

# Initial prey abundances for copepod grazing experiments in the Kaneohe Bay, HI, May-June 2013 (MEPS 2017) (EAGER: Copepod nauplii project)

**Website:** <https://www.bco-dmo.org/dataset/712626>

**Data Type:** experimental

**Version:** 1

**Version Date:** 2017-09-01

## Project

» [New molecular methods for studying copepod nauplii in the field](#) (EAGER: Copepod nauplii)

Contributors	Affiliation	Role
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## Abstract

This dataset reports initial abundance and biomass of size classes of prey items as measured by Coulter Counter, flow cytometer, inverted and epi-fluorescence microscopes and as chlorophyll concentration. These data are published in MEPS (2017) and are the result of M. Jungbluth's Ph.D. thesis work.

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## Coverage

**Spatial Extent:** Lat:21.432 Lon:-157.78

**Temporal Extent:** 2013-05-27 - 2013-06-05

## Acquisition Description

From Jungbluth et al. 2017 – MEPS

## Prey size spectra and abundance (Coulter Counter)

Initial and final time-point Coulter Counter (CC) samples were taken for prey particle spectra by gently pouring 20 ml from each incubation bottle through a 35 µm cap filter into a clean beaker, then gently back-washing the filter into the experimental bottle to return any nauplii using a small volume of 0.2 µm filtered seawater. From this subsample, triplicate 2 ml volumes were measured with a Beckman Coulter Multisizer III CC with a 100 µm orifice tube, yielding a spectrum of particle sizes from 2–35 µm ESD, as

well as quantitative abundance data. These raw data were further processed in R (R Core Team 2016) to streamline binning of prey size groups, for calculations of clearance and ingestion rates, and for statistical analyses.

Prey ESD was converted to biovolume (BV;  $\mu\text{m}^3$ ), then to carbon (C;  $\text{pg C cell}^{-1}$ ), using the relationship  $C = 0.216 \times \text{BV}^{0.939}$ , which applies well to taxonomically diverse protists (Menden-Deuer & Lessard 2000). Averages of cell abundance and biomass were binned into 5 prey size groupings (2–5, 5–10, 10–15, 15–20, and 20–35  $\mu\text{m}$ ). The binned, averaged data for initial and final time points for each control and treatment bottle were used to calculate carbon ingestion (I;  $\text{ng C nauplius}^{-1} \text{h}^{-1}$ ) and clearance rates (F,  $\text{ml nauplius}^{-1} \text{h}^{-1}$ ) on each prey size group using the equations of Frost (1972).

### **Photosynthetic eukaryotes (flow cytometry)**

Flow cytometry (FCM) samples (1.5 ml) for photosynthetic eukaryote (PEUK) abundance were taken from the 20 ml subsamples (as described above), preserved in 0.4% paraformaldehyde (final concentration), flash-frozen in liquid nitrogen and transferred to a  $-80^\circ\text{C}$  freezer until processing. Preserved, frozen FCM samples were thawed in batches, stained with the DNA dye Hoechst 34442 (1  $\mu\text{g ml}^{-1}$ , final concentration) (Campbell & Vaultot 1993, Monger & Landry 1993), and analyzed using a Beckman-Coulter Altra flow cytometer for phytoplankton population abundances using fluorescence signals from DNA, phycoerythrin and chl a. Data were grouped into relevant populations using FlowJo (Treestar). PEUK cell abundances were converted to biomass using data from parallel microscopy samples, which showed that the eukaryotic phytoplankton in these samples were dominated by 2 to 3  $\mu\text{m}$  ESD spherical cells, with an average biomass of 1.55  $\text{pg C cell}^{-1}$  (biomass conversions as in Menden-Deuer & Lessard 2000).

### **Nano- and microplankton abundance and biomass (microscopy)**

Initial and final samples for nano- and microplankton abundance by epifluorescence microscopy (EPI) were preserved (0.4% paraformaldehyde, final concentration), and kept in the dark and cold ( $4^\circ\text{C}$ ) until filtered within 24 to 48 h. EPI samples (25 or 50 ml) were stained with 0.5 nM proflavin (1 to 2 h prior to filtration), then filtered onto 0.8  $\mu\text{m}$  black polycarbonate filters (Midland Scientific), stained with 4',6-diamidino-2-phenylindole (DAPI) for 2 min and mounted on a slide. These slides were frozen at  $-80^\circ\text{C}$  until digitally imaged within 2 mo of collection.

Digital images of the slides were taken using a color camera (Olympus U-LH100HGAPO) attached to an epifluorescence microscope (Olympus Model BX51 TRF,  $400\times$  total magnification), and the software program Microfire<sup>TM</sup> (Optronics). For each slide, 3 sequential digital images were taken of 30 random fields, using 3 different excitation/emission filters; one each to illuminate chl a/proflavin (EX450-480; DM500,  $\text{EM} \geq 515$ ), phycoerythrin (primarily due to *Synechococcus*), and DNA (EX330-385, DM400,  $\text{EM} > 420$ ) fluorescence. Living cells were distinguished from dead cells and debris by the presence of nuclei, and autotrophic and heterotrophic cells were distinguished by the presence of chl a.

Images were analyzed by sizing, counting, and identifying autotrophic and heterotrophic cells 2–10  $\mu\text{m}$  in size until  $>100$  cells were characterized. To evaluate the large ( $>10 \mu\text{m}$ ) cell abundance and biomass, all  $>10 \mu\text{m}$  cells on  $\frac{1}{4}$  of one randomly selected control and treatment slide from each experiment were counted, identified as an autotroph or heterotroph, and measured using a calibrated ocular micrometer. Cell dimensions were used to estimate biovolume (oblate spheroid), and converted to biomass (Menden-Deuer & Lessard 2000). Diatoms  $>10 \mu\text{m}$  were also quantified, but they were never abundant during our experiments (see Table 2).

Ciliate biomass and abundance was estimated by inverted microscopy on samples pre-screened through a  $<35 \mu\text{m}$  mesh, preserved with a 1/20 dilution of acid Lugol's solution (Thronsdon 1978), and kept in the dark at room temperature until analysis ( $\sim 1$  yr later) by the Utermöhl technique (Sherr & Sherr 1993). Aliquots of 28 ml from randomly selected control and experimental treatments were settled and their entire contents examined (18 to 87 total cells in sample volume, median 55) with a Zeiss inverted microscope ( $400\times$  magnification), with digital images taken (Moticam camera and software) for subsequent dimensional analyses. The measured length and width of each cell was converted to biovolume based on the appropriate geometric shapes, and converted to carbon biomass (Menden-Deuer & Lessard 2000).

## Chl a determinations

For chl a, triplicate 305 ml samples were filtered onto GF/Fs (Whatman), flash-frozen (LN<sub>2</sub>), and kept at -80°C freezer until measurements were made 4 mo later. Chl a (and phaeopigment) was measured using a Turner Designs (model 10AU) fluorometer, using the standard extraction and acidification technique (Yentsch & Menzel 1963, Strickland & Parsons 1972).

For complete methodology, see the Supplemental Files section.

## Processing Description

### BCO-DMO Processing:

- added conventional header with dataset name, PI name, version date
- modified parameter names to conform with BCO-DMO naming conventions
- reformatted date from d-Mon-yy to yyyy-mm-dd

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## Related Publications

Jungbluth MJ (2016) Copepod nauplii and their roles in planktonic marine food webs. Oceanography Ph.D. Dissertation, University of Hawai'i at Manoa, Honolulu, Hawaii.

<https://pqdtopen.proquest.com/pubnum/10587374.html>

*Results*

Jungbluth, M., Selph, K., Lenz, P., & Goetze, E. (2017). Species-specific grazing and significant trophic impacts by two species of copepod nauplii, *Parvocalanus crassirostris* and *Bestiolina similis*. Marine Ecology Progress Series, 572, 57–76. doi:[10.3354/meps12139](https://doi.org/10.3354/meps12139)

*Results*

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## Related Datasets

### References

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Goetze, E. (2021) **Initial field conditions at Kane'ohē Bay, Oahu, Hawaii and abundances of *Parvocalanus crassirostris* and *Bestiolina similis* nauplii, May/June 2013 (EAGER: Copepod nauplii project)**. Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2017-08-07 doi:10.26008/1912/bco-dmo.712344.1 [[view at BCO-DMO](#)]

### IsRelatedTo

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Goetze, E. (2017) **Copepods *Parvocalanus crassirostris* and *Bestiolina similis* naupliar ingestion and clearance rates on natural prey assemblages from Kaneohe Bay, Oahu, 2013 (MEPS 2017) (EAGER: Copepod nauplii project)**. Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2017-09-01 <http://lod.bco-dmo.org/id/dataset/712293> [[view at BCO-DMO](#)]

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## Parameters

Parameter	Description	Units
experiment	experiment number	unitless
date_local	local date formatted as yyyy-mm-dd	unitless
lat	latitude; north is positive	decimal degrees
lon	longitude; east is positive	decimal degrees
prey_quant_method	Method used to determine abundance or biomass of prey: CC=Coulter counter measured prey; CHL=Chlorophyll a (	unitless
prey_type_size_range	type or size range of prey items: PEUK=photosynthetic eukaryote AUT=autotrophic HET=heterotrophic SYN=Synechococcus PRO=Prochlorococcus HBACT=Heterotrophic bacteria	micrometers
initial_cells	initial concentration of prey cells	cells/milliliter
initial_biomass	initial biomass of prey cells measured as either carbon or chlorophyll	micrograms Carbon/Liter or micrograms Chlorophyll/Liter

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## Instruments

<b>Dataset-specific Instrument Name</b>	
<b>Generic Instrument Name</b>	Turner Designs Fluorometer 10-AU
<b>Generic Instrument Description</b>	The Turner Designs 10-AU Field Fluorometer is used to measure Chlorophyll fluorescence. The 10AU Fluorometer can be set up for continuous-flow monitoring or discrete sample analyses. A variety of compounds can be measured using application-specific optical filters available from the manufacturer. (read more from Turner Designs, turnerdesigns.com, Sunnyvale, CA, USA)

<b>Dataset-specific Instrument Name</b>	Beckman Coulter Altra flow cytometer
<b>Generic Instrument Name</b>	Flow Cytometer
<b>Generic Instrument Description</b>	Flow cytometers (FC or FCM) are automated instruments that quantitate properties of single cells, one cell at a time. They can measure cell size, cell granularity, the amounts of cell components such as total DNA, newly synthesized DNA, gene expression as the amount messenger RNA for a particular gene, amounts of specific surface receptors, amounts of intracellular proteins, or transient signalling events in living cells. (from: <a href="http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm">http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm</a> )

<b>Dataset-specific Instrument Name</b>	
<b>Generic Instrument Name</b>	Inverted Microscope
<b>Generic Instrument Description</b>	An inverted microscope is a microscope with its light source and condenser on the top, above the stage pointing down, while the objectives and turret are below the stage pointing up. It was invented in 1850 by J. Lawrence Smith, a faculty member of Tulane University (then named the Medical College of Louisiana). Inverted microscopes are useful for observing living cells or organisms at the bottom of a large container (e.g. a tissue culture flask) under more natural conditions than on a glass slide, as is the case with a conventional microscope. Inverted microscopes are also used in micromanipulation applications where space above the specimen is required for manipulator mechanisms and the microtools they hold, and in metallurgical applications where polished samples can be placed on top of the stage and viewed from underneath using reflecting objectives. The stage on an inverted microscope is usually fixed, and focus is adjusted by moving the objective lens along a vertical axis to bring it closer to or further from the specimen. The focus mechanism typically has a dual concentric knob for coarse and fine adjustment. Depending on the size of the microscope, four to six objective lenses of different magnifications may be fitted to a rotating turret known as a nosepiece. These microscopes may also be fitted with accessories for fitting still and video cameras, fluorescence illumination, confocal scanning and many other applications.

<b>Dataset-specific Instrument Name</b>	
<b>Generic Instrument Name</b>	Fluorescence Microscope
<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.

<b>Dataset-specific Instrument Name</b>	Beckman Coulter Multisizer III Coulter Counter
<b>Generic Instrument Name</b>	Coulter Counter
<b>Dataset-specific Description</b>	100 µm orifice tube
<b>Generic Instrument Description</b>	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 <a href="https://en.wikipedia.org/wiki/Coulter_counter">https://en.wikipedia.org/wiki/Coulter_counter</a>

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## Deployments

### Goetze\_2012-2013

<b>Website</b>	<a href="https://www.bco-dmo.org/deployment/637678">https://www.bco-dmo.org/deployment/637678</a>
<b>Platform</b>	lab UHawaii_SOEST
<b>Start Date</b>	2012-03-16
<b>End Date</b>	2013-06-05
<b>Description</b>	microzooplankton studies

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## Project Information

### New molecular methods for studying copepod nauplii in the field (EAGER: Copepod nauplii)

**Coverage:** Kaneohe Bay, Oahu, Hawaii

*Description from NSF Award Abstract:*

The most abundant metazoans in the open sea are often the earliest developmental stages of copepods, their nauplii. Nauplii remain under-studied due to the limitations of conventional techniques and an historical emphasis on studying the larger mesozooplankton. However, there is increasing recognition that nauplii play important roles in food web dynamics, and considerable evidence that nauplii may be important trophic intermediaries between microbial and classical food webs due to their high abundance, high weight-specific ingestion rates, and ability to feed on relatively small particles. This team of investigators is developing a novel molecular approach to studying diverse populations of nauplii in mixed field samples based on quantitative Polymerase Chain Reaction (qPCR). They propose to complete development and validation of this qPCR-based technique for enumeration of nauplii, and evaluate its utility in the field. The specific objectives of this research are to identify and reduce technical and biological sources of error in the methodology, determine the accuracy of the method across a range of environmental conditions, and complete one paired field experiment that compares the grazing impact of naupliar and protozoan micro-grazers in a model subtropical coastal ecosystem.

**Note:** This project is funded by an NSF EAGER award.

*Related publications:*

Jungbluth, M.J., Goetze, E., and Lenz, P.H. 2013. Measuring copepod naupliar abundance in a subtropical bay using quantitative PCR. *Marine Biology*, 160: 3125-3141. doi: [10.1007/s00227-013-2300-y](https://doi.org/10.1007/s00227-013-2300-y)

Jungbluth, M.J., and Lenz, P.H. 2013. Copepod diversity in a subtropical bay based on a fragment of the mitochondrial COI gene. *Journal of Plankton Research*, 35(3): 630-643. doi: [10.1093/plankt/fbt015](https://doi.org/10.1093/plankt/fbt015)

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## Funding

Funding Source	Award
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-1255697</a>

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