Fluorescence per phytoplankton cell from each of six experiments quantifying the ingestion by copepods of marine snow and phytoplankton at different phytoplankton growth phases

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

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Abstract
Fluorescence per phytoplankton cell from each of six 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

Fluorescence per phytoplankton cell from each of six 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.

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 gut pigment analysis, two copepods were placed in 6-10 amber vials (depending on the total number of copepods recovered), which contained 3 mL of 90% acetone. A sonicator was used to break up the copepods at 40% amplitude for 5 seconds and release their gut content into the acetone solution. In addition, after each experiment water from each experimental tank was evenly mixed, and three subsamples of 25 mL of tank water was filtered onto a

GF/F filter and placed into 5 mL of acetone. After about a day in a -20°C freezer, the copepod and tank water samples were analyzed using a Triology Laboratory Fluorometer (Turner Designs) to measure the concentration of chlorophyll and pheophytin in the acetone solution.

Samples were also collected to calculate ingestion rate using stable analysis methods. For information on that and the associated data, see related dataset https://www.bco-dmo.org/dataset/824576

Processing Description

For gut pigment samples, total gut pigment concentration in units of µg pigment/copepod (combining chl *a* and pheophytin) was calculated according to EPA Method 445.0 as:

Gut Pigment Concentration = K (r/r-1) (r Ra - Ra) E / n

where K is the response factor from the fluorometer calibration, r is the before-to-after acidification ratio of a pure chlorophyll *a* solution, Ra is the fluorescence reading after acidification, E is the volume of acetone in L, and n is the number of copepods per sample (2 in our case).

To account for the fact that phytoplankton cells may fluoresce at differently when in aggregate form (because the aggregates were formed for three days in the dark prior to the experiment), fluorescence per cell (in units of µg pigment/cell) was calculated for the phytoplankton and aggregate treatments for each growth phase using the tank water that was filtered after each experiment (averaging both tanks for that treatment), as:

Fluorescence per Cell = K (r/r-1) (r Ra - Ra) E / S / C

where S is sample volume in L (i.e. the 0.025 L filtered), and C is the phytoplankton concentration in the tank (5,000,000 cells/L for *T. weissflogii* experiments and 9,750,000 cells/L for *S. marinoi* experiments).

In this data set, the fluorescence per cell data is provided. For the associated gut pigment concentration measurements used to calculate copepod ingestion, see related dataset https://www.bco-dmo.org/dataset/824364

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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) **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.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2021-02-23 http://lod.bco-dmo.org/id/dataset/824576 [view at BCO-DMO]

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Parameters

Parameter	Description	Units
Experiment	Experiment number	unitless
Growth_Phase	Phytoplankton growth phase: EarlyExp = Early Exponential; LateExp = Late Exponential; or LateStat = Late Stationary	unitless
Treatment	Treatment: Control; Photo = Individual Phytoplankton treatment; or Agg = Aggregate treatment	unitless
Fluorescence_Per_Cell	Fluorescence per phytoplankton cell (combined chlorophyll a and pheophytin)	micrograms (ug) of pigment per cell

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Instruments

Dataset-specific Instrument Name	Triology Laboratory Fluorometer (Turner Designs)
Generic Instrument Name	Fluorometer
Generic Instrument Description	A fluorometer or fluorimeter is a device used to measure parameters of fluorescence: its intensity and wavelength distribution of emission spectrum after excitation by a certain spectrum of light. The instrument is designed to measure the amount of stimulated electromagnetic radiation produced by pulses of electromagnetic radiation emitted into a water sample or in situ.

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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