PHOTOMOVEMENT OF TWO SPECIES OF *DUNALIELLA* TEOD. (*CHLOROPHYTA*)

Yuriy I. Posudin[1], Nadiya P. Massjuk[2], Galyna G. Llilitskaya[3]

[1]National University of Life and Environmental Sciences of Ukraine,
Geroiv Oborony st., 15, Kiev, 03041 Ukraine
posudin@yahoo.com

[2,3]M.G. Kholodny Institute of Botany of National Academy of Sciences of Ukraine,
2, Tereshchenkivska st., 01601, Kiev, Ukraine

This review is focused on photomovement of two species of green alga Dunaliella Teod., D. salina Teod. and D. viridis Teod., as the principal organisms investigated.

Problems in terminology and a logical basis for classification of photomovement in microorganisms are discussed. The results of experimental investigations on the critical factors controlling and modulating photomovement are described and include the effects of various abiotic factors, critical aspects of photomovement such as photoreception (i.e., location and structure of photoreceptor systems, composition of photoreceptor pigments, mechanisms of photoreception and photoorientation), sensory transduction of absorbed light into signals that govern the activity of the motor apparatus and flagellar activity.

Various aspects involved in the utilization of the species as models for studying photomovement, such as testing aquatic media and the effects of surface-active substances, salts of heavy metals and pesticides on algal photomovement parameters are described. Vector methods for testing are proposed for assessing the action of various chemicals. Likewise, the potential of using the two species as organisms for transgenic alteration, such as enhanced production of β-carotene, ascorbic and dehydroascorbic acids, glycerin and other valuable organic compounds are described. The main tendencies and perspectives for further photomovement investigations in flagellates are discussed.

Introduction

In a broad context the term *photomovement* encompasses any movement or its alteration induced by light. Photomovement is the result of the *photoregulation of movement* – which includes an entire complex of elementary processes caused by a light stimulus such as photoreception, primary reactions of the photoreceptor pigments, and the sensory transduction of the light stimulus into a physiological signal that governs the activity of the motor apparatus and results in the photoorientation of the organism.

The study of photomovement and the photoregulation of movement in microorganisms is of considerable interest due to the importance of these phenomena and that they are closely tied to fundamental biological processes such as photosynthesis, photoreception, energy transformation, membrane-coupled and membrane-mediated phenomena. The investigation of photomovement and its photoregulation are also closely tied to the elucidation of the basic principles of intracellular developmental processes, as well as ontogenesis, embryogenesis, and morphogenesis. A better understanding of light mediated responses impacts our understanding of light's role in the ecology and biocenology of these organisms since light is an important factor in their spatial and temporal distribution. While photomovement has an independent function, it also conveys information on the complexity of related environmental factors (e.g., temperature, pH, biogenesis of compounds, oxygen content, the presence of other microorganisms.

The investigation of photomovement mechanisms is also of interest from the standpoint of bionics, evolutionary biology, morphology, phylogeny, and systematics. It is known, for example, that the structure of the motor apparatus and photoreceptor is an important systematic character at higher taxonomic levels (divisions and classes) in phycology (Hoek Van den et al., 1995; Graham and Wilcox, 2000; Posudin et al., 2010). Thus, it is possible to assume the specificity of the mechanisms of photoperception and photoregulation of photomovement among members of different divisions or classes of algae. Finally, the study of photomovement has the potential for stimulating the practical application of this technology in areas such as biomonitoring of the environment, biotechnology, and the use of these organisms for the synthesis of useful natural products.

Our primary focus with regard to experimental and methodological approaches has been the investigation of the location and structure of the photoreceptor system, the composition of photoreceptor pigments, the mechanisms of photoreception and photoorientation, the processes of sensory transduction, and the activity of the motor apparatus in the two species. Comparison of photomovement parameters between two species of the same genus is likewise of taxonomic interest. The authors assessed the experimental and methodological techniques needed to facilitate understanding the key processes of photomovement in these species since they had not been previously studied. It was also imperative to understand the effect of environmental factors such as ultraviolet and visible radiation, temperature, pH, and electrical fields on the photomovement parameters in these species.

The potential of algal biotechnology is likewise addressed. Both species represent possible organisms for the commercial production of β-carotene (provitamin A), ascorbic and dehydroascorbic acids, glycerol, feed for fish production, and other products. Assessment of changes in photomovement by these organisms can also potentially be used as biosensors for assessing the composition of aquatic media.
A comparative analysis of both general and specific differences in photomovement among these flagellated algae species and representatives of different orders (classes) of algae is also reported.

The *main objective* of this review is to critique the current understanding of photomovement in the unicellular green alga *Dunaliella* Teod., particularly two species *D. salina* and *D. viridis*.
The *specific aims* of this work are:
1. Describe theoretical problems in terminology and the logic of the existing method for the classification of photomovement in these microorganisms;
2. Elucidate the primary characteristics of *D. salina* and *D. viridis*;
3. Critique the experimental methods utilized for the measurement of photomovement of these species and the effects of abiotic factors on photomovement;
4. Describe the processes of photoreception – location and structure of phootoreceptor systems, composition of photoreceptor pigments, mechanisms of photoreception, and photoorientation of the two species;

5. Describe the processes of sensory transduction of absorbed light into signals that govern the activity of the motor apparatus of the two species;
6. Assess the possible application of *D. salina* and *D. viridis* as models for testing the quality of aquatic media and estimating the effects of surface-active substances, salts of heavy metals, and pesticides on photomovement in algae;
7. Assess the potential of the two species of *Dunaliella* for transgenic alteration to enhance the synthesis of β-carotene, ascorbic and dehydroascorbic acids, glycerol and other valuable organic compounds;
8. Asses the implication of photomovement on evolutionary biology, phylogenetics, systematics and taxonomy, ecology and geography of algae;
9.Critique critical areas for future research on the biology of photomovement in flagellates.
All the results of our investigation of photomovement of *Dunaliella* Teod. are reflected in monograph (Posudin et al., 2010).

Characteristics of the Test Species
Unialgal cultures of two species of *Dunaliella*, *D. salina* Teod. strain №10 and *D. viridis* Teod. strain №42, from the collection of the N.G. Kholodny Institute of Botany, Ukrainian Academy of Sciences (Posudin et al., 2010), were used in this study. Information about the genus *Dunaliella* may be found in a monograph by Massjuk (1973) that contains extensive references detailing the major advances in research on the genus.

The species of *Dunaliella* described herein are distinguished by differences in the cell shape and size. The cells of *D. salina* are 5 to 29 μm in length and 4 to 20 μm wide, while *D. viridis* is 3 to 18 μm in length and 2 to 15 μm wide (Massjuk, 1973). The length of the flagella of *D. salina* is approximately equal to the length of the cell, while in *D. viridis* flagella are 1,3 times longer than the cell (Figure 1).

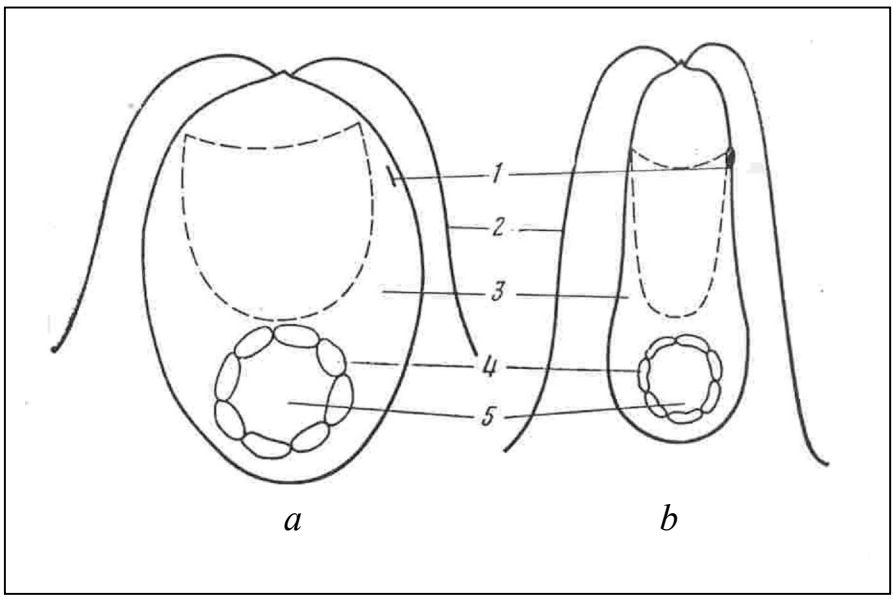
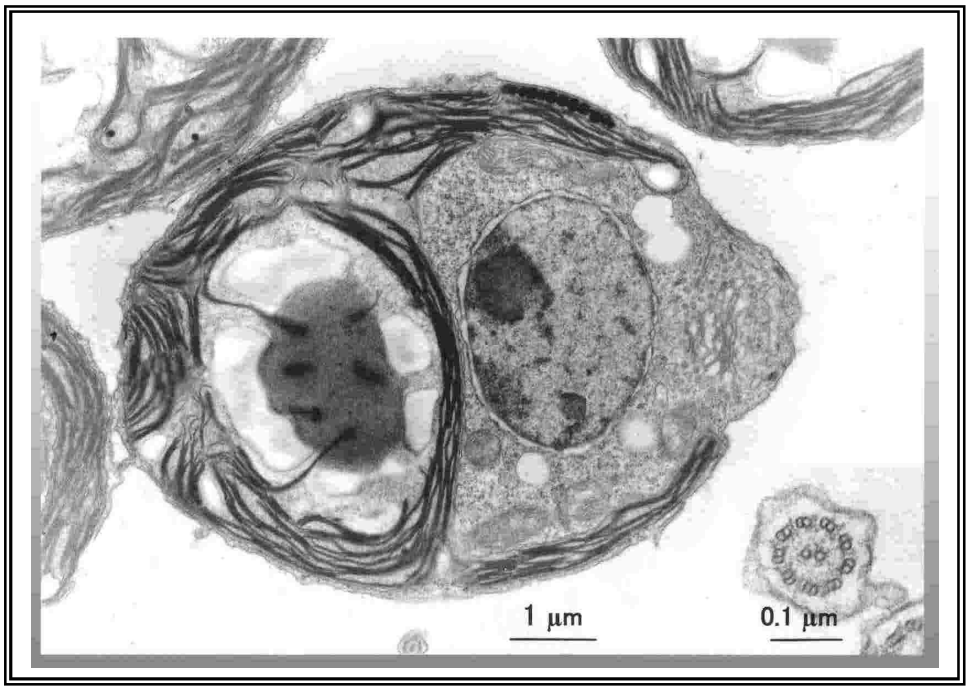


Figure 1. General schematic of two species of *Dunaliella*: *a – D. salina; b – D. viridis* where 1 – stigma; 2 – flagella; 3 – chloroplast; 4 – starch; 5 – pyrenoid (Posudin et al., 2010).

An image of *Dunaliella* sp. from an electron microscope is given in Photograph 1.



Photograph 1. An image of *Dunaliella* sp. from an electron microscope. Courtesy of Prof. Shogo Nakamura (Toyama University, Japan)

Experimental Installation

To study photomovement in *Dunaliell*a, a special experimental videomicrography system was developed (Figure 2). It allows the observation and measurement of the velocity and direction of movement of individual cells as modulated by light stimulus parameters. The system utilizes a microscope connected to a light source, monochromator and videosystem (Posudin et al., 2010).

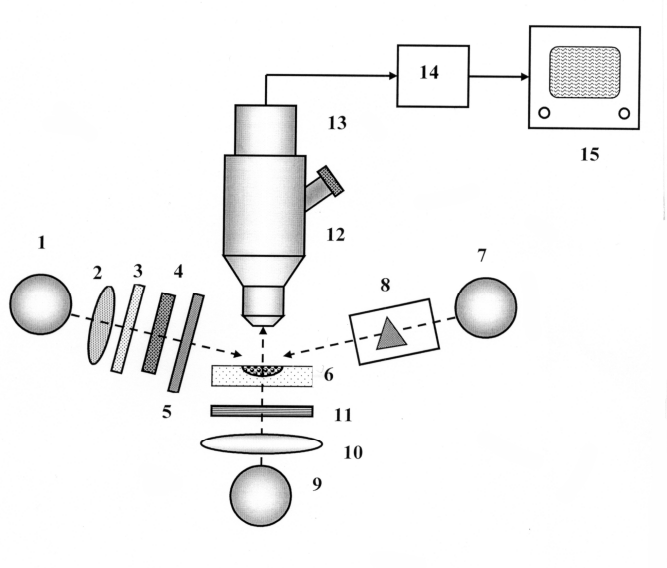


Figure 2. A schematic of experimental videomicrography for studying photomovement in algae: *1* – source of white light; *2* – collimator; *3* – glass infrared filter; *4* – liquid infrared filter; *5* – interference filter; *6* – sample in a concave slide; *7* – halogen lamp; *8* – monochromator; *9* – source of white light; *10* – condenser; *11* – polarizer; *12* – microscope; *13* – videocamera; *14* – coupling unit; *15* – monitor [Posudin et al., 1992, 1996].

Problems of terminology

Critical considerations in the terminology and classification of different types of light-induced behavior of freely motile organisms have shown that the existing classification systems create considerable terminological confusion. As a consequence, we developed a *parametrical classification system* for the light-dependent behavior of either individual motile cells (individual effect or micro-effect) or their aggregations (group effect or macro-effect).

It is known, that the light stimulus can be characterized by parameters such as intensity (I), direction ($\vec{s}$), the spectral composition (λ), polarization (P), duration and frequency of light pulses, etc. Similar to the movement parameters, the light parameters may be divided into: the intensity of light that is characterized by absolute magnitude (I) and its gradient in space (dI/dx) and time (dI/dt). According to the parametrical character of either movement of organisms or light stimulus we propose the classification of phototaxes (as the dependence of the organism movement on the light stimulus) on the basis of the *parametrical principle* for both freely single cells, which is presented schematically in Tables 2.1 (so-called individual or microeffect) and 2.2 (group or macroeffect). To this end, we retained the original meaning of the term *phototaxis* as any light-induced movement of freely motile organisms in space. Light-dependent *reactions* of motile organisms (*photoresponse, photoreaction*) are considered any immediate motion responses by the organism to any change in the light stimulus.

2.1. Photoresponses of Individual Organisms

Parameters of light stimulus / Parameters of movement	Intensity I	Gradients of intensity dI/dt	Gradients of intensity dI/dx	Direction $\vec{s}$	Wavelength λ	Polarization P
Velocity of individuals: ■ linear $\vec{\upsilon}$ ■ angular ω (frequency of spatial changes of trajectory of individuals: oscillations, rotations, trembling)	$\vec{\upsilon}\ (I)$ $\omega(I)$	$\vec{\upsilon}\ (dI/dt)$ $\omega(dI/dt)$	$\vec{\upsilon}\ (dI/dx)$ $\omega(dI/dx)$	$\vec{\upsilon}\ (\vec{s}\ )$ $\omega(\vec{s}\ )$	$\vec{\upsilon}\ (\lambda)$ $\omega(\lambda)$	$\vec{\upsilon}\ (P)$ $\omega(P)$
Direction of movement of individual $\vec{r}$	$\vec{r}\ (I)$	$\vec{r}\ (dI/dt)$	$\vec{r}\ (dI/dx)$	$\vec{r}\ (\vec{s}\ )$	$\vec{r}\ (\lambda)$	$\vec{r}\ (P)$
Trajectory of movement of individual l	$l(I)$	$l(dI/dt)$	$l(dI/dx)$	$l(\vec{s}\ )$	$l\ (\lambda)$	$l(P)$
Frequency of spatial changes in trajectory of individual (oscillations, trembling, rotations) n	$n(I)$	$n(dI/dt)$	$n(dI/dx)$	$n(\vec{s}\ )$	$n(\lambda)$	$n(P)$

2.2. Photoresponses of Populations and Colonies

Parameters of light stimulus / Parameters of movement	Intensity I	Gradients of intensity dI/dt	Gradients of intensity dI/dx	Direction $\vec{s}$	Wavelength λ	Polarization P
Concentration N of individuals (optical density) in population or colony	$N\ (I)$	$N\ (dI/dt)$	$N\ (dI/dx)$	$N(\vec{s}\ )$	$N(\lambda)$	$N(P)$
Shape (spatial distribution) of individuals in	$S\ (I)$	$S\ (dI/dt)$	$S\ (dI/dx)$	$S(\vec{s}\ )$	$S\ (\lambda)$	$S(P)$

population or colony S						
Trajectory of movement of population or colony L	$L(I)$	$L(dI/dt)$	$L(dI/dx)$	$L(\vec{s})$	$L(\lambda)$	$L(P)$
Relative number of individuals performing a response N/N_0	$N/N_0(I)$	$N/N_0(dI/dt)$	$N/N_0(dI/dx)$	$N/N_0(\vec{s})$	$N/N_0(\lambda)$	$N/N_0(P)$

Motility of biological objects is a special case in the general physical phenomenon of movement (mobility). Therefore, motility of organisms can be described by such well known parameters as speed or velocity ($\vec{v}$), direction (r), and trajectory (l) of movement. The light stimulus, in turn, can be characterized by such parameters as intensity (I), direction (s), spectral composition (λ), and polarization (P) of light and by the duration, frequency, and the shape of light pulses. Considering the parametrical characteristics of both factors (light and movement), we believe that any classification of the dependence of movement (phototaxis) of microorganisms on light should be based on a parametrical principle. We therefore propose defining *photokinesis* as any dependence of speed of individual organisms or their groups on any parameters of a light stimulus. *Phototopotaxis* is any dependence of the direction of movement of individual organisms or their groups on any light parameter.

The proposed classification can be further developed in greater detail by accounting for additional parameters of movement and light (e.g., the rhythm of a light flux), their possible interactions (e.g., wavelength and intensity of light, velocity and direction of movement), or specific features of certain parameters (e.g., velocity of movement can be linear or angular, light intensity can be characterized by its absolute value (*I*) or its gradient in space (*dI/dx*) and time (*dI/dt*)). The suggested principles not only promote an improvement in the existing terminology but also facilitate planning further research (Massjuk et al., 1991; Massjuk and Posudin, 2002).

Phenomenology of Photomovement

Species of *Dunaliella*, as well as other flagellate algae, move freely in an aquatic environment under the influence of light, a response termed *photomovement* (*phototaxis*). Photomovement involves a translational movement of a cell that is accompanied by rotation of the cell around its longitudinal axis, sidewise turns from the main direction, and oscillatory movements ("staying in one place"). Sometimes the cell is attached by the distal ends of the flagella to the substratum, convulsively twitching around the attachment site. When the cell becomes detached from the substratum, it continues free "navigation".

In contrast to the pattern observed in *Chlamydomonas reinhardtii* P.A. Dang. and *Euglena gracilis* G.A. Klebs, only the ciliary type of flagella movement is observed in *Dunaliella* during photomovement. Only in unusual cases, when mechanical obstacles

are present and/or when a *Dunaliella salina* Teod. cell becomes constrained in a very thin preparation between the cover glass and the slide, does it switch to a more ancient undulating type of flagella movement.
The trajectory of locomotion of a cell resembles a sine wave and its plane projection looks like a non-uniform zigzag. It is believed that, similar to *Chlamydomonas*, *Dunaliella* performs rotary cell movements around its longitudinal axis that are mediated by the beating of its flagella in the three-dimensional space. The sinusoid movement is a result of an almost synchronous but unequal number of beatings between the cell's two flagella. In contrast to the pattern observed in *Chlamydomonas,* when a change occurs in the movement direction, one flagellum of the *Dunaliella* cell ceases beating, while the second continues to beat, causing the cell to turn. Subsequently, the first flagellum renews its beating and the cell moves in the new direction.

Photoreactions
With regard to photoreactions, algae may be expressed through: 1. a change in the velocity (*kinetic reaction*); 2. a change in the direction and trajectory of movement (*vector reaction*) and 3. a simultaneous change in the velocity and direction of movement (*photophobic reaction*).
Species *of Dunaliella* are capable of photokinetic and vector reactions. In contrast, the presence of photokinetic reactions in *Chlamydomonas* has been challenged in the literature. We did not observe photophobic reactions in *Dunaliella*, though they are present in *Chlamydomonas* and *Euglena*. There are, however, several publications indicating the possibility of such reactions in *Dunaliella* (Wayne et al., 1991).

Photokinesis
The locomotion velocity of *Dunaliella* cells (*photokinesis*) depends on the intensity of the light stimulus and the environmental conditions, such as temperature, strength of electric and electromagnetic fields, intensity and duration of ionizing radiation, and the presence and concentration of calcium channel blockers (such as isoptin and cinnarizine), sodium aside, surfactants, salts of heavy metals, and pesticides. Likewise, a combination of factors can modulate velocity. We did not find the locomotion velocity of individual *Dunaliella* cells depended on the wavelength of the light, dosage of preliminary UV-irradiation, concentration of cobalt and calcium ions, the presence of ionophores that increase the permeability of the cellular membrane to calcium ions, and the presence of ionotropic preparations that stimulate Na^+-K^+-ATPase.

The average cell velocity of translational movement of hyperhalobic species of *Dunaliella* was 36±2 μm/s for *Dunaliella viridis* and 48±2 μm/s for *D. salina*. The average velocity of movement for these two closely related species among experiments varied over a wide range, indicating that distinctions between the species based on velocity probably are not valid. The modal value for the average velocity of movement of the marine species *Dunaliella bioculata* Butcher was 105±5 μm/s. The values for the *Dunaliella* species exceed by 1-3 orders of magnitude those of microorganisms not possessing a flagellar apparatus and are within the limits known for others flagellates, both prokaryotic and eukaryotic. However, the average locomotion velocity of hyperhalobic species of *Dunaliella* is lower (sometimes by an

order of magnitude) than in the marine species *D. bioculata* and the freshwater species *C. reinhardtii* and *E. gracilis*. These differences may be caused by variation in the viscosity of the media.

The average cell velocities for rotary movement in hyperhalobic species of *Dunaliella* were nearly identical [i.e., 0.52±0.04 rotations (revolutions) per second in *D. salina* and 0.54±0.04 rotations per second in *D. viridis*]. These values, however, were lower than in the freshwater algae *C. reinhardtii*. The maximum average velocity values for forward and rotary cell photomovement in *Dunaliella* were observed under the following conditions: white-light intensity of 0.22–0.81 W/m^2, illuminance of 150–550 lx, temperatures of 20–30 °C, and pH 8.

The rhythmic regulation of cell movement in *Haematococcus pluvialis* Flotow is caused by rhythmic pulses, presumably related to the functioning of contractile vacuoles (Sineshchekov, 1991). However, in hyperhalobic species *of Dunaliella*, the contractile (pulsating) vacuoles are absent and the beating rhythm of flagella is apparently related to other oscillators. The flagellar beating frequency in hyperhalobic species of *Dunaliella* is 25–50 Hz while in freshwater *C. reinhardtii* it is as much as 64 Hz.

Phototopotaxis

The direction of cell movement of *Dunaliella* in relation to the light source (*phototopotaxis*) depends on the parameters of the light signal (e.g., light intensity, spectral composition, gradients of intensity in space and time, polarization) and environmental conditions (e.g., temperature, intensity of electric fields, level of ionizing and UV radiation, concentration of Ca^{2+}, Co^{2+}, cinnarizine, isoptin, sodium aside, surfactants, salts of heavy metals and pesticides), and combinations of these factors. Phototopotaxis of *Dunaliella* species, however, is not modulated by ionotropic preparations that stimulate a membrane Na^+-K^+-ATPase.

Phototopotaxis of both hyperhalobic species of *Dunaliella* in laboratory cultures was observed at an illuminance of 500 lx (positive) and 40,000 lx (negative). Transition from positive to negative phototopotaxis was observed at 1,500 lx. These parameters are within the limits known for other algae. However, sensitivity thresholds to weak and strong illuminance, and transitions from positive to negative phototopotaxis differ substantially among algal species. They allow assessing shade-tolerance, sun-tolerance, and resistance to high-level light exposure of these species.

D. viridis is more sensitive to weak light (30 lx) than *D. salina*, which corresponds to behavioral peculiarities between the two species in nature. In laboratory culture, both species are more sensitive to high illuminance than *C. reinhardtii*; in the latter, the transition to negative phototopotaxis occurred at 100,000 lx.

The transition of *Dunaliella* species from positive to negative phototopotaxis differs from that in chlamydomonads. In contrast to chlamydomonads, the change in flagellar beating from a ciliary to an undulate mode was not observed in the *Dunaliella* species. The beating of only one flagellum was observed, which caused turning of the cell followed by its subsequent movement in the opposite direction to the light

source.
Under the conditions found in Crimean hyperhaline watersheds with high illuminance (i.e., above 100,000 lx), natural populations of *D. salina* are found in the “red form” and display a complete absence of negative phototopotaxis. Thus, hyperhalobic species *D. salina* and *D. viridis* differ in their degree of sensitivity to both high- and low-light intensity which explains differences in the ecological niches occupied by the species in nature.

The maximum values for positive and negative phototopotaxis were found at 20–30 °C and the maximum value for positive phototopotaxis was at pH 7.35. Electric fields suppress phototopotaxis in *Dunaliella* as well as in other algae, which indicates the participation of bioelectric potentials in this process. Increasing the temperature from 18 °C up to 30 °C removes the inhibitory influence of the electric field density and stimulates phototopotaxis in both hyperhalobic species of *Dunaliella.* Ionizing and UV irradiation inhibit phototopotaxis in *D. salina* and *D. viridis.* The inhibiting effect depends on the irradiation dosage; in the case of UV-irradiation, the response also depends on the wavelength. We described for the first time in *Dunaliella* the transformation from positive phototopotaxis to negative due to the influence of UV-irradiation; at high levels of irradiation, phototopotaxis can be completely blocked.

The maximum values for phototopotaxis in hyperhalobic species of *Dunaliella* were observed when $CaCl_2 \cdot 6H_2O$ was in the medium at concentrations between 10^{-5} and 10^{-3} M. Increasing the calcium chloride concentration up to 10^{-2} M suppressed phototopotaxis by 10–20 %. The addition of the ionophore A23187 to the medium increased the permeability of the cellular membrane to calcium ions causing a complete inhibition of phototopotaxis both in *D. salina* and *D. viridis*. The addition of $CoCl_2$, that blocks membrane calcium channels at concentrations from 10^{-6} to 10^{-3} M, suppressed phototopotaxis in both species of *Dunaliella*. Other calcium channel blockers, such as cinnarizine, isoptin, and sodium aside, elicit a similar effect on phototopotaxis in *Dunaliella.* Ouabain, which stimulates Na^+-K^+-ATPase, does not influence phototopotaxis in *Dunaliella.*

The phototopotaxis action spectrum for the two hyperhalobic species of *Dunaliella* was identical. It occurred between 400 and 520 nanometers (nm) and has maxima at 410–415 nm and 465–475 nm. The action spectrum for phototopotaxis in *Dunaliella* differs somewhat from those in *Chlamydomonas reinhardtii* and *Haematococcus pluvialis* which display a wide band in the 400–600 nm range, with the maximum at 500 nm. In contrast to the situation observed in representatives of the *Chlorophyceae*, *Tetraselmis viridis* Rouch. (*Chlorodendrophyceae*) exhibited phototopotaxis in the UV region of the electromagnetic spectrum. In *E. gracilis* (*Euglenophyta*), phototopotaxis occurs in the 300–550 nm range with two basic maxima at 385 nm and 460 nm and two smaller maxima at 410 nm and 490 nm. Thus, representatives of different genera, classes, and divisions differ distinctly in their action spectra for phototopotaxis indicating differences in their photoreceptor systems and the composition of photoreceptor pigments.

Motility

One parameter of photomovement is the motility of cells or the relative number of motile cells (N_m/N_0), where N_m is the number of motile cells, and N_0 – the total number of motile and non-motile cells. In populations of *Dunaliella* this varies from 0 to 100 % and displays the same dependence on the characteristics of the light stimulus and environmental conditions as phototopotaxis. The number of motile cells differs with the presence or absence and degree of dependence due to certain factors (e.g., wavelength of light, dose of preliminary ionizing and UV irradiation, concentration of compounds opening or blocking calcium channels) on the velocity of individual cells (photokinesis).

Photoreceptor System

The photoreceptor system of *Dunaliella* species, as well as that of other green algae, consists of a photoreceptor, presumably located in plasmalemma and membranes of the chloroplast (in the area near the stigma), and a stigma that consists in different species of one to two layers of lipid globules located in the peripheral zone of the plastid. It has been shown that, in contrast to algae such as *E. gracilis*, species of *Dunaliella* do not possess a photoreceptor with a dichroic structure.

Mechanisms of Photoreception

Photoreception in *Dunaliella* and probably in certain other motile microorganisms with flagella, is based on the interaction of several mechanisms: modulation, diffraction, and interference. The modulation mechanism is due to the rotary movement of the cell around its longitudinal axis during which the stigma modulates the light signal affecting the photoreceptor. With the presence of more than one layer of pigmented globules in the stigma, an interference mechanism in photoreception is possible (similar to that in chlamydomonads). We believe the photoreception diffraction mechanism is universal for all flagellates having a globular stigma structure (Figure 3).

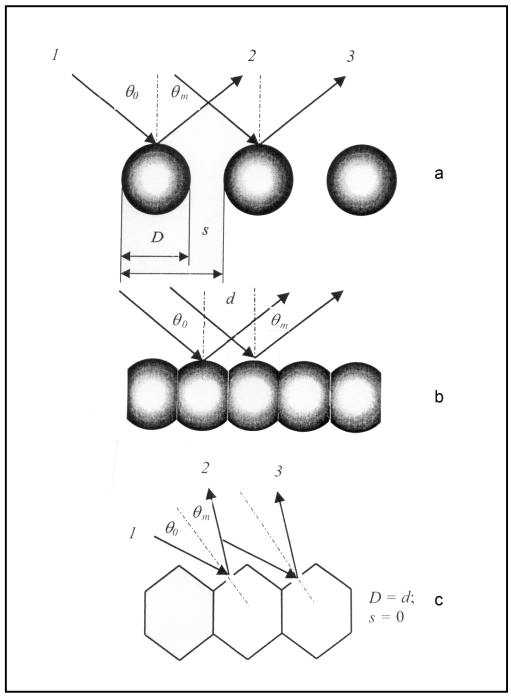


Figure 3. Schematic of the optical phenomena that occur during the interaction of light with the structure formed by spherical (*a,b*) or hexagonal (*c*) globules that are densely packed due to mutual compression, where: θ_0 and θ_m are the angles of incidence and diffraction, respectively; $d = D + s$ − period of diffraction grating (*D* is diameter of the globule and *s* is the interval between globules); *1* − incident beam of light; *2,3* − diffracted beams of light (Posudin and Massjuk, 1997).

Thus, during photoreception in these flagellates (including *Dunaliella*), there is a cooperative effect during the simultaneous functioning of several mechanisms, increasing the efficiency level of the light signal.
Our data provide evidence that the composition of photoreceptor pigments in *Dunaliella* is neither flavins (as in *E. gracilis*) nor rhodopsin (as in *E. gracilis*, *C. reinhardtii* and *H. pluvialis*), but is other carotenoids or carotenoproteins (Posudin et al., 2010), though some authors (Wayne et al., 1991) believe there is participation of rhodopsin in the photoreception of *D. salina.*

With regard to photoreception mechanisms, we accept Nultsch's hypothesis (1983) that the absorption of a quantum of light by a photoreceptor molecule is accompanied by its excitation and conformational changes in the photoreceptor protein(s). We proposed as further development of this hypothesis a photoregulation model for the movement of flagellate algae that is based on conformational changes in protein molecules that are components of either the photoreceptor system or the flagellar apparatus. The absorption of light by photoreceptor molecules is accompanied by the excitation of exciton or soliton conditions in α-spiral sites of membrane proteins. Reorganization of the configuration of the membrane protein results in the formation of ion channels through which calcium ions freely diffuse inside the cell thus stimulating locomotory activity. The discovery of the contractile protein *centrin* in the fibrous structures of the flagellar apparatus in *Dunaliella* indicates its possible participation in photo-orientation of basal bodies in these algae, as occurs in other flagellates.
Our experiments using imposing electric fields provide evidence that in the processes of photoreception the *Dunaliella* species, as well as in other green algae, light-induced changes of membrane potentials play a role.

Sensory Transduction of the Light Signal
As it has been demonstrated with ions of calcium and cobalt, and substances blocking or stimulating ion channels, sensory transduction of an absorbed quantum of light into a locomotory reaction in species of *Dunaliella,* as well as in other green algae, is most likely of ionic nature, thus confirming the critical role of Ca^{2+} in these processes. At the same time, Na^{+}-K^{+}-ATPase does not participate in the photoregulation of locomotion in *Dunaliella* cells nor does ouabain have an effect on photomovement parameters. It is assumed that the intake of calcium ions into the cells of these algae is controlled not by a Na^{+}-K^{+} pump, as is the case in *E. gracilis*, but probably occurs directly, through light-induced membrane channels, as in *Chlamydomonas.*
In contrast to *C. reinhardtii,* in many cases the *non-specific* reaction (autotomy of flagella) of *Dunaliella* cells to substances stimulating or blocking ionic channels does not result in the loss of flagella. Therefore, their locomotory reactions can be regarded as a *specific* response to the blocking or stimulating of ionic processes.
We demonstrated for the first time that different photomovement parameters in *Dunaliella* (phototopotaxis and the relative number of motile cells, on the one hand, and photokinesis, on the other hand) are controlled by different mechanisms. This is confirmed by the presence/absence or different degrees of dependence of these parameters on such environmental factors as the wavelength of incident light, dose of

preliminary ionizing and UV-irradiation, concentration of ions of calcium or cobalt, and compounds stimulating or blocking calcium channels, etc.

Importance of data on algal photomovement for related fields of science

Results of studies of photomovement processes in microorganisms are of interest not only for photobiology but also for related fields of science, such as evolutionary biology, phylogenetics, systematics, ecology, and applied aspects of biology. Comparative studies of photomovement in two closely related species of *Dunaliella* (i.e., *D. salina* and *D. viridis*) have shown that they generally do not differ from each other in key parameters. They also further substantiate the structure of their photoreceptor systems and photoregulation mechanisms for cell locomotion, that have developed as a result of a long process of joint evolution. At the same time, the species differ in their sensitivity to white light at low and high intensities and also to the threshold for the transition from positive to negative phototopotaxis. The two species also react differently to the interaction of some factors correlated with light (temperature, electric field, and intensity of light). In contrast to *D. viridis*, *D. salina* under conditions of natural hyperhaline watersheds does not display negative phototopotaxis at an illuminance above 100,000 lx due to the protective function of β-carotene that accumulates in its cell. The differential in sensitivity between the two species of *Dunaliella* to light is a result of their adaptation to the differing ecological niches for light. Such adaptation provides an opportunity for the two species to coexistence in the same reservoirs but in different niches: *a*) on the brightly illuminated surface of the salt solution (*D. salina*) and *b*) in more shaded benthic layers (*D. viridis*).

The sensitivity of *D. salina* and *D. viridis* to ionizing radiation also differs, which is most likely caused by differences in the size of their photoreceptor systems as targets responsible for effecting locomotory reactions. Thus, intrageneric differences do not involve structural features of the photoreceptor systems and mechanisms of photoreception and sensory transformation of a light signal into locomotory reactions. The differences are the result of ecological adaptations or are caused by dimensional characteristics of cells and their organelles. The ability of one species to accumulate large quantities of β-carotene plays an important protective function.

Critical differences in photobehaviour are found among representatives of various genera belonging to different orders of green algae in the class *Chlorophyceae*: species of *Dunaliella* (*Dunaliellales*) on the one hand, and *C. reinhardtii* and *H. pluvialis* (*Chlamydomonadales*), on the other hand. Differences in the action spectra and maxima for phototopotaxis leads to the assumption that there are different sets of photoreceptor pigments: carotenoids and carotenoproteins in *Dunaliella* and rhodopsin as the basic photoreceptor pigment in *C. reinhardtii* and *H. pluvialis.*

Taking into account differences in the stigma structure between the species of *Dunaliella* and *C. reinhardtii*, it is probable that photoreception in the former, in addition to the modulation mechanism, light diffraction plays an essential role, while in the latter, the critical mechanism involves interference in light flux.

Introduction of chemical compounds into the medium that stimulate or block membrane ionic channels causes in the *Dunaliella* species specific locomotory reactions, while in *C. reinhardtii* the reactions are nonspecific (autotomy of flagella). Likewise, in the

Dunaliella species photophobic reactions like those in *Chlamydomonas* have not been observed, while the presence of photokinetic reactions, so well expressed in *Dunaliella,* are questionable in *Chlamydomonas*.

Photomovement in *Chlamydomonas* and *Dunaliella* differ in the beating mode of their flagella. Forward movement of a *Chlamydomonas* cell is caused by ciliary beating of the flagella, while backward movement utilizes undulate beating. Since the cells of *Dunaliella* are not capable of photophobic reactions, the undulate mode of flagella beating has not been observed in the genus. *Chlamydomonas* cells change direction by using unequal beating frequencies between the *cis*- and *trans*-flagella. In contrast, *Dunaliella* cells temporarily stop the beating of one flagellum.
Thus, species of *Dunaliella* differ from *C. reinhardtii* in a complex array of fundamental features that include the composition of their photoreceptor pigments, mechanisms of photoreception, certain details in the sensory transduction of the light signal into a locomotory reaction, the presence or absence of photophobic and photokinetic reactions, functioning of the flagellar apparatus, etc. The results obtained correlate with data of molecular cladistics. The data of molecular phylogeny demonstrate that *C. reinhardtii,* as a representative of the heterogeneous genus *Chlamydomonas,* belongs to the group of chlamydomonads (which is closely related to colonial *Volvocales)* that is very distant from another group that is closer to *Dunaliella* (e.g., *Chlamydomonas applanata* Pringsh.). The evolutionary distance between these two groups is comparable to the distance between soybeans and cycads.
Interesting data are available on photomovement of *Tetraselmis viridis* (Roukhiyajnen) Norris et al., a representative of a separate class of green algae, the *Chlorodendrophyceae* (Massjuk, 2006. In: Posudin et al., 2010). Photomovement in the ultraviolet region of the spectrum and the presence of a phototopotaxis maximum in that area indicates the possible presence of flavins and/or pterins as part of the composition of photoreceptor pigments of this species. Thus, evolution of photoreceptor systems, in particular sets of photoreceptor pigments, may occur in different ways and in various branches (clades) of the phylogenetic tree of green plants.
Even greater differences in the structure of the photoreceptor were found between *Dunaliella* spp. and *E. gracilis*, a representative of the division *Euglenophyta.* According to some authors, *Euglena* and *Dunaliella* belong to different kingdoms: *Euglenozoa* Cavalier-Smith, 1981, or *Euglenobionta* Kussakin et Drozdov, and *Plantae* Leedale, 1974, or *Viridiplantae* Cavalier-Smith, 1981, respectively. Other authors place them in different super-kingdoms of the organic world: *Discicristata* (Mirabdullaev) Leontiev and Akulov (2002) ex Zmitrovich, 2003 and *Lamellicristata* (Taylor) Starobogatov (1986) emend. Zmitrovich (2003).
Dunaliella differs from *Euglena* in its low speed of cell movement, higher sensitivity to low and high light intensities, and the lower threshold for the transition from positive to negative phototopotaxis, which is probably partly explained by the differing ecological requirements at the species level. Until now the transition from positive to negative phototopotaxis with the subsequent complete suppression of phototopotaxis in response to UV irradiation was observed only in species of *Dunaliella*. Differences in the phototopotaxis action spectra also indicate differences in photoreceptor pigments. There are also distinct differences in the structure of the photoreceptor: crystal and dichroic in *Euglena* and non-crystal and non-dichroic in *Dunaliella.*

Taking into account the peculiarities of the photoreceptor system of euglenoid algae, in particular, their stigma structure, it is possible to believe that they possess only the most ancient, basic modulation mechanism for photoreception inherited from prokaryotes or common ancestors of these groups. In contrast, in green algae three mechanisms (modulation, diffraction, and interference) can function simultaneously, increasing the level of the light signal absorbed by a photoreceptor. Though processes of sensory transduction of the light signal into a locomotory reaction are most likely of an ion nature in all flagellate algae, in *E. gracilis,* in contrast to green algae, a Na^+-K^+-pump participates in the control of these processes. *Euglena* also differs from species of *Dunaliella* and *C. reinhardtii* in the unique functioning of its flagellar apparatus. During beating, the flagellum of *E. gracilis* looks like a "broken (interrupted) spiral" where the spiral parts of the flagellum are interrupted by its straight parts. Neither typical ciliary nor undulate type flagella beating are observed in euglenids.

Thus, the greater the phylogenetic distance between taxa, the greater number and degree of distinctions such as differences in their photobehaviour, structure and functioning mechanisms in the photoreceptor systems, sensory transduction of the light signal into the different types of locomotory reaction and the functioning of the flagellar apparatus. These differences make it possible to use variation in the photomovement traits as additional diagnostic criteria in the *evolutionary biology*, *phylogenetics*, *systematics*, and *taxonomy* of algae.

Along with the differences in photomovement processes and its photoregulation, representatives of different taxa of flagellates have common features. These include the structure of the photoreceptor system (which, with a few exceptions, consists of the photoreceptor and stigma), the primarily modulation mechanism in photoreception (which in representatives of different taxa, depending on the stigma structure, can be associated with additional mechanisms, such as diffraction and interference), the ionic nature of photoreception and sensory transduction of the light signal into a locomotory reaction coupled with the dominant role of calcium ions in these processes, the possible participation in these processes in light-induced changes in membrane electric potentials, conformational changes in protein molecules as the basis of photoreception and sensory transduction, and the participation of centrin in photoorientation of basal bodies.

At the same time, it is evident that the current experimental data on algal photomovement are far from complete and are based on only a small number of model organisms. Too often the studies are not carried out according to a coherent plan at an appropriate methodical level. Therefore, the results obtained in studies using different model organisms and by representatives of different photobiology schools are not always comparable. As a consequence, the conclusions made here must continue to be considered preliminary and represent a critique of the initial results of ongoing studies. It is anticipated that future studies will add to, modify, or even negate one or more of these conclusions.
Studies of peculiarities in photomovement are of interest with respect to the *ecology* and *geography* of algae, in particular, for *autecology*. Such studies allow

specifying characteristics of selected species focusing on their reaction to parameters of light, determining optimum, maximum and minimum values of these parameters among species, promoting their sub-dividing the species into groups of shade-preferring, shade-resistant, photophilous, and light-resistant organisms, and enhancing our understanding of laws governing their distribution on the planet Earth.

Applied importance of data on the photomovement of algae

The applied importance of studying photomovement parameters in species of *Dunaliella* is determined mainly by the position of the genus in the kingdom of green plants *(Viridiplantae)*. Plants dominate our planet and provide the everyday needs of mankind. Hyperhalobic species of *Dunaliella* are classical models for studying the mechanisms of salt tolerance, osmotic regulation, permeability of membranes, and processes governing the biosynthesis of carotene in plants and photomovement. Due to their copious synthesis of β-carotene, osmotically active compounds of strategic importance, and high content of additional physiologically active substances, species of *Dunaliella* are valuable organisms for *biotechnology* (Photograph 2).

Photograph 2. A general view of the reactor used for biomass production of *Dunaliella* near the city Eilat (courtesy of Prof. A. Ben-Amotz).

Their high sensitivity to environmental factors provides an opportunity for their use as *biomonitoring* test organisms. The advantages of these organisms include their microscopic sizes, high rate of reproduction, active motility, and photokinetic and photovector reactions.

In our experiments, the sensitivity of various parameters of photomovement in *D. salina* and *D. viridis* were studied in the presence of surface-active substances in the environment (surfactants: cation-active catamin, anion-active sodium

dodecylsulfonate, non-ionactive hydropol, a natural polysaccharide compound isolated from blue-green algae, agents "water bloom" in the Dnieper reservoirs, and also their combinations in the concentration range from 1 mg/L to 40 mg/L), salts of heavy metals [$CuSO_4 \cdot 5H_2O$, $CdCl_2$, and $Pb(NO_3)_2$ in the 10^{-7}-10^{-2} M concentration range] and pesticides (Acetal 55 %, Acetazine 50 %, Alachlor 45 %, Arylon 75 %, Basta 20 %, Dual 96 %, Harmoni 75 %, Tecto 45 % in concentrations from 10^{-7} to 10^{-2} M). The data obtained indicate the possibility of using the velocity of forward and rotary movement of the cells, the frequency of their flagella beating, and phototopotaxis values in the species of *Dunaliella* as biological monitors to assess the health of aquatic environments. The simultaneous assessment of several photomovement parameters has the potential to significantly increase the sensitivity of the method. The vector method of biotesting is proposed for assessing the concentration of various toxicants in aquatic environments and allows simultaneously monitoring of two or more movement parameters (Figure 4).

The method facilitates processing the data from large-scale measurements, allows obtaining a quantitative estimation of toxicant concentration, and provides the potential of identifying the toxicant.

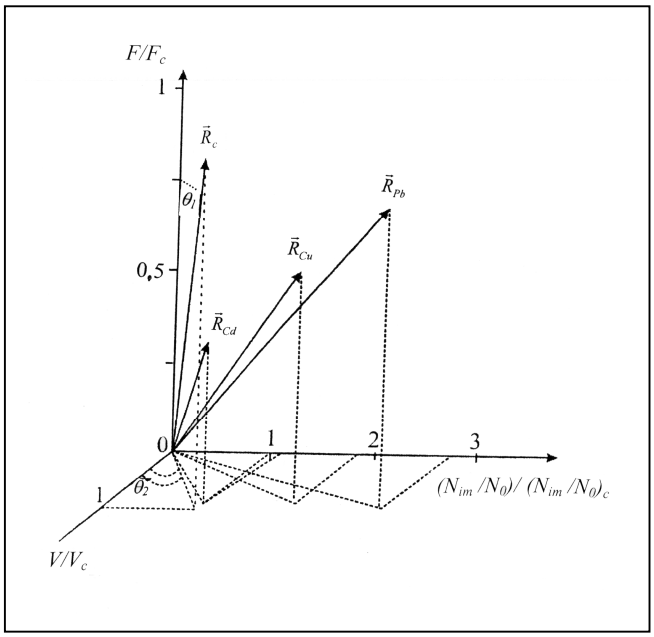


Figure 4. The dependence of the value *r* and direction (θ_1 and θ_2) of the vector $\vec{R}$ in a three-dimensional system of coordinates (u/u_c; F/F_c; $(N_{im}/N_0)/(N_{im}/N_0)_c$) under the effect of heavy metals (Cd, Cu, and Pb). Here: *u* is the velocity of movement; *F* is phototopotaxis level; N_{im}/N_0 is motility. Index *c* corresponds to the control values of photomovement parameters [Posudin et al., 1996].

Species of *Dunaliella* are excellent sources of β-carotene, ascorbic and dehydroascorbic acids, glycerin and other valuable organic compounds and are therefore candidates for biotechnological manipulation. Strains of *Dunaliella* are grown in many countries on an industrial scale for the production of β-carotene for use in the food and pharmaceutical industries and in medicine for the prevention and treatment of tumors, cardiovascular and ophthalmic diseases, avitaminosis, arthrosis, and other pathologies. Studying photomovement regularities and peculiarities in species of *Dunaliella* can help solve certain technological problems currently confronting the production of carotene from these algae.
The main tendencies and perspectives for further photomovement investigations in flagellates are discussed (Posudin et al., 1992, 2010).

References

Graham, L.E. and Wilcox, L.W.(2000). Algae. Prentice Hall. 650 pp.

Hoek Van den, C., Mann, D.G., Jahns, H.M. (1995). Algae. An Introduction to phycology. Cambridge, Univ. Press.

Massjuk, N.P., Posudin, Yu. I., Lilitskaya, G. G. (2006) Photomovement of algae: a comparative taxonomic aspect, Intern.J. on Algae. 7(4), 310-333.

Massjuk, N.P. (1973). Morphology, Systematic, Ecology, Geographical Distribution of the Genus Dunaliella Teod and Perspectives of its Practical Utilization. Kiev, Naukova Dumka. (In Russian).

Massjuk, N.P. (2006). Chlorodendrophyceae class. nov. (*Chlorophyta*, *Viridiplantae*) in flora of Ukraine.I. Capacity, phylogenitic links, systematic position. Ukr.Bot.J. 63(5), 662−675. (In Ukrainian).

Massjuk, N.P. and Posudin, Y.I. (2002). Rigorous fundamentals of classification of light-induced behaviour from freely motile microorganisms. Acta Botanica Malacitana. 27, 147−158.

Massjuk, N.P., Posudin, Y.I., Radchenko, M.I., and Sheiko, A.N. (1991). The parametrical principle of the classification of photomovement of organisms. J. Photochem. Photobiol. 10, 269−271.

Nultsch, W. (1983). The photocontrol of movement of *Chlamydomonas*. In: The Biology of Photoreception. D.J. Cosens and D. Vince-Price (Eds.), Soc. Experiment. Biology, Symp. XXXVI, 521-539.

Posudin, Yu.I., Massjuk, N.P. (1997). Diffractional mechanism of photoreception in green unicellular flagellated algae. Hydrobiol. J. 33(6), 124-131.

Posudin Yu.I., Massjuk N.P., Lilitskaya G.G. (2010) *Photomovement of Dunaliella* Teod. Vieweg+Teubner Verlag/Springer Fachmedien Wiesbaden.

Posudin, Y.I., Massjuk, N.P., and Lilitskaya, G.G. (1996). Videomicrography of algae photomovement and vectorial method of biomonitoring. Proceedings of SPIE-The International Society for Optical Engineering 2628 (Optical and Imaging Techniques for Biomonitoring), 382−388.

Posudin, Y.I., Massjuk, N.P., Lilitskaya, G.G., and Radchenko, M.I. (1992). Photomovement of two species of *Dunalialla* Teod. (*Chlorophyta*). Algologia 2(2), 37−47.

Sineshchekov, O. A. (1991). Electrophysiology of photomovements in flagellated algae. In Biophysics of Photoreceptors and Photomovements in Microorganisms. F. Lenci, F. Ghetti, G. Colombetti, D.-P. Haeder, and P.-S. Song, editors. Plenum Press, New York. 191–202.

Wayne, R., Kadota, A., Watanabe, M., and Furuya, M. (1991). Photomovement in *Dunaliella salina*: fluence rate-response curves and action spectra. Planta 184, 515−524.