

Multiyear RNA-Seq of *Neocalanus flemingeri* stages CV and Adult Female from the R/V Tiglax and R/V Sikuliaq in the Northern Gulf of Alaska from 2015-2022

Website: <https://www.bco-dmo.org/dataset/922330>

Data Type: Cruise Results, experimental

Version: 1

Version Date: 2024-05-30

Project

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

» [Collaborative Research: Molecular profiling of the ecophysiology of dormancy induction in calanid copepods of the Northern Gulf of Alaska LTER site](#) (Diapause preparation)

Contributors	Affiliation	Role
Lenz, Petra H.	University of Hawaii at Manoa (PBRC)	Principal Investigator
Roncalli, Vittoria	University of Hawaii at Manoa (PBRC)	Scientist
Cieslak, Matthew C.	University of Hawaii at Manoa (PBRC)	Technician, Data Manager
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Abstract

High-throughput sequencing study of field-collected *Neocalanus flemingeri* pre-adults (stage CV) and adult females between 2015 and 2022. Dataset includes information and accession numbers of the raw sequence reads. Zooplankton collections were made in the northern Gulf of Alaska in collaboration with the Seward Long-term Monitoring Program and the northern Gulf of Alaska Long-term Ecological Research Program (LTER). Pre-adults were collected during the spring from multiple stations, sorted from net collections and immediately preserved. Adult females were collected mostly from Prince William Sound, but also on one occasion from the Gulf of Alaska. Adult females were collected in depth during diapause and preserved upon net retrieval. In addition, time series data were generated in three different years to characterize the post-diapause period through the spawning phase. The purpose of the data collection is to generate gene expression profiles during different years and seasons to evaluate developmental stage and physiological state.

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Coverage

Location: Northern Gulf of Alaska

Spatial Extent: N:60.8243 E:-145.4238 S:56.2321 W:-149.7195

Temporal Extent: 2015-05-06 - 2022-05-06

Methods & Sampling

In collaboration with the Seward Long-Term Observation Program (LTOP) (<http://www.sfos.uaf.edu/sewardline/>) and the NGA LTER Program (<https://nga.lternet.edu/>), we obtained *N. flemingeri* individuals during the annual April-May and September oceanographic cruises. During the spring cruises stage CV individuals were collected from four to six locations: spanning the inner shelf to outer shelf gradient along the Seward Line in the northern Gulf of Alaska and at least one station in adjoining Prince William Sound. Samples were collected using a CaVET net (53- μ m mesh) towed vertically from 100 m depth to surface. In September, diapausing *N. flemingeri* adult females were sorted from collections obtained with a Midi Multinet towed vertically. Individuals were sorted from the 300-700 depth collections. In three years (2015, 2016, 2017) additional adult females were incubated for different lengths of time and preserved after a specified interval as indicated in the sample information. In July, 2019 we participated in a ship of opportunity cruise to the Gulf of Alaska Seamounts (SKQ201916S) and preserved diapausing adult females collected between 1000-2000 meters from two offshore stations (GAK19 and DeepQuinn). Mixed plankton samples were immediately diluted with surface seawater, and maintained at $\sim 5^{\circ}\text{C}$ prior to and during sorting. From each station actively swimming (healthy) *N. flemingeri* CVs were rapidly sorted under the microscope and preserved within 15 minutes to 1hr of the tow in RNeasy Lysis Reagent (QIAGEN).

RNA extraction, gene library preparation and RNA-seq

Total RNA was extracted from individual CV from each station using QIAGEN RNeasy Plus Mini Kit (catalog # 74134) in combination with a Qias shredder 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). For each station, total RNA from three of the ten individuals with high quality RNA yields were selected for RNA-seq and shipped on dry ice to the University of Georgia Genomics 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 beads), and then fragmented and reverse transcribed into double-stranded complementary DNA. Each sample was tagged with an indexed adapter and they were simultaneously paired-end sequenced (PE150 or PE75 bp) using an Illumina NextSeq 500 instrument using High-Output Flow Cell. Data in NCBI are the raw sequence reads.

Data Processing Description

Raw sequence reads have been deposited with links to BioProject accession numbers PRJNA324453, and PRJNA324453 in the NCBI (National Center for Biotechnology Information) BioProject database (<https://www.ncbi.nlm.nih.gov/bioproject/>).

BCO-DMO Processing Description

Imported data from source file CV-2015-2022-BCO-DMO.xlsx sheet name "Sheet 1 - sra_result" into the BCO-DMO data system.

Imported data from source file AdultFem-2015-2021-BCO-DMO.xlsx sheet name "Sheet 1 - sra_result (1)" into the BCO-DMO data system.

Renamed parameter names according to BCO-DMO naming standards by replacing spaces with underscores.

A date column was added with the parameter name "Date" and format %Y-%m-%d

Renamed the parameter Depth to Depth_Range to convey the parameter represents a range of depths.

The two datasets were then concatenated together to form the final dataset file named 922330_v1_rna_seq_flemingeri_cv_and_adult_female_2015-2022.csv.

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Related Publications

Roncalli, V., Cieslak, M. C., Castelfranco, A. M., Hopcroft, R. R., Hartline, D. K., & Lenz, P. H. (2021). Post-diapause transcriptomic restarts: insight from a high-latitude copepod. *BMC Genomics*, 22(1).

<https://doi.org/10.1186/s12864-021-07557-7>

Results

Roncalli, V., Cieslak, M. C., Germano, M., Hopcroft, R. R., & Lenz, P. H. (2019). Regional heterogeneity impacts gene expression in the subarctic zooplankter *Neocalanus flemingeri* in the northern Gulf of Alaska. *Communications Biology*, 2(1). <https://doi.org/10.1038/s42003-019-0565-5>

<https://doi.org/10.1038/s42003-019-0565-5>

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., Niestroy, J., Cieslak, M. C., Castelfranco, A. M., Hopcroft, R. R., & Lenz, P. H. (2022). Physiological acclimatization in high-latitude zooplankton. *Molecular Ecology*, 31(6), 1753–1765. Portico.

<https://doi.org/10.1111/mec.16354>

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>

<https://doi.org/10.1038/S41598-018-30873-0>

Results

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Related Datasets

IsRelatedTo

University of Hawaii at Manoa (2016). *Neocalanus flemingeri*, *Neocalanus flemingeri* adult females. 2016/06. NCBI:BioProject: PRJNA324453 [Internet]. Bethesda, MD: National Library of Medicine (US), National Center for Biotechnology Information; Available from: <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA324453>

University of Hawaii at Manoa (2018). *Neocalanus flemingeri*, *Neocalanus flemingeri* pre adult (CV). 2018/10. NCBI:BioProject: PRJNA496596 [Internet]. Bethesda, MD: National Library of Medicine (US), National Center for Biotechnology Information; Available from: <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA496596>.

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Parameters

Parameter	Description	Units
Experiment_Accession	Experiment metadata table (SRX) accession number in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA).	unitless
Experiment_Title	Title of the the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) experiment metadata table (SRX). Descriptive name of individual samples. All samples of stage CV individuals were preserved immediately. All samples of adult females labeled either Week 0 or Time 0 were preserved right after collection, samples with other time stamps (weeks or hours) were incubated in the laboratory for the specified amount of time prior to preservation	unitless
Organism_Name	species name (Crustacea: Copepoda: Calanoida)	unitless
Station	Station identifier	unitless
Latitude	Sampling location latitude, south is negative	decimal degrees
Longitude	Sampling location longitude, west is negative	decimal degrees
Date	Collection date of organism	unitless
Year	Collection year of organism	unitless
Month	Collection month of organism	unitless
Day	Collection day of organism	unitless
Depth_Range	Depth range of collection using depth stratified vertical net collection	meters (m)
Life_Stage	Life stage (CV or Adult)	unitless
Sex	Sex	unitless
Sample_Accession	NCBI SRA Sample accession (SRS)	unitless
Study_Accession	NCBI SRA study accession (SRP)	unitless
Study_Title	Title of the NCBI SRA study	unitless
BioProject	NCBI BioProject accession	unitless

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Instruments

Dataset-specific Instrument Name	Illumina NextSeq 500
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	Desktop sequencer. Illumina NextSeq 500 instrument using High-Output Flow Cell.
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	Agilent Model 2100 Bioanalyzer
Generic Instrument Name	Electrophoresis Chamber
Dataset-specific Description	Agilent Technologies, Inc., Santa Clara, CA, USA The 2100 Bioanalyzer system is an established automated electrophoresis tool for the sample quality control of biomolecules.
Generic Instrument Description	General term for an apparatus used in clinical and research laboratories to separate charged colloidal particles (or molecules) of varying size through a medium by applying an electric field.

Dataset-specific Instrument Name	Midi MultiNet
Generic Instrument Name	MultiNet
Dataset-specific Description	0.25 m ² mouth area; 150 µm mesh nets
Generic Instrument Description	The MultiNet© Multiple Plankton Sampler is designed as a sampling system for horizontal and vertical collections in successive water layers. Equipped with 5 or 9 net bags, the MultiNet© can be delivered in 3 sizes (apertures) : Mini (0.125 m ²), Midi (0.25 m ²) and Maxi (0.5 m ²). The system consists of a shipboard Deck Command Unit and a stainless steel frame to which 5 (or 9) net bags are attached by means of zippers to canvas. The net bags are opened and closed by means of an arrangement of levers that are triggered by a battery powered Motor Unit. The commands for actuation of the net bags are given via single or multi-conductor cable between the Underwater Unit and the Deck Command Unit. Although horizontal collections typically use a mesh size of 300 microns, mesh sizes from 100 to 500 may also be used. Vertical collections are also common. The shipboard Deck Command Unit displays all relevant system data, including the actual operating depth of the net system.

Dataset-specific Instrument Name	CaVET QuadNet
Generic Instrument Name	Plankton Net
Dataset-specific Description	53 µm mesh nets
Generic Instrument Description	A Plankton Net is a generic term for a sampling net that is used to collect plankton. It is used only when detailed instrument documentation is not available.

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Deployments

TXS15

Website	https://www.bco-dmo.org/deployment/917221
Platform	R/V Tiglax
Start Date	2015-05-05
End Date	2015-05-11

TXF15

Website	https://www.bco-dmo.org/deployment/852877
Platform	R/V Tiglax
Start Date	2015-09-09
End Date	2015-09-21
Description	Latitude North boundary (decimal degrees): 60.5298 Latitude South boundary (decimal degrees): 57.7747 Longitude West Boundary (decimal degrees): -149.4755 Longitude East Boundary (decimal degrees): -147.5105

TXF16

Website	https://www.bco-dmo.org/deployment/852880
Platform	R/V Tiglax
Start Date	2016-09-16
End Date	2016-09-19
Description	Latitude North boundary (decimal degrees): 60.5317 Latitude South boundary (decimal degrees): 57.745 Longitude West Boundary (decimal degrees): -149.4807 Longitude East Boundary (decimal degrees): -147.5788

TXF17

Website	https://www.bco-dmo.org/deployment/852883
Platform	R/V Tiglax
Start Date	2017-09-09
End Date	2017-09-22
Description	Latitude North boundary (decimal degrees): 60.6753 Latitude South boundary (decimal degrees): 57.7923 Longitude West Boundary (decimal degrees): . -149.4853 Longitude East Boundary (decimal degrees): -147.503

TXF18

Website	https://www.bco-dmo.org/deployment/910684
Platform	R/V Tiglax
Report	https://nga.itsnet.edu/wp-content/uploads/2019/04/Cruise-Report-TXF18.pdf
Start Date	2018-09-11
End Date	2018-09-25
Description	NGA LTER Fall cruise

TXS19

Website	https://www.bco-dmo.org/deployment/910688
Platform	R/V Tiglax
Report	https://nga.lternet.edu/wp-content/uploads/2019/10/Cruise-Report-TXS19.pdf
Start Date	2019-04-26
End Date	2019-05-08
Description	NGA LTER Summer cruise

TXF19

Website	https://www.bco-dmo.org/deployment/910759
Platform	R/V Tiglax
Start Date	2019-09-11
End Date	2019-09-26
Description	Northern Gulf of Alaska Long-Term Ecological Research (NGA-LTER) Fall cruise

TXS17

Website	https://www.bco-dmo.org/deployment/922412
Platform	R/V Tiglax
Start Date	2017-05-01
End Date	2017-05-09

SKQ201810S

Website	https://www.bco-dmo.org/deployment/922368
Platform	R/V Sikuliaq
Start Date	2018-04-18
End Date	2018-05-05
Description	Coordinates for this deployment can be found in R2R: https://www.rvdata.us/search/cruise/SKQ201810S

SKQ201916S

Website	https://www.bco-dmo.org/deployment/922370
Platform	R/V Sikuliaq
Start Date	2019-07-21
End Date	2019-08-03
Description	Coordinates for this deployment can be found in R2R: https://www.rvdata.us/search/cruise/SKQ201916S

SKQ202006S

Website	https://www.bco-dmo.org/deployment/922372
Platform	R/V Sikuliaq
Start Date	2020-05-04
End Date	2020-05-11
Description	Coordinates for this deployment can be found in R2R: https://www.rvdata.us/search/cruise/SKQ202006S

SKQ202106S

Website	https://www.bco-dmo.org/deployment/922374
Platform	R/V Sikuliaq
Start Date	2021-04-23
End Date	2021-05-06
Description	Coordinates for this deployment can be found in R2R: https://www.rvdata.us/search/cruise/SKQ202106S

TXF21

Website	https://www.bco-dmo.org/deployment/922408
Platform	R/V Tiglax
Start Date	2021-09-10
End Date	2021-09-27

SKQ202207S

Website	https://www.bco-dmo.org/deployment/922377
Platform	R/V Sikuliaq
Start Date	2022-04-21
End Date	2022-05-07
Description	Coordinates for this deployment can be found in R2R: https://www.rvdata.us/search/cruise/SKQ202207S

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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.

Collaborative Research: Molecular profiling of the ecophysiology of dormancy induction in calanid copepods of the Northern Gulf of Alaska LTER site (Diapause preparation)

Coverage: Northern Gulf of Alaska LTER

NSF Award Abstract:

The sub-arctic Pacific sustains major fisheries with nearly all commercially important species depending either directly or indirectly on lipid-rich copepods (*Neocalanus flemingeri*, *Neocalanus plumchrus*, *Neocalanus cristatus* and *Calanus marshallae*). In turn, these species depend on a short-lived spring algal bloom for growth and the accumulation of lipid stores in order to complete an annual life cycle that includes a period of dormancy. The intellectual thrust of this project measures how the timing and magnitude of algal blooms affect preparation for dormancy using a combination of field and experimental observations. The Northern Gulf of Alaska - with four calanid species that experience dormancy, steep environmental gradients, well-described phytoplankton bloom dynamics, and a concurrent NSF-LTER program - provides an unusual opportunity to identify the factors that affect dormancy preparation. Education and outreach plans are integrated with the research. Educational efforts focus on interdisciplinary opportunities for undergraduate, graduate and post-doctoral trainees. The project will generate content for existing graduate and undergraduate courses. U. of Alaska Fairbanks and U. Hawaii at Manoa are Alaska Native and Native Hawaiian Serving Institutions, and students from these groups will be recruited to participate in the project. Because fishing is a major industry in the Gulf of Alaska, outreach will communicate the role copepods play in marine ecosystems using the concept of a dynamic food web tied to production cycles.

Diapause (dormancy) and the accompanying accumulation of lipids in copepods have been identified as key drivers in high latitude ecosystems that support economically important fisheries, including those of the Gulf of Alaska. While the disappearance of lipid-rich copepods has been linked to severe declines in fish stocks, little is known about the environmental conditions that are required for the successful completion of the copepod's life cycle. A physiological profiling approach that measures relative gene expression will be used to test two

alternative hypotheses: the lipid accumulation window hypothesis, which holds that individuals enter diapause only after they have accumulated sufficient lipid stores, and the developmental program hypothesis, which holds that once the diapause program is activated, progression occurs independent of lipid accumulation. The specific objectives are: 1) determine the effect of food levels during *N. flemingeri* copepodite stages on progression towards diapause using multiple physiological and developmental markers; 2) characterize the seasonal changes in the physiological profile of *N. flemingeri* across environmental gradients and across years; 3) compare physiological profiles across co-occurring calanid species (*N. flemingeri*, *Neocalanus plumchrus*, *Neocalanus cristatus* and *Calanus marshallae*); and 4) estimate the reproductive potential of the overwintering populations of *N. flemingeri*. The broader scientific significance includes the acquisition of new genomic data and molecular resources that will be made publicly available through established data repositories, and the development of new tools for routinely obtaining physiological profiles of copepods.

This award reflects NSF's statutory mission and has been deemed worthy of support through evaluation using the Foundation's intellectual merit and broader impacts review criteria.

NOTE: Petra Lenz is a former Principal Investigator (PI) and Andrew Christie is a former Co-Principal Investigator (Co-PI) on this project (award #1756767). Daniel Hartline is the PI listed for the award #1756767 and is now a former Co-PI on this project.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1459235
NSF Division of Ocean Sciences (NSF OCE)	OCE-1459826
NSF Division of Ocean Sciences (NSF OCE)	OCE-1756767
NSF Division of Ocean Sciences (NSF OCE)	OCE-1756859

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