Estimated feeding rates of adult and nauplius copepod species on three phytoplankton species from the San Francisco estuary in 2013

Website: https://www.bco-dmo.org/dataset/546440
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

We estimated feeding rates of each copepod species and stage on three phytoplankton species that were ingested during the GPI (gut pigment index) experiments: the prasinophyte *Tetraselmis suecica*, the cryptomonad *Rhodomonas salina*, and the diatom *Thalassiosira pseudonana*.

Related Reference:

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

Starved copepods were incubated in bottles with each phytoplankton, then gut pigment was analyzed on a microplate reader. Microplate readers are capable of measuring low levels of fluorescence on small volumes of sample, allowing for feeding estimates on individual or relatively small groups of copepods (5–50).

All experiments were conducted at 19 ± 1°C. Copepods were sorted from the culture and allowed to evacuate their guts as above. Copepods were then pipetted individually into clean polystyrene Nalgene® bottles (*P. marinus* adults: 2 L; *O. davisae* adults: 1 L; all nauplii: 500 mL) containing fresh GF/F culture water. For *P. marinus*, four bottles containing 15 adult females each and six bottles containing 40 nauplii each were used. For *O. davisae*, four bottles containing 50 adult females each and six bottles containing 175 nauplii each were used.

Phytoplankton culture was added to the bottles to attain a final concentration of approximately 500 µg C L-1, and inoculations of each bottle were staggered by 10 min to allow for processing time for each bottle. The

same phytoplankton culture was used for both adults and nauplii.

Incubations were conducted as in the GPI experiment except that durations were 30 min for *P. marinus* and 60 min for *O. davisae*, based on the results of preliminary experiments (see below). Longer incubation times were chosen for *O. davisae* since this copepod is smaller than *P. marinus*, and more feeding time was needed for this species to ingest detectable amounts of pigment. Incubations were terminated as for the GPI experiments. Copepods were processed for analysis individually (*P. marinus* adults) or in groups (5 *P. marinus* nauplii; 5 *O. davisae* adults, 50 nauplii).

Under dim light, copepods were pipetted onto ethanol-rinsed, 1x1 cm, 20 µm pore size nylon mesh filter squares. Excess moisture was wicked away with a Kimwipe™. Filter squares were then put into 1.5 mL microcentrifuge tubes that were immediately placed on dry ice in a dark container and stored at -80°C. Processing times averaged two min per bottle.

A control group was processed with each experiment consisting of copepods that were treated identically to the experimental group, except that the incubation was terminated immediately after the phytoplankton was added. The purpose of this control was to account for any sources of contamination (e.g., phytoplankton cells that may have stuck to the copepod).

One hundred µL of 95% ethanol was dispensed into each microcentrifuge tube and samples were extracted for 24 h. Ethanol was used as the extraction solvent because it does not damage polystyrene microplates. Filters were removed using forceps rinsed with 95% ethanol between samples.

A Tecan Infinite F200 or Biotek Synergy 2 microplate reader was used for each analysis. Each microplate reader contained a 430/20 EX, 680/20 EM filter pair for chlorophyll a. The microplate reader was kept in a dark room maintained at 14ºC to minimize pigment loss and evaporation of solvent during analysis.

A Chl a standard was prepared by dissolving commercially available pure Chl a (source: *Anacystis nidulans* cyanobacteria, Sigma Chemical: C6144) in 95% ethanol. The concentration of the standard was measured on a Agilent 8453 spectrophotometer (Agilent Technologies) and calculated using published equations (Ritchie 2008).

The Chl a-ethanol stock standards were dispensed in 5 mL aliquots into 5 mL glass ampoules. The headspace was displaced using a commercially available 99.9% argon gas dispenser. The ampoules were flame sealed in dim light and on dry ice in a ventilated fume hood, then placed in a darkened container and kept in a -20 ºC freezer. The concentration of the standards was checked every 1–2 months by measuring the Chl a concentration of the stock standards on a spectrophotometer periodically for one year. No degradation in the standards was detected during that period. A standard dilution series for each experiment was prepared by dispensing known volumes of Chl a standard into 95% ethanol to achieve final concentrations of 0.05, 0.1, 0.3, 1, and 2 ng Chl a (100 µL)-1, the range of pigment concentrations in the copepod samples.

For each assay, standards, sample extracts, and 95% ethanol blanks were dispensed in 60 µL aliquots into white, skirted 96-well microplates (Bio-Rad, model HSP-9601) fitted with Optical Flat 8-cap strips (Bio-Rad, model TCS-0803). White plates provided the highest sensitivity for the assay. All samples and materials were allowed to acclimate to the temperature of the room before analysis. The microplate reader settings were: 25 flashes, optimal gain, center read.

Each microplate was read once, and then all samples were acidified with 1.0 µL of 1 mol L-1 HCl using a 0.1–2 µL multichannel pipettor. The microplate was then re-read with the gain set manually to the value set automatically by the instrument for the initial readings. The plate was read repeatedly for 10 min until readings stabilized, which typically occurred in under three min, but some samples took ~5–7 min to completely stabilize. The last readings in the 10-min cycle were used as the post-acidification fluorescence values to ensure that steady state had been reached.

Data Processing Description

Fluorescence measurements in the samples were converted to total chlorophyll using published equations (12.2 and 12.3 in Arar and Collins 1997). The average values of the control groups were subtracted from the estimates for the experimental groups to yield the adjusted total chlorophyll, expressed as ng individual (ind-1) consumed during the incubation.

A known volume of each phytoplankton culture used in the feeding experiment was filtered onto a GF/F filter,

then extracted in 90% acetone and measured on Turner 10AU fluorometer, calibrated using a pure chlorophyll standard (Arar and Collins 1997). The cell concentration of the culture estimated by counting cells on a hemocytometer was used to calculate chlorophyll cell-1. The microplate data were converted to an ingestion rate (cells copepod-1 h-1) by multiplying the chlorophyll content in each copepod sample by the estimate of total pigment per phytoplankton cell and dividing by the number of copepods and duration of the experiment. Clearance rates were calculated by dividing the ingestion rate by the estimated initial cell concentration in the incubation bottles. Daily ration estimates on each phytoplankton were also computed by converting the number of cells consumed by copepods to carbon and dividing that value by copepod body carbon and experimental duration.

BCO-DMO Processing:
- added conventional header with dataset name, PI name, version date, reference information
- renamed parameters to BCO-DMO standard
- replaced blanks with underscores
- replaced Oithona with Oithona_davisae, Pmarina with Pseudodiaptomus_marinus; Tetraselmis with Tetraselmis suecica; Rhodomonas with Rhodomonas salina; Thalassiosira with Thalassiosira pseudonana

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Data Files

File
2_feeding.csv (Comma Separated Values (.csv), 13.13 KB) MD5:52ec4dacddd38af3d3ed88891bd7704e Primary data file for dataset ID 546440

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Parameters

Parameter	Description	Units
copepod_species	copepod species name	unitless
stage	copepod life stage: N=nauplius	unitless
algae_species	alga species name	unitless
chl_equiv_ng	gut pigment content	nanograms chlorophyll-a equivalent
time_incub	incubation time	minutes

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Instruments

Dataset-specific Instrument Name	Fluorometer
Generic Instrument Name	Fluorometer
Dataset-specific Description	Turner 10AU fluorometer
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 EM filter pair for chlorophyll 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.

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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

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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
NSF Division of Ocean Sciences (NSF OCE)	OCE-0929075

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