Copepod gut pigment and tank water pigment data from a set of 4 experiments quantifying the foraging behavior and ingestion by copepods within different distributions of marine snow

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Data Type: experimental
Version: 1
Version Date: 2022-09-20

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

Contributors	Affiliation	Role
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Abstract
This dataset results from a set of four experiments conducted to quantify the foraging behavior and ingestion by copepods within different distributions of marine snow. Experiments were conducted at the University of San Diego in the Prairie research lab. This data set includes the copepod gut pigment and tank water pigment data from these experiments.

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Methods & Sampling

During the autumn of 2020, four experiments were conducted in which copepods of the species *Calanus pacificus* were exposed to four different feeding environments (treatments). These four treatments were: a tank with a marine snow layer, a tank with a homogenous distribution of marine snow, and two control treatments without food – one with a density gradient and one with no density gradient. Copepods were recorded with two cameras, allowing us to reconstruct 2D and 3D copepod tracks. From these tracks, we determined vertical distributions of copepods and quantified behavioral properties, including swimming velocity, a measure of path linearity, and jump frequency. We also measured copepod ingestion in the four treatments using gut pigment analysis.

C. pacificus was collected using a small boat near Scripps Canyon in La Jolla, CA (32° 51.720’ N, 117° 16.816’ W) 11-22 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 *Thalassiosira weissflogii* . Twenty-four hours prior to each experiment, a total of 120 copepods were starved (30 copepods for each treatment). The copepods for each treatment were placed in separate 100 mL beakers filled with filtered seawater and stored in complete darkness at room temperature (~21 ˚C) until just prior to the experiment. For each treatment, the copepods were starved in filtered seawater with a density equal to that of the top layer fluid for that treatment, which differed between treatments because of how each feeding environment was created. This allowed for the copepods to be acclimated to the density of the top layer fluid in their assigned

treatment tank to limit any shock or behavioral reaction at the beginning of the experiment.

Sixteen days prior to each experiment, four identical cultures of *T. weissflogii* were started with a concentration of 10,000 cells/mL in 1.8 L of f/2 media (two each for the layer treatment and the homogenous treatment). These cultures were kept on a 12:12 hour light:dark cycle at room temperature and phytoplankton concentration was measured every other day with a particle counter (Beckman Coulter). The phytoplankton cultures were grown for a total of thirteen days, corresponding to roughly the middle of their exponential growth phase.

After the phytoplankton cultures grew for thirteen days, and three days prior to the experiment, each *T. weissflogii* culture was diluted to 32,500 cells/mL and transferred into a cylindrical acrylic tank with a volume of 2.2 L and circumference of 51 cm. The cylindrical tanks used to form aggregates designated for the layer treatment were filled with seawater with density equal to the top layer fluid for that treatment. The tanks used to form aggregates designated for the homogenous treatment were filled with seawater that had a density ~0.0020 g/cm3 less (i.e. a salinity of ~1.3 psu less) than the fluid for that treatment; this was done to allow for slower aggregate settling speeds in that treatment, based on observations in preliminary experiments. The cylindrical tanks were placed on a roller table and were allowed to rotate at a speed of 4.3 RPM for 3 days in the dark to form aggregates.

A single experiment consisted of a set of four different treatments. These treatments were done one after the other, in the following order: control with gradient, layer, homogenous, and control with no gradient. The four treatments were all created in a rectangular acrylic tank of dimensions 10 cm x 10 cm x 50 cm. In each treatment, 25 copepods were allowed to feed in the tank for between 4-8 minutes.

The control with gradient treatment and the layer treatment required the formation of a density gradient. This density gradient allowed for the formation of an ephemeral marine snow layer (lasting about 8 minutes) in the layer treatment. An identical density gradient was created in the control with gradient treatment so we could account for any potential changes in copepod behavior in response to the change in density. Filtered seawater was used for the bottom layer fluid, which had a density between 1.0233-1.0236 g/cm3 (salinity between 33.6-34.0 psu) at room temperature, ~21 ˚C. Filtered seawater was diluted with DI water to a target density of 0.0040 g/cm3 less (or salinity of 5.3 psu less) than the bottom layer fluid to create the top layer fluid. To form the density gradient, we filled the tank with bottom layer fluid to ~25 cm from the bottom of the tank (approximate halfway mark). In order to get rid of any bubbles adhering to the sides of the tank, which might obstruct a clear camera view, we ran a sponge along the inside tank walls. Then, top layer fluid was carefully poured on top of the bottom layer fluid through a diffuser made from a sponge that had been soaked in top layer fluid. This diffuser floats at the surface of the water column and prevents mixing at the density interface as the less dense top layer fluid is added to the tank slowly with a pump (Micropump Model GB-P23.JVS.A.B1). The homogenous treatment and the control with no density gradient treatment did not require a density gradient, and instead were filled entirely with undiluted filtered seawater (i.e., bottom layer fluid from the other treatments).

Just prior to starting each treatment, the starved copepods that were set aside for each treatment were transferred from their 100 mL beakers into 10 mL beakers. This made it possible to pour the entire beaker into a ladle which was used to transfer the copepods into the tank. For treatments that required marine snow aggregates (the layer treatment and the homogenous treatment), the cylindrical tank containing aggregates was carefully taken off the roller table and placed upright so that the aggregates slowly settled to the bottom of the tank. The marine snow aggregates were then carefully transferred into a small glass vial, so as not to break up the fragile aggregates, such that the total volume of aggregates in the vial was equal to roughly 5 mL.

Once the tank was set up, the copepods and aggregates were added accordingly, based on the treatment. For both control treatments, the copepods were placed into the tank by transferring them with a ladle, which was gently placed at the surface of the water and tilted so the copepods were released into the tank. For the layer treatment, the aggregates were pipetted into the tank just below the surface, such that they had a relatively even horizontal distribution. The copepods were ladled into the tank once a distinct marine snow layer had formed. For the homogenous treatment, about half of the aggregates were pipetted just below the surface into the tank, again such that the distribution of aggregates horizontally was fairly homogenous. Then all 25 copepods were ladled into the tank, with the remaining aggregates added afterwards. This method created a roughly homogenous distribution of aggregates sinking around the copepods throughout the time of camera recording.

The experimental tank was set up on a table and was lit from below with a near-infrared light-emitting diode (LED) aimed upwards through a Fresnel lens through a cut-out in the table with an overlying piece of clear plexiglass. Two near-infrared sensitive cameras (Point Grey Grasshopper Camera Model GS3-U3-41C6NIR-C) were set up at 90˚ angles facing two neighboring sides of the tank. During the experiments, the cameras

recorded at 12 frames per second. The recording started immediately prior to adding the marine snow aggregates to the tanks (or the copepods in the case of the control treatments). For the two control treatments, the recording was stopped once the copepods had been in the tank for approximately 8 minutes. For the layer treatment, the recording was stopped once the aggregates started falling out of the layer. For the homogenous treatment, the recording was stopped once the aggregates started sinking out of the bottom of the field of view to limit the amount of time that the animals may be feeding on aggregates sitting on the bottom of the tank. The images had a field of view of ~30 cm x 10 cm. This field of view is vertically centered, so that it is ~10 cm from the bottom of the tank and ~10 cm from the top of the tank. The density gradient, which is located ~25 cm from the bottom of the tank, is located at roughly the halfway mark in the field of view (~15 cm from both the bottom and top of field of view).

Once copepod behavior had been recorded, the cameras were turned off and the copepods were carefully siphoned out of the tank onto a 100 µm mesh sieve. All the water from the tank was saved in a bucket for filtering after the experiments. Copepods were then collected for gut pigment analysis. After the copepods were removed from each treatment tank, using both cameras we recorded images of a ruler aligned vertically in the center of the tank, which were used for image calibration.

After the copepods were siphoned out of the tank and onto the sieve, pairs of copepods were added to amber vials filled with 3mL of 90% acetone (10 per treatment), recovering 20 of the copepods in each treatment. Copepods in each vial were sonicated at 40% amplitude for 5 seconds to break up the organisms and release gut content into the acetone. The amber vials were then placed in a -20˚C freezer overnight. The water of each treatment tank was also filtered and analyzed for chlorophyll concentration. Prior to filtering, the water from each tank was well mixed, and then three subsamples of 25 mL each were filtered onto a GF/F filter. These filters were then placed in amber vials filled with 5 mL of 90% acetone to extract the chlorophyll into solution and the vials were placed into a -20˚C freezer overnight. The following day, the copepod gut and tank water samples were analyzed using a fluorometer (Trilogy, Turner Designs) to measure the concentration of chlorophyll and pheophytin in the acetone solution.

This data set includes the copepod gut pigment and tank water pigment data from these experiments. For the copepod track data from the same experiments collected from the cameras, see related dataset https://www.bco-dmo.org/dataset/880645.

Data Processing Description

Data Processing:
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 = Fs (r/r-1) (r Ra - Ra) E DF / n

where Fs 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, DF is the dilution factor, and n is the number of copepods per sample (2 in our case). Samples with the GF/F filters with the treatment tank water were similarly measured for concentration of total pigment (in units of µg pigment/L) using EPA Method 445.0.

BCO-DMO Processing Description

- concatenated separate data sheets into one dataset; created column for experiment number.
- saved the final file as "880669_v1_copepod_ingestion.csv".

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Data Files

File
880669_v1_copepod_ingestion.csv (Comma Separated Values (.csv), 9.05 KB) MD5:72ccddd50cb72802adcaf6379c52e5ed Primary data file for dataset ID 880669, version 1

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Related Datasets

IsRelatedTo

Prairie, J. (2023) **Copepod track data from a set of 4 experiments quantifying the foraging behavior and ingestion by copepods within different distributions of marine snow.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2022-09-19 doi:10.26008/1912/bco-dmo.880645.1 [view at BCO-DMO]

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Parameters

Parameter	Description	Units
experiment	Experiment number (1, 2, 3, or 4)	unitless
treatment	The experimental treatment, which include Control with Gradient, Layer, Homogenous, and Control with No gradient	unitless
sample_type	The sample type, which can be either copepod (indicating copepod gut pigment measurement) or tank (indicating measurement of total pigment from the experimental tank water)	unitless
replicate	The replicate number for that treatment and sample type	unitless
total_pigment	The total pigment (combined chlorophyll a and pheophytin) in units of ug pigment/copepod (if it is a copepod sample) or ug pigment/L (if it is a tank sample)	micrograms (ug) pigment per copepod or per liter (L)

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Instruments

Dataset-specific Instrument Name	near-infrared sensitive cameras (Point Grey Grasshopper Camera Model GS3-U3-41C6NIR-C)
Generic Instrument Name	Camera
Generic Instrument Description	All types of photographic equipment including stills, video, film and digital systems.

Dataset-specific Instrument Name	Beckman Coulter particle counter
Generic Instrument Name	Coulter Counter
Generic Instrument Description	An apparatus for counting and sizing particles suspended in electrolytes. It is used for cells, bacteria, prokaryotic cells and virus particles. A typical Coulter counter has one or more microchannels that separate two chambers containing electrolyte solutions. from https://en.wikipedia.org/wiki/Coulter_counter

Dataset-specific Instrument Name	incubator
Generic Instrument Name	In-situ incubator
Generic Instrument Description	A device on a ship or in the laboratory that holds water samples under controlled conditions of temperature and possibly illumination.

Dataset-specific Instrument Name	near-infrared light-emitting diode (LED)
Generic Instrument Name	LED light
Generic Instrument Description	A light-emitting diode (LED) is a semiconductor light source that emits light when current flows through it. Electrons in the semiconductor recombine with electron holes, releasing energy in the form of photons.

Dataset-specific Instrument Name	333 micrometer 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.

Dataset-specific Instrument Name	Micropump Model GB-P23.JVS.A.B1
Generic Instrument Name	Pump
Generic Instrument Description	A pump is a device that moves fluids (liquids or gases), or sometimes slurries, by mechanical action. Pumps can be classified into three major groups according to the method they use to move the fluid: direct lift, displacement, and gravity pumps

Dataset-specific Instrument Name	fluorometer (Trilogy, 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.

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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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