Protist carbon from microscopy samples collected in the Argo Basin of the Eastern Indian Ocean on R/V Roger Revelle cruise RR2202 from Feb to Mar 2022

Website: https://www.bco-dmo.org/dataset/944892
Data Type: Cruise Results, Other Field Results
Version: 1
Version Date: 2024-12-03

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
Landry, Michael R.	University of California-San Diego Scripps (UCSD-SIO)	Principal Investigator
Stukel, Michael R.	Florida State University (FSU)	Co-Principal Investigator
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Abstract
This dataset is from CTD-based water collections of samples for phytoplankton in the Argo Basin in the Eastern Indian Ocean aboard the R/V Roger Revelle cruise in Feb-March 2022 led by Dr. Michael Landry to investigate the plankton community composition and impacts on growth and survival of larval Southern Bluefin Tuna (SBT). These samples were stained and fixed for epifluorescence microscopy analysis. The microscopy results include the abundance and carbon-based biomass estimates of nano (2 to 20 µm size) and microplankton (>20 µm) sized autotrophs and heterotrophs.

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Coverage

Location: Northwest Australia, Argo Basin, 11-17S, 114-124E, depth 5000m
Spatial Extent: **N**:-15.3494 **E**:118.1424 **S**:-17.1263 **W**:114.1351
Temporal Extent: 2022-02-03 - 2022-02-22

Methods & Sampling

Field Collection

We conducted four Lagrangian experiments (hereafter referred to as “cycles”) that lasted three to five days. The mixed layer was followed in a Lagrangian frame of reference using subsurface drogues attached to satellite-tagged marker buoys (Landry et al. 2009; Stukel et al. 2015). Six depths were chosen to span the euphotic zone (based on chlorophyll fluorescence measured during the conductivity-temperature-depth (CTD) downcast profiles).We used a 12-place 6-L Niskin bottle rosette equipped with a Seabird Conductivity, Temperature, and Depth (CTD) sensor and profiling chlorophyll fluorometer to sample water daily at six depths spanning the euphotic zone for phytoplankton community samples.

The RR2201 cruise report can be found at http://hdl.handle.net/1834/43464.

Epifluorescence Microscopy Sampling

From each depth, two different volumes of water were sampled: 50 mL for nanoplankton- epifluorescence microscopy (filtered through a 0.8-µm pore-size black polycarbonate filter) and 400 mL for microplankton epifluorescence microscopy (filtered through an 8-µm pore-size filter). Utilizing two different sized filters and sampling volumes allowed for appropriate, adjustable filtered volumes and avoid overlapping cells on the slides. 20 µm backing filters were utilized as data has indicated that they support the membrane filters and ensure even dispersal of sample on the filter (Kemp et. al., 1993; Taylor et al., 2015). The samples were preserved using buffered formalin, alkaline Lugol’s solution, and sodium thiosulfate then stained using proflavine and 4’, 6-diamidino-2phenylindole (DAPI) (modified protocol from Sherr and Sherr, 1993 in Kemp et. al., 1993). During and immediately after filtration, filters were covered with tin foil to prevent photochemical quenching. Filters were mounted onto a glass slide and frozen in a -80º C freezer for later analysis.

Data Processing Description

Slides were imaged using an Olympus Microscope DP72 Camera on an Olympus BX51 fluorescence microscope. Slides were calibrated from pixels to microns for both 0.8 µm and 8 µm to determine what length of pixels equates to microns. Our data includes conversion ratios (0.0012 for 0.8 µm and 0.0072 for 8 µm), which should be applied when determining biomass or abundance, along with the volume filtered. These ratios account for the area of the images taken and represent the proportion of cells actually counted for the 0.8 µm and 8 µm samples, and they have already been incorporated into the dataset presented.

Twenty 8-µm images (20x objective lens) and thirty 0.8-µm images (60x objective lens) were captured using filter sets that were suited for proflavine (green fluorescence with 482 nm excitation, 536 nm emission filters), auto-chlorophyll fluorescence (red fluorescence with 450-490 nm excitation, 660-680 nm emission) and DAPI (blue fluorescence with 350 nm excitation, 465 nm emission) to capture the fluorescence of cell proteins, chlorophyll and DNA. The images were subsequently analyzed to identify phototrophs and heterotrophs, with cells marked based on the presence of both fluorescence signals (indicating phototrophs) or only proflavine fluorescence (indicating heterotrophs).ImageJ image analysis software (v 1.52a or 1.53c) was utilized for processing images, involving manually outlining cells in order to calculate feret length, area and width. Cells that were >50% out of frame or broken were not included in analyses. Images were calibrated to microns and a conversion factor was applied to include the number of images, area of the slide and area imaged with the specific camera/lens combination.

To determine the true filtration diameter, a light microscope was used to examine a 25 mm glass fiber filter (GF/F) filter that had a small amount of dyed water filtered through. It was discovered that the filter funnel blocks roughly 12% of the 25-mm filter and the filtered region had a diameter of 22 mm. Equations 1-5 show the equations used to calculate cell width, biovolume, ESD (equivalent spherical diameter) and biomass, where * implies we assumed that cell height = cell width/2. ESD was used as a consistent measure of mean cell size since many plankton have an irregular shape. The height of a cell was assumed to be roughly equivalent to half of the cell width since cells are often flattened during filtration (Taylor et al., 2011) with the exception of diatoms. The biomass of diatoms (which were the only taxon we could conclusively identify) was estimated allometrically using equation 5 while all other cell biomass (non-diatoms) was estimated allometrically using equation 4. (Menden-Deuer and Lessard, 2000).

Equation 1: Cell width = (4/π) x (Area of the cell/Feret length of the cell)

Equation 2: Biovolume = (4/3)(π) x (Feret Length/2) x (Cell Width/2) x (Cell Height*/2)

Equation 3: ESD = 2 x (3 x Biovolume/4π)^(1/3)

Equation 4: Biomass (non-diatoms) = 0.216 x Biovolume^0.939

Equation 5: Biomass (diatoms) = 0.288 x Biovolume^0.811

BCO-DMO Curation Notes

-imported "BF_IO_Epifluroescence_Microscopy_Carbon_Data.xlsx" without excel formatting into BCO-DMO system
- split "Cycle,Day" column on commas into "Cycle" and "Day_Cycle"
- removed T00:00:00 from "Date" field values
- rounded biomass fields to 3 places
- renamed fields for BCO-DMO system requirements and clarity
- exported "944892_v1_protist_carbon.csv"

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Data Files

File
944892_v1_protist_carbon.csv (Comma Separated Values (.csv), 9.64 KB) MD5:f6726d28d79c2017896cc46630ee230b Primary data file for dataset ID 944892, version 1

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Related Publications

Kemp, P., B. F. Sherr, E. B. Sherr, and J. J. Cole (Eds.). (1993). Handbook of methods in aquatic microbial ecology. Lewis Publishing, Boca Raton, FL 33431, 777 pp. https://isbnsearch.org/isbn/0-873-71564-0
Methods

Landry, M. R. (2025). R/V Roger Revelle Cruise RR2201 BLOOFINZ-IO Cruise Report. Eastern Indian Ocean larval tuna habitat study 20 January – 14 March 2022. http://hdl.handle.net/1834/43464
General

Landry, M. R., Ohman, M. D., Goericke, R., Stukel, M. R., & Tsyrklevich, K. (2009). Lagrangian studies of phytoplankton growth and grazing relationships in a coastal upwelling ecosystem off Southern California. Progress in Oceanography, 83(1-4), 208–216. doi:10.1016/j.pocean.2009.07.026
Methods

Menden-Deuer, S., & Lessard, E. J. (2000). Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. Limnology and Oceanography, 45(3), 569–579. doi:10.4319/lo.2000.45.3.0569
Methods

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

Sherr, B. F. and E. B. Sherr (1993) Preservation and storage of samples for enumeration of heterotrophic protists. In Kemp, P. F., Sherr, B. F., Sherr, E. B. and Cole, J. J. (eds.) Handbook of Methods in Aquatic Microbial Ecology. CRC Press, pp. 207-212 https://isbnsearch.org/isbn/9780367449858
Methods

Stukel, M. R., Kahru, M., Benitez-Nelson, C. R., Décima, M., Goericke, R., Landry, M. R., & Ohman, M. D. (2015). Using Lagrangian-based process studies to test satellite algorithms of vertical carbon flux in the eastern North Pacific Ocean. Journal of Geophysical Research: Oceans, 120(11), 7208–7222. Portico. https://doi.org/10.1002/2015jc011264
Methods

Taylor, A. G., Landry, M. R., Selph, K. E., & Wokuluk, J. J. (2015). Temporal and spatial patterns of microbial

community biomass and composition in the Southern California Current Ecosystem. Deep Sea Research Part II: Topical Studies in Oceanography, 112, 117–128. doi:10.1016/j.dsr2.2014.02.006
Methods

Taylor, A. G., Landry, M. R., Selph, K. E., & Yang, E. J. (2011). Biomass, size structure and depth distributions of the microbial community in the eastern equatorial Pacific. Deep Sea Research Part II: Topical Studies in Oceanography, 58(3-4), 342–357. doi:10.1016/j.dsr2.2010.08.017
Methods

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Parameters

Parameter	Description	Units
Cruise	R/V Revelle cruise ID number	unitless
Station_Event	Station designation incorporating date and UTC time	unitless
DateTimeUTC	Date and time (UTC) in ISO 8601 format of collection	unitless
Date	Date of collection	unitless
Latitude	Latitude; negative values = South	decimal degrees
Longitude	Longitude; positive values = East	decimal degrees
Cycle	Lagrangian experiment number	unitless
Day_Cycle	Day of Lagrangian cycle	unitless
CTD_Cast	Sequential CTD number on the cruise	unitless
Depth	Depth of water collection	meters (m)
Autotrophic_nanoplankton_abundance	The number of autotrophic nanoplankton (2 to 20 µm in size)	cells/mL
Autotrophic_nanoplankton_biomass	The biomass of autotrophic nanoplankton (2 to 20 µm in size)	µg C L-1 (micrograms of carbon per liter)
Heterotrophic_nanoplankton_abundance	The number of heterotrophic nanoplankton (2 to 20 µm in size)	cells/mL

Heterotrophic_nanoplankton_biomass	The biomass of heterotrophic nanoplankton (2 to 20 µm in size)	µg C L-1 (micrograms of carbon per liter)
Autotrophic_microplankton_abundance	The number of autotrophic microplankton (>20 µm in size)	cells/mL
Autotrophic_microplankton_biomass	The biomass of autotrophic microplankton (>20µm in size)	µg C L-1 (micrograms of carbon per liter)
Heterotrophic_microplankton_abundance	The number of heterotrophic microplankton (>20 µm in size)	cells/mL
Heterotrophic_microplankton_biomass	The biomass of heterotrophic microplankton (>20µm in size)	µg C L-1 (micrograms of carbon per liter)

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Instruments

Dataset-specific Instrument Name	Olympus Microscope DP72 Camera
Generic Instrument Name	Camera
Dataset-specific Description	Slides were imaged using an Olympus Microscope DP72 Camera on an Olympus BX51 fluorescence microscope.
Generic Instrument Description	All types of photographic equipment including stills, video, film and digital systems.

Dataset-specific Instrument Name	Seabird Conductivity, Temperature, and Depth (CTD) sensor
Generic Instrument Name	CTD Sea-Bird 911
Dataset-specific Description	We used a 12-place 6-L Niskin bottle rosette equipped with a Seabird Conductivity, Temperature, and Depth (CTD) sensor and profiling chlorophyll fluorometer to sample water daily at six depths spanning the euphotic zone for phytoplankton community samples.
Generic Instrument Description	The Sea-Bird SBE 911 is a type of CTD instrument package. The SBE 911 includes the SBE 9 Underwater Unit and the SBE 11 Deck Unit (for real-time readout using conductive wire) for deployment from a vessel. The combination of the SBE 9 and SBE 11 is called a SBE 911. The SBE 9 uses Sea-Bird's standard modular temperature and conductivity sensors (SBE 3 and SBE 4). The SBE 9 CTD can be configured with auxiliary sensors to measure other parameters including dissolved oxygen, pH, turbidity, fluorescence, light (PAR), light transmission, etc.). More information from Sea-Bird Electronics.

Dataset-specific Instrument Name	subsurface drogues attached to satellite-tagged marker buoys
Generic Instrument Name	Drifter Buoy
Dataset-specific Description	The mixed layer was followed in a Lagrangian frame of reference using subsurface drogues attached to satellite-tagged marker buoys (Landry et al. 2009; Stukel et al. 2015).
Generic Instrument Description	Drifting buoys are free drifting platforms with a float or buoy that keep the drifter at the surface and underwater sails or socks that catch the current. These instruments sit at the surface of the ocean and are transported via near-surface ocean currents. They are not fixed to the ocean bottom, therefore they "drift" with the currents. For this reason, these instruments are referred to as drifters, or drifting buoys. The surface float contains sensors that measure different parameters, such as sea surface temperature, barometric pressure, salinity, wave height, etc. Data collected from these sensors are transmitted to satellites passing overhead, which are then relayed to land-based data centers. definition sources: https://mmisw.org/ont/ioos/platform/drifting_buoy and https://www.aoml.noaa.gov/phod/gdp/faq.php#drifter1

Dataset-specific Instrument Name	Olympus BX51 microscope with a Olympus DP72 camera
Generic Instrument Name	Fluorescence Microscope
Dataset-specific Description	Epifluorescence microscope: Olympus BX51 microscope with a Olympus DP72 camera and Exfo X-cite Series 120 mercury bulb with a FITC filter for green fluorescence.
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of fluorescence and phosphorescence instead of, or in addition to, reflection and absorption of visible light. Includes conventional and inverted instruments.

Dataset-specific Instrument Name	profiling chlorophyll fluorometer
Generic Instrument Name	Fluorometer
Dataset-specific Description	We used a 12-place 6-L Niskin bottle rosette equipped with a Seabird Conductivity, Temperature, and Depth (CTD) sensor and profiling chlorophyll fluorometer to sample water daily at six depths spanning the euphotic zone for phytoplankton community samples.
Generic Instrument Description	A fluorometer or fluorimeter is a device used to measure parameters of fluorescence: its intensity and wavelength distribution of emission spectrum after excitation by a certain spectrum of light. The instrument is designed to measure the amount of stimulated electromagnetic radiation produced by pulses of electromagnetic radiation emitted into a water sample or in situ.

Dataset-specific Instrument Name	6-L Niskin bottle
Generic Instrument Name	Niskin bottle
Dataset-specific Description	We used a 12-place 6-L Niskin bottle rosette equipped with a Seabird Conductivity, Temperature, and Depth (CTD) sensor and profiling chlorophyll fluorometer to sample water daily at six depths spanning the euphotic zone for phytoplankton community samples.
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
Report	http://hdl.handle.net/1834/43464
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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