## 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)

Website: https://www.bco-dmo.org/dataset/712293 Data Type: experimental Version: 1 Version Date: 2017-09-01

#### Project

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

| Contributors         | Affiliation   | Role                   |
|----------------------|---|------------------------|
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#### Abstract

This dataset reports naupliar prey ingestion, clearance rates, and biomass ingestion rates by two species of calanoid copepods, Parvocalanus crassirostris and Bestiolina similis. These data are published in Jungbluth et al., (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

#### Methods & Sampling

From Jungbluth et al. 2017 - MEPS:

A series of 5 bottle incubation experiments (hereafter referred to as E1 to E5; see Table 1) were conducted over a 10 d period (27 May to 5 June 2013) to measure grazing by copepod nauplii on the natural prey assemblage collected from Stn S3, located in the southern semi-enclosed basin of Kane'ohe Bay, Oahu, Hawaii (21° 25' 56" N, 157° 46' 47"W; Jungbluth & Lenz 2013). The copepods were N3 and N4 stage nauplii of Parvocalanus crassirostris and Bestiolina similis. Concurrent experiments were run to measure microzooplankton community grazing, and to quantify in situ predator and prey abundan ces. The results from these experiments were used to correct for multiple trophic interactions within the bottle incubations, as these interactions can mask the effect of metazoan grazing (Nejstgaard et al. 2001). Salinity and temperature in the field were measured using a YSI 6600V2 sonde prior to collecting water for bottle incubations. Daily rainfall estimates were obtained from a rain gauge located at Luluku (<u>www.prh.noaa.gov</u>), and weather station data from the Hawaii Institute of Marine Biology (HIMB) (<u>www.himb.hawaii.edu/weatherstation/</u>) were used for estimates of the wind magnitude, wind direction, and solar irradiance.

Copepod nauplii used in the incubations were obtained from laboratory culture populations of P. crassirostris and B. similis established from animals previously collected in Kane'ohe Bay (P. Lenz lab). Both species are capable of completing naupliar development in less than 3 d and reaching the adult stage (C6) in approximately 7 to 8 d (McKinnon et al. 2003, VanderLugt et al. 2009). Use of these monospecific cultures enabled us to produce high abundance naupliar cohorts of a specific age for grazing incubations. To produce these cohorts, adults of each species were isolated and fed  $1 \times 106$  cells ml-1 Tisochrysis lutea (formerly Isochrysis galbana Tahitian strain; Bendif et al. 2013) 18 h prior to the start of each experiment to increase naupliar production. The adults were removed 6 h later, resulting in a cohort of nauplii (N3 and N4; note that N3 are the first feeding stage of these nauplii) raised at the experimental temperature of 21°C by the beginning of each experiment. Sets of ~50 nauplii were isolated into small volumes (<10 ml) of 0.2 µm filtered seawater and held for 1 to 3 h prior to the start of each grazing experiment. This procedure resulted in minimal exposure of the N3 and N4 nauplii to prey prior to the start of the grazing experiments. Seawater for the prey assemblage was collected from 2 m depth using a 5 I General Oceanics Niskin bottle deployed by hand line, with the contents gently added (silicone tubing) to two 20 I polycarbonate carboys.

Separate experiments indicated that longer incubation times decreased ingestion estimates within the grazing treatments, likely due to the fast development rates of our nauplii (< 24 h inter-molt period) and the diverse and rapidly changing prey community in this relatively warm (> 20°C) system (Jungbluth et al. 2017). Thus, 6 h incubations were chosen to give the most representative view of naupliar grazing rates on natural prey, with conditions closest to those in situ, and in order to minimize nutrient remineralization and other food web interaction effects that can be significant during longer incubations (Roman & Rublee 1980).

Grazing incubations were performed in pre-washed (10% HCl rinse, followed by 3 rinses with ambient 0.2  $\mu$ m seawater) polycarbonate bottles (total volume: 1120 ml) with 35  $\mu$ m gently pre-screened bulk seawater offered as prey. It is possible that our nauplii would consume prey > 35  $\mu$ m given the opportunity, however the small size of the copepod species in our study (~40  $\mu$ m wide, ~70  $\mu$ m long; P. crassirostris N1 dimensions) necessitated the re moval of prey > 35  $\mu$ m to ensure removal of other nauplii from the field. Our initial expectations were that the optimum prey size for our species would be 2 to 7  $\mu$ m (Berggreen et al. 1988, Hansen et al. 1994), therefore the prey included here (< 35  $\mu$ m) should represent a majority consumed naturally by our species.

The experimental nauplii were transferred into the 1120 ml grazing bottles at 2 densities (42-51 [moderate] and 81-95 [high] nauplii; see Table 1) and placed on a bottle roller (Wheaton) at 5 rpm in the dark for 6 h. The 2 nauplius densities were tested to ensure that we could detect removal of prey cells relative to controls over our incubation period, since a predator density that is too low may result in insignificant prey removal relative to control bottles. Removal of cells in treatments relative to control bottles was detected in the moderate density treatments and ingestion rate estimates were comparable to those from higher density bottles. Since results were comparable between moderate and high density treatments, results reported here focus on bottles with ~50 nauplii l-1, also because treatment replication was better with moderate density bottles (n = 3 per experiment) than high density bottles (n = 2). This density of nauplii is well within the range of total nauplius concentrations reported in previous studies in Kane'ohe Bay (7 to 68 total nauplii l-1; Hoover et al. 2006) and within the range of each species abundance we have previously measured following storm runoff events in the bay (M.J.J. pers. obs.).

Treatment bottles were run in triplicate, with 2 or 3 no-nauplii control bottles for each experiment (2 for E1 to E2, 3 for E3 to E5). Experiments were incubated at 21°C, which is within the range of the annual temperature fluctuation in Kane'ohe Bay (20 to 29°C during the previous 5 yr; HIMB weather data). No nutrients were added to the bottles, because controls and experimental bottles were considered approximately equally influenced by nitrogen remineralization from grazing processes, due to the presence of other < 35  $\mu$ m microzooplankton grazers in all bottles and the short incubation times (6 h). Nauplii are known to have low expected nitrogen remineralization (~10- fold lower than adults) due to their small biomass compared to adults (Vidal & Whitledge 1982, Mauchline 1998); at 50 nauplii I-1, excretion rates were estimated to be 2 to 3 orders of magnitude below the in situ average nitrogen concentrations in Kane'ohe Bay (0.2 to 1.0  $\mu$ M; Drupp et al. 2011). Initial and final time-point measurements included samples to quantify particle size and abundance in the 2-35  $\mu$ m size range from the Coulter counter (CC), as well as samples for specific prey types, including chlorophyll a (chl a) and the abundance and biomass of types of nano- and microplankton. Prey types and CC-quantified potential prey were not expected to be equal; some prey types include cells < 2  $\mu$ m, while the lower

limit of the CC was 2 µm. Nauplii were re covered at the end of the experiments to check their condition (alive/dead; no dead nauplii were found), then preserved in 10% paraformaldehyde, stained with 1% Rose Bengal, and enumerated using microscopy for use in clearance and ingestion rate estimates.

For complete methodology, see the Supplemental Files section.

#### **Data 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
- cells containing only hyphen replaced with no data value 'nd'
- replaced spaces with underscores in species names
- added date\_local, lat, and lon columns

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#### Data Files

File

grazing\_MEPS.csv(Comma Separated Values (.csv), 14.13 KB) MD5:48ef05ccb7f0f2572668e4bb1771ae97

Primary data file for dataset ID 712293

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## **Supplemental Files**

| File   |
|--|
| Full Methodology - naupliar grazing expts.<br>filename: Jungbluth_etal_MEPS_2017_Methodology.pdf <sup>(Portable Document Format (.pdf), 599.55 KB)</sup><br>MD5:12d9d0250b1a8a2e3bac5812c5dd2b6a |
| Methodology for datasets from project "New molecular methods for studying copepod nauplii in the   |
| field" (EAGER: Copepod nauplii) https://www.bco-dmo.org/project/473049   |
|  |

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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. <u>https://pqdtopen.proquest.com/pubnum/10587374.html</u> *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:<u>10.3354/meps12139</u> *Results* 

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#### **Related Datasets**

#### IsRelatedTo

Goetze, E. (2021) **Initial field conditions at Kane'ohe Bay, Oahu, Hawaii and abundances of Parvocalanus crassirostris and Bestilina 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]

Goetze, E. (2021) **Initial prey abundances for copepod grazing experiments in the Kaneohe Bay, HI, May-June 2013 (MEPS 2017) (EAGER: Copepod nauplii project).** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2017-09-01 doi:10.26008/1912/bcodmo.712626.1 [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          |
| species                   | species name  | unitless                 |
| prey_quant_method         | method used to measure number of prey   | unitless                 |
| prey_type_size_range      | type or size range of prey: PEUK=photosynthetic<br>eukaryote AUT=autotrophic HET=heterotrophic<br>SYN=Synechococcus PRO=Prochlorococcus<br>HBACT=Heterotrophic bacteria | micrometers (um)         |
| ingestion_rate            | ingestion rate by nauplius; measured by abundance of prey   | cells/nauplius/day       |
| ingestion_rate_se         | standard error of ingestion rate by nauplius; measured by abundance of prey   | cells/nauplius/day       |
| clearance_rate            | clearance rate by nauplius  | milliliters/nauplius/day |
| clearance_rate_se         | standard error of clearance rate by nauplius  | milliliters/nauplius/day |
| biomass_ingestion_rate    | biomass ingestion rate by nauplius; reported as either carbon or chlorophyll  | micrograms/nauplius/day  |
| biomass_ingestion_rate_se | standard error of biomass ingestion rate by nauplius;<br>reported as either carbon or chlorophyll   | micrograms/nauplius/day  |

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#### Instruments

| Dataset-<br>specific<br>Instrument<br>Name |  |
|--|--|
| Generic<br>Instrument<br>Name              | Coulter Counter  |
| Dataset-<br>specific<br>Description        | Used to quantify particle size and abundance in the 2–35 $\mu m$ size range.   |
| Generic<br>Instrument<br>Description       | 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> |

| Dataset-<br>specific<br>Instrument<br>Name |  |
|--|--|
| Generic<br>Instrument<br>Name              | Flow Cytometer   |
| Dataset-<br>specific<br>Description        | For photosynthetic eukaryote (PEUK) abundance  |
| Generic<br>Instrument<br>Description       | 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: http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm) |

| Dataset-<br>specific<br>Instrument<br>Name | epifluorescence microscope  |
|--|---|
| Generic<br>Instrument<br>Name              | Fluorescence Microscope   |
| Dataset-<br>specific<br>Description        | For nano- and microplankton abundance.  |
| Generic<br>Instrument<br>Description       | Instruments that generate enlarged images of samples using the phenomena of fluorescence<br>and phosphorescence instead of, or in addition to, reflection and absorption of visible light.<br>Includes conventional and inverted instruments. |

| Dataset-<br>specific<br>Instrument<br>Name | General Oceanics Niskin bottle  |
|--|---|
| Generic<br>Instrument<br>Name              | Niskin bottle   |
| Dataset-<br>specific<br>Description        | Five liter size, deployed by hand line to collect seawater  |
| Generic<br>Instrument<br>Description       | A Niskin bottle (a next generation water sampler based on the Nansen bottle) is a cylindrical,<br>non-metallic water collection device with stoppers at both ends. The bottles can be attached<br>individually on a hydrowire or deployed in 12, 24, or 36 bottle Rosette systems mounted on a<br>frame and combined with a CTD. Niskin bottles are used to collect discrete water samples for a<br>range of measurements including pigments, nutrients, plankton, etc. |

| Dataset-<br>specific<br>Instrument<br>Name |  |
|--|--|
| Generic<br>Instrument<br>Name              | Turner Designs Fluorometer 10-AU   |
| Dataset-<br>specific<br>Description        | To measure chlorophyll-a concentration   |
| Generic<br>Instrument<br>Description       | 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) |

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## Deployments

#### Goetze\_2012-2013

| Website     | https://www.bco-dmo.org/deployment/637678 |
|-------------|---|
| Platform    | lab UHawaii_SOEST                         |
| Start Date  | 2012-03-16                                |
| End Date    | 2013-06-05                                |
| Description | microzooplankton studies                  |

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## **Project Information**

# EAGER: 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: <u>10.1007/s00227-013-2300-y</u>

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: <u>10.1093/plankt/fbt015</u>

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## Funding

| Funding Source                           | Award              |
|--|--------------------|
| NSF Division of Ocean Sciences (NSF OCE) | <u>OCE-1255697</u> |

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