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The effect of irradiance on the xanthophyll composition of *Skeletonema marinoi* (Bacillariophycae), *Teleaulax* sp., *Rhodomonas* sp. (Cryptophyceae), and *Heterocapsa triquetra* (Dinophyceae)

by

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Abstract

The aim of the research was to determine the effect of irradiance on the content of carotenoids in the natural algae community occurring in the Baltic Sea: diatom *Skeletonema marinoi*, cryptophytes *Teleaulax* sp., *Rhodomonas* sp., and dinoflagellate *Heterocapsa triquetra*.

In the natural population of *Skeletonema marinoi*, the highest fucoxanthin content was observed in the morning and afternoon, unlike with diatoxanthin+diadinoxanthin, where a mean of 0.008 pg cell^{-1} was found at dawn and at dusk, whereas maximum values were observed at noon (mean 0.017 pg cell^{-1}). Similar tendencies related to diurnal variations in the content of xanthophylls involved in the xanthophyll cycle occurred also in dinoflagellate *Heterocapsa triquetra*.

In cryptophytes *Teleaulax* sp. and *Rhodomonas* sp., no carotenoids of the xanthophyll cycle were detected. The content of alloxanthin showed diurnal variation from 0.048 pg cell^{-1} to 0.085 pg cell^{-1} and was not clearly correlated with the irradiance.

Key words: xanthophylls, irradiance, phytoplankton, the Gulf of Gdańsk

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Introduction

The xanthophyll cycle occurs in many photosynthesizing organisms, in which a reversible and light-independent transformation between epoxy- and non-epoxyxanthophylls occurs. The existence of two types of such cycles has been proved. The first cycle leads from violaxanthin via antheraxanthin to zeaxanthin and is found in the vascular plants, green algae, and brown algae, whereas the second one leading through diadinoxanthin to diatoxanthin occurs in Heterokontophyta (Bacillariophyceae, Pelagophyceae, Xanthophyceae), Haptophyta (Pavlophyceae, Prymnesiophyceae), Dinophyta and Euglenophyta (e.g. Yamamoto 1979, Demming-Adams & Adams III 1993, Demming-Adams et al. 1996, Lohr & Wilhelm 1999, Roy et al. 2011). About fifty xanthophylls with epoxy bonds are known to function in plants (Straub 1987). Their role seems to be similar to the roles described above. According to some authors, the origin of this extremely conservative process dates back to ca. 3.5 Ga and is attributed to photosynthesizing organisms that first inhabited the Earth and survived unchanged till the present days (Demming-Adams & Adams III 1996, Lohr & Wilhelm 1999).

Carotenoids of the xanthophyll cycle are strictly related to photosynthesis. The proper utilization of the solar energy necessitates the presence of mechanisms in order to achieve a safe dissipation of the excessive energy supplied. The diverse control of this process most effectively leads to the dissipation of the energy excess into the cell, resulting from the illumination stress. The excess of energy reaching the cell may result in photooxidative impairment of the photosynthetic apparatus (Demming-Adams & Adams III 1996, Frank & Cogdell 1996, Niyogi et al. 1997, Takaichi 2011).

The xanthophyll cycle displays an ideal combination of a series of sensitive reactions, allowing the most efficient utilization of energy, in the case of both energy deficiency and its excess. Numerous studies have proved that the increase in the zeaxanthin content in cells is correlated with the enhancement of the dissipation reaction measured as the magnitude of the chlorophyll fluorescence quenching (Demming et al. 1987, Gilmore & Yamamoto 1993, Mohanty & Yamamoto 1995, Pfűndel & Bilger 1994). Zeaxanthin in the violaxanthin cycle and diatoxanthin in the diadinoxanthin cycle have turned out to be the final products, which develop during the transformation of the energy excess into heat. In the case of energy deficiency, the xanthophyll cycle effectively intensifies the chlorophyll fluorescence by increasing the amount of the singlet form of chlorophyll, while at light excess, it maintains this form at a safe level. This mechanism protects the organism from a rapid and uncontrolled energy emission inside the photosynthetic apparatus (Demming-Adams 1990).

Reports of Frank (Frank et al. 1994), Owens (1996), and Cogdell (Cogdell et al. 2000) were important for the understanding of the role of this process in the physiology of plants. The authors have found that carotenoids present in the vascular plants differ in structure from those in numerous algae and bacteria. Carotenoids in algae also play an additional role, i.e. they capture and transfer the energy to the photosynthetic apparatus, because the necessity of the energy excess quenching is much more rare in the aquatic environment compared to the terrestrial one.

Factors stimulating the progress of the xanthophyll cycle have not been fully solved by now. As demonstrated to date, this cycle is affected by light intensity, nitrogen and phosphorus contents, as well as by certain metals (Skoda 1997, Anning et al. 2000, Bertrand et al. 2001).

Furthermore, the major role of xanthophylls is to absorb light for photosynthesis, e.g. fucoxanthin and peridinin have a significant function in extending the light-harvesting spectrum in the phytoplankton, thus ensuring optimal absorption efficiencies (Barlow et al. 2002). These carotenoids are classified as photosynthetic pigments (PSC). Carotenoids that serve to protect the photosynthetic apparatus against the effect of high irradiances, may be termed photoprotective pigments (PPC) (Anning et al. 2000, Goss & Jakob 2010, Stoń-Egiert et al. 2012).

Materials and Methods

The study material consisted of natural populations of the diatom *Skeletonema marinoi*, cryptophytes *Teleaulax* sp. and *Rhodomonas* sp., and the dinoflagellate *Heterocapsa triquetra,* collected during their mass occurrence in the Gulf of Gdańsk (the Baltic Sea) at the end of the Sopot pier. Water

samples were collected from the subsurface layer (at a depth of 20-30 cm) using a closing glass bottle (analogous to the Ruttner Water Sampler) during three following days of blooms. Data on the examined species and hydrological conditions are summarized in Table 1.

Detailed analyses were carried out only for those samples, in which the biomass of a single species exceeded 80%. The material was collected in the daytime, from dawn until dusk, at 3-hour intervals, during sunny days to obtain the greatest possible difference in the irradiance values measured at sunrise/sunset and at noon. The radiation values (photosynthetically active radiation PAR) were measured above the water surface by the quantum light meter (Li-190 Quantum Sensor, LI-COR, USA) (Table 1). Each time, the subsurface water was collected and a certain amount (depending on the bloom intensity) was filtered through Whatman GF/F glass filters, 10-15 minutes after sampling. The filters were stored at -80°C for less than 2 weeks before analysis.

Phytoplankton cells were determined using Utermöhl's sedimentation techniques (Utermöhl, 1958) and an inverted microscope (Axiovert 35, Carl Zeiss, Germany). Phytoplankton count calculations were performed according to the Manual for Marine Monitoring in the COMBINE Programme of HELCOM (2001). Cell volumes were converted to carbon (carbon biomass) with formulae based on the studies of Menden-Deuer & Lessard (2000).

Carotenoids and chlorophylls were analyzed by reversed-phase high performance liquid chromatography (RP-HPLC) using an HPLC system Hewlett-Packard 1050. The system was equipped with an HP 1050 pump, an HP1046 fluorescence detector, an HP1100 diode array detector, and a Rheodyne injector (100 µm loop). The samples were injected onto

Table 1

Data characterizing the investigation material collected

Species forming the bloom	Time of collection	Irradiance (µmol $m^{-2} s^{-1}$)			Temperature of water (°C)			Salinity		
Skeletonema marinoi Sarno et Zingone in Sarno et al. 2005 (Bacillariophyceae)	28-30.02.2003	28.02	29.02	30.02	28.02	29.02	30.02	28.02	29.02	30.02
	6:30 AM	2	1	5	1.8	1.7	1.9	7.1	7.1	7.0
	9:30 AM	360	460	509	1.8	1.8	1.8	7.0	7.2	7.1
	12:30 PM	834	734	818	2.0	1.8	1.8	7.1	7.1	7.2
	3:30 PM	320	220	347	2.1	1.9	1.9	7.1	7.1	7.0
	5:30 PM	25	20	15	2.1	1.9	2.0	7.0	7.2	7.0
Teleaulax sp., *Rhodomonas* sp. (Cryptophyceae)	26-28.07.2009	26.07	27.07	28.07	26.07	27.07	28.07	26.07	27.07	28.07
	4:00 AM	0	0	4	15.8	15.7	14.9	6.6	6.7	6.7
	7:00 AM	356	398	245	16.1	16.0	15.7	6.6	6.7	6.7
	10:00 AM	792	805	812	17.6	17.1	16.0	6.6	6.6	6.8
	1:00 PM	1223	1044	1100	19.5	18.7	17.9	6.6	6.7	6.8
	4:00 PM	589	612	609	19.4	18.8	18.0	6.7	6.7	6.7
	8:00 PM	107	55	90	18.9	17.0	17.5	6.6	6.7	6.7
Heterocapsa triquetra (Ehrenberg) Stein 1883 (Dinophyceae)	16-18.07.2012	16.07	17.07	18.07	16.07	17.07	18.07	16.07	17.07	18.07
	5:00 AM	97	117	115	14.1	14.0	15.1	6.9	6.8	6.9
	9:00 AM	420	550	722	15.2	15.5	16.1	6.8	6.9	6.8
	12:00 AM	991	1212	1015	16.4.	17.3	16.9	6.8	6.9	6.7
	3:00 PM	1004	1118	997	16.5	16.5	17.1	6.9	6.9	6.8
	6:00 PM	679	722	646	16.6	16.4	16.7	6.8	6.8	6.7
	9:00 PM	123	95	102	16.2	16.3	15.6	6.7	6.8	6.7

a precolumn with a LichroCARD™ LiChrospher™ 100 RP18e analytical column (dimensions 250 × 4 mm, particle size 5 µm, Merck). The carotenoid extraction and chromatographic analysis were performed in accordance with a modified Mantoura procedure (Mantoura & Llewellyn 1983), described in detail by Stoń & Kosakowska (2000, 2002). Calibration curves of defined amounts of pigment standards were used for a quantitative estimation of pigments. The carotenoids and chlorophylls standards obtained from The International Agency for ^{14}C Determination, DHI Institute for Water and Environment in Denmark were applied.

The importance of the obtained results was analyzed using the Student t-test.

Results

The study of the phytoplankton abundance during mass occurrence of *Skeletonema marinoi* in February 2003 showed a high percentage of this species in the total abundance of phytoplankton – above 90% (Fig. 1a) and in terms of biomass from 91 to 98% (not presented). The abundance of *Skeletonema marinoi* ranged from 8.7×10^6 to 20.9×10^6 cells dm^{-3} and the biomass varied from 138 to 331 µg C dm^{-3}. A clear trend in the daily fluctuations of this species was not observed.

The quantitative analysis of the algae species composition at the time of the study has demonstrated high diurnal fluctuations in the number of *Teleaulax* sp., *Rhodomonas* sp., and *Heterocapsa triquetra* (Fig. 1b, c). In the case of cryptophytes, compared to morning hours, this quantity dropped during the day almost by two orders of magnitude (Fig. 1b). The abundance of cryptophytes ranged from 2.1×10^6 to 50.1×10^6 cells dm^{-3} and the biomass varied from 29 to 689 µg C dm^{-3}. Then a decrease in the number of cryptophytes in the analyzed material and an increase in the percentage of other organisms, mainly nanoplanktonic species: *Woronichinia* sp., *Cyanodiction* sp. (Cyanobacteria), and *Pyramimonas* sp. (Prasinophyceae) were observed. Their percentage

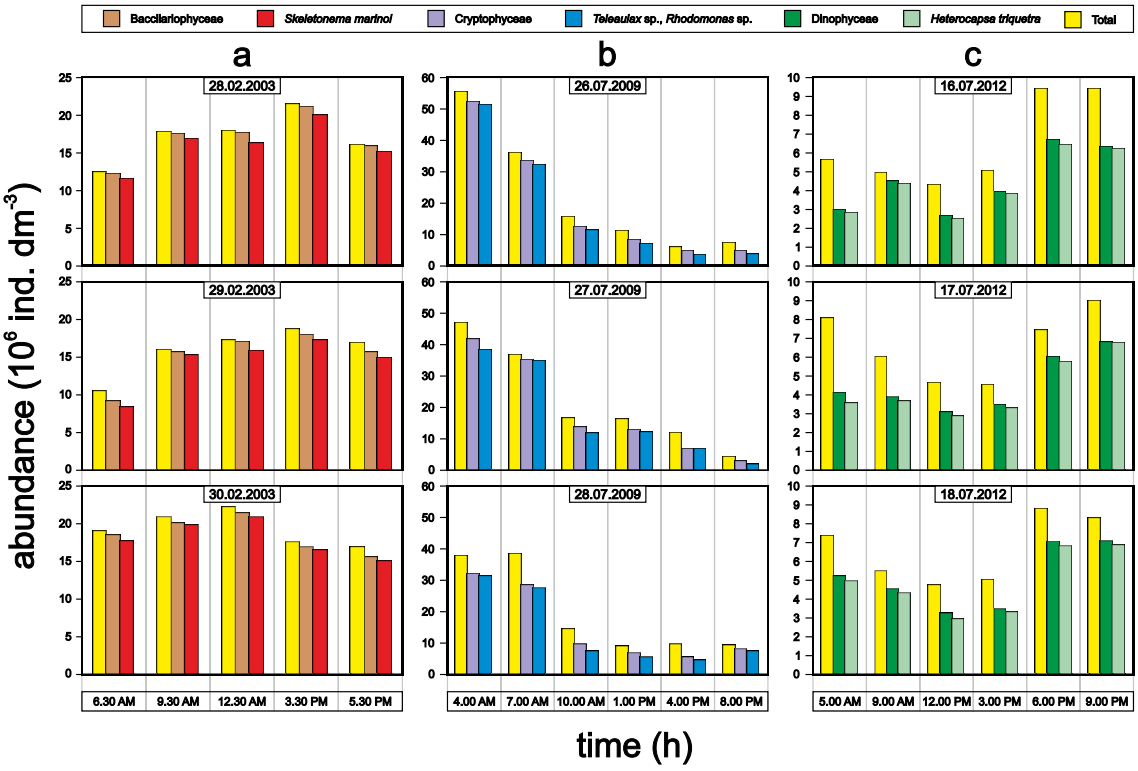

Figure 1

Content of phytoplankton collected during mass occurrence of diatom Skeletonema marinoi (a), cryptophytes Rhodomonas sp., Teleaulax sp. (b) and dinoflegellate Heterocapsa triquetra (c) in the Gulf of Gdańsk

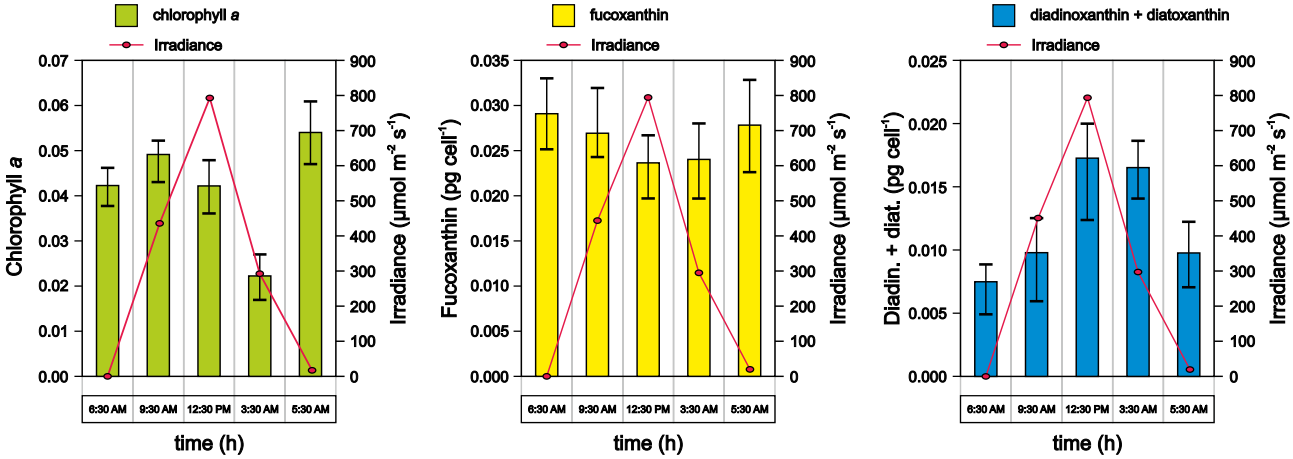

Figure 2

Diurnal changes in the content of chlorophyll *a*, fucoxanthin and diadinoxanthin +diatoxanthin during a bloom of *Skeletonema marinoi* (the Gulf of Gdańsk, 28-30.02.2003). Error bars indicate ±1 SE

of the biomass varied from 3.5 to 21.0% (with the exception of the samples collected on 27 July 2009 at 4.00 p.m., where the contribution of cryptophytes in the total phytoplankton biomass decreased to 63%). During the mass occurrence of *Heterocapsa triquetra,* in July 2012, numerous *Eutreptiella* sp. (Euglenophyceae) and *Myrionecta rubra* (Ciliata) were reported, but their biomass was not high and ranged from 3.7 to 16.0%. The abundance of *H. triquetra* ranged from 2.5 × 10^6 to 6.8.1 × 10^6 cells dm^{-3} (Fig. 1c) and the biomass was in the range from 432 to 1046 µg C dm^{-3}.

The HPLC analyses showed a significant, diurnal variation of the pigment concentration in the algae cells. The chlorophyll *a* content in the natural population of the diatom *Skeletonema marinoi* varied during the day from 0.022 to 0.054 pg $cell^{-1}$ (Fig. 2). The greatest differences ($P<0.01$) were observed between chlorophyll *a* concentrations at 3 p.m., when a clear minimum occurred, and the values measured at other times of the day. Regarding fucoxanthin, the concentration of this pigment dropped at noon and at 3 p.m., whereas in the morning and in the evening it ranged from 0.027 to 0.029 pg $cell^{-1}$, showing no statistically significant differences. The concentration of carotenoids directly involved in the xanthophyll cycle (diato- and diadinoxanthin) also varied significantly throughout the day, being almost two times higher at the strongest irradiance than at other times of the day ($P<0.001$).

In the examined material containing two species of cryptophytes (*Teleaulax* sp. and *Rhodomonas* sp.), alloxanthin – the predominant carotenoid, as well as α-carotene and crocoxanthin were present. The alloxanthin varied considerably in the daytime from 0.048 to 0.085 pg $cell^{-1}$ (Fig. 3). The correlation coefficient between daily distribution of the alloxanthin content and light intensity amounted to r = 0.68.

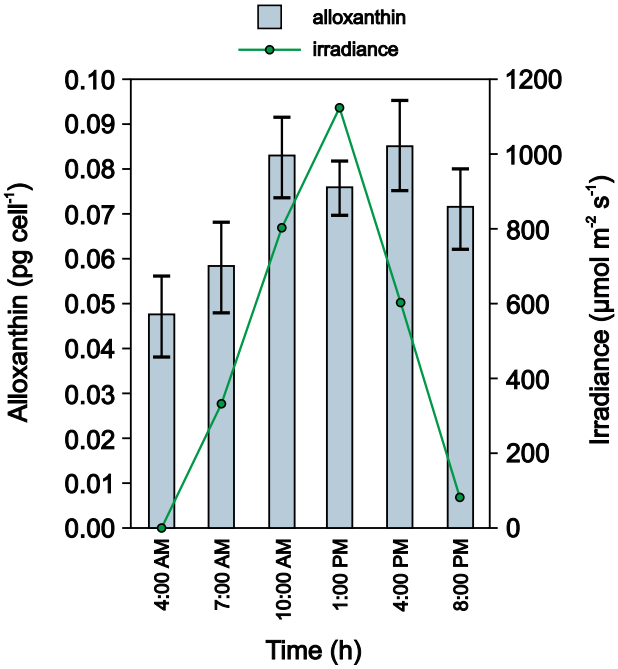

Figure 3

Diurnal changes in the content of alloxanthin in the cryptophytes *Teleaulax* sp. and *Rhodomonas* sp. (the Gulf of Gdańsk, 26-28.07.2009.). Error bars indicate ±1 SE

In the case of dinoflagellates *Heterocapsa triquetra*, the content of diatoxanthin and diadinoxanthin varied daily from 0.46 to 0.72 pg cell^{-1} (Fig. 4). The maximum values were observed at 9 a.m. and at noon, whereas changes in other concentration values were statistically insignificant ($P<0.1$). Peridinin is a carotenoid characteristic of dinoflagellates, including *H. triquetra*. The content of this carotenoid in cells clearly increased to 1.09 pg cell^{-1} at low light intensity, and dropped to 0.71 pg cell^{-1} upon exposure to intensive sunlight.

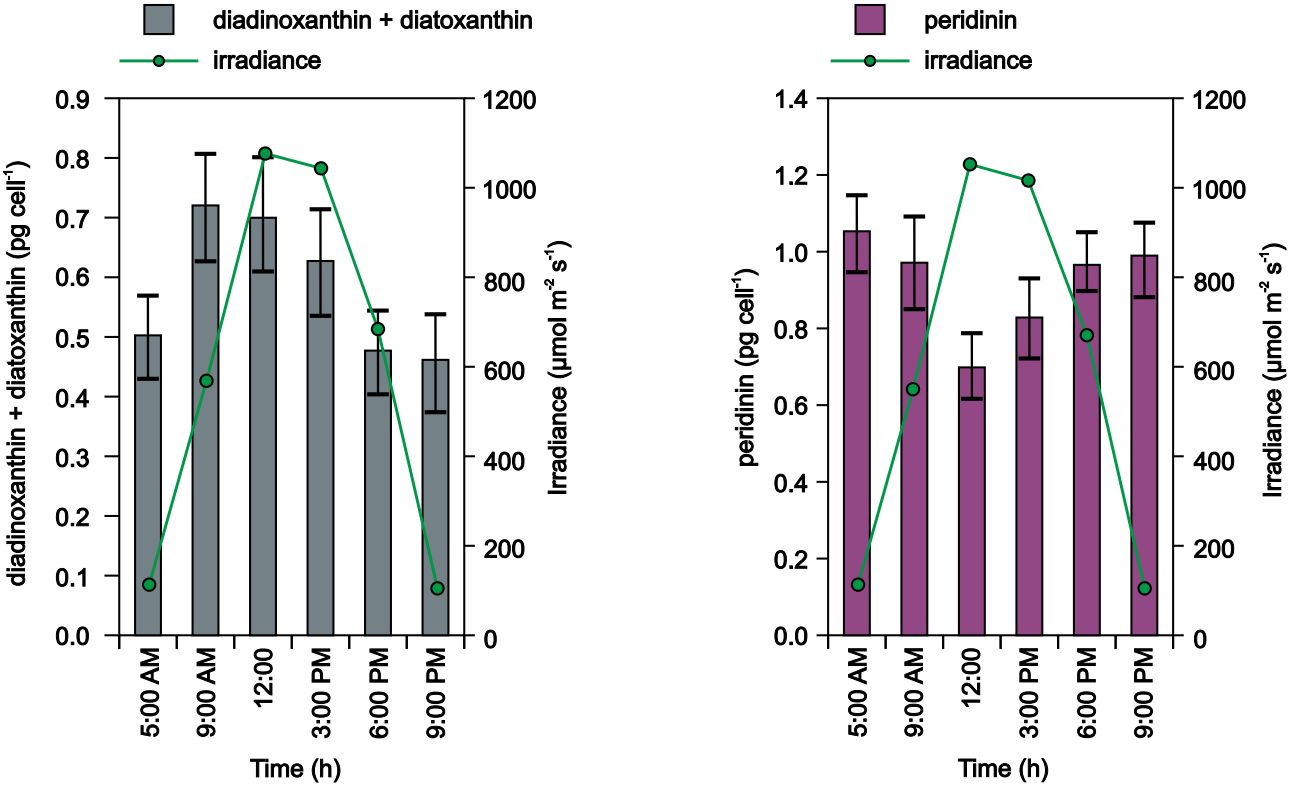

Figure 4

Diurnal changes in the content of diadinoxanthin+diatoxanthin and peridinin in the natural population of *Heterocapsa triquetra* (the Gulf of Gdańsk, 16-18.07.2012.). Error bars indicate ±1 SE

Discussion

The obtained results indicate that xanthophylls (including carotenoids participating in the xanthophyll cycle) play a dual role in algae. Apart from protecting intracellular structures against too strong illumination, they assist the photosynthetic process at low light intensity. The studies of other authors carried out on both micro- and macroalgae support this hypothesis and prove that in the aquatic environment, the assistance of the xanthophyll cycle in photosynthesis is more important than its protective action (Anning et al. 2000). This is indicated by a much more efficient process of photosynthesis measured in terms of the quantity of carbon absorbed at low illumination levels, compared to the case of illumination exceeding the optimum of photosynthesis (Anning et al. 2000). In the terrestrial environments, this cycle takes over the role of protection against too strong solar radiation much more frequently (Demming-Adams & Adams III 1996).

The obtained results have shown that the low illumination level corresponded to a low concentration of diato- and diadinoxanthin. The increase in the illumination resulted in the increase of their content in the cells of algae. Subsequent decrease in the light intensity caused a drop in the content

of these xanthophylls. Such a relationship may be accounted for by the necessity of rapid quenching of the energy excess. However, there are suggestions that the low level of diato- and diadinoxanthin in the case of insufficient illumination may be caused by the fact that both pigments are precursors in the synthesis of fucoxanthin (Goericke & Welschmeyer 1992, Lohr & Wilhelm 1999). According to Anning et al. (2000), the accumulation of diato- and diadinoxanthin when diatoms are exposed to strong light, and rapid conversion to fucoxanthin after transferring to low light may be advantageous in phytoplankton exposed to large diurnal variations at a mixed layer depth, such as those occurring during the spring blooms. A reverse situation was observed with fucoxanthin and peridinin, the content of which increased in cells while reducing the light intensity, thus manifesting that xanthophylls assist the photosynthesis under insufficient illumination. These pigments are classified as light-harvesting pigments (Latasa 1995). Analogously, the chlorophyll *a* content decreased in *Skeletonema marinoi* with increasing irradiance. However, this result is biased as it incorporates both the chlorophyll *a* of *S. marinoi* and other species present in the sample.

In the case of algae, which do not have such an efficient protection system as the xanthophyll cycle, this function can be taken over by other xanthophylls. Although no such high correlation was found between the content of alloxanthin in cryptophytes and the irradiance as in other analyzed cases, the relationship seems to be maintained. Perhaps this process is not as fast and efficient and therefore, these organisms have to migrate to deeper and darker water layers during daytime. The study by Funk et al. (2011) and Kaňa et al. (2012) showed that cryptophytes do not have any carotenoids involved in the xanthophyll cycle (e.g. zeaxanthin, diadinoxanthin, diatoxanthin).

On the other hand, some carotenoids have more than one role in the photosynthetic prokaryotes, e.g. β-carotene in the reaction-center complexes of photosynthesis might have protective functions, and in the peripheral photosystem II – mainly light-harvesting functions (Porra et al. 1997, Takaichi 2011). According to some authors, alloxanthin can be classified as photosynthetic pigments (Bonilla et al. 2009), according to others – it is one of the photoprotecitive xanthophylls (Roy et al. 2011, Stoń-Egiert et al. 2012). The role of alloxanthin in cryptophytes is poorly understood. Schlüter et al. calculated the increasing alloxanthin: chlorophyll *a* ratio obtained for *Rhodomonas marina* and *Rhodomonas salina* with increasing irradiance (Schlüter et al. 2000). These observations suggest a photoprotective role of this pigment. However, in the case of *Rhodomonas marina*, the increased alloxanthin:chlorophyll *a* ratio was caused by a reduction in cellular chlorophyll *a* rather than by an increase in alloxanthin. In addition, the increase in this ratio with the increasing irradiance is not common in cryptophytes as demonstrated by almost identical ratios under low- and strong-light conditions in *Plagioselmis prolonga* (Schlüter et al. 2000, Henriksen et al. 2002). Consequently, the explanation of this issue requires further detailed studies.

It seems that many environmental factors, e.g. light, temperature, nutrient availability, toxic compounds, affect the pigment composition (Demming-Adams et al. 1999). Nonetheless, the mechanism of interaction between other factors, apart from light, with xanthophylls has not been definitively resolved so far.

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