Adenosine triphosphate in the marine boundary layer in the southern Baltic Sea

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Abstract

Changes in adenosine triphosphate (ATP) concentration were measured in the offshore and coastal waters of the Gdańsk Basin in spring. As regards the vertical distribution, it was found that high ATP concentrations occurred mainly in the euphotic layer (above the thermocline) and near the bottom (below the halocline). The high concentrations of ATP in the euphotic layer resulted from primary and secondary production, while the other maximum was due to the presence of bacteria actively degrading organic matter. Changes in ATP concentration in the euphotic layer were closely correlated with the phase of the day. An increase in ATP concentrations in the surface microlayer was observed in the evening and at night, probably as a result of heterotroph proliferation. During daylight, ATP production was inhibited by increasing radiation, hence its concentrations in the sea surface microlayer were considerably lower. Strong winds exerted a significant influence on ATP concentrations in the surface microlayer and in the subsurface water. Windstress depressed ATP concentrations. The biomass of living microorganisms in the microlayer was comparable with the microbiomass beneath the halocline.

1. Introduction

A substantial amount of oceanographic research looks into the biomass present in the marine ecosystem, especially that of microorganisms at the base of the food chain. A compound fundamental to the energy metabolism of all living organisms, adenosine triphosphate (ATP) is applicable as

a bioindicator. So long as cells are alive, synthesis and degradation of ATP is continuous. After their death, production of ATP ceases and the compound decomposes rapidly. The amount of ATP in particulate material is thus a valuable indicator of the biomass of live microorganisms, including bacteria, phytoplankton and microzooplankton. On this assumption, ATP concentration can be used as a biomass parameter in the same way as, for example, chlorophyll, which is used as a relative measure for the standing crop of plants (Vosjan et al. 1987). Holm-Hansen & Booth (1966) were the precursors of the use of ATP as a biomass parameter, and their studies were continued by Karl (1980), who wrote a detailed review of their research. At present, the determination of ATP is considered a quick and easy method for evaluating the living biomass distribution, since only small sample volumes are required.

As far as the Gdańsk Basin is concerned, Falkowska (2001) conducted a pilot study on ATP concentration in the sea surface microlayer; her work, however, did not include a detailed investigation of ATP distribution. The aims of the present project were thus as follows: to survey ATP concentration ranges in the waters of the Gdańsk Basin; to analyse the vertical distribution of ATP as an indicator of the living biomass in the water column; to examine the effect of solar radiation on the ATP concentration within the euphotic layer and the sea surface microlayer; to determine the effect of meteorological factors on the ATP concentration in the sea surface microlayer.

2. Materials and methods

Water samples were collected from on board the Polish Navy vessel ORP 'Kopernik' in offshore waters, at station P1 ($\phi = 55°1'$N, $\lambda = 19°10'$E), and at station P2 in the coastal zone off the Hel Peninsula (Fig. 1). For the analysis of the ATP concentration in the sea surface microlayer, water samples were collected at 2 or 4 h intervals in four sampling periods (Table 1). Samples from the whole water column in the Gdańsk Basin (Station P1) were taken at 4 h intervals solely on 19 and 20 June 1996.

Samples of the sea surface microlayer (average thickness 10 μm) were collected using the teflon plate technique described earlier by Falkowska (1999). Subsurface water samples were taken from a depth of 15 cm below the sea surface into a polyethylene sampler. Water samples from 5 m depth down to the near-bottom layer were taken with a rosette sampler (Ocean Test Equipment Inc.), and the measurements of water temperature, conductivity and depth were done with a CTD profiler (Falmouth Scientific Inc.).

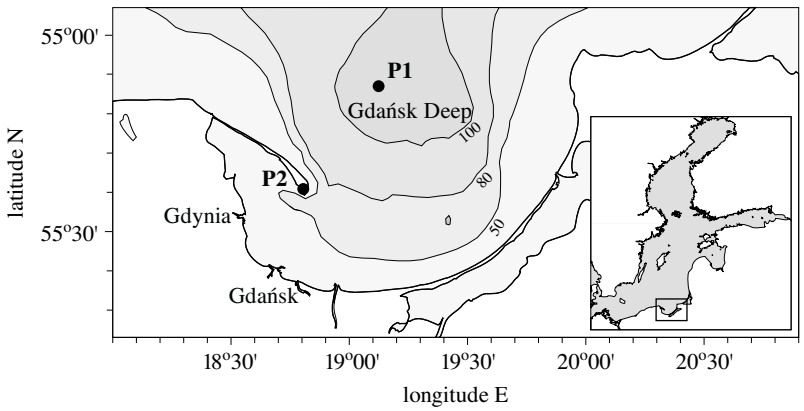

Fig. 1. Location of the sampling stations: P1 – Gdańsk Deep, P2 – coastal station off the Hel Peninsula

Table 1. List of water samples collected from the subsurface layer for ATP determination

Date	Sampling station	Water layer	Sampling period
18–21 June 1996	P1	10 μm (TPM), 15 cm (UWL)	4 h interval
6–8 May 1997	P1	10 μm (TPM), 15 cm (UWL)	2 h interval
10–18 May 2000	P1	10 μm (TPM), 15 cm (UWL)	2 h interval
19–21 June 2000	P2	10 μm (TPM), 15 cm (UWL)	4 h interval

Symbols: TPM – Teflon Plate Microlayer (10 μm thickness), UWL – Underwater Layer (15 cm depth).

On removal of the sample, 25 to 50 cm^3 of the sea water was filtered through 0.22 μm pore acetate filters (Millipore). ATP was determined using the method of Holm-Hansen & Booth (1966) with modifications by Bulleid. The method has been described in detail by Parsons et al. (1984). Thus, ATP was extracted from the suspended matter into boiling TRIS buffer (93350 Fluka) (buffer pH = 7.4–7.5), and a 4 cm^3 aliquot of this extract was then deep frozen (–20°C) until subsequent analysis in the laboratory on land. After the aliquots had been thawed, 0.2 cm^3 of the extract was transferred into scintillation vials to which 0.2 cm^3 of lucipherine-lucipherase enzyme solution (L1761Sigma) was added. The light emission of the samples was measured in a Beckman LS 6000 TA scintillation counter. The detection limit of the method was set at 1 ng dm^{-3}, and the precision within the studied range of concentrations was 50 ng dm^{-3}.

In June 1996, the radiation within the 300–1000 nm band [W m^{-2}] at the sea surface was measured using a spherical CM 5 probe (Kipp & Zonen).

In May 1997 and May 2000, PAR radiation (400–700 nm) was measured using an SKP 210/I 0896 13595 Eijkelkamp probe. In May 2000 there was an additional measurement of UV–B radiation (280–315 nm), using an SKU 430 0497 14854 Eijkelkamp probe.

During each measurement period the standard meteorological observations were carried out.

3. Results

Comparison of the three periods of measurements shows clearly that surface water ATP concentrations were high in June 1996 and May 1997, but very low in May and June 2000 (Table 2). The concentrations in 1996 and 1997 were one order of magnitude higher than those in 2000. In 1996 and 1997, high concentrations of ATP were found in the microlayer (TPM) and in the subsurface water (UWL), even greater than 8.00 μg dm^{-3}, whereas in 2000 the maximum concentrations of ATP did not exceed 1.30 μg dm^{-3}. Despite this considerable variability in ATP concentrations in the sea surface microlayer, enrichment coefficients (EF_{ATP}) were of a similar magnitude in all three measurement periods, their mean values being 1.48 ± 1.26 (June 1996, May 1997) and 1.48 ± 1.50 (May 2000, June 2000).

Table 2. Statistical characteristics of ATP concentrations [μg dm^{-3}] and enrichment factors (EF_{ATP}) in the surface waters of the Gdańsk Deep – station P1 (18–21 June 1996, 6–9 May 1997, 12–18 May 2000), and coastal station P2 (Hel 19–21 June 2000)

	1996, 1997			2000		
	TPM	UWL	EF_{ATP}	TPM	UWL	EF_{ATP}
N	41	42	25	44	51	42
X	1.96	2.28	1.48	0.13	0.10	1.48
SD	2.17	2.43	1.26	0.29	0.21	1.50
Min	0.03	0.02	0.12	0.01	0.01	0.08
Max	9.81	9.71	4.29	1.29	1.13	8.30

Symbols: N – number of data, X – mean value, SD – standard deviation, Min – minimum value, Max – maximum value, TPM – Teflon Plate Microlayer (10 μm thickness), UWL – Underwater Layer (15 cm depth).

In 1996, ATP concentrations in the surface water but also in the water column from 5 m down to the bottom most frequently (33.9% of cases) ranged from 0.00 to 1.00 μg dm^{-3} (Figs. 2 a, b). In May 1997 (Fig. 2c) the number of measurements in the 0.00–1.00 μg dm^{-3} range rose to 60%; at the same time, the number of maximum concentrations

in the 9.00–10.00 μg dm^{-3} range also increased (8.6%). In May 2000, by contrast, only 5.7% of the results were > 1.00 μg dm^{-3}, and the majority of measurements (78.3%) lay within the 0.00–0.10 μg dm^{-3} range (Fig. 2d). In all the samples from the coastal station off the Hel Peninsula, ATP concentrations were < 0.18 μg dm^{-3}, and 88.4% were values < 0.10 μg dm^{-3} (Fig. 2e).

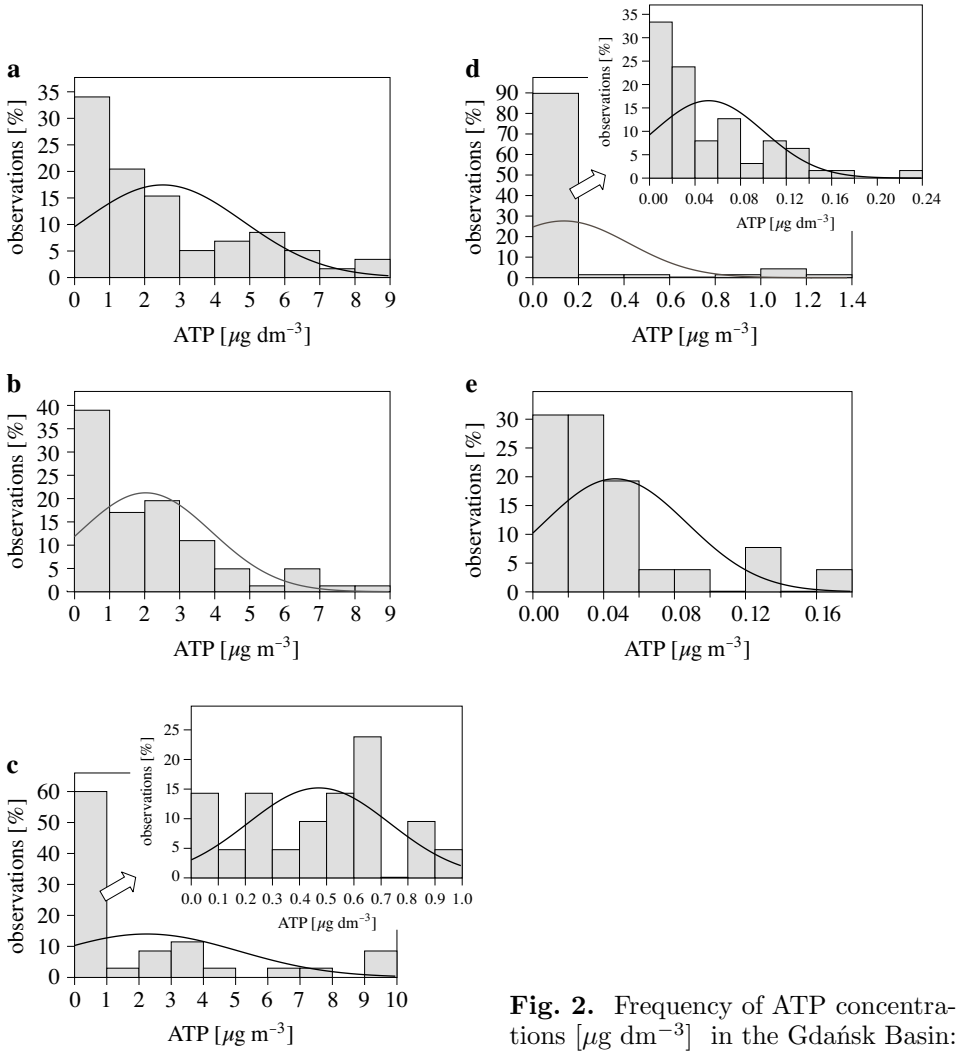

Fig. 2. Frequency of ATP concentrations [μg dm^{-3}] in the Gdańsk Basin:
(a) in the water column (from 5 m depth down to the bottom – P1 (21 June 1996),
(b) microlayer and subsurface water – P1 (18–21 June 1996),
(c) microlayer and subsurface water – P1 (6–8 May 1997),
(d) microlayer and subsurface water – P1 (12–18 May 2000),
(e) microlayer and subsurface water – P2 (19–21 June 2000)

As regards the distribution of ATP in the water column of the Gdańsk Deep (19–20 June 1996), the concentrations became increasingly variable in the vicinity of the thermocline and halocline (Table 3). At 15 m depth, ATP concentrations reached 3.69 ± 2.74 μg dm^{-3}, and at 90 m were as high as 6.27 ± 3.04 μg dm^{-3}. The intermediate water layer between the thermocline and halocline was characterised by very low ATP contents and highly variable readings.

Table 3. Statistical characteristics of ATP concentrations [μg dm^{-3}] in the water column of the Gdańsk Deep (18–21 June 1996)

Deep	N	X ± SD	Min	Max
0	20	1.84 ± 1.70	0.02	6.31
5	9	2.06 ± 2.25	0.03	6.15
10	7	2.08 ± 1.02	1.11	4.24
15	8	3.69 ± 2.74	0.55	7.50
20	8	1.91 ± 1.99	0.06	6.00
30	7	2.53 ± 1.81	0.06	4.25
50	5	1.89 ± 2.17	0.12	5.39
70	6	1.59 ± 2.24	0.17	5.98
90	4	6.27 ± 3.04	2.04	8.79
bottom	5	1.76 ± 1.06	0.50	2.71

Symbols as in Table 2.

4. Discussion

The ATP concentration in the sea surface microlayer underwent short-term fluctuations according to weather conditions. Air temperature, wind speed and cloud cover (cloudiness) exerted a considerable influence on the ATP concentration in the microlayer (TPM) and in the subsurface water (UWL). Regression analysis indicated the adverse effect of wind on ATP concentrations in the microlayer (TPM) ($r = -0.60$) and in the subsurface (UWL) water ($r = -0.61$) (Fig. 3). An increase in wind speed gave rise to a more homogeneous distribution of organisms. Moreover, it induced a change in the 'living' nucleotide content, because under turbulent mixing conditions the microorganisms had to engage their entire energy available in the form of ATP in order to sustain their life functions. Karl (1980) and Vosjan et al. (1990b) reported similar observations.

Solar radiation also caused ATP concentrations to drop. ATP concentrations in the surface microlayer fell to a minimum at noon, when the level of solar radiation is high (Figs. 4 and 5). Maximum concentrations,

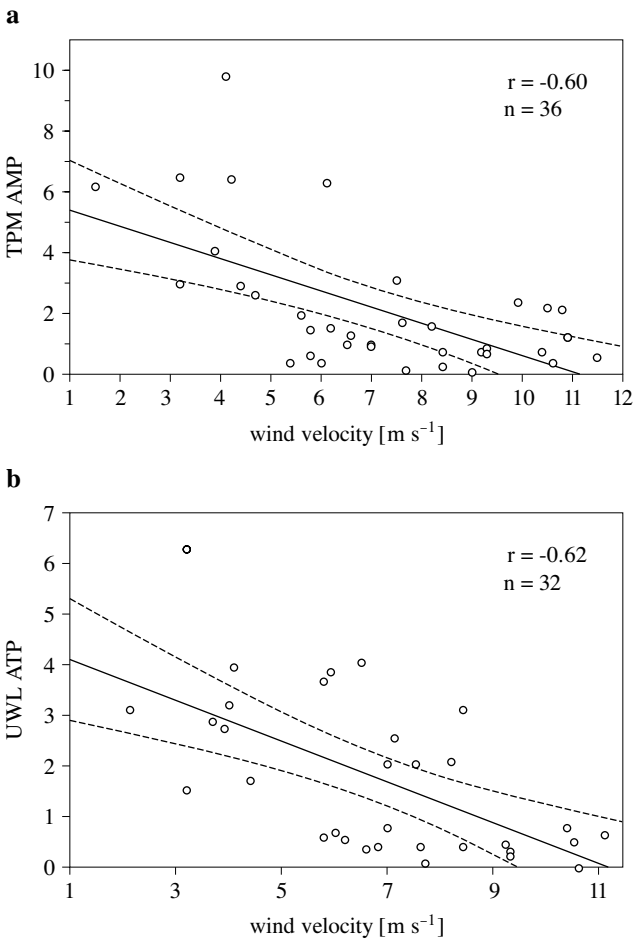

Fig. 3. The effect of wind velocity [m s^{-1}] on ATP concentration [μg dm^{-3}] (confidence coefficient 95%) (18–21 June 1996):
(a) teflon plate microlayer (TPM) – thickness 10 μm,
(b) underwater layer (UWL) – 15 cm depth

indicative of favourable conditions for plankton growth, were noted in the morning (at around 04:00 hrs) and in the evening (16:00 to 20:00 hrs). Cloud cover turned out to be the parameter governing the quantity of available radiation, and therefore facilitating life functions within the surface microlayer. As the sky became more overcast as the afternoon of 17 May 2000 progressed, the abundance of planktonic organisms, as indicated by ATP levels, rose distinctly (Fig. 5). This significant increase in the 'living' nucleotide content in the evening could thus have been a reflection of the photorepair mechanism operating over a short time-scale: this supposition was confirmed by microbiological analysis (Herndl et al. 1993).

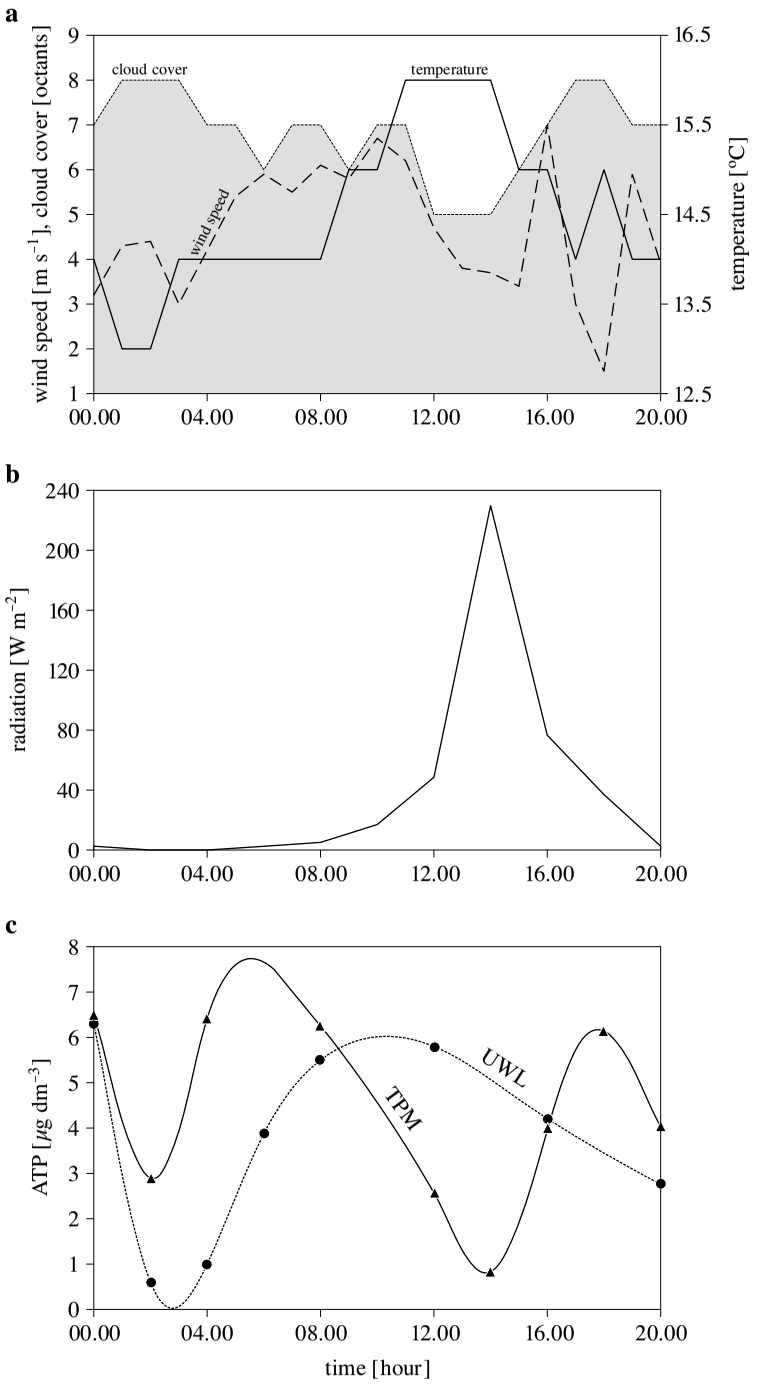

Fig. 4. Temporal changes in the analysed parameters in the Gdańsk Deep (21 June 1996): (a) weather conditions: wind speed [m s^{-1}], air temperature [°C] cloud cover [octants], (b) solar radiation (300–1000 nm) incident on the sea surface [W m^{-2}], (c) ATP concentration [μg dm^{-3}] in the sea surface waters

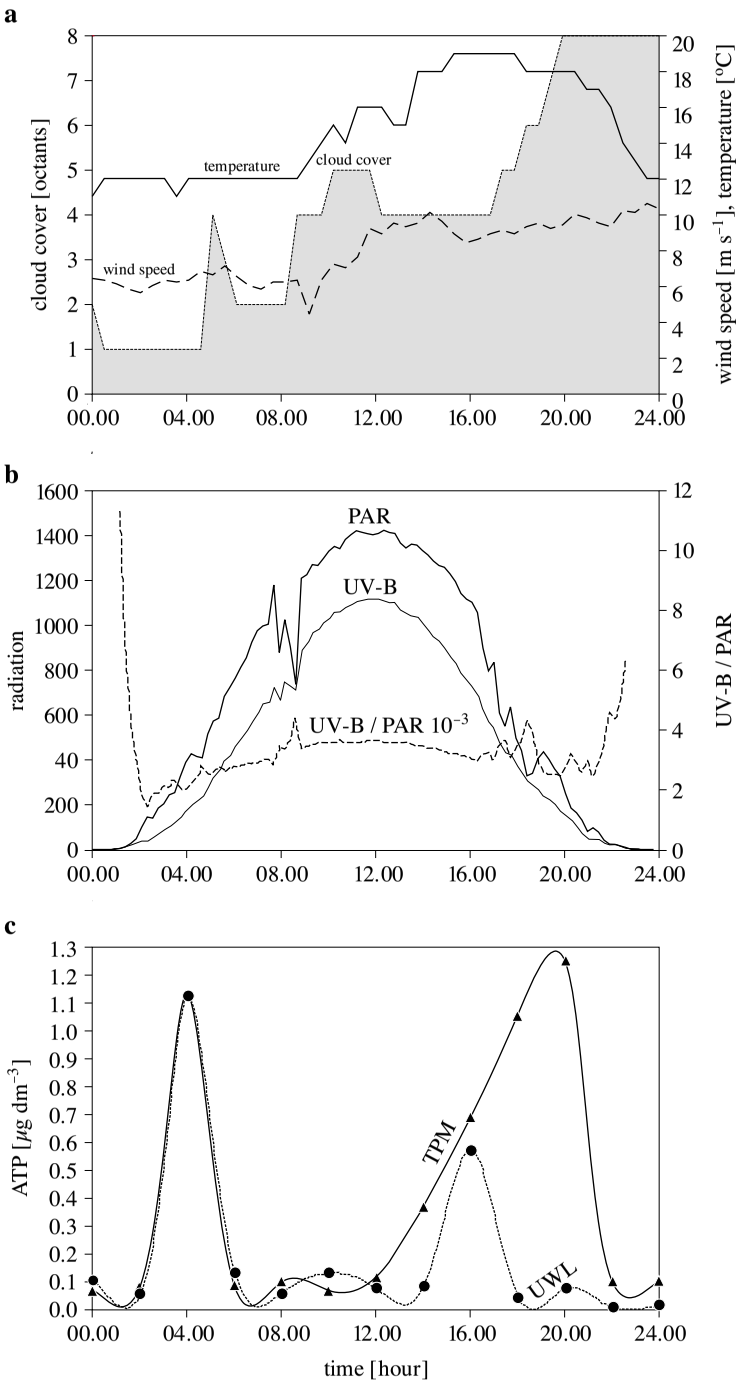

Fig. 5. Temporal changes in the analysed parameters in the Gdańsk Deep (17 May 2000):
(a) weather conditions: wind speed [m s^{-1}], air temperature [°C] cloud cover [octants],
(b) irradiance conditions: PAR radiation (400–700 nm) incident on the sea surface [μmol m^{-2} s^{-1}], UV–B radiation (280–315 nm) [mWat m^{-2}] and UV–B/PAR ratio, (PAR is given in [W m^{-2}] with 0.219 factor),
(c) ATP concentration [μg dm^{-3}] in the sea surface waters

Intense solar radiation leads to stress in the organisms, which depletes the total pool of ATP available to them by weakening their physiological condition or can finally lead to death with subsequent rapid destruction of ATP (Karl 1980). It is generally agreed that, of the entire radiation spectrum reaching the sea surface, the one mostly responsible for the destruction of plankton organisms is UV–B (Vosjan et al. 1990a, Hessen et al. 1995). Laboratory experiments have shown that after 5 h exposure of sea water to light of intensity 1.35 W m^{-2} in the UV–B band (290–320 nm), ATP concentrations drop by 75% (Vosjan et al. 1990a). In the natural environment, the level of UV radiation is lower owing to strong attenuation, but in the surface film and in the sea surface microlayer, where neuston (algae and bacteria) is prevalent, the effect of radiation can even be magnified by multiple reflections brought about by wave action (Regan et al. 1992). In the current project, the UV–B radiation measured at noon in May 2000 attained a level of 1.2 W m^{-2}, hence it was very close to that generated in the laboratory experiment of Vosjan and co-workers (Fig. 5).

ATP concentrations in the sea surface microlayer and the euphotic layer varied according to the diurnal rhythm (Fig. 6). During the hours of darkness, ATP concentrations in were high in the surface microlayer,

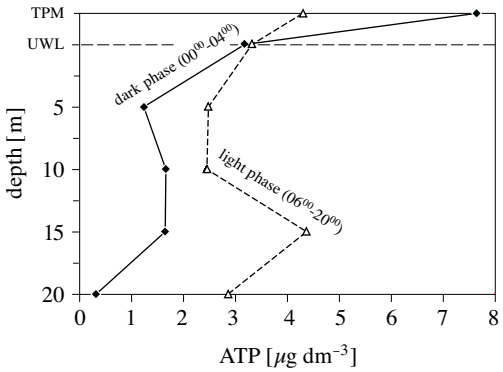

Fig. 6. Vertical profile of ATP concentrations [μg dm^{-3}] in the euphotic zone of the Gdańsk Deep (P1) (the sea surface microlayer is highlighted) during different phases of the day (June 1996) (dark phase from 21:00 to 04:00 hrs when the radiation level within the 300–1000 nm band did not exceed 2.5 W m^{-2}; light phase from 06:00 to 20:00 hrs, when the solar radiation was > 2.5 W m^{-2})

whereas during the daytime higher concentrations of ATP were recorded in the euphotic layer. It seems that during the hours of daylight and darkness heterotrophs and autotrophs are alternately predominant. Weiler & Karl (1979) reported a decrease in the internal ATP concentration in algae cells during the dark period, whereas Vosjan et al. (1990b) pointed out

that bacteria living in surface waters show a clear tendency to increase their biomass at night. The increase in ATP concentration in the surface microlayer during the dark phase could be caused by the proliferation of bacteria, which utilise the suspended and dissolved organic matter accumulated there. During a study in the Gdańsk Basin from May to July, Witek (1995) observed alterations in the bacterioplankton biomass in the euphotic layer and suggested that this was inversely correlated with phytoplankton abundance.

High concentrations of ATP were recorded not only in the microlayer, but also in the boundary layers delimited by the temperature and salinity gradients (Fig. 7):

– above the thermocline, at 15 m depth (3.69 ± 2.74 μg dm^{-3}),

– below the halocline, at 90 m depth (6.27 ± 3.04 μg dm^{-3}).

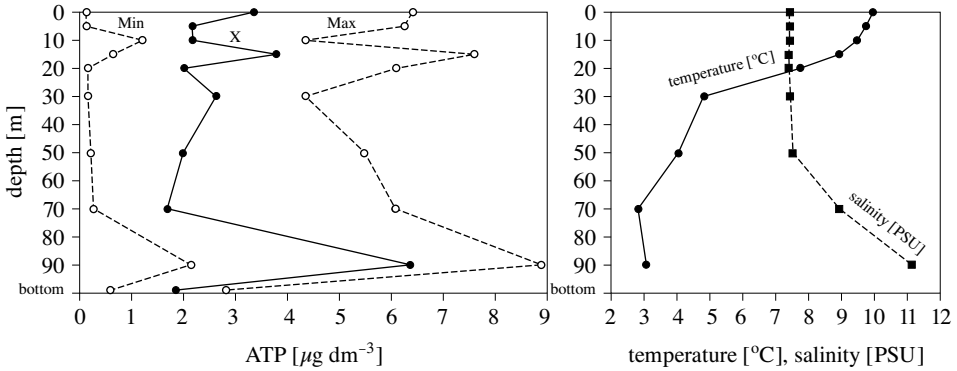

Fig. 7. Vertical profile of the analysed parameters in the waters of the Gdańsk Deep (P1) in June 1996:
(a) ATP concentrations [μg dm^{-3}] (X – mean value), (Min – minimum value) (Max – maximum value),
(b) temperature [°C] and salinity [PSU]

Maximum ATP concentrations were recorded in the near-bottom water layer, where phaeophytins make up a considerable part ($> 80\%$) of the total photosynthetic pigment content (paper in preparation). Below the halocline, the higher water density retards detritus sedimentation and accumulation of organic material there is increased. Because of the rich store of food available in this layer, conditions are optimal for the increase in the biomass of heterotrophs, and of bacteria in particular. The abundance and biomass of bacteria also rise near the sea floor under anaerobic conditions (Witek 1995). Hence, one can conclude that high ATP concentrations in the near-bottom layer are largely a reflection of heterotroph biomass.

5. Conclusions

The changes in adenosine triphosphate (ATP) concentrations in the water column in spring can be characterised as follows:

- A distinct rise in ATP concentration was observed in the sea surface microlayer, probably as a result of an increase in the numbers of bacterioplankton.
- The high concentration of ATP in the euphotic layer above the thermocline was caused by the presence of planktonic organisms.
- The high ATP concentration in the near-bottom layer (below the halocline) is due to the activity of heterotrophs participating in the degradation of organic matter.

The biomass of living microorganisms in the microlayer was comparable with the microbiomass beneath the halocline. In both cases it probably consisted of proteolytic bacteria.

The variations in ATP concentration in the euphotic layer turned out to be closely related to the time of day. During daylight, ATP production is inhibited and concentrations are much lower in the sea surface microlayer; at the same time, ATP concentrations in the subsurface water increase. During darkness, ATP concentrations in the surface microlayer rise as a result of heterotroph proliferation.

Wind speed exerted a significant effect on ATP concentrations in the sea surface microlayer and in the subsurface water; wind-stress depressed ATP concentrations.

Acknowledgements

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