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NANNOCHLORIS SPEC. AS A FOOD ORGANISM IN MARINE AQUACULTURE. CULTURE TECHNIQUES, PRODUCTION AND CONSUMPTION BY SECONDARY PRODUCERS [x]

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Abstract

The difficulties in transferring monocultures of marine microalgae from small-scale laboratory to large-scale open air conditions were overcome by the introduction of the small coccale green algae Nannochloris spec., which was found to dominate in highly eutrophic waters of low salinity as well as in sea water/waste water mixtures. This species proved to be eurythermic, euryhaline and insensitive to changing nutrient and light conditions, the maximum growth rate lying between 10 and 20 °/oo. Because of its high productivity, Nannochloris was never overgrown, though sometimes contaminated by other algae in aerated outdoor tanks during the course of the year. Inspite of its small size (2 - 6 µm cell diameter), it proved a suitable food item for secondary producers such as rotifers (Brachionus), copepods (Eurytemora), and oysters (Crassostrea). Its adaptability, rapid growth rate and high nutritional value qualify Nannochloris as an optimal primary producer in marine aquaculture enterprises.

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Introduction

A major problem in marine aquaculture is the production of suitable microalgae in sufficient quantities to serve as live food for the second link in the food chain. It is only with complicated technical and consequently costly means that pure cultures of microalgae can be maintained in outdoor tanks over a longer period.

Preliminary experiments with a view to selecting those of the commonly known 'food algae' (Monochrysis, Isochrysis, Tetraselmis, Dunaliella, Amphidinium) best adapted to open-air conditions showed that, unless the medium is previously filtered, all monocultures become overgrown with other species within a short time. We therefore tried to find an alga already optimally adapted to open-air conditions in respect of salinity, temperature and irradiation and at the same time suitable as a food item.

In the natural mixed populations of the highly eutrophic Schlei Fjord and in cultures fertilized with secondarily treated waste water, a small (2 - 6 µm diameter) coccale Chlorophycea Nannochloris spec. was found to be a dominant organism. We observed mass cultures of this species for over a year in aerated 4 and 28 m^2 outdoor tanks. No succession took place during this period. The cultures were sometimes contaminated by the diatoms Skeletonema costatum and especially Phaeodactylum tricornutum, which however, did not become dominant.

Laboratory and field experiments were conducted to gain information on the physiological, ecological and general suitability of this species as a food item with a view to creating the best possible growth conditions for the mass culture with regard to type and dosage of fertilizer, the aim being an optimal yield.

Methods

Nannochloris cultures were obtained from mixed cultures from the Schlei Fjord by means of serial dilution. In no case were sterile cultures used. The nutrients were biologically treated waste water from the sewage plant of Kiel and the agricultural

fertilizer 'Nitrophoska blau spezial(R)' containing N in the form of NH_4^+ and NO_3^-, PO_4^{---}, (N:P at = 11:1), Fe, Si and other trace elements. This fertilizer possesses the one drawback of not being fully soluble in water. A fully soluble fertilizer of similar composition would, though, be far more expensive.

Measurements: Cell counts with the Thoma haemocytometer, protein according to LOWRY et al. (1951), NH_4-N, NO_3-N, inorganic dissolved PO_4, total phosphorus according to GRASSHOFF (1976), seston according to LENZ (1971), chlorophyll a after UNESCO (1966).

Laboratory tests: In addition to the fertilizers mentioned above, tests were conducted with pure nutrients. Seawater and waste water were filtered through 0,45 µm membrane filters. Light sources were Philips fluorescent tubes TL 40 W, the light intensity was 9 - 15 W/m^2. The experiment temperature was 20° C. In the case of flow cultures, the nutrient was added with a peristaltic pump.

Outdoor experiments: The outdoor tanks are situated on the premises of the municipal sewage plant near Kiel. The tanks are of two sizes, 4 m^2 and 28 m^2, with a water depth of 25 - 50 cm. Turbulence and CO_2 input were accomplished by means of a Siemens cycloblower through perforated tubes running along the tank bottom. Preliminary experiments were conducted to find the most suitable aeration system with a sufficient input of CO_2 together with high turbulence (BARTELS, SCHRÖDER, 1978). The perforated tubes were compared with air-stones and air-lifts. Algae production was significantly lower especially when the air-lifts were used. For our purposes the perforated tubes with holes of 0,5 mm in diameter proved to be the least complicated and cheapest system. The seawater (15 - 20 °/oo salinity) is sucked up through a 1.5 m thick bed of gravel and pumped directly from the Baltic through a pipeline into two storage tanks, from where it flows by gravity into the culture tanks. Heating pipes running along the bottom of the tanks, through which warm (15° C) waste water is pumped, provide a heating system during winter time.

Results

Experiments with varying salinities (0 - 30 °/oo) have shown Nannochloris to be a decidedly euryhaline organism (Fig. 1). It grows within the entire salinity spectrum tested, the growth maximum lying at a salinity between 10 and 20 °/oo. How eurythermic it is may be seen from the fact that even at temperatures of 1° C and with very little irradiation (January), we observed doubling times of 2 - 3 days (cell concentrations 2 - 3 x 10^6/ml).

In experiments dealing with diurnal growth rhythms (light: dark hours 14:10), the cells were observed to grow from 2 - 3 µm to 4 - 6 µm diameter during daylight hours; during the dark phase, cells adapted to this rhythm showed a nearly synchronous division rate. The cells divided into two and also four. Parallel to cell count increase, chlorophyll a production also takes place during the dark hours (Fig. 2). For this reason the cell volume is very variable, a circumstance that hampers any attempt at calculating the exact biomass on the basis of cell counts, as shown in Table I. An exact measurement is not possible during routine counts of the very small size of the organisms.

From a fairly large number of routine measurements and by comparing cell counts, seston, protein and chlorophyll a we arrived at an average cell diameter of 3,45 µm during the morning hours. In laboratory cultures the cells tend to be somewhat larger (Tab. II). Moreover, cell volume also depends on nutrient concentration and irradiation.

Several experiments with fertilizers have shown that when offered NO_3-N and NH_4-N, Nannochloris prefers the NH_4 component. A comparison between filtered waste water and Nitrophoska as nutrient source showed no difference in growth and chlorophyll a content as long as the culture water contained enough ammonium. In cultures where the fertilizer used contained NO_3-N as the sole N component, the chlorophyll a as well as the protein content in the cells was observed to decrease (Tab. II). Since the measurement of dry matter as seston is not ideal (C/N analyses would be preferable), the values applying to dry matter should be considered only as

guidelines. The fertilizer requirement is calculated on the basis of the cell count. To find the exact doses of N and P required for the production of a certain biomass (number of cells), experiments were conducted with the aim of avoiding waste of fertilizer when cultures are harvested. The daily harvesting of algae as feed for the secondary producers is carried out as follows: when cell concentration in the culture has reached the required density of 8 - 20 million cells per ml, part of the crop is harvested, the amount varying with weather and season, and comprising roughly one-third to half of the volume in summer. The quantity of the crop removed daily is calculated in such a way that when the original volume is restored after harvesting, the former cell density is arrived at again the following day. This pattern can be repeated, using the required fertilizer amount as calculated according to the expected increase, until algal growth is inhibited by grazers. The proliferation of grazers makes it necessary to clean the tanks every 2 - 3 weeks and start new cultures. Therefore spare tanks are required to maintain the food supply.

Production capacity

The average yearly production calculated on the basis of cell count increase ranges from approximately 14 g dry matter $m^{-2}d^{-1}$ in June (max. 25 g $m^{-2}d^{-1}$) to 1 g dry matter $m^{-2}d^{-1}$ in January. Thus production of algae without artificial light is only worthwhile from April to September in our latitudes. It should be mentioned that the values obtained by means of the O_2 light-dark bottle or ^{14}C method are naturally higher than those yielded by cell counting. Losses through sedimentation, grazing and nocturnal respiration are not considered here.

Feed utilization

The algae have so far been fed to rotifers (Brachionus plicatilis), copepods (Eurytemora affinis), mysids (Neomysis integer) and molluscs (Crassostrea gigas). Mass cultures of Brachionus at densities of approx. 150 individuals per ml were reared without difficulty for several months on Nannochloris, both in the laboratory as well as outdoors (Fig. 3). In an

experiment of one month's duration, the average daily harvest was about 50 million individuals from a tank of 1.5 m^3 volume. Eurytemora showed a good production rate in outdoor tanks, maximum densities being 3000 adults and copepodites and 5000 nauplii per litre. This year we started a new experiment with Crassostrea spat from Scotland. After a high mortality (ca. 30 %) during the first few days, caused by the 20 hour long transport, the oysters are growing quite well considering the unusually low temperatures, which were not higher than 15° C during most of the observation time (Fig. 4).

Discussion

The results of the experiments so far conducted appear to indicate that Nannochloris is a suitable food item for mass cultures. Its advantages over other organisms tested lie in its excellent adaptability to environmental conditions, as evidenced by its dominance in outdoor cultures throughout the year. Of particular relevance are the positive results obtained from feeding experiments with Brachionus and Eurytemora, both of which play an important role as live food in our experiments with raising turbot larvae. Since we worked without pre-treated water, thus avoiding the high costs involved, our type of mass culture has the disadvantage of contamination by grazers, especially with Brachionus, the ciliate Euplotes and various heterotrophic flagellates. A mass development of these grazers can destroy a culture within a few days. To avoid this, it is advisable to harvest the algae crop in time. A treatment with 20 - 30 ppm of 40 % formalin is fairly effective against ciliates and heterotrophic flagellates without damaging the algae (ROTHBARD 1975).

For practical reasons we decided to work with batch cultures, harvesting not continuously but at intervals. Flow cultures (chemostat) are in theory superior to batch cultures as far as production capacity is concerned, but are very difficult to maintain outdoors on account of the variable environmental conditions. A further problem arises in connection with harvesting the exact feed dosages for the secondary producers.

The most economic fertilizer would be purified waste water. However, the quality fluctuates depending on the efficiency of the waste water plant. Moreover, there is again the danger of contamination through grazers. Toxic substances may be present and harm sensitive organisms. The more thoroughly the waste water is treated, the greater would be the quantity required on account of the low nutrient content, the result being an undesirable dilution of the seawater.

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Table I: Relation between dry weight and cell diameter in *Nannochloris spec.*

(Calculation presuming dry weight to be 25% of fresh weight, specific weight to be 1 and cell shape spherical).

Cell diameter (µm)	Vol. (μm^3)	Cell count 10^{-6}/ mg dry matter	Dry matter µg/ 10^6 cells
3,0	14,1	283,7	3,5
3,5	22,4	178,6	5,6
4,0	33,5	119,4	8,4
5,0	65,4	61,2	16,3
6,0	113,0	35,4	28,2

Table II: Effect of various N-containing fertilizers on growth and composition of cells, showing difference between laboratory and outdoor cultures (average values for morning measurements)

	Cell conc. 10^{-6}/mg dry matter	Chl.a % in dry matter	Prot. % in dry matter	P % in dry matter	N consumption µg/mg dry matter	P consumption µg/mg dry matter	N/P at/at
NH_4	72	0,87	31	0,85	57	8,0	16,0
NO_3	49	0,42	20	0,77	32	7,9	8,9
NH_4+NO_3	60	1,06	29	0,96	49	9,7	13,5
outdoors NH_4+NO_3	185	1,60	32	-	76	16,8	10,0

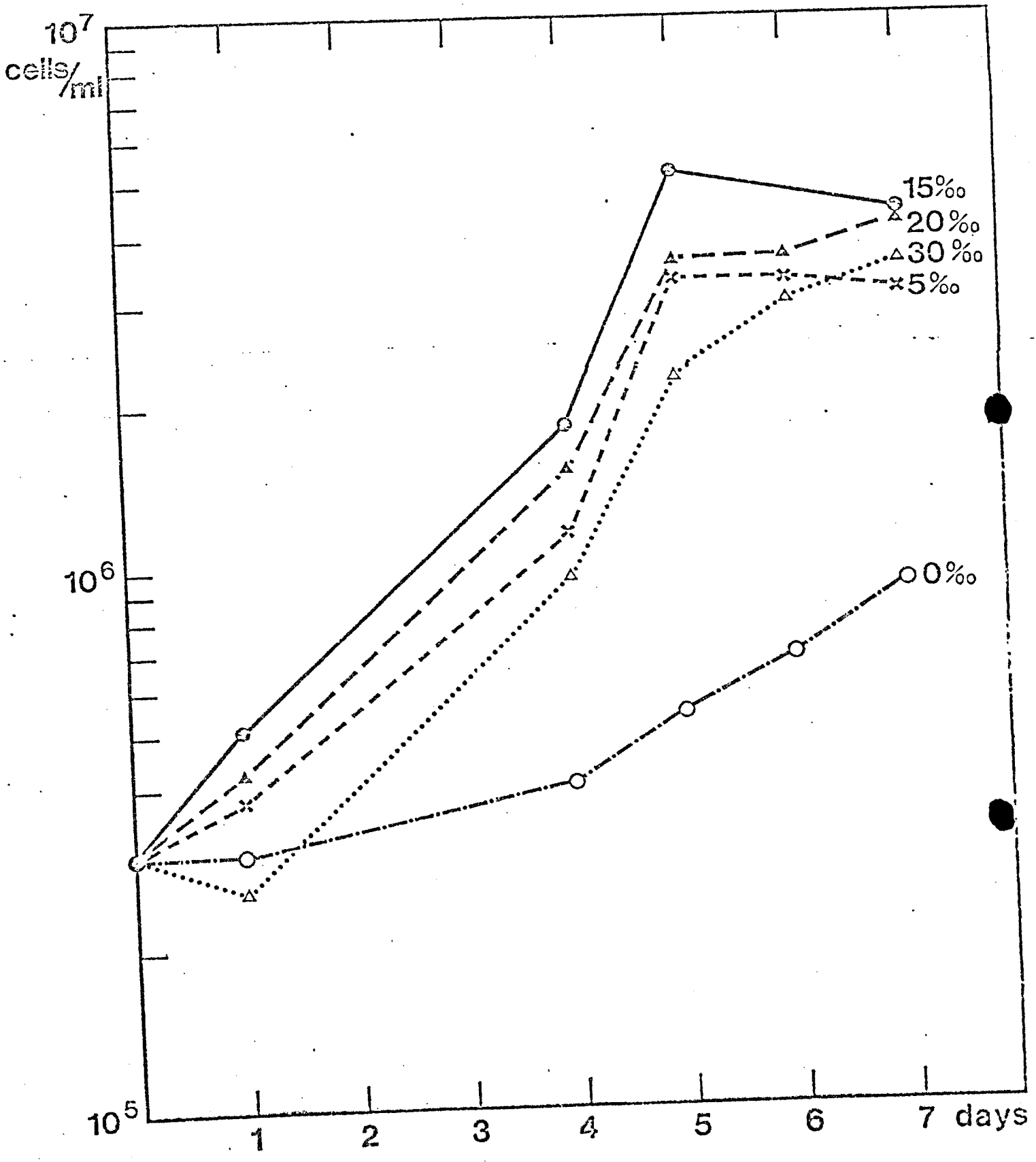

Fig. 1 Growth of Nannochloris at different salinities (temperature 20° C)

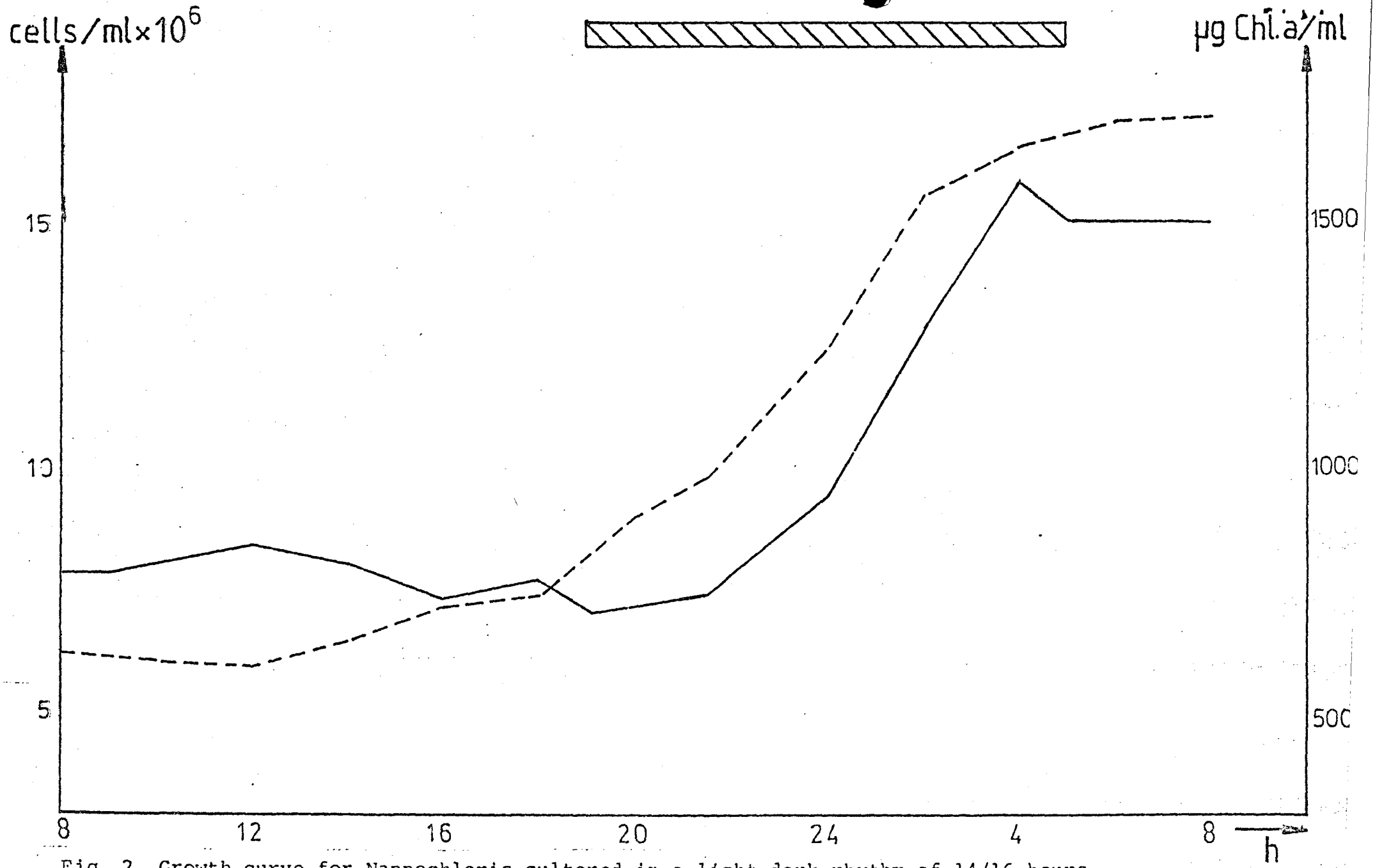

Fig. 2 Growth curve for Nannochloris cultered in a light-dark rhythm of 14/16 hours. Cell number (——), chlorophyll a (----), hatched bar indicates the night hours

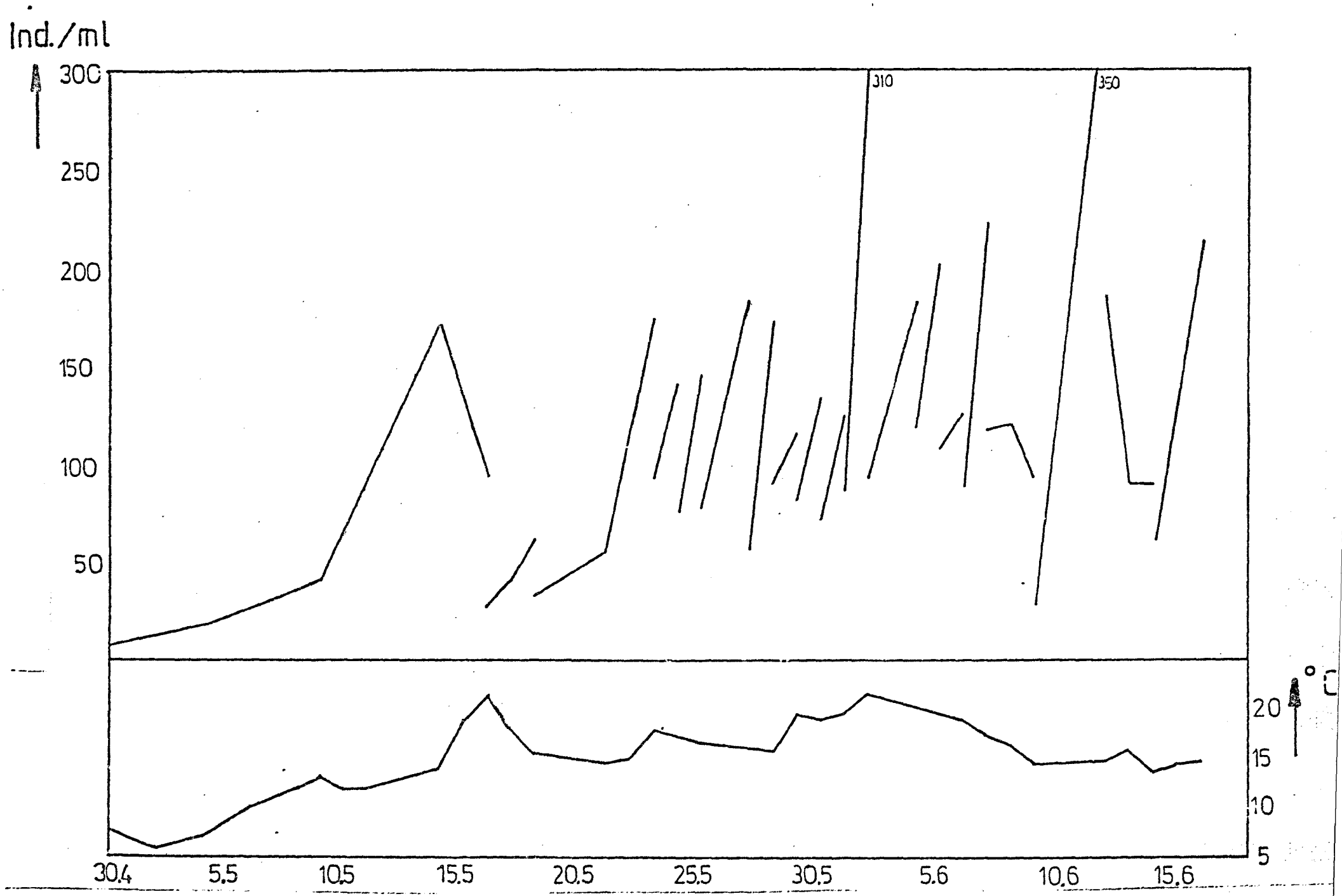

Fig. 3 Growth curve of a Brachionus culture harvested periodically. The interruptions show the dilution with algae medium after harvesting. Below the temperature variation in the outdoor tank.

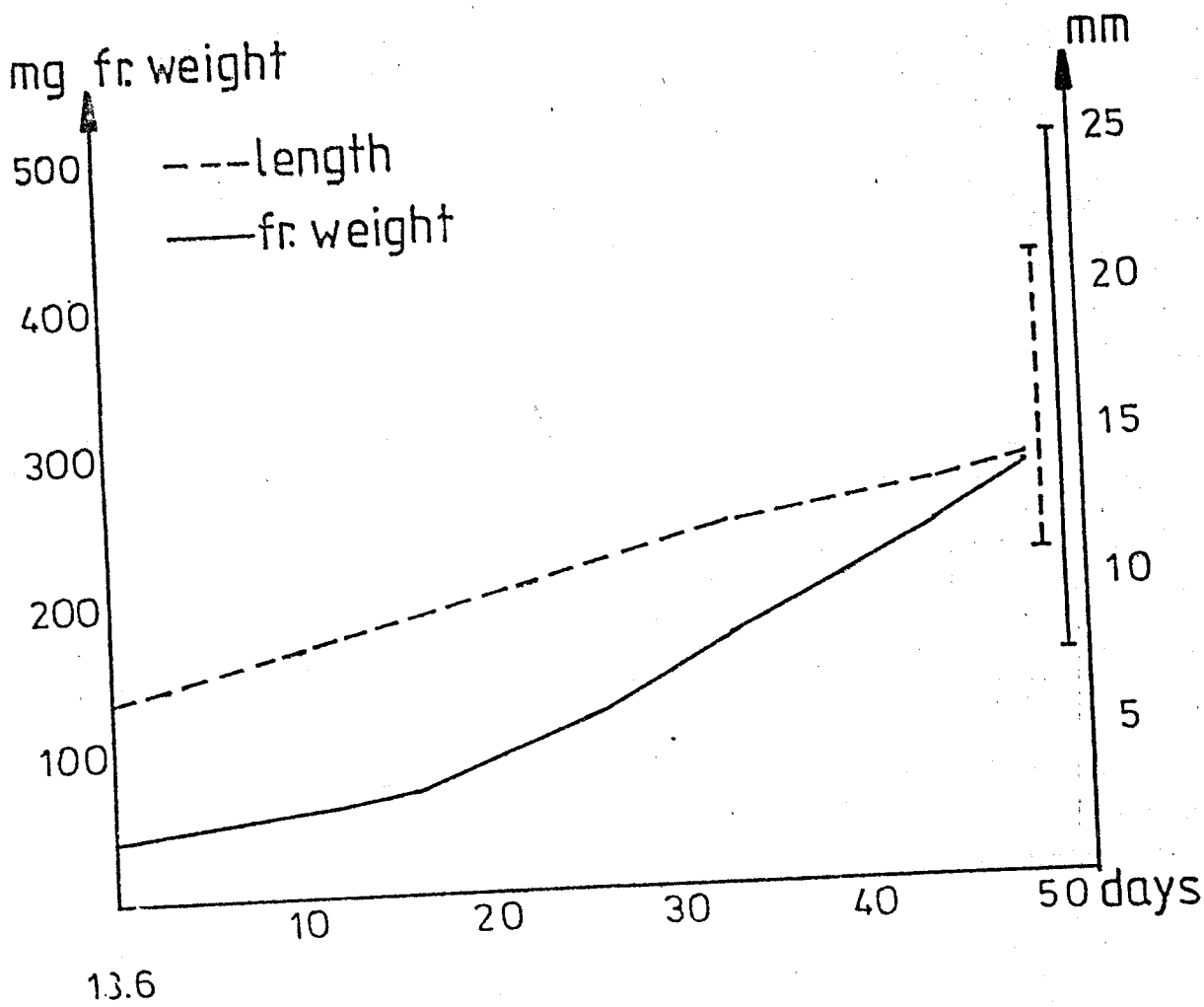

Fig. 4 Growth of Crassostrea gigas spat fed with Nannochloris (average value for more than 100 ind. with the length and weight variation at the end of observation period). Temperature varied around 15° C during the observation period