Phosphorus uptake kinetics by microbes to whole communities from R/V Atlantic Explorer AE1206, AE1319 in the Sargasso Sea; Bermuda Atlantic Time-Series Station from 2012-2013 (Biological C:N:P ratios project)

Website: https://www.bco-dmo.org/dataset/537996 Data Type: Cruise Results Version: 1 Version Date: 2014-11-10

Project

» Biological Controls on the Ocean C:N:P ratios (Biological C:N:P ratios)

Programs

- » Dimensions of Biodiversity (Dimensions of Biodiversity)
- » Ocean Carbon and Biogeochemistry (OCB)

Contributors	Affiliation	Role
Lomas, Michael W.	Bigelow Laboratory for Ocean Sciences	Principal Investigator
<u>Bonachela, Juan A.</u>	University of Strathclyde (U Strathclyde)	Co-Principal Investigator
Levin, Simon	Princeton University	Co-Principal Investigator
<u>Martiny, Adam</u>	University of California-Irvine (UC Irvine)	Co-Principal Investigator
Copley, Nancy	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract

Phosphorus uptake kinetics by microbes to whole communities from R/V Atlantic Explorer AE1206, AE1319 in the Sargasso Sea; Bermuda Atlantic Time-Series Station from 2012-2013 (Biological C:N:P ratios project)

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Coverage

Spatial Extent: N:55 E:-45 S:21.67 W:-65.967 Temporal Extent: 2011-09-28 - 2013-09-08

Dataset Description

Phosphorus uptake kinetics by microbes to whole communities from R/V Atlantic Explorer AE1206, AE1319 in the Sargasso Sea; Bermuda Atlantic Time-Series Station from 2012-2013. This data was published in Lomas et al. 2014 PNAS.

Methods & Sampling

Sample collection:

The data presented in this study were collected on 7 cruises throughout the Western North Atlantic Ocean (cruise X0606, X0705, X0804, BVAL 39, BVAL 46, AE1206, and AE1319). Data for kinetics experiments were collected from throughout the western North Atlantic, from roughly 550N in the Labrador Sea to ~210N, just north of Puerto Rico. All sample depths were <200m. All samples for Pi uptake rates and kinetics experiments were collected in acid-cleaned Niskin bottles attached to a CTD rosette and kept in subdued lighting until experiments were initiated (< 1 h). Samples for whole community ambient uptake rates were collected from ~4 depths in the upper 60 m, while samples for taxon-specific ambient uptake rates were collected from 5 m, 40 m, and the deep chlorophyll maximum (DCM; ranging from 80 to 120 m) (27). *Trichodesmium* colonies were collected from the near surface (roughly within the top 20 m) by vertically hauling a handheld 100 μ m net. Single colonies were transferred a second time into fresh 0.2 μ m-filtered water to reduce contamination of closely associated organisms, and subsequently separated by morphotype (either 'puff' with radial trichomes or 'raft' with parallel trichomes); only data for 'rafts' are presented here.

Dissolved inorganic and organic, and particulate nutrients:

Samples for NO3-/NO2-, NO2- and PO4-3 are gravity filtered through 0.8 µm Nucleopore polycarbonate filters using acid cleaned in-line polycarbonate filter holders, then frozen (-20oC) in HDPE bottles until analysis (Dore et al., 1996). Tests of frozen versus refrigerated samples have indicated no significant difference between storage methods (Dore et al., 1996). Nutrient samples prior to ~2003 were analyzed on a modified Technicon Autoanalyzer and samples post ~2003 were analyzed on an Alpkem Flow Solution IV; both instrumental setups have comparable sensitivity and method detection limits validated by 6-month sample overlap on both instruments. During every sample run, commercially available certified standards, OSIL and Wako Chemical, are analyzed to maintain data quality, as well as 'standard water' from 3000 m which serves as an internal standard.

Soluble reactive phosphate (SRP) concentrations in the euphotic zone of the Sargasso Sea are below analytical detection limits (~20 nmol kg-1) of standard nutrient autoanalyzer configurations. To resolve the low concentrations of SRP in the surface waters at BATS the Magnesium Induced Co-precipitation method, referred to as MAGIC-SRP measurements (Karl and Tien, 1992; Rimmelin and Moutin, 2005), was used starting in late 2004. Several modifications to the method were made and detailed in Lomas et al. (2010a). Sample accuracy was checked on each run with a certified OSIL nutrient standard. The method detection limit following this protocol is ~1 nmol kg-1 with a precision of + 5% at 5 nmol kg-1.

Particulate organic carbon (POC) and nitrogen (PON) samples are filtered on precombusted (450oC, 4h) Whatman GF/F filters and frozen until analysis Steinberg et al., 2001a. Samples are analyzed on a Control Equipment 240-XA or 440-XA elemental analyzer standardized to acetanilide. Particulate phosphorus samples (PPhos) are analyzed using a ash-hydrolysis method (Lomas et al., 2010a). Oxidation efficiency and standard recovery is tested with each sample run using an ATP standard solution and a certified phosphate standard (OSIL Phosphate Nutrient Standard Solution). Method precision is ~9% at 2.5 nmol kg-1 (the lowest concentrations typically observed well below the euphotic zone), and ~1% at 15 nmol kg-1 (typical euphotic zone concentrations). The method detection limit, defined as three times the standard deviation of the lowest standard (2.5 nmol kg-1) is ~0.5 nmol kg-1.

33Phosphate incubations:

The approach for ambient whole community and population-specific uptake rate measurements were previously published (Casey et al, 2009). Briefly, duplicate aliquots of 10 ml seawater were amended with 0.15 μ Ci (~80 pmol L-1) additions of H333PO4 (3000 Ci mol-1; PerkinElmer, USA), and incubated for 30 - 60 min in subdued lighting (~100 μ mol photons m-2 s-1) at ~23oC. This temperature was within ~3oC of the coolest/warmest in situ temperature from which the samples were collected. The duration of each incubation varied depending on turnover time of the added isotope, such that efforts were made to keep uptake to <25% of the tracer added. Duplicate killed control incubations were conducted for each station. Killed controls were amended with paraformaldehyde (0.5% final concentration) for 30 min prior to the addition of isotopic tracer and incubation. Whole community incubations were terminated by filtration onto 0.2 μ m polycarbonate filters that were subsequently placed in glass scintillation vials. Population-specific ambient uptake incubations were terminated by the addition of paraformaldehyde (0.5% final concentration), and stored at 4oC until sorting (<12 h) as described in the next section.

Whole community and population-specific kinetics experiments were conducted by adding 0.15 μ Ci (~80 pM) of H333PO4 to ~10 replicate 10 ml seawater samples that were further amended by increasing additions of 'cold' KH2PO4 up to 100 nM. Samples were incubated as above, but the incubations were terminated by the addition of KH2PO4 to a final concentration of 100 μ M (28). Whole community samples were filtered onto 0.2 μ m polycarbonate filters, and rinsed with an oxalate wash (29). Surface bound phosphate in population-specific samples was accounted for by subtracting 33P counts for sorted populations to which 100 μ M phosphate had been added prior to addition of the isotopic tracer. It is assumed that addition of such a high level of phosphate would result in negligible uptake of radioactive phosphate and thus any signal was attributed to surface

absorption; this correction was always <2-3%. Population-specific kinetics experiments for samples collected in the deep chlorophyll maximum were first gravity concentrated and resuspended in phosphate-free Sargasso Sea surface water prior to incubation as described. Population-specific samples were stored at 4oC in the dark until sorting (<3 h) as described in the next section. Kinetics experiments for Trichodesmium spp. were conducted in the same manner as above for whole community samples but with picked and rinsed colonies and increasing additions of 'cold' KH2PO4 up to 1000 nM.

Flow cytometry analysis and cell sorting:

Samples were sorted on an InFlux cell sorter (BD, Seattle, WA) at an average flow rate of ~40 μ L min-1. Samples were sorted for *Prochlorococcus*, *Synechococcus*, and an operationally defined eukaryotic algae size fraction (eukaryotes >2 μ m). A 100 mW blue (488 nm) excitation laser was used. After exclusion of laser noise gated on pulse width and forward scatter, autotrophic cells were discriminated by chlorophyll fluorescence (>650 nm), PE (585/30 nm), and granularity (side scatter). Sheath fluid was made fresh daily from distilled deionized water (Millipore, Billerica, MA) and molecular grade NaCl (Mallinckrodt Baker, Phillipsburg, NJ), pre-filtered through a 0.2 μ m capsule filter (Pall, East Hills, NY), and a STERIVEX sterile 0.22 μ m inline filter (Millipore, Billerica, MA). Mean coincident abort rates were < 1% and mean recovery from secondary sorts (n = 25) was 97.5 ± 1.1% (data not shown). Spigot[™] (BD Seattle, WA) and FCS Express V3[™] (DeNovo Software, Seattle, WA) were used for data acquisition and post acquisition analysis, respectively. Sorted cells from each sample were gently filtered onto 0.2 μ m Nucleopore polycarbonate filters, rinsed with copious amounts of 0.2 μ m filtered seawater, an oxalate wash(29), and placed in a 7 ml scintillation vial for liquid scintillation counting.

Data Processing Description

P uptake Rate Calculations:

Whole community and taxon-specific assimilation rates were calculated using the same equation as follows: -- [see formula at: <u>https://datadocs.bco-</u>

dmo.org/docs/302/lomas/DimBiodiv_CNP/data_docs/Metadata_Data_processing_section_formula.jpg]

where Vpi is the cell-specific utilization rate (amol 33Pi cell-1 hr-1); ßsample and ßTA are the ß-emission activities (counts min-1) for the sorted sample and the total activity added, respectively; n is the number of cells sorted; Delta-T is the elapsed time from 33P isotopic tracer addition to counting; To is the incubation duration; Lambda is the decay constant of 33P (half-life = 25.4 d); P is the ambient concentration of the P source (nmol L-1). The method detection limit following this protocol is ~0.5 nM with a precision of + 5% at 5 nM.

Data analysis:

Parameters for the hyperbolic nutrient uptake curves from all samples were estimated in SigmaPlot (Systat Software, San Jose, CA, Version 10) using an iteratively fit hyperbolic equation.

BCO-DMO Processing Notes:

- added conventional header with dataset name, PI name, version date
- renamed parameters to BCO-DMO standard
- replaced spaces and other special characters with underscore

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Data Files

```
File

kinetics.csv(Comma Separated Values (.csv), 3.36 KB)

MD5:70dac592f4d504cabc5dd059b0d7ed4d

Primary data file for dataset ID 537996
```

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Related Publications

Casey, J., Lomas, M., Michelou, V., Dyhrman, S., Orchard, E., Ammerman, J., & Sylvan, J. (2009). Phytoplankton taxon-specific orthophosphate (Pi) and ATP utilization in the western subtropical North Atlantic. Aquatic Microbial Ecology, 58, 31–44. doi:<u>10.3354/ame01348</u>

General

Dore, J., Houlihan, T., Hebel, D., Tien, G., Tupas, L., and Karl, D. (1996) Freezing as a method of sample preservation for the analysis of dissolved inorganic nutrients in seawater. Marine Chemistry 53, 173-185. <u>ftp://soest.hawaii.edu/dkarl/misc/dave/Reprints/1996MarChem53-173-185.pdf</u> *Methods*

Karl, D. M., & Tien, G. (1992). MAGIC: A sensitive and precise method for measuring dissolved phosphorus in aquatic environments. Limnology and Oceanography, 37(1), 105–116. doi:<u>10.4319/lo.1992.37.1.0105</u> *Methods*

Lomas, M. W., Bonachela, J. A., Levin, S. A., & Martiny, A. C. (2014). Impact of ocean phytoplankton diversity on phosphate uptake. Proceedings of the National Academy of Sciences, 111(49), 17540–17545. doi:<u>10.1073/pnas.1420760111</u> *Methods*

Lomas, M. W., Burke, A. L., Lomas, D. A., Bell, D. W., Shen, C., Dyhrman, S. T., & Ammerman, J. W. (2010). Sargasso Sea phosphorus biogeochemistry: an important role for dissolved organic phosphorus (DOP). Biogeosciences, 7(2), 695–710. doi:<u>10.5194/bg-7-695-2010</u> *Methods*

Rimmelin, P., & Moutin, T. (2005). Re-examination of the MAGIC method to determine low orthophosphate concentration in seawater. Analytica Chimica Acta, 548(1-2), 174–182. doi:<u>10.1016/j.aca.2005.05.071</u> *Methods*

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Related Datasets

IsRelatedTo

Lomas, M. W., Bonachela, J. A., Levin, S., Martiny, A. (2020) **Bulk phosphorus uptake by microbes from cruises in the NW Atlantic and western Sargasso Sea 2006-2013 (Biological C:N:P ratios project).** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2014-11-19 doi:10.26008/1912/bco-dmo.538027.1 [view at BCO-DMO]

Lomas, M. W., Bonachela, J. A., Levin, S., Martiny, A. (2020) **Taxon-specific phosphorus uptake by microbes from NW Atlantic and western Sargasso Sea from 2007-2013 (Biological C:N:P ratios project).** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2014-11-10 doi:10.26008/1912/bco-dmo.538053.1 [view at BCO-DMO]

Lomas, M. W., Bonachela, J. A., Levin, S., Martiny, A. (2021) **Biogeochemistry of microbial phosphorus uptake from cruises in the Sargasso Sea; Bermuda Atlantic Time-Series Station from 2011-2013 (Biological C:N:P ratios project).** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 2) Version Date 2021-02-05 doi:10.26008/1912/bco-dmo.538091.2 [view at BCO-DMO]

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Parameters

Parameter	Description	Units
cruise_id	cruise identification	unitless
month	month sample was collected	unitless
year	year sample was collected	уууу
station	station number or identification	unitless
lat	latitude; north is positive	decimal degrees
Depth_Loc	description of sample collection depth	unitless
population	phytoplankton group for which kinetics value is reported	unitless
Pmax	calculated maximum uptake; see Pmax_units units column in dataset for correct units	amol/cell/h or pmol/cell/h
Pmax_error	estimated error on uptake rate; see Pmax_units column in dataset for units	amol/cell/h or pmol/cell/h
Pmax_units	units differ between groups and so are listed in this column	unitless
Ks	calculated half saturation concentration	nM
Ks_error	estimated error on half saturation concentration	nM
R_squared	R^2 value of the fit	unitless

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Instruments

Dataset- specific Instrument Name	Flow Cytometer
Generic Instrument Name	Flow Cytometer
Dataset- specific Description	InFlux cell sorter (BD, Seattle, WA)
Generic Instrument Description	Flow cytometers (FC or FCM) are automated instruments that quantitate properties of single cells, one cell at a time. They can measure cell size, cell granularity, the amounts of cell components such as total DNA, newly synthesized DNA, gene expression as the amount messenger RNA for a particular gene, amounts of specific surface receptors, amounts of intracellular proteins, or transient signalling events in living cells. (from: <u>http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm</u>)

Dataset- specific Instrument Name	Niskin bottle
Generic Instrument Name	Niskin bottle
Dataset- specific Description	Acid washed Niskin bottles attached to a CTD rosette
	A Niskin bottle (a next generation water sampler based on the Nansen bottle) is a cylindrical, non- metallic water collection device with stoppers at both ends. The bottles can be attached individually on a hydrowire or deployed in 12, 24, or 36 bottle Rosette systems mounted on a frame and combined with a CTD. Niskin bottles are used to collect discrete water samples for a range of measurements including pigments, nutrients, plankton, etc.

Dataset- specific Instrument Name	Nutrient Autoanalyzer
Generic Instrument Name	Nutrient Autoanalyzer
Dataset- specific Description	Modified Technicon Autoanalyzer and an Alpkem Flow Solution IV
Generic Instrument Description	Nutrient Autoanalyzer is a generic term used when specific type, make and model were not specified. In general, a Nutrient Autoanalyzer is an automated flow-thru system for doing nutrient analysis (nitrate, ammonium, orthophosphate, and silicate) on seawater samples.

Instrument Name	Plankton Net
Generic Instrument Name	Plankton Net
Dataset-specific Description	Handheld 100 µm net
Generic Instrument Description	A Plankton Net is a generic term for a sampling net that is used to collect plankton. It is used only when detailed instrument documentation is not available.

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Deployments

AE1206		
Website	https://www.bco-dmo.org/deployment/58935	
Platform	R/V Atlantic Explorer	
Start Date	2012-03-14	
End Date	2012-03-23	
Description	AE1206 was the third in a series of four cruises for the Trophic BATS project. On each cruise, sampling was conducted at three stations: the center and edge of a mesoscale eddy and at one station outside of the eddy. Core CTD casts to ~2000 meters and pre-dawn 'Productivity' CTD casts were made at each station. Cruise information and original data are available from the NSF R2R data catalog.	

AE1319

Website	https://www.bco-dmo.org/deployment/537979
Platform	R/V Atlantic Explorer
Report	http://dmoserv3.whoi.edu/data_docs/Bio_CNP_Ratios/AE1319_Cruise_Report_09182013_reduced2.pdf
Start Date	2013-08-14
End Date	2013-09-11
Description	Cruise for project 'Dimensions of Biodiversity: Biological Controls on the Ocean C:N:P ratios'.

AE1123

Website	https://www.bco-dmo.org/deployment/538493	
Platform	R/V Atlantic Explorer	
Start Date	2011-09-27	
End Date	2011-10-19	
Description	Lomas_BATS_validation_2011: sampling for nutrient study	

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Project Information

Biological Controls on the Ocean C:N:P ratios (Biological C:N:P ratios)

Coverage: western North Atlantic; 60N to 20N along 66W longitude; 20N to 15S in the tropical Pacific

One of the fundamental patterns of ocean biogeochemistry is the Redfield ratio, linking the stoichiometry of surface plankton with the chemistry of the deep ocean. There is no obvious mechanism for the globally consistent C:N:P ratio of 106:16:1 (Redfield ratio), especially as there is substantial elemental variation among plankton communities in different ocean regions. Thus, knowing how biodiversity regulates the elemental composition of the ocean is important for understanding the ocean and climate as a whole -- now and in the future.

The conceptual hypotheses for this study are as follows: 1. The C:N:P ratio of a cell is constrained by its broad taxonomic group, which determines, for example, whether it has an outer shell, its size, functional metabolism, membrane lipid composition. 2. Within a taxon, there is high genetic diversity. Some of this genetic diversity is potentially laterally transferred, or can be lost within taxa, and confers various functional abilities (organic phosphate assimilation, nitrate assimilation, photoheterotrophy, etc.). Functional diversity provides the cell with further flexibility, such as the ability to respond to varying nutrient supply rates/ratios, and affects a cell's C:N:P ratio within the range specified by the taxon. 3. Given these taxonomic and genetic constraints, a cell is physiologically plastic and modifies how it allocates cellular resources in response to nutrient supply rates/ratios in the environment. 4. The microbial diversity (taxonomic, genetic, and functional) of the surface ocean varies over time and space, driven by many factors in addition to nutrients. The sum of this mixture composes the ecosystem C:N:P, the ratio that Redfield described.

Based on this framework, the CoPIs will make field observations of taxon-specific stoichiometry and growth rates, genomic analyses, and conduct laboratory chemostat experiments to improve understanding of how ocean taxonomic, genetic, and functional biodiversity control the stoichiometry of the surface ocean plankton. Their analyses of these data would lead to a mechanistic understanding of variations in the Redfield ratio, both spatially and temporally.

This study will greatly expand knowledge of the genomic diversity among ocean microbes and how this diversity affects biogeochemistry. The stoichiometry of the ocean's microbes is a parameter that nearly every chemical or biological oceanographer uses, from converting measurements made in one element to another, to estimating regional and global nitrogen budgets. The research also has important implications for the global carbon budget and any changes that might result from climate change.

To understand mechanistically temporal and spatial variability of the plankton C:N:P ratio, biodiversity must be

studied not only at the traditional taxonomic level, but at the genetic and functional levels which dictate organism response to their environment. Data will be integrated into a combined ocean ecological, evolutionary, and biogeochemical model, with flexible stoichiometry, including cellular biochemical allocations. Seeding a coupled physical-biological model of the oceans with multiple competing genotypes enables the exploration of ecological and evolutionary patterns of resource acquisition and C:N:P ratios. Developing a more mechanistic examination of the course of ecology and evolution, in which laboratory and field data define tradeoffs between different growth and nutrient acquisition strategies, would estabblish the framework of adaptive dynamics for determining "evolutionarily convergence". Finally, model outcomes will be evaluated against field data.

The field work planned for this project includes several cruises: BV46 (September/October 2011), BV48 (September 2012), a June 2013 cruise from Bermuda to the Labrador Sea, and a cruise from Hawaii to Tahiti (May 2014). Additionally, samples will be be acquired during cruises of opportunity.

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Program Information

Dimensions of Biodiversity (Dimensions of Biodiversity)

Website: http://www.nsf.gov/funding/pgm_summ.jsp?pims_id=503446

Coverage: global

(adapted from the NSF Synopsis of Program)

Dimensions of Biodiversity is a program solicitation from the NSF Directorate for Biological Sciences. FY 2010 was year one of the program. [MORE from NSF]

The NSF Dimensions of Biodiversity program seeks to characterize biodiversity on Earth by using integrative, innovative approaches to fill rapidly the most substantial gaps in our understanding. The program will take a broad view of biodiversity, and in its initial phase will focus on the integration of genetic, taxonomic, and functional dimensions of biodiversity. Project investigators are encouraged to integrate these three dimensions to understand the interactions and feedbacks among them. While this focus complements several core NSF programs, it differs by requiring that multiple dimensions of biodiversity be addressed simultaneously, to understand the roles of biodiversity in critical ecological and evolutionary processes.

Ocean Carbon and Biogeochemistry (OCB)

Website: http://us-ocb.org/

Coverage: Global

The Ocean Carbon and Biogeochemistry (OCB) program focuses on the ocean's role as a component of the global Earth system, bringing together research in geochemistry, ocean physics, and ecology that inform on and advance our understanding of ocean biogeochemistry. The overall program goals are to promote, plan, and coordinate collaborative, multidisciplinary research opportunities within the U.S. research community and with international partners. Important OCB-related activities currently include: the Ocean Carbon and Climate Change (OCCC) and the North American Carbon Program (NACP); U.S. contributions to IMBER, SOLAS, CARBOOCEAN; and numerous U.S. single-investigator and medium-size research projects funded by U.S. federal agencies including NASA, NOAA, and NSF.

The scientific mission of OCB is to study the evolving role of the ocean in the global carbon cycle, in the face of environmental variability and change through studies of marine biogeochemical cycles and associated ecosystems.

The overarching OCB science themes include improved understanding and prediction of: 1) oceanic uptake and release of atmospheric CO2 and other greenhouse gases and 2) environmental sensitivities of biogeochemical cycles, marine ecosystems, and interactions between the two.

The OCB Research Priorities (updated January 2012) include: ocean acidification; terrestrial/coastal carbon fluxes and exchanges; climate sensitivities of and change in ecosystem structure and associated impacts on biogeochemical cycles; mesopelagic ecological and biogeochemical interactions; benthic-pelagic feedbacks on biogeochemical cycles; ocean carbon uptake and storage; and expanding low-oxygen conditions in the coastal and open oceans.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	<u>OCE-1046001</u>
NSF Division of Ocean Sciences (NSF OCE)	<u>OCE-1046368</u>
NSF Division of Ocean Sciences (NSF OCE)	OCE-1046297
NSF Division of Ocean Sciences (NSF OCE)	OCE-1045966

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