Photobehavior for octopus ('Octopus bimaculatus') and squid ('Doryteuthis opalescens') paralarvae during exposure to 9 light irradiance levels and 4 oxygen conditions in trials conducted in April of 2019

Website: https://www.bco-dmo.org/dataset/835968 Data Type: experimental Version: 1 Version Date: 2021-08-30

Project

» <u>Vision-mediated influence of low oxygen on the physiology and ecology of marine larvae</u> (Vision under hypoxia)

Contributors	Affiliation	Role
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Abstract

This dataset gives the results of photobehavior experiments under different oxygen conditions in larvae of the market squid ('Doryteuthis opalescens') and two-spot octopus ('Octopus bimaculatus'). Photobehavior experiments were conducted to determine whether the impairment of visual physiology observed in marine invertebrate larvae (McCormick et al., 2019) is subsequently affecting visual behavior in marine larvae.

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Coverage

Temporal Extent: 2019-04

Methods & Sampling

This dataset gives the results of photobehavior experiments under different oxygen conditions in larvae of the market squid (Doryteuthis opalescens) and two-spot octopus (Octopus bimaculatus). Photobehavior experiments were conducted to determine whether the impairment of visual physiology observed in marine invertebrate larvae (McCormick et al., 2019) is subsequently affecting visual behavior in marine larvae.

Briefly, animals were placed in a 20cmx4cmx5cm clear plexiglass chamber filled with seawater to 16cm inside a light-tight box with an overhead light stimulus. After animals were placed in the chamber, the oxygen and temperature were measured, and then a 15-minute dark adaptation period was started. The experiment consisted of a series of a 10-s light stimulus followed by 3-minute dark period; this was repeated at 9 different irradiance levels (1= lowest, 9= highest; 0.062-2.45 mol photons/m^2/s) and at 4 different oxygen levels.

Oxygen levels were selected based on the 3 visual metrics that were calculated to quantify the effects of reduced pO2 on retinal function (McCormick et al., 2019). V90, V50, and V10 were calculated for each individual larva as the oxygen where there was 90%, 50%, and 10% retinal function remaining, in respect to retinal responses in normoxia (surface-ocean oxygen levels). For squid, oxygen levels were 1) Normoxia (21.1 kPa); 2) V50 (12.9 kPa); 3) V10 (6.6 kPa); and 4) Low (4.2 kPa). For octopus, oxygen levels were 1) Normoxia (21.3 kPa); 2) V90 (10.7 kPa); 3) V50 (7.4 kPa); and 4) V10 (5.8 kPa). All experiments were video recorded and analyzed using Noldus EthoVision XT software (version 15).

Oxygen and temperature were measured using a Microx4 (PreSens) oxygen meter and a Pst-7 oxygen optode probe.

Data Processing Description

All experiments were video recorded using Bonsai and later analyzed using Noldus EthoVision XT software (version 15). Analysis of videos allowed for 16 individuals to be tracked at once. Vertical position data is in mm from +80mm which is the surface of the water (closest to the light stimulus) and -80 which is the bottom of the chamber; 0 indicates the center of the chamber. Oxygen data was analyzed using PreSens Measurement Studio 2. Post-processing analysis was completed in R Studio (version 3.3.3).

BCO-DMO Data Manager Processing Notes (v1, 2021-08-30):

* Data from files VisualBehavior_Dopalescens_Vision3.txt and VisualBehavior_Obimaculatus_Vision3.txt imported in the BCO-DMO data system and combined into one data table.

* Data files imported with missing data identifier "NA". The missing data value served from this page will depend upon the data format (e.g. Matlab files will have missing data values as NaN).

* column 30s_bin changed to "bin_30s" to conform with BCO-DMO naming convention (can't start with a number)

* Files were originally submitted and archived as version 1 on 2021-03-16 but were later revised and resubmitted to BCO-DMO while the dataset was still embargoed. The final version 1 is version date is 2021-08-30 for the archvied version of this dataset (DOI: 10.26008/1912/bco-dmo.835968.1).

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Data Files

File
Details for all squid and octopus experimental trials for photobehavior experiments filename: VisualBehavior_ExperimentalTrials2.txt MD5:b9209fd59ccccd6a797b5cc8f88b037
Experimental conditions (age of larvae, pH, temperature, oxygen conditions) for all photobehavior experiments for both squid ('Doryteuthis opalescens') and octopus ('Octopus bimaculatus') paralarvae during exposure to 9 light irradiance levels in 4 different oxygen conditions.
Parameters:
"Species": In form Genus_species
"Oxygen_level": Experimental oxygen condition (see explanation below)
"Age_mean": Mean age of all paralarvae in the chamber on the day of testing
"Age_stdev": Standard deviation of the age of paralarvae in the chamber
"pO2_mean": Mean partial pressure of oxygen (pO2; in kPa) in the chamber
"pO2_stdev": Standard deviation of partial pressure of oxygen (pO2; in kPa) in the chamber
"Temp_mean": Mean temperature (deg. Celcius) in the chamber during experiment
"Temp_stdev": Standard deviation of temperature (deg. Celsius) in the chamber during experiment
"pH_mean": Mean pH in the chamber during experiment
"pH_stdev": Standard deviation of pH in the chamber during experiment

Raw output from analysis of videos of photobehavior for larvae of both Doryteuthis opalescens and Octopus bimaculatus paralarvae

filename: ceph_trial_raw_analysis_files.tar.gz

(GZIP (.gz), 2.27 GB) MD5:de8a745eeea63d165ccceab818a9c24b

This zip file contains are raw output files in Excel .xlsx format from analyzed videos of photobehavior for larvae of both Doryteuthis opalescens and Octopus bimaculatus using the Ethovision XT (v15) software. Experiments were conducted to test the photobehavior to 9 different irradiance stimuli. Videos begin with a 3-minute period of darkness, followed by a 10-s light stimulus and a 3-minute period of darkness repeated for each stimuli (x9). The trials end with a 2-minute period of darkness, for a total of 30 minutes. Files include raw data from every video frame. Experiments were conducted for 4-different oxygen levels for each species.

Files are split up by species, trial, and oxygen exposure. Oxygen exposures are normoxia (Ctrl), V90, V50, V10, or Low (more details in metadata form). The file names indicate the data, for example: "Raw data-Squid24_V50_New16.xlsx" indicates that this is Squid (D. opalescens) trial 24, measuring the V50 oxygen level. "New16" refers to a more recent analysis, and the number of individuals tracked in each trial. These files also show other metrics calculated, in addition to the vertical position (analyzed here in the main dataset). For the octopus trials, the file naming is the same: "Raw data-Obimac5_Ctrl_New6.xslx" indicates this is Octopus trial 5 (Obimac5), for the normoxia oxygen level (Ctrl), with 6 individuals tested (_New6_).

The datafiles are arranged with tabs for each individual larva that was tracked (e.g., Subject01, Subject02, etc.). Each tab is arranged in the exact same format. Files have 33 lines of header data, including the analysis date for the file. The main dataset has 42 columns, immediately below the column label is the unit of measurement (where applicable).

* Trial Time: Raw video time in seconds

Recording time: Time from 0 the file started the analysis in seconds

- * X Center: X position of the tracked object in mm
- * Y Center: Y position of the tracked object in mm
- * Area: Area of the tracked object in mm²
- * Areachange: Change in area of the tracked object in mm^2
- * Elongation: NA not useable for this analysis
- * Distance moved: The distance the target moved since the previous frame in mm
- * Velocity: The velocity of the target (in mm/s)
- * S1, S2, S3, S4, S5: Total count of individuals tracked in 5 even zones of the chamber. (S1 is the top and S5 is the bottom of the chamber).
- * In Zone: Which of the 5 zones (from above) the individual currently being tracked is in
- * Distance to point (Arena(Center/Center-point): Distance of the target to the center of the chamber in mm
- * Heading: Heading of the object in degrees (values >90 indicate heading to the left of the Y axis, < 90 indicates to the right of the Y axis, positive values are above X axis, negative values are below X axis)
- * Heading to point: Heading of the object (as above) to the center of the chamber in degrees
- * Acceleration: Acceleration in mm^2/s
- * Heading to point 2: Heading of the object (as above) to the center surface of the chamber in degrees
- * Distance to point (Surface /Center-point): Distance of the target to the surface of the chamber in mm

* The remaining columns represent the stimulus categories in their order of experiment (e.g., DkCtrl1, Light9, Dark9, Light8, Dark8, etc.). These values are binary: 1 indicates that stimulus is on/present, 0 indicates absence of the stimulus. For example, during the Light9 stimulus, there would be a 1 for that video frame; all other stimuli would show 0.

- ** DkCtrl1 is the 3-minute period of darkness before the experiment starts
- ** Light 9 Light 1: the 9, 10-second stimuli
- ** Dark 9-Dark 1: the 9, 3-minute dark period immediately after the light stimulus
- ** DkCtrl2 is the 2-minute period of darkness after the experiment ends
- ** Extra is the stimulus category for extra video that was recorded, but not part of the experiment

Octopus files are set up exactly the same, except they will have S1-S5 followed by a Bottom column. This shows the number of individuals on the bottom of the chamber.

File	
Video files of photobehavior in squid and octopus larvae filename: ceph_trial_videos.tar.gz	(GZ IP (.gz), 3.78 GB) MD5:9229e4bf4810e55163ae9dddc5a1c782
Video files for all photobehavior experiments. Experiments were conducted in larvae of squid ('Doryteuthis opa bimaculatus'). For a detailed description of experiments, please see Dataset Metadata Page https://www.bco-dr	lescens') and octopus ('Octopus no.org/dataset/835968.
Briefly, larvae were placed in the chamber, and after 3 minutes in darkness, exposed to 9, 10-second light stim by 3 minutes after each in darkness. The trial ends with a 2-minute period in darkness. This was completed for species [Normoxia ("Ctrl"), V50, V10, and Low for squid, and Normoxia, V90, V50, and V10 for octopus]. Oxygen found in VisualBehavior_ExperimentalTrials.txt. Files are named for the experiment type (Beh_), date (format YY Squid8, or Obimac1), and oxygen level. So for example, Beh_190419_Squid18_Ctrl_VID_conv.avi is the file for th on 4-19-2019. "Squid" refers to 'Doryteuthis opalescens' and "Obimac" refers to the octopus 'Octopus bimacula light, with the exception of the light stimulus, which was at 525 nm.	nuli of decreasing irradiance followed r 4 different oxygen levels per a and temperature for each trial is (MMDD), species and trial (e.g., ne Squid18 trial, tested in normoxia, atus'. All videos are recorded under IR
light, with the exception of the light stimulus, which was at 525 nm.	aus . An videos are recorded under IK

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NeuroGEARS. (n.d.). Bonsai. Retrieved March 18, 2021, from <u>https://bonsai-rx.org/</u> Software

Noldus Information Technology (n.d.). EthoVision XT - video tracking software (Version 15). Retrieved March 18, 2021, from https://www.noldus.com/ethovision-xt/ Software

PreSens Measurement Studio 2. (n.d.). Retrieved March 18, 2021, from <u>https://www.presens.de/products/detail/presens-measurement-studio-2</u> Software

R Core Team (2017). R: A language and environment for statistical computing. R v3.3.3. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/ Software

RStudio Team (2019). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA. URL <u>http://www.rstudio.com/</u>. *Software*

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Parameters

Parameter	Description	Units
Trial	Reference to the specific trial the data are obtained from. Format is SquidX_O2, where X is the trial number and O2 is the oxygen condition (see methodology for more details). "Squid" refers to 'Doryteuthis opalescens'. "Obimac" refers to 'Octopus bimaculatus'.	unitless
02	Oxygen level for the given trial (see methodology for details)	unitless
Time_Bin	Time of the experiment in seconds. Data are binned for every second.	seconds
Y_All1	Vertical position data in mm for larva number 1 of the 16 tracked squid for the entire chamber (or 5-6 tracked octopus larvae, see Trial name). See methodology for explanation of analysis methods. Blank cells indicate the larva was not being tracked during that time. Vertical position is between -80 (bottom of the chamber) to 80 (top of the chamber). 0 is the center of the chamber.	unitless

Y_All2	Vertical position data in mm for larva number 2 of the 16 tracked squid for the entire chamber (or 5-6 tracked octopus larvae, see Trial name). See methodology for explanation of analysis methods. Blank cells indicate the larva was not being tracked during that time. Vertical position is between -80 (bottom of the chamber) to 80 (top of the chamber). 0 is the center of the chamber.	unitless
Y_All3	Vertical position data in mm for larva number 3 of the 16 tracked squid for the entire chamber (or 5-6 tracked octopus larvae, see Trial name). See methodology for explanation of analysis methods. Blank cells indicate the larva was not being tracked during that time. Vertical position is between -80 (bottom of the chamber) to 80 (top of the chamber). 0 is the center of the chamber.	unitless
Y_A114	Vertical position data in mm for larva number 4 of the 16 tracked squid for the entire chamber (or 5-6 tracked octopus larvae, see Trial name). See methodology for explanation of analysis methods. Blank cells indicate the larva was not being tracked during that time. Vertical position is between -80 (bottom of the chamber) to 80 (top of the chamber). 0 is the center of the chamber.	unitless
Y_All5	Vertical position data in mm for larva number 5 of the 16 tracked squid for the entire chamber (or 5-6 tracked octopus larvae, see Trial name). See methodology for explanation of analysis methods. Blank cells indicate the larva was not being tracked during that time. Vertical position is between -80 (bottom of the chamber) to 80 (top of the chamber). 0 is the center of the chamber.	unitless
Y_All6	Vertical position data in mm for larva number 6 of the 16 tracked squid for the entire chamber (or 5-6 tracked octopus larvae, see Trial name). See methodology for explanation of analysis methods. Blank cells indicate the larva was not being tracked during that time. Vertical position is between -80 (bottom of the chamber) to 80 (top of the chamber). 0 is the center of the chamber.	unitless
Y_A 7	Vertical position data in mm for larva number 7 of the 16 tracked squid for the entire chamber (or 5-6 tracked octopus larvae, see Trial name). See methodology for explanation of analysis methods. Blank cells indicate the larva was not being tracked during that time. Vertical position is between -80 (bottom of the chamber) to 80 (top of the chamber). 0 is the center of the chamber.	unitless
Y_All8	Vertical position data in mm for larva number 8 of the 16 tracked squid for the entire chamber (or 5-6 tracked octopus larvae, see Trial name). See methodology for explanation of analysis methods. Blank cells indicate the larva was not being tracked during that time. Vertical position is between -80 (bottom of the chamber) to 80 (top of the chamber). 0 is the center of the chamber.	unitless
Y_A119	Vertical position data in mm for larva number 9 of the 16 tracked squid for the entire chamber (or 5-6 tracked octopus larvae, see Trial name). See methodology for explanation of analysis methods. Blank cells indicate the larva was not being tracked during that time. Vertical position is between -80 (bottom of the chamber) to 80 (top of the chamber). 0 is the center of the chamber.	unitless
Y_A 10	Vertical position data in mm for larva number 10 of the 16 tracked squid for the entire chamber (or 5-6 tracked octopus larvae, see Trial name). See methodology for explanation of analysis methods. Blank cells indicate the larva was not being tracked during that time. Vertical position is between -80 (bottom of the chamber) to 80 (top of the chamber). 0 is the center of the chamber.	unitless

Y_All11	Vertical position data in mm for larva number 11 of the 16 tracked squid for the entire chamber (or 5-6 tracked octopus larvae, see Trial name). See methodology for explanation of analysis methods. Blank cells indicate the larva was not being tracked during that time. Vertical position is between -80 (bottom of the chamber) to 80 (top of the chamber). 0 is the center of the chamber.	unitless
Y_All12	Vertical position data in mm for larva number 12 of the 16 tracked squid for the entire chamber (or 5-6 tracked octopus larvae, see Trial name). See methodology for explanation of analysis methods. Blank cells indicate the larva was not being tracked during that time. Vertical position is between -80 (bottom of the chamber) to 80 (top of the chamber). 0 is the center of the chamber.	unitless
Y_A 13	Vertical position data in mm for larva number 13 of the 16 tracked squid for the entire chamber (or 5-6 tracked octopus larvae, see Trial name). See methodology for explanation of analysis methods. Blank cells indicate the larva was not being tracked during that time. Vertical position is between -80 (bottom of the chamber) to 80 (top of the chamber). 0 is the center of the chamber.	unitless
Y_A 14	Vertical position data in mm for larva number 14 of the 16 tracked squid for the entire chamber (or 5-6 tracked octopus larvae, see Trial name). See methodology for explanation of analysis methods. Blank cells indicate the larva was not being tracked during that time. Vertical position is between -80 (bottom of the chamber) to 80 (top of the chamber). 0 is the center of the chamber.	unitless
Y_A 15	Vertical position data in mm for larva number 15 of the 16 tracked squid for the entire chamber (or 5-6 tracked octopus larvae, see Trial name). See methodology for explanation of analysis methods. Blank cells indicate the larva was not being tracked during that time. Vertical position is between -80 (bottom of the chamber) to 80 (top of the chamber). 0 is the center of the chamber.	unitless
Y_All16	Vertical position data in mm for larva number 16 of the 16 tracked squid for the entire chamber (or 5-6 tracked octopus larvae, see Trial name). See methodology for explanation of analysis methods. Blank cells indicate the larva was not being tracked during that time. Vertical position is between -80 (bottom of the chamber) to 80 (top of the chamber). 0 is the center of the chamber.	unitless
Y_mean	Mean vertical position for all individuals in the trial for each given time bin. Chamber positions are the same as above (range -80 to 80).	unitless
Stimulus	The experimental light stimulus the data are from. The stimulus will either be Light for during a light stimulus (1-9), or Dark for the dark period (no light) immediately after the light stimulus (1-9). DkCtrl1 and DkCtrl2 refer to the 2-3 minute periods of darkness before and after each experiment that serve as a control for normal swimming behavior.	unitless
bin_30s	The 30 second time bin of analysis before the termination of the light stimulus and 30 seconds (s) after the termination of the light stimulus. The 30 s before the light stimulus refers to 20 s in the darkness + the 10 s light stimulus. Values are similar to irradiance levels (e.g. 9.1 is the 30 s before the termination of Light9 and 8.9 is the 30 s after the termination of Light9 (see "Stimulus" description)	unitless
Irrad	Irradiance of the 9 experimental light stimuli. 0 refers to darkness.	mol photons/m^2/s

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Dataset-specific Instrument Name	Video Camera
Generic Instrument Name	Camera
Generic Instrument Description	All types of photographic equipment including stills, video, film and digital systems.

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Project Information

Vision-mediated influence of low oxygen on the physiology and ecology of marine larvae (Vision under hypoxia)

Coverage: Southern California Bight, Northeast Pacific Ocean

NSF abstract:

Oxygen is being lost in the ocean worldwide as a result of ocean warming and the input of nutrients from land. Vision requires a large amount of oxygen, and may be less effective or require more light when oxygen is in short supply. This is especially true for active marine animals with complex eyes and visual capabilities, including active arthropods (crabs), cephalopods (squid), and fish. The California coastal waters exhibit a sharp drop in oxygen and light with increasing water depth. This project examines how visual physiology and ecology in young (larval) highly visual marine animals respond to oxygen loss, with a focus on key fisheries and aquaculture species. Experiments and observations will test the hypothesis that oxygen stress will change the light required for these organisms to see effectively, influencing the water depths where they can live and survive. The project will provide interdisciplinary experiences to students and an early career scientist and inform both the public (through outreach at the Birch Aquarium at Scripps Institution of Oceanography) and policy makers about the effects of oxygen decline in the ocean.

Negative effects of oxygen loss on vision have been described for humans and other terrestrial organisms, but never in the marine environment, despite the large changes in oxygen that can occur with depth and over time in the ocean, and the high metabolic demand of visual systems. This project will test the effects of low oxygen on vision in 3 combinations of eye design and photo-transduction mechanisms: compound eye with rhabdomeric photoreceptors (arthropods), simple eye with rhabdomeric photoreceptors (cephalopods), and simple eye with ciliary photoreceptors (fish). A series of oxygen- and light-controlled laboratory experiments will be conducted on representative taxa of each group including the tuna crab, Pleuroncodes planipes; the market squid, Doryteuthis opalescens, and the white sea bass, Atractoscion nobilis. In vivo electrophysiology and behavioral phototaxis experiments will identify new oxygen metrics for visual physiology and function, and will be compared to metabolic thresholds determined in respiration experiments. Hydrographic data collected over 3 decades by the CalCOFI program in the Southern California Bight will be evaluated with respect to visual and metabolic limits to determine the consequences of oxygen variation on the critical luminoxyscape (range of oxygen and light conditions required for visual physiology and function in target species) boundary in each species. Findings for the three vision-based functional groups may test whether oxygen-limited visual responses offer an additional explanation for the shoaling of species distributions among highly visual pelagic taxa in low oxygen, and will help to focus future research efforts and better understand the stressors contributing to habitat compression with expanding oxygen loss in the ocean.

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.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1829623

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