



Dinoflagellate cell density limits explored using *Scrippsiella lachrymosa* cultured in flow-through cages

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Abstract

Dinoflagellates constitute one of the most important groups of primary producers and micro-zooplankton on earth, common in both marine and freshwater environments. Despite their prominent position among phytoplankton, they are difficult to grow into dense cultures in the laboratory. This discrepancy between field and laboratory indicates serious limitations caused by the laboratory culturing conditions. A difficult to study but important factor is the constraints of enclosure in a limited volume of water. We conducted an experiment wherein the dinoflagellate *Scrippsiella lachrymosa* was grown in “flow cells” – 100 cm³ cylindrical cages constructed from plankton net, inserted in larger volumes of growth medium, allowing an exchange of medium without dilution of the culture. Cell numbers far exceeding the normal for culturing of this species and dinoflagellates in general were attained, even though the experiment was terminated before cultures reached stationary phase. A cell number ten times higher than under regular batch culturing was achieved (up to 340,000 cells mL⁻¹). Pattern formation was distinct in cultures when cells were plentiful and water movements caused cell accumulation, not dispersion. High cell density concurrent with access to new growth medium promoted induction of the sexual cell cycle. The results indicate serious limitations to growth set by enclosure in a limited water volume in laboratory experiments; thus, maximum growth rates of dinoflagellates in favourable field conditions may be vastly underestimated. Cell accumulation behavior of dinoflagellates during the sexual life cycle may together with physical transport by larger forces in nature explain sudden bloom occurrences.

Keywords Culturing · Dinoflagellate · Gamete · Life stage · Red tide · *Scrippsiella lachrymosa*

Introduction

Dinoflagellates are important primary producers in both marine and freshwater ecosystems, ranging in size from several hundred micrometers to less than 2 μm (Le Bescot et al. 2016), and are represented by both autotrophic primary producers and heterotrophic microzooplankton species, as well as many mixotrophs (Jeong et al. 2010). Perhaps they

are most well-known for causing harmful algal blooms (“red tides”) in the oceans worldwide (e.g. Anderson et al. 2012). The majority of dinoflagellate species are harmless, but among harmful phytoplankton, the dinoflagellates dominate (75% of harmful species are dinoflagellates according to McLean 2013). Even though they sometimes occur in large quantities that color the water red-brown at daytime and/or bioluminescent at night, dinoflagellates can also cause toxicity in shellfish at low cell-numbers, not seen as color by the naked eye. Sometimes a cell density of only 200 cells L⁻¹ can result in shellfish toxicity and is considered dangerous (Dyhrman et al. 2006). Dinoflagellates are grown in laboratories around the world where research on harmful algal blooms is conducted and usually as “batch cultures” (an inoculum is taken from an old culture and placed in fresh growth medium). There are large difficulties in creating continuous culturing methods; slow vegetative growth combined with mechanical sensitivity demands great care in handling (most dinoflagellates cannot withstand shaking, mixing, or aeration especially well; García Camacho et al.

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2011; Gallardo-Rodríguez et al. 2016). Production of self-inhibiting substances such as waste products and substances for chemical defense (e.g. Gentien et al. 2007; Tillmann et al. 2007) results in a low steady-state cell number, but semi-continuous (e.g. García Camacho et al. 2011; Beuzenberg et al. 2012) and continuous (e.g. Corcoran et al. 2014) methods have been used. Generally, culture growth stops at approximately $10,000 \text{ cells mL}^{-1}$ in batch culture for the vast majority of dinoflagellates in culture, even if nutrients are available and irrespective of nutrient medium, container, lighting regime, etc. (Table S1, supplement). These numbers are comparable to the highest records from field measurements of dinoflagellate blooms (Table S2, supplement), but represent maximum cell numbers at steady state, after a period of growth, not a high biomass productivity. The biomass of dinoflagellates possible to produce in culture per unit volume and time is generally very low compared to what is common for phytoplankton cultures used in aquaculture for feed or biofuel production (e.g. Shah et al. 2016). For the above reasons dinoflagellates are generally not used as or considered as suitable for feed in aquaculture, but mostly grown for research purposes. Many dinoflagellates have a sexual life cycle that is induced when nutrients become limiting, and a batch culture in stationary phase very often consists of a mixture of different sexual and asexual life stages (personal observations). Different life stages of dinoflagellates have different roles and different behaviour; the asexual, vegetative cells divide into identical cells that perform daily vertical migrations (e.g. MacIntyre et al. 1997). Gametes – sexual cells – are formed at the end of the growing season and have a characteristic swimming pattern (Persson et al. 2013 and references therein); they strive to mate with each other and thus swarm. It is likely that gametes form substances that facilitate attraction (sex pheromones), adhesion, and fusion. Fusion of cells is a complex process that is highly regulated (Epan 2000) and an old culture with low nutrient content and high levels of waste products and inhibitory substances may not offer the ideal conditions for either continued vegetative growth or successful completion of the sexual cycle.

It is possible that dinoflagellates could thrive, at least temporarily, in dense cultures also in the laboratory if the medium could be changed fast enough without diluting the cells. If the sexual phase was induced by bringing cells close to each other, without nutrient limitation, this would indicate “quorum sensing” (the ability to sense a high enough number of conspecific individuals) as an additional important signal inducing sexuality in dinoflagellates. Quorum sensing is well known for bacteria (March and Bentley 2004). In early studies on resting cyst formation in dinoflagellates, cell density as a signal for the induction of sexual behaviour was investigated (e.g. Anderson et al. 1984). This, however, could not be proven to be of importance as a higher cell

number did not result in higher encystment success. Many cells together in a closed environment quickly use up nutrients needed for successful completion of a sexual cycle and produce toxins and waste products that inhibit further growth. Cell density as a factor inducing sexuality has not been tested in an environment where cells had the chance to proliferate.

Using 100-mL cages of plankton-net (“flow cells”) inserted into 1-L beakers, we performed a growth experiment with an exchange of medium without cell dilution using the cyst-forming homothallic dinoflagellate *Scrippsiella lachrymosa*. We achieved unprecedented high cell densities in the absence of nutrient deficiency and observed distinct pattern formation of contact-seeking cells under these conditions.

Materials and methods

The experiment was performed in April 2012 at NOAA/NFSC Milford Laboratory using *Scrippsiella lachrymosa* (strain B-10 from the Woods Hole Oceanographic Institution). This homothallic and cyst-producing dinoflagellate has been used in several studies on sexual life stages and cyst formation (Smith and Persson 2004, 2005; Persson et al. 2016).

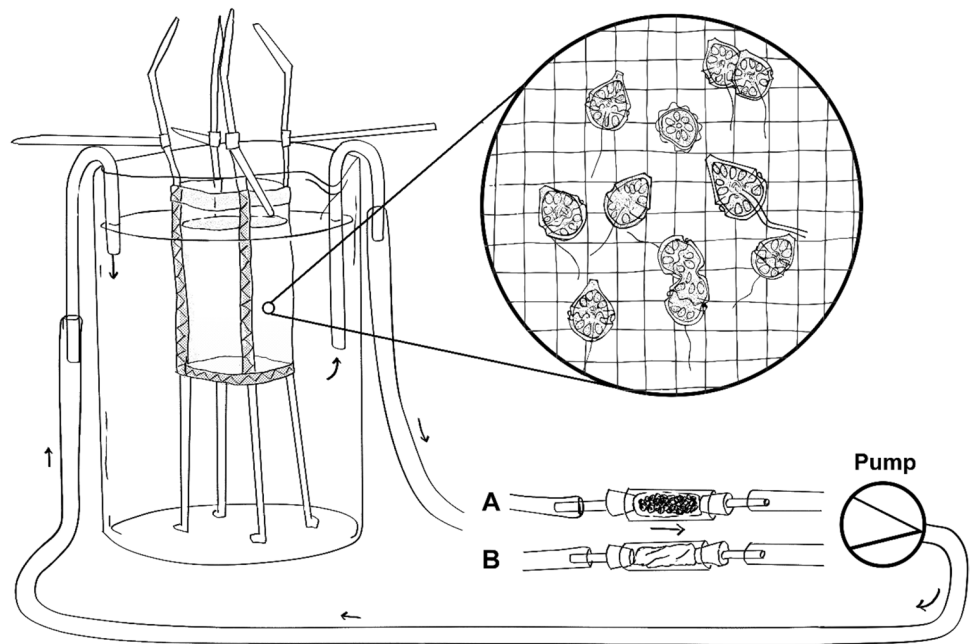
As high a flow and as little barrier as possible between the concentrated cell population and the surrounding (exchangeable) medium was desired. Accordingly, we constructed cages from plankton net with a screen size as large as possible that would contain the cells.

The experiment was started at a cell density near the maximum cell density achieved in batch culture, but with cells in exponential growth. *f/2*-enriched (Guillard and Ryther 1962) Milford Harbor seawater without added silicon at a salinity of 26‰ was used as growth medium.

Cages were made of 10- μm nylon Nitex screen and seams sealed with aquarium silicone on both inside and outside. Three replicates were used for each treatment. The total volume of *f/2* medium was one liter per replicate and the flow rate (in each replicate) was 40.0 mL min^{-1} (Ismatec ICP, 12 channel standard speed planetary gear driven pump, Cole-Parmer). The experimental set-up is shown in Fig. 1. Tubing used was 3.17-mm inside diameter (Tygon R3607). Pyrex glass tubing used had 6 mm ID and 9 mm OD. The effect of water ‘cleaning’ was tested by using filters made of 7 cm long \times 2.5 cm diameter cylindrical Pyrex glass tubes filled with 4.6 g activated carbon. Ends were plugged with #4 rubber stoppers; Nitex screen (10- μm) kept carbon particles within the container (Fig. 1).

To acquire a dense starting culture of exponentially-growing cells, several exponentially-growing cultures were added together, and the old medium was carefully removed using

Fig. 1 Experimental set-up in 1-L beaker. The cage made of 10 μm Nitex screen contains the *S. lachrymosa* culture, *f/2* medium flows in the direction of arrows through either an activated carbon filter (A) or the same construction without activated carbon (B),



a sterile pipette with the tip covered with a piece of sterilized, 10- μm plankton screen. The stock cultures were kept on glass shelves in a culturing room with similar conditions to those used in the experiment (determining the choice of experimental conditions).

Two starting cell densities were used: 33,000 cells mL^{-1} (high) and 16,500 cells mL^{-1} (low) (Table 1). The “high” cell density treatment received 100 mL of the starting culture, and the “low” cell density treatment received 50 mL. Cages were placed in 1-L beakers, and the total volume of culture + medium was 1 L for experimental treatments and 100 mL for batch cultures/controls in small beakers. For experimental treatments in cages, the medium was circulated through a cylinder with or without activated carbon. Each treatment had three replicates. The experiment was conducted in a lighted bio-incubator at $370 \pm 15 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, 16 °C, 12:12 h L:D (light 8 am to 8 pm), and the beakers were covered with plastic foil.

Samples were taken at the start and at the same time of day (at 14:00) on each sampling occasion; on day 3, 6 and 10 after start (0.5 mL for flow cytometry and 1 mL for microscopic cell counts). Flow cytometry was always performed at 14:30

to avoid differences caused by the time of day. Flow cytometric detectors used were forward scatter (FSC), side scatter (SSC) and chlorophyll *a* (red fluorescence at 650 nm; FL3). Samples for cell counts were preserved with iodine crystals ($\sim 5 \text{ mg mL}^{-1}$) and counted microscopically. Separate counts were made for small cells (gametes and vegetative cells), mating pairs, zygotes, and resting cysts. The experiment was terminated after 12 days. As the escape of cells outside cages was evident, samples for flow cytometry and cell counts were at the termination taken from both inside and outside cages. Flow cytometry was performed, and samples preserved with iodine crystals and counted in the microscope. Further, samples for nutrient analysis of the cell-free medium were filtered (GF/F filters, 20 mL per sample) and frozen at -80 °C.

Nutrient analysis

Samples for nitrate + nitrite, nitrite, and phosphate were frozen immediately after sample collection until batch analysis. All nutrient protocols used were developed by the Royal Netherlands Institute for Sea Research and analysis was conducted using a Quattro autoanalyzer (Seal

Table 1 Experimental treatments

Treatment	Starting cell density	Carbon filter	Container	Volume	Replicates
High + carbon	33,000 cells mL^{-1}	yes	cage	100 mL	3
High—carbon	33,000 cells mL^{-1}	no	cage	100 mL	3
Low + carbon	16,500 cells mL^{-1}	yes	cage	100 mL	3
Low—carbon	16,500 cells mL^{-1}	no	cage	100 mL	3
High beaker	33,000 cells mL^{-1}	no	beaker	100 mL	3
Low beaker	16,500 cells mL^{-1}	no	beaker	100 mL	3

Analytical, USA). Nitrate + nitrite was determined using the red azo dye method, with a detection limit of $0.02 \mu\text{mol L}^{-1}$ and a standard deviation of $0.03 \mu\text{mol L}^{-1}$ (Method NO Q-068–05 Rev. 4). Nitrite also used the red azo dye method, with a detection limit of $0.01 \mu\text{mol L}^{-1}$ and a standard deviation of $0.04 \mu\text{mol L}^{-1}$ (Method NO Q-070–05 Rev. 4). Phosphate was determined using method Q-064–4; a reduced phosphorous-molybdenum blue complex was read at 880 nm for phosphate measurements.

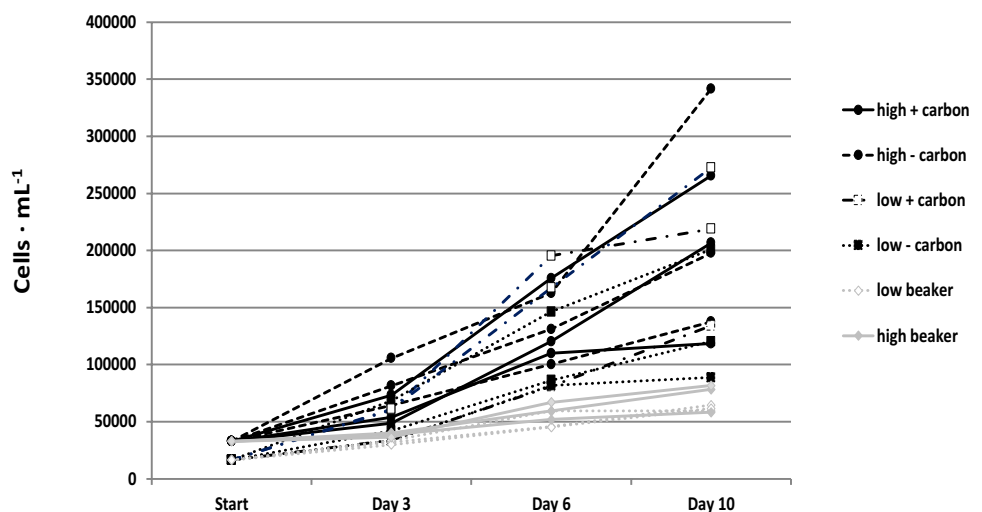
Statistical analysis

One-way analysis of variance (ANOVA) was done using StatGraphics Plus 5.1. (Statpoint Technologies, Inc., USA), with the significance level at $p < 0.05$. The method used to discriminate between means was Fisher's least significant difference (LSD) procedure.

Results

Very high cell densities were reached within cages that did not permit escape (Fig. 2), the highest count being $342,000 \text{ cells mL}^{-1}$. The specific growth rate was highest in caged treatments with low starting cell number ($\mu = 0.37 \pm 0.11 \text{ day}^{-1}$ with a carbon filter and $\mu = 0.34 \pm 0.12 \text{ day}^{-1}$ without). Growth rate decreased over time to only $\sim 0.1 \text{ day}^{-1}$ in caged cultures and $\sim 0.05 \text{ day}^{-1}$ in controls at day 10. Cultures started with high or low cell numbers were different for only a very short time when a large volume of circulating medium was available. After three days no differences in cell density between treatments with different starting cell number could be seen in the flow treatments. Cultures in beakers had much lower growth rate and the starting cell number continued to be of importance throughout the experiment.

Fig. 2 *S. lachrymosa* cell densities in cages (high started with twice the cell number of low) and controls over time. Flow treatments with the circulation of the nutrient medium through an activated carbon filter (+ carbon) or not (-carbon)



We did not manage to construct escape-free cages (Fig. 3). The total number of cells (inside and outside cages) was measured at the termination of the experiment after 12 days. As is clear from Fig. 4, very rapid growth occurred

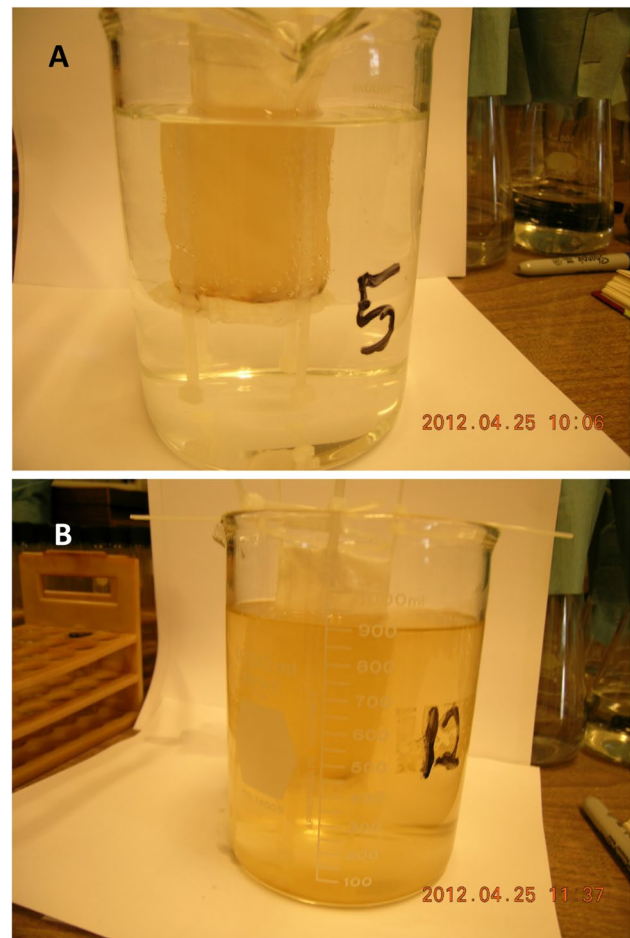
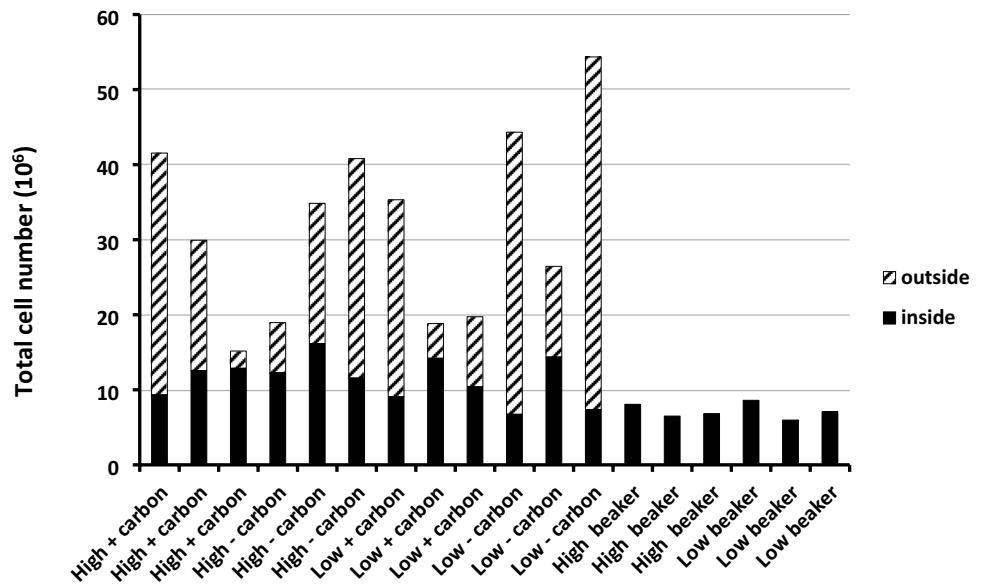


Fig. 3 At end of *Scrippsiella lachrymosa* experiment. (A) Beaker with few escaped cells. (B) Beaker with many escaped cells

Fig. 4 The total cell number inside and outside cages. “High” and “Low” means started with high or low cell number; “filter” means carbon filter. Cells that had ‘escaped’ exhibited strong growth outside the cage, while the total number of cells remained limited inside the cage. Controls in beakers, representing normal batch culturing, had a significantly lower cell number after the same amount of time



when cells “escaped” outside the cage; whereas, the total cell number remained limited when most cells stayed very close to each other within the cage. Cultures in 100-mL beakers which represented normal batch culturing had a significantly lower cell number after the same amount of time.

Flow cytometry results: No significant differences in FL3 (Chlorophyll *a* content), FSC (cell size) or internal complexity (SSC) were found between treatments when compared over time, but a comparison of cells from inside and outside cages on the last day (termination) of the experiment showed significant differences. Cells were of the same size (FSC, Fig. 5A), but inside cages, they had more chlorophyll *a* (FL3, Fig. 5B) and higher internal complexity (side scatter, SSC, Fig. 5C). Cells inside cages were thus different from those outside cages (in the same beaker).

Cells inside the cages were similar to cells in control beakers regarding chlorophyll *a* content and internal complexity (Fig. 6). Cells outside cages, in contrast, had significantly lower chlorophyll *a* content, as well as significantly lower internal complexity (Fig. 6).

The use of carbon filters did not lead to any significant differences between treatments.

Nutrient analysis: Nutrients were depleted in beakers, especially those started with high cell numbers (Table S3, supplement). For flow cell cultures, nutrients were not exhausted, but the content was much lower where cells had managed to escape and grow outside the flow cell.

Observations

Macroscopic: the cultures were very dense and formed patterns of accumulation (Fig. 7).

Microscopic: the bottom of control beakers could easily be observed through an inverted microscope, revealing an enormous amount of empty cell walls on the third day of the experiment (Fig. 8). At the same time, gamete swimming behavior was evident near the beaker wall. The cells that were seen mating were not particularly small, but lacked the characteristic drop shape (with a pointed apex) of vegetative cells. Several large elongated cells without wall were seen (seeming to have recently abandoned thecas) and interpreted as zygotes in the process of theca change. The proportion of mating pairs, zygotes and cysts increased quickly towards the end of the experiment (Fig. 9).

Discussion

Dinoflagellate blooms and laboratory studies

As a dinoflagellate bloom is a natural, environmental phenomenon, it is very difficult to study in the laboratory. The scales involved (tiny cells in a large body of water) make it impossible, and all aspects of a bloom (bacteria, viruses, parasites, grazers, competing phytoplankton, water movements, stratification, nutrients, rain, land runoff, season, temperature changes, pheromones, toxins, allelopathy etc. etc.) cannot be mimicked simultaneously. Different aspects are studied separately, and the laboratory environment necessitates keeping cultures in enclosures to enable quantitative studies on the factors of interest. The present study shows that the effect of confinement is probably much greater than previously known. Most dinoflagellate species cannot be grown into dense cultures; they stop growing at a cell density far from what is common for other cultivable

Fig. 5 Flow cytometric measurements of (A) cell size (FSC); (B) Chlorophyll *a* content (FL3); (C) internal complexity (SSC); a comparison of cells inside and outside cages at the termination of the experiment. High and Low are inoculum at start and + and – carbon is with or without carbon in the filter. Scale bars show standard deviation

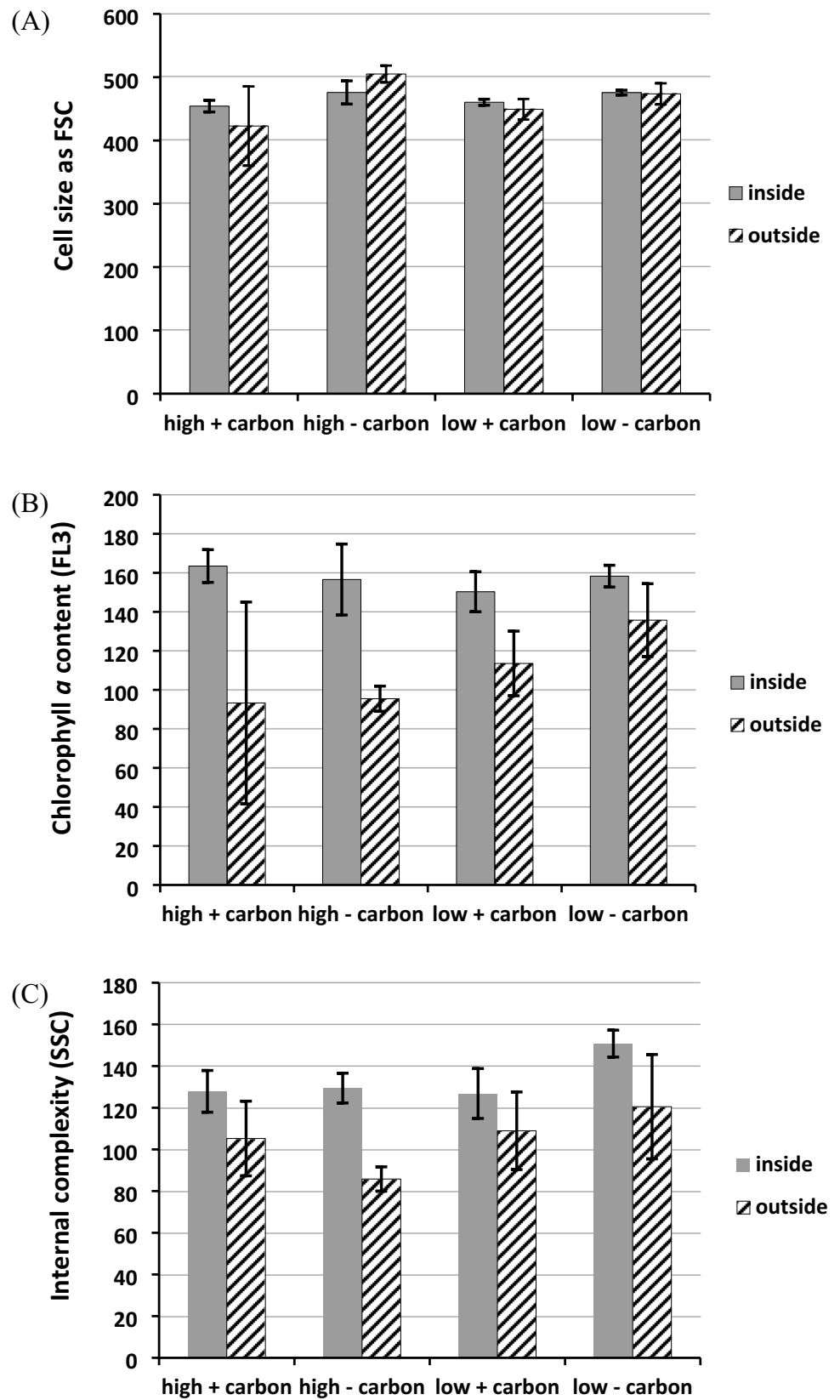


Fig. 6 Comparison between cells inside and outside cages indicate that cells inside the cages were very much comparable to cells in control beakers; whereas, cells outside cages, in a larger volume of medium, appeared to grow much faster. Not only did they have a higher number of cells, but these cells had a lower chlorophyll *a* content and lower internal complexity. Error bars show standard deviation

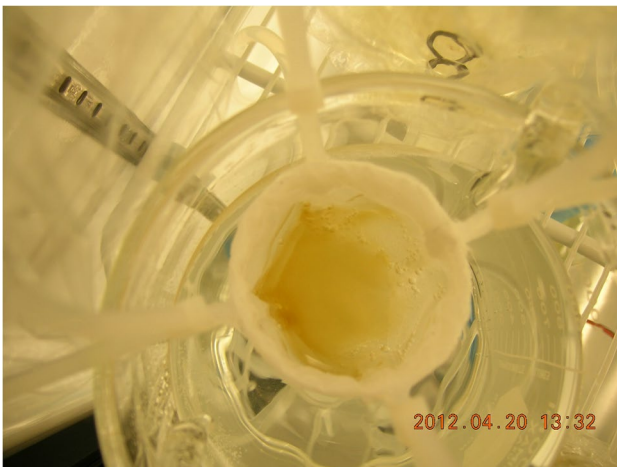
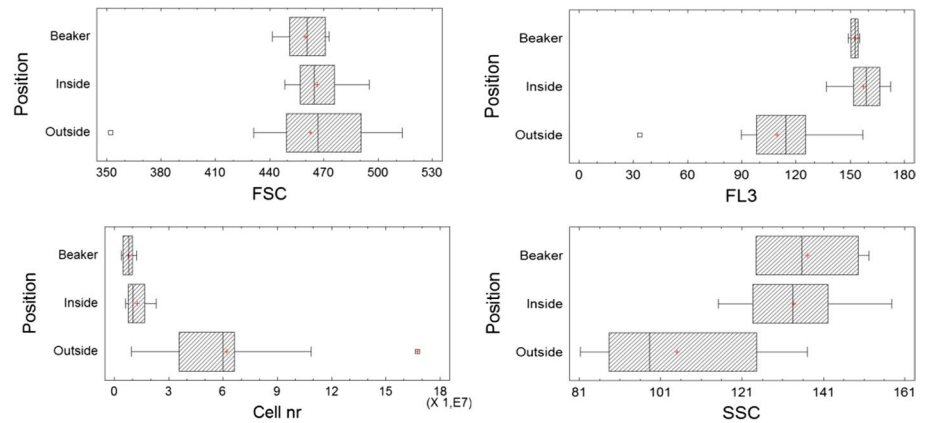


Fig. 7 Cells accumulated in patterns within cages

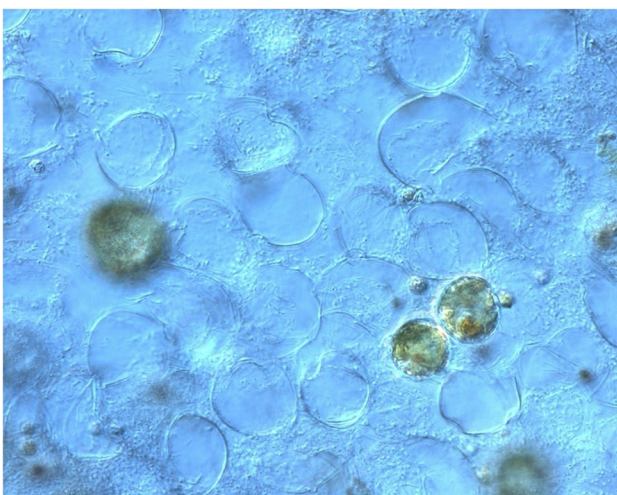


Fig. 8 Many empty thecae were found in control beakers on the third day. Observation through inverted microscope

phytoplankton. We used cages of plankton screen inserted in a larger volume of circulating medium to avoid confinement in a small volume of medium while still enabling the study of dense cultures. The experiment was inoculated with high densities of cells in the exponential vegetative growth stage, but exponential growth of cultures was not seen, nor lag phase or stationary phase. A high cell density simultaneously with access to fresh growth medium accelerated gamete formation rather than suppressing it. The results from the present experiment show two very important aspects of dinoflagellate growth:

- 1) Dinoflagellates could grow faster and into a ten times denser culture with flow-cell culturing (confinement quickly restricts growth)
- 2) Dense cell accumulations triggered induction of the sexual phase without nutrient limitation, suggestive of “quorum sensing” in dinoflagellates

The importance of cell proximity

Very high cell densities were reached with flow cell culturing, the highest measurement representing 342,000 cells mL⁻¹. Thus, *S. lachrymosa* cells can grow very dense, but only when the medium is changed. The cells became much more numerous when they escaped outside the cage, which means that escaping cells grew faster than cells still inside the cage. Both cell density (closeness to other cells) and availability of nutrients/flow is apparently important. Cages with few escaping cells had fewer cells in total, even though the flow was fast and nutrients in the added medium not limiting. Was the medium the same or different outside the cage? The results indicate that it was probably different because of fast changes in nutrient uptake and/or waste disposal causing the environment to become different in the high cell density environment inside cages despite the fast flow of the medium. Cell–cell proximity (quorum sensing) was very important; it induced sexuality and inhibited

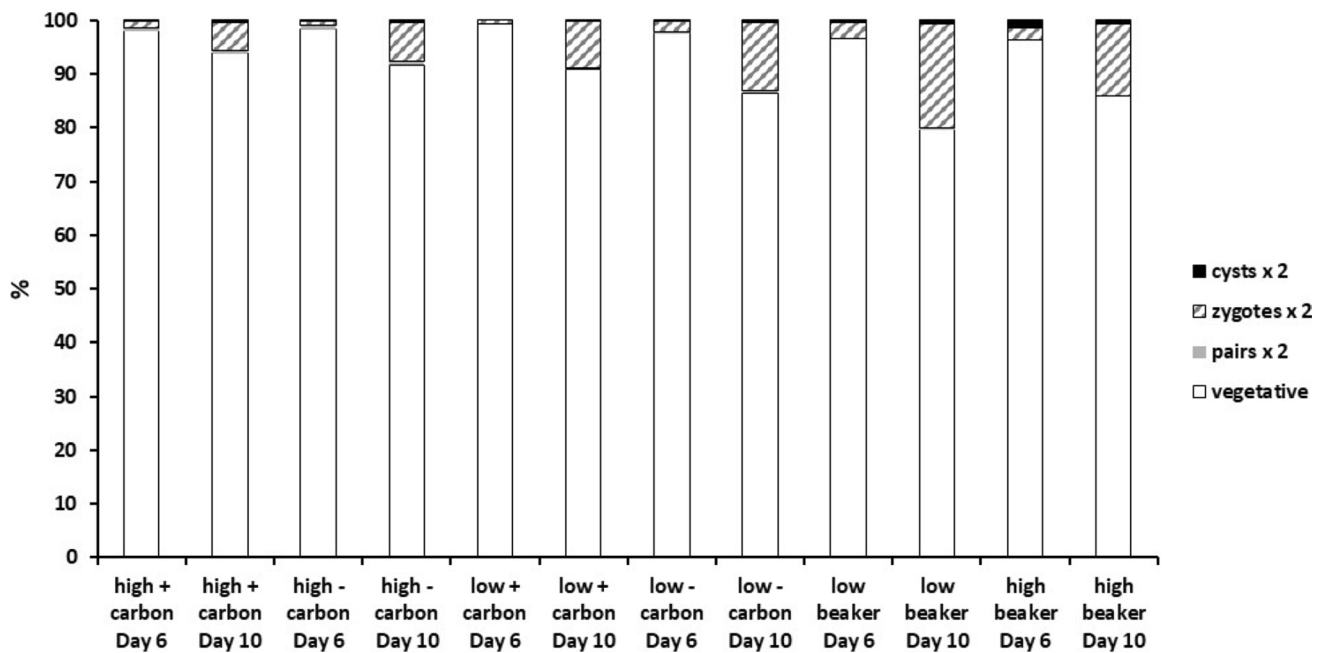


Fig. 9 The proportion of mating pairs, zygotes, and cysts increased towards the end of the experiment in all treatments. Zygotes, pairs and resting cysts were counted as two since they each consist of two cells

continued vegetative growth. Because of the low Reynolds numbers, each cell is surrounded by a boundary layer of water (Breckels et al. 2010 and references therein) in which exudates have a higher concentration and nutrients are of lower levels than in the “free” water, and close proximity between cells has disadvantages at the same time as it is necessary for enabling gamete fusion. Carbon filters are used for cleaning STX from cyanobacteria in freshwater in Australia (e.g. Falconer et al. 1989; Orr et al. 2004). We hypothesized that circulation through a filter with activated carbon could remove inhibitory substances and enhance growth, but this was not confirmed the present experiment. No significant differences could be found between treatments with and without carbon filter.

Growth phases and swimming behavior

Cultures did not reach stationary phase even though cell densities were very high, and we do not know where maximum cell density could be with the continued addition of nutrients (exchange of medium). The sexual phase was clearly initiated and the possibility of continued growth into even denser cultures remains unexplored. Experiments were also performed in the same way with *A. catanella* during methods development for the present experiment, but the high cell densities attained (up to 250,000 cells mL⁻¹) caused fear of exposure to high toxin levels and were discontinued. *A. catanella* demonstrated so sharp and fast pattern formation that mixing of samples by

hand stirring (with a glass rod or pipette tip) into an even distribution of cells was not possible. Cells, when pattern forming, used every water movement as an opportunity to assemble and formed patterns of accumulation. This behavior is probably very important in nature; the cells ‘use’ water movements for accumulation and transport. This behavior can, together with oceanographic factors such as stratification and movement of water masses, explain bloom formation. The pattern forming behavior observed macroscopically, occurred (in this experiment and all our previous, published and unpublished experiments) simultaneously with gamete formation as observed microscopically (described for *S. lachrymosa* in Smith and Persson 2005, and for *A. fundyense* (now *A. catanella*) in Persson et al. 2013 and Persson and Smith 2013).

Swimming together

The induction of gamete formation by bringing cells close to each other without nutrient limitation, shown here, suggests “quorum sensing” in dinoflagellates. The term quorum sensing is most often used to describe a form of cell-to-cell communication by which bacteria (by secreting signalling molecules) can regulate cell density- or growth-phase-dependent processes (e.g., Portugal 2013). When cells form patterns, a very high cell density is achieved within the pattern itself. Perhaps access to fresh medium at the same time as high cell numbers is something that occurs in nature but has never before been studied in the

laboratory. We speculate that quorum sensing substances are formed rapidly and diffuse rapidly. Enclosed in a small container, these substances remain within the enclosure. The bloom-forming dinoflagellates we studied can swim and in nature stay in a gathered assembly of cells by swimming together, and substances produced diffuse away in the vast water mass. A high concentration of substances needed to attract co-specific gametes and deter predators and competitors requires continuous regeneration to counteract diffusion. This can lead to very high levels of such substances in a "batch culture", which in turn may explain the limitation in cell density (i.e., through auto-inhibition). Alternations between asexual growth and sexual reproduction are widespread in nature and very common in phytoplankton (Fryxell 1983). Induction of sexual behavior is often triggered by environmental cues when, or slightly before, environmental conditions deteriorate for the asexually dividing stages. In dinoflagellates, it is known that N-limitation alone can trigger gamete formation in the laboratory (e.g., Persson et al. 2008 and references therein). In nature, however, it is reasonable to assume that several signals together (light, temperature, stratification, nutrients from land run-off, etc.) make up a "seasonal cue" (Persson et al. 2008; Figueroa et al. 2011). Optimal timing of sexual reproduction is important in nature as sexual reproduction instead of asexual means major changes in metabolism and behavior that must be consistent with seasonal weather patterns that for dinoflagellates can be, for example, rain with land-runoff and stratification and/or water movements allowing accumulation and transport. Accumulating evidence points to "blooms", e.g., densely accumulated cells, as consisting of sexual stages forming patterns of accumulation involving chemical attraction (Persson and Smith 2013; Persson et al. 2013; Wyatt and Zingone 2014; Brosnahan et al. 2015), and these cell accumulations being transported by hydrodynamic action into even denser patches or layers. It is well documented that high numbers of dinoflagellates can be found in thin layers during calm weather after rainfall with land runoff when water mass is density-stratified (e.g. Smayda 1997). Such water masses can subsequently be transported to the surface driven by shifts in meteorological forcing variables (Raine 2014). Surface blooms often form when wind causes upwelling and transport of water masses containing accumulated cells with currents into nearshore areas (Anderson et al. 2012; Ruiz-de la Torre et al. 2013; Lai and Yin 2014; McGillicuddy et al. 2014; Raine 2014). The cells in a bloom are thus accumulated by swimming behaviors and physical transport by larger forces in nature, and the cells in a sample from a bloom can originate from (have grown in) a much larger volume of water (and elsewhere) than the volume of the sample taken.

Environment determines growth

A comparison between cultures started with high or low cell numbers of *Scippsiella lachrymosa* shows clearly that the environment (availability of nutrients and elimination of waste substances) is much more important than the starting number of cells. Also, in the field the same phenomenon occurs; the number of germinated cysts or starting cell number is far less important than growth conditions in the water (Martin et al. 2014). It is difficult to estimate the size of a background population that is thinly distributed in a large area and volume in the field. If conditions are favorable for growth, there is a possibility that growth can continue undiscovered until a seasonal signal induces sexuality and accumulation of cells, which are further concentrated by cell behavior and weather patterns as described above. A seemingly low-nutrient or low background population situation can thus still lead to dense dinoflagellate blooms, depending on local weather patterns and transport. Can growth rate in nature be vastly underestimated from lab experiments because of limitations set by culturing conditions? Our results show declines in growth (decreasing growth rate), even though the medium was exchanged and nutrients not limiting. When studying growth curves in published dinoflagellate experiments one can see that the exponential growth phase is very short. An extrapolation of the exponential phase imagining a period of optimum conditions answers our question. The growth rate in nature during optimum conditions can probably be much higher than previously thought. This is also suggested by Brosnahan et al. (2015) and Anderson et al. (2012).

Mass culturing of bloom-forming dinoflagellates – not an easy task

Many have wished to mass culture dinoflagellates to enable extraction of toxins and other valuable substances in large scale. The small heterotrophic dinoflagellate *Cryptocodinium cohnii* is cultured in industrial scale for the extraction of docosahexaenoic acid (DHA), an omega-3 fatty acid, for infant formula (Mendes et al. 2009). The vast majority of dinoflagellates are, however, impossible to grow in large scale even though they are known to cause dense blooms. An understanding of the causes underlying dinoflagellate blooms in nature is necessary. Dinoflagellate blooms do not represent cells that have grown where they are found in large amounts. Underlying factors are, as discussed above, seasonal accumulation (possibly by sexual life stages) in water masses that are secondarily transported by upwelling and currents into the areas where blooms eventually are found.

Conclusions

Scrippsiella lachrymosa could grow to a cell number more than ten times higher than normal using the flow-through cage culturing system described here. At high cell densities of this resting cyst-producing species, sexuality was induced and gametes formed. Cells accumulated in patterns that seen microscopically consisted of contact seeking gametes. The mating gamete pairs formed zygotes and resting cysts.

“Flow-cell” culturing can be improved and used for further studies of dinoflagellate cells in high cell density without nutrient limitation. Pattern formation at high cell densities could be studied, as well as gamete behavior, but flow-cell culturing is not suggested as an economically viable method for mass culturing of dinoflagellates without modification. Vegetative growth was limited by high cell numbers despite non-limiting nutrient conditions. The maximum growth rate of dinoflagellates in nature is likely vastly underestimated from laboratory experiments due to the limitations set by the necessity of enclosures.

Finding many dinoflagellates in the same place (bloom in layer or patches) should not lead to the conclusion that the cells have grown where they are found or that they thrive that densely within the volume they occupy at bloom conditions. Dense accumulations of dinoflagellates may be more connected to seasonal sexual reproduction than to vegetative growth; they are not passive victims of their surroundings but use water movements for their accumulation, reproduction, and transport.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10811-023-02910-7>.

Authors' contributions All authors contributed to the study conception and design. Material preparation and data collection were performed by all authors, and analysis was performed by Agneta Persson. The first draft of the manuscript was written by Agneta Persson and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. We are grateful to Dr. Shannon Meseck, NOAA/NMFS Milford Laboratory for nutrient analyses, to Karl Persson for help with illustration and to Dr. Gary H. Wikfors for manuscript advice. We also thank the reviewers for in-depth constructive advice. Mention of trade names does not imply endorsement.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors have no competing interests to declare that are relevant to the content of this article. Mention of trade names does not imply endorsement.

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