

# Presence of dissolved nucleotides in the North Pacific Subtropical Gyre and their role in cycling of dissolved organic phosphorus

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**ABSTRACT:** Dissolved and particulate nucleotide triphosphate (NTP) concentrations were measured in the upper 1000 m of the water column during 3 summer and 3 winter months at Stn ALOHA (22.75° N, 158° W) in the oligotrophic North Pacific Subtropical Gyre. In the euphotic zone (0 to 175 m) particulate adenosine-5'-triphosphate (P-ATP) and dissolved ATP (D-ATP) concentrations were positively correlated in summer (0 to 175 m;  $r^2 = 0.61$ ,  $p < 0.001$ ,  $n = 24$ ), but not in winter ( $r^2 = 0.02$ ,  $n = 24$ ). D-ATP comprised >65 % of the total ATP (T-ATP) pool in the summer, and ~50 % in winter. Dissolved guanosine-5'-triphosphate (D-GTP) inventories were 5- to 6-fold greater in July and August than those observed in mid-June ( $1.1 \pm 0.1$  vs.  $8.0 \pm 0.6$  and  $6.5 \pm 0.3$   $\mu\text{mol D-GTP m}^{-2}$ , respectively) and winter concentrations were on average lower than the mean summer concentrations. The particulate GTP (P-GTP) inventories were almost twice those measured in winter (mean  $1.1 \pm 0.4$  in summer vs.  $0.6 \pm 0.1$   $\mu\text{mol P-GTP m}^{-2}$  in winter,  $n = 3$ ). These results are consistent with higher microbial growth rates in summer. Uptake of D-ATP showed multi-phasic kinetic patterns. The half-saturation constants ( $K_i$ ) ranged from 1 to 26 nM at D-ATP concentrations of 0.2 to 30 nM, and  $V_{\text{max}}$  ranged from 0.3 to 1.4  $\text{nM d}^{-1}$ . At concentrations >30 nM,  $K_i$  exceeded 100 nM and  $V_{\text{max}}$  was 10.4  $\text{nM d}^{-1}$ . The calculated net production rates of D-ATP ranged from 40 to 150  $\text{pM d}^{-1}$ , and the turnover time of the ambient D-ATP pool was estimated to be 1 to 2 d. The P flux through the D-ATP pool could potentially be 5 times faster than that of the bulk DOP pool, implying that P derived from nucleotides may be an important pathway in the P cycle of oligotrophic oceans.

**KEY WORDS:** North Pacific · ATP · GTP · Nucleotides · DOM · Microbial ecology

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## INTRODUCTION

Dissolved nucleic acids, nucleotides and nucleotide derivatives are ubiquitous components of the dissolved organic matter (DOM) pool in aquatic environments (Minear 1972, Azam & Hodson 1977, Karl & Bailiff 1989). Dissolved adenosine-5'-triphosphate (D-ATP) has been reported to occur in a wide variety of aquatic habitats ranging from eutrophic, freshwater lakes to temperate, marine coastal regions and Antarctic seawaters (Azam & Hodson 1977, Riemann 1979, Hodson et al. 1981a, McGrath & Sullivan 1981, Maki et al. 1983, Nawrocki & Karl 1989). Contrary to many other biomolecules present in marine environments, the ability to quantify both intra- and extracellular concentrations of nucleotides gives them a unique position in

elucidating nutrient and energy fluxes through the microbial community. Nucleotide inventories and fluxes are potentially useful characteristics in marine ecology due to their universal occurrence in the environment and their obligate role in life. Despite this potential, few studies have been carried out in marine environments.

In the North Pacific Subtropical Gyre (NPSG) inorganic nutrient supply is chronically low, and concentrations of dissolved organic nutrients are frequently an order of magnitude higher (Karl & Tien 1992, Karl & Björkman 2002). Hence, the ability to utilize the organically bound nutrients should alleviate severe nutrient stress. Nucleotides are enriched in both nitrogen (N) and phosphorus (P) relative to carbon (C), compared to the average elemental ratio of plankton (Redfield et al.

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1963), and could constitute a source for nutrients as well as being valuable biosynthetic precursors. The bioavailability of dissolved organic phosphorus (DOP) in marine environments has rarely been studied directly, largely due to limited knowledge of the chemical composition of the natural pool and, therefore, the lack of a universal tracer. Some studies have assessed the bioavailability of selected compounds in P amendment experiments (Ammerman & Azam 1985, Björkman & Karl 1994). Alternatively, the ability of communities to hydrolyze DOP via exo-enzymatic reactions such as alkaline phosphatase has been studied (Perry 1972, Kobori & Taga 1978). In the only direct assessment of DOP availability, Björkman & Karl (2003) showed that the extant microbial community could derive up to 50% of their P from the ambient DOP pool. The ability to track a known DOP compound, such as ATP, at natural abundance through its dissolved and particulate pools would elucidate the production and regeneration of DOP and lead to a better understanding of P dynamics in oligotrophic oceans.

In the present study a novel method for the measurement of D-ATP and dissolved guanosine-5'-triphosphate (D-GTP) in seawater samples was used (Björkman & Karl 2001). We also investigated the uptake kinetics of radiolabeled ATP, and estimated the production rates of D-ATP by natural assemblages of marine microorganisms. Our results indicate the usefulness of nucleotides as tracers in nutrient dynamics studies, as well as the ecological implications of high flux through dissolved nucleotides and their potential contribution to the DOP pool and their role in the P dynamics in the oligotrophic NPSG.

## MATERIALS AND METHODS

**Station location and sample collections.** Seawater samples were collected between June 1998 and February 1999 at Stn ALOHA (22.75°N, 158.00°W; Karl & Lukas 1996) as part of the Hawaii Ocean Time-series (HOT; cruise numbers 94, 95, 96, 100, 101 and 102). Seawater samples for D-ATP and D-GTP were collected from 11 depths (5, 25, 45, 75, 100, 125, 150, 175, 250, 500 and 1000 m) into acid-washed, high-density polyethylene bottles. The samples were immediately vacuum filtered through 0.2 µm polycarbonate membrane filters (Nuclepore or Poretics) at a pressure differential between 5 and 15 cm Hg, to remove particles. Duplicate samples were typically collected for the top 2 or 3 depths. The filtrate (450 ml) was collected and stored frozen (–20°C) until analyzed for D-ATP and D-GTP, usually within 10 d of collection. The seawater samples were then treated with a 0.5% (v/v) addition of 1 M NaOH, to initiate Mg(OH)<sub>2</sub> formation (MAG-

nesium-Induced Co-precipitation-MAGIC; Karl & Tien 1992), which quantitatively co-precipitates D-ATP and D-GTP and was used to effect a 200-fold concentration of both nucleotides (Björkman & Karl 2001). These concentrated samples were stored at –20°C until analyzed, as described in Björkman & Karl (2001). The GTP concentrations were based on the difference between ATP, calculated using the peak height, versus 'ATP' concentration in the same sample, using the integrated light-emission values of the firefly bioluminescence assay (Karl 1978). If only ATP is present in the sample, both estimates are identical (i.e. the difference is zero). If non-adenine nucleotide triphosphates, such as GTP, are present, the integral ATP (ATP<sub>int</sub>) estimate will exceed the peak ATP (ATP<sub>peak</sub>) when the assay employs FLE-50 (Sigma). This crude extract preparation of firefly enzymes also contains the enzyme nucleoside diphosphokinase (NDPK), which catalyzes the equilibrium reaction of GTP to ATP. Karl (1978) showed that the difference between these 2 measurements, termed ΔATP [ $\Delta\text{ATP} = (\text{ATP}_{\text{int}} - \text{ATP}_{\text{peak}})$ ], is highly correlated to GTP concentrations for a variety of natural samples. Björkman & Karl (2001) also showed that ΔATP was equivalent to GTP in natural seawater samples at concentrations up to 50 pM GTP, after which ΔATP underestimated GTP by 3 to 5%.

Experiments designed to estimate the total intracellular adenine pool turnover (Karl & Bossard 1985), D-ATP uptake kinetics and D-ATP production rates involved time-course incubations of surface seawater (5 m). These were collected on several occasions from September 1998 to August 1999 (HOT-96, -97, -98, -99, -100, -104, -105, -107). Either <sup>32</sup>P<sub>4</sub> (orthophosphoric acid, carrier free; ICN Radiochemicals, No. 64014 L) or <sup>3</sup>H-ATP (2,8-<sup>3</sup>H-adenosine-5'-triphosphate, specific activity 1.2 TBq mmol<sup>-1</sup>; ICN Radiochemicals, No. 24009) was added to the incubation bottles. Samples for particulate ATP (P-ATP) were from 1 to 2 l of seawater filtered onto glass fiber filters (Whatman GF/F) and extracted in 5 ml of boiling Tris buffer (20 mM, pH 7.4; Karl & Holm-Hansen 1976).

Measurements of chlorophyll *a* (chl *a*), inorganic phosphate (Pi), DOP, particulate P (P-PO<sub>4</sub>) and primary production (PP), also collected on GF/F filters, were available as part of the HOT core measurement program. Information about HOT core measurements is available at [http://hahana.soest.hawaii.edu/hot/hot\\_jgofs.html](http://hahana.soest.hawaii.edu/hot/hot_jgofs.html).

**<sup>3</sup>H-ATP uptake experiments.** Surface seawater was placed into 500 ml polycarbonate (PC) incubation bottles and spiked with <sup>3</sup>H-ATP to a final activity ranging from 0.18 to 8.9 MBq l<sup>-1</sup> and with an ATP addition of approximately 0.2 to 7.3 nM ATP over a background seawater level of approximately 0.1 nM ATP. Non-labeled ATP was also added (range 10 to 250 nM ATP)

to samples with a constant radioactivity of approximately 8.9 MBq l<sup>-1</sup>. The bottles were incubated on deck under natural sunlight and at ambient sea surface temperature. Subsamples were taken 5 to 6 times over a 10 to 12 h daylight period. A 20 ml portion was filtered through 0.2 µm PC filters (Nuclepore) for particulate activity, and 1 ml of whole water was taken at each sampling point for total radioactivity. Triplicate samples were taken at 1 of the time points to assess the variability among subsamples. The samples were placed into borosilicate scintillation vials and stored frozen for later analysis of <sup>3</sup>H-activity by liquid scintillation counting (LSC). Radioactivity was determined using a Packard Tri-Carb<sup>®</sup> LSC. Aquasol II (Packard, No. NEF 952) was used as the scintillation cocktail, and samples were quench corrected using Packard instruments SIE protocols. Uptake rates were calculated from the linear portion of the time course expressed as Bq l<sup>-1</sup> d<sup>-1</sup>. Turnover time (*T*) was calculated as:

$$T = t/r \quad (1)$$

where *t* is the total <sup>3</sup>H-ATP activity (Bq l<sup>-1</sup>) added to the samples and *r* is the uptake rate into the particulate fraction (Bq l<sup>-1</sup> d<sup>-1</sup>). These data were used to analyze uptake kinetics based on the turnover rate at different concentrations of ATP and using the modified Eadie–Hoftsee transformation (Azam & Hodson 1981) to calculate the half-saturation constant (*K<sub>t</sub>* + *S<sub>n</sub>*) and maximum uptake rate (*V<sub>max</sub>*) as:

$$1/TA = V_{\max} - 1/T(K_t + S_n) \quad (2)$$

where 1/*T* is the turnover rate (h<sup>-1</sup>) and *A* is the ATP concentration in the incubations. Plotting (1/*T* × *A*) against 1/*T* (*x*-axis) yields a straight line relationship, with the *y*-axis intercept equivalent to *V<sub>max</sub>* and a slope equivalent to *K<sub>t</sub>* + *S<sub>n</sub>*. This transformation is sensitive to multiphasic uptake kinetics and results in non-linearity of the curve when uptake systems of different or variable *V<sub>max</sub>* and *K<sub>t</sub>* are present (Azam & Hodson 1981). In our case *S<sub>n</sub>* was measured, thus *K<sub>t</sub>* could be calculated.

**P-ATP pool turnover experiments.** Several experiments were conducted to measure the labeling kinetics of the ATP pool following the addition of <sup>32</sup>PO<sub>4</sub> (8.9 MBq l<sup>-1</sup>) to seawater samples (Karl & Bossard 1985). The 2 terminal P groups (β and γ) of ATP have 'high-energy' anhydride bonds that readily exchange during cellular energy transfers. The <sup>32</sup>P tracer will rapidly and uniformly incorporate into the β and γ positions. The kinetics of this labeling of ATP can be used to assess the turnover time of the internal ATP pool (Karl & Bossard 1985). Samples were collected 8 to 9 times over a 12 h incubation period. At each sampling time the content of an entire incubation bottle

(450 ml) was filtered through a Whatman GF/F filter and extracted in boiling Tris buffer, as described above for P-ATP samples. The extract was then stored frozen until it could be processed further, following procedures described elsewhere (Karl & Bossard 1985, Bossard & Karl 1986, Karl et al. 1987, Karl 1993). Briefly, the Tris extract was concentrated by vacuum evaporation, and the ATP was purified using polyethyleneimine (PEI) thin-layer chromatography. To estimate the respective radioactivities of the α, β and γ positions of ATP as a function of time, the isolated P-ATP<sup>32</sup>P was eluted from the PEI matrix and treated with apyrase (Sigma Chemical, No. A-6132) to selectively hydrolyze the 2 terminal P groups (β and γ) of ATP (Karl & Bossard 1985). Extraction with activated charcoal suspended in 0.1 M H<sub>3</sub>PO<sub>4</sub> separated the inorganic and organic <sup>32</sup>P activities. The data were fitted using a non-linear function formula, similar to PROCNLIN (Karl et al. 1987), to estimate the maximum specific radioactivity of the γ P position of ATP. This value was used to estimate the production of D-ATP (Eq. 4; below).

**Production of D-ATP<sup>32</sup>P.** Seawater incubated with <sup>32</sup>PO<sub>4</sub>, as described above, was used for D-ATP<sup>32</sup>P determination. The D-ATP produced will originate from P-ATP before entering the dissolved fraction and, hence, will have the same specific activity as that of the P-ATP pool from which it was derived. The seawater samples were filtered through 0.2 µm PC filters (Nuclepore); 450 ml of the filtrate was collected and immediately frozen at -20°C. These samples were later concentrated using the MAGIC procedure, as described for D-ATP samples above. To separate radioactivity stemming from co-precipitated inorganic <sup>32</sup>P from the AT<sup>32</sup>P signal, the concentrated samples were purified by PEI column chromatography using miniature columns prepared in standard Pasteur pipettes. A glass wool plug was placed in the lower end, and the columns were packed with a PEI slurry to a height of 2.5 cm. The PEI slurry was prepared from powder (Sigma Chemical, No. P-6883, fine mesh <74 µm). Fine particles were removed by washing in several volumes of distilled water (dH<sub>2</sub>O) followed by 0.1 M HCl (Magnusson et al. 1976). Concentrated samples were diluted 10-fold with dH<sub>2</sub>O prior to loading onto the columns to avoid interference by high sample concentrations of MgCl<sub>2</sub> with the binding of ATP to the PEI. Final sample volumes were usually 2 ml, but up to 5 ml was loaded (HOT-104). The columns were eluted stepwise with LiCl (Magnusson et al. 1976), followed by MgCl<sub>2</sub> (2 ml per fraction and 3 fractions per concentration step—LiCl: 0.1 M, 0.3 M, 1 M; MgCl<sub>2</sub>: 0.35 M, 0.7 M). Each fraction was collected directly into glass scintillation vials, and <sup>32</sup>P activity was analyzed using Cerenkov radiation. Counting efficiency was deter-

mined to be 43%. Control samples containing only  $^{32}\text{PO}_4$  and ATP labeled at the gamma position ( $\gamma\text{-AT}^{32}\text{P}$ ) were used to monitor compound elution patterns. The aliquots containing  $\text{AT}^{32}\text{P}$  activity, as determined from the  $\gamma\text{-AT}^{32}\text{P}$  elution profile, were split equally. One sample portion was treated with apyrase and the other remained untreated. Both sample splits were subsequently extracted with activated charcoal, as described above. The fraction ( $F$ ) of the total activity in the samples attributed to  $\text{AT}^{32}\text{P}$  was calculated as:

$$F = (\text{Ap} - \text{NAP})/\text{TA} \quad (3)$$

where TA is the total activity in the eluted fraction, NAP is the activity in the supernatant of charcoal-treated samples without apyrase and Ap is the activity in the supernatant of charcoal-treated samples after apyrase hydrolysis. This gives an estimate of the  $^{32}\text{P}$  labeling of the  $\beta$  and  $\gamma$  phosphates of ATP. These corrections were necessary due to the large signal originating from inorganic  $^{32}\text{P}$  and the fact that a small portion of inorganic  $^{32}\text{P}$  co-eluted in all fractions (~0.1% of the total activity loaded). The total activity in 2 ml of eluate was multiplied by  $F$ , estimated from the apyrase and charcoal treatments and corrected to the original seawater sample volume as  $\text{Bq l}^{-1}$ .

The net D-ATP production rates were calculated from the  $\text{AT}^{32}\text{P}$  in the eluates and by assuming that D- $\text{AT}^{32}\text{P}$  has the same specific activity as that of the P-ATP fraction, as described above. From time-course incubations, production rates of D- $\text{AT}^{32}\text{P}$  were calculated from the activity of the  $\beta$  and  $\gamma$  phosphates and

corrected for  $T_0$  activity. This production rate ( $\text{Bq l}^{-1} \text{h}^{-1}$ ) was converted to  $\text{pM ATP d}^{-1}$  using the following calculation:

$$\text{D-ATP}_p = \text{PR}/S_A \quad (4)$$

where  $\text{D-ATP}_p$  is the dissolved ATP production, PR is the production rate of  $\text{AT}^{32}\text{P}$  in the eluted fraction and  $S_A$  is the specific activity ( $\text{Bq pmol ATP}^{-1}$ ) of the  $\beta$  and  $\gamma$  phosphates of the P-ATP pool.

On 1 occasion (HOT-104), the ATP concentration in the 0.35 M  $\text{MgCl}_2$  eluates was measured using the firefly bioluminescence assay. These data were used to calculate the  $S_A$  in the respective fractions as a comparison to the  $S_A$  of the P-ATP pool.

## RESULTS

### Dissolved and particulate nucleotide concentrations at Stn ALOHA

D-ATP and P-ATP concentrations measured during the period June 1998 to February 1999 at Stn ALOHA revealed that the dissolved pools consistently exceeded the particulate pools, with the exception of February 1999. Similarly, D-GTP concentrations were generally higher than those observed in the respective particulate fractions, except for June 1998 (Table 1). There was an apparent seasonal variability in the pool sizes with relatively larger dissolved fractions during the summer/fall months for both ATP and GTP

Table 1. Dissolved and particulate nucleotide concentrations and related biological parameters for samples collected at Stn ALOHA in different months during different cruises (HOT-94 to HOT-102). Values shown are mean concentrations  $\pm$  SD (in parentheses) integrated over 0 to 100 m depth, except for PP/chl *a*, which is presented as an average value over the upper 0 to 25 m of the water column where production is light saturated. P-ATP: particulate adenosine-5'-triphosphate; D-ATP: dissolved adenosine-5'-triphosphate; P-GTP: particulate guanosine-5'-triphosphate; D-GTP: dissolved guanosine-5'-triphosphate; SRP: soluble reactive phosphorus; DOP: dissolved organic phosphorus; P- $\text{PO}_4$ : particulate phosphorus; chl *a*: chlorophyll *a*; PP: primary production; PP/chl *a*: primary production per unit chlorophyll *a*. D-ATP% is the proportion D-ATP of the total ATP (D-ATP/[D-ATP + P-ATP])

Parameter	Summer			Winter		
	Jun 1998 HOT-94	Jul 1998 HOT-95	Aug 1998 HOT-96	Dec 1998 HOT-100	Jan 1999 HOT-101	Feb 1999 HOT-102
P-ATP ( $\mu\text{mol m}^{-2}$ )	6.4 $\pm$ 0.2	4.8 $\pm$ 0.5	8.5 $\pm$ 0.6	3.8 $\pm$ 0.2	5.3 $\pm$ 0.3	6.5 $\pm$ 0.5
D-ATP ( $\mu\text{mol m}^{-2}$ )	11.8 $\pm$ 0.4	13.9 $\pm$ 0.5	15.5 $\pm$ 0.4	7.2 $\pm$ 0.1	6.5 $\pm$ 0.3	3.4 $\pm$ 0.1
D-ATP%	65	74	65	65	55	34
P-GTP ( $\mu\text{mol m}^{-2}$ )	1.8 $\pm$ 0.5	1.0 $\pm$ 0.1	0.4 $\pm$ 0.1	0.6 $\pm$ 0.0	0.6 $\pm$ 0.1	0.6 $\pm$ 0.1
D-GTP ( $\mu\text{mol m}^{-2}$ )	1.1 $\pm$ 0.1	8.0 $\pm$ 0.6	6.5 $\pm$ 0.3	1.8 $\pm$ 0.2	1.3 $\pm$ 0.3	1.1 $\pm$ 0.1
SRP ( $\text{mmol m}^{-2}$ )	6.8	4.0	9.8	7.4	12.3	12.8
DOP ( $\text{mmol m}^{-2}$ )	19.3	23.7	32.5	23.7	28.7	18.4
P- $\text{PO}_4$ ( $\text{mmol m}^{-2}$ )	1.3	1.4	1.5	1.4	1.5	0.9
Chl <i>a</i> ( $\text{mg m}^{-2}$ )	10.8 $\pm$ 0.1	10.8 $\pm$ 0.3	15.9 $\pm$ 0.3	11.4 $\pm$ 0.1	13.4 $\pm$ 0.3	16.9 $\pm$ 0.1
PP ( $\text{mg C m}^{-2} \text{d}^{-1}$ )	595 $\pm$ 7	622 $\pm$ 11	611 $\pm$ 40	174 $\pm$ 15 <sup>a</sup>	400 $\pm$ 1	390 $\pm$ 4
PP/chl <i>a</i> ( $\text{g C g}^{-1} \text{chl a h}^{-1}$ )	3.8 $\pm$ 0.1	8.4 $\pm$ 0.5	4.8 $\pm$ 0.8	2.4 $\pm$ 0.3	2.4 $\pm$ 0.0	4.0 $\pm$ 0.0

<sup>a</sup>The PP incubations were terminated approximately 3 h short of the routine dawn to dusk PP protocol

(Fig. 1A,C). The particulate nucleotide pools were also larger in the summer/fall relative to winter/spring months (Fig. 1B,D). The highest D-ATP and D-GTP concentrations were found in the upper water column (>175 m), similar to the P-ATP and particulate GTP (P-GTP) distributions. Below the euphotic zone, both the dissolved and particulate nucleotide concentrations were low and relatively invariant with season (Fig. 1). A significant positive correlation ( $p < 0.001$ ) was observed between P-ATP and D-ATP in the summer (Fig. 2A), when D-ATP concentrations in the top 175 m of the water column were on average 1.5 times greater than those of P-ATP ( $D\text{-ATP [pM]} = 1.50 \times P\text{-ATP [pM]} + 35$ ,  $r^2 = 0.61$ ,  $n = 24$ ). In the 3 winter months sampled, however, the particulate and dissolved fractions were more variable and there was no significant correlation between the 2 pools ( $D\text{-ATP [pM]} = 0.18 \times P\text{-ATP [pM]} + 41$ ,  $r^2 = 0.02$ ,  $n = 24$ ; Fig. 2B).

Depth-integrated D-ATP inventories over the upper euphotic zone (0 to 100 m) were about twice as high during summer compared to winter ( $13.8 \pm 1.1 \mu\text{mol ATP m}^{-2}$ ,  $n = 3$ , vs.  $5.7 \pm 1.1 \mu\text{mol ATP m}^{-2}$ ,  $n = 3$ ). The average differences in P-ATP were less pronounced between seasons, but lower in winter (summer:  $6.5 \pm 1.0 \mu\text{mol ATP m}^{-2}$ ,  $n = 3$ ; winter:  $5.2 \pm 0.8 \mu\text{mol ATP m}^{-2}$ ,  $n = 3$ ; Fig. 3A). In contrast, D-GTP and P-GTP concentrations varied much more between seasons and among cruises (Table 1, Fig. 3B), with a summer maximum in D-GTP 8-fold higher than the winter minimum. Again the particulate concentrations were lower than the dissolved concentrations, but the peak summer values were about 3 times higher than those observed during the winter (Table 1, Fig. 3B). The mean proportion ( $\pm 1$  SD) of the total ATP (T-ATP) pool (i.e. [P-ATP + D-ATP]) contributed by D-ATP in depth-integrated samples (0 to 100 m) was  $68 \pm 11\%$  (range 65 to 74%) for the summer cruises and  $52 \pm 13\%$  (range 34 to 66%) in the winter (Table 1).

The D-GTP/D-ATP ratios in depth-integrated samples (0 to 100 m) were

significantly higher during the late summer cruises (0.4 to 0.6 [mol/mol]; Fig. 3C), with a 4- to 6-fold increase from June to July/August. However, P-GTP concentrations and P-GTP/P-ATP ratios were highest

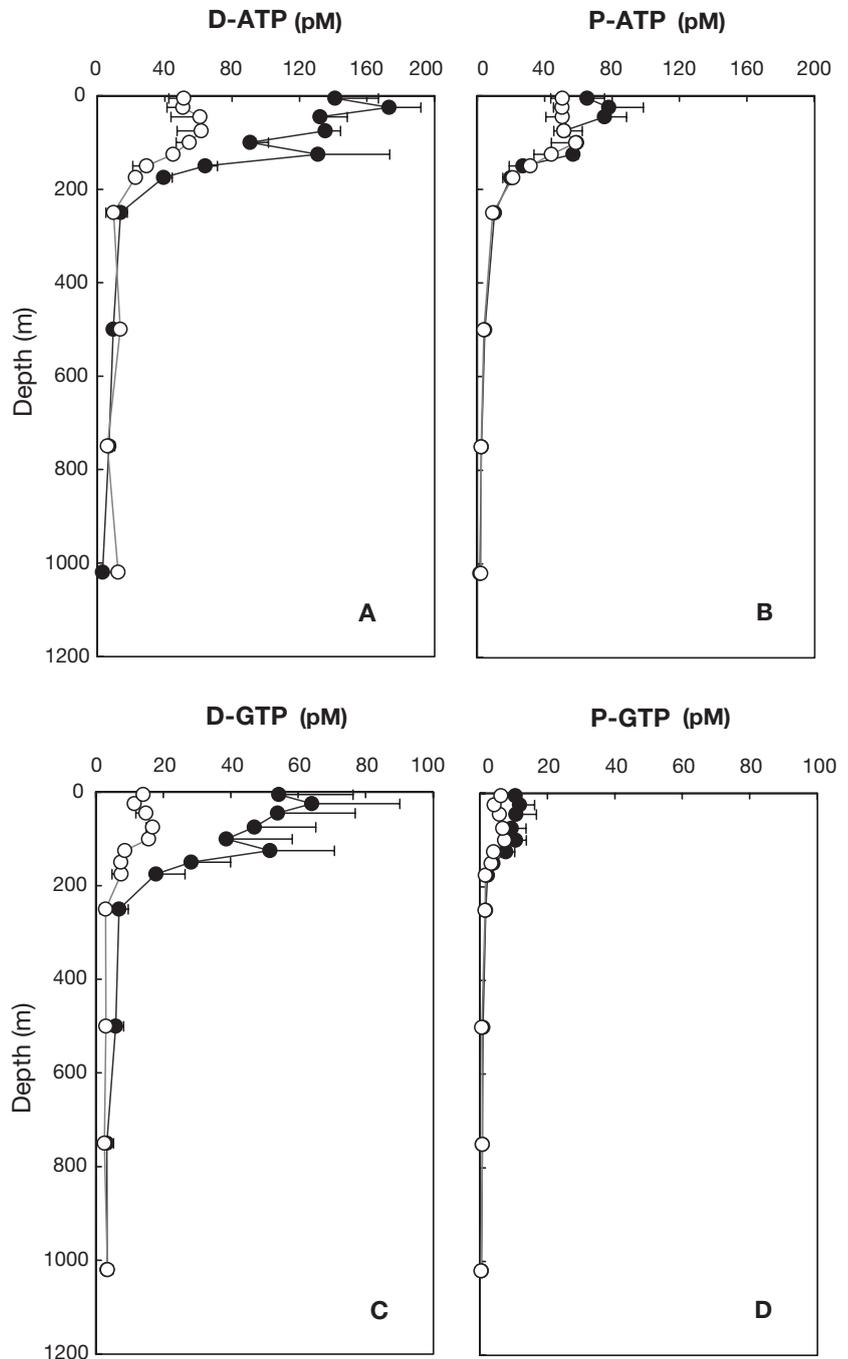


Fig. 1. Depth distributions of dissolved and particulate nucleotide concentrations at Stn ALOHA. ●: summer months, ○: winter months. Values are mean concentrations for 3 cruises for each season; error bars are  $\pm 1$  SE,  $n = 3$ : (A) dissolved adenosine-5'-triphosphate (D-ATP), (B) particulate adenosine-5'-triphosphate (P-ATP), (C) dissolved guanosine-5'-triphosphate (D-GTP) and (D) particulate guanosine-5'-triphosphate (P-GTP)

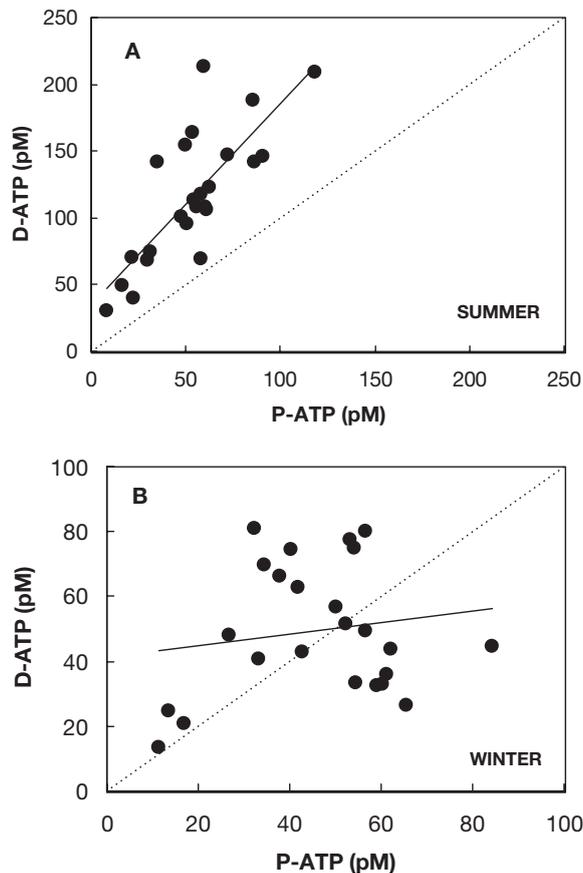


Fig. 2. Correlation between P-ATP and D-ATP concentrations within the euphotic zone (0 to 175 m) at Stn ALOHA: (A) summer period ( $D\text{-ATP} = 1.50 P\text{-ATP} + 35$ ,  $r^2 = 0.61$ ,  $n = 24$ ) and (B) winter period ( $D\text{-ATP} = 0.18 P\text{-ATP} + 41$ ,  $r^2 = 0.02$ ,  $n = 24$ ). The dotted line is the 1:1 relationship; the solid line is the linear regression solution

in June and lowest in August. During the winter cruises, overall GTP/ATP ratios were lower and less variable than in the summer (dissolved: 0.2 to 0.3; particulate:  $<0.2$ ) and may be consistent with overall lower microbial activities in winter (Fig. 3C). Furthermore, there appears to have been a lag period in the summer D-GTP concentrations relative to the increase in the particulate fraction in June. By the following month, the D-GTP concentrations had risen dramatically and remained high in August, even though the P-GTP pool concentration had fallen sharply from a high of  $1.8 \mu\text{mol m}^{-2}$  in June to a low of  $0.4 \mu\text{mol m}^{-2}$  in August (Table 1).

#### D-ATP uptake and turnover as measured by $^3\text{H-ATP}$

Uptake rates, based on  $^3\text{H-ATP}$  turnover time of the D-ATP pool concentrations, ranged from 0.05 to

$2.32 \text{ nM d}^{-1}$ . When investigating the kinetics of the uptake by modified Eadie-Hofstee plots (Eadie 1952, Hofstee 1952), as described by Azam & Hodson (1981), it was evident that there were multiphasic uptake systems within the extant microbial community. The half-saturation constant ( $K_i$ ) values ranged between 1.0 and  $26.4 \text{ nM}$  in the lower range of ATP concentrations, and could be as high as  $1.9 \mu\text{M}$  at a D-ATP concentration of  $\sim 260 \text{ nM}$ .  $V_{\text{max}}$  was also variable, and ranged between 0.30 and  $0.83 \text{ nM d}^{-1}$  in the low concentration range and between 6.8 and  $10.4 \text{ nM d}^{-1}$  in the high region (Table 2).

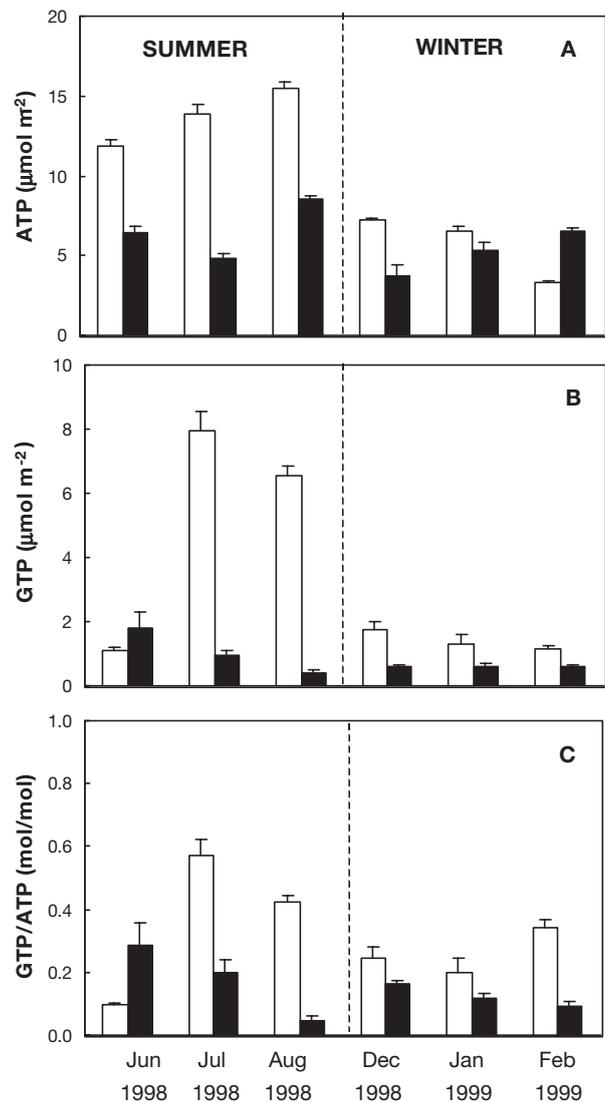


Fig. 3. Inventories of (A) P-ATP and D-ATP, (B) P-GTP and D-GTP and (C) GTP/ATP concentration ratios in the upper water column (depth integrated from 0 to 100 m) at Stn ALOHA for 3 summer and 3 winter cruises from 1998 to 1999 (open bars: dissolved nucleotides; solid bars: particulate nucleotides). Error bars are  $\pm 1$  SD of the integral inventories

### D-ATP production measured by $^{32}\text{PO}_4$ uptake, incorporation and release

With the PEI column elution protocol used here the recovery of the total dissolved  $^{32}\text{P}$  activity loaded on the columns was 100%. The fractions in which  $^{32}\text{PO}_4$  eluted (0.3 M LiCl) were well separated from those in which ATP eluted (0.35 M  $\text{MgCl}_2$ ). The D-AT $^{32}\text{P}$  data

Table 2. Uptake kinetics and turnover times of  $^3\text{H}$ -ATP at increasing concentrations of D-ATP by natural assemblages of microorganisms in seawater samples collected at 5 m at Stn ALOHA (22.75°N, 158.00°W) in the North Pacific Subtropical Gyre. Uptake rate designates the D-ATP concentration/turnover time.  $K_t$ : half-saturation constant for ATP uptake derived from modified Eadie–Hofstee transformation of Michaelis–Menten kinetics (Azam & Hodson 1981). Values listed are the lowest and highest estimates, due to non-linear kinetics. HOT-107 (Aug 1999) showed multiple uptake phases.  $V_{\text{max}}$ : maximum uptake velocity of ATP; estimates derived as above for  $K_t$

Date/ Cruise	[D-ATP] (nM)	Turnover time (d)	Uptake rate (nM d <sup>-1</sup> )	$K_t$ (nM)	$V_{\text{max}}$ (nM ATP d <sup>-1</sup> )
Nov 1998/ HOT-99	7.4	65	0.11	26.4	0.51
	57.4	191	0.30		
	107	230	0.47		
	257	211	1.22		
Dec 1998/ HOT-100	5.2	53	0.10	12.4	0.33
	15.2	85	0.18		
	30.2	128	0.24		
	55.2	113	0.49		
	105	149	0.71		
May 1999/ HOT 105	1.3	16.5	0.08	20.1	1.36
	2.4	15.1	0.16		
	3.2	20.0	0.17		
	28.3	34.0	0.83		
	53.4	54	0.98		
	103	44	2.32		
Aug 1999/ HOT 107	0.2	4.2	0.05	1.0	0.30
	0.4	4.7	0.08		
	0.9	6.5	0.14		
	1.8	12.2	0.15		
	3.6	15.8	0.23		
	13.6	34	0.40		
	28.5	32	0.88		

Table 3. Polyethyleneimine (PEI) column separation of D-AT $^{32}\text{P}$  in natural seawater samples collected during 4 cruises at 5 m at Stn ALOHA in the North Pacific Subtropical Gyre. P-ATP SA: the maximum specific  $^{32}\text{P}$  activity of the terminal phosphates [ $\beta$  and  $\gamma$ ] in the particulate ATP [P-ATP] pool; D-ATP production rate: D-AT $^{32}\text{P}$  production rate/P-ATP SA, assuming that D-ATP produced will have the same SA as the P-ATP

Cruise/Date	P-ATP SA (Bq pM <sup>-1</sup> P-ATP)	D-AT $^{32}\text{P}$ production rate (Bq l <sup>-1</sup> h <sup>-1</sup> )	D-ATP production rate (pM h <sup>-1</sup> )
HOT-96/Aug 1998	164	655	4.0
HOT-97/Sep 1998	72	125	1.7
HOT-98/Oct 1998	88	554	6.5
HOT-104/Apr 1999	45	194	4.3

obtained from time-course incubations were used to calculate the production rates of D-ATP (pM h<sup>-1</sup>), as discussed above (Eqs. 3 & 4). The incorporation into the particulate fraction and the subsequent appearance in the dissolved pool revealed that the D-ATP produced varied among cruises and ranged from 1.7 to 6.5 pM h<sup>-1</sup> (Table 3). The production rates of P-ATP, estimated from specific  $^{32}\text{PO}_4$  incorporation into P-ATP (Karl & Bossard 1985) were approximately  $28 \pm 10$  pM h<sup>-1</sup>, indicating that the net D-ATP production may constitute 6% to 23% of the corresponding P-ATP production rates.

## DISCUSSION

Azam & Hodson (1977) first developed a method for the measurement of D-ATP in seawater using a mixture of activated charcoal and celite as an adsorption matrix, ammoniacal-ethanol solvent elution to desalt and evaporation to concentrate D-ATP prior to measurement by firefly bioluminescence. This method has been applied only rarely in marine ecological studies (Hodson et al. 1981a, McGrath & Sullivan 1981, Nawrocki & Karl 1989), despite the potential of D-ATP as a model compound. These studies were all conducted in relatively nutrient-rich, high-productivity regions with P-ATP concentrations ranging from 400 to >2000 pM and D-ATP commonly well above 200 pM. By comparison, P-ATP concentrations in the subtropical North Pacific Ocean rarely exceed 100 pM, even in the most productive portions of the water column (e.g. HOT-1 to HOT-100; 0 to 100 m, mean = 68.4 pM, SE = 30.7 pM, median 61.3 pM, <15% of distribution >100 pM, n = 493). The method used in this study relies on a pH-induced formation of the mineral brucite ( $\text{Mg}[\text{OH}]_2$ ), which co-precipitates a variety of organic and inorganic phosphorus-containing compounds from seawater (Karl & Tien 1992, Karl & Yanagi 1997, Thomson-Bulldis & Karl 1998). D-ATP and D-GTP are among the compounds that are quantitatively co-precipitated (Björkman & Karl 2001).

Both ATP and GTP play integral roles in the regulation of metabolic activities in all living cells. ATP functions in the energy transfer for anabolic and catabolic cellular processes and, together with ADP and AMP, for controlling the energy status of the cell (Karl 1980). However, many biosynthetic reactions rely on energy derived from non-adenine nucleotide triphosphates. For example, GTP is required in protein synthesis as the energy donor in RNA transcription and in tRNA binding and translocation. GTP and ATP are also necessary components in the biosynthesis of both RNA and, in their deoxyribose forms, DNA. P-ATP concentrations will then be a measure of the living biomass, or size of the standing stock of microorganisms, whereas P-GTP concentrations indicate the relative amount of protein synthesis. Karl (1978, 1979) found that the P-GTP/P-ATP ratio in microorganisms was positively correlated to growth rate, suggesting that the P-GTP/P-ATP ratio could be useful in comparing microbial community growth rates in nature.

We hypothesized that the pattern of increasing P-GTP/P-ATP ratios through June and July indicated a more actively growing microbial population compared to the other months studied (Fig. 3). This was not immediately reflected in standing stocks of chl *a* or P-ATP; however, an increase in the PP per chl *a* concentration, an indicator of relative productivity, in the top 25 m of the water column was apparent (Table 1). Although the standing stocks of D-ATP, P-ATP and chl *a* reached their highest values in August, the P-GTP and P-GTP/P-ATP ratio were at their minimum in the same month. PP also remained high; however, the PP per unit chl *a* decreased by 30% relative to June and July (Table 1), possibly indicating a less actively growing microbial community as suggested by the low P-GTP/P-ATP ratio. There was also a shift in the phytoplankton population structure from the typically dominant prokaryotic *Prochlorococcus* sp. community (Karl et al. 2001a) in July and August, when large eukaryotic phytoplankton (>5  $\mu\text{m}$ ) were more abundant in the mixed layer (approx. 0 to 45 m) compared to the previous months. This may have caused the changes in the GTP/ATP structure too. The increase in the D-GTP pool from June to July and its persistence even after P-GTP concentrations had fallen off implies that the D-GTP pool was turning over more slowly than the intracellular pools. In comparison, the D-ATP and P-ATP pools did not vary as dramatically as the GTP pools (Fig. 3).

The release of organic compounds into the DOM pool is the combined result of exudation from healthy cells, losses from grazing activities, leaching from dead cells and/or cell lysis caused by viral infections (Sharp 1977, 1993, Proctor & Fuhrman 1990, Williams 1990, Wood & van Valen 1990). We suggest that the dis-

solved nucleotide signal in seawater could be useful for elucidating the specific production mechanism for DOM. Healthy, actively growing cells contain higher ATP and GTP concentrations compared to senescent or moribund populations, which contain only minimal amounts of ATP and GTP, as well as having a low GTP/ATP ratio (Karl 1979, 1980). We hypothesize, from the dissolved nucleotide signature observed here, that the dominant mechanism leading to the release of DOM during the summer cruises was grazing activity rather than leakage from decaying or lysed cells.

The variations in both the dissolved and particulate nucleotide concentrations between summer and winter months may also be indicative of seasonal changes in microbial biomass and activities. Although it should be kept in mind that these observations are based on only a few data and that it will be necessary to collect samples over several years to establish whether these are true seasonal trends. Nevertheless, the NPSG is now known to exhibit seasonal changes in a variety of parameters (Karl et al. 1998, Karl 1999, Hebel & Karl 2001), much like the well-documented seasonal cycles of temperate ecosystems, although of lesser magnitude. In other habitats experiencing more dramatic seasonal variations in light and nutrient fluxes, such as off the Antarctic Peninsula, both positive and negative correlations for D-ATP versus P-ATP have been observed, as well as conditions with apparently no correlation between the 2 parameters (Nawrocki & Karl 1989). Furthermore, depth-integrated concentrations (0 to 50 m) for both D-ATP and P-ATP could vary by an order of magnitude in a single season (December to March; Nawrocki & Karl 1989), a degree of variability not unexpected considering the temporal changes and spatial heterogeneity of this environment.

The high partitioning towards dissolved nucleotides relative to particulate pools, with summertime values of ~70% of the total ATP being dissolved, may indicate that the NPSG is fundamentally different in this regard from coastal marine and eutrophic freshwater ecosystems. For example, in the coastal waters off the Antarctic Peninsula, samples within the euphotic zone had an average ratio of  $[\text{D-ATP}]/[\text{D-ATP} + \text{P-ATP}]$  of  $0.29 \pm 0.15$  ( $n = 17$ , median 0.23; Nawrocki & Karl 1989). Surface seawater from the continental shelf and Gulf Stream had ratios of  $0.12 \pm 0.07$  ( $n = 14$ , median 0.08; Hodson et al. 1981b) and freshwater systems showed ratios between 0.14 and 0.45, with values reaching 0.76 during a bloom event in a eutrophic lake (Riemann 1979). This may reflect differences in ecosystem dynamics, especially the intensity of nutrient recycling. Ecosystems that are recycling intensive, like oligotrophic gyres, might be expected to have greater nucleotide fluxes and higher ambient dissolved concentrations relative to the particulate pools, in the

same way as dissolved organic N (DON) and DOP dominate the total N and P pools (Karl et al. 2001b).

Another important aspect of a potentially high nucleotide flux into the DOM pool is their chemical composition with respect to C, N and P. Nucleotides are enriched in both N and P relative to C compared to average microbial cells and to bulk DOM. Consequently, they may constitute a preferential source of macro-nutrients for the microbial community.

Nucleotides appear to be readily assimilated by aquatic microorganisms (Azam & Hodson 1977) and are often preferentially utilized relative to other bioavailable DOP compounds (Björkman & Karl 1994, Björkman et al. 2000). ATP uptake studies in eutrophic freshwater systems (Riemann 1979, Maki et al. 1983) indicated that D-ATP was taken up rapidly by the extant microbial community. In lakes, the rate of ATP uptake can exceed  $1.35 \text{ nM h}^{-1}$ , with turnover times of  $<10 \text{ h}$  (Riemann 1979). Similar turnover times have been observed in Antarctic waters and in temperate coastal marine habitats (Hodson & Azam 1977, Hodson et al. 1981a,b, Nawrocki & Karl 1989). The higher rates of D-ATP uptake were frequently correlated with high concentrations of both P-ATP and D-ATP.

From the experiments conducted here on the uptake kinetics of D-ATP it was apparent that the extant microbial community had the capacity to turn over the dissolved nucleotide pool very rapidly. The D-ATP concentrations tested were higher than ambient concentrations, and, consequently, the D-ATP uptake rates were also higher (Table 2) than what would be expected at natural concentrations. However, even at the lowest rate observed, the entire D-ATP pool in this oligotrophic system could turn over in 1 to 2 d. In contrast, the intracellular ATP pool was estimated to turn over approximately 10 times  $\text{d}^{-1}$  (turnover time  $2.5 \pm 1 \text{ h}$ ,  $n = 8$ ). Considering that the intracellular level of ATP could be in the range of 1 to 5 mM (Karl 1980), it is plausible that ephemeral patches with significantly higher concentrations of D-ATP than the average could be present. At higher concentrations of D-ATP the uptake rate would increase and it is likely that these patches are rapidly exploited. Furthermore, the lack of a lag period at any given concentration of exogenous ATP (Fig. 4) indicates that the respective uptake systems were active immediately and not induced in response to increased concentrations of the substrate. However, as these experiments were conducted using unfractionated seawater, it cannot be determined whether the increase in uptake rate was the result of multiple uptake systems of lower substrate affinity within 1 organism group, or a response by other community members. The observation that natural microbial assemblages possess high-capacity uptake systems for D-ATP strengthens the suggestion

that there are conditions under which dissolved nucleotide concentrations are well above the average ambient level and that the extant microbial community has the ability to utilize this situation efficiently.

In an environment presently believed to be under P control (Karl 1999, Karl et al. 2001b) nucleotides may be important in sustaining the P cycle. Recent studies in the NPSG have shown nucleotides to be among the most bioavailable DOP compounds, and they have shown that the release of orthophosphate from dissolved nucleotides could exceed the rate of phosphorus uptake by as much as 50-fold (Björkman et al. 2000). This indicates a potentially significant role for nucleotides in P dynamics in these oligotrophic environments. These observations are consistent with the phosphohydrolytic activities of 5'-nucleotidase (Ammerman & Azam 1985), a ubiquitous enzyme of marine bacteria. This ectoenzyme has been shown to have the capacity to regenerate P at a rate that frequently vastly exceeds microbial P uptake rates (Siuda & Güde 1994) and has been hypothesized to provide sufficient P to satisfy microbial requirements (Tamminen 1989, Ammerman 1991). Consequently, dissolved nucleotides may be important in microbial metabolism, and the measurement of the fluxes of ATP and GTP through the microbial community could be a powerful tool for understanding interactions among the particulate and dissolved matter fractions, especially, but not exclusively, to the dynamics of the P pool.

At Stn ALOHA, the net production rates of DOP have been estimated to be  $0.6 \text{ to } 2.5 \text{ nM P d}^{-1}$  (mean  $1.4 \pm 0.6 \text{ nM P d}^{-1}$ ,  $n = 6$ ; Björkman et al. 2000), with mean

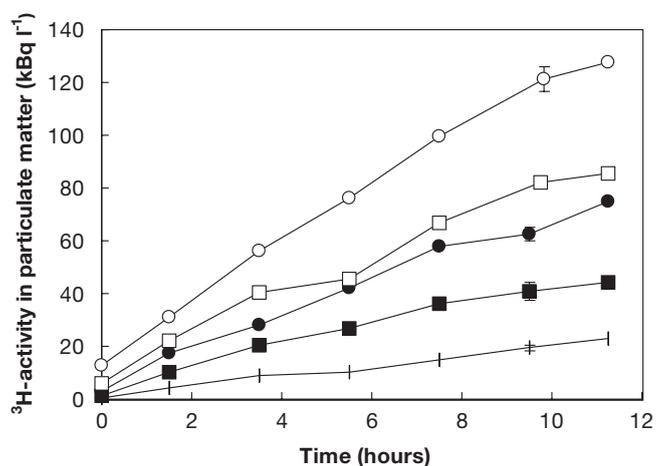


Fig. 4. Time-course experiment with dissolved  $^3\text{H}$ -ATP. Uptake into the particulate fraction (retained on  $0.2 \mu\text{m}$  PC filters) at increasing concentrations and activities of ATP ( $\square$ :  $0.19 \text{ MBq l}^{-1}$  [ $0.2 \text{ nM ATP}$ ];  $\blacksquare$ :  $0.42 \text{ MBq l}^{-1}$  [ $0.3 \text{ nM ATP}$ ];  $\bullet$ :  $1.06 \text{ MBq l}^{-1}$  [ $0.9 \text{ nM ATP}$ ];  $\square$ :  $2.1 \text{ MBq l}^{-1}$  [ $1.8 \text{ nM ATP}$ ];  $\circ$ :  $4.4 \text{ MBq l}^{-1}$  [ $3.6 \text{ nM ATP}$ ]). Error bars at  $\sim 10 \text{ h}$  are  $\pm 1 \text{ SD}$  ( $n = 3$ ).

turnover times of the bulk DOP of 40 to 300 d. These turnover rates are consistent with those recently reported by Benitez-Nelson & Karl (2002). In comparison to the ATP flux rates presented herein, 7 to 33% (mean  $28 \pm 18\%$ ) of the net DOP production could be attributed to net D-ATP production. Furthermore, while the standing stock of D-ATP is only a small fraction of the DOP pool ( $\sim 0.2\%$  in terms of P, Table 1), the flux and turnover rates of D-ATP imply that 0.13 to  $0.6 \text{ nM P d}^{-1}$  could be delivered to the SRP pool. This would be equivalent to  $\sim 1\%$  of the standing stock of SRP and up to 10% of the SRP uptake flux. The P flux through D-ATP can thus be up to 5-fold faster than for bulk DOP compounds, indicating a potentially important role of nucleotides in the P dynamics of the oligotrophic ocean.

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