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Effect of temperature on the aggregation of *Skeletonema costatum* (Bacillariophyceae) and the implication for carbon flux in coastal waters

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ABSTRACT: *Skeletonema costatum* (Greville) Cleve was isolated from a mucilaginous algal bloom in the Northern Adriatic Sea during April 1993. In nitrate-limited continuous culture the formation of aggregations of cells was positively correlated with temperature. Raising the temperature from 10 to 20°C caused cells to aggregate without a change in culture biomass. Reducing the temperature to 10°C caused washout of the aggregates, again with no change in biomass. In batch culture there was a positive relationship between aggregate concentration and temperature at 10, 15, 20 and 25°C. Aggregates and the cell surface of *S. costatum* adsorbed Alcian blue, a stain for acid and sulphated polysaccharides. Disruption of the aggregates on addition of 0.2 M Na_2EDTA indicated that cross-linking between polysaccharides by divalent cations (probably Ca^{2+} in seawater) bound the aggregates together. Aggregates may form *in situ* under conditions of temperature increase and high phytoplankton biomass. Surface water temperature increased by 0.2°C d^{-1} in the second half of July 1992 in the Northern Adriatic, coinciding with a chlorophyll *a* increase of 10 to 50 µg l^{-1}. A temperature difference of 1.2°C was observed at 2 stations 4 m apart in May 1992 on either side of a plume front associated with the River Po; transfer of phytoplankton in eddies from cold to warm waters may lead to the formation of aggregates. Aggregate disruption may occur with the sinking of aggregates from surface waters to the relatively cool waters below the thermocline. The temperature decrease through the thermocline varied between 2.7 and 4.1°C in May 1992. The relationship between aggregation and temperature may be an important factor in determining the flux rate of fixed carbon and nutrients from the photic zone to deeper waters and the sea bed. Aggregated cultures had significantly higher sinking rates than unaggregated cultures.

KEY WORDS: Diatom · Aggregation · Temperature Continuous culture · Marine snow · *Skeletonema costatum*

INTRODUCTION

In both coastal seas and the open ocean macroscopic aggregates, known as marine snow, form from detritus, living organisms and inorganic matter. Aggregates are an ubiquitous feature of the water column in marine systems. Aggregates are also found in limnetic systems; Grossart & Simon (1993) described the occurrence of lake snow in Lake Constance, Germany. The aggregation of cells in diatom blooms is an important source of marine snow of potential global significance (Alldredge & Silver 1988). Aggregation and the sinking of aggregates have been suggested to play a role in the life histories of diatoms (Smetacek 1985, Logan & Alldredge 1989), representing the transition from a growing to a resting stage.

Aggregates are ecologically significant for 2 reasons: they function as transport agents and form microenvironments in the water column. Large (>100 µM) particles sinking through the water column are responsible for most of the downward vertical mass flux in the sea (Fowler & Knauer 1986). This mass flux or transport will result in the removal of carbon fixed in the photic zone from the sea surface to the sea bed. Sinking of

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aggregates leads to the removal of nutrients such as nitrogen and phosphorus from the photic zone. These elements are essential for the growth of phytoplankton. Aggregation increases the rate of downward flux of elements, as larger particles will sink through the water column faster than smaller particles of the same density. Mass sinking of diatoms is accelerated by cell protuberances and mucous secretions which lead to entanglement and aggregation. Aggregates tend to collect particulate material from the water column and may have sinking rates of up to 100 m d^{-1} (Smetacek 1985). Jackson (1995) emphasised the importance of the coagulation of algae in marine ecosystems and noted that aggregates are necessary to explain the disappearance of algae from the surface waters and their appearance in the benthos. The microenvironment within aggregates serves as a habitat containing enriched microbial communities in which the rates of processes such as photosynthesis, decomposition and nutrient regeneration occur at highly elevated levels (Alldredge & Silver 1988). There are pronounced chemical gradients within this microenvironment; Shanks & Reeder (1993) found strong reducing microzones in marine snow, even when the surrounding water column was well oxygenated.

The 4 major mechanisms which bring particles together in the ocean are Brownian motion, fluid shear, differential settlement and animal feeding (McCave 1984). Diatoms are too large to be affected by Brownian motion. A major feature of recent studies of diatom aggregation in the laboratory has been the measurement of coagulation efficiency or stickiness (α, the probability that the cells will stick together on collision). Diatom aggregation may occur by 2 mechanisms: cell-cell binding as observed in *Skeletonema costatum* (Kiørboe & Hansen 1993) or the binding of cells to transparent exopolymeric particles (TEP) produced by the diatoms. Cell-TEP binding has been shown to occur in *Chaetoceros affinis* (Kiørboe & Hansen 1993) and *Chaetoceros gracilis* (Crocker & Passow 1995). By carrying out flocculation experiments with mixtures of 2 species, Crocker & Passow (1995) showed that there was preferential aggregation of some species and those with a high stickiness (α) occurred at higher frequencies in aggregates.

There have been many field observations of diatom aggregation and mass sinking. Alldredge & Gotschalk (1989) followed the mass flocculation of diatom blooms in the surface waters of the Santa Barbara Channel, California, USA, during 1986 and 1988. The aggregates contained living diatoms, predominantly *Chaetoceros* sp. and *Nitzschia* sp.; 9 other diatom genera were present including *Skeletonema* sp. Alldredge & Gotschalk (1989) found that the *in situ* settling velocity of aggregates, formed predominantly from *Chaetoceros* sp., was 2 orders of magnitude faster than unaggregated *Chaetoceros* sp. at 117 ± 56 m d^{-1} (mean ± SD). Riebesell (1991a, b) monitored aggregates at a fixed station in the southern North Sea for 6 wk. The aggregates were mainly formed from diatoms, including *Chaetoceros* sp., *Nitzschia* sp. and *Skeletonema costatum*. Physical coagulation processes, rather than inorganic nutrient limitation, appeared to control the termination of the bloom. The aggregates were mucus rich, composed of algal and detrital particles, with large numbers of attached bacteria (Riebesell 1991b). In both 1990 and 1991 the collapse of a spring bloom of *S. costatum* in the Firth of Clyde, Scotland was associated with the sedimentation of aggregates dominated by *S. costatum* (Napier 1995). Stachowitsch et al. (1990) suggested that the mucous aggregates often observed during summer in the Northern Adriatic are initially produced by diatoms.

During a study investigating the cause of mucilaginous blooms in the Northern Adriatic we observed that batch cultures of *Skeletonema costatum* removed from relatively cool culture conditions (10 to 15°C) to room temperature (20 to 25°C) formed visible aggregates (<1 mm in diameter). There have been no laboratory studies on the effect of temperature on the aggregation of diatoms. The aim of this work was to test the hypothesis that aggregation was a direct consequence of temperature rise on the diatom. The waters of coastal seas, including the Northern Adriatic, may be considered as a mosaic of water masses with different properties. River plumes, areas of mixing by tidal currents and stratified areas contribute to this mosaic of water masses between which there are often sharp gradients of temperature on horizontal and vertical scales. The transfer of phytoplankton biomass across sharp gradients may lead to aggregation on transfer to warmer waters or disaggregation on transfer to cooler waters.

METHODS

Organism. *Skeletonema costatum* (Greville) Cleve (Bacillariophyceae) was isolated from a bloom in the Northern Adriatic in April 1993. The water temperature was 10°C. At this time *S. costatum* was the dominant component of the microflora (>99% of the cell density) and mucilage was observed in the water column. *S. costatum* has been observed in previous studies (Stachowitsch et al. 1990) in association with mucous aggregates in the Northern Adriatic. *S. costatum* often dominates the spring bloom in coastal waters (Han et al. 1992).

Medium. The broad spectrum artificial seawater of Harrison et al. (1980) was used to grow *Skeletonema costatum* in batch and continuous culture. To ensure

there was a single nitrogen source, $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ was replaced with $FeCl_3$ to produce the same Fe concentration (6.6 µM). Silicon (141 µM) was provided as $Na_2SiO_3 \cdot 5H_2O$, phosphorus as $Na_2glyceroPO_4$ and nitrogen as $NaNO_3$. Concentrations of N and P were manipulated and are given in the appropriate section.

Batch culture. The batch cultures used to inoculate the chemostats were grown under continuous light at 10°C with a photon flux density (p.f.d.) of 70 µmol m^{-2} s^{-1} with nitrate and phosphate concentrations of 100 µM.

Continuous culture. Continuous cultures such as chemostats are open systems in which growth rates (μ) are controlled by the rate of supply of a limiting nutrient. The rate of biomass change is given by:

$$\frac{dX}{dt} = \mu X - DX \quad (1)$$

where X is biomass concentration, D is dilution rate and t is time. At steady state, X is constant and therefore $\mu = D$. The cells produced at steady state are of similar biochemical composition and physiological state. The theory of chemostats has been reviewed by Tempest (1970) and their application to algal cultures by Fogg & Thake (1987). Chemostat cultures were used to test the following hypotheses:

Hypothesis 1: Aggregation is a function of the rate of temperature increase. Once the organism has adapted to the higher temperature, the aggregates will wash out at the dilution rate.

Hypothesis 2: Aggregate concentration is proportional to temperature. Aggregate concentration will increase with a rise in temperature and decrease with a drop in temperature.

The chemostat system used in this work has been described by several workers (Wymer & Thake 1980, Santillo 1993, Thornton 1996). Cultures of 1.5 l were grown in 2 l glass reaction vessels. Medium was supplied via silicone rubber tubing through a 22-channel Carlo Erba peristaltic pump from a medium reservoir of Pyrex glass. Overflow was collected via a glass tube and passed to a waste container. Filtered air from a compressor was supplied to the cultures to produce a slight positive pressure which ensured that excess culture was removed via the overflow, CO_2 supply was maintained and that the culture was mixed. Stirring was provided by magnetic stirrers and magnetic followers at a rate of 150 rpm. Temperature of the culture was maintained by a temperature controlled (± 0.5°C) water jacket connected to flow heater/cooler systems (Grant Instruments Ltd, Cambridge, UK). Continuous light was provided on 2 sides of the culture by two 4 ft (ca 1.2 m) 40 W fluorescent tubes (F40W/DL-RS, Sylvania, UK). Photon flux density at the surface of the vessels was 80 to 110 µmol m^{-2} s^{-1}. If light was passed through the culture from 1 side, the p.f.d. in the centre of the culture was 40 to 70 µmol m^{-2} s^{-1}, depending on the culture conditions. Actual p.f.d. in the centre of the vessels would be higher, as light was entering the culture vessels from 2 sides. Light saturation of growth (μ = 2.9 d^{-1}) was observed at 25 to 30 µmol m^{-2} s^{-1} for the isolate used in this work (Santillo 1993, pers. comm.).

Cell counts. Samples were taken with sterile pipettes from sampling ports of the chemostats and were preserved with Lugol's iodine (Parsons et al. 1984). Counts were made in a haemocytometer with modified Fuchs-Rosenthal ruling. Aggregates were broken up without cell damage by passing through a 23 gauge hyperdermic needle. *Skeletonema costatum* is a chain-forming diatom; therefore, counts were made of cell chains rather than individual cells, as these were the randomly distributed units on the haemocytometer. The mean cell chain length was less than 2 cells per chain. 400 cell chains were counted to give a precision (95% confidence) of ± 10% (Guillard 1973).

Bacteria were stained with the DNA specific stain DAPI (4'6-diamidino-2-phenylindole dihydrochloride) and filtered onto a membrane filter (Porter & Feig 1980). The DNA-DAPI complex fluoresces bright blue, producing a glow which allows cells smaller than the limit of resolution (generally <1 µm) to be seen and counted under fluorescence with the light microscope.

Aggregate analysis. 100 aggregates were counted (giving a standard deviation of ± 10 aggregates) using a Sedgewick-Rafter counting slide and a light microscope.

The chelating agent Na_2EDTA (ethylenediaminetetraacetic acid disodium salt) was used to test the hypothesis that binding between *Skeletonema costatum* cells to form aggregates is facilitated by divalent cations such as Ca^{2+} and Mg^{2+}. If cations are involved in the binding, then the addition of Na_2EDTA will cause the disruption of the aggregates. 1 ml of 0.8 M Na_2EDTA was added to 3 ml of Lugol preserved culture to produce a final concentration of 0.2 M.

Sinking rates. Sinking rates were measured at room temperature (ca 20°C) in 25 ml glass burettes using a method based on that of Bienfang (1981). The burettes were filled at t_0 (time zero) and the optical density of the culture was measured at 664 nm spectrophotometrically. The burettes were covered in aluminium foil to exclude light and left in a vertical position for several hours, ensuring that a significant portion of the biomass remained in suspension. At the end of the experiment (t_t) the optical density of 4 ml from the bottom of the burette was measured. This 4 ml included the settled out biomass. Settling rate was calculated from the following equation:

$$V = \frac{B_s}{B_t} \times \frac{L}{t} \quad (2)$$

where B_t = total biomass = ($b_t \times v_t$); B_s = settled biomass = ($b_s \times v_s - b_t \times v_s$) ($b_t$ = biomass ml^{-1} at start, v_t = total volume in burette, b_s = biomass ml^{-1} at end of settling time, v_s = settled volume removed from the burette [4 ml]); L = depth of liquid in burette, t = settling time (h).

To test the hypothesis that aggregated *Skeletonema costatum* would sink faster than less aggregated *S. costatum* an aggregated culture was divided into 2 aliquots, one of which was passed through a hypodermic needle to reduce the number of aggregates. This produced 3100 ± 390 aggregates ml^{-1} in the aggregated and 690 ± 200 aggregates in the less aggregated aliquot (mean ± SD, n = 3). There was a significant difference between the mean number of aggregates in the 2 treatments ($p < 0.001$, Student's t-test). A settling experiment was set up with 11 replicates from each aliquot.

Batch experiment. *Skeletonema costatum* was grown in a 1 l culture with nitrate and phosphate concentrations of 500 µM. Aliquots (50 ml) of cultures in exponential phase were placed in 60 ml tissue culture vessels in either the light (70 µmol m^{-2} s^{-1}) or dark (by wrapping in aluminium foil) and incubated for 50 h. The diatoms were maintained in suspension by swirling the bottles 3 times a day. Incubations were carried out in triplicate at 4 temperatures (10, 15, 20 and 25°C). The bottles were sampled for cell counts and aggregate concentrations.

Chemostat experiments. The chemostats were grown under nitrate limitation as this was found to be the nutrient limiting algal growth during field bioassays carried out in 1992 (data not presented). Chemostats were used in the experimental work as they allowed for control of growth rates. Specific chemostats are referred to by name (N1–N14). All chemostats were grown at a dilution rate of 0.3 d^{-1} with nitrate and phosphorus concentrations of 100 µM and an initial temperature of 10°C. Small samples, such as those used for cell counts, were removed from the chemostat with a sterile pipette via a sampling port in the lid of the vessel. Larger samples were taken via the overflow by tilting the chemostat vessel. The maximum volume removed from the vessel at 1 sampling was 250 ml.

Data analysis. Statistical analysis was carried out on logarithmic transformed data [$X' = \log(X+1)$] to remove heteroscedasticity from the data set. Linear regressions were fitted using Fig.P (Fig.P Sofware Corporation 1992) and multiple comparisons were made by analysis of variance (ANOVA) followed by post hoc Tukey tests (SYSTAT Products 1997). Data in the figures are presented in an untransformed state. Percentage data was arcsine transformed before analysis; the back transformed data are presented.

RESULTS

Batch experiment

Aggregate concentration increased with temperature (Fig. 1a) in both the light and dark incubated *Skeletonema costatum* cultures. In the light the mean

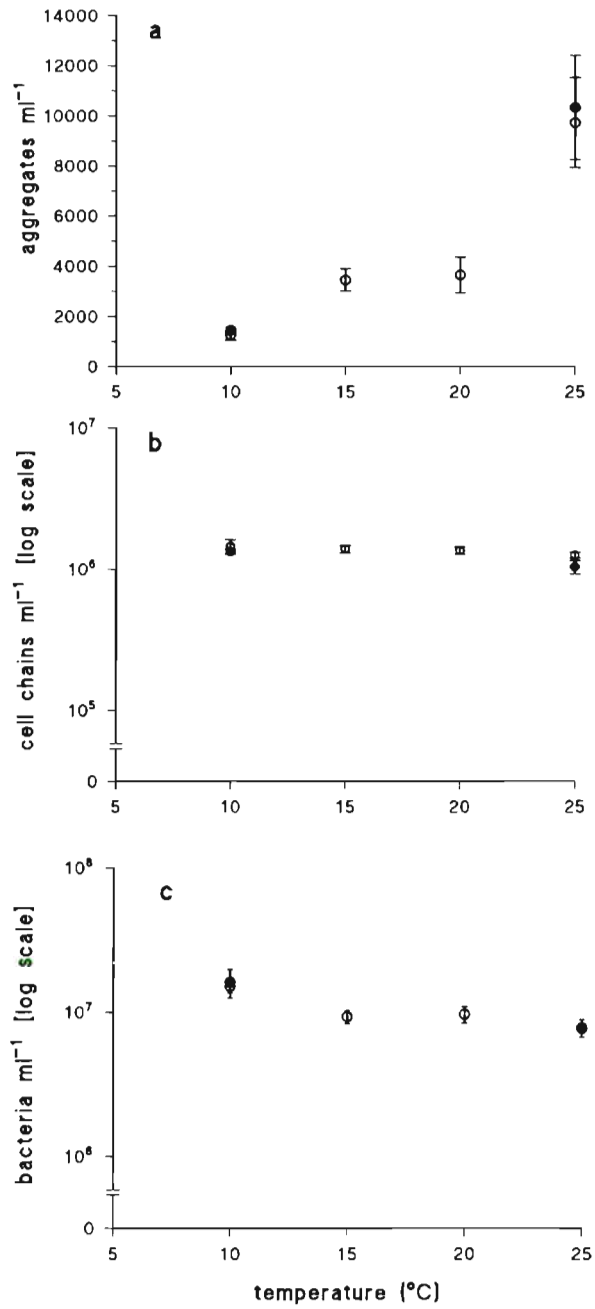

Fig. 1. *Skeletonema costatum*. Aggregation 50 h after transfer from 10°C to 1 of 4 temperatures. (a) Aggregate concentration, (b) cell chain concentration and (c) bacteria concentration. Incubations were carried out in the light (○) and dark (●). Values are means ± SE (n = 3)

concentration at 10°C was 1260 ± 200 aggregates ml^{-1} (mean ± SE, n = 3) increasing to 9740 ± 1800 aggregates ml^{-1} at 25°C (Fig. 1a). There was a significant difference between aggregate concentration at the 4 temperatures in the light ($F_{3,8} = 21.2$, $p < 0.001$). The increase in aggregate concentration was not due to an increase in growth rate at higher temperatures resulting in a higher biomass. Yields were the same after 50 h (Fig. 1b), irrespective of temperature or light regime. Therefore, the increase in aggregate concentration at higher temperatures was due to a greater relative aggregation of the biomass. There was a significant difference in the yield of bacteria ($F_{3,7} = 5.68$, $p < 0.05$) at the 4 temperatures in the light. A post hoc Tukey test showed that the significant ($p < 0.05$) source of variation was between cultures at the 2 extremes of the temperature range, 10 and 25°C. At 10°C the mean number of bacteria was 1.2×10^7 bacteria ml^{-1}, and at 25°C there were 7.7×10^6 bacteria ml^{-1}. A significant linear regression was fitted to aggregate concentration against time ($A = bT + c$, where A is aggregates ml^{-1} and T is temperature [°C]); the results of this fit were $A = 513T - 4450$ ($r^2 = 0.69$; $p < 0.001$, Table B.17 in Zar 1996).

Chemostat experiments

Increase in temperature

Production of aggregates in chemostats N1–N4 is shown in Fig. 2. When temperature was stepped up to 20 from 10°C, there was a highly significant ($F_{11,36} = 18.3$, $p < 0.001$) increase in aggregate concentration (Fig. 2a) from 2870 ± 391 (mean ± SE, n = 24) to 22 600 ± 1720 (n = 24) aggregates ml^{-1}. A post hoc Tukey test showed that the source of this variation was between sampling points before and after Day 50, the day on which the temperature was raised ($p < 0.05$). The sample on Day 50 was taken before the temperature was raised. The post hoc Tukey test showed that there was no significant difference between aggregate concentrations on different days at either 10 or 20°C. The increase in aggregate concentration was not due to an increase in cell concentration (Fig. 2b). There was a highly significant variation between aggregate concentration normalised to biomass with time ($F_{11,36} = 14.3$, $p < 0.001$).

Fig. 3 shows the effect of raising the temperature from 10 to 20°C and from 20 to 30°C. Fig. 3a shows that there was a significant difference between the aggregation concentration at 10 and 20°C, but not between 20 and 30°C. There was a highly significant difference between aggregate concentration with time ($F_{9,40} = 8.97$, $p < 0.0001$) (Fig. 3b). A post hoc Tukey test showed that the source of variation was between 10°C and the other temperatures (20 and 30°C) ($p < 0.05$). There was no significant difference between aggregate concentrations at 20 and 30°C.

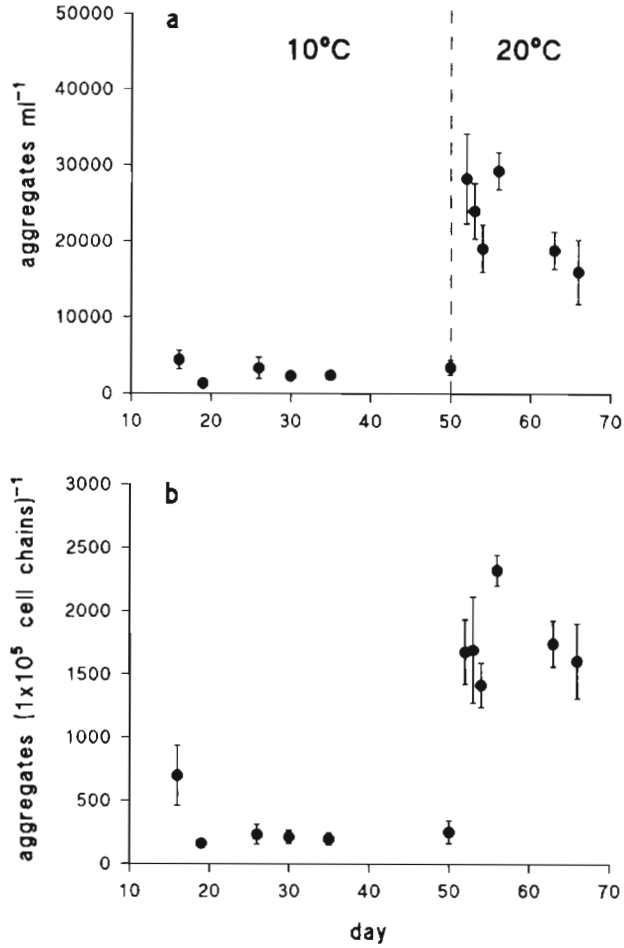

Fig. 2. *Skeletonema costatum*. Production of aggregates in nitrogen-limited continuous cultures (N1–N4) at 10 and 20°C with a dilution rate of 0.3 d^{-1}. (a) Aggregate production against time. Values are means ± SE (n = 4). (b) Aggregate production normalised to cell chain concentration against time. Values are means ± SE (n = 4)

Increase in temperature followed by a decrease

An increase in temperature from 10 to 20°C followed by a decrease in temperature back to 10°C resulted in a higher aggregate concentration at 20°C, with a return to the original aggregate concentration after the decrease in temperature (Fig. 4). At 10°C there were 1010 ± 213 (mean ± SE, n = 30) aggregates ml^{-1}, increasing to 3390 ± 704 (n = 15) aggregates ml^{-1} at 20°C and decreasing to 798 ± 104 (n = 10) aggregates ml^{-1} when the temperature was dropped back to 10°C. A

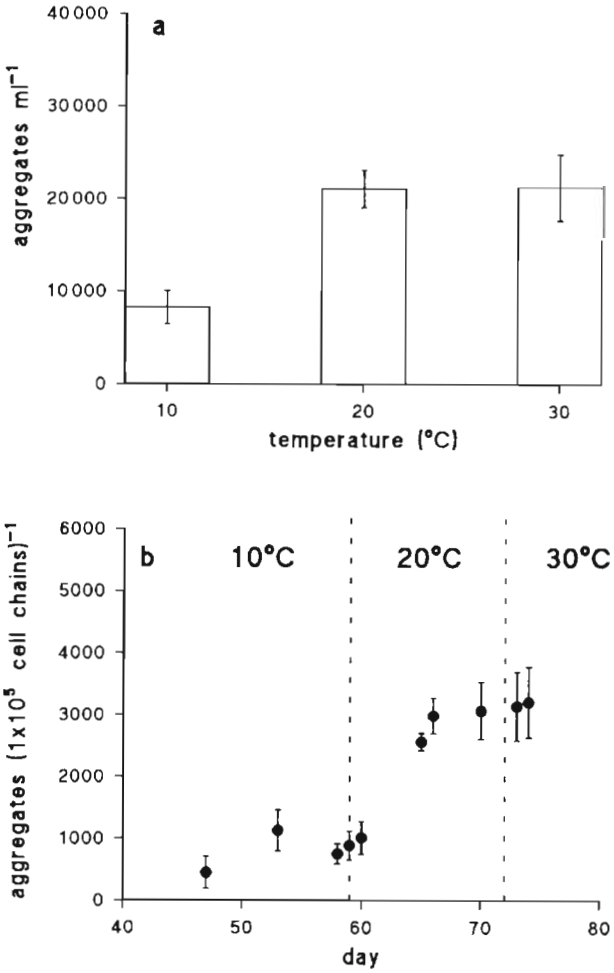

Fig. 3. *Skeletonema costatum*. Aggregate production in nitrogen-limited continuous cultures (N5–N9) grown at a dilution rate of 0.3 d^{-1} (a) Aggregate concentration at 10 (n = 15), 20 (n = 20), 30°C (n = 5). Values are means ± SE. (b) Aggregate concentration normalised to cell chain concentration with time. Values are means ± SE (n = 5)

post hoc Tukey test showed that all the significant ($p < 0.05$) variation lay between aggregate concentrations at 10 and 20°C; there was no significant difference between aggregate concentrations at 10°C on either side of the 20°C period.

Relationship between aggregate concentration and temperature

The relationship between mean daily temperature and aggregate concentration in chemostat N12 is shown in Fig. 5. Results from correlation analysis (Table 1) showed that there was a significant positive relationship between aggregate concentration and mean daily temperature, except in chemostat N10.

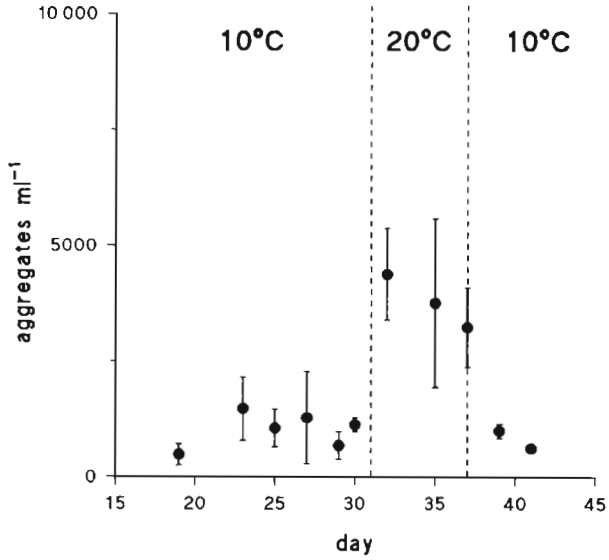

Fig. 4. *Skeletonema costatum*. Production of aggregates in nitrogen-limited continuous cultures (N10–N14) at 10 and 20°C with a dilution rate of 0.3 d^{-1}. Values are means ± SE (n = 5)

Properties of the aggregates

The aggregates were relatively small, as indicated by the high concentrations of aggregates ml^{-1}. Mean aggregate length was 75 ± 25 µm (± SD). Aggregates were composed of short chains of *Skeletonema costatum*. The aggregates were irregular with a dense core and a loose outer edge from which distinct cell chains

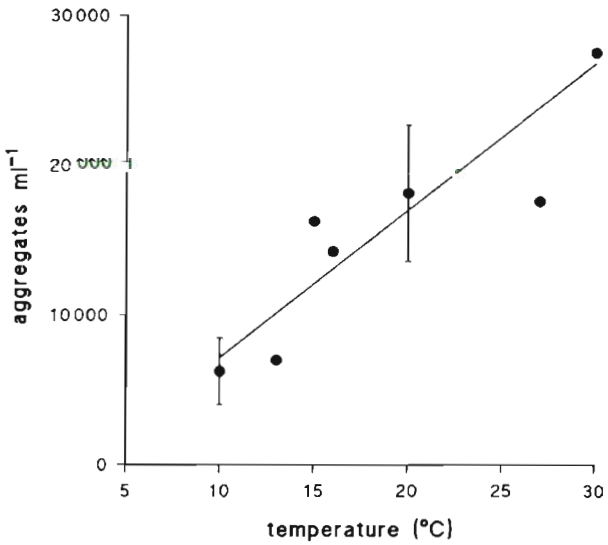

Fig. 5. *Skeletonema costatum*. Relationship between mean daily temperature and aggregate concentration in a nitrogen-limited continuous culture (N12). Values are means ± SE (n = 1–4)

Table 1. *Skeletonema costatum*. Positive correlation between mean daily temperature and aggregate number in nitrate-limited chemostats (N10–N14). Correlation coefficients were calculated using Spearman's coefficient of rank correlation. Significance of the correlations was obtained from Table C.11 in Rees (1987) where n = 12. Significance were ranked as follows: not significant (–), significant (•), and highly significant (•••)

Chemostat	Correlation coefficient	Significance level (p)	
N10	0.37	>0.05	–
N11	0.57	<0.05	•
N12	0.95	<0.005	•••
N13	0.78	<0.005	•••
N14	0.90	<0.005	•••

projected (Fig. 6). There was no visible extracellular matrix holding the cells together. Staining with Alcian blue revealed a faint halo of blue around cell chains.

The addition of Na_2EDTA (0.2 M) to aggregated samples caused disaggregation. The mean reduction in the number of aggregates was 74 ± 4 % (mean ± SE, n = 3). The addition of Na_2EDTA also caused lysis of some cells, the break-up of aggregates was a function of cell lysis as well as the chelation of divalent cations. Na_2EDTA caused a mean reduction in cell chain numbers of 24 ± 1 % (mean ± SE, n = 3).

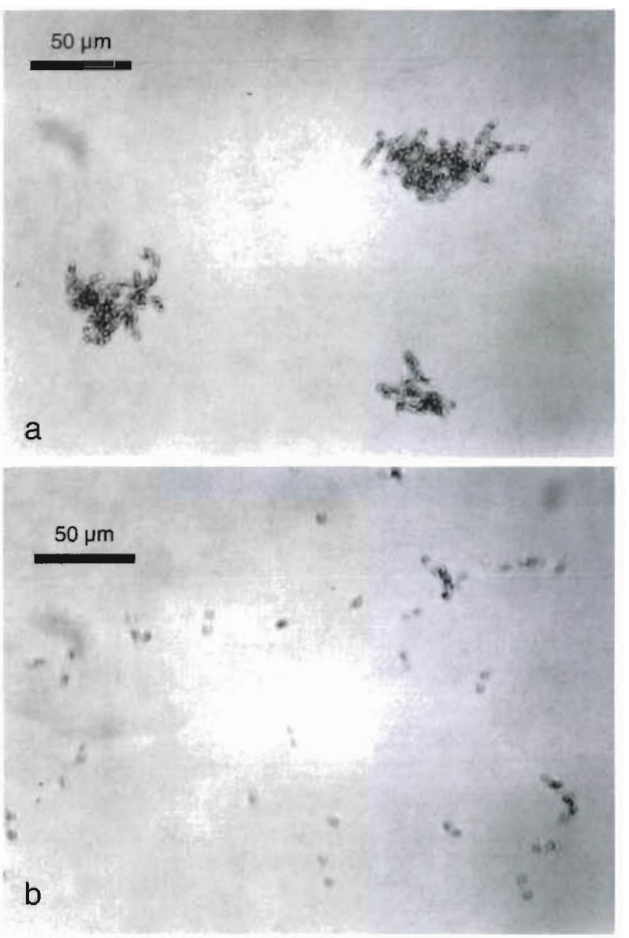

Fig. 6. *Skeletonema costatum*. Light micrograph taken from a nitrate-limited chemostat culture (N9). (a) Aggregated at 20°C and (b) unaggregated at 10°C

Aggregated cultures had a faster sinking rates than cultures in which aggregate concentration had been reduced ($p < 0.001$, Student's *t*-test). The mean settling rates were 0.62 ± 0.03 cm h^{-1} in aggregated and 0.37 ± 0.02 cm h^{-1} (mean ± SE, n = 11) in unaggregated cultures. The settling rate in aggregated culture was almost twice that in unaggregated culture at 0.15 and 0.09 m d^{-1} respectively.

DISCUSSION

Aggregate concentration was positively correlated with temperature in batch cultures of *Skeletonema costatum* incubated at 10, 15, 20 and 25°C. In the chemostat cultures, the aggregation of *S. costatum* was positively correlated with temperature; a rise in temperature caused an increase in aggregate concentration which decreased when temperature was lowered (hypothesis 2). As aggregate concentration reached a steady state as well as cell numbers, aggregation must have occurred at approximately the same rate as cell growth, which was dictated by the dilution rate of the chemostat. Therefore continued aggregation was dependent on continued growth. New aggregates could only form with the generation of new biomass, unless there was a rise in temperature. Aggregation was not a function of rate of temperature increase (hypothesis 1) as the aggregates did not wash out at the dilution rate once temperature had stabilised. The formation of new aggregates was likely to be a result of growth within the aggregates and the attachment of free cell chains until such a size was reached that the shear stress within the chemostat caused fragmentation into 2 or more smaller aggregates.

To increase the chance of aggregation there must either be an increased probability of 2 chains colliding, an increase in the probability (α) of 2 cell chains becoming stuck together on collision, or a combination of the 2 processes. As there was no increase in cell numbers with temperature, the observed increase in aggregation was due to an increase in α. In non-axenic cultures of *Skeletonema costatum*, α was measured as 0.02 to 0.25 by Kiørboe & Hansen (1993); the wide range of observed values show that there is a potential for significant changes in α. An increase in α must have been due to a change in the surface properties of the cells. No changes in cell size with increasing temperature were observed during this work. We propose that

the surface of the cells became more sticky with increased temperature. The cationic dye Alcian blue may be used to stain negatively charged polysaccharides (Decho 1990, Alldredge et al. 1993). In this work both aggregates and free suspensions of *S. costatum* were observed to take up the stain, which was clearly associated with the cell surface. The addition of Na_2EDTA caused aggregates to break up; Na_2EDTA is a strong chelating agent, known to disrupt cationic bridges (Ca^{2+} and Mg^{2+}) between exopolymers (Decho 1990). Alldredge et al. (1993) found that laboratory-made aggregates of *Chaetoceros gracilis* broke up immediately on suspension in 1 M EDTA. Break-up of aggregates occurred at 0.2 M Na_2EDTA in this work. Alldredge et al. (1993) do not comment on the effect of EDTA on cell integrity, but it is highly likely that cell lysis occurred within the aggregates, which may have caused some of the observed disaggregation. In this work the lysis of cells was insufficient to account for the 74 % reduction in aggregate concentration and it was concluded that the cationic bridges binding the cells together were disrupted.

The turbulent regime within the chemostats was one of the factors dictating the size of the aggregates formed within the chemostat system. The stirring regime and vessel size of all the chemostats was equal; therefore, it was unlikely that there were any differences in turbulence between chemostats. An increase in temperature will result in a decrease in the viscosity of the medium and a resulting increase in turbulence; therefore, the contact rate between cell chains will increase. This was not an important factor in this investigation owing to the high degree of turbulence imparted by the vigorous mixing regime within the chemostats. There was no significant difference in aggregate size at different temperatures. Owing to the vigorous mixing regime aggregates were relatively small, mean aggregate length was 75 ± 25 µm (± SD). *In situ* turbulence is likely to be lower than that in the chemostats; therefore, it is likely that larger aggregates will form.

Bacteria were present in the isolate from the Adriatic Sea. In the batch experiment there were significantly fewer bacteria in cultures at 25°C compared to 10°C; the greatest aggregation occurred at 25°C. In batch culture the presence or absence of bacteria did not effect growth of *Skeletonema costatum*. We have demonstrated that the growth rates of axenic batch cultures did not have significantly different growth rates from cultures with natural background levels of bacteria or cultures to which up to 50 times the background concentration of bacteria were added after concentration through a flow cell (data not presented). These experiments showed no relationship between aggregate formation and presence or absence of bacteria. Other work has shown that bacteria were not important during the aggregation of *S. costatum*; Kiørboe et al. (1990) found that the stickiness of *S. costatum* was not dependent on bacterial numbers or the ratio of bacterial numbers to algal biomass in batch cultures.

Skeletonema costatum used in this work was isolated from the Northern Adriatic Sea, where we observed temperature gradients in both time and space. A combination of a high phytoplankton biomass and rapid temperature change may lead to aggregation *in situ*. Field observations in July 1992 showed a rapid increase in surface water temperature during the second half of July (0.2°C d^{-1}) from 25 to 28.4°C. There were sharp thermal gradients on horizontal scales. A plume front was observed in May 1992 off the river Po delta in the Northern Adriatic; there was a 1.2°C difference in surface temperature at 2 stations 4 m apart on either side of the front. Phytoplankton carried across the boundary within small eddies from cold to warm would be exposed to rapid temperature increase. Even greater thermal gradients were observed across thermoclines measured in the Northern Adriatic in May 1992. The decrease in temperature with depth at the thermocline was between 2.7 and 4.1°C. The sinking of aggregates through the thermocline may lead to their disaggregation.

Associated with the temperature rise in July 1992 was an increase in chlorophyll *a* (chl *a*) from 10 to 50 µg l^{-1}, which indicated an increase in cell density and therefore a greater potential for contact between cells and thus aggregation. In the chemostat cultures chl *a* concentrations ranged between 20 to 50 µg l^{-1}, indicating that the *in situ* biomass was of a sufficient density to aggregate with an increase in temperature. Exceptionally high chl *a* concentrations have been recorded in the Northern Adriatic; in 1984 concentrations up to 850 µg l^{-1} were observed off the Po river delta (Regione Emilia Romagna 1985, cited by Marchetti 1990).

Skeletonema costatum was grown under nitrate limitation in the chemostat cultures as nitrogen was found to be the nutrient limiting growth in the field during 1992. Nutrient limitation may be an important factor during aggregation. Kiørboe et al. (1990) showed that the stickiness of *Thalassiosira pseudonata* increased by 2 orders of magnitude as cell growth ceased and the diatom became nutrient limited. The stickiness of *S. costatum* was shown to be highest during the transition between the exponential and stationary growth phases (Kiørboe et al. 1990).

The aggregation of *Skeletonema costatum* with temperature increase may potentially explain the observed aggregation of diatom blooms *in situ* and subsequent sinking out of the water column. In this work

the sinking rate of aggregated *S. costatum* was twice that of unaggregated cells. The aggregation of a diatom bloom associated with a temperature rise will lead to a rapid increase in the flux rate of organic carbon and other nutrients from the photic zone to deeper waters and the sea bed. In shallow sea ecosystems such as the Northern Adriatic the flux of aggregates to the sea bed may have a severe impact on the ecosystem, including bottom water anoxia and the death of benthic organisms (Marchetti 1990, Stachowitsch et al. 1990). The seasonal pulse of phytodetritus to the abyssal and the sea bed is recognised as a world-wide phenomena (Smith et al. 1996) of importance to the carbon cycle and the supply of fixed carbon to the vast areas of the abyssal plain. The temperature facilitated aggregation of diatoms into fast sinking aggregates may have an importance not only in coastal zones but also in the world ocean.

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