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Impact of Microphytobenthos Photosynthesis on the Characteristics of the Echo Signal from Baltic Sandy Sediments

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The understanding the influence of biological processes on the characteristics of the signals backscattered by the sea floor is crucial in the development of the hydroacoustical benthic habitat classification techniques. The impact of the microphytobenthos photosynthesis on the acoustical backscattering properties of the Atlantic sandy sediments was previously demonstrated by Holliday *et al.* (2004) and Wildman and Huettel (2012).

To account for the sensitivity of the hydroacoustical classification techniques to the backscattering properties of local marine sediments, it is important to understand the microphytobenthos photosynthesis impact for the Baltic Sea where the techniques are being actively developed now. This is the main motivation of the paper.

In the paper the influence of the microphytobenthos photosynthesis on the characteristics of the echo signals reflected by sandy sediments in the typical Baltic temperature and the salinity conditions is discussed. The interdisciplinary multiday laboratory experiment was conducted to study the impact of benthic microalgal photosynthesis on the characteristics of the echo signal reflected by sandy sediments. Hydroacoustical data were collected under controlled constant light, temperature and salinity conditions. The oxygen content at different levels of the water column was simultaneously monitored.

Keywords: hydroacoustics, backscattering, microphytobenthos photosynthesis, Baltic Sea.

1. Introduction

The use of hydracoustical techniques to classify and map benthic fauna and flora and to observe benthic biological processes (e.g. benthic photosynthesis) is one of the leading challenges in the present marine research. The progress in the development of the hydroacoustical methods for examining living benthic communities and benthic biological processes is reported in numerous journal papers (Anderson *et al.*, 2002; Sabol *et al.*, 2002; Holliday *et al.*, 2003; Kenny *et al.*, 2003; Klusek *et al.*, 2003; Pinn, Robertson, 2003; Tęgowski *et al.*, 2003; Brown *et al.*, 2005; Ehrhold *et al.*, 2006; Gorska, Kowalska, 2012), conference papers (Faghani *et al.*, 2004; Holliday *et al.*, 2004; Kruss *et al.*, 2006; Tęgowski *et al.*, 2007), monographs and reports (Hermand, 2003, International Council for the Exploration of the Sea [ICES] Cooperative Report, 2007).

The further development and improvement of the techniques needs deeper knowledge of the acoustical backscattering properties of the benthic flora and fauna and advances in the understanding of how biological processes such as benthic photosynthesis affect the characteristics of the signals backscattered by the sea floor (ICES Cooperative Report, 2007). The physics of sound scattering from benthic animals and plants was studied mainly theoretically (numerical modelling) by Shenderov (1998), Stanton *et al.* (2000), Stanton (2000), Stanton and Chu (2004), Gorska and Kowalska (2012). The impact of the benthic photosynthesis on the sandy sediment acoustical backscattering properties was studied both in the laboratory (Holliday *et al.*, 2004; Wildman, Huettel, 2012) and *in situ* (Hermand, 2003; Wildman, Huettel, 2012) as well. The key finding was that the photosynthesis of the microphytobenthos (microscopic algae and cyanobacteria inhabiting the seafloor; MacIntyre *et al.*, 1996) can significantly influence the echo signal characteristics (Holliday *et al.*, 2004; Wildman, Huettel, 2012).

In approximately one third of the continental shelf area light reaching the sea bed is sufficient for the benthic photosynthesis (Gattuso *et al.*, 2006) responsible for oxygen supersaturation in sediment pore water and formation of numerous bubbles. Approximately 50,000 bubbles m^{-2} were common at study site in St. Joseph Bay, FL, in the research conducted by Wildman and Huettel (2012), and these bubbles significantly affect the sound propagation and scattering (Leighton, 1997; Medwin, 2005; Ainslie, Leighton, 2011). Therefore, the understanding of the benthic photosynthesis influence on the backscattering properties of the marine sediments under different local environmental conditions is a crucial task.

The studies of Holliday *et al.* (2004) and Wildman and Huettel (2012) were conducted in hydrophysical conditions for sandy sediments types and microphytobenthos species typical of Atlantic Ocean (Gulf of Mexico). These factors are different in the southern Baltic Sea, the area of significantly lower marine water salinity and lower temperature. Accounting for their potential impact on the microphytobenthos phothosynthesis, including photosynthetic oxygen production and the oxygen bubbles generation in seabed sandy sediments and water, it is important to understand the microphytobenthos phothosynthesis effect on the acoustical backscattering from the southern Baltic sandy sediments.

The main objective of this paper is to demonstrate the impact of the photosynthetic activity of the southern Baltic benthic microalgae on the echo signal characteristics (echo energy and arrival time). Changing the light conditions (light/dark (L/D) photocycles) the backscattering data were collected in the aquarium with sandy bottom under controlled constant temperature and salinity conditions during a multiday laboratory experiment. Additionally, the oxygen content was registered and the microphytobenthos community was studied using light microscopy.

The methodology of the conducted research is presented in details in **Materials and Methods** section. The influence of the benthic photosynthesis on the characteristics of the echo signals reflected by sandy sediments is discussed in *Results and discussion* section. The main results are summarized in *Summary* part.

2. Materials and methods

2.1. Measurements

An interdisciplinary multiday laboratory experiment was conducted to study the impact of benthic microalgal photosynthesis on the characteristics of the echo signal reflected by sandy sediments. Changing the light conditions (modelling day and night illumination, L/D photocycles) allowed the diel variability of the echo signal to be analyzed. Hydroacoustical and oxygen concentration measurements were carried out under controlled light, temperature and salinity conditions. The experimental setup (including aquarium, size: 50 × 50 × 50 cm) is presented in Fig. 1.

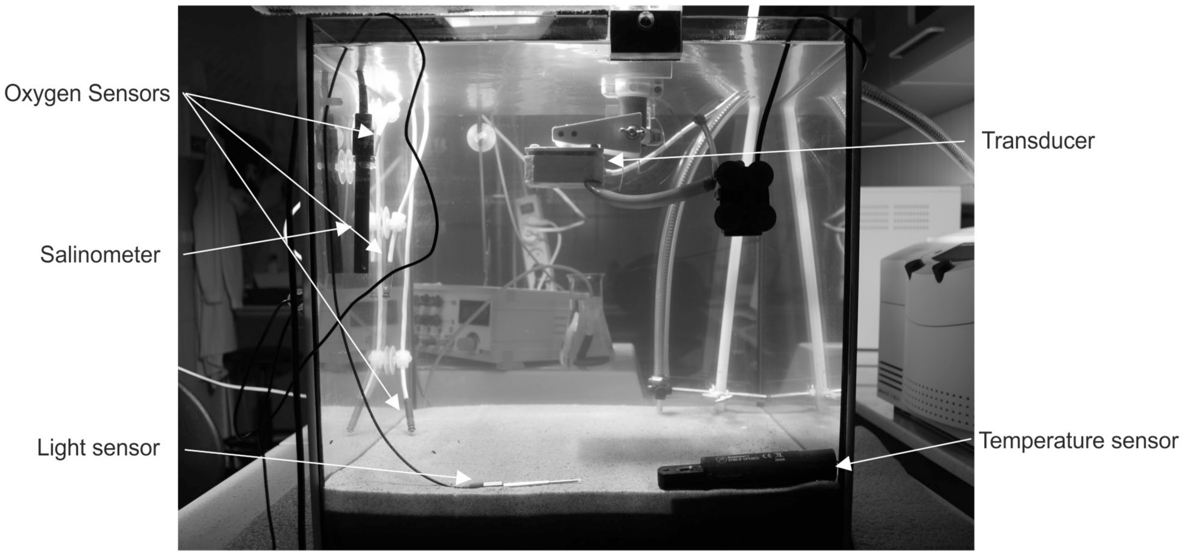

Fig. 1. A photograph of the aquarium in which the measurements were carried out.

The sediment samples were collected in the vicinity of Władysławowo (45° 50′ 2″ N, 18° 18′ 56″ W). The sample place is located in the western, internal part of the Puck Bay (the Gulf of Gdańsk, southern Baltic Sea, Poland). During sampling, upper 2-cm thick layer of sediment was collected from the area, where the water column depth was 20–40 cm. The basic readings of light intensity at the seabed surface, water temperature and salinity were also taken. The sediment sample contained medium sand (diameter of 69% of sandy grains varied within the range from 250 to 500 μm). After sampling, the sand was transported to the laboratory and wet sieved through a 2 mm screen in order to remove macrozoobenthos.

The sediment was fairly mixed in order to obtain equally distributed algal biomass and then placed in the aquarium forming 7 cm thick bed of sand. The sediment surface was smoothed with a straight edge and the tank was filled with artificial sea water of 6.7 PSU (value characteristic for the waters of the Gulf of Gdańsk).

The experiment was conducted in a darkened air-conditioned laboratory in the period from 30.08.13 to 10.09.13. In order to recreate day and night conditions (L/D photocycles), the Philips 400W sodium lamp located above the aquarium was used. To keep constant temperature and to avoid upper water layer overheating during the illumination period, an additional cooling system was employed.

The experiment was conducted at the photosynthetically active radiation (PAR) intensity: 500 ± 15 $\mu\mathrm{mol\,m^{-2}\,s^{-1}}$ and stable temperature: 18°C. The photoperiod (L:D cycle, duration of light and dark periods in hours) was 9:15. During three days before the measurements' beginning, the sediment was adapted to the laboratory conditions (including: light conditions, photoperiod, temperature, etc.).

2.1.1. Hydroacoustical measurements

The hydroacoustical system is presented in Fig. 2. The vertically down-looking transducer operated in pulse-echo mode emitting acoustical pulses and receiving the echoes.

It was mounted 29 cm above the sand surface (Fig. 1). The electric signal produced by the Tektronix AFG 3011 generator was sent to the transducer through the transmitter/receiver unit which powered the emitting mode of the transducer. Over the entire working frequency range (from 200 to 700 kHz) at each frequency f the electric pulse length was equal to $10/f$ (ten periods). The signal reflected by the aquarium sandy bottom was registered by the transducer, which was run as a receiver by the transmitter/receiver unit, and then amplified. Then the backscattered data were visualized, partially processed and acquired at the Tektronix TPS 2024B oscilloscope. The hydroacoustical

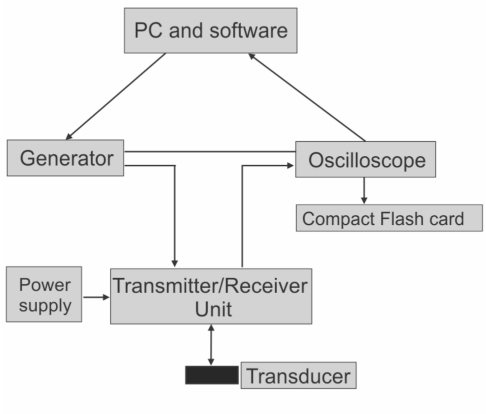

Fig. 2. The hydroacoustical measurement scheme.

measurements were governed by the specialized software created in MATLAB codes.

During one measurement series, which lasted about 11–12 minutes, the transducer operated consecutively at the frequencies from 200 to 700 kHz with the step of 10 kHz. This frequency range was chosen because it was over this frequency range that the significant impact of the benthic microalgal photosynthetic process on the acoustical backscattering properties of the sandy sediments was demonstrated by Holliday *et al.* (2004). Over the entire frequency range at each individual frequency 64 pulses were emitted. It means that one pulse per approx. 0.22 sec was generated. The echo signal averaged over these 64 echo pulses was saved at the oscilloscope compact flash card. As a result of one measurement series the 51 averaged echo signals (at 51 frequencies) were logged at the frequency range from 200 to 700 kHz. In our measurements at each frequency an echo signal was sampled with the frequency 2.5 MHz.

The transducer directivity pattern was narrow over the entire frequency range: its half width decreased from approximately 4.2^o at 200 kHz to approximately 1.2° at 700 kHz (Marszal, 1992). It provided comfort conditions excluding the multi reverberation which typically disturbs the hydroacoustical measurements in a small aquarium (Bobber, 1970). The hydroacoustical measurements were conducted in the far field (Salamon, 2006).

A pump system was used to gently circulate water in the aquarium during the experiments. The flow was directed across the face of the transducer. This swept the bubbles from the face when they grew large enough and rose from the sandy bottom toward the water surface.

2.1.2. Accompanying measurements

Three oxygen electrodes YSI 5331 probes were used to measure the oxygen concentration changes that could be induced by the photosynthesis and the respiration of benthic microalgae. They were located at three different depths in the aquarium: near the sandy bed, at the middle depth and just below the water surface – respectively 1 cm, 15 and 35 cm above the sandy bottom (Fig. 1). To monitor salinity and temperature of water in the tank inoLAB COND LEVEL 1 salinometer was employed (it is shown in the left side of the tank in Fig. 1). A Submersible Spherical Micro Quantum Sensor (Walz GmbH, Effeltrich, Germany) connected to the LI-COR LI-189 light meter (LI-COR Biosciences, Lincoln, Nebraska, USA) was used to control photosynthetically active radiation (PAR) intensity. The collected data were logged once per hour. In order to control temperature stability the additional temperature logger (the small cylinder on the tank floor, right corner) was also used. The data collected by this sensor were logged with higher frequency (once per ten minutes). All sensors were placed around the area being acoustically sampled to avoid mutual interference.

The small sediment samples were taken from the aquarium after the experiment in order to analyze microphytobenthos species composition. The species identification was made using a Nikon Eclipse 80i microscope equipped with a 4× objective.

2.2. Analysis of the backscattered data

Bearing in mind the prior observations of diel oscillations of the backscattered energy due to the microphytobenthic photosynthesis (Holliday *et al.*, 2004; Wildman, Huettel, 2012), the collected data were processed to reveal the diel fluctuation of the reverberation from the sandy bed. The example of acoustical echo (at 250 kHz) from the sediment-water interface is demonstrated in Fig. 3. As it was mentioned above it is the result of the averaging over the 64 succeeding echo pulses recorded at the frequency 250 kHz during the one measurement series.

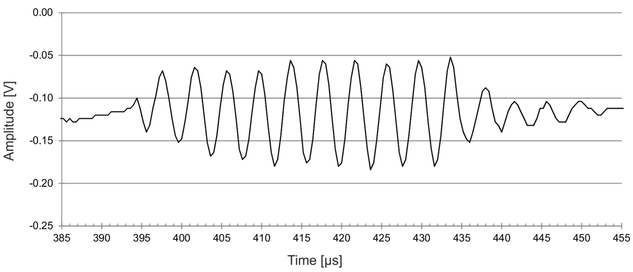

Fig. 3. Analyzed data set. Example of echo signal from the sediment-water interface at frequency 250 kHz. Over the entire frequency range at each individual frequency 64 pulses were emitted and the echo signal averaged over these echo pulses was saved. The presented pulse is averaged over the 64 successive pulses.

In order to characterize the strength of the backscattering at each frequency the parameter called below "echo energy" was calculated basing on the equation:

$$E_i = 10\log_{10}\left(\sum_{j=n_1}^{j=n_2} U_{ij}^2\right), \tag{1}$$

where E_i describes the individual pulse of number i. The parameter U_{ij} denotes the voltage of sample of number j in the echo ping of number i . Here n_1 and n_2 describe respectively the number of the first and last samples corresponding to the start and the end of the time window selected for the analysis.

The start points and the end points of the time window used in the echo energy calculation (n_1 and n_2 in the Eq. (1)) were determined at each individual frequency over the range from 200 to 700 kHz. The entire set of the individual echoes collected during the multiday experiment was analyzed and the sensitivity of the echo energy to the specific choice of the window boundaries was tested. At each individual frequency selecting the upper window boundary (n_2 in the Eq. (1)), it was controlled that the analyzed signal being reflected from the sand was not "contaminated" by the echo from the base of the tank (from sand – glass interface). In our measurements it was qualified due to the appropriate relation between the thicknesses of sandy layer at the aquarium's bottom and the echosounder sampled volume, which is determined by the acoustic pulse duration (Simmonds, MacLennan, 2005). The parameters:

$$\tau_w = Tc_w/2 \tag{2}$$

and

$$\tau_s = Tc_s/2 \tag{3}$$

describe the thickness of the sampled volumes in the water and sand respectively, and characterize the vertical resolution of the measurements. Here T is the pulse length and symbols c_w and c_s describe the sound speed in water column and sandy bottom, respectively.

At the frequency 250 kHz the parameters τ_w and τ_s are approximately equal 3.0 and 3.2 cm, respectively and are less than the 7 cm-thickness of sandy layer at the aquarium bottom. The values of $c_w = 1484$ cm/s (the temperature 18°C and salinity 6.7 PSU) and $c_s = 1600$ m/s (medium sand) were taken in the calculations.

It was demonstrated that the choice $n_1 = 970$ and $n_2 = 1130$ was reasonable in the analysis of the echoes at the frequency 250 kHz. The value n_1 provides the start point of the time window close to the time of the arrival of the front of the pulse reflected from the sediment-water interface:

$$t_1 = 2H/c_w. \tag{4}$$

Here $t_1 \approx 390$ μsec ($H = 29$ cm is the distance between echosounder face and the boundary). The end point (n_2) corresponds to the time $t_2 \approx 450$ μsec. It can be shown that for this time moment the information was collected within the sampled volume located between 1.5 and 4.7 cm beneath the sediment-water interface. It was also checked that the slight shift to the right of the upper window boundary does not impact on the echo energy (due to the decreasing amplitudes).

Summarizing, it can be concluded that the information contained in the analyzed part of the echo signal is collected not deeper than approximately 4.7 cm below the sediment-water interface and that it is not "polluted" by the echo from the base of the aquarium which is located 7 cm beneath the interface. Moreover, the part of the echo from the glass base of the aquarium started in the time:

$$t_3 = 2H/c_w + 2d/c_s\,, \tag{5}$$

where d is the thickness of the sandy layer at the aquarium bottom. t_3 approximately equals to 478 μsec. It corresponds to the sample number approx. equal to 1200, which is larger than chosen n_2 (the right end of the window). It once more confirms that the echo from the aquarium glass base was not considered in data analysis at 250 kHz.

At higher frequencies the same value of $n_1 = 970$ determining the window start point was used. Of course, over the entire frequency range, the n_2 value was carefully chosen and left smaller than 1200 corresponding to the arrival of the echo from the base of the aquarium.

Taking in to account that:

- the parameters τ_w and τ_s decrease with frequency growth,
- for smaller frequencies (example 250 kHz was considered above) the echo is not contaminated by the reflection from the aquarium base,

it can be certain that the reflection from the aquarium base did not impact on the analyzed signal at higher frequencies.

Using Eq. (1) with the reasonably chosen time window (n_1 and n_2 values), the diel echo energy variation over a few days measurements was studied.

Accounting for that the growth of oxygen content and number of bubbles in the water column during the light time could decrease the sound propagation speed and increase the signal propagation time (the effect was observed by Wildman and Huettel (2012)) the difference in the arrival times between light and dark periods was also analyzed.

3. Results and discussion

3.1. The diel variation of the echo energy

The diel variation of the echo energy and its mean values for the dark and the light periods (diel cycles) are presented in Figs. 4a and 4b, respectively at the frequency 250 kHz. In the figures white and grey strips refer to the "day" and "night" periods respectively. In Fig. 4a for each diel cycle the energy was normalized in respect to the energy at the start of the night measurements in order to demonstrate the echo energy difference at the dark and the light periods.

The mean values of the echo energy were calculated separately for each consecutive light and dark period. Accounting for that the echo energy increased

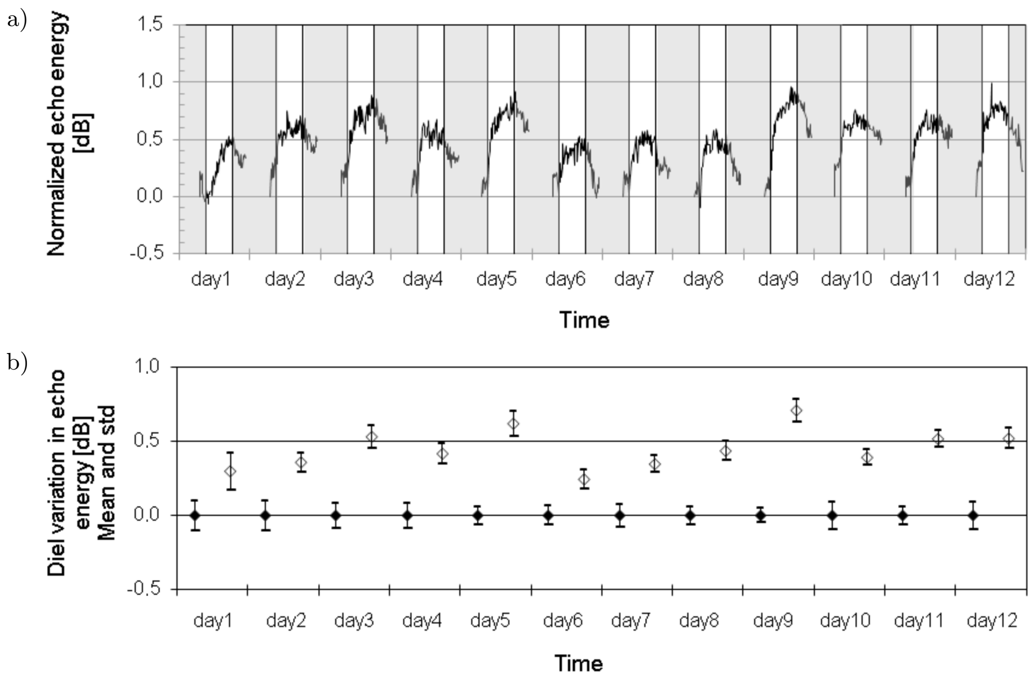

Fig. 4. Time variation of the normalized echo energy at 250 kHz frequency backscattered from a sandy sediment layer (a). Temporal dependence of the mean echo energy at 250 kHz (b).

before reaching the some stabilized level during the first 2–3 hours after switching on the sodium lamp (Fig. 4a), the echo energy data over this initial period were not considered in the averaging. To understand the diel variation of the echo energy, for each diel cycle the echo energy means both, for "day" and prior "night" times, were reduced by the mean echo energy of this dark period. The differences in the mean echo energy between each light period and the prior darkness were presented in Fig. 4b by white rhombs, while the black rhombs correspond to the dark periods. Whiskers in Fig. 4b represent the standard deviation values.

Figure 4a demonstrates that the echo energy increased after the sodium lamp had been turned on and decreased when it had been turned off. The echo energy was distinctly larger during the "day" comparing to the values measured at "night". The "day / night" difference in the echo energy was not more than 1 dB. The results presented in Fig. 4b also confirm the difference in the echo energy level between the light and the dark periods.

Figure 5 demonstrates the diel echo energy variability over the entire frequency range from 200 to 700 kHz. Each pixel in the figure corresponds to one measured frequency and to one experimental day. Its colour corresponds to the difference in the mean echo energy between the light period and the prior darkness (in dB). The colour scale bar is presented in right plot side and the light green colour refers to a 0 dB-difference.

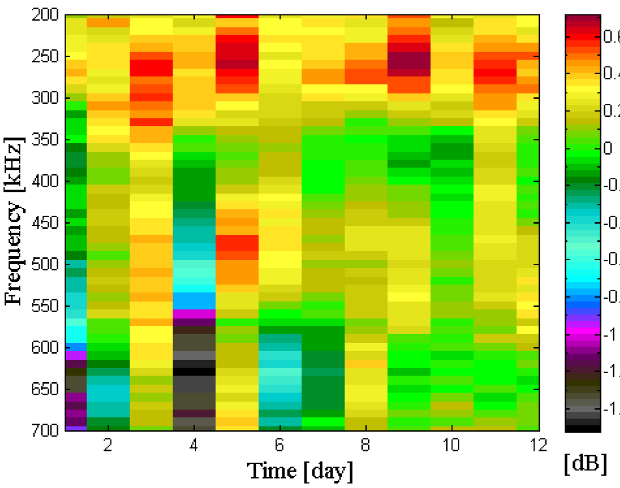

Fig. 5. The diel variability in the mean echo energy over the entire frequency range.

The figure demonstrates that at 250 kHz the echo energy increases in light periods. The larger "day / night" differences were observed in third, fifth and ninth days (orange and red pixels). It is in agreement with the results presented in Fig. 4b. The similar pattern (larger echo energy during the light period) was observed over the frequency range from 200 to approx. 300 kHz. The diel patterns are different for the larger frequencies: for some diel cycles the echo energy difference between light and dark periods was not observed (green pixels) and for others – the echo energy in "day" time was smaller than during the "night" (blue, ping, grey and black pixels). To explain the diel variation in the acoustical backscattering observed over the frequency range from 200 to 300 kHz it is important to notice that in the measurements series the bubbles (approximately 1 mm diameter) were frequently visible during the light time at the sediment-water interface and rising from the sandy bed within the water column. The photographs, presented in Fig. 6, are only the qualitative documentation of the bubble presence at the sediment surface during a "day" time. The detailed study of the size distribution of bubbles at the surface and within the sandy layer has not been carried out.

The bubbles disappeared at the dark period. Accounting for the fact that the gas bubbles, even in small numbers, can strongly modify the sound propagation and scattering conditions (Leighton, 1997; Medwin, 2005; Ainslie and Leighton, 2011) it can be concluded that their cyclical growth (during the "day") and disappearing (at "night") were responsible for the diel variation of the echo energy. Based on the experience of Holliday *et al.* (2004) and Wildman and Huettel (2012) it can be also assumed that the interstitial bubbles were present in the sediments and had the impact on the echo signal.

The variation of the visibly observed bubble number was also correlated with the oxygen content change. As it was mentioned in the methodological part of the paper three sensors were mounted at different depths (Fig. 1) in order to collect the oxygen data. The values, registered by different sensors, varied within a very narrow range. Therefore, only the readings logged by the sensor near the bottom are presented in Fig. 7. The figure demonstrates the diel variation of the oxygen content: the "day" growth changed approximately from 12 to 20%.

The synchronization of the variation in the echo energy, the oxygen content and the visible bubbles number with the light cycle provides convincing evidence that the increase in the echo energy during the light period can be explained by the microphytobenthos photosynthesis. It went on during the light time and was accompanied by the oxygen production and therefore the increase occurred in the number of the bubbles, which impacted on the echo energy. It is also essential to note that the microscopic observations confirmed that the healthy benthic microalgae community was present in the sediment and that it was dominated by diatoms.

It is not clear why the diel patterns of the echo energy variation at individual frequencies differ for higher

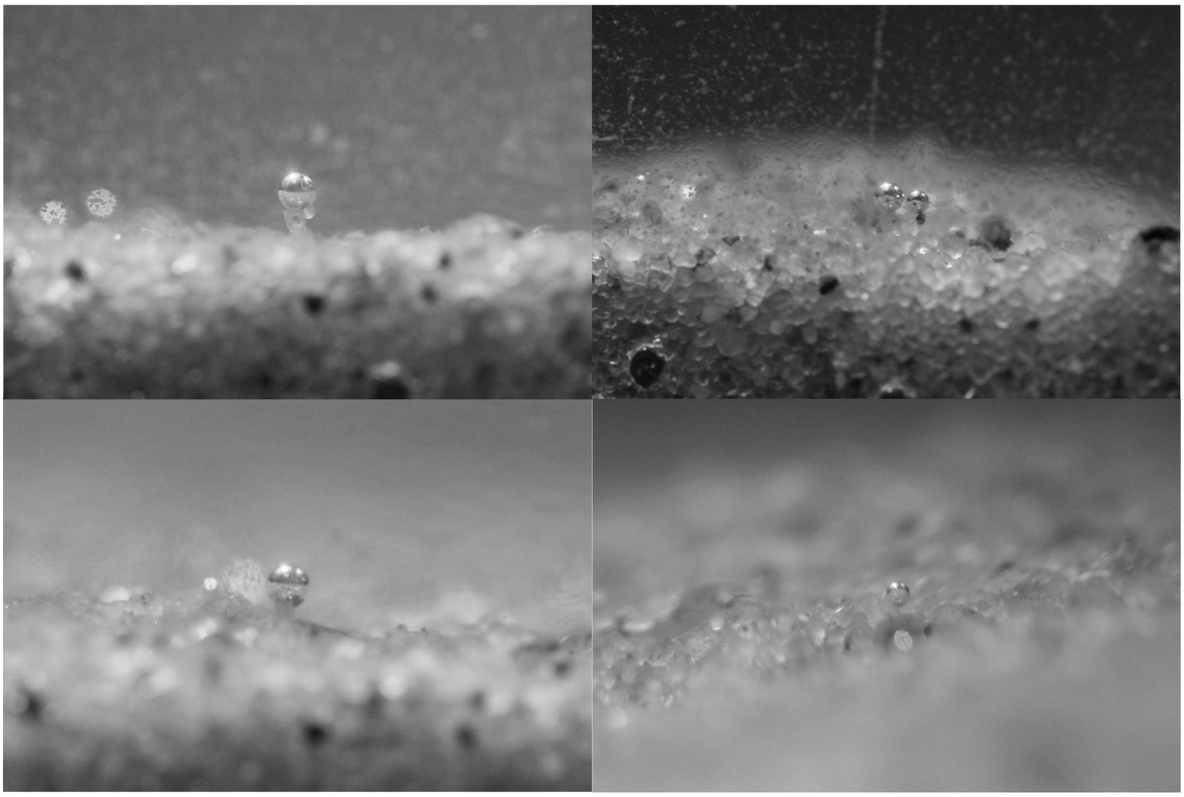

Fig. 6. Bubbles are visible on the sandy sediment surface during the light time (four images).

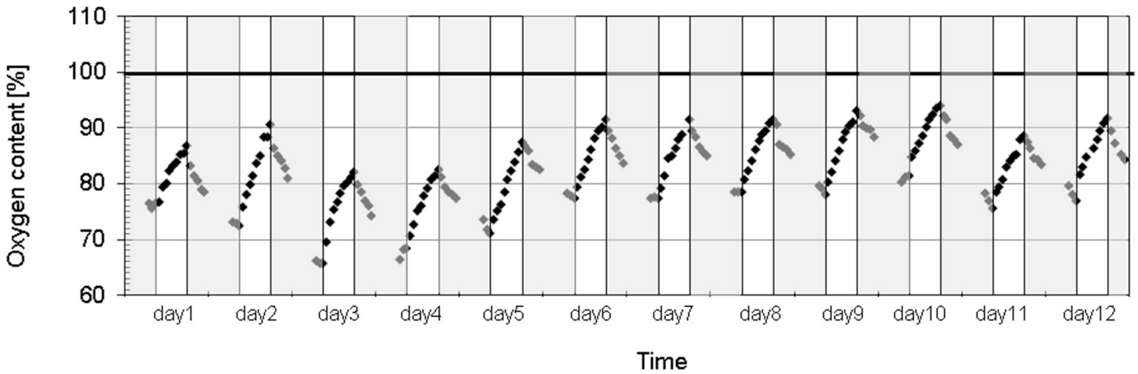

Fig. 7. Temporal dependence of the oxygen content.

and lower frequencies (respectively larger or less than approx. 300 kHz). Accounting for that the bubble impact on the acoustical wave is controlled by the parameter ka, where k is the wave number and a is the characteristic radius of the bubble (Leighton, 1997; Medwin, 2005; Ainslie and Leighton, 2011), it is important to provide the information on the size distribution of the bubbles within the sediment and at the surface.

It is interesting to compare the obtained result with the study results of Holliday *et al.* (2004) and Wildman and Huettel (2012). Similarly to our study they conducted multiday laboratory measurements and analyzed the difference in the echo signal reflected from the sandy sediments between the illumination and the dark periods. In order to recreate "day" and "night" conditions a 400-W metal halide lamp (Hamilton Technologies) and natural illumination were used respectively by Wildman and Huettel (2012) and Holliday *et al.* (2004). The transducer, positioned at a 45° angle, was used by Holliday *et al.* (2004) to transmit and receive reverberant echoes, while Wildman and Huettel (2012) applied vertically sounding transducer as it was done in our measurements. In our study it was operated over the similar band of frequencies as Holliday *et al.* (2004), while Wildman and Huettel (2012) used two higher frequencies: 1 MHz and 2.27 MHz.

Both scientific teams conducted measurements using sand samples collected near Florida beach: the water edge on beaches at Panama City, FL (Holliday et al., 2004) and Bald Point State Park, FL (Wildman, Huettel, 2012). In the first experiment the benthic microflora were characterized as an extremely diverse community, in the second – the diatoms were dominant, similarly as it was observed in our case.

The both experiments strongly differed from our study by hydrophysical conditions. The salinity was 35 PSU in both measurements, while it was 6.7 PSU in our study. The temperature varied from 22°C during the night to 27°C during the day in the Holliday *et al.* (2004) study, while it was close to stable about 26.5°C in the experiments conducted by Wildman and Huettel (2012). The temperature was stable with a good accuracy and lower (18°C) in our measurements. If the photosynthetically active radiation (PAR) during the day time in the experiment of Holliday *et al.* (2004) was comparable with our records at the illumination periods, the radiation could be approximately 2–4 times higher in the measurements of Wildman and Huettel (2012).

The both previous experiments demonstrated larger diel changes in acoustical backscattering than it was observed in our experiment. In Holliday *et al.* (2004) measurements the echo energy increase during the day approached 20 dB and the backscattering decreased during the dark, night time (Fig. 3 and Fig. 4 of their paper). Wildman and Huettel (2012) demonstrated that the maximum amplitude of the echo signal measured at illumination period was about 3 dB higher than at the dark period at 1 MHz frequency (it could be concluded analyzing Fig. 4 of the paper of Wildman and Huettel (2012)). The diel variation of the acoustical backscattering was explained based on their temporal correlation with the ambient light intensity, the presence of a healthy community of benthic microalgae known to produce oxygen by photosynthesis and the direct observation of gas bubbles escaping the sediment during the day time.

One explanation for the stronger effect demonstrated in the previous studies could be the higher temperature and the salinity. The oxygen solubility sensitive to the temperature and salinity (the higher the temperature and the salinity, the lower the solubility (Benson, Krause, 1984)) could be responsible for the fact that the water was more bubbly during the light period in the experiments with higher temperature. Thus the diel variation of the echo energy was stronger in two previous experiments

It is difficult to explain completely the difference in the impact between our study and previous measurements because of the lack of:

1) the complete size bubble data that makes impossible to evaluate the parameter *ka* controlling the bubble size regime, i.e. the bubble impact on the acoustic wave (Leighton, 1997; Medwin, 2005; Ainslie, Leighton, 2011),
2) understanding how the impact of the microphytobenthos photosynthesis on the acoustical backscattering is sensitive to the inclination of the transducer acoustical axis.

3.2. The arrival time of the echo signal

The exemplary acoustical data sets collected at 250 kHz are presented in Fig. 8. In the plot the continuous and dotted black lines correspond to the "night" and "day" measurements respectively. The zoom is presented for the temporal range: from 425 to 430 μs. Comparison of the amplitudes of the echo signal oscillations for the "day" and "night" showed that they were slightly higher during the "day", which coincided with the diel variation of the echo energy shown in Figs. 4a and 4b.

In the plot the echo signal peaks corresponding to the illumination period "appeared" slightly later than the peaks referring to the dark time. There is the slight (approximately 0.5 μs) time shift between the data sets corresponding to the light and dark periods. Since the distance between transducer and the sediment-water interface was the same during the illumination and the dark periods, the difference in the arrival time can be explained by the difference in the sound speed. The oxygen content growth and the increase of bubbles number in water column during the light period, discussed in the previous subsection, can be responsible for the lower sound speed during the "day" time. It is important to note that the temperature was kept stable during the entire experiment (the stability was carefully checked) and so it is not responsible for the diel variation of the arrival time.

The impact of the bubbles of photosynthetic origin on the echo signal arrival time was also shown by Wildman and Huettel (2012). In the upper panel of Fig. 4 of the paper (Wildman, Huettel, 2012), the results of the measurements conducted from the midnight to 7 a.m. at the frequency 1 MHz were presented. For each successive sound record most peaks occurred slightly later. The time shift was about 0.25 μs. Since the distance between the transducer and the sand did not change and T increased by $\leq$ 0.8°C over more than 6 h due to heating by the lamp, this implies that bubbles reduced sound speed propagation. In the experiment two factors impacted on the sound speed during the day time: increasing temperature and increasing number of bubbles in water column. The first was responsible for the sound speed growth, the second resulted in the sound speed decrease. In our measurements, in which the temperature stability was carefully controlled, only one factor was important – the bubbles decreasing the sound speed. It could be one of the reasons why the delay is larger in our experiment.

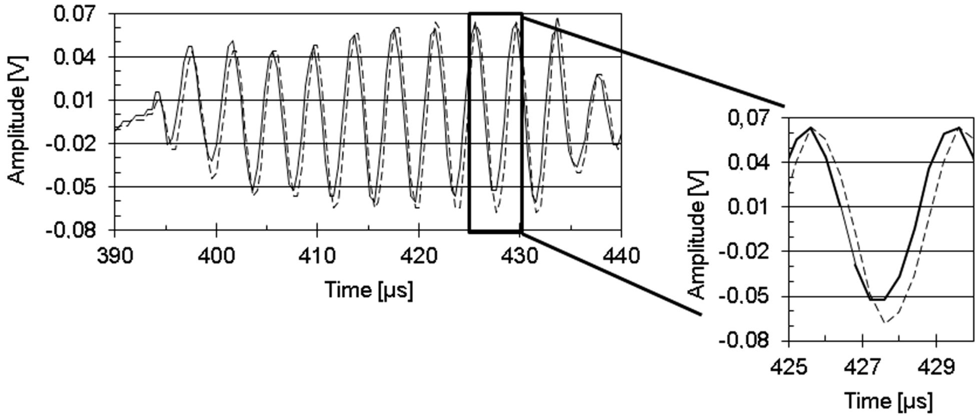

Fig. 8. The exemplary data sets (transducer operated at frequency of 250 kHz). The results of the measurements conducted during the "day" (dotted black line) and "night" (continuous black line) are presented. Over the entire frequency range at each individual frequency 64 pulses were emitted and the echo signal averaged over these echo pulses was saved. Each of the presented pulses is averaged over the 64 successive pulses.

4. Summary

The interdisciplinary multiday laboratory experiment was conducted to study the impact of benthic microalgal photosynthesis on the characteristics of the echo signal reflected by Baltic sandy sediments. Modelling "day" and "night" cycles in a darkened air-conditioned laboratory, diel variability of the echo signal was registered using hydroacoustical equipment originally designed, operated over the broad frequency range from 200 to 700 kHz and characterized by narrow directivity pattern over the entire frequency band. The diel variation of the echo energy and the arrival time of the echo signal were analyzed. The hydroacoustical measurements were carried out under controlled light, temperature and salinity conditions. The oxygen content was also monitored.

Summarizing, the following results were obtained:

1. The diel regular changes in acoustical backscattering were observed: over the frequency range from 200 kHz to approx. 300 kHz: the increase in the echo energy during the illumination time and the decrease during the dark hours. The increase was not larger than 1 dB.
2. It was shown that in the darkness echo signal propagates with higher sound speed as long as temperature remains constant during the diel cycle.
3. In considering the following observations:
   - the synchronization of the changes in sound backscattering, the oxygen content, the number of visible gas bubbles in aquarium with the light cycle,
   - the presence of the healthy diatom community indicated by microscope analysis,

   it can be concluded that there was the relationship between the acoustical backscattering (over the frequency range from 200 to approx. 300 kHz) and the benthic microalgal photosynthesis in the sediment or in other words at microscopic level the acoustical backscattering properties of the sea floor were controlled by the photosynthesis – a biological process induced by the microphytobenthos community.

In order to deepen the understanding:

- the relationship between the acoustical frequency used and the diel variation in the backscattering,
- the difference between our results and the results of the previous studies (Holliday *et al.*, 2004; Wildman, Huettel, 2012),

the gas babble size distribution control is recommended in the future experiments.

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