CRUISE PLAN - CH-02-12_Stewart

This cruise will involve a combination of metagenomic sampling, gene expression profiling, and shipboard microcosm experiments to characterize microbial sulfur cycling and microbial community transcriptional responses to oxygen depletion in the hypoxic "dead zone" on the Louisiana Shelf west of the Mississippi River. A primary objective is to sample along gradients (vertical and longitudinal) extending from oxygen-enriched waters to the most oxygen-depleted regions of the hypoxic zone. Size predictions for the 2012 hypoxic zone west of the Mississippi are variable (~1200-6200 square miles) and the full extent of the zone will not be known until completion of the LUMCON monitoring survey (July 22-31), which will be conducted concurrent with our cruise. Near-real time monitoring of the LUMCON data will be possible and may help guide our sampling objectives. However, in the initial absence of these data, our proposed sampling will primarily target near-shore (<40 miles) sites that, based on prior years' data, are likely to be within the shelf hypoxic zone. Specifically, sampling will encompass ~8 stations (<50m water depth) on a rough west-east transect paralleling the Louisiana Coast, beginning at ~29.2°N, 94° W and ending ~28.6°N, 88.9° W. Time and weather permitting, approximately ~3-4 additional stations will be sampled along a north-south transect extending off the slope and shelf (~120 miles), beginning at ~ 28.9 °N, 89.6°W. Ideally, 1-2 of these stations in will be in deep water (>1500m). Please see attached schematic.

Water column sampling – <u>all stations</u>: At each station, general water column parameters will be assessed via vertical depth surveys using a CTD equipped with sensors for fluorometry, PAR, and dissolved oxygen. Seawater collections for nutrients (nitrate, nitrite, ammonium, phosphate), DNA, RNA, and single-cell genomics will be done via rosette casts to multiple depths (4-12 depth anticipated per profile) from the surface to the sediment-water interface (<30m depth at most shelf stations). At deeper slope stations (>300m) on the north-south transect, water column sampling for RNA may necessitate multiple single-depth casts. This is necessary to minimize time between bottle firing and arrival on deck, and thereby avoid changes in the RNA sample due to sample collection. At a subset (3-4) of the stations, additional seawater casts (likely to a single depth) will be required to collect water for shipboard microcosm experiments conducted over 2-3 days per experiment in the cold van. We anticipate that rosette-based water collection will require ~6 hours at the deeper stations involving multiple casts, and ~2-3 hrs at the on-shelf stations.

At one of the on-shelf stations we would like to spend additional time testing a recently developed device for collecting and preserving bacterioplankton RNA samples in-situ. This device has been engineered to mount within the rosette space normally occupied by a standard 10L Niskin bottle. The sampler operates via connections to standard CTD electronics. When triggered, the sampler begins pumping water through a set of two filters. After a specified time, the device then preserves the filtered biomass by pumping in a small amount of RNA stabilizing reagent. The sampler has been constructed (at Woods Hole) over the past few months but has not yet been deployed in situ. This cruise would be an ideal venue for testing and using the device. To do so, we anticipate deploying the sampler on a series of shallow-water rosette casts at one of the on-shelf stations sampled earliest in the cruise, potentially station 29.2°N, 93.9°W – please see attached. Ideally, these casts will take place every four hours over a 24-48 hr, in order to capture a diel transcriptional time-series. However, these intervals can adjusted to accommodate ongoing activities at this station (e.g., sediment coring).

Sediment coring – <u>shelf stations only</u>: Sediment core samples will be collected at each on-shelf station using a gravity corer and/or multi-corer. We anticipate collecting 1-2 cores per station, with an estimated prep and deployment time of ~5 hrs.

As the goals of the cruise participants may shift in response to field conditions and observations, we anticipate that the exact number and location of sampling stations might vary. We will (to the extent possible, and as determined by consultation with the captain and crew) try to accommodate the interests and objectives of all groups.