Molecular identification of genetic variants of Neocalanus flemingeri in the Gulf of Alaska from samples collected from 2015 to 2023

Website: https://www.bco-dmo.org/dataset/954181
Data Type: Cruise Results
Version: 1
Version Date: 2025-02-21

Project
» Collaborative Research: Zooplankton restarts in a high-latitude marine ecosystem: species-specific recruitment and development in early spring (Zooplankton recruitment)

Contributors	Affiliation	Role
Lenz, Petra H.	University of Hawaiʻi at Mānoa	Principal Investigator
Hartline, Daniel K.	University of Hawaiʻi at Mānoa	Co-Principal Investigator
Roncalli, Vittoria	University of Hawaiʻi at Mānoa	Scientist
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Abstract
The subarctic Pacific is inhabit by three copepod congeners in the genus Neocalanus with an overlapping biogeographic range that includes the open ocean, marginal seas and fjord systems. Two distinct genetic variants of Neocalanus flemingeri have been reported from the western Pacific: the “small form” with an annual life cycle is found throughout the region, while the “large form” population with a 2-year life cycle is centered in the Sea of Okhotsk. Using a molecular approach, this study examined the genetic composition of N. flemingeri populations in the Gulf of Alaska from multiple stations over an eight-year period using existing nucleotide sequence data from RNA-Seq, Sanger sequencing and metabarcoding data. This is the first report for the occurrence of the large form in the eastern Pacific with a significant presence in fjord systems.

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Coverage

Location: Gulf of Alaska, sub-arctic Pacific
Spatial Extent: **N**:59.8443 **E**:-145.4238 **S**:56.2321 **W**:-149.4838
Temporal Extent: 2015-05-01 - 2023-03-24

Dataset Description

The raw sequence data are available through the National Center for Biotechnology Information (NCBI):
RNA-Seq data: BioProjects: PRJNA324453, PRJNA662858, PRJNA496596, PRJNA807352
* Supplemental file "954181_v1_sra-run-table.csv" for SRA accession and BioSample collection information for the four bioprojects.

Methods & Sampling

Collections and sample preservation

Collections during the spring and fall were made during oceanographic cruises of the Seward Line Long-term Observation Program (LTOP) and northern Gulf of Alaska Long Term Ecological Research programs (https://nga.lternet.edu/) between 2015 and 2022. Additional samples were collected at nearshore stations (GAK1 and RES2.5) in 2019 and 2023, and in 2019 in the Gulf of Alaska Seamount region from below 1000 m (see map, Supplemental File "collections_regional_map.png").

RNA-Seq Data

Pre-adult *Neocalanus flemingeri* stage CV were collected from the upper 100 m in April and May from 5 to 6 stations. Upon retrieval of the net (QuadNet, 53 µm mesh), the plankton collection was live sorted under the microscope and preserved in RNALater. In 2019, *N. flemingeri* were collected in mid-April for an incubation experiment with individuals maintained in the laboratory for up to 2 months before preservation for RNA-Seq (Roncalli et al., 2023). In September, samples were collected from depth (300 to 700 m) using a 0.25 m^2 Multinet (Hydrobios), live sorted and preserved for RNA-Seq immediately or after laboratory incubation. Fall collections were primarily from Prince William Sound (stations PWS2 and KIP2) with the exception of July 2019, when adult females were collected from depth (>1000 m) in the Gulf of Alaska (stations GAK19 and “Quinn deep”).

Sample processing (RNA extraction, library preparation and high-throughput sequencing) has been described previously (Roncalli et al., 2018, 2019, 2021). Paired-end sequencing was done on the Illumina Platform (NextSeq) and short-sequence read lengths were set to either 75 or 150 bp with sequencing depth of 10M or greater. Raw sequence data are available through National Center for Biotechnology Information with additional metadata available through BCO-DMO datasets ids 922330 and 914459 (Lenz et al., 2024). Raw sequence data were quality checked, sequences with phred scores below 30 were removed, and sequences were trimmed to remove adapters and the first 9 bp (Roncalli et al., 2018). In addition, rRNA transcripts were removed using SortMeRNA (version 4.2.0) (Kopylova et al., 2012). Lineage identification was based on cytochrome *c* oxidase subunit-1 (mtCOI) reference sequences that were used to establish the sequence differences between the two forms (Machida and Tsuda, 2010). For the RNA-Seq data, a mtCOI reference database was generated using full length sequences for small-form and large-form *N. flemingeri*, as well as *N. plumchrus*, *N. cristatus*, *Calanus marshallae*, *Eucalanus bungii* and *Metridia pacific* (see Supplemental File "species_list_copepods.csv" for additional copepod species name information and taxonomic identifiers). The reference consisted of consensus sequences obtained by comparing sequences downloaded from NCBI and from *de novo* assemblies (Hartline et al., 2023; Hartline et al., 2024 [BCO-DMO dataset 908689]). RNA-Seq libraries from each individual were mapped against the mtCOI reference using Bowtie2 (version 2.3.5.1, default options in paired end mode) as described previously for an approach that was originally developed to identify and quantify *Calanus* congeners in RNA-Seq data (Lenz et al., 2021a,b). Since mtCOI mapping is highly specific, this approach successfully distinguishes between closely related species and genetic lineages. Cross-mapping is minimal, and even between the two *N. flemingeri* lineages is typically below 2% (maximum 7%). Data analysis involved tallying the number of individuals that mapped to each lineage for each station/year collection. Analysis for spatial and temporal patterns of the number of small vs large form individuals was done by combining data by year from multiple locations, and by combining regional data for both pre-adult and adult individuals to examine interannual differences.

DNA Sequencing

In 2016, additional individuals were collected during the spring and fall cruises for DNA extraction (n=123), amplification of the mtCOI and Sanger sequencing. Individual *N. flemingeri* were preserved in RNALater prior to DNA extraction using the DNEasy Blood and Tissue Kit (Qiagen). Extracted DNA was amplified using universal DNA primers, LCO1490 and HCO2198, which consistently amplify a 710-bp region of the mitochondrial cytochrome oxidase subunit I gene from a variety of metazoan invertebrates (Folmer et al, 1994). PCR products were checked for expected size using gel electrophoresis, and purified using Qiagen`s Purification Kit prior to Sanger sequencing at the Advanced Studies in Genomics, Proteomics and Bioinformatics (ASGPB) at the University of Hawai'i at Mānoa. Sequence data were edited for quality using Geneious and searched on NCBI for the closest match using BLAST (Altschul et al., 1990). Top *N. flemingeri* hits were compared to published lineage-specific sequences (Machida and Tsuda, 2010) for small form vs. large form identification.

Metabarcoding

DNA metabarcoding of bulk samples containing nauplii of *N. flemingeri* were collected in Resurrection Bay between January and March, 2023, size fractionated and preserved in ethanol (Block, 2024). The small size-fraction of the bulk samples (53 – 210 µm fraction) were extracted for DNA using the Qiagen DNeasy Blood and Tissue Mini Kit with an extended 24-hour proteinase K incubation to ensure adequate lysis. The mtCOI was amplified using the mlCOIintF and jgHCO2198 primers (Leray et al., 2013), sequenced and processed using established pipelines as described elsewhere (Block, 2024). Amplicon sequences were quantified, clustered and identified to lineage using reference sequences downloaded from the MetaZooGene database (Bucklin et al., 2021). The full MZGdb (downloaded July 14, 2023), included global sequences of marine invertebrates, vertebrates, and microbes (https://metazoogene.org/, MetaZooGene & O'Brien, T. (2023)). The two *N. flemingeri* genetic lineages were represented by two distinct operational taxonomic units (OTUs) that differed by ca. 3%. 10 diagnostic base pairs out of 307 are indicated by the highlighting in the fasta sequences:

>Nf1_Neocalanus flemingeri OTU 1 (small form)

GTCTAGAAATATTGCCCATGCGGGAGGTTCTGTAGACTTCGCTATTTTCTCACTTCATTT

AGCAGGTGTGAGATCTATTTTAGGGGCCGTAAACTTCATTAGAACCCTCGGAAACTTACG

AGTATTTGGTATATTATTAGACCGAATACCTTTATTTGCCTGAGCTGTTCTTATTACTGC

TGTTCTCCTTCTCCTGTCTTTACCAGTATTAGCTGGAGCTATTACAATATTGTTAACAGA

TCGTAACCTAAATACTTCTTTTTATGATGTTGGCGGGGGCGGTGACCCTATTCTGTACCA

GCATCTA

>Nf2_Neocalanus flemingeri OTU2 (large form)

CTCTAGAAATATTGCCCATGCGGGAGGTTCTGTAGACTTCGCTATTTTCTCACTTCACTT

GGCAGGTGTGAGATCTATTTTAGGGGCCGTAAACTTCATTAGGACCCTGGGAAACTTGCG

AGTATTTGGTATATTATTAGACCGAATACCTTTATTTGCCTGAGCTGTTCTTATTACTGC

TGTTCTCCTTCTCCTGTCTTTACCGGTATTAGCTGGAGCTATTACAATATTGTTAACAGA

TCGTAACCTAAATACTTCTTTCTATGATGTTGGGGGGGGCGGTGACCCTATTCTATACCA

GCATCTA

The proportion of the two lineages was estimated from the relative counts of each OTU.

Cruise identifiers and Sampling dates:

This list includes cruise identifiers also entered in the "Deployments" section of this metadata page as well as description of additional small boat deployments without formal cruise identifiers.

TXS15, Russell Hopcroft, 5/10/15 - 5/11/15
TXF15, Russell Hopcroft, 9/13/15 - 9/20/15
TXS16, Russell Hopcroft, 4/29/16 - 5/6/16
TXS16, Russell Hopcroft, 4/29/16 - 5/6/16
TXF16, Russell Hopcroft, 9/15/16 - 9/20/16
TXS17, Russell Hopcroft, 5/3/17 - 5/9/17
TXF17, Russell Hopcroft, 9/15/17 - 9/22/17
SKQ201810S, Russell Hopcroft, 4/17/18 - 5/6/18
TGX201809, Russell Hopcroft, 9/11/18 - 9/25/18
TGX201904, Russell Hopcroft, 4/26/19 - 5/9/19
SKQ201916S, Russell Hopcroft, 7/21/19 – 8/3/19
TGX201909, Russell Hopcroft, 9/10/19 - 9/26/19
SKQ202006S, Russell Hopcroft, 5/3/20 -5/10/20
SKQ202106S, Russell Hopcroft, 4/19/21 -5/7/21
TGX202109, Russell Hopcroft, 9/10/21 - 9/27/21
SKQ202207S, Ana Aguilar Islas, 4/19/22 - 5/8/22
Day-trips aboard the M/V Dora 4/15/2019 to GAK1 and the R/V Nanuq in Resurrection Bay, AK between January and March, 2023
NOAA Ocean Exploration, Gulf of Alaska Seamounts 2019, Russell Hopcroft, 7/21/2019 – 8/3/2019

Data Processing Description

The presence and relative occurrence of the two genetic variants were based on differences in the cytochrome *c* oxidase subunit-1 sequence (mtCOI) (Machida & Tsuda 2010, Lenz et al. 2021). A mtCOI reference database was generated from the unique sequences for the two variants as well as from those of other common copepod species from the Gulf of Alaska (Hartline et al. 2023). To distinguish between the genetic variants, short-read RNA-Seq data from live-sorted *N. flemingeri* were mapped against this mtCOI reference (Bowtie2, vs. 2.3.5.1). The variant (or species) receiving the most mapped reads, usually in great excess, was taken as the individual's identity. This was also used to identify and remove individuals that had been misidentified in the original morphological-based sorting.

BCO-DMO Processing Description

* Submitted files were imported into the BCO-DMO data system for this dataset:
CopepodSpeciesFilter.csv (will appear in this dataset as Data File: 954181_v1_copepod_species_filter.csv)
Bowtie-Mapping results.csv (will appear in this dataset as Data File: 954181_v1_bowtie-mapping-results.csv)
Suitos-BLAST-data.csv (will appear in this dataset as Data File: 954181_v1_suitos-blast-data.csv)
* submitter noted that the bowtie-mapping results 954181_v1_bowtie-mapping-results.csv were to be considered the primary data table of this dataset. As such all other

tables were attached as supplemental files.

* Column names adjusted to conform to BCO-DMO naming conventions designed to support broad re-use by a variety of research tools and scripting languages. [Only numbers, letters, and underscores. Can not start with a number]

* Non-standard whitespace characters replaced with standard space character.
* Character Ü was removed for values in COI_location and Notes columns of this dataset. Data provided that was for internal use and could be removed from the published dataset.

* Unique species names extracted from "CopepodSpeciesFilter.csv" and matched to identifiers at World Register of Marine Species (WoRMS, marinespecies.org on 2025-02-27). ScientificName and Life Science Identifer (LSID) column added for the identifiers for the species names used within "Species" categories in this table. Supplemental file for copepod species list added.
* Species names in the column descriptions for 954181_v1_bowtie-mapping-results.csv (Parameters section) were updated with the correct spelling of the currently accepted names.

* Additional supplemental file added with combined information from all SRA Run tables from all three associated NCBI BioProjects which includes SRA run, experiment, and BioSample collection metadata. This was added as Supplemental File: 954181_v1_sra-run-table.csv

* lat_lon field split into lat, lon columns and standardized to decimal degree format for geospatial research-ready capability. No lat_lon was provided for samples in BioProject PRJNA496596, PRJNA807352, PRJNA662858, and some were missing in PRJNA324453. The data submitter noted that the lat lons are available two BCO-DMO datasets. These datasets were added to the "Related Datasets" section of this page for easier discoverability:

Dataset (922330)
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.
BCO-DMO page: https://www.bco-dmo.org/dataset/922330

Dataset (914459):
Gene expression profiles for Neocalanus flemingeri pre adults (CV) exposed to four different experimental food conditions collected from the M/V Dora in the Gulf of Alaska at station GAK1 from April 2019
BCO-DMO page: https://www.bco-dmo.org/dataset/914459

* Several columns in sra_run table were not able to be typed as numerc such as depth (due to some being ranges in single value) and temp which included String units in the values.

* The citation in metadata on this dataset "(see map, SupplementS2)" was replaced with "(see map, Supplemental File "collections_regional_map.png")" since the submitter supplied this map. The supplementS2 reference was explained as a reference to a manuscript under review.

* Invalid sampling date in metadata text "TXS15, Russell Hopcroft, 5/103/15 - 5/11/15" changed to "5/10/15 - 5/11/15" as cruise dates were only between "05-05-2015 to 05-11-2015"

Problem Description

Read mapping to the mitochondrial COI revealed that some individuals that were originally identified as N. flemingeri during live sorting were mis-identified as shown in data file.

Missing Latitudes and longitudes in the supplemental file: 954181_v1_sra-run-table.csv are due to missing latitudes and longitudes for the BioSample accessions at NCBI. The locations can be found in related BCO-DMO datasets. See "Related Datasets" section of this page.

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Data Files

File
mtCOI Bowtie Mapping results filename: 954181_v1_bowtie-mapping-results.csv (Comma Separated Values (.csv), 19.69 KB) MD5:f812d8e2a20c2261b148d365516408eb Mapping results for RNA-Seq data showing short-sequence read counts aligned to mtCOI sequences from six different species. This is the primary data table of this BCO-DMO dataset (953181 version 1).

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Supplemental Files

File
Collections - Regional Map filename: collections_regional_map.png (Portable Network Graphics (.png), 192.85 KB) MD5:3fc728adfc8024fae83b140c7cb06094 A regional map of the eastern Pacific and locates the collection area. These locations were previously published as part of Figure 1 of Pinchuk, et al. (2008).
Copepod species list and taxnomic identifiers filename: species_list_copepods.csv (Comma Separated Values (.csv), 687 bytes) MD5:e3784dc8a603ff9527c6de1b5f6d66fb Copepod ScientificNames and taxnomic identifiers. Columns: Name_in_Dataset, Category name as it appears in 954181_v1_copepod_species_filter.csv column "Species" ScientificName, The scientific name within "Species" column. AphiaID, World Register of Marine Species identifier (AphiaID) for the ScientificName LSID, Life Science Identifier (LSID) for the ScientificName
mtCOI reference file filename: 954181_v1_copepod_species_filter.csv (Comma Separated Values (.csv), 12.34 KB) MD5:466fc154634cc819d3027d51dd35b241 Reference mtCOI sequences and their source used in the species identification using Bowtie mapping. Sequences are provided in FASTA format. Column (parameter) information: Identifier,COI sequence identifier description ,Unitless, NCBI Accession,NCBI Accession number available at the National Center for Biotechnology Information,Unitless, COI location,The location of the reference sequence in relation to the entire sequence in the NCBI accession,integer range, Species,Genus and species,Unitless, Notes,Special note on entry,Unitless, Sequence,Nucleotide sequence in fasta format,Unitless, ScientificName, The Genus and species within the "Species" column LSID, The Life Science Identifier (LSID) for the ScientificName
Sanger sequencing results filename: 954181_v1_suitos-blast-data.csv (Comma Separated Values (.csv), 6.84 KB) MD5:31ee327ae961c61d171f144e4663ade2 Search results and identification to genetic variant of mtCOI sequences obtained from a ca. 600 bp amplicon from DNA extracted from individual Neocalanus flemingeri. Column (parameter) information Station,GAK = numbering on Seward Line; PWS & KIP2 = Prince William Sound,Unitless, Sample,Sample sequence number; gaps in numbering due to failed sequencing reaction,Unitless, Date,Collection date,Day-Month-Year, Query Cover,Column label for NCBI BLAST indicating how much of the Query sequence was represented in the Subject,Percent, E-Value,Column label for NCBI BLAST indicating the E-value for the Query-to-Subject matrch (0 for E < 1e-180),Probability, Ident,Identity between Query and Subject in aligned portion,Percent, Base Pairs,Length in nucleotide bases of aligned portion,Integer, Accession,Accession number for the best-match Subject,Unitless, Species,Species of the bast-match Subject,Unitless, Form,Identified variety of bast-match Subject Large or Small,Unitless,
SRA Run and Biosample collection metadata filename: 954181_v1_sra-run-table.csv (Comma Separated Values (.csv), 238.83 KB) MD5:a12d504f3416f22aea35ec74afc0432e Supplemental table containing additional information related to the SRA accessions and BioSamples referenced in this dataset. These tables are the results of the NCBI SRA Run Selector for each of the BioProjects referenced in this dataset (PRJNA324453, PRJNA662858, PRJNA496596, PRJNA807352). See https://www.ncbi.nlm.nih.gov/Traces/study/#

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Related Publications

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. Journal of Molecular Biology, 215(3), 403–410. doi:10.1016/s0022-2836(05)80360-2 https://doi.org/10.1016/S0022-2836(05)80360-2
Methods

Block, L. N. (2024). Evaluating Species-Specific Naupliar Recruitment During the Winter-to-Spring Transition in the Northern Gulf of Alaska Using Molecular Tools (Master's thesis, University of Hawai'i at Manoa). Available from https://hdl.handle.net/10125/108679
Results

Bowtie 2: fast and sensitive read alignment. (n.d.). Retrieved from https://bowtie-bio.sourceforge.net/bowtie2/index.shtml
Software

Bucklin, A., Peijnenburg, K. T. C. A., Kosobokova, K. N., O'Brien, T. D., Blanco-Bercial, L., Cornils, A., Falkenhaug, T., Hopcroft, R. R., Hosia, A., Laakmann, S., Li, C., Martell, L., Questel, J. M., Wall-Palmer, D., Wang, M., Wiebe, P. H., & Weydmann-Zwolicka, A. (2021). Toward a global reference database of COI barcodes for marine zooplankton. Marine Biology, 168(6). https://doi.org/10.1007/s00227-021-03887-y
Methods

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular marine biology and biotechnology, 3(5), 294–299. PMID: 7881515. https://www.researchgate.net/publication/15316743_DNA_primers_for_amplification_of_mitochondrial_Cytochrome_C_oxidase_subunit_I_from_diverse_metazoan_invertebrates
Methods

Geneious | Bioinformatics Software for sequence data analysis. (n.d.). Retrieved from https://www.geneious.com/
Software

Hartline, D. K., Cieslak, M. C., Castelfranco, A. M., Lieberman, B., Roncalli, V., & Lenz, P. H. (2023). De novo transcriptomes of six calanoid copepods (Crustacea): a resource for the discovery of novel genes. Scientific Data, 10(1). https://doi.org/10.1038/s41597-023-02130-1
Results

Kopylova, E., Noé, L., & Touzet, H. (2012). SortMeRNA: fast and accurate filtering of ribosomal RNAs in metatranscriptomic data. Bioinformatics, 28(24), 3211–3217. https://doi.org/10.1093/bioinformatics/bts611
Software

Lenz, P. H., Block, L. N., Cieslak, M. C., Suitos, J., Roncalli, R., Castelfranco, A. M., and Hartline, D. K. (n.d.) Genetic divergence in a marine copepod associated with marginal habitats across the subarctic Pacific. Marine Ecology Progress Series, in review
Results

Lenz, P. H., Roncalli, V., Cieslak, M. C., Tarrant, A. M., Castelfranco, A. M., & Hartline, D. K. (2021). Diapause vs. reproductive programs: transcriptional phenotypes in a keystone copepod. Communications Biology, 4(1). https://doi.org/10.1038/s42003-021-01946-0
Methods

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Methods

Leray, M., Yang, J. Y., Meyer, C. P., Mills, S. C., Agudelo, N., Ranwez, V., Boehm, J. T., & Machida, R. J. (2013). A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. Frontiers in Zoology, 10(1), 34. https://doi.org/10.1186/1742-9994-10-34
Methods

Machida, R. J., & Tsuda, A. (2010). Dissimilarity of Species and Forms of Planktonic Neocalanus Copepods Using Mitochondrial COI, 12S, Nuclear ITS, and 28S Gene

Sequences. PLoS ONE, 5(4), e10278. https://doi.org/10.1371/journal.pone.0010278
Methods

Northern Gulf of Alaska Long Term Ecological Research. (2025, January 27). Northern Gulf of Alaska LTER - Northern Gulf of Alaska. Northern Gulf of Alaska. https://nga.lternet.edu/
Methods

Pinchuk, A. I., Coyle, K. O., & Hopcroft, R. R. (2008). Climate-related variability in abundance and reproduction of euphausiids in the northern Gulf of Alaska in 1998–2003. Progress in Oceanography, 77(2–3), 203–216. https://doi.org/10.1016/j.pocean.2008.03.012
Methods

Roncalli, V., Block, L. N., Niestroy, J. L., Cieslak, M. C., Castelfranco, A. M., Hartline, D. K., & Lenz, P. H. (2023). Experimental analysis of development, lipid accumulation and gene expression in a high-latitude marine copepod. Journal of Plankton Research, 45(6), 885–898. https://doi.org/10.1093/plankt/fbad045
Methods

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
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

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Related Datasets

IsRelatedTo

Block, L. N., Lenz, P. H. (2025) **CTD (temperature, salinity and fluorescence) from bi-weekly vertical profiles in Resurrection Bay, AK from January to March of 2023.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2025-02-21 doi:10.26008/1912/bco-dmo.954156.1 [view at BCO-DMO]
Relationship Description: Related data collected as part of the same study published in Block, L. N. (2024, https://hdl.handle.net/10125/108679).

Block, L. N., Lenz, P. H. (2025) **Chlorophyll a and flow cytometry data from bi-weekly vertical profiles in Resurrection Bay, AK from January to March of 2023 from bi-weekly vertical profiles in Resurrection Bay, AK from January to March of 2023.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2025-02-21 doi:10.26008/1912/bco-dmo.954173.1 [view at BCO-DMO]
Relationship Description: Related data collected as part of the same study published in Block, L. N. (2024, https://hdl.handle.net/10125/108679).

Block, L. N., Lenz, P. H. (2025) **Microplankton microscopy and biovolume analysis from Lugol's samples collected in Resurrection Bay, AK from January to March of 2023.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2025-02-21 doi:10.26008/1912/bco-dmo.954189.1 [view at BCO-DMO]
Relationship Description: Related data collected as part of the same study published in Block, L. N. (2024, https://hdl.handle.net/10125/108679).

Hartline, D. K., Lenz, P. H., Cieslak, M. C. (2024) **Annotated de novo transcriptomes generated from six co-occurring species of calanoid copepods from the R/V Tiglax TXF18, TXS19, TXF15, TXF17 in the Gulf of Alaska from 2015-2019.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-07-02 doi:10.26008/1912/bco-dmo.908689.1 [view at BCO-DMO]
Relationship Description: Cited in methods: "...The reference consisted of consensus sequences obtained by comparing sequences downloaded from NCBI and from de novo assemblies (Hartline et al., 2023; Hartline et al., 2024 [BCO-DMO dataset 908689]). "

Lenz, P. H., Hartline, D. K., Roncalli, V., Block, L. N., Niestroy, J. L., Cieslak, M. C. (2024) **Gene expression profiles for Neocalanus flemingeri pre adults (CV) exposed to four different experimental food conditions collected from the M/V Dora in the Gulf of Alaska at station GAK1 from April 2019.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-05-30 doi:10.26008/1912/bco-dmo.914459.1 [view at BCO-DMO]
Relationship Description: The dataset "Neocalanus flemingeri incubation experiment: gene expression (914459)" contains latitudes and longitudes for collections in this dataset "Molecular identification of genetic variants of Neocalanus flemingeri (954181)"

Lenz, P. H., Roncalli, V., Cieslak, M. C. (2024) **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.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-07-26 doi:10.26008/1912/bco-dmo.922330.1 [view at BCO-DMO]
Relationship Description: The dataset "Multiyear RNA-Seq of Neocalanus flemingeri stages CV and Adult Female (922330)" contains latitudes and longitudes for collections in this dataset "Molecular identification of genetic variants of Neocalanus flemingeri (954181)"

IsDerivedFrom

MetaZooGene & O'Brien, T. (2023). MetaZooGene Atlas & Database (mzgdb): A collaborative product of SCOR WG157 (MetaZooGene) and Todd O'Brien. https://metazoogene.org/ accessed July 14, 2023. https://metazoogene.org/database/

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Parameters

Parameter	Description	Units
Year	Sample collection year	unitless
Stage	Developmental stage: pre-adult copepodite stage CV or adult female	unitless
SRX	NCBI SRX (SRA experiment) accession number of individual's reads file used for Bowtie mapping	unitless
Nf_Small	Neocalanus flemingeri (urn:lsid:marinespecies.org:taxname:353708) small form	count
Nf_Large	Neocalanus flemingeri (urn:lsid:marinespecies.org:taxname:353708) large form	count
Np	Neocalanus plumchrus (urn:lsid:marinespecies.org:taxname:196772)	count
Nc	Neocalanus cristatus (urn:lsid:marinespecies.org:taxname:104470)	count
Cg	Calanus glacialis (urn:lsid:marinespecies.org:taxname:104465) / Calanus marshallae (urn:lsid:marinespecies.org:taxname:196770)	count
Eb	Eucalanus bungii (urn:lsid:marinespecies.org:taxname:196775)	count
Mp	Metridia pacifica (urn:lsid:marinespecies.org:taxname:196784)	count
BioProject	NCBI BioProject containing the SRX set	unitless

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Instruments

Dataset-specific Instrument Name	Illumina MiSeq
Generic Instrument Name	Automated DNA Sequencer
Generic Instrument Description	A DNA sequencer is an instrument that determines the order of deoxynucleotides in deoxyribonucleic acid sequences.

Dataset-specific Instrument Name	Illumina Next-Seq 500
Generic Instrument Name	Automated DNA Sequencer
Generic Instrument Description	A DNA sequencer is an instrument that determines the order of deoxynucleotides in deoxyribonucleic acid sequences.

Dataset-specific Instrument Name	
Generic Instrument Name	Bongo Net
Dataset-specific Description	Bongo Nets, 30-cm diameter equipped with 53-µm mesh nets
Generic Instrument Description	A Bongo Net consists of paired plankton nets, typically with a 60 cm diameter mouth opening and varying mesh sizes, 10 to 1000 micron. The Bongo Frame was designed by the National Marine Fisheries Service for use in the MARMAP program. It consists of two cylindrical collars connected with a yoke so that replicate samples are collected at the same time. Variations in models are designed for either vertical hauls (OI-2500 = NMFS Pairovet-Style, MARMAP Bongo, CalVET) or both oblique and vertical hauls (Aquatic Research). The OI-1200 has an opening and closing mechanism that allows discrete "known-depth" sampling. This model is large enough to filter water at the rate of 47.5 m3/minute when towing at a speed of two knots. More information: Ocean Instruments, Aquatic Research, Sea-Gear

Dataset-specific Instrument Name	General Oceanics flowmeters
Generic Instrument Name	Flow Meter
Generic Instrument Description	General term for a sensor that quantifies the rate at which fluids (e.g. water or air) pass through sensor packages, instruments, or sampling devices. A flow meter may be mechanical, optical, electromagnetic, etc.

Dataset-specific Instrument Name	
Generic Instrument Name	Folsom Plankton Splitter
Generic Instrument Description	A device for sub-sampling of plankton and ichthyoplankton samples by splitting, developed by Dr. Folsom of the Scripps Institute of Oceanography. Ideally suited for splitting plankton samples with minimal debris. A measured volume of plankton sample is placed in the undivided section of the drum. This is rotated 120 degrees to divide the stirred sample with a separating blade. Standard Methods suggests splitting until a subsample of 200-500 individuals is obtained.

Dataset-specific Instrument Name	Dissecting microscope, Leica MZ16 and Olympus SZN
Generic Instrument Name	Microscope - Optical
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	Midi Multinet, Hydro-Bios
Generic Instrument Name	MultiNet
Dataset-specific Description	Midi Multinet, Hydro-Bios (0.5 m2 cross-sectional 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 m2), Midi (0.25 m2) and Maxi (0.5 m2). 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	General Oceanics messenger with double trip mechanism
Generic Instrument Name	no_bcodmo_term
Generic Instrument Description	No relevant match in BCO-DMO instrument vocabulary.

Dataset-specific Instrument Name	Elongated QuadNet
Generic Instrument Name	Plankton Net
Dataset-specific Description	Elongated QuadNet net equipped with two 53-µm and two 150-µm mesh nets (25 cm diameter)
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
Report	https://www.ncei.noaa.gov/access/ocean-carbon-acidification-data-system/oceans/Coastal/seward.html
Start Date	2015-05-05
End Date	2015-05-11

TXF15

Website	https://www.bco-dmo.org/deployment/852877
Platform	R/V Tiglax
Report	https://www.ncei.noaa.gov/access/ocean-carbon-acidification-data-system/oceans/Coastal/seward.html
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

TXS16

Website	https://www.bco-dmo.org/deployment/922410
Platform	R/V Tiglax
Report	https://www.ncei.noaa.gov/access/ocean-carbon-acidification-data-system/oceans/Coastal/seward.html
Start Date	2016-04-30
End Date	2016-05-27

TXF16

Website	https://www.bco-dmo.org/deployment/852880
Platform	R/V Tiglax
Report	https://www.ncei.noaa.gov/access/ocean-carbon-acidification-data-system/oceans/Coastal/seward.html
Start Date	2016-09-15
End Date	2016-09-20
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

TXS17

Website	https://www.bco-dmo.org/deployment/922412
Platform	R/V Tiglax
Report	https://www.ncei.noaa.gov/access/ocean-carbon-acidification-data-system/oceans/Coastal/seward.html
Start Date	2017-05-01
End Date	2017-05-09

TXF17

Website	https://www.bco-dmo.org/deployment/852883
Platform	R/V Tiglax
Report	https://www.ncei.noaa.gov/access/ocean-carbon-acidification-data-system/oceans/Coastal/seward.html
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

SKQ201810S

Website	https://www.bco-dmo.org/deployment/922368
Platform	R/V Sikuliaq
Report	https://nga.lternet.edu/wp-content/uploads/2019/04/Cruise-Report-SKQ201810S.pdf
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

TXF18

Website	https://www.bco-dmo.org/deployment/910684
Platform	R/V Tiglax
Report	https://nga.lternet.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

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

TXF19

Website	https://www.bco-dmo.org/deployment/910759
Platform	R/V Tiglax
Report	https://nga.lternet.edu/wp-content/uploads/2020/02/Cruise-Report-TXF19.pdf
Start Date	2019-09-11
End Date	2019-09-26
Description	Northern Gulf of Alaska Long-Term Ecological Research (NGA-LTER) Fall cruise

SKQ202006S

Website	https://www.bco-dmo.org/deployment/922372
Platform	R/V Sikuliaq
Report	https://nga.lternet.edu/wp-content/uploads/2020/07/Cruise-Report-SKQ202006S_v2.pdf
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
Report	https://nga.lternet.edu/wp-content/uploads/2022/03/Cruise-Report-TGX202109.pdf
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 Research: Zooplankton restarts in a high-latitude marine ecosystem: species-specific recruitment and development in early spring (Zooplankton recruitment)

Coverage: Sub-arctic marine ecosystem, Gulf of Alaska

NSF Award Abstract

Global climate change and associated extreme weather events are increasingly impacting marine communities at all trophic levels and leading to shifts in the timing of life history events. This project is investigating the annual restart of the spring zooplankton community in the Gulf of Alaska in order to determine the timing of species-specific recruitment and growth. Zooplankton are small pelagic animals that are a critical link between microalgae and protozoans and higher levels in the food web including economically important fishes, birds and marine mammals. While their abundances and species composition have been documented over part of the annual cycle between late spring and fall, this project focuses on winter and early spring. The project integrates traditional methods with modern molecular approaches to characterize the diversity, development, feeding and physiology of zooplankton, especially the early developmental stages of copepods (small crustaceans). The goal is to determine which species are there, how many are present and where they are in the water column, and to reveal indicators of their health. Broader impacts include research training for three graduate students and at least four undergraduates in biological oceanography and physiological ecology. Outreach activities are focusing on broadening the public’s understanding of plankton ecology. An illustrated zooplankton guide for the Gulf of Alaska and plankton module for school teachers and students is being produced in collaboration with the Center for Alaskan Coastal Studies. Other plans include sponsorship of nature-drawing workshops on zooplankton and the production of an Art & Science traveling exhibit.

This project is tracking zooplankton population abundances, species composition and developmental stages through the spring restart in a high-latitude fjord in the northern Gulf of Alaska. While the entire zooplankton community is being characterized, the main focus is on the difficult-to-assess early developmental stages of copepods, which dominate the late spring biomass in the region. Three central hypotheses guide the research: 1) high abundances of copepod nauplii are present before any measurable increases in food in surface waters; 2) species diversity increases between winter and spring, with nauplii from large lipid-rich capital-breeding species appearing first, followed by those from income- and hybrid-strategy species and finally nauplii that emerge from dormant eggs; 3) prior to the appearance of food resources, nauplii from capital-breeding species conserve resources by delaying development and entering a state of dormancy in the second and third naupliar stages. The project entails intensive depth-stratified field sampling to characterize the wild community, in combination with laboratory experiments on nauplii to determine their responsiveness to food. The prey are being characterized by measuring chlorophyll a, dietary and prey community DNA sequencing and flow cytometry to establish diversity and abundances. Size-fractionated zooplankton samples are being analyzed using microscopy and community DNA sequencing to ascertain species diversity, developmental stage distribution and abundances. Feeding activity is being measured using dietary DNA sequencing of nauplii followed by comparisons with the prey field. Dormancy in nauplii is being determined by differential gene expression of target genes (RT-qPCR) and high-throughput sequencing of mRNA of individuals (transcriptomics) and community samples (meta-transcriptomics). Short-term and long-term effects of food availability on dormancy, development and growth are being quantified in laboratory experiments. Broader impacts are focused on training of students in interdisciplinary research and state-of-art techniques, and public outreach to introduce plankton ecology to broader audiences.

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Funding

Funding Source	Award
NSF Office of Polar Programs (formerly NSF PLR) (NSF OPP)	OCE-2222376
NSF Office of Polar Programs (formerly NSF PLR) (NSF OPP)	OCE-2222592
NSF Office of Polar Programs (formerly NSF PLR) (NSF OPP)	OCE-2222558

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