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Research Article

**DENITRIFICATION IN THE SEDIMENTS OF THE INNER PUCK BAY
-PRELIMINARY RESULTS¹**

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Abstract

Denitrification was measured in the sediments of the Inner Puck Bay using the acetylene inhibition technique and pertinent environmental data. This process was not found to be particularly significant for the nitrogen budget of the ecosystem. The rate ranged from 0 to 40.2 $\mu\text{mol N m}^{-2} \text{h}^{-1}$, which is equivalent to 151 t N per year. This constitutes 6.5% of the external nitrogen input to the investigated water body. The denitrification rate reached the highest values in spring, after the first algal blooms, when relatively fresh organic matter had amassed in the sediments and nitrate concentrations in interstitial waters peaked. The rates were almost sixfold lower than the maximal value in summer and fall. The main nitrogen source for denitrification in the investigated area was nitrification within the sediments. The deciding parameter determining the variability of the denitrification rate was the nitrate concentration in interstitial waters.

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INTRODUCTION

Denitrification is the only biological process that produces free nitrogen. It removes fixed nitrogen compounds and, therefore, exerts negative feedback on eutrophication making it a crucial process (Hansen & Zabel 2000). In estuaries and coastal and shelf sea areas, up to 80% of external N input has been found to be released via denitrification (Christensen *et al.* 1996, Jørgensen & Sørensen 1988, Stockenberg & Johnstone 1997). Accordingly, denitrification does not only counteract eutrophication; it might also affect pelagic production (Nixon 1981). Denitrification in the Baltic Sea has been estimated from nutrient budget calculations (Shafer & Rønner 1984, Stigebrand & Wulff 1987, Witek *et al.* 2003, Wulff & Stigebrand 1989) and ammonium fluxes (Conley *et al.* 1997). Direct measurements of denitrification in Baltic sediments were conducted by Tuominen *et al.* (1998), Rønner (1985), and Stockenberg & Johnstone, (1997), among others. The most frequently used methods of direct denitrification rate measurements in sediments include the determination of totally produced N₂ gas under incubation, isotope pairing, and the acetylene inhibition technique. More detailed overviews of this subject can be found, for example, in Koike & Sørensen (1988), Setzinger (1988), and Setzinger *et al.* (1993).

The current study presents the first direct measurements of denitrification in the sediments of the Inner Puck Bay. The acetylene inhibition technique (Christensen *et al.* 1989) was used to quantify denitrification at three different stations during the vegetation season. An attempt was made to evaluate the impact of selected environmental factors on the denitrification rate and the significance of this process in the nitrogen budget.

The Inner Puck Bay (102.7 km²), located in the western part of the Gulf of Gdańsk (Baltic Sea), is a shallow water basin with mean and maximal depths of 3 and 9.4 m, respectively (Nowacki 1993). Mixing in the water column reaches the bottom due to the shallow depth of the bay; the intensity of mixing varies depending on the season (Klekot 1980). The study area is separated from the open waters of the Baltic Sea by the Hel Peninsula. Water flow only occurs through the Outer Puck Bay; it is also limited by an underwater sandy bank known as the Rybitwia Mielizna. The bottom sediments of the study area are very diverse. They are comprised of sands of various particle size, sludge, silts, and organic matter (Pieczka 1974; Musielak 1984; Kępińska & Wypych, 1990), and their distribution on the surface is related distinctly to their morphology. The limiting factor of primary production in the bay is nitrogen (Bolałek *et al.* 1993). The production season in the study area generally lasts from March-April through September (Latała 1993). In this period, a decrease in nutrient concentrations in the water column is observed. In early spring, there is high

input of flood waters that carry large loads of nitrogen and phosphorus compounds washed out from farmlands (Bolałek *et al.* 1993). The Inner Puck Bay is intensely exploited economically and for tourism; however, despite its advanced degradation, it is still the region with the highest species diversity on the Polish Baltic coast, which makes it particularly valuable (Andrulewicz 1993). The prerequisite of properly protecting the ecosystem is to thoroughly identify the processes that take place within it. Therefore, it is fully justified to assess the role of denitrification in the nitrogen budget, particularly since nitrogen is a factor limiting primary production in the study area.

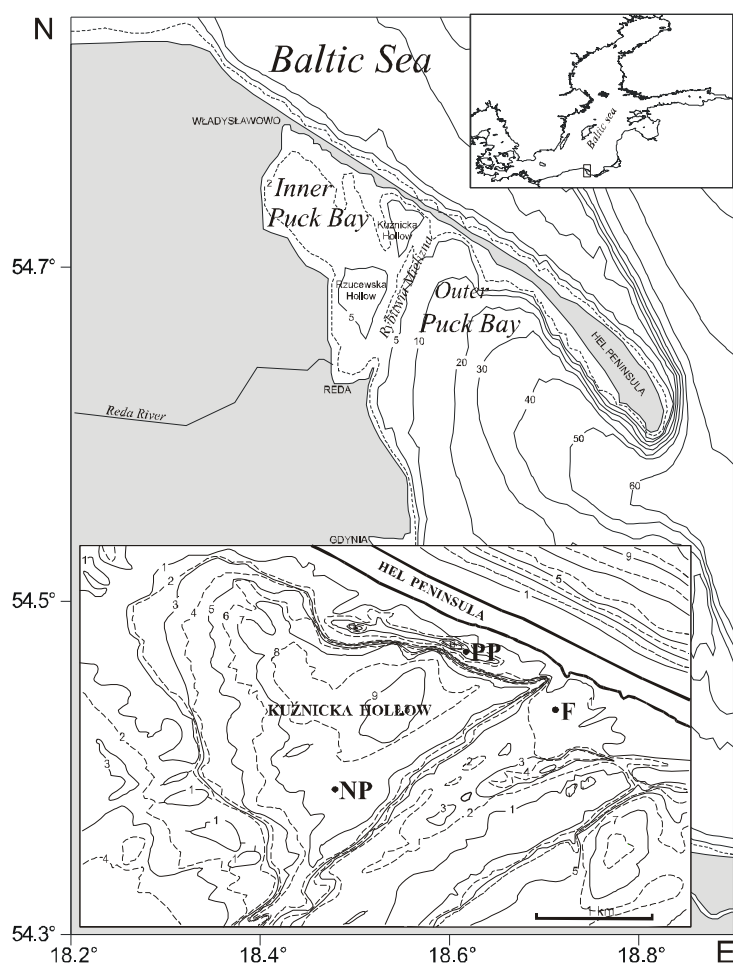


Fig. 1. Location of sampling sites in the Inner Puck Bay

Table 1

General physical and chemical characteristics of analyzed sediments (0-5 cm)

Station	depth (m)	Sediment type		bulk	Water	LOI	Porosity	C	N
				density	content				
				g dm^{-3}	%			%	
F	2.0	medium-sands	n ^{a)}	8	8	8	8	24	24
			mean	2.01	22	0.4	0.42	0.14	0.01
			minimum	1.93	20	0.3	0.39	0.05	0.00
			maximum	2.05	26	0.5	0.48	0.21	0.02
			standard deviation	0.05	3	0.07	0.04	0.08	0.01
NP	7.5	sandy-mud	n	8	8	8	8	12	12
			mean	1.47	53	4.5	0.74	1.77	0.18
			minimum	1.43	48	3.9	0.71	1.23	0.15
			maximum	1.52	64	4.8	0.82	2.42	0.21
			standard deviation	0.04	7	0.44	0.06	0.53	0.03
PP	9.0	sandy-mud	n	8	8	8	8	12	12
			mean	1.39	64	13.6	0.79	5.90	0.70
			minimum	1.06	32	1.9	0.55	1.28	0.10
			maximum	1.79	93	36.0	0.97	13.71	1.60
			standard deviation	0.32	26	15.40	0.18	5.70	0.68

n^{a)} - number of samples**MATERIALS AND METHODS**

Sampling took place four times a year in the 2001-2002 period in June-July (25-04), September-October (30-03), March (27-29), and April (13-17). The samples were collected at three stations in the Inner Puck Bay (Fig. 1). The shallowest station F (depth 2 m) was representative of the shallow areas, while station NP at a depth of 7.5 m represented the deep areas. Station PP (depth 9.5 m), located within the Kuźnica II post-dredging pit, was also investigated. The characteristics of the sediments in the study area are presented in Table 1.

The sampling was carried out by scuba divers using a plexiglass tube 3.6 cm in diameter, whereas samples of surface and near-bottom seawater were

collected directly into plastic and glass bottles. Interstitial water was obtained *in situ* with a syringe fitted with a 5 cm long pumice cube at the needle end. To avoid aspirating water from the near-bottom layer and deeper sediments, the top and bottom of the pumice were covered with silicone. In addition, interstitial water was obtained by centrifuging (3500 x g, 15 min) sediment (5 cm layers of intact sediment). The difference in concentrations of nutrients obtained by the two methods was within the error limit of both procedures.

MEASUREMENTS IN WATERS

Temperature, salinity, and nitrate ($\text{NO}_3^- + \text{NO}_2^-$) concentrations were measured in surface and near-bottom waters, while the nitrate concentrations in interstitial waters were determined. Salinity was measured with a WTW inoLAB Conductivity Meter, and Winkler titration was performed to determine dissolved O_2 concentrations (Strickland & Parsons 1972). The nitrate concentrations were determined spectrophotometrically (Grasshoff, 1983). Chemical analyses were done in duplicate.

MEASUREMENTS IN SEDIMENT

Water content (W %) was determined by drying sediment samples at 120°C until a constant mass reading was reached. Loss on ignition (LOI %) was measured by heating sediment samples to 500°C for 8 h. These two parameters were examined in 0-5 cm slices of sediment. Organic carbon (C) and total nitrogen (N) were determined with a CHN autoanalyser (Perkin Elmer 2400 series) in the following sediment slices: 0-1, 1-3 cm, and 3-5 cm. Samples were dried to a constant mass and homogenized, then the calcium carbonate was removed (Hedges & Stern 1984). The bulk density (0-5 cm) was measured with a cutting ring (Myślińska 1998). All determinations were done in duplicate.

The denitrification rate in the sediments was measured with the acetylene inhibition technique (Christensen *et al.* 1989). This method is based on inhibiting the final step of denitrification using acetylene, *i.e.*, converting N_2O to N_2 . The sediment cores incubated with acetylene contain amounts of N_2O that are proportional to the denitrification rate. The quantification of N_2O was performed with gas chromatography.

High purity acetylene, which had been additionally purified by scrubbing it in concentrated H_2SO_4 and 5 M NaOH, was used (Hyman & Arp 1987). Four to ten undisturbed sediment cores were taken for each determination of denitrification activity. The sediment and the adjacent near-bottom water (70-90 cm^3) were incubated together. A certain volume of air (approximately 25-30 cm^3)

was left above the water to compensate for oxygen use during incubation. Small magnetic stirring bars were placed underneath the caps to mix the water phase. Acetylene-saturated distilled water was injected (150 μ l) through a vertical series of silicone-filled holes (i.d. 3 mm) placed at 5 mm intervals along the four side of the tubes. The water and air phase also received C_2H_2 -saturated water and the final acetylene pressure in all incubated matrices, *i.e.*, air, water and sediment, equaled approximately 10 kPa. The two additional sediment cores received no acetylene and served as controls. The cores were incubated in the dark at the *in situ* temperature using a thermostat water bath. The maximal incubation time was 120 minutes; samples for N_2O determination were taken every 10-20 minutes from the consecutive cores. Nitrous oxide in the gas phase was analyzed after transferring a 3 cm^3 sample into an evacuated vacutainer (Terumo, Belgium). Nitrous oxide in the overlying water was analyzed after transferring a 10 cm^3 sample into a 22 cm^3 extraction vial. The vial was shaken vigorously for 10 minutes to equilibrate headspace and water, and a 3 cm^3 sample of the gas phase was transferred into an evacuated vacutainer (Terumo, Belgium). A similar procedure was applied to sediment samples, except that they were shaken inside the tubes in which incubation had been conducted after removing the water. The nitrous oxide in the vacutainers was analyzed on a Hewlett Packard 5890 Series II gas chromatograph equipped with an electron capture detector. The concentration of N_2O in the water was calculated using the solubility coefficients of N_2O in seawater (Weiss & Preis 1980). The denitrification rate was calculated from the total increase of nitrous oxide in the pore water, overlying water, and the air of individual cores.

STATISTICAL METHODS

Multiple regression analysis and the U Mann-Whitney test in the software package STATISTICA were used to statistically analyze the data.

RESULTS

Interstitial and near-bottom water

Surface water temperature ranged from 6°C in March to 20.6°C in July, and in near-bottom waters from 5°C in March to 18°C in July (Table 2). Salinity in the water column varied slightly from 6.5 to 6.9 PSU (Table 2). The relatively comparable temperature and salinity values in surface and near-bottom waters are indicative of intense mixing processes that are typical for shallow coastal waters.

Table 2

Temperature ($^{\circ}\text{C}$), salinity (PSU), oxygen content ($\text{cm}^3 \text{dm}^{-3}$), and nitrite concentration ($\text{NO}_3^- + \text{NO}_2^-$) in surface, near-bottom, and interstitial water

<i>Season</i>	<i>Station</i>		T	S	O ₂	NO ₃ ⁻ + NO ₂ ⁻
			<i>°C</i>	<i>PSU</i>	<i>cm³ dm⁻³</i>	<i>μmol dm⁻³</i>
<i>June-July</i>	<i>F</i>	<i>s.w.</i> ^{a)}	16.6	6.9	6.79	0.04
		<i>nb.w.</i> ^{b)}	17.0	6.9	6.93	0.03
		<i>int.w.</i> ^{c)}				1.74
	<i>NP</i>	<i>s.w.</i>	18.0	6.9	6.13	0.19
		<i>nb.w.</i>	17.0	6.9	1.12	0.56
		<i>int.w.</i>				1.73
	<i>PP</i>	<i>s.w.</i>	20.6	6.9	6.07	0.15
		<i>nb.w.</i>	18.0	6.9	5.56	0.46
		<i>int.w.</i>				3.45
<i>September-October</i>	<i>F</i>	<i>s.w.</i>	12.8	6.5	7.66	0.04
		<i>nb.w.</i>	12.0	6.6	8.08	0.00
		<i>int.w.</i>				1.73
	<i>NP</i>	<i>s.w.</i>	13.1	6.5	6.81	0.20
		<i>nb.w.</i>	12.0	6.6	7.20	0.18
		<i>int.w.</i>				3.19
	<i>PP</i>	<i>s.w.</i>	16.6	6.5	6.99	0.12
		<i>nb.w.</i>	13.0	6.5	7.53	0.20
		<i>int.w.</i>				0.23
<i>March</i>	<i>F</i>	<i>s.w.</i>	8.0	6.5	8.64	0.71
		<i>nb.w.</i>	8.0	6.5	8.58	0.64
		<i>int.w.</i>				5.80
	<i>NP</i>	<i>s.w.</i>	7.0	6.6	6.88	0.51
		<i>nb.w.</i>	7.0	6.5	4.56	0.62
		<i>int.w.</i>				8.47
	<i>PP</i>	<i>s.w.</i>	7.0	6.6	7.21	0.85
		<i>nb.w.</i>	6.0	6.6	9.27	1.37
		<i>int.w.</i>				4.98
<i>April</i>	<i>F</i>	<i>s.w.</i>	6.0	6.8	9.19	2.68
		<i>nb.w.</i>	6.0	6.8	8.97	2.78
		<i>int.w.</i>				6.38
	<i>NP</i>	<i>s.w.</i>	7.0	6.9	8.31	2.37
		<i>nb.w.</i>	5.0	6.8	8.73	2.18
		<i>int.w.</i>				3.17
	<i>PP</i>	<i>s.w.</i>	7.0	6.9	8.48	3.36
		<i>nb.w.</i>	5.0	6.8	8.91	2.75
		<i>int.w.</i>				5.81

a) -surface water

b) -near-bottom water

c) -interstitial water

Nitrate concentrations in the water column were low, and there was no vertical variability in nitrate distribution in any of the seasons (Table 2, Fig.2a). The highest concentration in both waters was observed in April ($2.4 \pm 0.2 \mu\text{mol dm}^{-3}$) and the lowest in June-July and September-October ($<0.6 \mu\text{mol dm}^{-3}$) (Fig.2a). These generally low concentrations probably resulted from phytoplankton nitrate consumption, as the samples were collected during the production season in the Inner Puck Bay. Nitrate concentrations in interstitial water reached higher values than in near-bottom water (Table 2). The values observed in March ($6.4 \pm 1.8 \mu\text{mol dm}^{-3}$) and April ($5.1 \pm 1.7 \mu\text{mol dm}^{-3}$) were almost three-fold higher than those in June-July ($2.3 \pm 1.0 \mu\text{mol dm}^{-3}$) and September-October ($2.2 \pm 0.9 \mu\text{mol dm}^{-3}$) (Fig.2b).

The oxygenation of water was good ($4.6\text{-}9.3 \text{ cm}^3 \text{ dm}^{-3}$) (Table 2). Only once did oxygen content decrease to $1.12 \text{ cm}^3 \text{ dm}^{-3}$ at station NP in July.

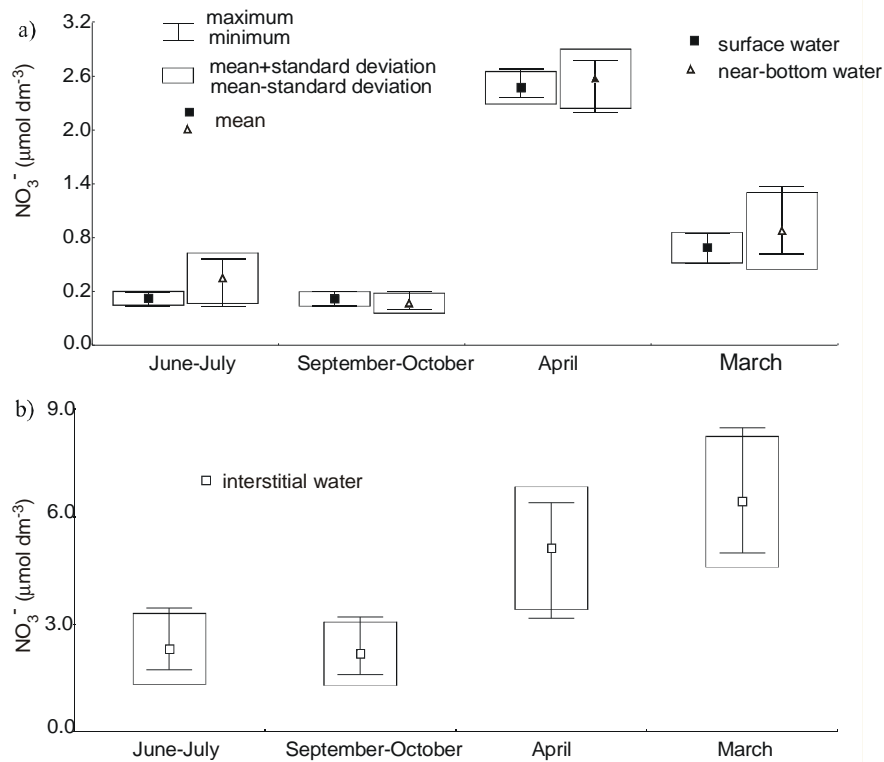


Fig. 2. Temporal changes of nitrate concentration in a) surface and near-bottom water, b) interstitial water (number of samples $n=6$)

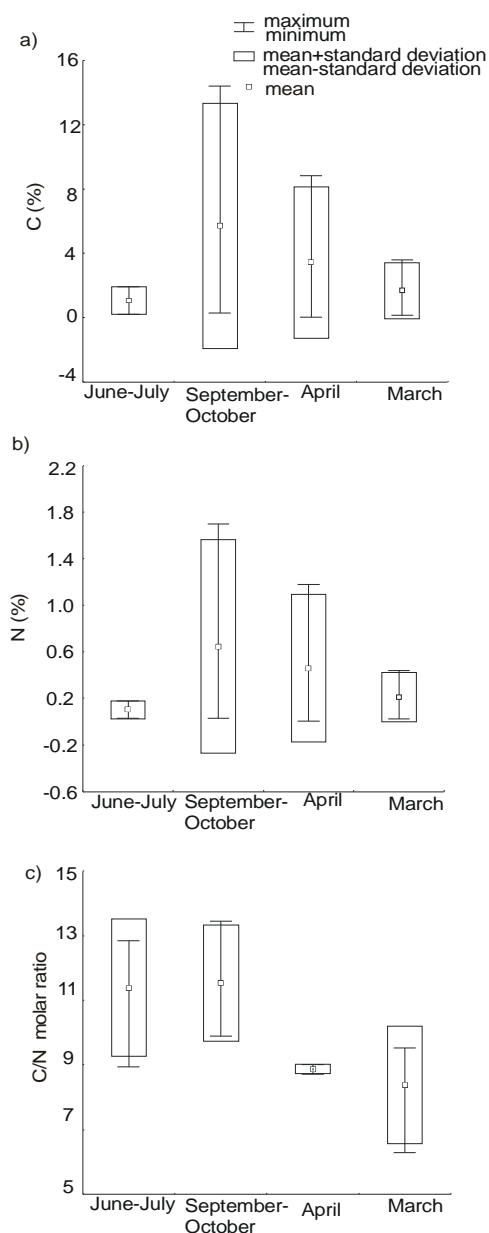


Fig. 3. Temporal changes of a) carbon, b) nitrogen, c) molar ratio of C/N in the surface layer of sediment (0-1 cm) (number of samples n=6)

Carbon and nitrogen in sediment

The content of organic carbon and total nitrogen in the sediments varied widely depending on sediment type as well as the time period of the growing season (Table 1, Fig. 3a, b). The lowest values occurred in fine grained sands at the shallowest station (F). Mean C_{org} and N_{tot} contents in these sediments were 0.14 ± 0.01 and 0.02 ± 0.01 . The sediments at the deeper station NP, covered by sandy-mud, were notably richer in carbon and nitrogen. Mean C_{org} and N_{tot} contents were 1.57 ± 0.42 and 0.18 ± 0.02 respectively. The sediments in the post-dredging pit were characterized by the highest organic carbon and total nitrogen content; the mean C_{org} and N_{tot} contents in the 5-cm sediment layer were 5.25 ± 5.02 and 0.62 ± 0.61 respectively, and, were an average of 2.5 times higher in comparison with station NP. Considering the mean values from the three stations, the highest carbon and nitrogen content in the surface sediment layer (0-1 cm) was observed in September-October ($C=5.70 \pm 7\%$, $N=0.65 \pm 0.91\%$) while the lowest were recorded in June-July ($C=1.08 \pm 0.84\%$, $N=0.1 \pm 0.76\%$) (Fig. 3a, b). In the same periods, the C/N ratios in the sediment were higher (11.5 ± 1.8) compared to those observed in spring (March-April, 8.5 ± 1.3) (Fig. 3c). Most likely, relatively fresh organic matter from diatom blooms that are usually observed

in this area reached the sediments in spring.

Denitrification in sediment

Denitrification ranged from 0 to 40.2 with a mean of $11.0 \mu\text{mol N m}^{-2} \text{h}^{-1}$ (Fig. 4). The most intense denitrification occurred in March at station NP ($40.2 \mu\text{mol N m}^{-2} \text{h}^{-1}$). No denitrification was detected during summer (June-July) within the post-dredging pit area (station PP). Denitrification was most intense in deeper regions, *i.e.*, at stations NP and PP in March, and in April in a shallow area (station F). Considering the mean values from the three stations, denitrification was most intense in March ($25.5 \pm 16.1 \mu\text{mol N m}^{-2} \text{h}^{-1}$); it later decreased by almost 50% in April ($11.3 \pm 9.2 \mu\text{mol N m}^{-2} \text{h}^{-1}$). In June-July and September-October, the values were 15-16% of the rates recorded in March at 4.1 ± 4.8 and $3.7 \pm 1.8 \mu\text{mol N m}^{-2} \text{h}^{-1}$, respectively.

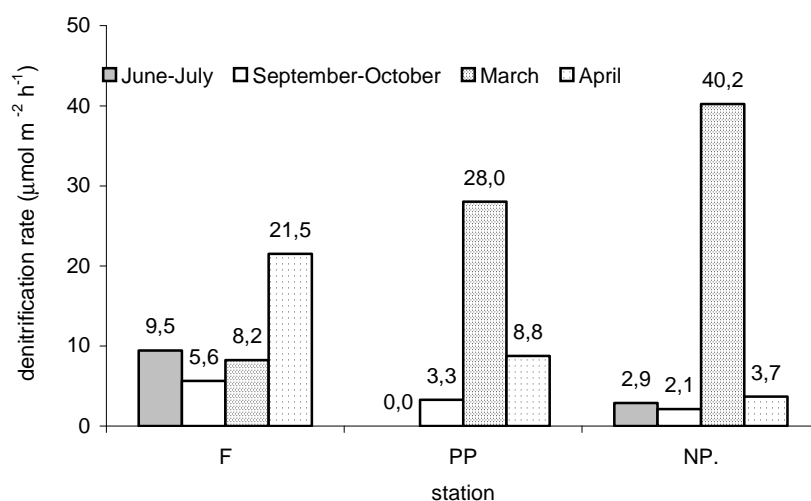


Fig. 4. Spatial and temporal changes in the denitrification rate in the sediment of the Inner Puck Bay

DISCUSSION

Temporal variability of denitrification

Quantitative and qualitative seasonal changes in the species composition of phytoplankton of the Puck Bay have been described thoroughly (Pliński 1994).

Diatom maxima occur in the spring and fall, with the former being much more intense. A dinoflagellate bloom accompanies the summer bloom of blue-green algae. When diatoms are predominant, the sedimentation rate increases significantly (Enoksson *et al.* 1990). Diatoms reach deeper water layers easily because they lack adaptations that prevent sedimentation (Witek *et al.* 1993). The sharp increase of denitrification in the sediments, observed in the study area in early spring, seemed to be directly related to the sedimentation of the preceding diatom blooms. Spring (and occasionally fall) maximums of denitrification activity are rather common for coastal marine environments (Jensen *et al.* 1988, 1996, Jørgensen & Sørensen 1988, Kemp *et al.* 1990, Stockenberg & Johnstone 1997).

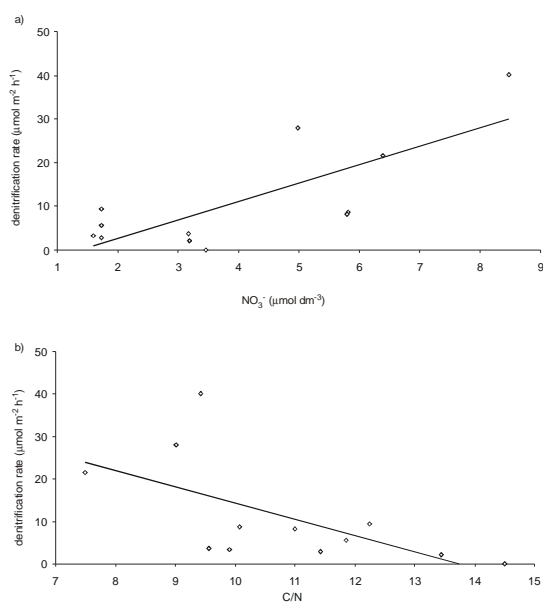


Fig. 5. Relationship between denitrification rate and a) concentration of nitrates in interstitial water, b) molar ratio of C/N in sediment

within the sediments was the predominant nitrate source for denitrification in the study area. Accordingly, denitrification was described by an approximately linear function of nitrate in interstitial water (Fig. 5a). Furthermore, no statistically significant relationship between denitrification rate and nitrate concentrations in near bottom water was found.

In the marine environment the main nitrate source for denitrification is usually nitrification in the sediments (Wang *et al.* 2003). However, in highly eutrophic areas characterized by very high nitrate concentrations in the water column, the near-bottom waters may be a basic nitrate source for this process (Middelburg *et al.* 1996). Nitrate concentrations in the water column during the study period were low and did not exceed the long-term average typical for the warm season (3.87 ± 2.60 , Bolalek *et al.* 1993). A higher nitrate concentration in interstitial water as compared to near-bottom water, and the net efflux of nitrate across the sediment-water interface (Graca *et al.* 2004) imply that nitrification

The lowest nitrate concentrations in near-bottom and interstitial waters, and the ensuing low denitrification rates, were observed in summer and fall. Denitrification rates in this period were statistically different from those in other months (U Mann-Whitney test, $p < 0.05$). Low denitrification activity during summer has been observed often and ascribed to the inhibition of nitrification by decreasing O_2 penetration into the sediment, thus suppressing coupled nitrification/denitrification (Kemp *et al.* 1990).

The availability of organic matter is a prerequisite for denitrification to occur. Hensen & Zabel (2000) stated that the most suitable conditions for denitrification exist at intermediate carbon availability levels when carbon is not limiting for oxic respiration and nitrification but sulfate reduction is still low or absent. In the investigated area, no statistically significant temporal differences in C and N contents in sediments were found (U Mann-Whitney test, $p < 0.05$). However, such differences were detected for the C/N ratios. In summer (June-July) and fall (September-October), the ratios were higher and statistically different from those observed in spring (March and April; U Mann-Whitney test, $p < 0.05$). A general decrease of denitrification rates with increasing C/N ratios in the sediments was noted (Fig. 5b). Graca *et al.* (2004) demonstrated that high C/N ratios in the sediments in the study area were accompanied by increased ammonium concentrations in interstitial water and by large ammonium fluxes into near-bottom water, which indicated intensified ammonification during these seasons.

Special attention should be given to the results obtained from the post-dredging pit which is characterized by the lowest mean denitrification rate ($10.0 \mu\text{mol m}^{-2} \text{h}^{-1}$) of the three investigated areas of the bay. However, differences among the areas are not statistically significant (U Mann-Whitney test, $p < 0.05$). Very low denitrification rates in the pit area were anticipated due to the low abundance of bioturbations (Szymelfenig, unpublished data; Wawrzyniak *et al.* 1993) and the high rates of sulfate-reduction in the sediments and the resulting occurrence of hydrogen sulfide (Bolałek *et al.* 1996; Graca *et al.* 2004). Low concentrations of hydrogen sulfide alone completely inhibit nitrification and in turn, hamper denitrification; whereas denitrification itself can only be directly inhibited at H_2S concentrations exceeding $300 \mu\text{mol dm}^{-3}$ (Sørensen *et al.* 1980). Denitrification in the pit sediments was not detected during summer when the highest H_2S concentration was measured ($49.9 \mu\text{mol dm}^{-3}$; Graca *et al.* 2004). Most likely denitrification occurred very close to the sediment surface where nitrification also had a chance to occur, while sulfate-reduction occurred in deeper sediment layers (a 5-cm thick sediment layer was analyzed).

Denitrification in the nitrogen budget

The acetylene inhibition technique applied in this study is relatively simple, sensitive, and inexpensive. It is, however, biased by certain flaws; the basic ones are the incomplete inhibition of N_2O to N_2 and the inhibition of nitrification (Knowles 1990; Lohse *et al.* 1996; Seitzinger 1988). During the consecutive experiments, the nitrate pool in the interstitial water was not exhausted since short incubation times (10-20 minutes) were applied. In effect, approximately constant N_2O production was observed. One exception was the experiment conducted within the post-dredging pit area in the summer when no N_2O production was detected. However, there is the possibility of the incomplete inhibition of N_2O reduction to N_2 . Therefore, it was concluded that the current measurements of denitrification with the acetylene inhibition method probably consistently underestimated the actual rates.

Denitrification rates in the Baltic Sea and adjacent areas vary considerably. The highest rate of this process (up to $416 \mu\text{mol N m}^{-2} \text{h}^{-1}$) was observed in very shallow areas rich in organic matter that also have an external nitrate supply (Andersen *et al.* 1984; Jørgensen and Sørensen 1988). However, when rates from these regions are eliminated, the majority of values did not exceed $50 \mu\text{mol N m}^{-2} \text{h}^{-1}$. For example, the denitrification activity in the northern Baltic Proper ranges from 6.3 to $27.1 \mu\text{mol N m}^{-2} \text{h}^{-1}$ (Tuominen *et al.* 1998). In the Gulf of Bothnia it is from 0.0 to $39.2 \mu\text{mol N m}^{-2} \text{h}^{-1}$ (Stockenberg & Johnstone 1997), while figures in the Gulf of Finland vary from 6.6 to $11.5 \mu\text{mol N m}^{-2} \text{h}^{-1}$ (Conley *et al.* 1997). In the Gulf of Gdańsk it is $53 \mu\text{mol N m}^{-2} \text{h}^{-1}$ (Witek *et al.* 2003). Denitrification rates in sediments obtained in this study (0 - $40.2 \mu\text{mol N m}^{-2} \text{h}^{-1}$) fall within the range of values typical for the marine environment, although they are at the lower end of the spectrum (50% of the values fall in the range of 3.0 - $15.5 \mu\text{mol N m}^{-2} \text{h}^{-1}$).

Assuming that the mean annual denitrification rates at stations F ($11.2 \mu\text{mol N m}^{-2} \text{h}^{-1}$) and NP ($12.2 \mu\text{mol N m}^{-2} \text{h}^{-1}$) are representative of shallow (depths $\leq 3\text{m}$) and deep (depths $\geq 3\text{m}$) bay areas, respectively, it was estimated that approximately 151 t N undergoes denitrification per annum in the Inner Puck Bay. The figures of the surface area over the considered depths were taken from Nowacki (1993), and the area of the post-dredging pit ($h > 9\text{m}$) was estimated to be 28764 m^2 (Łęcznyński, pers. comm.). The study period was only from March through October, and extreme values of denitrification rates are usually observed during this time (Jensen *et al.* 1988, 1996; Jørgensen & Sørensen 1988; Law *et al.* 1991). Therefore, the omission of the winter values should not significantly affect the calculations.

The external nitrogen input into the Inner Puck Bay is 2275 t yr⁻¹ (181 μmol N m⁻² h⁻¹; load from air, wastewater, and rivers) (Pempkowiak 1994). Denitrification is capable of removing the equivalent of up to approximately 6.5% of the discharged nitrogen. This relatively low value indicates that the denitrification pathway in the Inner Puck Bay is not particularly significant for the nitrogen budget. It is likely that high external nitrogen input affects nitrogen cycling processes in proportion to the input. Kelly *et al.* (1985) found that denitrification rates increased with higher N loading to the system but the ratio of N₂ to NH₄⁺ fluxes to the overlying water decreased with increased nutrient inputs. This indicates that the role of denitrification is rather diminishing relative to that of nitrate ammonification. An extreme situation occurred in the Chesapeake Bay where NH₄⁺ produced by organic decomposition was no longer transformed to N₂ but was recycled back into the overlying water to support primary production (Kemp *et al.* 1990). It is likely that in the Inner Puck Bay the increased production and consumption of organic matter associated with eutrophication led to a reduction in rates of nitrification and subsequently coupled denitrification.

Environmental factors versus denitrification rate

Denitrification is a complicated process shaped by a series of interconnected factors. The physico-chemical parameters most frequently cited in the literature as significant for denitrification processes are temperature, nitrate concentration in near-bottom and interstitial waters, and organic matter and oxygen content (Knowles 1982; Nowicki 1994; Seitzinger 1988). Multiple linear regression can assign certain weight to the following parameters: C/N ratio in sediment, nitrate concentration in near-bottom and interstitial waters, temperature and oxygen content in near-bottom water. Temperature and oxygen displayed a strong linear relation and were also correlated with other independent variables, therefore they have been removed from the statistical model. The multiple regression equation is as follows:

$$DR = 40.909 + 4.747 \cdot {}_{in}NO_3 - 7.584 \cdot {}_{nb}NO_3 - 3.700 \cdot C/N \quad (\pm 4.538) \quad (1)$$

(12.646) (0.775) (1.877) (0.971)

where:

DR - denitrification rate (μmol m⁻² h⁻¹); *in*NO₃ - nitrate concentration in interstitial water (μmol dm⁻³); *nb*NO₃ - nitrate concentration in near-bottom water (μmol dm⁻³); C/N - molar C/N ratio in the sediment; 4.538 - standard error of the predicted value. The standard errors of regression parameters are given in brackets. The calculated regression coefficients were statistically significant (p<0.05).

The variability of independent variables in the model explained over 87% of the variability of the denitrification rate (adjusted coefficient of multiple determination, $R^2=0.87$). The values of semi-partial correlation coefficients (r_s) indicated that nitrate concentration in interstitial water alone explains 46% ($r_s = 0.68$) of the denitrification rate variance, while nitrate concentration in near-bottom water and the molar C/N ratio are responsible for 20% ($r_s = -0.42$) and 18% ($r_s = -0.45$), respectively. An increase of nitrate concentration in interstitial water by one unit resulted in the increase of the denitrification rate by approximately $4.7 \mu\text{mol m}^{-2} \text{h}^{-1}$. A one-unit increase of the C/N ratio and nitrate concentration in the near-bottom water caused a decrease in denitrification rate by 3.7 and $7.6 \mu\text{mol m}^{-2} \text{h}^{-1}$. It is questionable whether the decrease of denitrification rate is related to the increased nitrate concentration in near-bottom water. It is probable that the physical transport of nitrate to active sites of denitrification is a more important factor than is the nitrate concentration in overlying water. Intensive transport stimulates denitrification and decreases nitrate concentration in near-bottom water.

The standard error of predicted value, which reflects how the mean standard deviation of the observed denitrification rate in a sample varies from that predicted by the statistical model, is 4.538 (equation 1). Despite small violations of the statistical model assumptions and the small sample size, the model had good fit with regard to the observed and predicted values (Fig. 6). Considering that the number of factors influencing the precision of direct measurements of denitrification is large (Hansen and Zabel, 2000), the statistical model presented in this paper has adequately good fit for particular applications in the researched area, *i.e.*, the shallow waters of the Inner Puck Bay.

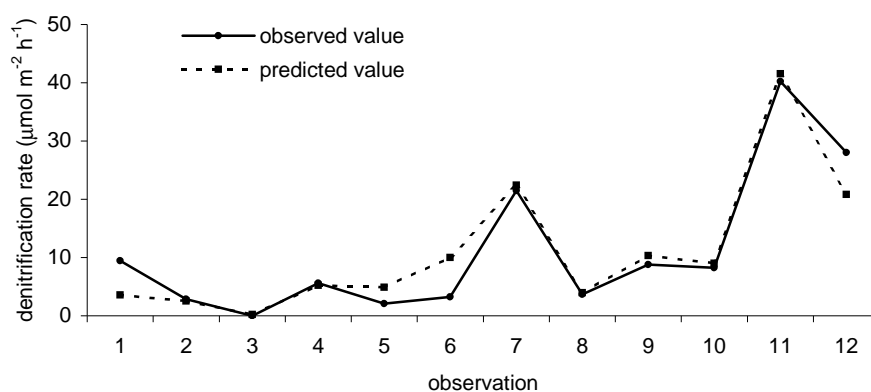


Fig. 6. Observed and predicted (with the statistical model) denitrification rates in the sediment

CONCLUSIONS

The denitrification pathway in the Inner Puck Bay is not particularly significant for the nitrogen budget. The process rate ranged from 0 to 40.2 $\mu\text{mol N m}^{-2} \text{ h}^{-1}$ which is the equivalent of 151 t N per year. This amount constitutes 6.5% of the external nitrogen input to the investigated water body. The highest rates of denitrification were observed in early spring after the first algal blooms. The main source of nitrates for denitrification was nitrification in sediments. The nitrate concentration in interstitial water was responsible for 46% of the variability of the denitrification rate, 20% to the nitrate concentration in the near-bottom water, and 18% to the type of organic matter, *i.e.*, C/N ratio. High nitrate concentrations in interstitial water were accompanied by increased denitrification rates, while the presence of highly mineralized organic matter in the sediment and high nitrate concentrations in near-bottom water hampered denitrification.

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