Bacterial abundance Thymidine & Leucine incorporation from R/V Thomas G. Thompson cruises TT007, TT011 in the Equatorial Pacific in 1992 during the U.S. JGOFS Equatorial Pacific (EqPac) project

Website: https://www.bco-dmo.org/dataset/2641
Version: August 27, 2001
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Project
» U.S. JGOFS Equatorial Pacific (EqPac)

Program
» U.S. Joint Global Ocean Flux Study (U.S. JGOFS)

Contributors	Affiliation	Role
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Dataset Description

Bacterial abundance Thymidine & Leucine incorporation

Methods & Sampling

See Platform deployments for cruise specific documentation

Data Processing Description

Samples were processed immediately following collection. Short-term incorporation assays followed procedures described in Ducklow et al. (1992a). Duplicate 30 ml samples were amended with methyl-H-thymidine (New England Nuclear, sp. act. >75 Ci mmol; 10 nM final concl.) and incubated at or near in situ water temperatures in screwtop polycarbonate centrifuge tubes in chilled water bath incubators. Following incubation periods of ca. 1--3 h, the incubation was terminated with the addition of 0.5 % formalin. To measure nonspecific incorporation, these samples were filtered onto Sartorius cellulose nitrate membranes (0.22 um pore size, extracted by rinsing the filters over a vacuum three times with ice-cold 5 % trichloroacetic acid (TCA) and three times with 80 % ethanol, as suggested in Wicks and Robarts (1988). To measure incorporation into DNA only, separate parallel samples were extracted in 0.25 n NaOH (final conc.) and chilled on ice. These samples were stored on ice for up to 48 hours, then neutralized with ice-cold 100 % TCA (final conc. 20 %), and filtered onto 22 mm dia. 0.2 um cellulose nitrate membrane filters. Finally the samples wre extracted on the filter holders by rinsing three times each with 50 % chloroform-phenol (a 1:1 c/c mixture of liquified phenol and chloroform) and with 80 % ethanol to purify the labelled DNA (Wicks and Robarts, 1987). Zero-time controls

were subtracted to correct for adsorption and other abiotic effects. The cellulose nitrate filters were packed tightly into 7 ml glass scintillation vials and dissolved in 1.0 ml of ethyl acetate, prior to addition of Ultima Gold biodegradeable scintillation cocktail (Packard). Samples were counted aboard ship on the T.G. Thompson scintillation counter.

H-leucine incorporation was estimated in parallel incubations of samples inoculated with 0.5 nM H-leuchine (New England Nuclear, sp. act. 153 Ci mmol) and 10 nM unlabeled leucine (Kirchman et al., 1985), for a final leucine concentration (hot plus cold) of 10.5 nM 30 ml leucine samples were filtered onto replicate 22 mm dia. 0.22 um cellulose nitrate filters and extracted with ice cold 5 % TCA and ethanol as described above.

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Parameters

Parameter	Description	Units
event	event number per event log	YYYYMMDD
sta	station number from event log	dimensionless
cast	TM or CTD cast number from event log	dimensionless
bot	rosette bottle number	dimensionless
depth_n	nominal sample depth	meters
thy_incorp	Thymidine incorporation	picomoles/liter/hour
leuc_incorp	Leucine incorporation	picomoles/liter/hour
bact_het_orig	heterotrophic bacteria abundance, original units; microscopy	cells/milliliter *10^6
bact_het_mic	heterotrophic bacteria abundance, DMO converted units; microscopy	cells/milliliter

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Instruments

Dataset-specific Instrument Name	Niskin Bottle
Generic Instrument Name	Niskin bottle
Dataset-specific Description	CTD clean rosette (Niskin) bottles were used to collect water samples.
Generic Instrument Description	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	Trace Metal Bottle
Generic Instrument Name	Trace Metal Bottle
Dataset-specific Description	Trace metal (TM) clean rosette bottles were used to collect water samples.
Generic Instrument Description	Trace metal (TM) clean rosette bottle used for collecting trace metal clean seawater samples.

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Deployments

TT007

Website	https://www.bco-dmo.org/deployment/57728
Platform	R/V Thomas G. Thompson
Start Date	1992-01-30
End Date	1992-03-13

Description	Purpose: Spring Survey Cruise; 12°N-12°S at 140°W TT007 was one of five cruises conducted in 1992 in support of the U.S. Equatorial Pacific (EqPac) Process Study. The five EqPac cruises aboard R/V Thomas G. Thompson included two repeat meridional sections (12°N - 12°S), 2 equatorial surveys, and a benthic survey (all at 140° W). The scientific objectives of this study were to observe the processes in the Equatorial Pacific controlling the fluxes of carbon and related elements between the atmosphere, euphotic zone, and deep ocean. As luck would have it, the survey window coincided with an El Nino event. A bonus for the research team. **Methods & Sampling** PI: David Kirchman of: University of Delaware dataset: Bacterial abundance Thymidine & Leucine incorporation dates: February 04, 1992 to March 08, 1992 location: N: 11.972 S: -12.2083 W: -140.7452 E: -134.7269 project/cruise: EQPAC/TT007 - Spring Survey ship: Thomas Thompson DMO cautionary note: The DMO suspects a depth error in event 02261720 bottle number 22 depth 20 meters. Murray's final bottle cast file reports this bottle tripping at a nominal depth of 30 meters. The DMO suspects that event number 03071545 should be changed to 03061530. Doing so would correct for bottle number and depth misalignments. Methodology PI-Notes EqPac bottle quality review summary from DMO Samples from the upper 200 m were collected during hydrocases with a trace-metal-free rosette (Moss Landing). Samples for estimation of bacterial abundance and biovolume (20--100 ml, depending on depth) were preserved with particle-free 1.0 % glutaraldehyde then filtered within 24 h onto black Poretics polycarbonate filters (0.2 ï¿½m pore size), stained with acridine orange (Hobbie et al., 1977) and mounted in Cargille Type A immersion oil on slides and stored frozen until examination. Samples for microscopy were not replicated. All samples were enumerated using a Zeiss Axiophot microscope (final magnification 1613 x). Biovolume was estimated using the 386-based Zeiss VIDAS VIDEOPLAN Image Analysis system which acquired images from a Dage-MTI Nuvicon video camera connected to the Axiophot microscope through a Dage gen-II image intensifier. In our configuration this imaging system projects 0.2 ï¿½m spheres onto an area of approximately 17 pixels. We measure length and width (D and D), perimeter and area of approximately 300 cells in each sample. The measurements are calibrated by measuring fluorescent spheres of various sizes (Polysciences Corp.). **Processing Description** Samples were processed immediately following collection. Short-term incorporation assays followed procedures described in Ducklow et al. (1992a). Duplicate 30 ml samples were amended with methyl-H-thymidine (New England Nuclear, sp. act. >75 Ci mmol; 10 nM final concl.) and incubated at or near in situ water temperatures in screwtop polycarbonate centrifuge tubes in chilled water bath incubators. Following incubation periods of ca. 1--3 h, the incubation was terminated with the addition of 0.5 % formalin. To measure nonspecific incorporation, these samples were filtered onto Sartorius cellulose nitrate membranes (0.22 ï¿½m pore size, extracted by rinsing the filters over a vacuum three times with ice-cold 5 % trichloroacetic acid (TCA) and three times with 80 % ethanol, as suggested in Wicks and Robarts (1988). To measure incorporation into DNA only, separate parallel samples were extracted in 0.25 n NaOH (final conc.) and chilled on ice. These samples were stored on ice for up to 48 hours, then neutralized with ice-cold 100 % TCA (final conc. 20 %), and filtered onto 22 mm dia. 0.2 ï¿½m cellulose nitrate membrane filters. Finally the samples wre extracted on the filter holders by rinsing three times each with 50 % chloroform-phenol (a 1:1 c/c mixture of liquified phenol and chloroform) and with 80 % ethanol to purify the labelled DNA (Wicks and Robarts, 1987). Zero-time controls were subtracted to correct for adsorption and other abiotic effects. The cellulose nitrate filters were packed tightly into 7 ml glass scintillation vials and dissolved in 1.0 ml of ethyl acetate, prior to addition of Ultima Gold biodegradeable scintillation cocktail (Packard). Samples were counted aboard ship on the T.G. Thompson scintillation counter. H-leucine incorporation was estimated in parallel incubations of samples inoculated with 0.5 nM H-leuchine (New England Nuclear, sp. act. 153 Ci mmol) and 10 nM unlabeled leucine (Kirchman et al., 1985), for a final leucine concentration (hot plus cold) of 10.5 nM 30 ml leucine samples were filtered onto replicate 22 mm dia. 0.22 ï¿½m cellulose nitrate filters and extracted with ice cold 5 % TCA and ethanol as described above.

TT011

Website	https://www.bco-dmo.org/deployment/57730

Platform	R/V Thomas G. Thompson
Start Date	1992-08-05
End Date	1992-09-18
Description	Purpose: Fall Survey; 12°N-12°S at 140°W TT011 was one of five cruises conducted in 1992 in support of the U.S. Equatorial Pacific (EqPac) Process Study. The five EqPac cruises aboard R/V Thomas G. Thompson included two repeat meridional sections (12°N - 12°S), 2 equatorial surveys, and a benthic survey (all at 140° W). The scientific objectives of this study were to observe the processes in the Equatorial Pacific controlling the fluxes of carbon and related elements between the atmosphere, euphotic zone, and deep ocean. As luck would have it, the survey window coincided with an El Nino event. A bonus for the research team. **Methods & Sampling** PI: David Kirchman of: University of Delaware dataset: Bacterial abundance Thymidine & Leucine incorporation dates: August 10, 1992 to September 14, 1992 location: N: 12.025 S: -11.9717 W: -140.67 E: -134.95 project/cruise: EQPAC/TT011 - Fall Survey ship: Thomas Thompson Comments on Bacterial Data from TT007, First EqPac Survey Cruise The data on bacterial abundance can be used to calculate bacterial biomass (the common assumption is 20 fg C/cell) and the incorporation rates of thymidine (TdR) and leucine (Leu) can be used to calculate bacterial production. Any one using these data are encouraged to contact D.Kirchman (U.Delaware) for an update on proper factors for converting incorporation rates into bacterial production. The depths indicate in this data set are those original targeted for the particular casts. That is, none of them have been changed to reflect current guesses of where the samples were actually taken. Also, we noted some samples that were clearly anomalous, but again we did not delete or change these. Some of these anomalous values may be due to problems with the CTD bottles, but others are clearly not. - Dave Kirchman Measurement of Bacterial Biomass and Production (EqPac) Hugh W. Ducklow and David L. Kirchman Thymidine and Leucine Incorporation Samples from the upper 200 m were collected during hydrocases with a trace-metal-free rosette (Moss Landing) and processed immediately following collection. Short-term incorporation assays followed procedures described in Ducklow et al. (1992a). Duplicate 30 ml samples were amended with methyl-H-thymidine (New England Nuclear, sp. act. >75 Ci mmol; 10 nM final concl.) and incubated at or near in situ water temperatures in screwtop polycarbonate centrifuge tubes in chilled water bath incubators. Following incubation periods of ca. 1--3 h, the incubation was terminated with the addition of 0.5 % formalin. To measure nonspecific incorporation, these samples were filtered onto Sartorius cellulose nitrate membranes (0.22 µm pore size, extracted by rinsing the filters over a vacuum three times with ice-cold 5 % trichloroacetic acid (TCA) and three times with 80 % ethanol, as suggested in Wicks and Robarts (1988). To measure incorporation into DNA only, separate parallel samples were extracted in 0.25 n NaOH (final conc.) and chilled on ice. These samples were stored on ice for up to 48 hours, then neutralized with ice-cold 100 % TCA (final conc. 20 %), and filtered onto 22 mm dia. 0.2 µm cellulose nitrate membrane filters. Finally the samples wre extracted on the filter holders by rinsing three times each with 50 % chloroform-phenol (a 1:1 c/c mixture of liquified phenol and chloroform) and with 80 % ethanol to purify the labelled DNA (Wicks and Robarts, 1987). Zero-time controls were subtracted to correct for adsorption and other abiotic effects. The cellulose nitrate filters were packed tightly into 7 ml glass scintillation vials and dissolved in 1.0 ml of ethyl acetate, prior to addition of Ultima Gold biodegradeable scintillation cocktail (Packard). Samples were counted aboard ship on the T.G. Thompson scintillation counter. H-leucine incorporation was estimated in parallel incubations of samples inoculated with 0.5 nM H-leuchine (New England Nuclear, sp. act. 153 Ci mmol) and 10 nM unlabeled leucine (Kirchman et al., 1985), for a final leucine concentration (hot plus cold) of 10.5 nM 30 ml leucine samples were filtered onto replicate 22 mm dia. 0.22 µm cellulose nitrate filters and extracted with ice cold 5 % TCA and ethanol as described above. Bacterial Abundance and Biomass Samples for estimation of bacterial abundance and biovolume (20--100 ml, depending on depth) were preserved with particle-free 1.0 % glutaraldehyde then filtered within 24 h onto black Poretics polycarbonate filters (0.2 µm pore size), stained with acridine orange (Hobbie et al., 1977) and mounted in Cargille Type A immersion oil on slides and stored frozen until examination. Samples for microscopy were not replicated. All samples were enumerated using a Zeiss Axiophot microscope (final magnification 1613 x). Biovolume was estimated using the 386-based Zeiss VIDAS VIDEOPLAN Image Analysis system which acquired images from a Dage-MTI Nuvicon video camera connected to the Axiophot microscope through a Dage gen-II image intensifier. In our configuration this imaging system

	projects 0.2 µm spheres onto an area of approximately 17 pixels. We measure length and width (D and D), perimeter and area of approximately 300 cells in each sample. The measurements are calibrated by measuring fluorescent spheres of various sizes (Polysciences Corp.). Biovolumes (V) are calculated using an algorithm (Baldwin and Bankston, 1988) which derives linear dimensions from the image analyzer's estimates of cellular perimeter, (C) and area, (A): V = 4/3(r) + 2h, where (1) _____ (C ± C -4A) r (cell radius) = _____ and (2) 2 A - r h (height) = _____ (3) 2r To estimate bacterial production rates from the incorporation results, conversion factors and derived empirically, loosely following the experimental design first proposed by Kirchman et al. (1982), and described more fully in Ducklow et al. (1992b). Cell volume data can be converted to biomass using various factors centering around 20 fg C 0.1 µm. NB: These protocols closely follow methods used in JGOFS NABE by the same PI's and are quite similar to protocols in use in BATS. Literature Cited Baldwin, W.W. and P.W. Bankston (1988). Measurement of live bacteria by Nomarski interference microscopy and stereologic methods as tested with macroscopic rod-shaped models. Applied and Environmental Microbiology, 54: 105--109. Ducklow, H.W., D.L. Kirchman, H.L. Quinby, C.A. Carlson and H.G. Dam (1992a). Response of bacterioplankton to the spring phytoplankton bloom in the eastern North Atlantic Ocean. Deep-Sea Research, (in press). Ducklow, H.W., D.L. Kirchman and H.L. Quinby (1992b). Bacterioplankton cell growth and macromolecular synthesis in seawater cultures during the North Atlantic spring phytoplankton bloom, May 1989. Microbial Ecology, (in press). Hobbie, J.E., R.J. Daley and S. Jasper (1977). Use of Nuclepore filters for counting bacteria by fluorescence microscopy. Applied and Environmental Microbiology, 33: 1225--1228. Kirchman, D.L., H.W. Ducklow and R. Mitchell (1982). Estimates of bacterial growth from changes in uptake rates and biomass. Applied and Environmental Microbiology, 44: 1296--1307. Kirchman, D.L., E. K'nees and R. Hodson (1985). Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural waters. Applied and Environmental Microbiology, 49: 599--607. Wicks, R.J. and R.D. Robarts (1987). The extraction and purification of DNA labelled with [methyl-H] thymidine in aquatic bacterial production studies. Journal of Plankton Resesearch, 9: 1167--1181. Wicks, R.J. and R.D. Robarts (1988). Ethanol extraction requirement for purification of protein labeled with [H] leucine in aquatic bacterial production studies. Applied and Environmental Microbiology, {f 54(12):} 3191--3193.

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Project Information

U.S. JGOFS Equatorial Pacific (EqPac)

Website: http://usjgofs.whoi.edu/research/eqpac.html

Coverage: Equatorial Pacific

The U.S. EqPac process study consisted of repeat meridional sections (12°N -12°S) across the equator in the central and eastern equatorial Pacific from 95°W to 170°W during 1992. The major scientific program was focused at 140° W consisting of two meridional surveys, two equatorial surveys, and a benthic survey aboard the R/V Thomas Thompson. Long-term deployments of current meter and sediment trap arrays augmented the survey cruises. NOAA conducted boreal spring and fall sections east and west of 140°W from the R/V Baldridge and R/V Discoverer. Meteorological and sea surface observations were obtained from NOAA's in place TOGA-TAO buoy network.

The scientific objectives of this study were to determine the fluxes of carbon and related elements, and the processes controlling these fluxes between the Equatorial Pacific euphotic zone and the atmosphere and deep ocean. A broad overview of the program at the 140°W site is given by Murray et al. (Oceanography, 5: 134-142, 1992). A full description of the Equatorial Pacific Process Study, including the international context and the scientific results, appears in a series of Deep-Sea Research Part II special volumes:

Topical Studies in Oceanography, A U.S. JGOFS Process Study in the Equatorial Pacific (1995), Deep-Sea Research Part II, Volume 42, No. 2/3.

Topical Studies in Oceanography, A U.S. JGOFS Process Study in the Equatorial Pacific. Part 2 (1996), Deep-Sea

Research Part II, Volume 43, No. 4/6.

Topical Studies in Oceanography, A U.S. JGOFS Process Study in the Equatorial Pacific (1997), Deep-Sea Research Part II, Volume 44, No. 9/10.

Topical Studies in Oceanography, The Equatorial Pacific JGOFS Synthesis (2002), Deep-Sea Research Part II, Volume 49, Nos. 13/14.

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Program Information

U.S. Joint Global Ocean Flux Study (U.S. JGOFS)

Website: http://usjgofs.whoi.edu/

Coverage: Global

The United States Joint Global Ocean Flux Study was a national component of international JGOFS and an integral part of global climate change research.

The U.S. launched the Joint Global Ocean Flux Study (JGOFS) in the late 1980s to study the ocean carbon cycle. An ambitious goal was set to understand the controls on the concentrations and fluxes of carbon and associated nutrients in the ocean. A new field of ocean biogeochemistry emerged with an emphasis on quality measurements of carbon system parameters and interdisciplinary field studies of the biological, chemical and physical process which control the ocean carbon cycle. As we studied ocean biogeochemistry, we learned that our simple views of carbon uptake and transport were severely limited, and a new "wave" of ocean science was born. U.S. JGOFS has been supported primarily by the U.S. National Science Foundation in collaboration with the National Oceanic and Atmospheric Administration, the National Aeronautics and Space Administration, the Department of Energy and the Office of Naval Research. U.S. JGOFS, ended in 2005 with the conclusion of the Synthesis and Modeling Project (SMP).

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