STANDARD OPERATING PROCEDURES (SOP)

for the

Northern Gulf of Mexico Hypoxia Shelf-Wide Survey Cruise

in support of the

Mississippi River/Gulf of Mexico Hypoxia Task Force Annual Monitoring Metric

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This SOP would not be possible without the many hypoxia colleagues and research associates that have participated in the cruise over the years, beginning in 1985.

Contents

I. INTRODUCTION

Hypoxia, typically regarded as low dissolved oxygen less than 2 mg l^{-1}, occurs throughout the world's coastal oceans. Hypoxic and anoxic waters (i.e., the absence of dissolved oxygen) can occur naturally, but its prevalence in shallow coastal and estuarine areas appears to be increasing, most likely accelerated by human activities (Diaz and Rosenberg 2008, Rabalais et al. 2010, 2014). For example, increases in nutrient inputs are directly linked to the population density in watersheds draining to coastal areas (Howarth et al. 1996, Caraco and Cole 1999) and increases in nutrient loads from agricultural activities (Turner and Rabalais 1991, Goolsby et al. 1999, Galloway et al., 2008, Rabalais et al. 2010, 2014, Turner et al. 2012).

The largest area of human-caused oxygen-depleted coastal waters in the United States is in the northern Gulf of Mexico on the Louisiana continental shelf, and it ranks second globally in bottom area covered among coastal areas. The threshold used to define hypoxia in the northern Gulf of Mexico are oxygen levels below 2 mg l^{-1}, because trawlers do not capture any shrimp or demersal fish in their nets below that value (Renaud 1986, Rabalais et al. 2001, 2010). The Gulf of Mexico hypoxic zone reached a record size of 22,700 km^2 in 2017 and has averaged 14,042 km^2 over the last five years (Figure 1) (http://www.gulfhypoxia.net), which is more than two and a half times the target size of the Hypoxia Action Plan goal of 5,000 km^2 (Mississippi River/Gulf of Mexico Watershed Nutrient Task Force 2001, 2008, 2015).

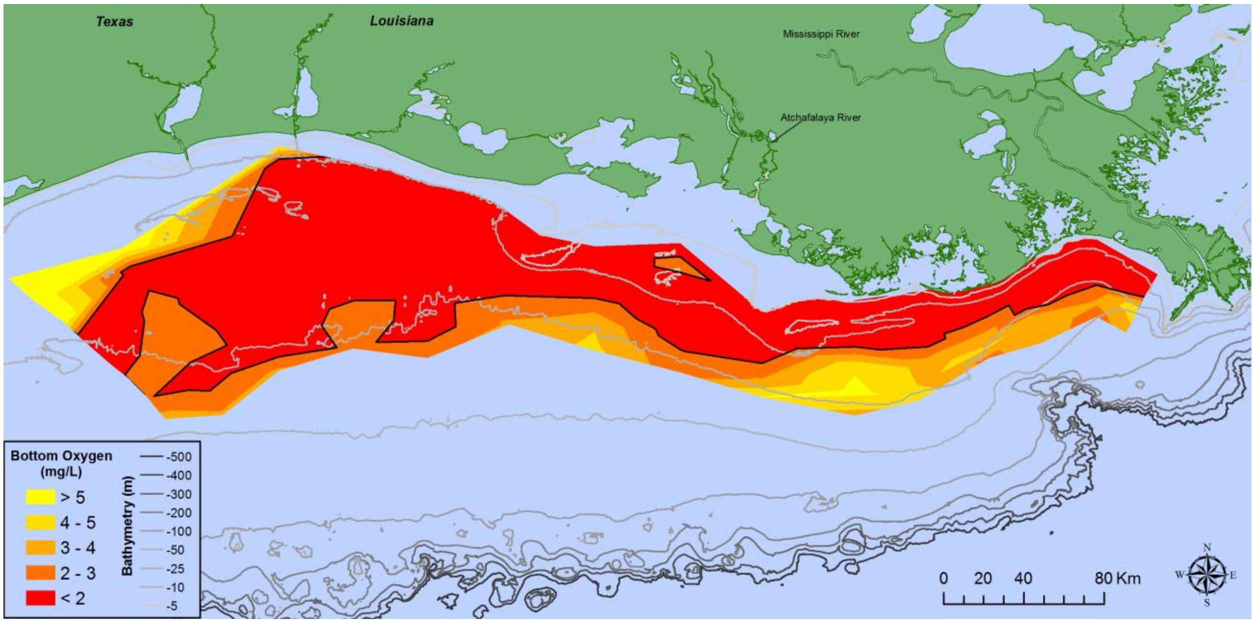

Figure 1. Size of bottom-water hypoxic area in 2017 with black line contour representing dissolved oxygen levels less than 2 mg l^{-1}.

A clearer understanding of the causes, consequences, and mitigation strategies associated with hypoxia in the Gulf of Mexico has emerged over the past three decades through numerous peer-reviewed publications, reports, and a U.S. EPA Hypoxia Assessment Panel (EPA-SAB-08-004, December 2007). An hypoxia advisory panel (SAB, 2007) recommended a reduction of 45-50% of nitrogen and phosphorus inputs into the Gulf of Mexico to meet the Action Plan goal of 5,000 km^2. The recently revised Action Plan (2015) maintains the original 5,000 km^2 goal, but the date to accomplish the goal has been revised to 2035, with a 20% interim nutrient reduction goal by 2025. The Action Plan goal incorporates an adaptive management approach that integrates monitoring, research, modeling, and education and outreach. As part of these efforts, continued observational studies have occurred for several decades and continue to be required as critical components for informing potential hypoxia mitigation and restoration efforts.

The first shelf-wide cruise to document the size of bottom-water hypoxia occurred in July 1985, as the second-ever cruise on LUMCON's R/V *Pelican*. The original cruise participants were Nancy Rabalais (LUMCON), Don Boesch (LUMCON), R. Eugene Turner (LSU) and William J. Wiseman, Jr. (LSU). Nancy Rabalais (LSU) and R. Eugene Turner (LSU) have continued to serve as the lead investigators or chief scientists on the cruises to date. Most cruises have used the R/V *Pelican*, but other vessels, including the R/V *Acadiana*, R/V *Tommy Munro*, and others, contracted by multiple hypoxia researchers, have surveyed the hypoxic zone over the 34 year history of the cruise. Original funding came from NOAA's National Ocean Service and has persisted over the years, primarily through competitive research awards from NOAA's National Ocean Service Center for Coastal Ocean Research and currently from NOAA's National Centers for Coastal Ocean Science via the Northern Gulf Institute. Other NOAA offices and funding agencies have also provided ad hoc support funding such as NOAA's National Undersea Research Program, Louisiana Sea Grant College Program, the US Environmental Protection Agency Gulf of Mexico Program, and the Louisiana Board of Regents Support Fund.

The main purpose of the summer cruise is to map the extent of hypoxia and the associated physical, chemical and biological parameters and to link the size with conditions in the Mississippi River watershed. The area of bottom-water hypoxia during the summer, has typically been mapped in late July from 1985 through 2018, except for 1989 and 2016. Hypoxic conditions were mapped in 1989, but for only half of the study area, due to lack of funds and a functional vessel was not available in 2016 to conduct the cruise. A grid of stations involving transects (lines of stations) provides the foundation for the annual cruise and has been modified and expanded over the years to expand west and east of the original grid to capture the full extent of hypoxia for a given year. Over the years, the cruise has provided an ideal platform (i.e., ship of opportunity) to leverage other studies and to work with collaborators to fully understand the dynamics of hypoxia in the Gulf of Mexico.

The number of historical summer hypoxia cruises (Figure 2) began as a minimal effort during the 1985-1997 timeframe and then expanded from 1998- 2007 with additional NOAA, MMS, and EPA funding and from other cruises for directed hypoxia-related research. Since then, funding has steadily decreased, returning to minimal levels in 2015 (Figure 2; Matli et al. 2018). The mid-summer cruise has been a core component that has remained throughout this period and represents the sole metric used by the Hypoxia Task Force to measure progress toward achieving it nutrient reduction loading goals to the northern Gulf of Mexico. Without the annual cruise survey and the long-history of measurements developed so far, the ability to detect changes, within the context of the natural variability of the system, would not be possible. Given the long history of the current methodology, any new benchmarks that may be developed for future metrics will have to be carefully evaluated to ensure transferability, consistency and reproducibility.

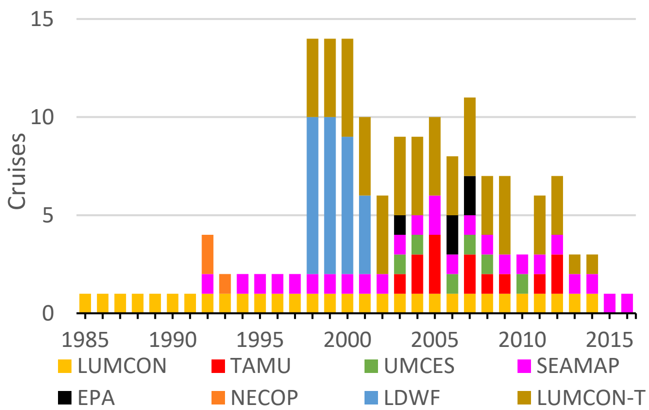

Figure 2. Figure taken from Matli et al. 2018. The number of State, Federal and university cruises associated with hypoxia measurements in the northern Gulf of Mexico from 1985 to 2016. LUMCON = Louisiana Universities Marine Consortium; SEAMAP = Southeast Area Monitoring and Assessment Program; TAMU = Texas A&M University; UMCES = University of Maryland Center for Environmental Studies; EPA = U.S. Environmental Protection Agency; NECOP = Nutrient Enhanced Coastal Ocean Productivity; LDWR = Louisiana Department of Wildlife Research; LUMCON-T = transects sampled during the year by LUMCON. The graph was created by Rohith Matli and Dan Obenour, Department of Civil, Construction, and Environmental Engineering, North Carolina State University, and used with permission. Data not represented include LUMCON 2015 and 2017, and LUMCON-T for 2016.

II. OBJECTIVES

The objectives of this document are to:

1. Provide a consistent methodology to assess the spatial and temporal extent of hypoxia in the Northern Gulf of Mexico;
2. Maintain a sequential series of long-term data that document the temporal and spatial extent of hypoxia in the region; and
3. Ensure consistent analysis of collected data and area calculation of the annual long-term metric (benchmark) used by the Mississippi River Nutrient/Gulf of Mexico Hypoxia Task Force.

In the following sections, we describe the current methodology and instrumentation used to assess the extent of bottom-water hypoxia and ancillary environmental parameters in the northern Gulf of Mexico west of the Mississippi River. These should provide a methodology available to current or future researchers determining the bottom-water hypoxia size for the federal/state Hypoxia Task Force. Should the methodology change, new procedures should be employed along with a new benchmark.

Data and metadata (compliant with ISO19115 standards) can be accessed through databases with the National Oceanic and Atmospheric Administration (NOAA) and the National Center for Environmental Information (formerly, the National Oceanographic Data Center).

III. VESSEL REQUIREMENTS AND PRE-CRUISE ACTIVITIES

The annual hypoxia cruise survey, used as the HTF metric, is consistently collected during the third or fourth week of July to coincide with the expected maximal extend of hypoxia in the Gulf of Mexico. Other cruises collected outside of this time period (e.g., in June and August) typically do not obtain as large an area of mapped bottom-water hypoxia as in the latter half of July (Rabalais et al. 2017). Recent work, Matli et al. 2018, identifies how often the peak period in hypoxia occurs historically.

Several pre-cruise activities are essential weeks to months ahead of the July survey:

- Procure an appropriate research vessel that can operate in the northern Gulf of Mexico west of the Mississippi River and access depths as shallow as 5 m. Another consideration is that the vessel has the authority to operate among the many large (manned) and small (unmanned) oil platforms present in the mapping area. If the vessel is a member of the UNOLS (University National-Oceanographic Laboratory System) fleet, the requests for time on the vessel should be made several years in advance.
- Establish a research cruise date as close as possible to the last week in July for maximal comparative analyses with prior years. It will be necessary to work with the Operator of the vessel on alternate dates, even when already scheduled.
- Develop a Pre-Cruise Plan with the Operator, including request for on-board instrumentation (Appendices A.1 and A.2), electronic technician coverage for a 24-h day, and scientific berths.
- Develop a list of cruise activities relevant to the mapping of the bottom-water dissolved oxygen levels and ancillary environmental parameters. Determine and develop configuration of inside the vessel space for research activities.
- Explore opportunities for additional researchers and investigations to conduct ancillary and complementary sampling outside of hypoxia parameters.
- Make sure the appropriate instrumentation, chemicals, and supplies are available.
- Designate a co-Chief Scientist, and work with that individual prior to and during the cruise. Determine crew assignments for 24-h coverage of activities (Appendix A.3).
- Develop appropriate chemical SDS sheets to take on the cruise.
- A binder of necessary information and log sheets is prepared before each summer cruise and taken onboard. It includes general information, such as Mississippi River discharge at time of departure (Appendix A.4), marine forecast at time of departure (Appendix A.5),

prior cruise maps, blank station logs (Mississippi River and routine stations; Appendix B.2), procedures for laboratory measurements, log sheets for data determined on board, list of scientific party with shift and duties, and SDS sheets for all chemicals brought on board by scientific party. The purpose of this notebook is to serve as a central location to house data as it is collected (especially Station Logs and color graphs of CTD parameters), to which all scientific staff can refer to during the cruise.

- Review cruise policy to ensure safety equipment and personnel documentation will be available in accordance with all organizations that will attend the cruise.
- The usual chartered vessel, the RV *Pelican*, is a UNOLS vessel and meets high safety standards of the academic fleet and all U.S. Coast Guard Regulations for a 300-ton vessel. A required vessel safety meeting is conducted prior to departure, usually late evening of night of departure, for safety equipment and its location and use, space restrictions, man overboard protocols, fire drill protocols, sounding of general alarm, introduction of crew, and general vessel protocols. A medical form is filled out for each scientific crew participant.
- A briefing by the Chief Scientist is also conducted prior to departure to describe the cruise objective, shifts, protocols for being on station, general operations, and science crew responsibilities and obligations.

IV. CRUISE PROTOCOLS

General Protocols

The goal of the shelf-wide hypoxia cruise is to map the bottom-water hypoxic area within the shortest time available, making it quasi-synoptic, or until the research vessel is to be returned to the Port of Call, Cocodrie (based on available funding).

Operations are 24-h with two shifts of 7am – 7pm and 7pm – 7am, coinciding with timing of sunup and sundown for Secchi disk depth measurements. Each shift includes a ship electronic technician (Marine Tech) responsible for operation of the CTD and other instrumentation, a designated shift leader (Chief scientist or co-Chief Scientist), and a minimum of four individuals for deck CTD operations, other water collections, and running the necessary underway laboratory analyses (Appendix A.3).

Time on station is approximately 30 min, time between stations is approximately 1 h, and time between transects is approximately 2 h (Note: time between stations and transects closest to the Mississippi River, then west to south of Cocodrie is much shorter). The return time to the dock needs to be estimated as the ship moves further to the west. The time can be as much as 20 to 24 h. Estimating the time to return to dock is necessary to meet the requirements of the vessel for other charters.

The number of transects and stations determines whether the total area is mapped. The Chief Scientist determines the selection of stations/transects that can best overlap with previous year’s mapping as well as complete a quasi-synoptic survey in the time allotted. The ability to identify stations to collect data is based on several prior years of research data. There is a balance between getting the most bottom-water data as possible with continuing to re-occupy prior years' stations for a longer-term data base. The Chief Scientist determines the transects and station progression and provides guidance to the co-Chief Scientist on the opposite 12-h shift for which transects/stations should be occupied. The Chief Scientist is on call 24-h to help guide station selection, or other problems that might arise.

The sequence of stations consists of western movement in an offshore or inshore direction until there is no longer bottom-water hypoxia or a routinely collected station is occupied. If the bottom-water dissolved oxygen at the offshore end of a transect is less than 2 mg l^{-1}, the ship continues to move offshore to determine the end of the bottom-water hypoxia. Once bottom-water dissolved oxygen is greater than 2 mg l^{-1} in the offshore direction for two consecutive stations (a general rule), the ship moves west to an adjacent transect to occupy a similar dissolved oxygen concentration at the bottom (a station of similar depth is targeted). Inshore movement along a transect continues to the nearshore depth to which the R/V *Pelican* can operate (4-5 m). Approach to the shallow stations is slow so that the water column is not mixed. Hypoxia is often encountered at these depths, and a complete transect to delineate inshore hypoxia extent is not always possible due to the depth limitation of the vessel.

In each summer cruise, there is an effort to document all stations less than 2 mg l^{-1}, but logistics and time do not always allow for this (Figure 4). If the time remaining to determine the size of bottom-water hypoxia becomes limited, some stations may be skipped to determine a better defined map. Decisions are made during the cruise that may or may not determine a better-defined bottom-water hypoxic area. Thus, the summer estimate (http://www.gulfhypoxia.net) is always defined as an 'estimate' and not a 'definite' delineation of summer hypoxia.

Layout of Transects and Stations

We routinely depart from the Cocodrie dock of the R/V *Pelican* at 0000 of the first day of the shelf-wide cruise to arrive at the mouth of Southwest Pass of the Mississippi River delta early that morning.

The goal at Southwest Pass is to generate a salinity mixing diagram with Mississippi River nutrient concentrations for conservative versus non-conservative mixing. Stations are determined by the R/V *Pelican* MIDAS flow through system within a range of salinities of 0 to 35 psu. As useful a range of salinities as can be obtained is a mixture of the research vessel's ability to find adequate salinities and the research team's determination of onboard salinity and other environmental parameters. After an adequate salinity range has been established, the research vessel moves to station A′1, the most nearshore station adjacent to the Mississippi River delta. If an adequate range of salinities is not determined within 3 to 4 h, then the ship departs to the first station.

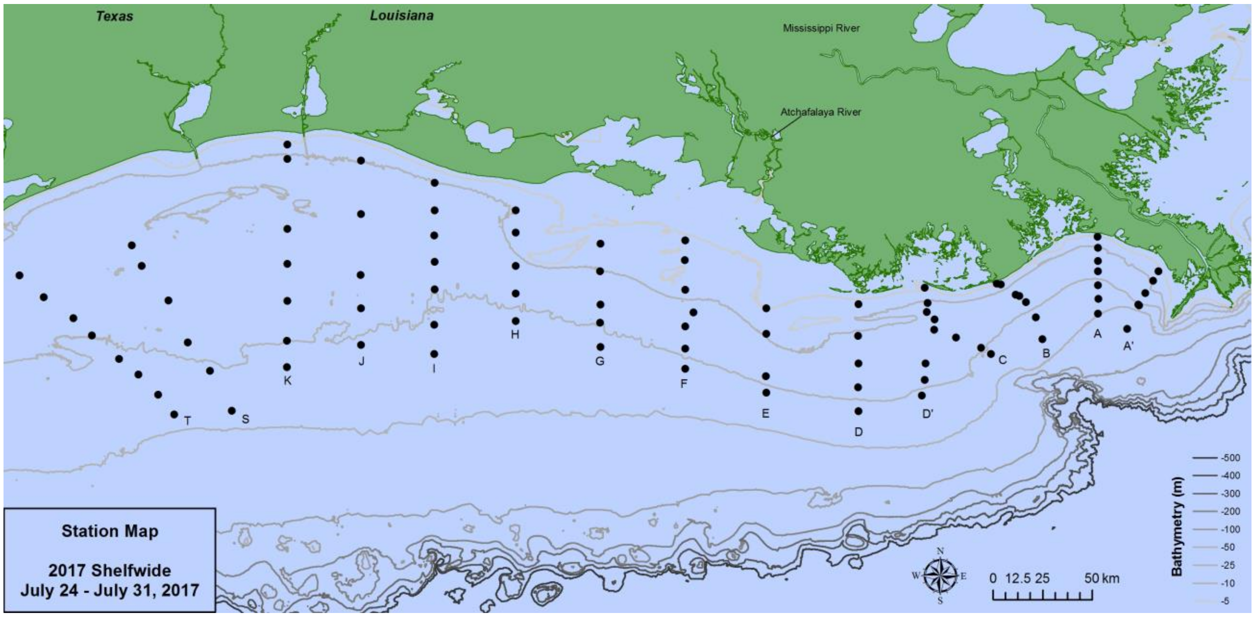

Figure 3. Map of shelf-wide sampling locations.

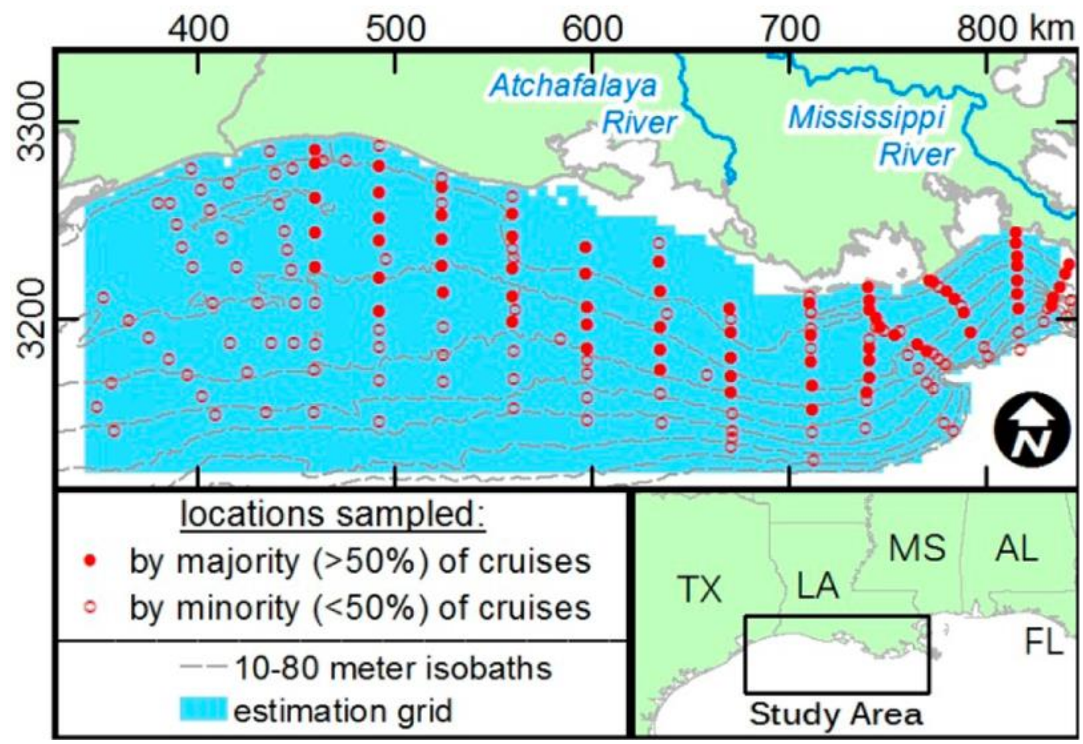

Figure 4. Stations most often sampled and those less frequently sampled (from Obenour et al. 2013).

During the July shelf-wide cruise, stations are sampled along 15 generally perpendicular-to-shore (mostly north-south) transects across the Louisiana and Texas coastal shelves (Figure 3) to delimit and describe the area of midsummer bottom dissolved oxygen less than 2 (mg l^{-1}). An electronic file is maintained of all station locations and edited as necessary (Appendix B.1).

Transects are sampled typically moving from east to west. On rare occasions (e.g., 2010), due to hurricanes and tropical storms in the east, western transects may be visited first. Typically, the first stations sampled are those located within the mouth of the Mississippi River. From there the research vessel travels to station A'1 and southward along the A' transect. Once the southern end of the A' transect is reached, the research vessel travels west to the offshore end of the A transect and samples from south to north. Most stations through transect K are visited each year (Figure 4) regardless of the presence of hypoxia for long-term continuity of data and documentation of shelf hydrology, with or without hypoxia. Transects to the west of the K transect may be occupied if the hypoxia continues to be well-developed on the western end of the study area.

Multiple instruments are used to ensure sampling locations are properly logged. SeaBird times and locations of operations are logged from R/V *Pelican*'s Multiple Instrument Data Acquisition System (MIDAS), which is maintained by the ship's Electronic Technician. Navigational data are acquired from a Starlink differential GPS (http://www.starlinkdgps.com/) or a Trimble GPS (http://www.trimble.com/) with a Micronet Receiver Station with sub 5-meter accuracy. Station depths are logged from the ship's Odom Echotrac II (http://www.odomhydrographic.com/) fathometer.

At each station, positions are logged from the R/V *Pelican*'s differential GPS at the beginning of sampling operations. GPS manufacturer's accuracy claim is 1-5 meters 95% of the time. Wind, current, and tidal forces may cause vessel drift from the beginning position. The ship is moved back to the designated lat/lon if it has drifted more than 500 m away. A Captain's log also hand notes the location and activities. A handwritten log of CTD operations and any technological issues is maintained by the Electronic Technician. The Chief Scientist determines the protocol for the CTD (Appendix B.4) and also maintains a hand-written log (Appendix B.3) of some of the instrument data, depth of Niskin bottle collections, and information from the YSI 6820**.**

V. PRE-CRUISE SENSOR CALIBRATION OF SEABIRD AND YSI 6820

Several instruments are calibrated in accordance with the manufacturer recommendations. SeaBird SBE91+ CTD probes are factory calibrated. Before each cruise, the YSI 6820 multimeter sonde (YSI 6820) is calibrated for specific conductivity (SpC), salinity, oxygen, pH, and chlorophyll according to the manufacturer's instructions (Appendix B.6). The YSI 6820 calibration is not usually changed during the cruise, but the sonde is post-calibrated in the laboratory.

Because the R/V *Pelican* routinely has charters that work in very deep offshore waters, where exact measures of depth are not so critical, we found it good practice to have the Marine Technician zero the pressure sensor located on the CTD rosette before Hypoxia Cruise departure. This ensures that we could more accurately profile and then target thin layers of low oxygen for water collection.

SeaBird CTD probes are returned to the factory at least annually for inspection and service. The YSI sonde and/or individual probes are returned to the factory for inspection and repair, as necessary. After calibration, but prior to departure, the YSI 6820 and the SeaBird CTD data are compared with Winkler titrations. All instruments must be within acceptable error ranges of each other before the cruise departs. Winkler titrations are used to determine which SeaBird oxygen probe (primary or secondary) is the most consistent with the Winkler measurements. Measurements from both the primary and the secondary oxygen probes are recorded automatically in the CTD data files during the cruise, but the sensor most consistent with the pre-cruise titration comparison is used for preliminary mapping of the distribution of bottom-water dissolved oxygen concentrations.

Key Points: It is important that the pressure sensors are carefully zeroed pre-cruise to ensure sampling location depths are accurate. It is also imperative to have a dialogue with the winch operator regarding hand signals to ensure sampling occurs right at the bottom and at the correct depths above the water column. It is also valuable to bring the sensor just off the bottom, usually dropping the equipment until a weight, located just below the equipment, hits the bottom and then pulling up very slightly, so that sediment is not captured in the Niskin sample. Any Niskin sample that captures sediment must be resampled. Outlined below is a list of measured parameters along with an overview of the calibration process and acceptable ranges for each parameter.

Oxygen calibration

SeaBird oxygen probes are calibrated by the manufacturer. The YSI 6820 is calibrated with a one-point calibration in air-saturated water, using internal instrument temperature and salinity readings with a manually input barometric pressure reading (Appendix B.6). This calibration is then compared and verified against a theoretical DO value determined using the following USGS site and methodology (https://water.usgs.gov/software/DOTABLES/). Before ship departure, a comparison is conducted between the SeaBird oxygen probes (primary and secondary), the 6820 oxygen probe, and a Winkler sample drawn from a plastic tub where the two instruments are placed and allowed to equilibrate (Appendix C.4 and C.5). The Winkler method has an accuracy level of ±0.02 mg l^{-1}. Accuracy of oxygen readings for the YSI 6820 is ±0.4 mg l^{-1} and ±0.4%. Accepted range of accuracy between all three readings is ±0.4 mg l^{-1}. During the cruise, additional water samples for Winkler analysis are collected to confirm the initial cross check and to be used to develop regressions against YSI 6820 and SeaBird CTD data to correct the final oxygen data (Appendix C.8). The water for Winkler analysis during the cruise is collected from a parcel of water that is as uniform in dissolved oxygen as possible, across a range of 0 to an upper limit that may be as high as 14 mg l^{-1}. Pycnoclines and oxyclines are avoided.

Specific Conductivity and Salinity

Specific conductivity and salinity for the YSI 6820 are calibrated using a 50mS/cm KCl solution standard purchased from YSI. The accepted range of accuracy between the CTD and 6820 is ±0.9 mS cm^{-1} and ±0.4 ppt for SpC and salinity, respectively. During the cruise, discrete salinity samples from surface and bottom water are collected at Mississippi River stations and the C and

F transects for post-cruise analysis on a bench top salinometer for potential post correction of data from either instrument (Appendix D.1. and D.2.).

Temperature

Temperature for the SeaBird CTD and YSI 6820 sensors are factory calibrated. Accepted range between the two instruments is ±0.30 ^{o}C.

pH

pH is calibrated for the YSI 6820 using a two-point calibration. 7.0 and 10.0 pH are calculated with buffer solutions purchased from YSI. Accuracy of the 7.0 pH buffer is ±0.01. Accuracy for the 10.0 pH buffer is ±0.02.

Chlorophyll

Chlorophyll is calibrated for the YSI 6820 using a one-point calibration, calibrated for zero (using de-ionized water). Accepted resolution of this parameter is 0.1 µg l^{-1}, and has a capable range of 0-400 µg l^{-1}. The Turner Model 10 AU fluorometer is periodically calibrated against a Chl *a* standard measured on a spectrophotometer and diluted to a different concentration. A secondary standard is used on each cruise to check for a decline in the precision of the fluorometer signal (Appendix E.3. and E.4.).

VI. UNDERWAY VESSEL SAMPLING

R2R (Rolling Deck to Repository)

The UNOLS academic fleet, of which the R/V *Pelican* is a member, routinely receives underway (navigation, MIDAS, flow-through, ADCP, cruise track, and meteorological) data from the vessel at the end of each cruise (http://www.rvdata.us/overview). Data are classified by the Chief Scientist as either “open” or “closed.” “Closed” data are typically “opened” by the Chief Scientist within the next year. R2R expects that the CTD data will eventually be submitted to a national repository along with the appropriate metadata that complies with the ISO19115 standard protocol. All of the data and metadata from the LUMCON and LSU shelf-wide cruises (unless still under QA/QC) have been deposited in NOAA’s National Center for Environmental Information (formerly, National Oceanographic Data Center).

ADCP

The Acoustic Doppler Current Profiler (ADCP) measures currents (speed and direction) beneath a ship while underway. Sound signals sent from the moving ship bounce back to receivers aboard the ship. This provides a profile of water movement relative to the ship-precise modern navigation, allowing the ship's motion to be subtracted from the data.

MIDAS

A PC-based Multiple Instrument Data Acquisition System (MIDAS) controlled by a rack mounted dual processor Digital Server 3000R with 21" monitor, and 10 gigabyte hard drive collects multiple data streams coordinated with the GPS. A National Instruments 16 port serial expansion board allows modular integration of instrumentation, rapid sampling rates, and the maintenance of a real-time data display. Analog sensor signals are converted to a serial format for output to the host PC using R.M. Young A/D devices. The controlling software was developed using National Instruments LabVIEW, to allow accessibility and ease of modification. A real time graphical display provides charting and data display to the ships two labs as well as anywhere else on the ships network. Navigational data are acquired from a Starlink differential GPS or a Trimble GPS with a Micronet Receiver Station. The Micronet Receiver Station is a land based differential system privately maintained (provided for LUMCON's use by Doug Chocrane Technologies, Lafayette, Louisiana), with sub 5-meter accuracy and available out to 300 miles in the Gulf of Mexico. A sea-water flow-through system provides sea surface temperature, conductivity, chlorophyll fluorescence, and transmissometry data using: a Sea-bird Electronics SBE 21 Thermosalinograph; a Sea-Bird Electronics SBE 38 Remote Digital Immersion Thermometer; Turner Designs Model 10 Series Fluorometers; and a WETLabs 10 centimeter or 25.0-centimeter path length transmissometer. MIDAS also integrates the data from the ships meteorological suite into the data set and display. The meteorological suite consists of a R.M. Young 05103 Wind Monitor, a R.M. Young model 61201 Barometric Pressure sensor, a R.M. Young TS05327 Temperature and Relative Humidity sensor and photo synthetically active radiation (PAR) is measured with a LI-COR LI-190SZ Quantum Sensor.

VII. ON STATION MEASUREMENTS (Appendix B.10) AND WATER SAMPLES

Secchi Disk

Secchi disk depths are measured by hand. The disk is weighted to ensure, as much as possible, a straight descent. The observer should deploy the disk from as close to the water as possible. Observations are taken on the sunny side of the vessel. If the current pulls the disk under the ship, then different sunny locations on the ship are tried. The observer does not wear sunglasses. The line is marked in 0.5-m increments for the first 5 m, then in 1-m increments. Any relevant conditions, such as cloudy skies, too early, too late, or descent not straight, are noted on the station log sheet. The Secchi should only be completed in a 12-hour window, including the 6-hour period on either side of peak sunlight. The peak sunlight is calculated evenly between official sunrise and sunset. This means at many sites there will not be a consistent sample taken.

SeaBird CTD/Rosette Cast

The SeaBird CTD unit is attached to a 12-carousel rosette with 5-l Niskin bottles and deployed from 1.5 to 2 m below the surface to within 1.5 to 2 m of the bottom. The CTD operator will try to bring the altimeter to within 1 m of the bottom if the seas are calm. 5-l Niskin bottles are preferred over 12-l bottles, because the depth of water column over which the Niskin collects is much less than a 12-l bottle. Collections of water near pycnoclines and oxyclines are avoided. If larger volumes of water are needed, multiple Niskin bottles are tripped.

Prior to performing a CTD cast, the SeaBird CTD deck unit is set to one "number of scans" to average. At the beginning of each hydrocast, the entire CTD/Rosette package is soaked while submerged 0.5- to 1.0-m below the surface until pump flow and oxygen values observed via the SeaBird deck unit (in Marine Tech lab) indicate the system is operating properly. Sensor packages are located below the Niskin bottle rosette sampler, with the exception of PAR which is located at the top of the carousel frame. The chief scientist works with the Marine Technician to ensure that the pressure setting is set at the water level for the sensor on the instrument. This maximizes the collection of similar data between the CTD and the YSI6820 at similar depths.

To minimize the effect of delays in oxygen sensor response time caused by temperature, sensor condition and plumbing configuration, the CTD package is lowered at a rate of 1 m s^{-1}. The CTD package is raised at the same speed it was lowered so that appropriate upcast data can be used for the ALIGN CTD function of data post-processing. The Chief Scientist marks suitable profiles that might serve this feature (Appendix B.5). These are usually profiles that have a similar time lapse over a uniform depth. If a second cast is necessary to collect additional water and a good profile has been obtained, the speed of the CTD lowering and raising is faster.

Mid-water column samples are collected with Niskin bottles on the rosette. Depths for mid-water samples are taken from corresponding SeaBird data, adjusting for the SeaBird pressure sensor located approximately 0.75 meters below the mid-point of the 5-l Niskin. Mid-water samples may be used for Winkler calibrations or divided amongst other researchers onboard for additional research projects. For each set of bottle depths, the CTD is usually raised 1 m to record the units most likely to correspond to the middle of the Niskin bottle water collection. Additional mid-water samples were historically collected at Station C6C through 2016 (3 m, 12 m, 19 m to correspond with deployed continuously recording oxygen meters) for analysis of chlorophyll, nutrients, and suspended sediment. The graph of each CTD cast is printed, in color, and added to the cruise notebook.

YSI Cast

A YSI 6820 (or Hydrolab equivalent for historic data) is deployed to capture water quality data closer to the sea surface and bottom than the CTD rosette can be deployed (due to sensor placement restrictions). Surface depth is set as close to 0.1 m with the probes in the water and is checked periodically through the cruise. (See Appendices B.6 and B.7 for YSI 6820 operations).

The YSI 6820 is lowered to the sea floor on a separate hydrowire with a lead weight attached by a chain (See Appendix B.11 for requirements). The YSI probes are located approximately 0.5 meters above the ocean floor when the lead weight is resting on the sea floor. If bottom currents are strong, it is sometimes necessary to nudge the YSI sensor array upward or downward to ensure that the package is as close to the bottom as possible (by watching real time depth data displayed on the YSI handheld). When the oxygen sensor stabilizes, a data record of bottom water values is stored electronically on the YSI handheld. The YSI 6820 is then raised in 0.5-meter increments, and after DO sensor stabilization at each depth, data records are stored at each increment. After recording data for the few meters closest to the ocean floor (to overlap with CTD bottom depth), the Shift Leader will target any mid-depths where water may have been collected by the CTD rosette for Winkler titrations. These depths are bracketed to provide another oxygen measure to compare later to CTD and Winkler titrations. If no mid-depths were collected, the YSI 6820 is raised to 2 m to 3 m from the surface and a data record is saved. The final record is stored when the YSI 6820 is as close to the surface as possible with the sensors still submerged (typically 0.1 m). A full YSI 6820 depth profile is not taken because of time constraints.

Bucket Sample

Following the “all clear” from the captain to the shift leader via radio, the shift leader lets the deck crew know that surface water samples can be collected. They are collected with a twice-rinsed surface bucket placed over the side of the ship to capture steep gradients of surface water parameters in the uppermost sea surface. These samples are given the depth of “0”. Water samples are transferred from the bucket into 500-ml or 1000-ml acid-washed polypropylene bottles, after triple-rinsing with bucket water. The determination of salinity, chlorophyll, phaeopigments, suspended sediments, and nutrient characteristics are provided in following sections and associated appendices. Samples are taken as quickly as possible to avoid particles settling in the bucket.

Bottom Niskin

Bottom-water samples are collected using a single 5-l Niskin bottle on the same hydrowire used for the YSI 6820 deployment to capture bottom water as close to the seafloor as possible (See Appendix B.11 for requirements). The chain length of the separate hydrowire is shortened (to allow the bottom of the Niskin bottle to get as close to the seabed as possible), and the lead weight is lowered carefully to the bottom so as not to disturb the sediments. Care must be exercised to make sure the weight is resting on the bottom (not bouncing due to wave action) and the cable tension is suitable for smooth deployment of the messenger for proper triggering of the bottle. The chief scientist on duty and the winch operator work together so that they both understand and can complete the operation. If the water captured by the Niskin has suspended sediments in it (apparent when a bottle is filled for chlorophyll or other samples, the Niskin deployment is abandoned and a second deployment is made). Water samples from this Niskin bottle correspond to the deepest depth recorded from the YSI 6820. Bottom water samples are collected for chlorophyll, nutrient, salinity measurements at select stations and water for Winkler analyses as appropriate. If a sample for Winkler analysis is to be collected, it is collected first, then the other sample containers are filled.

Departure from Station

Once the shift leader is sure that all instruments and collection samplers are on board, they notify the Captain/First Mate that the ship can depart to the next station. The shift leader identifies that station, and it is verified by the Captain/First Mate.

VIII. ANALYTICAL METHODS FOR WATER SAMPLE PROCESSING

Detailed sample processing methods are kept in the cruise notebook, but abbreviated posted versions as well as a check list of parameters collected at each station, are posted in the laboratory for reference by the scientific crew. These posted instructions are in the appendices according to the methodology.

Winkler Sample Collection and Processing

Water is collected from Niskin bottles into a 300-ml glass BOD bottle via tygon tubing. Samples are drawn to avoid air bubbles in the water sample. The BOD bottle is overflowed several times and capped. Titration reagents are added immediately to fix the oxygen.

Winkler titrations are conducted onboard with a Mettler Toledo G20S Compact Titrator using dissolved oxygen determination methods outlined in Appendix C.1, "A Practical Handbook of Seawater Analysis" by Strickland and Parsons (1977). Shipboard Winkler titrations (Appendix C.2 and C.3) during the cruise are used to develop regressions against YSI 6820 and SeaBird CTD data to correct the oxygen data (see Appendix C.8 for detailed instructions).

Chlorophyll and Phaeopigment Sample Collection & Processing

Water for chlorophyll and phaeopigment analysis (10 - 100 ml) is filtered on board through 25-mm GF/F (0.7-micron) filters and then fixed in 5 ml of DMSO/90% acetone (40/60) solution. Filters are allowed to extract for at least two hours in the dark. The solution is measured pre- and post-acidification (2 drops of 10% HCl) on a Turner Model 10 AU fluorometer. Details are available in Appendix E.1.

The Turner Designs model 10 AU fluorometer is periodically calibrated against a Chl *a* standard obtained from a chemical supply house measured on a spectrophotometer. Each time the fluorometer is used, it is checked with a Turner 10-AU solid standard that tracks drift, to ensure calibration is maintained (Appendix E.3 and E.4). Additionally, during the cruises, the fluorometer is zeroed with a blank solution of DMSO/Acetone daily in accordance with Turner Designs recommended procedures.

Suspended Sediment Sample Collection & Processing

Water samples (approximately 50 to 1500 ml) collected for suspended sediments are filtered onboard through 47-mm GF/F filters (pre-combusted, pre-weighed) and rinsed with distilled water. The filters are placed in Petri dishes and frozen for later laboratory analysis.

As needed, these chlorophyll data can be used for post-calibration of the YSI 6820 and SeaBird CTD sensors.

In the laboratory, filters are thawed, dried in a convection oven overnight at 60°C, and weighed. The filters are then ashed in a muffle furnace at 400°C for 12 hours and re-weighed. The difference in the dried and ashed samples represents the difference in the inorganic and organic contribution to the total suspended sediments. Details are available in Appendix F.

Nutrient Sample Collection and Processing

Best management practices during nutrient sample collection dictate that water sampling is done with clean hands that avoid touching the water sample. Gloves are worn. Caps and vials (15 ml) are triple rinsed with water sample before vial filling, to within 1 cm of the top, and closing. Replicates of surface- and bottom-water are taken and numbered sequentially (two each) to coincide with the Station Log sheet numbers. Samples are not filtered; sample vials are frozen for later analysis in the laboratory. (See Appendix G)

Nutrient concentrations (µM) are determined from frozen, unfiltered water samples analyzed using EPA methodology (353.2, 350.1, and 365.2) on a Lachat auto-analyzer II system (8000 series) equipped with an autosampler (ASX-400 series). Ammonium concentration are determined using Lachat Instrument's QuikChem method 31-107-06-1-B. Phosphates are determined by Lachat Instrument's QuikChem Method 31-115-01-1-H. Silicates are measured using Lachat Instrument's Method 31-114-27-1-C. TKN are determined using Lachat Instrument's QuikChem Method 10-107-06-2-H. Nitrates and Nitrites are determined using Lachat Instrument's Method 31-107-04-1-C.

Salinity Sample Collection and Processing

Water samples for salinity analysis are collected during the cruise in an acid-washed, triple-rinsed 500-ml Nalgene jar from a twice-rinsed bucket of surface water or a bottom-deployed 5-l Niskin bottle (Appendix D.1). The sample jar lid is secured tightly to minimize evaporation. After the cruise, a volume of each sample is removed, placed in a YSI 6820 calibration cup, rinsed three times, and read with the YSI 6820. The remaining sample volumes are analyzed with a Portasal or AutoSal bench top salinometer. The lab-based 6820 and salinometer data are compared with the Seabird CTD salinity data from the time of the original sample collection to develop regressions to correct the final salinity data. An example of corrections of SeaBird and YSI 6820 salinity data is provided in Appendix D.2.

IX. QUALITY CONTROL

CTD Data Processing and QC (Appendix H)

Sea-Bird SBE 13-01 dissolved oxygen sensors, the Paroscientific Digiquartz(r) pressure sensor, the SBE 3-01/F temperature sensors, SBE 5-01 pumps, and the SBE 4-01/0 Conductivity sensors are factory tested and calibrated at Sea-Bird (http://www.seabird.com/) at recommended intervals and maintained and serviced by R/V *Pelican* Electronic Technical support staff in accordance with Sea-Bird procedures. The Chelsea Instruments Aquatraka III Chlorophyll a sensor was factory tested and calibrated at recommended intervals and maintained and serviced by R/V *Pelican* Electronic Technical support staff in accordance with Chelsea Instruments procedures. Note: WET Labs acquired SEA Tech June 1, 1998, (http://www.wetlabs.com/). The Wet Labs C Star 10-cm path transmissometer was maintained by R/V *Pelican* Electronic Technical support staff in accordance with Wet Labs recommendations.

Sea-Bird CTD data are acquired onboard using Seasoft and processed after the cruise using SBE Data Processing-Win32 software. All scans are processed without averaging or interpolation (i.e. a bin size of one scan).

In order to compensate for the delay in oxygen sensor response time and improve the alignment between oxygen sensor values and temperature and conductivity sensor values, the Seasoft module ALIGNCTD is used (see example case in Appendix B.5). After alignment, the DERIVE function is used to calculate Depth (m), Salinity (psu), Density sigma-t (kg/m^3), Oxygen concentration ($mg\ l^{-1}$), and Oxygen percent saturation (%). Final data are exported as ascii files.

A MATLAB script is used to processes these ascii files to select a water column profile comprised of scans from the downcast of discrete 1-meter (or 0.1-m) measurements. The MATLAB program selects scans using the following criteria: 1) Data from Seabird warm up period sitting on the sea surface are removed; 2) Upcast data are removed; 3) Data scans are selected at 1.0 meter increments through the water column; 4) When DO values changed significantly (> 1.0 mgO_2 l^{-1}) within 1-meter, scans at 0.1-m intervals are selected within that meter; and 5) Minimum oxygen and maximum depth scans are selected.

Dissolved oxygen concentrations (mg l^{-1}) from both the SBE sensors are corrected as necessary with data from Winkler titrations (Appendix C.8); salinity data are corrected with PortaSal (AutoSal equivalent for historical) salinity determinations (Appendix D.2). Note that if oxygen data are corrected, oxygen % data are re-calculated, and similarly if salinity data are corrected oxygen % and density data are re-calculated.

Final Seabird profiles are reviewed by hand across parameters for outliers, spikes, or a decreasing/increasing trends that are not consistent. Data that do not meet quality control thresholds are not included with reported data, but are noted in the metadata.

YSI Data Processing and QC

Dissolved oxygen concentrations (mg l^{-1}) from the YSI sensor are corrected as necessary with data from Winkler titrations (Appendix C.8); salinity data are corrected with PortaSal (AutoSal equivalent for historical) salinity determinations (Appendix D.2.). Note that if oxygen mg l^{-1} data and/or salinity are corrected, oxygen % data are re-calculated.

Final YSI profiles are reviewed by hand across parameters for outliers, spikes, or a decreasing/increasing trend that is not consistent. Data that do not meet quality control thresholds are not included with reported data, but are noted in the metadata.

QA/QC of Water Sample Data

Water sample data are quality controlled by examining values +/- 2 SD from the mean. Data are manually inspected for values inconsistent with replicates, outliers in a continuous string, and for values outside the range of a functional instrument. Additionally, relationships between water sample and sensor (YSI & Seabird) data are evaluated. Suspected data values are compared with station log notes to ensure proper data entry.

Oxygen Corrections

The quality of dissolved oxygen data is paramount to the long-term monitoring of the Gulf of Mexico's hypoxic zone, its formation, maintenance, and dissipation. As such special care is taken in determining that oxygen data are of the highest quality (Appendix C). All Seabird CTD DO probes are factory calibrated and all YSI 6820 DO probes are laboratory calibrated. A Winkler titration is run to compare sensor function in a large water bath on the deck that can accommodate the SeaBird rosette and the YSI 6820. During the cruise two DO probes are utilized on the Seabird CTD in case one of the probes begins to malfunction. Both DO probes from the SeaBird and YSI 6820 are calibrated against a Winkler titration, which is accurate to 0.04 mg l^{-1}, and data are corrected post-cruise (Appendix C.8) as necessary by a regression equation. The data from the Seabird DO probe that is best aligned with the Winkler samples is used as the final oxygen dataset. Corrected oxygen data from the Seabird and YSI are examined for outliers, spikes, or a decreasing/increasing trend that is not consistent. Data that do not meet quality control thresholds are not included with reported data, but are noted in the metadata.

X. MAPPING ANNUAL HYPOXIA AREAL EXTENT

Hand Contoured Map and Area Estimate

The bottom-water area of hypoxia (km^2) is determined by interpolating between sampling locations and hand-contouring parallel to isobaths over a calibrated (planimeter) grid. Bottom oxygen (mg l^{-1}) concentration data from the YSI 6820 are used for each sampling location.

Note: An alternative approach is the geostatistical interpolation approach, which is very comparable to the Rabalais long-term, hand contoured method (Obenour et al. 2013). At times, within the historical data, the bottom-water dissolved oxygen data from the CTD and the hand-held YSI 6820 equivalent were not collected together. When only the CTD data were available, its bottom-water oxygen values are adjusted to extend from the CTD measured depth (roughly 2-m above the bottom) to a bottom depth more similar to the YSI according to relationships between the dual CTD and YSI data (Obenour et al., 2013). If these calculations changed any of the original spatially generated maps of Rabalais, then the estimate of bottom-water hypoxia was re-calculated. The percentage difference of those not changed and those changed over a 27-y period was 0.006%. Recalculated area estimates by Rabalais are used in the analysis found on (http://www.gulfhypoxia.net).

GIS Created map

Contour maps are created in ArcGIS v10.3.1 using the 3D Analyst Toolbox Triangulated Irregular Network (TIN) interpolation method. The TIN method interpolates data between points by creating a series of non-overlapping triangles and interpolating linearly along each edge.

XI. PRESS RELEASE

Separate press releases are prepared by NOAA and the Chief Scientist with the same area size of the bottom-water hypoxia for the yearly cruise. The release of the press items is coordinated and serves as a key public outreach tool and as the official reporting of the preliminary size of the hypoxia zone used in the annual metric for that year. The official size of the hypoxic area is updated at (http://www.gulfhypoxia.net) once all the data are quality controlled.

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