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

Website: https://www.bco-dmo.org/dataset/907880
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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Abstract
Morphometric data on Neocalanus flemingeri females were made on individuals collected in diapause in June and September, 2019 and after incubation in the laboratory for up to 4.5 weeks. Collections were made from depth in Prince William Sound in the Gulf of Alaska during two NGA LTER cruises. After sorting, females were incubated in flasks at 4-6ºC for up to 4.5 weeks. Individuals were removed for imaging and experimental incubations to study oocyte production. Morphometric analysis was used to measure prosome length and area as well as lipid sac area for lipid fullness and lipid volume calculations. Lipid consumption during diapause was minimal and modest initially.

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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 m2 mouth area; 150 µm mesh nets) towed vertically from near the bottom to the 163 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 emperatures 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 (see related dataset https://www.bco-dmo.org/dataset/908514).

Experimental design

For the timeline, sets of females were removed from the incubation flasks for imaging and/or incubations in low concentrations of 5-Ethynyl-2'-deoxyuridine (EdU). Individuals that were imaged as well as incubated in EdU are identified in the dataset and provide identifications used in the related dataset.

Data Processing Description

Lipid sac and prosome length imaging

A total of 204 females were imaged for body measurements, with 31 of these females being also part of EdU experiments. Live females were placed in a chilled embryo dish with a small drop of seawater. Females were imaged laterally at 32x magnification using a Leica MZ16 microscope equipped with a 12 MPx Spot Insight camera. Using ImageJ light images were analyzed manually for three measurements: prosome length in mm, area of the lipid sac in mm2, and area of the prosome in mm2. Prosome length was measured by placing a line from the anterior to posterior tip of the prosome, measurements were rounded to the nearest 0.1 mm. Lipid sac and prosome area were measured by outlining their perimeters. Total lipid content in milligrams (mg) was estimated using the area of the lipid sac (equation: TL=0.197A1.38, where A is the lipid sac area and TL is total lipid). This relationship was established by comparing lateral images of the lipid sac to gas-chromatographic lipid measurements of three *Calanus* spp. that generated an equation that used lipid sac area as a proxy for lipid content. Measured areas of the lipid sac and prosome were also used to compute a lipid fullness percentage (lipid sac area/prosome sac area × 100).

BCO-DMO Processing Description

Processing steps to create the final dataset named "907880_v1_morphometric_measurements_and_lipid_calculations.csv" using the BCO-DMO data processor laminar.

1. Loaded the submitted file of body measurements named body_measurements_nflem_2019.csv into laminar.
2. Renamed fields to follow BCO-DMO parameter naming conventions by replacing spaces with underscores and removing punctuation marks and parenthesis.
3. Removed units in the parameter names which will be noted in parameter definitions on the dataset page.
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.
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 '907880_v1_morphometric_measurements_and_lipid_calculations.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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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

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. Nature Methods, 9(7), 671–675. https://doi.org/10.1038/nmeth.2089
Software

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Related Datasets

IsRelatedTo

Lenz, P. H., Hartline, D. K., Monell, K. J. (2024) **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.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-06-13 http://lod.bco-dmo.org/id/dataset/908514 [view at BCO-DMO]
Relationship Description: Study of the formation of oocytes in post-diapause Neocalanus flemingeri females during which morphological changes and female size and lipid sac area measurements were made. 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 name 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	Species of organism	unitless
Sex	Sex of organism	unitless
EdU_sample_ID_number	Sample tube identification. If female was used in EdU experiments, then her sample ID number is listed	unitless
Used_in_EdU_experiments	Yes or no question asking if female with a light microscope image was also used for EdU incubations	unitless
Length_of_EdU_incubation_in_hours	Length in hours of EdU incubation	hours
Image_number_light_microscope	images were taken in a series; each image was given an identification number	unitless
Date_image_was_taken	date when light microscope image was taken to be used for body size measurements	unitless
Time_point	experimental time point written out as hours or weeks; experiment start for body measurements was when females were collected	hours or weeks
Time_point_in_days	experimental time point written out as days after females were collected	days
Prosome_length	measured prosome length of each female	millimeters (mm)
Prosome_area	measured prosome area of each female	square millimeters (mm^2)
Lipid_area	measured lipid sac area of each female	square millimeters (mm^2)
Lipid_volume	lipid volume calculated as described in methods	milligrams (mg)
Lipid_fullness_percentage	lipid fullness percentage of each female; lipid fullness was measured by dividing lipid area by prosome area and multiplying by100	percent (%)

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Instruments

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

Dataset-specific Instrument Name	Leica MZ16 microscope
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
Generic Instrument Name	MultiNet
Dataset-specific Description	Mesh nets with 0.25 m2 mouth area and 150 µm mesh
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.

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Deployments

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

SKQ201915S

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

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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-1756767
NSF Division of Ocean Sciences (NSF OCE)	OCE-1756859

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