Yuriy I. Posudin | Nadiya P. Massjuk | Galyna G. Lilitskaya

Photomovement of *Dunaliella* Teod.

VIEWEG+TEUBNER RESEARCH

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Bibliographic information published by the Deutsche Nationalbibliothek
The Deutsche Nationalbibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data are available in the Internet at http://dnb.d-nb.de.

1st Edition 2010

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Editorial Office: Ute Wrasmann | Anita Wilke

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Springer Fachmedien is part of Springer Science+Business Media.
www.viewegteubner.de

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Cover design: KünkelLopka Medienentwicklung, Heidelberg
Printing company: STRAUSS GMBH, Mörlenbach
Printed on acid-free paper
Printed in Germany

ISBN 978-3-8348-0974-2

In Memory of Professor Nadiya Massjuk
1931-2009

One of the authors of this monograph, Professor Nadiya Massjuk, Dr. Sci. Biol. and Leading Researcher of M. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine, passed away on 13 March, 2009.

Her scientific interests were related to algology, particularly biodiversity, flora, systematics, ecology, geography, origin, evolution, phylogeny, the role of algae in the world of living organisms, and applied algology.

She was interested in the biology of algal photomovement from the point of view of diversity, phylogeny of phytoflagellates, classification, biotechnology of cultivation of carotene-containing algae, and carotenoid production.

Dr. Massjuk was an author of the classic monograph "Morphology, Systematic, Ecology, Geographical Distribution of Genus *Dunaliella* Teod and Perspectives of its Practical Applications" (Kiev, Naukova dumka, 1973) in which the results provide essential basic information on the genus *Dunaliella*, the main principles of systematics of the genus and elucidation of its species, subspecies, versions and forms.

Dr. Massjuk's published work (over 260 books and articles) and her impact on her friends and collegues has left an invaluable and lasting legacy to the scientific community.

May she rest in peace. Colleagues

Preface

This monograph represents 30 years of scientific cooperation on the study of the basic biology of photomovement in algae between the National University of Life and Environmental Sciences of Ukraine (Prof. Yuriy Posudin) and the M.G. Kholodny Institute of Botany of National Academy of Sciences of Ukraine (Prof. Nadiya Massjuk and Dr. Galyna Lilitskaya). It reviews the historical development and current state of the art in the biology of photomovement in algae. Problems in terminology and a logical basis for classification of photomovement in microorganisms are discussed. The research has focused on two species of *Dunaliella* Teod., *D. salina* Teod. and *D. viridis* Teod., as the principal organisms investigated.

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 these 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 results of photomovement investigations are assessed relative to the evolutionary biology of algae and their phylogenetics, systematics, taxonomy, ecology and geography. Critical aspects of photomovement biology that remain to be investigationed in flagellates are discussed.

The monograph is intended for algologists, protistologists, hydrobiologists, biophysicists, physiologists, ecologists and biotechnologists, teachers, post-graduate students and students of related biological specialities.

The authors express their deep and sincere gratitude to Professor Francesco Lenci and Doctor Giuliano Colombetti (Institute of Biophysics CNR, Pisa, Italy) for stimulating our interest in the photobiology of microorganisms and introducing the authors to the fascinating world of algal photomovement.

The authors are grateful to Professor Felix Litvin (Moscow State University, Russia) and Professor Boris Gromov (St.-Pertersbourg State University, Russia) for their continued interest in the investigation of photomovement in *Dunaliella* and fruitful discussions of the results.

The authors are much indebted to Prof. D.P. Häder (Friedrich-Alexander University, Erlangen, Germany), Prof. A. Flores-Moya (University of Malaga, Malaga, Spain), Prof. H. Kawai (Kobe University, Kobe, Japan), Prof. C. Wiencke (Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany) and Prof. D. Hanelt (Hamburg University, Hamburg, Germany) for providing the opportunity to conduct research on the photobiology and photomovement of algae in their laboratories.

The authors would like to express their grateful thanks to Prof. Ami Ben-Amotz (National Institute of Oceanography, Israel) for illustrative materials and Prof. Shogo Nakamura (Toyama University, Japan) for an electron micrograph of *Dunaliella*.

Special gratitude to Dr. Igor Zaloilo for developing the computer versions of figures in the book.

The authors would like to express their very grateful thanks to Professor Stanley J. Kays and Betty Schroeder (The University of Georgia, USA) for technical assistance.

Yuriy Posudin
Nadiya Massjuk
Galyna Lilitskaya

Contents

List of Figures and Photographes

.

List of Tables

"The only generalization that can be made for photomovement is its diversity"

W. Haupt, 1983

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 [Kritsky, 1982; Sineschekov and Litvin, 1982]).

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 [Sedova, 1977; Topachevsky and Massjuk, 1984; van den Hoek et al., 1995; Graham and Wilcox, 2000; Massiuk and Kostikov, 2002]. 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.

There have been a number reviews on light induced movement of microorganisms [Halldal, 1958, 1961; Haupt, 1959, 1983; Bendix, 1960; Hand and Davenport, 1970; Nultsch, 1975; Wolken, 1977; Lenci and Colombetti, 1978; Miyoshi, 1979; Nultsch and Häder, 1979, 1988; Diehn, 1979, 1980; Feinleib, 1980; Colombetti and Lenci, 1982; Lenci, 1982; Poff and Hong, 1982; Sineshchekov and Litvin, 1982, 1988; Häder, 1987*a,* 1987*b,* 1987*c*; 1994; 1996*a*; Lenci et al., 1984; Colombetti and Petracchi, 1989; Doughty, 1991; Nultsch and Rueffer, 1994; Donk and Hessen, 1996; Häder and Lebert, 2000; Lebert and Häder, 2000; Sineshchekov and Govorunova, 2001*a*; Hegemann and Deininger, 2001; Hegemann et al., 2001; Williams and Braslavsky, 2001; Sgarbossa et al., 2002; Checcucci et al., 2004]. In addition, there have been a number of scientific conferences and schools that communicate recent advances in our fundamental understanding of the subject (e.g., "Biophysics of Photoreceptors and Photobehaviour of Microorganisms" (Pisa, 1975), "Photoreception and Sensory Trans-

Y. I. Posudin et al., *Photomovement of Dunaliella Teod.,*
DOI 10.1007/978-3-8348-9765-7_1,
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duction in Aeural Organisms" (N.Y., 1980), "Sensory Perception and Transduction in Aeural Organisms" (N.Y., 1985), "Biophysics of Photoreceptors and Photomovement in Microorganisms" (Tirrenia, 1990), "Light as Energy Source and Information Carrier in Plant Photophysiology" (Volterra, 1994); International Conferences "Actual Problems of Algology" (Chercassy, 1987; Kiev, 1999); "Photosensory Receptors & Signal Transduction" (Ventura, 2004), just as periodical Congresses of the European Society for Photobiology that are organized each two years since 1986, and annual meetings of American Society for Photobiology and of the Japanese Society for Photomedicine and Photobiology). Likewise, several conferences were dedicated to algal biotechnology (Third Asia-Pacific Conference on Algal Biotechnology, 1997, Phuket, Thailand and "Algae and Their Biotechnological Potential", 2000, Hong Kong).

New strains of motile microorganisms continue to be identified. Experimental analysis of photomovement includes methods such as videomicrography, phototaxigraphy, Doppler laser spectroscopy, high-speed cinematography, and electrophysiological measurements. An automated system of registration of different photomovement characteristics and the collection and analysis of information utilized to assess differences in photomovement of organisms are now widely used. Meanwhile, the development and application of new experimental approaches and instrumentation to assess photomovement have stimulated considerable interest.

The study of the photomovement of microorganism is confronted with number of problems due in part to the great diversity in types of photoreactions and photoreceptor systems within and among various microorganisms, variation in the absorption spectra of photoreceptor pigments, and the difficulty in isolation of these pigments.

The study of sensory transduction of a quantum of light absorbed by a pigment molecule and its conversion into a signal that controls the movement of the cell is extremely complicated. As a consequence, the mechanism of photoregulatory control of movement in microorganisms is sometimes referred to as a "black box" due to the mysteries that remain to be elucidated.

While many well-known photobiological processes, such as photosynthesis or the biophysics of vision, are sufficiently uniform that they allow making generalizations about many of the details across a diverse range of organisms, the situation is quite different for photomovement of organisms. The elucidation of the basic photoregulatory biology of one type of microorganism is not necessarily applicable to another. This situation was most aptly described by the prominent photobiologist W. Haupt: "The only generalization that can be made for photomovement is its diversity" [Haupt, 1983].

Due to the tremendous diversity among organisms in their biology of photomovement, we have focused on theoretical, experimental and applied problems that are related to the photomovement of unicellular green alga of *Dunaliella salina* Teod. and *Dunaliella viridis* Teod.

Intense investigation in any field usually results in the enrichment, revision, and alteration of old terminology since new information often requires new terms to be properly understood. At the present, alterations in terminology are occurring in the biology of microorganism photomovement. As a consequence, we have paid special attention to both the terminology and classification of photomovement.

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 monograph is to critique the current understanding of photomovement in the unicellular green algae species *D. salina* and *D. viridis*.

The *specific aims* of this work are:

1. Review the historical development and current state of the art of investigations on algal photomovement;

2. Describe theoretical problems in terminology and the logic of the existing method for the classification of photomovement in these microorganisms;

3. Elucidate the primary characteristics of *D. salina* and *D. viridis*;

4. Critique the experimental methods utilized for the measurement of photomovement of these species and the effects of abiotic factors on photomovement;

5. Describe the processes of photoreception – location and structure of phootoreceptor systems, composition of photoreceptor pigments, mechanisms of photoreception, and photo-orientation of the two species;

6. Describe the processes of sensory transduction of absorbed light into signals that govern the activity of the motor apparatus of the two species;

7. 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;

8. 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;

9. Assess the implications of photomovement on evolutionary biology, phylogenetics, systematics and taxonomy, ecology and geography of algae;

10. Critique critical areas for future research on the biology of photomovement in flagellates.

Chapter 1

Photomovement of Algae – Historical Overview of Research and Current State of the Art

Interest in understanding the mystery surrounding the movement of living organisms dates from ancient times. The first published work in the field [De Motu Animalium ("On the Motion of Animals")] was by Aristotle (384–322 B.C.) who was interested in similarities in motion among animals. Leonardo da Vinci (1452–1519), a distinguished painter, architect and engineer, also studied the mechanics of movement in organisms (biomechanics). His Codex on the Flight of Birds was a precise study of the mechanics of flight and air movement. The same problems captured the interest of Giovanni Alfonso Borelli (1608–1679), a famous Italian mathematician, astronomer and compatriot of Galileo Galilei. He authored the first book on biomechanics [De Motu Animalium I and De Motu Animalium II ("On the Motion of Animals"), 1679] that was dedicated to muscular movement and body dynamics. He also studied bird flight and the swimming of fish [Thurston, 1999].

The nature and mechanisms of movement of living organisms preoccupied the attention of many famous scientists – I.M. Sechenov (1829-1905), I.P. Pavlov (1849–1936), P.F. Lestgaft (1837–1930), A.A. Ukhtomsky (1875–1942), N.A. Bernstein (1896–1966) and others.

There has been a progressive increase in interest in motile behaviour of microorganisms since 1674 when Antonie van Leeuwenhoek [Mosolov and Belkin, 1980] first observed, using a microscope he developed, the movement of *Euglena* and *Volvox* [cited by: Wolken, 1975]. An article by Ludolph Christian Treviranus (1779–1864), a German botanist, was the first work dedicated to the investigation of algae. Zoospores of *Draparnaldia glomerata* (Vaucher) CA Agardh and *Ulothrix subtilis* Kutzing accumulated near the illuminated edge of the vessel or at the opposite side [Treviranus, 1917].

Christian Gottfried Ehrenberg (1795–1876), a German scientist, studied over a 30 years period thousands of new species, including flagellates such as *Euglena*, ciliates such as *Paramecium aurelia* Müller and *Paramecium caudatum* Ehr., a group of unicellular protists called diatoms, and many species of radiolaria. Of particular interest was his manuscript published in 1838 describing the red eye (eyespot) or stigma of *Euglena*, an organelle that plays an important role in the photomovement of the algae.

Charles Darwin wrote in 1872 "*How a nerve comes to be sensitive to light, hardly concerns us more than how life itself originated; but I may remark that, as some of the lowest organisms, in which nerves cannot be detected, are capable of perceiving light, it does not seem impossible that certain sensitive elements in their sarcode should become aggregated and develop into nerves, endowed with this special sensibility*".

Experiments by F. Cohn (1865*a*) demonstrated that zoospores of some algae, just as the cells of *Euglena*, exhibited phototaxis in response to blue-green but not red light. This was the first indication of spectral sensitivity in microorganism photomovement.

A.S. Famintzin (1843–1918) published "Action of light on algae and some other organisms close to them" (St.-Petersbourg, 1866) and was conferred the title of Doctor of Botany. The author distinguished two types of locomotion in protozoa; those that have cilia (zoospores) and pseudopodia (amoeboid organisms). Cilia are present in flagellates such as: *Volvox*, *Gonium*, *Stephanosphaera*, *Euglena*, and *Chlamydomonas*.

Y. I. Posudin et al., *Photomovement of Dunaliella Teod.*,
DOI 10.1007/978-3-8348-9765-7_2,
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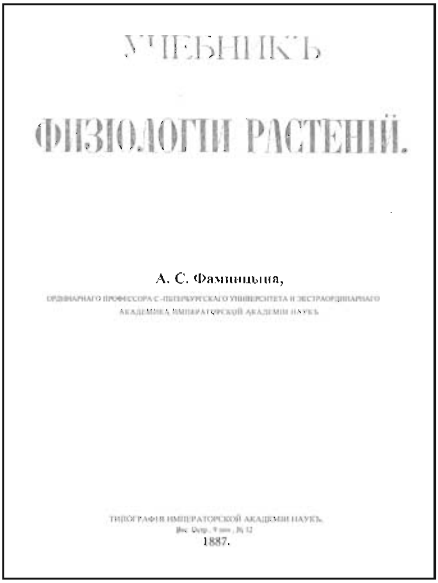

УЧЕБНИКЪ

ФИЗІОЛОГІИ РАСТЕНІЙ.

А. С. Фаминцына,

ОРДИНАРНАГО ПРОФЕССОРА С.-ПЕТЕРБУРГСКАГО УНИВЕРСИТЕТА И ЭКСТРАОРДИНАРНАГО АКАДЕМИКА ИМПЕРАТОРСКОЙ АКАДЕМІИ НАУКЪ.

ТИПОГРАФІЯ ИМПЕРАТОРСКОЙ АКАДЕМІИ НАУКЪ.

1887.

Fig.1.1. A. Famintzin "Text-Book of Plant Physiology" (St.-Petersbourg, 1887)

Famintzin (1887*a*, c. 19) further characterized this phenomenon: "*It was observed long ago that lateral illumination of the vessel with liquid, where zoospores are swimming, provoked the accumulation of them along the edge of the vessel forming a green strip*" (translation of Yu. Posudin). Famintsin concluded that light induced the movement of zoospores and that the light-induced movement of algae depended upon the light intensity, temperature, and the composition of aquatic medium [Famintzin 1867*a*, *b*, 1887*a*]. A number of articles describing photomovement in desmids and blue-green algae were published in the 1880s which represented the general understanding of the biology of photoorientation mechanisms during that time period.

Eduard Adolf Strasburger (1844–1912), a famous Polish-German Professor of Botany, confirmed that various microorganisms use different mechanisms of photoorientation. He believed that zoospores of *Haematococcus* respond to light gradients, while the motile reproductive cells of *Botrydium* respond to the direction of the light. Strasburger was the first to use the term "phototaxis" to distinguish between the light-induced transfer of mobile (phototaxes) and fixed (phototropisms) organisms and to distinguish between positive and negative phototaxes. He also was the first to use coloured glass filters to study spectral peculiarities of zoospore photomovement and likewise discovered the ability of colourless microorganisms to respond to light [Strasburger, 1878].

Theodor Wilhelm Engelmann (1843–1909), a German botanist, physiologist, and microbiologist, published in 1882 the effects of different wavelengths (or colors of light) on photosynthetic activity and showed that the conversion of light energy to chemical energy took place in the chloroplast [Drews, 2005; Engelmann, 1882*a*,*b*]. Engelmann also made a number of valuable contributions to the investigation of photomovement in algae (1882*a*,*b*). Using the technique "projected microspectrum", he demonstrated the dependence of the photoreaction in microorganisms on the wavelength of the light stimulus. Despite the limited qualitative precision of these early experiments, it was possible to estimate the action spectra