

# Initial prey abundances for copepod grazing experiments in the Kaneohe Bay, HI, March-April 2015 (JEMBE 2017) (EAGER: Copepod nauplii project)

**Website:** <https://www.bco-dmo.org/dataset/714278>

**Data Type:** experimental

**Version:** 1

**Version Date:** 2017-09-06

## 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. These data are published in JEMBE (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:** 2017-03-10

## Acquisition Description

From Jungbluth et al. 2017 – JEMBE:

Naupliar grazing rates were measured on field-collected prey assemblages in bottle incubation experiments in the laboratory. Nauplii used in these experiments were derived from laboratory culture populations of *Parvocalanus crassirostris*, originally established from animals collected in Kaneohe Bay. At 18-h prior to the start of each experiment, adults were isolated and fed *Tisochrysis lutea* (formerly *Isochrysis galbana* Tahitian strain [Bendif et al., 2013]) at a concentration of 105–106 cells mL<sup>-1</sup>. After 6-h, adults were removed, and eggs and nauplii were allowed to develop for 12-h in order to produce a cohort of mid-stage nauplii (N3-N4) with a narrow age-range at the beginning of each experiment. Sets of approximately 50 nauplii were isolated into small volumes (< 10 mL) of 0.2 µm filtered seawater 1–2 h prior to the start of each grazing experiment.

Seawater for the prey assemblage was collected from the central basin of the southern semi-enclosed region of Kane'ohe Bay, Oahu, Hawai'i (21°25'56"N, 157°46'47"W) on two dates: 10 March 2015 (Experiment: E1) and 22 April 2015 (Experiment: E2). Seawater was collected from ~ 2 m depth using a 5 L General Oceanics Niskin bottle deployed by hand line, and gently transferred using acid-washed silicon tubing directly from the Niskin bottle into 20 L covered (dark) polycarbonate carboys. The seawater was transported to the laboratory within 2-h of collection. The collected water was gently pre-screened (35 µm Nitex mesh), which was intended to remove all in situ nauplii and other large grazers, so that the only metazoan grazers in the bottles were the added nauplii. The < 35 µm incubation water was added to pre-washed (10% HCL rinse, followed by 3 rinses with experimental seawater) 1 L polycarbonate bottles (total volume: 1120 mL).

Nutrients were not amended in control or treatment bottles due to the expected low rates of excretion by these small biomass nauplii over the incubation duration as compared with baseline levels in Kane'ohe Bay, and also in order to minimize development of artificially high nutrients given prevailing oligotrophic conditions in the study area. Excretion rates of copepods are a function of biomass (Vidal and Whitley, 1982, Mauchline, 1998), with excretion by nauplii roughly an order of magnitude lower than conspecific adults. At a nauplius grazer concentration of 50 nauplii in a 1 L volume, excretion rates result in values 2 to 3 orders of magnitude below the average nitrogen concentrations of 0.2–1.0 µM in Kane'ohe Bay (Drupp et al., 2011). Therefore, excretion rates in bottle incubations were expected to have negligible impacts on prey growth rates in experimental bottles, and nutrient amendment would have only altered the prey community further away from in situ conditions.

The isolated N3-N4 nauplii were transferred into triplicate < 35 µm incubation water bottles (grazing treatments) and placed on a bottle roller (4–6 rpm) to maintain prey in suspension for the duration of the incubation period. Parallel triplicate control treatments (incubation water without added nauplii) were also placed on the bottle roller. Grazing rates were measured using two densities of naupliar grazers: high (N = 92–97 nauplii L<sup>-1</sup>) and moderate (N = 45–50 nauplii L<sup>-1</sup>) densities. All incubations were run for a total of 24-h in the dark, with subsamples taken every six hours to examine changes in ingestion rates over time. Experiments were run at 21 °C, which is at the low end of the range of annual temperature fluctuations for this region of Kane'ohe Bay (20–29 °C in prior 5 years [Franklin et al., 2015]).

During the course of the incubation, triplicate 2-mL volumes of each subsample were measured with a Coulter Counter (Beckman-Coulter Multisizer III) with a 100 µm orifice tube, yielding a spectrum of particle sizes from 2 to 35 µm ESD, as well as quantitative abundance data. In a diverse environment with a variety of autotrophic and heterotrophic pico- to microplankton, standard cell quantification methods (e.g. epifluorescence microscopy, inverted microscopy) do not reliably preserve some components of the community (Omori and Ikeda, 1984, Sherr and Sherr, 1993), requiring a patchwork of methods to quantify the full potential suite of prey items. In the absence of large cells or of abiotic particles that may result in unreliable quantification (e.g. Harbison and McAlister, 1980), the Coulter Counter is an appropriate and more reliable means of describing how the abundance of different sized cells change over the duration of grazing incubations (Paffenhöfer, 1984), with results comparable to methods based on gut fluorescence and egg production (Kiørboe et al., 1985). Water subsamples for Coulter Counter measurements were taken directly from experimental bottles upon addition of nauplii at the start of each experiment (time 0) and at each six-hour time point, being careful to retain nauplii as experimental grazers by recovery of animals on a 35 µm cap filter and washing them back into bottles during subsampling with a small volume of filtered seawater.

Data on prey size (ESD) and abundance from the Coulter Counter were further processed using R (Core Team, 2013). Prey ESD was converted to biovolume (BV, µm<sup>3</sup>), then to carbon (C, pg C cell<sup>-1</sup>) using the relationship  $C = 0.216 \times BV^{0.939}$  (Menden-Deuer and Lessard, 2000). Averages (triplicate Coulter Counter measurements) were binned into 5 functionally relevant prey size groupings (2–5, 5–10, 10–15, 15–20, and 20–35 µm), chosen to ensure comparable data to a prior study of adult copepod grazing in Kane'ohe Bay (Calbet et al., 2000). The binned data for initial and final time points for each control and treatment bottle were used to calculate carbon ingestion (I, ng C grazer<sup>-1</sup> h<sup>-1</sup>) and clearance (F, mL grazer<sup>-1</sup> h<sup>-1</sup>) rates on each prey size group using the equations of Frost (1972), and are reported here only where F or I > 0.

Linear regressions were used to evaluate whether there was a relationship between control bottle prey

biomass and incubation duration, and between measured ingestion rates (I) and incubation time. An analysis of covariance (ANCOVA) was used to test for significant ( $p < 0.05$ ) effects of incubation time, predator treatment (P<sub>low</sub>, P<sub>high</sub>), and experiment (E1, E2) on carbon ingestion rates (I), and for interactions between variables, accounting for random error due to differences between replicate bottles. The ANCOVAs were performed for each prey size group or total prey using the aov function in the package stat with time, predator treatment, and experiment as potentially interacting factors, and incorporating bottle replicate error as a random effect. The coefficient of variation (CV, %) was calculated for cell abundance estimates and followed by a two-way ANOVA and post-hoc Tukey test to evaluate for the effects of prey size group and incubation time on variation in cell abundance. In many studies of zooplankton grazing (e.g., Atienza et al., 2006, Calbet et al., 2009, Almeda et al., 2011), significant differences in prey growth rates between control and treatment bottles were tested, and then only significantly different conditions were considered in further interpretations of I. Here, significant differences (t-test,  $p < 0.05$ ) between treatment and control prey growth rates were used to evaluate whether the significance of this test was affected by the duration of incubation. All statistical analyses were conducted using the program R (stats package) (R core team, 2016).

## 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

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## Related Publications

Almeda, R., Calbet, A., Alcaraz, M., Saiz, E., Trepas, I., Arin, L., ... Saló, V. (2011). Trophic role and carbon budget of metazoan microplankton in northwest Mediterranean coastal waters. *Limnology and Oceanography*, 56(1), 415–430. doi:[10.4319/lo.2011.56.1.0415](https://doi.org/10.4319/lo.2011.56.1.0415)

*Related Research*

Atienza, D., Calbet, A., Saiz, E., Alcaraz, M., & Trepas, I. (2006). Trophic impact, metabolism, and biogeochemical role of the marine cladoceran *Penilia avirostris* and the co-dominant copepod *Oithona nana* in NW Mediterranean coastal waters. *Marine Biology*, 150(2), 221–235. doi:[10.1007/s00227-006-0351-z](https://doi.org/10.1007/s00227-006-0351-z)

*Related Research*

Bendif, E. M., Probert, I., Schroeder, D. C., & de Vargas, C. (2013). On the description of *Tisochrysis lutea* gen. nov. sp. nov. and *Isochrysis nuda* sp. nov. in the Isochrysidales, and the transfer of *Dicrateria* to the Prymnesiales (Haptophyta). *Journal of Applied Phycology*, 25(6), 1763–1776. doi:[10.1007/s10811-013-0037-0](https://doi.org/10.1007/s10811-013-0037-0)

*Methods*

Calbet, A., Atienza, D., Henriksen, C. I., Saiz, E., & Adey, T. R. (2009). Zooplankton grazing in the Atlantic Ocean: A latitudinal study. *Deep Sea Research Part II: Topical Studies in Oceanography*, 56(15), 954–963. doi:[10.1016/j.dsr2.2008.10.009](https://doi.org/10.1016/j.dsr2.2008.10.009)

*Related Research*

Calbet, A., Landry, M., & Scheinberg, R. (2000). Copepod grazing in a subtropical bay: species-specific responses to a midsummer increase in nanoplankton standing stock. *Marine Ecology Progress Series*, 193, 75–84. doi:[10.3354/meps193075](https://doi.org/10.3354/meps193075)

*Related Research*

De Carlo, E. H., Hoover, D. J., Young, C. W., Hoover, R. S., & Mackenzie, F. T. (2007). Impact of storm runoff from tropical watersheds on coastal water quality and productivity. *Applied Geochemistry*, 22(8), 1777–1797. doi:[10.1016/j.apgeochem.2007.03.034](https://doi.org/10.1016/j.apgeochem.2007.03.034)

*Related Research*

Franklin, E., Jokiel, P.L., Rodgers, K. (2015) Weather station: Hawaii: Oahu: coconut island.  
<http://www.pacioos.hawaii.edu/metadata/AWS-HIMBagg.html>

*Related Research*

Frost, B. W. (1972). EFFECTS OF SIZE AND CONCENTRATION OF FOOD PARTICLES ON THE FEEDING BEHAVIOR OF THE MARINE PLANKTONIC COPEPODCALANUS PACIFICUS<sup>1</sup>. *Limnology and Oceanography*, 17(6), 805–815. doi:[10.4319/lo.1972.17.6.0805](https://doi.org/10.4319/lo.1972.17.6.0805)

*Methods*

Harbison, G. R., & McAlister, V. L. (1980). Fact and artifact in copepod feeding experiments<sup>1</sup>. *Limnology and Oceanography*, 25(6), 971–981. doi:[10.4319/lo.1980.25.6.0971](https://doi.org/10.4319/lo.1980.25.6.0971)

*Methods*

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. J., Selph, K. E., Lenz, P. H., & Goetze, E. (2017). Incubation duration effects on copepod naupliar grazing estimates. *Journal of Experimental Marine Biology and Ecology*, 494, 54–62.

doi:[10.1016/j.jembe.2017.05.005](https://doi.org/10.1016/j.jembe.2017.05.005)

*Results*

Kjørboe, T., Møhlenberg, F., & riisgård, H. U. (1985). In situ feeding rates of planktonic copepods: A comparison of four methods. *Journal of Experimental Marine Biology and Ecology*, 88(1), 67–81.

doi:[10.1016/0022-0981\(85\)90202-3](https://doi.org/10.1016/0022-0981(85)90202-3)

*Methods*

Mauchline, J. (Ed.) (1998). *Adv. Mar. Biol. 33: The biology of calanoid copepods. Advances in Marine Biology, 33.* Academic Press: London. ISBN [0-12-026133-2](https://doi.org/10.1016/0022-0261(1998)33:1-2). X, 170 pp. Part of: *Advances in Marine Biology.* Academic Press: London, New York. ISSN 0065-2881; e-ISSN 2162-5875

*General*

Menden-Deuer, S., & Lessard, E. J. (2000). Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. *Limnology and Oceanography*, 45(3), 569–579. doi:[10.4319/lo.2000.45.3.0569](https://doi.org/10.4319/lo.2000.45.3.0569)

*Methods*

Omori, M., Ikeda, T. (1984) *Methods in Marine Zooplankton Ecology.* Krieger Pub Co, Melbourne, Florida, U.S.A. ISBN 10: 0471801070 ISBN, 13: 9780471801078 <https://isbnsearch.org/isbn/10:0471801070>

*General*

Paffenhöfer, G. A. (1984). Food ingestion by the marine planktonic copepod *Paracalanus* in relation to abundance and size distribution of food. *Marine Biology*, 80(3), 323–333.

<https://link.springer.com/content/pdf/10.1007/BF00392828.pdf>

*Methods*

R Core Team (2016) *R: A language and environment for statistical computing.* R Foundation for Statistical Computing. Vienna, Austria. <https://www.r-project.org>

*Software*

Sherr, B. F. and E. B. Sherr (1993) Preservation and storage of samples for enumeration of heterotrophic protists. In Kemp, P. F., Sherr, B. F., Sherr, E. B. and Cole, J. J. (eds.) *Handbook of Methods in Aquatic Microbial Ecology.* CRC Press, pp. 207–212 <https://isbnsearch.org/isbn/9780367449858>

*Methods*

Team, R. C. (2013). *R: A language and environment for statistical computing.* R Foundation for Statistical Computing, Vienna, Austria (<http://www.R-project.org/>)

*Software*

Vidal, J., & Whitley, T. E. (1982). Rates of metabolism of planktonic crustaceans as related to body weight and temperature of habitat. *Journal of Plankton Research*, 4(1), 77–84. doi:[10.1093/plankt/4.1.77](https://doi.org/10.1093/plankt/4.1.77)

*General*

## Related Datasets

### IsRelatedTo

Goetze, E. (2017) **Copepods Parvocalanus crassirostris naupliar ingestion and clearance rates on natural prey assemblages from Kaneohe Bay, Oahu, 2015 (JEMBE 2017) (EAGER: Copepod nauplii project)**. Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2017-09-06 <http://lod.bco-dmo.org/id/dataset/714300> [[view at BCO-DMO](#)]

## Parameters

Parameter	Description	Units
experiment	experiment number	unitless
incubation_time	duration of incubation	hours
expt_type	whether control or treatment	unitless
prey_size_range	size range of prey items	micrometers
prey_concentration	initial concentration of prey cells	cells/milliliter
prey_concentration_se	standard error of initial concentration of prey cells	cells/milliliter
prey_biomass	initial biomass of prey cells	micrograms Carbon/Liter
prey_biomass_se	standard error of initial biomass of prey cells	micrograms Carbon/Liter

## 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>

## 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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