FACTORS CONTROLLING THE WINTER DOMINANCE OF NANOFLAGELLATES IN SAANICH INLET, BRITISH COLUMBIA

by

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ABSTRACT

The alternation between a summer diatom population and a winter nanoflagellate population in Saanich Inlet is documented. Factors controlling the winter dominance by nanoflagellates, as well as the spring and fall transitions, are considered. Field monitoring of temperature, salinity, light, nitrate and silicate concentrations, and zooplankton size and abundance was conducted for the year November, 1975 to October, 1976 and compared with the pattern of phytoplankton succession during that time. This was supplemented with laboratory experimentation on the effects of light, temperature and photoperiod, as well as metabolic excretions and hydrocarbon pollution, on the growth of diatoms and nanoflagellates in unialgal culture and natural populations.

Factors which were considered to be non-contributory to the winter dominance by flagellates included nutrient concentrations, grazing, excretions, and hydrocarbon pollution. Factors of some importance included temperature, photoperiod and water stability. The single factor of major importance was light intensity. Diatoms were found to be incapable of growth at winter light levels while flagellates were able to do so, due to their ability to maintain themselves high in the water column, and possibly due to a capacity for heterotrophy.

A qualitative model is presented which relates the succession of phytoplankton in Saanich Inlet to temporal changes in various environmental parameters.

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INTRODUCTION

It is recognized that diatoms and nanoflagellates (flagellated phytoplankters less than 20 µm in their greatest diameter[1]) possess certain inherent physiological differences which allow one group or the other to predominate in a given area. Ryther (1969), for example, described three generalized plankton communities from three different zones of the ocean (oceanic, continental shelf and upwelled), each of which was characterized by a specific type of phytoplankton. The low-nutrient, stable conditions of the open ocean, he predicted, would favour nanoflagellate populations, while the nutrient-rich, turbulent environment of upwelled zones would support macrophytoplankton, including large, chain-forming diatoms and dinoflagellates. A continental shelf type of community would be intermediate. On the basis of experiments with diatoms and nanoflagellates Parsons and Takahashi (1973a) similarly predicted that, depending on three major factors--nutrient concentration, stability of the water column, and light intensity--either a diatom or a nanoflagellate community would develop. Other instances may be found in the literature which correlate the relative abundances of diatoms and nanoflagellates (sometimes

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[1] a working definition which I have evolved from that given by Parsons and Takahashi (1973b), citing Dussart (1965). They define "nanoplankton" as plankton within the size range 2-20 µm. Since phytoflagellates smaller than 2 µm were rarely encountered in my work, I have omitted the lower size limit from my definition.

approximated as the ratio of net- and nanoplankton) with specific environmental parameters (e.g., Yentsch and Ryther, 1959; Malone, 1971; Eppley, 1972; Durbin *et al.*, 1975).

Any factor in the environment can potentially regulate the ratio of diatoms to nanoflagellates. However if one were to consider the properties of the two groups some factors would intuitively become more important than others. Clearly the stability of the water column must have some effect. If the water is stable, as is the case of highly stratified environments, the nanoflagellates would benefit by their motility which would enable them to remain within the euphotic zone. In upwelled or otherwise mixed conditions it is not intuitively obvious which group would be favoured, as cells of both diatoms and nanoflagellates could be maintained in suspension. The importance of sinking, upwelling and other mixing phenomena has been discussed by Smayda (1970) and Schöne (1970).

A second factor of intuitive significance is the concentration of silicate in the water. Relatively few nanoflagellates (with the obvious exception of the silicoflagellates, chrysophytess possessing an internal siliceous skeleton) require silicate, while diatoms are clearly dependent on it.

Munk and Riley (1952) predicted that when any nutrient at all is reduced to limiting concentrations, surface area-to-volume relationships permit more efficient uptake by small cells. This prediction, as well as the significance of water stability, is borne out by the observation that the oceanic environment, which is typically stable and oligotrophic, is dominated by nanoflagellates (Wood and Davis, 1956; Holmes *et al.*, 1958; Bernard and Lecal, 1960; Wood, 1963a).

Several other environmental factors have been considered. Yentsch and Ryther (1959) and Durbin *et al.* (1975) observed changes in net- to nanoplankton ratios corresponding to temperature variations off New England. Light intensity has been mentioned as one of three factors thought to be significant by Parsons and Takahashi (1973a). Zooplankton are known to graze selectively with respect to food size (Mullin, 1963; Parsons *et al.*, 1967; Richman and Rogers, 1969; Hargrave and Geen, 1970) and therefore could conceivably regulate phytoplankton cell size. Smayda (1963) stressed the importance of cell metabolites as growth stimulators and inhibitors, and their role in succession. In pollution experiments, low levels of hydrocarbons (Lee and Takahashi, 1977; Lee *et al.*, in press) and copper (Thomas and Seibert, 1977) have been found to favour the growth of nanoflagellates. Some investigators have made the generalization that smaller cells tend to grow intrinsically more quickly than larger cells (Odum, 1956; Williams, 1964; Saijo and Takesue, 1965), although this observation is subject to question (as will be seen in this study).

A complete evaluation of all of these factors has not been attempted for any one location. The purpose of the present investigation was to attempt to do this for Saanich Inlet, B.C. This north temperate fjord has an annual cycle of phytoplankton which is broadly characterized by the presence of nanoflagellates in the winter and diatoms throughout much of the remainder of the year, not an unusual pattern for these waters (e.g., Buchanan, 1966). To simplify the problem, the investigation was restricted primarily to the winter population of nanoflagellates. The question became, "Which factors are critical to the winter dominance of nanoflagellates over diatoms in Saanich Inlet?" and in particular, "What changes occur in the fall and spring which bring about the shift from diatoms

to nanoflagellates and vice versa?" A combination of empirical and experimental approaches was used. All pertinent parameters were measured for a one-year cycle and compared with phytoplankton composition. Those which were considered to show some correlation with the diatom-to-nanoflagellate ratio were further investigated by means of controlled experiments. Factors such as metabolites and pollution effects were studied in laboratory situations only.

Qualitative observations such as these could provide the basis for quantitative modelling and ultimately the ability to manipulate the composition of the phytoplankton in an area such as Saanich Inlet.

DESCRIPTION OF THE STUDY AREA

Saanich Inlet is a fjord located at the southeast end of Vancouver Island (Fig. 1). It is 24 km long and 7.2 km wide at its widest point. There is a submerged sill at the mouth, approximately 75 m deep, behind which the basin deepens to 234 m. Above the sill depth the properties of the water are continuous with those in the approaches, but below the sill depth the water is isolated, oxygen-deficient, and usually contains hydrogen sulphide. Freshwater runoff into the inlet is negligible, and originates mainly from the approaches. It provides a weak, counterclockwise estuarine circulation above the sill depth. The water below the sill is flushed rarely, only when dense water from the approaches is able to cascade over the sill into the deep basin.

A positive salinity gradient, ranging from $14^{o}/oo$ to $29^{o}/oo$ at the surface to $31.2^{o}/oo$ in the deep basin, exists year-round. The waters

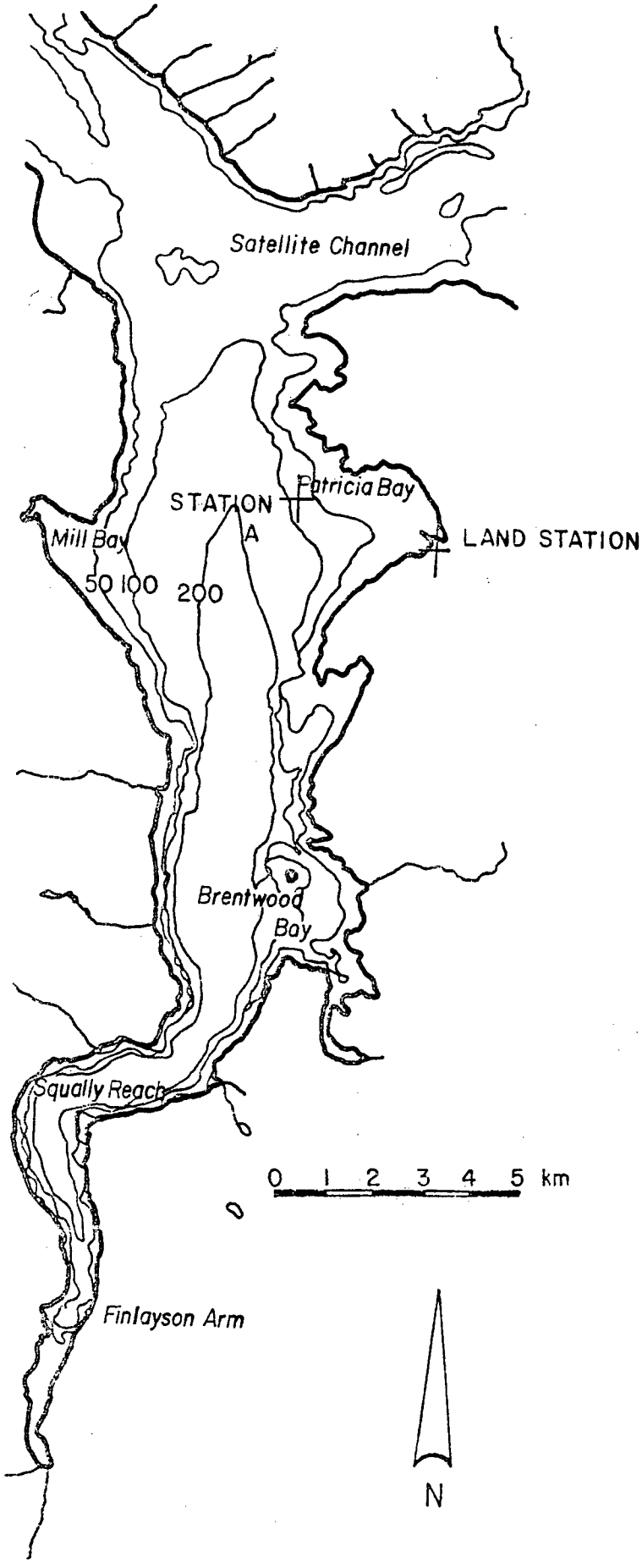

Figure 1. Location of the study area. Depths indicated on the contours are in meters.

of the inlet are thus stratified at all times. A sharp surface pycnocline persists year-round, and is associated chiefly with the thermocline in summer and the halocline in winter. Winds are evidently not sufficient to produce a mixed layer in the inlet. Occasional storms do not result in turbulent mixing, but rather displacement of surface layers, followed by seiche-type oscillations.

The hydrography of Saanich Inlet has been described more completely by Herlinveaux (1962, 1968, 1972).

MATERIALS AND METHODS

A. Field Measurements

Observations of temperature, salinity, light, silicate, nitrate, phytoplankton and zooplankton were made continuously for the year November, 1975 - October, 1976 at station A (Fig. 1).

Total photosynthetically active radiation (PAR) was measured continuously at the land station using a quantum scalar irradiance meter (Booth, 1976), totalled daily and expressed as an average for each 24-hour period. Light penetration into the water column was measured weekly at approximately noon using a 4π quantum meter (Booth, 1976). By combining data on total PAR and penetration it was possible to produce light profiles which approximated daily averages (probably overestimated due to the use of noon light penetration data). *In situ* temperature profiles were taken weekly using a thermistor which was incorporated into the quantum meter assembly.

Water samples for salinity, nutrient and phytoplankton analyses were collected using a diaphragm type pump (JABSCO Products ITT Model 34600), at a flow rate of 1-5 gal/min. Samples were either taken from discrete depths or integrated over desired intervals. Salinity was measured using an Autosal salinometer (Guildline Instruments Model 8400). Nitrate and silicate concentrations were analysed weekly following the methods given by Strickland and Parsons (1972). Water for both analyses was prefiltered through Gelman type A-E glass fibre filters. Nitrate was always measured immediately following water sampling; water for silicate analysis was frozen if not measured immediately.

Phytoplankton samples for floristics analysis were preserved with Lugol's iodine, concentrated by settling (Lund *et al.*, 1958; Utermöhl, 1958; Uehlinger, 1964), and counted using inverted, phase microscopy. Cell counts were converted to carbon according to Strathman (1967).

Zooplankton were sampled from 25-0 m using 20 cm bongo nets fitted with 202 µm Nitex mesh, preserved with 10% buffered formalin, split (if required) using a Folsom plankton splitter, and counted under a dissecting microscope.

B. Experimental

Natural phytoplankton populations and all seawater used were collected by diaphragm pump as described previously. Seawater used for culture media was filtered through 0.45 µm Millipore filters and autoclaved for 1 hour at low temperature and pressure to avoid precipitation of silicate. Unialgal cultures of the following species were maintained in E.S. Medium (Provasoli, 1968; Appendix 1) at $12^{o}C \pm 1C^{o}$, illuminated from the side by cool white fluorescent lamps (3-5 klux or 70-120 µE m^{-2} sec^{-1}) on a 16-$\bar{8}$ (16 h light, 8 h dark) light-dark cycle:

Species	Source of Culture	Strain No.
Bacillariophyceae		
Skeletonema costatum (Grev.) Cleve	(1)	NEPCC 18
Thalassiosira nordenskioldii Cleve	(1)	NEPCC 252
Thalassiosira sp. 1	(2)	
Thalassiosira sp. 2	(2)	
Prymnesiophyceae (=Haptophyceae)		
Chrysochromulina kappa Parke & Manton	(1)	NEPCC 188
Cryptophyceae		
Chroomonas salina (Wisl.) Butcher	(2)	
Cryptomonas profunda Butcher	(1)	NEPCC 65
Dinophyceae		
Katodinium rotundatum (Lohm.) Loeblich III	(1)	NEPCC 44
Euglenophyceae		
Eutreptiella cf. *gymnastica* Throndsen	(1)	NEPCC A2
Eutreptiella sp.	(2)	

(1) Northeast Pacific Culture Collection (NEPCC), UBC

(2) Saanich Inlet, isolated by author

Bioassays were conducted to test the effects of metabolites and hydrocarbons, neither of which was monitored in the field, on diatoms and flagellates. In addition, experiments on the effects of light intensity, photoperiod and temperature were performed to assess their roles in determining phytoplankton composition in the winter.

1. Metabolite experiment

The purpose of this experiment was to determine whether or not the presence of a diatom population stimulated or inhibited the growth of

a flagellate population (and vice versa) in the presence of excess nutrients and otherwise favourable conditions for growth. The experimental set-up is shown in Fig. 2. Sections of Fisherbrand cellulose dialysis tubing (pore size 4.8 nm or approximately 12,000 daltons molecular weight) were cleaned[1] and sealed at one end. These were filled with 150 ml of cultures of either *Thalassiosira nordenskioldii* or *Chrysochromulina kappa*. Another dialysis tubing "bag" was filled with equal amounts of *Thalassiosira* and *Chrysochromulina*. The total biomass (as measured by *in vivo* fluorescence) was approximately equal in all "bags." These were sealed with plastic clips and suspended in clean 5% E.S. medium (Appendix 1) in 4 1-l beakers as follows:

beaker 1: *Thalassiosira* alone

beaker 2: *Chrysochromulina* alone

beaker 3: *Thalassiosira* and *Chrysochromulina* in separate bags

beaker 4: *Thalassiosira* and *Chrysochromulina* mixed in the same bag

Stirrers were added to the beakers to prevent the formation of gradients near the bag walls. All cultures were then allowed to grow under continuous illumination by fluorescent light at 3-5 klux (70-120 $\mu E\ m^{-2}\ sec^{-1}$) at $12^{o}C$

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[1] Dialysis tubing cleaning procedure, used to remove heavy metals and UV-absorbing impurities:

1. Boil tubing 3 h in 5% Na_2CO_3.
2. Rinse in running tap water.
3. Repeat.
4. Boil in 50 mM EDTA, pH 8.0.
5. Store in 0.1 mM EDTA.
6. Before use, boil and rinse in distilled water.
7. Handle with gloves to prevent contamination.

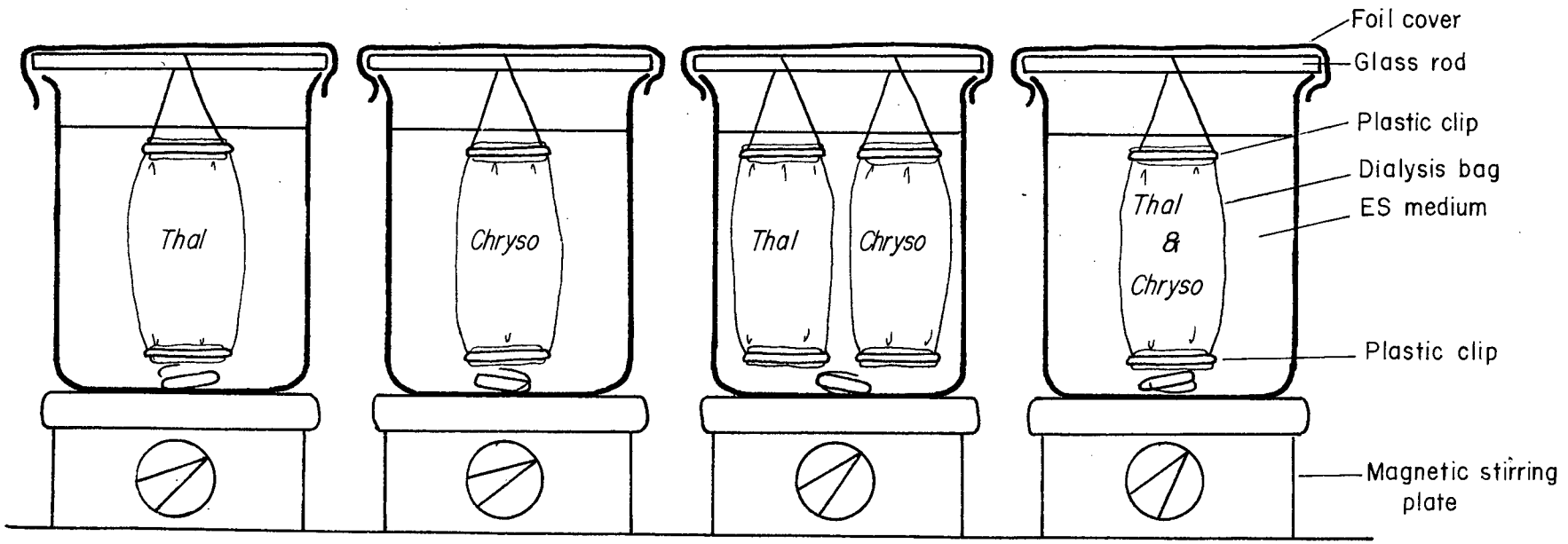


Figure 2. Set-up for the metabolite experiment.

± 1C°. Bags containing *Thalassiosira* were mixed once daily by inverting gently several times, in order to prevent settling of the cells. *In vivo* fluorescence was measured daily using a Turner Model 111 fluorometer and growth rates calculated from the equation (Parsons and Takahashi, 1973b):

$$\mu = \frac{1}{t} \log_2 \left(\frac{N_t}{N_o} \right) \qquad (1)$$

where N_o is the initial biomass as estimated by *in vivo* fluorescence (or cell counts), N_t is the biomass at time t, and μ is the specific growth rate in doublings per day. For the mixed culture cell counts were performed daily using inverted, phase microscopy, and growth rates calculated from equation (1).

2. Hydrocarbon experiment

Bioassays tested the effects of No. 2 Fuel Oil (a crude oil) and 1-methylnaphthalene (a member of the toxic naphthalene fraction of oils) on the growth of diatoms and nanoflagellates in culture and in mixed natural populations. Species assayed in culture included the diatoms *Thalassiosira* sp. 1 and *Skeletonema costatum*, and the flagellates *Chroomonas salina*, *Cryptomonas profunda*, *Eutreptiella gymnastica*, *Eutreptiella* sp. and *Katodinium rotundatum*. The natural population was obtained by Niskin bottle from 1 m at station "A" in Saanich Inlet, in November, 1976.

A seawater extract of the No. 2 Fuel Oil was made by adding 4 ml of the fuel oil to 200 ml of autoclaved, filtered sea water in a 500 ml flask and stirring gently for 12 h. The mixture was transferred to a separatory funnel and allowed to separate for 2 h, after which time the water phase was drawn off, giving a final concentration of 9 mg volatile

hydrocarbon per litre (Lee, personal communication).

1-methylnaphthalene (Eastman-Kodak, Rochester, New York) was diluted with ethanol to give a stock solution of 1 μg μl^{-1}. In a separate assay ethanol was shown to have no measurable effect on the algae up to the maximum concentration used, 960 μl/200 ml.

The 1-methylnaphthalene and fuel oil extract were added to sterile glass bottles containing the cultures (or natural sea water) and immediately sealed. Final hydrocarbon concentrations ranged from 0 to 4800 μg l^{-1} 1-methylnaphthalene and 0 to 500 μg l^{-1} No. 2 Fuel Oil.

All bottles were incubated at 12°C, under fluorescent light, 25-30 klux (350-425 μE m^{-2} sec^{-1}), with a 12-$\overline{12}$ photoperiod. *In vivo* fluorescence was measured daily, and microscope counts were made on the natural seawater samples at the beginning and end of the experiment. Growth rates were calculated from equation (1) (see metabolite experiment).

3. Light and temperature experiments

Bioassays tested the effects of varying light intensity, photoperiod and temperature on the growth of diatoms and nanoflagellates in pure culture, and of natural summer and winter populations.

a. *Pure cultures.* Species used included the diatoms *Skeletonema costatum* and *Thalassiosira* sp. 1 and 2 and the nanoflagellates *Katodinium rotundatum*, *Eutreptiella* sp., *Cryptomonas profunda* and *Chrysochromulina kappa*. To avoid any possible effects of the unnaturally high nutrient concentrations present in culture medium (see, for example, Maddux and Jones, 1964; McAllister *et al.*, 1964), the experiments were run using 5% E.S. medium (nitrate, approximately 27 μg-at N l^{-1}), plus 10% silicate mixture for diatoms (approximately 99 μg-at Si l^{-1}; see Appendix 1 for concentrations of other nutrients). Cells were grown to logarithmic phase (5 to 10 days)

in the dilute medium. Light intensities at this time ranged from 2.7 to 3.9 klux (39 to 55 μE m^{-2} sec^{-1}) for *Katodinium*, *Cryptomonas* and *Chrysochromulina*, and 5.0 to 5.2 klux (72 to 74 μE m^{-2} sec^{-1}) for *Skeletonema*, *Thalassiosira* and *Eutreptiella*.

During the experimental period, various light levels were produced by fitting light screens consisting of one or more layers of black or white netting over the bottom of 125-ml BOD bottles and blackening the rest of the bottle. The resulting light intensities inside the bottles ranged from 100% to 0.50% of the incident light, or 27 to 0.14 klux (388 to 1.94 μE m^{-2} sec^{-1}) in the incubator described below.

One hundred and twenty ml aliquots of the cultures were dispensed into the bottles and incubated at 12°C or 6°C, illuminated from below by a bank of 6 cool white 40w fluorescent lamps with a 12-$\overline{12}$ photoperiod. *In vivo* fluorescence was measured daily and growth rates calculated (equation 1). In the 12°C experiment, pigments (spectrophotometric method--Strickland and Parsons, 1972), ^{14}C productivity, and cell size and appearance were analysed on day 0 and after the cells had reached logarithmic phase of their growth in their new light regimes.

b. *Natural populations*. Water was collected from station "A" from 1 m on December 1, 1976, 15 m on May 5, 1977, and 5 m on August 19, August 25, and September 8, 1977. With the exception of the December 1, 1976 collection (in which nutrient concentrations were high--nitrate >20 μg-at N l^{-1}, silicate >50 μg-at Si l^{-1}) the water was enriched with 5% E.S. medium with silicate (nitrate *ca.* 20 μg-at N l^{-1}, silicate *ca.* 49 μg-at Si l^{-1}). One hundred and twenty ml aliquots were dispensed into the BOD bottles described above and incubated as summarized in Table I, illuminated as before from below by a bank of 6 cool white 40w fluorescent lamps.

Table I. Summary of light and temperature experiments using natural phytoplankton populations.

Experiment	Source of Water			T°C	Photoperiod	Culture Medium	Duration of Experiment (days)
1	Stn.A	1m	1/12/76	12	12-$\overline{12}$	none added	8
2	Stn.A	15m	12/5/77	12	24- $\overline{0}$	5% E.S.+Si	4-14
3	Stn.A	5m	19/8/77	12	12-$\overline{12}$	5% E.S.+Si	5
4	Stn.A	5m	25/8/77	6	12-$\overline{12}$	5% E.S.+Si	8
5	Stn.A	5m	8/9/77	6	12-$\overline{12}$	5% E.S.+Si chemostat	25

In the fifth experiment (Table I) a crude chemostat system was used in order to maintain steady, non-nutrient-limited growth. A 4-ml aliquot from each sample was removed daily and replaced with an equal quantity of E.S. medium with silicate (an addition of 18, 33 and 1 μM of nitrate, silicate and phosphate respectively). At 1-week intervals, larger volumes were removed (10 ml on days 7 and 21, 28 ml on day 14) and replaced with 5% E.S. medium with silicate. Growth was maintained in this way for 25 days.

Measurements of *in vivo* fluorescence were taken daily and growth rates calculated (equation 1) for all 5 experiments. Floristics analyses were performed at the beginning and the end of all experiments, and additionally on days 7 and 14 for experiment no. 5.

RESULTS

A. Field Observations

1. Temperature and salinity

Fig. 3 shows the temperatures in the top 20 m at station "A" for the year November, 1975 to October, 1976. There was a significant seasonal variation at all depths, ranging from a mean of $7^{o}C$ for the top 20 m in the winter to about $12^{o}C$ in the summer.

Salinity profiles (Fig. 4) show that there was a year-round gradient in salinity in the top 20 m, which would tend to maintain the stability of this layer. In winter and early spring there was a layer of markedly lower salinity in the top 2 m which disappeared during the summer.

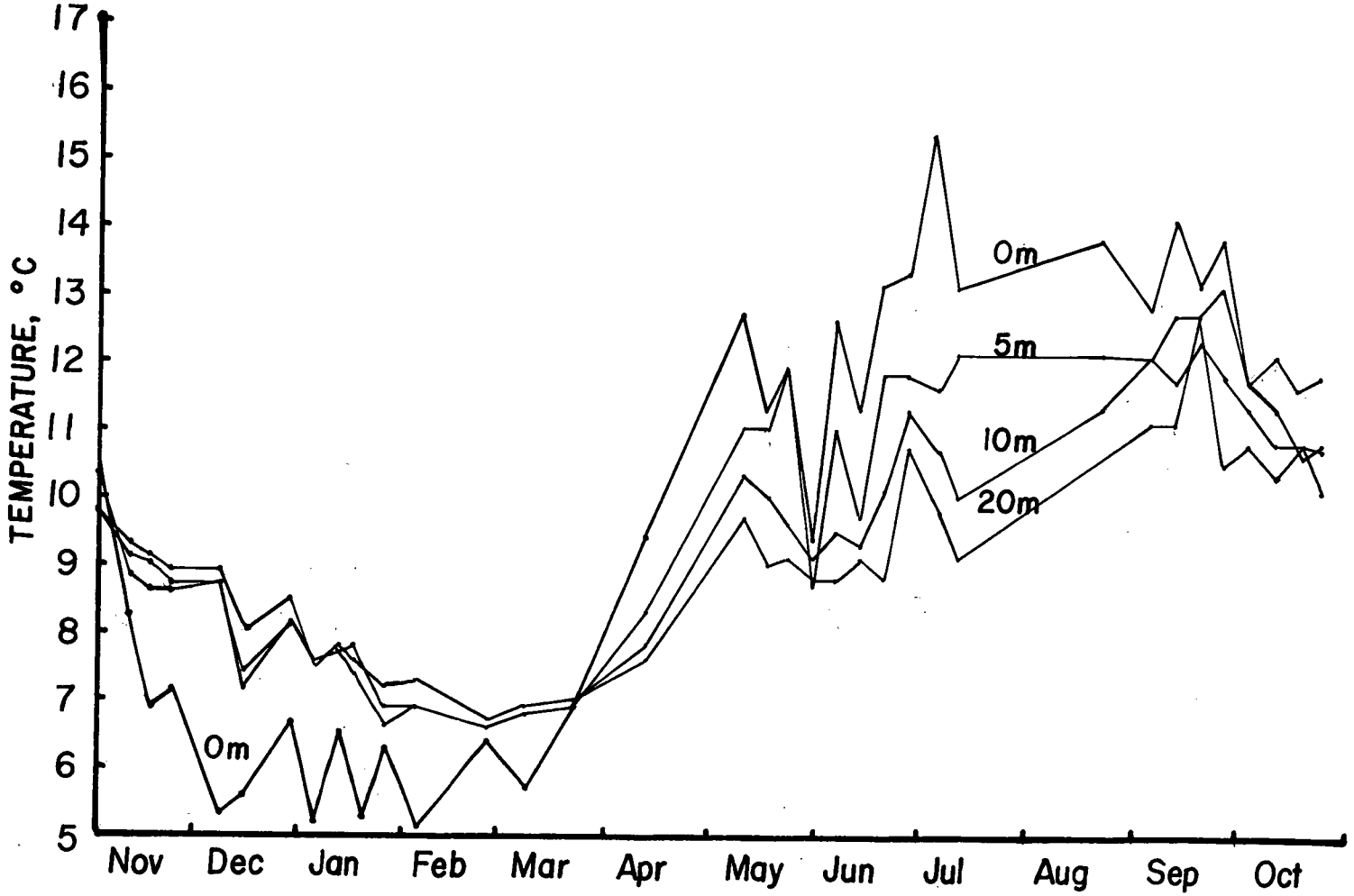


Figure 3. Temperatures at 0, 5, 10, and 20 m at station "A," November, 1975 - October, 1976.

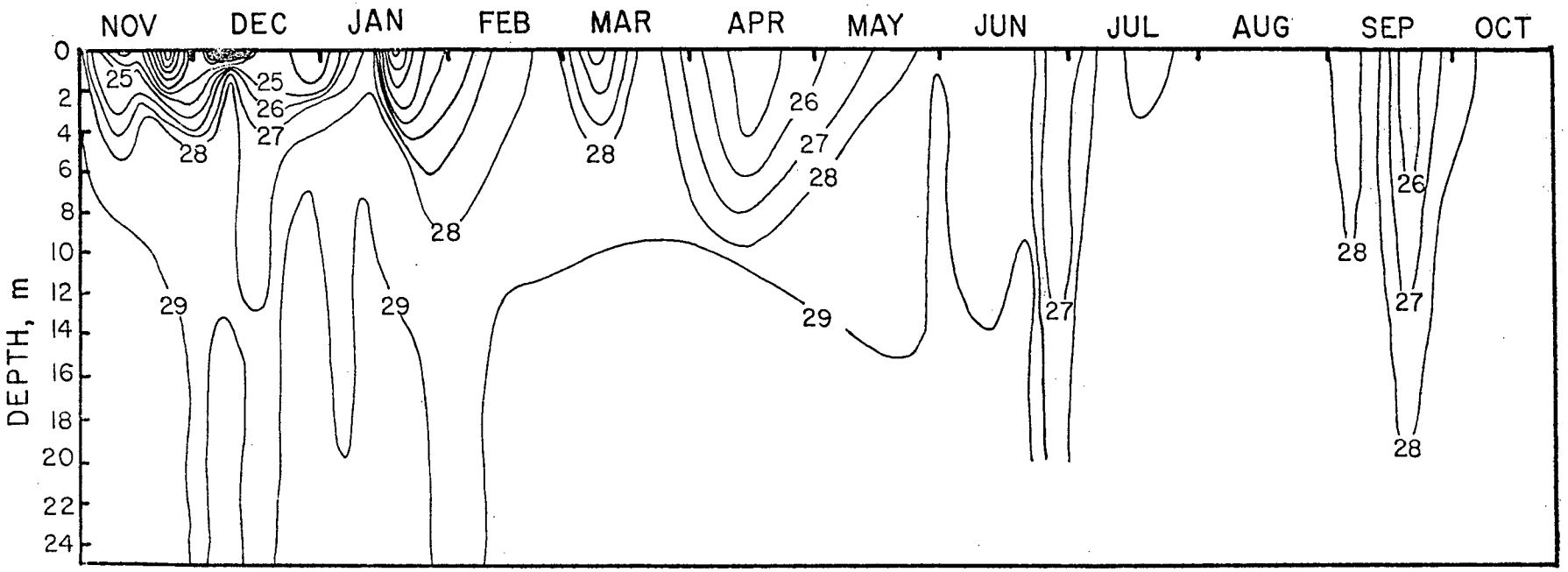


Figure 4. Salinity structure at station "A," November, 1975 - October, 1976. Salinities are shown in parts per thousand.

This layer was 1 to 2C^{o} cooler than the underlying water and probably originated as river runoff (and direct precipitation). It was physically distinct from the subsurface water, as evidenced by the sharp temperature and salinity clines at the boundary.

2. Light

PAR varied seasonally over an order of magnitude, from a winter minimum of 20 μE m^{-2} sec^{-1} to a summer maximum of 200 μE m^{-2} sec^{-1} (Fig. 5). Winter water was generally much clearer than summer, but because of the large difference in incident light, the quanta available at a given depth was much less (Fig. 6). The only exceptions to this were during intense summer blooms when the turbidity occasionally rose enough to reduce light at 20 m to winter levels. These were strictly short-term reductions, however.

3. Nutrients

Nitrate, silicate and phosphate concentrations were high and uniform with depth all winter (greater than 20 μg-at l^{-1}, 40 μg-at l^{-1} and 1.5 μg-at l^{-1} respectively in the top 20 m; see Fig. 7,8). Beginning in March surface levels showed a rapid decline and remained low until September when they gradually began to increase to winter levels. Occasional upwelling events following storms renewed surface nutrient supplies during the summer.

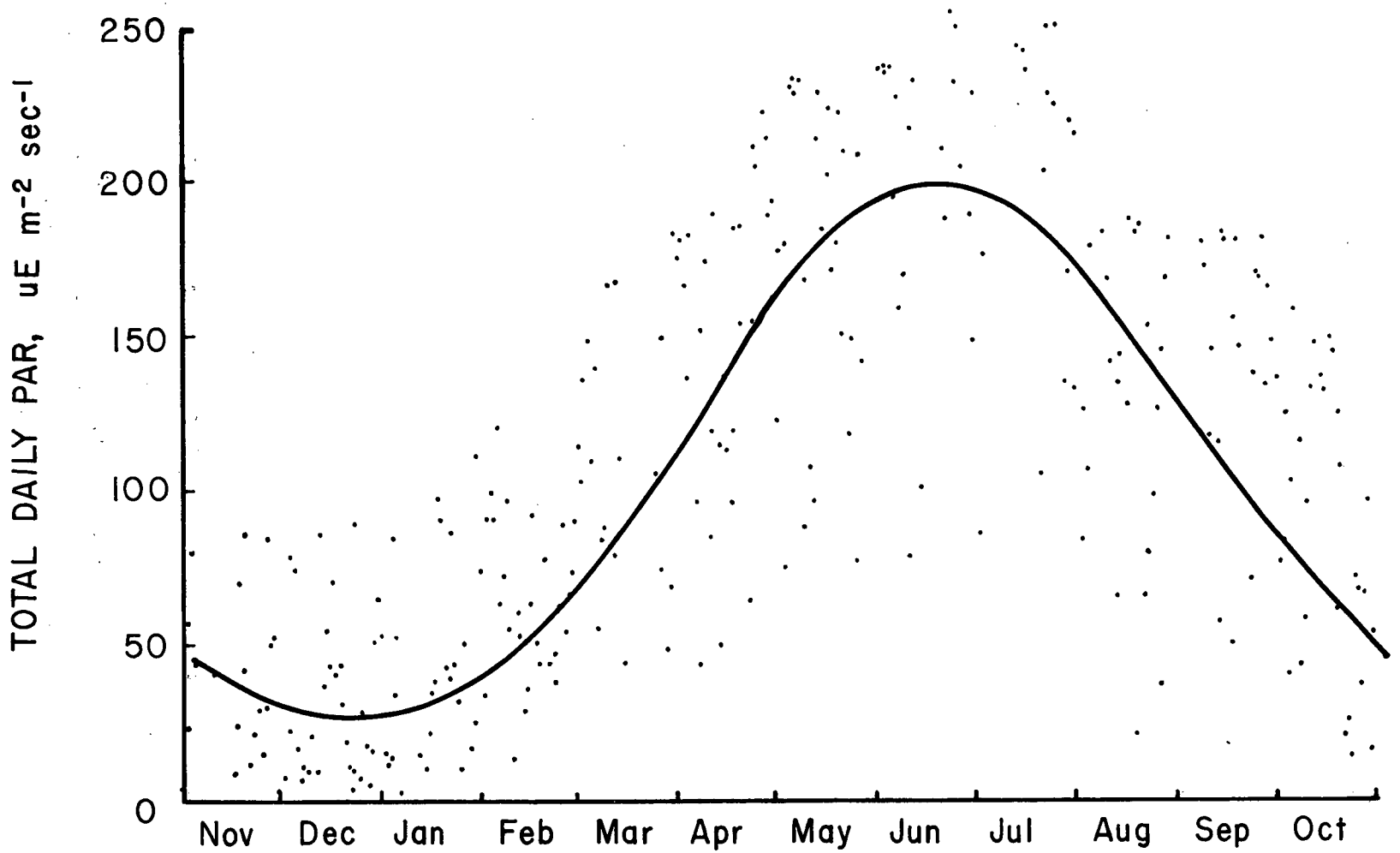


Figure 5. Total daily photosynthetically active radiation (PAR), 400 - 700 nm, measured at the land station, November, 1975 - October, 1976.

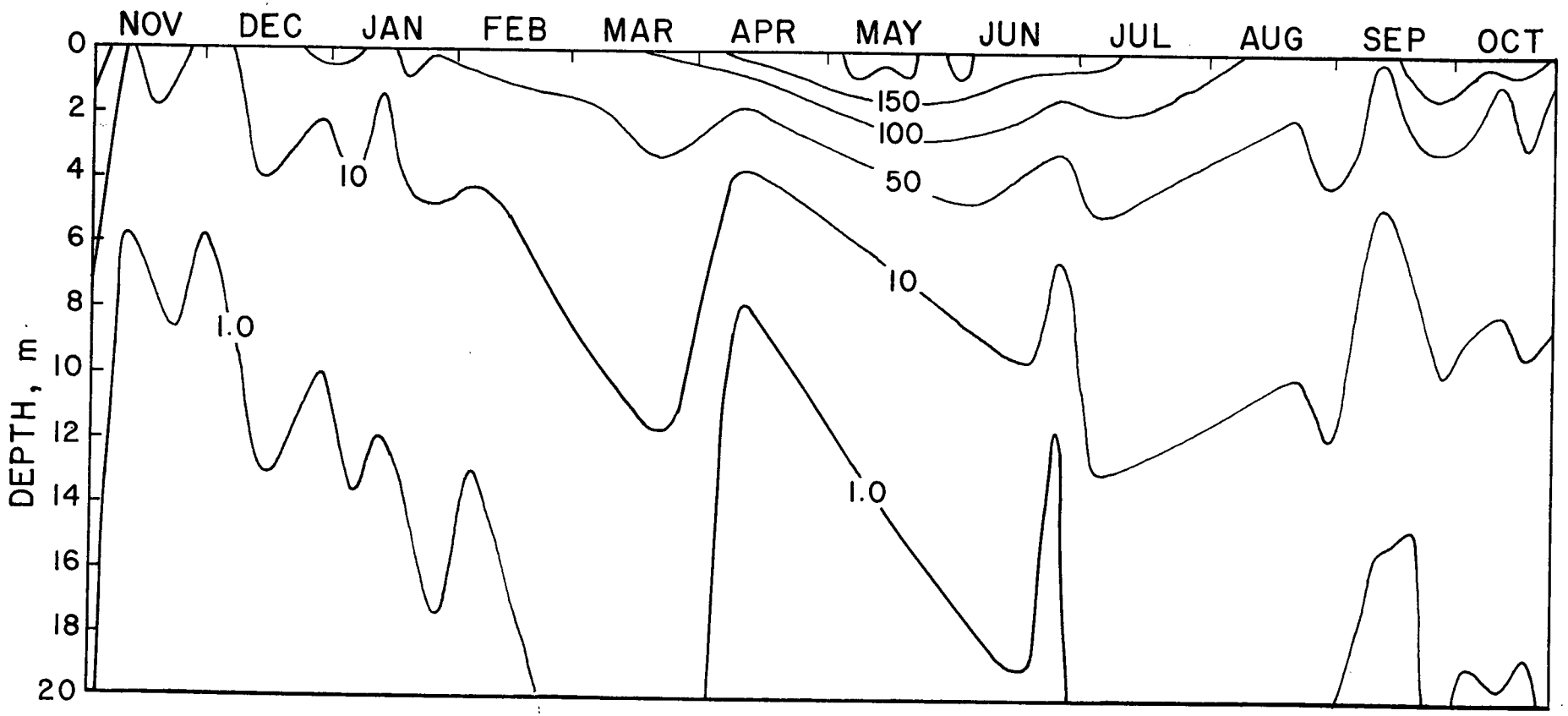


Figure 6. Depth profiles of light intensity ($\mu E\ m^{-2}\ sec^{-1}$) at station "A," November, 1975 - October, 1976.

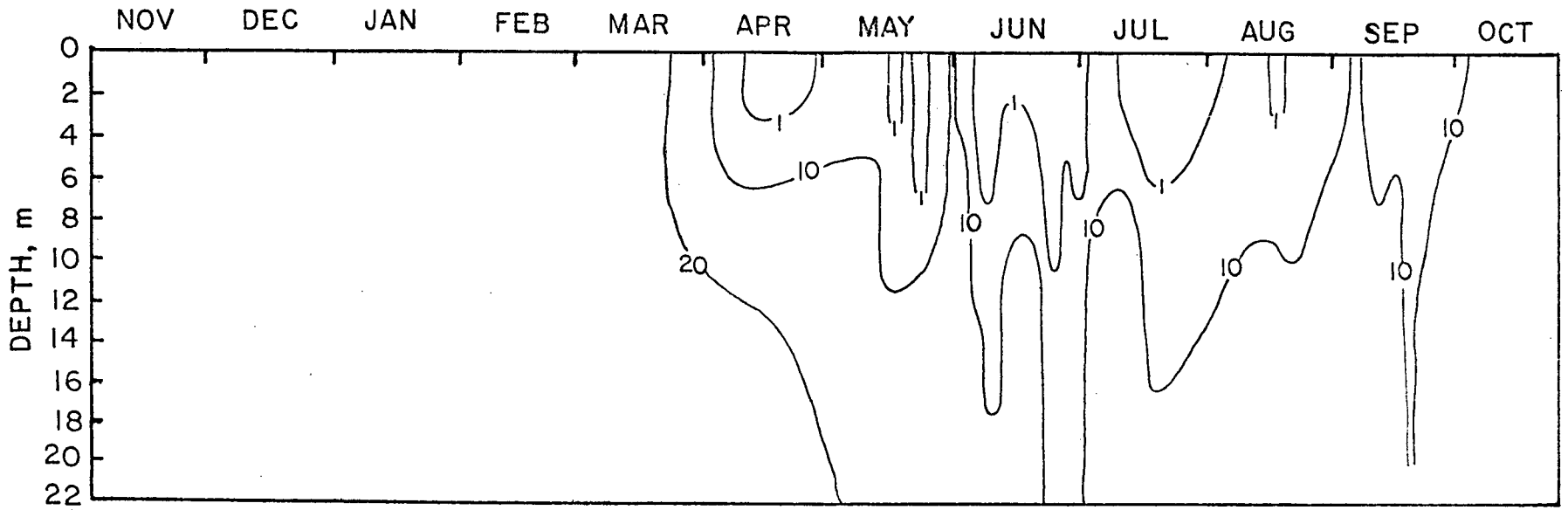


Figure 7. Depth profiles of nitrate concentration (μg-at N l^{-1}) at station "A," November, 1975 - October, 1976.

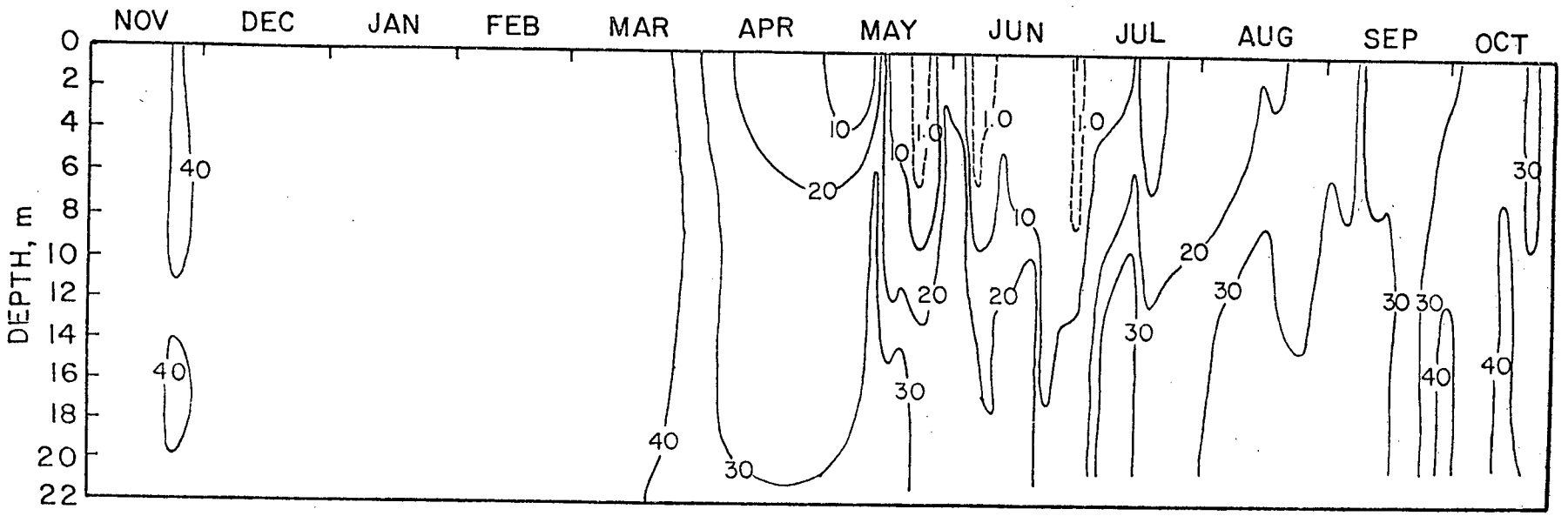


Figure 8. Depth profiles of silicate concentration (μg-at Si l^{-1}) at station "A," November, 1975 - October, 1976.

4. Phytoplankton

Diatoms and nanoflagellates alternately form the dominant Saanich Inlet phytoplankton in summer and winter respectively (Fig. 9). Larger flagellates, chiefly dinoflagellates, seldom dominate the phytoplankton, and although they formed, at times, as much as 40% of the total biomass during the summer of 1976, they were always outnumbered by diatoms.

Fig. 9 shows the pattern of phytoplankton biomass composition by group. The end of the 1975 cycle of diatom dominance is seen on November 4. This was followed by a rapid decline in the diatom population which did not recover again until mid-April, 1976. The winter (November to March) nanoflagellate population included a mixture of cryptomonad genera, including *Cryptomonas*, *Chroomonas*, *Hemiselmis* and *Plagioselmis*, haptophytes --*Chrysochromulina* and *Dicrateria*, the prasinophyte *Pyramimonas*, the chrysophyte *Apedinella*, and a small dinoflagellate, *Katodinium rotundatum*. The biomass all winter was very low (10 µg C l^{-1}, 5 µg chl *a* l^{-1} or less). The beginning of the spring bloom was marked by a sharp increase in the nanoflagellate biomass in early March, followed by a diatom (and, secondarily, dinoflagellate) increase in early April which rapidly outgrew the flagellate bloom. This initial diatom burst was dominated by *Thalassiosira* spp. (68.7%), *Skeletonema costatum* (11.2%), and *Chaetoceros* spp. (3.0%). By mid-May, *Chaetoceros* spp. comprised more than 90% of the total biomass and remained dominant for most of the summer (Fig. 10). Following the diatom crash at the end of August the population was briefly dominated by flagellates, although the total phytoplankton biomass was very low. A final autumn diatom bloom, composed exclusively of *Chaetoceros* spp., occurred in September, and by mid-October the winter phytoplankton community was re-established (Fig. 10).

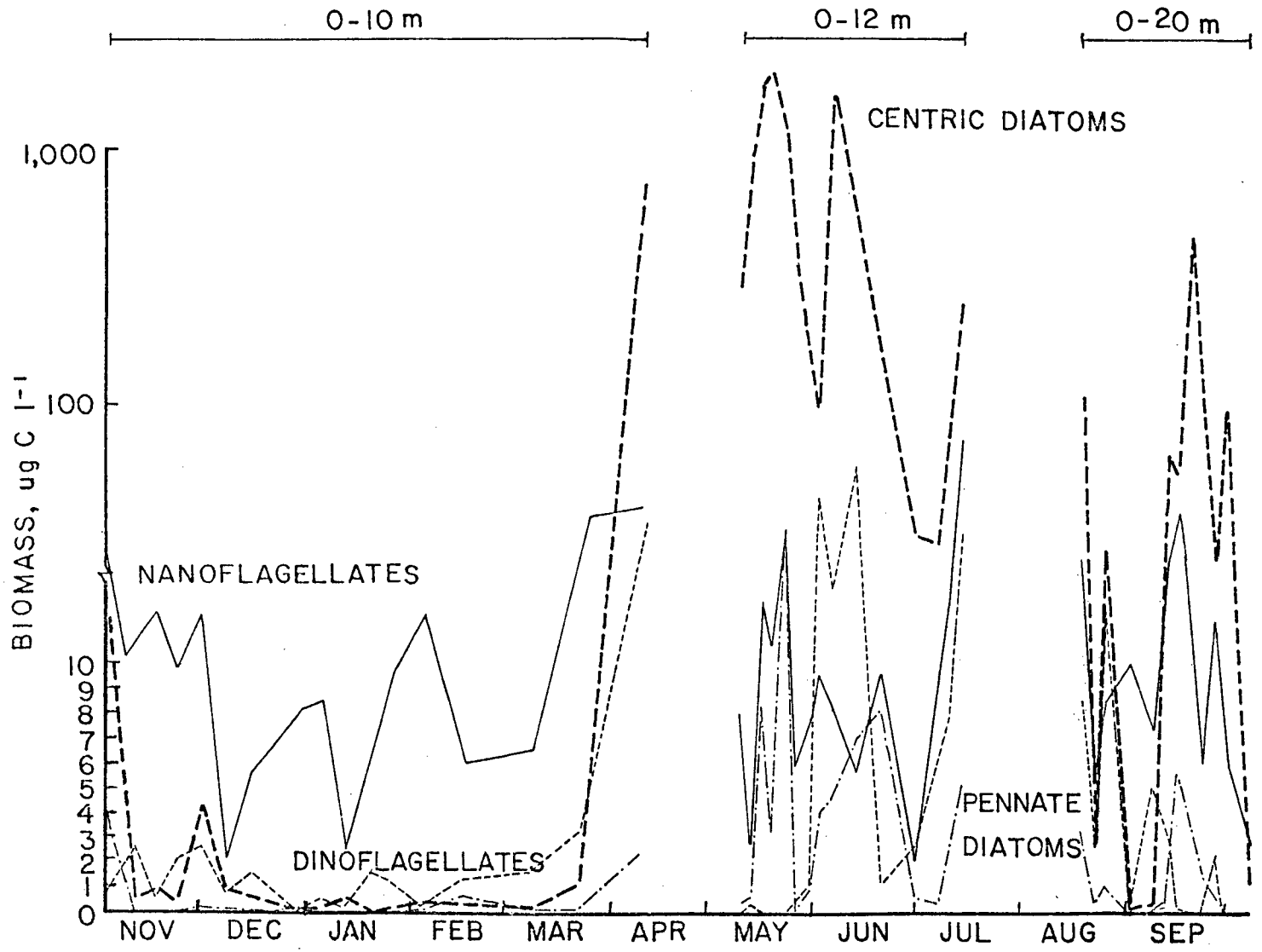


Figure 9. Biomass of major phytoplankton groups at station "A," November, 1975 - October, 1976. Bars indicate depths over which samples were taken at various times of the year.

—— nanoflagellates

— — — centric diatoms

—·—·— pennate diatoms

------- dinoflagellates

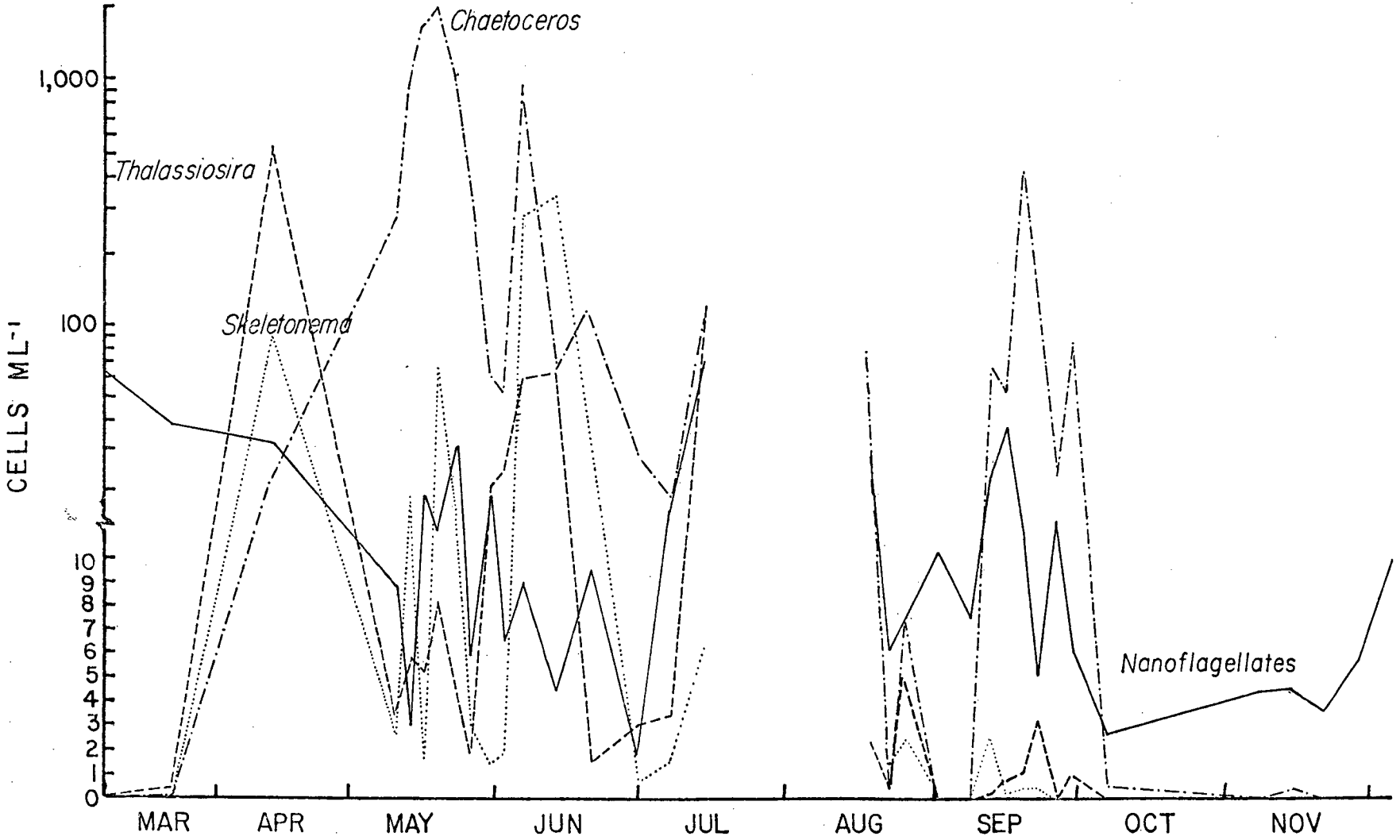


Figure 10. Species succession in phytoplankton at station "A," March - December, 1976. Bars indicate depths over which samples were integrated at different times of the year. ——— nanoflagellates, —·—·— *Chaetoceros*, ------ *Thalassiosira*, ····· *Skeletonema*.

5. Zooplankton

Fig. 11 shows abundances over the year December, 1975 - December, 1976 for three size classes of copepods (defined in Table II). Copepods smaller than 2.0 mm in length were present year-round but were most abundant during the period April - November. Copepods larger than 2.0 mm were present only from March to November; abundances greater than 100 m^{-3} occurred only from April through August and again in late October and early November. The periods of greatest abundance for small copepods and of presence for large copepods correlate with and lag slightly behind the summer and fall blooms of phytoplankton.

B. Experimental

1. Metabolite experiment

Fig. 12 shows the growth of *Thalassiosira nordenskioldii* and *Chrysochromulina kappa* when grown separately (physically and chemically), together (physically and chemically), and together chemically but separated physically. When the two species were separated physically the growth rates in both cases were unchanged whether or not they were allowed to interact chemically. *Thalassiosira* grew slightly faster than *Chrysochromulina* (doubling rates were 1.82-1.87 and 1.38-1.44 doublings day^{-1} respectively). When the two species were mixed together (in the same dialysis bag), *Thalassiosira* grew more quickly than it had alone, while *Chrysochromulina* grew more slowly (growth rates were 2.56 and 1.13 doublings day^{-1} respectively). However, *Thalassiosira* senesced in the mixed culture three days after the initiation of the experiment, while *Chrysochromulina* maintained logarithmic growth until the conclusion of the experiment. Thus it appears that intimate contact of cells of the two species may affect their growth rates while simply sharing the same medium does not.

Table II. Copepod size classification (based on body lengths obtained from Fulton, 1972).

Body Length	Species and Stages
<1.0 mm	*Pseudocalanus minutus* I-III
	Paracalanus parvus I-V
	Metridia pacifica I-II
	Corycaeus cf. *anglicus*
1.0-2.0 mm	*Calanus* spp. I-III
	Metridia pacifica III-V
	Pseudocalanus minutus IV-adult
	Paracalanus parvus adult
	Microcalanus sp.
	Scolecithricella
	Acartia
	Tortanus
	Oithona
	Oncea
	Centropages
>2.0 mm	*Calanus* spp. IV-adult
	Metridia pacifica adult
	Epilabidocera

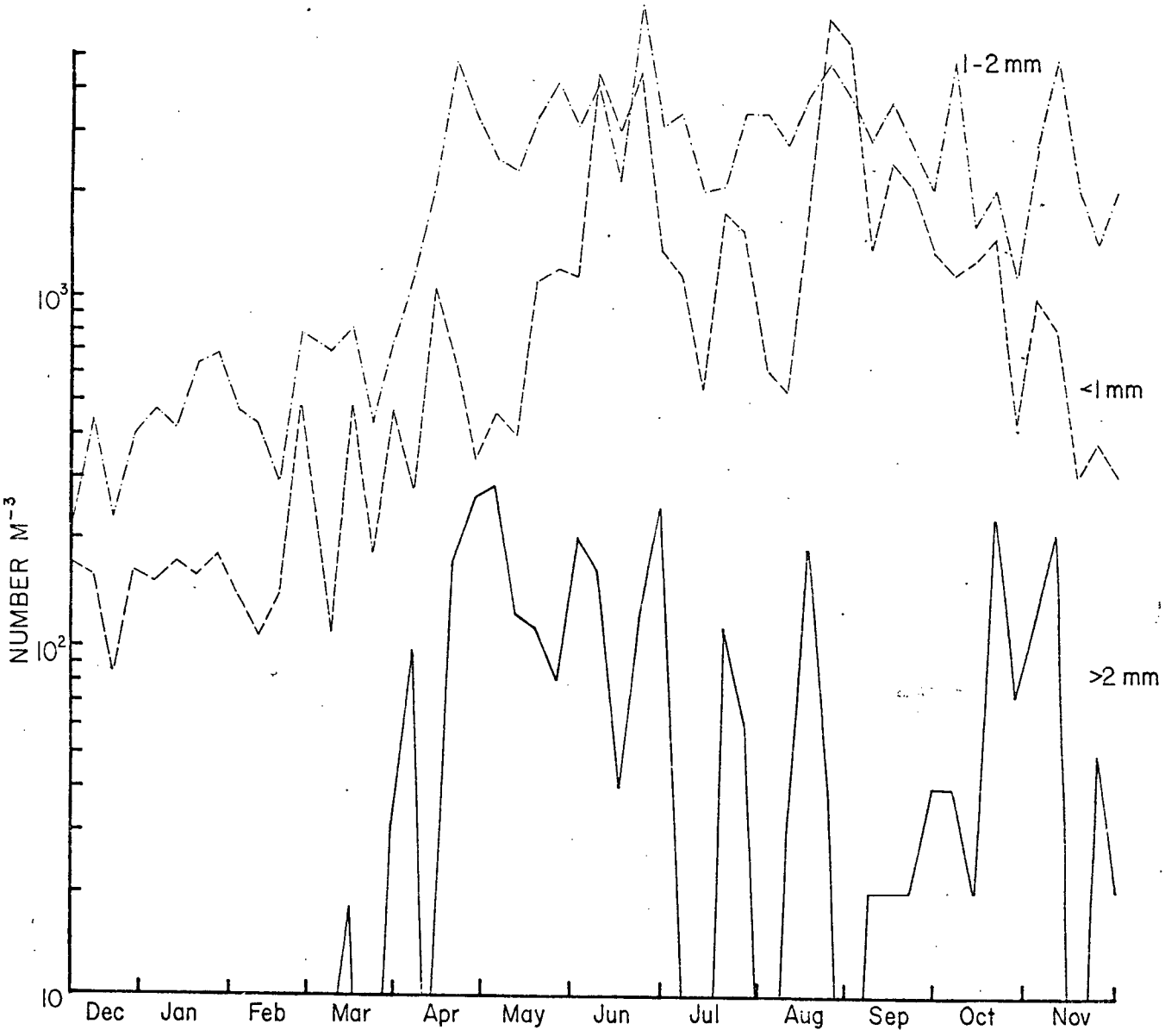


Figure 11. Abundance of three size classes of copepods sampled from 25-0 m at station"A," December, 1975 - December, 1976. ----- <1.0 mm, -·-·- 1.0-2.0 mm, ——— >2.0 mm total body length.

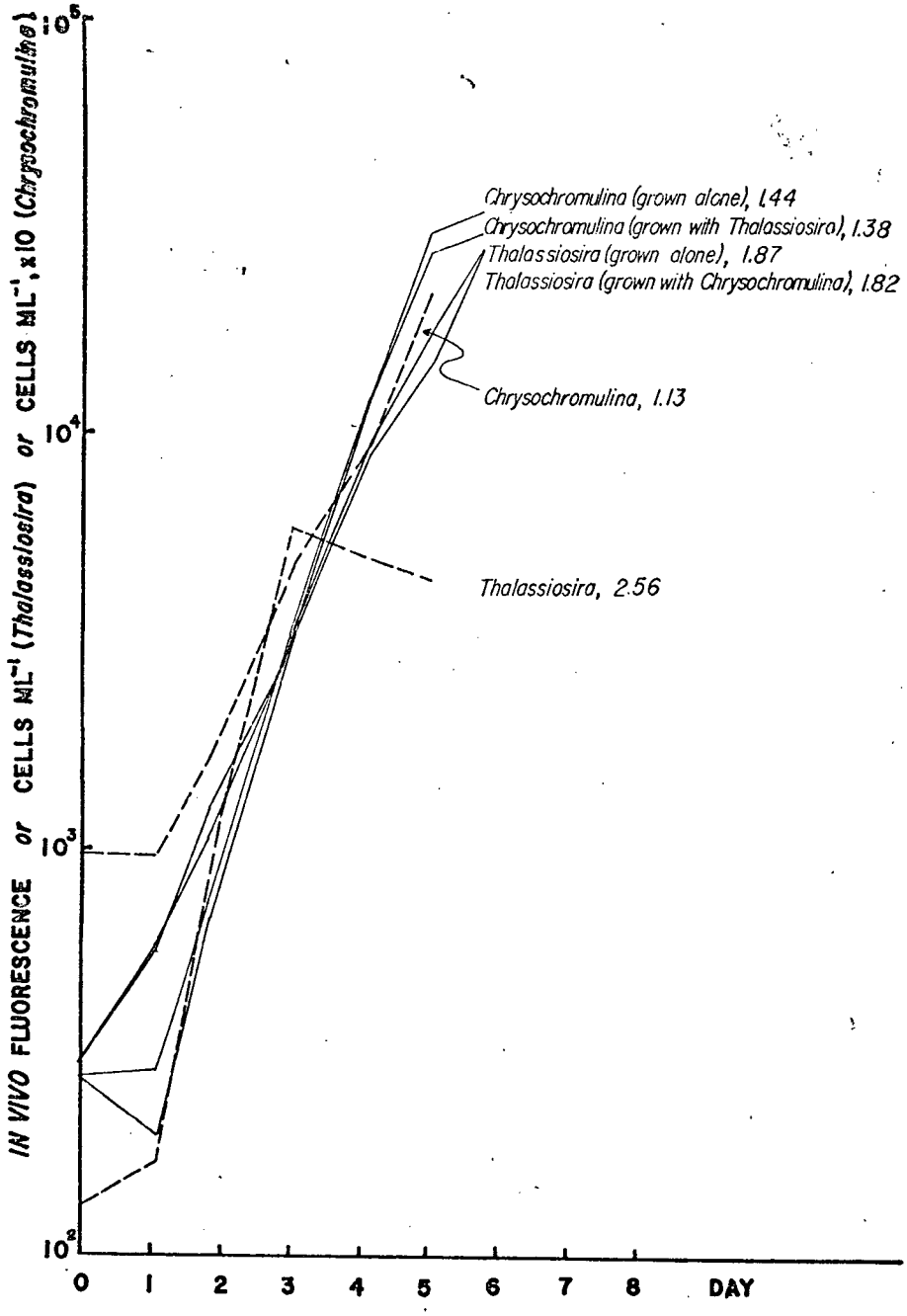


Figure 12. Growth of *Chrysochromulina kappa* and *Thalassiosira nordenskioldii* in the metabolite experiment. ——— *in vivo* fluorescence, measured for cells grown in individual dialysis bags, ----- cell count, measured for cells in mixed culture.

2. Hydrocarbon experiment

Hydrocarbons affected two aspects of growth in phytoplankton: they inhibited (or occasionally stimulated) the doubling rate of the cells during logarithmic growth, or they influenced the length of the lag phase of growth prior to the onset of logarithmic growth (see Fig. 13). In all but one instance (experiments involving the dinoflagellate *Katodinium rotundatum*), 1-methylnaphthalene had the former effect only, while No. 2 Fuel Oil affected both aspects of growth. It is likely that inhibition of the logarithmic doubling rate of the cells represents a true toxic effect of the hydrocarbon resulting in an inability to reproduce at a normal rate, while the delay of growth represents a waiting period during which the cells manage to resist the toxic effects of the hydrocarbon until volatilization, removal by bacterial activity, or adsorption has reduced the concentrationstooanacceptableelevel. The latter situation would be expected to arise more often with a hydrocarbon mixture (as in the case of a fuel oil) than with a pure compound: one can envision the volatile toxic aromatic fractions being removed after a few days (depending on initial concentration), followed by phytoplankton growth limited by the presence of less toxic, less volatile naphthalenes.

In Fig. 14 doubling rate has been plotted against hydrocarbon concentration for individual species and the natural population. The duration of the lag phase is plotted for No. 2 Fuel Oil only. From the monospecific experiments it can be seen that despite the small number of species used there was not general enhancement of flagellate species with respect to diatom species, either in terms of logarithmic doubling rate or duration of the lag phase, involving 1-methylnaphthalene or No. 2 Fuel Oil. No flagellates were ever stimulated by either hydrocarbon relative

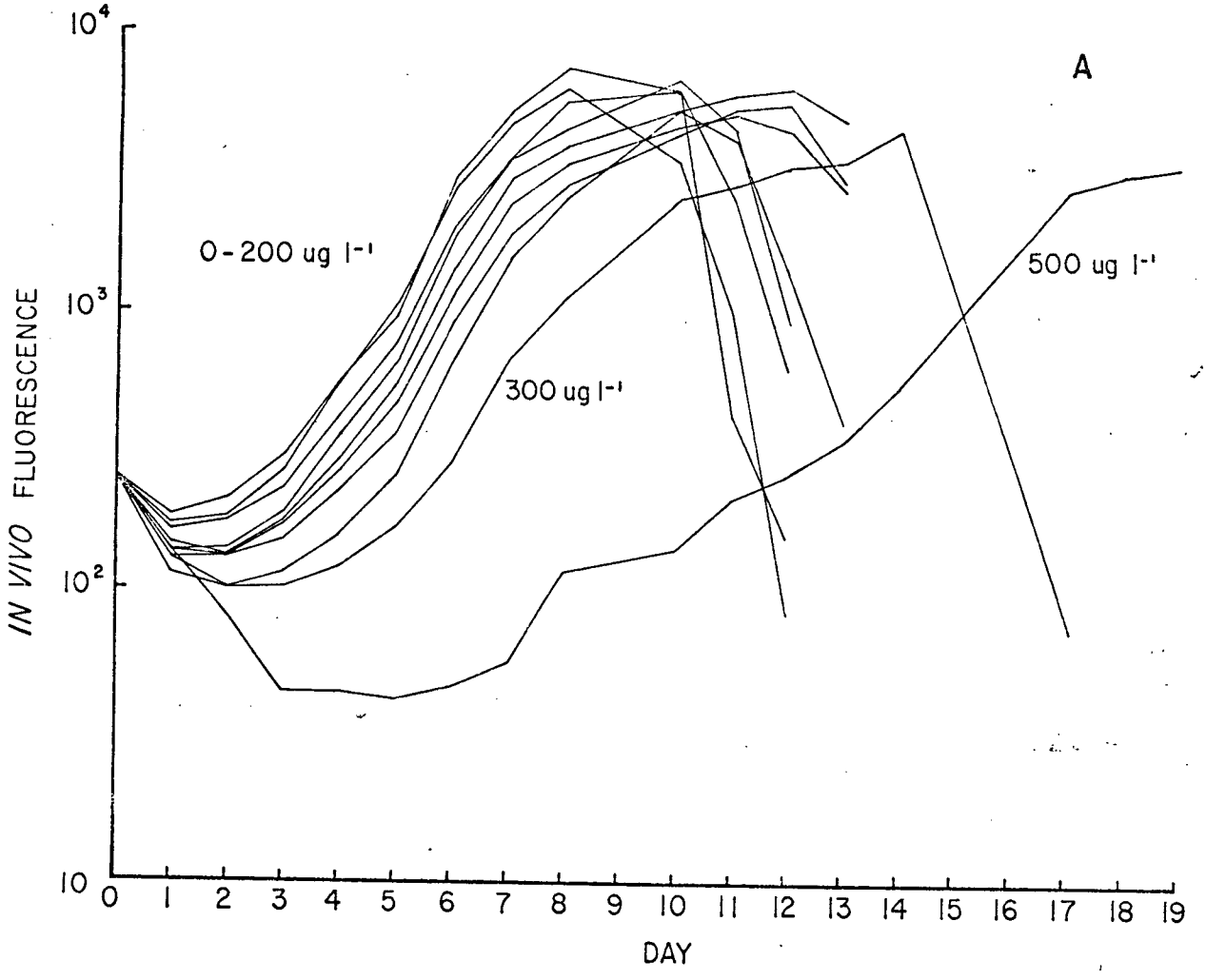
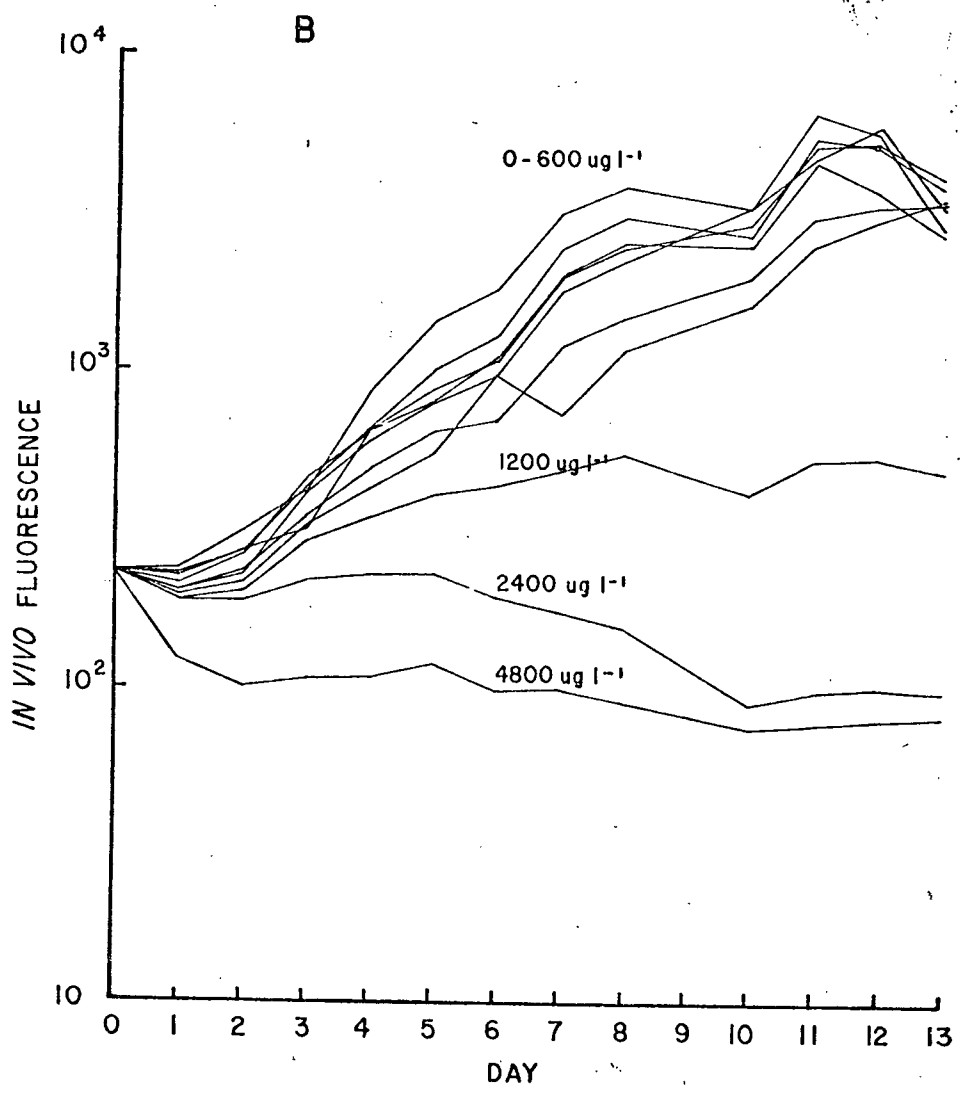


Figure 13. Two types of effects of hydrocarbons on the growth of phytoplankton. A. The response of *Katodinium rotundatum* to No. 2 Fuel Oil: the length of the lag phase of growth increased with increasing hydrocarbon concentration. B. The response of *Thalassiosira* sp. 1 to 1-methylnaphthalene: growth rate decreased with increasing hydrocarbon concentration.


B
10^4
10^3
10^2
10
IN VIVO FLUORESCENCE
0-600 ug l-1
1200 ug l-1
2400 ug l-1
4800 ug l-1
0 1 2 3 4 5 6 7 8 9 10 11 12 13
DAY

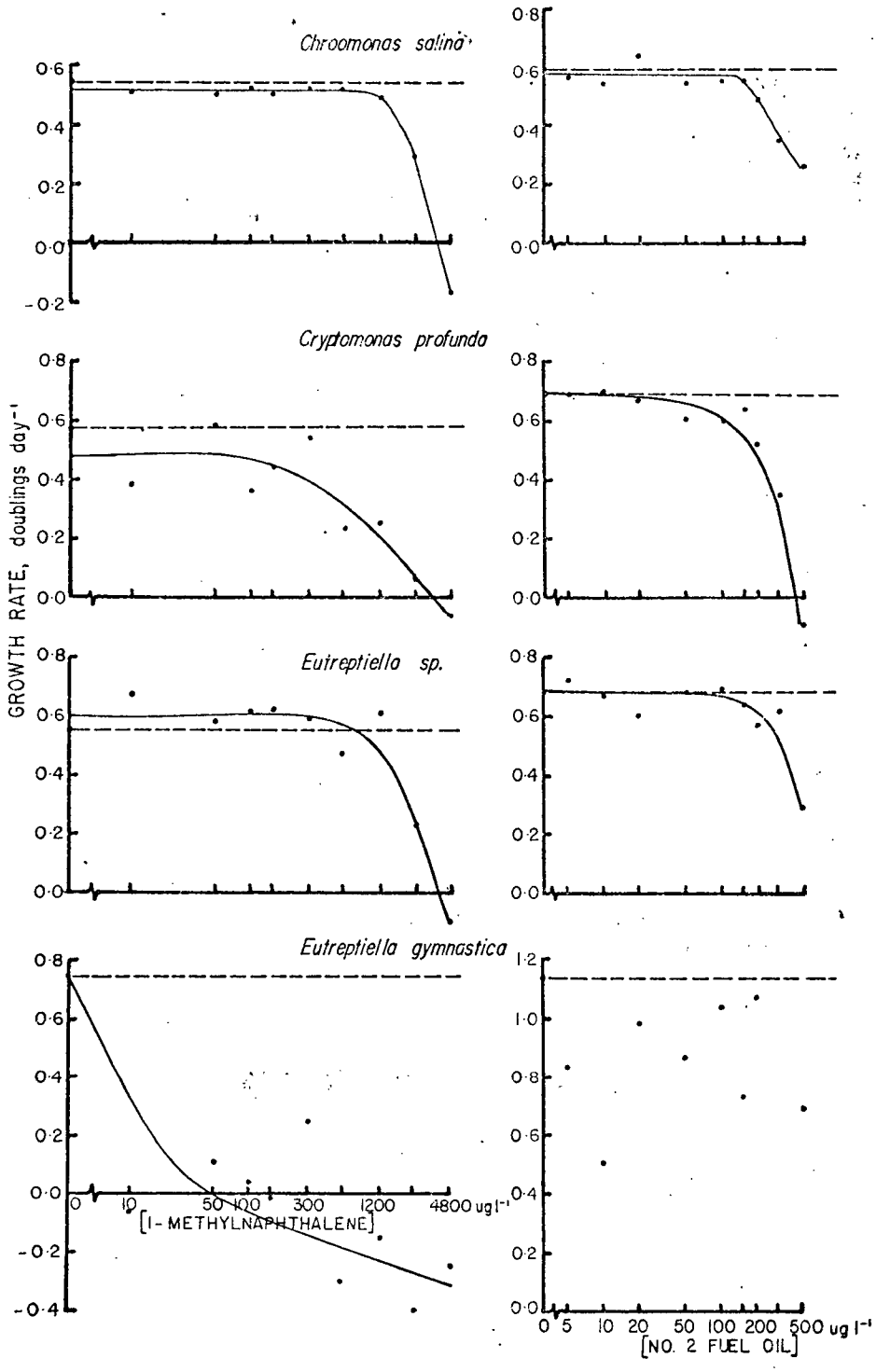
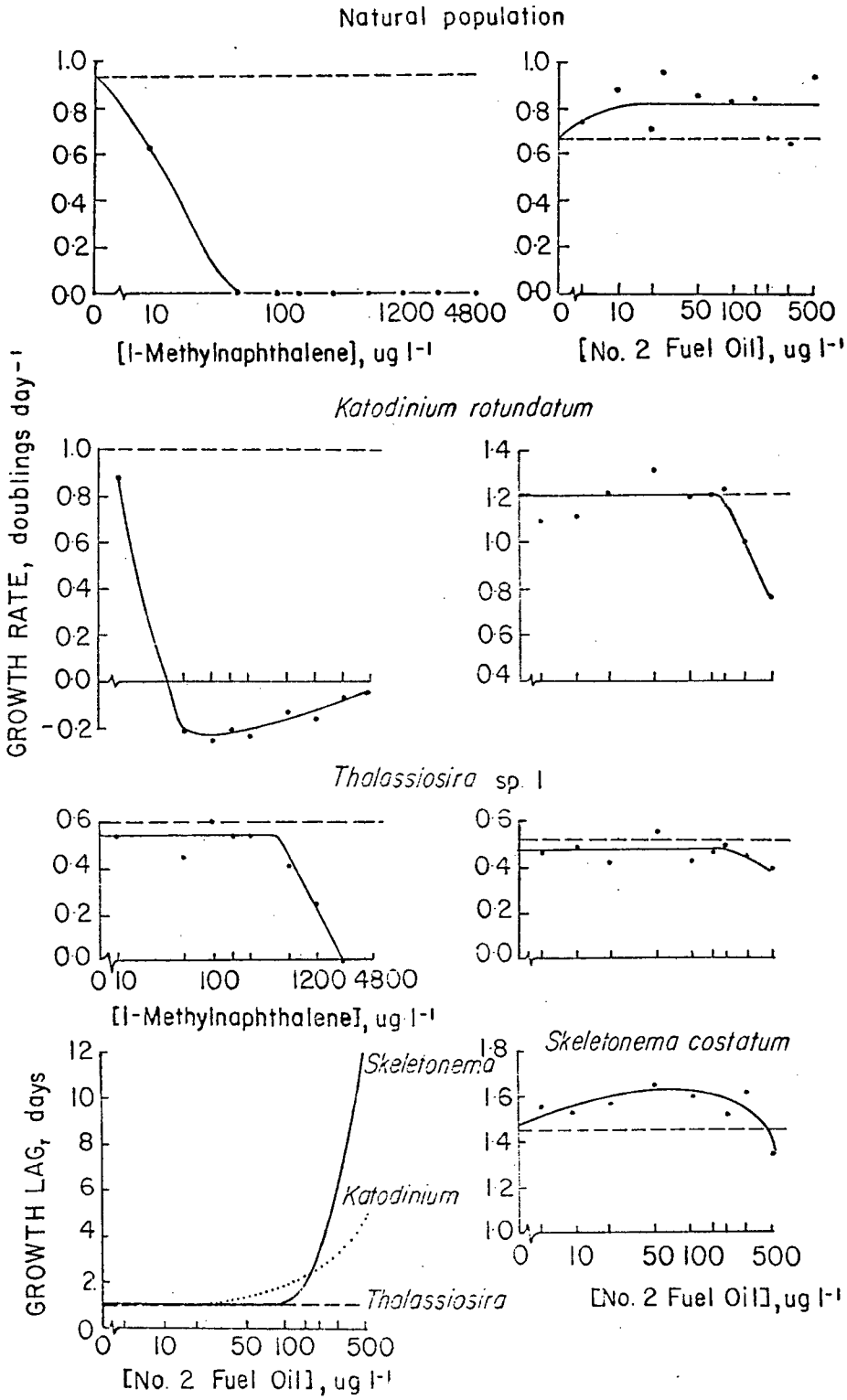


Figure 14. The effects of No. 2 Fuel Oil and 1-methylnaphthalene on the growth rate and growth lag in 7 species of phytoplankton and in a natural population. Dashed lines represent growth in the controls.


Natural population
1.0
0·8
0·6
0·4
0·2
0·0
0 10 100 1200 4800
[1-Methylnaphthalene], ug l-1
0 10 50 100 500
[No. 2 Fuel Oil], ug l-1
GROWTH RATE, doublings day-1
Katodinium rotundatum
-0·2
1·4
1·2
1·0
0·8
0·6
0·4
Thalassiosira sp. 1
0·10 100 1200 4800
[1-Methylnaphthalene], ug l-1
GROWTH LAG, days
12
10
8
6
4
2
0
Skeletonema
Katodinium
Thalassiosira
0 10 50 100 500
[No. 2 Fuel Oil], ug l-1
Skeletonema costatum
1·8
1·6
1·4
1·2
1·0
0 10 50 100 500
[No. 2 Fuel Oil], ug l-1

to the controls, while *Skeletonema costatum* grew slightly faster at No. 2 Fuel Oil concentrations less than or equal to 300 $\mu g\ l^{-1}$ than with no hydrocarbon added. The fact that there was no similarity in the responses of two species of the same genus of euglenoflagellate (see Fig. 14: *Eutreptiella* sp. and *E.* cf. *gymnastica*) suggests that there are no broad generalizations that can be made about the responses to hydrocarbons by different taxonomic groups, and that resistance to or enhancement by hydrocarbons varies at the species level at least.

Incubation of a natural winter population with varying concentrations of No. 2 Fuel Oil did not produce any significant change in the 8-day species composition with increasing hydrocarbon concentration (Table III). The population did shift from a flagellate-dominated situation to a diatom-dominated one, but this occurred in the controls as well as the treated samples, indicating that factors other than hydrocarbons were functional in bringing about the change.

3. Light and temperature experiments

a. *Pure cultures*. Growth rate versus light intensity curves are given in Fig. 15. At 12°C (Fig. 15A), approximately a mean summer temperature for the upper 20 m of Saanich Inlet (see Fig. 3), *Skeletonema costatum* grew more quickly than any flagellate or diatom tested at all light intensities except 0.14 klux (1.94 $\mu E\ m^{-2}\ sec^{-1}$). At this intensity, *Eutreptiella* sp. was the only species demonstrating a positive growth rate, possibly due to heterotrophic capabilities which are known to be common among euglenophytes (Droop, 1974). An intensity of 0.14 klux (1.94 $\mu E\ m^{-2}\ sec^{-1}$) is very low, even for winter conditions (see Fig. 6). According to these data, at 12°C, *Skeletonema* should outgrow any of the

Table III. Species composition of a natural phytoplankton population before and after 8 days' incubation with varying concentrations of No. 2 Fuel Oil.

Species	Cells ml^{-1} Day 0	Day 8 0	5	50	500 $\mu g\ l^{-1}$
Flagellates					
Pyramimonas sp.	9	-	-	-	-
Chrysochromulina kappa	63	-	-	-	-
Chrysochromulina ericina	5	-	-	-	-
Chrysochromulina sp.	5	-	-	-	-
Apedinella sp.	8	-	-	-	-
Eutreptiella sp.	+[1]	-	-	-	-
Cryptomonads	137	-	-	-	-
Katodinium sp.	25	-	-	-	-
Unidentified dinoflagellates	4	-	-	-	-
Total flagellates	256	0	0	0	0
Diatoms					
Skeletonema costatum	+	220,000	85,000	130,000	77,000
Chaetoceros spp.	+	-	-	1,400	-
Chaetoceros spores	+	4,600	1,200	4,900	2,900
Thalassiosira sp.	5	4,200	4,400	9,900	21,000
Coscinodiscus sp.	+	-	350	940	-
Nitzschia delicatissima	+	2,100	2,600	8,900	2,600
Cylindrotheca sp.	+	-	-	240	-
Thalassionema sp.	+	1,400	180	1,200	-
Unidentified pennates	7	5,700	5,100	2,100	6,200
Total diatoms	12	238,000	98,830	159,580	109,700

[1] (+) present
(-) absent

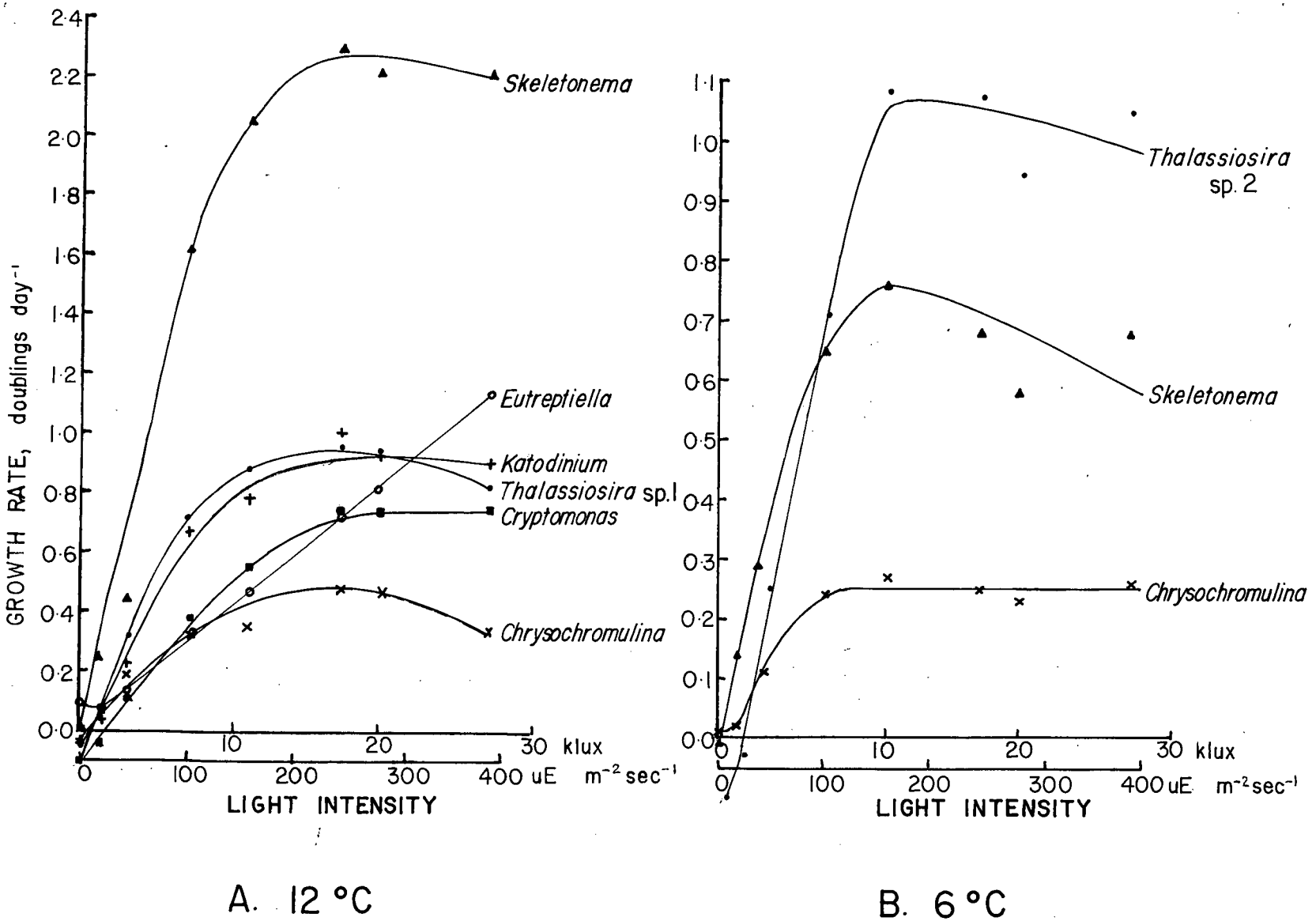


Figure 15. Growth rate versus light intensity curves for phytoplankton in culture. A. 12°C. B. 6°C.

other five species tested, in the absence of other limiting factors.

At 6°C (Fig. 15B), a typical winter temperature (see Fig. 3), *Skeletonema costatum* again outgrew the flagellate *Chrysochromulina kappa* at all intensities greater than 0.14 klux (1.94 μE m^{-2} sec^{-1}), although the growth rates of both species were reduced at the lower temperature (Fig. 16). *Thalassiosira* sp. 2 grew faster than *Skeletonema* at intensities greater than about 6 klux (86 μE m^{-2} sec^{-1}). The prediction one would make on the basis of these data resembles that at 12°C: in the absence of other limiting factors, diatoms should be able to outgrow flagellates at light intensities much above 0.14 klux (1.94 μE m^{-2} sec^{-1}) (with a 12-$\overline{12}$ photoperiod). In other words diatoms should outgrow flagellates at all times of the year, if light intensity alone is the critical factor.

The curves for photosynthetic rate versus light intensity in non-adapted cells (adapted to the temperature and nutrient regimes, but not adapted to the various light levels[1]) closely resemble the curves obtained for growth rate versus light intensity (Fig. 17A). The only exceptions are *Eutreptiella* which seemed to be able to grow relatively quickly on a low photosynthetic rate compared to the other species (due to alternate modes of nutrition?), and *Katodinium* which grew more slowly thank would be expected from the relative photosynthetic rates of the six species.

In adapted cultures (cultures which were allowed to attain logarithmic growth at each of the various light levels, usually 5-10 days),

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[1] Pre-experimental light intensities were low compared to the test intensities, and ranged from 2.7 to 5.2 klux (39-74 μE m^{-2} sec^{-1}), depending upon the species. Refer to methods, section B3a.

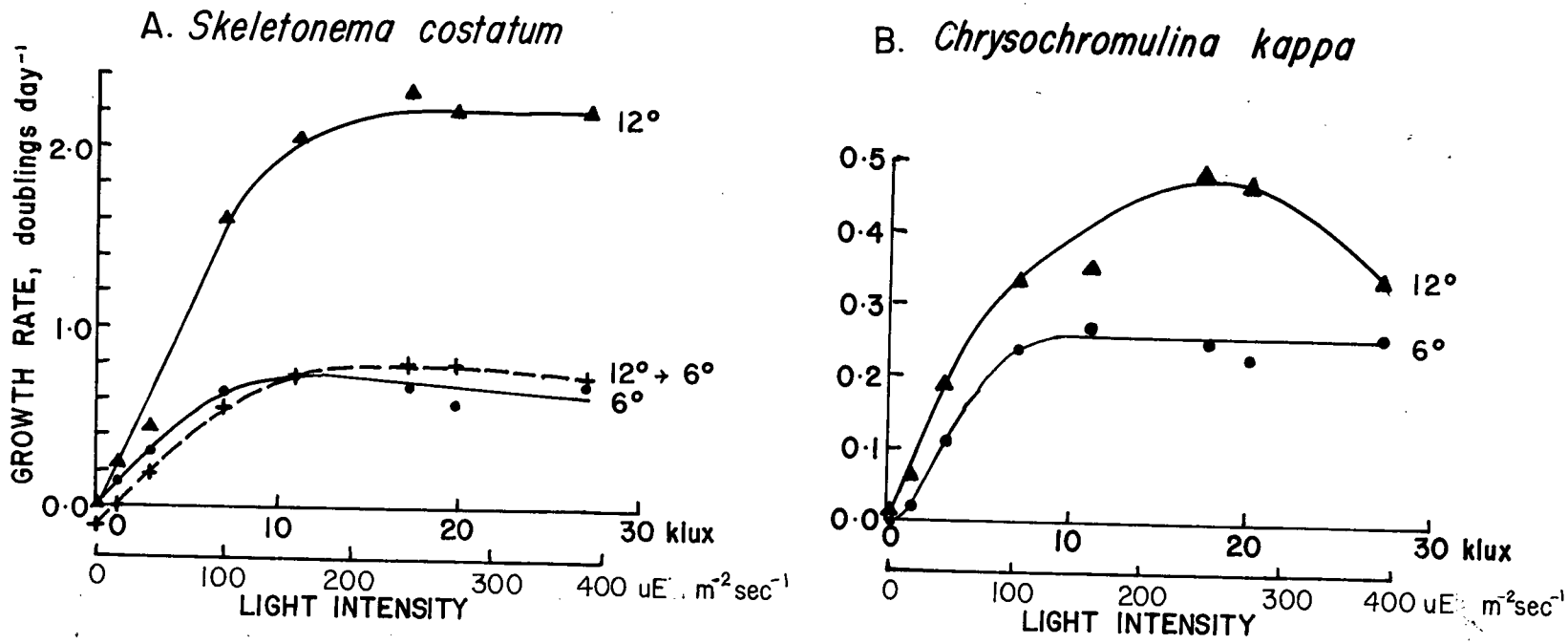


Figure 16. The effect of temperature on growth versus light intensity curves of algae in culture. A. *Skeletonema costatum*. B. *Chrysochromulina kappa*.

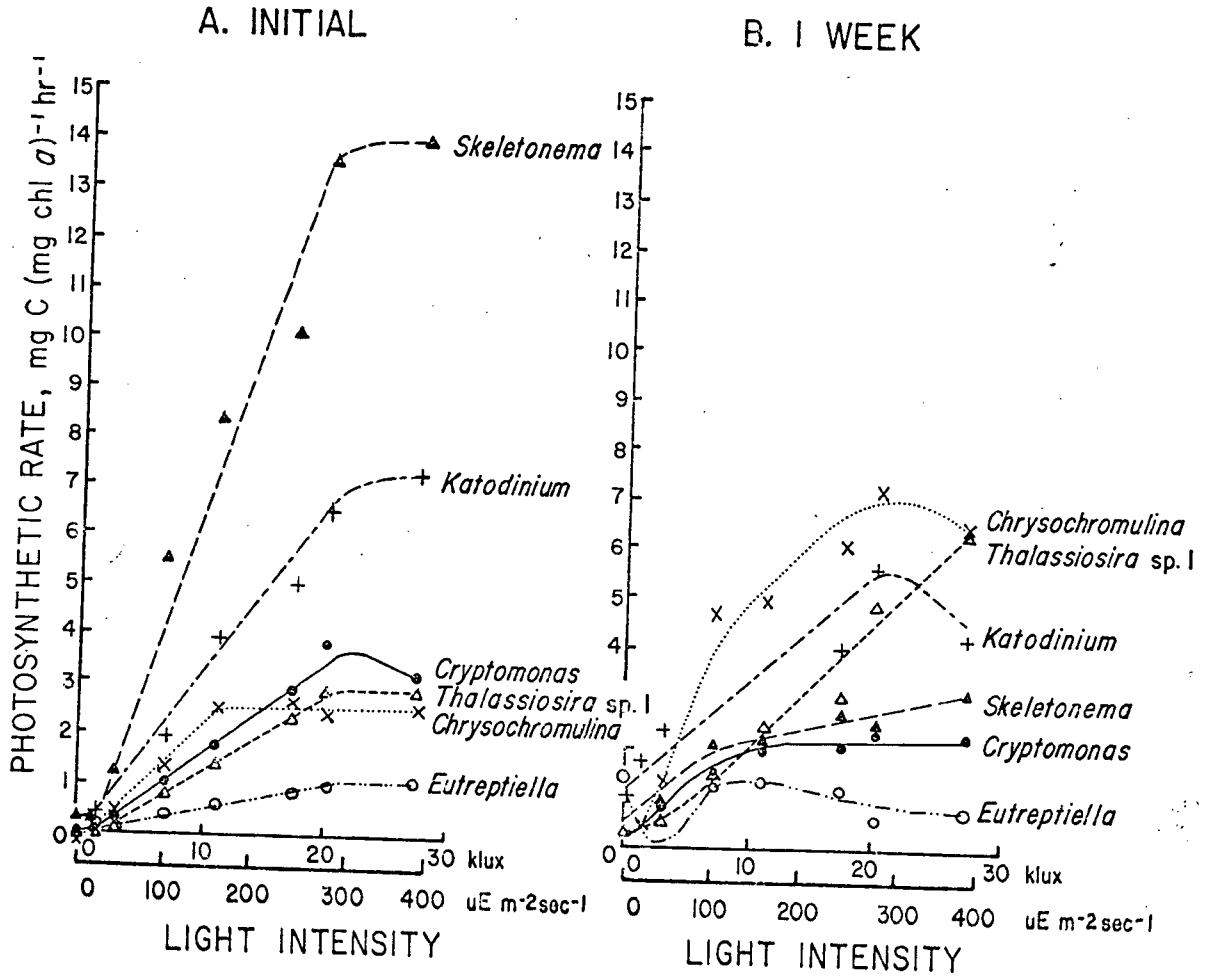


Figure 17. Photosynthetic rate versus light intensity curves for phytoplankton in culture. A. Cells not adapted to light conditions, previously grown at 2.7-5.2 klux (39-74 μE m^{-2} sec^{-1}). B. Cells grown one week at individual light intensities.

some of the curves changed drastically (Fig. 17B). The light-saturated photosynthetic rate of *Chrysochromulina* increased greatly, as did $\frac{\Delta P}{\Delta I}$. Additionally there is evidence in the adapted cells of photoinhibition at the highest intensity, although this may be artifactual, since it is represented by a single data point. A similar development of photoinhibition was observed in *Katodinium*. There was little change in the curves for either *Eutreptiella* or *Cryptomonas*, although in *Cryptomonas* photoinhibition was lost at high intensities.

The I_k values (Talling, 1957) of the two diatom species increased as they adapted to the various light intensities. The $\frac{\Delta P}{\Delta I}$ (and presumably P_{max}) value for *Thalassiosira* sp. 1 increased, but for *Skeletonema* $\frac{\Delta P}{\Delta I}$ decreased. This was rather unexpected, and may represent senescence in the *Skeletonema* cultures. This is rather unfortunate, as there is a strong possibility that, had the culture not senesced, the overall picture of flagellate and diatom growth rates in adapted cultures would have been very different.

From Fig. 17B it appears that, once cells have adapted to the various light intensities, some flagellates--*Chrysochromulina* in particular --can photosynthesize more efficiently than diatoms at light intensities much below about 27 klux (388 $\mu E\ m^{-2}\ sec^{-1}$). At intensities less than about 10 klux (143 $\mu E\ m^{-2}\ sec^{-1}$) only *Eutreptiella* was less efficient than *Thalassiosira*. If the curves are extrapolated one can see that above 27 klux (388 $\mu E\ m^{-2}\ sec^{-1}$) *Thalassiosira* would overtake *Chrysochromulina* which was saturated and perhaps inhibited at that intensity. However due to the unconvincing nature of the *Skeletonema* data this line of reasoning may or may not be warranted.

Comparing the P vs. I curves with the μ vs. I curves (Fig. 17 and 15A respectively) it appears that the growth rates represented in Fig. 15A are more closely linked to the photosynthetic rates in unadapted cells than in adapted cells. Since growth rates were determined for the days between measurements of photosynthetic rates on unadapted and adapted cells, and not following adaptation, this is entirely possible. Determination of μ vs. I curves would probably have been better conducted on light-adapted cells.

Several biochemical and morphological changes were associated with growth at varying light intensities. Volumes of the light-adapted cells (i.e., grown to the onset of stationary phase at the experimental intensities, or about 5-10 days, depending on the intensity and the species) were calculated for samples of 20 cells per light intensity per species, by approximating the shape of the cells of each species to a geometrical figure and microscopically measuring the appropriate dimensions: *Skeletonema* and *Thalassiosira* cells were likened to cylinders, *Chrysochromulina* to spheres, and *Cryptomonas*, *Eutreptiella* and *Katodinium* to ellipsoids. In all species except *Thalassiosira*, cell volume decreased with decreasing light intensity, although in *Chrysochromulina* this tendency was very slight and cells of *Eutreptiella* were extremely variable in size (Fig. 18). With the exception of *Thalassiosira* sp. 1 all species tested showed a predictable increase in surface area-to-volume ratio at low light intensities. This could be an adaptation to maximize the illumination of the chloroplasts within the cells. Alternately, smaller cells with reduced energy demands could mean increased efficiency at low light levels. I am unable to suggest a reason for the reversal of this trend in *Thalassiosira*. (Note that the volume of *Skeletonema* cells was considerably greater at 0.14 klux--1.94

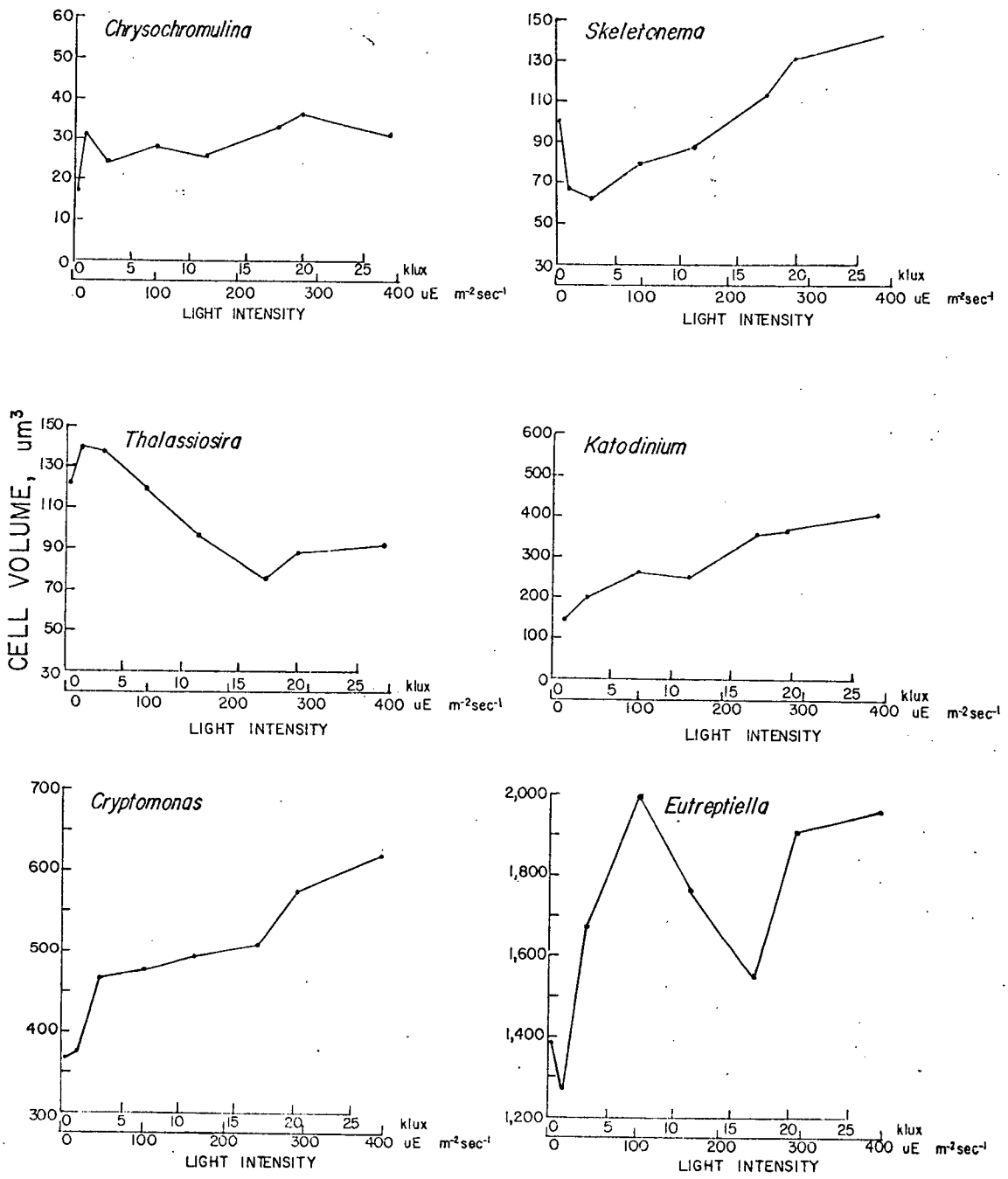


Figure 18. Effect of light intensity on cell volume.

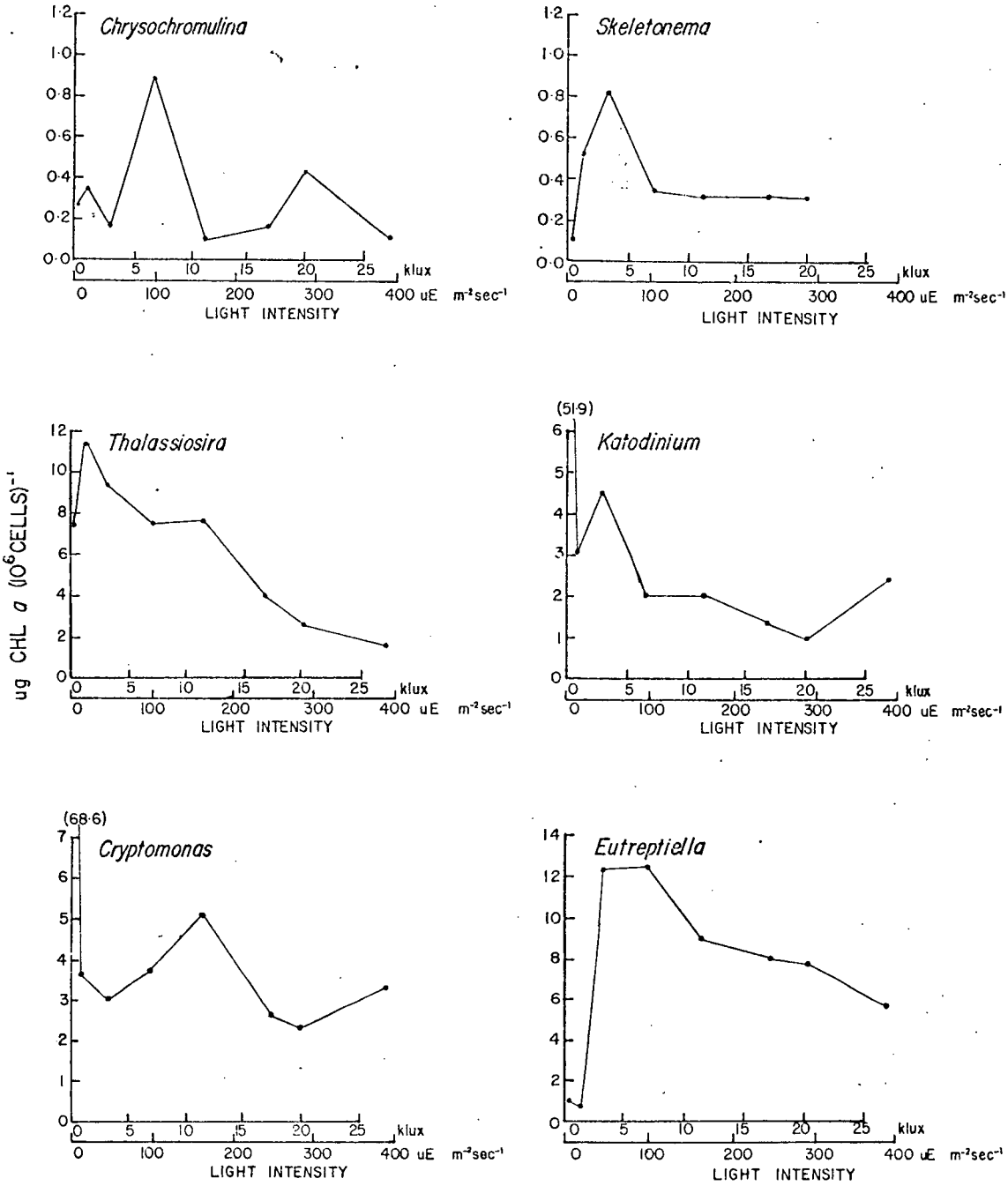


Figure 19. Effect of light intensity on chlorophyll content.

$\mu E\ m^{-2}\ sec^{-1}$--than at any of the intensities immediately higher. This is doubtlessly due to the fact that no growth occurred at this light intensity; in fact cells died very early in the experiment. Therefore the cells measured in this case would represent the original inoculum of relatively large cells.)

In *Eutreptiella* and *Thalassiosira* cells there was a marked increase in the chlorophyll content per cell as light intensity decreased (Fig. 19). This is consistent with the theory that cells tend to increase the concentration of their photosynthetic pigments in an effort to use more efficiently the decreased available light. The chlorophyll content of *Skeletonema*, *Katodinium* and *Cryptomonas* showed no change over the brighter range of intensities but quickly increased as light decreased to below a threshold value which was different for each species. This probably represents the same effect as was observed with *Eutreptiella* and *Thalassiosira*, except that these three species can withstand much lower intensities before chlorophyll concentration is increased. The chlorophyll content of *Chrysochromulina* cells was variable and showed no dependence on light intensity over the range tested.

b. *Natural populations*. Results obtained using pure cultures were somewhat confusing due to the apparent importance of adaptation, and the intrinsic problems in dealing with cultures. A rather tentative conclusion could be drawn to the effect that light is not important in regulating diatom-flagellate abundances, as diatoms seem to be able to grow faster than flagellates at any normally encountered intensity. However, the possibility that these relative growth rates can change as the cells adapt to their new environments places the whole hypothesis in doubt.

Experiments with natural populations produced more consistent results. When a natural summer population of phytoplankton, comprised chiefly of the diatoms *Skeletonema costatum*, *Nitzschia delicatissima*, *Chaetoceros* spp., *Thalassiosira* spp. and the nanoflagellate *Chrysochromulina* spp., was incubated at 12°C and varying light levels (experiment 2, Table I) there was a clear tendency for the diatom species to outnumber the flagellates at high intensities and for the flagellates to dominate at low intensities (Fig. 20). Note that each species had its own light optimum: *Skeletonema* at about 2.9 klux (41.5 μE m^{-2} sec^{-1}), *Nitzschia* at about 6.9 klux (99.3 μE m^{-2} sec^{-1}), *Chaetoceros* at 27 klux (388 μE m^{-2} sec^{-1}) or higher. It is interesting that the optimum for *Chrysochromulina* was at about 17 klux (244 μE m^{-2} sec^{-1}) although it was not dominant at this intensity. Instead it was outnumbered by the diatoms *Nitzschia* and *Chaetoceros*. In the region where it dominated it seemed to do so only "by default:" it dominated only because of its tolerance to low light levels where the diatoms could not grow.

A plot of flagellate-to-diatom ratios (by cell numbers) is shown in Fig. 21. The downward peak at 2.9 klux (41.5 μE m^{-2} sec^{-1}) represents a peak in diatom abundance, specifically *Skeletonema costatum*, as can be seen by comparing the curve with Fig. 20. The beginnings of another diatom peak, partly due to *Chaetoceros* and partly due to the decrease in flagellate abundance, can be seen above 20 klux (300 μE m^{-2} sec^{-1}). Below 1.2 klux (17.3 μE m^{-2} sec^{-1}) the flagellate-to-diatom ratio increases rapidly, not due to any increase in flagellate number, but rather to a dropoff in diatom abundance.

This basic relationship seems to be independent of a number of other factors, including the initial population, photoperiod, and temperature.

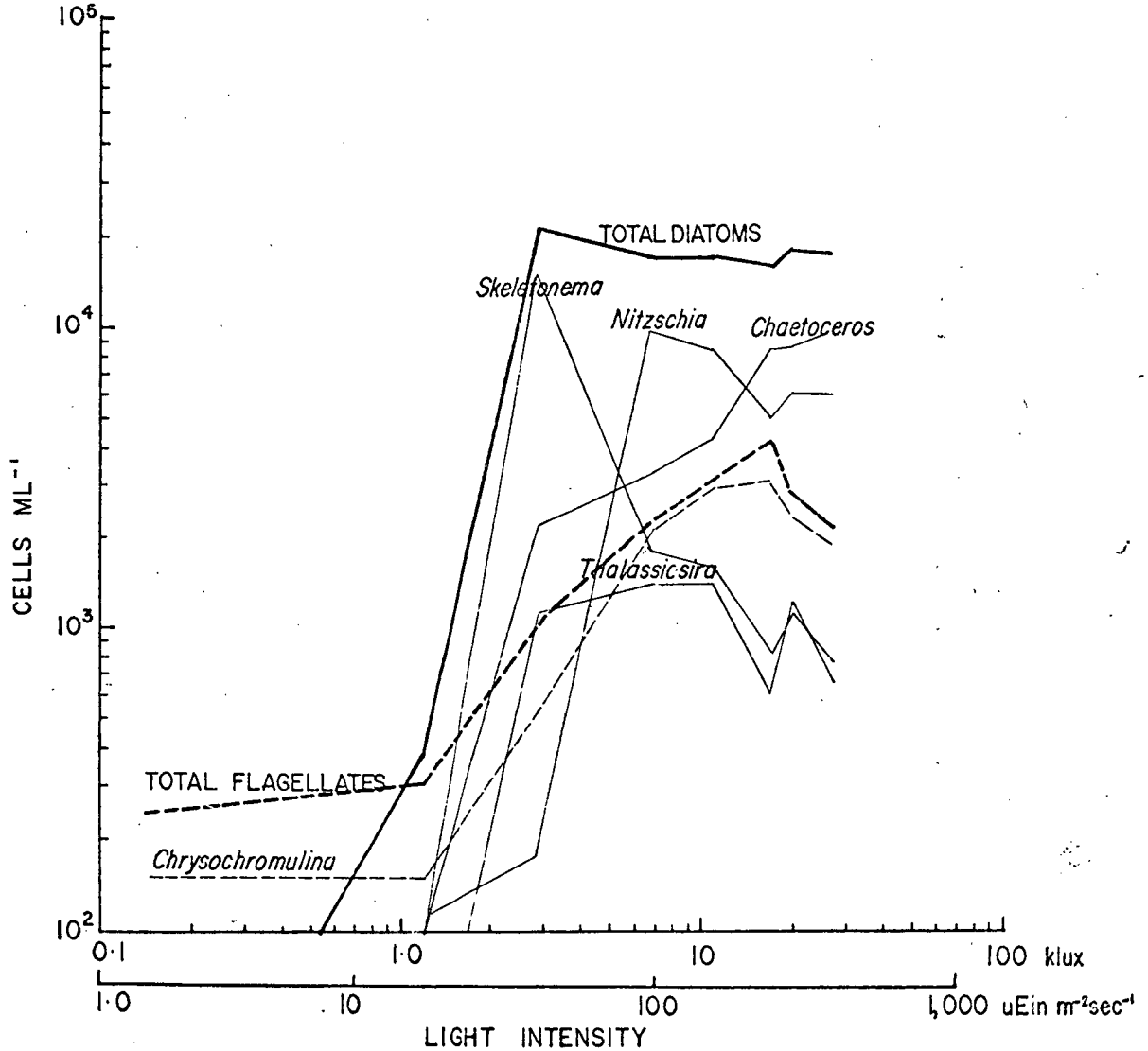


Figure 20. Effect of light intensity on the composition of a natural phytoplankton population: cells ml^{-1} after 4-14 days' growth at 12°C and continuous illumination at various light intensities.

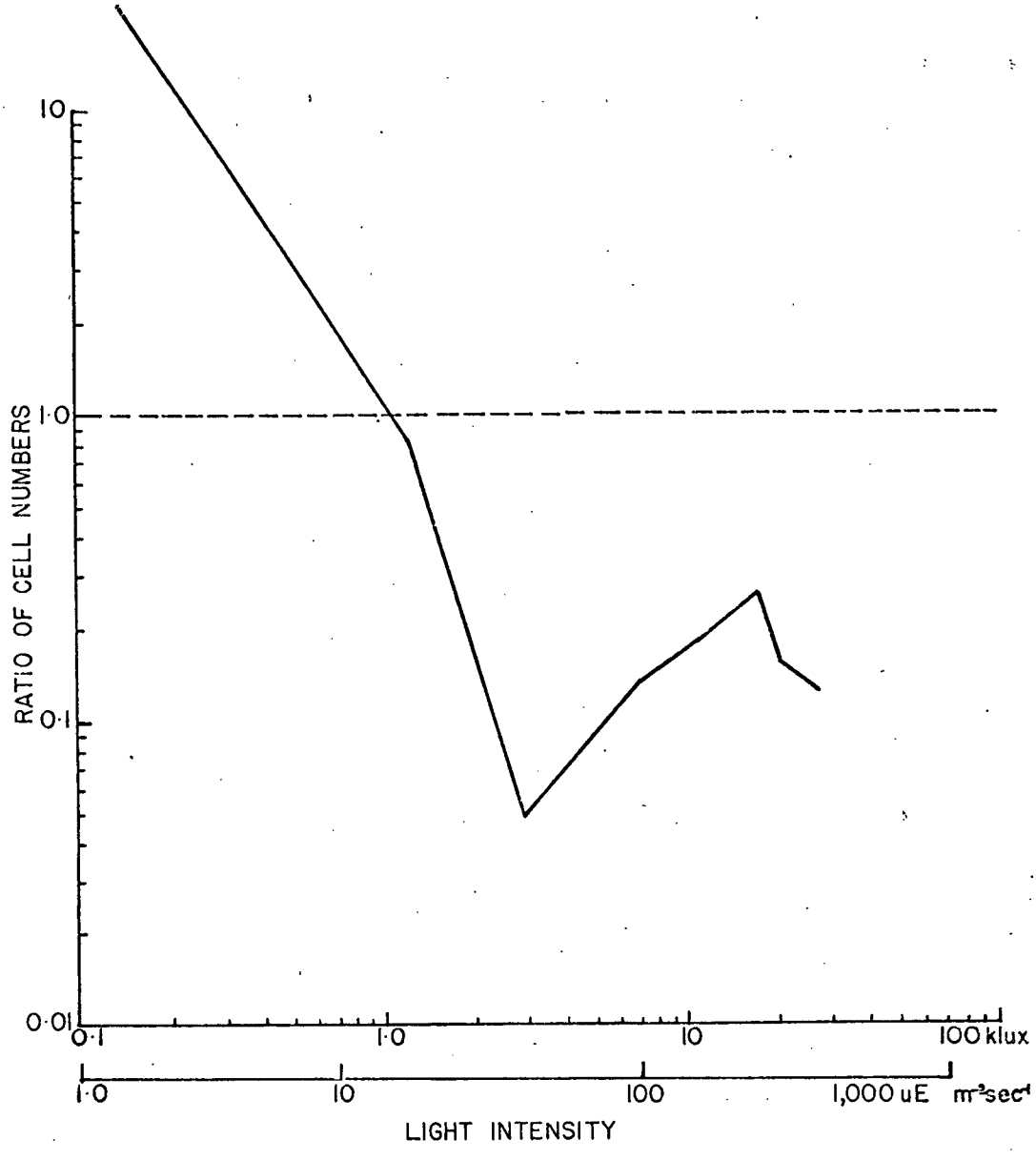


Figure 21. Effect of light intensity on the ratio (by cell numbers) of flagellates to diatoms after 4-14 days' growth at 12°C and continuous illumination at various light intensities.

Fig. 22 illustrates two experiments run under identical conditions (12°C, 12-$\overline{12}$), using natural populations collected in December and August (experiments 1 and 3, Table I). Although the absolute value of the ratios varies between the two populations, the shapes of the two curves are almost identical, indicating that light had the same effect on two very different phytoplankton populations.

Fig. 23A shows the influence of photoperiod on the basic flagellate-diatom light effect. The two curves illustrate the same general principle: decreased light intensity results in an increase in the flagellate-to-diatom ratio, while higher light intensities allow diatoms to increase in abundance. Longer photoperiods also allow single diatom species to grow at lower light intensities than possible with shorter photoperiods. For example, the *Skeletonema* "peak" at 12-$\overline{12}$ is at 17 klux (244 μE m^{-2} sec^{-1}) while at 24-$\overline{0}$ the peak is at 2.9 klux (41.5 μE m^{-2} sec^{-1}). Apparently dim light can (to a point) be integrated over time to produce an effect similar to brighter light of shorter duration. This effect can be seen in growth rate versus intensity curves as well (Fig. 23B). Here the growth rates for populations grown (to stationary phase) with a 24-$\overline{0}$ photoperiod are generally higher than those grown (to stationary phase) with a 12-$\overline{12}$ photoperiod. However, it also seems from Fig. 23B that if the intensity is insufficient to support a positive growth rate longer photoperiods make little difference.

The fact that there were more flagellates overall in the populations which wase illuminated continuously is likely a function of the original compositions of the two populations rather than an effect of photoperiod. The fact that the flagellate-to-diatom ratio was much higher at low intensities in the continuously illuminated cultures than in the 12-$\overline{12}$ cultures may indicate that the total light energy available with a 12-$\overline{12}$

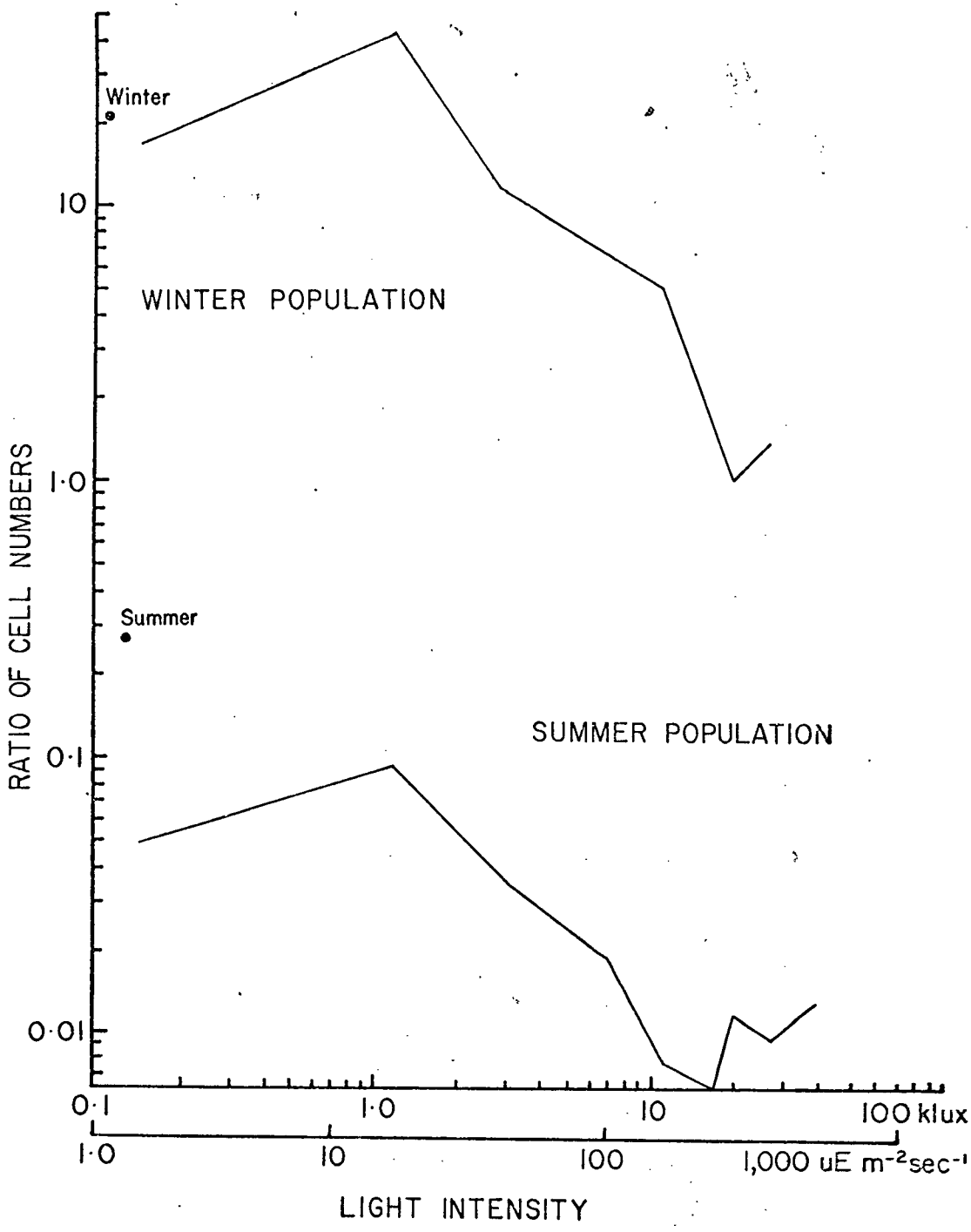


Figure 22. Effect of varying the initial composition of a natural phytoplankton population on its response to light intensity. Both populations were grown 5-8 days at 12°C with a 12-12 photoperiod. Points show ratios of flagellates to diatoms (by cell numbers) in the initial populations.

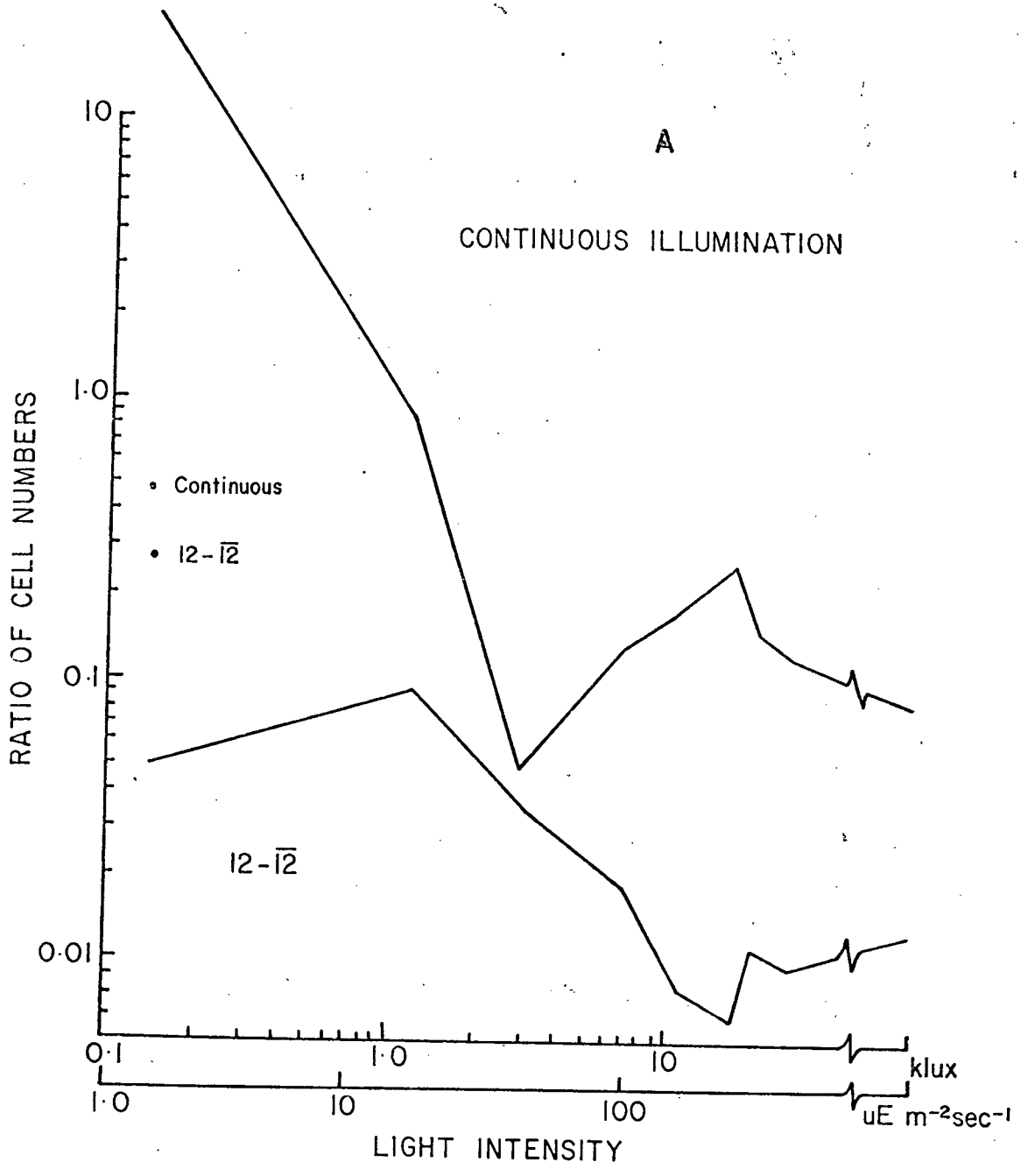
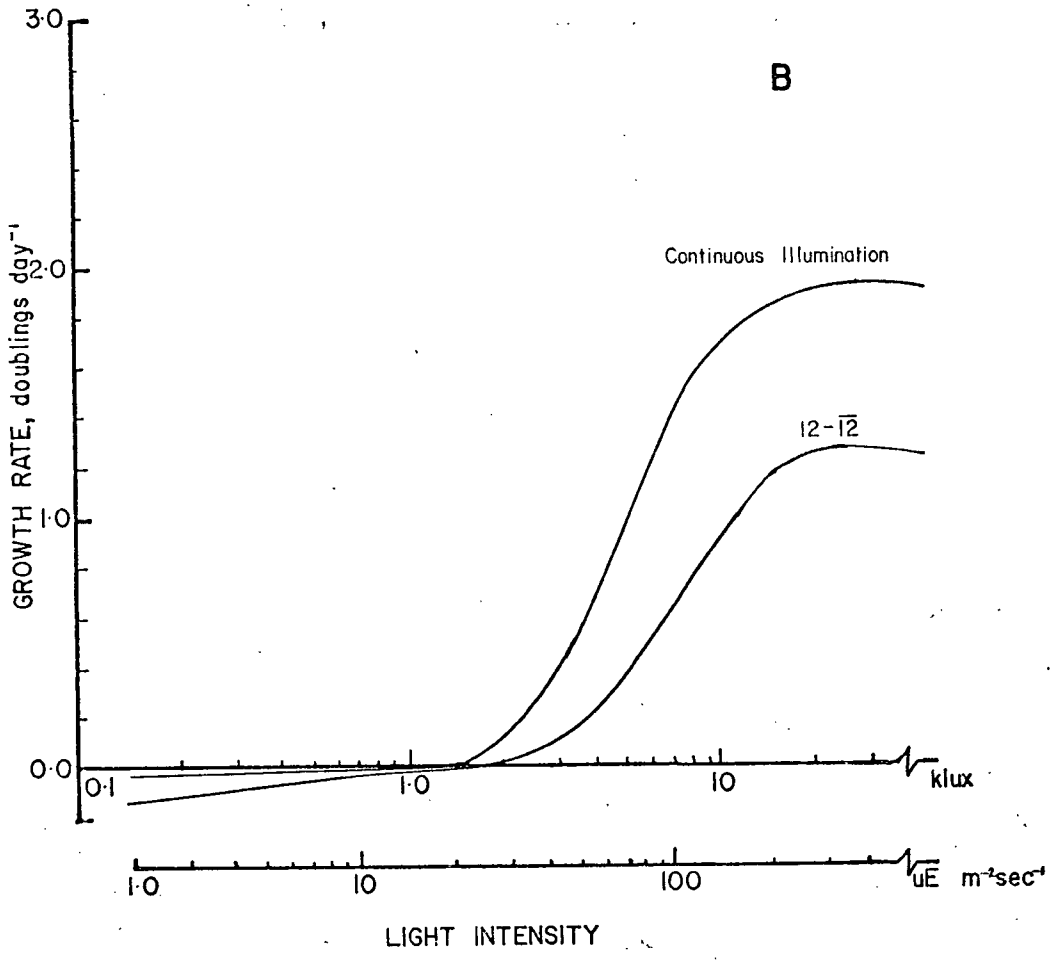


Figure 23. Effect of photoperiod on the response of natural populations of phytoplankton to light intensity. A. Ratio of flagellates to diatoms after 4-14 days' growth at 12°C. Points show ratios in the initial populations. B. Growth rates under continuous illumination or 12-$\overline{12}$.


B
3·0
2·0
1·0
0·0
GROWTH RATE, doublings day⁻¹
Continuous Illumination
12-12
0·1
1·0
10
klux
1·0
10
100
uE m⁻²sec⁻¹
LIGHT INTENSITY

photoperiod is insufficient to support basal metabolism in all cells, so that cells die and the flagellate-to-diatom ratio becomes variable. On the other hand continuous low level light may support basal cell maintenance in flagellates but not diatoms, thereby increasing the flagellate-to-diatom ratio.

The effect of temperature on the growth rates of natural populations is shown in Fig. 24A. The population grown at 12°C achieved a higher light-saturated growth rate than did the 6°C population. Under favourable conditions growth rate is a function of photosynthetic rate, and since the light-saturated photosynthetic rate of a population is a function of temperature, it is not surprising that the light-saturated growth rate should also be temperature-dependent. The rate of increase in growth rate with an increase in light intensity ($\frac{\Delta\mu}{\Delta I}$), on the other hand, does not appear to be temperature-dependent. This again is not unexpected, since $\frac{\Delta P}{\Delta I}$ does not vary with temperature.
does not

One consideration which is not shown in Fig. 24A is the duration of the lag period between the beginning of the experiment and the initiation of growth. At 12°C there was no lag--growth commenced immediately--while at 6°C there was a 4-day lag period. Since the initial populations were collected during the summer (mean ambient temperature, 12°C) the lag period which occurred when the temperature was reduced to 6°C doubtlessly represents an adaptation phase.

The ratios of flagellates to diatoms after one week's growth at 12°C and 6°C are shown in Fig. 24B. The two curves are very similar in shape, with diatom peaks at 17.1 klux (244 $\mu E\ m^{-2}\ sec^{-1}$) and flagellate numbers increasing above and below this point. However, the 6°C curve is more pronounced--that is, the diatom peak is better defined and there are

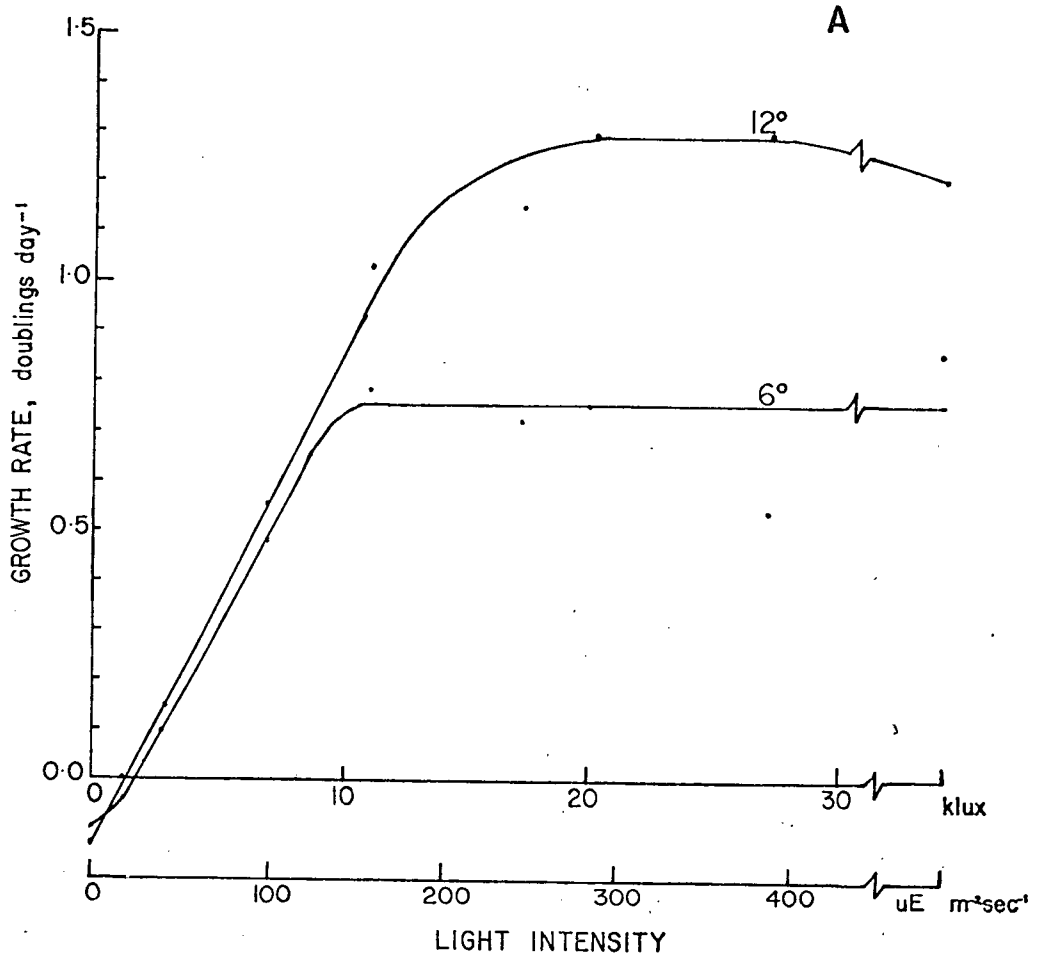
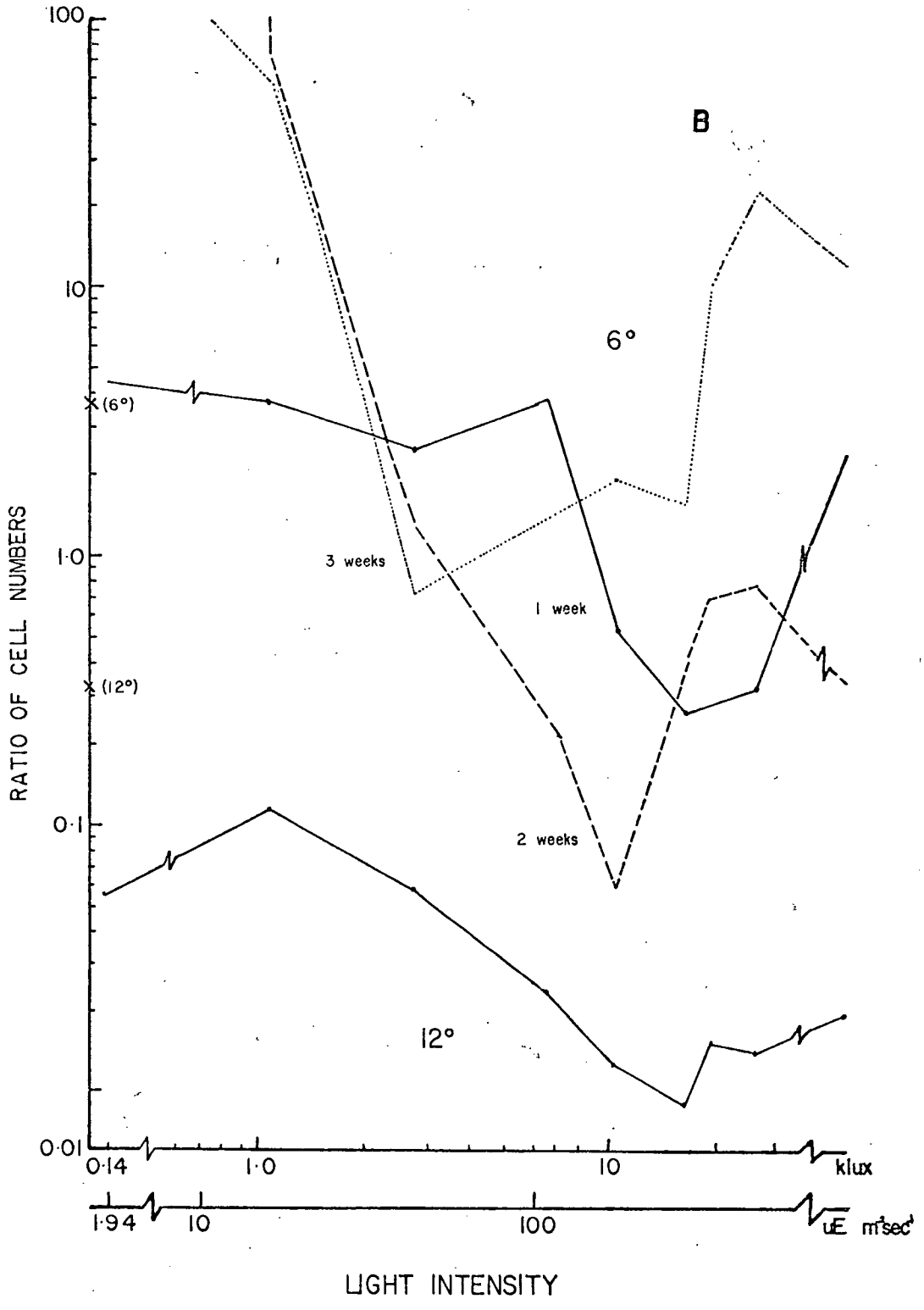


Figure 24. Effect of temperature on the response of natural populations of phytoplankton to light intensity. A. Growth rates. B. Flagellate-to-diatom ratios. Points show ratios in the initial populations. ——— 1-week curves, – – – – 2 weeks, ····· 3 weeks.


B
100
10
1·0
0·1
0·01
RATIO OF CELL NUMBERS
(6°)
(12°)
6°
12°
3 weeks
1 week
2 weeks
0·14
1·0
10
klux
1·94
10
100
uE m²sec¹
LIGHT INTENSITY

no anomalous values at the lowest light intensity. Secondly, at 6°C the whole curve is shifted toward flagellate dominance. The latter observation may be due to the fact that diatoms were initially far more abundant in the 12°C experiment than in the 6°C experiment--and this is probably a major consideration--however both observations may also be accounted for by the possibility that diatoms grow better at 12°C than at 6°C. Perhaps at 12°C they are less sensitive to light intensity changes because they are at a favourable temperature. At 6°C they may be temperature stressed and so are more sensitive to sub-optimal light intensities. It may be argued that, while this may be true, it is also true that the original populations were growing at 12°C and that therefore they would of course by stressed at 6°C. However after two and even three weeks the sharpness of the diatom peak and the overall increase in flagellates had increased and not decreased. Were this the case one would expect that after two or three weeks the populations would have adapted to the new temperature and the curve would have begun to look more like the 12°C curve instead of less like it. Therefore it seems reasonable to conclude that Saanich diatoms intrinsically prefer 12°C to 6°C, and that at 6°C they are stressed and restricted to narrower light intensity ranges than they would be at 12°C.

In Fig. 24B it is possible to see the progress of light adaptation in diatoms. The peak in all curves is due to the diatom *Skeletonema costatum*. One can see how, with time, the peak shifted to the left--that is, *Skeletonema* gradually adapted to dimmer and dimmer light intensities. However this adaptation had a lower limit, as is evidenced by the fact that the relative abundance of flagellates below 2.9 klux (41.5 $\mu E\ m^{-2}\ sec^{-1}$) increased with time. Therefore it seems reasonable to conclude that at 6°C *Skeletonema* will never grow at intensities less than 2.9 klux (41.5 $\mu E\ m^{-2}$

sec^{-1}). Secondly, it is important to note that the *Skeletonema* peak did not widen to include the lower light intensities but in the first two weeks the upper tolerance limit decreased as well as the lower limit. This seems to indicate some synergistic effect of temperature and light intensity such that at low temperatures the light intensity optimum is also lower. This may relate to a general slowing down of the whole physiology of the cell with decreased temperature.

DISCUSSION

The winter dominance in Saanich Inlet by flagellates has been documented for the period 1975-1976. This is known to occur on a regular basis (Takahashi, personal communication). Buchanan (1966) has documented a similar pattern for Indian Arm, another British Columbia fjord. On the basis of experimental findings some conclusions may now be drawn as to the relative importance of various physical, chemical and biological factors involved in mediating this phenomenon, as well as the crash of diatoms in the fall and their return in the spring. Factors under consideration include temperature, light intensity, photoperiod, water stability, nutrient concentrations, pollution by hydrocarbons, grazing, chemical interactions among species, and what I have called "intrinsic growth rates," which refers to the success of species under optimal growth conditions.

A. Non-Contributing Factors

Certain factors may be eliminated at the outset as unimportant to the control of the flagellate-diatom cycle. These include nutrient concentrations, grazing, chemical interactions among species, and hydrocarbon

pollution.

1. Nutrient concentrations

Despite the fact that competition for nutrients has classically been considered an important determinant of phytoplankton succession (e.g., Dugdale, 1967; Eppley *et al.*, 1969; Semina, 1972; Parsons and Takahashi, 1973a; Titman and Kilham, 1976), it is unlikely that it plays such a role in the spring and fall diatom-flagellate changeovers in Saanich Inlet. Nitrate, silicate and phosphate levels were high and consistent before, during and after the period of flagellate dominance. Decreases in nutrient concentrations occurred only after the spring diatom bloom was initiated, indicating that the decreases were caused by diatom uptake, rather than the diatom bloom being brought about by decreases in nutrient levels. Similarly, nutrient levels were high well before the fall diatom crash and the shift to a flagellate population. Moreover, the models of both Semina (1972) and Parsons and Takahashi (1973a) predict that increased nutrient concentrations favour the growth of large cells. Ryther's (1969) model predicted that nutrient-rich upwelled waters would favour large, chain-forming diatoms while oligotrophic oceanic waters would favour nanoflagellates.

On the other hand, summer in Saanich Inlet is a period of highly changeable nutrient concentrations. Nitrate in particular frequently reaches limiting concentrations in the surface layers. It is possible that at this time nutrients could play an important role in regulating succession among diatom species or allowing occasional flagellate blooms to occur. However, this role of nutrients is beyond the scope of the present discussion which is limited to the major changes which occur in the spring and fall, from a flagellate-dominated winter population to a diatom-dominated summer population and vice versa.

2. Grazing

The purpose of monitoring zooplankton abundance was to determine whether or not herbivores have a regulating effect on the relative abundances of diatoms and nanoflagellates in the inlet. Since copepods are by far the most important grazers in Saanich Inlet, only these were considered. In order to evaluate their potential effects on phytoplankton they were grouped into three size classes: less than 1.0 mm total body length, 1.0 to 2.0 mm, and greater than 2.0 mm. Copepods of different sizes are known to prefer different sizes of food, even within a single species (Mullin, 1963; Nival and Nival, 1973, 1976; Lamport, 1974). The smallest category of copepods (less than 1.0 mm), including copepodite stages of *Paracalanus parvus*, *Pseudocalanus minutus* and *Metridia pacifica*, were assumed to be incapable of subsisting on a diet of large diatoms, the chain lengths of which may exceed 1.0 mm. These animals would be largely restricted to a nanoflagellate diet. The largest category (greater than 2.0 mm), including adult *Metridia pacifica* and *Calanus* spp., was considered to be capable of filtering the larger diatoms as well as the nanoflagellates, but unable to live on nanoflagellates alone unless present in great numbers. For example, Raymont and Gross (1942) showed that adult *Calanus finmarchicus* could subsist on a diet of small phytoplankton (1-3 μm) but that their survival was greatly improved by the presence of larger diatoms. The intermediate category (1.0-2.0 mm), including juvenile stages of the larger copepods and adult stages of small copepods, was considered to be capable of using phytoplankton of all sizes. Adult *Pseudocalanus minutus*, for example, were found by Poulet (1974) to be opportunistic, feeding on anything between 3.57 and 57 μm depending on abundance. However there is evidence that larger phytoplankton are selected when available (Mullin, 1963; Hargrave and Geen, 1970).

Fig. 11 shows that copepods in the two smaller size categories were present year-round while the larger copepods "disappeared" from December to March, almost exactly when the diatoms "disappeared" (Fig. 9). Were grazers responsible for the changes in diatom-to-nanoflagellate ratios the expected pattern would be reversed: that is, diatoms would be grazed down to low numbers when large grazers appeared and bloom following the disappearance of large grazers. It is therefore apparent that copepod life cycles are timed to phytoplankton cycles and not vice versa.

3. Chemical interactions

Several authors have suggested that excretions of algal and bacterial cells may stimulate or inhibit the growth of other algal species and thereby influence succession patterns (Johnston, 1963; Provasoli, 1971; Provasoli and Carlucci, 1974). This has fostered the concept of water "preconditioning" which implies that one species of algae (or bacteria) must release some organic compound, such as a vitamin, into the water before the succeeding species can grow. Alternatively one algal species may excrete compounds which inhibit the growth of competitors. The possibility that such a mechanism is operational in the diatom-flagellate succession pattern in Saanich was tested in the metabolite experiment, in which the most common winter nanoflagellate, *Chrysochromulina kappa*, and the first spring diatom, *Thalassiosira*, were grown together and separately. When the two species were placed in separate dialysis bags but in the same culture medium, free exchange of substances such as vitamins, amino acids, simple proteins, sugars and so on (any substance with a molecular weight of 12,000 daltons, the pore size of the dialysis membrane, or less) could occur between the two species, although they were kept apart physically. The fact that there was no difference in growth rate whether or not the two species

were allowed to exchange metabolites in this manner indicates there was probably no biochemical interaction between them which involved molecules smaller than 12,000 daltons. This size range includes most of the growth regulators known to be present in plants: vitamins, auxins, gibberellins, kinetins, and cytokinins.

When *Thalassiosira* and *Chrysochromulina* were grown in mixed culture the growth rate of *Thalassiosira* was enhanced and that of *Chrysochromulina* was reduced. This could be due to a marked difference in nutrient uptake rates at the concentration of nutrients used. If *Thalassiosira* cells were taking up nutrients more quickly than the *Chrysochromulina* cells, this could have created a microenvironment in which nutrient levels were reduced (relative to the situation where *Chrysochromulina* was growing alone), and hence caused a reduction in its growth rate. The *Thalassiosira* cells, on the other hand, were, in effect, in more dilute culture than they were when grown alone (since the total biomass in each dialysis bag was held constant). If *Chrysochromulina* were indeed a poorer competitor for nutrients than other *Thalassiosira* cells, the growth rate of the *Thalassiosira* cells would be enhanced in mixed culture. The fact that the cells in the dialysis bags were very concentrated (e.g., 50,000 cells *Chrysochromulina* and 6,000 cells *Thalassiosira* per ml on day 3; see Fig. 12) increases the possibility that such a mechanism was involved. This concept of micro-scale nutrient limitation has been discussed by Gavis (1976) and Pasciak and Gavis (1974).

It was also observed in mixed culture that *Thalassiosira* senesced before *Chrysochromulina*, suggesting that it had a greater uptake threshold (higher K_s) for the limiting nutrient than *Chrysochromulina*. *Chrysochromulina*, with its apparently lower threshold for uptake, was able to continue to grow. Alternately, perhaps *Chrysochromulina* was able to utilize alternate nitrogen sources, such as organic nitrogen, when inorganic sources were depleted, whereas *Thalassiosira* was not.

If chemical interactions were involved in either of these two instances, for example a release by *Chrysochromulina* of some inhibitor which affected *Thalassiosira* after 3 days, one would expect that these same observations would have been made in the situation where *Chrysochromulina* and *Thalassiosira* were grown in separate dialysis bags but sharing the same medium, that is, interacting chemically but not physically. The fact that the growth of the two species in this situation resembled much more closely the patterns observed when they were completely separated, suggests that either metabolic interactions were not occurring, or that the substance in question was too large to pass through the dialysis bag walls. However it has already been shown that most growth regulators known to be present in plants will readily pass through the dialysis bag walls.

My conjectures as to the nutrient kinetics of diatoms and flagellates are consistent with reports in the literature. Caperon and Meyer (1972) measured nitrate uptake rates in steady-state cultures of *Dunaliella*, *Monochrysis* and *Cyclotella*. Maximum uptake rates per unit carbon (V_{max}) were 0.0182, 0.0158 and 0.0858 g-at N (g-at C)$^{-1}$ h^{-1} respectively: the diatom took up nitrate at a rate five times faster than the green or the chrysophyte. Eppley *et al.* (1969) measured maximum nitrate uptake rates in four diatoms which ranged from 0.285-227 x 10^{-7} mole cell^{-1} h^{-1} and in the flagellate *Emiliana huxleyi* 0.046 x 10^{-7} mole cell^{-1} h^{-1}: again the diatoms could take up nitrate 6-5,000 times more quickly than the flagellate. These findings agree with the hypothesis that, under crowded conditions, *Thalassiosira* might have been able to outcompete *Chrysochromulina* for nutrients and therefore grew faster.

At low nutrient concentrations, on the other hand, there is evidence that small flagellates have an advantage. Half-saturation constants for

nitrate determined by Eppley *et al.* (1969) ranged from 0.4-9.3 μM for four species of diatoms but the K_s value for the flagellate *Emiliana huxleyi* was equal to 0.10 μM. Thus at low nitrate concentrations the flagellate was more efficient. The ability of flagellates to outcompete diatoms for organic nitrogen sources has been observed by Ryther (1954). was due to the greater e[illegible] of cells

These observations support the hypothesis that *Chrysochromulina* was able to outlast *Thalassiosira* in mixed culture due to its ability to function at the reduced nitrate levels which would have been present after several days' growth.

Admittedly this discussion is based on the results of a single experiment under a single set of favourable conditions. Therefore it would be presumptuous to generalize and to state that metabolites are definitely not involved in flagellate-diatom interactions. However, the purpose of the experiment was to determine whether or not metabolites play a functional role in the spring and fall changeovers from flagellate to diatom and back to flagellate populations in Saanich Inlet. Conditions at both of these times are comparable to those used in this experiment: nutrients are abundant, light levels are somewhat low but able to support growth in both species (see light experiments), temperatures are moderate, and so on. As well, the species used were isolated from Saanich Inlet, and represent the most common winter flagellate and the first spring diatom. On this basis it seems reasonable to conclude that the conditions chosen for the experiment bear enough resemblance to the natural situation that we can state that metabolites are probably not important to the spring and fall population changes which occur in Saanich Inlet. Moreover, the fact that the same shifts occur yearly, regardless of the species composition of the phytoplankton, is

further reason to suppose that metabolites do not play a major role in the observed seasonal shifts in Saanich Inlet phytoplankton.

4. Hydrocarbon pollution

In experiments with large plastic containers in Saanich Inlet, Lee (Lee and Takahashi, 1977; Lee *et al.*, in press) found that low level hydrocarbon pollution favoured the growth of nanoflagellates. Experiments conducted in the present investigation revealed no such enhancement of nanoflagellates by hydrocarbons, either in pure culture or mixed natural populations. It is possible that in the case reported by Lee, the flagellates observed were colourless heterotrophs living off the organics released by dying phytoplankters, as no distinction was made between colourless and photosynthetic flagellates. Colourless flagellates are abundant in Saanich Inlet and commonly outnumber the phytoflagellates by as much as an order of magnitude. In the present study the only species found to be enhanced by the presence of hydrocarbons was in fact a diatom, *Skeletonema costatum*, whose growth was enhanced by the presence of No. 2 Fuel Oil in concentrations less than or equal to 300 $\mu g\ l^{-1}$. No algae (with the possible exception of one species of green soil algae-see Kruglov and Paromenskaya, 1970) have been found to metabolize hydrocarbons, and the probable mechanism for stimulation appears to be the improvement of membrane permeability which would affect equally phytoplankters offall taxonomic groups (Dunstan *et al.*, 1975).

B. Factors Contributing to Winter Flagellate Dominance

1. Light

Of all the factors which combine to produce flagellate dominance in the winter, light appears to be the single most important factor.

Parsons and Takahashi in their 1973(a) model of factors controlling cell size concluded that light was one of three important factors, along with nutrient concentration and sinking rate. Takahashi *et al.* (in press) showed that light was the sole factor responsible for depressed primary productivity in the winter in Saanich Inlet. Steemann-Nielsen (1955) observed that "in highly productive regions having a rich supply of nutrient salt, light intensity is the most important limiting factor. It is also limiting in high latitudes during winter in regions that lack stability of the water column...."

Growth versus intensity and photosynthesis versus intensity relationships derived for various Saanich phytoplankters in pure culture did not show this. Results were inconsistent, probably as a result of adaptation problems. There are numerous reports of values for I_k, I_c, I_{sat} and so on, in the literature; however results here are just as inconsistent, due to the wide variety of units used for measuring light intensity and the difficulty in interconversion, as well as preconditioning and adaptation problems with the cells themselves. For example, in three separate reports, values for I_{sat} for *Skeletonema costatum* at 20°C varied from 64 to 400 $\mu E\ m^{-2}\ sec^{-1}$ (Curl and McLeod, 1961; McAllister *et al.*, 1964; Jorgensen, 1970). From the cumulated results of many studies there are no apparent trends in responses to light intensity among species of diatoms, dinoflagellates or nanoflagellates (Table IV).

Despite the scatter of values for various growth constants, certain consistencies do exist in the literature with respect to morphological and chemical responses of algae to light intensity. Results from the present study and others have shown that within a single species, it is common for small cells to be favoured (e.g., Winokur, 1948; Brown and

Table IV. Summary of literature on light constants for various species of algae. All values were converted to $\mu E\ m^{-2}\ sec^{-1}$ using the following conversion factors:

1 klux = 92.9 ft-c
= 5×10^{-3} ly min^{-1} (Westlake, 1965)

1 ly min^{-1} = 1 g cal $cm^{-2}\ min^{-1}$
= 6.97×10^{4} $\mu W\ cm^{-2}$
= 6.97×10^{2} kerg $cm^{-2}\ sec^{-1}$
= 3200 $\mu E\ m^{-2}\ sec^{-1}$ (Hollaender, 1956)

Species	Preconditioning I	Preconditioning T°C	Exptal T°C	I_c	I_k	I_{sat}	I_{opt}	I_{inhib}	Basis	Reference
Avg. of 7 greens	160	20	20		80				μ	Ryther, 1956
Euglena						51		517	μ	Cook, 1963
Chlorella						18			μ	Myers, 1946; Stepanova, 1963
Nannochloris sp. 582	86	21	27-29	hetero.		138			μ	Thomas, 1966
Dunaliella tertiolecta	320-480	25	25	42		400			ps	McAllister *et al.*, 1964
"	960-1280	25	25	42		960			ps	McAllister *et al.*, 1964
Monochrysis lutheri			20	32		320			ps	McAllister *et al.*, 1964
Isochrysis galbana						32			?	Kain and Fogg, 1958
Amphidinium carteri				32		400			ps	McAllister *et al.*, 1964
"							320-1280		μ	Jitts *et al.*, 1964
Gymnodinium splendens							32-128		μ	Thomas *et al.*, 1973
G. simplex							96-320		μ	Thomas, 1966
Gymnodinium sp. 581	86	21	23-26	6.1		130			μ	Thomas, 1966
Gymnodinium sp. 582	86	21	23-26	6.1		130			μ	Thomas, 1966
Gonyaulax polyedra							80-160		μ	Hastings & Sweeney, 1964
Prorocentrum gracile							>74		μ	Barker, 1935
P. micans							>96		μ	Kain and Fogg, 1960
"							>74		μ	Barker, 1935
Peridinium							>74		μ	Barker, 1935
Avg. of 4 dinos	160	20	20		400				μ	Ryther, 1956
Skeletonema costatum	160	19-21	5-18	16		192-256			ps	Curl and McLeod, 1961
"	160	19-21	>18	16		64-96			ps	Curl and McLeod, 1961
"	48	20	20		112-208				ps	Jorgensen, 1970
"			20	19		400			ps	McAllister *et al.*, 1964

Table IV (continued)

Species	Preconditioning I	Preconditioning T°C	Exptal T°C	I_c	I_k	I_{sat}	I_{opt}	I_{inhib}	Basis	Reference
Chaetoceros affinis	86	14-16	14-16.5		74-123			448-928	ps	Talling, 1960
Chaetoceros sp. 581	86	21	23-26	1.8		104			μ	Thomas, 1966
Coscinodiscus excentricus						192		512	?	Jenkins, 1937
Biddulphia regia						192		512	?	Jenkins, 1937
Asterionella japonica						112			?	Kain and Fogg, 1958
"			8		64			320	ps	Steemann-Nielsen, 1949
Asterionella sp.			16			288		640	?	Talling, 1957
Nitzschia dissipata						256			?	Wassink and Kersten, 1945
N. closterium f. *minutissima*				4.5		9.3			ps	Mann and Myers, 1968
Avg. of 3 diatoms	160	20	20		400				μ	Ryther, 1956
Natural, northern, October						96				Steemann-Nielsen, 1937
Natural, Bergēn, May						192				Berge, 1957
W. Pacific						448				Steemann-Nielsen and Al Kholy, 1956

Richardson, 1968; Jorgensen, 1970) and for pigment content to increase at low intensities (Sargent, 1940; Myers, 1946; Kratz and Myers, 1955; Brody, 1958; Steemann-Nielsen *et al.*, 1962; Jorgensen, 1964; Waaland *et al.*, 1974). Such observations are consistent with a strategy to maximize utilization of available light by increasing the amount of sensitive material and increasing the area illuminated-to-volume ratio. According to such a strategy, low light levels should favour the growth of nanoplankton.

Unlike studies with pure cultures, incubations of natural populations consistently showed that diatoms have narrow tolerance ranges to light relative to flagellates, and that the lower thresholds of diatoms are higher than those of flagellates. A schematic representation of this is given in Fig. 25A. If for a simple, hypothetical phytoplankton community of three diatoms and one flagellate, D_1, D_2 and D_3 represent the growth versus light intensity curves for the three diatom species, and F_1 the curve for the flagellate species, then between points A and C, and above D, diatoms will dominate, and below A, and between C and D, flagellates will dominate. Note that although diatom D_2 and the flagellate have similar light optima, the diatom will always dominate at those intensities due to a higher intrinsic growth rate. In those regions where it is dominant the flagellate appears to do so only "by default:" that is, there is no particular reason why it should dominate except that there is no diatom which will grow properly at those intensities. According to findings in the present study this simplified scheme is not unrealistic. Although the shapes of the individual curves may vary with other environmental conditions such as temperature, photoperiod and nutrient concentration, the basic relationships represented in Fig. 25A seem to hold.

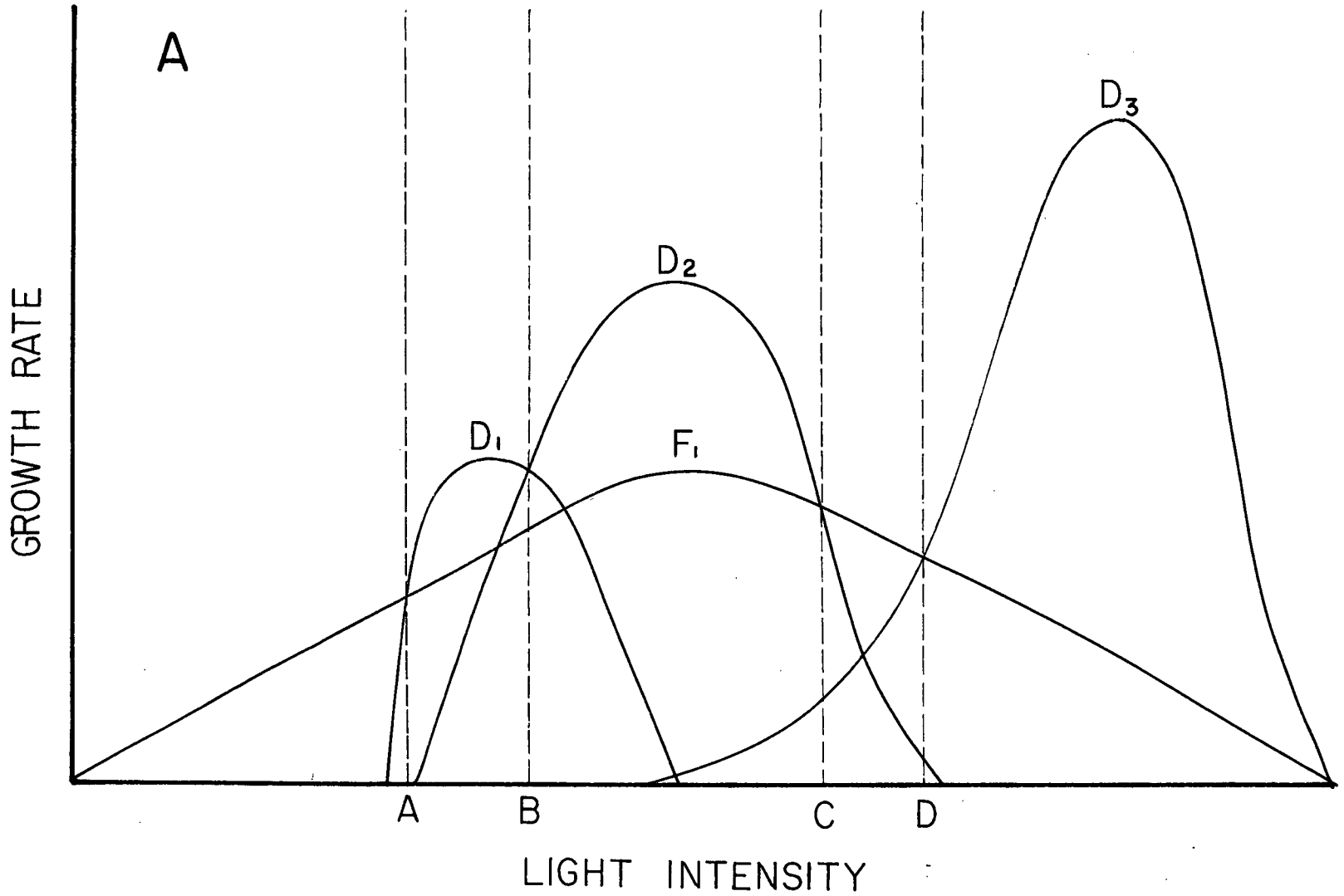
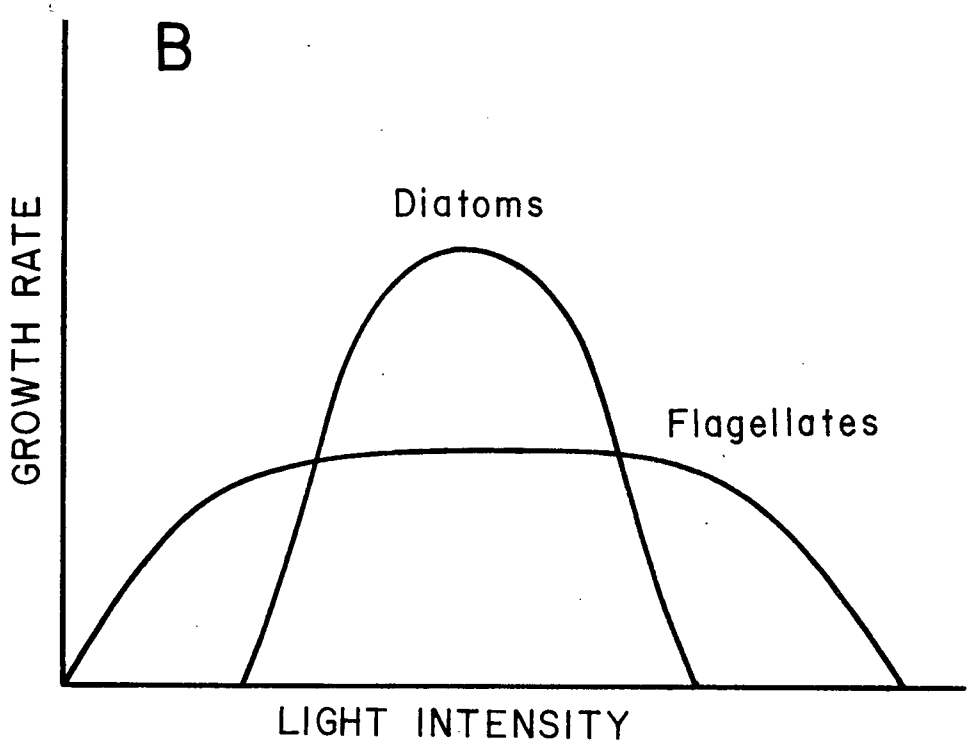


Figure 25. Schematic representation of the relative light tolerances of diatoms and flagellates. A. Growth rate versus light intensity curves for a hypothetical phytoplankton community of 3 species of diatoms and 1 flagellate. B. Generalized curves which include all species of diatoms and flagellates.


B
Diatoms
Flagellates
GROWTH RATE
LIGHT INTENSITY

If we were to extrapolate to include all diatoms and flagellates, the relationship would probably resemble that shown in Fig. 25B. As a group, flagellates can grow over a much wider range of light intensities than diatoms, but within their narrow "corridor" of suitable light intensities, diatoms have higher growth rates than flagellates and therefore would be expected to dominate a phytoplankton community living within the "corridor." Parsons refers to this corridor in a recent model of diatom-flagellate interactions (Parsons, *et al.*, in press).

Why should such a situation exist? What allows flagellates to be so much more independent of light intensity? Why should diatoms have intrinsically higher growth rates? Flagellates are of course motile and in many cases phototactic, so that it is possible that they are able to seek out depths in the water column where optimum conditions of light intensity exist. Diurnal vertical migration is known to exist among dinoflagellates (Holmes *et al.*, 1967; Eppley *et al.*, 1968) and has been reported for natural phytoplankton populations (Wood, 1963b). The fact that many flagellates will actively seek out their own preferred light conditions (i.e., avoid both dim and overly bright light intensities) is commonly used to separate them from diatoms and other non-motile forms, as well as from each other, in culture studies (e.g., Halldal, 1958; Tsuji, 1973).

Clearly this is not the sole mechanism for flagellate tolerance of wide ranges of light intensity, since in determinations of photosynthesis versus intensity and growth versus intensity curves they are held in bottles at fixed light intensities. Brown and Richardson (1968), working with 18 species of algae, concluded that "the degree to which light intensity is an influence can be directly related to the degree to which an alga is a photoautotroph. We can simulate a series with increasing dependence upon light

among the algae tested as follows: *Astasia, Ochromonas, Euglena, Chlorococcum*,..." Among centric diatoms the capability for facultative heterotrophy is rare (Lewin, 1963; Lylis and Trainor, 1973; Droop, 1974). The only species which have been reported to have this ability are *Cyclotella cryptica* and *Coscinodiscus* sp. on glucose (Kuenzler, 1965; Hellebust, 1971; White, 1972). Among pennates the ability to use organic substrates is common (Lewin and Lewin, 1960; Lewin, 1963; Chansang, 1975). The majority of euglenoids are obligate heterotrophs (Droop, 1974), and facultative heterotrophy has been demonstrated among pigmented haptophytes (Rahat and Jahn, 1965; Rahat and Spira, 1967; Provasoli and Pintner, 1968), cryptophytes (Antia *et al.*, 1969), chrysophytes (Pringsheim, 1952; Aaronson and Baker, 1959), chlorophytes (Chodat and Schopfer, 1960; Shihara and Krauss, 1965), prasinophytes (Turner, 1970) and blue-greens (Kenyon *et al.*, 1972). It is entirely possible that at limiting light intensities many flagellates can supplement their photoautotrophic nutrition with heterotrophic nutrition and thus are able to maintain growth during the winter where diatoms cannot.

On a broader scale it is intuitively reasonable that flagellates as a group should have wider tolerances to light intensity, as they represent a taxonomically much more diverse assemblage than do diatoms. Diatoms all belong to the class Bacillariophyceae in the phylum Chrysophyta, while flagellates include members of many algal phyla, including not only the Chrysophyta but the Chlorophyta, Euglenophyta, Haptophyta (sometimes considered a class of Chrysophyta), Cryptophyta, Pyrrophyta and Rhaphidophyta. Thus the diversity of pigment spectrum and internal structure is considerably greater among the flagellates than the diatoms, and therefore it might be expected that as a group they would be suited for a wider range of conditions

than would diatoms. Brown and Richardson (1968) showed that in general chlorophyll *c*, fucoxanthin and peridinin-containing algae grow best at intensities of 780 ft-c (8.4 klux or approximately 130 μE m^{-2} sec^{-1}), while phycobilin-containing algae grow best at 400 ft-c (4.3 klux or approximately 70 μE m^{-2} sec^{-1}), and chlorophyll *b*-containing algae at 1000 ft-c (10.8 klux or approximately 170 μE m^{-2} sec^{-1}). Flagellates are represented in all three of these categories while no diatoms contain phycobilins or chlorophyll *b*. This corresponds well with the "diatom corridor" concept, mentioned earlier, which refers to the narrow, moderate-level range of light intensity where diatoms will grow, as compared to the much broader range over which flagellates will grow.

Given that flagellates are adapted to much broader ranges of light conditions than are diatoms, how then do diatoms compete at all? It was observed that within their optima diatoms grow much more quickly thankdo flagellates. The same observation has been made by Eppley (personal communication), although it conflicts with reports made by others (Odum, 1956; Williams, 1964; Saijo and Takesue, 1965). It is possible that the metabolic costs of locomotion are such that the amount of energy devoted to growth and reproduction is reduced in flagellates, whereas in non-motile diatoms relatively more energy can be devoted to growth. Knoechel (personal communication) has suggested that flagellates suffer greater losses on the basis of cell numbers, due to their fragility: apparently following cell division lysis of 1 of the daughter cells is not uncommon. Diatoms, with their sturdier construction, would be less subject to such losses. Alternatively, perhaps when cells are light saturated some other cell process such as nutrient uptake becomes limiting. Perhaps faster uptake and assimilation of nutrients is what

enable diatoms to grow more quickly than flagellates. There was some indication of this in the metabolite experiment.

2. Photoperiod

Many researchers have reported increased growth rates at a given light intensity as photoperiod is increased (e.g., Talling, 1955; Castenholz, 1964; Eppley and Sloan, 1966; Paasche, 1967). In some cases an increase in photoperiod reduced the compensation light intensity (e.g., Paasche, 1968), and often the threshold for photoinhibition (e.g., Castenholz, 1964; Paasche, 1968). Typically however the saturating light intensity does not change (e.g., Castenholz, 1964; Paasche, 1967), although in *Ditylum brightwellii* apparently it does (Paasche, 1968). The growth rate of *Biddulphia aurita* is independent of daylength within the range 9 to 15 hours light per 24 hours (Castenholz, 1964). In most cases reported in the literature, then, as well as in the present study, increasing the photoperiod increases growth rate, lowers I_c and I_{inhib} but has little or no effect on I_{sat}. Decreasing the photoperiod of course would have the opposite effect. In the natural population incubation studies, increasing the photoperiod had the effect of shifting the species dominance relations such that individual species peaks occurred at lower intensities. Presumably shortening the day would again have the opposite effect.

A typical winter photoperiod in Saanich Inlet might be $8\text{-}\overline{16}$, a typical summer photoperiod about $16\text{-}\overline{8}$. The change in photoperiod probably has the effect of exaggerating the seasonal variations in available light. Therefore an intensity of 100 $\mu E\ m^{-2}\ sec^{-1}$ which in the summer may be able to support the growth of species A, may be insufficient to do so in the winter. In experiments conducted in the laboratory the

photoperiod used was 12-$\overline{12}$, so that light optima determined for individual species may be underestimated for winter conditions and overestimated for summer.

Photoperiod has been reported to have regulatory effects on cellular functions such as pigment synthesis (e.g., Sorokin, 1957; Lorenzen, 1959; Cook, 1961; Castenholz, 1964; Jorgensen, 1966) and cell division (e.g., Tamiya *et al.*, 1953; Sweeney and Hastings, 1962; Jorgensen, 1966; Pirson and Lorenzen, 1966; Paasche, 1967; Eppley *et al.*, 1967; Tanoue and Aruga, 1975), but there is no evidence that diatoms and flagellates differ in this respect.

3. Temperature

Winter temperatures reduced the growth rates of both diatoms and flagellates in pure culture (Fig. 16), but diatoms were judged to be more sensitive to the lower temperatures than were the flagellates. This conclusion was made on the basis of experiments with natural populations, in which gradual increases in the flagellate-to-diatom ratio were seen in a summer population placed at 6°C. These changes were slow, however, on the order of weeks. By comparison, light-induced population shifts occurred in days, much more quickly than temperature-induced shifts, so that temperature must be considered a secondary factor in the diatom-to-flagellate shift which occurs in the fall.

Unfavourably low temperatures caused the light tolerance ranges of diatoms to narrow. The combined effects of decreasing light intensity, narrowed light tolerances ranges and decreasing temperature would serve to hasten the fall crash of diatoms, while the more tolerant flagellates would persist, though suffering somewhat from the poorer growth conditions.

Curiously, although present experiments showed consistently that flagellates are able to survive at lower temperatures than diatoms, several reports in the literature show the opposite to be true (Yentsch and Ryther, 1959; Durbin *et al.*, 1975; see review by Eppley, 1972). It seems the "diatom corridor" concept applies not only to light but to temperature and other factors as well.

4. Water stability

Salinity data showed that a positive salinity gradient is maintained year-round in Saanich Inlet. This gradient is sufficient to maintain the stability of the water column. Two properties of the inlet show how unusually stable the water of Saanich Inlet is. Below the sill depth (about 75 m) the inlet is anoxic, the oxygen supply being replenished only occasionally when dense outside water spills over the sill into Saanich. Secondly, winds, which normally cause turbulent mixing in the surface layers of the water column, in Saanich do so only to a minimal extent. Instead the effect of winds is to push the surface "skin" of low salinity water toward one end or out of the inlet (depending on wind direction), resulting in the upwelling of subsurface water, and followed by seiche-type oscillations upon cessation of the winds (Herlinveaux, 1962). Such events could only occur in an unusually stable body of water.

Vertical water motion in Saanich is minimal. Two possible mechanisms exist for upward transport, both weak. The first is the upwelling mentioned above which follows storms. The second is entrainment by the surface freshwater layer. This however is sporadic and restricted primarily to winter and spring due to the weak nature of the estuarine circulation in the inlet (Herlinveaux, 1962).

Sinking is therefore a major consideration for phytoplankton cells in Saanich Inlet. Flagellates have a tremendous advantage in this respect. Diatoms could be severely restricted by vertical mixing events. It is easy to see how in a stable environment such as Saanich Inlet diatoms with good flotation mechanisms would have an advantage over more rapidly sinking species.

If experimentally determined values for saturating light intensity and compensation light intensity of various diatom species are compared with light profiles in Saanich, some indication can be obtained of favourable and compensation depths for each species. The compensation light intensity for *Skeletonema costatum* (calculated as the mean value of I_c measured in the experiments conducted in this study) was 17.3 $\mu E\ m^{-2}\ sec^{-1}$. Since this species had the lowest measured value for I_c of all diatoms encountered, its compensation intensity must represent the compensation intensity for Saanich Inlet diatoms as a whole. Comparing the value of 17.3 $\mu E\ m^{-2}\ sec^{-1}$ with the light profiles in Fig. 6, we obtain compensation depths of 2.5 m on December 16, and 7.5 m on June 21. Saturating light intensities for *Nitzschia delicatissima*, *Thalassiosira* spp., *Skeletonema costatum*, and *Chaetoceros* spp. ranged from 183 to 263 $\mu E\ m^{-2}\ sec^{-1}$. These intensities exist in Saanich Inlet only during the summer in a surface "skin" less than 1 m deep.[1] Under these circumstances a diatom in Saanich Inlet is rarely light saturated but may easily become light limited. In order to survive, it must remain above its compensation depth of 2.5 m during the winter or 7.5 m [illegible]

[1] This calculation is based on data from Fig. 6. Since light values in this figure represent 24-hour averages, it is entirely possible that saturating light intensities could exist below this surface layer for several hours around mid-day.

compensation depth of 2.5 m during the winter or 7.5 m in the summer.[1] (Note that the concept of critical depth does not apply here as there is no mixed layer.) If a moderate sinking rate of 1 m day^{-1} is assumed, survival can only be maintained if upwelling events occur every 2.5 days in the winter and 7.5 days in the summer. If sinking rates are greater than 1 m day^{-1} or if cells are not brought to the surface then of course upwelling must occur more often.

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[1] Values calculated for survival do not account for processes such as encystment. Actual values for survival in total darkness may be as great as 9 weeks at 10°C for *Skeletonema costatum* and more than 30 weeks at 10°C for *Chaetoceros gracilis* (Antia, 1976).

If cells have been surviving at sub-compensation intensities, growth does not begin immediately upon reillumination. Smayda and Mitchell-Innes (1974) observed a lag period of 5 days for *Ditylum brightwellii* at 15°C after 46 days in the dark, and longer for *Skeletonema costatum*. Due to slower metabolic rates one would expect this lag to be even longer at lower temperatures. Thus in winter if a cell is brought to the surface following a storm it must be able to remain afloat long enough to go through the growth-initiating processes which occur during lag phase before increases in numbers can occur. Clearly, neither is there suffieient upwelling nor are diatoms able to remain afloat long enough for this to occur in the winter. The fact that water maintained at surface light intensities produced healthy diatom cultures following incubations of 1 week supports this hypothesis.

The absence of large dinoflagellates during the winter months may be a function of temperature. The small *Katodinium rotundatum* appears to be an exception, since it is a common winter flagellate. However, larger dinoflagellates, despite their capability for phototaxis, may be limited by the cold temperatures of the winter euphotic zone.

C. Summary of Phytoplankton Dynamics

It is now possible to construct a qualitative model of the seasonal events in Saanich Inlet. Light appears to be the single most important consideration. The unusually stable nature of the water column and the seasonal change in photoperiod and temperature contribute to reinforce the light effect. Nutrient concentrations may be important during the summer only. Grazing, pollution and chemical interactions among species are of minimal significance.

1. Winter

In winter reduced solar radiation is the overall limiting factor to primary productivity in Saanich Inlet. Additionally, shortened days effectively reduce the available light even further, and low temperatures decrease the tolerance of diatoms to sub-optimal light intensities. The range of favourable intensities exists in a shallow layer of water, perhaps 5 m deep, in the surface of the inlet. The upper 2 m of this is most frequently occupied by water up to $10^{o}/oo$ less saline than that immediately below. For flagellates it is fairly simple to remain within that narrow subsurface band of water where favourable conditions exist. For diatoms it is virtually impossible. While storms may occasionally re-seed the surface with dormant diatom cells or spores, they still require a period of up to 5 days or more before active growth can be initiated. Well before that time they have usually sunk to below their compensation depths. The low biomass of diatoms which does exist in the winter must consist of these dormant cells, along with perhaps some very small cells with exceedingly slow sinking rates, such as small, single-celled *Chaetoceros danicus* or *Thalassiosira*, as well as pennates which are able to rely in part on heterotrophy.

Flagellates, less affected by low light intensities, and some with the ability to live heterotrophically, can grow in the winter even if they are not phototactic. *Chrysochromulina kappa*, for example, a common winter flagellate, is not phototactic but is known to assimilate several organic substances in dim light (Pintner and Provasoli, 1968).

2. Spring

A number of changes occur in the spring. Solar radiation increases, deepening the euphotic zone. Calmer seas increase penetration

of sunlight at the sea surface, and daylength increases; both of these events effectively deepen it further. As temperatures rise diatoms become better able to survive at previously limiting light intensities. The first indication of the spring bloom, however, comes before the diatoms begin to grow. As was seen earlier, although flagellates grow in the winter their growth is improved in warmer, brighter conditions. As spring approaches then, the first event is an increase in flagellate numbers. Gradually light increases to a point where the euphotic zone is deep enough to compensate for thessinking rates of the diatoms. Note that in the case of a very windy spring, or a spring with large volumes of snow melt (and hence increased entrainment in the estuary) upwelling may cause the bloom to arrive earlier than in a calm spring. An uncommonly cloudless spring may have the same effect. The first diatoms to bloom should be those with lowest light requirements. Among those, faster growth rates and simply presence in the water will determine exactly which species will bloom. From experiments in this study, *Thalassiosira* spp., *Skeletonema costatum* and *Nitzschia delicatissima* were all found to have similar, low requirements for light. Looking at the data for 1975-1976 phytoplankton (Fig. 10), *Nitzschia* was never present, and *Thalassiosira* and *Skeletonema* bloomed concurrently. *Chaetoceros*, with higher light requirements, bloomed much later.

3. Summer

Summer conditions were never examined in the present study, except as a basis for comparison with the winter. However, on the basis of some experimental findings and by looking at the trends in the field monitoring program, some indication can be gained of the dynamics of summer phytoplankton succession in Saanich Inlet.

In summer growth conditions were favourable for both diatoms and flagellates. However due to their apparent ability to grow faster than flagellates under favourable conditions (whatever the mechanism), diatoms dominated the phytoplankton almost without exception.

Diatoms appeared to be regulated by the availability of nitrate. When light conditions became favourable in the spring the diatoms bloomed, but soon the nitrate supply was depleted, and they and the flagellates crashed. Wind then became instrumental in upwelling new nutrient supplies (as well as seed diatom stock) which enabled production to be renewed (see Fig. 26).

Flagellates were no less abundant in the summer than in the winter. Although it is likely that they were outcompeted by diatoms for nitrate, they may have been able to utilize alternate, less abundant sources of nitrogen, such as organic nitrogen. There is some evidence that flagellates can outcompete diatoms for this source (Ryther, 1954). Alternately there exists the possibility that flagellates and diatoms were separated vertically in the water column, although there was no indication of this in Saanich Inlet profiles (Fig. 27).

In many instances during the summer a diatom bloom was preceded by a small flagellate increase which decreased again as the diatoms began to grow (Fig. 26). It may be that flagellates are quicker to respond to a boost in the nutrient supply but are eventually overtaken by diatoms. There was no evidence during the summer studied, to support the idea that flagellates proliferate between diatom blooms during periods of nitrogen depletion.

It is interesting to note that, approaching the summer solstice (June 21), the overall nitrate level in the top 20 m gradually decreased,

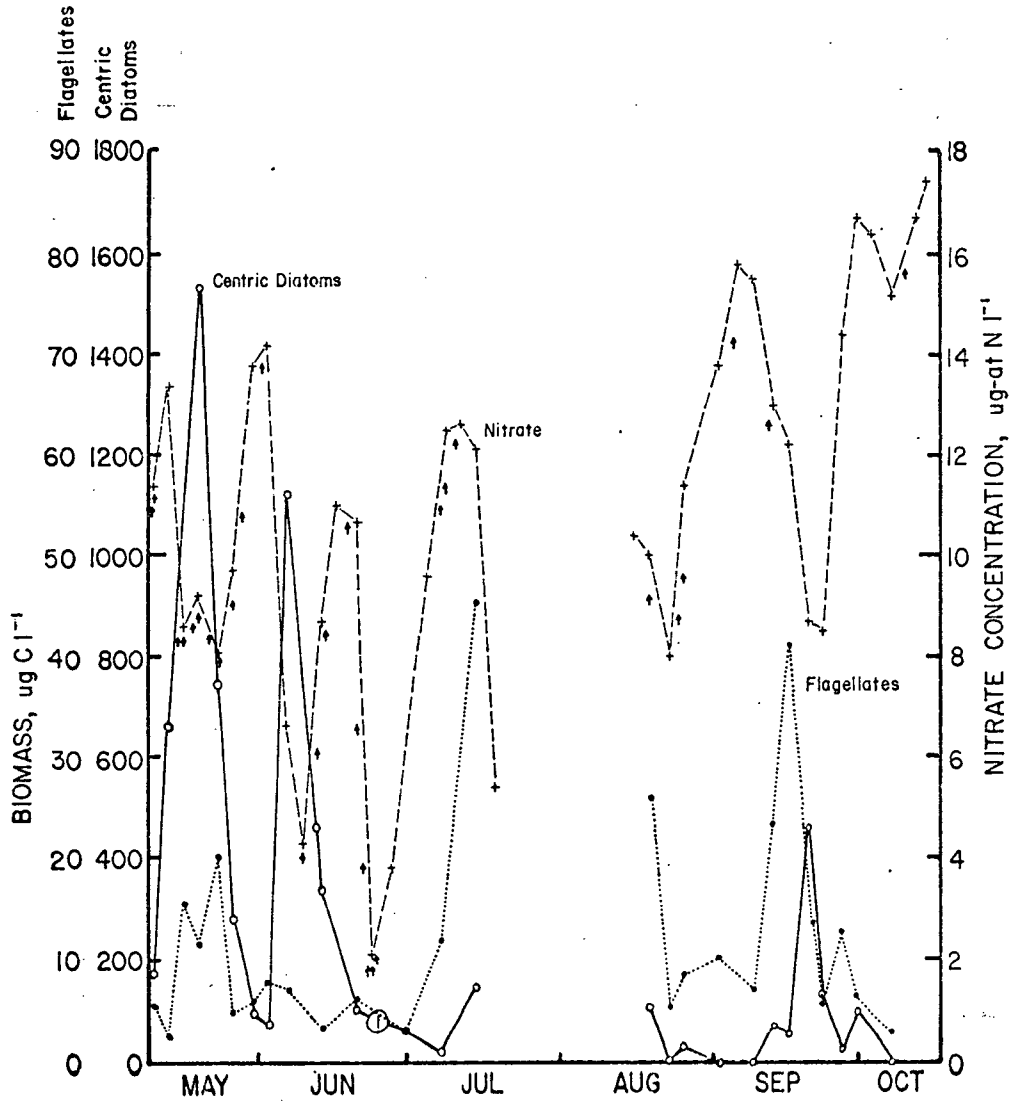

Figure 26. Nitrate concentration and the changes in biomass of centric diatoms and flagellates in the top 20 m during the summer of 1976. ---- nitrate, ——— centric diatoms, ····· flagellates. ↑ average wind speed >10 km h^{-1}. At ①, a bloom (of diatoms?) may have occurred but may have not been observed: chlorophyll *a* which was sampled during this period rose from 2.70 µg l^{-1} on June 21 to 10.9 and 8.04 µg l^{-1} on June 24 and 28 respectively, then fell back to 1.54 µg l^{-1} on July 1.

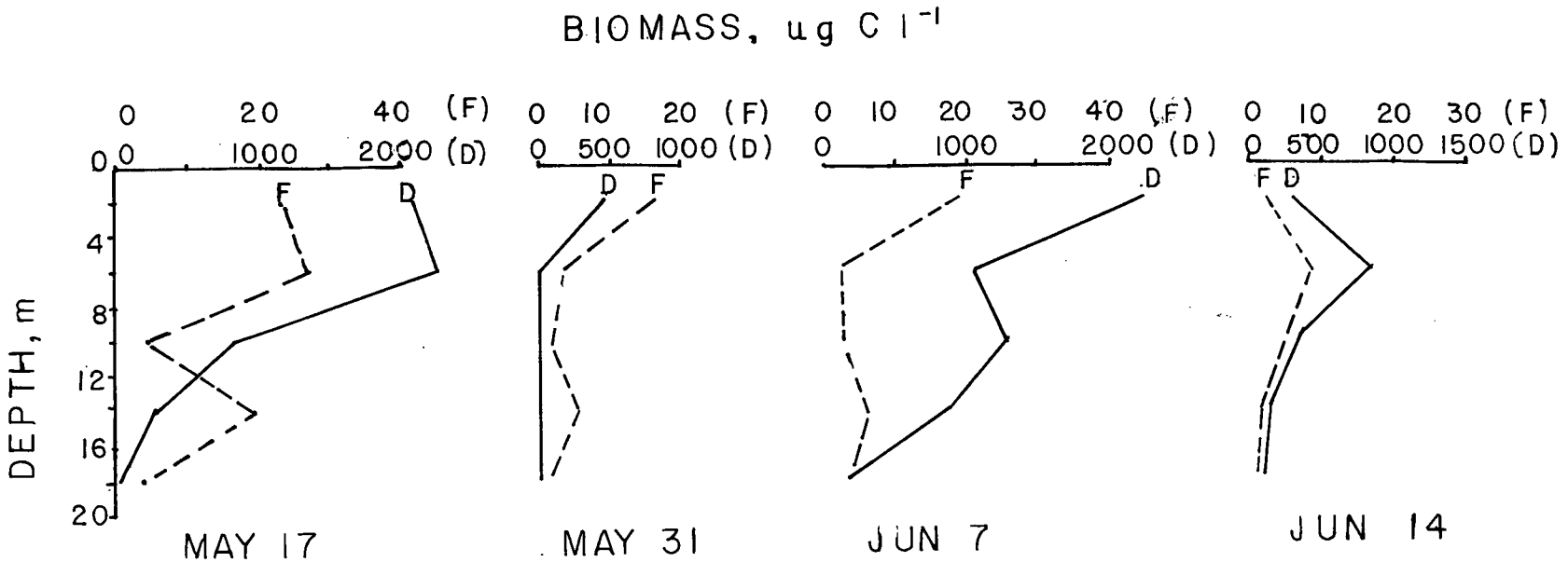

Figure 27. Vertical distributions of centric diatoms and flagellates on days of maximum and minimum diatom numbers.

and following the solstice it gradually increased again (Fig. 26). In Fig. 7 it can be seen that this was due to changes in the depth of the nitrate depleted layer. This undoubtedly relates to the deepening of the euphotic zone to its maximum around the solstice and its shallowing after that date. As the euphotic zone deepened, diatom productivity could occur deeper, and hence nitrate was depleted down to greater depths; as the euphotic zone became shallower so did the nitrate depleted layer. The effect of light can be seen even in the summer.

4. Fall

The sequence of events in the fall is essentially the reverse of spring. The euphotic zone gradually shallows; temperature and daylength become more restrictive. Perhaps diatoms survive longer than might be expected from thresholds demonstrated in the spring or in laboratory experiments, as has been shown to be possible. Eventually they must crash[1] however, and then the flagellates take over "by default." Although it was not observed in 1976 it is conceivable that a period of sunny and/or windy weather could produce an isolated fall bloom, probably of a low-light tolerante species such as *Skeletonema*, *Thalassiosira* or *Nitzschia*, after the main diatom crash; however it would likely be short-lived.

An indication of the critical light intensities in the spring and fall can be obtained by comparing Fig. 5 and 9. A line drawn through the graph of total daily solar radiation (Fig. 5) at about 2100 $\mu E\ m^{-2}\ sec^{-1}$ intersects the curve at almost precisely the onset of the spring diatom

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[1] The decline in diatom numbers is truly a "crash," occurring within a week or less. See for example the rapid decrease in diatom biomass at the beginning of November, 1975, in Figure 9.

bloom and the fall crash. Therefore, in Saanich Inlet, depending upon conditions of cloud cover, wind mixing and seed species, the spring diatom bloom and fall crash should occur when the daily solar radiation levels reach 100 $\mu E\ m^{-2}\ sec^{-1}$.

D. Problems

Several approximations and sacrifices in methodology acted as sources of error in the present study.

1. Light profiles were calculated from available data on total daily solar radiation and percent transmission of light through the water column at noon. Since the amount of light reaching any depth is a function of sun angle, sea conditions and cloud cover, a profile calculated from data taken at noon may not be representative of the day: in fact it undoubtedly overestimated the average light penetration for the day due to variations in sun angle. Diurnal variationsiin intensity are not considered: all data were given as averages over a 24-hour period. Actual intensities varied from perhaps three times higher than those given in Fig. 6 at noon to zero at night. Since light varied seasonally over an order of magnitude, the problem is likely not serious.

2. Different quantum meters were used to measure light in the laboratory and the field, although their sensitivity ranges were comparable. The light sources were also different. For these reasons and that presented above, data from the laboratory and field are only roughly comparable.

3. Samples for phytoplankton counting were integrated over varying depths, making it difficult to make comparisons from day to day.

4. Under ideal circumstances all light experiments should have been conducted concurrently, especially those involving natural populations. However since only a single incubator capable of maintaining a single temperature and photoperiod was available this was impossible. Fortunately the composition of the population did not affect the outcome of the experiments.

5. Experiments involving phytoplankters in pure culture produced inconsistent results. From reports in the literature as well as the present study it appears that depending upon the clone and its physiological state the behaviour within a single species may vary widely (see Table IV). For this reason it appears wise to regard the interpretation and ecological significance of culture studies with some caution.

6. Adaptation seems to be a problem with any physiological study. This was encountered with the pure culture experiments, and there is no reason to expect that it should not also arise with natural populations. However the fact that results proved to be fairly consistent whether the actual population was obtained during the summer or winter suggests that the observations made were applicable year-round. The process of adaptation was observed in the long-term experiment, and although changes did occur, the basic light-growth relationships persisted.

7. Many of the conclusions were based on a single year's field study. Data obtained over several years would have provided a superior basis for study.

E. Proposed Research

Further research is indicated on the role of nutrients and upwelling in regulating summer succession. Davis and Harrison (unpublished) have shown in preliminary work with continuous culture of natural populations that reduced nitrogen turnover rates favour the growth of flagellates. Work of this type with natural populations may be of greater ecological value than pure culture determinations of nutrient kinetics.

Further experimentation with light thresholds as described in this study may provide the basis for a mathematical model which compares those thresholds with measured sinking rates, cell survival and light levels

to predict the timing and sequence of the spring diatom bloom and fall crash. If this is combined with knowledge obtained on nutrient effects it should become possible to manipulate phytoplankton populations.

CONCLUSION

The competition between diatoms and flagellates in Saanich Inlet seems to be not so much a competition but rather a matter of survival for diatoms. Flagellates have wide tolerance ranges for a number of environmental factors and can grow year-round. However their growth rates are slow. Diatoms on the other hand can grow quickly but only under limited circumstances. Therefore when we consider factors regulating the ratio of diatoms to flagellates in Saanich Inlet, we are in reality looking at factors which regulate diatoms. When they are favourable, diatoms grow and outnumber the flagellates; when they are unfavourable the diatoms die and only the flagellates remain.

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APPENDIX I

Composition of enriched sea water (E.S.) culture medium for algae (Provasoli, 1968).

E.S. Enrichment

1.	Distilled water	100 ml
2.	$NaNO_3$	350 mg
3.	Na_2 glycerophosphate	50 mg
4.	Tris buffer	500 mg
5.	Adjust pH to 7.6	
6.	Autoclave	
7.	Fe (as EDTA, 1:1 molar)[1]	2.5 mg
8.	P II metals[2]	25 ml
9.	Vitamin B_{12} (autoclave)	10 µg
	Thiamine (autoclave)	0.5 mg
	Biotin (prepare aseptically)	5 µg

(These may be prepared as a single solution. Keep frozen.)

[1] Dissolve 351 mg $Fe(NH_4)_2(SO_4)_2.6H_2O$ and 330 mg Na_2EDTA in 500 ml distilled water; 1 ml of this solution = 0.1 mg Fe. Autoclave.

[2] P II Metal Mix (directions for making 100 ml of metal mix)

	1 ml contains	quantity for 100 ml	coefficient for salt	quantity of salts to add to 100 ml	
1.	Na_2EDTA 1 mg	100 mg		100 mg	Na_2EDTA
2.	Fe 0.01 mg	1 mg	x 4.9	4.9 mg	$FeCl_3.6H_2O$
3.	Mn 0.04 mg	4 mg	x 4.1	16.4 mg	$MnSO_4.4H_2O$
4.	Zn 0.005 mg	0.5 mg	x 4.4	2.2 mg	$ZnSO_4.7H_2O$
5.	Co 0.001 mg	0.1 mg	x 4.77	0.48 mg	$CoSO_4.7H_2O$
6.	B 0.2 mg	20 mg	x 5.7	114 mg	H_3BO_3

Autoclave.

To obtain E.S. medium add 2 ml E.S. enrichment to 100 ml filtered sea water. For bacteria-free cultures sterilize enrichment in tubes, add aseptically to filter-sterilized or autoclaved sea water.

Silicate enrichment for diatoms: Add 1 ml $Na_2SiO_3.9H_2O$ (2.8 g/100 ml) solution and 0.2 ml 1 N HCl, admixed, per 100 ml of medium.

Approximate enrichment of nutrients obtained by using E.S. culture medium (concentrations in μM unless otherwise specified)

Nutrient	100% E.S.	5% E.S.
Nitrate	538	27
Silicate	985	49
Phosphate	30	1.5
Tris buffer	415	21
EDTA	15	0.73
Iron	6.4	0.32
Manganese	24	1.2
Zinc	0.25	0.013
Cobalt	0.055	0.0028
Boron	661	3.0
Vitamin B_{12}	0.97 nM	0.048 nM
Thiamine	0.19	0.0097
Biotin	2.6 nM	0.13 nM