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The time for sex: A biennial life cycle in a marine planktonic diatom

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Abstract

Whereas it is accepted that the population dynamics of higher organisms is strongly grounded in life history processes, such as the time taken to mature and reproduce, regulation in the abundance of aquatic protists is generally attributed to proximate properties of the environment in which they grow. We used 10 yr of data from the Gulf of Naples to determine the life history of a planktonic diatom, *Pseudo-nitzschia multistriata*, through the analysis of cell abundances and cell size patterns. Asexual and sexual phases recurred with remarkable regularity. Maximum abundance occurred annually, between the end of summer and the beginning of autumn, but cohorts of large cells, derived from sexual events, were found only every second year. We developed a model of population growth, based on parameters determined from laboratory cultures, with further tuning using information from natural populations. The model simulated the birth, maturation, and disappearance of age classes like those in natural populations, but only when we imposed seasonal variation in the rate of cell division and timed sexual reproduction during the stationary phase of a bloom. The model predicts that *P. multistriata* will become locally extinct if sexual reproduction does not occur within 4 yr. Our data and model show that coherent life cycle properties can emerge in natural populations of unicellular organisms, analogous to those in multicellular organisms, as a result of finely tuned regulation of cell division and sexual competence.

Different life cycle strategies allow species to regulate their population dynamics, to cope with external constraints, and to perpetuate the genetic pool, in tune with the environment. Conceptual models of the population dynamics of multicellular organisms are strongly grounded in life cycle processes, which determine the spatial and temporal structure of natural populations (Caswell 2001). In these models, life history processes account for a strong tendency towards population synchrony in spite of the fact that environmental fluctuations can be both synchronic and chaotic (Benton et al. 2001; Bjørnstad and Grenfell 2001). Conversely, the dynamics of phytoplankton populations are generally thought to be driven by external factors, such as the availability of nutrients and light, or grazing (Reynolds 2006; Beninca et al. 2008). Nonetheless, complex life cycles have been described for several microalgae, including stages with different morphology, ploidy, and function (Montresor and Lewis 2006). The adaptive role of this complexity is far from being elucidated, but the multiphased character of their life histories is suggestive of a strong organization and a fine tuning with the environment (Matrai et al. 2005; Frada et al. 2008).

The complexity of the diatom life cycle has been well known since the beginning of the 19th century (Geitler 1932). As in the majority of unicellular microalgae, two main distinct but interconnected phases are recognized: a vegetative one, in which mitotic divisions lead to an increase in cell number, and a sexual one, in which meiosis and genetic recombination occur. Sexual reproduction has a major role in modulating the rate of adaptation (Colegrave 2002), pruning the genome from deleterious mutations (Kondrashov 1988), and coping with the evolution of parasites (Hamilton et al. 1990), which provides possible evolutionary explanations for its occurrence in diatoms, as in the vast majority of unicellular and multicellular lineages. The most distinctive property of the diatom life cycle is a progressive reduction in cell size during the vegetative phase, caused by the way diatom cells divide (Round et al. 1990). Although some species have evolved vegetative cell enlargement to escape extreme miniaturization, many diatoms restore their largest size only during the sexual phase (Fig. 1; Chepurnov et al. 2004). In these cases, sex is the only means to avoid death, in contrast to other protists, in which cells can keep on dividing asexually for an unlimited period. The aging of cohorts of large cells originating from sexual events can be tracked from the pace of cell size reduction, which can be used to estimate the rate of cell division. Therefore, cell size dynamics in diatoms is a reliable proxy of life cycle processes (Mann 1988; Jewson 1992) and allows the use of a demographic approach similar to that applied to higher organisms (Caswell 2001).

Our study concerns the marine planktonic diatom *Pseudo-nitzschia multistriata* (Takano) Takano, which is like most other diatoms in requiring sexual reproduction to regain maximum size (D'Alelio et al. 2009*a,b*). *Pseudo-nitzschia* species are needle-shaped, chain-forming diatoms widespread from polar to tropical latitudes and are significant contributors to phytoplankton blooms in coastal and oceanic waters (Hasle 2002). They thrive also in iron-poor regions, probably because of their capability to produce ferritin, a protein that stores iron inside the cell (Marchetti et al. 2009). The genus *Pseudo-nitzschia* has

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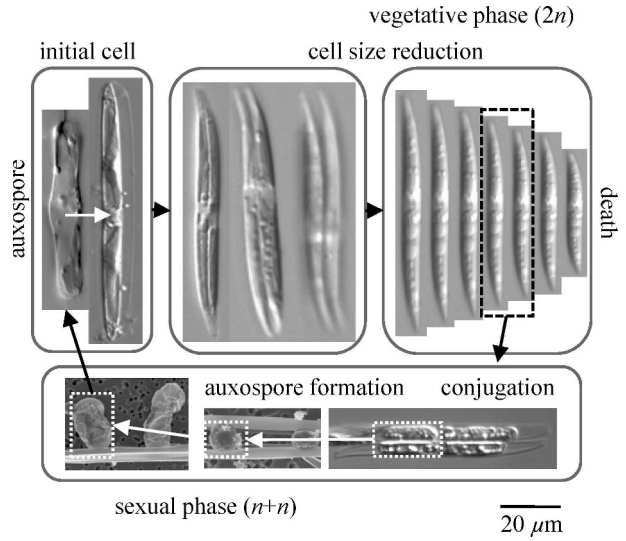

Fig. 1. The life cycle of the pennate diatom *P. multistriata* (modified from D'Alelio et al. 2009*b*). The peculiar structure and division modality of diatom cells imposes a progressive reduction in the apical axis (=cell length) in the population along its vegetative growth. Below the cell size threshold for gametogenesis (55 μm), gametangia differentiate, each generating two haploid gametes. Conjugation follows the pairing of gametangia of opposite mating type and produces two diploid zygotes. The zygote, now called an auxospore, elongates while depositing weakly silicified perizonial bands and hosts the formation of a long initial cell.

gained considerable attention over the last decade because some species can produce the neurotoxin domoic acid that causes amnesic shellfish poisoning (Fryxell and Hasle 2003).

We present the results of a decade-long demographical study carried out at the Long-Term Ecological Research station MareChiara (LTER-MC; Ribera d'Alcalà et al. 2004) in the Gulf of Naples, in which we integrated physiological and morphometric information to decipher the life cycle of the marine toxic diatom *P. multistriata* (Fig. 1). By using cell abundance and size, we tracked birth, aging, maturation, and sex in natural populations. We conclude that the biennial timing of sexual reproduction events and the predictable dynamics of the vegetative phase provide evidence for a coherent and periodic life cycle of this marine diatom.

Methods

Biological data—Water samples were collected weekly with Niskin bottles in the upper layer (1 m) of the water column at the LTER-MC station (40°48.5′N, 14°15′E; Ribera d'Alcalà et al. 2004), located 2 miles offshore in the Gulf of Naples (Tyrrhenian Sea, Mediterranean Sea) from 1996 to 2006. Additional samples were collected weekly with a net of 20-μm mesh size in 2005–2006. For *P. multistriata* cell counts and measurements, samples were fixed with neutralized formaldehyde solution (1.6% final concentration) and stored at 4°C. Enumerations were performed with an inverted microscope at 400× magnification, after sedimentation of variable volumes of seawater (1–100 mL), depending on cell concentration (Utermöhl 1958). The apical length of 200 cells was measured for each sample in which *P. multistriata* was present using a Zeiss Axiovert microscope at a magnification of 400×, approximating the measurements to the minimum unit of the micrometric ocular, 2.5 μm. A lower number of cells were measured only in a few samples collected during non-bloom periods, when cell abundances were extremely low. Cell size reduction in *P. multistriata* occurs mainly along the apical axis, and we thus used cell length as a proxy for cell size. Cell size frequency distributions were analyzed with the open source program FisatII (http://www.fao.org/fi/statist/fisoft/fisat/index.htm). The Lomb-Scargle periodogram (Press and Rybicki 1989) was used to detect periodic variability in three distinct time series: (1) the mean cell size from natural samples, (2) the mean cell size simulated by the model, and (3) the values extracted from the time series of the mean cell size simulated by the model, for the period of sampling of the natural populations. When two statistically separated subpopulations occurred in the same sample, the highest mean cell size value was used for the analysis of periodic variability. The significance of periodogram peaks and associated frequencies was tested with the modified Scargle method under the null hypothesis that a given peak is random (Glynn et al. 2006).

The growth rate (*gr*) of natural populations was calculated by scaling the reduction rates of the mean cell size between two sampling intervals with the cell size reduction rates observed in cultured strains and expressed as μm × division^{-1} (D'Alelio et al. 2009*b*). Cell size reduction (*r*) was calculated as $r = [mean\ size(time_1) - mean\ size(time_2)] \times (time_2 - time_1)^{-1}$. The division rate (*D*, division × d^{-1}) was calculated as $D = (r \times R^{-1})$, where *R* (μm × division^{-1}) is the rate of cell size decrease associated to each cell division as estimated in cultured strains. The absolute growth rate (*gr*, d^{-1}) was calculated as $gr = D \times 0.6931$, where the constant number is a factor of conversion between the unit "divisions d^{-1}" and d^{-1}.

Model implementation—The life cycle model is a matrix model in which growth, cell size reduction, and mortality rates are parameterized for each size class based on observations made in culture (D'Alelio et al. 2009*b*) unless otherwise specified. The sensitivity of the model was tested by changing parameters and comparing the actual simulations with the real data. In this paper we illustrate simulations only from the final model and justify our choice of input parameters.

The parameters used in the model (Table 1) are described in the following. (1) Size of the initial cells (*Init size*): We used a fixed value of 80 μm, which was among the highest values observed in mating experiments. (2) Size of the smallest cells (*Small size*): We used a fixed value of 30 μm, below which cell death in culture was generally observed. (3) Parameterized growth rate (*Pgr*): The growth rate (d^{-1}) of each cell of size *s* was parameterized as $Pgr = k \times gr$, with *gr* being the range of growth capability of the population during bloom or non-bloom phases and *k* being

Table 1. Life cycle parameters used to implement the model, the abbreviation used in the text, and the literature reference.*

	Parameter	Abbreviation	Value or formula	Data source
1	Size of the initial cells	*Init size*	=80 μm	D'Alelio et al. 2009*b*
2	Size of the smallest cells	*Small size*	=30 μm	D'Alelio et al. 2009*b*
3	Parameterized growth rate	*Pgr*	$=gr \times (0.25 + 0.04 \times s - 0.0005 \times s^2)$; *gr* ranging from 0.07 to 1.40 d^{-1}, *s* (cell size) ranging from 30 to 80 μm	Amato et al. 2005; D'Alelio et al. 2009*b*
4	Parameterized death rate	*Pdr*	$=dr \times (0.4 + 0.04 \times s - 0.0005 \times s^2)$; *dr* ranging from 0.05 to 1.00 d^{-1}, *s* (cell size) ranging from 30 to 80 μm	This paper
5	Rate of cell size decrease	*Red*	$=0.0000001 \times s^{2.5}$	D'Alelio et al. 2009*b*
6	Gametangia threshold size	*Sex size*	=55 μm	D'Alelio et al. 2009*b*
7	Probability of forming gametes	*Sex prob*	=2%	D'Alelio et al. 2009*b*; this paper
8	Timing of sexual reproduction	*Sex time*	Continuous, annual, biennial, multiannual	This paper

* *gr*, growth rate; *dr*, death rate.

the growth rate variation of *P. multistriata* with cell size described by the polynomial equation $k = 0.25 + 0.04 \times s - 0.0005 \times s^2$, where *s* is the cell size class. This trend was inferred from experimental data on *P. multistriata* (D'Alelio et al. 2009*b*) and *Pseudo-nitzschia delicatissima* (Amato et al. 2005). The dataset for the latter species was more complete and was hence used to find the best fit for the equation for *k*. According to the final formulation of the equation, the growth rate was very low in the longer cells and gradually (linearly) increased until 60% of the specific cell length was reached. The parameter *gr* varied in the course of the simulations, reproducing the alternation of periods of fast and slow growth, which were inferred from the rate of decrease of cell size in natural samples. The values for *gr* used in the final version of the model (i.e., the one that definitely matched the environmental data) were 0.69–1.40 d^{-1}, corresponding to 1.0–2.0 divisions d^{-1}, during the bloom exponential phase, and 0.07–0.20 d^{-1}, corresponding to 0.1–0.3 divisions d^{-1}, during the stationary and non-bloom phases. Higher growth rates (up to 2.5 divisions d^{-1}) observed by D'Alelio et al. (2009*b*) were also tested in the simulations, but they did not allow reproduction of the natural patterns. A sudden collapse in growth rate was imposed for cells smaller than 40 μm, because they were approaching the smallest size, 30 μm, at which growth rate was zero. In the course of model implementation, we also used other equations to model the relation between growth rate and cell size (i.e., linear positive and negative, constant relationship) but the results did not match the natural patterns. (4) Parameterized death rate (*Pdr*): The mortality factor (d^{-1}) was calculated as $Pdr = k' \times dr$, where k' is the size-dependent mortality, $k' = 0.4 + 0.04 \times s - 0.0005 \times s^2$, with *s* being the cell size and *dr* the death rate. In the final version of the model we computed *Pdr* using an equation similar to the one used for *Pgr*. The polynomial equation used for both rates was in the form of $k = a + b \times cell\ size - c \times cell\ size^2$. The constant values *b* and *c* were the same in both rates, whereas *a* was slightly higher in the *Pdr* equation (0.4 vs. 0.25), thus generating a death rate lower than the growth rate, in order to avoid the population's going extinct in the model simulation. Values for *dr* varied in parallel with *gr* over the simulation, ranging from 0.05 to 1 d^{-1} (i.e., they were always below 50% of *gr*). To prevent an exponential increase of cell abundance and to reduce the simulation time, some cells were randomly removed at each time step (corresponding to 1 d), keeping the total number of cells always below a preassigned threshold of 10,000. Simulations with *dr* constant for all cell sizes failed to reproduce the observed cell size pattern. (5) Cell size reduction $\times$ generation^{-1} (*Red*): The rate of cell size (*s*) decrease, expressed as $\mu m \times division^{-1}$, was provided by the equation $Red = 0.0000001 \times s^{2.5}$. The equation takes into account that in *P. multistriata* cell size reduction at each mitotic division decreased with cell size. Using other equations to model the relation between cell size reduction and cell size (i.e., linear positive and negative, or constant relationship), the natural pattern was not reproduced. (6) Gametangia threshold size (*Sex size*): We used 55 μm as the cell size threshold for sexual reproduction, because it was the maximum length for gametangia in culture conditions. With a threshold size higher than 55 μm, the model failed to reproduce the natural pattern. (7) Probability of forming gametes (*Sex prob*): The proportion of cells below the fertile cell size threshold (55 μm) turning into gametangia was not more than 2%, which corresponds to the maximum production rate of sexual stages in mating experiments performed in culture. With higher percentages of gamete formation, the output of the model did not fit the real pattern. In the model, the success of auxospore development was considered to be 100%, i.e., all auxospores developed into initial cells. The process was also set to occur instantaneously, though it takes 2–3 d in culture (D'Alelio et al 2009*b*). (8) Timing of sexual reproduction (*Sex time*): The model was run using different timings of sexual reproduction: continuous (sexual reproduction always possible within *Sex size*), annual, biennial, and multiannual (sexual reproduction limited to the bloom period every year, in alternate years, or with longer periodicity, respectively). Model simulations were run with sex occurring at the bloom exponential or stationary phases.

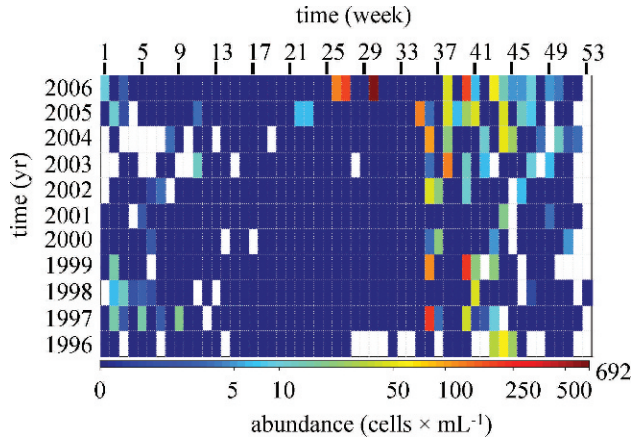

Fig. 2. The annual timing of the *P. multistriata* bloom at LTER-MC. Logarithmically transformed abundance plot of *P. multistriata* over the period 1996–2006. White squares indicate no sampling.

The model reproduces the development of cell cohorts of different sizes over time. At each time step, a cell can either divide (at a rate set by *Pgr*) or die (at a rate set by *Pdr*). Once a cell divides, two new cells are produced, one of the same size and the other of reduced size according to *Red*. Among cells smaller than the size threshold for gametogenesis (*Sex size*), a number of pairs are randomly selected according to the parameter *Sex prob*, and two cells of maximum size are produced from each pair. Sexual reproduction occurs only within the temporal window for sex imposed by the parameter *Sex time*. Every 10 time steps (=every 10 d) the model provides a discrete size spectrum having a size step of 2.5 μm (corresponding to the resolution of the measurements, *see* above).

Results

P. multistriata was found in surface samples collected between 1996 and 2006 in the Gulf of Naples throughout the year, with the exception of the periods March–May and from the end of July to the end of August. Cell abundance ranged from undetectable to 700 cells mL^{-1} (July 2006), with long-lasting non-bloom periods and short blooms generally occurring somewhere between the end of August and the end of October (Figs. 2, 3a).

Cell sizes ranged from 75 to 30 μm. Large-sized cells, pinpointing the occurrence of sex, appeared every 2 yr and decreased in size over the following period (Fig. 3b). There was an alternation in successive years between bi- and unimodal size distributions (Fig. 3b). The maximum cell size—i.e., the upper extreme of the cell size distribution—showed a biennial oscillation, with phases of reduction and subsequent restoration occurring every 2 yr.

Intensified sampling in 2005–2006 allowed detection and measurement of *P. multistriata* cells during non-bloom periods over these 2 yr (Fig. 3b). Over those 2 yr, size reduction was slow and gradual over the non-bloom phase, whereas it increased during the bloom. Rates calculated on the basis of observed reductions in cell size were not constant over the annual cycle. Its values calculated based on the observed cell size reduction were low over most of the year (non-bloom phase; mean growth rate = 0.3 ± 0.2 divisions d^{-1}, n = 6). Division rates increased steeply to 2.6 divisions d^{-1} in late summer until the annual peak was reached (bloom exponential phase; mean ± standard deviation = 1.1 ± 0.6 divisions d^{-1}, n = 11), and suddenly decreased afterwards while cell concentrations were still high (bloom stationary phase).

Given estimates of key life cycle processes obtained from cultured strains (threshold cell size for sexual reproduction, size range of initial cells, cell size reduction rate over the species size range; D'Alelio et al. 2009*b*), the field data can be interpreted as a biennial sequence, which can be summarized as follows (Fig. 4): (1) vegetative cells belonging to a population with a unimodal cell size produce the bloom in late summer; (2) during the bloom, a small fraction of the population below the threshold size for sex (55 μm) differentiates into gametes, which eventually conjugate and form auxospores; (3) the auxospores give birth to long initial cells (72–82 μm), rarely detected in natural samples; (4) over the long (8–10 months) subsequent non-bloom season, the young large cells divide at a very low pace along with the old generation, both nevertheless continuing to decrease in cell size; (5) during the bloom phase of the next year, both the new-generation cells, now in the range between 55 and 65 μm, and the old population with a smaller size enhance their growth rates; (6) over the next long non-bloom phase, the cohort with a larger cell size proceeds towards the next bloom period (phase 1), while the older cohort keeps on reducing in cell size while dividing and eventually fades away.

We tested this conceptual scheme with the individual-based model described in the methods. The model was initialized with one cell of the maximum size and run for five annual cycles with different combinations of the following parameters: growth rates constantly high, low, or variable over the annual cycle, occurrence of sex during the exponential or stationary phase, and bloom timing of sexual events (continuous, annual, biennial, or multi-annual). The model only fitted real data, producing an alternation of uni- and bimodal cell size distributions, when (1) simulation started with one maximum size cell at the end of the bloom, (2) cell growth rate varied through the year, and (3) biennial sexual reproduction was assumed to take place during the bloom stationary phase (Fig. 5a). Using this combination of life cycle parameters, we also simulated the dynamics of cell size over an 11-yr period after initializing the model with the cell size distribution detected at the beginning of the time series (22 October 1996). The model output matched natural size spectra well and reproduced the 10-yr sequence of seven cohorts of cells (Fig. 3c). Periodograms for (1) the mean cell size from natural samples, (2) the mean cell size simulated by the model, and (3) values extracted from the mean cell size simulated by the model for the period of the actual sampling showed periods of oscillation of 110.7, 103.3, and 107.7 weeks, respectively, which do not differ significantly from 2 yr ($p < 0.005$).

There were two occasions in which the model failed to reproduce the real data: the average cell sizes of the smaller

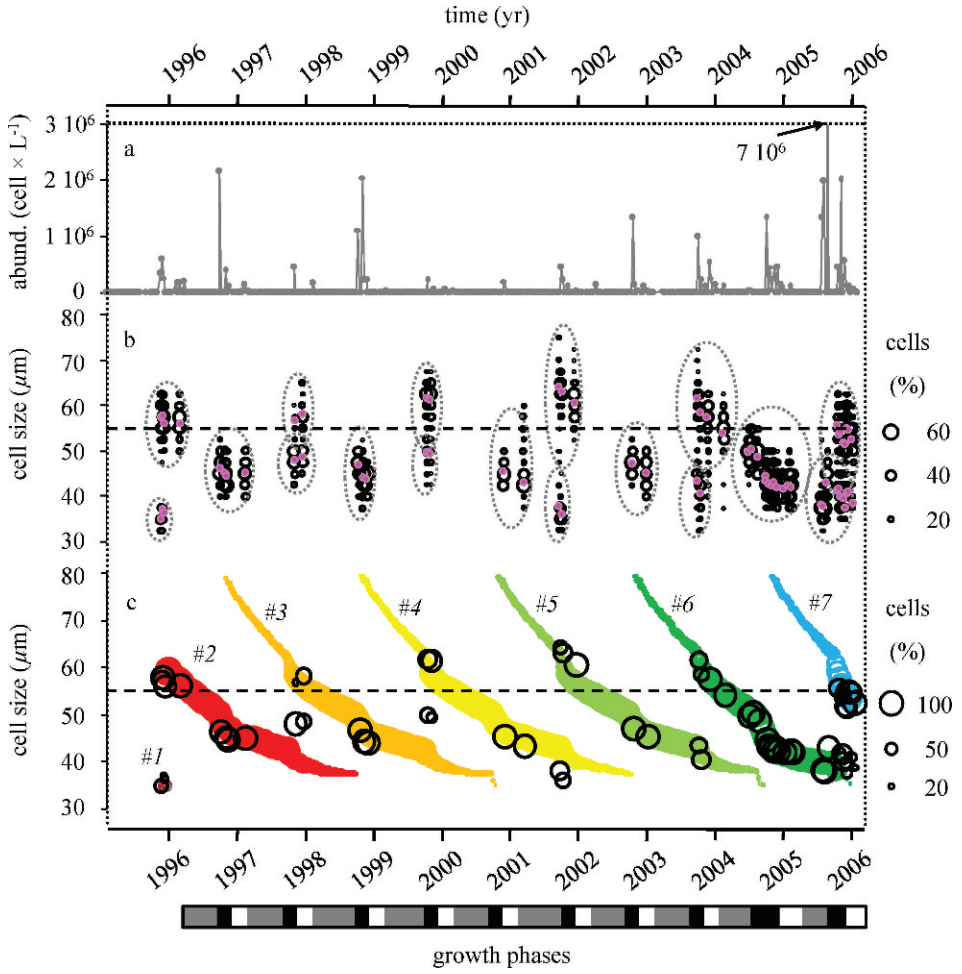

Fig. 3. Cell size dynamics of *P. multistriata* (1996–2006). (a) Cell abundance. (b) Cell size distribution: grey dashed ovals delimit the cell size sub-distributions; black circles represent the cell percentage within each size class; pink dots represent the mean cell size for each sub-distribution; the horizontal dashed line indicates the size threshold for gametogenesis. (c) Comparison between the mean natural population cell size (black circles) and the simulation output (colored circles); the horizontal dashed line indicates the size threshold for gametogenesis. The bar below the graphs represents different growth phases: grey = non-bloom phase, black = bloom exponential phase, and white = stationary phase.

virtual populations No. 2 and No. 3 were smaller than the natural ones in 1998 and 2000 respectively. This mismatch could be due either to a much lower growth rate, and consequently a slower cell size reduction in the field populations as compared to the model prediction, or to the possible formation of initial cells of two different sizes in the preceding year.

Alternative scenarios of life cycle organization were incompatible with the pattern in cell size observed in the field. Sex occurring during every annual bloom (Fig. 5b) or unconstrained in time (Fig. 5c) produced patterns in cell size different from those observed in the natural environment. Constant growth rates coupled with biennial sex also failed to reproduce the observed patterns (Fig. 5d–f). In addition, the model predicted that the species would become locally extinct if sex did not occur within 4 yr.

Discussion

Natural populations of *P. multistriata* collected at LTER-MC in the Gulf of Naples over 11 yr showed surprisingly coherent and predictable dynamics in terms of the succession and timing of different life cycle phases. Our results strongly suggest that the local populations synchronously undergo cycles of low and high growth rates, responsible for biomass increase and cell size reduction, and sexual events, which allow for the restoration of the cell size. The planktonic environment is highly unstable and unicellular organisms living therein are dispersed and drift with the water masses. In this scenario, a recurrent temporal pattern of life cycle phases is remarkable, and even more so at our study site, which is located in a boundary area under the influence of contrasting hydrodynamic and trophic regimes (Marino et al. 1984). Because

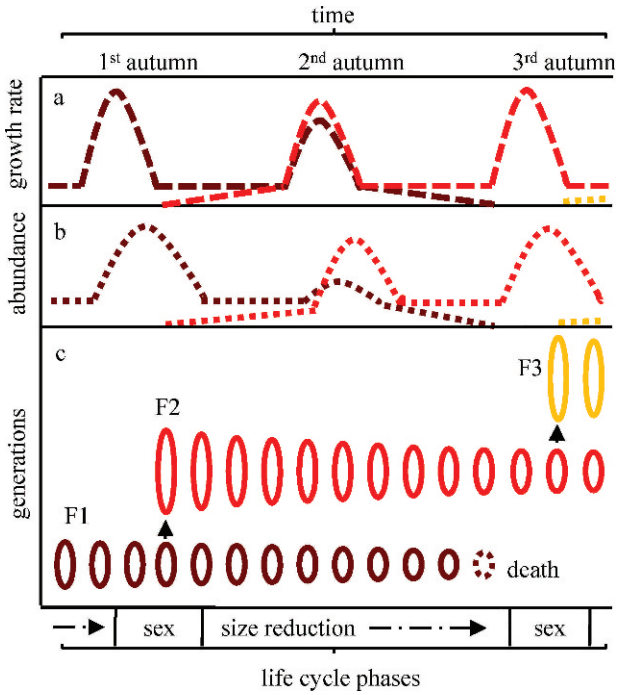

Fig. 4. Conceptual model of population dynamics in *P. multistriata*. (a, b) Non-dimensional schemes for the annual oscillation in the species growth rate and abundance, respectively, as derived from field observations. (c) Schematic view of the alternation of different subpopulations or generations, where each oval represents a cell. In the three panels, brown refers to the oldest generation (F1), which undergoes sex at the end of the first autumnal bloom in the scheme, then shows slight size reduction during the following bloom and reaches death without reproducing sexually again; red refers to the F2 generation, which arises from sex performed by F1, undergoes marked size reduction, flanks F1 in the second bloom, and finally undergoes sex in the third autumn, giving rise to the F3 generation (yellow).

it is unlikely that we were tracking the cell size evolution of single clones over time, the traceable succession of distinct cohorts can be explained only assuming that *P. multistriata* populations were synchronized over a wide area.

We explain the interannual oscillation between bimodal and unimodal cell size distributions in *P. multistriata* as caused by a biennial periodicity of sex. In theory, a similar pattern could be due to the existence of cryptic species with different cell sizes in the same area. However, genetic analyses coupled with mating experiments showed that the populations sampled at LTER-MC over time belong to a single interbreeding species (D'Alelio et al. 2009*a*). Moreover, the progressive cell size reduction of natural populations observed during the last 2 yr of our study closely matches the cell size reduction observed in all clonal strains of *P. multistriata* examined in laboratory experiments (D'Alelio et al. 2009*b*) and can only be explained by assuming the existence of a single species. Cells of the maximum size have been obtained in the laboratory when crossing strains of opposite mating types, because the species is heterothallic.

Life cycle and population dynamics—The duration of the life cycle can be seen as the average time, or number of mitotic divisions, between the origin of a new generation of cells (produced by a single initial cell) and its loss through sexual reproduction. To date, a biennial life cycle has been hypothesized for two diatoms, the freshwater planktonic *Stephanodiscus* Ehrenberg sp. (Round 1982) and the marine epiphytic *Cocconeis scutellum* Ehrenberg (Mizuno and Okuda 1985), although in both cases the natural populations were followed for only a short period (1 and 2 yr, respectively). The biennial occurrence of sex was inferred from cell size reduction rates recoded in situ, which did not allow the production of a population of sexually inducible cells on an annual basis.

However, studies in which cell size distribution was monitored over time suggest that the length of the life cycle can be longer in other diatom species. D. G. Mann (1988) reanalyzed cell size data for the freshwater pennate diatom *Tabellaria fenestrata* (Lyngbye) Kützing in laminated sediments of the Zürichsee spanning over 27 yr and deduced that sexual reproduction would have to occur every 7–8 yr to account for the observed patterns. A similar timing (6 yr) was detected in a natural population of the benthic freshwater pennate *Nitzschia sigmoidea* (Nitzsch) W. Smith (Mann 1988) and the freshwater centric diatom *Aulacoseira subarctica* (O. F. Müller) E. Y. Haworth, which undergoes sex with a rhythm of 4–6 yr (Jewson 1992).

Unfortunately, we never detected *P. multistriata* auxospores in natural samples. Edlund and Stoermer (1997) attempted to group diatom auxosporulation in two main categories: "synchronous," limited to a short time interval and involving a variable proportion of the population, and "asynchronous," not constrained in time. In both cases, sexual reproduction can occur during periods of either strong or weak vegetative growth, i.e., at the peak or at the end of a bloom, respectively. According to this framework, *P. multistriata* auxosporulation is constrained to a short time period every 2 yr, most likely at the end of the bloom. In addition, this strategy should not allow massive auxosporulation, which has been associated mainly with synchronous sexual events occurring at the peak of large blooms (Edlund and Stoermer 1997).

Because diatoms are subject to progressive cell size reduction and have to reproduce sexually to restore maximal cell size, it is surprising that sexual stages are so rarely reported in nature. The few reports of auxospore production by planktonic diatoms in the natural environment (Crawford 1995; Edlund and Stoermer 1997; Assmy et al. 2006; Sarno et al. in press) suggest that this phenomenon could (1) occur at very low rates, (2) be extremely restricted in time, or (3) occur in layers of the water column that are extremely difficult to sample. Evidence for the first case is provided by the freshwater centric diatom *A. subarctica* (O. Müller) Haworth, in which only 0.16% of the whole population was involved in sexual reproduction (Jewson 1992), and by the oceanic pennate diatom *Fragilariopsis kerguelensis* (O'Meara) Hustedt, in which auxospores accounted only for 0.03–0.4% of the total cell number (Assmy et al. 2006). However, auxospores are also an ephemeral stage in the diatom life cycle—their

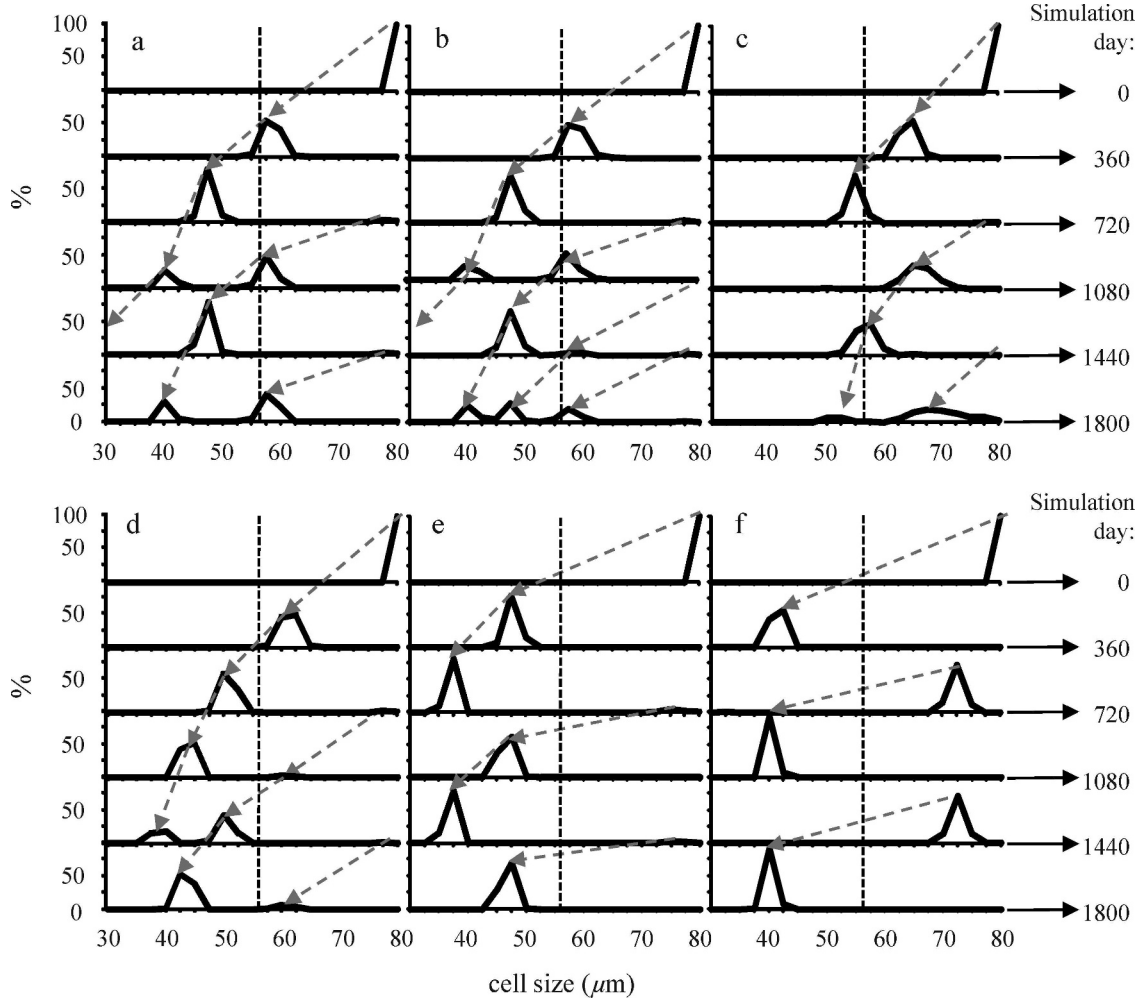

Fig. 5. Model outputs of cell size distribution in *P. multistriata* under different settings. (a–c) Growth rate modulated over the year (0.2, 1.0, and 0.2 divisions d^{-1} during the non-bloom, bloom exponential, and bloom stationary phases, respectively) and (a) biennial, (b) annual, and (c) continuous timing of sex. (d–f) Biennial timing of sex and growth rates constant over the year of (d) 0.2, (e) 0.6, and (f) 1.0 divisions d^{-1}. Arrows indicate cell size reduction trajectories; vertical dashed lines indicate the size threshold for gametogenesis.

persistence lasts for only a few days—and they could be produced asynchronically, thus making their detection in the natural environment extremely challenging. Over more than 20 yr of regular sampling at LTER-MC in the Gulf of Naples, only once have we detected major production of auxospores by planktonic diatoms (in this case by two different *Pseudo-nitzschia* species), and this was restricted to a single sampling (Sarno et al. in press). The finding that *Pseudo-nitzschia* species often aggregate in thin layers of physical discontinuity in the water column (Rines et al. 2002; Velo-Suarez et al. 2008) suggests that these peculiar environments might provide the ideal conditions for sexual reproduction to occur. In the case of *P. multistriata* we only have evidence of a regular appearance of cohorts of large cells over 2 yr. Cells in the larger size range, which are derived from auxospores, are not numerically abundant, and we therefore assume that auxosporulation in this species occurs at relatively low rates. According to our model, very low concentrations of initial cells, down to 0.05% of the parental population, are also enough to recruit new cohorts that become numerically comparable to the parental ones at the following bloom, notwithstanding death rates accounting for 50% of the growth rates (Fig. 3c).

In their seminal papers, W. M. Lewis, Jr. (1983, 1984), D. G. Mann (1988), and D. H. Jewson (1992) presented a conceptual framework for diatom life histories rooted in (1) supra-annual duration of the life cycle (2–40 yr), (2) long-term asexual reproduction, (3) low-frequency, short-term, and synchronic sexual phase, and (4) cell size reduction process acting as a chronometer for sex. This chronometer might regulate both annual and supra-annual sexual cycles, and would be particularly effective in species with sex occurring with a period longer than 1 yr, which is decoupled from any predictable environmental periodicity. In most of the diatoms studied so far, sex can only be induced within a species-specific cell size window (Geitler 1932; Chepurnov et al. 2004). However, cell size is not the only factor that allows sexual reproduction. Reasonably, a threshold cell concentration is required in order to allow

vegetative cells' encounter and subsequent gametogenesis. This requirement is met at the end of the exponential growth phase of the bloom, when cell concentration is at its maximum. Considering that gamete production implies the interruption of growth for a fraction of the population, the best solution to balance this "cost of sex" is to localize the sexual phase after the growth pulse responsible for the bloom, when cells are not actively dividing any longer (Lewis 1983). Based on these considerations, we can hypothesize that the biennial periodicity of sex in *P. multistriata* is because of the fact that only once every 2 yr a sufficient concentration of sexually mature cells occurs. This should happen when a single cohort of cells with a medium size—and thus in the size window for sex—was detected. Conversely, in the years when two cohorts were present, sex did not occur, possibly because the cohort with a largest size was not mature yet, whereas the one with the smallest size—though within the size window for sex—was constituted of weak cells close to death. We do not have direct evidence for our hypothesis; however, the production of allelopathic metabolites (Cembella 2003) or pheromone-like compounds (Haché 2000) only within a specific cell size interval might be responsible for providing aggregation and/or mutual coordination in diatom cells.

Ecological implications—The coherent pattern in *P. multistriata* population dynamics that we observed in nature over a decade, and modeled with the support of laboratory studies, reveals a fine-tuned organization of the life cycle. In multicellular organisms, the synchronization of growth, reproduction, and senescence phases is generally kept by entrainment to external cues showing annual or shorter periodicities (Schwartz 2003). On the other hand, in some cases of multiannual life cycle periodicities, such as reproduction of cicadas (Williams and Simon 1995) and flowering in bamboos (Janzen 1976), where the link to exogenous triggers is less clear, internal rather than environmental control is invoked as a matter of regulation. Our results also indicate that growth in *P. multistriata* is not constant along the annual cycle, with a brisk acceleration of growth rate during the bloom phase. This was shown by cell size variations over time and confirmed by the simulations, which reproduced the observed patterns only when a time-dependent growth rate was imposed. A similar modulation of growth was inferred for few freshwater planktonic diatoms by applying the same indirect approach (Mann 1988; Jewson 1992). The ephemeral nature of phytoplankton species blooms, normally interspersed with long periods of apparent absence from the water column, is often explained with the formation of resting stages that settle out of the photic layer, spending most of their life in the sediments (Marcus and Boero 1998). However, not all species produce benthic resting stages, and the seasonal dynamics can be the result of a different strategy, such as the annual modulation of growth rates discovered in *P. multistriata*. Interestingly, this species had its minimum growth in spring–early summer, which is a favorable period for a large part of the phytoplankton assemblage in the study area in terms of light, nutrients, and thermal stability (Ribera d'Alcalà et al. 2004), whereas in the laboratory it shows high growth rates under spring-simulating settings (D. D'Alelio unpubl.). This indicates that single-species seasonality may not be exclusively regulated by proximate factors such as light and nutrients, and that life cycle dynamics could play a major role in determining population periodicity, although we cannot exclude that other factors not considered in our studies, e.g., allelopathic interaction, competition, predators, and other mortality agents, can contribute to the observed seasonal pattern.

We have provided evidence for a coherent and periodic population dynamics in a planktonic diatom in the natural environment, comprising a rhythmic alternation of fast and slow growth phases, regularly interspersed with sexual events occurring on a biennial basis. The regularity of these processes reveals a strong tendency towards order in the pace of the life cycle of this diatom. Our findings also suggest that unicellular organisms are tuned to their environment through life cycle traits selected over evolutionary time scales and that their wax and wane is not merely the response to short-term variability of proximate factors.

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