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ECOLOGICAL PHYSIOLOGY  
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## Physiological Mechanism of Dinoflagellate Survival under a Biogenic Limitation

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**Abstract**—The physiological mechanism of the long-term survival of dinoflagellates under a biogenic limitation has been studied using the cultures of three common species from the Black Sea—*Prorocentrum cordatum*, *Prorocentrum micans*, and *Gyrodinium fissum*—as an example. The transfer of *P. cordatum* and *G. fissum* cells, which have a maximum intracellular pool of nutrients, to seawater depleted in nutrients decreases their specific growth rate and chlorophyll *a* content per cell. Due to the intracellular pool of nutrients, *P. cordatum* performs 3.3 cell divisions in 9 days and *G. fissum* performs 2.3 cell divisions in 4 days. After the exhaustion of the intracellular reserves of nutrients in the culture of *G. fissum* for 16 days, the growth of its cells ceases and the specific content of chlorophyll *a*, as well as the efficiency of the photosystem 2, decreases. In the *P. micans* culture, which is under the conditions of the most severe biogenic limitation throughout the 20-day experiment, against the background of an almost constant number of cells, an alternation of low positive and negative values of the specific growth rate is observed, as well as a low content of chlorophyll *a* per cell. However, the efficiency of photosystem 2 remains at a sufficiently high level (0.27–0.41) and exceeds the control values most of the time. This study reveals a high degree of heterogeneity of the functional activity of this species cells and the death of some of them under biogenic stress. The survival of *P. micans* under biogenic stress is probably ensured by the nutrients that enter the water during the death of the least viable part of its cells and serves as a source of organic matters for the physiologically active cells of microalgae.

**Keywords:** Black Sea, phytoplankton, dinoflagellates, biogenic limitation

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### INTRODUCTION

Dinoflagellates are considered one of the most important components of phytoplankton. In the marine environment, they account for a considerable—and occasionally larger—portion of primary production and biomass of phytoplankton and are a source of food for representatives of higher trophic levels. (Anderson et al., 2008; Thompson et al., 2008). “Blooms” caused by the high rate of growth of some dinoflagellate species are often harmful for hydrobionts and humans because they produce a wide range of toxic substances in the process of blooming (Yang et al., 2011).

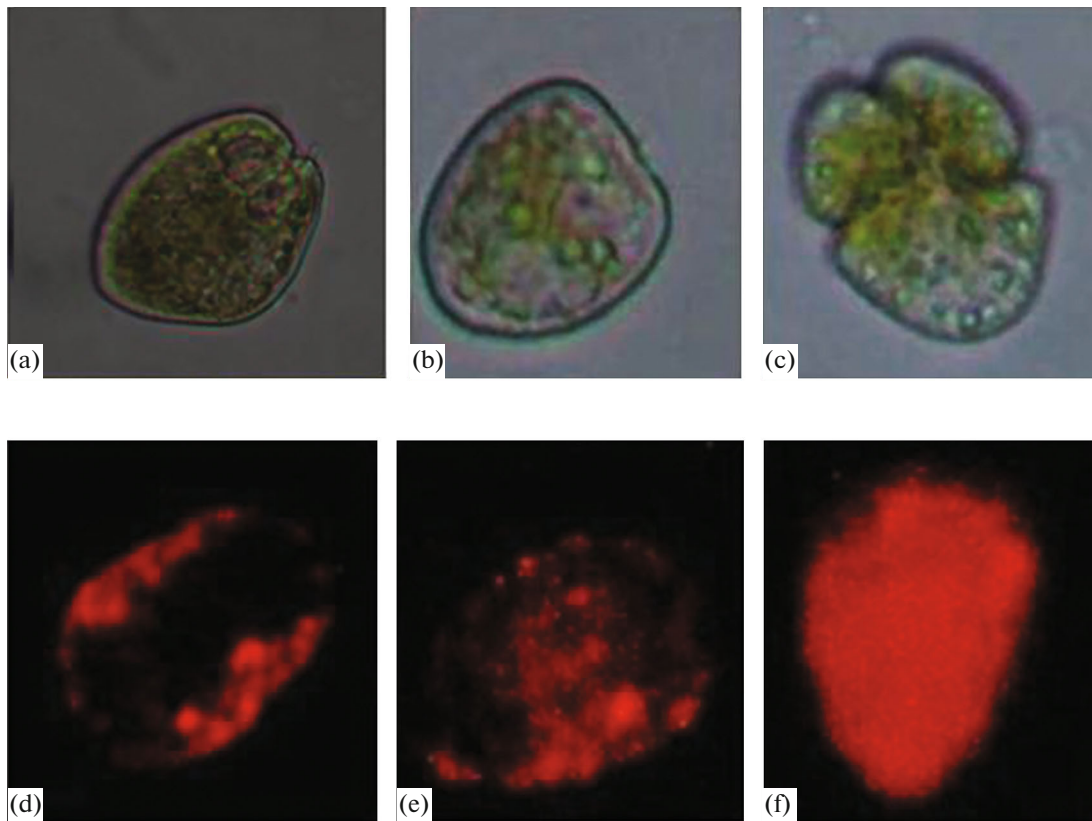
Over the past century, seawater temperature has increased by ~1°C on a global scale due to climate change (Häder and Gao, 2015), which has intensified the thermal stratification of the waters and weakened the upward flow of biogenic substances (Behrenfeld et al., 2006). Similarly, since 1991 onwards, a positive trend has been recorded in regard to a change in the

temperature of surface layer of the deepwater part of the Black Sea (Oguz and Glibert, 2007). The supply of biogenic substances from the deep waters to the photosynthetic zone continues to decrease gradually as a result of the strengthened thermal stratification of Black Sea waters during warm season (Mikaelyan et al., 2018; Pakhomova et al., 2014), while the content of these substances in the sea surface layers frequently drops to zero (Stelmakh and Gorbunova, 2019). Therefore, along with the representatives of other taxonomic groups of phytoplankton, dinoflagellates have to be adapted to survival under the conditions of biogenic limitation. Experiments with the involvement of cultures of particular marine microalgae species can serve as a methodological basis for the identification of adaptation mechanisms in phytoplankton to the deficiency of mineral nutrients.

The goal of this work is to study the survival mechanism of Black Sea dinoflagellates under conditions of a deficiency of biogenic substances in the water and cells.

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**Abbreviations:** Chl *a*—chlorophyll *a*; C/Chl *a*—ratio between organic carbon and Chl *a*;  $F_v/F_m$ —efficiency of photosystem 2;  $\mu$ —specific daily growth rate of culture.



**Fig. 1.** Cells of algae (a) *Prorocentrum micans*, (b) *P. cordatum*, and (c) *Gyrodinium fissum* under a light microscope; fluorescence of cells *Prorocentrum micans* (d) and (e) cultured under the conditions of biogenic limitation and (f) at high content of biogenic substances in the medium; magnification  $\times 400$ .

## MATERIALS AND METHODS

Research involved three unialgal cultures of dinoflagellates *Prorocentrum cordatum* (Ostenfeld) J.D. Dodge, *P. micans* Ehrenberg, and *Gyrodinium fissum* (Levander) Kofoid & Swezy isolated from the Black Sea plankton and reposit in the collection of the Department of Ecological Physiology of Algae in the Federal Research Center Institute of Biology of the Southern Sea (Figs. 1a–1c). These species are common representatives of Black Sea phytoplankton and grow well under conditions of culture. Initial mean cell volumes are  $1000 \pm 150 \mu\text{m}^3$  for *Prorocentrum cordatum*,  $5200 \pm 1300 \mu\text{m}^3$  for *P. micans*, and  $18900 \pm 3100 \mu\text{m}^3$  for *Gyrodinium fissum*.

Prior to the experiment, each culture was adapted for several days for a continuous light of  $70\text{--}80 \mu\text{E} (\text{m}^{-2} \text{s})$  intensity, which corresponds to light saturation by growth for the studied algal species (Mansurova, 2013). Control and experimental flasks 250 mL in volume were placed on an optical lattice and illuminated from underneath using light-emitting diodes. Illuminance was measured using a U-116 lux meter; the coefficient of conversion from illuminance in lux to light intensity is  $1000 \text{ lx} = 17 \mu\text{E} (\text{m}^{-2} \text{ s})$  (Parsons et al.,

1982). A water temperature of  $22\text{--}23^\circ\text{C}$  is optimal for dinoflagellates (Stelmakh et al., 2014).

Cultures of *G. fissum* and *Prorocentrum cordatum* were adapted for light conditions of the experiment for 3 to 8 days and were in exponential growth phase. Culture *P. micans* was adapted for continuous light for 12 days. By the end of this period, it was in the stationary phase, which was evidenced by the cessation of algal cell growth during the past 5 days of adaptation. Next, each species of algae was transferred to sterile seawater without being supplemented by additional biogenic substances (experiment) and with seawater with the addition of the f/2 culture medium (control) with a final concentration of nitrates of  $800 \mu\text{M}$  and phosphates of  $36 \mu\text{M}$  (Guillard and Rither, 1962). The seawater used in the study originally contained nitrates and phosphates of  $\leq 0.05 \mu\text{M}$ .

Throughout the experiment, aliquots from the flasks containing microalgae culture were taken daily to measure the cell number, their volume, chlorophyll *a* concentration,  $F_v/F_m$  (efficiency of photosystem 2), and red fluorescence intensity of chlorophyll *a*.

To determine the chlorophyll *a* concentration, triplicate 2–14 mL aliquots were taken from flasks of algae and precipitated on membrane Sartorius filters

with pores 0.8  $\mu\text{m}$  in diameter. The filters were placed in a 90%-acetone aqueous solution. The pigments were extracted for 12 h at 8°C. The chlorophyll *a* concentration was determined using a laboratory fluorometer, which was calibrated with a pure chlorophyll *a* (*Protocols for JGOFS*, 1996). The relative measurement error was less than 10%.

The cellular carbon (C) content in the dinoflagellates was calculated based on their volume from the equation reported in (Menden-Deuer and Lessard, 2000). The linear dimensions of cells were determined using a ZEISS Primo Star light microscope in 20 replicates at a total magnification of the system of  $\times 100$ . The volume of cells was estimated according to (Bryantsev et al., 2005) based on a principle of geometric similarity. The cell number was counted in the Goriaev chamber in three replicates. The coefficient variation of the mean largely remained within 2–15%.

Daily specific growth rate of culture was calculated based on an incremental growth of cell numbers in samples from the equation

$$\mu = \ln(N_t - N_0),$$

where  $\mu$  is specific growth rate of algae,  $\text{day}^{-1}$ , and  $N_0$  and  $N_t$  are the initial cell count and their quantity a day later.

The red fluorescence of chlorophyll *a* in cells of *P. micans* in the dark band was recorded using a Micromed 3LUM light microscope additionally equipped with a fluorescent block, containing a 100-watt mercury lamp, and a Toupcam UCMOS14000 KPA camera. The fluorescence excitation light in algal cells ranged from 500 to 550 nm, which was provided by green light filter. The feasibility of the use of the latter for exciting red chlorophyll *a* fluorescence in dinoflagellates was demonstrated in the paper (Stelmakh and Mansurova, 2019). The red radiance of the object in reflected light after passing through the barrier (emission) filter (590 nm) was observed in the band of 590–700 nm. The color and brightness characteristics of “light-emitting” cells in *P. micans* were determined using Adobe Photoshop, which had been employed earlier to measure the color characteristics of the Black Sea zooplankton (Litvinyuk, 2015). Each algal cell was analyzed separately. The entire light-emitting portion of a cell was isolated by a lasso tool; its color and brightness characteristics were averaged and measured. The averaging was done using the “average blur” filter (menu Filter—Blur—Average); the eyedropper tool served to measure the brightness and color characteristics. The quantitative assessment of fluorescence intensity was performed using parameter B (brightness) from the HSB model (H for *hue*, S for *saturation*, and B for *brightness*) of the program. It is measured in percentage and can serve as a quantitative parameter of fluorescence intensity of particular cells of microalgae. In each sample, between 50 and 70 cells were analyzed to determine the intensity of fluorescence of particular cells.

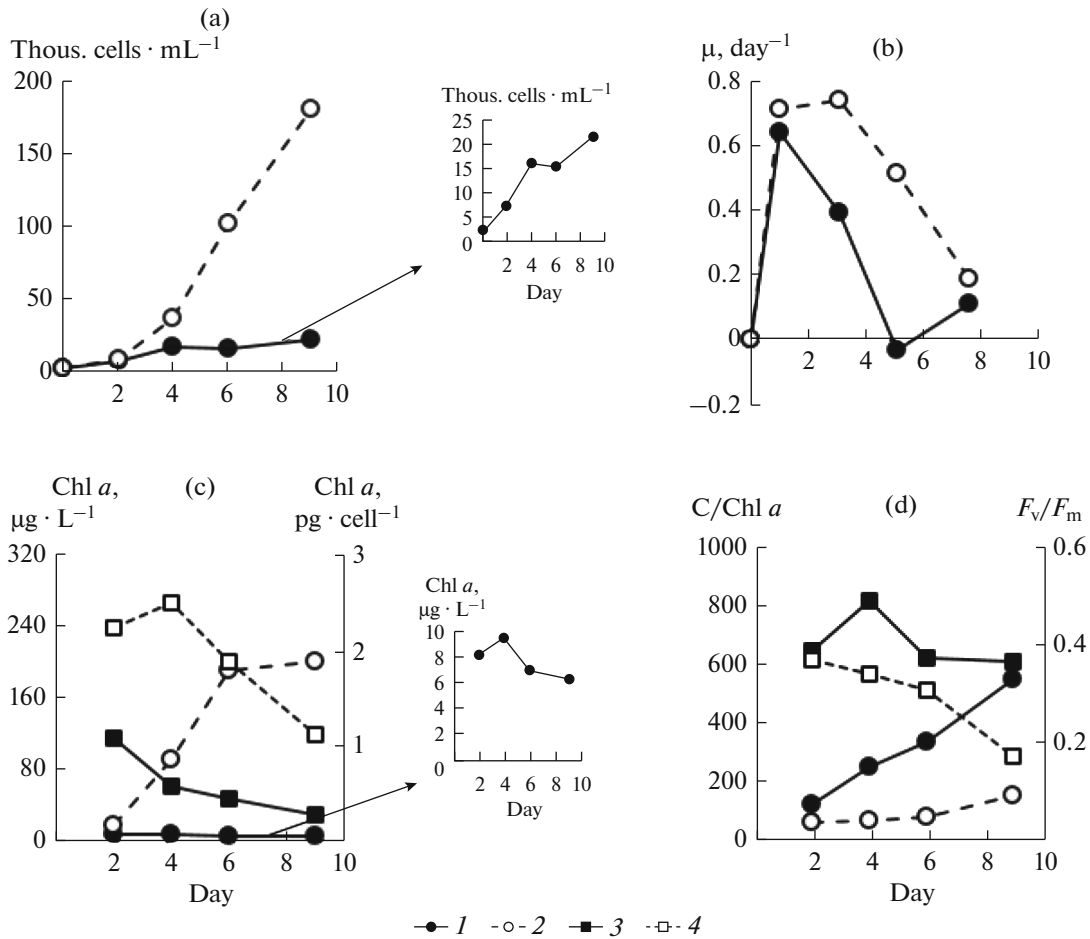
The  $F_v/F_m$  ratio of microalgae was determined on double-flash fluorometer, the principle of operation and configuration of which were developed at the Chair of Biophysics of the Faculty of Biology at Moscow State University (Pogosyan et al., 2009). The measurements were taken after the algae had been dark-adapted for 30 min for all reaction centers of photosystem 2 to transit into an open state. The fluorometer employs a technique of measuring the initial level of variable fluorescence ( $F_0$ ) through short probe flashes and its maximum level ( $F_m$ ) with saturating flash. The measured parameters assisted in calculating the variable fluorescence  $F_v = F_m - F_0$  and  $F_v/F_m$ . Relative measurement error of  $F_v/F_m$  was less than 5%.

Data processing and graph plotting were done in Excel 2013 for Windows.

## RESULTS

*Experiment on Prorocentrum cordatum culture.* The growth was observed in the exponential phase cells both when transferred to the seawater and fresh culture medium for all 9 days of the experiment (Fig. 2a). The end concentrations of the cells in the experiment, however, was lower by a factor of nine compared with the control due to substantial differences in the specific growth rate (Fig. 2b). The estimations revealed that *P. cordatum* performed 3.3 cell divisions in 9 days in the medium depleted in biogenic substances. Along with this, the absolute chlorophyll *a* concentration varied little, whereas the specific one (on a per cell basis) declined by a factor of 3.7 by the end of the experiment (Fig. 2c). In the control, the chlorophyll *a* content in the culture volume unit increased by an order of magnitude on the ninth day relative to the initial value, while its intracellular concentration decreased by 50%. Therefore, the ratio between organic carbon and chlorophyll *a* (C/Chl *a*) in culture under the conditions of biogenic limitations increased fourfold, whereas under conditions of high biogenic substances content it was only 2.4-fold (Fig. 2d). No appreciable change in  $F_v/F_m$  was established in the experiment (Fig. 2d); its value averaged  $0.41 \pm 0.06$ . The latter parameter was observed to decline gradually in the control from 0.4 to  $<0.2$  throughout the experiment.

*Experiment on Gyrodinium fissum culture.* Cells in the exponential phase further placed into seawater not supplemented with additional nutrients exhibited incremental growth during first 4 days. By the end of this period, their number peaked at 8800 cells  $\cdot \text{mL}^{-1}$ , which exceeded the initial value fivefold (Fig. 3a). Incremental growth of the number of the algae (Fig. 3b) produced  $\mu$ , which was equal at this time to  $0.5 \text{ day}^{-1}$ . Altogether, cells of culture *G. fissum* performed 2.3 divisions over 4 days. An alternation between periods of weak growth and cell death was recorded subsequently. This provided for maintaining the cell num-



**Fig. 2.** Dynamics of (a) the number of cells, (b)  $\mu$ , (c) concentration of chlorophyll *a*, and (d) C/Chl *a* ratio and  $F_v/F_m$  in the *Prorocentrum cordatum* culture. (a, b): (1) Experiment and (2) control; (c) absolute concentration chlorophyll *a* ( $\mu\text{g} \cdot \text{L}^{-1}$ ) in (1) experiment and (2) control, normalized to a cell ( $\text{pg} \cdot \text{cell}^{-1}$ ) in (3) experiment and (4) control; (d) C/Chl *a* ratio in (1) the experiment and (2) the control; and  $F_v/F_m$  in (3) the experiment and (4) the control.

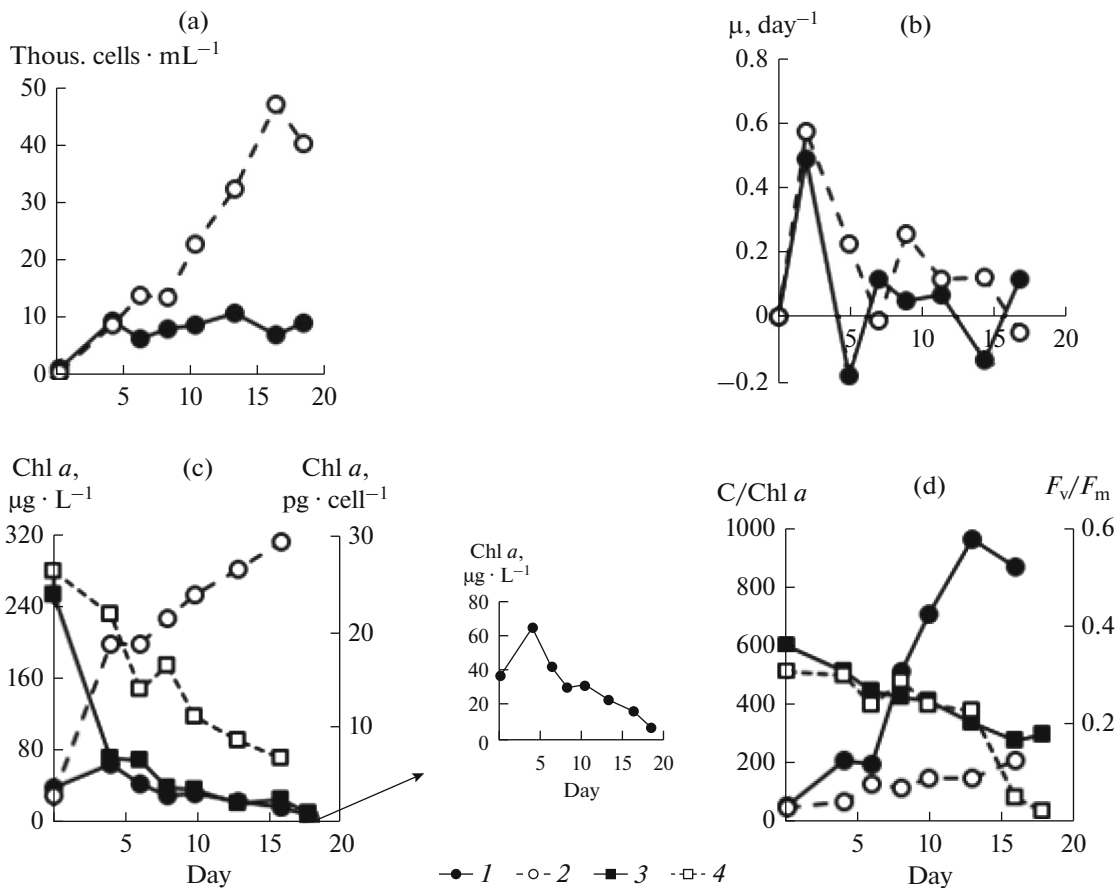
ber at a nearly constant level for 20 days, i.e., until the end of the experiment. Incremental growth of the *G. fissum* cell number was observed in the control on the f/2 culture medium for 16 days, which is determined by the higher  $\mu$  values in this species compared with the experiment. As a result, the maximum number of algae was nearly five times as great as the experimental number.

The concentration of chlorophyll *a* in the culture volume unit was growing during the first 4 days along with the number of cells, while its specific content (on a per cell basis) dropped by a factor of 3.6 (Fig. 3c). The parameters continued to decline during the subsequent period. In the control, absolute chlorophyll *a* content increased during the entire period, whereas the specific one decreased. The rate of the latter decrease, however, was considerably lower relative to the experiment.

Over the entire study period, the *G. fissum* culture kept under conditions of severe deficiency of biogenic

substances in the water exhibited an increase in the C/Chl *a* ratio from 50 to 900–1000, which did not exceed 200 under high content of biogenic substances (Fig. 3d). The  $F_v/F_m$  value gradually decreased nearly half of the initial in the experiment (0.36). During the first 2 weeks, the  $F_v/F_m$  did not fall below 0.20, following which it experienced a dramatic drop to values close to zero in the control (Fig. 3d).

*Experiment on Prorocentrum micans culture.* The species culture reached the late stationary phase during adaptation and, when transferred to the seawater, did not display any appreciable growth (Fig. 4a) due to  $\mu$  tending to zero in general (Fig. 4b). A slight incremental growth of the cells was recorded as late as on the tenth day due to  $\mu$  rising to  $0.2 \text{ day}^{-1}$ . Subsequently,  $\mu$  dropped to negative values, while the total cell number decreased to the initial level. Altogether, the mean number of cells in the *P. micans* culture was  $5800 \pm 900 \text{ cells} \cdot \text{mL}^{-1}$  over 20 days. In the control, the cell number was rising over the first 10 days; after



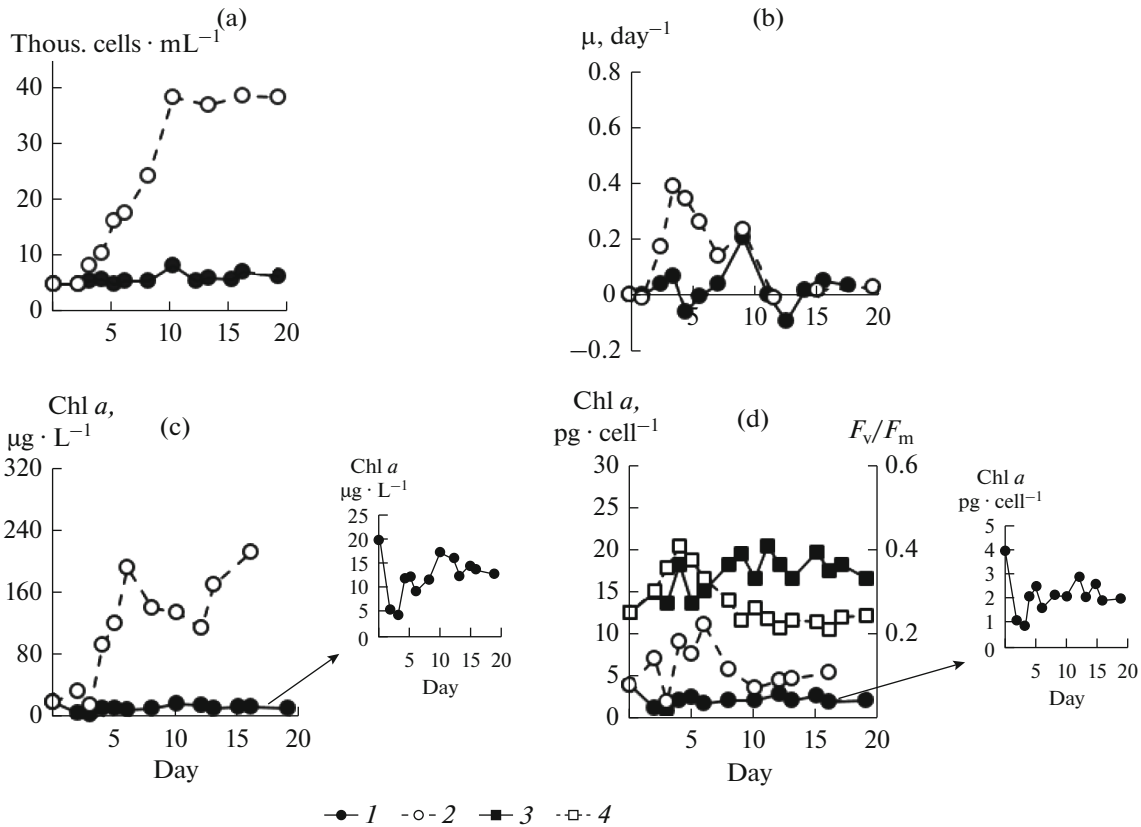
**Fig. 3.** Dynamics of (a) the number of cells, (b)  $\mu$ , (c) concentration of chlorophyll *a*, and (d)  $C/Chl\ a$  ratio and  $F_v/F_m$  in the *Gyrodinium fissum* culture; a, b: (1) Experiment and (2) control; (c) absolute concentration of chlorophyll *a* in (1) the experiment and (2) the control, normalized to a cell in (3) experiment and (4) control; (d)  $C/Chl\ a$  ratio in (1) the experiment and (2) the control;  $F_v/F_m$  in (3) the experiment and (4) the control.

attaining the maximum number of  $38\,500\ \text{cell} \cdot \text{mL}^{-1}$ , no increase in the cell number was detected in the culture due the specific growth rate dropping nearly to zero. Subsequently, the cell number remained at the same level.

As *P. micans* was maintained in the seawater depleted in biogenic substances, the concentration of chlorophyll *a* on a per culture volume unit and cell bases dramatically dropped on the first and second day of the experiment. No unidirectional variations of these parameters were subsequently detected; periods of increase alternated with periods of decrease (Figs. 4c). Similar variations were observed for the  $F_v/F_m$  value (Fig. 4d), which fell within the limits of 0.27–0.41 throughout 20 days. In the control, the chlorophyll *a* content increased until day 6, followed by a gradual decline as the enrichment culture continued its incremental growth. After attaining the maximum (0.41) on the fourth day, an increase in the  $F_v/F_m$  values turned to a smooth decline to reach 0.2 by the middle of the experiment; no further changes were detected.

The initial intensity or brightness (B) of cell fluorescence of *P. micans* culture, exhibiting the late sta-

tionary growth phase, averaged 9% (Fig. 5a). This parameter varied severalfold during the experiment. In seawater depleted in biogenic substances, the parameter was recorded to display maximum values on the 2nd and 16th days (18 and 11%, respectively) and minimum values (6–7%) between the 4th and 8th days and at the end of the experiment. Overall, the brightness of cells in seawater averaged  $9 (\pm 3)\%$  throughout the entire study period. The coefficient of variation of this parameter remained relatively high (55–100%) throughout the experiment and averaged  $77 (\pm 13)\%$  (Fig. 5b). This suggests a high degree of heterogeneity in fluorescence intensity of this species of algae and, therefore, the concurrent occurrence of cells with both high and low viability in the culture. The intensity of cell fluorescence in the control was considerably higher than in the experiment (Figs. 1d–1f). During the first 3 or 4 days, it grew from 10 to 33%, the whereas coefficient of variation of this parameter mean fell from 60 to 20%. This may be indicative of a high rate of recovery of the viability of cells in this suspension on the culture medium after long-term starvation. The stationary growth phase of the control cul-



**Fig. 4.** Dynamics of (a) the number of cells, (b)  $\mu$ , (c) concentration of Chl *a*, and (d) specific concentration of chlorophyll *a* and  $F_v/F_m$  in the *Prorocentrum micans* culture: (a, b) Experiment (1) and control (2); (c) absolute concentration of Chl *a* in the experiment (1) and the control (2), (d) specific content of Chl *a* in the experiment (1) and the control (2);  $F_v/F_m$  in experiment (3) and control (4).

ture was characterized by a reduction in cell brightness and increase in the coefficient of variation of its mean value. Cell brightness averaged 20 ( $\pm 9$ )% in the control throughout the observation period with a brightness coefficient of variation at 38 ( $\pm 13$ )%.

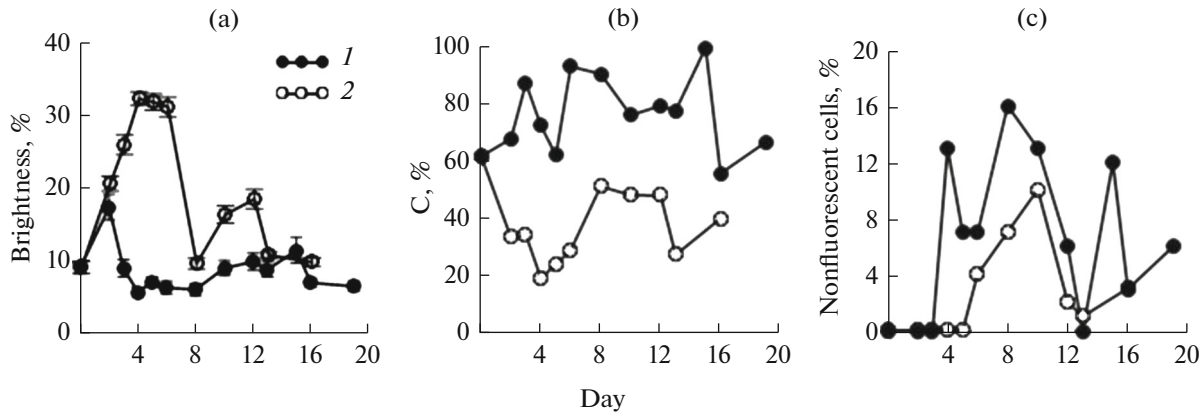
During the experiment, the proportion of *P. micans* cells that were nonfluorescent in the dark-field microscopy but noticeable in the light field varied in both the seawater depleted in biogenic substances and the culture medium. Periods of an increase in percentage of nonfluorescent cells in the culture alternated with periods of decrease under the conditions of biogenic limitation. The behavior of a portion of the nonfluorescent *P. micans* cells on the culture medium was characterized by a unimodal curve with a peak on the tenth day, falling within a period of the completion of exponential growth of the culture. The value of this parameter averaged 6 ( $\pm 6$ )% in the experiment and was lower by a factor of three in the control.

## DISCUSSION

Light, temperature, and biogenic substances are known to be the primary factors regulating the growth of microalgae. The deviation of one of these factors

from optimal delays the growth of algae, while approaching the tolerance limits causes growth cessation. Provided that there is a sufficient amount of biogenic substances in the environment and optimal light and temperature conditions, the algal growth rate attains maximum values. In enrichment cultures, this process is observed in the exponential phase, during which the growth of algae occurs due to supplying their cells with mineral nutrients from the external environment. The latter are used to synthesize the organic algal cell components in a process of a wide range of enzymatic reactions. Biogenic substances, however, continue to be transported from the environment to the cell via its plasma membrane after the maximum growth rate of the algae had already been attained (Baek et al., 2008). This indicates a capacity in marine planktonic algae, including dinoflagellates, for intracellular accumulation (e.g., in a vacuole) of a particular reserve of nutrients, which should be viewed as a strategy for the survival of algae under conditions unfavorable in terms of biogenic substances (Baek et al., 2008; Aldridge et al., 2014). Our experiments show that dinoflagellates *P. cordatum* and *G. fissum*, which had been cultured first under conditions of a high content of biogenic substances in the medium,





**Fig. 5.** Dynamics of cell brightness (a), coefficient (C) of variation of mean values of cell brightness (b) and proportion of non-fluorescent cells (c) in the *Prorocentrum micans* culture on the seawater (1) and culture medium (2). There is standard error of the mean value in Fig. 5a.

continued their growth for a number of days even after being transferred to depleted seawater. In the absence of external sources of mineral forms of nitrogen and phosphorus, it appears to have occurred due to the intracellular reserve of nutrients. In the process of their growth, owing to the intracellular reserves, algae first consume the so-called easy-to-assimilate nutrients, such as inorganic forms of nitrogen and phosphorus and, then, “nonreadily utilizable” nutrients, such as amino acids, nucleic acids, some proteins, and organic forms of phosphorus (Bronk et al., 2007; Girault et al., 2013; Palabhanvi et al., 2014). Thereafter, the vegetative growth of algae ceases. The amount of nutrients inside cells is apparently different in different species, inasmuch as *Prorocentrum cordatum* could perform 3.3 cell divisions, whereas *Gyrodinium fissum* performed slightly more than 2 time. In the studied species, under these conditions, values of  $\mu$  were several-fold lower than the maximum values produced under optimal conditions of the medium (Mansurova, 2013). A delay in the growth of algae should be viewed as one of the mechanisms or ways of survival to maintain functional activity for several days without additional external inputs of nitrogen and phosphorus. A decline in the growth of *Prorocentrum cordatum* culture was accompanied by a reduction of specific chlorophyll *a* content in the cells, whereas  $F_v/F_m$  varied little if any and reached 0.4–0.5, which is characteristic of the Black Sea dinoflagellates not exposed to a limitation in terms of biogenic substances (Akimov and Solomonova, 2019). Apparently, intracellular pool of biogenic substances was not fully utilized in *P. cordatum* cells by the end of the 9-day experiment. In the longer running experiment, the *Gyrodinium fissum* culture, which exhausted the intracellular reserves of nutrients, against the background of cell growth cessation exhibited both a reduction in the intracellular content of chlorophyll *a* and a decrease in  $F_v/F_m$ , which had values of 0.18–0.19 by the 20th day. The juxtaposition of

our data to those reported in (Aldridge et al., 2014) for dinoflagellates *Neoceratium hexacanthum* (Gourret) F. Gomez, D. Moreira & P. Lopez-Garcia and *N. candelabrum* (Ehrenberg) F. Gómez, D. Moreira & P. López-Garcia suggests that this level of  $F_v/F_m$  values may potentially allow for the complete restoration of the functional activity of algae providing the emergence of an external source of biogenic substances. Note that, on the 20th day of the experiment, the  $F_v/F_m$  value in the “starving” *Prorocentrum micans* culture was 1.5 times as great as in *Gyrodinium fissum*, which may point to a high degree of its capacity for survival under conditions of a deficiency of biogenic substances.

Investigations into the dynamics of functional activity of *P. micans* culture and its individual cells were conducted to detect possible causes of the long-term preservation of the viability of dinoflagellates under conditions of the most severe mineral nutrient limitation. The exhaustion of intracellular reserves of biogenic substances in this species and their extremely low content in the water leads to a conclusion that the algae were under biogenic stress throughout the experiment. The intensity of red autofluorescence of chlorophyll *a* was used as an indicator of the functional activity of individual cells of algae. The fluorescence intensity of the individual cells varied against the background of cessation of the *P. micans* cell-number growth in the Black Sea water depleted in nitrates and phosphates. There were viable cells with relatively high fluorescence intensity, weakly fluorescent cells, and cells with no fluorescence (nonviable) present in the suspension over 20 days. The number of the latter periodically increased in the experiment, which was accompanied by a certain increase in chlorophyll *a* content on a per cell basis,  $F_v/F_m$ , and  $\mu$ . Apparently, during their disintegration, dying cells released into external environment the organic matters and served as a source of nutrients for the most viable *P. micans*

cells and promoted their activity. Bacteria present in experimental flasks appear to improve the assimilation of organic matter by cells of this species, which is corroborated by the results of studies on other dinoflagellate species (Bronk et al., 2007; Girault et al., 2013).

In addition to the aforementioned physiological mechanisms of survival under the conditions of deficiency of mineral nutrients, marine dinoflagellates employ other mechanisms, including vertical migrations, which are signalled to intensify by a deficiency of biogenic substances in the water (Jephson and Carlsson, 2009); phagotrophy—in other words, engulfing bacteria, other species of algae, and protozoa (Schnepf and Elbrächter, 1992; Legrand et al., 1998); and an uptake in organic compounds of phosphorus and nitrogen dissolved in water (Richardson and Fogg, 1982).

Data reported by (Aldridge et al., 2014) and obtained therewith allow for the conclusion that the successful recovery of dinoflagellates after biogenic stress requires a periodic access of their cells (no less than in 20 days) to external source of mineral forms of biogenic substances. This can be ensured due to their upward flow as a result of strengthened wind activity and weakening of thermal stratification of the water column, upwelling, or with the strengthened activity of gyre circulations, as well as other dynamic processes (Karl, 1999). The formation of cysts or encystment is the only long-term survival mechanism for dinoflagellates if the environmental conditions fall outside of their tolerance limits. They can retain their vital capacity for years and produce vegetative cells if the conditions become favorable (Chen et al., 2015).

## CONCLUSIONS

This work investigated the physiological mechanism of survival in cultures of three dinoflagellate species common in the Black Sea—*P. cordatum*, *P. micans*, and *Gyrodinium fissum*—under conditions of biogenic limitation. The mechanism relies on processes of a decrease in the growth rate of the algae, the efficiency of the photosynthetic apparatus performance, and the specific content of chlorophyll *a* in cells to levels which will allow for the complete restoration of the physiological functions under optimal conditions. In Black Sea water depleted in biogenic substances, cultures *Prorocentrum cordatum* and *Gyrodinium fissum*, which had the intracellular pool saturated with biogenic substances, displayed a gradual decline in the growth rate, a reduction in specific chlorophyll *a* content on a per cell basis, and a decrease in  $F_v/F_m$  in *G. fissum*. The intracellular reserve of nutrients provided for 2.3–3.3 cell divisions in these species. After the reserve exhaustion, the incremental growth of cells in *G. fissum* and *Prorocentrum micans* ceased, while the specific chlorophyll *a* content continued to decrease. In this setting,  $F_v/F_m$  dropped to

0.2 in *Gyrodinium fissum* by the end of the experiment, whereas it was nearly 1.5 times as great in *Prorocentrum micans*. The case of *P. micans* culture reveals that the long-term exposure of dinoflagellates to the conditions of the most severe biogenic limitation leads to an increased heterogeneity of the functional state of cells and death in part of them. The survival of the most viable cells in the biogenic stress conditions appears to be ensured by the entry into the external environment of organic matter from dying cells. The complete restoration of the functional activity of dinoflagellates requires access to an external source of mineral forms of nutrients at least once every 20 days.

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## COMPLIANCE WITH ETHICAL STANDARDS

*Conflict of interests.* The authors declare that they have no conflict of interest.

*Statement on the welfare of animals.* This article does not contain any studies involving animals performed by any of the authors.

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