[DRAFT] Physiological measurements from iron incubation experiment using upwelled waters in the California Current System during the PUPCYCLE II cruise in May and June 2023r]

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Data Type: Cruise Results, experimental
Version: 1
Version Date: 2026-03-12

Project
» CAREER: An integrated molecular and physiological approach to examining the dynamics of upwelled phytoplankton in current and changing oceans (Upwelled Phytoplankton Dynamics)

Contributors	Affiliation	Role
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Abstract
This dataset includes physiological measurements from an iron incubation experiment using upwelled waters sampled in the California Current System (CCS) during the PUPCYCLE II cruise with Chief Scientist Adrian Marchetti. PUPCYCLE II (Phytoplankton response to the UPwelling CYCLE) took place onboard the R/V Sally Ride from May 29th to June 10th, 2023. Seawater for the incubation experiment was collected within the northern CCS, off the southern coast of Oregon at 43°02'42.7"N, 124°33'07.2"W. The collected seawater was deemed as freshly upwelled water and taken from a depth of 55 m, then placed into designated cubitainers. Three cubitainers were immediately harvested for the initial timepoint (T0). The remaining twenty-seven cubitainers were assigned treatments: nine were unamended (Ctrl), nine were amended with 5 nM FeCl2 (Fe), and nine were amended with 200 nM Desferrioxamine B, a strong iron chelator that inhibits dissolved iron uptake (DFB). The cubitainers were placed in an on-deck incubator covered with neutral density screening to simulate 25% irradiance supplied with flow-through surface seawater to maintain ambient surface temperature. Three cubitainers from each treatment were harvested for each of the three subsequent timepoints: 48 hours (T1), 168 hours (T2), and 264 hours (T3) after incubation. Harvested measurements included dissolved nutrients, size-fractionated chlorophyll a, nitrate and carbon isotope uptake rates, flow cytometry, FlowCam imaging, fluorescently labelled particle incubation experiments, and RNA-seq. RNA-seq analyses conducted provided relative taxonomic compositions for each sample and multiple differential expression data frames for mixotrophs and diatoms between Fe and DFB treatments at T1 and T2. These datasets provide vital information on phytoplankton communities in the CCS in response to variable iron regimes and are crucial for metatranscriptomics studies aimed to understand mixotrophy versus phototrophy dynamics under nutrient limitation.

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Coverage

Location: California Current System, off the southern coast of Oregon at 43°02'42.7"N, 124°33'07.2"W, depth 55m
Spatial Extent: **N**:44.62701922 **E**:-123.867405 **S**:37.71706162 **W**:-125.492809
Temporal Extent: 2023-05-29 - 2023-06-10

Dataset Description

This dataset is one of many generated from an iron incubation experiment conducted as part of the PUPCYCLE II cruise in May and June 2023.

- Physiology
- Plankton Reads Taxonomy
  - Protist reads
  - Mixotroph read
  - Diatom reads
- RNA-Seq Diff Exp Mixotroph Pfam
- RNA-Seq Diff Exp Diatom Pfam
- RNA-Seq Differential Expression: KEGG Pathways
  - Mixotroph KEGG pathways
  - Diatom KEGG pathways

Methods & Sampling

Data collection took place on the R/V Sally Ride from May 29th to June 10th, 2023. To simulate upwelling conditions under different iron treatments, an onboard incubation experiment was conducted. Seawater for the incubation experiment was collected within the northern CCS, off the southern coast of Oregon at 43°02'42.7"N, 124°33'07.2"W. The collected seawater was deemed as freshly upwelled water and taken from a depth of 55 m – which corresponds to a depth slightly below the euphotic zone receiving less than 1% irradiance – using trace-metal clean techniques on May 30th, 2023, 13:30 GMT. The seawater was pumped and homogenized using trace metal clean techniques, then transferred into a total of thirty 20 L low-density polyethylene cubitainers. Three cubitainers were immediately harvested for the initial timepoint (T0). The remaining twenty-seven cubitainers were assigned treatments: nine were unamended (Ctrl), nine were amended with 5 nM FeCl2 (+Fe), and nine were amended with 200 nM desferrioxamine B, a strong iron chelator that inhibits dissolved iron uptake (DFB). The cubitainers were placed in an on-deck incubator covered with two layers of neutral density screening to simulate 26% of incidence irradiance supplied with flow-through surface seawater to maintain ambient surface temperature. Three cubitainers from each treatment were harvested for each of the three subsequent timepoints: 48 hours (T1), 168 hours (T2), and 264 hours (T3) after incubation.

Iron Incubation Experimental Set-Up. The seawater for the iron incubation experiment was pumped into a positive pressure trace metal clean laboratory and then into 50-gallon acid-washed high-density polyethylene (HDPE) drums. These drums homogenized the seawater using a Wilden air-operated double diaphragm pump made of polytetrafluoroethylene and acid-washed HDPE tubing. The seawater was then transferred into a total of thirty 20 L low-density polyethylene cubitainers.

Dissolved inorganic nutrients. Dissolved inorganic nutrient measurements were collected by filtering 30 mL of seawater through a GF/F filter into an acid-rinsed polypropylene scintillation vial. The filtrate was immediately frozen and then sent to Wetland Biogeochemistry Analytic Services at Louisiana State University and analyzed via their OI Analytical Flow Solutions IV auto analyzer for nitrate + nitrite, nitrite, phosphate (PO43-), and silicic acid (Si(OH)4). Detection limits were 0.09 µM for nitrate + nitrite, 0.02 µM for PO43-, and 0.02 µM for Si(OH)4. Nitrate (NO3-) concentrations were derived by subtracting the concentration of nitrite from nitrate + nitrite. NO3- concentrations were also used in calculations for NO3- uptake rates.

Size-fractionated chlorophyll a. Chlorophyll *a* (chl *a*) measurements were collected by gravity filtering 250 mL of seawater through 5 µm Isopore membrane filters (47 mm) to collect large cells (≥ 5 µm), then vacuum filtering the remaining seawater onto GF/F (25 mm) filters under 100 mmHg of vacuum pressure to collect small cells (< 5 µm). Filters were then rinsed with filtered seawater and stored at -20°C. Chl *a* was extracted on

the ship using 90% acetone solution at -20°C for 24 hours, then measured on a 10-AU fluorometer (Turner Designs, San Jose, CA) using the acidification method (1).

Size-fractionated isotope uptake rates. Isotope uptake rates of dissolved inorganic carbon (DIC) and NO3- were assessed by collecting seawater into 1 L polycarbonate bottles (filled to the top, resulting in a total volume of 1.09 L). Each bottle was immediately spiked with NaH13CO3 and Na15NO3, with spiking concentrations varying depending on the ambient DIC and NO3- concentrations. The ambient DIC concentrations were estimated at 2000 µM based on literature values (2), while the NO3- ambient concentrations were estimated using the Submersible Ultraviolet Nitrate Analyzer (SUNA) (3). These estimates resulted in adding 200 µM of 13C to every sample as well as 2, 2, 0.50, and 0.20 µM of 15N at T0, T1, T2, and T3 respectively, to achieve approximate additions of 10% of the ambient DIC and NO3- concentrations.

Samples were then placed in the ship incubator for 6 hours, simulated under 25% of incident irradiance. After incubation, the seawater from each bottle was gravity filtered onto 5 µm Isopore membrane filters (47 mm) to collect large cells and vacuum filtered onto pre-combusted (450°C for 5 hours) GF/F filters to collect small cells. Large cells were then rinsed off the Isopore filter using 0.2 mm filtered seawater and vacuum filtered onto pre-combusted (450°C for 5 hours) GF/F filters. The standard filtering volume was 1.09 L, but due to accumulation of biomass hindering filtering at the later timepoints, all samples in T2 and T3 had a filtering volume of 0.5 L. Filters were stored in small petri dishes at -20°C until lab preparation.

To prepare for lab analysis, filters were dried at 60°C for 24 hours, encapsulated in tin, and pelletized. Pelletized samples were sent to the UC Davis Stable Isotope Facility, where they were analyzed for particulate organic carbon (POC), particulate organic nitrogen (PON), and atom percentages of 13C and 15N using an isotope ratio mass spectrometer (EA-IRMS). POC and PON concentrations (µM) were calculated by dividing the mass of POC/PON (µg) by the respective atomic mass of carbon and nitrogen over the volume filtered. Absolute uptake rates (ρ) of DIC and NO3- were calculated using the constant transport model and biomass-normalized uptake rates (*V*) of DIC and NO3- were calculated using the specific uptake model; these calculations utilize actual dissolved NO3- concentrations (4).

Flow Cytometry. Flow cytometry was performed on a Guava easyCyte HT flow cytometer live on the cruise. The Guava easyCyte HT was outfitted with a blue 488 nm laser. Gain settings were forward scatter (FSC) 4, side scatter (SSC) 1, green fluorescence 2.95, yellow fluorescence 98.7, and red fluorescence 4. Communities of interest were gated based on community recommendations (5). *Synechococcos* were detected based on high yellow fluorescence and low FSC, picoeukaryotes (~1-3 µm) were detected based on medium yellow and red fluorescence, and nanoeukaryotes (~3-20 µm) were detected based on high red fluorescence. Samples for bacteria abundance were preserved with 0.25% glutaraldehyde and frozen at -80 ºC. Samples were then stained SYBR Green I (Thermo Fisher; 10,000x stock) diluted to a final concentration of 1x and measured on the Guava EasyCyte HT flow cytometer.

FlowCAM Analysis. FlowCAM imaging was performed for T2 samples of the iron incubation experiment based on the protocol provided by Pierce et al. 2023 using a Portable Series FlowCam connected to a peristaltic pump (6). A 10x FlowCell objective was used on autoimage mode for samples preserved in Lugols. The sets of images produced were analyzed using the Yokogawa Fluid Imaging VisualSpreadsheet software. Any images not containing particles (such as bubbles or blurry images) and/or less than 15 µm were deleted from the dataset. For each sample, the first 300 identifiable diatom cells were counted and categorized based on cell morphology and assigned to taxa at the genus level when possible. Final categories included: *Asterionellopsis* spp., *Chaetoceros* spp., *Pseudo-nitzschia* spp., other centric diatoms, and other pennate diatoms. To calculate concentrations of each diatom taxonomy within each sample (cells/L), the following equation was used:

(# of cells counted * image factor) / volume ran through FlowCAM

The image factor was determined by taking the total number of images for the sample (after quality control) divided the number of images counted for all taxonomy. The volume processed through the FlowCAM was 25 mL for all samples.

FLP Experiments for Mixotroph Grazing. Fluorescently labeled particle (FLP) incubations were performed and analyzed for the iron incubation experiment based on the protocol provided and used by Cook et al. (6). 500 mL of seawater was pre-screened through 200 µm mesh to remove mesozooplankton. Fluorescently labeled surrogate microspheres (0.5 µm diameter, Fluoresbrite, Polysciences) were spiked into the volume at concentrations of 105 particles mL-1. Immediately after surrogate prey addition, a time zero (Tinitial) sample was taken and preserved. For preservation, 10-30 mL were fixed with 4% glutaraldehyde, incubated at 4 ºC for 30 minutes, filtered onto 3 µm polycarbonate filters, mounted using DAPI Vectashield (Vector Laboratories), and frozen at -20 ºC. Incubations were carried out for one hour in a clear plexiglass flow-through incubator with mesh bags emulating *in situ* light availability. After an hour incubation, another sample (Tfinal) was taken

and preserved in the same manner as Tinitial.

FLP incubation analysis was performed using an Olympus CKX53 inverted microscope at 400x total magnification. The field-of-view counting method with a calibrated reticle was used. At least 300 chloroplast-containing cells were enumerated on every slide to ensure statistical reliability. Cell specific grazing rate (CSGR, bacteria mixotroph-1 hr-1), was calculated using the following equation:

CSGR = (((Cingested / Cphoto)Tfinal - (Cingested / Cphoto)Tinitial) / t) * (Cbacteria / CFLP)

Cingested is the count of ingested beads divided by the count of all pigmented phototrophic cells (Cphoto), with Tinitial values subtracted from Tfinal values to account for non-ingestion adsorption. Values were divided by time of incubation in hours (t) then multiplied by the Bacteria:FLP ratio. Cbacteria is the natural concentration of heterotrophic bacteria measured via flow cytometry and CFLP is the concentration of FLPs spiked into the sample (105 particles mL-1).

RNA Collection and Metatranscriptomics. Cubitainers were harvested for RNA by collecting and filtering approximately 2.5 to 4 L of each sample onto 0.8 µm Pall Supor filters (142 mm) using a peristaltic pump; samples were then immediately flash frozen in liquid nitrogen and stored at -80°C. RNA was extracted using the RNAqueous-4PCR kit, per instructions from the manufacturer with few modifications – filters were cut up due to their large size, 200 µL of glass beads were added, and 3 mL of lysis buffer was added. RNA samples were sent to GENEWIZ for library preparation and sequencing with PolyA tail selection. Sequencing was performed on an Illumina HiSeq 4000 with a 2x150 bp configuration. GENEWIZ provided raw paired-end read sequences for each sample.

Raw reads were trimmed using Trim Galore v0.6.10. and quality control was assessed through FastQC (8). A *de novo* metatranscriptome assembly was conducted using rnaSPAdes v3.15.5 for individual assemblies (9) and CD-HIT v4.8.1 for a grand assembly (10). TransDecoder v5.7.1 was used to determine the most likely open reading frame (ORF) for each contig (11). ORFs were annotated using the Marine Functional Eukaryotic Reference Taxa (MarFERReT) database v1.1.1 (e-value < 1e-06) (12) for NCBI taxonomies (13), PR2 taxonomies (14) and Pfam 34.0 functions (15). To remove any potential bacterial contamination, ORFs were also annotated against the PhyloDB v1076 database using EUKulele (e-value < 1e-06) (16). Any ORF with a higher bitscore for a bacteria or virus compared to its MarFERReT annotation was removed (resulting in < 3% of ORFs removed). To maximize functional annotation coverage, ORFs were annotated using eggNOG-mapper v2.1.12 (17) to obtain annotations from the Kyoto Encyclopedia of Genes and Genomes (KEGG) (18). Trimmed samples were then aligned to the grand assembly using Salmon v1.10.3 (19) and exported into a comprehensive counts table using tximport (20). Contigs with no taxonomic annotation or an Opisthokonta annotation were removed before downstream analysis.

DESeq2 was used to obtain normalized counts and conduct differential expression analyses (21). DESeq2 analyses (for Pfam protein domain, KEGG gene, and KEGG pathway differential expression) were performed for reads mapping to mixotroph genera and reads mapping to the lineage Bacillariophyta (referred to as diatoms). Mixotrophs were identified based on constitutive mixotroph genera within the Mixoplankton Database (MDB) (22). Reads were summed based on annotation ID prior to entering DESeq2 analysis; if a contig annotated to multiple IDs, its reads were evenly distributed between the mapped annotations.

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Related Publications

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Software

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experimental considerations1,2. Limnology and Oceanography, 31(4), 673–689. Portico. https://doi.org/10.4319/lo.1986.31.4.0673
Methods

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Methods

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Results

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Methods

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Parameters

Parameters for this dataset have not yet been identified

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Deployments

SR2311

Website	https://www.bco-dmo.org/deployment/994610
Platform	R/V Sally Ride
Start Date	2023-05-29
End Date	2023-06-10
Description	California Current System, off the southern coast of Oregon at 43°02'42.7"N, 124°33'07.2"W, depth 55m

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Project Information

CAREER: An integrated molecular and physiological approach to examining the dynamics of upwelled phytoplankton in current and changing oceans (Upwelled Phytoplankton Dynamics)

Coverage: California Upwelling Zone

NSF Award Abstract:
Upwelling zones are hotspots of photosynthesis that are very dynamic in space and time. Microsocopic algae, known as phytoplankton, bloom when deep, nutrient-rich waters are upwelled into sunlit surface layers of the ocean, providing nourishment that supports productive food webs and draws down carbon dioxide (CO2) from the atmosphere to the deep ocean. Photosynthetic microbes in these regions must constantly adapt to changes in their chemical and physical environments. For example, subsurface populations respond to changes in light as they approach the surface. When upwelled waters move offshore, cells sink out of the illuminated zone, establishing seed populations that remain inactive until the next upwelling event. This process is called the upwelling conveyor belt cycle (UCBC). How phytoplankton respond to these changes in environmental conditions and how they may influence their nutrient requirements remains unknown. With future ocean changes predicted to alter seawater chemistry, including ocean acidification and decreased iron

availability, some phytoplankton groups may be more vulnerable than others. Accompanying educational activities provide learning experiences to enhance understanding and awareness of marine microbes. The development of a research hub at UNC aims to provide infrastructure and support for scientists and students conducting research on environmental genomics. A laboratory component for an upper-level undergraduate course focused on marine phytoplankton is being developed. Educational outreach activities to broader communities include creation of a lesson plan on phytoplankton in upwelling zones and a virtual research cruise experience for middle-school students, as well as a hands-on lab activity for a local museum focused on marine phytoplankton and the important roles they play in shaping our planet.

The project examines how phytoplankton respond at the molecular and physiological level to the different UCBC stages, which seed populations (i.e., surface versus subsurface) contribute most to phytoplankton blooms during upwelling events of varying intensity, how phytoplankton elemental compositions are altered throughout UCBC stages, and how future predicted ocean conditions will affect the phytoplankton responses to UCBC conditions. This project contains both laboratory and fieldwork. In the laboratory, phytoplankton isolates recently obtained from upwelling regions are exposed to simulated UCBC conditions to examine changes in gene expression, growth and photosynthetic characteristics and elemental composition. Cultures are subjected to both current and future ocean conditions, including reduced iron availability and higher CO2. In the field, research cruises within upwelling regions study the dynamics of natural phytoplankton communities (both surface and subsurface) experiencing upwelling and relaxation and within simulated upwelling incubation experiments. Knowledge of how phytoplankton are affected by UCBC conditions at an integrated molecular, physiological and elemental level under both current and future scenarios is imperative for the proper conservation and management of these critically important ecosystems.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1751805

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