

Protist carbon from microscopy samples collected in the Gulf of Mexico on R/V Nancy Foster cruises in May 2017 and May 2018

Website: <https://www.bco-dmo.org/dataset/851302>

Data Type: Cruise Results

Version: 1

Version Date: 2021-06-04

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
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Abstract

This dataset is from CTD hydrocasts in the Gulf of Mexico from R/V Nancy Foster cruises in May 2017 and May 2018, which were part of a NOAA RESTORE project (aka: BLOOFINZ-GoM) to investigate the epipelagic marine nitrogen cycle, plankton dynamics, and impacts on growth and survival of larval Atlantic Bluefin Tuna (ABT). These data are meant to be used in inter-species, interregional comparisons to data from the BLOOFIN-IO study of larval Southern Bluefin Tuna in the Indian Ocean spawning region.

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Coverage

Spatial Extent: N:28.3358 E:-87.3032 S:25.4092 W:-89.6769

Temporal Extent: 2017-05-11 - 2018-05-19

Acquisition Description

This dataset is from CTD hydrocasts in the Gulf of Mexico from R/V Nancy Foster cruises in May 2017 and May 2018, which were part of a NOAA RESTORE project (aka: BLOOFINZ-GoM) to investigate the epipelagic marine nitrogen cycle, plankton dynamics, and impacts on growth and survival of larval Atlantic Bluefin Tuna (ABT). These data are meant to be used in inter-species, interregional comparisons to data from the BLOOFIN-IO study of larval Southern Bluefin Tuna in the Indian Ocean spawning region.

Seawater samples (500 mL) for analysis by epifluorescence microscopy (EPI) were preserved with 260 µL of alkaline Lugol's solution, 10 mL 0.08M borax-buffered 10% formalin and 500 µL 0.19M sodium thiosulfate (Sherr and Sherr, 1993), and stained with 1 mL of proflavin (0.33% w/v) and 1 mL of DAPI (0.01 mg mL⁻¹) prior to filtering. Subsamples of 50 mL were filtered onto 25-mm, black, 0.8-µm pore polycarbonate filters to enumerate small cells at 630X magnification. The remaining 450 mL was filtered onto 25-mm, black, 8.0-µm pore polycarbonate filters to count larger cells at 200X. Each filter was mounted onto a glass slide using Type DF immersion oil and a No. 2 cover slip.

The slides were imaged and digitized using an automated Zeiss Axiovert 200M inverted epifluorescence microscope, with an AxioCam MRc black and white 8-bit CCD camera (Taylor et al., 2016). Fifty random positions were imaged for each slide, with each position consisting of four fluorescent channels: Chla, DAPI (DNA stain), FITC (proflavin protein stain, cell outline) and phycoerythrin (PE). In addition, 6–7 Z-plane images were acquired at each position for each fluorescence channel. The resulting z-stack images were combined using an extended depth of field algorithm to produce one in-focus image for each position and channel (Chla, DAPI, FITC, PE). These were then false colored (red, blue, green and orange, respectively) and combined into a single composite 24-bit RGB image for each position. Cell biovolumes (BV; µm³) were determined from length (L) and width (W) measurements according to Taylor et al. (2011) from images that passed quality inspection. Image processing and analysis was carried out in Image Pro software. Carbon (C; pg cell⁻¹) biomass was computed from BV from the equations: $C = 0.216 * BV^{0.939}$ for non-diatoms, and $C = 0.288 * BV^{0.811}$ for diatoms (Menden-Deuer and Lessard, 2000).

Seawater samples (150 mL) were also preserved with 5% acid Lugol's solution for separate analyses of ciliates, concentrated onto 25-mm 8.0-µm polycarbonate membranes and prepared as slides according to the protocol of Freibott et al. (2014). The slides were imaged on a Zeiss AxioVert 200 M inverted microscope at 200X magnification using brightfield illumination and processed using Image Pro software as described for EPI microscopy. Length and width measurements were used to calculate cell biovolumes (BV, µm³) based on the most appropriate cell shape, and carbon biomass was calculated as $pg\ C = 0.19 \times BV$ (Putt and Stoecker, 1989).

Processing Description

Data Processing:

Image Pro software.

BCO-DMO Processing:

- changed date form from MM/DD/YY to YYYY-MM-DD.

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Related Publications

Freibott, A., Linacre, L., & Landry, M. R. (2014). A slide preparation technique for light microscopy analysis of ciliates preserved in acid Lugol's fixative. *Limnology and Oceanography: Methods*, 12(1), 54–62.

doi:[10.4319/lom.2014.12.54](https://doi.org/10.4319/lom.2014.12.54)

Methods

Landry, M. R., Selph, K. E., Stukel, M. R., Swalethorp, R., Kelly, T. B., Beatty, J. L., & Quackenbush, C. R. (2021). Microbial food web dynamics in the oceanic Gulf of Mexico. *Journal of Plankton Research*.

doi:[10.1093/plankt/fbab021](https://doi.org/10.1093/plankt/fbab021)

Results

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](https://doi.org/10.4319/lo.2000.45.3.0569)

Methods

Putt, M., & Stoecker, D. K. (1989). An experimentally determined carbon : volume ratio for marine

“oligotrichous” ciliates from estuarine and coastal waters. *Limnology and Oceanography*, 34(6), 1097–1103. doi:[10.4319/lo.1989.34.6.1097](https://doi.org/10.4319/lo.1989.34.6.1097)

Methods

Selph, K.E., Swalethorp, R., Stukel, M.R., Kelly, T.B., Knapp, A.N., Fleming, K., Hernandez, T., & Landry, M.R. (2021). Phytoplankton community composition and biomass in the oligotrophic Gulf of Mexico. *Journal of Plankton Research*. doi:[10.1093/plankt/fbab006](https://doi.org/10.1093/plankt/fbab006)

Results

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

Taylor, A. G., Landry, M. R., Freibott, A., Selph, K. E., & Gutiérrez-Rodríguez, A. (2015). Patterns of microbial community biomass, composition and HPLC diagnostic pigments in the Costa Rica upwelling dome. *Journal of Plankton Research*, 38(2), 183–198. doi:[10.1093/plankt/fbv086](https://doi.org/10.1093/plankt/fbv086)

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](https://doi.org/10.1016/j.dsr2.2010.08.017)

Methods

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Parameters

Parameter	Description	Units
Cruise	Cruise identifier	unitless
Station	Station number	unitless
Date	Sampling date (Central Standard (GMT-6)); format: YYYY-MM-DD	unitless
Lat	Latitude	degrees North
Long	Longitude	degrees East
Cycle	Cycle number	unitless
CTD	CTD cast number	unitless
Exp	Experiment number	unitless
Depth	Depth of sample	meters (m)
A_Diat	Diatom carbon biomass (ug C/L) from epifluorescence (EPI) microscopy	micrograms Carbon per liter (ug C/L)
A_Dino	Phototrophic Dinoflagellate carbon (ug C/L) from EPI with chloroplasts, includes mixotrophs	micrograms Carbon per liter (ug C/L)
A_Prym	Identifiable Prymnesiophyte carbon (ug C/L) from EPI with chloroplasts, includes mixotrophs	micrograms Carbon per liter (ug C/L)
A_Crypt	Identifiable Cryptophyte carbon (ug C/L) from EPI with chloroplasts, includes mixotrophs	micrograms Carbon per liter (ug C/L)
A_Other	Other Phototrophs (ug C/L), cells from EPI and	micrograms Carbon per liter (ug C/L)

H_Dino	Heterotrophic Dinoflagellate carbon (ug C/L), cells without chloroplasts from EPI	micrograms Carbon per liter (ug C/L)
H_Other	Other Heterotrophs (ug C/L), cells without chloroplasts from EPI	micrograms Carbon per liter (ug C/L)
H_Cil	Ciliate carbon (ug C/L) from inverted microscopy of acid Lugol's preserved samples	micrograms Carbon per liter (ug C/L)
A_Euk_lt_2_um	Carbon biomass (ug C/L) of < 2-um phototrophic eukaryotes from flow cytometry	micrograms Carbon per liter (ug C/L)
A_Euk_2_5_um	Carbon biomass (ug C/L) of 2-5 um phototrophic eukaryotes from flow cytometry	micrograms Carbon per liter (ug C/L)
A_Euk_5_10_um	Carbon biomass (ug C/L) of 5-10 um phototrophic eukaryotes from EPI	micrograms Carbon per liter (ug C/L)
A_Euk_10_20_um	Carbon biomass (ug C/L) of 10-20 um phototrophic eukaryotes from EPI	micrograms Carbon per liter (ug C/L)
A_Euk_20_40_um	Carbon biomass (ug C/L) of 20-40 um phototrophic eukaryotes from EPI	micrograms Carbon per liter (ug C/L)
A_Euk_gt_40_um	Carbon biomass (ug C/L) of >40 um phototrophic eukaryotes from EPI	micrograms Carbon per liter (ug C/L)
H_Euk_2_5_um	Carbon biomass (ug C/L) of 2-5 um heterotrophic eukaryotes from EPI	micrograms Carbon per liter (ug C/L)
H_Euk_5_10_um	Carbon biomass (ug C/L) of 5-10 um heterotrophic eukaryotes from EPI	micrograms Carbon per liter (ug C/L)
H_Euk_10_20_um	Carbon biomass (ug C/L) of 10-20 um heterotrophic eukaryotes from EPI	micrograms Carbon per liter (ug C/L)
H_Euk_20_40_um	Carbon biomass (ug C/L) of 20-40 um heterotrophic eukaryotes from EPI	micrograms Carbon per liter (ug C/L)
H_Euk_gt_40_um	Carbon biomass (ug C/L) of >40 um heterotrophic eukaryotes from EPI	micrograms Carbon per liter (ug C/L)
Tot_A_Euk	Total carbon biomass (ug C/L) of phototrophic eukaryotes	micrograms Carbon per liter (ug C/L)
Tot_H_Euk	Total carbon biomass (ug C/L) of heterotrophic eukaryotes	micrograms Carbon per liter (ug C/L)

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Instruments

Dataset-specific Instrument Name	Niskin bottles
Generic Instrument Name	Niskin bottle
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.

Dataset-specific Instrument Name	CTD
Generic Instrument Name	CTD profiler
Generic Instrument Description	The Conductivity, Temperature, Depth (CTD) unit is an integrated instrument package designed to measure the conductivity, temperature, and pressure (depth) of the water column. The instrument is lowered via cable through the water column and permits scientists observe the physical properties in real time via a conducting cable connecting the CTD to a deck unit and computer on the ship. The CTD is often configured with additional optional sensors including fluorometers, transmissometers and/or radiometers. It is often combined with a Rosette of water sampling bottles (e.g. Niskin, GO-FLO) for collecting discrete water samples during the cast. This instrument designation is used when specific make and model are not known.

Dataset-specific Instrument Name	Zeiss AxioCam MRc black and white 8-bit CCD camera
Generic Instrument Name	Camera
Generic Instrument Description	All types of photographic equipment including stills, video, film and digital systems.

Dataset-specific Instrument Name	Zeiss Axiovert 200M inverted compound microscopy
Generic Instrument Name	Inverted Microscope
Generic Instrument Description	An inverted microscope is a microscope with its light source and condenser on the top, above the stage pointing down, while the objectives and turret are below the stage pointing up. It was invented in 1850 by J. Lawrence Smith, a faculty member of Tulane University (then named the Medical College of Louisiana). Inverted microscopes are useful for observing living cells or organisms at the bottom of a large container (e.g. a tissue culture flask) under more natural conditions than on a glass slide, as is the case with a conventional microscope. Inverted microscopes are also used in micromanipulation applications where space above the specimen is required for manipulator mechanisms and the microtools they hold, and in metallurgical applications where polished samples can be placed on top of the stage and viewed from underneath using reflecting objectives. The stage on an inverted microscope is usually fixed, and focus is adjusted by moving the objective lens along a vertical axis to bring it closer to or further from the specimen. The focus mechanism typically has a dual concentric knob for coarse and fine adjustment. Depending on the size of the microscope, four to six objective lenses of different magnifications may be fitted to a rotating turret known as a nosepiece. These microscopes may also be fitted with accessories for fitting still and video cameras, fluorescence illumination, confocal scanning and many other applications.

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Deployments

NF1704

Website	https://www.bco-dmo.org/deployment/834975
Platform	R/V Nancy Foster
Report	https://datadocs.bco-dmo.org/docs/302/BLOOFINZ_IO/data_docs/cruise_reports/NF1704_CRUISE_REPORT.pdf
Start Date	2017-05-07
End Date	2017-06-02
Description	R/V Nancy Foster cruise in May 2017 as part of a NOAA RESTORE project (aka: BLOOFINZ-GoM).

NF1802

Website	https://www.bco-dmo.org/deployment/834976
Platform	R/V Nancy Foster
Report	https://datadocs.bco-dmo.org/docs/302/BLOOFINZ_IO/data_docs/cruise_reports/NF1802_CRUISE_REPORT.pdf
Start Date	2018-04-27
End Date	2018-05-20
Description	R/V Nancy Foster cruise in May 2018 as part of a NOAA RESTORE project (aka: BLOOFINZ-GoM).

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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 ^{14}C productivity, N_2 fixation, $^{15}\text{NO}_3^-$ 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), ^{15}N -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-1851558
National Oceanic and Atmospheric Administration (NOAA)	NA15OAR4320071

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