# Bacteria abundance and thymidine/leucine incorporation from R/V Thomas G. Thompson TT043, TT045, TT049, TT054 cruises in the Arabian Sea in 1995 (U.S. JGOFS Arabian Sea project)

**Website**: https://www.bco-dmo.org/dataset/2518
**Version**: final
**Version Date**: 2002-02-27

**Project**
» U.S. JGOFS Arabian Sea (Arabian Sea)

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

| Contributors | Affiliation | Role |
|---|---|---|
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| Smith, David C. | University of California-San Diego (UCSD-SIO) | Co-Principal Investigator |
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## Table of Contents



## Dataset Description

Bacteria abundance and thymidine/leucine incorporation

### Methods & Sampling

See Platform deployments for cruise specific documentation

### Data Processing Description

# Methods for the bacterial component of US JGOFS Arabian Sea Process 1 and Process 7 Cruises

Bacterial Abundance

Bacterial abundance was determined by epifluorescence microscopy of

acridine orange- stained bacteria on Nuclepore filters ( Hobbie et al. 1977 ). Samples of between 50 to 100 ml of seawater for bacteria counts were fixed with 0.2 m-filtered, EM-grade glutaraldehyde (1.25% final concentration; Ted Pella). Subsamples of 2 to 50 ml were vacuum filtered (< 150 mm Hg) onto 0.2 m pore size, black, polycarbonate membrane filters (Nuclepore). During filtration, when a sample volume had been reduced to 2 ml, 200 l of an 0.2 m-filtered acridine orange working solution (0.05%) was added to the sample in the tower for a final concentration of 0.005%. Filtration rate was kept low enough to allow at least 5 minutes of contact with the stain before filtration was complete. Blanks were 0.2 m-filtered seawater or deionized, Milli-Q-treated water fixed and processed in the same manner as the samples. After filtration, filters were transferred quickly (while still moist) onto fogged glass slides. Before the filters could dry, a coverslip with a drop of paraffin oil on the underside was placed over the filter. Slides were stored at approximately 4 degrees C for less than 1 day, then transferred to a -20 degrees C freezer for longer term storage. Bacteria were counted at 1250' magnification by epifluorescence microscopy with an oil immersion objective. Each sample was counted until either 20 fields or 200 cells had been enumerated.

Bacterial Production

Bacterial production was estimated using both the 3H-thymidine ( Fuhrman and Azam 1980; Fuhrman and Azam 1982 ) and the 3H-leucine (Kirchman et al. 1985 ) incorporation methods as modified for processing by microcentrifugation by Smith and Azam (1992) . Aliquots of seawater (1.7 ml) were incubated with 20 nM 3H-leucine or 3H-thymidine in the dark. The length of incubation ranged from 1 to 6 hours depending on the depth from which the sample was collected. The incubations were terminated with the addition of trichloroacetic acid (TCA; 5% v/v final). Samples which received the addition of TCA prior to the radioisotope served as blanks. The samples were centrifuged at 16000 x g for 7 min and the supernatant was removed. The pellet was washed once with 5% TCA and once with 80% ethanol. Liquid scintillation cocktail was added directly to the centrifuge tube which were then radioassayed.

References


Fuhrman, J. A. and F. Azam 1980. Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica and California. Appl. Environ. Microbiol. 39: 1085-1095.

Fuhrman, J. A. and F. Azam 1982. Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. Mar. Biol. 66: 109-120.

Hobbie, J. E., R. J. Daley and S. Jasper 1977. Use of nuclepore filters for counting bacteria by epifluorescence microscopy. Appl. Environ. Microbiol. 33: 1225-1228.

Kirchman, D., E. K'Nees and R. Hodson 1985. Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. Appl. Environ. Microbiol. 49: 599-607.

Smith, D. C. and F. Azam 1992. A simple, economical method for measuring bacterial protein synthesis rates in seawater using 3H-leucine. Mar. Microb. Food Webs. 6: 107- 114.


[ table of contents | back to top ]

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## Parameters

| Parameter | Description | Units |
|---|---|---|
| event | event number, from event log | YYYYMMDD |
| sta | station number, from event log | dimensionless |
| sta_std | Arabian Sea standard station identifier | dimensionless |
| cast | CTD cast number, from event log consecutive within station | dimensionless |
| bot | CTD rosette bottle number | dimensionless |
| press | sample depth | decibars |
| bact_het_orig | heterotrophic bacteria abundance, original units; microscopy | cells/liter *10^9 |
| bact_het_se | standard error for heterotrophic bacteria cell counts; original units | cells/liter *10^9 |
| bact_het_mic | heterotrophic bacteria abundance; DMO converted units, microscopy | cells/milliliter |
| thy_incorp | thymidine incorporation | picomoles/liter/hr |
| thy_sd | standard deviation thymidine incorporation | picomoles/liter/hr |
| leuc_incorp | leucine incorporation | picomoles/liter/hr |
| leuc_sd | standard deviation leucine incorporation | picomoles/liter/hr |

[ table of contents | back to top ]

## Instruments

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|---|---|
| **Dataset-specific Instrument Name** | Niskin Bottle |
| **Generic Instrument Name** | Niskin bottle |
| **Dataset-specific Description** | Niskin bottles were mounted on the CTD rosette. |
| **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. |

[ table of contents | back to top ]

## Deployments

**TT043**

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| **Website** | https://www.bco-dmo.org/deployment/57704 |
| **Platform** | R/V Thomas G. Thompson |
| **Report** | http://osprey.bcodmo.org/datasetDeployment.cfm?ddid=2580&did=353&flag=view |
| **Start Date** | 1995-01-08 |
| **End Date** | 1995-02-05 |
| **Description** | Purpose: Process Cruise #1 (Late NE Monsoon)<br><br>**Methods & Sampling**<br>PI: Farooq Azam and David Smith of: Scripps Institute of Oceanography dataset: Bacteria abundance and thymidine/leucine incorporation dates: January 09, 1995 to January 31, 1995 location: N: 22.483 S: 10 W: 57.2999 E: 68.75 project/cruise: Arabian Sea/TTN-043 - Process Cruise 1 (Late NE Monsoon) ship: Thomas Thompson Sampling Seawater samples (250 ml) were taken from the Niskin bottles mounted on the CTD rosette and transferred directly into brown high-density polyethylene bottles. The bottles were maintained within 2 degrees C of the in situ temperature until the start of the incubations or fixing.<br><br>**Processing Description**<br>Methods for the bacterial component of US JGOFS Arabian Sea Process 1 and Process 7 Cruises Bacterial Abundance Bacterial abundance was determined by epifluorescence microscopy of acridine orange- stained bacteria on Nuclepore filters ( Hobbie et al. 1977 ). Samples of between 50 to 100 ml of seawater for bacteria counts were fixed with 0.2 m-filtered, EM-grade glutaraldehyde (1.25% final concentration; Ted Pella). Subsamples of 2 to 50 ml were vacuum filtered (< 150 mm Hg) onto 0.2 m pore size, black, polycarbonate membrane filters (Nuclepore). During filtration, when a sample volume had been reduced to 2 ml, 200 l of an 0.2 m-filtered acridine orange working solution (0.05%) was added to the sample in the tower for a final concentration of 0.005%. Filtration rate was kept low enough to allow at least 5 minutes of contact with the stain before filtration was complete. Blanks were 0.2 m-filtered seawater or deionized, Milli-Q-treated water fixed and processed in the same manner as the samples. After filtration, filters were transferred quickly (while still moist) onto fogged glass slides. Before the filters could dry, a coverslip with a drop of paraffin oil on the underside was placed over the filter. Slides were stored at approximately 4 degrees C for less than 1 day, then transferred to a -20 degrees C freezer for longer term storage. Bacteria were counted at 1250' magnification by epifluorescence microscopy with an oil immersion objective. Each sample was counted until either 20 fields or 200 cells had been enumerated. Bacterial Production Bacterial production was estimated using both the 3H-thymidine ( Fuhrman and Azam 1980; Fuhrman and Azam 1982 ) and the 3H-leucine (Kirchman et al. 1985 ) incorporation methods as modified for processing by microcentrifugation by Smith and Azam (1992) . Aliquots of seawater (1.7 ml) were incubated with 20 nM 3H-leucine or 3H-thymidine in the dark. The length of incubation ranged from 1 to 6 hours depending on the depth from which the sample was collected. The incubations were terminated with the addition of trichloroacetic acid (TCA; 5% v/v final). Samples which received the addition of TCA prior to the radioisotope served as blanks. The samples were centrifuged at 16000 x g for 7 min and the supernatant was removed. The pellet was washed once with 5% TCA and once with 80% ethanol. Liquid scintillation cocktail was added directly to the centrifuge tube which were then radioassayed. References Fuhrman, J. A. and F. Azam 1980. Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica and California. Appl. Environ. Microbiol. 39: 1085-1095. Fuhrman, J. A. and F. Azam 1982. Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. Mar. Biol. 66: 109-120. Hobbie, J. E., R. J. Daley and S. Jasper 1977. Use of nuclepore filters for counting bacteria by epifluorescence microscopy. Appl. Environ. Microbiol. 33: 1225-1228. Kirchman, D., E. K'Nees and R. Hodson 1985. Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. Appl. Environ. Microbiol. 49: 599-607. Smith, D. C. and F. Azam 1992. A simple, economical method for measuring bacterial protein synthesis rates in seawater using 3H-leucine. Mar. Microb. Food Webs. 6: 107- 114. |

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| **Website** | https://www.bco-dmo.org/deployment/57706 |
| **Platform** | R/V Thomas G. Thompson |
| **Start Date** | 1995-03-14 |
| **End Date** | 1995-04-10 |
| **Description** | **Methods & Sampling**<br>PI-provided images: Surface leucine incorporation Surface thymidine incorporation Surface bacterial abundance Surface mean cell volume PI: Hugh Ducklow of: Virginia Institute of Marine Science dataset: Bacterial abundance, cell volume, thymidine and leucine incorporation dates: March 15, 1995 to April 07, 1995 location: N: 22.4858 S: 9.9993 W: 57.3007 E: 68.7532 project/cruise: Arabian Sea/TTN-045 Process Cruise 2 (Spring Intermonsoon) ship: R/V Thomas Thompson<br><br>**Processing Description**<br>Methods: Bacteria were enumerated essentially as described in our EqPac data documentation and the article in the EQPAC I DSR volume. Multiple images of all samples were acquired, processed and analyzed with Zeiss software. We used a new algorithm which removed most, but possibly not all of the prochlorophytes, so these data represent heterotrophic bacterial counts only. We will be comparing samples with Rob Olson and David Caron to constrain the bacteria counts. We also include estimates of the mean volume of cells in each sample. Bacterial abundance times cell volume yields total biovolume per liter, an index of bacterial biomass, to which carbon per unit volume factors can (and will) be applied. All raw images have been archived and can be made available by separate arrangement. Bacterial production was estimated by the incorporation of 3H-thymidine and 3H-leucine into cold, 5% TCA-insoluble extracts, essentially as described in our earlier JGOFS work, although using the new method of Smith & Azam (see the Azam data documentation for Process-1). Production estimates are forthcoming. Analytical precision for bacterial abundance and cell volume. We do not routinely perform replicate determinations of bacterial counts. However, reanalysis of 92 slides from the March series gave the following coefficients of variation (sd/mean) for bacterial abundance and cell volume: property mean st.dev. CV (%) n - - - - - - - - - - - - - abundance* 0.53 0.061 11.7 92 cell volume 0.037 0.006 16.4 92 - - - - - - - - - - - - - *units for abundance are x 10^9 cells/liter. volumes are um3/cell; these are mean values for 92 replicate determinations of each property. Hugh Ducklow tel 804-642-7180 Virginia Institute of Marine Sciences fax 804-642-7293 Box 1346 email duck@vims.edu Gloucester Point, VA 23062-1346 |

**TT049**

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| **Website** | https://www.bco-dmo.org/deployment/57710 |
| **Platform** | R/V Thomas G. Thompson |
| **Start Date** | 1995-07-17 |
| **End Date** | 1995-08-15 |
| **Description** | **Methods & Sampling**<br>PI: Hugh Ducklow of: Virginia Institute of Marine Science dataset: Bacterial abundance, cell volume, thymidine & leucine incorporation dates: July 18, 1995 to August 13, 1995 location: N: 22.5268 S: 9.911 W: 57.2997 E: 68.7507 project/cruise: Arabian Sea /TTN-049 Process cruise 4 (Middle SW Monsoon) ship: R/V Thomas Thompson<br><br>**Processing Description**<br>Hugh Ducklow's notes on methodology Comments: Incorporation data are only available for stations 13 - 30 as Omani customs would not allow our isotopes into the country. Methods: Bacteria were enumerated essentially as described in our EqPac data documentation and the article in the EQPAC I DSR volume. Multiple images of all samples were acquired, processed and analyzed with Zeiss software. We used a new algorithm which removed most, but possibly not all of the prochlorophytes, so these data represent heterotrophic bacterial counts only. We will be comparing samples with Rob Olson and David Caron to constrain the bacteria counts. We also include estimates of the mean volume of cells in each sample. Bacterial abundance times cell volume yields total biovolume per liter, an index of bacterial biomass, to which carbon per unit volume factors can (and will) be applied. All raw images have been archived and can be made available by separate arrangement. Bacterial production was estimated by the incorporation of 3H-thymidine and 3H-leucine into cold, 5% TCA-insoluble extracts, essentially as described in our earlier JGOFS work, although using the new method of Smith & Azam (see the Azam data documentation for Process-1). Production estimates are forthcoming. Analytical precision for bacterial abundance and cell volume. We do not routinely perform replicate determinations of bacterial counts. However, reanalysis of 92 slides from the March series gave the following coefficients of variation (sd/mean) for bacterial abundance and cell volume: property mean st.dev. CV (%) n - - - - - - - - - - - - - abundance* 0.53 0.061 11.7 92 cell volume 0.037 0.006 16.4 92 - - - - - - - - - - - - - *units for abundance are x 10^9 cells/liter. volumes are um3/cell; these are mean values for 92 replicate determinations of each property. Hugh Ducklow tel 804-642-7180 Virginia Institute of Marine Sciences fax 804-642-7293 Box 1346 email duck@vims.edu Gloucester Point, VA 23062-1346 |

**TT054**

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| **Website** | https://www.bco-dmo.org/deployment/57715 |
| **Platform** | R/V Thomas G. Thompson |
| **Start Date** | 1995-11-30 |
| **End Date** | 1995-12-28 |

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|---|---|
| **Description** | **Methods & Sampling**<br>PI: Farooq Azam and David Smith of: Scripps Institute of Oceanography dataset: Bacteria abundance and thymidine/leucine incorporation dates: December 01, 1995 to December 26, 1995 location: N: 22.5011 S: 9.9789 W: 57.302 E: 68.7849 project/cruise: Arabian Sea/TTN-054 - Process Cruise 7 (Early NE Monsoon) ship: Thomas Thompson Sampling Seawater samples (250 ml) were taken from the Niskin bottles mounted on the CTD rosette and transferred directly into brown high-density polyethylene bottles. The bottles were maintained within 2 degrees C of the in situ temperature until the start of the incubations or fixing.<br><br>**Processing Description**<br>Methods for the bacterial component of US JGOFS Arabian Sea Process 1 and Process 7 Cruises Bacterial Abundance Bacterial abundance was determined by epifluorescence microscopy of acridine orange- stained bacteria on Nuclepore filters ( Hobbie et al. 1977 ). Samples of between 50 to 100 ml of seawater for bacteria counts were fixed with 0.2 m-filtered, EM-grade glutaraldehyde (1.25% final concentration; Ted Pella). Subsamples of 2 to 50 ml were vacuum filtered (< 150 mm Hg) onto 0.2 m pore size, black, polycarbonate membrane filters (Nuclepore). During filtration, when a sample volume had been reduced to 2 ml, 200 l of an 0.2 m-filtered acridine orange working solution (0.05%) was added to the sample in the tower for a final concentration of 0.005%. Filtration rate was kept low enough to allow at least 5 minutes of contact with the stain before filtration was complete. Blanks were 0.2 m-filtered seawater or deionized, Milli-Q-treated water fixed and processed in the same manner as the samples. After filtration, filters were transferred quickly (while still moist) onto fogged glass slides. Before the filters could dry, a coverslip with a drop of paraffin oil on the underside was placed over the filter. Slides were stored at approximately 4 degrees C for less than 1 day, then transferred to a -20 degrees C freezer for longer term storage. Bacteria were counted at 1250' magnification by epifluorescence microscopy with an oil immersion objective. Each sample was counted until either 20 fields or 200 cells had been enumerated. Bacterial Production Bacterial production was estimated using both the 3H-thymidine ( Fuhrman and Azam 1980; Fuhrman and Azam 1982 ) and the 3H-leucine (Kirchman et al. 1985 ) incorporation methods as modified for processing by microcentrifugation by Smith and Azam (1992) . Aliquots of seawater (1.7 ml) were incubated with 20 nM 3H-leucine or 3H-thymidine in the dark. The length of incubation ranged from 1 to 6 hours depending on the depth from which the sample was collected. The incubations were terminated with the addition of trichloroacetic acid (TCA; 5% v/v final). Samples which received the addition of TCA prior to the radioisotope served as blanks. The samples were centrifuged at 16000 x g for 7 min and the supernatant was removed. The pellet was washed once with 5% TCA and once with 80% ethanol. Liquid scintillation cocktail was added directly to the centrifuge tube which were then radioassayed. References Fuhrman, J. A. and F. Azam 1980. Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica and California. Appl. Environ. Microbiol. 39: 1085-1095. Fuhrman, J. A. and F. Azam 1982. Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. Mar. Biol. 66: 109-120. Hobbie, J. E., R. J. Daley and S. Jasper 1977. Use of nuclepore filters for counting bacteria by epifluorescence microscopy. Appl. Environ. Microbiol. 33: 1225-1228. Kirchman, D., E. K'Nees and R. Hodson 1985. Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. Appl. Environ. Microbiol. 49: 599-607. Smith, D. C. and F. Azam 1992. A simple, economical method for measuring bacterial protein synthesis rates in seawater using 3H-leucine. Mar. Microb. Food Webs. 6: 107- 114. |

[ table of contents | back to top ]

## Project Information

**U.S. JGOFS Arabian Sea (Arabian Sea)**

**Website**: http://usjgofs.whoi.edu/research/arabian.html

**Coverage**: Arabian Sea

The U.S. Arabian Sea Expedition which began in September 1994 and ended in January 1996, had three major components: a U.S. JGOFS Process Study, supported by the National Science Foundation (NSF); Forced Upper Ocean Dynamics, an Office of Naval Research (ONR) initiative; and shipboard and aircraft measurements supported by the National Aeronautics and Space Administration (NASA). The Expedition consisted of 17 cruises aboard the R/V Thomas Thompson, year-long moored deployments of five instrumented surface buoys and five sediment-trap arrays, aircraft overflights and satellite observations. Of the seventeen ship cruises, six were allocated to repeat process survey cruises, four to SeaSoar mapping cruises, six to mooring and benthic work, and a single calibration cruise which was essentially conducted in transit to the Arabian Sea.

[ table of contents | back to top ]

## 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).

[ table of contents | back to top ]

## Funding


| Funding Source | Award |
|---|---|
| National Science Foundation (NSF) | unknown Arabian Sea NSF |


[ table of contents | back to top ]