obvious from Fig. 2 that the pattern of increase and decrease of the *Ceratium* population was independent of the growth rate and exclusively controlled by losses. Temporal variability of the growth rate is small, from 0.12 day^{-1} to 0.29 day^{-1}, while temporal variability of the loss rate is much higher. The loss rate (λ) can be obtained by equation 8:

$$\lambda = \mu - k$$

Unfortunately we have no estimate for the growth rate on August 3, but if we assume that it has been within the range of the other data, then the range for λ for the entire period of study extends from 0 to 0.53 (lowest estimate) or 0.68 d^{-1} (highest estimate; close to one halving per day). It has been subject to much speculation, whether phytoplankton succession is predominantly controlled by the growth rate or by the loss rate (Kalff & Knoechel, 1978). Obviously it is the balance between both, that decides about wax and wane of a population, but the rate with the higher temporal variability has the bigger influence on the temporal pattern of population development.

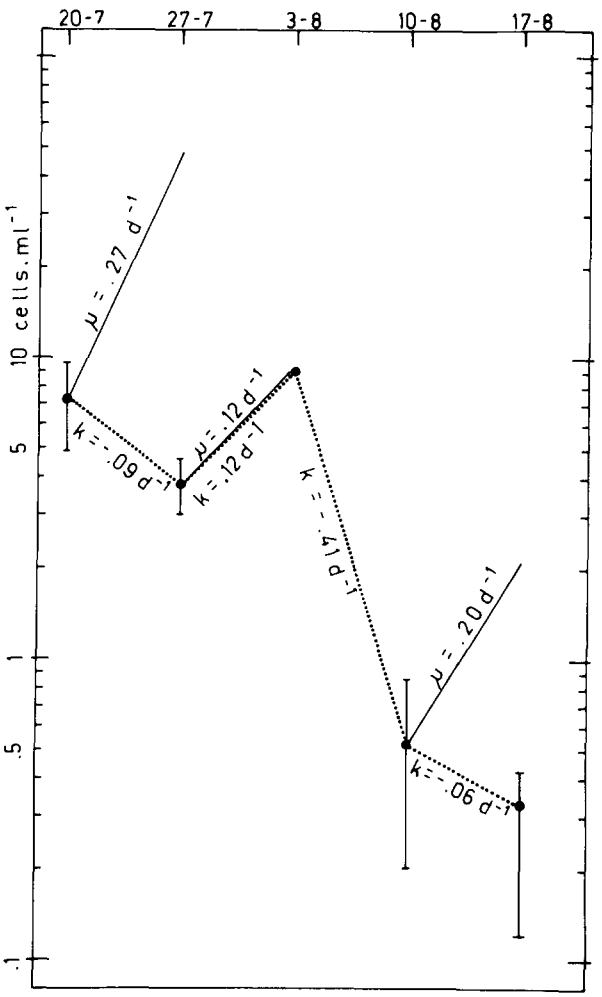

Fig. 2. Comparison of observed population growth (dotted line) with potential growth derived from growth rate u (solid line). Vertical lines indicate standard deviation of population density.

The high loss rates observed in 1982 completely disagree with our experience from the previous years in which the net rates of decrease during the decline phase of the population were between 0.09 day^{-1} and 0.17 day^{-1}, with the exception of phases of cyst-formation (Sommer, 1981). Consequently *Ceratium hirundinella* has been classified as a K-strategist characterized by slow growth but high resistance against losses. There is general agreement that *Ceratium hirundinella* is not grazed by herbivorous Cladocera, the dominant filter feeders in Lake Constance, and that it is not subject to sedimentation (Heaney & Talling, 1980), which are, according to Reynolds *et al.* (1982), the two most

important factors of loss in phytoplankton. We also can exclude the possibility of cyst formation as a source of loss, because no cysts have been found in the free water samples and only extremely few cysts in the sediment traps that were exposed at the same time in the lake. Although there are no reliable counts of cysts in the sediment traps – because of their scarcity – it can be said with certainty that cyst formation and subsequent sinking cannot account for more than 5% of the losses observed.

The most probable source of losses during the period of this study was fungal parasitism. We found numerous empty *Ceratium* cells bearing spherical particles, that were stained brown with Lugol's iodine solution. Comparisons with literature (for a review see Canter, 1979) suggested, that these cells might have been victims of fungal parasitism. 7 to 69% of all cells (live and dead together) were found to belong to this category. Afterwards the samples were reexamined for signs of parasitism on live cells. We found ovoid sporangia of 15–30 μm attached on 2 (July 27/28) to 10% (August 10/11) of the live cells. In a few cases rhizoids were clearly visible. According to literature these sporangia were probably chytried zoosporangia. The brown shaerical particles mentioned above resembled the resting spores described by Canter (1963) for the chytrid *Zygorhizidium cystogenum,* parasitizing on *Dinobryon.* Although a later stage in the development of chytrid parasites, there was no time shift between the frequency maxima of the zoosporangia and the resting spores. Probably temporal resolution of one week is not sufficient to observe this time shift. Species determination of the parasites was not successful. Since the counts of the dead cells with the resting spores were more accurate than the counts for live cells with zoosporangia, the numerical degree of parasitism given in Fig. 3 was derived from the first ones. The highest proportion of parasitized cells occurred at the end of the period with the highest loss rate. Unfortunately we have no knowledge about the time required from infection to cell-death. Therefore we are unable to convert the percent values of parasitized cells into exponential death rates. Nevertheless the observed similarity in the time sequence of loss rates and degree of parasitism (Fig. 3) supports our interpretation of *Ceratium* losses being mainly due to parasitism. Parasitism as a controlling factor for algal population dynamics has been reported for diatoms (Canter & Lund, 1948) and desmids (Canter & Lund, 1969), but not yet for dinoflagellates.

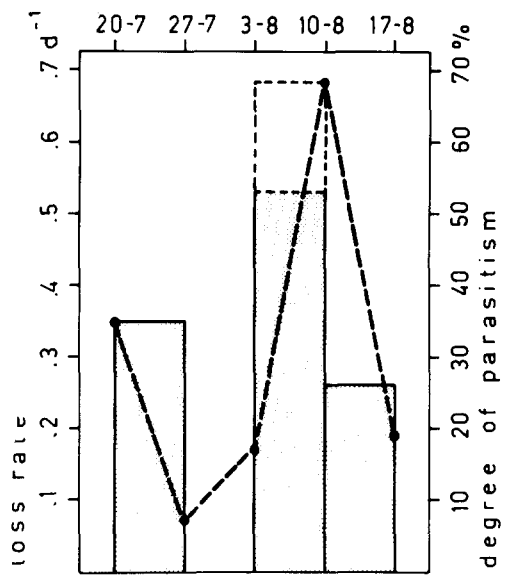

Fig. 3. Comparison of loss rates (shaded columns) with depree of parasitism (infected cells in % of total cells number, thick broken line).

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