

Bioavailability of dissolved organic phosphorus in the Baltic Sea

Monika Nausch*, Günther Nausch

Baltic Sea Research Institute Warnemünde, Seestrasse 15, 18119 Rostock-Warnemünde, Germany

ABSTRACT: We determined bioavailable, dissolved organic phosphorus (BAP) by measuring changes in dissolved organic phosphorus (DOP) in experiments using 0.8 μm filtered seawater, representing the free bacteria fraction, with the addition of carbon and nitrogen. Experiments were conducted at 3 stations in the central Baltic Sea from May to July 2004, a period characterized by a decrease in phosphate concentrations, and the development of diazotrophic cyanobacteria blooms. DOP concentrations ranged between 0.32 and 0.52 μM in May, and declined to 0.20 μM in July. Changes in DOP were caused by fluctuations in the BAP decreasing from 0.34 μM in May to 0.03 μM in July. The refractory DOP pool ranged from 0.14 to 0.21 μM , and did not vary seasonally. The DOP turnover and P release from BAP was calculated using 2 different approaches: (1) [γ - ^{33}P]ATP hydrolysis time, and (2) the time needed for DOP degradation in the experiments. Based on [γ - ^{33}P]ATP hydrolysis times, BAP is recycled several times per day, except at 2 stations in May when the time is longer. According to the second approach, BAP is recycled within 3 or 4 d without seasonal differences. The fast [γ - ^{33}P]ATP turnover indicates that BAP comprises a fraction of compounds (such as ATP) that are degraded very rapidly. Another pool of BAP compounds exists which are turned over at a slower rate. Thus, the [γ - ^{33}P]ATP does not represent the total BAP turnover.

KEY WORDS: Bioavailable dissolved organic phosphorus · BAP · DOP turnover · [γ - ^{33}P]ATP hydrolysis · Baltic Sea

—Resale or republication not permitted without written consent of the publisher—

INTRODUCTION

Dissolved inorganic phosphorus (DIP) and dissolved organic phosphorus (DOP) are the main phosphorus (P) sources that sustain phytoplankton and bacterial growth in aquatic ecosystems. If available, DIP is preferred to DOP (Bentzen et al. 1992, Nausch & Nausch 2004). DOP becomes the most important source under DIP-depleted conditions (Thingstad et al. 1993, Monagha & Ruttenberg 1999, Cavender-Bares et al. 2001) and can prevent or delay phosphorus limitation. Therefore, when discussing reservoirs of bioavailable P, DOP has to be taken into account, as otherwise a serious underestimation of the reservoir and the resulting productivity can occur (Monagha & Ruttenberg 1999). However, DOP is not completely bioavailable. It is partly comprised of inert compounds (Jackson & Williams 1985, Hino 1989) that persist over longer periods.

Consequently, in addition to DIP concentrations, data on the amount of bioavailable DOP (BAP) are necessary to determine if phosphorus limits plankton production.

Karl & Björkman (2002) reviewed DOP distribution on a global scale. With few exceptions, clear depth profiles exist, with highest concentrations in the surface layer (Jackson & Williams 1985, Karl & Björkman 2002, Pöder et al. 2003). According to the review by Karl & Björkman (2002), DOP concentrations range between 0.10 and 0.44 μM in the open Pacific and Atlantic Oceans, and the continental shelf regions. Mulholland et al. (2002) and van der Zee & Chou (2005) found higher DOP concentrations between 0.35 and 0.87 μM off the coast of northern Australia, and from 0.20 to 0.80 μM in the Belgian coastal zone. DOP concentrations up to 0.60 μM were found in semi-enclosed bays such as the Chesapeake Bay (Conley et

*Email: monika.nausch@io-warnemuende.de

al. 1995), Xiamen Bay (Huang & Hong 1999) and Tokyo Bay (Suzumura et al. 1998). For the Baltic Sea, DOP concentrations between 0.48 and 0.98 μM have been given for the Aarhus Bay (Thingstad et al. 1996) and 0.50 and 0.90 μM for the Gulf of Riga (Pöder et al. 2003). In contrast, in the central Baltic Sea, spring to summer concentrations in 2001 ranged between 0.20 and 0.23 μM , except in buoyant cyanobacterial surface blooms, where higher concentrations were recorded (Nausch et al. 2004).

Whereas information about DOP concentrations in marine systems is relatively abundant, data on the bioavailable fraction are rare. Reynolds & Davies (2001) defined the BAP as 'those fractions of the total mass of phosphorus present in a system that are readily assimilable by organisms, or are made more assimilable through the activities of the organisms themselves (for instance, through the production of phosphatases) and that portion of phosphorus which has been already assimilated and is intracellular'. However, at the time of estimation, the assimilated phosphorus is attributed to the particulate portion and is not a portion of DOP. Techniques to determine the bioavailable amount of DOP or the usage of DOP by microorganisms include DIP increase after enzyme (phosphatase, phosphodiesterase) additions (Strickland & Solórzano 1966, Hino 1989,

Suzumura et al. 1998, Hernandez et al. 2000), and the inhibition of [^{33}P]- PO_4 uptake by selected organic phosphorus compounds (mostly phosphoesters, nucleic acids) (Berman 1988, Björkman & Karl 1994, Hernandez et al. 1996, Huang & Hong 1999). Björkman et al. (2000) used specific labeling of ATP in the γ position to assess the size of the bioavailable pool. All these techniques may detect the amounts and usage of selected compounds, rather than the total BAP pool. More directed to the detection of total BAP is the method used by Stepanauskas et al. (2002), who incubated water samples with the addition of cultured bacteria. Carbon and nitrogen was supplied to obtain a P-limited medium, and the bioavailable amount was derived from bacterial growth.

Previous investigations (Nausch & Nausch 2004), demonstrated that experiments with 0.8 μm filtered seawater, incubated with an additional carbon and nitrogen supply, are well suited for the determination of BAP. In contrast to the approach of other authors, BAP is calculated directly from changes in DOP. A similar method was used by Søndergaard et al. (1995) to detect the bioavailable amount of DOC.

In the present study, we determined the BAP in the central Baltic Sea using this experimental design. The investigations were conducted during a period characterized by a DIP decline to the detection limit, and the development of a nitrogen fixing cyanobacteria bloom. For the first time BAP data for the central Baltic Sea are presented, and the role of DOP as a potential source for diazotrophic cyanobacterial growth and nitrogen fixation is suggested.

MATERIALS AND METHODS

Sampling sites. During 4 cruises from May until July 2004, 12 experiments were conducted at 3 central stations in the Baltic proper: Stn 213 in the Bornholm Basin, Stn 259 in the southeastern Gotland Basin, and Stn 271 in the eastern Gotland Basin (Fig. 1). An additional experiment was conducted in the eastern Gotland Basin at Stn L1 during the third cruise at the beginning of July.

Water was sampled at a depth of 1 to 2 m using a rosette sampler (Hydrobios) combined with a Seabird SBE911 equipped with sensors for conductivity, temperature and depth (CTD). Temperature, salinity and ambient nutrient concentrations during water sampling are summarized in Table 1.

Experimental design. Immediately after sampling, water for the time-course experiments was filtered through 0.8 μm cellulose nitrate filters using a vacuum of -200 mbar. The filters were tested for P release and rinsed additionally with Milli-Q water. The filtrate was bottled in precleaned (1 M HCl and Milli-Q water) and

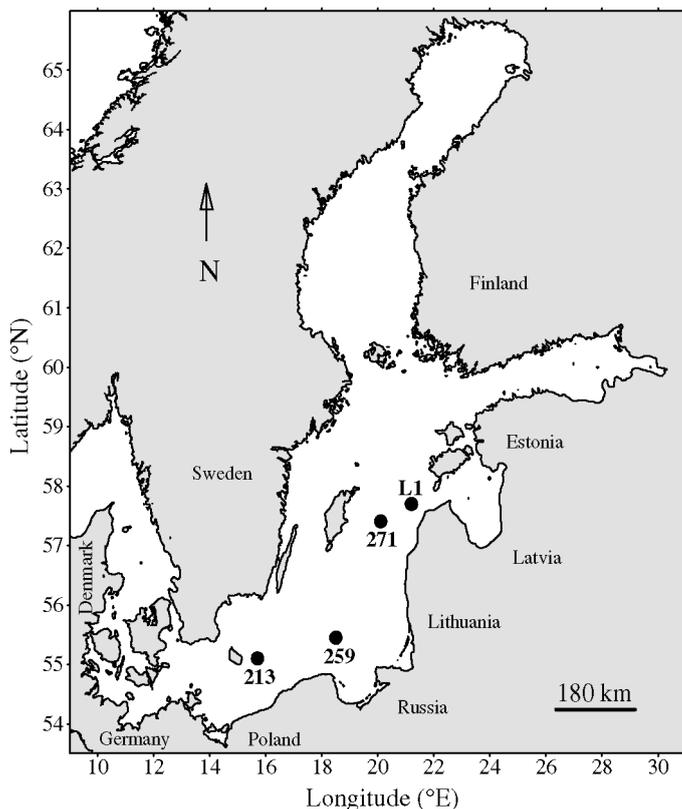


Fig. 1. Baltic Sea, and the 4 sampling stations

Table 1. Hydrographical conditions, inorganic nutrient and chl *a* concentrations, and bacterial abundance in ambient water during sampling

Date (2004)	Stn	Salinity	<i>T</i> (°C)	DIP (μM)	NH ₄ (μM)	NO ₂ +NO ₃ (μM)	Chl <i>a</i> (μg l ⁻¹)	Bacterial counts (10 ⁶ cells ml ⁻¹)
3 May	213	7.4	6.3	0.29	0.23	0.00	3.47	0.3
3 May	259	7.0	7.7	0.05	0.29	0.05	13.43	1.3
4 May	271	7.1	6.8	0.15	0.29	0.04	5.63	0.4
18 Jun	213	7.4	11.9	0.15	0.15	0.05	1.23	1.4
18 Jun	259	7.1	10.2	0.13	0.17	0.05	3.18	1.9
19 Jun	271	7.1	11.8	0.05	0.12	0.05	3.86	2.1
29 Jun	213	7.4	13.2	0.18	0.20	0.07	1.61	2.5
30 Jun	271	6.8	13.7	0.05	0.22	0.06	3.58	3.5
2 Jul	L1	6.3	13.5	0.01	0.17	0.07	4.12	3.4
5 Jul	259	7.1	13.9	0.14	0.24	0.13	2.55	1.2
16 Jul	213	7.4	14.8	0.21	0.17	0.00	1.66	1.4
17 Jul	259	7.2	14.7	0.34	0.14	0.01	1.67	1.4
18 Jul	271	6.6	15.6	0.01	0.18	0.01	1.87	3.4

sterilized Duran flacons, 5 l in each. Ammonium chloride (Merck 10145) and D-(+)-glucosemonohydrate (Merck 4074) were added at concentrations of 7 μM and 1 mg l⁻¹ (≡ 30 μM C), respectively, resulting in a C:N addition of 4.3:1. The bottles were incubated in the dark for 4 to 6 d at room temperature (ca. 16°C), and aerated with air passed through a 0.2 μm filter. Experiments were performed in duplicate.

Duplicate subsamples of each flacon were taken for determination of DIP, ammonium (NH₄), DOP and bacterial P immediately after substrate addition, and daily during the incubation period.

Analysis of ambient water samples. Analyses of inorganic nutrients (dissolved inorganic nitrogen [DIN], DIP) were done with standard colorimetric methods (Rohde & Nehring 1979, Grasshoff et al. 1983) employing either manual methods for unfiltered water or the autoanalyzer system 'Evolution III' with GF/F filtered water immediately after sampling.

For chl *a* determination, samples were filtered onto Whatman GF/F-filters and stored in liquid nitrogen. After extraction with 96 % ethanol for at least 4 h, fluorescence was measured at an excitation wavelength of 450 nm and an emission of 670 nm (HELCOM 2001, available at www.helcom.fi/Monas/combineManual2/CombineHome.htm).

Phytoplankton composition and biovolumes were determined according to HELCOM (1988). Phytoplankton was preserved with acetic Lugol's solution (KI/I₂) and counted under an inverted microscope (Leica) (Utermöhl 1958). The cell volume was calculated from the size measurements by using the appropriate stereometric formula. It was converted to wet weight (WW, mg m⁻³), assuming that the density of the plasma is equal to that of water (approx. 1 mg mm⁻³).

For bacterial abundances, samples (4 ml) were fixed with 50 μl formaldehyde (0.5 % v/v final concentration), shock frozen in liquid nitrogen and stored at -70°C until measurement. Abundances were analyzed using a flow cytometer (FacsCalibur, Becton Dickinson) according to Gasol & del Giorgio (2000). SYTO-13 (Molecular Probes) at a final concentration of 5 μM were used to stain the bacteria. After addition of fluoresbrite microspheres (Polysciences), measurements were performed at a low flow rate. Measurements and calculations were done using the software program 'CellQuestPro'.

[γ-³³P]ATP hydrolysis was measured according to Ammerman (1993). Triplicate 40 ml subsamples were incubated with 185 kBq [γ-³³P]ATP (Hartmann Analytix; specific activity = 92 TBq mmol⁻¹) corresponding to a final concentration of 50 pM [γ-³³P]ATP. Incubation was in the dark for 1 h, and was stopped by the addition of 100 μM of carrier-free ATP. A 10 ml sample was filtered through a 0.2 μm polycarbonate filter. The filter and 1 ml of the filtrate (first filtrate) were counted in a liquid scintillation counter. The other 9 ml of the first filtrate were mixed with 20 mg of activated charcoal and 1 ml 0.03 N H₂SO₄, filtered through a 0.45 μm filter (second filtrate containing only DIP), and 1 ml was counted. For blanks, 100 μl formaldehyde was added 15 min before the addition of the radiotracer. ATP hydrolysis was calculated using the equations of Ammerman (1993):

$$\text{ATP hydrolysis (\% h}^{-1}\text{)} = \frac{(\text{counts on filters} + \text{counts of second filtrate})}{(\text{counts on filters} + \text{counts of first filtrate})} \times 1 \text{ h}$$

$$\text{Uptake of P released by ATP hydrolysis} = \frac{\text{counts on filters}}{(\text{counts on filters} + \text{counts of first filtrate})} \times 1 \text{ h}$$

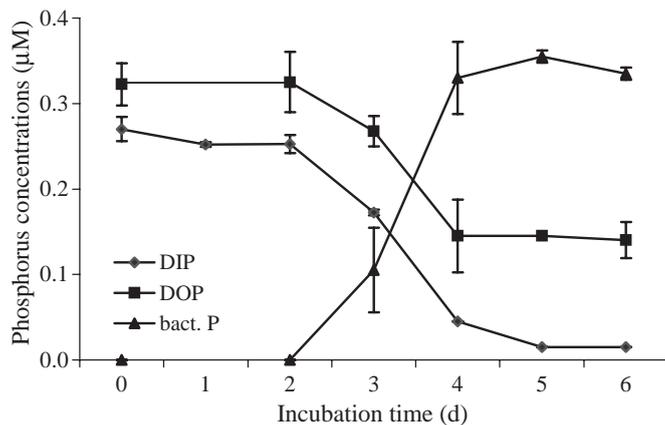


Fig. 2. Experiment at Stn 213 in May 2004, shown as example of change in phosphorus pools (mean \pm SD, $n = 4$) during the time course experiments. DIP, DOP: dissolved inorganic and organic phosphorus, respectively; bact. P: bacterial phosphorus

Analysis during experiments. During the course of the experiments, DIP and NH_4 were measured manually immediately after subsampling. Bacterial P and DOP were determined using different filtrate fractions: the 0.8 μm filtrate included bacteria, DOP and DIP; additional filtration through 0.2 μm cellulose nitrate filters yielded a fraction that contained only DOP and DIP. Samples (40 ml) of each fraction were stored at -20°C until determination. The thawed samples were oxidized with potassium peroxodisulfate in an alkaline medium (Grasshoff et al. 1983). The subsequent DIP determination was done using a 10 cm cuvette. The detection limit was 0.01 μM . Determinations were performed in duplicate from each incubation flagon. Bacterial P was determined as the difference between the P concentrations in the 0.8 μm prefiltered and in the 0.2 μm filtered water. DOP was calculated as the difference between the total P concentration in the 0.2 μm filtered water and the DIP concentration.

The amount of BAP was defined as that part of DOP which is decomposed by bacteria. It was calculated as the difference between the DOP concentration at the beginning of the experiment and that at the time the DOP concentration was lowest. The remaining DOP is the 'refractory' DOP part. The period of DOP decrease is called degradation time. Photochemical DOP degradation, e.g. by UV light (Francko & Heath 1979, 1982, Wetzal et al. 1995), was not considered.

RESULTS

Change in P pools during time course experiments

An example of the changes in DIP, DOP and phosphorus in the bacterial fraction during the course of the experiments is given in Fig. 2. DIP and DOP concentrations decreased in parallel with bacterial P increase. After reaching maximum values, usually after 3 to 5 d, P in the bacterial fraction decreased slightly in some experiments, resulting in a marginal increase in DIP and DOP concentrations. In the example shown in Fig. 2, DOP declined between the second and the fourth day from 0.32 to 0.14 μM , resulting in a BAP of 0.18 μM and a degradation time of 48 h.

DOP concentration and bioavailable DOP

Ambient DOP concentrations in seawater samples of 0.32 to 0.52 μM were measured in May. They declined to concentrations between 0.20 and 0.24 μM in July, a pattern that was apparent at all 3 stations (Fig. 3, Table 2).

The amount of BAP was strongly correlated with the ambient DOP concentrations at the beginning of the experiments ($r^2 = 0.948$, $p < 0.01$, $n = 13$) (Fig. 3). The relationship between them is given by the linear equation $\text{BAP} = 1.006 \times \text{DOP} - 0.175$. The regression slope of 1.006 indicates that all DOP above the intercept with the abscissa is bioavailable (Fig. 3). In May, BAP accounted for 0.18 and 0.20 μM at Stns 213 and 271, respectively, and 0.34 μM at Stn 259, where a phyto-

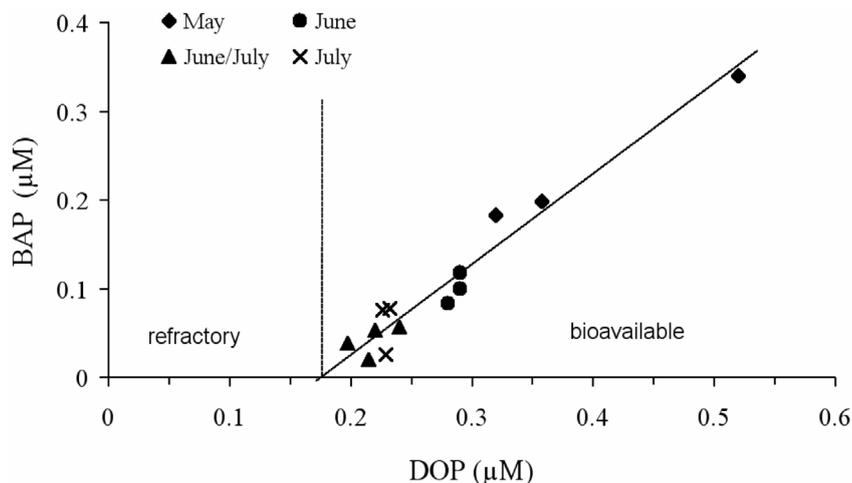


Fig. 3. Relationship between ambient DOP and dissolved bioavailable organic phosphorus (BAP) concentrations (determined from time-course experiments) in the central Baltic Sea from May to July 2004. Slope of 1.006 of regression line indicates that variations of DOP are caused by fluctuations in BAP; intercept with x-axis marks amount of refractory DOP

Table 2. Dissolved inorganic (DIP) and organic (DOP) phosphorus concentrations at beginning of experiments, and BAP (absolute and as percentage of total DOP) and refractory (Refr.) DOP. Data are means \pm SD (n = 4)

Date (2004)	Stn	DIP (μM)	DOP (μM)	BAP		Refr. DOP (μM)
				(μM)	% of total DOP	
3 May	213	0.27 \pm 0.01	0.32 \pm 0.01	0.18 \pm 0.03	56.8 \pm 4.9	0.15 \pm 0.00
3 May	259	0.05 \pm 0.00	0.52 \pm 0.03	0.34 \pm 0.01	65.4 \pm 3.0	0.18 \pm 0.02
4 May	271	0.18 \pm 0.00	0.36 \pm 0.00	0.20 \pm 0.00	55.2 \pm 0.4	0.16 \pm 0.00
18 Jun	213	0.17 \pm 0.00	0.29 \pm 0.03	0.12 \pm 0.02	40.3 \pm 4.0	0.18 \pm 0.02
18 Jun	259	0.15 \pm 0.00	0.30 \pm 0.01	0.10 \pm 0.02	33.3 \pm 3.6	0.20 \pm 0.01
19 Jun	271	0.06 \pm 0.00	0.28 \pm 0.01	0.08 \pm 0.02	29.1 \pm 6.0	0.20 \pm 0.00
29 Jun	213	0.18 \pm 0.00	0.22 \pm 0.01	0.02 \pm 0.01	9.1 \pm 3.8	0.20 \pm 0.01
30 Jun	271	0.05 \pm 0.00	0.24 \pm 0.01	0.06 \pm 0.01	23.6 \pm 2.4	0.19 \pm 0.01
2 Jul	L1	0.02 \pm 0.00	0.22 \pm 0.02	0.05 \pm 0.01	23.7 \pm 4.5	0.17 \pm 0.01
5 Jul	259	0.16 \pm 0.00	0.20 \pm 0.01	0.04 \pm 0.01	18.8 \pm 3.2	0.16 \pm 0.01
16 Jul	213	0.18 \pm 0.00	0.23 \pm 0.01	0.08 \pm 0.01	33.7 \pm 6.8	0.15 \pm 0.02
17 Jul	259	0.30 \pm 0.01	0.23 \pm 0.00	0.03 \pm 0.01	8.5 \pm 8.1	0.21 \pm 0.03
18 Jul	271	0.02 \pm 0.00	0.23 \pm 0.01	0.08 \pm 0.00	33.3 \pm 2.0	0.14 \pm 0.03

plankton bloom was observed. In May, the proportion of BAP ranged between 55.2 and 65.4% (Table 2). From the end of June onward, the BAP declined to between 0.02 and 0.08 μM , and its proportion ranged between 8.5 and 33.7%.

The remaining refractory DOP pool varied between 0.14 and 0.21 μM (mean 0.17 μM). This concentration range was similar during all experimental periods, indicating that this fraction remains more or less constant (Table 2). In Fig. 3, the refractory fraction is indicated by the intercept with the x-axis.

Relationship between BAP, nutrients and chl *a* during sampling

In 2004, a DIP decrease to the detection limit (as reported for previous years for the whole central Baltic Sea) was only observed in the eastern Gotland Basin (Stn 271), where DIP concentrations of 0.15 μM measured in May had declined to 0.01 μM in July. In the SE Gotland Basin (Stn 259), lowest values were measured in May, as a result of an intense phytoplankton bloom (Table 1), increasing to 0.34 μM in mid July. In the Bornholm Basin (Stn 213), DIP was never depleted, with concentrations ranging between 0.15 and 0.29 μM . DIN concentrations were low at all stations during the whole investigation period.

Chl *a* was highest in May (3.47 to 13.43 $\mu\text{g l}^{-1}$) at all stations, and declined to 1.66–1.87 $\mu\text{g l}^{-1}$ in mid July. The extremely high concentration of 13.43 $\mu\text{g l}^{-1}$ measured at Stn 259 was caused by a dinophyceae bloom associated with autotrophic ciliates of the genus *Mesodinium*. During the third cruise in June/July, chl *a* concentrations in the eastern Gotland Basin

(Stns 271 and L1), exceeded those at the other stations (Table 1). Here, blooms of filamentous cyanobacteria occurred, reaching a biomass of 300 mg m^{-3} WW (Stn 271) and 806 mg m^{-3} WW (Stn L1). The blooms consisted of the heterocystous species *Aphanizomenon baltica*, *Nodularia spumigena* and *Anabaena* sp., as well as the non-heterocystous species *Planktolyngbia* sp. and *Pseudoanabaena* sp. At the other stations during this cruise and during the other cruises, abundances of filamentous cyanobacteria remained at a lower level, with biomasses ranging between 0.5 and 170 mg m^{-3} WW. Cyanobacteria never formed a buoyant surface bloom, as they were dispersed in the surface layer.

Heterotrophic bacteria were found at relatively constant abundances of 1.3 to 1.9 $\times 10^6$ cells ml^{-1} at Stn 259 during all investigation periods. At the other stations, they reached abundances of 3.4 $\times 10^6$ cells ml^{-1} toward the end of June and the beginning of July, when diazotrophic cyanobacteria were present (Table 1). They remained at this high level after the collapse of the cyanobacterial bloom in the middle of July.

No relationship could be established between total DOP and DIP in the ambient water, nor between BAP and DIP. Spearman rank correlation shows a relationship between temperature and DOP ($p < 0.01$), as well as between temperature and BAP ($p < 0.01$) (Fig. 4), consistent with the view that degradation processes were enhanced in summer. There were elevated BAP values at high chl *a* concentrations, but no correlation was found between them (Fig. 4). It is evident from Table 2, that BAP was low in summer, independent of the concentrations of filamentous or nitrogen fixing cyanobacteria. Furthermore, DOP and BAP did not show any relationship with bacterial abundance.

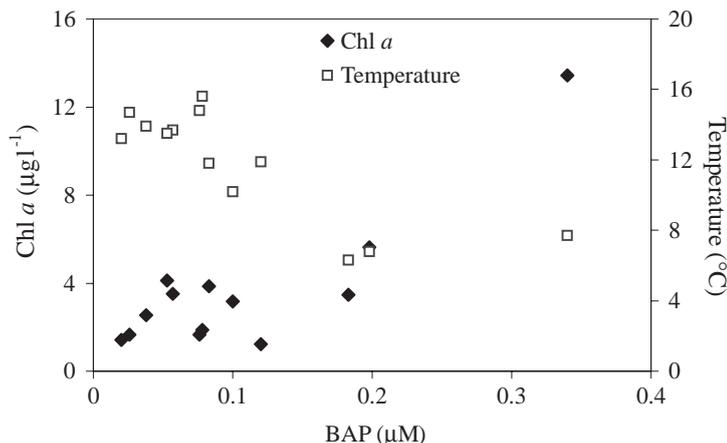


Fig. 4. Relationship between BAP determined in experiments and ambient chl *a* and temperature during water sampling

Calculated DOP turnover

DOP turnover was calculated using 2 different approaches: (1) the $[\gamma\text{-}^{33}\text{P}]\text{ATP}$ hydrolysis rate and (2) the DOP degradation time during the course of the experiments.

The $[\gamma\text{-}^{33}\text{P}]\text{ATP}$ hydrolysis rate was investigated in samples from surface waters at the same stations during 3 of the 4 cruises. The calculation was based on 3 assumptions: (1) that $[\gamma\text{-}^{33}\text{P}]\text{ATP}$ hydrolysis is representative of the total BAP hydrolysis; (2) that BAP is completely hydrolyzed; and (3) phosphorus from DOP must be released before it can be taken up. According to the $[\gamma\text{-}^{33}\text{P}]\text{ATP}$ hydrolysis time (Table 3), BAP had to be converted once within 5 to 11 h, except on 2 dates in May, when the conversion time was longer. The calculated P released from DOP ranged between 0.7 and

41.0 nM h^{-1} , meaning that between 0.2 and 20.5% h^{-1} of DOP was degraded. The highest value of 41.0 nM h^{-1} was calculated during the phytoplankton spring bloom. When cyanobacteria were present in June/July at Stns 271 and L1, 11.9 and 8.1 nM h^{-1} were released from DOP (Table 3).

According to the second approach, DOP degradation times ranged between 70 and 96 h in most experiments, with 3 exceptions when the time was shorter (Table 3). A P release ranging between 0.4 and 4.9 nM h^{-1} (= 10 and 116 nM d^{-1}) was calculated, meaning that between 0.2 and 1.2% h^{-1} of the DOP was converted. These results indicate that more P was released from DOP in May than in June and July (i.e. summer). The P release from DOP in summer was in the same range at stations where diazotrophic cyanobacteria occurred and at stations where the cyanobacteria did not dominate the phytoplankton.

It is evident from the comparison of both methods (Table 3), that the DOP turnover estimated by $[\gamma\text{-}^{33}\text{P}]\text{ATP}$ hydrolysis rates is 1 order of magnitude shorter, with 1 exception in May. P release and the amount of converted DOP calculated by this method were 2 to 18 times higher.

DISCUSSION

The nutrient cycle from spring to summer in the central Baltic Sea has for many years been characterized by a more rapid decrease of inorganic nitrogen compounds than of phosphate, as a result of the low DIN/DIP ratio of winter surface waters (Matthäus et al.

Table 3. DOP turnover and P release from dissolved bioavailable organic phosphorus (BAP) calculated from $[\gamma\text{-}^{33}\text{P}]\text{ATP}$ hydrolysis time, and time required for DOP degradation. DOP conver.: proportion of total DOP converted to BAP; nd: not determined

Date (2004)	Stn	— $[\gamma\text{-}^{33}\text{P}]\text{ATP}$ hydrolysis —			— DOP degradation —		
		Time (h)	P release (nM h^{-1})	DOP conver. (% h^{-1})	Time (h)	P release (nM h^{-1})	DOP conver. (% h^{-1})
3 May	213	276	0.7	0.2	48	3.8	1.2
3 May	259	8	41.0	7.9	70	4.9	0.9
4 May	271	30	6.6	1.8	72	2.8	0.8
18 Jun	213	nd	—	—	70	1.7	0.6
18 Jun	259	nd	—	—	72	1.4	0.5
19 Jun	271	nd	—	—	72	1.1	0.4
29 Jun	213	11	1.8	0.8	24	0.8	0.4
30 Jun	271	5	11.9	4.9	48	1.3	0.5
2 Jul	L1	6	9.0	20.5	96	0.5	0.2
5 Jul	259	11	3.8	1.9	96	0.4	0.2
16 Jul	213	5	15.0	6.5	96	0.8	0.4
17 Jul	259	9	3.3	1.4	72	0.4	0.2
18 Jul	271	5	17.5	5.5	72	1.1	0.5

2001). The DIN concentrations were completely exhausted after the spring bloom, whereas a distinct DIP reservoir of around 0.10 μM remained. Between May and July, this excess DIP is normally consumed in parallel with the growth of nitrogen fixing cyanobacteria (Matthäus et al. 2001). C:P ratios reaching values of 300 or higher (e.g. Larsson et al. 2001, Nausch et al. 2004) are indicative of P-limitation of cyanobacteria. The high alkaline phosphatase activity associated with the cyanobacteria when DIP is depleted (Paasche & Erga 1988, Graneli et al. 1990) is interpreted as P-stress of the organisms and as the ability to use DOP as P source (Connors et al. 1996, Reynolds & Davies 2001, Mulholland et al. 2002). During our 2004 study, the inorganic nutrient status differed somewhat from that in previous years. The usual decrease in DIP concentrations down to the detection limit (Matthäus et al. 2001, Nausch et al. 2004) in parallel with the development of diazotrophic cyanobacteria was observed only in the eastern Gotland Basin (Stns 271, L1) and did not occur at the other stations.

The amount of DOP and of BAP declined at all stations from spring to summer, independent of the variation in DIP concentrations. In May, DOP concentrations exceeded those previously measured in the open waters of the Baltic Sea (between 0.20 and 0.30 μM , with no strong interannual fluctuations: own unpubl. data). Nevertheless, higher values can occur temporally and spatially, as evidenced by the present study and by measurements made in 2001 of buoyant cyanobacterial surface blooms (Nausch et al. 2004). Furthermore, Pöder et al. (2003) reported higher concentrations in the Gulf of Riga from May to July 1999. According to the results of the present study, variations within the DOP pool may have been due to fluctuations in the bioavailable fraction. The refractory DOP of 0.14 to 0.21 μM is relatively constant. It is unclear whether the refractory DOP is inert or merely not utilizable for bacteria within the short period covered by our experiments. DOP in the deep ocean below 1000 m can be assumed to be inert. The remaining DOP concentrations in our experiments are in the same range as in some regions of the deep ocean (Karl & Björkman 2002), an indication that the refractory DOP in the Baltic Sea could really be inert.

The elevated DOP concentrations at Stn 259 in May 2004 can be attributed to a spring phytoplankton bloom. Extremely high DOP concentrations were observed in cyanobacterial surface blooms in summer 2001 (Table 4). Therefore, BAP in the surface layer of the open Baltic Sea may be predominantly a result of autochthonous production, although other influences such as lateral exchange cannot be excluded. Phytoplankton as the main DOP source was also reported by Karl & Björkman (2002) and Pöder et al. (2003). From

Table 4. Calculation of BAP at Stn 271 in 2001 using equation ($y = 1.006 \text{ DOP} - 0.175$) established in present study. Blooms: buoyant cyanobacterial surface blooms

Date (2001)	DOP (μM)	BAP calculated (μM)
9 – 15 May	0.25	0.07
9 – 12 Jun	0.23	0.06
10 – 14 Jul	0.22	0.04
2 – 05 Aug	0.2	0.03
10 July	Bloom A	0.58
	Bloom B	0.18

May to July, the composition of the phytoplankton community changed. In May, dinophyceae dominated the plankton and filamentous cyanobacteria were sparse, whereas at the beginning of July, filamentous cyanobacteria occurred in high concentrations in the eastern Gotland Basin. In contrast to the spring phytoplankton, no elevated DOP or BAP concentrations were observed when diazotrophic cyanobacteria were dispersed in the euphotic layer. Either the cyanobacteria release lower amounts of DOP or degradation and uptake processes balance out in summer. The inverse relationship between temperature and BAP concentrations and the short [$\gamma\text{-}^{33}\text{P}$]ATP hydrolysis times recorded in our study suggest the latter. High DOP concentrations were measured when cyanobacteria accumulated in buoyant surface blooms, as observed in 2001 (Nausch et al. 2004). It is often speculated that cyanobacteria in the buoyant surface blooms are senescent. If so, DOP is mainly released when they die.

The BAP concentrations measured in the central Baltic Sea in 2004 are similar or higher than the values of 13 to 111 nM reported for the surface layer of the North Pacific Subtropical Gyre (Björkman et al. 2000), where BAP accounted for between 9 and 38% of the total DOP. According to Stepanauskas et al. (2002), about 75% of the DOP was bioavailable in rivers entering the Baltic Sea. In 2001, we measured DOP concentrations at Stn 271 during the same period of year (Nausch et al. 2004) (Table 4). Comparison of the 2 years shows that lower DOP concentrations occurred in May 2001, whereas DOP concentrations were similar in summer. Applying the equation for the relationship between DOP and BAP ($\text{BAP} = 1.006 \times \text{DOP} - 0.175$) established in this study, the calculated BAP concentrations differed only marginally from May to July 2001 (Table 4).

The DOP concentrations measured are the net result of production and consumption processes. This raises the question of BAP turnover rates. The results arising from the 2 approaches used in our calculations differed. The method using [$\gamma\text{-}^{33}\text{P}$]ATP hydrolysis times indicated that BAP is converted several times a day.

The long hydrolysis times in May at Stns 271 and 213 could be due to the low water temperatures of 6 to 7°C, higher BAP concentrations than in summer and low heterotrophic bacterial numbers (data not shown), yielding a calculated P release rate of between 0.7 and 6.6 nM h⁻¹. However, high BAP concentrations and low temperatures are not always reflected in longer turnover times. This was evident in the spring bloom at Stn 259, where a high BAP concentration was coupled with a hydrolysis time similar to that in summer, resulting in a high P release of 41 nM h⁻¹. In summer, short hydrolysis times were combined with increased P release, whereby BAP concentrations were relatively low. The DOP degradation experiments indicated that BAP was converted mostly within 3 or 4 d, and this time period was not shorter in summer. The amount of released P varied between 0.4 and 4.9 nM h⁻¹. Because of decreasing BAP concentrations, a trend to lower P release was evident in summer; an exception seems to be Stn 271, where the P release was relatively constant in June and July.

Both calculation methods indicated elevated P release during the phytoplankton spring bloom. The largest differences occurred in summer at low BAP or DOP concentrations. The [γ -³³P]ATP method resulted in an overestimation of BAP turnover. ATP and other nucleotides—substrates of the [γ -³³P]ATP assay—comprise only a small fraction of BAP that is converted rapidly. According to Ammerman & Azam (1985), 5'-nucleotide concentrations in seawater ranged between 10 and 20 nM. Karl & Björkman (2002) reported dissolved ATP concentrations between 400 and 800 pM in the upper 100 m depth at 2 stations in the North Pacific. Assuming similar concentrations in the Baltic Sea, 5'-nucleotide and dissolved ATP concentrations amount for less than 10 and 0.5% of DOP, respectively. However, the time course experiments showed that there is another pool of BAP that is converted much slower than ATP or nucleotides. According to our results, the ATP turnover is not representative of the total DOP pool. In the time course experiments, grazers of bacteria were excluded, and nitrogen and carbon was added to avoid limitation by these elements. Supply of these substrates can induce a higher P demand than under unaffected conditions, as shown in Nausch & Nausch (2004). Therefore, the utilization of DOP by bacteria may be lower under *in situ* conditions than in our experiments.

With respect to the importance of DOP as supplier of P to diazotrophic cyanobacteria, no elevated DOP or BAP concentrations occurred when cyanobacteria were abundant in the euphotic layer, except when cyanobacteria accumulated in buoyant surface blooms. Seasonal changes in ATP turnover indicated that ATP or nucleotides may serve as a preferential P source for cyanobacteria in the Baltic Sea.

Acknowledgements. We thank the crews of RV 'Professor Albrecht Penck' and RV 'Gauss' for their help during sampling. S. Busch, A. Grützmüller, R. Hansen, D. Setzkorn, I. Topp and Å. Welz are thanked for the intensive support during field work or in the laboratory with the different analyses, K. Jürgens and N. Wasmund are thanked for many fruitful discussions.

LITERATURE CITED

- Ammerman JW (1993) Microbial cycling of inorganic and organic phosphorus in the water column. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) Handbook of methods in aquatic microbial ecology. Lewis, Boca Raton, p 649–660
- Ammerman JW, Azam F (1985) Bacterial 5'-nucleotidase in aquatic ecosystems: a novel mechanism of phosphorus regeneration. *Science* 227:1338–1340
- Bentzen E, Taylor WD, Millard ES (1992) Importance of dissolved organic phosphorus to phosphorus by limnetic plankton. *Limnol Oceanogr* 37:217–231
- Berman T (1988) Differential uptake of orthophosphate and organic phosphorus substrates by bacteria and algae in Lake Kinneret. *J Plankton Res* 10:1239–1249
- Björkman K, Karl DM (1994) Bioavailability of inorganic and organic phosphorus compounds to natural assemblages of microorganisms in Hawaiian coastal waters. *Mar Ecol Prog Ser* 111:265–273
- Björkman K, Thomson-Buldis AL, Karl DM (2000) Phosphorus dynamics in the North Pacific Subtropical gyre. *Aquat Microb Ecol* 22:185–198
- Cavender-Bares KK, Karl DM, Chisholm SW (2001) Nutrient gradients in the western North Atlantic Ocean: relationship to microbial community structure and comparison to patterns in the Pacific Ocean. *Deep-Sea Res I* 48: 2373–2395
- Conley DJ, Smith WM, Cornwell JC, Fisher TR (1995) Transformation of particle bound phosphorus at the land–sea interface. *Estuar Coast Shelf Sci* 40:161–176
- Connors SD, Auer MT, Effler SW (1996) Phosphorus pools, alkaline phosphatase activity, and phosphorus limitation in hypereutrophic Onondaga Lake. *Lakes Reserv Manag* 12:47–57
- Francko DA, Heath RT (1979) Functionally distinct classes of complex phosphorus compounds in lake water. *Limnol Oceanogr* 24:463–473
- Francko DA, Heath RT (1982) UV-sensitive complex phosphorus: association with dissolved humic material and iron in a bog lake. *Limnol Oceanogr* 27:564–569
- Gasol JM, del Giorgio PA (2000) Using flow cytometry for counting natural planktonic bacteria and understanding the structure of planktonic bacterial communities. *Sci Mar* 64:197–224
- Graneli E, Wallström K, Larsson U, Graneli W, Elmgren R (1990) Nutrient limitation of primary production in the Baltic Sea area. *Ambio* 19:142–151
- Grasshoff K, Ehrhardt M, Kremling K (eds) (1983) Methods of seawater analysis, 2nd edn. Verlag Chemie, Weinheim
- HELCOM (1988) Guidelines for the Baltic Sea Monitoring Programme for the third stage. *Baltic Sea Environ Proc* 27D
- Hernandez I, Hwang SA, Heath RT (1996) Measurement of phosphomonoesterase activity with a radiolabelled glucose-6-phosphate. Role in the phosphorus requirement of phytoplankton and bacterioplankton in a temperate mesotrophic lake. *Arch Hydrobiol* 137:265–280

- Hernandez I, Pérez-Pastor A, Lloréns JLP (2000) Ecological significance of phosphomonoesters and phosphomonoesterase in a small Mediterranean river and its estuary. *Aquat Ecol* 34:107–117
- Hino S (1989) Characterization of orthophosphate release from dissolved organic phosphorus by gel filtration and several hydrolytic enzymes. *Hydrobiologia* 174:49–55
- Huang B, Hong H (1999) Alkaline phosphatase activity and utilization of dissolved organic phosphorus by algae in subtropical coastal waters. *Mar Pollut Bull* 39:205–211
- Jackson GA, Williams PM (1985) Importance of organic nitrogen and phosphorus to biological nutrient cycling. *Deep-Sea Res A* 32:223–235
- Karl DM, Björkman K (2002) Dynamics of DOP. In: Hansell AD, Carlson GA (eds) *Biogeochemistry of marine dissolved organic matter*. Academic Press, Amsterdam, p 250–366
- Larsson U, Hajdu S, Walve J, Elmgren R (2001) Baltic Sea nitrogen fixation estimated from the summer increase in upper mixed layer total nitrogen. *Limnol Oceanogr* 46: 811–820
- Matthäus W, Nausch G, Lass HU, Nagel K, Siegel H (2001) The Baltic Sea in 2000—low inflow activity caused an increase in stagnation of the central Baltic deep water. *Meereswiss Ber* 45:89–98
- Monagha EJ, Ruttenberg KC (1999) Dissolved organic phosphorus in the coastal ocean: reassessment of available methods and seasonal phosphorus profiles from the Eel River Shelf. *Limnol Oceanogr* 44:1702–1714
- Mulholland MR, Flöge S, Carpenter EJ, Capone DG (2002) Phosphorus dynamics in cultures and natural populations of *Trichodesmium* spp. *Mar Ecol Prog Ser* 239:45–55
- Nausch M, Nausch G (2004) Bacterial utilization of phosphorus pools after nitrogen and carbon amendment and its relation to alkaline phosphatase activity. *Aquat Microb Ecol* 37:237–245
- Nausch M, Nausch G, Wasmund N (2004) Phosphorus dynamics during the transition from nitrogen to phosphate limitation in the central Baltic Sea. *Mar Ecol Prog Ser* 266: 15–25
- Paasche E, Erga SR (1988) Phosphorus and nitrogen limitation of phytoplankton in the inner Oslofjord (Norway). *Sarsia* 73:229–243
- Pöder T, Maestrini SY, Balode M, Lips U, Béchemin C, Andrushaitis A, Purina I (2003) The role of inorganic and organic nutrients on the development of phytoplankton along a transect from the Daugava River mouth to the open Baltic in spring and summer 1999. *ICES J Mar Sci* 60: 827–835
- Reynolds CS, Davies PS (2001) Sources and bioavailability of phosphorus fractions in fresh-waters: a British perspective. *Biol Rev* 76:27–64
- Rohde KH, Nehring D (1979) Ausgewählte Methoden zur Bestimmung von Inhaltsstoffen im Meer- und Brackwasser. *Geod Geoph Veröff R IV* 27:1–68
- Søndergaard M, Hansen B, Markager S (1995) Dynamics of dissolved organic carbon lability in a eutrophic lake. *Limnol Oceanogr* 40:46–54
- Stepanauskas R, Jørgensen NOG, Eigard OR, Žvikas A, Tranvik LJ, Leonardson L (2002) Summer inputs of riverine nutrients to the Baltic Sea: bioavailability and eutrophication relevance. *Ecol Monogr* 72:579–597
- Strickland JDH, Solórzano L (1966). Determination of monoesterase hydrolysable phosphate and phosphomonoesterase activity in sea water. In: Barnes H (ed) *Some contemporary studies in marine science*. Allen, London, p 665–674
- Suzumura M, Ishikawa K, Ogawa H (1998) Characterization of dissolved organic phosphorus in coastal seawater using ultrafiltration and phosphohydrolytic enzymes. *Limnol Oceanogr* 43:1553–1564
- Thingstad TF, Skjoldal EF, Böhne RA (1993) Phosphorus cycling and algal–bacterial competition in Sandsfjord, western Norway. *Mar Ecol Prog Ser* 99:239–259
- Thingstad TF, Riemann B, Havskum H, Garde K (1996) Incorporation rates and biomass content of C and P in phytoplankton and bacteria in the Bay of Aarhus (Denmark) June 1992. *J Plankton Res* 18:97–121
- Utermöhl H (1958) Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. *Mitt Int Ver Theor Angew Limnol* 9:1–38
- van der Zee C, Chou L (2005) Seasonal cycling of phosphorus in the southern bight of the North Sea. *Biogeosciences* 2:27–42
- Wetzel RG, Hatcher PG, Bianchi TS (1995) Natural photolysis by ultraviolet irradiation of recalcitrant dissolved matter to simple substrates for rapid bacterial metabolism. *Limnol Oceanogr* 40:1369–1380

Editorial responsibility: Otto Kinne (Editor-in-Chief), Oldendorf/Luhe, Germany

*Submitted: November 4, 2005; Accepted: December 22, 2005
Proofs received from author(s): August 18, 2006*