

# Particle size, volume, and converted biomass from the gut contents of salps imaged via scanning electron microscopy on the "SalpPOOP" cruise on R/V Tangaroa during October and November 2018

**Website:** <https://www.bco-dmo.org/dataset/880927>

**Data Type:** Cruise Results

**Version:** 1

**Version Date:** 2023-05-10

## Project

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

Contributors	Affiliation	Role
<a href="#">Fender, Christian</a>	Florida State University (FSU)	Principal Investigator
<a href="#">Decima, Moira</a>	University of California-San Diego (UCSD-SIO)	Co-Principal Investigator
<a href="#">Stukel, Michael</a>	Florida State University (FSU)	Co-Principal Investigator
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## Abstract

This dataset includes particle size, volume, and converted biomass from the gut contents of salps imaged via scanning electron microscopy on the "SalpPOOP" cruise on R/V Tangaroa during October and November 2018.

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## Coverage

**Spatial Extent:** N:-42.6648 E:-178.75 S:-44.6417 W:179.4748

**Temporal Extent:** 2018-10-30 - 2020-08-31

## Dataset Description

Note: A more condensed dataset containing the average abundance and biomass contribution of each particle size class is also available in the related dataset "Salp Gut Carbon Content" (<https://www.bco-dmo.org/dataset/881172>).

## Methods & Sampling

58 salps representing the species *Salpa thompsoni*, *Pegea confoederata*, *Thalia democratica*, *Soestia zonaria*, *Thetys vagina*, *Salpa fusiformis*, and *Ihlea magalhanica*, including both solitary and aggregate stages of the first four and a distribution of sizes for the first two, were collected during the "SalpPOOP" cruise on R/V Tangaroa. Salps were collected via twice daily oblique Bongo tows down to 200 meters as well as daily ring net surface tows with a 30-liter non-filtering cod-end. Once onboard, salps from ring net and Bongo tows were identified to the species level, sorted by life stage (i.e., solitary or aggregate), measured, and sexed (Foxton et al. 1966; Luskow et al. 2020). Triplicate representative samples for each salp species from a total of 10 of these casts were preserved in 5% formalin < 30 minutes after collection the first time each species was encountered.

Once ashore, SEM samples were prepared from each preserved organism by excising guts under a HEPA-filter equipped laminar flow exhaust hood using acid-cleaned plastic dissection equipment to minimize contamination. Guts were then placed in either 15 or 50 mL plastic Falcon tubes with a small volume of brine, lacerated, and then vortex mixed for two minutes to release gut contents into solution while minimizing damage to the more fragile phytoplankton (Jung et al. 2010; von Harbou et al. 2011; Ahmad-Ishak 2017). An aliquot of this solution was then filtered onto a 0.2 µm Nuclepore filter, followed by six rinses of decreasing salinity in 5 ppt increments for a minimum of 5 minutes each with the final MilliQ water rinse performed twice. This was immediately followed by a dehydration series of increasing ratios of Ethanol:MilliQ to purge water from the sample, with the final 100% anhydrous ethanol step again performed twice. Finally, a substitution series of increasing ratios of the chemical drying agent hexamethyldisilazane (HMDS):anhydrous ethanol was conducted with each step lasting a minimum of 10 minutes, with the final HMDS step being allowed to air dry. Each step was conducted under either a light vacuum or gravity filtration depending on material concentration to minimize loss between treatments. The dried filter was then affixed to an aluminum SEM stub using carbon conductive adhesive tabs and further grounded with a thin piece of carbon conductive tape touching the edge of the filter and the bottom of the stub. Samples were then sputter coated with 10 nm iridium and visualized using an FEI Nova 400 NanoSEM set to an accelerating voltage of 10 kV. Twenty random regions of each filter were imaged at 3 different magnifications: ~500x, ~2,500x, and ~12,000x to target microplankton (20-200 µm), nanoplankton (2-20 µm), and picoplankton (<2 µm), respectively.

Particles in SEM images were manually outlined using ImageJ (v. 1.52a or 1.53c) to extract the maximum feret length (Feret.um), minimum feret length, and area (Area.um2). These measurements were used to estimate equivalent spherical diameter (ESD.um), biovolume (BV), and carbon biomass assuming a prolate spheroid. To avoid overestimating the size of irregularly shaped particles, we calculated width (Width.um) for a prolate spheroid of measured area and length such that:

$$\begin{aligned}
 1) \quad & \text{Width.um} = \frac{4}{\pi} \times \frac{\text{Area.um}^2}{\text{Feret.um}} \\
 2) \quad & \text{ESD.um} = \sqrt[3]{\text{Feret.um} \times \text{Width.um} \times \frac{\text{Width.um}}{2}} \\
 3) \quad & \text{BV} = \frac{4\pi}{3} \times \frac{\text{Feret.um}}{2} \times \frac{\text{Width.um}}{2} \times \frac{\text{Width.um}}{4}
 \end{aligned}$$

(Note: The equations in this image are also attached as Supplemental File "[SalpGut\\_Particles\\_Equations.JPG](#)".)

Note that because we estimated the three-dimensional size of particles using a two-dimensional image, we determined the height of each particle from its width. To account for the ~50% flattening of height caused by filtration (Taylor et al. 2011), we furthermore assumed height to be equivalent to half of the width. This correction was only applied for soft-bodied groups such as picoplankton and most nanoplankton, while for incompressible particles such as diatoms and dinoflagellates no correction was applied.

Since sufficient structural detail could not be observed to definitively identify very small particles, spherical particles within the size range of ~0.4-1.5 µm and resembling control images from lab cultures of *Prochlorococcus sp.* and *Synechococcus sp.* are instead referred to as bacteria-like particles (PartType "BLP"). Other PartTypes include: "WS" for white spheres assumed to be partially digested nanoflagellates; "Unknown" for nondetrital particles for which a classification could not be provided; "Alex" for *Alexandrium spp.*-like dinoflagellates; "ProB" for *Prorocentrum minimum*; "ProD" for *Prorocentrum dentratum*; "UnkDino" for unknown dinoflagellates; "Cyst" for particles resembling the resting stage cysts of diatoms; "Oxy" for *Oxytoxum spp.*; "Cerat" for *Ceratium spp.*; "CeratSpike" for only a portion of a *Ceratium* horn; "Tin" for tintinnids; "Pennate" for unknown pennate diatoms; "Dicty" for *Dictyocha speculum*; "UnkCocc" for particles similar to coccolithophores but with distinct holes at regular intervals (possibly just digestion artefacts); "Centric" for unknown centric diatoms; "SmallPen" for smaller, possibly nitzschiaform diatoms that smaller and

fatter about the center than "Pennate"; "Diplo" for *Diplopelta spp.*; "Cocco" for unknown coccolithophores; "Frag" for *Fragilariopsis spp.*; "Radio" for unknown polycystine radiolarians; "Pnut" for unknown pennate diatoms with shape reminiscent of a peanut; "Pyro" for *Pyrodinium spp.*; "RaphDia" for unknown pennate diatom with distinct raphid ridge; "Dinoph" for *Dinophysis spp.*; "Ciliate" for unknown ciliates. Each class may also be preceded by a "B", connotating the particle was considered broken or missing more than  $\frac{3}{4}$  its true size. "CeratSpike" was considered broken as well.

The biomass of formalin-preserved ciliates was estimated as  $0.14 \text{ pg C } \mu\text{m}^{-3}$  (Putt and Stoecker 1989) while rhizarians were  $0.001 \text{ pg C mm}^{-3}$  (Stukel et al. 2018). The biomass of diatoms was estimated allometrically as  $0.288 \cdot \text{BV}^{0.811}$  while other protists and unidentified particles were estimated using  $0.216 \cdot \text{BV}^{0.939}$  (Menden-Deuer and Lessard 2000). Because we could not differentiate between types of prokaryotes in the SEM images, we calculated a single average biomass conversion for all bacteria-like particles in the salp guts using published allometric relationships weighted by the ratio of each of the key bacterial groups to each other in the water column from flow cytometry data for a given cycle.

## Data Processing Description

### BCO-DMO Processing:

- started with file named "SalpGut\_RawV2.xlsx" received February 2023;
- converted dates to YYYY-MM-DD format;
- converted times to hh:mm format;
- renamed fields to comply with BCO-DMO naming conventions;
- added date-time fields in ISO8601 (UTC) format: YYYY-MM-DDThh:mmZ;
- converted latitudes and longitudes to decimal degrees.

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## Data Files

File
<b>salp_gut_raw.csv</b> (Comma Separated Values (.csv), 12.43 MB) MD5:7f0bb49fbbdb83e5d99cf91fe1127f110 Primary data file for dataset ID 880927.

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## Supplemental Files

File
<b>SalpGut_Particles_Equations.JPG</b> (JPEG Image (.jpg), 21.87 KB) MD5:868b5a9f7813b406caa217bc0d8862a6 Equations used in calculating width, equivalent spherical diameter (ESD), and biovolume (BV) from particle SEM images. Associated with datasets 880927 and 881172, "Salp Gut Particle Sizes" and "Salp Gut Carbon Content", from Christian Fender.

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## Related Publications

Ahmad Ishak, N. H., Clementson, L. A., Eriksen, R. S., van den Enden, R. L., Williams, G. D., & Swadling, K. M. (2017). Gut contents and isotopic profiles of *Salpa fusiformis* and *Thalia democratica*. *Marine Biology*, 164(6). <https://doi.org/10.1007/s00227-017-3174-1>  
*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*

Foxton, P. (1966). The distribution and life history of *Salpa thompsoni* Foxton with observations on a related species, *Salpa gerlachei* Foxton. *Discovery Rep* 34:1-116.

*Methods*

Jung, S. W., Joo, H. M., Park, J. S., & Lee, J. H. (2009). Development of a rapid and effective method for preparing delicate dinoflagellates for scanning electron microscopy. *Journal of Applied Phycology*, 22(3), 313-317. <https://doi.org/10.1007/s10811-009-9461-6>

*Methods*

Lüskow, F., Pakhomov, E. A., Stukel, M. R., & Décima, M. (2020). Biology of *Salpa thompsoni* at the Chatham Rise, New Zealand: demography, growth, and diel vertical migration. *Marine Biology*, 167(12).

<https://doi.org/10.1007/s00227-020-03775-x>

*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*

Putt, M., & Stoecker, D. K. (1989). An experimentally determined carbon : volume ratio for marine "oligotrichous" ciliates from estuarine and coastal waters. *Limnology and Oceanography*, 34(6), 1097-1103. doi:[10.4319/lo.1989.34.6.1097](https://doi.org/10.4319/lo.1989.34.6.1097)

*Methods*

Schneider, C. A., Rasband, W. S., ... (n.d.). ImageJ. US National Institutes of Health, Bethesda, MD, USA.

Available from <https://imagej.nih.gov/ij/>

*Software*

Stukel, M. R., Biard, T., Krause, J., & Ohman, M. D. (2018). Large Phaeodaria in the twilight zone: Their role in the carbon cycle. *Limnology and Oceanography*, 63(6), 2579-2594. Portico. <https://doi.org/10.1002/lno.10961>

*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*

von Harbou, L., Dubischar, C. D., Pakhomov, E. A., Hunt, B. P. V., Hagen, W., & Bathmann, U. V. (2011). Salps in the Lazarev Sea, Southern Ocean: I. Feeding dynamics. *Marine Biology*, 158(9), 2009-2026.

<https://doi.org/10.1007/s00227-011-1709-4>

*Methods*

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## Related Datasets

### IsSourceOf

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Fender, C., Decima, M., Stukel, M. (2023) **Size-binned particle abundance and biovolume of the gut contents of salps imaged via scanning electron microscopy on the "SalpPOOP" cruise on R/V Tangaroa during October and November 2018**. Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2023-05-08 doi:10.26008/1912/bco-dmo.881172.1 [[view at BCO-DMO](#)]

*Relationship Description: The companion dataset "Salp Gut Carbon Content" (881172) is derived from this dataset.*

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## Parameters

Parameter	Description	Units
Label	Running particle identifier	unitless
Species	Species of salp particle originates from. IM=Ihlea magalhanica, SF=Salpa fusiformis, ST=Salp thompsoni, TD=Thalia democratica, SZ=Soestia zonaria, TV=Thetys vagina, PC=Pegea confoederata.	unitless
Life_Stage	Life stage of salp particle originates from. B=Blastozoid, O=Oozoid.	unitless
Date_Caught_NZST	Date salp sample was collected in NZST time zone	unitless
Start_Time_NZST	Start time of net trawl salp was caught in (NZST)	unitless
End_Time_NZST	End time of net trawl salp was caught in (NZST)	unitless
Start_ISO_DateTime_UTC	Date and start time (in UTC) of net trawl salp was caught in as ISO8601 format	unitless
End_ISO_DateTime_UTC	Date and end time (in UTC) of net trawl salp was caught in as ISO8601 format	unitless
Cycle	Lagrangian experiment number	unitless
Station	Deployment number	unitless
Start_Latitude	Latitude at which net trawl began; negative values = South	decimal degrees North
Start_Longitude	Longitude at which net trawl began; negative values = West	decimal degrees East
End_Latitude	Latitude at which net trawl ended; negative values = South	decimal degrees North
End_Longitude	Longitude at which net trawl ended; negative values = West	decimal degrees East
Start_water_depth	Depth at location which net trawl began	meters (m)
End_water_depth	Depth at location which net trawl ended	meters (m)
Date_Prepped_EST	Date of gut excision and preparation for SEM; in EST time zone	unitless
TL_After_mm	Salp length after removal from formalin	millimeters (mm)
Aliquot_Fraction	Fraction of whole gut suspension filtered for SEM	unitless
Gut_Major_Axis	Length of longest side of excised gut	millimeters (mm)
Gut_Minor_Axis	Length of shortest side of excised gut	millimeters (mm)
Area	Particle area	square pixels (px <sup>2</sup> )
Perimeter	Particle perimeter	square pixels (px <sup>2</sup> )
Feret	Maximum caliper width	pixels (px)
MinFeret	Minimum caliper width	pixels (px)
Sample	Identifier for individual salps	unitless
Image	Which of 20 images taken per gut per magnification particle was observed in	unitless
Scale	Approximate magnification at which particle was observed	unitless

PartType	Functional group classification of particle (see Acquisition Description)	unitless
Scalar	Pixel to micrometer (um) conversion scalar	pixels per micrometer (px/um)
Scalar2	Square pixel to square micrometer conversion scalar	square pixels per square micrometer (px <sup>2</sup> /um <sup>2</sup> )
Area_um2	Metric particle area	square micrometers (um <sup>2</sup> )
Feret_um	Metric maximum caliper width	micrometers (um)
Width_um	Metric width estimated assuming prolate spheroid of observed area and feret length	micrometers (um)
BV	Metric volume of the particle	cubic micrometers (um <sup>3</sup> )
ESD_um	Metric equivalent spherical diameter	micrometers (um)
CountVal	Adjusted particle count values. Due to the 3 magnifications used, particles counted only at 12000x and 2500x are multiplied by 25 to be comparable to those counted at 500x. This column is essentially a workaround to avoid replicating each row 25-625 times.	unitless
TotCount	Final count values accounting for magnification, aliquot, and fraction of filter imaged.	unitless
TotBiomass	Final biomass accounting for magnification, aliquot, and fraction of filter imaged.	picograms Carbon (pg C)

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## Instruments

<b>Dataset-specific Instrument Name</b>	Bongo tows
<b>Generic Instrument Name</b>	Bongo Net
<b>Generic Instrument Description</b>	A Bongo Net consists of paired plankton nets, typically with a 60 cm diameter mouth opening and varying mesh sizes, 10 to 1000 micron. The Bongo Frame was designed by the National Marine Fisheries Service for use in the MARMAP program. It consists of two cylindrical collars connected with a yoke so that replicate samples are collected at the same time. Variations in models are designed for either vertical hauls (OI-2500 = NMFS Pairovet-Style, MARMAP Bongo, CalVET) or both oblique and vertical hauls (Aquatic Research). The OI-1200 has an opening and closing mechanism that allows discrete "known-depth" sampling. This model is large enough to filter water at the rate of 47.5 m <sup>3</sup> /minute when towing at a speed of two knots. More information: Ocean Instruments, Aquatic Research, Sea-Gear

<b>Dataset-specific Instrument Name</b>	ring net surface tows
<b>Generic Instrument Name</b>	Ring Net
<b>Generic Instrument Description</b>	A Ring Net is a generic plankton net, made by attaching a net of any mesh size to a metal ring of any diameter. There are 1 meter, .75 meter, .25 meter and .5 meter nets that are used regularly. The most common zooplankton ring net is 1 meter in diameter and of mesh size .333mm, also known as a 'meter net' (see Meter Net).

<b>Dataset-specific Instrument Name</b>	FEI Nova 400 NanoSEM set to an accelerating voltage of 10 kV
<b>Generic Instrument Name</b>	Scanning Electron Microscope
<b>Generic Instrument Description</b>	A scanning electron microscope (SEM) scans a focused electron beam over a surface to create an image. The electrons in the beam interact with the sample, producing various signals that can be used to obtain information about the surface topography and composition.

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## 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

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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.

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## 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>

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