Data resulting from testing dilution experiments conducted in the development of four assays to quantify copeod naupliar biomass in Kaneohe Bay, Hawaii

Website: https://www.bco-dmo.org/dataset/869011
Data Type: Other Field Results, experimental
Version: 1
Version Date: 2022-02-01

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

Contributors	Affiliation	Role
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Abstract
Quantitative PCR data from developing four assays to quantify copepod naupliar biomass in Kaneohe Bay, Hawaii. This dataset is from testing dilutions of focal species' DNA into non-focal species' DNA.

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Coverage

Spatial Extent: **Lat**:21.45 **Lon**:-157.8
Temporal Extent: 2014-06 - 2014-06

Methods & Sampling

Methodology:
Complete methodology is detailed in Jungbluth et al. (accepted 2022, Limnology and Oceanography Methods). Briefly, qPCR assays were developed for four copepod species: *Parvocalanus crassirostris*, *Bestiolina similis, Oithona simplex,* and *Oithona attenuata.* The assays were optimized and applied to mixed field samples to estimate biomass of nauplii of each species, over 5 orders of magnitude in length-based biomass and DNA copy number measured with qPCR.

Sampling and Analytical Procedures:
Zooplankton samples were either collected from the field with a Niskin bottle or 150 µm mesh plankton net with filtering cod end, or cultured in the laboratory, then preserved in 95% molecular ethyl alcohol and stored in the freezer until processing. Field samples were collected from southern Kāneʻohe Bay on the island of

Oahu, Hawai'i. DNA was extracted from individual copepods of all focal and non-focal species from the area and used to assess whether our qPCR assays were working as expected. Dilutions of the various focal and non-focal species were tested to look at the range of focal species DNA that was detectible with the assays. Finally, the DNA copy number measurements were compared with length-based and laboratory-measured carbon biomass of each species to assess the accuracy of using qPCR to estimate copepod naupliar biomass from mixed field samples.

Data Processing Description

Data Processing:
Light Cycler 96 software (Roche, v. 1.1.0.1320)
R (R core team, 2017), packages *stats* (base R) and *car* (Fox and Weisberg 2019)

BCO-DMO Processing:
- renamed fields to comply with BCO-DMO naming conventions.

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Data Files

File
dilution_test.csv (Comma Separated Values (.csv), 1.70 KB) MD5:cea38b6042359b6b63fdc18590a3fb4b Primary data file for dataset ID 869011

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Related Publications

Jungbluth, M. J., Hanson, K. M., Lenz, P. H., Robinson, H. E., & Goetze, E. (2022). Species-specific biomass estimation from gene copy number in metazoan plankton. Limnology and Oceanography: Methods. Portico. https://doi.org/10.1002/lom3.10487
Results

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Related Datasets

IsRelatedTo

Goetze, E., Lenz, P., Selph, K. E. (2022) **Measurements of individual body size and qPCR estimates of DNA copy number per individual from the development of four assays to quantify copeod naupliar biomass in Kaneohe Bay, Hawaii.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2022-02-02 doi:10.26008/1912/bco-dmo.869085.1 [view at BCO-DMO]

Goetze, E., Lenz, P., Selph, K. E. (2022) **Measurements of mixed field samples used for qPCR estimates and estimating biomass of DNA copy number in bulk samples.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2022-02-03 doi:10.26008/1912/bco-dmo.869225.1 [view at BCO-DMO]

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Parameters

Parameter	Description	Units
FocalSpecies	Species that is the target of the assay	unitless
Experiment	Corresponding experiment ID	unitless
Sample_Name	Sample name and dilution level	unitless
TotalFocal_DNA	Amount of focal species DNA diluted into non-focal species DNA	nanograms per microliter (ng/uL)
Cq_Mean	Cycle threshold measured in qPCR, mean	unitless
SD_Cq	Cycle threshold measured in qPCR, standard deviation	unitless
Concentration_Mean	Mean concentration of DNA measured in qPCR, ng/ul	nanograms per microliter (ng/uL)
SD_Concentration	Standard deviation of concentration measured in qPCR	nanograms per microliter (ng/uL)

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Instruments

Dataset-specific Instrument Name	Applied Biosystems 3730XL
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	Applied Biosystems 3730XL, with BigDye terminator chemistry v3.1, was used for sequencing species and qPCR products.
Generic Instrument Description	General term for a laboratory instrument used for deciphering the order of bases in a strand of DNA. Sanger sequencers detect fluorescence from different dyes that are used to identify the A, C, G, and T extension reactions. Contemporary or Pyrosequencer methods are based on detecting the activity of DNA polymerase (a DNA synthesizing enzyme) with another chemoluminescent enzyme. Essentially, the method allows sequencing of a single strand of DNA by synthesizing the complementary strand along it, one base pair at a time, and detecting which base was actually added at each step.

Dataset-specific Instrument Name	Exeter Analytical CE 440
Generic Instrument Name	Elemental Analyzer
Dataset-specific Description	Exeter Analytical CE 440 was used for CHN analysis of copepods.
Generic Instrument Description	Instruments that quantify carbon, nitrogen and sometimes other elements by combusting the sample at very high temperature and assaying the resulting gaseous oxides. Usually used for samples including organic material.

Dataset-specific Instrument Name	Qubit Fluorometer (Invitrogen)
Generic Instrument Name	Fluorometer
Dataset-specific Description	A Qubit Fluorometer (Invitrogen) with the HS Assay was used for measurement of total DNA.
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	Olympus dissection microscope (model SZX16)
Generic Instrument Name	Microscope - Optical
Dataset-specific Description	An Olympus dissection microscope (model SZX16) fitted with Luminera Infinity-3 was used for sorting and sizing copepod nauplii.
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of reflection and absorption of visible light. Includes conventional and inverted instruments. Also called a "light microscope".

Dataset-specific Instrument Name	Light Cycler 96 Quantitative PCR Thermalcycler (Roche)
Generic Instrument Name	qPCR Thermal Cycler
Dataset-specific Description	A light Cycler 96 Quantitative PCR Thermalcycler (Roche) was used for qPCR measurements.
Generic Instrument Description	An instrument for quantitative polymerase chain reaction (qPCR), also known as real-time polymerase chain reaction (Real-Time PCR).

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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: 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

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1255697

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