

# Experimental results describing *Pseudodiaptomus marinus* gut area measured in pixels from San Francisco State University in 2013

**Website:** <https://www.bco-dmo.org/dataset/546575>

**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
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## Dataset Description

Adult and nauplius *Pseudodiaptomus marinus* gut area were measured using epifluorescent microscopy.

### Related Reference:

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*

Li, A., et al. (1996). Ingestion of fluorescently labeled and phycoerythrin-containing prey by mixotrophic dinoflagellates." *Aquatic Microbial Ecology* 10(2): 139-147.

Merrell, J. R. and D. K. Stoecker (1998). Differential grazing on protozoan microplankton by developmental stages of the calanoid copepod *Eurytemora affinis* Poppe. *Journal of Plankton Research* 20(2): 289-304.

Vogt, R.A., T.R. Ignoffo, L.J. Sullivan, J. Herndon, J.H. Stillman, and W. Kimmerer. 2013. Feeding capabilities and limitations in the nauplii of two pelagic estuarine copepods, *Pseudodiaptomus marinus* and *Oithona davisae*. *Limnology and Oceanography* 58: 2145-2157

## Methods & Sampling

Copepods were incubated with phytoplankton for 1 hour. Incubations were terminated by gently pouring the contents of incubation bottles through a 53 mm sieve, then rinsing the copepods into a petri dish with GF/F filtered water. A total of 12 copepods were sampled from each stage for each phytoplankton and for the controls.

Copepods were sorted under a dissecting microscope and immediately transferred to glass microscope slides. Excess water was removed with a fine-tipped Pasteur pipette to immobilize the copepod for optimal viewing. To

minimize pigment degradation, slides containing the copepods were transferred to the microscope as quickly as possible, always within 2 min of sorting.

A Nikon model E400 epifluorescence microscope at 20X magnification, outfitted with a Nikon B-2A long-pass filter cube (470EX/515LP, Nikon) and a custom band-pass filter cube (430EX/680EM, Omega Optical) was used. The longpass filter pair provided a brighter overall image than the band-pass filter pair and also fluoresced non-chlorophyll structures (e.g., the copepod exoskeleton fluoresces green with blue excitation). The band-pass filter cube showed fluorescence from only chlorophyll and phaeopigments. Photographs of the copepods were taken at each filter setting using a Canon Digital model T3i single-lens reflex camera (ISO 6400, F 1/15) remotely controlled with the Canon Electro-Optical System Utility software to produce clear images of the epifluorescence (Vogt, 2013, Fig. 1).

Images were processed with Photoshop CS6 (Adobe). Total gut area was manually digitized using the lasso and measurement tools on the long-pass-filtered images. The mean gut area of 12 individuals for each copepod species and stage was used for calculations. The threshold tool was used to select areas of the long-pass-filtered images containing pigment (gut pigment area) that exceeded baseline fluorescence. Signal intensity was not used in the calculation of the GPI (Gut Pigment Index) because pigment content and composition differed among phytoplankton taxa. Pixels of the band-pass-filtered images exceeding the threshold value were selected and their area estimated using the measurement tool. The gut-pigment area was then divided by the total gut area, yielding a relative GPI for each copepod. The same procedure was used to process images of copepods sampled from the control bottles. Copepods with <5% GPI were assumed not to have fed, as the GPI values in those samples were within the range of indices estimated from the controls.

## Data Processing Description

### 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
- reformatted date from mmm/dd/yyyy to yyyy-mm-dd
- added mean values after each stage

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## Data Files

File
<b>gut_area.csv</b> (Comma Separated Values (.csv), 5.22 KB) MD5:6ea59b7b86e4f0189bcd1d87eba6ad0
Primary data file for dataset ID 546575

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## 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
area	area of gut	pixels

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## Instruments

<b>Dataset-specific Instrument Name</b>	camera
<b>Generic Instrument Name</b>	Camera
<b>Dataset-specific Description</b>	Canon® Digital model T3i single lens reflex (SLR) camera (ISO 6400, F 1/15) remotely controlled with the Canon Electro-Optical System (EOS) Utility software.
<b>Generic Instrument Description</b>	All types of photographic equipment including stills, video, film and digital systems.

<b>Dataset-specific Instrument Name</b>	Epifluorescence Microscope
<b>Generic Instrument Name</b>	Fluorescence Microscope
<b>Dataset-specific Description</b>	Nikon model E400 epifluorescence microscope at 20X magnification, outfitted with a Nikon B-2A longpass filter cube (470EX/515LP, Nikon) and a custom bandpass filter cube (430EX/680EM, Omega Optical).
<b>Generic Instrument Description</b>	Instruments that generate enlarged images of samples using the phenomena of fluorescence and phosphorescence instead of, or in addition to, reflection and absorption of visible light. Includes conventional and inverted instruments.

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## Deployments

### Kimmerer\_2013

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

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## 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 \(pdf\)](#)

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## Funding

Funding Source	Award
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-0929075</a>

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