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Ecological peculiarities of natural populations of hyperhalobe microalga *Dunaliella salina* Teod. in solar salt work ponds of the South of Ukraine and Russia

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The paper presents the results of expedition research of some solar salt works of the South of Ukraine (Kherson region, AR Crimea) and lake Baskunchak (Astrachan region, Russia), as well as stationary observations on populations of the microalga *Dunaliella salina* Teod. in the ponds of Heroyske salt works (Gola Prystan' district, Kherson region) that we carried out in 2005–2008. We discuss the approaches to modeling natural environment in *D. salina* laboratory culture to develop and optimize the process of industrial culturing in the open culture.

Key words: *Dunaliella salina, β-carotene, salt works, natural culture.*

Экологические особенности природных популяций гипергалобной микроводоросли *Dunaliella salina* Teod. в бассейнах солепромыслов юга Украины и России

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Представлены результаты экспедиционных обследований ряда солепромыслов юга Украины (Херсонская область, АР Крым) и оз. Баскунчак (Астраханская область, Россия), а также стационарных наблюдений за популяциями микроводоросли *Dunaliella salina* Teod. в бассейнах солепромысла ОПП «Геройское» (Голопристанский район, Херсонская область), проведенных в 2005–2008 годах. Обсуждаются подходы к моделированию природных условий в лабораторной культуре *D. salina* с целью разработки и оптимизации процесса промышленного культивирования в открытой культуре.

Ключевые слова: *Dunaliella salina, β-каротин, солепромысел, природная культура.*

Екологічні особливості природних популяцій гіпергалобної мікроводорості *Dunaliella salina* Teod. в басейнах солепромислів півдня України і Росії

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Представлено результати експедиційних обстежень низки солепромислів півдня України (Херсонська область, АР Крим) і оз. Баскунчак (Астраханська область, Росія), а також стаціонарних спостережень за популяціями мікроводорості *Dunaliella salina* Teod. в басейнах солепромислу ДПП «Геройське» (Голопристаньський район, Херсонська область), що було проведено в 2005–2008 роках. Обговорюються підходи до моделювання природних умов в лабораторній культурі *D. salina* з метою розробки та оптимізації процесу промислового культивування у відкритій культурі.

Ключові слова: *Dunaliella salina, β-каротин, солепромисел, природна культура.*

Introduction

Biotechnology exploits the hyperhalobe microalga *Dunaliella salina* Teod. because of its natural ability to develop massively (causing water "bloom") and accumulate β-carotene (changing cells color from green to orange-red). Natural populations of *D. salina* can provide the information necessary to develop and optimize β-carotene biosynthesis technology. The monograph of N.P.Massyuk (1973) summarizes the results of extensive work of domestic algologists on this topic during the 60s of the last century. Despite these and numerous subsequent domestic and international researches on the ecology and physiology of *D. salina*, the technology of *D. salina* industrial cultivation has not been implemented in Ukraine and Russia. The mechanism of β-carotene accumulation seems to be explained long ago as the result of "uncoupling the

processes of cell division and photosynthesis" (Semenenko, Abdullaev, 1980), but the literature lacks a method of *D. salina* cultivation able to provide stable and reliable high β-carotene yield.

The reason, in our view, is that the majority of the laboratory experiments on *D. salina* were conducted under significantly deviated from the natural conditions that made impossible to uniquely identify the environmental factors influencing cell reproduction and β-carotene accumulation in this microalga in the nature.

The objective of this work was to identify the environmental characteristics of natural populations of *D. salina* in hyperhaline waters of Ukraine and Russia to model in the laboratory for the purpose of further experimental research and development of the technology of industrial biosynthesis of β-carotene.

Materials and methods

Field observations were made and 60 live samples were collected during expeditions to the ponds of 5 salt works in different seasons of 2004–2008. Almost all at that time operating solar salt works in Ukraine were investigated: Heroyske and Genichesk salt works (Kherson region), "Halite" cooperative (Sasyk-Sivash, AR Crimea), ponds of Crimean Soda Plant (the western part of the Gulf of Siwash, AR Crimea). In addition, several samples were collected from Baskunchak salt works (Astrakhan region, Russia). Stationary observations, during which more than 300 live samples were analyzed, were carried out in 2006–2008 at Heroyske salt works.

Samples were scooped from salt works ponds. Sampling points were located around the perimeter of a pond (in each corner and in the middle of each side of a pond). Agglomerates of cells caused by wind were avoided at sampling for more accurate estimates of cell numbers. Brine was vertically mixed at a sampling point before sampling. Data on 8 samples collected along the perimeter of a pond were averaged, or one sample obtained by mixing equal volumes of them was analyzed to ensure the representativeness. To evaluate the natural turbidity of brine Secchi disk was used.

Samples were assayed on the day of collection. Preference is given to simple and fast methods convenient for field studies, mass laboratory analyzes and rapid control of biotechnological process. Concentration of cells was counted using Goryaev haemocytometer and expressed in thousands of cells per 1 ml of brine. To determine β-carotene concentration 2.5 ml of brine was shaken vigorously with the equal volume of ethyl acetate, and then the sample was allowed to stand for phase separation. The upper phase of ethyl acetate containing extracted pigments was transferred into the cuvette of КФК-2МП photometer and the extinction was measured at 440 nm. This rapid method was preliminary proved to give the results comparable with the standard procedure of β-carotene quantification in *D. salina* after fractionating carotenoids by thin layer chromatography as described in (Bozhkov, Komaristaya, 2003). β-carotene content was calculated using the reference extinction coefficient $E_{1cm}^{1\%} = 2500$ (IARC, 1998) and expressed in pg per cell.

Brine density was determined with areometer and expressed in g/cm^3.

For nitrate and phosphate quantification, brine was centrifuged at 3000 rpm to precipitate algal cells, and supernatant aliquots were taken for analysis.

Nitrate nitrogen was determined with salicylic acid according to the procedure described in (Cataldo et al., 1975). To improve the sensitivity of the method the volume of aliquots was increased to 0.4 ml and the concentration of NaOH – to 8N, the volume of 5% salicylic acid was decreased to 0.4 ml and the volume of NaOH – to 4 ml. Extinction at 400 nm was measured. The method was calibrated by KNO_3.

Inorganic phosphate was determined with molybdate and ascorbic acid (Fogg, Wilkinson, 1958), also in our modification. All laboratory glassware for the analysis was pre-washed with saturated potassium dichromate in H_2SO_4. To 1.5 ml aliquot there were added in succession: 0.75 ml of 10 N H_2SO_4, 0.5 ml of 7,5% ammonium molybdate in 3N H_2SO_4, and 0.2 ml of freshly prepared ascorbic acid solution (15 mg/ml). Samples were incubated in boiling water bath for 10 minutes, cooled to room temperature, and the extinction was determined at 670 nm. The method was calibrated by K_2HPO_4.

Analyses were run in the field laboratory of "Betacar-XP" LLC as the part of pilot project on *D. salina* mixed carotenoids and β-carotene manufacturing (Betacar-X, 2009). The data obtained were considered as general population characteristics for *D. salina* inhabiting the reservoirs investigated. In the figures the raw data are given. Spearman nonparametric test was used to investigate the correlation between brine density and cell number increase, and between cellular β-carotene content and air temperature.

Results

D. salina in salt works ponds at the South of Ukraine and Russia. We discovered the alga *D. salina* in its vegetative monadic “red” form in all the samples collected from all the points in all the seasons, including winter. Only a few spring (April) samples from the lake Sasyk-Sivash contained besides vegetative form cysts of vegetative origin (differed from zygotes by thick slightly granulose orange-colored shell), often germinating by two cells (comparing with zygotes, which germinate by up to 32 cells) (photo 1). It is noteworthy that the "green" form (usual in laboratory culture) was not observed in any sample from natural habitats. All the brine samples had reddish hue, although it often (at densities about 1.240 g/cm^3) differed from the reddish-orange color of the cells themselves by raspberry tone that should be attributed to the development of halobacteria.

Photo 1. *D. salina* cyst of vegetative origin (A); germinating by two cells cyst (B)

D. salina remained viable over a wide range of salinities. Brine densities from April to August varied at different sampling places and ranged from 1.100 to 1.250 g/cm^3. From September to November, almost all samples had a density of more than 1.200 g/cm^3 (technological storage bittern density in winter), and from December to March the density of collected samples was lower (due to rainfall and snowmelt) (fig. 1).

Concentration of *D. salina* cells in natural populations varied from several thousands to several tens of thousands (usually up to 40) per 1 ml. Salt works operating season (April-August) was characterized by higher values and range of cells concentrations than winter. Cells concentrations did not correlate with brine densities (fig. 1).

Denser cell agglomerates formed occasionally at the brine surface in pond corners as wind wash. Stratification of ponds after rainfall contributed to formation of the agglomerates. On the surface of dense brine, rain or meltwater formed a film easily driven by wind. *D. salina* cells migrated into freshened surface layers, and before different density layers mixed up, huddled in lee pond corners into agglomerates which persisted long enough (until wind direction change). Cell concentration in one of such agglomerates (the corner of crystallization pond 5 of Heroyske salt works, 18.12.2006) reached 595 thousand per 1 ml, in the other (the corner of one Crimea soda plant pond, 12.07.2008) – 2.050 million per 1 ml. In such agglomerates, cells lost their motility, their surface became rough, that could indicate lesions of membrane integrity and cell death. So, high cell concentrations are not typical for natural populations under normal conditions, though they are very often used in laboratory studies.

D. salina "red" form color was due to wide range of cellular β-carotene content: from 15 to 100 pg per cell, rarely (March to July) – up to 150 pg per cell (fig. 1). Cellular β-carotene content and cells number or brine density did not correlate.

Brine nitrate concentrations never exceeded 80.8 mg/l calculated as KNO_3, and phosphate concentrations – 1.4 mg/l calculated as K_2HPO_4. In the most samples the latter was even below the detection limit. In the standard laboratory medium for culturing *D. salina* (Massyuk modification of Artari medium), the concentration of nitrate (2.5 g/l as KNO_3) exceeds the natural values more than 30 times, and the concentration of phosphate (200 mg/l as K_2HPO_4) – hundreds of times.

Natural brine had relatively low transparency: Secchi disk became invisible at the depth of 9–10 cm, i.e., 95% of light was completely absorbed at this depth, while the pond depth at sampling points ranged from 25 to 50 cm.

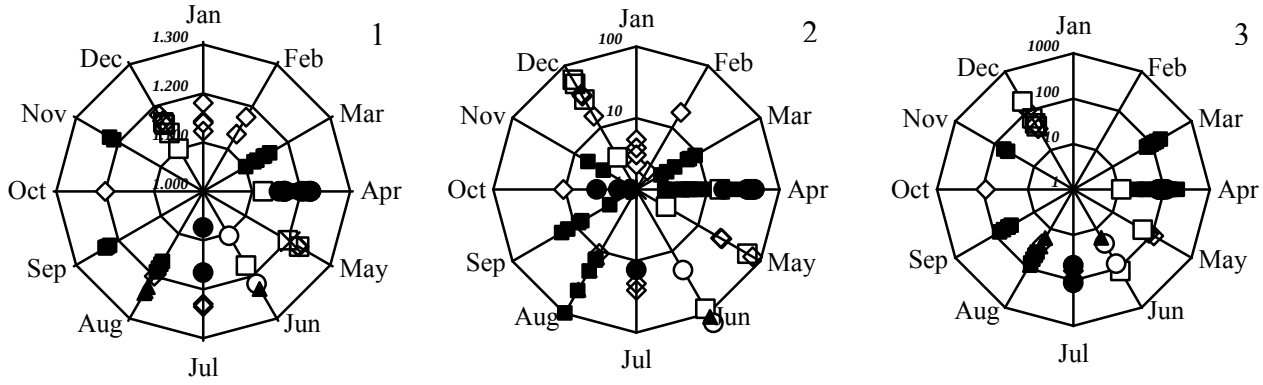

Fig. 1. Annual dynamics of brine density (g/cm^3) (1), *D. salina* cells concentration (thousand/ml) (2) and cellular β-carotene content (pg per cell) (3) in solar salt works ponds:

■ – *lake Sasyk-Sivash (45°11'36.91"N, 33°26'27.83"E)*
◊ – *lake Zmievo (46°29'19.84"N, 31°54'15.96"E)*
□ – *lake Garkushovo (46°28'54.04"N, 31°54'25.23"E)*
○ – *lake Dubove (46°29'9.67"N, 31°52'58.71"E)*
▲ – *lake Genicheske (46° 3'54.21"N, 34°45'40.99"E)*
● – *the western part of Sivash gulf (46° 5'38.33"N, 33°53'32.94"E)*
× – *lake Baskunchak (48°10'43.69"N, 46°53'37.75"E)*

No peculiarities associated with geographic location or specific reservoirs were revealed.

D. salina in salt works ponds of different technological purposes. To establish how *D. salina* population dynamics is associated with salt manufacturing process, the ponds of Heroyske salt works of different technological purposes were examined in detail. Salt works ponds system included the lake Zmievo broken by wood and gley banks and bridges into separate ponds, communicating through ditches, cut off by wooden shutters (fig. 2). The process of salt manufacturing is known from antiquity and based on the phenomenon of sequential fractionate salts crystallization at seawater evaporation (Baas-Becking, 1931). The technological cycle at Heroyske salt works began with pumping or drifting water to the pickle pond (N7, fig. 2) from nearby lakes connected with the sea via a series of lagoons fed by water of Yagorlytskiy bay of Black Sea. At natural water evaporation to brine density 1.120 g/cm^3, there occurred precipitation of poorly soluble calcium salts – carbonate (aragonite, $CaCO_3$), sulfate (gypsum, $CaSO_4$) and hydrophosphate ($CaHPO_4$). By that brine was purified from calcium salt impurities, undesirable, particularly in the food salt. Purified brine, which reached 1.200 g/cm^3 density, flowed by gravity through a system of ditches and shutters, into the crystallization ponds – salterns (NN5, 6 and 9, fig. 2), where, at further evaporation, NaCl crystallized. NaCl deposition was carried out until the brine density reached 1.240-1.250 g/cm^3, because at higher densities more bitter Mg salts admixed to the precipitate (Jakimov et al., 1934). During the operating season the cycle was performed repeatedly, new brine portions passed through the gypsum ponds into the crystallization ones diluting the brine to the density of pure NaCl crystallization. At the end of the season, dense brine, so-called bittern, was discharged from the crystallization into the bittern reserve ponds (NN10, 12, fig. 2) and salt was harvested by salt harvester machine. After harvesting salt and repairing pond banks and bottom, the gypsum and crystallization ponds were filled with bittern to prevent their damage and to maintain during winter salinity gradient necessary for the next season. Before the beginning of the next operating season (May-June), all the ponds were filled with reserve brine, prepared in the last season, slightly diluted because of rainfall and snowmelt. In the reserve ponds (NN10, 12) bittern was kept the whole operating season and, if necessary, was used to adjust the density of the brine in the gypsum and crystallization ponds in case of dilution by heavy rain.

Everyday operations at Heroyske salt works could depart from above described rough scheme, depending on weather conditions and ponds state, but the brine density values were generally consistent with the season and the technological purpose of the ponds. In March, the density of the brine in all the ponds was minimal – about 1.150 g/cm^3. The brine density in the reserve and gypsum ponds did not exceed 1.200 g/cm^3. In the crystallization ponds during summer months the brine density reached 1.250 g/cm^3 (fig. 2).

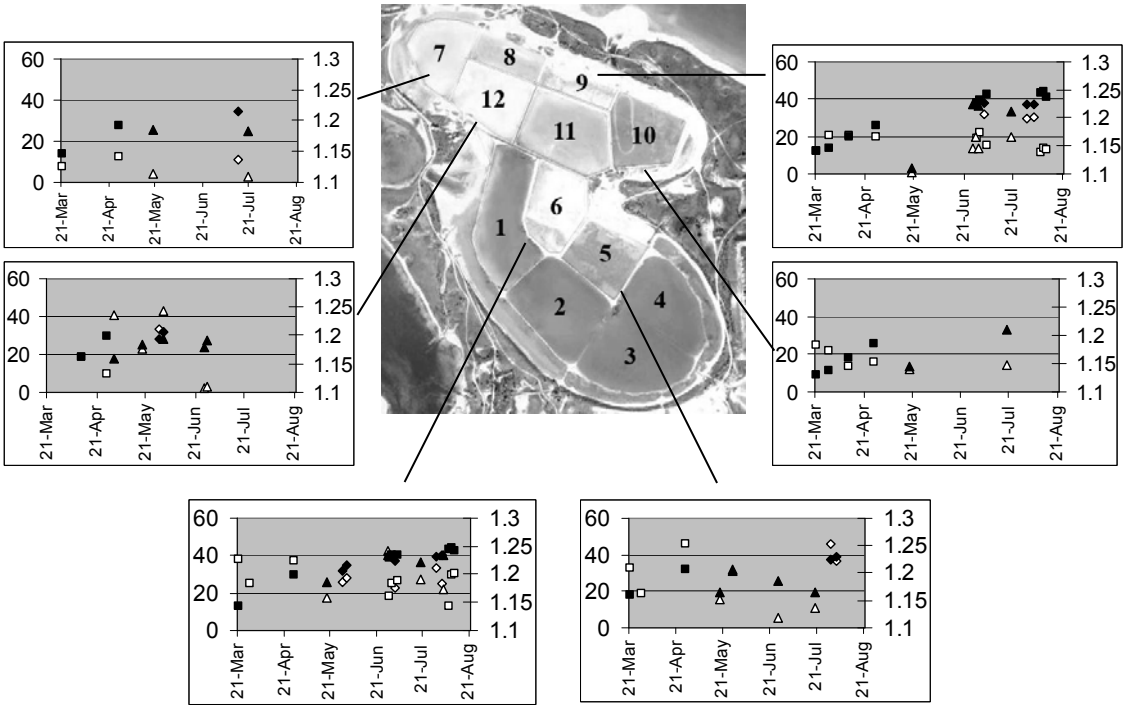

Fig. 2. Dynamics of brine density (♦,■,▲, g/cm^3, right scale) and *D. salina* cells concentrations (◊, □, Δ, thousand per 1 ml, left scale) in the ponds of Heroyske salt works in the season 2006 (♦,◊), 2007 (■,□) and 2008 (▲,Δ): *1–4 – the ponds were not exploited at the observation period; 5–6, 8–9, 11 – crystallization ponds; 7– preparation ponds; 10, 12 – bittern storage ponds.*

The gypsum and reserve ponds often contained relatively low number of *D. salina* cells (about 20 thousand per ml). In these ponds there was noticed massive development of the crustacean *Artemia salina* L., which feeds on algae and causes the decrease in its population. Large concentrations of *D. salina* cells (over 20 thousand per ml) were more frequently observed in the crystallization ponds (fig. 2). Here, at a relatively high salinity, *A. salina* did not develop. Furthermore, in the crystallization ponds algal populations might grow due to physical process of concentrating by brine evaporation, or as a result of cell divisions.

The range of β-carotene content variation in *D. salina* cells in Heroyske salt work ponds was consistent with that in the various reservoirs (fig. 1). Any pattern related to pond technological purpose, season, brine density and cells concentrations was not established.

Dependence of *D. salina* population dynamics on brine density and temperature. The two ponds of Heroyske salt works, N8 and N11 (fig. 2) were, with the kind permission of the salt works administration, temporarily withdrawn from the manufacturing process. Deliberate addition of fresh water from an artesian well compensated water evaporation in these ponds. Salinity value was adjusted to different levels and resulted from the balance between downhole pump operation time and evaporation rate, which itself depended on weather conditions (temperature, cloud cover, wind speed) (fig. 3). Experimental harvesting of the biomass caused periodic decreases of cells concentrations (fig. 3). Restoring cells concentrations significantly correlated with the brine density decrease below 1.17 g/cm^3. At higher salinities cell population did not grow. At that, for the 8th pond there were observed one-day lag time after brine dilution and moderate negative correlation between brine density and cells concentration increase (Spearman coefficient -0.36). For the 11th pond lag time reached 3 days and the correlation was strong (Spearman coefficient -0.84). Specificity of population responses in the different ponds could be due to their different geometry, which might affect homogeneity and oscillations amplitude of the conditions in the ponds. The 8th pond had smaller area (1 ha) and greater depth (35 cm), approximately the same in all parts of the pond. The 11th pond had larger area (2.5 ha) and smaller average depth (25 cm). The coastal areas of the 11th pond were quite shallow (3–5 cm), the conditions in the 11th pond were less homogeneous, and locally could change more

dramatically, but level slowly. All that could result in the relatively slow reaction but more pronounced response of the 11th pond population to brine dilution.

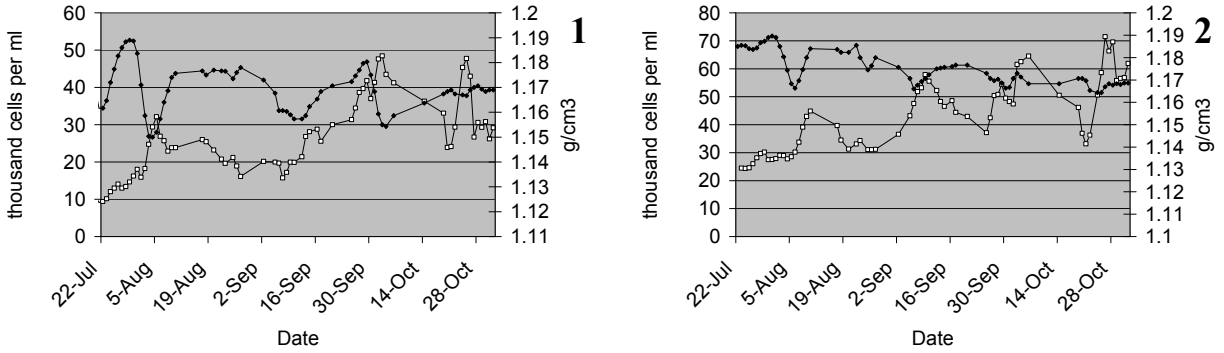

Fig. 3. Dynamics of brine density (♦) and *D. salina* cells concentration (□) in "Heroyske" salt works ponds with fresh water addition: 1 – pond N8, 2 – pond N11 (in 2006)

Contrary to the prevailing notions (Massyuk, 1973; Semeneko, Abdullaev, 1980) that the accumulation of β-carotene and *D. salina* cell division are strongly negatively correlated, in the both ponds we observed cell divisions of the "red" form, with high (more than 20 pg) β-carotene content per cell (fig. 4). Significant correlations between brine density, cells number and cellular β-carotene content were not revealed. In September-October cellular β-carotene content changed synchronously in the both ponds, with the sharper decline in the relatively shallow and heterogeneous pond N11. We hypothesized that those changes may relate to the temperature regime. To test this hypothesis, we took archival weather data for Gola Pristan' region during the observation period and calculated correlation coefficient between β-carotene content in microalgal cells and average, maximum and minimum temperatures. For a deeper, smaller in area and homogeneous by depth pond N8 significant correlations were not found. For shallower and diverse by depth pond 11 the significant relationship was established between the content of β-carotene in *D. salina* cells and the course of temperatures with the lag-time: reducing the average daily temperature below 20°C resulted in cellular β-carotene decrease in 3–4 days (Spearman correlation coefficient 0,64-0,69) (fig. 5).

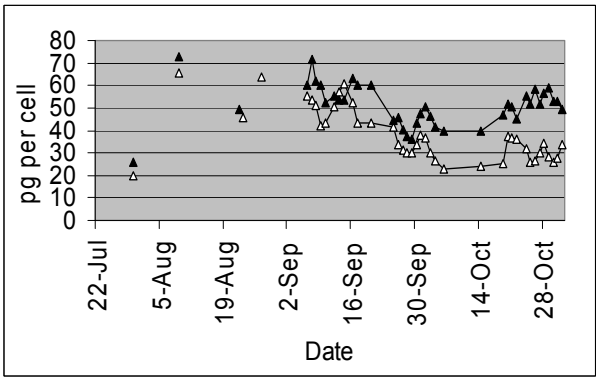

Fig. 4. Cellular β-carotene content in *D. salina* in "Heroyske" salt works ponds with fresh water addition: ▲ – *pond N8,* Δ – *pond N11 (in 2006)*

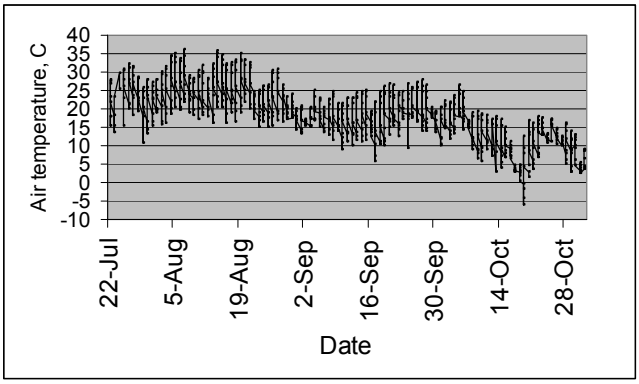

Fig. 5. Diurnal temperature range during stationary research at Heroyske salt works ponds N8 and N11 in 2006 *(according to the weather archive data at www.rp5.ru)*

When brine temperature dropped below 10°C (late October – early November) the algae cells migrated to the pond bottom and accumulated in the bottom layers of brine. In the beginning of March, the entire brine depth warmed to 12–15 °C and the algal cells left the bottom layers. We can conclude that at the South of Ukraine the active vegetation season of *D. salina* lasts on the average 8 months (from early March

to late October).

Discussion

Our observations of *D. salina* natural populations showed that most laboratory studies do not reproduce natural habitat of this species; especially the concentration of cells and the nutrient medium composition.

Initial culture density of millions of cells per 1 ml might rapidly inhibit cell division and biosynthesis as a result of self-shadowing, self-poisoning by exometabolites or contact inhibition, that does not occur under natural conditions (except very specific conditions in agglomerates of cells resulted from wind wash). Unbalanced and tens and hundreds times greater than the natural level, the content of nutrients in the culture medium might lead to artifacts in metabolism studies or even to changes in genotype of cells in culture due to selection of forms adapted to innormaly high trophic levels.

In reservoirs of the South of Ukraine and Russia, natural ability of *D. salina* cells to accumulate β-carotene reaches, according to our data, up to 100–150 pg per 1 cell. Open culture remains the most cost-effective way of industrial algae cultivation (Ben-Amotz, 2009). Finding ways to reliably control *D. salina* natural ability to accumulate β-carotene remains an urgent task that can only be resolved in the laboratory experiment. Field experiments, like our studies on brine dilution, are technically difficult, conclusions are subject to mixing effects of several factors (pond geometry, weather conditions, etc.). Laboratory experiment gives a researcher the opportunity to study simultaneously large number of factors and their combinations with maximum control of all the rest. Experimental conditions must be adjusted to the data on the species ecology in nature – the algae must be grown in so called natural culture. So, as a result of adjusting cell concentration and nutrients levels, we established an independent influence of nitrogen and phosphorus deficiency on β-carotene accumulation, ceteris the other optimal culture conditions: salinity, light, and temperature (Komaristaya et al., 2010). From the natural culture approach, investigation of sulfur deficiency (Giordano et al., 2000) can only have theoretical value, because natural brine – the product of sea water concentrating – is rich in sulfates, and sulfur deficiency conditions are not typical of salt works waters.

Brine salinity is another important environmental factor in determining *D. salina* habitat specificity. And, although *D. salina* remained viable through wide range of salinities – up to NaCl crystallization – noticeable cells number increase was observed only at brine densities below 1.170 g/cm^3.

Intense red "bloom" of salt works reserve ponds (Massyuk, 1973), in which brine density most of the time exceeds 1.170 g/cm^3 might have less to do with *D. salina* cells division as with physical concentration of algal cells during salt works operation. Brine concentrates approximately 2-fold at Ca salts precipitation in gypsum ponds, and 10-fold – at NaCl crystallization in salterns (Yakimov et al., 1934). Given new portions of prepared brine enters each crystallization pond several times per season (at least once every 2 weeks under favorable weather conditions), *D. salina* cells massively concentrate and accumulate in salterns. Similarly, spring outbreak of red "bloom" in bittern (Massyuk, 1973) can mostly be explained by cells accumulation during previous season of salt manufacturing, their wintering at the bottom and further distribution over pond depth when the temperature rises again in spring. Unbiased effect of salinity should be thoroughly studied in the laboratory experiment in all the natural range for *D. salina* habitats: 1.100–1.250 g/cm^3.

The most difficult is to recreate natural irradiation conditions with artificial light sources, so in this respect the laboratory experiment inevitably remains an approximation to natural conditions. This applies to light intensity, light spectral composition, photoperiod, daily and seasonal changes in irradiation. This problem could theoretically be solved by the methods of modeling, model parameters and their statistical significance should be determined experimentally. A priori from the literature data (Ben-Amotz, Avron, 1983), it can be concluded that the total amount of light energy received by the cells will have the greatest impact on their vital activity. Close to the latitude of the habitats studied, the maximum irradiance occurs at noon on a clear day in late June, and comprises just over 90 klux (Sharonov, 1953; Leman, 1976).

In the laboratory we managed to create irradiances from 1 to 15 klux using «Maxus» lamps with the color temperature 2700 K (warm white light close to natural sunlight) and reflective foil screens. Layer thickness in the culture flasks did not exceed 2 cm. Irradiance at horizontal surface in summer cloudy day may drop to 24 klux or even down to 2 klux on the dawn or sunset (Sharonov, 1953). Taking into account high opacity of natural brine (95% of light absorption occurs at 10 cm depth by Secchi disk), irradiance in our laboratory experiments can be considered close to natural.

Temperature, according to our observations, impacts β-carotene content in *D. salina* cells and their vital activity. It is possible that low and negative temperatures influence the formation of cysts in *D. salina*, as

we observed germinating cysts in spring samples. All the natural temperature range can be modeled and should be studied in the laboratory.

Although our findings were limited geographically and in time, hyperhaline lakes exist in the arid zone around the globe, and salt works operate already thousands of years. These habitats are unique in their typology, but very stable and similar. In this regard salt work ponds at the South of Ukraine and Russia do not differ from other reservoirs of the same typology in the world.

Comparable values of β-carotene content (85–185 pg per 1 cell) were indicated for *D. salina* in ponds of Eilat salt works in Israel. Cell numbers on 23.03.1992 was low (0.16–1.02 thousand per 1 ml) (Oren et al., 1992), as in our reservoirs in March. In solar salterns near Alicante (Spain) in May *D. salina* cell numbers varied from approximately from 5 to 10 thousand per 1 ml (Joint et al., 2002) and in August – from 10^3 to 10^5 cells per ml (Rodriguez-Valera et al., 1985), the same range that we observed in our reservoirs. Apparently, very high concentrations of cells (up to 970 thousand per 1 ml) in the ponds of salt works "Halite" (lake Sasyk-Sivash) in 2008–2009 indicated in the work (Gudvilovich, 2010) corresponded to local agglomerates of cells in the leeward pond corners.

Salterns worldwide vary in brine nutrients concentrations: nitrate and ammonium nitrogen in the range 0–500 μM (0–50 mg/l as KNO_3), phosphate in the range 0–60 μM (0–10 mg/l as K_2HPO_4) (Oren, 2009), that is also close to our data. Revealed relatively low concentrations of nutrients in the ponds are likely due to the activity of algo-bacterial mats. Maintaining active vegetative benthic communities in gypsum ponds comprises part of the biological management of salt works and plays an important role in manufacturing salt of high quality (Gongora et al., 2005).

Special attention should be paid to the possibility of using salt works of the South of Ukraine as a base for industrial cultivation of *D. salina* without violation of salt manufacturing technology. Salt works ideally feet for *D. salina* industrial cultivation by their intended purpose (artificial technical reservoirs), sanitary requirements (designed to produce food-grade salt), and infrastructure (means of brine salinity control). It is obvious that *D. salina* population grows actively at gypsum ponds salinity, but not in crystallization ponds. Therefore, maximum biomass growth requires the other conditions in gypsum ponds made optimum (determined experimentally in the laboratory), including adding fertilizers and inoculum pre-grown in the laboratory. Adding fertilizers is not something inadmissible as it is a usual measure of biological manadgement of salterns to enhance the development of benthic and planctonic microbial communities that play an important role in salt quality (Davis, 1993). *D. salina* biomass could be collected from crystallization ponds that would purify salt from organic admixtures improving crystals size and quality.

D. salina remains one of the few alternatives to synthetic β-carotene in food ingredients market. Development of measures for the effective management of β-carotene biosynthesis in the culture will allow this product to compete by the price with synthetic β-carotene. Replacing synthetic ingredients for the natural ones reduces undesirable impurities (by-products of chemical synthesis) in human diet and promotes quality of life.

Our survey of natural habitats of *D. salina* showed that for simulation of this alga natural populations in the laboratory experiment the initial concentration of cells should be set in the range from several tens to about 100 thousand cells per 1 ml, nitrogen concentrations (as KNO_3) – within a few tens of mg per 1 liter, phosphorus concentrations (as K_2HPO_4) – several mg per 1 liter. Natural irradiance levels, the average for the entire thickness of shallow salt works ponds, could be achieved in the laboratory, using light sources that provide on the surface of 2 cm thick culture illumination of 1–15 klux. It is also advisable to investigate the effects of a wide range of salinity (brine density in the range from 1.100 to 1.250 g/cm^3), and the temperature including low and negative values.

Thus, field and stationary studies could contribute to laboratory experimental research.

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