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Research Article

SHORT-TERM FLUCTUATIONS OF CHLOROPHYLL *A* FLUORESCENCE VERSUS DIURNAL VARIATIONS OF SOLAR RADIATION IN THE SURFACE WATER OF THE GDAŃSK BASIN

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Abstract

Measurements of chlorophyll *a* fluorescence accompanied by solar radiation measurements were carried out during two spring cruises in the region of the Gdańsk Basin. Chlorophyll *a* fluorescence, similarly to that of chlorophyll *a* concentration in the seawater, showed considerable diurnal variability. The measurements provided statistically significant negative correlation coefficients that indicated that chlorophyll *a* fluorescence is inhibited as solar PAR and UV radiation intensity increases. The approximate range of radiation intensity that corresponded to a clearly marked decline in chlorophyll *a* fluorescence and chlorophyll *a* concentration was determined. It was revealed that the decline in chlorophyll *a* concentration in the surface water layer resulted from the photodegradation of chlorophyll *a* as well as phytoplankton grazing. In both cases, the resulting observation was an increase of pheophytine *a* concentration. The vertical migration of phytoplankton was identified as an additional process induced by high radiation levels.

INTRODUCTION

Solar radiation exerts a considerable influence on a number of biological and chemical processes that take place in the marine environment. Although it stimulates the development of marine organisms, inhibition is also possible. Solar radiation plays a particular role in the surface layer of the sea where organic matter is being transformed (Wängberg *et al*. 1998; Mopper and Kieber 2000; Falkowska 2001; Kumar *et al*. 2003).

Many authors have indicated that intense UV radiation causes the degradation of intercellular structures and molecules and decreases the rate of photosynthesis (Cullen and Lesser 1991; Brachner and Wiencke 2000). UV-B radiation induces changes in many intercellular processes of autothrophic and heterothrophic organisms (Häder *et al*. 1998; Neale 2001). It can cause the bleaching of chlorophyll *a* (Renk 1997; Holm-Hansesn *et al*. 2000; Vernet 2000; Falkowska 2001). Phytoplankton is equipped with variable photo-protecting mechanisms that guard against the negative effects of solar radiation, especially within the UV-B band. An important role is attributed to the ability of phytoplankton to produce carotenoid pigments of the group PPC (photo-protecting carotenoids), which protect cells against excessive radiation. Phytoplankton organisms are also capable of migrating in the water column to avoid exposition to harmful radiation (Sinha *et al*. 1998; Vernet 2000).

The aim of the present project was to confirm the observation that chlorophyll *a* fluorescence, similarly to chlorophyll *a* concentration, presents a good measure of phenomena relating to phytoplankton biomass in the surface water layer. An additional aim was to identify the parameters which are decisive as regards the short–term fluctuations of chlorophyll *a* fluorescence in the marine environment.

MATERIAL AND METHODS

Measurements of chemical and physical parameters were conducted during two cruises aboard the navy vessel ORP *Kopernik*. The measurements were carried out within the area of the Gdańsk Deep at station P1 (φ = 54°52'N, λ = 19°10'E) located about 80 km from the mouth of the Vistula River over the deepest spot in the Gdańsk Basin.

In 2001 the measurements were carried out between 1-5 June, and in 2003 from 9 to 14 May. Surface water samples used to determine chlorophyll *a* concentration and pheophytine *a* were collected every two or four hours. Chlorophyll *a* concentration was determined using the spectrophotometric method (Edler 1979). Seawater samples were filtered through a GF/F fiberglass filter, followed by filter extraction with 90% acetone. Chlorophyll *a* concentration was calculated from the measurement of absorbance of the resulting extract in a Beckman DU 530 and Cadas 200 spectrophotometers at preselected light wavelengths.

Pheophytine *a* concentration was determined in the same extract by the additional measurement of absorbance after the sample had been acidified with 0.4 M HCl (Parsons *et al*. 1985). Both methods are recommended by HELCOM for routine measurements within the HELCOM Baltic Monitoring Programme. The standard error of the average is ±4%.

Chlorophyll *a* fluorescence was measured every 10 min with a Minitrack fluorimeter (serial number 175089), which was deployed permanently at a depth of 0.5 m. The level of solar radiation on the sea surface was measured with equal frequency at a height of 10 m above sea level. An SKP 210/I 0896 13595 Eijkelkamp probe was used to measure photosynthetically active radiation (PAR; 400-700 nm), and an SKU 430 0497 14854 probe by the same manufacturer was used to measure UV-B radiation (280-315 nm). On 4 June 2001 the registering unit malfunctioned, so irradiation measurements were not begun until the afternoon.

Table 1

Statistical characteristics of chlorophyll *a* and pheophytine *a* concentration [mg m^{-3}], chlorophyll *a* fluorescence [V] in surface seawater between 1-5 June 2001 and 9-14 May 2003

	chlorophyll *a*	chlorophyll *a* fluorescence	pheophytine *a*
N	131	1437	121
X	1,339	0,388	0,289
SD	0,784	0,140	0,197
Min	0,047	0,035	0,010
Max	3,822	0,833	0,946
25%	0,724	0,275	0,142
75%	1,908	0,476	0,408

N – number of data, X-mean value, SD – standard deviation, Min – minimum value, Max – maximum value, 25% - lower quartile, 75% - upper quartile

RESULTS

During the measurements conducted in the Gdańsk Deep region in spring 2001 and 2003, considerable variability of chlorophyll *a* concentrations were observed and confirmed by the calculated standard deviation (Table 1). Diurnal amplitudes of chlorophyll *a* concentrations reached maximal values of the order of 3.7 mg m^{-3}, although at the beginning of June 2001 the variability of concentrations was more intense than in May 2003. On several occasions in June 2001, a 2 dm^3 seawater sample was insufficient to detect chlorophyll *a* and the sample volume had to be increased. In May 2003 chlorophyll *a* concentration did not fall below such a low level. In both measurement periods the diurnal minimum of chlorophyll *a* concentrations was observed during intense solar radiation. In general, the maximal diurnal concentrations of chlorophyll *a* occurred during periods of low solar radiation (sunrise or sunset) or even at night.

Pheophytine *a* concentrations were characterized by lower variability as compared to chlorophyll *a* (Table 1). In 2001, the maximal concentration of pheophytine *a* was 0.822 mg m^{-3}, while in 2003 it was 0.946 mg m^{-3}. In both measurement periods pheophytine *a* concentrations showed considerable variability (SD_{2001}=0.156 mg m^{-3}; SD_{2003}=0.190 mg m^{-3}), and, similarly to chlorophyll *a* concentrations, they indicated the complexity of processes influencing the variability of organic suspensions in the surface sea layer.

Chlorophyll *a* fluorescence was measured at high frequency, so even small changes that occurred were registered. The fluorescence of chlorophyll *a* showed considerable fluctuations, as did the variability of chlorophyll *a* concentration (Table 1). The maximal diurnal amplitudes of chlorophyll *a* fluorescence were up to 0.7 V. In 2001, the maximal level of chlorophyll *a* fluorescence reached 0.833 V and was recorded in the late evening. The minimal value, 0.035 V, was registered in the afternoon. The mean fluorescence value in 2001 was 0.410±0.15 V, while in 2003 it was only slightly lower (0.375±0.12V) and less variable.

The solar radiation that reached the sea surface was measured at two wave bands: UV-B (280-315 nm) and PAR (photosynthetically active radiation - 400-700 nm). In 2001, the maximal value of PAR intensity was 1757 μmol m^{-2} s^{-1}, which was registered at noon on 3 June 2001, while the maximal intensity of UV-B at 1053 mW m^{-2} was recorded in the afternoon two days later.

The 2003 measurements were carried out three weeks earlier than those in 2001. This meant that there was two hours fewer daylight than in 2001, and daytime lasted for fourteen hours. The maximal level of PAR radiation was 1515 μmol m^{-2} s^{-1}, while that of UV-B was 946 mW m^{-2}. Solar radiation was characterized by high, short-term fluctuations (Fig.1). In one hour, the ampli

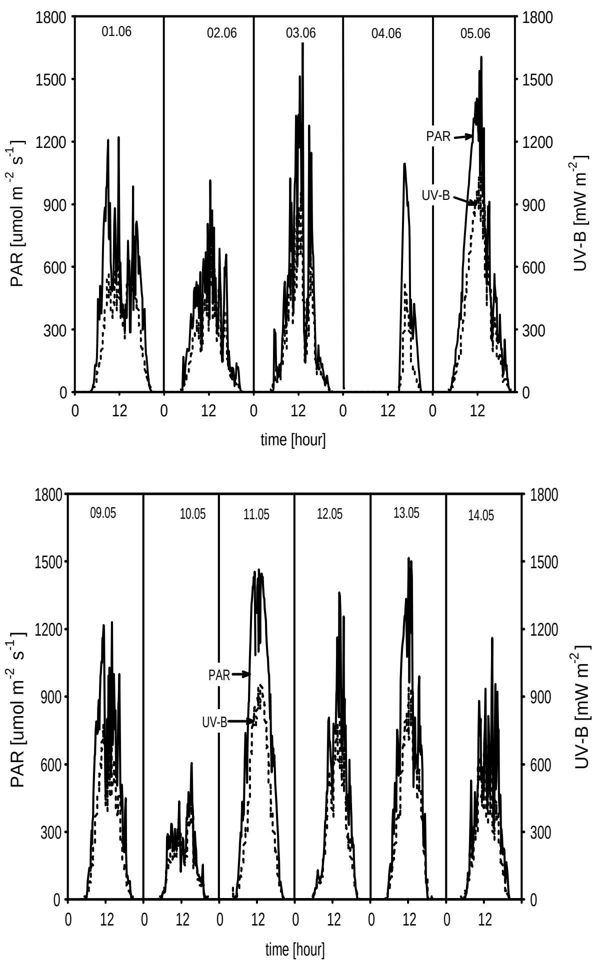

Fig. 1. Changes in radiation coming into the sea surface between: a) 15 June 2001 b) 9-14 May 2003.

tude of radiation intensity in the PAR band could change within 1500 μmol m^{-2} s^{-1} and within the UV-B band up to 800 mW m^{-2}.

Radiation fluctuations in the PAR band were frequently at the level of 500-600 μmol m^{-2} s^{-1}, and, correspondingly, the most frequent UV-B changes were found to be within the range of 300-400 mW m^{-2}. However, there were periods when radiation intensity increased or decreased systematically without strong fluctuations.

The results of measurements of chlorophyll *a* concentration and chlorophyll *a* fluorescence were analyzed to establish a relation between the two parameters. It was revealed that they are related by a linear correlation that is statistically significant at a confidence level $p<0.05$. Chlorophyll *a* measurements performed using classic immersible fluorimeters comprised the total fluorescence within the red section of the spectrum, where the fluorescence from chlorophyll *a* plays the dominant role. Holm-Hansen *et al*. (2000) used 934 data pairs from depths ranging from 5 to 100 m to analyze the linear regression between chlorophyll *a* concentration and chlorophyll *a* fluorescence in the coastal zone of the Antarctic. The correlation coefficient of the order of $r=0.88$ indicated that there was highly statistically significant correlation between

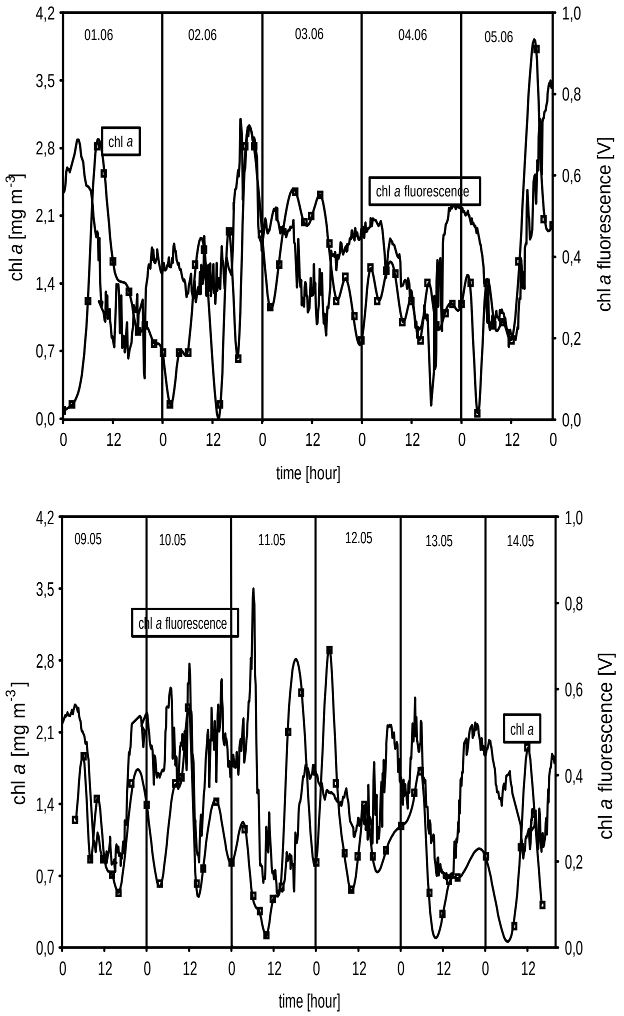

Fig. 2. Changes in chlorophyll *a* concentration and chlorophyll *a* fluorescence between a) 1-5 June 2001and b) 9-14 May 2003.

them. In the current study the correlation coefficient was much lower, but this could be a reflection of the considerable contribution of heterothrophs in the suspended matter as well as the negative effect of natural radiation. Solar radiation (natural background) causes the closure of a certain amount of photosynthetic traps in the phytoplankton, and they do not assimilate the energy of excitation (Ostrowska 2001). To analyze the effect of natural irradiation on chlorophyll *a* fluorescence measurements, the data were divided into two groups - night samples (measurements performed between 22:00 and 6:00) and daylight samples (between 8:00 and 20:00). In the first group the correlation coefficient was $r=0.68$, $N=38$ and $p<0.05$. In the daylight sample group the correlation coefficient was lower at $r=0.56$, $N=69$ and $p<0.05$. In both cases the correlation coefficients were statistically significant, and this confirmed that chlorophyll *a* fluorescence as well as chlorophyll *a* concentration can be used as good indicators of phytoplankton biomass fluctuations.

DISCUSSION

The chlorophyll *a* concentration and chlorophyll *a* fluorescence determined in the two measurement periods showed significant variability (Fig.2). On the majority of days, decreases in chlorophyll *a* concentration and chlorophyll *a* fluorescence in the surface water layer was observed at the peak of solar radiation. At night, at sunrise or sunset, an increase of chlorophyll pigment concentration and its fluorescence was noted. Earlier studies indicated that solar radiation exerts a decisive impact on chlorophyll *a* concentration in the surface layer of the sea (Falkowska and Latała 1995; Renk 1997; Falkowska 2001).

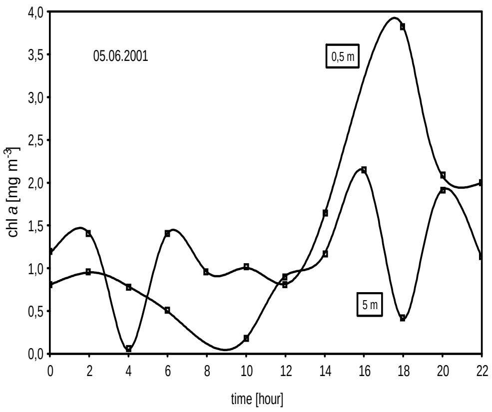

Fig. 3. Changes in chlorophyll *a* concentration at depths of 0.5 and 5 m.

Changes in chlorophyll *a* concentration in the surface sea layer and at a depth of 5 m were compared, and some of the fluctuations noted were indicative of vertical phytoplankton migrations (Fig. 3). The migrations took place within the time span of several hours. A decrease in chlorophyll *a* concentration in the surface water layer occurred between 8:00 and 12:000, while at a depth of 5 m an increase in chlorophyll *a* concentration occurred around 16:00. In the seawater of the Gulf of Gdańsk, Renk *et al*. (1985) estimated that phytoplankton cells are capable of covering a distance of about 1 m in one hour, thus the results obtained in the present study seem quite realistic. This is also supported by Sinh *et al*. (1998), who expressed the opinion that certain phytoplankton species are able to migrate vertically in the water column and that these phototactic movements are a protective measure against excessive solar radiation.

Solar energy definitely stimulates living processes; nonetheless, it can also have inhibitory effects. Both PAR and UV radiation exert inhibitory effects on photosynthesis or phytoplankton biomass increments. Intense solar radiation can cause decreased chlorophyll *a* concentrations and fluorescence (Cullen and Lesser 1991; Neale *et al*. 1998; Bracher and Wiencke 2000; Neale 2001). The inhibition of chlorophyll *a* fluorescence was observed in Antarctic regions even at depths of 30-40 m (Holm-Hansen *et al*. 2000). This inhibitory effect is confirmed by a close relation between chlorophyll *a* fluorescence and PAR and

UV-B radiation (Fig.4). On the basis of the negative (statistically significant) correlation coefficients, it was found that increased solar radiation resulted in decreased chlorophyll *a* fluorescence. Additionally, an increase of radiation in both bands resulted in the results scattering less. The observed inhibitory effect in the sea surface layer was the result of the full spectrum influence of solar radiation; however, numerous authors are of the opinion that the UV-B band is the most effective inhibitor.

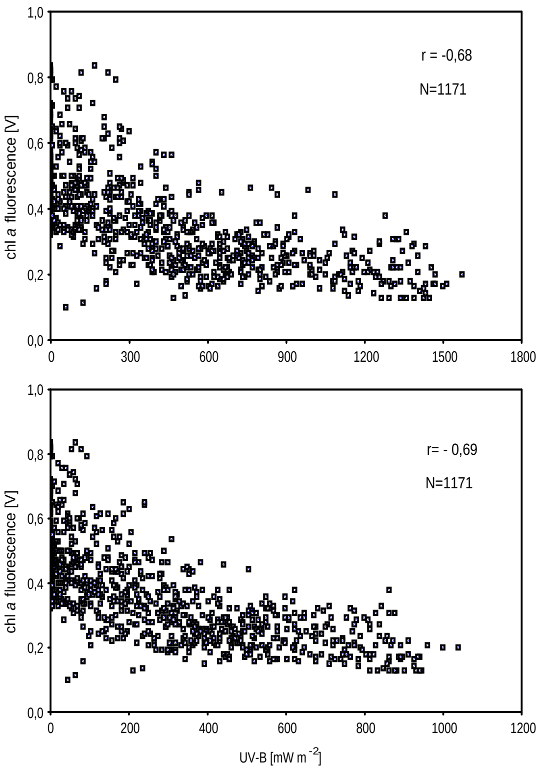

Fig. 4. Relation between chlorophyll *a* fluorescence and solar radiation in the band: a) PAR and b) UV-B at a probability level of p<0.05.

The solar radiation observed during both measurement periods showed considerably high, though extremely variable, intensity levels (Fig. 1a,b; 5a,b). The results of daily measurements were used to estimate the approximate range of radiation that corresponded to marked decreases in chlorophyll *a* concentration and fluorescence; in the PAR band it was 350 to 550 μmol m^{-2} s^{-1}, and in the UV-B band it was 200-400 mW m^{-2}. Therefore, wide radiation ranges are indicative of the complexity of the studied processes. In the natural environment the phytoplankton community is composed of many different species, and the common reaction of phytoplankton to light intensity is the result of the reactions of particular organisms. Additionally, it has to be taken into account that the phytoplankton as a mass was in a defined physiological state and also that the organisms were in different developmental stages. All of these factors have to be accounted for; therefore, the radiation levels that caused decreases in chlorophyll *a* concentration and fluorescence in the current study have to be treated with caution.

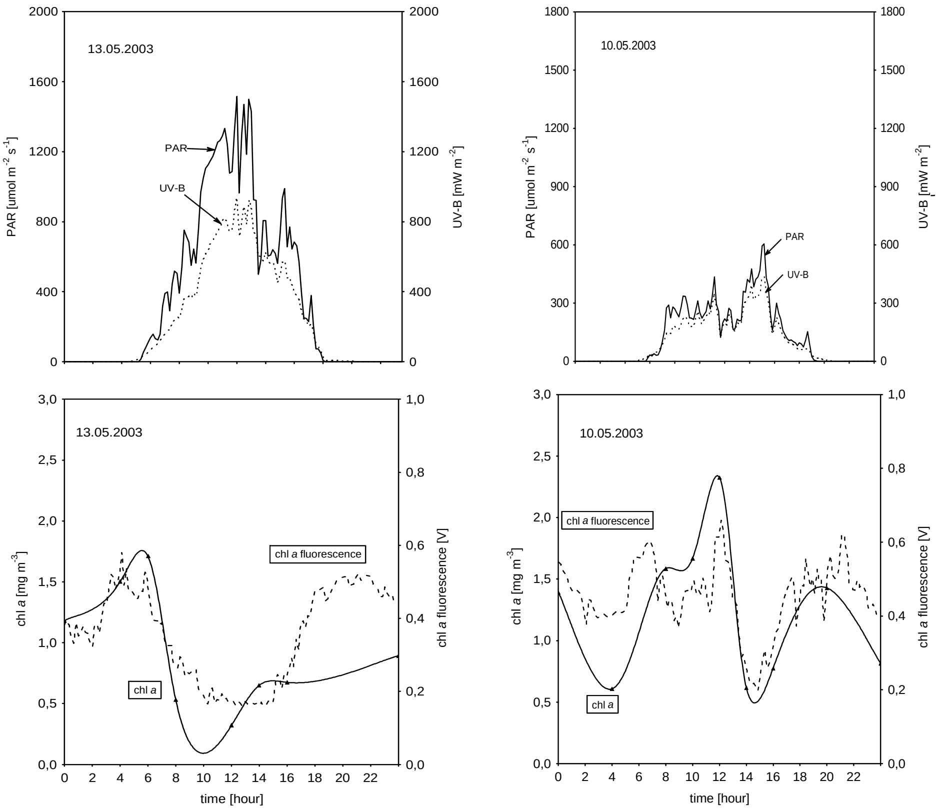

Fig. 5. Changes in solar radiation and chlorophyll *a* concentration and fluorescence a) at relatively high radiation levels and b) at relatively low radiation levels.

When radiation reached low levels, no marked decreases in chlorophyll *a* concentration or fluorescence were observed (Fig.5b). This situation was noted in the morning of 10 May 2003. Living processes are inhibited in phytoplankton by absorbed doses of radiation (*i.e.*, as a result of radiation intensity and irradia tion duration). Some authors believe that markedly more negative effects are observed when an organism is exposed to lower intensity radiation for a longer period than to high intensity radiation for a short time (Cullen and Lesser 1991; Neale *et al*. 1998;). A decrease in chlorophyll *a* concentration occurred in the second part of the day (10 May 2003), shortly before 14:00 when PAR and UV-B radiation were characterized by slight but sufficiently large increments that exceeded in a short time the range of critical values presented above (Fig. 5). Although the increased radiation intensity lasted only a few hours, the response

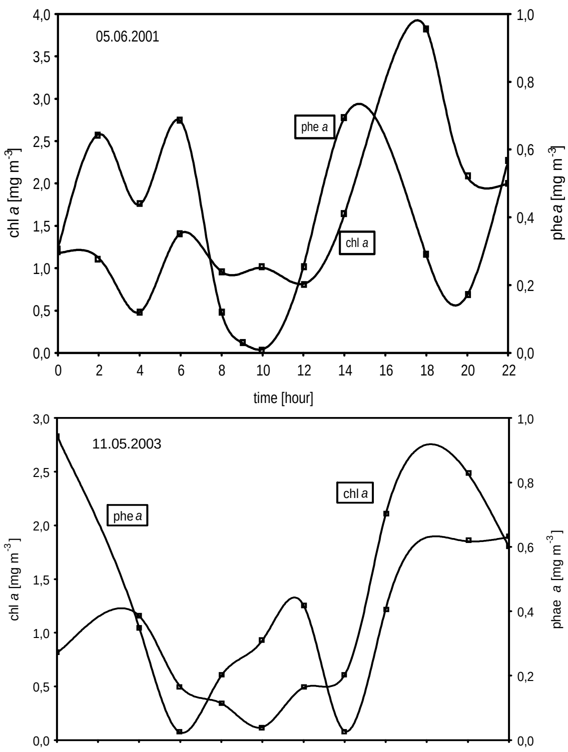

Fig. 6. Diurnal variations in chlorophyll *a* and pheophytine *a* concentration a) 11 May 2003 and b) 05 June 2001.

of the phytoplankton was immediate and was manifested in a decrease of chlorophyll *a* concentration by about 2 mg m^{-3}.

During increased irradiation between 08:00 and 12:00 on 11 May 2003, a gradual increase in pheophytine *a* concentration was observed (Fig.1, 6a). The phae *a*/chl *a* ratio was considerably high under intense radiation and reached nearly 2.5. This was the maximal value recorded during the daily series of measurements. A relatively high concentration of pheophytine *a* in comparison to chlorophyll *a* was also observed before and at sunrise between 02:00 and 06:00 (Fig.6b). The phae *a*/chl *a* ratios at 02:00, 04:00 and 06:00 were 0.58, 0.91, and 0.48, respectively. In the first case, this could be the effect of chlorophyll *a* degradation, as has been suggested in works by Vincent and Neale (2000) and Falkowska (2001). In the second case, these ratio values could have been indicative of zooplankton grazing considerably on phytoplankton. This is another process that influences chlorophyll *a* fluorescence. Zooplankton is known for its markedly cyclic appearance; the maximal abundance of zooplankton in the surface waters of the Gdańsk Deep is usually observed at sunrise and at twilight before sunset (Ciszewski *et al*. 1983; Renk 1997).

CONCLUSIONS

Changes in chlorophyll *a* concentration and chlorophyll *a* fluorescence were strongly correlated to changes in PAR and UV-B radiation. Intense solar radiation that reached the surface water layer caused either a loss of pigmentation or pigment bleaching. While short-term fluctuations of chlorophyll *a* fluorescence registered in the sea surface layer were the direct effect of solar radiation; they also resulted from the occurrence of zooplankton in this water layer at defined times of the day. In both cases, increases of pheophytine *a* concentration were observed as a result of phytoplankton mortality. Enhanced levels of radiation could have induced phototactic migrations of phytoplanktonic organisms.

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