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)

Website: https://www.bco-dmo.org/dataset/905170
Data Type: Cruise Results
Version: 1
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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 µm), nanoplankton (2-20 µm) and picoplankton (<2 µm) and diatoms. Our results suggest that the subtropical waters are dominated by nanoplankton (35.2 ± 4.6 µg C/L). Offshore subantarctic waters were dominated by picoplankton (24.7 ± 2.1 µg C/L) while microplankton dominated in coastal subantarctic waters (21.7 ± 2.2 µg 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

Spatial Extent: **N**:180 **E**:-42.6622 **S**:174.095 **W**:-45.5557
Temporal Extent: 2018-10-21 - 2018-11-21

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-µm pore-size black polycarbonate filter) and 400 mL for microplankton epifluorescence microscopy (filtered through an 8-µm 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 µm 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 -80º C freezer for later analysis.

Data Processing Description

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) n 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 µm filters while 20x magnification was used to image slides with 8 µm 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. Conversions 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/π) x (Area of the cell/Feret length of the cell)

Equation 2: Biovolume = (4/3)(π) x (Feret Length/2) x (Cell Width/2) x (Cell Height*/2)

Equation 3: ESD = 2 x (3 x Biovolume/4π)^(1/3)

Equation 4: Biomass (non-diatoms) = 0.216 x Biovolume^0.939

Equation 5: Biomass (diatoms) = 0.288 x Biovolume^0.811

For every cycle and depth, biomass size spectra were calculated for nanoplankton and microplankton biomass. Two different pore sizes were used to capture a range of cell sizes; 0.8 µm pore size filters captured 2 to 12 µm cells and 8 µm pore size filters captured >12 µm cells. First only cells that were in the size range of 2 to 12 µm were included for the 0.8 µm samples and cells >12 µm were included for the 8 µm samples. Next, biomass was calculated into units of pg C/mL by dividing biomass by the volume filtered and the conversion ratio. Then each biomass value was separated by ESD into different size bins and summed in each size bin. Size bins were created in octave spacing, e.g. 0.25 to 0.5 µm, 0.5 to 1 µm, 1 to 2 µm, etc., where the width of a size bin would be the difference of the values, e.g. size bin of the 1 to 2 µm would be 1 µm. A similar approach was done for abundance size spectra calculations for nanoplankton and microplankton abundance. Only cells in the appropriate ranges (2-12 µm for 0.8 µm; >12 µm for 8 µm samples) were sorted by ESD into different size bins, counted and then divided by the volume filtered and the conversion ratio. Next both sample sets would be combined for each size bin to give one set of values. Cells less than 2 µm were analyzed using flow cytometry (also shown in this project dataset).

Problems/Issues

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. Several slides broke during travel in cycle 3 and 4 therefore there is data missing in depth profiles and only slides that had both 0.8 µm samples and 8 µm samples were included in the analysis. In addition, some 8 µm samples had higher biomass and slow filtration rates therefore a known amount of volume was removed from the sample to speed up filtration time and to avoid over-layering cells. This is why some volume filtering times are 325 mL in place of 400 mL. This was not an issue with 0.8 µm samples as 50 mL filtered at a normal rate for all samples.

BCO-DMO Processing Description

Rounded latitude and longitude fields to 6 decimal places.

Removed special characters from column header names; this includes periods from float numbers in column names. See parameter descriptions for full details.

Added Abundance_ and Biomass_ prefixes to relevant column header names.

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]

Related Datasets

IsDerivedFrom

Yingling, N., Selph, K. E., Stukel, M. (2023) **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 2023-07-24 http://lod.bco-dmo.org/id/dataset/905060 [view at BCO-DMO]

[table of contents | back to top]

Parameters

Parameter	Description	Units
Cycle	Lagrangian experiment number	unitless

Cast	CTD deployment number	unitless
Depth	Depth the sample originated in meters	meters
Lat	Latitude in decimal degrees; a negative value indicates a Northern coordinate	decimal degrees
Lon	Longitude in decimal degrees; a positive value indicates an Eastern coordinate	decimal degrees
Date	Date in New Zealand Standard Time	unitless
Abundance_025_to_05	Abundance of cells in the 0.25 to 0.5 µm range; blank cells represent size ranges that had no cells present	microns (µm)
Abundance_05_to_1	Abundance of cells in the 0.5 to 1 µm range; blank cells represent size ranges that had no cells present	microns (µm)
Abundance_1_to_2	Abundance of cells in the 1 to 2 µm range; blank cells represent size ranges that had no cells present	microns (µm)
Abundance_2_to_4	Abundance of cells in the 2 to 4 µm range; blank cells represent size ranges that had no cells present	microns (µm)
Abundance_4_to_8	Abundance of cells in the 4 to 8 µm range; blank cells represent size ranges that had no cells present	microns (µm)
Abundance_8_to_16	Abundance of cells in the 8 to 16 µm µm range; blank cells represent size ranges that had no cells present	microns (µm)
Abundance_16_to_32	Abundance of cells in the 16 to 32 µm µm range; blank cells represent size ranges that had no cells present	microns (µm)
Abundance_32_to_64	Abundance of cells in the 32 to 64 µm range; blank cells represent size ranges that had no cells present	microns (µm)
Abundance_64_to_128	Abundance of cells in the 64 to 128 µm range; blank cells represent size ranges that had no cells present	microns (µm)
Abundance_128_to_256	Abundance of cells in the 128 to 256 µm range; blank cells represent size ranges that had no cells present	microns (µm)
Abundance_256_to_512	Abundance of cells in the 256 to 512 µm range; blank cells represent size ranges that had no cells present	microns (µm)
Abundance_512_to_1024	Abundance of cells in the 512 to 1024 µm range; blank cells represent size ranges that had no cells present	microns (µm)
Abundance_1024_to_2048	Abundance of cells in the 1024 to 2048 µm range; blank cells represent size ranges that had no cells present	microns (µm)
Biomass_025_to_05	Biomass of cells in the 0.25 to 0.5 µm range; blank cells represent size ranges that had no cells present	picogram of carbon per mL (pg C/mL)
Biomass_05_to_1	Biomass of cells in the 0.5 to 1 µm range; blank cells represent size ranges that had no cells present	picogram of carbon per mL (pg C/mL)
Biomass_1_to_2	Biomass of cells in the 1 to 2 µm range; blank cells represent size ranges that had no cells present	picogram of carbon per mL (pg C/mL)
Biomass_2_to_4	Biomass of cells in the 2 to 4 µm range; blank cells represent size ranges that had no cells present	picogram of carbon per mL (pg C/mL)
Biomass_4_to_8	Biomass of cells in the 4 to 8 µm range; blank cells represent size ranges that had no cells present	picogram of carbon per mL (pg C/mL)
Biomass_8_to_16	Biomass of cells in the 8 to 16 µm µm range; blank cells represent size ranges that had no cells present	picogram of carbon per mL (pg C/mL)
Biomass_16_to_32	Biomass of cells in the 16 to 32 µm µm range; blank cells represent size ranges that had no cells present	picogram of carbon per mL (pg C/mL)
Biomass_32_to_64	Biomass of cells in the 32 to 64 µm range; blank cells represent size ranges that had no cells present	picogram of carbon per mL (pg C/mL)

Biomass_64_to_128	Biomass of cells in the 64 to 128 µm range; blank cells represent size ranges that had no cells present	picogram of carbon per mL (pg C/mL)
Biomass_128_to_256	Biomass of cells in the 128 to 256 µm range; blank cells represent size ranges that had no cells present	picogram of carbon per mL (pg C/mL)
Biomass_256_to_512	Biomass of cells in the 256 to 512 µm range; blank cells represent size ranges that had no cells present	picogram of carbon per mL (pg C/mL)
Biomass_512_to_1024	Biomass of cells in the 512 to 1024 µm range; blank cells represent size ranges that had no cells present	picogram of carbon per mL (pg C/mL)
Biomass_1024_to_2048	Biomass of cells in the 1024 to 2048 µm range; blank cells represent size ranges that had no cells present	picogram of carbon per mL (pg C/mL)

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	Epifluorescence microscope: Olympus BX51 microscope with a Olympus DP72 camera and Exfo X-cite Series 120 mercury bulb with a FITC filter for green fluorescence
Generic Instrument Name	Fluorescence Microscope Image Analysis System
Dataset-specific Description	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.
Generic Instrument Description	A Fluorescence (or Epifluorescence) Microscope Image Analysis System uses semi-automated color image analysis to determine cell abundance, dimensions and biovolumes from an Epifluorescence Microscope. An Epifluorescence Microscope (conventional and inverted) includes a camera system that generates enlarged images of prepared samples. The microscope uses excitation ultraviolet light and the phenomena of fluorescence and phosphorescence instead of, or in addition to, reflection and absorption of visible light.

[table of contents | back to top]

Deployments

TAN1810

Website	https://www.bco-dmo.org/deployment/757070
Platform	R/V Tangaroa
Start Date	2018-10-23
End Date	2018-11-21

[table of contents | back to top]

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.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1756465
NSF Division of Ocean Sciences (NSF OCE)	OCE-1756610

[table of contents | back to top]