

# Epifluorescence Microscopy Water Column Samples from R/V Tangaroa TAN1810 in the Chatham Rise (Subtropical and Sub-Antarctic waters off of New Zealand) from October to November 2018 (Salp Food Web Ecology project)

**Website:** <https://www.bco-dmo.org/dataset/905060>

**Data Type:** Cruise Results

**Version:** 1

**Version Date:** 2023-07-24

## Project

» [Collaborative Research: Quantifying trophic roles and food web ecology of salp blooms of the Chatham Rise](#)  
(Salp Food Web Ecology)

Contributors	Affiliation	Role
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## Abstract

The structure of the phytoplankton community is crucially important to pelagic food webs, biogeochemical processes, and carbon (C) cycle. This study quantifies C-based size spectra, phytoplankton community composition across subtropical and subantarctic waters east of New Zealand. Depth-resolved water column samples were analyzed using epifluorescence microscopy at 15 different sampling locations. Samples were analyzed for biomass and abundance of microplankton ( $>20\ \mu\text{m}$ ), nanoplankton ( $2\text{--}20\ \mu\text{m}$ ) and picoplankton ( $<2\ \mu\text{m}$ ) and diatoms. Our results suggest that the subtropical waters are dominated by nanoplankton ( $35.2 \pm 4.6\ \mu\text{g C/L}$ ). Offshore subantarctic waters were dominated by picoplankton ( $24.7 \pm 2.1\ \mu\text{g C/L}$ ) while microplankton dominated in coastal subantarctic waters ( $21.7 \pm 2.2\ \mu\text{g C/L}$ ). Overall, our study helps provide important insights into the structure of phytoplankton communities, their biomass distribution and their contribution to carbon sequestration in the subtropical and subantarctic waters east of New Zealand, highlighting the dominance of nanoplankton in subtropical waters and picoplankton in offshore subantarctic waters.

## Table of Contents

- [Coverage](#)
- [Dataset Description](#)
  - [Methods & Sampling](#)
  - [Data Processing Description](#)
  - [BCO-DMO Processing Description](#)
- [Related Publications](#)
- [Related Datasets](#)
- [Parameters](#)
- [Instruments](#)
- [Deployments](#)
- [Project Information](#)
- [Funding](#)

## Coverage

**Spatial Extent:** N:180 E:-42.6621666666667 S:174.095333 W:-45.5556666666667

**Temporal Extent:** 2018-10-25 - 2018-11-18

## Methods & Sampling

## Field Collection

Data was collected in the Chatham Rise section of the Southern Ocean, located east of Aotearoa New Zealand, as part of the SALPOOP ('Salp Particle expOrt and Ocean Production') voyage during October to November 2018. ). We conducted five Lagrangian experiments (hereafter referred to as "cycles") that lasted four to eight days (Décima et al., 2023). There were three cycles that were sampled in SA waters (1, 2 and 5) and two cycles in ST waters (3 and 4) while salps were only present in three cycles (1, 2 and 4). Six depths were chosen to span the euphotic zone (based on chlorophyll fluorescence measured during the conductivity-temperature-depth (CTD) downcast profiles).

## Epifluorescence Microscopy Sampling

From each depth, two different volumes of water were sampled: 50 mL for nanoplankton- epifluorescence microscopy (filtered through a 0.8- $\mu\text{m}$  pore-size black polycarbonate filter) and 400 mL for microplankton epifluorescence microscopy (filtered through an 8- $\mu\text{m}$  pore-size filter). Utilizing two different sized filters and sampling volumes allowed for appropriate, adjustable filtered volumes and avoid overlapping cells on the slides. 20  $\mu\text{m}$  backing filters were utilized as data has indicated that they support the membrane filters and ensure even dispersal of sample on the filter (Kemp et al., 1993; Taylor et al., 2015). The samples were preserved using buffered formalin, alkaline Lugol's solution, and sodium thiosulfate then stained using proflavine and 4', 6-diamidino-2phenylindole (DAPI) (modified protocol from Sherr and Sherr, 1993 in Kemp et al. 1993). During and immediately after filtration, filters were covered with tin foil to prevent photochemical quenching. Filters were mounted onto a glass slide and frozen in a -80 $^{\circ}\text{C}$  freezer for later analysis.

## Data Processing Description

### Processing Notes from Researcher:

#### Epifluorescence Microscopy Analysis

Phytoplankton images were captured with an Olympus Microscope DP72 Camera using an Olympus BX51 fluorescence microscope. 20 images were taken under the fluorescence of FITC (green fluorescence) in order to capture the fluorescence of cell proteins. Proflavine overstained cells to the point that we were not able to confidently differentiate heterotrophic cells from autotrophic cells, therefore only biomass and abundance values were able to be calculated. A 60x magnification lens was used to image slides with 0.8  $\mu\text{m}$  filters while 20x magnification was used to image slides with 8  $\mu\text{m}$  filters. Images were then processed using the ImageJ image analysis software (v 1.52a or 1.53c). Cells were manually outlined using the freehand tool and approximate feret cell length and cell area were calculated by ImageJ in pixels and then converted to microns using a calibration scale. To avoid biasing the examined cell size, cells that were roughly greater than 50% out of frame and cells that were broken or fragmented were not included in analysis. Conversion factors were applied to account for volume filtered and percentage of the filter area analyzed. To determine the true filtration diameter, a light microscope was used to examine a 25 mm glass fiber filter (GF/F) filter that had a small amount of dyed water filtered through. It was discovered that the filter funnel blocks roughly 12% of the 25-mm filter and the filtered region had a diameter of 22 mm. Equations 1-5 show the equations used to calculate cell width, biovolume, ESD (equivalent spherical diameter) and biomass, where \* implies we assumed that cell height = cell width/2. ESD was used as a consistent measure of mean cell size since many plankton have an irregular shape. The height of a cell was assumed to be roughly equivalent to half of the cell width since cells are often flattened during filtration (Taylor et al., 2011) with the exception of diatoms. The biomass of diatoms (which were the only taxon we could conclusively identify) was estimated allometrically using equation 5 while all other cell biomass (non-diatoms) was estimated allometrically using equation 4. (Menden-Deuer and Lessard, 2000).

Equation 1: Cell width =  $(4/\pi) \times (\text{Area of the cell}/\text{Feret length of the cell})$

Equation 2: Biovolume =  $(4/3)(\pi) \times (\text{Feret Length}/2) \times (\text{Cell Width}/2) \times (\text{Cell Height}*/2)$

Equation 3: ESD =  $2 \times (3 \times \text{Biovolume}/4\pi)^{(1/3)}$

Equation 4: Biomass (non-diatoms) =  $0.216 \times \text{Biovolume}^{0.939}$

Equation 5: Biomass (diatoms) =  $0.288 \times \text{Biovolume}^{0.811}$

## BCO-DMO Processing Description

Spaces in column name headers replaced by underscores ("\_").

Dates converted from %m/%d/%y format to %Y-%m-%d format.

Latitude and longitude coordinates rounded to 6 decimal places.

[ [table of contents](#) | [back to top](#) ]

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## Related Publications

Décima, M., Stukel, M. R., Nodder, S. D., Gutiérrez-Rodríguez, A., Selph, K. E., dos Santos, A. L., Safi, K., Kelly, T. B., Deans, F., Morales, S. E., Baltar, F., Latasa, M., Gorbunov, M. Y., & Pinkerton, M. (2023). Salp blooms drive strong increases in passive carbon export in the Southern Ocean. *Nature Communications*, 14(1).

<https://doi.org/10.1038/s41467-022-35204-6>

*Methods*

Fender, C. K., Décima, M., Gutiérrez-Rodríguez, A., Selph, K. E., Yingling, N., & Stukel, M. R. (2023). Prey size spectra and predator to prey size ratios of southern ocean salps. *Marine Biology*, 170(4).

<https://doi.org/10.1007/s00227-023-04187-3>

*Results*

Kemp, P., B. F. Sherr, E. B. Sherr, and J. J. Cole (Eds.). (1993). *Handbook of methods in aquatic microbial ecology*. Lewis Publishing, Boca Raton, FL 33431, 777 pp. <https://isbnsearch.org/isbn/0-873-71564-0>

*Methods*

Menden-Deuer, S., & Lessard, E. J. (2000). Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. *Limnology and Oceanography*, 45(3), 569–579. doi:[10.4319/lo.2000.45.3.0569](https://doi.org/10.4319/lo.2000.45.3.0569)

*Methods*

Taylor, A. G., Landry, M. R., Selph, K. E., & Yang, E. J. (2011). Biomass, size structure and depth distributions of the microbial community in the eastern equatorial Pacific. *Deep Sea Research Part II: Topical Studies in Oceanography*, 58(3-4), 342–357. doi:[10.1016/j.dsr2.2010.08.017](https://doi.org/10.1016/j.dsr2.2010.08.017)

*Methods*

[ [table of contents](#) | [back to top](#) ]

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## Related Datasets

### IsSourceOf

Yingling, N., Stukel, M. R., Selph, K. E. (2023) **Reduced Epifluorescence Microscopy Water Column Samples from R/V Tangaroa TAN1810 in the Chatham Rise (Subtropical and Sub-Antarctic waters off of New Zealand) from October to November 2018 (Salp Food Web Ecology project)**. Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2023-07-26 <http://lod.bco-dmo.org/id/dataset/905170> [[view at BCO-DMO](#)]

[ [table of contents](#) | [back to top](#) ]

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## Parameters

Parameter	Description	Units
ID	Unique number given to each individual cell measured	unitless

Cycle	Lagrangian experiment number	unitless
Cast	CTD deployment number	unitless
Particle_Number	Number assigned to each particle imaged in a particular image	unitless
Filter_Pore_Size	Pore size of filters that were used to filter the sample	microns ( $\mu\text{m}$ )
Volume_Filtered	Volume of water filtered for that slide in mL	mL
Depth	Depth the sample originated in meters	meters
Lat	Latitude of sample location in decimal degrees; negative values indicates a Northern coordinate	decimal degrees
Long	Longitude of sample location in decimal degrees; a positive value indicates an Eastern coordinate	decimal degrees
Date	Date in New Zealand Standard Time	unitless
Area	Area of the cell	microns squared ( $\mu\text{m}^2$ )
Width	Width of the cell	microns ( $\mu\text{m}$ )
Feret_Length	Feret length of the cell	microns ( $\mu\text{m}$ )
Conversion_Ratio	Conversion ratio that incorporates area of the image taken and provides a single value for the ratio of cells actually counted (note: this value changes based on pore size of the filter)	unitless
Calculated_Width	Calculated width (equation one found within the data processing description of this metadata page)	microns ( $\mu\text{m}$ )
Biovolume	calculated biovolume (equation two found within the data processing description of this metadata page)	$\mu\text{m}^3$
ESD	Equivalent Spherical diameter (equation three found within the data processing description of this metadata page) in $\mu\text{m}$	microns ( $\mu\text{m}$ )

Biomass	Biomass in units of pg C (If a non-diatom, use equation four found within the data processing description of this metadata page; if diatom, use equation 5)	picogram of carbon per cell (pg C cell <sup>-1</sup> )
Final_Biomass	Biomass in units of pg C/mL (note: this value is the biomass (pg C) divided by the volume filtered (mL) and the conversion ratio)	picogram of carbon per mL (pg C/mL)
Diatoms	0 represents that this cell is not a diatom, 1 represents this cell is a diatom	unitless

[ [table of contents](#) | [back to top](#) ]

## Instruments

<b>Dataset-specific Instrument Name</b>	Olympus DP72 camera
<b>Generic Instrument Name</b>	Camera
<b>Dataset-specific Description</b>	Slides were calibrated from pixels to microns for both 0.8 µm and 8 µm to determine what length of pixels equates to µm thus only micron area, width and feret length are show in the raw datasheet. Our data also includes conversation ratios in the raw excel sheet. This ratio should be included when determining biomass in units of pg C/mL or abundance in #/mL, along with volume filter, as this ratio incorporates the area of the images taken and gives a value that states the ratio of the cells that were actually counted for 0.8 µm and 8 µm samples.
<b>Generic Instrument Description</b>	All types of photographic equipment including stills, video, film and digital systems.

<b>Dataset-specific Instrument Name</b>	Epifluorescence microscope: Olympus BX51 microscope with a Olympus DP72 camera and Exfo X-cite Series 120 mercury bulb with a FITC filter for green fluroescence
<b>Generic Instrument Name</b>	Fluorescence Microscope
<b>Dataset-specific Description</b>	Slides were calibrated from pixels to microns for both 0.8 µm and 8 µm to determine what length of pixels equates to µm thus only micron area, width and feret length are show in the raw datasheet. Our data also includes conversation ratios in the raw excel sheet. This ratio should be included when determining biomass in units of pg C/mL or abundance in #/mL, along with volume filter, as this ratio incorporates the area of the images taken and gives a value that states the ratio of the cells that were actually counted for 0.8 µm and 8 µm samples.
<b>Generic Instrument Description</b>	Instruments that generate enlarged images of samples using the phenomena of fluorescence and phosphorescence instead of, or in addition to, reflection and absorption of visible light. Includes conventional and inverted instruments.

[ [table of contents](#) | [back to top](#) ]

## Deployments

## TAN1810

<b>Website</b>	<a href="https://www.bco-dmo.org/deployment/757070">https://www.bco-dmo.org/deployment/757070</a>
<b>Platform</b>	R/V Tangaroa
<b>Start Date</b>	2018-10-23
<b>End Date</b>	2018-11-21

[ [table of contents](#) | [back to top](#) ]

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## Project Information

### **Collaborative Research: Quantifying trophic roles and food web ecology of salp blooms of the Chatham Rise (Salp Food Web Ecology)**

**Coverage:** East of New Zealand, Chatham Rise area

#### *NSF Award Abstract:*

Salps are unique open-ocean animals that range in size from a few millimeters to greater than twenty centimeters, have a gelatinous (jelly-like) body, and can form long chains of many connected individuals. These oceanic organisms act as oceanic vacuum cleaners, having incredibly high feeding rates on phytoplankton and, unusual for consumers of their size, smaller bacteria-sized prey. This rapid feeding and the salps' tendency to form dense blooms, allows them move substantial amounts of prey carbon from the surface into the deep ocean, leading to carbon dioxide removal from the atmosphere. However, salps are often considered a trophic dead-end, rather than a link, in the food web due to the assumption that they themselves are not consumed, since their gelatinous bodies are less nutritious than co-occurring crustacean prey. Along with this, salp populations are hypothesized to be increasing due to climate change. This proposal addresses these questions: 1) Do salps compete primarily with crustaceans (as in the prevailing paradigm) or are they competitors of single-celled protists, which are the dominant grazers of small phytoplankton? 2) Do salp blooms increase the efficiency of food-web pathways from tiny phytoplankton to fisheries production in nutrient-poor ocean regions?

This project will support the interdisciplinary education of a graduate student who will learn modeling and laboratory techniques in the fields of biological and chemical oceanography and stimulate international collaborations between scientists in the United States and New Zealand. Additionally, several Education and Outreach initiatives are planned, including development of a week-long immersive high school class in biological oceanography, and education modules that will serve the "scientists-in-the schools" program in Tallahassee, FL.

It is commonly assumed that salps are a trophic sink. However, this idea was developed before the discovery that protists (rather than crustaceans) are the dominant grazers in the open ocean and was biased by the difficulty of recognizing gelatinous salps in fish guts. More recent studies show that salps are found in guts of a diverse group of fish and seabirds and are a readily available prey source when crustacean abundance is low. This proposal seeks to quantify food web flows through contrasting salp-dominated and salp-absent water parcels near the Chatham Rise off western New Zealand where salp blooms are a predictable phenomenon. The proposal will leverage previously obtained data on salp abundance, bulk grazing impact, and biogeochemical significance during Lagrangian experiments conducted by New Zealand-based collaborators. The proposal will determine 1) taxon- and size-specific phytoplankton growth rate measurements, 2) taxon- and size-specific protozoan and salp grazing rate measurements, 3) compound specific isotopic analysis of the amino acids of mesozooplankton to quantify the trophic position of salps, hyperiid amphipods, and other crustaceans, 4) sediment traps to quantify zooplankton carcass sinking rates, and 5) linear inverse ecosystem modeling syntheses. Secondary production and trophic flows from this well-constrained ecosystem model will be compared to crustacean-dominated and microbial loop-dominated ecosystems in similarly characterized regions (California Current, Costa Rica Dome, and Gulf of Mexico).

This award reflects NSF's statutory mission and has been deemed worthy of support through evaluation using the Foundation's intellectual merit and broader impacts review criteria.

## Funding

Funding Source	Award
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-1756465</a>
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-1756610</a>