

# Oocytes formation in post-diapause *Neocalanus flemingeri* females from the R/V Sikuliaq and the R/V Tiglax in the Northern Gulf of Alaska from 2019-06-30 to 2019-09-13

**Website:** <https://www.bco-dmo.org/dataset/908514>

**Data Type:** Cruise Results, experimental

**Version:** 1

**Version Date:** 2024-06-13

## 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
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<a href="#">Monell, Kira J.</a>	University of Hawai'i at Mānoa (PBRC)	Scientist, Student, Data Manager
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## Abstract

These data are from a study on the formation of oocytes in post-diapause *Neocalanus flemingeri* females collected from depth in Prince William Sound in the Gulf of Alaska. Collections were made during two NGA LTER cruises. After sorting, females were incubated in flasks and removed for experimental incubations and imaging. Oocyte production by post-diapause females that involved DNA replication in the ovary and oviducts was examined using incubation in 5-Ethynyl-2'-deoxyuridine (EdU). Both oogonia and oocytes incorporated EdU, with the number of EdU labeled cells peaking at 72 hours following diapause termination. Cells labeling with EdU remained high for two weeks, decreasing thereafter with no labeling detected by four weeks post diapause, and three to four weeks before spawning of the first clutch of eggs. By limiting DNA replication to the initial phase, the females effectively separate oocyte production from oocyte provisioning.

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## Coverage

**Location:** Gulf of Alaska, sub-arctic Pacific

**Spatial Extent:** N:60.535 E:-147.803 S:60.278 W:-147.987

**Temporal Extent:** 2019-06-30 - 2019-09-13

## Methods & Sampling

### Sample collection and sorting

Copepods were collected in Prince William Sound, Alaska in the summer and fall of 2019 during the Northern Gulf of Alaska Long Term Ecological Research (NGA LTER) cruises (<https://nga.lternet.edu/>). Females “PWS2/June” were collected on June 30th, 2019 at the sampling site PWS2 (Latitude 60° 32.1’N; Longitude 147° 48.2’W) (R/V Sikuliaq, cruise number: SKQ201915S), and the “Pleiades/September” females were collected on September 12th and 13th, 2019 at PWS2 and near the Pleiades Islands (Latitude 60° 16.7’N; Longitude 147° 59.2’W) (M/V Tiglax, cruise number: TGX201909). Copepods were collected with a Midi MultiNet (0.25 m<sup>2</sup> mouth area; 150 µm mesh nets) towed vertically from near the bottom to the surface at 0.5 m/sec (PWS2: 798 m; KIP2: 588 m). Upon retrieval, net samples were immediately diluted using filtered seawater collected from depth and kept between 4-6°C to minimize thermal stress. All females selected for the experiments were sorted under a dissecting microscope. Females were placed in groups of three into 750 mL Falcon tissue-culture flasks and incubated under dim light in an incubator for up to 4.5 weeks. Experimental temperatures were at or below deep-water temperatures in Prince William Sound (temperature settings: 4°C for June and 6°C for September). A subset of females was used in the DNA replication experiments; the remaining females were imaged for measurements of prosome length and lipid sac area.

### Experimental design and timeline

For the timeline, two to four females were incubated in low concentrations of 5-Ethynyl-2'-deoxyuridine (EdU) for 24 hours at eight time points in June (Figure 2, 0-24, 24-48, 36-60, 72-96 hours and 2, 3, 4, 4.5 weeks), and at six time points in September (0-24, 24-48, 72-96 hours and 1, 2, 3 weeks) to track the numbers of cells with DNA replication in the ovary and oviducts from collection (diapause) to 4.5 weeks post-collection. Furthermore, after checking the females from the June experiment, three time points were added in the first 24 hours with shorter EdU incubation periods (0-3, 0-6, 0-14 hrs) to establish the start of DNA replication post-diapause. Prior to preservation and processing for confocal microscopy, females were examined by light microscopy for any visible 199 morphological changes and imaged for female size and lipid sac area measurements.

See the related dataset <https://www.bco-dmo.org/dataset/907880> for the morphological changes and female size and lipid sac area measurements.

### EdU protocol

The EdU incubations at the time points listed above were used to obtain a timeline of the formation of oocytes post-diapause. For each experimental time point two to four females were carefully pipetted out of the experimental flasks, imaged, and transferred into well plates with 2 ml of filtered seawater with 0.5 mg of EdU per copepod in June. This concentration was found to be high, and the EdU concentration was adjusted to decrease labeling brightness. Thus, in September, the concentration of EdU was decreased to 0.06 mg of EdU per copepod. The lower concentration improved viewing in the confocal microscope. Females were incubated in this solution for 24 hours except for the first three September time points (0-3, 0-6, 0-14 hrs). After the incubation, females were removed from the EdU, fixed in 4% paraformaldehyde in Sorensen's Phosphate Buffer pH 7.2 (PB) and labeled using a ThermoFisher Click-iT EdU Alexa Fluor 594 Imaging Kit (catalog number: C10639) following the manufacturer's instructions. Samples were washed for 15 minutes thrice in PB then in 0.5% Triton X-100 in PB for three 15-minute long permeabilization washes. EdU labeled cells were fluorescently tagged with Alexa Fluor 594 dye using a copper-catalyzed click reaction. Three additional 15-minute washes in PB were done before samples were stored in VECTASHIELD Antifade Mounting Medium containing DAPI, a nuclear DNA counter-label to EdU. Samples were stored at 4°C until mounting and imaging. Because DAPI in VECTASHIELD frequently did not permeate into the ovary, dilutions of VECTASHIELD with DAPI or Hoechst 3342 in phosphate-buffered saline were used to fully label the ovary prior to imaging on the confocal microscope.

### Data Processing Description

Confocal imaging and quantification of cell division Females were mounted in VECTASHIELD with DAPI with their left lateral side facing up except for three individuals that were mounted dorsally. Samples were imaged using a Leica SP8 X Confocal Laser Scanning microscope with a × 20 glycerol immersion lens and a white light laser.

DAPI has an excitation peak of 359 nm and emission peak of 457 nm, while Alexa fluor 594 has an excitation peak of 590 nm and an emission peak of 617 nm. Imaging was optimized through gating out of some wavelengths to decrease background and autofluorescence. Samples were imaged by tile scanning through each copepod to locate the entire ovary. Z-stack sections were 1.04  $\mu\text{m}$  apart to ensure that no cells were missed due to large imaging gaps. Whole-mount females were imaged from the start of the ovary and oviducts until the depth at which resolution was lost due to insufficient laser penetration. Using Leica's merge software, multiple regions with individual z-stacks were merged to form a single z-stack of an ovary larger than the lens' field of view.

## BCO-DMO Processing Description

Processing steps to create the final dataset named "908514\_v1\_oocyte\_production.csv" using the BCO-DMO data processor laminar.

1. Loaded the submitted oocytes data file named EdU\_information\_2019\_Nflem.csv into laminar.
2. Renamed parameter fields to BCO-DMO naming conventions. Replaced spaces with underscores.
3. Renamed the parameter 'Sample ID' to 'EdU\_sample\_ID\_number' to match a column name in a related dataset for the same experiment.
4. Added latitude and longitude values to new latitude and longitude columns for stations PWS2 and Pleiades. Each station has singular values which come from the methods description for the dataset. Latitude and longitude values used in the table were calculated by converting the degrees and minutes values to decimal degrees with three digit precision after discussion with the submitter.
5. Capitalized the month names.
6. Converted the dates to ISO date format of %Y-%m-%d.
7. Added a parameter column named Species with the value 'Neocalanus flemingeri'.
8. Added a parameter column named Sex with the value 'female'.
9. Reordered the fields so that the sample collection information is at the front followed by columns about EdU incubation. And then the results columns.
10. Table saved to final dataset file named '908514\_v1\_oocyte\_production.csv'.
11. Taxonomic names in the dataset were checked using the World Register of Marine Species (WoRMS) taxa match tool. The species name matched the accepted name exactly as of 2024-05-27. A unique species list with associated AphiaID and LSID identifiers was added as a supplemental file.

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## Data Files

File
<b>908514_v1_oocyte_production.csv</b> (Comma Separated Values (.csv), 5.58 KB) MD5:49ede6f930d74a610404fd87236be03d
Primary data file for dataset ID 908514, version 1

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## Supplemental Files

File
<b>Species WoRMS taxonomy</b> filename: species_list.csv (Comma Separated Values (.csv), 211 bytes) MD5:0996e3c2eab4418bf250f26afec882aa
Species WoRMS taxonomy table with columns: ScientificName, AphiaID, LSID, Authority, Class, Order, Family, Genus, Species

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## Related Publications

Monell, K. J., Roncalli, V., Hopcroft, R. R., Hartline, D. K., & Lenz, P. H. (2023). Post-Diapause DNA Replication during Oogenesis in a Capital-Breeding Copepod. Integrative Organismal Biology, 5(1).  
<https://doi.org/10.1093/iob/obad020>  
*Results*

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## Related Datasets

### IsRelatedTo

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Lenz, P. H., Hartline, D. K., Monell, K. J. (2024) **Post-diapause *Neocalanus flemingeri* females morphometric measurements and calculations of lipid fullness and lipid volume taken from the R/V Sikuliaq and the R/V Tiglax in the Northern Gulf of Alaska from 2019-06-30 to 2019-09-13.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-06-12 doi:10.26008/1912/bco-dmo.907880.1 [[view at BCO-DMO](#)]  
*Relationship Description: Morphological changes and female size and lipid sac area measurements in post-diapause *Neocalanus flemingeri* females as part of an experiment to also study the formation of oocytes in post-diapause *Neocalanus flemingeri* females. Copepods were collected from depth in Prince William Sound in the Gulf of Alaska in June and September, 2019.*

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## Parameters

Parameter	Description	Units
Station	Station of plankton tow, stations were decided by the Northern Gulf of Alaska Long Term Ecological Research group	unitless
Latitude	Sampling location latitude, south is negative	decimal degrees
Longitude	Sampling location longitude, west is negative	decimal degrees
Date_collected	Collection date of organism	unitless
Collection_month	Collection month of organism. Month is the full name.	unitless
Species	Organism species	unitless
Sex	Organism sex	unitless
EdU_sample_ID_number	Sample tube identification. If female was used in EdU experiments, then her sample ID number is listed	unitless
Amount_of_EdU	Milligrams of EdU added to each female's well during the incubation step	milligrams (mg)
Incubation_date_at_start	Date when the EdU incubation started	unitless
Length_of_EdU_incubation_in_hours	Duration of EdU incubation	hours
Time_point_in_days	The experimental time point in days after collection, time point is counted as the end of the EdU incubation so the zero hour experimental time point is 1 day after collection	days
Number_of_cells_replicating	Number of EdU-labeled cells that were counted within the ovary and oviducts of a female	unitless

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## Instruments

<b>Dataset-specific Instrument Name</b>	Spot Insight camera
<b>Generic Instrument Name</b>	Camera
<b>Dataset-specific Description</b>	12 MPx resolution
<b>Generic Instrument Description</b>	All types of photographic equipment including stills, video, film and digital systems.

<b>Dataset-specific Instrument Name</b>	Leica SP8 X Confocal Laser Scanning microscope
<b>Generic Instrument Name</b>	Confocal Laser Scanning Microscope
<b>Generic Instrument Description</b>	A laser scanning confocal microscope is a type of confocal microscope that obtains high-resolution optical images with depth selectivity, in which a laser beam passes through a light source aperture and then is focused by an objective lens into a small (ideally diffraction-limited) focal volume within or on the surface of a specimen. The confocal microscope uses fluorescence optics. 'Confocal' means that the image is obtained from the focal plane only, any noise resulting from sample thickness being removed optically. 'Laser scanning' means the images are acquired point by point under localized laser excitation rather than full sample illumination, as in conventional widefield microscopy.

<b>Dataset-specific Instrument Name</b>	Leica MZ16 microscope
<b>Generic Instrument Name</b>	Microscope - Optical
<b>Generic Instrument Description</b>	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".

<b>Dataset-specific Instrument Name</b>	Midi MultiNet
<b>Generic Instrument Name</b>	MultiNet
<b>Dataset-specific Description</b>	0.25 m2 mouth area; 150 µm mesh nets
<b>Generic Instrument Description</b>	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.

## Deployments

### SKQ201915S

<b>Website</b>	<a href="https://www.bco-dmo.org/deployment/910757">https://www.bco-dmo.org/deployment/910757</a>
<b>Platform</b>	R/V Sikuliaq
<b>Report</b>	<a href="https://nga.lternet.edu/wp-content/uploads/2020/03/Cruise-Report-SKQ201915S_v3.pdf">https://nga.lternet.edu/wp-content/uploads/2020/03/Cruise-Report-SKQ201915S_v3.pdf</a>
<b>Start Date</b>	2019-06-29
<b>End Date</b>	2019-07-18
<b>Description</b>	Northern Gulf of Alaska Long-Term Ecological Research (NGA-LTER) See more cruise details on R2R <a href="https://www.rvdata.us/search/cruise/SKQ201915S">https://www.rvdata.us/search/cruise/SKQ201915S</a>

### TXF19

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

## 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
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-1459235</a>
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-1756767</a>
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-1756859</a>

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