Filmography data from a set of 3 experiments of copepod and phytoplankton aggregate micro-scale interactions using high-speed filmography in 2020

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Data Type: experimental
Version: 2
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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
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Abstract
Filmography data from a set of 3 experiments of copepod and phytoplankton aggregate micro-scale interactions using high-speed filmography in 2020. This dataset includes videos and supplemental tables containing file inventories, interaction type, and effect on aggregate for each video of an observed copepod-aggregate interaction.

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Coverage

Location: Experimental animals collected near Scripps Canyon in La Jolla, CA
Spatial Extent: **Lat**:32.8566111 **Lon**:-117.2666944
Temporal Extent: 2020-10 - 2020-12

Methods & Sampling

Included are 33 video files (MP4) from three experiments conducted in 2020. 26 of the videos show interactions between individual tethered copepods and sinking phytoplankton aggregates from above. 7 of the videos are control observations with tethered copepods.

The filenames of each copepod-aggregate interaction video have the following format:
Exp#_copepod#_aggregate#

The filenames of each control video have the following format:
Exp#_copepod#_control_abbreviation
where the abbreviation describes the type of control that is described further in Table 1 (see Supplemental Files).

Video files are available in file bundles (zip), in the "Data Files" section:
control_copepod_videos.zip
Exp1_copepod_videos.zip
Exp2_copepod_videos.zip
Exp3_copepod_videos_part1.zip
Exp3_copepod_videos_part2.zip

Sampling and analytical procedures:

Three experiments were conducted between October and December of 2020 (Experiment 1 on October 2, Experiment 2 on November 21, and Experiment 3 on December 19), in which individual, tethered *Calanus pacificus* female copepods were exposed to sinking aggregates in order to determine if copepods are capable of modifying phytoplankton aggregate properties. Each copepod was exposed to 1-4 aggregates in order to increase the chances of observing a direct interaction between female *C. pacificus* copepods (2.6-3.4 mm prosome length) and phytoplankton aggregates (~ 3-5 mm major axis length, ~2-4 minor axis length).

Aggregates were formed in the lab from non-axenic cultures of *Thalassiosira weisflogii* grown in f/2 media at room temperature. Cylindrical acrylic tanks (volume 2.2 L) were prepared with these cultures at concentrations of 30,000 cells per mL. These cylindrical tanks were placed on a roller table and allowed to rotate at 3.3 revolutions per minute for 48 hours to form aggregates. Aggregate formation occurred in the dark to arrest phytoplankton growth. Aggregates were always formed during the exponential growth phase of the cultures and new cultures were used for each experiment.

C. pacificus copepods were collected with a 300 µm mesh plankton net (0.5 m diameter mouth) using a small boat near Scripps Canyon in La Jolla, CA (32° 51' 23.8" N, 117° 16' 00.1" W) 6-7 days before each experiment. 5-6 oblique tows were taken per sampling trip to a depth of at least 40 m and for a duration of 3 to 5 minutes. Zooplankton samples were sorted in the lab to isolate adult *C. pacificus*. Copepods were maintained with regular water changes in an incubator in the dark at 18 degC until the experiment and fed a diet of *Thalassiosira weissflogii*.

Copepods were starved for 24 hours prior to each experiment by transferring 20-25 *C. pacificus* individuals (adult females) into 1-2 200 mL beakers (depending on copepods available for the experiment). Each beaker was wrapped in aluminum foil and placed in a dark room to maintain darkness, and was kept at room temperature.

Copepods were pipetted out of the 200 mL one at a time 10-20 minutes before the experiment and tethered to a 3-4 cm hair strand glued to a plastic straw on one end (taking the shape of a garden pick). To tether the copepod to the far end of the hair strand, an individual copepod was placed on a Petri dish with the least possible amount of water to limit the copepod movement. The far end of the hair strand was dipped into a drop of cyanoacrylate glue and immediately attached to the back of the copepod prosome (as if poking the copepod). Once tethered, the copepod was submerged in filtered seawater at room temperature and was observed for a few minutes to ensure that the copepod was still alive and no appendages had been damaged (typically the long antennules can break during this process). If the copepod was in good shape and still active, it was used for the experiment. This mode of tethering allows the copepod to still perform forward escape jumps or other movements since all the appendages (sensory, locomotory and feeding appendages) were free of movement.

Two experimental tanks were used for this experiment. One tank had a square base (10 cm × 10 cm) and a height of 50 cm, and a second tank had a rectangular base (16 cm x 8 cm) and a height of 30.5 cm. For the experiments, a tank filled with filtered natural seawater was set up on a table. At the top of the tank there was an adjustable rig used to attach and suspend the straw with the tethered copepod. This allowed us to set the copepod at a consistent distance away from the walls and directly under a small funnel used to introduce individual aggregates, one at a time. Importantly, the tether was used to constrain the movement of the copepod so that it remained in the camera's field of view (FOV).

A high-speed camera (Photron FASTCAM Mini AX50) was placed facing a side of the tank in a way that the copepod appeared within the camera's FOV (~2 cm x 2 cm) and on a sideview body position so the camera could capture all the copepod appendages (antennule, feeding and swimming appendages). This allowed us to capture the copepod appendages when it was still as well as when it performed escape jumps. These jumps were limited by the hair tether, but constrained within the FOV to observe when the copepod made contact with the aggregate passing by on its downward trajectory. A 730 nanometer near-infrared light-emitting diode (M730L4 730 nm, 515 mW Mounted LED, Thorlabs) was set up facing straight into the lens but on the opposite side of the tank; this created a shadowgraph-type image. A near-infrared light was used because copepods, like most crustaceans and marine animals, are not sensitive to this wavelength. This was done to

avoid light-induced responses by the copepods. All recordings were filmed at 2000 frames per second (fps), but the videos were compressed to 30 fps for visual processing. All experiments were conducted inside a dark room.

Before switching to a new copepod for recording, the tank was emptied and replaced with fresh filtered seawater to remove any residual aggregate material from the tank. Video recordings were only kept if the aggregate successfully sank within the field of the view of the camera (thus allowing a possible interaction to occur). So, for example, in Experiment 1, there is a recording for Copepod #3, Aggregate #3, but no other aggregates, indicating that Aggregate #1, #2, and #4 did not successfully sink within the field of view of the camera.

Data Processing Description

Images of individual copepod-aggregate interactions were analyzed by visually observing the recordings of copepod behaviors when the aggregate was above, near and below the animal. The video feed was slowed down to annotate the type of interaction that occurred (or did not occur) and what effect that interaction had on the aggregate (see Table 2 in "Supplemental Files" and the associated keys).

Interaction Type Key

DI = Direct Interaction
IT = Indirect Interaction
NI = No Interaction*
*When the aggregate landed on the tether and therefore behind copepod, the video was not included in analysis.

Effect on Aggregate Key

FG = Fragmentation**
DT = Distortion (Deformation)**
NE = No observed Effect*
*When the aggregate landed on the tether and therefore behind copepod, the video was not included in analysis.
**Note that it was possible for both fragmentation and distortion to occur, in which case both are indicated for that interaction.

BCO-DMO Data Manager processing notes:
* Copepod videos bundled into zip files. And a full inventory of files, size, and which bundle they are in was attached as a supplemental table.
* Additional supplemental tables extracted from metadata form text and formatted as a table.

BCO-DMO Processing Description

Version 1 2022-06-28
* Submitted files were attached to this dataset. Video files were bundled into zip files and attached (see file descriptions for details of each file). File inventories with associated metadata were constructed and also attached as supplemental files.

Note on 2025-02-11 about Version 2:
This dataset has not been reviewed by the data submitter and has been temporarily taken down for further QA assessment, error checking, and file transfer to BCO-DMO's contemporary data system.
An error was previously detected in one of the zip files and was corrected in 2024-04-03 but the dataset hadn't been finalized.

Note on 2025-02-13 about version 2:
There are no known issues with the dataset but it has not been marked "final." The data submitter is being contacted for their review ahead of publication.

Data files were restored to the public page as of 2025-02-13 (corresponding to dataset version 2) after revising the zip files and file inventories. Each video was assessed on a windows PC and was playable.

To ensure the integrity of the video files, the .zip packages were re-made and added to the page along with a revised supplemental file inventory "copepod_video_inventory.csv." The updated file inventory includes checksums for each video file (as provided by the submitter from dropbox share). The checksums (UNIX command md5sum) can be used to verify the video file integrity after download or file transfers.

Note on 2025-02-18 about version 2: Data have been reviewed by the data submitter and no further changes were requested ahead of publication on this date.

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Data Files

File
Copepod Videos (Control) filename: control_copepod_videos.zip (ZIP Archive (ZIP), 26.72 GB) MD5:9708b75e8dbfd5b94fc2a266d708b8e3 See related Supplemental tables for file inventories and a description of the experimental design for each control. This file bundle contains all control videos (.mp4) for all experiments (1,2,3).
Copepod Videos (Exp1) filename: Exp1_copepod_videos.zip (ZIP Archive (ZIP), 25.29 GB) MD5:a6bb80ca270e0015823e00475f53d455 This file bundle contains videos (.mp4) of copepod-aggregate interactions for Experiment 1 (Exp1). Control videos for this experiment are within the separate bundle control_copepod_videos.zip See related Supplemental tables for file inventories and information about the interaction type, and effect on aggregate for each video.
Copepod Videos (Exp2) filename: Exp2_copepod_videos.zip (ZIP Archive (ZIP), 19.55 GB) MD5:97772a7573c8959a0ee3de459677df85 This file bundle contains videos (.mp4) of copepod-aggregate interactions for Experiment 2 (Exp2). Control videos for this experiment are within the separate bundle control_copepod_videos.zip See related Supplemental tables for file inventories and a information about the interaction type, and effect on aggregate for each video.
Copepod Videos (Exp3 part1) filename: Exp3_copepod_videos_part1.zip (ZIP Archive (ZIP), 26.38 GB) MD5:6a92ec3b835baddcbfd0437396ef62ef This file bundle contains videos (.mp4) of copepod-aggregate interactions for Experiment 3 (Exp3) copepods 1-4 of 8. Control videos for this experiment are within the separate bundle control_copepod_videos.zip See related Supplemental tables for file inventories and a information about the interaction type, and effect on aggregate for each video.
Copepod Videos (Exp3 part2) filename: Exp3_copepod_videos_part2.zip (ZIP Archive (ZIP), 22.06 GB) MD5:edd9856e9ecbb3c605ecfc89a3f654c8 This file bundle contains videos (.mp4) of copepod-aggregate interactions for Experiment 3 (Exp3) copepods 5-8 of 8. Control videos for this experiment are within the separate bundle control_copepod_videos.zip See related Supplemental tables for file inventories and a information about the interaction type, and effect on aggregate for each video.

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Supplemental Files

File

Copepod video inventory (.mp4 file list)

filename: copepod_video_inventory.csv

(Comma Separated Values (.csv), 3.49 KB)
MD5:7ce81cdb6838ac051b12f83edf062b0e

This table contains a full inventory for all copepod videos including control and interaction videos, it also includes which file bundle (zip) each video is in.

Columns:
Filename filesize_GB md5sum file_bundle_name

Filename, Filename including extension
filesize_bytes, Filesize in bytes as reported by unix command find (%s)
Filesize_GB, Filesize in GB (storage size in GB)
md5sum, Checksum (unix command md5sum) which can be used to verify the integrity of the transferred file.
file_bundle_name, the name of the file bundle each mp4 file is within. These file bundles are available for download in the "Data Files" section.

Table 1. File size and description of control observation videos

filename: Table1_control_observation_videos.csv

(Comma Separated Values (.csv), 1.25 KB)
MD5:5b7889ed075d44935151b16c1fdf696f

Table 1. File size and description of control observation videos.

Data table contains columns:

Filename,file name without extension (add extension .mp4 for full filename)

Video File Size (GB),File size in GB

Description, Description of the control experimental design

Table 2. File size, interaction type, and effect on aggregate for each video of an observed copepod-aggregate interaction.

filename: Table2_copepod_aggregate_interaction_videos.csv

(Comma Separated Values (.csv), 1.03 KB)
MD5:e19b5dd70342c6ed9062d77b2acc8d2a

Table 2. File size, interaction type, and effect on aggregate for each video of an observed copepod-aggregate interaction.

Columns in data table:

Filename,file name without extension (add extension .mp4 for full filename)

Video File Size (GB),File size in GB

Interaction Type,Code for interaction type (e.g. DI). See key below.

Effect on Aggregate,Code for effect on aggregate (e.g. FG). See key below.

Interaction Type Key:

DI = Direct Interaction

IT = Indirect Interaction

NI = No Interaction*

*When the aggregate landed on the tether and therefore behind copepod, the video was not included in analysis.

Effect on Aggregate Key:

FG = Fragmentation**

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*When the aggregate landed on the tether and therefore behind copepod, the video was not included in analysis.

**Note that it was possible for both fragmentation and distortion to occur, in which case both are indicated for that interaction

Parameters

Parameters for this dataset have not yet been identified

Instruments

Dataset-specific Instrument Name	Photron FASTCAM Mini AX50
Generic Instrument Name	high-speed camera
Dataset-specific Description	high speed camera: Photron FASTCAM Mini AX50
Generic Instrument Description	A high-speed imaging camera is capable of recording rapid phenomena with high-frame rates. After recording, the images stored on the medium can be played back in slow motion. The functionality in a high-speed imaging device results from the frame rate, or the number of individual stills recorded in the period of one second (fps). Common video cameras will typically record about 24 to 40 fps, yet even low-end high-speed cameras will record 1,000 fps.

Dataset-specific Instrument Name	M730L4 730 nm, 515 mW Mounted LED, Thorlabs
Generic Instrument Name	LED light
Dataset-specific Description	A near-infrared light-emitting diode: M730L4 730 nm, 515 mW Mounted LED, Thorlabs
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.

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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