PE23_20_Thrash: MDOR (Microbial DO Respir)
Detailed cruise plan

Ship/port
R/V Pelican
Departure: Louisiana Universities Marine Consortium (LUMCON), Cocodrie, LA
Return: LUMCON

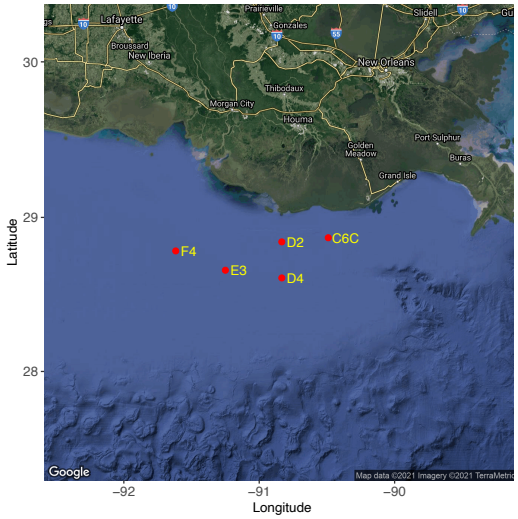
Dates
Mobilization: 05/01/23
Departure: 05/02/23
Return: 05/07/23
Demobilization: 05/08/23
Duration: 6 days at sea

Science party
Thrash Lab, University of Southern California
J. Cameron Thrash, Chief Scientist
Jordan T. Coelho, Ph.D. Candidate
Shelby Barnes, Research Technician
Zack Henning, Ph.D. Student

Baker Lab, University of Texas at Austin
Valarie De Anda, Research Associate
Kathryn Appler, Ph.D. Candidate
Emily Aguilar-Pine, Undergraduate Researcher

Waypoints

Station	Lat	Long	Depth (m)
C6C	28.8686	-90.4903	19.2
D2	28.8417	-90.8333	15.6
D4	28.6083	-90.8333	19.1
E3	28.6583	-91.25	22
F4	28.7833	-91.6167	24.2



Station map

Cruise track and timing
We will proceed from LUMCON to station C6C, conduct science operations (detailed below), then proceed to D2, D4, E3, and finally F4, repeating scientific operations at each station. After operations are concluded at station F4, we will sail back to LUMCON. The table below contains approximate distances and travel times for this cruise track.

Start	End	Approx. distance (n miles)	Approx. transit time (@ 8 kts)
LUMCON	C6C	26	3 hr 15 min
C6C	D2	18	2 hr 20 min
D2	D4	14	1 hr 45 min
D4	E3	22	2 hr 45 min
E3	F4	21	2 hr 40 min
F4	LUMCON	72	9 hr

Thrash Lab Science operations
At each station, we will be performing casts of the CTD/Niskin rosette to collect a station water column profile and discreet water samples at the bottom (variable) and at the surface (2 m). We will use half the Niskins for bottom water collection, and half for surface water collection.

For the University of Southern California team, water will be apportioned for filtration, flow cytometry, bottle incubations, and cryostocks, according to the **Science operations schematic** on the next page. We will be conducting bottom water incubations in an electric dry cold incubator that we will bring with us. Surface water incubations will be conducted in a light-proof tub connected to the surface water flow through system in the aft starboard section of the main wet lab.

The longest incubations will be for the respiration measurements (up to 24 hrs), but we have two sets of bottles so that we can begin a second set of incubations before the first set concludes. Thus, our only limiting factor for collection at a new station is whether the incubations from two prior stations have finished. However, given the relatively short transit times and the fact that we only have 5 planned stations, we anticipate staying on station for most of each day, and we may elect to perform additional CTD/Niskin rosette casts for additional sample filtration.

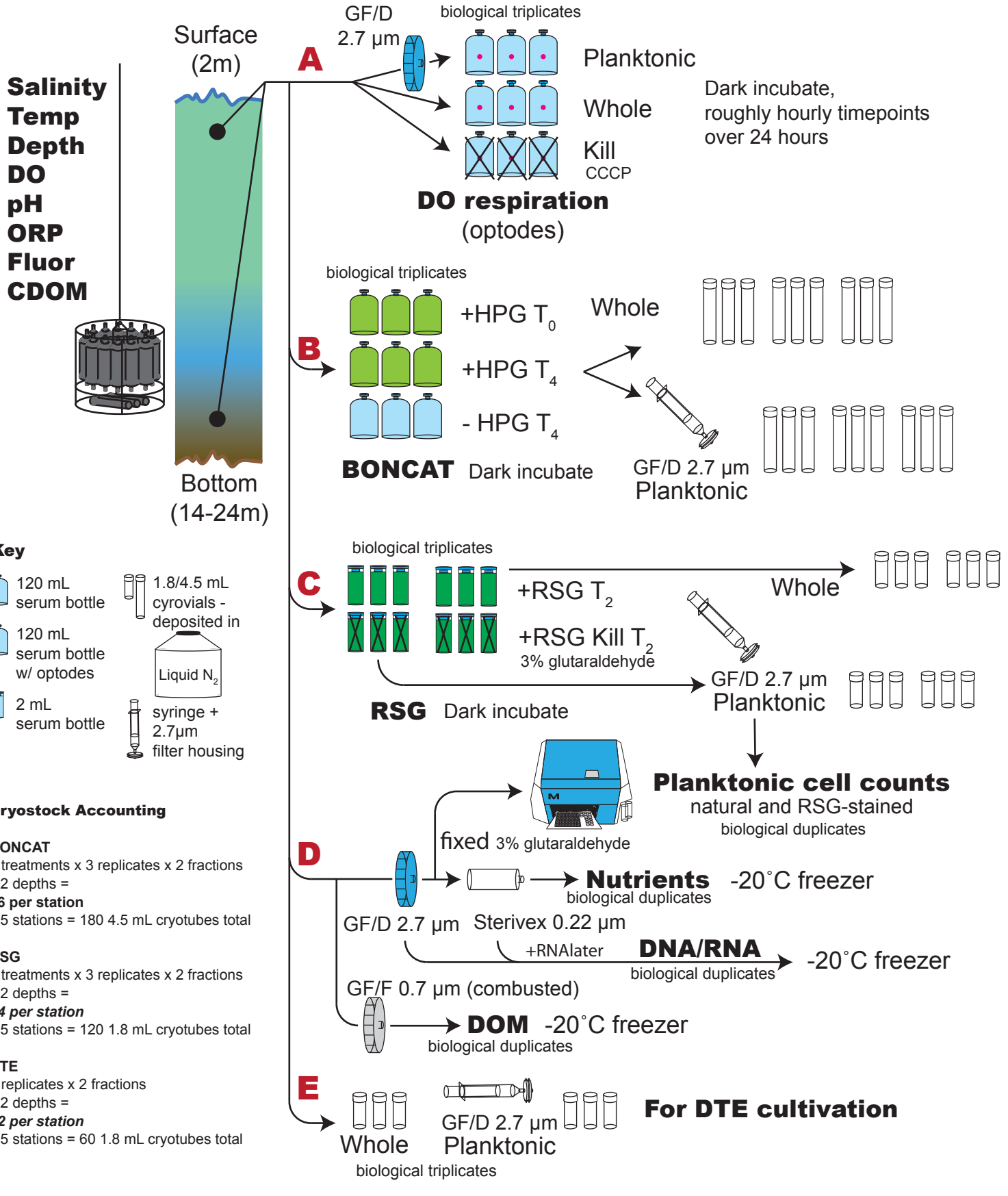
The University of Texas at Austin team would like to collect surface sediment samples as described below when there is a convenient time in between CTD casts. These sediment samples will be used for nucleic acid extraction aboard ship, ideally in the smaller dry lab (see **Baker Lab** information below).

Liquid N_2. Many of our material collections will be placed in cryovials and then stored in dry shippers. Dry shippers are charged with liquid N_2, but don't contain any liquid N_2, and will remain in the main lab. We may bring an additional liquid N_2 dewar for extra storage. These will be filled on the dock during mobilization from a large liquid N_2 supply dewar. We would like to bring the large parent liquid N_2 dewar aboard the ship for easy recharging of the storage dewars. This will also prevent the need to order a second supply dewar. Ideally, a LUMCON representative will order the liquid N_2 supply dewar for delivery prior to mobilization.

Hazardous Materials. In addition to the liquid N_2, the other hazardous materials we will be bringing are glutaraldehyde (25%), hydrochloric acid (38%), and sodium hydroxide (5N). Glutaraldehyde will be used as a fixative for negative controls in our respiration and redox sensor green incubations. It will be diluted into stock solutions, and these will be added to incubation bottles either in the fume hood or outside on the deck. Hydrochloric acid and sodium hydroxide will be diluted to 0.1N for use in cleaning/sterilizing filtration tubing and incubation bottles. All three of these chemicals will be stored in secondary containment in their concentrated form.

Thrash Lab shipboard science operations, PE23-20, R/V Pelican

This schematic illustrates the science activities to be conducted aboard the R/V Pelican during the May, 2023 Microbial DO Respiration cruise (PE23-XX) at each station by the Thrash Lab members. Five CTD casts will be conducted to examine water column stability throughout a ~16 hour period, with water for sections A-E used from the first cast. Data and research products of different types are noted in bold black. T_x designations indicate collection timepoints at X hours.



Cryostock Accounting

BONCAT
3 treatments x 3 replicates x 2 fractions x 2 depths =
36 per station
x 5 stations = 180 4.5 mL cryotubes total

RSG
2 treatments x 3 replicates x 2 fractions x 2 depths =
24 per station
x 5 stations = 120 1.8 mL cryotubes total

DTE
3 replicates x 2 fractions x 2 depths =
12 per station
x 5 stations = 60 1.8 mL cryotubes total

Baker Lab

Science operations

At each station (previously described), we will use the available **multi-corer** (Ocean Instruments MC-800 Multi-corer Deep Ocean Sediment Sampler) and, if necessary, the available **box corer** (0.1 m2 Gomex type Stainless Steel Box Grab) to collect sediment samples between CTD cast measurements. We will need 2-3 casts of the multi-corer, which collects 8 sediment cores per cast, depending on the depth of core samples. Depending on the quality of the multi-corer samples, we may request an additional box core to ensure an adequate amount of sediment is collected.

Samples format

These sediments will be collected and apportioned for nucleic acid extraction (DNA and RNA), fluorescence in situ hybridization (FISH) fixation, live culture preparation (glycerols), and sediment sample preservations in available lab space. We will collect pore water from one of the segmented cores to preserve for viral filtration and analysis. For each site, we plan to have 20 x 2 mL DNA, 20 x 2 mL RNA, >10-20 x 15 mL FISH, 20 x 2 mL live culture preps, 6 x 15 mL pore water samples, and remaining sediment preserved for future analysis (See schematic below for more detailed information). In total for the expedition, we plan to have 100 x 2 mL DNA, 100 x 2 mL RNA, >50-100 x 15 mL FISH, 100 x 2 mL live culture preps, 30 x 15 mL pore water samples, and all remaining sediment preserved in 15 mL tubes.

The University of Texas at Austin team will bring a microcentrifuge and vortex mixer for extractions and preservations. The anticipated one day per station will be ample time for the University of Texas at Austin team to perform sediment collections and lab work for each station's samples. We will finish FISH preparation steps the following day or during the return to port.

Liquid N_2. In addition to the University of Southern California's dry shippers, the University of Texas at Austin team will bring their own dry shipper for storage (SIZE) for cryovial samples. The remaining fixed samples will be stored in the -20 C freezer. Dry shippers are charged with liquid N_2, but don't contain any liquid N_2, and will remain in the main lab.

Hazardous Materials. In addition to the liquid N_2, we will bring absolute ethanol and paraformaldehyde (4%) in PBS. Our team will use absolute ethanol in the FISH fixation steps and a diluted stock solution as a cleaning agent (70%). Paraformaldehyde (4%) in PBS will also be used in the FISH fixation steps. Below we provide a list of all chemicals to be brought onboard with the amount and quantities (Tables 1-4). We will include hardcopies of the SDS sheets and specific protocols in the containers transporting the listed chemicals. Chemicals will be transported on board as 6 bottles, two spray bottles, and 4 extraction kits (boxes). This will be roughly 6L of fluids transported in addition to chemicals contained in the kits.

Table 1: Chemicals to bring onboard for extractions and preservation.

Item Name	CAS	Catalog #/Product #	Vendor	Amount	Quantity	SDS	Intended Use
Paraformaldehyde (PFA), 4% in PBS	see components ;SDS	J19943.K2	ThermoFisher	1 L	1	sds	FISH microscopy
absolute ethanol	64-17-5	61509-0010	Acros Organics N.V.	1L	1	sds	FISH microscopy, etc.
Phosphate-buffered saline (PBS,10x), pH 7.6	see components ;SDS	J62692-K3	ThermoFisher	2L	1	sds	FISH microscopy
ZR Soil/Fecal Microprep Kit 50 Preps	see SDS	76020-638	Qiagen	NA	2	sds	RNA extraction
Dneasy PowerSoil Pro Kit (50)	see SDS	47014	Qiagen	NA	2	sds	DNA extraction
Glycerol 99%	58-81-5	A16205-AP	ThermoFisher	500 ml	1	sds	sample preservation
Decontaminant RNAse Away Spray	NA	21-402-178	ThermoFisher	475 ml	1	NA	RNA/DNA extraction decontamination
Pre-prepped medium	see components ;SDS	NA	NA	1L	2	NA	live culture prep

Table 2: Components of the PFA 4% in PBS.

Components: Paraformaldehyde (PFA), 4% in PBS	CAS
Water	7732-18-5
Paraformaldehyde	30525-89-4
Sodium Chloride	7674-14-5
Sodium phosphate dibasic	7558-79-4
Dihydrogen potassium phosphate	7778-77-0
Potassium chloride	7447-40-7

Table 3: Components of the Phosphate-buffered saline.

Components: Phosphate-buffered saline (PBS,10x), pH 7.6	CAS
Water	7732-18-5
Sodium Chloride	7674-14-5
Sodium phosphate dibasic	7558-79-4
dihydrogen potassium phosphate	7778-77-0
potassium chloride	7447-40-7

Table 4: Components of the pre-prepped medium for live culture preservation.

Components: Pre-prepped medium	CAS
Difoco Marine broth 2216	10043-52-4
Glycerol	58-81-5

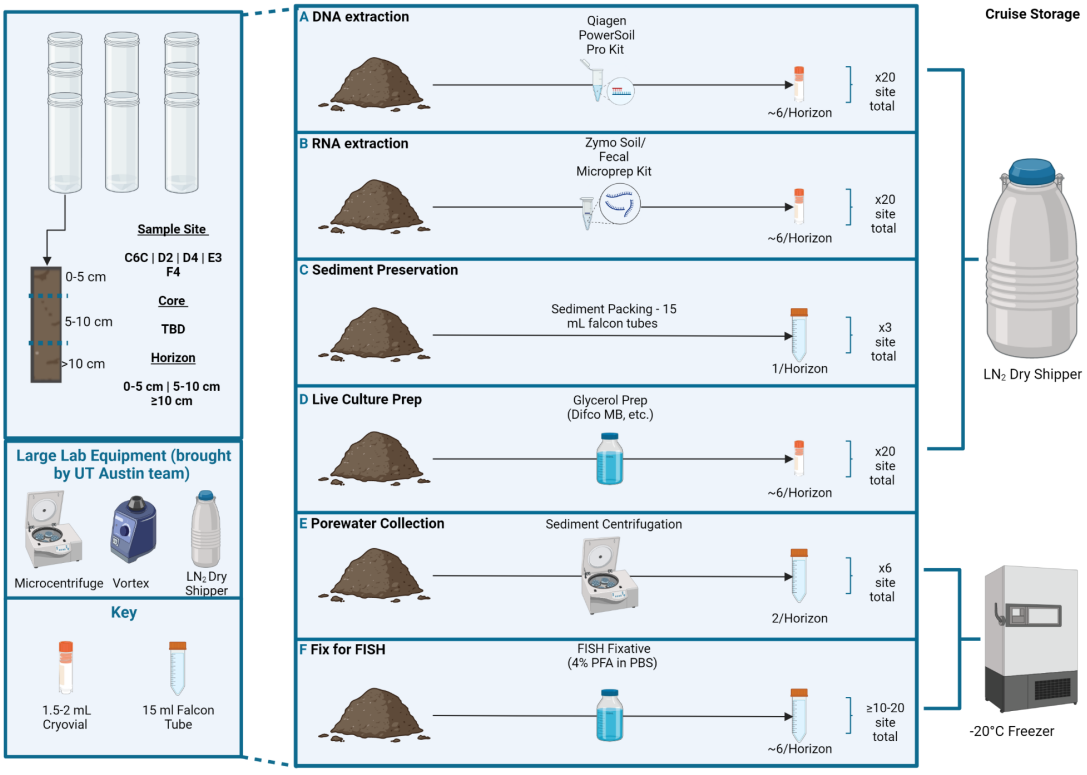


Figure 1: Schematic displaying the sampling, extraction, and preservation of samples from RV Pelican Cruise with all amounts of materials are shown per station. May 1-8, 2023 by the University of Texas at Austin Team.