

A modelling approach to the interplay of carbohydrates, bacteria and non-pigmented flagellates in a controlled ecosystem experiment with *Skeletonema costatum*

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ABSTRACT: The role of heterotrophic nano-flagellates in the process of bacterial removal in marine plankton was investigated by developing a predator-prey model based on Monod kinetics. Four state variables were defined: total dissolved carbohydrates, and total populations of bacteria, flagellates and ciliates. Growth kinetic parameters were optimized by using experimental data from an *in situ* culture experiment with *Skeletonema costatum* in a controlled ecosystem. The successive developments of heterotrophs and the consumption of dissolved carbohydrates were simulated very well by the model. Potential biomass production through the detrital food chain was also calculated and compared to estimates based on experimental observations. Bacterial production was partly due to decomposition of amino acids and proteins in the early phase of growth. It is concluded that bacteria were the primary consumers of dissolved carbohydrates, while phagotrophy by heterotrophic flagellates caused the subsequent rapid decline in the bacterial population.

INTRODUCTION

In recent years some investigators have pointed to the colorless nano-flagellates in marine plankton as important grazers on bacterioplankton (Sorokin, 1977; Sieburth et al., 1978; Sieburth, 1979; King et al., 1980). Species with cell diameters ranging from 1.5 to 5 µm are common within the families Bodonidae, Amphimonadidae and Monadidae (Sieburth, 1979), which have been shown to feed voraciously on marine bacteria in culture (Haas and Webb, 1979; Laake et al., in prep.). Non-photosynthetic flagellates are commonly present in coastal plankton in the northern hemisphere (Thronsdon, 1970). Their trophic status is not fully understood, although traditionally they are

believed to be organotrophic and as such competing with bacteria for dissolved substrates. They are commonly regarded as phytoplankton by taxonomists, although they definitely belong to the heterotrophs (Sieburth, 1979). During the review of the present study, Fenchel (1982a, b, c and d) published important papers on the ecology of heterotrophic flagellates in aquatic environments that clearly demonstrates their function as bacteriovores in plankton.

Controlled ecosystem experiments were carried out in Rosfjord, southern Norway, from March to April 1979 (Brockmann et al., 1981). In one experiment the growth of *Skeletonema costatum*, re-inoculated into filtered seawater, was followed, as well as the dynamics of carbohydrate concentrations and heterotrophic organisms (Eberlein et al., 1983). From these observations it was concluded that the rapidly growing bacteria probably consumed the dissolved carbohydrates, and were in turn grazed upon by small, heterotrophic flagellates. To further test this hypothesis a model has been constructed, based on modified Monod growth kinetics (Caperon, 1967).

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Jost et al. (1973) developed 2 models for grazing of bacteria by ciliates based on Monod-type kinetics, and reported these to fit very well with data obtained from culture experiments (Proper and Garver, 1966; Hamilton and Preslan, 1970; Tsuchiya et al., 1972). Later developments in predator-prey systems modelling seem to confirm this statement (Dubois, 1979; Graham and Canale, 1982). Fenchel (1982b) found that exponential growth rates as function of bacterial density fitted to a Michaelis-Menten form equation in growth experiments with 5 different species of bacteria-feeding flagellates.

MATERIALS AND METHODS

The experimental set-up is described in detail by Eberlein et al. (1983). On March 16, 1979, seawater from 5 m depth in Rosfjord, southern Norway, with a salinity of 29.6 ‰, was pressure-filtered through a cellulose plate filter (average pore size 0.2 μm at the start of filtration) and filled into a 1 m diameter, 20 m deep plastic bag of transparent polyethylene/polypropylene film. The bag was supported by a floating platform, open at the surface and closed at the bottom (Brockmann et al., 1977; Brockmann et al., 1982). The prefiltration removed approximately 80 % of the total population of bacteria present, as judged from epifluorescent counts, and practically all larger organisms. Nutrients for phytoplankton growth were mixed evenly into the water to approach a normal pre-bloom level, and the following concentrations were obtained: 11.2 $\mu\text{g-at nitrate-N dm}^{-3}$, 0.8 $\mu\text{g-at orthophosphate-P dm}^{-3}$, and 7.5 $\mu\text{g-at silicate-Si dm}^{-3}$. The initial ammonium concentration was approximately 1 $\mu\text{g-at ammonium-N dm}^{-3}$.

On March 18 the bag was re-inoculated with a monoculture of *Skeletonema costatum* (Jahnke et al., 1983), and on March 21 with 5 isolates of organotrophic bacteria (Laake et al., 1983), and the inoculates homogeneously distributed in the water column. *S. costatum* started to grow exponentially, but at a very low rate (see Jahnke et al., 1983, for further details). Temperature varied from 1° to 5°C throughout the experiment, which lasted until April 5.

The water was analysed for macronutrients, total dissolved carbohydrates and organic C and N (Eberlein et al., 1983). Phytoplankton growth was followed by chlorophyll *a*, particle counts and specific counting of *Skeletonema costatum* by microscopy (Jahnke et al., 1983). Particle counts and size distribution were monitored using a Coulter Counter TA II with a 100 μm orifice tube, from which also the growth of the nanoflagellates present could be followed. Net primary production was measured by $^{14}\text{C-CO}_2$ fixation. Total

numbers of bacteria were followed by acridine orange staining and epifluorescence microscopy on black 0.4 μm Nuclepore polycarbonate filters (Hobbie et al., 1977). In this context only data from 3 m depth will be reported, which is justified by the fact that the development of bacteria and flagellates was virtually independent of depth (Eberlein et al., 1983) and almost identical in a second bag inoculated with *Thalassiosira nordenskiöldii* (Laake et al., 1983). Model calculations have been carried out at the Computing Center Blindern/Kjeller, University of Oslo, Norway.

MODEL DEVELOPMENT

From particle counts (Fig. 1) and other data (Eberlein et al., 1983) it is evident that heterotrophic flagellates were present and grew rapidly from Day 6 through Day 11 of the experiment. Neither chlorophyll *a* nor photosynthesis increased in this period (Jahnke et al., 1983).

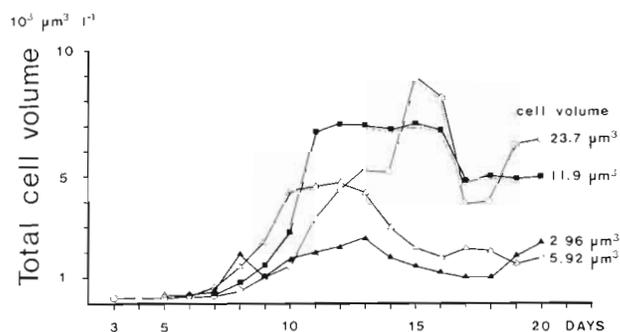


Fig. 1. Growth of nanoflagellates in the controlled ecosystem, measured as total particle volume in 4 size classes. Particle volumes were measured on fresh samples with a Coulter Counter TA II fitted with a 100 μm orifice tube

Although no direct evidence for the presence of ciliates was obtained, their relatively small numbers may have been included as an insignificant part of the phytoplankton particle counts (see Discussion for details). Small ciliates (e.g. *Uronema* spp.; Hamilton and Preslan, 1969) which graze on bacteria and/or flagellates are thus included in the model.

Four state variables have been defined: bacteria (B); flagellates (F); ciliates (C); and dissolved substrate, i.e. total dissolved carbohydrates (S). It was assumed that grazing pressure on the prey organism is directly proportional to predator density and growth rate. Dependence of predator growth on prey density, on the other hand, was assumed to follow Monod kinetics. Removal by sinking was assumed to be negligible. The following set of equations was then derived (see Table 1 for list of symbols):

$$\dot{B} = \mu_B B - Y_{B/F} \mu_F F - Y_{B/C} W_B \mu_C C \quad (1)$$

$$\dot{F} = \mu_F F - Y_{F/C} w_F \mu_C C \quad (2)$$

$$\dot{C} = \mu_C C - D_C C \quad (3)$$

$$\dot{S} = a R_P P_N - Y_{S/B} \mu_B B \quad (4)$$

Thus the time derivative of bacterial population density (\dot{B}) was considered a function of growth on available substrates, minus grazing by flagellates and ciliates (Eq. 1). Flagellates similarly depend for growth on available substrates (i.e. bacteria) and are grazed by ciliates (Eq. 2). Since no grazers on ciliates had been introduced to the model, the ciliates were assigned a specific death rate (D_C) to balance their growth on available substrates (i.e. bacteria and flagellates) (Eq. 3). The change in total dissolved carbohydrates (\dot{S}) was considered a function of net photosynthesis minus consumption by bacteria (Eq. 4). Expressions for the specific growth rates (μ) and grazing selectivity coefficients (w) for ciliates are given below.

The substrates available to bacteria were predominantly dissolved monomeric or polymeric carbohydrates (Eberlein et al., 1983), and as a simplification only this pool is included in the model. Further simplifications are made by assuming that flagellates obtain all their energy from grazing of bacteria; organotrophy and phagotrophy of particulate detritus is considered negligible. Ciliates, however, are allowed to graze both on bacteria and flagellates. Based on these simplifications the following equations for specific growth rates were derived:

$$\mu_B = \hat{\mu}_B \frac{(S - S_{\min}) / K_{S/B}}{(S - S_{\min}) / K_{S/B} + 1} \quad (5)$$

$$\mu_F = \hat{\mu}_F \frac{(B - B_{\min, F}) / K_{B/F}}{(B - B_{\min, F}) / K_{B/F} + 1} \quad (6)$$

$$\mu_C = \hat{\mu}_C \frac{(B - B_{\min, C}) / K_{B/C} + (F - F_{\min}) / K_{F/C}}{(B - B_{\min, C}) / K_{B/C} + (F - F_{\min}) / K_{F/C} + 1} \quad (7)$$

It was necessary to modify the Monod equations by introducing minimum concentrations of substrates, below which the organisms can gain no net energy for growth. A fairly large fraction of carbohydrates resistant to microbial attack was thus accounted for, while the other minimum values are represented by the observed minima in the experimental results during the observation period. It was also necessary to separate the grazing pressure by ciliates on bacteria and on flagellates by introducing selectivity coefficients (w) to the respective rate equations, as being a function of the prey minimum concentration and a half saturation constant for grazing. Vanderploeg and Scavia (1979) developed a somewhat similar expression for zooplankton having a selection of several prey organisms on their diet. Thus bacteria and flagellates are grazed

in proportion to their abundance, scaled by the ciliate half-saturation constants for grazing. The sum of selectivity coefficients is one ($w_B + w_F = 1$).

$$w_B = \frac{(B - B_{\min, C}) / K_{B/C}}{(B - B_{\min, C}) / K_{B/C} + (F - F_{\min}) / K_{F/C}} \quad (8)$$

$$w_F = \frac{(F - F_{\min}) / K_{F/C}}{(B - B_{\min, C}) / K_{B/C} + (F - F_{\min}) / K_{F/C}} \quad (9)$$

RESULTS

Functional evaluations of alternative models

Three conceptual models were evaluated for their ability to reproduce a succession of events similar to those observed in the experiment: I, with flagellates only as grazers on bacteria; II, with ciliates in addition grazing on flagellates; and III, with ciliates grazing on both flagellates and bacteria. To test for this, numeric values estimated from the experimental results or based on theoretical considerations, were assigned to all growth constants and coefficients not given by any function. Maximum specific growth rates were derived from semi-log plots for bacteria and flagellates. Half saturation constants were estimated by adding to the minimum concentration observed, approximately 30 % of the difference between minimum and maximum values (e.g. $K_{S/B} = S_{\min} + 0.3 [S_{\max} - S_{\min}]$), as given in Table 1. At present there are very few experimental values of relevance in the literature. Yield coefficients were assigned low and high values (Table 2) from theoretical calculations on small and large cell dimensions within each group of organisms (Table 3), assuming 30 % biomass transfer efficiency, as reported for bacteria growing on extracellular products from algae (Bell and Sakshaug, 1980). The yield coefficients were later optimized to make the simulated values for state variables fit the experimental results, as discussed later.

Initial runs with Model I with 1 day time intervals in the numeric procedure resulted in delayed maxima in B and F, and a continued increase in F after 20 d. Damped oscillations occurred in S until stabilisation at S_{\min} . By reducing the time interval to 1 h, the damped oscillations in S were removed, B reached a maximum at Days 9 to 10, and F flattened out at Day 17. Later runs confirmed the 1 h time interval to be optimal.

The next refinements were to introduce ciliates as grazers on flagellates (Model II), assign minimum values to B and F, and to introduce the net primary production (P_N) as a forcing function supplying new substrate to bacteria, by assuming a proportional release of carbohydrates (R_P). Experimental values for

Table 1. Growth parameters and selected start conditions in the final simulation with the grazing model

Yield factors	$\left\{ \begin{array}{l} Y_{S/B} = 0.00067 \\ Y_{B/F} = 100 \\ Y_{B/C} = 120.000 \\ Y_{F/C} = 1000 \end{array} \right.$	$\mu\text{mol glc. eqv. } 10^{-6} \text{ cells}$ cells B cell F^{-1} cells B cell C^{-1} cells F cell C^{-1}
Half saturation constants for growth	$\left\{ \begin{array}{l} K_{S/B} = 0.50 \\ K_{B/F} = 200 \\ K_{B/C} = 200 \\ K_{F/C} = 3 \end{array} \right.$	$\mu\text{mol glc. eqv. } l^{-1}$ $10^6 \text{ cells B } l^{-1}$ $10^6 \text{ cells B } l^{-1}$ $10^6 \text{ cells F } l^{-1}$
Maximum specific growth rates	$\left\{ \begin{array}{l} \hat{\mu}_B = 0.80 \\ \hat{\mu}_F = 0.44 \\ \hat{\mu}_C = 0.20 \\ D_C = 0.06 \end{array} \right.$	d^{-1} d^{-1} d^{-1} d^{-1}
Death rate ciliates	$\left\{ \begin{array}{l} w_B = (\text{eq. 8}) \\ w_F = (\text{eq. 9}) \end{array} \right.$	(dimensionless) (dimensionless)
Ciliate selectivity coefficients for prey	$\left\{ \begin{array}{l} S_{min} = 1.00 \\ B_{min} = 100 \\ F_{min} = 1 \end{array} \right.$	$\mu\text{mol glc. eqv. } l^{-1}$ $10^6 \text{ cells B } l^{-1}$ $10^6 \text{ cells F } l^{-1}$
Minimum substrate and prey concentrations for growth	$\left\{ \begin{array}{l} S = 2.50 \\ B = 200 \\ F = 0.5 \\ C = 0.005 \end{array} \right.$	$\mu\text{mol glc. eqv. } l^{-1}$ $10^6 \text{ cells B } l^{-1}$ $10^6 \text{ cells F } l^{-1}$ $10^6 \text{ cells C } l^{-1}$
Start concentrations	$\left\{ \begin{array}{l} TD_F = 0 \\ TD_C = 9 \\ \Delta T = 1 \end{array} \right.$	d d h
Time delay before start of growth	$\left\{ \begin{array}{l} P_N = (\text{measured}) \\ R_p = 0.10 \end{array} \right.$	$\mu\text{g C } l^{-1} \text{ h}^{-1}$ C release C fixation $^{-1}$
Simulation time step	$a = 72$	$\mu\text{g C } \mu\text{mol glc. eqv.}^{-1}$
Net primary production		
Photosynthetic release coefficient		
Conversion factor		

P_N were used, except at start when low, constant values were assigned to account for a daily cycle observed in dissolved carbohydrates when no P_N was measured (Eberlein et al., 1983). From Day 18 a linear decrease in P_N was assumed until Day 32. The model now simulated the real events qualitatively and with approximate timing of minima and maxima.

However, knowing that ciliates may be efficient grazers on bacteria, this function was added in the third generation (Model III). To account for grazing on ciliates by larger zooplankton and to balance their proliferation, a death rate for ciliates (D_C) was intro-

duced. When allowed independent simultaneous grazing on bacteria and flagellates, ciliates outcompeted the flagellates and reached unrealistic numbers. Thus abundance-dependent selective grazing was assumed and accounted for by introducing the selectivity-coefficients (w_F and w_B). Their numeric values are governed mainly by the ciliate half-saturation constants for growth on flagellates ($K_{F/C}$) and bacteria ($K_{B/C}$). A functionally realistic model which simulated very well the succession of events observed in S, B and F, was then obtained.

Table 2. Estimated yield factors for growth of bacteria, flagellates and ciliates, on their respective available substrates

State variable	Estimated yield factor ⁽²⁾		Unit
	Symbol	Low High ⁽³⁾	
Bacteria (B) ⁽¹⁾	$Y_{S/B}$	0.35 - 1.0	$\mu\text{mol glc. eqv.}/10^9 \text{ cells}$
Flagellates (F)	$Y_{B/F}$	70 - 470	cells B/cell F
Ciliates (C)	$Y_{F/C}$	900 - 8000	cells F/cell C
	$Y_{B/C}$	50 000 - 560 000	cells B/cell C

(1) Assuming that $1/3$ of substrate carbon is converted to new cells
(2) Assuming $1.21 \cdot 10^{-13} \text{ g C } \mu\text{m}^{-3}$ cell volume, as reported for bacteria (Watson et al., 1977), equivalent to $121/72 \cdot 10^{-15} \text{ mol glc. eqv. } \mu\text{m}^{-3}$
(3) Low and high estimates calculated from extreme combinations of predator-prey cell volumes (Table 3)

Table 3. Theoretical cell volumes for small and large bacteria, flagellates and ciliates

State variable	Method of estimation	Cell volume (μm^3)	
		small	large
Bacteria (B)	Sphere $d = 0.5 \mu\text{m}$	0.07	
	Cylinder, $d = 0.5 \mu\text{m}$, $h = 1 \mu\text{m}$		0.20
Flagellates (F)	Sphere $d = 2 \mu\text{m}$	4.2	
	Sphere $d = 5 \mu\text{m}$		66
	Geometric mean volume*		
	Channel 2: $d = 1.6-2.0 \mu\text{m}$	2.96	
	Channel 5: $d = 3.17-4.0 \mu\text{m}$		23.7
	Arithmetic mean measured on Day 5: minimum value on Day 15: maximum value	4.70	11.0
Ciliates (C)	Rotation ellipsoid $\frac{4}{3} \pi \frac{a}{2} (\frac{b}{2})^2$		
	$b = 16 \mu\text{m}$, $a = 25 \mu\text{m}$	3350	
	$b = 25 \mu\text{m}$, $a = 40 \mu\text{m}$		13 100

* Calibrated mean volumes for Channel 2, resp. Channel 5, on Coulter Counter TA II, $100 \mu\text{m}$ orifice

Sensitivity tests with the complete model

A factorial experiment (Andersin and Sulonen, 1974) was conducted with the model to reveal relative effects of changes in $Y_{S/B}$, $Y_{B/F}$ and $Y_{F/C}$, and interactions of these parameters, on fitting the model to experimental data. Positive factors with values from 0 to 1 indicate how the model reacted to changes in numeric values of the parameters. A high factor value for one particular yield coefficient in comparison with others shows that the model is sensitive to changes in the numeric value of this parameter. Low and high numeric values for the yields were selected (Table 2), and the following three criteria for success in curve-fitting were formulated: (1) B_{max} , and (2) F_{max} should be as close to the experimental values as possible, and (3) S should not be reduced below $2.0 \mu\text{mol glc. eqv. dm}^{-3}$ before Day 8.

Given these sensitivity measures the following evaluations were obtained:

- high $Y_{B/F}$ has the most positive effect (factor 0.44);
- high $Y_{S/B}$ has a positive but weaker effect (0.28), because a positive effect on B is counteracted by a negative effect on S ;
- high $Y_{F/C}$ has no or a slightly negative effect (-0.04);
- high $Y_{B/F}$ has its most positive effect when $Y_{S/B}$ is low;
- $Y_{F/C}$ has almost no influence on the effects of the other two yields.

The half-saturation constants for growth ($K_{S/B}$, etc.) were shown to be of much less significance than the yield factors, except for the competition between ciliates and flagellates for bacteria.

Curve fitting to experimental data

In optimizing the growth-kinetic constants in the model, we attempted to make B and F approach the observed values, and as shown above, the yield factors were of most importance in this regard. The combined effects of $Y_{S/B}$ and $Y_{B/F}$ on the simulated values for B_{max} and F_{max} , respectively, are shown in Fig. 2. An optimal combination of numeric values existed, which resulted in correct maxima for B and F , with values still within the realistic range (Table 2). The minimum concentrations of substrate or prey organisms were chosen as equivalent to the minima observed in the field experiment and not further optimized.

When population sizes and yield factors are given in terms of numbers of cells, and not in biomass units, the estimated values are susceptible to variations in cell size distributions. A functional relationship between measured cell size distributions of flagellates (from data in Fig. 1) and biomass might have been included in the model to convert from numbers to biomass units, but since these data were unobtainable for the bacteria, this was not done here. However, the average cell size of the flagellates was fairly constant throughout the experiment, with cell volume gradually increasing from 4.7 to $11.0 \mu\text{m}^3$ (Table 3).

Following optimization of the yields, μ_C was derived by relating the simulated reduction in F to the experimental values. A specific growth rate of 0.20 d^{-1} was chosen. This may be compared to the value 0.17 d^{-1} calculated from Heinbokel's (1978) data for tintinnids and corrected for temperature. Finally, the yield coefficients were further optimized by a few

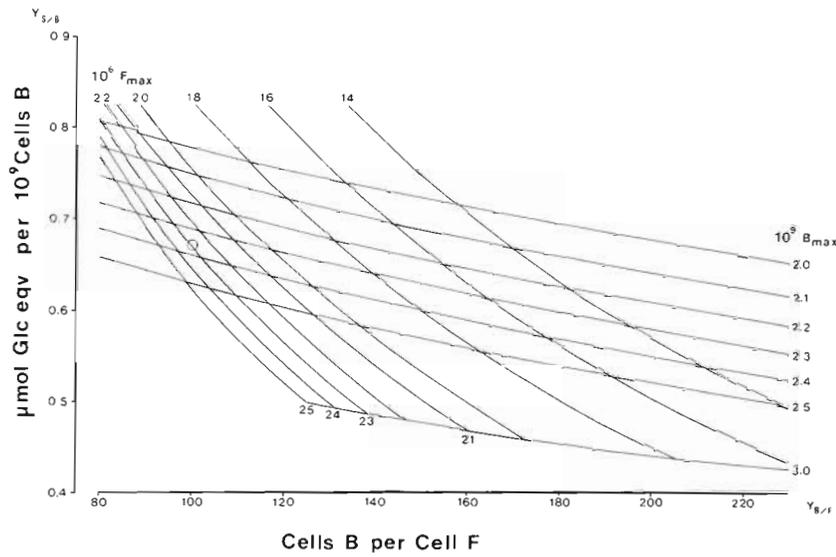


Fig. 2. Interactions of yield factors for growth of bacteria ($Y_{S/B}$) and flagellates ($Y_{B/F}$), with respect to population maxima of bacteria (B_{max}) and flagellates (F_{max}). O = optimal choice of yield factors

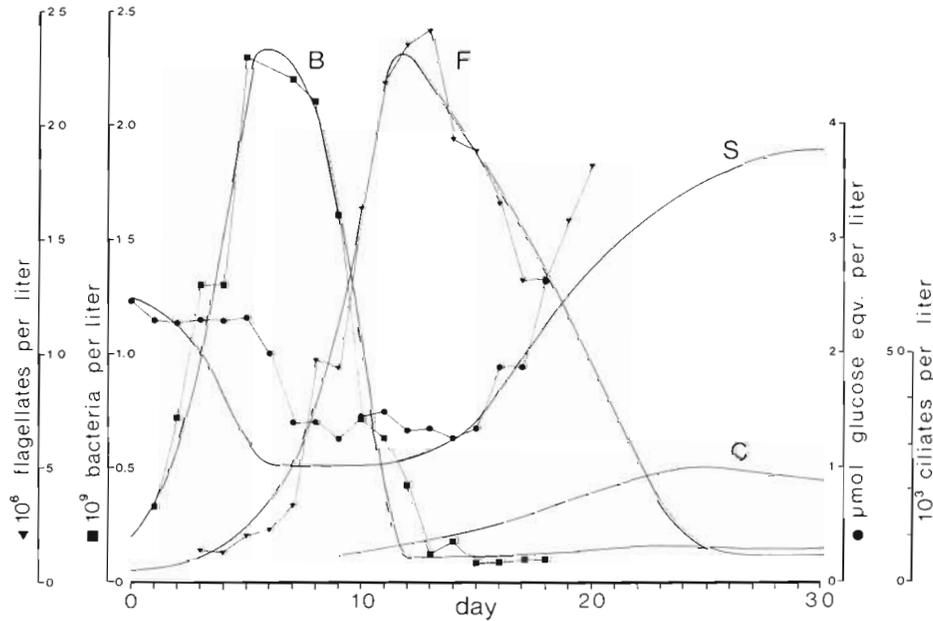


Fig. 3. Simulated values for organic substrate (S), bacteria (B), flagellates (F) and ciliates (C), compared to experimental data from the controlled ecosystem as a function of time. ● total dissolved carbohydrates (S); ■ total counts of bacteria by acridine orange staining (B); ▼ total numbers of nano-flagellates (F) with cell diameters from 1.6 to 4.0 μm . Measurements of ciliate populations were not available

simulations using the same criteria, until the numeric values in Table 1 were obtained. In Fig. 3 the simulated state variables are compared to the experimental results.

Production of heterotrophs

Gross production of heterotrophic microorganisms was calculated by not taking grazing and death into account (Eq. 1, 2 and 3); Fig. 4 gives the cumulative production of B, F and C after transformation into biomass units (Watson et al., 1977). Consumption of substrate was calculated similarly from Eq. 4. The gross production of bacteria is not far from the value calculated from consumption of carbohydrates in the actual experiment (Eberlein et al., 1983).

It is interesting to note that the simulated ciliates competed efficiently with flagellates for the bacterial production, because they also controlled the flagellate population and were not preyed upon by efficient grazers. In the actual experiment, however, flagellates increased significantly in numbers after Day 18 (Fig. 3), which may indicate that ciliates, if present, were less competitive than assumed by the model. The flagellates probably responded to an increased bacterial production, shown by simulation to occur after Day 16.

However, the increased bacterial production was much too low to compensate for the increased supply of substrates released by photosynthesis, as also observed in the experiment. This is due to the excess grazing pressure keeping the bacterial population very low,

which again must be due to the lack of efficient grazers on the bacteria-feeding protozoans. In neither model nor experiment were copepods or other metazoans present. Because of this, the normally rapid dissipation of dissolved substrates by the bacteria was blocked, and the carbohydrates released by *Skeletonema costatum* were allowed to fluctuate to abnormally high values (Eberlein et al., 1983).

DISCUSSION

Some small species of phagotrophic nanoflagellates may have passed through the cellulose plate filter used in the pre-filtration procedure, and started to grow when the bacterial population had regained. Although there is no direct evidence for ciliates being present in the planktonic community studied, it is not very likely that small species were completely eliminated through pre-filtration. Ciliates and flagellates may also have been introduced by aerosols or contamination during the filling procedure and gradually have re-established their populations. The bag was frequently sampled by a modified Niskin bottle, and since only a temperature gradient was established with depth, a rather efficient vertical migration of protozoa most probably was accomplished.

The cell numbers of ciliates necessary to account for the observed reduction in flagellates at Day 17 is still only about 10^4 cells $^{-1}$, and their growth may in the actual period have been masked by the diatom development measured as particle counts. Ciliates were not observed by microscopy on preserved samples, but are seldom well preserved by direct fixation with formaldehyde and more suitable techniques for the observation of ciliates were not included in the program. Considering these arguments the Model III approach, which includes ciliates as grazers both on bacteria and flagellates, is still the most realistic one

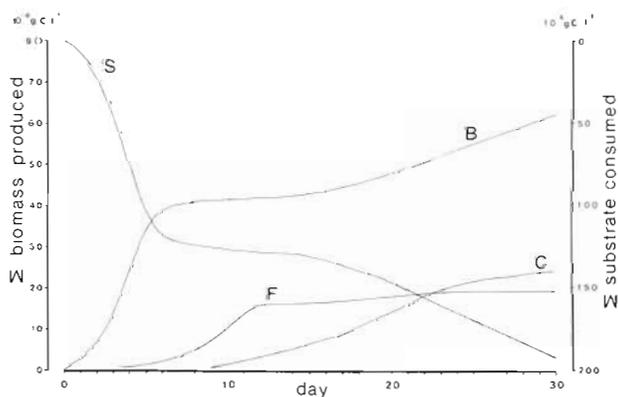


Fig. 4. Simulated gross biomass production as a function of time, for bacteria (B), nanoflagellates (F), and ciliates (C), compared to simulated consumption of total dissolved carbohydrates (S)

and offers an explanation for the observed reduction in flagellate cell counts, which is difficult to account for by other mechanisms.

The purpose of this model was to study a transient state situation shortly after the beginning of a succession of organisms. In the conventional predator-prey models more often the stability of a steady state is analysed. The present model does not necessarily represent a system that may achieve a realistic steady state between substrates and populations without further refinements, as demonstrated by the failure of bacterial numbers to respond to increased supply of carbohydrates (Fig. 3). Arbitrary but decreasing values for net photosynthesis were chosen for the last 10 d of the simulation, assuming a depletion of phytoplankton nutrients. The simulation was therefore extended only 10 d beyond the observation period, but is still believed to give a fairly correct picture of the expected development in this simplified planktonic ecosystem, however with some exceptions as discussed later. The concept of minimum concentrations of the state variables is believed to be critical in models attempting to achieve a stable steady state with complex systems (Jost et al., 1973).

Without detailed information on the concentrations of individual carbohydrate species in the total dissolved pool it was necessary to assume only one $K_{S/B}$ as representative, given in total concentration units and assigned to the average bacterium. Similar simplifications were made also for the flagellates and ciliates, assuming non-discriminative grazing within one class of prey, and an average cell size throughout the observation period. However, single substrate Monod kinetics is validated by the very good overlap of simulated and observed variations in the state variables, and more refined kinetics would have given only marginal improvements. The estimated numeric values may be fairly inaccurate and still give reasonable growth rate functions. Growth-kinetic data on flagellates have been published by Fenchel (1982b); his yield factors are in principle accordance with the range and optimized values obtained by us (Fig. 2). We estimated a half-saturation constant $K_{B/F}$ of $2 \cdot 10^8$ cells $B \text{ dm}^{-3}$, which is significantly lower than the range $1.3 \cdot 10^9$ to $3.8 \cdot 10^{10}$ cells $B \text{ dm}^{-3}$ reported by Fenchel (1982b). Our value was, however, based on assumptions and the observed very low minimum concentrations of bacteria (around 10^8 cells $B \text{ dm}^{-3}$), and may be too low. Fenchel (1982b) does not give safe estimates of minimum prey concentrations, but seems to assume higher bacterial densities, while his estimate of gross growth efficiencies of 34 to 43 % as cell carbon fits well with our estimate of 30 % originally derived for marine organotrophic bacteria (Bell and Sakshaug, 1980). Due to the sensitivity tests performed we assume that deviations

in any half-saturation constants had only marginal effects on the simulation.

Recalculations from the optimized yield factors gave corresponding average cell volumes of $0.129 \mu\text{m}^3$ for bacteria, $5.15 \mu\text{m}^3$ for flagellates, and $5140 \mu\text{m}^3$ for ciliates; this is reasonably within the ranges given (Table 3). However, the ratio $1.21 \cdot 10^{-13} \text{ gC } \mu\text{m}^{-3} \text{ cell volume}$, derived for marine bacteria (Watson et al., 1977) which corresponds to approximately 20 % dry weight in the cells, is probably too high for flagellates and ciliates. The corresponding cell volumes are more likely 8 to $10 \mu\text{m}^3$ and 8000 to $10\,000 \mu\text{m}^3$, respectively, as indicated in Fig. 1 for flagellates.

Eberlein et al. (1983) discuss some possible mechanisms for the observed very fast removal of carbohydrates following release by *Skeletonema costatum* in late afternoon during the last 5 d of observations. Even if one assumes a similarly rapid daily cycle in the bacterial population caused by sequential growth and grazing, it is difficult to account for more than 10 to 20 % consumed by bacteria due to their low biomass. Some bacteria attached to algal cells may not have been counted, but epifluorescence microscopy indicated that the diatom cell surface generally was free from bacteria. This is in agreement with recent evidence suggesting that organotrophic bacteria are repelled by the cell surface, and free-living to a large extent within a phycosphere of approximately $100 \mu\text{m}$ diameter (Azam and Ammerman, 1982). Given the low competition from bacteria, organotrophy by nanoflagellates or resorption by *S. costatum* itself seem to have dominated in this case.

In the first 18 d of observations there is a generally good agreement between simulated and observed variables. However, the plateau in total dissolved carbohydrates observed at start could not be reproduced. This probably reflects low availability of predominantly polymeric carbohydrates until sufficient hydrolytic enzyme activities were produced by the bacteria. In the first 4 d the rapid growth of bacteria may, as discussed by Eberlein et al. (1983), have been due to consumption of low molecular weight proteins and amino acids, probably released from plankton biomass through the pressure filtration. These pools were not included as substrates in the model, and the simulation thus points to these sources as being of major importance. Sinking was assumed negligible for the development of heterotrophs, and this is most probably a valid assumption considering the very small particle sizes that dominated before *Skeletonema costatum* developed. No copepod fecal pellets or large particulate debris were present. In a natural system, however, sinking of bacteria associated with particulates may be considered important for removal of bacterial production and included in the calculations.

After Day 18, however, the observed flagellates increased in numbers compared to the simulation, probably due to higher bacterial production (Fig. 4). Lack of grazing by ciliates must also be assumed in that case though, and the previous rapid decrease in flagellate cell numbers therefore explained by other mechanisms. Another more likely explanation is interference in the data from growth of photosynthetic flagellates. The increase was largely due to a rapid growth in the 3.2 to $4.0 \mu\text{m}$ diameter cell population (Fig. 1), while smaller cells did not increase significantly. At that time chlorophyll *a* concentration and net primary production also increased rapidly, mainly due to the growth of *Skeletonema costatum* (Jahnke et al., 1983). There is obvious need for reliable methods to enumerate photosynthetic and non-photosynthetic flagellates separately in field samples.

Growth and removal of bacteria and flagellates are in general very well represented by the model, and the succession of events strongly supports the theory of bacterial grazing by flagellates as being the major mechanism of bacterial removal in this experiment (Eberlein et al., 1983). The simulation also shows that the observed flagellate maximum can be explained from grazing of bacteria only, with growth yields still being within the realistic ranges. Partial growth by phagotrophy on particulate debris or by organotrophy cannot be excluded, however. The nutritional biology of non-pigmented flagellates commonly present in the sea is still open to debate, but as elegantly demonstrated by Fenchel (1982a, b), they most probably are voracious grazers on bacteria by phagotrophy. A number of marine species have now been demonstrated to feed on bacteria in culture (Haas and Webb, 1979; Fenchel, 1982a; Laake et al., in prep.). Nanoflagellates may in turn be efficiently grazed upon by ciliates, copepods and planktonic larvae, and thus represent a missing link in the detrital food chain in marine plankton (Sorokin, 1977; Sieburth et al., 1978; King et al., 1980; Fenchel, 1982d). In our opinion the congruency of experimental and simulated events observed here strongly supports this theory. By applying natural selection of species contained in a controlled ecosystem incubated *in situ* we have also demonstrated that phagotrophic nanoflagellates actually have the potential of controlling bacterial populations in plankton.

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