Effect of Marine Snow Distribution on Copepod Ingestion of Marine Snow Experiments 2018

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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
These data are from a set of 2 experiments quantifying the ingestion by copepods of marine snow with different food distributions (layer vs. homogenous). Experiments were conducted in July 2018 in the Prairie research lab at the University of San Diego, California, USA.

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Coverage

Spatial Extent: **Lat**:32.862 **Lon**:-117.280267
Temporal Extent: 2018-07-02 - 2018-07-13

Methods & Sampling

During the summer of 2018, two experiments were conducted to investigate the effect of marine snow distribution on the ingestion rage of the copepod *Calanus pacificus*: Experiment 1 was conducted on July 2 and Experiment 2 was conducted on July 13. Each experiment consisted of three treatments: a control (in which copepods were placed in a tank with no marine snow), a layer treatment (in which copepods were placed in a tank with a layer of marine snow in the middle), and a homogenous treatment (in which copepods were placed in a tank with a roughly homogenous distribution of marine snow).

C. pacificus was collected for both experiments using a small boat near Scripps Canyon in La Jolla, CA (32° 51.720' N, 117° 16.816' W) on June 28th, 2021 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 *Thalassiosira weissflogii*. Copepods were starved for 24 hours prior to each experiment by transferring copepods into beakers with filtered seawater which were wrapped in aluminum foil to maintain darkness and kept at room temperature.

Prior to each experiment, less dense filtered seawater was mixed by diluting filtered seawater with distilled (DI) water to be used as the top layer fluid in the control treatment and layer treatment (both of which had a density gradient). The density of the top layer fluid was 0.004 g/cm3 less than the bottom layer fluid. Densities of the different seawaters were measured using a handheld density meter (DMA 35, Anton Paar), and were kept roughly consistent for the two experiments.

Also before each experiment, two phytoplankton cultures of the species *T. weissflogii* were started in 2L flasks and were grown in f/2 media at room temperature under 24 hour light for 6 days corresponding to the exponential growth phase. Three days before each experiment, the cultures were diluted to 32,500 cell/mL to form aggregates. The filtered seawater used to dilute these cultures had different densities depending on the treatment: aggregates for the layer treatment were rolled in the top layer fluid (with a density 0.004 g/cm3 less than the experimental bottom layer fluid) and aggregates for the homogenous treatment were rolled in seawater with a density 0.001 g/cm3 less than the experimental bottom layer fluid. The diluted cultures were then added to cylindrical acrylic tanks (each with a volume of 2.2 L) and these cylindrical tanks were allowed to rotate in the dark on roller table for 3 days at a rate of 3.3 rpm to form aggregates.

The experimental tank had a square base (10 cm × 10 cm) and a height of 50 cm. For the stratified treatments (control treatment and layer treatment) density gradients were established by filling the tank with ~2.5 L of bottom layer fluid, followed by ~2.5 L of less dense top layer fluid. Top layer fluid was slowly pumped on top of the bottom layer fluid through a diffuser to minimizing mixing. The non-stratified homogenous treatment was set up by pouring ~5 L of filtered seawater/bottom layer fluid into the tank.

Experimental treatments were done in succession, with the control treatment first, followed by the layer treatment, and then the homogenous treatment. Once each tank was set up, aggregates were pipetted into the top of the tank (except for the control treatment). For the layer treatment, ~5 mL of aggregates were pipetted into the top of the tank at once, allowing to settle and form a layer at the density gradient. For the homogenous treatment, a similar total volume of aggregates were pipetted into the tank but were pipetted individually evenly across the surface of the tank and throughout the duration of the experiment. For each treatment, 20-25 copepods (a mixture of adult males, adult females, and copepodite-V stage copepods) were carefully pipetted into the top of the tank. For the layer treatment, copepods were added after the majority of aggregates had settled at the density gradient. For the homogenous treatment, copepods were added after about half of the aggregates had been added to the tank and the first aggregates had settled about halfway through the tank.

Copepods were allowed to forage in the tank until aggregates in the layer and homogenous treatment started reaching the bottom of the tank (since we wanted to avoid measuring any bottom feeding). The total time that the copepods were in the tank for these treatments was between 4-7 minutes. The total time the copepods were in the tank for the control treatment was about 9-10 minutes.

The copepods were removed from the experimental tank with gentle suctioning of water onto a sieve. For gut pigment analysis, two copepods were placed in 8-10 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 Trilogy Laboratory Fluorometer (Turner Designs) to measure the concentration of chlorophyll and pheophytin in the acetone solution. Tank fluorometer measurements are given in the attached Supplemental File "Tank_Fluorescence_Measurements.csv".

Data Processing Description

Data Processing:
For gut pigment samples, copepod gut chlorophyll *a* content and copepod gut pheophytin *a* content in units of µg pigment/copepod were calculated according to EPA Method 445.0 as:

Chlorophyll Gut Content = K (r/r-1) (Rb - Ra) E / n

Pheophytin Gut Content = K (r/r-1) (r Ra - Rb) 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, Rb and Ra are the fluorescence readings before and after acidification respectively, E is the volume of acetone in L, and n is the number of copepods per sample (2 in our case). Total gut pigment

content was then calculated as the sum of the chlorophyll gut content and pheophytin gut content.

The concentration of chlorophyll and pheophytin (in units of *u*g/L) in the tank water after experiments was calculated from the GF/F filters according to EPA Method 445.0 as:

Tank chlorophyll concentration = K (r/r-1) (Rb - Ra) E / s

Tank pheophytin concentration = K (r/r-1) (Rb - Ra) E / s

with s representing the volume (in L) that was filtered onto the GFF and the other variables are the same as above. Similarly total pigment concentration was calculated from the sum of chlorophyll concentration and pheophytin concentration.

BCO-DMO Processing:
- concatenated data from two separate Excel sheets into a single dataset.

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Data Files

File	
marine_snow_copepod_ingestion.csv	(Comma Separated Values (.csv), 4.92 KB) MD5:1e959bd54bfbf491912be7580ea3e53f
Primary data file for dataset ID 860674	

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Supplemental Files

File	
Tank_Fluorescence_Measurements.csv	(Comma Separated Values (.csv), 955 bytes) MD5:6ae758786916b60dbb6e1a0271508675

Measurements of pigment concentration from the experimental tank water (measured after the experiment) are provided for each experiment and treatment. The columns in this file are:

1. Experiment: gives the Experiment number.

2. Treatment: gives the treatment name (either Control, Layer, or Homogenous).

3. Replicate: gives the replicate sample number for each treatment.

4. Chorophyll: gives the concentration of chlorophyll a in units of µg/L.

5. Pheophytin: gives the concentration of pheophytin a in units of µg/L.

6. TotalPigment: gives the concentration of total pigment (chlorophyll a and pheophytin a combined) in units of µg/L.

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Parameters

Parameter	Description	Units
Experiment_Number	exeriment number	unitless
Experiment_Date	date of experiment; format: YYYY-MM-DD	unitless
Treatment	treatment name (Control, Layer, or Homogenous)	unitless
Replicate	replicate sample number for each treatment	unitless
Chlorophyll	copepod gut chlorophyll a content	micrograms (ug) chlorophyll per copepod
Pheophytin	copepod gut pheophytin a content	micrograms (ug) pheophytin per copepod
TotalPigment	copepod total pigment content (chlorophyll a and pheophytin a combined)	micrograms (ug) pigment per copepod

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Instruments

Dataset-specific Instrument Name	hand-held density meter: DMA 35, Anton Paar
Generic Instrument Name	density meter
Generic Instrument Description	Portable or bench-top instruments to measure density of liquids. [lab]

Dataset-specific Instrument Name	333 µm mesh plankton net (0.5 m diameter mouth)
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.

Dataset-specific Instrument Name	cylindrical acrylic tanks (each with a volume of 2.2 L)
Generic Instrument Name	Roller Tank
Generic Instrument Description	Rolling tanks, which keep particles in suspension, thus simulating aggregate formation in situ. Marine snow experiments are conducted in roller tanks, which turn continuously, keeping marine snow in suspension. It is important for marine snow not to touch surfaces. The rolling tanks, which keep particles in suspension, thus simulate aggregate formation in situ. Marine snow formation due to different types of oil was tested. Some treatments are easily identifiable as containing oil by their color (middle). UCSB, CA 2012.

Dataset-specific Instrument Name	Trilogy Laboratory Fluorometer (Turner Designs)
Generic Instrument Name	Turner Designs Trilogy fluorometer
Generic Instrument Description	The Trilogy Laboratory Fluorometer is a compact laboratory instrument for making fluorescence, absorbance, and turbidity measurements using the appropriate snap-in application module. Fluorescence modules are available for discrete sample measurements of various fluorescent materials including chlorophyll (in vivo and extracted), rhodamine, fluorescein, cyanobacteria pigments, ammonium, CDOM, optical brighteners, and other fluorescent compounds.

Dataset-specific Instrument Name	sonicator
Generic Instrument Name	ultrasonic cell disrupter (sonicator)
Generic Instrument Description	Instrument that applies sound energy to agitate particles in a sample.

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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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