

The effect of nutrient availability and temperature on chain length of the diatom, *Skeletonema costatum*

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We determined the effects of temperature and nutrients on the chain length of a diatom, Skeletonema costatum, in batch culture and enclosure experiments with estuarine water from San Francisco Bay, USA, using the recently developed CytoBuoy flow cytometer. Determination of the number of cells per diatom chain by CytoBuoy flow cytometer and associated software correlated well with but was much more precise and time efficient than microscopic quantification. Increasing temperatures (from 6, 8 to 17 °C) and nutrient concentrations induced high growth rates and dominance by longer chains in a cultured S. costatum strain that was originally acclimatized to a temperature range of 11–30 °C. Similarly, a positive correlation between growth rate and chain length was observed in S. costatum in batch culture and natural communities in enclosure experiments. Maximal chain lengths of S. costatum were greater in natural populations than in the batch culture. Longer chains affect sinking rates and thus likely help the diatom remain suspended in the upper part of the water column where physical and chemical parameters are more favorable for growth.

INTRODUCTION

Marine diatoms exhibit a large morphological variability. All species of marine diatoms, by definition, possess a siliceous cell wall, but their morphology can vary from solitary to chain or colony forming with each cell shape ranging from spherical to cylindrical and elongate pennate. Each shape and size of diatoms has apparent advantages and disadvantages in terms of suspension in the water column, nutrient uptake, light absorption and predator avoidance. For instance, smaller cells can remain suspended in the euphotic zone more efficiently than larger cells, as the rate of sinking for a spherical particle is directly proportional to the square of the radius (Smayda, 1970). However, larger cells may experience decreased predation rate compared with the small cells and can form resistance to settling by departing from a spherical shape to a more elongated rod shape (Fogg, 1991). Although Waite *et al.* (Waite

et al., 1997) found no correlation between cell volume and sinking rate, chain length and appendage shapes contribute to resistance against sinking. Some of the common appendages possessed by diatoms are siliceous spines. These spines, while increasing the chance of encounter with sensory and feeding currents and capture appendages of the herbivores, can decrease the diatoms' sedimentation rate by increasing their surface area to volume ratio. Likewise, forming chains of cells can also aid against sinking. Smayda and Boleyn (Smayda and Boleyn, 1966) found that the sinking rate of a chain-forming diatom *Skeletonema costatum* was inversely related to colony length irrespective of culture age. *S. costatum* forms rigid chains comprised of relatively small, elliptical cells joined by long, marginal, siliceous processes that can exceed the length of the cells (Cupp, 1943). In this genus, the chain formation is facilitated by the production of

specialized valves in the heterovalvate terminal cells (Fryxell, 1976).

Most chain-forming diatoms including *S. costatum* can exist solitarily and in chains of various lengths. What then determines the length of these diatom chains? Despite the large number of studies on the morphology of diatoms, this question remains largely unanswered. We studied the effect of nutrient availability and temperature on the chain length of the diatom, *S. costatum* in batch culture and in natural populations that developed in enclosure experiments using the estuarine water of San Francisco Bay. With the aid of a recently developed flow cytometer, CytoSense and its analytical software packages, CytoSift and CytoClus (CytoBuoy b.v., Nieuwerbrug, Netherlands; Dubelaar and Gerritzen, 2000), which makes enumeration of diatom chains and individual cells less laborious than the traditional visual examination by microscopy, we addressed the question of whether nutrient availability and temperature affect diatom chain length.

METHODS

Batch culture of *S. costatum*

For the first experiment, monocultures of *S. costatum* (CCMP 1132; Provasoli-Guillard National Center for Culture of Marine Phytoplankton, ME, USA) were grown in media with different combinations of nitrate and ammonium (non-nitrogenous nutrients were the same as *f/2* media; Guillard, 1975) at 17 °C (Table I). For the second batch culture experiment, the same strain of *S. costatum* was grown in *f/2* medium, containing 880-μM nitrate and no ammonium, at 6, 8 and 17 °C (Table I). For all treatments, cells were acclimatized to the conditions for five subcultured batch generations. In both experiments, the cells were cultured in an incubator (Hotpack, Warminster, PA, USA) with illumination from two sides by four Vita-Lite Plus Power Twist fluorescent tubes (Duro-Test Corporation; Color rendering Index-91) to give 60-μmol photons m⁻² s⁻¹ in 12 h:12 h L:D (light:dark) cycles and were stirred three times a day by gentle agitation. Twenty millilitres of the culture was

taken from each treatment on each day of the experiment and analysed (see following) in triplicate using a CytoSense flow cytometer (CytoBuoy).

Phytoplankton in enclosure experiments

Estuarine water, containing a natural plankton assemblage and collected from the surface in central San Francisco Bay, was incubated in two 200-L high-density polyethylene enclosures during 19–23 April 2004. Sodium nitrate (NaNO₃) was added to one enclosure to make an initial enclosure concentration of 50 μM, whereas the other enclosure (control) had nothing added. The water in both enclosures received unobstructed ambient light [mean integrated photosynthetically active radiation (PAR) over 6-h experiment period each day for 5 days = $1.4 \times 10^3 \pm 3.6 \times 10^2$ μmol photons m⁻² s⁻¹], thorough hourly mixing using a paddle, and was maintained at ambient San Francisco Bay temperature (mean temperature over 6-h experiment period 17.4 ± 2.9 °C) with a water jacket around the enclosures. Samples for particle profile analysis were pumped to the CytoSense flow cytometer using a peristaltic pump (Cole Parmer MasterFlex L/S Economy Drive Peristaltic Pump) with tubing (1/4" diameter, Bev-A-Line IV, Cole Parmer) placed in the dark at the flow rate of 400 mL min⁻¹. Under CytoSift software control, the water was subsampled by the instrument automatically each hour with particle acquisition parameters optimized for diatom cells (see below). As there were no consistent diel patterns, the hourly measured cytometric data were pooled for every 24 h for analyses.

Nutrient concentration analyses

Concentrations of nitrate, phosphate and silicate in the culture media and enclosures were measured after filtration (GF/F filters) with an Auto-Analyzer (Auto-Analyzer 2; Bran and Luebbe), as previously described (Wilkerson *et al.*, 2002). The concentration of ammonium was measured using a modified protocol of Solórzano (Solórzano, 1969; Wilkerson *et al.*, 2002).

Table I: Nutrient concentrations and temperatures of *Skeletonema costatum* in culture experiments

| | Control | A | B | C | D | E | F | G |
|---|---------|-----|-----|-----|-----|--------------------|--------------------|--------------------|
| Temperature (°C) | 17 | 17 | 17 | 17 | 17 | 6 | 8 | 17 |
| NO ₃ ⁻ concentration (μM) | 0 | 300 | 300 | 400 | 400 | 880 (<i>f/2</i>) | 880 (<i>f/2</i>) | 880 (<i>f/2</i>) |
| NH ₄ ⁺ concentration (μM) | 0 | 5 | 50 | 5 | 50 | 0 | 0 | 0 |

Cytometry

The CytoSense is a scanning flow cytometer that yields one-dimensional resolution profiles (see Fig. 1) obtained from particles pumped in single file within a particle-free sheath fluid past a detection laser beam (green laser at 532 nm; Dubelaar and Gerritzen, 2000). Digital data acquisition is initiated as a particle enters the laser beam and is terminated when the particle is no longer detected. The length of the particle is determined by the time of passage by forward scatter (FWS) measurement. The light scattered from each passing particle is measured at two angles [FWS and sideward scatter (SWS)] to provide information on size and shape of the particles. In addition to the scatter, the fluorescence emitted by photosynthetic pigments in algal cells is detected at three different wavelengths [536–601 nm for phycoerythrin, 601–668 nm for phycocyanin and 668–734 nm for chlorophyll *a* (Chl *a*)]. These fluorescence signatures of Chl *a*, phycoerythrin and phycocyanin, displayed as red, orange and yellow, respectively, assist in determining the algal type of each particle. A profile of a particle, in this case a long chain of the diatom, *S. costatum*, is shown in Fig. 1 where individual cells can be seen as peaks in the FWS signal. This chain (Fig. 1) is 172 μm long and is made up of 11 cells. Accompanying each FWS peak is the chlorophyll fluorescence (in red) of each cell. As this is a diatom profile, there are no fluorescent signatures for phycoerythrin (in orange) or phycocyanin (in yellow). The CytoSift software provided with the CytoSense enables selective data acquisition, and the CytoClus software can be used to characterize the particles that have been counted and analysed. Consequently, data for the number of particles, their size, shape and fluorescence characteristics can be obtained using the CytoSense flow cytometer and associated software (Dubelaar *et al.*, 2004).

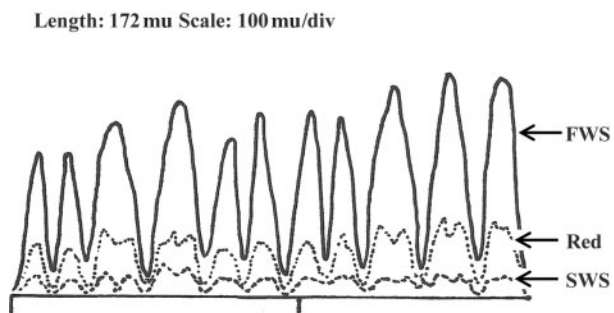


Fig. 1. An example of simultaneous chl *a* fluorescence (red), forward scatter (FWS), and sideward scatter (SWS) profiles on a log scaled Y-axis of an 11-cell long chain *S. costatum* of 172 μm in total length. Redrawn in monochrome from the color original as shown in Cytosense output available as supplementary material at www.plankt.oxfordjournals.org.

The CytoSift software allows the selective data acquisition by setting boundaries for fluorescence amplitude, particle length and sampling time (Dubelaar *et al.*, 2004). In all experiments described here, the CytoSift parameters used were ones that had been empirically optimized for live diatom cells (A. Marchi and K. Lew, San Francisco State University, unpublished results). These parameters (in arbitrary units) were 0 for minimum particle length, 80 for minimum threshold red fluorescence (designated as “top level” in CytoSift software) and 15 min for maximum sampling time. Despite this maximum sampling time, routine sampling only took <2 min. A highly concentrated number of particles per volume will fill the data acquisition buffer and sample too small a volume for good data reproducibility. Thus, concentrated cultures were diluted with autoclaved nutrient-deplete artificial seawater so that the sufficient volume (>20 μl) could be analysed.

The data, acquired using CytoSift, were further analysed using the CytoClus software to characterize the particles according to their shape, size and fluorescence (Dubelaar *et al.*, 2004). Two sets of criteria (“selection sets” in CytoClus) for counting the frequencies of size classes (one-cell, two-cells, three-cells, etc.) of *S. costatum* were determined. The first criterion used was the ratio of orange fluorescence to red fluorescence (“FLO:FLR”). Debris or phytoplankton containing phycobilins can have smaller red/orange fluorescence ratio than algae without these accessory pigments. To eliminate these particles from our analyses, a maximum FLO:FLR of 0.125 was set as a maximum threshold. This FLO:FLR threshold was determined empirically by examination of each particle ($n > 500$), dead or alive, for its relative phycocyanin and phycoerythrin fluorescence signals (FLO) to Chl *a* signal (FLR), using the data-viewing software, Dataview (CytoBuoy). This selection set (minimum FLO:FLR of 0.125) was therefore used as a fingerprint for live diatoms and dinoflagellates in both culture and environmental samples. The particles, selected by the above selection set, were then further sorted through the second selection set which binned chains consisting of different cell numbers into size classes based on FWS ranges. These FWS ranges representing size classes of one cell, two cells, three cells and so on to eight or more cells per chain were determined by the frequency distribution analysis by manual inspection of each particle with CytoClus (Table II). These size class ranges in each class were sufficiently robust to accommodate the changes in the cell size (e.g., length of pervalvar axis or distance between the two valves) previously noted during rapid population growth (Joint *et al.*, 1987). FWS, SWS and chlorophyll fluorescent peak profiles of the particles in the eight or more cell size class were individually

Table II: Forward scatter (FWS) ranges for size class of Skeletonema costatum chains determined by the frequency distribution analysis by manual inspection of each particle with CytoClus

| Chain size class (number of cells per chain) | FWS range (µm) |
|---|----------------|
| 1 | 0–12.4 |
| 2 | 12.5–24.4 |
| 3 | 24.5–27.9 |
| 4 | 28.0–37.9 |
| 5 | 38.0–43.9 |
| 6 | 44.0–52.9 |
| 7 | 53.0–58.9 |
| 8 | 59.0–69.9 |
| >9 (profiles manually inspected) | >70 |

inspected to record the number of cells per chain as the correlation between FWS and cell number per chain became weak above this level due to the accumulation of size deviation of cells and inter-cellular spaces.

The frequency distribution of size classes (one cell, two cells, three cells and so on up to 17 cells per chain) of the *S. costatum* chains in the present study was determined using either the above-described selection sets in CytoClus or for longer chains, by manually counting the FWS peaks (i.e., cells per chain) for each particle. Daily growth rate was calculated as \log_2 of the ratio between final and initial cell densities divided by the number of days elapsed.

Comparison of CytoSense measurements with microscopic counts

To truth the cytometric measurements obtained by the CytoSense system, the diatom cell concentration and chain length were analysed simultaneously using CytoSense and the Utermöhl method (Utermöhl, 1958) to obtain microscopic counts. Samples were settled in 25-mL chambers and enumerated on grids using an inverted phase-contrast Nikon Type 180 microscope. The changes in cell concentration in an f/2 culture of a single-celled diatom, *Thalassiosira pseudonana* (CCMP 1335) were analysed daily for 7 days. In addition, for enumeration of cells per chain, the samples of *S. costatum* from the control enclosure during the first 3 days of the enclosure experiment

were analysed using both approaches. All statistical analyses in this and other sections were performed using Minitab 14.20 (Minitab, Pennsylvania, USA).

RESULTS

Comparison of CytoSense measurements with microscopic counts

Cell concentrations measured using CytoSense and visual Utermöhl microscopic counts were well correlated and equivalent ($r^2 = 0.98$, $P < 0.01$, slope = 1.19; Fig. 2A) all through the 7-day growth in a *T. pseudonana* culture. This correlation became less clear at higher cell concentrations in other experiments (data not shown). The average numbers of cells per *S. costatum* chain in the control enclosure during the first 3 days of the enclosure experiment, measured using CytoSense and visual Utermöhl microscopic counts, were also well correlated and equivalent ($r^2 = 0.98$, $P < 0.01$, slope = 1.06; Fig. 2B). Count variability among replicates ($n = 12$) was smaller in CytoSense measurements than visual microscopic counts ($n = 3$) for both cell concentrations and chain length (Fig. 2).

Changes in *S. costatum* chain length in batch culture

In the batch culture of *S. costatum* kept at 17°C (treatments A–D and the control), the concentration of cells in culture increased exponentially with the peak growth rate between 72 and 120 h in all nitrogen (N)-replete treatments (Fig. 3A). Ammonium was depleted by 168 h, which coincided with the start of a significant nitrate uptake in all N treatments (Fig. 3B and C). Silicate and phosphate were removed from the media in a similar fashion to nitrate but were not completely depleted by the end of the experiment (Fig. 3D–E). The mean number of cells per chain of *S. costatum* peaked during the mid-log phase around 120 h (Fig. 3F). This peak is also reflected by a decrease in the mean percentages of the population occupied by chains shorter than two cells in N-replete treatments, compared with the control which shows no change (Fig. 4). When cultures were grown at different temperatures (6, 8 and 17°C; treatments E–G) in f/2 medium, the mean chain length (cells per chain) in the batch cultures of *S. costatum* all increased in logarithmic fashion (Fig. 5). The maximum mean chain length and slope achieved in these cultures increased with temperature up to 17°C. The published temperature range for this strain of *S. costatum* is 11–30°C (Provasoli-Guillard National Center of Culture of Marine Phytoplankton).

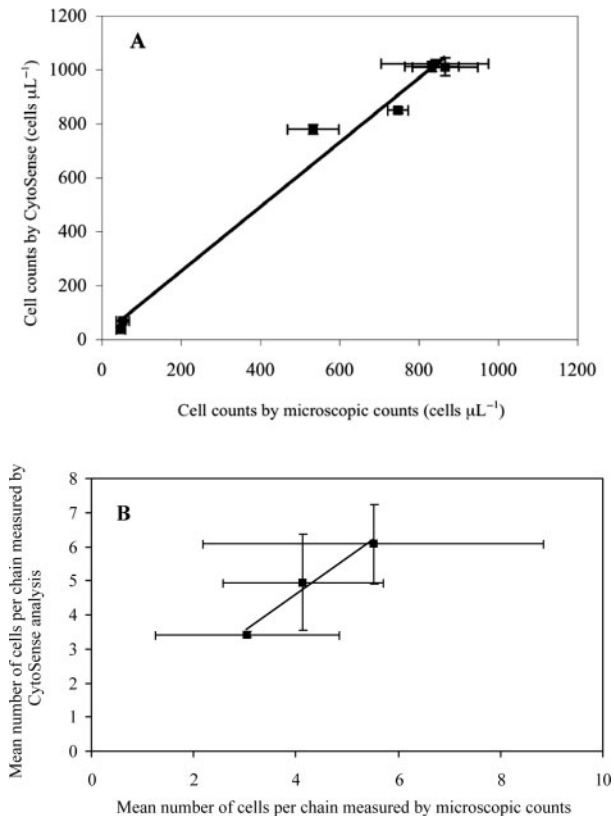


Fig. 2. Correlation between CytoSense measurements with Utermöhl microscopic measurements of (A) mean ($n = 12$ for CytoSense and $n = 3$ for microscopic measurements \pm SD) cell concentrations measured during a 7-day growth of a diatom, *Thalassiosira pseudonana* maintained in f/2 media and (B) mean ($n = 3$, \pm SD) number of cells per chain of *Skeletonema costatum* in the control enclosure for the first 3 days of the experiment.

Although the mean chain length of *S. costatum* grown at 8 and 17°C did not differ at the end of the experiment (360 h), the cells at 17°C achieved this maximum length (3.5 cells/chain) earlier (180 h) compared with the cells grown at 8°C (Fig. 5). The dominance of longer chains in the population in N-replete treatments and optimal temperature treatment (17°C) coincides with a peak in growth rate during the exponential growth, which is shown in a positive correlation ($r^2 = 0.55$, $P < 0.01$) between the growth rate and mean number of cells per chain (and therefore chain length; Fig. 6).

Changes in the length of chain-forming diatoms in enclosure experiments

A phytoplankton bloom was observed in the enclosed water drawn from San Francisco Bay. Daily visual observation and enumeration using microscopy indicated that *S. costatum* was the only chain-forming diatom found in the enclosed water and represented 73–83% of the total

phytoplankton population throughout the duration of the experiment (A. Lassiter, San Francisco State University, unpublished results). It was therefore feasible to assume that all chain-forming particles detected with a strong red fluorescence compared with orange or yellow fluorescence (i.e., FLO:FLR < 0.125) by the CytoSense flow cytometer were *S. costatum* in this enclosure. We could not differentiate among single-celled diatoms and dinoflagellates, as they all had FLO/FLR of below 0.125 and similar “shape” determined by their FWS and SWS profiles. Single-celled particles were therefore excluded from the analyses of *S. costatum* cells from the enclosures. This did not alter the patterns in the analyses described below.

The number of cells observed in *S. costatum* chains that were longer than two cells increased in an exponential manner as expected in both nitrate-enriched and control enclosures over 4 days (Fig. 7A). The growth in both enclosures had the same mid-log growth rate as each other but began their plateau phases when nitrate in each respective enclosure was depleted (Fig. 7B). The mean chain length of *S. costatum* (excluding the single-celled diatoms) peaked simultaneously in the control and enriched enclosures after 3 days (Fig. 7C). This peak coincided with the beginning of the population growth plateau in the nitrate-enriched treatment but was 24 h after the population plateau in the control enclosure. Chain size of *S. costatum* in the control and enriched treatments remained very similar to each other throughout the experiment, despite the differences in the timing of nitrate depletion and the population size achieved in the two treatments. The correlation between growth rate and changes in mean number of cells per chain was strong ($r^2 = 0.94$) in the enriched treatment but not in the control ($r^2 = 0.22$) because of the 24-h lag between population growth plateau and the decrease in chain length observed in the control. The chain length of *S. costatum* in the enclosures of natural San Francisco Bay water was generally longer than those found in culture with the natural *S. costatum* achieving a mean chain length of 8.0 cells (Fig. 7C) at their peak compared with 3.8 cells in culture (Figs 3F and 5). This pattern held even when the single cells were excluded from the counts in cultures, which made the maximum mean chain length to 4.2 cells (data not shown).

DISCUSSION

Many colony-forming diatoms exist in chains of extensive lengths. What determines chain length or number of cells per chain in a given species has not been clearly explained. However, both advantageous and

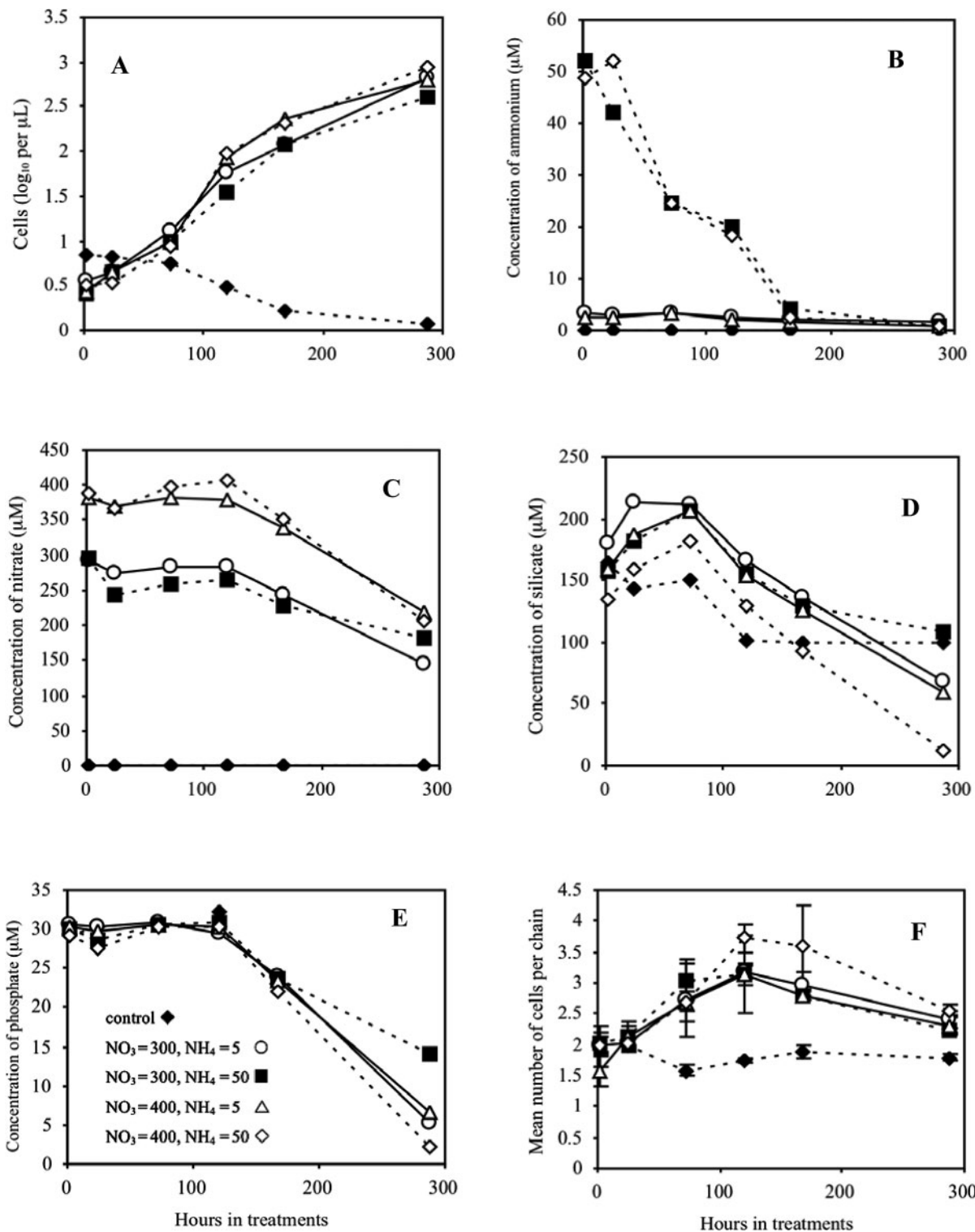


Fig. 3. Results from the batch cultures of *Skeletonema costatum* grown at 17°C with varying nutrient concentrations in treatments A–D and the control. The initial concentrations in µM of NO₃⁻ and NH₄⁺ in the media are indicated next to the treatments in the legend. (A) Mean (±SD) number of cells (on log₁₀ scale) per µL. (B) Concentrations of nitrate, (C) ammonium, (D) phosphate and (E) silicate in the medium of nutrient treatments. (F) Mean (±SD) number of cells per chain.

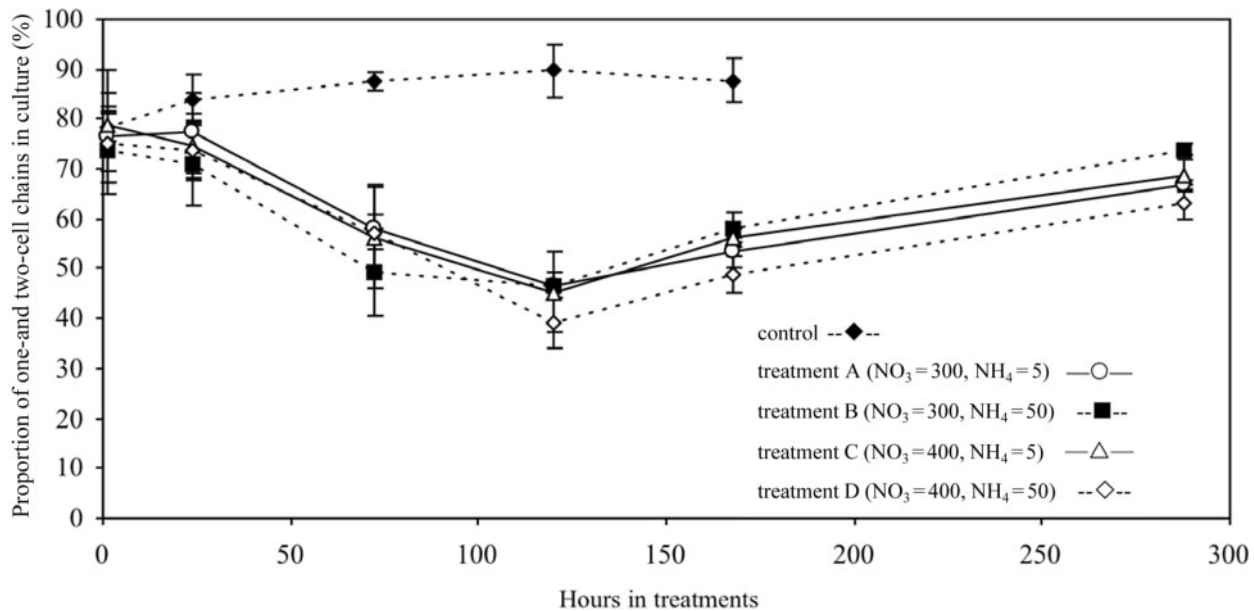


Fig. 4. Proportion (mean \pm SD) of one- and two-cell chains *Skeletonema costatum* in monoculture grown in nitrogen (N)-replete treatments A–D. The initial concentrations in μM of nitrate and ammonium in the media are indicated.

disadvantageous reasons for chain formation by diatoms have been put forward. Previous observations suggest specific factors which influence chain length (or number of cells per chain) of diatoms of the genus *Skeletonema* to include temperature, duration of daylight and rate of cell division (Smayda and Boleyn, 1966; Brockmann *et al.*, 1977; Kiss *et al.*, 1994). We examined the effect of population growth rate on the chain length of *Skeletonema costatum* by altering nutrient availability and temperature conditions. The enumeration and analysis of the morphological composition of chain-forming diatoms were done using a novel instrument and software package, Cytosense. We observed that the diatom chain length increased under favorable growth conditions indicated as higher growth rates. Higher growth rates resulting in longer chains of *S. costatum* were observed in natural estuarine water enclosures compared with the batch cultures as well as observed in cultures grown at a higher temperature compared with lower temperatures than their optimum range.

Smayda and Boleyn (Smayda and Boleyn, 1966) noted that the sinking rate of *S. costatum* was inversely related to the number of cells per chain irrespective of culture age. Longer chains assist diatoms to remain in the euphotic zone by decreasing their sedimentation rate, which is achieved by increased surface area to volume ratio. Remaining in the euphotic zone is advantageous for the diatoms' growth as there is sufficient light for photosynthesis especially when the nutrient concentrations are

increased by upwelling. A positive correlation between growth rate and chain length was observed in our batch culture experiment and the enriched enclosure (Figs 6 and 7). Nutrient availability and a higher temperature both enhanced growth rate and the mean chain length of cultured *S. costatum* (Figs 4–5). Contrary to a previous study that observed diurnal variations of cell size and chain length (Brockmann *et al.*, 1977), we did not detect any diurnal change in chain length in our enclosures (data not shown).

One disadvantage of colony formation to diatoms is that longer colonies, as compared with solitary cells, can cause a greater local depletion of nutrients in the water by creating shear flow (Pahlow *et al.*, 1997). Certain morphological features such as spaces between cells in some chain-forming diatoms may compensate for this event, allowing them to attain even a higher nutrient supply than single cells (Pahlow *et al.*, 1997). *S. costatum* has long, marginal siliceous processes connecting the cells in the chains that can exceed the length of the cells themselves (Cupp, 1943), thus producing intercellular spaces that would decrease the biomass per unit of chain length. These spaces are probably able to counteract the disadvantage of chain formation against shear flow.

Longer chains, in turn, could provide *S. costatum* with greater intercellular volumes to maximize their uptake of nutrients under enriched conditions. Once the nutrients are depleted, however, long chains become

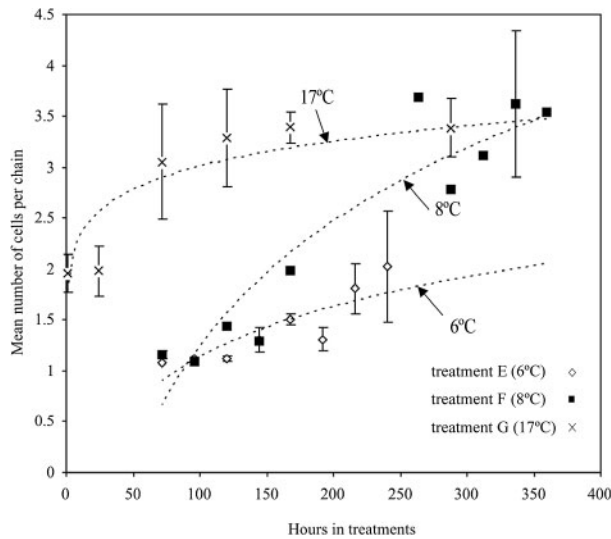


Fig. 5. Relationship between mean number of cells per *Skeletonema costatum* chain and daily growth rate (μ) in batch cultures under different nutrient and temperature treatments. The linear correlation of all data is overlaid as a dotted line ($y = 1.44x + 2.06$; $r^2 = 0.55$, $P < 0.01$).

disadvantageous, and thus diatom populations will shift toward shorter chains and solitary cells. This idea is supported by a previous observation that the chain length of *S. costatum* decreased with increasing culture age, resulting in the dominance of the population by

solitary cells and two-celled chains after 16 days, although nutrient concentrations of the cultures were not reported (Smayda and Boleyn, 1966). *S. costatum* colonies in the enclosures showed decreased chain length once the growth rate plateaued following nutrient depletion (Fig. 7). Interestingly, the shift to shorter chains in response to decreases in growth rate occurred earlier in the batch cultures than in enclosures. The cultivated *S. costatum* colonies began to show decreased chain length following a peak in growth rate although nitrate, silicate and phosphate in the media were not completely depleted at that stage (Fig. 3C-E). There are two possible reasons for this. The first is that in the enclosures, the enhanced grazing pressure following the increase in diatom population selectively eliminated the smaller chains of *S. costatum* (Fogg, 1991), allowing the mean chain length to remain high longer. There were no grazers in the batch cultures; thus, the broken, shorter chains were detected earlier than in the enclosures. The second reason for the difference between the enclosure and batch cultures is the decrease in the shear turbulence around the long curved chains that has been described in relatively stagnant batch cultures (Karp-Boss and Jumars, 1998), leading to depletion of nutrients in the region immediately surrounding the cells instead of overall depletion of nutrients in the medium. The decreased shear flow in the intermittently stirred batch culture

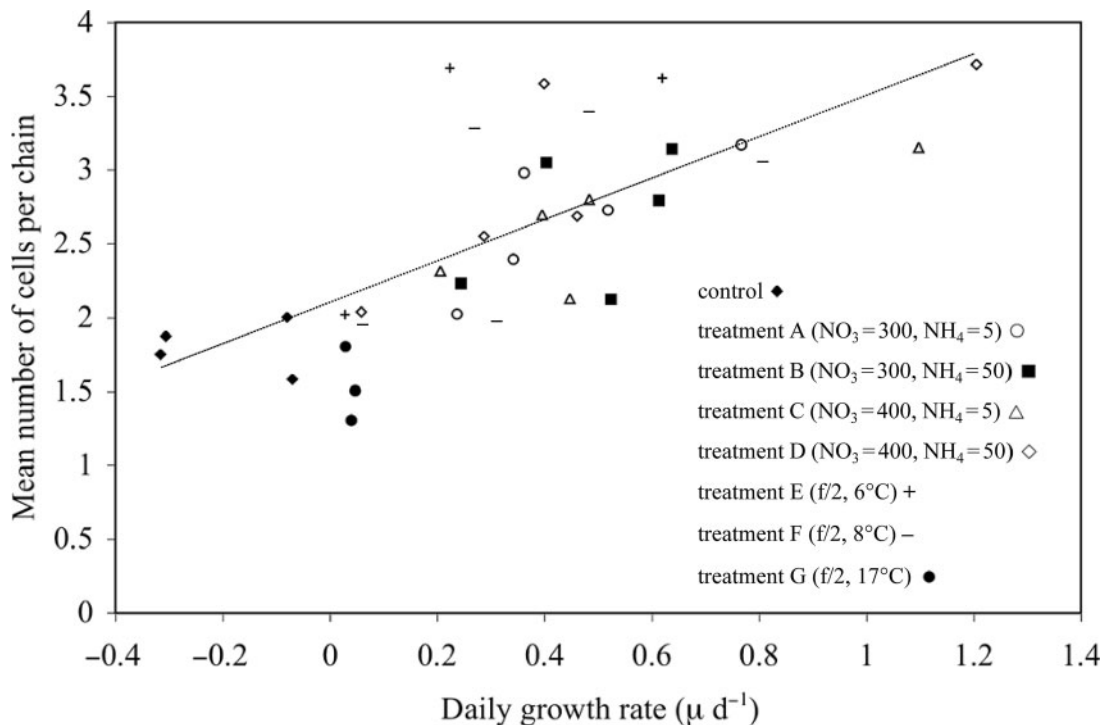


Fig. 6. Mean number of cells per chain of *Skeletonema costatum* grown in *f/2* media at 6, 8 and 17°C as a function of time in batch culture (treatments E-G). Logarithmic functions of best fit are overlaid with $r^2 = 0.73$, 0.87 and 0.76 for 6, 8 and 17°C, respectively.

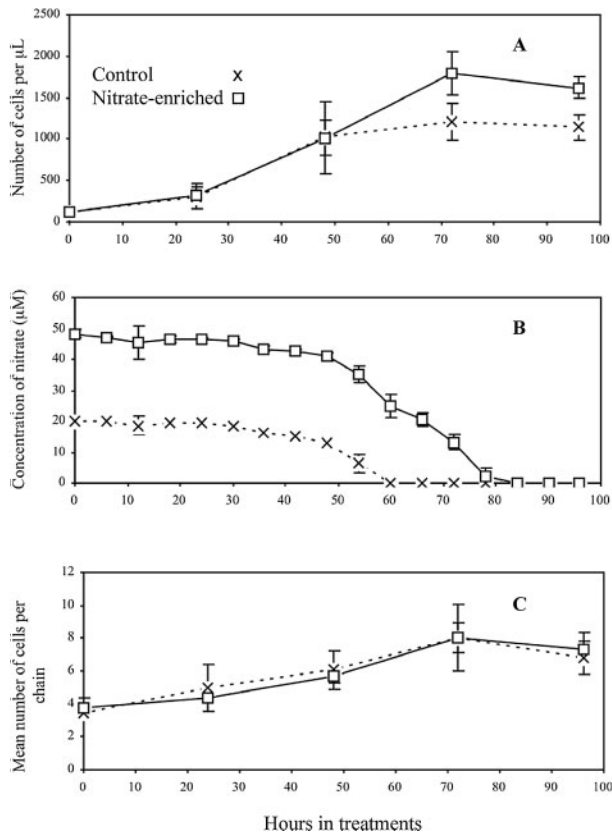


Fig. 7. Changes in (A) population size of *Skeletonema costatum*, (B) nitrate concentration, and (C) mean number of cells per chain of *S. costatum* in control and nitrate-enriched enclosures of San Francisco Bay water during spring 2004.

might also have contributed to the shorter maximum chain length of *S. costatum* achieved in culture compared with the regularly mixed water of the enclosures. The effect of water motion in conjunction with nutrient limitation on diatom chain size needs to be further examined. The chain length of *S. costatum* in enclosures with and without nitrate addition changed almost in identical pattern over time despite that the diatom growth rate and nutrient depletion peaked 24 h earlier in the control compared with N-replete enclosure (Fig. 7). It is likely that in the natural enclosure with more natural irradiance conditions, there was an insufficient difference in the added nitrate concentrations to change the growth rate enough to produce a significant difference in the chain length.

Evaluation of the changes in cells per chain with time in natural populations may yield information on the growth rate or state of a phytoplankton bloom. The use of a modern flow cytometer designed for analysing particles of diatom size and the associated analytical software makes it practical to acquire the necessary data with relatively little effort and with greater accuracy

than microscopy counts. Combining size of cells, particle shapes and fluorescence characteristics make CytoSense technology a powerful tool for cytometric analyses of phytoplankton once these characteristics have been positively identified using pure cultures of particular types of phytoplankton. A library of characteristics detectable by CytoSense for phytoplankton species common in San Francisco Bay is currently being constructed (Marchi *et al.*, San Francisco State University unpublished results).

In conclusion, our analyses of cultured and natural populations of a chain-forming diatom, *S. costatum*, using a CytoSense scanning flow cytometer and accompanying analytical software packages (CytoSift and CytoClus), determined that cells with higher growth rates due to either increased temperature from 6, 8 to 17°C, the last being the only temperature within the strain's optimal range, or enhanced availability of nutrients led to populations dominated by longer chains. The elongation of chains continued until either nutrients were depleted or the decrease in shear turbulence caused by the long chains outweighed the benefits, before the diatom population shifted toward shorter chains. Longer chains are expected to aid the diatom to remain suspended in the upper part of the water column that has favorable physical and chemical parameters for growth.

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