Abundances of phytoplankton and non-pigmented bacteria determined by flow cytometry from water samples collected on R/V Roger Revelle cruise RR2201 in the Eastern Indian Ocean during February and March 2022

Website: https://www.bco-dmo.org/dataset/916288
Data Type: Cruise Results
Version: 1
Version Date: 2024-01-09

Project
» Collaborative Research: Mesoscale variability in nitrogen sources and food-web dynamics supporting larval southern bluefin tuna in the eastern Indian Ocean (BLOOFINZ-IO)

Program
» Second International Indian Ocean Expedition (IIOE-2)

Contributors	Affiliation	Role
Selph, Karen E.	University of Hawaii at Manoa (SOEST)	Principal Investigator
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Abstract
This dataset is from CTD-based water collections of samples for phytoplankton and non-pigmented bacteria in the Indian Ocean on an R/V Roger Revelle cruise in Feb-March 2022 led by Dr. Michael Landry to investigate the plankton dynamics and impacts on growth and survival of larval Southern Bluefin Tuna (SBT). These flow cytometry results include abundances of phytoplankton taxa (Prochlorococcus, Synechococcus, photosynthetic eukaryotes), non-pigmented bacteria (HBACT), heterotrophic eukaryotes (HEUK), and potential mixotrophic eukaryotes (MEUK). Photosynthetic eukaryote (PEUK) abundance includes the MEUK cells reported (e.g., MEUK are a subset of the PEUK cells with chlorophyll and acidic-vacuoles). Note that MEUK cells likely also include some HEUK with intact chlorophyll-bearing prey, but there is no way to definitively separate these cells from each other.

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Coverage

Spatial Extent: **N**:-13.174 **E**:121.49767 **S**:-17.12632 **W**:114.13514
Temporal Extent: 2022-02-03 - 2022-03-02

Methods & Sampling

Flow cytometry samples were collected from each CTD rosette or individual Go-Flo bottles. All samples were

analyzed on board the ship with a Beckman Coulter CytoFlex S flow cytometer, equipped with 4 lasers (375 nanometers (nm), 405 nm, 488 nm, and 536 nm) and CytExpert software. Preserved (0.5% paraformaldehyde final) samples (500 microliters (µL)) were stained with Hoechst 34580 (1 microgram per milliliter (µg mL^{-1})) and run (50 microliters per minute (µl min^{-1})) within 3 hours of collection (Selph 2021). The data presented here for all populations, except heterotrophic eukaryotes (HEUK) and potential mixotrophs (MEUK), are from these preserved samples. At many stations, a live sample (500 µL) was also analyzed after staining with Hoechst (1 µg mL^{-1} final) and Lysotracker Green (LTG, acidic vacuole stain, 75 nanomolar (nM) final, Rose et al. 2004) to obtain estimates of HEUK and MEUK. Data files (Lismode 3.0) were generated and analyzed using FlowJo software (Tree Star, Inc.).

Data Processing Description

Gating analyses of the listmode files followed those in Selph 2021 for the Hoechst-stained samples. For samples stained with Hoechst and LTG, the gating involved many steps as follows. LTG positive and negative particles were separated assuming that the maximum negative signal was that of co-occurring prokaryotes (Prochlorococcus, Synechococcus, and non-pigmented bacteria). Thus, all particles that had LTG signals greater than that of prokaryotes were designated LTG POS. LTG POS particles were further separated by the signal of the forward light scatter (FSC) of a 1 micrometer (µm) yellow-green fluorescent plastic beads (Polysciences, Inc.). Those particles with less FSC than a 1 µm bead were designated "PICO" sized particles and plotted as chlorophyll vs. phycoerythrin to eliminate any cyanobacteria, whereas the remaining cells were designated as potential mixotrophic cells. Those with more scatter than a 1 µm bead were designated "NANO+" sized particles and plotted as a single parameter chlorophyll histogram, where those with more chlorophyll than Prochlorococcus were considered potential mixotrophic eukaryotic cells. LTG POS PICO and NANO+ particles comprise the MEUK category reported. NANO+ (LTG POS) particles with less chlorophyll than Prochlorococcus were designated as heterotrophic eukaryotes (HEUK).

BCO-DMO Processing Description

- Imported original file "Selph FCM IO.xlsx" into the BCO-DMO system.
- Flagged "nd" as a missing data value (missing data are blank/empty in the final CSV file).
- Renamed fields to comply with BCO-DMO naming conventions.
- Corrected Station numbers where needed so dates match those in Date column, as instructed by data provider.
- Converted the Date column to YYYY-MM-DD format.
- Created the ISO 8601 date-time column by extracting date-times from the Station column.
- Saved the final data file as "916288_v1_bloofinz-io_flow_cytometry.csv".

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Data Files

File	
916288_v1_bloofinz-io_flow_cytometry.csv	(Comma Separated Values (.csv), 15.53 KB) MD5:3d606be4cfddb78fe1175529662c5eda
Primary data file for dataset ID 916288, version 1	

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Related Publications

Rose, J., Caron, D., Sieracki, M., & Poulton, N. (2004). Counting heterotrophic nanoplanktonic protists in cultures and aquatic communities by flow cytometry. Aquatic Microbial Ecology, 35, 263–277. https://doi.org/10.3354/ame035263
Methods

Selph, K. E. (2021). Enumeration of marine microbial organisms by flow cytometry using near-UV excitation of Hoechst 34580-stained DNA. Limnology and Oceanography: Methods, 19(10), 692–701. Portico. https://doi.org/10.1002/lom3.10454
Methods

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Parameters

Parameter	Description	Units
Cruise	R/V Revelle cruise ID number	unitless
Station	Station designation incorporating date and UTC time	unitless
ISO_DateTime_UTC	Date and time (UTC) in ISO 8601 format	unitless
Date	Date	unitless
Latitude	Latitude; negative values = South	decimal degrees
Longitude	Longitude; positive values = East	decimal degrees
CTD_Number	Sequential CTD number on the cruise	unitless
Depth	Depth of water collection	meters (m)
PRO	Prochlorococcus abundance	cells per milliliter
SYN	Synechococcus abundance	cells per milliliter
PEUK	Photosynthetic Eukaryote abundance (includes MEUK abundance)	cells per milliliter
HBACT	Heterotrophic bacteria abundance	cells per milliliter
HEUK	Heterotrophic eukaryote abundance	cells per milliliter
MEUK	Mixotrophic eukaryote abundance	cells per milliliter

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Instruments

Dataset-specific Instrument Name	Beckman Coulter CytoFlex S
Generic Instrument Name	Flow Cytometer
Dataset-specific Description	Samples were analyzed with a Beckman Coulter CytoFlex S flow cytometer with a 96-well sample plate and 4 lasers (375 nm, 405 nm, 488 nm, and 536 nm).
Generic Instrument Description	Flow cytometers (FC or FCM) are automated instruments that quantitate properties of single cells, one cell at a time. They can measure cell size, cell granularity, the amounts of cell components such as total DNA, newly synthesized DNA, gene expression as the amount messenger RNA for a particular gene, amounts of specific surface receptors, amounts of intracellular proteins, or transient signalling events in living cells. (from: http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm)

Dataset-specific Instrument Name	Go-Flo bottles
Generic Instrument Name	GO-FLO Bottle
Dataset-specific Description	Samples were collected using a CTD-rosette with Niskin Bottles or Go-Flo bottles deployed by hand on a line.
Generic Instrument Description	GO-FLO bottle cast used to collect water samples for pigment, nutrient, plankton, etc. The GO-FLO sampling bottle is specially designed to avoid sample contamination at the surface, internal spring contamination, loss of sample on deck (internal seals), and exchange of water from different depths.

Dataset-specific Instrument Name	Niskin Bottles
Generic Instrument Name	Niskin bottle
Dataset-specific Description	Samples were collected using a CTD-rosette with Niskin Bottles or Go-Flo bottles deployed by hand on a line.
Generic Instrument Description	A Niskin bottle (a next generation water sampler based on the Nansen bottle) is a cylindrical, non-metallic water collection device with stoppers at both ends. The bottles can be attached individually on a hydrowire or deployed in 12, 24, or 36 bottle Rosette systems mounted on a frame and combined with a CTD. Niskin bottles are used to collect discrete water samples for a range of measurements including pigments, nutrients, plankton, etc.

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Deployments

RR2201

Website	https://www.bco-dmo.org/deployment/916293
Platform	R/V Roger Revelle
Start Date	2022-01-20
End Date	2022-03-14
Description	See more information at R2R: https://www.rvdata.us/search/cruise/RR2201

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Project Information

Collaborative Research: Mesoscale variability in nitrogen sources and food-web dynamics supporting larval southern bluefin tuna in the eastern Indian Ocean (BLOOFINZ-IO)

Coverage: Eastern Indian Ocean, Indonesian Throughflow area, and the Gulf of Mexico

NSF Award Abstract:
The small area between NW Australia and Indonesia in the eastern Indian Ocean (IO) is the only known spawning ground of Southern Bluefin Tuna (SBT), a critically endangered top marine predator. Adult SBT migrate thousands of miles each year from high latitude feeding areas to lay their eggs in these tropical waters, where food concentrations on average are below levels that can support optimal feeding and growth of their larvae. Many critical aspects of this habitat are poorly known, such as the main source of nitrogen nutrient that sustains system productivity, how the planktonic food web operates to produce the unusual types of zooplankton prey that tuna larvae prefer, and how environmental differences in habitat quality associated with ocean fronts and eddies might be utilized by adult spawning tuna to give their larvae a greater chance for rapid growth and survival success. This project investigates these questions on a 38-day expedition in early 2021, during the peak time of SBT spawning. This project is a US contribution to the 2nd International Indian Ocean Expedition (IIOE-2) that advances understanding of biogeochemical and ecological dynamics in the poorly studied eastern IO. This is the first detailed study of nitrogen and carbon cycling in the region linking Pacific and IO waters. The shared dietary preferences of SBT larvae with those of other large tuna and billfish species may also make the insights gained broadly applicable to understanding larval recruitment issues for top consumers in other marine ecosystems. New information from the study will enhance international management efforts for SBT. The shared larval dietary preferences of large tuna and billfish species may also extend the insights gained broadly to many other marine top consumers, including Atlantic bluefin tuna that spawn in US waters of the Gulf of Mexico. The end-to-end study approach, highlights connections among physical environmental variability, biogeochemistry, and plankton food webs leading to charismatic and economically valuable fish production, is the theme for developing educational tools and modules through the "scientists-in-the-schools" program of the Center for Ocean-Atmospheric Prediction Studies at Florida State University, through a program for enhancing STEM learning pathways for underrepresented students in Hawaii, and through public outreach products for display at the Birch Aquarium in San Diego. The study also aims to support an immersive field experience to introduce talented high school students to marine research, with the goal of developing a sustainable marine-related educational program for underrepresented students in rural northwestern Florida.

Southern Bluefin Tuna (SBT) migrate long distances from high-latitude feeding grounds to spawn exclusively in a small oligotrophic area of the tropical eastern Indian Ocean (IO) that is rich in mesoscale structures, driven by complex currents and seasonally reversing monsoonal winds. To survive, SBT larvae must feed and grow rapidly under environmental conditions that challenge conventional understanding of food-web structure and functional relationships in poor open-ocean systems. The preferred prey of SBT larvae, cladocerans and Corycaeidae copepods, are poorly studied and have widely different implications for trophic transfer efficiencies to larvae. Differences in nitrogen sources - N fixation vs deep nitrate of Pacific origin - to sustain new production in the region also has implications for conditions that may select for prey types (notably cladocerans) that enhance transfer efficiency and growth rates of SBT larvae. The relative importance of these N sources for the IO ecosystem may affect SBT resiliency to projected increased ocean stratification. This research expedition investigates how mesoscale variability in new production, food-web structure and trophic fluxes affects feeding and growth conditions for SBT larvae. Sampling across mesoscale features tests hypothesized relationships linking variability in SBT larval feeding and prey preferences (gut contents), growth rates (otolith analyses) and trophic positions (TP) to the environmental conditions of waters selected by adult spawners. Trophic Positions of larvae and their prey are determined using Compound-Specific Isotope Analyses of Amino Acids (CSIA-AA). Lagrangian experiments investigate underlying process rates and relationships through measurements of water-column 14C productivity, N2 fixation, 15NO3- uptake and nitrification; community biomass and composition (flow cytometry, pigments, microscopy, in situ imaging, genetic analyses); and trophic fluxes through micro- and mesozooplankton grazing, remineralization and export. Biogeochemical and food web elements of the study are linked by CSIA-AA (N source, TP), 15N-constrained budgets and modeling. The project elements comprise an end-to-end coupled biogeochemistry-trophic study as has not been done previously for any pelagic ecosystem.

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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Program Information

Second International Indian Ocean Expedition (IIOE-2)

Website: https://web.whoi.edu/iioe2/

Coverage: Indian Ocean

Description from the program website:
The Second International Indian Ocean Expedition (IIOE-2) is a major global scientific program which will engage the international scientific community in collaborative oceanographic and atmospheric research from coastal environments to the deep sea over the period 2015-2020, revealing new information on the Indian Ocean (i.e. its currents, its influence upon the climate, its marine ecosystems) which is fundamental for future sustainable development and expansion of the Indian Ocean's blue economy. A large number of scientists from research institutions from around the Indian Ocean and beyond are planning their involvement in IIOE-2 in accordance with the overarching six scientific themes of the program. Already some large collaborative research projects are under development, and it is anticipated that by the time these projects are underway, many more will be in planning or about to commence as the scope and global engagement in IIOE-2 grows.

Focused research on the Indian Ocean has a number of benefits for all nations. The Indian Ocean is complex and drives the region's climate including extreme events (e.g. cyclones, droughts, severe rains, waves and storm surges). It is the source of important socio-economic resources (e.g. fisheries, oil and gas exploration/extraction, eco-tourism, and food and energy security) and is the background and focus of many of the region's human populations around its margins. Research and observations supported through IIOE-2 will result in an improved understanding of the ocean's physical and biological oceanography, and related air-ocean climate interactions (both in the short-term and long-term). The IIOE-2's program will complement and harmonise with other regional programs underway and collectively the outcomes of IIOE-2 will be of huge benefit to individual and regional sustainable development as the information is a critical component of improved decision making in areas such as maritime services and safety, environmental management, climate monitoring and prediction, food and energy security.

IIOE-2 activities will also include a significant focus on building the capacity of all nations around the Indian Ocean to understand and apply observational data or research outputs for their own socio-economic requirements and decisions. IIOE-2 capacity building programs will therefore be focused on the translation of the science and information outputs for societal benefit and training of relevant individuals from surrounding nations in these areas.

A Steering Committee was established to support U.S. participation in IIOE-2. More information is available on their website at https://web.whoi.edu/iioe2/.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1851381

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