Copepod ingestion rate as calculated through stable isotope analysis from experiments quantifying the ingestion by copepods of marine snow and phytoplankton at different phytoplankton growth phases

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Data Type: Other Field Results, experimental
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Project
» CAREER: Small-scale plankton-aggregate dynamics and the biological pump: Integrating mathematical biology in research and education (PlanktonAggDyn)

Contributors	Affiliation	Role
Prairie, Jennifer	University of San Diego (USD)	Principal Investigator
Rauch, Shannon	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
Copepod ingestion rate as calculated through stable isotope analysis from experiments quantifying the ingestion by copepods of marine snow and phytoplankton at different phytoplankton growth phases.

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Coverage

Spatial Extent: **Lat**:32.862 **Lon**:-117.2803
Temporal Extent: 2018-06 - 2019-12

Dataset Description

Copepod ingestion rate as calculated through stable isotope analysis from experiments quantifying the ingestion by copepods of marine snow and phytoplankton at different phytoplankton growth phases.

Acquisition Description

During the summer of 2018 and fall of 2019, six experiments were conducted to investigate the effect of phytoplankton growth phase on the ingestion rage of the copepod *Calanus pacificus* on phytoplankton and marine snow aggregates (formed from the same phytoplankton). Each experiment consisted of three

treatments: a control (in which copepods were placed in tanks with filtered seawater and no food source), an individual phytoplankton treatment (in which copepods were placed in tanks with individual phytoplankton as a food source), and an aggregate treatment (in which copepods were placed in tanks with aggregates as a food source). The six experiments were: Experiment 1 conducted in June of 2018, Experiment 2 in July of 2018, Experiment 3 in September of 2019, Experiment 4 in October of 2019, Experiment 5 in November of 2019, and Experiment 6 in December of 2019. Ingestion rate was quantified in these experiments using two methods: gut pigment analysis and stable isotope analysis.

C. pacificus was collected using a small boat near Scripps Canyon in La Jolla, CA (32° 51.720' N, 117° 16.816' W) 5-20 days before each experiment with a 333 µm mesh plankton net (0.5 m diameter mouth). Samples were sorted in the lab to isolate individuals of the species *C. pacificus*. Copepods were maintained with regular water changes in an incubator in the dark at 18°C until the experiment and fed a mixed diet of *Thalassiosira weissflogii* and haptophytes (*Tisochrysis sp. and Pavlova sp.*) for the 2018 experiments and *Thalassiosira weissflogii* and *Skeletonema marinoi* for the 2019 experiments. Copepods were starved for 24 hours prior to each experiment by transferring 30 *C. pacificus* individuals (C5 copepodites and female adults) into a beaker corresponding to each treatment tank. Each beaker was wrapped in aluminum foil to maintain darkness, and was kept at room temperature.

Prior to each experiment, phytoplankton cultures of the species *T. weissflogii* (Experiments 1, 2, 3, and 5) or *S. marinoi* (Experiments 4 and 6) were started in 2L flasks. All cultures were grown in f/2 media at room temperature under 12:12 hour LED light:dark cycle. In total, two culture flasks were started for each growth phase: one flask for the aggregate treatment and one flask for the phytoplankton treatment. The aggregate treatment culture flasks were started three days prior to the phytoplankton treatment culture flasks, to account for the fact that the aggregate treatment culture flasks were stopped three days earlier to allow for aggregate formation on a roller table (described below). Experiments 1 and 2 were carried out for three different growth phases phases (with the corresponding phytoplankton culturing time shown in parentheses): Early Exponential (5 days), Late Exponential (13 days), and Late Stationary (20 days). Experiments 3, 4, 5, and 6 were carried out for Early Exponential (5 days) and Late Exponential (12 days) growth phases. To carry out ingestion rate measurements using stable isotope analysis, 1.7 mL of an N-15 nitrate solution (7.5 g/L N-15 potassium nitrate salt in DI water) was added to each phytoplankton culture 3 days before the culture was to be stopped (except for Experiment 1 where 12.75 mL of N-15 nitrate solution was added 2 days before each culture was stopped). Stable isotope analysis was not carried out for Experiment 2. Right before adding N-15 labeled nitrate to each culture, 25 mL of the culture was filtered onto a GF/F to be used as initial measurements of naturally occurring N-15 concentrations in the phytoplankton cultures.

Three days before each experiment, after the aggregate treatment phytoplankton cultures had grown for the time specified above (depending on growth phase), these cultures were diluted (to 20,000 cell/mL for *T. weissflogii* and 39,000 cells/mL for *S. marinoi*) and added to two cylindrical acrylic tanks (each with a volume of 550 mL). These cylindrical tanks were allowed to rotate in the dark on roller table for 3 days at a rate of 4.6 rpm to form aggregates.

On the day of each experiment for each growth phase, two replicate cylindrical tanks (each with a volume of 2200 mL) were prepared per treatment (control, individual phytoplankton, and aggregate), thus resulting in 6 tanks per experiment per growth phase. The control tanks were filled completely with filtered seawater along with a small amount of N-15 nitrate solution such that the final concentration of N-15 in the tank was comparable to that in the phytoplankton and aggregate treatment tanks. The phytoplankton treatment tanks were filled with the phytoplankton culture that was diluted with filtered seawater (to final concentration of 5,000 cells/mL for *T. weissflogii* and 9,750 cells/mL for *S. marinoi*). For the aggregate treatments, aggregates formed in the smaller cylindrical tanks were transferred to the experimental tank (which had four times the volume) along with the associated seawater; the rest of the volume of the experimental tank was filled with filtered seawater, thus resulting in an equivalent phytoplankton concentration (in cells/mL) between the phytoplankton and aggregate treatment tanks. For each treatment tank, 30 copepods were added and copepods were allowed to feed for an hour while the tank slowly rotating at ~1 rpm. Note that due to a lack of copepods, only one tank per treatment was run in the Late Stationary growth phase for Experiment 1 and only one control tank was run in the Late Stationary growth phase for Experiment 2.

Immediately after the one-hour incubation time had elapsed for each treatment, 40 mL of seltzer water was added to the tank to anesthetize the copepods. The copepods were removed from the cylindrical experimental tank with gentle suctioning of water onto a filter. For stable isotope analysis, immediately after the one-hour feeding experiment in each tank, five sets of two copepods were transferred by forceps into tin cups for each tank of each treatment (only 3 to 4 sets of two copepods were collected for Experiment 1, and no stable isotope analysis data was collected for Experiment 2 due to of lack of copepods). For each experiment, 5-6 sets of two unfed copepods (which were starved alongside experimental copepods but not used in any feeding tank) were transferred into tin cups to be used as initials, which were used to measure the natural concentration of N-15 in copepods before being exposed to phytoplankton grown in N-15 nitrate solution. Three replicates of 250 mL each of the remaining tank water for each treatment was filtered onto a GF/F and packed into a tin cup for N-15 measurements of the food that was fed to the copepods in each treatment. All samples were sent off to be processed at UC Davis Stable Isotope Facility (with the exception of Experiment 1, in which samples were processed on an Isotope Ratio Mass Spectrometer at Scripps Institution of Oceanography).

Samples were also collected to calculate ingestion rate using gut pigment methods. For information on that and the associated data, see related dataset https://www.bco-dmo.org/dataset/824364

Processing Description

For the stable isotope analysis, the copepod ingestion rate (in units of µg C/copepod) was calculated as (following Verschoor 2005):

Ingestion Rate = (Ms Fs - Fi Ms) / Ff / t

where Ms is the mass per copepod in the sample (in µg C, so divided by two since two copepods were processed in each sample) as provided by the stable isotope analysis results, t is the total time of the grazing experiment (1 hour), and Fs, Fi, and Ff are the isotopic fractions (in terms of N-15) of the copepod sample (Fs), the initial copepods (Fi), and the food source (Ff), respectively. Isotopic fractions were calculated from the stable isotope data of the respective samples as:

F = R / (R +1)

where R is the isotopic ratio of the sample calculated as:

R = (∂N-15/1000 +1) RR

where RR is the isotopic ratio of a reference standard.

BCO-DMO Processing:
- concatenated data from separate Excel sheets into one dataset;
- added columns for experiment number, month, year, and species (from sheet names);
- renamed columns.

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Related Publications

Verschoor, A. M., Boonstra, H., & Meijer, T. (2005). Application of Stable Isotope Tracers to Studies of Zooplankton Feeding, using the Rotifer Brachionus calyciflorus as an Example. Hydrobiologia, 546(1), 535–549. doi:10.1007/s10750-005-4296-x
Methods

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Related Datasets

IsRelatedTo

Prairie, J. (2021) **Copepod gut pigment data from each of six experiments quantifying the ingestion by copepods of marine snow and phytoplankton at different phytoplankton growth phases.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2021-02-26 http://lod.bco-dmo.org/id/dataset/824364 [view at BCO-DMO]

Prairie, J. (2021) **Fluorescence per phytoplankton cell from each of six experiments quantifying the ingestion by copepods of marine snow and phytoplankton at different phytoplankton growth phases.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2021-02-16 http://lod.bco-dmo.org/id/dataset/824665 [view at BCO-DMO]

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Parameters

Parameter	Description	Units
exp_number	Experiment number	unitless
month	Month of experiment	unitless
year	Year of experiment	unitless
species	Phytoplankton species used in the grazing experiment	unitless
IngestionRate	The copepod ingestion rate as calculated from stable isotope analysis (see Methodology section for more details)	micrograms carbon per copepod per hour (ug C/copepod/hour)
Growth_Phase	Phytoplankton growth phase: EarlyExp = Early Exponential; or LateExp = Late Exponential	unitless
Treatment	Treatment: Control; Photo = Individual Phytoplankton treatment; or Agg = Aggregate treatment	unitless
Tank	Tank number	unitless

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Instruments

Dataset-specific Instrument Name	333 µm mesh plankton net
Generic Instrument Name	Plankton Net
Generic Instrument Description	A Plankton Net is a generic term for a sampling net that is used to collect plankton. It is used only when detailed instrument documentation is not available.

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Project Information

CAREER: Small-scale plankton-aggregate dynamics and the biological pump: Integrating mathematical biology in research and education (PlanktonAggDyn)

NSF Award Abstract:
The global carbon cycle is in part modified by marine biological processes, which can impact the amount of carbon that is transported from surface waters to the deep ocean. This project will investigate interactions between planktonic grazers and marine aggregates - sinking particles that form in the surface ocean and have been shown to play an important role in marine food webs. The small scale of these biological processes makes them particularly challenging to study, but modern advances in mathematics and computer science have made direct observations of these interactions feasible. Experiments using high-resolution imaging will provide direct visual observations of zooplankton ingestion and the alteration of marine aggregates. These laboratory studies will guide the development of mathematical models to examine how these interactions affect particulate carbon sinking out of the surface ocean. This project will support an educational initiative focused on training undergraduate biology students in mathematical and computational techniques. This initiative includes the development of new interdisciplinary courses and undergraduate-focused independent research projects to help prepare the next generation of scientists in quantitative techniques that are essential to tackling the most challenging and complex biological problems.

Marine snow aggregates are particles that form in the surface ocean from organic and inorganic matter. These aggregates play a fundamental role in the biological pump, as sinking particles are a dominant contributor to the downward transfer of carbon in the ocean. However, much of the small-scale processes governing these particles and their role in the marine carbon cycle are still unknown. The goal of this project is to use mathematical and computational techniques to investigate interactions between aggregates and planktonic grazers, an understudied link in the planktonic food web that has important implications for carbon export. Three-dimensional trajectories of copepods within marine snow thin layers will be obtained to experimentally investigate copepod foraging behavior in response to patchy distributions of marine snow. In addition, high-speed imaging will allow for the direct observation of how copepods manipulate and ingest marine snow aggregates, thus affecting their size and settling velocity. Lastly, a mathematical model will be developed to study the impact of these small-scale interactions on large-scale carbon cycling and export. This project will also support the implementation of a comprehensive education plan focused on teaching undergraduate students how mathematical modeling and computational techniques can be used to address biological questions. This educational objective will be accomplished through the development of new courses in mathematical and computational biology and through the inclusion of undergraduate students in independent research projects.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1654276

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