Results from Calanus pacificus Acidification Laboratory Experiments from 2019-2020 (Zooplankton Swimming project)

Website: https://www.bco-dmo.org/dataset/926368
Data Type: experimental
Version: 1
Version Date: 2024-04-30

Project
» Causes and consequences of hypoxia and pH impacts on zooplankton: Linking movement behavior to vertical distribution. (Zooplankton Swimming)

Contributors	Affiliation	Role
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Abstract
We conducted laboratory experiments using stratified 1 m x 0.1 × 0.1 acrylic water columns to measure the effects of bottom water hypoxia and low pH on mortality, distribution, and swimming behaviors of the calanoid copepod Calanus pacificus. Calanus pacificus were collected from within Puget Sound, Washington, USA, between June and October of 2019 and 2020. Their behaviors and vertical distributions in response to either hypoxic or acidified bottom waters were observed in an array of 4 replicate columns. Swimming behavior was observed for 90 minutes using 5-megapixel IR USB cameras. Two front-facing cameras (‘bottom camera’, ‘surface camera’) recorded swimming in the X (left, right) and Z (up, down) directions. An upwards-facing ‘base camera’ was added to each tank in 2020 to improve tracking and behavioral analysis of copepods near the bottom. Base cameras recorded the bottom 2 cm of each tank in the X and Y (front, back) directions. The primary data file of this dataset contains collection and organism details for copepods used in acidification experiments (926368_v1_zooplankton_acidification_pH_experiment_overview.csv). This dataset also includes four Supplemental Files .csv files summarizing results from our acidification experiments (926368_v1_zooplankton_acidification_pH_chemistry.csv, 926368_v1_zooplankton_acidification_pH_moribundity.csv, 926368_v1_zooplankton_acidification_pH_mean_height.csv, 926368_v1_zooplankton_acidification_pH_speed.csv).

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Coverage

Location: University of Washington, Seattle, Washington, USA
Spatial Extent: **N**:48.023629 **E**:-122.313681 **S**:47.27327 **W**:-123.1076517

Temporal Extent: 2019-08 - 2020-10

Dataset Description

The latitude and longitude coordinates of this dataset represent the collection sites where zooplankton were collected for experiments that later took place in an onshore laboratory at the University of Washington. The time bounds of this dataset represent the dates experiments were conducted (from the Experiment_Date column), not the dates of zooplankton collection (Collection_Date column).

Methods & Sampling

Sample Collection

Zooplankton samples were collected using either a 60 cm diameter, 200 µm mesh ring net with a non-filtering cod- end or a 60 cm diameter, 335 µm mesh bongo net with non-filtering codends, lifted vertically from 10 m off the seafloor. Samples were stored in a cooler and air-bubbled for <24 h until actively swimming adult female *C. pacificus* were manually sorted under a microscope into 1 liter jars filled with 200 µm filtered seawater. The copepods were kept at 14°C and fed a pre-made mixture of five marine microalgae (*Isochrysis, Pavlova, Tetraselmis, Thalassiosira weissflogii* and *T. pseudonana*) daily, for no more than 2 wk until they were used in a single laboratory experiment.

Experiment Details

Water treatments of 2 different salinities (29 and 31) were made using Instant Ocean (~36 and ~39 g l^{-1}) and verified with a YSI Pro 2030 salinity probe. Replicate 2-layer water columns with stable haloclines were created by pumping light (low salinity) water into the bottom of the tanks using a peristaltic pump until the water level was 500 mm from the bottom. Then, heavy (high salinity) water was pumped slowly to avoid mixing into the bottom, displacing the light water up- wards until the water columns were 780 mm deep with haloclines located 280 mm above the bottom.

To manipulate pH conditions, bottom water was split into 2 buckets of equal volume to use in treatment and control tanks. Treatment bottom water was bubbled with CO2 until it reached the desired pH. In the first 5 pH experiments, treatment bottom water was bubbled with a 2000 ppm CO2−air mixture for approximately 40 min. The final pH in this treatment was approximately 7.6, as measured by a Star A221 pH meter with a Ross ultra gel pH/ATC electrode that was calibrated daily prior to use. In the last 5 experiments, treatment bottom water was made using a feedback-control system that supplied mixed lab air and pure CO2 gas at 3000 ppm at 4.1 l min^{-1} through an airstone. The pH was measured with a Sunburst AFT (assumed constant salinity of 31) during bubbling to ensure a pH of 7.4 was maintained.

C. pacificus behaviors and vertical distributions in response to either hypoxic or acidic bottom waters were observed in an array of 4 replicate 1 × 0.1 × 0.1 m acrylic tanks, installed in an environmental chamber set to 14°C. Stressful water layers (or non-stressful controls) were placed at the bottoms of salinity-stratified tanks, modeled after conditions experienced in the field. To start each experiment, 20 animals were gently introduced to the top of each of the 4 tanks. Swimming behavior was then observed for 90 min using 5-megapixel IR USB cameras. Experiments were run during the day in the dark, with the tanks backlit with IR LED strips behind and around the base of each tank. Two front-facing cameras ('bottom camera', 'surface camera') recorded swimming in the X (left, right) and Z (up, down) directions, observing true vertical motion and projected horizontal motion. An upwards-facing 'base camera' was added to each tank in 2020 to improve tracking and behavioral analysis of copepods near the bottom. Base cameras recorded the bottom 2 cm of each tank in the X and Y (front, back) directions and therefore observed true horizontal motion but not vertical motion.

Video output details

Videos were processed with the software Fosica (Wallingford Imaging) to distinguish moving copepods from stationary background and noise and to extract copepod pixel coordinates. Pixel coordinates were converted into physical space units and then assembled into individual swimming paths using the Matlab software package Tracker3D (Chan & Grünbaum 2010), neglecting parallax in the camera field of view. A smoothing spline was applied to remove features changing faster than 6 Hz, which were dominated by frame rate noise. X and Z (or, for the base cameras, X and Y) pixel coordinates for each object and the total projected speed and velocities were calculated at every frame for each swimming path. Swimming paths included only animals actively moving in the tank, excluding motionless (moribund) animals at the bottom of the tank. Copepod swimming paths were used to calculate the mean height from the bottom of the tank, mean number of copepod localizations per frame, and mean swimming speeds.

Our video system could not distinguish between individuals that were dead and those that were lying immobilized on the bottom for extended periods (a behavior leading, at least in hypoxia, to a high likelihood of eventual mortality). Therefore, for 2020 experiments, we developed a video-based metric using the base cameras to classify copepods at the bottom of tanks that were 'moribund.' Remaining motionless on the bottom of the tank is an uncommon behavior for *C. pacificus*, and we conservatively estimated that copepods motionless for a 2 minute threshold were in a 'moribund' or stressed state. To quantify moribundity, we calculated the mean brightness values for each pixel from frames in each of the last 1 minute sections (89th and 90th minutes). Because the videos were recorded in a dark field with IR back-lighting, copepods appeared as bright spots in the videos. The brightness of pixels representing a copepod in these 1 min means was a direct function of the number of frames in which it remained stationary. We then used a brightness threshold to classify copepods as moribund if pixel brightness indicated they had not moved during the last 2 minute of video observations.

More details on the methodology can be found here: Wyeth, A.C., Grünbaum D., Keister J.E. (2022). Effects of hypoxia and acidification on Calanus pacificus: behavioral changes in response to stressful environments. Marine Ecology Progress Series, 697: 15-29. https://doi.org/10.3354/meps14142.

Data Processing Description

Videos were processed with the software Fosica (Wallingford Imaging) to distinguish moving copepods from stationary background and noise and to extract copepod pixel coordinates. Pixel coordinates were converted into physical space units and then assembled into individual swimming paths using the Matlab software package Tracker3D (Chan & Grünbaum 2010).

BCO-DMO Processing Description

- Originally, this data and related Zooplankton Acidification Lab Result Data (see related datasets section of this metadata page) were contained in the same .csv file, these data from separate but related experiments were parsed out and served separately through BCO-DMO to describe and represent data and experiments more accurately
- Column names across the Supplemental data tables and primary data table were made the same across all files
- Blank spaces in column names replaced with underscores ("_")
- Date columns within the data file (Experiment_Date, Collection_Date, and Sort_Date) were converted from %m%d%y format to %Y-%M-%D format
- Replaced NA missing data values in the dataset with blank values ("")
- Column name weight.avg changed to mean_height
- Column name camera changed to camera_ID
- Special characters in column names changed to underscores ("_")
- Latitude and longitude values rounded to 6 degrees of precision

Problem Description

In 10 of the 23 experiments conducted in 2020, one of the 4 base cameras malfunctioned (for an equal number of experimental and control tanks across the 10 runs). In statistical analyses that required base camera video, those individual tanks were dropped.

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Data Files

File
926368_v1_zooplankton_acidification_ph_experiment_overview.csv (Comma Separated Values (.csv), 1.53 KB) MD5:05103fa15bcbd1f9906fc1490592deed Primary data file for dataset ID 926368, version 1

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Supplemental Files

File
926368_v1_acidification_pH_chemistry.csv (Comma Separated Values (.csv), 3.10 KB) MD5:5bc4ffc2302138ed63814991ac234a7a Chemistry results from pH experiments (original filename: pH_chemistry.csv).
926368_v1_zooplankton_acidification_pH_mean_height.csv (Comma Separated Values (.csv), 9.20 KB) MD5:851067e39fc8dcb4efcf3e6ceeafef21 Mean height results from pH experiments (original filename: pH_mean_height.csv).
926368_v1_zooplankton_acidification_pH_morbundity.csv (Comma Separated Values (.csv), 958 bytes) MD5:c630413e426ff1b4775fae71ac73d8c4 Morbundity results from pH experiments (original filename: pH_morbundity.csv).
926368_v1_zooplankton_acidification_pH_speed.csv (Comma Separated Values (.csv), 39.39 KB) MD5:9faffd071b1ed85df252d1341c59dc96 Speed results from pH experiments (original filename: pH_speed.csv).

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Related Publications

Chan, K., & Grünbaum, D. (2010). Temperature and diet modified swimming behaviors of larval sand dollar. Marine Ecology Progress Series, 415, 49–59. https://doi.org/10.3354/meps08744
Methods

Wyeth, A., Grünbaum, D., & Keister, J. (2022). Effects of hypoxia and acidification on Calanus pacificus: behavioral changes in response to stressful environments. Marine Ecology Progress Series, 697, 15–29. https://doi.org/10.3354/meps14142
Results

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Related Datasets

IsRelatedTo

Keister, J. E., Grunbaum, D. (2024) **Results from Calanus pacificus Hypoxia Laboratory Experiments from 2019-2020 (Zooplankton Swimming project).** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-04-30 doi:10.26008/1912/bco-dmo.926332.1 [view at BCO-DMO]
Relationship Description: Data from additional laboratory experiments that were conducted ahead of field operations to provide behavioral context to in-situ zooplankton responses to environmental stressors from the same project (Zooplankton Swimming project).

Keister, J. E., Grunbaum, D., Roberts, P. (2024) **In Situ Amphipod and Copepod Video Output Captured**

by the Hoodsport ORCA Profiling Mooring Mounted SPC-2 Zoocam in the Hood Canal, Puget Sound, Washington from August to September 2018 (Zooplankton Swimming project). Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-05-21 doi:10.26008/1912/bco-dmo.928222.1 [view at BCO-DMO]
Relationship Description: The field operations represented in this related dataset were conducted after these laboratory experiments. The data from associated lab experiments provide behavioral context to in-situ zooplankton responses to environmental stressors.

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Parameters

Parameter	Description	Units
Experiment_Date	Date when the experiment took place.	unitless
Collection_Date	Date zooplankton were collected from Puget Sound.	unitless
Sort_Date	Date zooplankton were sorted under the microscope and moved into artificial seawater.	unitless
Collector	Brief details highlighting who conducted the live zooplankton tow.	unitless
Collection_Site	Location of zooplankton tow.	unitless
Lifestage	Lifestage of sorted Calanus pacificus (all are adult).	unitless
Sex	Sex of sorted Calanus pacificus (all are female).	unitless
Collection_Lat	Latitude of zooplankton tow in decimal degrees; a positive value indicates a Northern coordinate.	decimal degrees
Collection_Long	Longitude of zooplankton tow in decimal degrees; a negative value indicates a Western coordinate.	decimal degrees
Experiment_Type	Indicates the experiment type; can be used to determine differences between experiment types if merged with related BCO-DMO dataset ZooplanktonHypoxia Lab Results (#926332).	unitless
Collection_Notes	Field notes on zooplankton collection including time of day, tow depth, and net type.	unitless
Experiment_Notes	Important experimental notes (if applicable).	unitless

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Instruments

Dataset-specific Instrument Name	Star A221 pH meter with a Ross ultra gel pH/ATC electrode
Generic Instrument Name	Benchtop pH Meter
Dataset-specific Description	Treatment pH conditions were measured as a part of experiment set-up by a Star A221 pH meter with a Ross ultra gel pH/ATC electrode that was calibrated daily prior to use.
Generic Instrument Description	An instrument consisting of an electronic voltmeter and pH-responsive electrode that gives a direct conversion of voltage differences to differences of pH at the measurement temperature. (McGraw-Hill Dictionary of Scientific and Technical Terms) This instrument does not map to the NERC instrument vocabulary term for 'pH Sensor' which measures values in the water column. Benchtop models are typically employed for stationary lab applications.

Dataset-specific Instrument Name	IR USB Camera
Generic Instrument Name	Camera
Dataset-specific Description	Copepod Swimming behavior was then observed for 90 min periods using 5-megapixel Infrared (IR) USB cameras with 8 mm wide angle lenses.
Generic Instrument Description	All types of photographic equipment including stills, video, film and digital systems.

Dataset-specific Instrument Name	YSI Pro 2030 Salinity Probe
Generic Instrument Name	Salinity Sensor
Dataset-specific Description	Water treatments of 2 different salinities (29 and 31) were made using Instant Ocean (~36 and ~39 g l−1) and verified with a YSI Pro 2030 salinity probe.
Generic Instrument Description	Category of instrument that simultaneously measures electrical conductivity and temperature in the water column to provide temperature and salinity data.

Dataset-specific Instrument Name	Sunburst AFT
Generic Instrument Name	Sunburst Autonomous Flow Through Instrument pH
Dataset-specific Description	Within experimental tanks while treatment bottom water was bubbled, pH was measured with a Sunburst AFT (with an assumed constant salinity of 31) to ensure a pH of 7.4 was maintained.
Generic Instrument Description	The Autonomous Flow Through Instrument pH (AFT-pH) measures pH in the 7-9 range (salinity 25-40), designed to accurately measure pH of flowing seawater or individual samples, packaged to be plumbed into a sea line on a research vessel or can be used for bottle samples in the lab, and has accuracy and precision of: +/- 0.003 pH units and < 0.001 pH units. More information from Sunburst Sensors: http://www.sunburstsensors.com/products/oceanographic-ph-sensor-benchtop...

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Project Information

Causes and consequences of hypoxia and pH impacts on zooplankton: Linking movement behavior to vertical distribution. (Zooplankton Swimming)

Coverage: Puget Sound, WA

NSF Award Abstract:
Low oxygen (hypoxia) and low pH are known to have profound physiological effects on zooplankton, the microscopic animals of the sea. It is likely that many individual zooplankton change vertical mirgration behaviors to reduce or avoid these stresses. However, avoidance responses and their consequences for zooplankton distributions, and for interactions of zooplankton with their predators and prey, are poorly understood. This study will provide information on small-scale behavioral responses of zooplankton to oxygen and pH using video systems deployed in the field in a seasonally hypoxic estuary. The results will deepen our understanding of how zooplankton respond to low oxygen and pH conditions in ways that could profoundly affect marine ecosystems and fisheries through changes in their populations and distributions. This project will train graduate students and will engage K-12 students and teachers in under-served coastal communities by developing ocean technology-based citizen-scientist activities and curricular materials in plankton ecology, ocean change, construction and use of biological sensors, and quantitative analysis of environmental data.

Individual directional motility is a primary mechanism underlying spatio-temporal patterns in zooplankton population distributions. Motility is used by most zooplankton species to select among water column positions that differ in biotic and abiotic variables such as prey, predators, light, oxygen concentration, and pH. Species-specific movement responses to de-oxygenation and acidification are likely mechanisms through which short-term, localized impacts of these stressful conditions on individual zooplankton will be magnified or suppressed as they propagate up to population, community, and ecosystem-level dynamics. This study will quantify responses by key zooplankton species to oxygen and pH using in situ video systems to measure changes in individual behavior in hypoxic, low- pH versus well-oxygenated, high-pH regions of a seasonally hypoxic estuary. Distributions and movements of zooplankton will be quantified using three approaches: 1) an imaging system deployed in situ on a profiling mooring over two summers in a hypoxic region, 2) imagers deployed on Lagrangian drifters to sample simultaneously throughout the water column, and 3) vertically-stratified pumps and net tows to verify species identification and video-based abundance estimates. These field observations will be combined with laboratory analysis of zooplankton movements in oxygen and pH gradients, and with spatially-explicit models to predict how behavioral mechanisms lead to large-scale impacts of environmental stresses.

The following deployments were conducted in 2017 and 2018:
CB1077: https://www.bco-dmo.org/deployment/735746
CB1072: https://www.bco-dmo.org/deployment/735748
Zoocam_ORCA_Twanoh_2017: https://www.bco-dmo.org/deployment/735762
RC0008: https://www.bco-dmo.org/deployment/775288
Mooring ORCA_Hoodsport; NANOOS-APL4: https://www.bco-dmo.org/deployment/775291

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1657992

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