Morphological and Morphometrical Features in *Dunaliella salina* (Chlamydomonadales, Dunaliellaceae) During the Two-phase Cultivation Mode

ANDREI B. BOROVKOV, IRINA N. GUDVILOVICH, OLGA A. MEMETSHAEVA, ANNA L. AVSIYAN*, ALEXANDER S. LELEKOV & TATYANA M. NOVIKOVA

A.O. Kovalevsky Institute of Biology of the Southern Seas of RAS, 2 Nakhimov av., 299011, Sevastopol, Russia

*Corresponding author: anna.l.avsiyan@gmail.com

Received 9 July 2019 │ Accepted by V. Pešić: 12 September 2019 │ Published online 17 September 2019.

Abstract
The paper presents studies of morphological and morphometrical characteristics of green halophilic carotenogenic microalga *Dunaliella salina* (Dunal) Teodoresco, 1905, from the south-west region of Crimean Peninsula. *D. salina* was cultivated in two-phase mode under conditions of natural illumination at the premises of A.O. Kovalevsky Institute of Biology of the Southern Seas of RAS (IBSS), Sevastopol, Russia. The maximum *D. salina* cell density was 1.69·10⁶ cell/ml in the “green” phase and 0.84·10⁶ cell/ml in the “red” growth phase. The maximum productivity by cell number reached 0.15·10⁶ cell/(ml·day) in the “green” phase while it was lower by 73% in the “red” phase (0.04·10⁶ cell/(ml·day)). Along with the maximum productivity, linear growth stage in the first phase was characterized by a maximum fraction of small (up to 500 μm³ in volume) cells (about 15-29%) and a decrease in cell volume by 40-45% as compared with initial value. The mean of *D. salina* cell volume in the “red” phase was 30% higher than in the “green” phase. At the same time, the large cell fraction in the “red” phase was consistently high (15-35%). The patterns of change in morphological and morphometrical cell parameters were in accordance to stage and conditions of growth. Thus, cell elongation was noted in the stage of linear growth, while under unfavorable conditions at growth-declining stage cells became more round-shaped, with orange and tile-red coloration and granulation of cell content. It was shown that morphological and morphometric cell parameters can serve as additional criteria for assessment of physiological condition in *D. salina* culture. The experiment demonstrated the prospects for two-stage *D. salina* cultivation in Crimea.

Key words: *Dunaliella salina*, batch culture, cell density, cell size measurements, productivity, carotenoids.

Introduction

Crimean Peninsula (the south of Russia) is region of *Dunaliella salina* (Dunal) Teodoresco, 1905 natural habitat. The abundance of hypersaline reservoirs provides favorable conditions for mass reproduction of this species in the summer time, while the impact of stress factors (high salinity, temperature, solar radiation) promotes β-carotene accumulation in the cells of microalgae (Massyuk 1973; Oren 2005; Posudin *et al.* 2010). Studies of natural reservoirs, as well as salt evaporation ponds, show that the concentration of
biodiversity and \( \beta \)-carotene in them is significantly less as compared with intensive \textit{Dunaliella} cultivation (Massyuk 1973; Oren 2014; Borovkov & Gudvilovich 2015).

\textit{Dunaliella salina} cells might belong to the “green” or the “red” type depending on environmental conditions. The cells of \textit{Dunaliella} have green coloration and reproduce predominantly by vegetative propagation under favorable for growth and reproduction external factors (Massyuk, 1973). Under influence of the stress factors the \( \beta \)-carotene synthesis is induced and \textit{Dunaliella} cells turn into a “red” phase (Ben-Amotz 1995; Borovkov & Gudvilovich 2015) that in turn is used for commercial production of \( \beta \)-carotene.

\textit{Dunaliella salina} cells have monad structure with two identical flagella at the apical side. Cells sizes are varying: from 2.8 to 40 \( \mu \)m in length, from 1.5 to 20 \( \mu \)m in width and from 8 to 4500 \( \mu \)m\(^3\) in volume (Massyuk 1973; Oren 2005; Borovkov 2005; Tafreshi & Shariati 2009; Posudin et al. 2010; Preetha et al. 2012). High species variability (plasticity level) is the main morphological characteristic of \textit{D. salina}, so that its cells change form and size easily in response to environmental changes (Oren 2005; Preetha et al. 2012; Srirangan et al. 2015). Cells form can vary from ovoid, pear-shaped, ellipsoid to fusiform and almost spherical depending on growth and development stage and on environmental conditions (Massyuk 1973). There is one cup-like chloroplast surrounded by starch with pyrenoid in the center. The abundant oil drops containing dissolved carotenoids, predominantly \( \beta \)-carotene (Tafreshi & Shariati 2009, Achour et al. 2019) accumulate in the chloroplast stroma under extreme conditions. As a result the cells acquire a yellow-brown, orange or tile-red coloration (Fu et al. 2014; Lv et al. 2016, Wei et al. 2017). Such colored cells usually contain carotene lipid globules not only in chloroplast, but also in cytoplasm.

The presence of the thin transparent plasmalemma lacking rigid cell wall that enables examining cell organelles during cytokinesis is one of sufficient features of genus \textit{Dunaliella} (Massyuk 1973). This feature allows quick response control of changes in the morphometric and physiological parameters in culture of \textit{D. salina} at different growth stages and under the action of various abiotic factors. This character allows the use of the microalga as a good model for studying the characteristics of carotenogenesis taking into account the growth of alga in a specific region. Two-phase cultivation in open ponds under natural illumination is convenient for study of morphometric and physiological characteristics in \textit{D. salina}, as it provides the observation of different growth stages, as well as the influence of nutrient supply and illumination increase due to culture dilution. In this regard, the purpose of our study was to investigate the physiological, morphological and morphometric characteristics of carotenogenic microalgae \textit{D. salina} during its two-phase cultivation in the south-west of Crimean Peninsula (Sevastopol region).

**Material and methods**

**Culturing conditions**

The investigation was carried out at the premises of Biotechnology and Phytoresources Department of IBSS, Sevastopol, Russia (located at 44°36'54.6"N 33°30'11.7"E) from May 24, 2018 to July 13, 2018. The unialgal culture of \textit{D. salina} (strain IBSS-2 from IBSS common use center “World Ocean hydrobionts collection”) was grown in modified culture medium of Shaish et al. (1990). The modification involved adding of sea salt (“Galit”, Russia) up to 120 g/L concentration. Algae were cultivated in algae-biotechnological greenhouse module made of polycarbonate that provided temperature maintaining and protection from possible atmospheric precipitation. Square ponds (1 \( \times \) 1 m) were covered with polyethylene film and layed on the leveled ground. Culture of \textit{D. salina} maintained in greenhouse module during previous winter and spring period was used as the inoculate, algal cells were at the “red” form. Temperature and illumination level were natural: the maximum illumination at the surface of the cultivators reached 80-90 klx on sunny days and 40-50 klx on the cloudy days; temperature in the ponds varied from 24 to 35 °C. Cell suspension was continuously stirred by aquarium pump Atman AT-201 (Chuangxing Electrical Appliances Co., Ltd, China).

The first phase duration was 29 days, the second phase duration was 21 days. In the first cultivation phase the inoculate and culture medium were mixed (with the ratio 1:1.5). In the second cultivation phase, culture of \textit{Dunaliella} was diluted twofold with fresh culture medium lacking nitrogen and phosphorous salts. Culture layer depth was 9 cm in the first stage and 4 cm in the second stage, culture volume was 90 L and 40 L respectively.
Measurements of growth parameters
Cell density ($N$, cell/ml) counts were made with hemo-cytomere (Gorjayev’s chamber, MiniMed, Russia) using light microscope Carl Zeiss Axioskop Plus (Carl Zeiss company, Oberkochen, Germany) (Absher 1973). Samples for cell count were taken in triplicate, sample volume was 5 ml.

Maximum productivity ($P_m$) was calculated through the approximation of growth curve in the linear growth stage area on the basis of cell number:

$$N = N_l + P_m \times (t - t_l)$$

where $N_l$ – cell density at the beginning of linear growth phase; $t_l$ is the time at the beginning of linear growth phase (Lelekov & Trenkenshu 2007).

In the linear growth phase cell division number and rate were determined during the night. Cell division number ($n$) was calculated by equation:

$$n = \frac{\log N - \log N_0}{\log 2},$$

where $N$ and $N_0$ are cell density in the end and in the beginning of the period.

Microscopy
Morphometrical characteristics of $D. salina$ in culture were analyzed using a microphotographs obtained with the help of light microscope Carl Zeiss Axioskop Plus (Carl Zeiss company, Oberkochen, Germany) equipped with digital camera Canon PowerShot a620 (Canon Inc., Tokyo, Japan), and software Micam (Science4all, 2009). The morphological characters such as size, shape and coloration of the cell, flagella occurrence, characteristics of stigma, pyrenoids, chloroplasts and other cytoplasmic inclusions, such as starch granules were estimated based on 100 randomly chosen cells. Cell length ($L$) and width ($D$) were measured and cell volume ($V$) was calculated by the formula of prolate spheroid (Sun & Liu 2003):

$$V = \frac{\pi}{6} \times D \times L \times H,$$

where $H$ is the third axis of ellipse and ellipsoid.

Dividing cells were determined by the occurrence of appropriate physiological changes, including pyrenoid, chloroplast and cytoplasm fission (cytokinesis).

Statistical analyses
Statistical data analysis was performed using Microsoft Excel software. Arithmetic mean ($\bar{x}$), standard deviation ($S$), error of the mean, confidence intervals for the mean ($\Delta \bar{x}$) were estimated. The significance of differences was determined by Student’s $t$-test (if the variances of the samples were assumed to be equal) or by unequal variances $t$-test (if samples have unequal variances). All calculations were made for the significance level $\alpha = 0.05$. The mean and confidence intervals ($\bar{x} \pm \Delta \bar{x}$) are given in the text and at the diagrams.

Results
“Green” phase
At the first, “green” phase, the favorable conditions for high cell division rate in $D. salina$ culture are established. Hence, the conditions, provided in our experiment, enabled increasing Dunaliella cell density from 0.23·$10^6$ cell/ml to 1.69·$10^6$ cell/ml (7.3-fold) during the first 13 days (Fig. 1 A). It should be noted that exponential growth phase was absent whereas linear growth phase lasted 6 days. In the latter phase the maximum productivity ($P_m$) was constant and reached 0.15·$10^6$ cell / (ml · day) by cell number. After 13 days, the gradual decrease in cell density of $D. salina$ was observed. By the end of the “green” phase cell density fell by a factor of 1.4 compared to the maximum value (Fig. 1 A).

Mean dividing cell percentage at the linear stage (0 – 6 days) decreased from 8 to 4 % and after that varied within 2 – 6 % until the end of the first growth phase (Fig. 1 A).

At the linear growth stage in the “green” culture, 0.51 cell divisions occurred during the night (average value for five days of linear phase) that correspond to growth rate of 0.039 divisions/h.
Study of *D. salina* morphometric parameters has demonstrated the decrease in mean cell size in 1.2 times during linear growth phase: from 17.8 to 15.34 μm in length and from 11.01 to 9.18 μm in width (Fig. 2 A). In the course of subsequent culturing (phase of growth declining and stationary phase) gradual increase of mean cell length and width was observed. The mean cell size in the “green” phase was 16.88 μm in length and 10.11 μm in width.

Mean cell volume in *D. salina* both dividing and non-dividing cells rapidly declined from the first to the 6th day of cultivation (Fig 2 C). Thus, non-dividing cells volume significantly decreased from 1159.7 ± 6 μm³ to 691.5 ± 5.4 μm³ (t=11.24 > t_05=1.66), and dividing cells from 1477.7 ± 4 μm³ to 830.9 ± 3.9 μm³ (t=3.91 > t_05=1.81) (1.7 and 1.8 times in comparison with initial cell volume, respectively). Mean cell volume exhibited increase gradually from the 7th day when the phase of of growth declining in culture was attained. Thus, non-dividing *D. salina* cells significantly expanded up to 1185.5 ± 5 μm³ (1.7 times) by the end of “green” phase and dividing cells increased their volume up to 1485.7 ± 3.8 μm³ (1.8 times) (t=2.37 > t_05=1.66) in comparison with the minimal values in the linear growth phase. The mean cell volume in the “green” phase was 1.3 times higher in dividing then in non-dividing cells (Fig 2 C).

It has been found that small cells (less than 500 μm³) fraction in *D. salina* increased significantly (from 1 to 29%) while large cells (more than 1500 μm³) fraction decreased (from 16 to 0%) during linear growth phase (Fig. 2 E). After linear growth phase small cells fraction progressively diminished and was not visible after the 28th day. At the same time, large cells fraction increased from 0 to 20% in the course of stationary growth phase.

Also, morphological changes were noted in *D. salina* culture in the “green” phase (Fig. 3 A). Early in the phase, the cells were green, with tight cell wall, the inclusions were absent in chloroplast, pyrenoid was clearly visible. After the 10th day of exposition (in the phase of growth declining and stationary phase) as the culture grew and got senescent, the small granules of lipid globules became visible in the cells. At the end of the phase, the granular chloroplast stood out in the cell structure while pyrenoid became invisible and cells gained orange color.

**“Red” phase**

At the second, “red” phase, the cell density of *D. salina* increased by 25% compared to initial number and reached the maximum value of 0.84·10⁶ cell/ml during the first 5 days (Fig. 1 B). The maximum productivity of *D. salina* (*P_m*) calculated based on cell number, was 0.04·10⁶ cell/ (ml·day). At the end of the second phase, the density of *D. salina* cells reduced by 78% in comparison with the maximum value (Fig. 1 B).

The mean percentage of dividing cells in the “red” phase generally averaged about 5% (Fig. 1 B). However, after the 15th day the portion of dividing cells was 4 times less than it was in linear growth phase that corresponds to the pattern of *D. salina* cell density change over this period.
Figure 2. Changes of *D. salina* morphometrical parameters: cell height and width (A, B), dividing and non-dividing cells volume (C, D) and percentage of cells with different volume (E, F) in “green” and “red” cultivation phase
In the conditions of “red” culture and in the phase of linear growth, 0.47 cell divisions occurred during the night that corresponds to growth rate of 0.036 divisions/h.

The estimation of *D. salina* morphometrical parameters in culture has demonstrated that cell length did not vary significantly at the beginning and in the end of “red” phase, averaged about 17.3 μm. At the 5th day of cultivation (at the end of linear growth phase), this parameter increased to 20.2 μm, by 20% in comparison with the initial value (Fig 2 B). The cell width remained almost constant over 21 cultivation days and averaged 11 μm. The *D. salina* cells became of oblate spheroid at the 5th day of cultivation and obtained the spheroidal shape at the end of the second growth phase.

The average volume both of dividing and non-dividing cells of *D. salina* increased from the beginning of the phase to the 5th day of cultivation. At the same time the volume of non-dividing cells significantly increased from 1188.5 ± 5 μm$^3$ to 1366.6 ± 6.1 μm$^3$ ($t=1.37 > t_05=1.65$), i.e. by 20%, whereas the volume of dividing cells changed from 1349.7 ± 1.8 μm$^3$ to 1659.0 ± 3.6 μm$^3$, but mean difference was not significant (Fig. 2 D). When algal culture had reached stationary phase the decreasing of cell volume was observed (981.6 ± 4.7 μm$^3$ for the cells in growth phase and 1106.4 ± 0.1 μm$^3$ for the cells in division phase, i.e. 1.4 and 1.5 times, respectively). It has been noted that the volume of *D. salina* dividing cells in the “red” phase was 20% higher than that of non-dividing cells.

As was shown the small cells fraction of *D. salina* (less than 500 μm$^3$) was practically absent at the second cultivation phase, comprising about 1%. The large cells fraction (more than 1500 μm$^3$), on the contrary, comprised about 30-35 % at the end of linear phase (3rd – 7th days) (Fig. 2 F).

Significant morphological changes in cells of *D. salina* were observed in the “red” phase, when stress conditions for the algae growth and reproduction take place (Fig. 3 B). The granulation of cytoplasm in the form of colored lipid globules had been seen from beginning of the second phase, had been pronounced at the 9th day and got distinct at the end of the experiment. At the same time the *D. salina* cells became more round-shaped and assumed the orange and tile-red coloration. In addition, some *Dunaliella* cells formed the pseudopodia-like processes (Fig. 3 B).

![Figure 3](https://example.com/figure3.png)  
**Figure 3.** Changes of morphology and coloration of *D. salina* cells in “green” (A) and “red” (B) cultivation phase. Scale is 10 μm.

**Discussion**

The peculiarities of morphology, morphometry and physiology in *D. salina* during its two-stage cultivation depend on the conditions at the first and second stages of cultivation.

It is known that illumination is a leading factor responsible for carotenogenesis induction in *D. salina* (Ben-Amotz 1995), therefore at the second growth phase we used earlier approved procedure of raising the irradiance level of cells. The method consists in dilution of culture media and reducing of culture layer depth twofold (Borovkov & Gudvilovich 2017) that enabled increasing the irradiance level by a factor of four. It
should be noted that the linear growth of the *D. salina* culture is not always observed at the second stage of growth because the stress factors might significantly reduce the rate of cell division. However, in our experiment the linear growth was exhibited during the first five days of the “red” phase, which is possibly due to the cloudy weather during this stage of cultivation. At the same time, the cells of *D. salina* apparently used residual amounts of mineral nutrients for its growth (Fig. 1 B).

Biogenic elements deficiency, especially in old cultures of algae, may disrupt the process of some cell organelles division, resulting in reduction of the cell division rate (Massyuk 1973; Davis *et al*. 2015; Lv *et al*. 2016). This circumstance allows explaining the fact, that dividing cells ratio did not decrease during 15 days of cultivation in the “red” phase, at the expense of prolonged cytokinesis of *D. salina* cells (Fig. 1 B).

It was observed that cell division in the linear growth phase took place both under the light and in the dark conditions (at “green” and “red” phases). This corresponds to the literature data on the division of *D. salina* cells under the conditions of a daily light-dark regime (Xu *et al*. 2016). According to our data, the cell division rate of *D. salina* by night did not differ significantly between the first and the second culturing stage 
\[ t=0.099 < t_{0.05}=2.306 \], amounting to 0.036-0.039 div/h despite the difference in the average apparent daily productivity. This may be due to the circumstance that the stress effect leading to cell death occurred only during the daytime under high illumination.

*D. salina* cell sizes of different strains (according to the literature) vary widely from 2.8 to 40 \( \mu \)m in length and from 1.5 to 20 \( \mu \)m in width (Massyuk 1973; Borovkov 2005; Oren 2005; Tafreshi & Shariati 2009, Preetha *et al*. 2012). The cell sizes determined in our experiment were 11-25 \( \mu \)m in length and 6-15 \( \mu \)m in width (Fig. 2 A, B), i.e. IBSS-2 *D. salina* strain has intermediate cell sizes. Generally, cell sizes did not vary significantly between “green” and “red” stages. Mean cell length was 16.88 and 17.3 \( \mu \)m; mean cell width was 10.1 and 11 \( \mu \)m in the “green” and “red” phases, respectively.

It should be noted that the length and width of *D. salina* cells at the first and the last days of batch cultivation did not differ significantly at the “green” and the “red” stages (Fig. 2 A, B). This was because the culture at “red” stage with large cells was used as inoculum. Cell length and width decreased by a factor of 1.2 at the first cultivation stage and increased by a factor of 1.2 at the second cultivation stage, which corresponded to the linear phase of culture growth in both cases. The differences of patterns of Dunaliella cell size changes are probably the result of significant differences in growth conditions at two experimental stages. As many authors (Massyuk 1973; Preetha *et al*. 2012; Davis *et al*. 2015) noted, the rounding of cells, i.e. a decrease in the length-to-width ratio, is a typical response to stressful environmental conditions and the initial marker of algae depressed condition, whereas the elongation of cells (increase in the length-to-width ratio) is characteristic response to increasing of nutrient concentrations. The cell length of *D. salina* increased during the first five days of “red” phase along with the increasing of its cell number (at linear growth phase). It is our point that this testifies that some amount of biogenic elements was present in the greenhouse complex and amounted about 10 \( \mu \)g in width (Lv 2016). This corresponds to the literature data on the division of *D. salina* culture grown at two

The mean *D. salina* cell volume was 30% higher in the “red” phase than in the “green” phase. The fraction of large cells in the “red” phase was consistently high (15-35%) (Fig. 2 D, F). The ratio between groups of cells of different volume changed over the entire time of cultivation, suggesting that changing of the age structure of the Dunaliella cell populations took place both at the “green” and the “red” growth phases. Hence, at the linear stage of the “green” phase, when productivity is constant, the small “young”, actively dividing cells dominate, which are at the growth stage. While large “old” cells are prevalent at limiting factor change and under negative growth rate.

The morphological changes in Dunaliella cells evident as manifestation of granulation and gaining of orange and tile-red coloration point to the increasing of carotenoids content value in culture (Fig. 3 B).

The optimal duration of cultivation phases was determined by way of experimental registration of morphological and morphometrical parameters in *D. salina* culture grown at two-phase mode of cultivation in greenhouse complex and amounted about 10-12 days both for the first and the second phase. The concentration of Dunaliella cells reached the maximal levels in particular at 10-12th days of each culturing stage (Fig. 1 A, B); at that the “large” cells fraction started increasing at the “green” stage and was enough high (18%) at the “red” stage, while “small” cells fraction was close to minimal values at both stages (Fig. 2 E, F). After 10 days of cultivation at the second stage the mean cell volume and “large” cells fraction decreased, the reduction of motor activity was noted, and some cells had formed the pseudopodia-like
processes. The mentioned modification is typical to old *D. salina* cultures as a response to extreme environmental conditions, such as the changes of salinity, mineral composition of the medium, temperature and illumination, as well as combination of specified parameters (Massyuk 1973; Oren, 2014; Lv *et al.* 2016).

The prolongation of the duration of first and second cultivation stages at more than 15 days was unfavourable, because the cell density did not increase at this period in the “green” phase and was significantly reduced in the “red” stage (by 75%). Moreover, just as the fraction of dividing cells significantly decreased (by 80%), so did their volume (by 15% from 10th to 22nd day) (Fig. 2 B, D, F).

**Conclusion**

Morphological and morphometrical characteristics of carotenogenic microalga *D. salina* have been studied during its two-phase cultivation in the south-west of Crimean Peninsula in summer. The obtained experimental data on the dynamics in cell density and size structure have indicated the significant influence of growth conditions in “green” or “red” phase as well as culture growth stage on the studied parameters. The relationship among *Dunaliella* cell size groups varied over all cultivation period, which indicates changes in the age structure of cell population both in the “green” and in the “red” growth phase. Changes in morphological and morphometrical parameters of *D. salina* cells can serve as an additional criterion for determining of durations in cultivation stages during the commercial cultivation of this alga. In conclusion, Crimea is not only a part of natural *D. salina* areal but also a prospective region for development of its biotechnology.

**Acknowledgements**

This study was supported by the grant № 18-44-920009 from Russian Foundation for Basic Research and the A.O. Kovalevsky Institute of Biology of the Southern Seas of RAS state project № AAAA-A18-118021350003-6.

**References**

Absher, M. (1973). Hemocytometer Counting. *In: Paul F. Kruse Jr. & M. K. Patterson Jr.* (Eds.), *Tissue Culture: Methods and Applications*. Academic Press., New York, USA, pp. 395–397.

Achour, H.Y., Doumandji, A., Bouras, N., Sabau, N., & Assunção, P. (2019). Isolation, Molecular Identification and The Carotenogenesis Process of the Microalga *Dunaliella salina* Strain DunaDZ1 Isolated from an Algerian Salt Lake. *Turkish Journal of Fisheries and Aquatic Sciences*, 19 (5), 399–407.

Ben-Amotz, A. (1995). New mode of *Dunaliella* biotechnology: two-phase growth for β-carotene production. *Journal of applied phycology*, 7(1), 65–68.

Borovkov, A.B. (2005) Green microalga *Dunaliella salina* Teod. (Review). *Ecology of the Sea*, 67, 5–17. (In Russian).

Borovkov, A.B., & Gudvilovich, I.N. (2017). The testing of two-stage system of semi-industrial cultivation of *Dunaliella salina* Teod. *Voprosy sovremennoy algologii*, 13 (1). (In Russian) Available at: http://algology.ru/1155 (Date of access: 13.06.2019).

Borovkov, A.B., & Gudvilovich, I.N. (2015). Intensive cultivation of *Dunaliella salina* as way of obtaining of biomass with elevated β-carotene content. Communication 2. Optimization of Cultivation Regime. *Hydrobiological Journal*, 51 (4), 31–38.

Davis, R.W., Carvalho, B.J., Jones, H.D.T. & Singh, S. (2015). The role of photo-osmotic adaptation in semi-continuous culture and lipid particle release from *Dunaliella viridis*. *Journal of Applied Phycolgy*, 27(1), 109–123.

Fu, W., Paglia, G., Magnúsdóttir, M., Steinarsdóttir, E. A., Gudmundsson, S., Palsson, B., … Brynjólfsson, S. (2014). Effects of abiotic stressors on lutein production in the green microalga *Dunaliella salina*. *Microbial Cell Factories*, 13(1), 3.
Lelekov A.S., Trenkenshu R.P. (2007). The simplest models of microalgae growth. 4. Exponential and linear growth phases. *Ecology of the Sea*, 74, 47–49. (In Russian)

Lv, H., Cui, X., Wahid, F., Xia, F., Zhong, C. & Jia, S. (2016). Analysis of the Physiological and Molecular Responses of *Dunaliella salina* to Macronutrient Deprivation. *PLoS ONE*, 11 (3): e0152226.

Massyuk, N.P. (1973). *Morphology, Taxonomy, Ecology and Geographic distribution of the Genus Dunaliella Teod. and prospectus for its potential utilization*. Naukova Dumka Press., Kiev, 487 pp. (In Russian)

Oren, A. (2005) A hundred years of *Dunaliella* research: 1905–2005. *Saline Systems*, 1 (1), 2.

Oren, A. (2014) The ecology of *Dunaliella* in high-salt environments. *Journal of Biological Research-Thessaloniki*, 21 (1), 23.

Posudin, Y. I., Massjuk, N. P., & Lilitskaya, G. G. (2010). *Photomovement of Dunaliella Teod.* Vieweg+Teubner Verlag | Springer Fachmedien Wiesbaden GmbH, Wiesbaden, 225 pp.

Preetha, K., John, L., Subin, C.S. & Vijayan, K.K. (2012). Phenotypic and genetic characterization of *Dunaliella* (Chlorophyta) from Indian salinas and their diversity. *Aquatic Biosystems*, 8 (1), 27.

Science4all (2009). Microscopy and Photography. Available from: http://science4all.nl/?Microscopy_and_Photography (Date of access: 02.09.2019)

Shaish, A., Avron, M., & Ben-Amotz, A. (1990). Effect of inhibitors on the formation of stereoisomers in the biosynthesis of β-carotene in *Dunaliella bardawil*. *Plant and cell physiology*, 31(5), 689–696.

Srirangan, S., Sauer, M.-L., Howard, B., Dvora, M., Dums, J., Backman, P. & Sederoff, H. (2015). Interaction of temperature and photoperiod increases growth and oil content in the marine microalgae *Dunaliella viridis*. *PLoS ONE*, 10 (5): e0127562.

Sun, J., & Liu, D. (2003). Geometric models for calculating cell biovolume and surface area for phytoplankton. *Journal of Plankton Research*, 25 (11), 1331–1346.

Tafreshi A. H. & Shariati, M. (2009). *Dunaliella* biotechnology: methods and applications. *Journal of Applied Microbiology*, 107(1), 14–35.

Wei, S., Bian, Y., Zhao, Q., Chen, S., Mao, J., Song, C., … Dai, S. (2017) Salinity-Induced Palmella Formation Mechanism in Halotolerant Algae *Dunaliella salina* Revealed by Quantitative Proteomics and Phosphoproteomics. *Frontiers in Plant Science*, 8, 810.

Xu, Y., Ibrahim, I. M., & Harvey, P. J. (2016). The influence of photoperiod and light intensity on the growth and photosynthesis of *Dunaliella salina* (Chlorophyta) CCAP 19/30. *Plant Physiology and Biochemistry*, 106, 305–315.