

- Ghadiri, M. R., Granja, J. R., Milligan, R. A., McRee, D. E. & Khazanovich, N. Self-assembling organic nanotubes based on a cyclic peptide architecture. *Nature* **366**, 324–327 (1993).
- Conn, M. M. & Rebek, J. Jr Self-assembling capsules. *Chem. Rev.* **97**, 1647–1668 (1997).
- Geib, S. J., Vincent, C., Fan, E. & Hamilton, A. D. A self-assembling, hydrogen-bonded helix. *Angew. Chem. Int. Edn Engl.* **32**, 119–121 (1993).
- Hanessian, S., Gontsyan, A., Simard, M. & Roelens, S. Molecular recognition and self-assembly by “weak” hydrogen bonding: unprecedented supramolecular helicate structures from diamine/diol motifs. *J. Am. Chem. Soc.* **116**, 4495–4496 (1994).
- Saurez, M., Branda, N., Lehn, J.-M., De Cian, A. & Fischer, J. Supramolecular chirality: chiral hydrogen-bonded supermolecules from achiral molecular components. *Helv. Chim. Acta* **81**, 1–13 (1998).
- Atwood, J. L. & MacGillivray, L. R. A chiral spherical molecular assembly held together by 60 hydrogen bonds. *Nature* **389**, 469–472 (1997).
- Rowan, A. E. & Nolte, R. J. M. Helical molecular programming. *Angew. Chem. Int. Edn Engl.* **37**, 63–68 (1998).
- Palmans, A. R. A., Vekemans, J. A. J. M., Havinga, E. E. & Meijer, E. W. Sergeants-and-soldiers principle in chiral columnar stacks of disc-shaped molecules with  $C_3$  symmetry. *Angew. Chem. Int. Edn Engl.* **36**, 2648–2651 (1997).
- Zarges, W., Hall, J., Lehn, J.-M. & Bolm, C. Helicity induction in helicate self-organisation from chiral tris(bipyridine) ligand strands. *Helv. Chim. Acta* **74**, 1843–1852 (1991).
- Woods, C. R., Benaglia, M., Cozzi, F. & Siegel, J. S. Enantioselective synthesis of copper(I) bipyridine based helicates by chiral templating of secondary structure: transmission of stereochemistry on the nanometer scale. *Angew. Chem. Int. Edn Engl.* **35**, 1830–1833 (1996).
- Mamula, O., Von Zelewsky, A. & Bernardinelli, G. Completely stereospecific self-assembly of a circular helicate. *Angew. Chem. Int. Edn Engl.* **37**, 289–293 (1998).
- Castellano, R. K., Kim, B. H. & Rebek, J. Jr Chiral capsules: asymmetric binding in calixarene-based dimers. *J. Am. Chem. Soc.* **119**, 12671–12672 (1997).
- Qiao, S., Choi, I. S. & Whitesides, G. M. Observation of diastereomers of the hydrogen-bonded aggregate  $\text{Hub}(\text{M})_3\text{CA}$  using  $^1\text{H}$  NMR spectroscopy when CA is an optically-active isocyanuric acid. *J. Org. Chem.* **62**, 2619–2621 (1997).
- Rivera, J. M., Martin, T. & Rebek, J. Jr Chiral spaces: dissymmetric capsules through self-assembly. *Science* **279**, 1021–1023 (1998).
- Vreekamp, R. H., Van Duynhoven, J. P. M., Hubert, M., Verboom, W. & Reinhoudt, D. N. Molecular boxes based on calix[4]arene double rosettes. *Angew. Chem. Int. Edn Engl.* **35**, 1215–1218 (1996).
- Timmerman, P. et al. Noncovalent assembly of functional groups on calix[4]arene molecular boxes. *Chem. Eur. J.* **3**, 1823–1832 (1997).
- Crego Calama, M., Fokkens, R., Nibbering, N. M. M., Timmerman, P. & Reinhoudt, D. N. Libraries of non-covalent hydrogen bonded assemblies; combinatorial synthesis of supramolecular systems. *Chem. Commun.* 1021–1022 (1998).
- Green, M. M. et al. A helical polymer with a cooperative response to chiral information. *Science* **268**, 1860–1866 (1995).
- Russell, K. C., Lehn, J.-M., Kyriakos, N., DeCian, A. & Fischer, J. Self-assembly of hydrogen-bonded supramolecular strands from complementary melamine and barbiturate components with chiral selection. *New J. Chem.* **22**, 123–128 (1998).
- Gulik-Krzywicki, T., Fouquey, C. & Lehn, J.-M. Electron microscopic study of supramolecular liquid crystalline polymers formed by molecular recognition-directed self-assembly from complementary chiral components. *Proc. Natl Acad. Sci. USA* **90**, 163–167 (1993).
- Prelog, V. & Helmchen, G. Basis of the CIP system and proposal for a revision. *Angew. Chem. Int. Edn Engl.* **21**, 567–594 (1982).
- Jolliffe, K. A. et al. Characterization of supramolecular hydrogen-bonded assemblies by MALDI-TOF mass spectrometry after  $\text{Ag}^+$ -labelling. *Angew. Chem. Int. Edn Engl.* **37**, 1294–1297 (1998).

Supplementary information is available on Nature's World-Wide Web site (<http://www.nature.com>) or as paper copy from the London editorial office of Nature.

**Acknowledgements.** We thank R. Hulst for the 2D ROESY experiments, R. Fokkens and N. M. M. Nibbering for the MALDI-TOF measurements, and E. W. Meijer for providing the use of the CD equipment. This work was supported by the Council for Chemical Sciences (CW) and the Technology Foundation (STW) of the Netherlands Organization for Scientific Research (NWO).

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## Variability of inorganic and organic phosphorus turnover rates in the coastal ocean

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Phosphorus is an essential nutrient in pelagic marine ecosystems. Phosphorus cycling in the upper ocean is, however, poorly understood, and few studies have directly investigated the biological utilization of this essential element<sup>1–4</sup>. Here, we have determined *in situ* phosphorus-turnover rates in a coastal marine environment by measuring the activities of two cosmogenic radionuclides ( $^{32}\text{P}$  and  $^{33}\text{P}$ , with half lives of 14.3 and 25.3 days, respectively) in dissolved inorganic, dissolved organic and total particulate phosphorus pools over a seasonal cycle. Phosphorus turnover rates within dissolved and particulate pools are rapid and vary over seasonal timescales, suggesting that low phosphorus concentrations can support relatively high primary production. Furthermore, picoplankton, such as bacteria, appear preferentially to

utilize certain dissolved organic phosphorus compounds to obtain other associated nutrients, such as carbon and nitrogen. It seems that the significance of the roles of both dissolved inorganic and organic phosphorus in supporting primary production—and, hence,  $\text{CO}_2$  uptake and particulate organic carbon export—has been hitherto underestimated.

The radionuclides  $^{32}\text{P}$  and  $^{33}\text{P}$  are produced primarily by cosmic ray interactions with atmospheric argon and enter the oceans predominantly in rain<sup>5–7</sup>. If the ratio of  $^{33}\text{P}/^{32}\text{P}$  in rain is known, then one can determine the relative ‘age’ of cosmogenic phosphorus, P, by measuring the  $^{33}\text{P}/^{32}\text{P}$  ratio in various biological pools. High  $^{33}\text{P}/^{32}\text{P}$  ratios indicate an older P pool. The inventories of  $^{32}\text{P}$  and  $^{33}\text{P}$  in the ocean are quite low, ranging from just tens to hundreds of disintegrations per minute per square metre ( $\text{d.p.m.m}^{-2}$ )<sup>1,8–10</sup>. Thus,  $^{32}\text{P}$  and  $^{33}\text{P}$  measurements require several thousand litres of sea water and extensive purification from other  $\beta$ -emitters. Previous investigations which sought to utilize these isotopes were hampered by a lack of known input fluxes, possible contamination and the inability to measure the low-energy  $\beta$ -emitter  $^{33}\text{P}$ , especially in coastal environments with high P concentrations<sup>1,8–10</sup>. This study is, to our knowledge, the first to constrain the  $^{32}\text{P}$  and  $^{33}\text{P}$  input flux<sup>6</sup> and to simultaneously measure both these isotopes in various dissolved inorganic, organic and particulate pools.

Sampling was conducted in Wilkinson basin in the Gulf of Maine ( $42^\circ 29.41' \text{N}$ ,  $69^\circ 45.02' \text{W}$ ) during four cruises in March, April, July and August 1997. This highly productive region supports one of the largest fisheries in North America<sup>11</sup>. Surface and deep particulate and total dissolved phosphorus (TDP) samples were collected by passing 4,000–6,000 l of sea water sequentially through a series of 10, 1.0 and 0.2  $\mu\text{m}$  cartridge prefilters followed by cartridges packed with iron-impregnated polypropylene filters. These filters have been demonstrated to collect TDP with close to 100% efficiency<sup>7</sup>. Separate surface samples were collected for soluble reactive phosphorus (SRP), as defined by the molybdenum blue method<sup>12</sup>, using acrilan filters and the technique developed by Lee *et al.*<sup>9</sup>. Plankton tows (nominally  $>102 \mu\text{m}$ ) were collected from various depths and sieved through a 335- $\mu\text{m}$  screen to collect different size classes. All samples were extensively purified to remove all other  $\beta$ -emitting radionuclides and counted using low-level liquid scintillation<sup>6</sup>. In March and April, deep-water P samples were taken just above the base of the mixed layer (defined by a change in density  $\geq 0.125 \text{ kg m}^{-3}$ ), whereas in July and August, deep-water samples were taken below both the mixed layer and the deep chlorophyll maximum.

The ratio of  $^{33}\text{P}/^{32}\text{P}$  measured in rain at Portsmouth, NH and Woods Hole, MA was flux weighted over a  $35 \pm 3$ -day period before the April, July and August cruises and over a 14-day period before the March cruise.  $^{33}\text{P}/^{32}\text{P}$  ratios averaged  $0.82 \pm 0.07$  (Fig. 1, Table 1). Thus, any ratio higher than this value must be due to radioactive decay. A non-continuous model can be used to determine the relative age of phosphorus in any particular reservoir:  $\tau_p = [\ln(R_p/R_s)]/(\lambda_{32} - \lambda_{33})$  where  $\tau_p$  is the age of phosphorus in the product material,  $R_p$  and  $R_s$  are the  $^{33}\text{P}/^{32}\text{P}$  ratio found in the product and source material, respectively, and  $\lambda_{32}$  and  $\lambda_{33}$  are the radioactive decay constants<sup>5</sup>. Using this model, phosphorus ages are resolved on timescales ranging from 1 to 100 days. In general, age estimate errors will increase with increasing  $^{33}\text{P}/^{32}\text{P}$  ratios as P activities decrease over time.

In March, April and July, ratios of  $^{33}\text{P}/^{32}\text{P}$  in particulate matter were similar between surface and deep waters, indicating rapid transport of sinking particulate material from the euphotic zone to depth (Fig. 1). In August, the activities in all particulate samples retrieved from deep waters were below detection, indicating that the source of sinking particulates had decreased.  $^{33}\text{P}/^{32}\text{P}$  ratios in total dissolved and small particulate ( $<102 \mu\text{m}$ ) surface pools measured during March, April and August were similar to those found in rain.

**Table 1**  $^{32}\text{P}$  and  $^{33}\text{P}$  inventories from rain and seawater measurements

Sample	Inventory expected from measured rain input (d.p.m. m <sup>-2</sup> )				Measured $^{32}\text{P}$ † seawater inventory (d.p.m. m <sup>-2</sup> )	Measured $^{33}\text{P}$ † seawater inventory (d.p.m. m <sup>-2</sup> )
	$^{32}\text{P}$		$^{33}\text{P}$			
	NH*	MA†	NH*	MA†		
March	73	168	98	236	204	206
April	72	272	98	435	157	142
July	122	54	148	84	89	89
August	133	66	196	129	59	67

\* NH represents Portsmouth, New Hampshire (43°04' N, 70°42' W).

† MA represents Woods Hole, Massachusetts (41°32' N, 70°39' W).

‡ Over upper 50 m at Wilkinson basin.

This indicates rapid P turnover rates on timescales of less than a day to just over a week (Figs 1 and 2a, b, and Supplementary Information). In July, however, the age of TDP increased to two weeks (Fig. 2a).

In July and August, the separation of TDP and SRP allowed for the estimation of the activity of both  $^{32}\text{P}$  and  $^{33}\text{P}$  in dissolved organic phosphorus DOP (= TDP - SRP). In contrast to previous investigations<sup>1,5,7-9</sup>, these results demonstrate the *in situ* temporal variability in the  $^{32}\text{P}$  and  $^{33}\text{P}$  activity and the P residence time of DOP. Surface DOP concentrations increased from a low of 0.04  $\mu\text{M}$  in April to 0.20  $\mu\text{M}$  in July, whereas SRP concentrations decreased from 0.56 to 0.26  $\mu\text{M}$ . In July, the relative age of the DOP pool was 28 days and the activity of  $^{32}\text{P}$  and  $^{33}\text{P}$  in the DOP pool was close to 50% of the measured  $^{32}\text{P}$  and  $^{33}\text{P}$  TDP activity (Fig. 2a). In contrast, the relative fraction of  $^{32}\text{P}$  and  $^{33}\text{P}$  activity in the August DOP pool was over a factor of two lower, whereas DOP concentrations decreased by less than 5%. The measured decrease in August DOP activity, however, could not be accounted for by radioactive decay alone. This implies that there was preferential remineralization of a small fraction of DOP compounds that were younger than the bulk DOP pool. The remaining DOP fraction must cycle over timescales longer than 100 days.

Concurrent with the decrease in DOP activity was an increase by a factor of five in both  $^{32}\text{P}$  and  $^{33}\text{P}$  activities within the picoplankton (0.2–1.0  $\mu\text{m}$ ) size class. Assuming that all the P uptake of the 0.2–1.0- $\mu\text{m}$ -size class was derived from the TDP pool, a picoplankton P turnover rate of approximately two days can be determined for August (Fig. 2b). This is comparable to a bacterial P residence time of several hours to several days found by previous investigations using incubation techniques<sup>2,3</sup>.

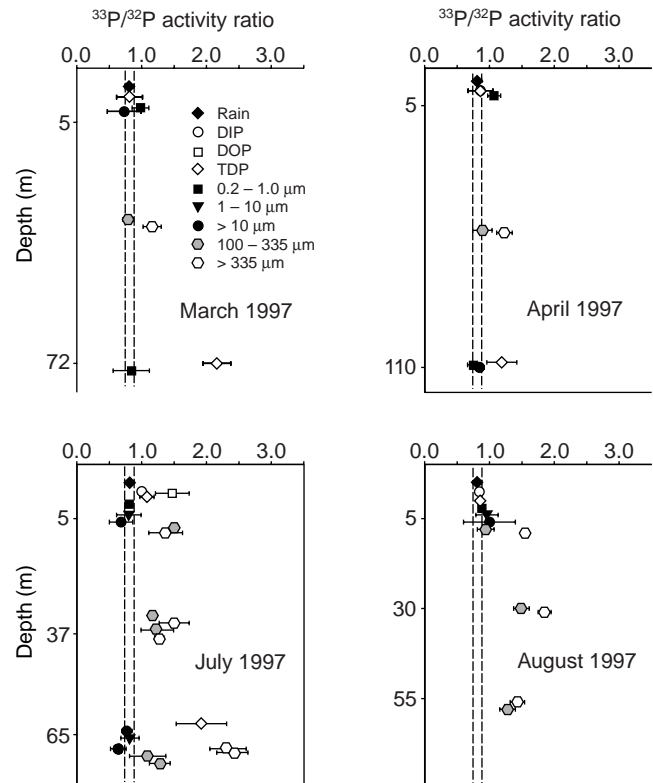
The relatively high  $^{32}\text{P}$  and  $^{33}\text{P}$  activities and low  $^{33}\text{P}/^{32}\text{P}$  ratio found within the August picoplankton (0.2–1.0  $\mu\text{m}$ ) size class also suggests that these organisms were consuming SRP and possibly small particulate P (1.0–10  $\mu\text{m}$  and 10–102  $\mu\text{m}$ ). Although low in  $^{32}\text{P}$  and  $^{33}\text{P}$  activity, rapid remineralization of the 0.1–10- $\mu\text{m}$  and 10–102- $\mu\text{m}$  P pools could support a significant fraction of the bacterial P uptake. Whereas previous studies have shown SRP and particulate P uptake in incubation experiments<sup>13–15</sup>, our results demonstrate the rapidity of these processes in the water column.

During uptake, P is incorporated into a wide variety of biological components. However, the rate of incorporation and the distribution of this uptake will vary depending on the environment and growth cycle of the organism. Preferential remineralization of DOP compounds with relatively high  $^{32}\text{P}$  and  $^{33}\text{P}$  activities suggest that these compounds were more bioavailable than the bulk DOP pool. Previous research has indicated that the proportion of phosphonates within the high molecular weight (1–100 nm) fraction of DOP was significantly higher than that found in natural assemblages of plankton<sup>16</sup>. Thus, it was hypothesized that the remineralization of other DOP compounds was preferred. Several studies have found that bacteria can readily hydrolyse organic phosphorus compounds, such as monophosphate esters and nucleotides, through enzymatic processes<sup>4,17–19</sup>. Our results suggest that bacteria are rapidly remineralizing these compound classes. If correct, our results also

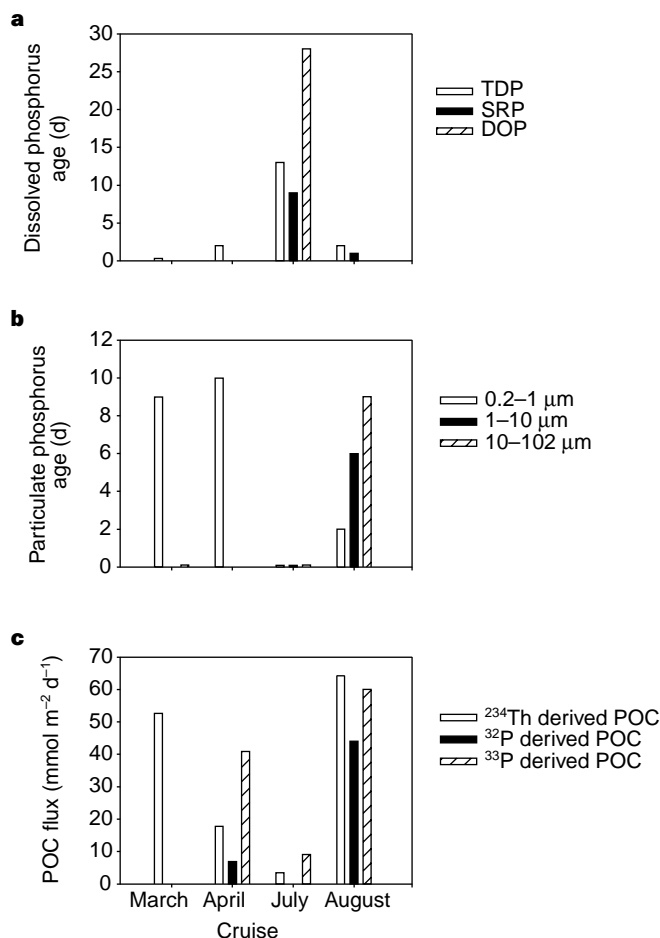
imply that phytoplankton rapidly incorporate P from outside the cell into nucleotides and monophosphate esters. These compounds are then released through grazing or virolytic processes, thereby providing a mechanism in which more bioavailable DOP compounds can contain higher  $^{32}\text{P}$  and  $^{33}\text{P}$  activities than the bulk DOP pool. Regardless, these results provide direct evidence that there is a more bioavailable pool of DOP.

The observation that SRP is also being consumed indicates that the picoplankton-size organisms must be expending energy in the hydrolysis of DOP for reasons other than P limitation. Dissolved organic carbon concentrations in surface waters decreased from 78.2  $\mu\text{M}$  in July to 64.5  $\mu\text{M}$  in August, while combined nitrate and nitrite levels averaged 0.1  $\mu\text{M}$ . Thus, we hypothesize that bacteria and picoplankton were hydrolysing specific DOP compounds for their carbon (C) and possibly nitrogen (N) content, presumably as structural biosynthetic precursors.

These results have several important implications concerning the P cycle in the open ocean. Our results demonstrate that the



**Figure 1**  $^{33}\text{P}/^{32}\text{P}$  ratios in rain and sea water. Seawater samples were obtained during March, April, July and August 1997 cruises in the Gulf of Maine. With the exception of the March and April plankton tows, samples were taken at the specific depth listed along the y-axis. Vertical separation is for clarity. In March and April, plankton tows were integrated over the depth of the mixed layer and are shown at the midpoint of depth collection.



**Figure 2** Phosphorus ages and particulate organic carbon (POC) fluxes. **a**, Dissolved phosphorus ages. Separate SRP samples were not taken during the March and April cruises. In August, a DOP age could not be determined owing to the large errors associated with the P activity measurements. **b**, Small particulate phosphorus ages. Bars not shown had P activities below detection. In April, the 0.2–1.0 μm and 1.0–10 μm fractions were combined before measurement. **c**, Radionuclide-derived POC export. Methods for the <sup>32</sup>P and <sup>33</sup>P derived POC fluxes are described in the text. POC fluxes derived from <sup>234</sup>Th were taken from the same cruises according to the methods described by Buesseler<sup>22</sup>.

residence times of SRP and DOP are distinct from one another and vary seasonally, suggesting that these two pools play distinct roles in biological production. Even at low concentrations, rapid turnover of SRP can support a much greater fraction of primary production than previously thought. This has significant implications for those studies that assume P limitation based only on P concentration (relative to N). In addition, bacteria and picoplankton can obtain not only additional P, but also C and N through the breakdown of DOP. The bioavailable DOP pool, however, represents a small fraction of bulk DOP within coastal environments. Nonetheless, picoplankton in the presence of DOP may substantially increase primary production in the open ocean.

In contrast to the 0.2–1.0 μm size class, <sup>32</sup>P and <sup>33</sup>P activities in the 1–10 μm and 10–102 μm size fractions had decreased by more than 50% between July and August, and the average residence time increased from less than two days to just over a week (Fig. 2b). This implies that both autotrophic and heterotrophic P consumption had dropped. Given the available SRP concentrations, a decrease in phytoplankton activity suggests limitation by trace metals and/or other nutrients. The reduction in heterotrophic activity, however, indicates that microzooplankton, such as ciliates, were inefficient grazers of picoplankton. This is in direct contrast to laboratory

studies which have found that microflagellate grazing plays a dominant role in both trace-metal and nutrient recycling under oligotrophic conditions<sup>20,21</sup>. Furthermore, although picoplankton can increase primary production, an increase in secondary production and particulate export does not always result.

Particulate export fluxes over the upper 50 m were determined for <sup>32</sup>P and <sup>33</sup>P assuming steady state and negligible advection and diffusion, as well as considering the difference between the average rain flux<sup>6</sup> at Woods Hole (Massachusetts) and Portsmouth (New Hampshire) and the measured seawater inventory (Table 1). Although <sup>33</sup>P/<sup>32</sup>P ratios did not vary significantly between the two sites, fluxes varied by as much as a factor of four. These large flux variations are most likely to be due to differences in the source of the scavenged air mass<sup>7</sup>. Because rainfall was not measured directly at our seawater sampling site, an average value between the two rain stations was used to determine the input of <sup>32</sup>P and <sup>33</sup>P. In March, the Portsmouth data were limited and suffered from rain-gauge inaccuracies associated with large snowfall<sup>7</sup>. As a result, <sup>32</sup>P and <sup>33</sup>P export fluxes could not be determined for the March cruise.

The flux of particulate organic carbon (POC) was found by multiplying the calculated radionuclide fluxes by the ratio of <sup>32</sup>P (and <sup>33</sup>P) to carbon on particulates (≥1 μm). Using an average <sup>32</sup>P and <sup>33</sup>P rain input gave POC fluxes which agreed well with those determined using the more commonly utilized<sup>22</sup> radioisotope POC flux tracer, <sup>234</sup>Th (Fig. 2c). Using an annual Gulf of Maine primary production estimate of 66.2 mmol C m<sup>-2</sup> d<sup>-1</sup> (from 1978–1980)<sup>11</sup> allows the calculation of an average ‘export’ ratio of 0.52. Given the low activities of <sup>32</sup>P and <sup>33</sup>P observed in the 1–10 μm and 10–102 μm size fractions, it is likely that the large increase in carbon export observed during the late summer is the result of zooplankton growth in July and mortality in August (Fig. 2c). This further indicates a lag period between changes in community structure and organic carbon export.

<sup>32</sup>P and <sup>33</sup>P activities within the larger microplanktonic pools (>102 μm) were an order of magnitude lower than those found in the smaller particles (<102 μm). In March and April, both the 102–335 μm and the >335-μm size classes were dominated by phytoplankton and had turnover rates which ranged from a day to as long as two weeks. In July and August, residence times of particles in the 102–335-μm class were longer, ranging from two to four weeks (mixed assemblages of copepods and dinoflagellates). The >335-μm size class, however, had residence times as long as seven weeks (dominated by mature copepods). These P residence time estimates are similar to the 19 days found by laboratory studies which fed copepods with <sup>32</sup>P-labelled phytoplankton<sup>23</sup> and to the 30–80 day range found in plankton tows using *in situ* measurements of <sup>32</sup>P and <sup>33</sup>P at Bermuda and off the coast of Southern California<sup>1,8,10</sup>. However, our study is one of the first to show a seasonal relationship between P ages and trophic level. Our results provide additional evidence that increasing primary production within the Gulf of Maine does not immediately result in an increase in macrozooplankton P uptake.

The measurement of <sup>32</sup>P and <sup>33</sup>P within dissolved and particulate pools has provided much-needed insight into the temporal variability of P cycling in the upper ocean. Further measurements of these isotopes, especially in regimes that appear to be P limited, will help to provide new constraints on models that seek to emulate primary production and particulate export. It is already clear that <sup>32</sup>P and <sup>33</sup>P have the unique ability to pinpoint the most biologically ‘active’ pools of dissolved inorganic, organic and particulate P pools. □

Received 5 June 1998; accepted 2 February 1999.

- Lal, D. & Lee, T. Cosmogenic <sup>32</sup>P and <sup>33</sup>P as tracers to study phosphorus recycling in the upper ocean. *Nature* **333**, 752–754 (1988).
- Orrett, K. & Karl, D. M. Dissolved organic phosphorus production and turnover in surface waters. *Limnol. Oceanogr.* **32**, 383–395 (1987).
- Cotner, J. B., Ammerman, J. W., Peele, E. R. & Bentzen, E. Phosphorus limited bacterioplankton growth in the Sargasso Sea. *Aquat. Microb. Ecol.* **13**, 141–149 (1997).

4. Karl, D. M. & Yanagi, K. Partial characterization of the dissolved organic phosphorus pool in the oligotrophic North Pacific Ocean. *Limnol. Oceanogr.* **42**, 1398–1405 (1997).
5. Waser, N. A. D., Bacon, M. P. & Michaels, A. P. Natural activities of  $^{32}\text{P}$  and  $^{33}\text{P}$  and the  $^{32}\text{P}/^{33}\text{P}$  ratio in suspended particulate matter and plankton in the Sargasso Sea. *Deep-Sea Res. II* **43**, 421–436 (1996).
6. Benitez-Nelson, C. R. & Buesseler, K. O. Measurement of cosmogenic  $^{32}\text{P}$  and  $^{33}\text{P}$  activities in rainwater and seawater. *Anal. Chem.* **70**, 64–72 (1998).
7. Benitez-Nelson, C. R. & Buesseler, K. O.  $^{32}\text{P}$ ,  $^{33}\text{P}$ ,  $^7\text{Be}$ , and  $^{210}\text{Pb}$ : Atmospheric fluxes and utility in tracing stratosphere troposphere exchange. *J. Geophys. Res.* (in the press).
8. Lee, T., Barg, E. & Lal, D. Studies of vertical mixing in the Southern California Bight with cosmogenic radionuclides  $^{32}\text{P}$  and  $^7\text{Be}$ . *Limnol. Oceanogr.* **36**, 1044–1053 (1991).
9. Lee, T., Barg, E. & Lal, D. Techniques for extraction of dissolved inorganic and organic phosphorus from large volumes of seawater. *Anal. Chim. Acta* **260**, 113–121 (1992).
10. Waser, N. A. D. & Bacon, M. P. Wet deposition fluxes of cosmogenic  $^{32}\text{P}$  and  $^{33}\text{P}$  and variations in the  $^{32}\text{P}/^{33}\text{P}$  ratios at Bermuda. *Earth Planet. Sci. Lett.* **133**, 71–80 (1995).
11. O'Reilly, J. E. & Busch, D. A. Phytoplankton primary production on the northwestern Atlantic Shelf. *Rapp. P.-v. Reun. Cons. Int. Explor. Mer.* **183**, 255–268 (1984).
12. Koroleff, F. in *Methods of Seawater Analysis* 2nd edn (eds Grasshoff, K., Ehrherd, M. & Kremling, K.) 125–135 (Verlag Chemie, Weinheim, 1983).
13. Currie, D. J. & Kalfif, J. The relative importance of bacterioplankton and phytoplankton in phosphorus uptake in freshwater. *Limnol. Oceanogr.* **29**, 311–321 (1984).
14. Wheeler, P. A. & Kirchman, D. Utilization of inorganic and organic nitrogen by bacteria in marine systems. *Limnol. Oceanogr.* **31**, 998–1009 (1986).
15. Cho, B. C. & Azam, F. Major role of bacteria in biogeochemical fluxes in the ocean's interior. *Nature* **332**, 441–443 (1988).
16. Clark, L. L., Ingall, E. D. & Benner, R. Marine phosphorus is selectively remineralized. *Nature* **393**, 426 (1998).
17. Ammerman, J. W. & Azam, F. Bacterial 5'-nucleotidase in aquatic ecosystems: A novel mechanism for phosphorus regeneration. *Science* **227**, 1338–1340 (1985).
18. Ammerman, J. W. in *Microbial Enzymes in Aquatic Environments* (ed. Chost, R. J.) 165–186 (Springer, New York, 1991).
19. Bjorkman, K. & Karl, D. M. Bioavailability of inorganic and organic phosphorus compounds to natural assemblages of microorganisms in Hawaiian coastal waters. *Mar. Ecol. Prog. Ser.* **111**, 265–273 (1994).
20. Anderson, O. K., Goldman, J. C., Caron, D. A. & Dennett, M. R. Nutrient cycling in a microflagellate food chain: III. Phosphorus dynamics. *Mar. Ecol. Prog. Ser.* **31**, 46–55 (1986).
21. Barbeau, K., Moffett, J. W., Caron, D. A., Croot, P. L. & Erdner, D. L. Role of protozoan grazing in relieving iron limitation of phytoplankton. *Nature* **380**, 61–64 (1996).
22. Buesseler, K. O. The decoupling of production and particle export in the surface ocean. *Glob. Biogeochem. Cycles* **12**, 297–310 (1998).
23. Marshall, S. M., Conover, R. J. & Orr, A. P. On the biology of *Calanus finmarchicus*. XII. The phosphorus cycle: excretion, egg production, and autolysis. The turnover of phosphorus by *Calanus finmarchicus*. *J. Mar. Biol. Assoc. UK* **41**, 463–488 (1961).

Supplementary information is available on Nature's World-Wide Web site (<http://www.nature.com>) or as paper copy from the London editorial office of Nature.

**Acknowledgements.** We wish to thank G. Crossin, J. Andrews, L. Ball and C. Tarr for help in sample preparation, collection and purification. We also thank the crew of the RV *Cape Hatteras*. The manuscript was greatly improved with the help of E. Ingall. This work was supported by NSF, EPA STAR Fellowship Program, and WHOI unrestricted funds.

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## Large-scale impoverishment of Amazonian forests by logging and fire

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Amazonian deforestation rates are used to determine human effects on the global carbon cycle<sup>1–3</sup> and to measure Brazil's progress in curbing forest impoverishment<sup>1,4,5</sup>. But this widely used measure of tropical land use tells only part of the story. Here we present field surveys of wood mills and forest burning across

Brazilian Amazonia which show that logging crews severely damage 10,000 to 15,000 km<sup>2</sup> yr<sup>-1</sup> of forest that are not included in deforestation mapping programmes. Moreover, we find that surface fires burn additional large areas of standing forest, the destruction of which is normally not documented. Forest impoverishment due to such fires may increase dramatically when severe droughts provoke forest leaf-shedding and greater flammability; our regional water-balance model indicates that an estimated 270,000 km<sup>2</sup> of forest became vulnerable to fire in the 1998 dry season. Overall, we find that present estimates of annual deforestation for Brazilian Amazonia capture less than half of the forest area that is impoverished each year, and even less during years of severe drought. Both logging and fire increase forest vulnerability to future burning<sup>6,7</sup> and release forest carbon stocks to the atmosphere, potentially doubling net carbon emissions from regional land-use during severe El Niño episodes. If this forest impoverishment is to be controlled, then logging activities need to be restricted or replaced with low-impact timber harvest techniques, and more effective strategies to prevent accidental forest fires need to be implemented.

Human uses of tropical forests vary greatly in their ecological impacts. Ranchers and farmers 'deforest' land in preparation for cattle pasture and crops by clear-cutting and burning patches of forest. Loggers do not clear-cut and burn, but perforate forests by harvesting or damaging many trees. Rubber tapping and similar activities use the forest at very low intensity through the harvest of animals, fruits, latex and other "non-timber products"<sup>8–11</sup>. Deforestation by ranchers and farmers has a greater effect on forest carbon content, forest hydrology, and the diversity of native plant and animal species than other forest uses<sup>9–14</sup> and has become the main parameter by which human effects on tropical forests are measured. Part of the appeal of this forest versus non-forest approach to assessing human effects on tropical forests is its tractability. Forest conversion to agriculture is readily monitored from space using imagery from the Landsat Thematic Mapper (TM) satellites, permitting the development of deforestation maps of large regions at a reasonable cost and speed<sup>4,5</sup>.

This binary approach to the analysis of human effects on tropical forests neglects those forest alterations that reduce tree cover, but do not eliminate it, such as logging and surface fires in standing forests. The forest openings created by logging and accidental surface fires are visible in Landsat TM images, but they are covered over by regrowing vegetation within 1 to 5 years, and are easily misclassified in the absence of accompanying field data<sup>15</sup>. Although logging and forest surface fires usually do not kill all trees, they severely damage forests. Logging companies in Amazonia kill or damage 10–40% of the living biomass of forests through the harvest process<sup>9,10,16</sup>. Logging also increases forest flammability by reducing forest leaf canopy coverage by 14–50%<sup>7,9,10,16,17</sup>, allowing sunlight to penetrate to the forest floor, where it dries out the organic debris created by the logging. Fires ignited on agricultural lands can penetrate logged forests<sup>7,17,18</sup>, killing 10–80% of the living biomass<sup>6,17</sup> and greatly increasing the vulnerability of these forests to future burning<sup>6,19</sup>. Fires from agricultural lands can also penetrate those undisturbed forests that have lost portions of their leaf canopies because of severe seasonal drought<sup>19</sup>.

We estimated the area of Brazilian Amazonian forest that is impoverished each year through logging by interviewing 1,393 wood mill operators, representing more than half of the mills located in 75 logging centres (Table 1); these logging centres are responsible for >90% of Amazonian timber production. In each interview, we obtained the mill's harvest records of roundwood (tree trunks) for 1996 and 1997 and the harvest rate (m<sup>3</sup> of timber per ha of forest), thereby calculating the forest area required to supply each centre's timber production. The accuracy of the roundwood harvest rates reported by mill operators was tested by comparing these interview data with direct measurements of roundwood harvest in