Experimental results describing Pseudodiaptomus marinus gut minimum fluorescence thresholds that were analyzed at San Francisco State University in 2013

Website: https://www.bco-dmo.org/dataset/546558
Data Type: experimental
Version: 2015-01-15

Project
» Feeding and food limitation in copepod nauplii, the neglected life stage (food limitation in copepod nauplii)

Contributors	Affiliation	Role
Kimmerer, William	San Francisco State University (SFSU)	Principal Investigator
Cohen, Sarah	San Francisco State University (SFSU)	Co-Principal Investigator
Copley, Nancy	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Table of Contents

Dataset Description

Minimum fluorescence thresholds were measured for adult and nauplius *Pseudodiaptomus marinus* guts.

Methods & Sampling

The copepod *Pseudodiaptomus marinus* was cultured at RTC and used in the experiments. The evening before an experiment the copepods were size fractioned to separate the adults from the nauplii (larval stage) and were placed in 0.45 µm filtered San Francisco Bay water with the alga *Rhodomonas salina,* to allow for feeding. We handpicked 25 adults and 50 nauplii (Naupliar stage 3 and 4) which were maintained in 3 L of previously described water. The following morning (0900) the copepods were rinsed into freshly filtered 0.45 µm San Francisco Bay water and held for three hours to allow the individuals to clear the contents of their guts. While the copepods cleared their guts, the ciliates (*Codonellopsis sp.* or *Favella sp.*) were stained with the fluorescent stain, CellTracker™ Green CMFDA (5-chloromethylfluorescein diacetate) (Li et al. 1996) for one hour at 1 µmolar solution. After staining was complete, the ciliates were rinsed from the stain by being rinsed over a 35µm mesh and gently dunked into clean 0.45 µm filtered San Francisco Bay water. The ciliates were then resuspended and introduced to the copepods, which were allowed to feed for 1 hour.

To obtain the best images of the copepod digestive track the individuals needed to be alive. After 1 hour of feeding the copepods were rinsed over a 100 µm mesh which allowed for the separation of the ciliates and copepods. Using a dissecting microscope, individuals were transferred to a microscope slide in a small amount of water, which held each individual on its side for the best image of the gut. Once in place the microscope slide was transferred to the Olympus IX83 Epifluorescence microscope, and each individual was imaged under two settings. The first setting was bright-field and the second setting was the GFP filter (395 nm excitation, 500 nm emission) which allowed for the detection of the stained ciliate in the digestive track. This experiment was repeated twice.

Our next approach to identify the grazing of copepods on ciliates focused on offering the copepods a natural assemblage of food that was spiked with a known amount of stained ciliates. Three liters of surface seawater was collected from the seawall at RTC, size fractioned by reverse filtration into a 20-50 µm cohort, which removed other large grazers yet maintained other species of ciliates and phytoplankton. Cultured *Favella sp.* was stained as previously described and then introduced to the natural assemblage. Two containers (1500 ml each) were made and the copepods were allowed to feed for 1 hour. At the end of the grazing period 3 replicate samples of 50 ml each were preserved with 0.1% Acid Lugol and 3 replicate samples of 50 ml each were preserved in 50% Glutaraldehyde.

Data Processing Description

The images were analyzed using Photoshop and Image J to calculate a Gut Fullness Index which was calculated as a percent. All images were processed identically including, calculation of total gut volume, a threshold correction to account for autofluorescence, and amount of gut full of the food item.

BCO-DMO Processing:
- added conventional header with dataset name, PI name, version date, reference information
- renamed parameters to BCO-DMO standard
- replaced space with underscore
- reformated date from mmm/dd/yyyy to yyyy-mm-dd

[table of contents | back to top]

Data Files

File
fluor_thresholds.csv (Comma Separated Values (.csv), 1.89 KB) MD5:2dd21b2974f04625f921e651bf9b6f67 Primary data file for dataset ID 546558

[table of contents | back to top]

Related Publications

Kamiyama, T. (2000). Application of a vital staining method to measure feeding rates of field ciliate assemblages on a harmful alga. Marine Ecology Progress Series, 197, 299–303. https://doi.org/10.3354/meps197299
Methods

Li, A., Stoecker, D., Coats, D., & Adam, E. (1996). Ingestion of fluorescently labeled and phycoerythrin-containing prey by mixotrophic dinoflagellates. Aquatic Microbial Ecology, 10, 139–147. https://doi.org/10.3354/ame010139
Methods

Merrell, J. R., & Stoecker, D. K. (1998). Differential grazing on protozoan microplankton by developmental stages of the calanoid copepod Eurytemora affinis Poppe. Journal of Plankton Research, 20(2), 289–304. https://doi.org/10.1093/plankt/20.2.289
Methods

Vogt, R. A., Ignoffo, T. R., Sullivan, L. J., Herndon, J., Stillman, J. H., & Kimmerer, W. J. (2013). Feeding capabilities and limitations in the nauplii of two pelagic estuarine copepods, Pseudodiaptomus marinus and Oithona davisae. Limnology and Oceanography, 58(6), 2145–2157. Portico. https://doi.org/10.4319/lo.2013.58.6.2145
Methods

[table of contents | back to top]

Parameters

Parameter	Description	Units
date	experiment data	yyyy-mm-dd
species	copepod species	unitless
stage	copepod life stage: N=nauplius	unitless
file_rep_num	file replicate number	unitless
threshold	the threshold for deciding if a pixel is or is not showing fluorescence beyond the background; determined by observations on copepods without food in their guts.	relative fluorescence units in the plate reader

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	Fluorometer
Generic Instrument Name	Fluorometer
Dataset-specific Description	Turner 10AU
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	
Generic Instrument Name	plate reader
Dataset-specific Description	Tecan Infinite F200 or Biotek Synergy 2 microplate reader was used for each analysis. Each microplate reader contained a 430/20 EX, 680/20 EMfilter pair for Chl a.
Generic Instrument Description	Plate readers (also known as microplate readers) are laboratory instruments designed to detect biological, chemical or physical events of samples in microtiter plates. They are widely used in research, drug discovery, bioassay validation, quality control and manufacturing processes in the pharmaceutical and biotechnological industry and academic organizations. Sample reactions can be assayed in 6-1536 well format microtiter plates. The most common microplate format used in academic research laboratories or clinical diagnostic laboratories is 96-well (8 by 12 matrix) with a typical reaction volume between 100 and 200 uL per well. Higher density microplates (384- or 1536-well microplates) are typically used for screening applications, when throughput (number of samples per day processed) and assay cost per sample become critical parameters, with a typical assay volume between 5 and 50 µL per well. Common detection modes for microplate assays are absorbance, fluorescence intensity, luminescence, time-resolved fluorescence, and fluorescence polarization. From: http://en.wikipedia.org/wiki/Plate_reader, 2014-09-0-23.

Dataset-specific Instrument Name	Spectrophotometer
Generic Instrument Name	Spectrophotometer
Dataset-specific Description	Agilent 8453 spectrophotometer (Agilent Technologies)
Generic Instrument Description	An instrument used to measure the relative absorption of electromagnetic radiation of different wavelengths in the near infra-red, visible and ultraviolet wavebands by samples.

[table of contents | back to top]

Deployments

Kimmerer_2013

Website	https://www.bco-dmo.org/deployment/546436
Platform	SFSU RTC
Start Date	2009-09-01
End Date	2014-08-31
Description	Copepod feeding studies

[table of contents | back to top]

Project Information

Feeding and food limitation in copepod nauplii, the neglected life stage (food limitation in copepod nauplii)

Coverage: San Francisco Estuary

This project will investigate feeding by copepod nauplius larvae, the most abundant metazoans in the sea. It will answer three questions: 1) How does food selection by adults and nauplii differ when they are fed multiple prey species in the laboratory? 2) How does food selection by adults and nauplii differ when they are feeding on natural prey assemblages? and, 3) How do growth, development, and survival differ between copepodites and nauplii when their growth is food limited? Comparative experiments and field-based measurements will contrast the food consumed, and the effects of food limitation, between nauplii and later life stages. This contrast will include attributes of food such as size, taxon, and motility, and will include experiments with cultured prey offered singly or in a mixture, and natural prey, and apply genetic techniques to determine prey consumption by a predatory copepod. Copepods will be collected from the San Francisco Estuary, with four species selected for experiments to span taxonomic groups, sizes, salinity ranges, and general feeding behavior. A variety of techniques will be applied to account for the inevitable biases and limitations of each; all but one have previously been applied in our laboratories. These will include laboratory feeding experiments using cultured prey individually and in mixtures, and experiments using natural prey. Consumption of prey in experimental bottles will be measured as chlorophyll concentration and through particle counts by microscopy and flow cytometry. Radioactively labeled prey will be used in short incubations to determine feeding on particular prey types. Samples from the field will be examined for gut fluorescence. Separate experiments will determine how nauplii and copepodites survive and grow at different concentrations of food. Investigations of feeding by a predatory copepod (Tortanus dextrilobatus) will use molecular techniques to identify mitochondrial and nuclear DNA from diverse suspected prey species. Specific primers will be developed for common zooplankton species consumed by T. dextrilobatus in the laboratory. General primers and screening protocols developed here will be useful for identifying food web interactions in other estuarine communities.

Copepod nauplii are important both in their diverse trophic roles in ocean foodwebs and in the population dynamics of copepods. Nauplii have a completely different feeding apparatus from later stages, and the first feeding stage can be very sensitive to starvation, making these life stages critical to population dynamics. Yet extant copepod population models treat nauplii as miniature adults. This work will provide valuable input to the growing efforts at modeling ocean ecosystems.

Further details from final report (pdf)

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-0929075

[table of contents | back to top]