

RNA-Seq sample information and accessions numbers for the copepods *Neocalanus flemingeri* (Prince William Sound, Gulf of Alaska)(2015-2017) and *Labidocera madurae* (Kane`ohe Bay, Oahu, Hawaii)(2017)

Website: <https://www.bco-dmo.org/dataset/821289>

Data Type: Other Field Results

Version: 1

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Project

» [Collaborative Proposal: Optimizing Recruitment of *Neocalanus* copepods through Strategic Timing of Reproduction and Growth in the Gulf of Alaska](#) (*Neocalanus* Gulf of Alaska)

Contributors	Affiliation	Role
Hartline, Daniel K	University of Hawaii at Manoa	Lead Principal Investigator
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Abstract

This dataset includes accession information for RNA-seq data for the copepods *Neocalanus flemingeri* and *Labidocera madurae* used to generate de novo reference transcriptomes and for gene expression analysis. *N. flemingeri* adult females (CVI) were collected in Prince Williams Sound (Gulf of Alaska) during the fall (September 2015 and 2017) oceanographic cruises of the Seward line long-term observation program (Itop)(<http://www.sfos.uaf.edu/sewardline/>) and Northern Gulf of Alaska Long-Term Ecological Research Program (NGA LTER). For each sample collection date and preservation dates are listed. *Labidocera madurae* adult females (CVI) and mixed copepodid stages (CIII-CV) were collected in Kane`ohe Bay, Oahu (Hawaii) in August 2015.

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Coverage

Spatial Extent: N:60.535 E:-147.8033 S:21.2833 W:-157.1167

Dataset Description

This dataset includes accession information for RNA-seq data for the copepods *Neocalanus flemingeri* and *Labidocera madurae* used to generate de novo reference transcriptomes and for gene expression analysis. *N. flemingeri* adult females (CVI) were collected in Prince Williams Sound (Gulf of Alaska) during the fall (September 2015 and 2017) oceanographic cruises of the Seward line long-term observation program (ltop)(<http://www.sfos.uaf.edu/sewardline/>) and Northern Gulf of Alaska Long-Term Ecological Research Program (NGA LTER). For each sample collection date and preservation dates are listed. *Labidocera madurae* adult females (CVI) and mixed copepodid stages (CIII-CV) were collected in Kane'ohe Bay, Oahu (Hawaii) in August 2015.

Acquisition Description

Materials and Methods

Copepod collections, transfer to laboratory and preservation

Neocalanus flemingeri adult females were collected in Prince Williams Sound during the fall oceanographic cruises of the Seward Line Long-term Observation Program (LTOP) (<http://www.sfos.uaf.edu/sewardline/>) and northern Gulf of Alaska Long-term Ecological Research Program (NGA LTER). Samples were collected between 700 and 400 m, using an opening and closing multiple plankton sampler (0.5 m² cross-sectional area; 153 µm mesh nets; Multinet, Hydro-Bios) towed vertically from 700 m depth. Plankton collections were immediately diluted with deep seawater, and a set of *N. flemingeri* adult females were sorted within 15 min (T0) or within 45 min (Wk1) of net retrieval and preserved individually in microcentrifuge tubes in RNeasy Lysis Reagent (QIAGEN). Additional females were stored in the cold and dark (5°C) prior to sorting and placed in incubation flasks (Falcon flasks, 750 ml) and maintained up to 9.5 weeks at 5-6°C before microscopic examination and preservation in RNeasy Lysis Reagent.

Labidocera madurae were live sorted from mixed zooplankton samples collected in Kane'ohe Bay, Oahu, Hawaii with a zooplankton net (30 cm diameter, 123 µm mesh) towed horizontally subsurface from a slowly moving boat. Collections were immediately diluted in seawater and transported to the laboratory. Adult females and mixed copepodid stages (CIII-CV) were live sorted within six hours of collection and either preserved in RNeasy Lysis Reagent, or immediately processed for total RNA extraction. Each sample consisted of a group of individuals.

RNA extraction, gene library preparation and RNASeq

Total RNA was extracted from individuals using QIAGEN RNeasy Plus Mini Kit (catalog # 74134) in combination with a Qiashredder column (catalog # 79654) following the instructions of the manufacturer and stored at -80°C. Total RNA concentration and quality were checked using an Agilent Model 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA, USA). Samples of extracted total RNA from individuals or group of copepods were shipped on dry ice to the University of Georgia Genomics and Bioinformatics Core (GGBC) Facility (dna.uga.edu). There, double-stranded cDNA libraries were prepared from total RNA extracted using the Kapa Stranded mRNA Seq kit (KK8420) following manufacturer's instructions. Briefly, RNA samples were first purified with two oligo-dT selection (polyA enrichment using oligodT beds), and then fragmented and reverse transcribed into double-stranded complementary cDNA. Each sample was tagged with an indexed adapter and paired-end sequenced (PE150 bp or PE75 bp) using an Illumina NextSeq 500 instrument using a High or Medium Output Flow Cells. Short-sequence reads (RNA-Seq) were submitted to the short read archive (SRA) database at the National Center for Biotechnology Information (NCBI) for public access (see BioProjects PRJNA324453 and PRJNA324849) .

Additional cruise data can be found at <https://portal.aos.org/> and <https://nga.lternet.edu/>.

Station information:

Gulf Of Alaska Stations:

PWS2 (Lat: 60° 32.1'N; Long: 147° 48.2'W)

KIP2 (Lat: 60°17'N; Long: 147° 59'W)

Hawai'i:

Kane'ohe Bay, Oahu (Hawaii) (Lat: 21°4'N; Long: 157°7'W)

Processing Description

BCO-DMO Data Manager Processing Notes:

- * Combined data submitted in files BCODMO-Hartline-Bioproject-PRJNA324453.csv and BCODMO-Hartline-PRJNA324849.csv. Added column BioProject.
- * added a conventional header with dataset name, PI name, version date
- * modified parameter names to conform with BCO-DMO naming conventions: only A-Za-z0-9 and underscore allowed. Can not start with a number. (spaces, +, and - changed to underscores).
- * blank values in this dataset are displayed as "nd" for "no data." nd is the default missing data identifier in the BCO-DMO system.
- * Converted various Date formats to ISO 8601 format YYYY-MM-DD and year, month to YYYY-MM.
- * Data from bioproject had Aug-15 in the Preservation Date and Collection Date columns so the column name was changed to Collection_mon_year and Preservation_mon_year.
- * Site "Kāne'ohe Bay" changed to "Kaneohe Bay" for interoperability purposes.
- * Site_Lat and Site_Lon added to dataset from coordinates provided and converted to decimal degrees.

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Related Publications

Lenz, P. H., & Roncalli, V. (2019). Diapause within the Context of Life-History Strategies in Calanid Copepods (Calanoida: Crustacea). *The Biological Bulletin*, 237(2), 170–179. doi:[10.1086/705160](https://doi.org/10.1086/705160)

Results

Roncalli, V., Christie, A. E., Sommer, S. A., Cieslak, M. C., Hartline, D. K., & Lenz, P. H. (2017). A deep transcriptomic resource for the copepod crustacean *Labidocera madurae*: A potential indicator species for assessing near shore ecosystem health. *PLOS ONE*, 12(10), e0186794. doi:10.1371/journal.pone.0186794
<https://doi.org/10.1371/JOURNAL.PONE.0186794>

Results

Roncalli, V., Cieslak, M. C., Hopcroft, R. R., & Lenz, P. H. (2020). Capital Breeding in a Diapausing Copepod: A Transcriptomics Analysis. *Frontiers in Marine Science*, 7. doi:10.3389/fmars.2020.00056
<https://doi.org/10.3389/FMARS.2020.00056>

Results

Roncalli, V., Cieslak, M. C., Sommer, S. A., Hopcroft, R. R., & Lenz, P. H. (2018). De novo transcriptome assembly of the calanoid copepod *Neocalanus flemingeri*: A new resource for emergence from diapause. *Marine Genomics*, 37, 114–119. doi:10.1016/j.margen.2017.09.002

<https://doi.org/10.1016/J.MARGEN.2017.09.002>

Results

Roncalli, V., Sommer, S. A., Cieslak, M. C., Clarke, C., Hopcroft, R. R., & Lenz, P. H. (2018). Physiological characterization of the emergence from diapause: A transcriptomics approach. *Scientific Reports*, 8(1). doi:10.1038/s41598-018-30873-0 <https://doi.org/10.1038/S41598-018-30873-0>

Results

University of Hawaii at Manoa (2016). *Labidocera madurae* Transcriptome or Gene expression. NCBI:BioProject: PRJNA324849. Bethesda, MD: National Library of Medicine (US), National Center for

Biotechnology Information; 2011-. Available from: <http://www.ncbi.nlm.nih.gov/bioproject/PRJNA324849>.

References

University of Hawaii at Manoa (2016). *Neocalanus flemingeri* adult females. NCBI:BioProject: PRJNA324453. Bethesda, MD: National Library of Medicine (US), National Center for Biotechnology Information; 2011-. Available from: <http://www.ncbi.nlm.nih.gov/bioproject/PRJNA324453>.

References

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Parameters

Parameter	Description	Units
BioProject	NCBI BioProject No. at the National Center for Biotechnology Information (NCBI).	unitless
Cruise_ID	cruise identifier. Collections were obtained during Seward Long-term Monitoring Program (2015 – 2017) and Northern Gulf of Alaska Long-term Ecological Research Program (starting in 2018). Additional cruised data are located at the following websites: https://portal.aocs.org/gulf-of-alaska#metadata/e25fe1f2-1c98-44f6-856f-... and https://nga.lternet.edu/	unitless
Station	station name. Latitude and longitude of the stations are included in the cruise data and they are listed in the relevant biosample information in National Center for Biotechnology Information (NCBI)	unitless
Collection_date	collection date in ISO 8601 format YYYY-MM-DD. Collection date is referenced to local time (Alaska daylight time, AKDT [UTC - 8 hr])	unitless
Preservation_date	preservation date in ISO 8601 format YYYY-MM-DD. Preservation is the date the individual sample was preserved in RNALater and frozen until further processing	unitless
Collection_mon_year	collection month and year (e.g. 'Aug-15'). Collection date is referenced to local time (Alaska daylight time, AKDT [UTC - 8 hr])	unitless
Preservation_mon_year	preservation month and year (e.g. 'Aug-15'). Preservation is the date the individual sample was preserved in RNALater and frozen until further processing	unitless
Sample	Sample. Sample is identified as time delay between collection and preservation (T0 = within 15 minutes following net retrieval; T#hr = hours post-collection; Wk# = weeks post-collection, with Wk0 = within 45 min of net retrieval)	unitless
Species_name	Species name. Scientific name (Genus species).	unitless
Developmental_Stage	Developmental stage. Number of individuals in sample and developmental stage (C = copepodid, CI - CVI; N = Nauplius, NI - NVI) and sex (adults only)	unitless
NCBI_Biosample_Acc_No	NCBI Biosample Acc. No. National Center for Biotechnology Information (NCBI) biosample accession number.	unitless
NCBI_SRX_Acc_No	NCBI SRX Acc. No. National Center for Biotechnology Information (NCBI) accession number for the raw RNA-Seq sequence reads stored in the sequence read archive (SRA) database.	unitless
Station_Lat	Station latitude	decimal degrees
Station_Lon	Station longitude	decimal degrees

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Project Information

Collaborative Proposal: Optimizing Recruitment of *Neocalanus* copepods through Strategic Timing of Reproduction and Growth in the Gulf of Alaska (*Neocalanus* Gulf of Alaska)

Coverage: Gulf of Alaska; Seward Line

NSF abstract: The Gulf of Alaska supports a diverse and productive marine community that includes many commercially important fishes. Toward the base of this food web are small planktonic crustaceans that serve as the primary food source for many of these fish, as well as seabirds and marine mammals. The copepod *Neocalanus flemingeri* is one of these crustaceans, and it experiences rapid population growth during each spring's algal, or phytoplankton, bloom. An apparent mismatch between the presence of the youngest stages of the copepod, or nauplii, in early winter and the unpredictable timing of the spring phytoplankton bloom several months later raises important questions about when females reproduce and how this relates to survival and growth of nauplii. Two types of dormancy, diapause in adult females and physiological quiescence in nauplii, may be the key to the success of this copepod species. Timing and duration of the egg-laying period by adult females is linked to emergence from diapause. In addition, nauplii may enter a state of physiological quiescence while food resources are low, resuming growth after phytoplankton levels increase. This research will address a long-standing goal of biological oceanographers to understand dormancy and its role in controlling population cycles in marine copepods. It will use new technologies in molecular biology called transcriptomics to catalog the messages used by the cells to control copepod life processes, in this case those related to dormancy in adults and nauplii. Undergraduate students and a postdoctoral investigator will be trained in interdisciplinary research, and students from Native Hawaiian and Native Alaskan groups will be targeted for participation. Fishing is a major industry in the Gulf of Alaska, and outreach will focus on communicating the role copepods play in marine ecosystems. New content, including images, will be generated for existing websites: the Seward Line long-term observation program, the Alaska Ocean Observing System and the Gulf Watch Alaska Program. Recruitment to the *Neocalanus flemingeri* spring population is dependent on successful emergence from diapause followed by reproduction, survival, and growth of the next generation. Individual-based models have made significant progress in predicting population growth in calanoid copepods using food, temperature, and advection as key environmental factors. Few of these models include predictors for naupliar recruitment, however, because little is known about this part of the life cycle given sampling difficulties and the lack of biomarkers to evaluate physiological state. This study will leverage existing monitoring efforts to track the *N. flemingeri* population during the winter and early spring. The research team will combine laboratory and field approaches to determine duration and synchronization of reproduction in emerging females and strategies for naupliar survival during low food conditions. Zooplankton samples will be processed to enumerate nauplii to species and to determine physiological condition of both nauplii and adult females. Gene expression studies will develop molecular markers for female dormancy and reproductive readiness and for naupliar growth and possible dormancy, which in turn will be used to evaluate field collected individuals. This will be the first comprehensive study to combine molecular and traditional tools to connect diapausing adults, naupliar production, and the resulting spring population of copepodites.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1459235

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