C-MORE 2013: The Hawaii Ocean Experiment – Phosphorus Rally (HOE-PhoR)

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Summary: This summer, C-MORE will conduct a series of experiments to observe and interpret the fundamental role of phosphorus (P) in the sea, using Station ALOHA as the open ocean benchmark. While there is a stated emphasis on the element P, the inextricable metabolic and biogeochemical links to C, N, S, O, H and many trace elements implies that the HOE-PhoR mission is a broad, multi-element, trans-disciplinary opportunity that should appeal to all C-MORE scientists whether you have a love affair with P, or not. Indeed the use of the term "rally" to describe this 2-cruise expedition is wholly appropriate: ***to summon or bring together for a common purpose*.** Scientific collaborations and partnerships, in this case through common sampling and shared experiments to test fundamental hypotheses, is the primary goal of HOE-PhoR. These coordinated activities will assess a range of scales from genes and genomes to populations and ecosystems, and will build on the fundamental understanding that has been achieved during the ongoing, 25-year Hawaii Ocean Time-series (HOT) program; indeed the efforts during HOE-PhoR will contribute to the year-long HOT silver anniversary and will also benefit from ongoing achievements of the successful HOE-DYLAN (2012) project. We seek volunteers to serve as Chief Scientists and Junior Chief Scientists for these important cruises, and look forward to your input, ideas, discussion, and especially your participation. The expedition acronym, HOE-PhoR, has real meaning and significance: HOE is Hawaiian for ***get to work, do your share, paddle a canoe,*** and PhoR is the gene designation for the histidine kinase, the enzyme that initiates the phosphorus starvation inducible response (also known as the Pho regulon) leading to many fundamental genetic and physiological changes in microorganisms.

Background: Cellular P metabolism is complex; P biogeochemistry and microbial ecology even more so. Tom Brock, a pioneer in aquatic microbial ecology, once pronounced that ecology was "physiology under the worst possible conditions," and this is still an accurate assessment. In November 2008, just as C-MORE entered its 3rd year, an important meeting was held at MIT to think broadly about C-MORE's mission and how we can build meaningful scientific partnerships. This ***C-MORE Futures Meeting*** (also known as the Kendall Conference) was organized by Dan Repeta and Ed DeLong, and included a series of presentations of on-going and future research activities followed by lively, far-ranging discussions. Four themes emerged: (1) **Phosphorus dynamics**, (2) Photoheterotrophy, (3) Model microbes, and (4) Diversity and time-series. A 21-page report and series of recommendations were prepared to promote future discussion and to facilitate Center collaboration. HOE-PhoR is being proposed, in part, to move forward on the Phosphorus dynamics recommendation. As stated in the report, "A large number of C-MORE investigators have research interests in P cycling, placing C-MORE in a unique position to foster collaborations directed at several outstanding issues in the P-cycle." However, as Riff Raff (Rocky Horror Picture Show) would say, *time is fleeting*, and this may be one of the last opportunities for C-MORE to sponsor a coordinated study of the marine microbial P-cycle.

Phosphorus is an essential element for life; indeed the entire biosphere is built around P. Phosphorus is important for cell structure (phospholipids), for the storage and expression of hereditary information (nucleic acids), for cellular energy transduction (nucleotides), and for

many metabolic regulatory functions. The marine environment, and especially the large anticyclonic subtropical gyres that dominate our planet, are in a state of chronic phosphate (P*i*) starvation, compared to growth conditions imposed on microorganisms during primary isolation or in most laboratory-based physiological studies. The P status of an ecosystem constrains solar energy capture, net primary production, fish production, and the potential for carbon dioxide sequestration. While selected microorganisms can partially substitute sulfur (S) for P in membrane lipids under conditions of severe P-deficiency, P can never be totally replaced.

In a now classic paper entitled "On protein synthesis," Francis Crick first hypothesized what has become known as the central dogma of biology, namely the unidirectional flow of genetic information from DNA to RNA to proteins (Crick 1958 *Symp. Soc. Exp. Biol.* XII, 139-163). He argued that the main function of proteins was to act as enzymes for the catalysis of nearly all cellular reactions, and that the main function of genetic material was to direct protein synthesis. Although there was sparse evidence at that time to support these claims, Crick concluded that "there was little point in genes doing anything else". The central dogma has been the guiding light in molecular biology for the past half-century, and during the past two decades remarkable progress has been made in our understanding of the microbial P-cycle as a consequence of novel discoveries in the areas of genomics, transcriptomics, and proteomics; many of these have been C-MORE contributions.

Pioneering research using *Escherichia coli* as a laboratory model documented an enhanced synthesis of the enzyme alkaline phosphatase (APase; encoded by the PhoA gene) upon P*i* limitation. APase is a relatively nonspecific phosphomonoesterase that allows *E. coli* to use a wide range of phosphate esters as alternate sources of P for biosynthesis. Subsequent laboratory efforts revealed that the control of APase synthesis was part of a much more complex P*i* starvation induction response that is now known to involve multiple genes and gene clusters (operons) that are all co-regulated with PhoA. This well orchestrated pattern of gene expression and synthesis of many key P-cycle proteins has been termed the Pho regulon. The knowledge gained in these laboratory studies, and others since then, can be used for ecological P-cycle hypothesis generation and testing under field conditions. While the nature of the P*i*-stress response in model marine microorganisms varies considerably, many marine microbial genomes and environmental metagenomes contain homologs to genes that are central to the *E. coli* Pho regulon. Despite significant advances in understanding over the past few decades, there are still many Pho regulated genes for which we have no assigned function, so it is likely that new P-metabolic and regulatory pathways are likely to be discovered.

Research Prospectus: There will be two separate legs during the 2013 HOE-PhoR expedition: 15-29 May (14 days) and 20 Sept – 02 Oct (12 days). Each will have the option for extensive on-deck and *in situ* experiments, including high resolution (vertical scale) free-drifting productivity and sediment trap arrays, full water column depth sampling, high volume – near surface pumps, and much more. We are looking into the feasibility of deploying large, *in situ* enclosures (big bags) for selected experiments, and the construction of several large 1 m^3 deck mesocosms (M^3 = marine microbial mesocosms) that could be run as semi-continuous cultures to investigate input nutrient ratio (N:P) control of microbial metabolism, selection, and competition, or used for other purposes. The cell-sorting flow cytometer will be available for live/real-time analyses, and other shipboard instrumentation can be brought aboard and supported. The timing

of these two legs correspond roughly to the start and to the end of Sta ALOHA summer, so there is an opportunity to investigate seasonal differences, while acknowledging the fact that Mother Nature is not always predictable. Indeed, the two decade-long downward trend in the inventory of phosphate in the surface waters at Sta. ALOHA (that has been the subject of several HOT/C-MORE publications) suddenly ended, and reversed its course during 2012 (just in time for HOE-DYLAN, of course!) and the euphotic zone phosphate inventory was restored to 1988 levels! A careful, systematic analysis of this phosphate pulse recorded during HOE-DYLAN, with conditions one year later, may be an unique scientific opportunity. In addition to these two legs, there are opportunities to join the regular monthly HOT program (4-day) cruises either before, after, or between the HOE-PhoR cruises, on a not-to-interfere/space available basis (if interested there is an online request form at: ***hahana.soest.hawaii.edu/hot/crequest/main.html***).

Our plan as it is now developing, is to host several coordinated experiments of various aspects of the marine microbial P-cycle. The design would be a team effort, and would address one or more fundamental P regulatory process, pathway, or control mechanism. Just thinking about and contributing to the design should be a blast; participation a thrill. So that we are all on the same page, I include a brief summary Table of previously published P-cycle research at Sta. ALOHA that I have compiled for a commissioned review article on the Marine Microbial P-cycle. If you see any published P-cycle activity that has been omitted from this Table, please let me know and I will add it. I have not included various modeling efforts that have employed Sta. ALOHA P-cycle observations, but once assembled I will post these materials, the draft review article, the DeLong-Repeta ***C-MORE Futures Report,*** and other related documents to the HOE-PhoR website. I hope that you will follow suit with your own recently completed or ongoing P-cycle manuscripts whether directly related to Sta. ALOHA, or not.

Sta. ALOHA: An accessible open ocean sentinel for marine microbial P-cycle research: Pioneering P-cycle research in the North Pacific Subtropical Gyre (NPSG) conducted by Mary Jane Perry in the early 1970s suggested that P*i* might control microbial growth and productivity in the surface waters. This conclusion was based on two independent lines of evidence: (1) a higher than anticipated C:P ratios in POM and (2) high biomass-normalized rates of APase activity. Subsequent P-cycle research in the NPSG conducted during the VERTEX program (Aug 1983) investigated dissolved organic P (DOP) cycling, as well as particulate organic P (POP) export and remineralization. However, an experimental assessment of the P*i*-control hypothesis was not conducted until the establishment of Sta. ALOHA in Oct 1988.

On approximately monthly intervals since 1988, HOT scientists have made systematic measurements of a suite of core parameters, including selected P pool inventories and fluxes at Sta. ALOHA. These observations, supplemented by hypothesis testing experimentation, have produced the most comprehensive microbial P-cycle data base for any oceanic ecosystem; selected results are presented in Table 1. Biogeochemical assessments at or near Sta. ALOHA range in scope from a complete 24-year (and counting) record of monthly P*i*, ATP, and POP concentrations as well as POP export from the euphotic zone, to a broad spectrum of more specialized measurements conducted for only brief portions of the total observation period, and (unfortunately) rarely at the same time. Examples include the partial chemical characterization of the DOP pool (Sep 1991 – Mar 1992), P flux estimates based on cosmogenic $^{32}P/^{33}P$ (Feb 1999 – Apr 2000), ^{32}P*i* uptake and biologically available P pool estimation (Oct 2000 – Nov

2001), and APase activity and kinetic characterization (May 2008 – Nov 2009). Other key measurements were conducted only once, and in some cases at a single depth (e.g., a global analysis of microbial community gene expression at 75 m on 9 March 2006 at 0330 hr local time). Unfortunately, because the P-cycle is not in long term steady-state at Sta. ALOHA, we are unable to integrate these independent P-cycle observations, so they should be viewed as single frames of the ongoing Sta. ALOHA motion picture. In addition to these field measurements and experiments, P-cycle data from Sta. ALOHA have been used in a number of conceptual and numerical modeling studies ranging from C-N-P remineralization stoichiometry, to the controls on and impacts of N_2 fixation, to *Trichodesmium* vertical migration and P*i*-mining, to potential consequences of artificial upwelling, to name a few examples. Finally, the establishment of novel DNA libraries and gene transcription patterns have provided unprecedented opportunities to explore and to model P-cycle mechanisms and pathways.

The emergent P-cycle data set from Sta. ALOHA is unique, robust, and rich with previously undocumented phenomena and ecological insights. Although the present ongoing ocean time-series study at Sta. ALOHA has certainly not resolved all of these important matters, it does provide an important data set to begin the next phase of hypothesis testing with numerous research opportunities. What is now needed, in our humble opinion, is an expedition focused specifically on the marine microbial P-cycle, where a complete set of measurements and experiments can be performed simultaneously. C-MORE's HOE-PhoR will build upon current knowledge to conduct the most comprehensive study of the microbial P-cycle even attempted. Please join in the adventure, it should be an exciting year.

Timetable (tentative):

1 Dec 2012 - 1 Jan 2013: circulate Version 1.0 of planning document; seek input on measurements, experiments, and hypotheses; determine level of interest within C-MORE and identify possible external collaborators; recruit expedition Chief Scientists (senior and junior); host one or more videoconferences to facilitate planning

15 Jan – 15 Feb 2013: prepare and circulate Versions x-y of planning document; host one or more videoconferences as needed; continue detailed expedition planning

15 Feb – 30 Mar 2013: Finalize expedition planning, including key leadership and support personnel; identify outstanding issues; prepare gear and materials for leg I

15-29 May 2013: HOE-PhoR leg I

Summer 2013: continue sample analysis from HOE-PhoR I, and finalize second expedition planning (may change depending on conditions observed during the first leg); host cruise related videoconferences as needed

20 Sept - 2 Oct 2013: HOE-PhoR leg II

Fall 2013 – Winter 2014: continue sample analysis and data interpretation; data exchange workshop in conjunction with 2014 Ocean Sciences mtg in Honolulu

Table 1: A chronology of marine microbial P-cycle related research at or near Sta. ALOHA in the North Pacific Subtropical Gyre (1988-present)

Sampling Date	Event and Primary Reference
Oct 1988 – present (250 cruises)	Establishment of Sta. ALOHA at 22°45'N, 158°W; monthly measurements of P*i* (0-4600 m), POP (0-1000 m), P-ATP (0-1000 m), and P-export at 125 m (*http://hahana.soest.hawaii.edu/hot/hot-dogs/interface.html*)
Aug 1989 (1 cruise)	Dissolved and particulate matter inventories and dynamics (including P*i*, DOP, POP, and ATP) during a large bloom of *Trichodesmium* (Karl et al. 1992)
Oct 1988 – Nov 1994 (59 cruises)	Development and field testing of MAGIC method for P*i* analysis (Karl and Tien 1992 and 1997)
Oct 1988 – Nov 1994 (59 cruises)	C, N, P export and solubilization length and time scale estimation (Christian et al. 1997)
Oct 1988 – Feb 2001 (123 cruises)	Water column profiles (0-1000 m) of DOP (Karl et al. 2001b; *http://hahana.soest.hawaii.edu/hot/hot-dogs/interface.html*)
Oct 1989 – Jul 1997 (17 cruises)	Comparison of P*i* estimation using standard technique, MAGIC, and modified-MAGIC methods, and determination of abyssal ocean DOP (Thomson-Bulldis and Karl 1998)
1989-1999 (104 cruises)	Multi-year variability in DOC, DON, and DOP inventories (Church et al. 2002)
May 1990 and Oct 1991	C:N:P elemental stoichiometry of isolated *Trichodesmium* colonies (Letelier and Karl 1996)
Sep 1991 – Mar 1992 (6 cruises)	Partial characterization of DOP using controlled UV light-induced photodecomposition (Karl and Yanagi 1997)
Jan-May 1997 (HALE ALOHA)	Observational data showing a strong and rapid coupling between nutrient (NO_3^- and P*i*) upwelling and microbial/biogeochemical processes (Letelier et al. 2000)
Jun 1992 – Oct 2004 (13 yrs)	Deep-sea (2,800 and 4,000 m) moored sediment trap collections for estimating POP export and remineralization rates(Karl et al. 2012)
Sep 1993 (1 cruise)	C:N:P stoichiometry of positively and negatively buoyant *Trichodesmium* colonies, and measurement of dark P*i* uptake of sinking colonies to test the "P-mining" hypothesis (Letelier and Karl 1998)
Jan 1994 – Dec 2005 (125 cruises)	Assessment of zooplankton migration as a vehicle for P export (Hannides et al. 2009)
Jul 1996 – Aug 1997 (5 cruises)	Light and dark P*i* uptake and regeneration rates, DOP production, BAP estimation, and selected DOP substrate bioavailability (Björkman et al. 2000)
Jun 1998 – Feb 1999 (6 cruises)	Measurement of dissolved and particulate ATP and GTP concentrations, and dissolved ATP metabolism (Björkman and Karl 2005)
Feb 1999 – Apr 2000 (10 cruises)	Cosmogenic ^{32}P / ^{33}P activity ratio determination, P*i* and DOP residence time estimation (Benitez-Nelson and Karl 2002)

Oct 1999 (1 cruise)	Tangential flow ultrafiltration / ^{31}P-NMR characterization of DOP and POP (Sannigrahi et al. 2006)
Nov 1999 (1 cruise)	C-N-P remineralization of particulate and dissolved organic matter (Kaiser and Benner 2012)
Jan 2000-Dec 2001 (21 cruises)	Comparison of P-dynamics and controls on N_2 fixation for samples and experiments at Sta. ALOHA to those in the Southeast Pacific Ocean BIOSOPE cruise and Southwest Pacific Ocean DIAPALIS cruise (Moutin et al. 2008)
Mar 2000 (1 cruise)	P*i*-δ^{18}O determination (Colman et al. 2005)
Mar 2000 – Nov 2001 (15 cruises)	Depth profiles (0-175 m) of light and dark ^{32}P*i* uptake (Duhamel et al. 2012)
Jul 2000 and Aug 2001 (2 cruises)	P*i* control of N_2 fixation (Zehr et al. 2007)
Oct 2000 – Nov 2001 (8 cruises)	Depth profiles (0-175 m) of ^{32}P*i* and total P uptake, BAP estimation, POP turnover time determination (Björkman and Karl 2003)
Jun 2002 – Nov 2003 (4 cruises)	Concentration-dependent P*i* uptake using ^{32}P radio-labeling and size fractionation (Björkman et al. 2012)
Oct 2002 (1 cruise)	Construction and analysis of microbial community DNA (fosmid) libraries for 7 samples from the surface (10 m) to the abyss (4,000 m) (DeLong et al. 2006)
Dec 2002 (1 cruise)	Concentration, production, and turnover of dissolved DNA (Brum 2005)
Dec 2002 (1 cruise)	Vertical profiles, production, and turnover of “virus-free” dissolved DNA concentrations (Brum 2005)
Jul 2003 (1 cruise)	Total microbial RNA synthesis rates using ^{32}P*i*, *Prochlorococcus* RNA synthesis using RIBOTRACE method, and nutrient controls of RNA synthesis (Van Mooy and Devol 2008)
Jul 2003 (1 cruise)	Measurement of S substitution for P in microbial membrane lipids and consequences of NH_4^+-amendments (Van Mooy et al. 2009)
Jun-Jul 2004 (VERTIGO cruise)	Comparison of C-N-P export using conventional surface tethered and neutrally buoyant sediment traps (Lamborg et al. 2008)
Jul and Nov 2004 (2 cruises)	Metabolic response of microbial assemblages to deep water nutrient (P*i*) enrichment (McAndrew et al. 2007)
Jul 2004 – May 2007 (10 cruises)	Temporal variability in phytoplankton response to deep water nutrient (P*i*) additions (Mahaffey et al. 2012)
Nov 2004 – Mar 2005 (3 cruises)	P*i* and Fe control and size distribution of N_2-fixation (Grabowski et al. 2008)
Nov 2004 – Sep 2007 (31 cruises)	Measurement of N_2 fixation (0-125 m) using $^{15}N_2$ (Church et al. 2009)
Jul 2005 (1 cruise)	Nutrient inventories and dynamics (including P*i*) and controls on diazotroph diversity, abundances, and N_2-fixation rates within a large anticyclonic eddy (Fong et al. 2008)
Mar 2006 (1 cruise)	DNA metagenomic and RNA transcriptomic sequence analysis of a 75 m depth sample (Frias-Lopez et al. 2008)
Oct 2006	Comparison of the relative frequency of occurrence for selected P-

(1 cruise)	cycle genes in samples collected from 0-100 m at the Bermuda and Hawaii sites (Coleman and Chisholm 2010)
Feb 2008 – Nov 2009 (6 cruises)	Depth profiles (0-120 m) of alkaline phosphatase activity and kinetic characterization using MUF-P substrate, P*i* uptake and turnover (Duhamel et al. 2011)
May-Jun 2008	Project OPPEX: A field test of the Karl-Letelier P-dependent, two stage phytoplankton bloom hypothesis (White et al. 2010)
May 2008 – Nov 2009 (5 cruises)	^{32}P*i* uptake, dissolved and particulate matter APase activity (MUF-P substrate), APase kinetic parameters (K_m and V_{max}), and estimates of DOP hydrolysis rate (Duhamel et al. 2011)
Jul-Aug 2008 (OPEREX cruise)	P*i* and DOP (MPn) control of inorganic C- and N_2-fixation (Watkins-Brandt et al. 2011)
Oct 2008 (1 cruise)	Depth profiles (0-175 m), size-fractionated, and flowcytometric cell sorted uptake of ^{32}P*i* and ^{32}P-ATP (Duhamel et al. 2012)
Jul-Aug 2008 (OPEREX cruise)	Assessment of the impacts of mesoscale eddies on P*i* inventories and related microbial and biogeochemical parameters (Guidi et al. 2012)
Sep 2009 (1 cruise)	Construction of 16s ribosomal DNA and RNA tag sequence (V6 to V8 region) libraries at 25 and 1000 m (Hunt et al. 2012)
Jul 2010 (1 cruise)	Assessment of the role of bacterial quorum sensing for P-acquisition in *Trichodesmium* consortia (Van Mooy et al. 2011)
Jul-Aug 2010 (2 cruises)	Taxon-specific ^{32}P*i* and ^{32}P-ATP uptake and kinetic characterization using flow cytometry (Björkman et al. 2012)

References

Benitez-Nelson, C. R. & Karl, D. M. Phosphorus cycling in the North Pacific Subtropical Gyre using cosmogenic ^{32}P and ^{33}P. *Limnol. Oceanogr.* **47,** 762-770 (2002).

Björkman, K. M. & Karl, D. M. Bioavailability of dissolved organic phosphorus in the euphotic zone at Station ALOHA, North Pacific Subtropical Gyre. *Limnol. Oceanogr.* **48,** 1049-1057 (2003).

Björkman , K. M. & Karl, D. M. Presence of dissolved nucleotides in the North Pacific Subtropical Gyre and their role in cycling of dissolved organic phosphorus. *Aquat. Microb. Ecol.* **39,** 193-203 (2005).

Björkman, K., Thomson-Bulldis, A. L. & Karl, D. M. Phosphorus dynamics in the North Pacific subtropical gyre. *Aquat. Microb. Ecol.* **22,** 185-198 (2000).

Björkman, K., Duhamel, S. & Karl, D. M. Microbial group specific uptake kinetics of inorganic phosphate and adenosine-5'-triphosphate (ATP) in the North Pacific Subtropical Gyre. *Frontiers Aquat. Microbiol.* **3,** doi: 10.3389/fmicb.2012.00189 (2012).

Brum, J. R. Concentration, production and turnover of viruses and dissolved DNA pools at Stn ALOHA, North Pacific Subtropical Gyre. *Aquat. Microb. Ecol.* **41,** 103-113 (2005).

Christian, J. R., Lewis, M. R. & Karl, D. M. Vertical fluxes of carbon, nitrogen and phosphorus in the North Pacific subtropical gyre near Hawaii. *J. Geophys. Res.* **102,** 15,667-15,677 (1997).

Church, M. J., Ducklow, H. W. & Karl, D. M. Multiyear increases in dissolved organic matter inventories at Station ALOHA in the North Pacific Subtropical Gyre. *Limnol. Oceanogr.* **47,** 1-10 (2002).

Church, M. J. *et al.* Physical forcing of nitrogen fixation and diazotroph community structure in the North Pacific Subtropical Gyre. *Global Biogeochem. Cycles* **23,** GB2020, doi:10.1029/2008GB003418 (2009).

Coleman, M. L. & Chisholm, S. W. Ecosystem-specific selection pressures revealed through comparative population genomics. *Proc. Natl. Acad. Sci. US* **107,** 18634-18639 (2010).

Colman, A. S., Blake, R. E., Karl, D. M., Fogel, M. L. & Turekian, K. K. Marine phosphate oxygen isotopes and organic matter remineralization in the oceans. *Proc. Natl. Acad. Sci. USA* **102,** 13023-13028 (2005).

DeLong, E. F. *et al.* Community genomics among stratified microbial assemblages in the ocean's interior. *Science* **311,** 496-503 (2006).

Duhamel, S., Björkman, K. M., Van Wambeke, F., Moutin, T. & Karl, D. M.. Characterization of alkaline phosphatase activity in the North and South Pacific Subtropical Gyres: implication for phosphorus cycling. *Limnol. Oceanogr.* **56,** 1244-1254 (2011).

Duhamel, S., Björkman, K. M. & Karl, D. M. Light dependence of phosphorus uptake by microorganisms in the subtropical North and South Pacific Ocean. *Aquat. Microb. Ecol.* **67,** 225-238 (2012).

Fong, A. A. *et al.* Nitrogen fixation in an anticyclonic eddy in the oligotrophic North Pacific Ocean. *ISME J.* **2**, 663-676 (2008).

Frias-Lopez, J. *et al.* Microbial community gene expression in ocean surface waters. *Proc. Natl. Acad. Sci. USA* **105,** 3805-3810 (2008).

Grabowski, M. N. W., Church, M. J. & Karl, D. M. Nitrogen fixation rates and controls at Stn ALOHA. *Aquat. Microb. Ecol.* **52,** 175-183 (2008).

Guidi, L. *et al*. Does eddy-eddy interaction control surface phytoplankton distribution and carbon export in the North Pacific Subtropical Gyre? *J. Geophys. Res.* **117,** G02024, doi:10.1029/2012JG001984 (2012).

Hannides, C. C. S. *et al.* Export stoichiometry and migrant-mediated flux of phosphorus in the North Pacific Subtropical Gyre. *Deep-Sea Res I* **56,** 73-88 (2009).

Hunt, D. E. *et al.* Relationship between abundance and specific activity of bacterioplankton in open ocean surface waters. *Appl. Env. Microbiol.* **79,** in press (2012).

Kaiser, K. & Benner, R. Organic matter transformations in the upper mesopelagic zone of the North Pacific: Chemical composition and linkages to microbial community structure. *J. Geophys. Res.* **117,** C01023, doi:10.1029/2011JC007141 (2012).

Karl, D. M. & Tien, G. MAGIC: A sensitive and precise method for measuring dissolved phosphorus in aquatic environments. *Limnol. Oceanogr.* **37,** 105-116 (1992).

Karl, D. M. & Tien, G. Temporal variability in dissolved phosphorus concentrations in the subtropical North Pacific Ocean. *Mar. Chem.* **56,** 77-96 (1997).

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).

Karl, D. M., Letelier, R., Hebel, D. V., Bird, D. F. & Winn, C. D. in *Marine Pelagic Cyanobacteria: Trichodesmium and Other Diazotrophs* (eds. Carpenter, E. J., Capone, D. G. & Rueter, J. G.), 219-237 (Kluwer Academic Publishers, The Netherlands, 1992).

Karl, D. M. *et al.* Ecological nitrogen-to-phosphorus stoichiometry at Station ALOHA. *Deep-Sea Res. II* **48,** 1529-1566 (2001b).

Karl, D. M., Church, M. J., Dore, J. E., Letelier, R. M. & Mahaffey, C. Predictable and efficient carbon sequestration in the North Pacific Ocean supported by symbiotic nitrogen fixation. *Proc. Natl. Acad. Sci. USA* **109,** 1842-1849 (2012).

Lamborg, C. H. *et al.* The flux of bio- and lithogenic material associated with sinking particles in the mesopelagic "twilight zone" of the northwest and North Central Pacific Ocean. *Deep-Sea Res. II* **55,** 1540-1563 (2008).

Letelier, R. M. & Karl, D. M. Role of *Trichodesmium* spp. in the productivity of the subtropical North Pacific Ocean. *Mar. Ecol. Prog. Ser.* **133,** 263-273 (1996).

Letelier, R. M. & Karl, D. M. *Trichodesmium* spp. physiology and nutrient fluxes in the North Pacific subtropical gyre. *Aquat. Microb. Ecol.* **15,** 265-276 (1998).

Letelier, R. M. *et al.* Role of late winter mesoscale events in the biogeochemical variability of the upper water column of the North Pacific Subtropical Gyre. *J. Geophys. Res.* **105,** 28,723-28,739 (2000).

Mahaffey, C., Björkman, K. M. & Karl, D. M. Phytoplankton response to deep seawater nutrient addition in the North Pacific Subtropical Gyre. *Mar. Ecol. Prog. Ser.* **460,** 13-34 (2012).

McAndrew, P. M. *et al.* Metabolic response of olitrophic plankton communities to deep water nutrient enrichment. *Mar. Ecol. Prog. Ser.* **332,** 63-75 (2007).

Moutin, T. *et al.* Phosphate availability and the ultimate control of new nitrogen input by nitrogen fixation in the tropical Pacific Ocean. *Biogeosci.* **5,** 95-109 (2008).

Sannigrahi, P., Ingall, E. D. & Benner, R. Nature and dynamics of phosphorus-containing components of marine dissolved and particulate organic matter. *Geochim. Cosmochim. Acta* **70,** 5868-5882 (2006).

Thomson-Bulldis, A. & Karl, D. Application of a novel method for phosphorus determinations in the oligotrophic North Pacific Ocean. *Limnol. Oceanogr.* **43,** 1565-1577 (1998).

Van Mooy, B. A. A. & Devol, A. H. Assessing nutrient limitation of *Prochlorococcus* in the North Pacific subtropical gyre by using an RNA capture method. *Limnol. Oceanogr.* **53,** 78-88 (2008).

Van Mooy, B. A. S. *et al.* Phytoplankton in the ocean use non-phosphorus lipids in response to phosphorus scarcity. *Nature* **458,** 69-72 (2009).

Van Mooy, B. A. S. *et al.* Quorum sensing control of phosphorus acquisition in *Trichodesmium* consortia. *ISME J.* **6,** 422-429 (2011).

Watkins-Brandt, K. S. *et al.* Addition of inorganic or organic phosphorus enhances nitrogen and carbon fixation in the oligotrophic North Pacific. *Mar. Ecol. Prog. Ser.* **432,** 17-29 (2011).

White, A. *et al.* An open ocean trial of controlled upwelling using wave pump technology. *J. Atmos. Ocean. Technol.* **27,** 385-396 (2010a).

Zehr, J. P. *et al.* Experiments linking nitrogenase gene expression to nitrogen fixation in the North Pacific subtropical gyre. *Limnol. Oceanogr.* **52,** 169-183 (2007).