

# Bacteria, Microzooplankton/Phytoplankton enumeration, production, grazing and growth from R/V Tangaroa cruise 61TG\_3052 in the Southern Ocean in 1999 (SOIREE project)

Website: <https://www.bco-dmo.org/dataset/2845>

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## Project

» [Southern Ocean Iron Release Experiment](#) (SOIREE)

## Program

» [Iron Synthesis](#) (FeSynth)

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## Dataset Description

SOIREE Bacterial Microzoop/Phytoplankton Enumeration, Production, Gazing and Growth

## Methods & Sampling

See [SOIREE Preliminary Voyage Report](#)

## Data Processing Description

See [SOIREE Preliminary Voyage Report](#)

## Bacteria enumeration

Bacterial abundance was determined by Flow Cytometry using a FACScan (15mW air-cooled argon-ion laser fixed at 488 nm) instrument (Becton Dickinson, Mountain View, Calif.) with CellQuest Vers. 3.1f software. The sheath fluid was 0.1 µm filtered sea water and the analysed volume was calculated using Trucount™ (Becton Dickinson, Mountain View, Calif.) beads as a tracer. The samples were frozen in liquid nitrogen and stained using SYBR11 stain (Molecular Probes Inc.) at a concentration of 10<sup>-4</sup> of stock solution and then incubated in

the dark for 10-15 mins before being analysed following the methods described in Lebaron et. al., (1998). Just prior to analysis, 100ml of Trucount™ beads were added to each sample as a tracer. Samples were run on the low flow setting (~12 µl/min) with a minimum of 10,000 counts per sample. The bacterial population was identified using a FL1 (green light, 530/30 BP filter, log scale) vs FSC (Cell size, linear scale) scatter plot. FL1 was set to a voltage of 520 with an amp gain of 1.0. FSC was set to the E02 voltage setting with an amp gain of 7.37. Cell carbon for bacteria was estimated to be 12.4 fg C per cell as reported by Fukuda et. al., (1998). Flow cytometric counts of both bacteria were verified by direct counts using epifluorescent microscopy following Hobbie et. al., (1977). The variations between cytometric counts and microscopy counts were within the range reported by both Lebaron et. al., (1998) and Del Giorgio et. al., (1996).

### **Eukaryotic picophytoplankton enumeration**

Eukaryotic picophytoplankton abundance was determined by Flow Cytometry using a FACScan (15mW air-cooled argon-ion laser fixed at 488 nm) instrument (Becton Dickinson, Mountain View, Calif.) with CellQuest Vers. 3.1f software. The sheath fluid was 0.1 µm filtered sea water and the analysed volume was calculated using Trucount™ (Becton Dickinson, Mountain View, Calif.) beads as a tracer. The samples were frozen in liquid nitrogen (Lebaron et. al., 1998) and were thawed immediately before counting and 50ml of Trucount™ beads were added as a tracer. Samples were run on the FACScan at Hi flow setting (~60 µl/min) with a minimum of 1500 counts per sample following methods based on Corzo et. al. (1999) and Li (1989). Eukaryotic picophytoplankton numbers were then determined under FL1 vs FL2 (orange/red light, 585/42 BP filter). FL1 was set to a voltage of 520 with an amp gain of 1.0. FL2 was set to a voltage of 420 with an amp gain of 1.0. To remove background electronic noise FL2 was also set at a threshold of 52. Cell carbon for <2 µm eukaryotic picophytoplankton was determined by assessing average spherical diameter by direct microscope observation using epifluorescence. This was then converted following Booth (1988) conversion for small phytoplankton (<4 µm) using 220 fg C µm<sup>3</sup> to yield a factor of 920 fg C per eukaryotic picophytoplankton. Flow cytometric counts of eukaryotic picophytoplankton were verified by direct counts using epifluorescent microscopy following Hall (1991). The variations between cytometric counts and microscopy counts were within the range reported by both Lebaron et. al., (1998) and Del Giorgio et. al., (1996).

### **Bacterial Production**

Heterotrophic bacterial productivity was measured using (methyl-3H) thymidine as detailed in Smith & Hall (1997). Incubations were conducted at in situ surface temperatures. The extraction procedure followed Wicks & Robarts (1987) modified TCA precipitate method, which involved rinsing the TCA precipitate with phenol-chloroform followed by ethanol. Tritium incorporation was determined with a liquid scintillation counter (LKB, 1217 Rackbeta) using OptiPhase HiSafe 3 (Wallac) as the scintillation fluor. Counts were corrected for quench by external standards. In order to estimate bacterial production we converted mol thymidine to g C using Fuhrman & Azam's (1982) conversion factor of 2.4 x 10<sup>18</sup> cells per mol thymidine incorporated, and Lee & Fuhrman's (1987) estimate of 20 x 10<sup>-15</sup> g C cell<sup>-1</sup>.

### **Nanoflagellate enumeration**

Samples collected for nanoflagellate enumeration were size fractionated through a 20 µm nylon mesh. The filtrate was then fixed 1:1 with ice cold glutaraldehyde (2% final concentration) for 1 hour (Sanders et. al., 1989). Fixed samples were filtered onto prestained 0.8 µm black Nuclepore filters, stained for five minutes with 2 ml primulin, rinsed with 2 ml Tris HCL, mounted on slides and stored frozen (Bloem et. al., 1986). Nanoflagellates were counted under UV excitation using a Leica compound microscope (BP 450-490 nm excitation, LP 520 barrier filter, FT 510 dichromatic beam splitter). Nanophytoflagellates were differentiated by chlorophyll a fluorescing red under blue light excitation (BP 450-490 excitation, LP 515 barrier filter, RPK 510 dichromatic beam splitter). Forty randomly selected fields were counted per filter. Nanoflagellate biovolumes were calculated using dimensions and approximated geometric shape (Chang 1988). Biovolumes were calculated from measurements on a minimum of 200 cells, collected at 20 m for each sampling. Cell carbon for phyto and heterotrophic nanoflagellate biomass was estimated to be 0.24 pg C per µm<sup>-3</sup> as reported by Verity et. al., (1992) for nanophytoflagellates.

### **Ciliate enumeration**

Samples for enumeration of ciliates were preserved in 1% Lugol's iodine. Samples were left to settle for 48 h and the supernatant removed. The remaining sample was transferred to a 25 ml Utermohl chamber. The microzooplankton were identified to genus where possible and enumerated using a Wild inverted microscope (James & Hall, 1995) but with no differentiation of plastidic ciliates. Ciliate biomass was estimated from dimensions of 10 to 20 randomly chosen individuals of each taxon. The volumes

were estimated from approximate geometric shapes and were converted to carbon biomass using a factor of 0.19 pg C  $\mu\text{m}^{-3}$  (Putt & Stoecker 1989). The use of Lugol's iodine for preservation may have resulted in an underestimation of biomass due to cell shrinkage.

### Microzooplankton grazing rates and phytoplankton growth rates

Microzooplankton grazing rates and phytoplankton growth rates were determined using methods based on the dilution technique of Landry & Hassett (1982), modified following the experimental protocols described in Gallegos et. al., (1996). Water for dilution was gravity filtered at 0.2  $\mu\text{m}$  through a pre-rinsed Gelman SuporCap™ 100. Filtration of the dilution water required about 1 h. The total standing time between water collection and the start of the experiments was a maximum 2 h. In a set of 12 acid washed, 2.4-litre polycarbonate bottles, <200  $\mu\text{m}$  screened water was diluted with 0.2  $\mu\text{m}$  filtered water to concentrations of ~10%, 40%, 70% and 100% (i.e. undiluted). The screening of the water at 200  $\mu\text{m}$  removed up to 40% of the phytoplankton particularly towards the end of the experiment when chains of large diatoms had formed. All bottles were then placed in an on deck incubator, with continuous sea water supply and covered with shade cloth that transmitted ~40% of the incident light, simulating ambient conditions. Incubations for all experiments were conducted in triplicate bottles for 24 h.

The dilution factor for each bottle was calculated by taking sub-samples for <200  $\mu\text{m}$  chlorophyll a at T0 and measuring the actual percentage of <200  $\mu\text{m}$  chlorophyll a in each treatment. Size fractionated chlorophyll a and picophytoplankton sub-samples were also taken from experiments at T24 from all dilutions, but only from 100% undiluted water at T0. To determine growth and grazing rates on phytoplankton, size fractionated chlorophyll a and picophytoplankton at T0 were then estimated for each dilution using the calculated dilution. In addition, to determine growth rate of grazers, we measured heterotrophic nanoflagellate and ciliate in only 100% undiluted water at T0 and T24.

Chlorophyll a was measured by filtering 500 mls of sample through a Whatman GF/F filter. Size fractionated chlorophyll a samples were collected using prefiltration at 20 and 2  $\mu\text{m}$ . The filters were stored frozen until analysed spectrofluorometrically on a Perkin-Elmer LS 50B (Strickland & Parsons 1972).

### BCO-DMO Processing Notes

Generated from original file Microbiology.xls provided on the Deep-Sea Research II 48 (2001) accompanying CD-Rom

### BCO-DMO Edits

- parameter names modified to conform to BCO-DMO convention
- date reformatted to YYYYMMDD
- Station Number changed to station
- added 'T' to CTD Station number for compatibility with events in other spreadsheets
- blank rows in original sheet removed
- 'nd' added to blank cells or cells with '-' in original file
- added Patch Location column and removed individual rows with 'Outside' and 'Inside'
- date\_UTC, time\_UTC, date\_local, time\_local, timezone, lon and lat added from CTD sampling spreadsheet

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## Data Files

File
<b>microbiology.csv</b> (Comma Separated Values (.csv), 14.50 KB) MD5:fdcaa4e0182b103017264b09e98c5cba
Primary data file for dataset ID 2845

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## Parameters

<b>Parameter</b>	<b>Description</b>	<b>Units</b>
station	Cruise Station Id	meters
Day	Experiment Day	integer
Patch	Patch Location (In/Out)	text
depth	Sample depth	meters
Bacteria	Bacteria	Cells ml-1
Biomass_Bacterial	Biomass Bacterial	ugC/ L
Pico_Ekary	Pico Ekary	Cells ml-1
Biomass_Pico_Ekary	Biomass Pico Ekary	ugC/ L
Bacterial_Prod	Bacterial Prod	ug C l-1 h-1
Biomass_Aut_Flag	Biomass Aut Flag	ugC/ L
Biomass_Het_Flag	Biomass Het Flag	ugC/ L
MeanBiovolume_Aut_Flag	MeanBiovolume Aut Flag	um <sup>3</sup>
MeanBiovolume_Het_Flag	MeanBiovolume Het Flag	um <sup>3</sup>
Aut_Flag	Aut Flag	Cells ml-1
Het_Flag	Het Flag	Cells ml-1
Aut_to_Het	Ratio of Aut to Het	dimensionless
Non_Cil	Non Cil	No's / L
Non_Lor_lt_20um	Non Lor	No's / L
Biomass_Non_Lor_lt_20um	Biomass Non Lor	ugC/ L

Non_Lor_gt_20um	Non Lor > 20um	No's / L
Biomass_Non_Lor_gt_20um	Biomass Non Lor > 20um	ugC/ L
Tintinnids	Tintinnids	No's / L
Biomass_Tintinnid	Biomass Tintinnid	ugC/ L
Biomass_Total_Ciliate	Biomass Total Ciliate	ugC/ L
Total_Ciliates	Total Ciliates	No's / L
Biomass_Total_Microzoop	Biomass Total Microzoop	ug/ L
date.UTC	UTC Date	YYYYMMDD
date_local	local date	YYYYMMDD
lat	latitude, negative denotes South	decimal degrees
lon	longitude, negative denotes West	decimal degrees
timezone	local time zone, +/- from GMT	integer
time.UTC	UTC time	HHMM
time_local	local time	HHMM

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## Deployments

61TG\_3052

<b>Website</b>	<a href="https://www.bco-dmo.org/deployment/57827">https://www.bco-dmo.org/deployment/57827</a>
<b>Platform</b>	R/V Tangaroa
<b>Report</b>	<a href="http://bcodata.whoi.edu/Fe_Synthesis/SOIREE/SOIREE_cruisereport.pdf">http://bcodata.whoi.edu/Fe_Synthesis/SOIREE/SOIREE_cruisereport.pdf</a>
<b>Start Date</b>	1999-01-31
<b>End Date</b>	1999-03-01
<b>Description</b>	Cruise to the Southern Ocean as part of the Fe Sythesis project whose aim was to maintain a coherent patch of iron-enriched seawater for the duration of SOIREE and to interpret any iron-mediated effects on the patch by conducting measurements and performing experiments during this period.

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## Project Information

### Southern Ocean Iron Release Experiment (SOIREE)

**Coverage:** Southern Ocean

Project in the Southern Ocean aimed at maintaining a coherent patch of iron-enriched seawater for the duration of project and to interpret any iron-mediated effects on the patch by conducting measurements and performing experiments during this period of the project.

The Southern Ocean Iron RElease Experiment (SOIREE), was the first in situ iron fertilization experiment performed in the polar waters of the Southern Ocean. SOIREE was an interdisciplinary study involving participants from six countries, and took place in February 1999 south of the Polar Front in the Australasian-Pacific sector of the Southern Ocean.

Approximately 3800 kg of acidified FeSO<sub>4</sub>.7H<sub>2</sub>O and 165 g of the tracer sulphur hexafluoride (SF<sub>6</sub>) were added to a 65-m deep surface mixed layer over an area of ~50 km<sup>2</sup>. Initially, mean dissolved iron concentrations were ~2.7 nM, but decreased to ambient levels within days, requiring subsequent additions of 1550-1750 kg of acidified FeSO<sub>4</sub>.7H<sub>2</sub>O on days 3, 5 and 7 of the experiment.

During the 13-day site occupation, there were iron-mediated increases in phytoplankton growth rates, with marked increases in chlorophyll a (up to 2 µg l<sup>-1</sup>) and production rates (up to 1.3 gCm<sup>-2</sup>d<sup>-1</sup>). These resulted in subsequent changes in the pelagic ecosystem structure, and in the cycling of carbon, silica and sulphur, such as a 10% drawdown of surface CO<sub>2</sub>.

The SOIREE bloom persisted for >40 days following our departure from the site, as observed via [SeaWiFS remotely sensed observations of Ocean Colour](#).

#### BCO-DMO Note:

All original data and metadata provided on a CD-Rom accompanying the Deep-Sea Research II 48 (2001) volume. The CD-Rom contains the main SOIREE datasets and ancillary information including the pre-experiment 'desktop' database study for site-selection, and satellite images of the SOIREE bloom.

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## Related files

[SOIREE Preliminary Voyage Report](#)

[SOIREE Introduction and Summary, Deep-Sea Research II 48 \(2001\) 2425-2438](#)

[SOIREE Cruise Track](#)

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## Program Information

### Iron Synthesis (FeSynth)

**Coverage:** Global

The two main objectives of the Iron Synthesis program (SCOR Working Group proposal, 2005), are:

1. Data compilation: assembling a common open-access database of the *in situ* iron experiments, beginning with the first period (1993-2002; Ironex-1, Ironex-2, SOIREE, EisenEx, SEEDS-1; SOFeX, SERIES) where primary articles have already been published, to be followed by the 2004 experiments where primary articles are now in progress (EIFEX, SEEDS-2; SAGE, FeeP); similarly for the natural fertilizations S.O.JGOFS (1992), CROZEX (2004/2005) and KEOPS (2005).

2. Modeling and data synthesis of specific aspects of two or more such experiments for various topics such as physical mixing, phytoplankton productivity, overall ecosystem functioning, iron chemistry, CO<sub>2</sub> budgeting, nutrient uptake ratios, DMS(P) processes, and combinations of these variables and processes.

SCOR Working Group proposal, 2005. "The Legacy of *in situ* Iron Enrichments: Data Compilation and Modeling".

[http://www.scor-int.org/Working\\_Groups/wg131.htm](http://www.scor-int.org/Working_Groups/wg131.htm)

See also: SCOR Proceedings Vol. 42 Concepcion, Chile October 2006, pgs: 13-16 2.3.3 Working Group on The Legacy of *in situ* Iron Enrichments: Data Compilation and Modeling.

The first objective of the Iron Synthesis program involves a data recovery effort aimed at assembling a common, open-access database of data and metadata from a series of *in-situ* ocean iron fertilization experiments conducted between 1993 and 2005. Initially, funding for this effort is being provided by the Scientific Committee on Oceanic Research (SCOR) and the U.S. National Science Foundation (NSF).

Through the combined efforts of the principal investigators of the individual projects and the staff of Biological and Chemical Oceanography Data Management Office (BCO-DMO), data currently available primarily through individuals, disparate reports and data agencies, and in multiple formats, are being collected and prepared for addition to the BCO-DMO database from which they will be freely available to the community.

As data are contributed to the BCO-DMO office, they are organized into four overlapping categories:

1. Level 1, basic metadata  
(e.g., description of project/study, general location, PI(s), participants);
2. Level 2, detailed metadata and basic shipboard data and routine ship's operations  
(e.g., CTDs, underway measurements, sampling event logs);
3. Level 3, detailed metadata and data from specialized observations  
(e.g., discrete observations, experimental results, rate measurements) and
4. Level 4, remaining datasets  
(e.g., highest level of detailed data available from each study).

Collaboration with BCO-DMO staff began in March of 2008 and initial efforts have been directed toward basic project descriptions, levels 1 and 2 metadata and basic data, with detailed and more detailed data files being incorporated as they become available and are processed.

### Related file

[Program Documentation](#)

The Iron Synthesis Program is funded jointly by the Scientific Committee on Oceanic Research (SCOR) and the U.S. National Science Foundation (NSF).



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