APPENDIX J

Appendix J contains copies of the Standard Operating Procedures (SOPs) developed by either of the two monitoring vessels during the period 8 May through 27 August 2010. They were developed as needed during the cruise, and some were modified over time. This appendix contains the version or versions which appear to have been in use most frequently.

Research Vessel Ocean Veritas and Brooks McCall Water Sampling SOP.

Purpose: Sample collections conducted from Nisken bottles fired at selected depths are stored and shipped to a laboratory facility for chemistry analyses. Samples are collected for PABDVAP full suite analysis. Surface absorbent pad for visible weathered oil is collected for PAH, aPAH, and Biomarker analyses.

Materials:

1 4"x4" absorbent pad in 8oz. jar

1 bucket with rope or other surface collection method

1-Liter bottle, 1 per sample (2 additional bottles to be used for MS/MSD and 1 additional bottle for a Field Duplicate samples every 20 bottles sampled from)

40mL vial with 0.5ml HCl, 3 per sample

40mL vial without preservative, 3 per sample

DO sample jars (unpreserved 4oz. jars), 1 per sample

Procedures:

1. Prior to cast make labels for each Nisken bottle which will include 3 40mL vials with 0.5ml HCl, 3 40mL vials without preservative and 1 1-L amber bottle. Each bottle will receive a sequential SW number and a NOAA ID number, i.e. SW-20100707-OV8-023 and OV09102-01. On the cap of the 1-Liters write the Nisken bottle associated to the ID number written on the 1-L. Separate the labeled 40ml vials in Ziploc bags with the number of the Nisken bottle written on the outside along with the matching 1-L into plastic containers.

2. While the CTD is on its up cast a surface sample is collected. First make sure bucket has been properly cleaned by rinsing the bucket with tap water then adding 1ml of liquid soap (Dawn) into the bucket and fill with tap water. Using a scrub brush, scrub the inside, outside, and bottom of the bucket, as well as the rope. Rinse the bucket and rope with tap water until all soap is gone. Rewash if necessary and then add some distilled water to the bucket and swirl. Pour out water and repeat 2 times.
    I. Holding the end of the rope, toss the bucket over the side of the ship. Be sure to toss bucket in a location away from any place where input from ship can affect samples (i.e. go up-current of and ship water discharge).

II. Pull bucket up and discard water. Repeat 2 times to rinse bucket with sample water.
III. Collect a water sample as described.
IV. If oil is visible, place an absorbent pad on sample water surface to collect oil. Place pad back in jar and seal.
V. Collect DO in an unpreserved 4oz. jar. Stick jar head down in water then scoop up sample.
VI. Collect water samples for any other group that asks for them (i.e. DFO, NOAA).

3. When CTD is on deck open the top of the Nisken bottle to check and record sheen and they should be categorized as light, medium, heavy, or none. To determine the classification for each please see chart below.
    I. Run water through the Nisken tube for a second to clean out any contaminates that it might have collected. Then collect samples for DFO and deliver them to the wet lab.
    II. Then fill the 1-Liter 1" from the top, and each 40mL fill completely until there is a meniscus on top of bottle before capping to prevent head space. After capping the vial tightly, turn it upside down and tap bottle to check for any air bubble in vial, if head space is present put a few more drops of water in the vial and try again. Additionally, collect 1 DO sample jar by tiling the jar so the water hits the side of the jar to prevent air mixing into the sample. Collected water samples for any other group that asks for them.
    III. While filling bottles, hold the caps downward to prevent exhaust fumes from the vessel adhering to the inside of the lid. Also, be sure the tube on the Nisken bottle does not touch the edge of the sample bottles/vials/jars.
    IV. For every 20 Nisken bottles sampled a QA/QC samples must be collected. On that bottle, collect 2 additional 1-Liter bottles. Label them like the parent sample in sequential order but with a different subsample number in the sample name code (i.e. OV09905-MS or OV09905-MSD). Also a Field Duplicate should be done for every 20 samples collected (best done on same cast) and should also be labeled like parent sample but with a sample name code like OV09907-FD.
    V. Additionally, once per day an equipment blank should be conducted. After completing Decon on Nisken bottles pour DI water into one of the bottles. Collect from that bottle, 1 1-Liter, 3 40mL with HCl and 3 40mL unpreserved samples. Label each sample as equipment blank with a sequential ID code and a sample name (i.e. OV09901-EB).
4. After samples are collected, bring them to the work desk and check the 1-L bottles to make sure the cap it on tightly and then rap electrical tape around lid. The bottle should be placed in a 1 gallon ziplock bag and sealed with the least amount of air possible then place it in an open large bubble wrap bag. The 40 ml vials should be placed in a large bubble wrap bag in a row of 6 then folded over tightly and sealed then placed in a ziplock back two at a time. After all samples are wrapped place all samples in the refrigerator set to 40 degree F by cast number until it is backed for shipping to the lab.

5. Use DO jars for Dissolved Oxygen analysis. Readings are taken with the Entrix provided DO meter (YSI EcoSense DO200) in the wet lab container. Before taking the first DO sample readings, the probe is calibrated following the instructions in the meter user manual (see DO200 manual pg. 5-6). Pressure is set to 1013 mBar and salinity is set to 36 ppt to approximate the atmospheric conditions and the salinity of seawater.

    I. To take DO readings, turn on the probe and push the mode button to measure DO in mg/L. Rinse the yellow membrane surrounded by a black cage with distilled water by pouring at least 20mL over the probe into the sink. Submerge the probe end in the sample water in a filled amber bottle and move the probe up and down at a rate of 30cm/sec to prevent the inherent consumption of oxygen by the membrane (see DO200 manual pg. 5).

    II. Sample water may spill out of the bottle because of the volume displacement by the probe.Once the value displayed on the DO meter stabilizes such that it is within 0.02 mg/L of a central value, record the mean value displayed. If the value displayed does not stabilize but rather steadily and slowly increases/decreases, record the lowest value displayed before the value started increasing. A steadily changing value may indicate that the dissolved oxygen in the water is moving towards equilibrium with the sea level atmospheric pressure and temperature. This is illustrated by the below graph showing that the readings taken by the field probe converge towards a central value while the CDT measurements at the underwater depths show a wider range of dissolved oxygen. Between each sample, rinse the probe with 20mL of distilled water. Store the DO meter in the provided plastic bottle with a moist sponge as outlined in the probe manual.

    III. DO readings are recorded in a field notebook and the remaining water is discarded. Once all DO readings have been taken the empty bottles are filled with 20mL of distilled water, caped, and swirled to remove sample residue. Discard distilled rinse water. A full decontamination of the bottles is completed once daily with 20mL potable water and one drop of dawn dish soap followed by a rinse with distilled water.

6. Upon completion of all sampling of Nisken bottles they should be Deconed. Dump any remaining water from the bottles, and then remove each Nisken bottle from Rosette. Add 1 gallon of water mixed with 1cc of Dawn, and shake bottle to wash inside. Return the bottle to the Rosette and open the caps to drain all soap from the bottle. Using the fresh water deck hose, wash down the inside and outside of the bottles to remove any soap residue. Rinse out collecting tubes on the bottles as well. Store with caps closed to prevent contamination between sampling stations.

7. Each night a COC and the Field Data form should be written for all samples including a trip blank (refer to MC252-SOP-05). The Field Data form and a daily report should be sent to the current project leader. Start over the next day the Sample ID code with 001.

Standard Operating Procedure

CTD Rosette Deployment and Depth Targeted Sampling and Monitoring Onboard the Research Vessels Brooks McCall and Ocean Veritas

Revision No: 0

Revision Date: July 11, 2010

TABLE OF CONTENTS

1.0 PURPOSE

The purpose of this Standard Operating Procedure (SOP) is to describe the requirements for operating the Conductivity Temperature Depth (CTD) Rosette and the collection targeted samples for both laboratory analysis and onboard in-situ analysis. The requirements of this procedure are applicable to the deployment of the CTD Rosette onboard the research vessel cruises of the Brooks McCall and Ocean Veritas. The Deepwater Dispersement Sampling Plan (DDSP) governs this plan and the frequency of implementation.

2.0 GENERAL CONSIDERATIONS

Potential hazards associated with the planned tasks should be thoroughly evaluated prior to conducting field activities. Refer to the site-specific Health and Safety Plan (HASP) for a description of potential hazards and associated safety and control measures and vessel-specific guidance documents.

Prior to commencing CTD Rosette deployment the manufacturer instruction manuals shall be reviewed for specific operating procedures including calibration and maintenance frequency. Appropriately sized Nisken bottles shall be selected that provide sufficient volume to conduct analytical sampling and monitoring activities identified in the DDSP. Additionally vessel-specific CTD deployment procedures and communication procedures shall be reviewed and understood by concerned parties (both top-side and bottom-side).

Teams aboard the vessels shall maintain field logbooks in accordance with the *Field Documentation* SOP (MC252-SOP-02). Field logbooks will be maintained by the Field Team Leader to record daily activities. The minimum requirements for field logbook documentation are discussed in MC252-SOP-02. The Field Team Leader will review the field logbook entries for completeness and accuracy and will indicate this review by initialing each page of the logbook. The Field Team Leader is responsible for completion of the required data collection forms.

2.1 Definitions

Station – The station is defined as the unique location where a CTD Rosette is deployed.
Cruise – The sequentially numbered event where each vessel was out at sea.
Nisken Bottle – The collection bottle attached to the CTD Rosette, typically there are 8 4-Liter Nisken bottles affixed to each CTD Rosette for depth-targeted sample collection.
Fluorometer – Refers to the fluorometer on the CTD Rosette.
Downcast – Refers to the acquisition of readings (of the CTD Rosette) from the surface to the bottom depth of deployment.
Upcast – Refers to the acquisition of readings (of the CTD Rosette) from the bottom depth of the downcast to the surface.
Cast – Refers to the composite readings of both the downcast and upcast.

3.0 PROCEDURES

Review the *Rosette Launch and Recovery Procedures* SOP (Attachment 1) for the activities and logistics involved with the launch and recovery of the CTD Rosette aboard the vessel. Additionally the *Onsite Procedures and Communications* SOP (Attachment 2) shall be reviewed for coordination between topside and bottomside for the deployment, sample collection, and retrieval of the CTD Rosette.

3.1 CTD Rosette Deployment

The CTD Rosette system is equipped with the following instrumentation:

- Seasave software
- Winch and slip ring system spooled with protected data cable
- Weighted deployment frame
- Sea-Bird 9 plus (SBE 9 plus) CTD
    - Equipped with a Seabird 43 (SBE 43) dissolved oxygen sensor
    - Equipped with a WET Labs ECO-CDOM fluorometer
    - Equipped with a Teledyne altimeter
- Sea-Bird 11 plus deck box (SBE 11 plus) for real-time data acquisition
- Sea-Bird 19 plus CTD (SBE 19 plus) for backup/comparison against SBE 9 plus
- Sea-Bird 32 Carousel Water Sampler (SBE 32) for remote firing of bottles
- (8) 4-liter Nisken bottles

Review to the manufacturers manuals for detailed specifications of instrumentation on the CTD Rosette. Associated calibration techniques and frequency shall be governed and performed by manufacturer recommendations. A brief description of manufacturer specifications of the instrumentation on the CTD Rosette is presented in Appendix A.

To initiate a CTD Rosette data acquisition session using the Seasave prior to deployment follow the below procedures.

- Open Seasave on desktop
- Select Real-Time Data
- Select Start
- Select Output Data File Name
    - SBE9_(Vessel Code)(cast number)_(date)
- Select Instrumentation File
    - BP CTD Con File 0454.xmlcon
- Select Start
- Enter Header Information
    - Vessel/Site ID
    - Start Time (GMT)
    - End Time (GMT)
    - Latitude
    - Longitude
    - NAD 27
- Select Start

At this point, the instrumentation aboard the CTD Rosette will start collecting data. Once the pump turns on, the winch operator starts the CTD Rosette deployment system's decent to the approximately 40 meters above the estimated seafloor depth. The CTD Rosette reports conductivity, temperature, dissolved oxygen (DO), practical salinity, and fluorescence by depth. At this point the downcast is complete and a screenshot of the instrumentation's downcast plot profile is captured for reporting purposes and sample location selection.

3.2 Depth Targeted Sample Location Selection

The chief scientist and Fugro GEOS party chief review the downcast and select depths at which samples shall be collected. Eight sample locations shall be chosen: three from depth-targeted locations (at 3 meters[m], 50 m, and at the bottom of the downcast), one from the depth corresponding to the lowest DO reading, and three locations from depths where fluorescence readings deflected the highest from background levels (or where other parameters indicate the presence of a plume). Once the depths have been established, the winch operator starts his ascent to the desired sample location depths. The CTD Rosette is stopped at each desired depth-targeted sample location and each Nisken bottle is sequentially "fired" to grab a water sample.

3.3 Rating Plume Strength from Fluorescence Signal

Fuorescence signal strength categories have been identified for the purpose of providing a standard approach and enable better categorization of the relative strength of the plume to assist in sample planning and reporting. These categories are intended to assist the science team identify the strongest plume signals in the field and is not intended for other uses. The categories have been selected based on a review of the fluorescence measurements collected on the Brooks McCall and the Ocean Veritas to date.

To provide a standard approach to plume rating (classified by the strength of the fluorescence signal) the categories below are based on fixed ranges above background fluorescence.

Plume Rating	Fluorescence Signal milligrams per cubic meter (mg/m^3)
No Plume	Background
Very Weak	Up to 1 mg/m^3 above background
Weak	Between 1 mg/m^3 to 5 mg/m^3 above background
Moderate	Between 5 mg/m^3 and 15 mg/m^3 above background
Strong	Greater than 15 mg/m^3 above background

The rating is based on a visual assessment of the data output from the CTD Rosette cast (comprised of the composite image of both the downcast and the upcast). Where a single spike in the fluorescence signal appears on one (but not both) of the downcast or upcast, and no other indication of a plume is present, the value is not used in the plume rating.

3.4 Sampling and Monitoring

Once the CTD Rosette is secured on the deck of the vessel, water samples are collected for laboratory analysis, on-board DO measurement, on-board Laser In-situ Scattering Transmissometry (LISST) measurements, and on-board Rotofer Toxicity Testing. Volumes of water are collected from each Niskin bottle for the previously listed analyses and measurements and further defined below and in associated SOPs.

3.4.1 CTD Rosette Data

The raw CTD Rosette data is collected by the Furgo team and processed by the onboard NOAA data manager and sample collection depth and time, and coordinate information are distributed to the members of the field team, the LISST team, the fluorescence team, and Rotofer toxicological team for inclusion in their respective data sets. Each of the previously identified teams shall use the sample collection depth and time, and coordinate information obtained from the NOAA data manager for consistency purposes.

3.4.1 Laboratory Analytical Analysis

Sample collection for laboratory analysis shall be governed by the most recent version of Analytical Request Form (ARF) 22. ARF 22 identifies analysis parameters (including the appropriate bottleware) and where electronic data shall be reported. See *Research Vessel Ocean Veritas and Brooks McCall Water Sampling* SOP (Attachment 3) for details of the sample collection activities for laboratory analytical analysis. Laboratory analytical data (reported by the laboratory) and field data (collected by the field team) are reported to the Equis database (see *Sample Management* SOP, MC252-SOP-03 for data format and transmission specifications).

3.4.2 DO Measurement

DO measurements are taken from water collected in each Niskin bottle on the deployed CTD Rosette. The field sampler collects DO measurements onboard the vessel for comparison to the DO measurements collected on the CTD Rosette. See the DO procedures in Attachment 3 for details of DO collection techniques and calibration frequencies. DO measurements are reported to the NOAA data manager (see *Guidance for Shipboard Data Management Coordinator* for data format and transmission specifications).

3.4.3 LISST Measurements

LISST measurements are taken from water collected in each Niskin bottle on the deployed CTD Rosette. The field sampler collects water for onboard LISST analysis for the purpose of identifying the plume and comparison with the laboratory analytical data and the fluorescence data (collected on the CTD Rosette). The LISST team operates and reports the LISST measurements collected onboard the vessel. See the *LISST-100X Particle Size Analyzer* SOP (Attachment 4) for details regarding onboard LISST measurements and calibrations. The LISST data is reported to the NOAA data manager (see *Guidance for Shipboard Data Management Coordinator* for data format and transmission specifications).

3.4.4 Onboard Fluorescence Measurements

Onboard fluorescence measurements are taken from water collected in each Niskin bottle on the deployed CTD Rosette. The water submitted to the personnel operating the LISST use the remainder of water in the sample container for LISST measurements for onboard fluorescence measurements. See the *SOP for the Quantech Fixed Wavelength Fluorometer* (Attachment 5) for details regarding the onboard fluorescence measurements and calibrations. The fluorescence measurements are collected for comparison to the CTD Rosette fluorescence measurements. Onboard fluorescence measurements are reported to the NOAA data manager (see *Guidance for Shipboard Data Management Coordinator* for data format and transmission specifications).

3.4.5 Rotofer Toxicity Tests

Rotofer Toxicity Testing is conducted from water collected in each Niskin bottle on the deployed CTD Rosette. The field sampler collects water for onboard Rotofer Toxicity Testing to collect toxicological data at the depth-targeted sample locations. See the *Standard Protocol for Shipboard Rotoxkit M Testing During MC252 Oil Spill Response* (Attachment 6) for details of the onboard Rotofer toxicological tests. The Rotofer toxicological data is reported to the NOAA data manager (see *Guidance for Shipboard Data Management Coordinator* for data format and transmission specifications).

4.0 REFERENCES

- BP, *Field Documentation* SOP, MC252-SOP (SOP-02), July 2010
- BP, Sample Management SOP, MC252-SOP (SOP-03), July 2010
- BP, Analytical Request Form, ARF-22REV4

APPENDIX A – CTD Rosette Instrumentation Specifications

SBE 9 plus

The SBE 9 plus can continually measure conductivity, temperature, and pressure, and parameters from up to eight auxiliary sensors, in marine or freshwater environments at depths up to 10,500 meters (34,400 feet). Designed for applications where vertical profiles of the measured parameters are required, the 9 plus samples at 24 scans per second (24 Hz). [cited from the SBE 9 manual]

The SBE 9 plus main housing contains the acquisition electronics, telemetry circuitry, and Paroscientific Digiquartz pressure sensor. The pressure sensor, ported to outside pressure through an oil-filled plastic capillary tube protruding from the bottom end cap, is available in five depth ranges to suit the operating depth requirement. Bulkhead connectors for the modular sensors are mounted on the main housing's end caps. [cited from the SBE 9 manual]

The SBE 9 plus uses the modular SBE 3 plus Temperature Sensor and SBE 4C Conductivity Sensor. The 9 plus also includes the SBE 5T Submersible Pump and TC Duct. The pump-controlled, TC-ducted flow significantly reduces salinity spiking caused by ship heave, and in calm waters allows slower descent rates for improved resolution of water column features. [cited from the SBE 9 plus manual]

The Ocean Veritas uses the SBE 9 plus to measure salinity in practical salinity units (PSU) within the range of 32.00-37.00 PSU, temperature in ITS-90 (degrees Celsius) within the range of 0.00-26.00 degrees Celsius, and depth in meters within a range of 0.00-1,700 meters.

Specifications

- Measurement Range
  - Conductivity 0 to 7 Siemens/meter (0-70 mmho/cm)
  - Temperature -5 to + 35 °C
  - Pressure 0 to full scale – 2000/3000/6000/10,000/15,000 psia (1400/2000/4200/6800/10500 m)
  - A/D inputs 0 to +5 volts
- Initial Accuracy
  - Conductivity 0.0003 S/m (0.003 mmho/cm)
  - Temperature 0.001 °C
  - Pressure 0.015% of full scale
  - A/D inputs 0.005 volts
- Typical Stability
  - Conductivity 0.0003 S/m (0.003 mmho/cm) per month
  - Temperature 0.0002 °C per month
  - Pressure 0.018% of full scale per year
  - A/D inputs 0.001 volts per month
- Resolution (at 24 Hz)
  - Conductivity 0.00004 S/m (0.0004 mmho/cm)
  - Temperature 0.0002 °C
  - Pressure 0.001% of full scale
  - A/D inputs 0.0012 volts
- Time Response 1
  - Conductivity 0.065 second
  - Temperature 0.065 second
  - Pressure 0.015 second
  - A/D inputs 5.5 Hz 2-pole Butterworth Low Pass Filter

- Master Clock Error Contribution 2
    - Conductivity 0.00005 S/m
    - Temperature 0.00016 °C
    - Pressure 0.3 dbar (for 6800 m [10,000 psia] pressure sensor)
- Dimensions, mm (inches)
    - 952 x 330 x 305 (37.5 x 13 x 12)
- Weight, kg (lbs), in air (in water)
    - 16 (35)

Calibration

Sea-Bird sensors are calibrated by subjecting them to known physical conditions and measuring the sensor responses. Coefficients are then computed, which may be used with appropriate algorithms to obtain engineering units. The conductivity, temperature, and pressure sensors on the SBE 9 plus are supplied fully calibrated, with coefficients printed on their respective Calibration Certificates and stored in the instrument configuration (.xmlcon or .con) file.

The SBE 9 plus is delivered from the factory calibrated. It must be returned to Sea-Bird for calibration.

SBE 43 Oxygen Sensor

The Ocean Veritas uses the SBE 43 oxygen sensor to measure saturated oxygen in mg/l within the range of 0.00-20.00 mg/l.

Specifications

- Measurement range: 120% of surface saturation in all natural waters, fresh and salt
- Initial accuracy: 2% of saturation
- Typical stability: 0.5% per 1000 hours (clean membrane)
- Input power: 6.5 - 24 VDC, 60 milliwatts
- Output signal: 0 - 5 VDC (SBE 43), frequency (SBE 43F)
- Housing/depth rating: 600-meter plastic or 7000-meter titanium housing (10,500-meter titanium housing available on request)
- Weight (in air): SBE 43 — 0.7 kg (1.5 lbs) with titanium housing, 0.5 kg (1.0 lb) with plastic housing
- SBE 43F — 0.4 kg (0.9 lbs) with titanium housing

Calibration

Calibration drift is caused primarily by membrane fouling from ocean contaminants, and less so by chemical processes inside the sensor. If the membrane is kept clean, the sensor's improved chemical stability yields demonstrated calibration drift rates of less than 0.5% over 1000 hours of operation (*on* time). It must be to Seabird for calibration.

ECO-CDOM Fluorometer

The ECO-CDOM fluorometer uses specific or broadband excitation sources and emission detectors to determine the fluorescent response of a substance or an environment. Generally the fluorescence response is linearly proportional to the concentration of the material, allowing

a fluorometeric approach to non-destructively measure the concentration of a particular fluorescent material. [cited from the ECO-CDOM manual]

The Ocean Veritas uses the fluorometer to measure fluorescence in mg/m^3 within a range of 0.00-50.00 mg/m^3.

The excitation of crude oil is 350 nm and the emission of crude oil is 505/190 nm.

Specifications

- Excitation: 370 nm
- Emission: 460 nm
- Sensitivity: 0.09 ppb QS
- Linearity: 99%R^2
- Range: 0–500 ppb

Calibration

The fluorometer is delivered from WET labs calibrated. It must be returned to WET labs for calibration.

Teledyne Benthos Altimeter

The Ocean Veritas uses the altimeter to find the depth of the seafloor within the range of 0.00-40.00 meters.

Calibration

The altimeter is delivered from Teledyne Benthos calibrated. It must be returned to Teledyne Benthos for calibration.

SBE 11 plus Deck Box

The SBE 11 plus Deck Unit includes RS-232 and IEEE-488 computer interfaces, a modem channel for real-time water sampler control (including water sampler control push buttons and status lights), NMEA 0183 interface for adding GPS position to CTD data, 12-bit A/D input channel for surface PAR sensor, switch-selectable 115/ 230 VAC operation, audio tape interface (data backup), LED readout for raw data, and audible bottom contact (or altimeter) alarm. The SBE 11 plus also provides a remote pressure output (useful as an input signal for towed vehicle control) and a programmable serial ASCII data output containing up to seven variables in computed engineering units. Calibration coefficients are stored in EEPROM, and a separate microcontroller converts raw CTD data to temperature, depth, salinity, etc. [cited from the SBE 11 plus manual]

SBE 19 plus CTD

The SBE 19 plus is designed to measure conductivity, temperature, and pressure in marine or fresh-water environments at depths up to 7000 meters (22,900 feet). The 19 plus operates in profiling mode for acquiring vertical profiles. The SBE 19 plus runs continuously, sampling at 4 scans per second (4 Hz). It can average up to

32,767 samples, storing and transmitting only averaged data. [cited from the SBE 19 plus manual]

The SBE 19 plus is self-powered and self-contained. It includes conductivity and temperature sensors and a precision, semiconductor, strain gauge pressure sensor. Nine D-size alkaline batteries provide 60 hours operation in profiling mode; the 64 Mbyte FLASH RAM records 400 hours of conductivity, temperature, and pressure data while sampling at four scans per second. User-selectable output format is raw data or engineering units, in hexadecimal or decimal form; XML output is also available. Setup, diagnostics, and data extraction are performed without opening the housing. [cited from the SBE 19 plus manual]

The Ocean Veritas uses the SBE 19 plus for backup and comparison against the SBE 9 plus.

Specifications

- Conductivity (S/m)
  - Measurement Range: 0 to 9
  - Initial Accuracy: 0.0005
  - Typical Stability: 0.0003/month
  - Resolution: 0.00005 (most oceanic waters; resolves 0.4 ppm in salinity)
- Temperature (°C)
  - Measurement Range: -5 to +35
  - Initial Accuracy: 0.005
  - Typical Stability: 0.0002/month
  - Resolution: 0.0001
- Pressure Strain Gauge
  - Measurement Range: 0 to 20/100/350/600/1000/2000/3500/7000 meters
  - Initial Accuracy: 0.1% of full scale range
  - Typical Stability: 0.1% of full scale range/year
  - Resolution: 0.002% of full scale range

Calibration

The SBE 19 plus is delivered from Seabird calibrated. It must be returned to Seabird for calibration.

SBE 32 Carousel Water Sampler

The SBE 32 Carousel Water Sampler is a versatile, reliable, continuously operating water sampling system. Each Carousel bottle position has its own lanyard release latch controlled by a magnetic trigger. When the microprocessor in the Carousel pylon receives a command to fire a bottle, it activates the magnetic trigger for the bottle position specified. Bottles may be fired sequentially or any order. The Carousel's design allows the lanyard release mechanism to be cocked with a touch of a finger before the lanyards are secured, permitting fast, convenient, safe, and reliable setup. Titanium, acetal plastic, and other corrosion-resistant materials are used in the latch and magnet assembly. [cited from the SBE 32 manual]

The energy used to trip the magnetic trigger that controls each release latch is stored in an internal capacitor. When a fire command is received, the Carousel switches the capacitor to the selected magnetic trigger for 15 milliseconds. A fire-confirm circuit detects current flowing through the circuit. Receipt of a fire-confirm message from the Carousel verifies the bottle position selected and that energy was delivered to the magnetic trigger. The capacitor is

charged to 75 volts with a current-limited DC/DC converter; time to recharge the capacitor is approximately 3 seconds. The Carousel electronics are electrically isolated from the CTD. [cited from the SBE 32 manual]

The SBE 32 carousel water sampler is set up for an 8 bottle configuration. It accepts standard water sample bottles, in sizes ranging from 1.2 to 10 liters.

The SBE 32 carousel water sampler is rated to a depth of 6800 meters.

Niskin Bottles

The Ocean Veritas uses 4-liter Niskin bottles. These bottles are installed onto the SBE 32 carousel water sampler for remote firing at desired depths. The Niskin bottle is basically a plastic cylinder with stoppers at each end, connected by an elastic cord. The stoppers are held open by plastic cords attached to an individual release mechanism on the SBE 32 carousel water sampler.

ROSETTE LAUNCH/RECOVERY PROCEDURES

PREPARATION

1) Turn on hydraulics (port aft center).
2) Make sure circuit breaker is on (outside laundry room).
3) Verify that switch is on for winch control

LAUNCH

Two persons man the tag lines. One person operates the A frame (port aft), and 1 Mate operates the winch (forward center). The Party Chief spots and controls the evolution with hand signals and radio.

1) Winch operator raises the rosette about 2 feet off deck and allows A frame to swing the rosette over the stern.
2) Rosette is lowered to just above water surface and winch read out is zeroed.
3) Rosette is lowered to a depth of 2 meters.
4) Upon command from the lab the rosette is lowered at a rate of 60 meters/minute
5) A stop depth is given for the winch. The lab will give a more specific number and count down when approaching maximum depth, usually within a few meters of winch reading.
6) At 50 meters from desired depth slow to about 30 meters / minute so as to not jolt the wire.

RECOVERY

1) Ascend at a rate of 60 meters / minute, stopping at selected depths. The winch rate should be slowed as the target depth is approached, using guidance from the lab.
2) Notify the Bridge when close to the surface. Stop a 2 meters.
3) Rosette is recovered in reverse of deployment.
4) Notify Bridge when rosette is on deck.

WINCH CONTROLS

The winch control lever operates in two ways. Move the lever to the neutral centered position to switch between the two methods.

1) Push lever away (aft) to let line out, or pull lever towards you (towards the bow) to retrieve line. ONLY A SLIGHT TOUCH WILL MOVE LINE RAPIDLY. A REAL POSSIBILITY OF TWO BLOCKING EXISTS DURING LAUNCH OR RECOVERY EXISTS. USE EXTREME CAUTION! When the lever is released it returns to the stopped position.
2) Twist the lever to change and maintain the line speed. Twist left (counterclockwise) to pay out, twist right (clockwise) to retrieve.

There is another control unit on the winch as well – it is not to be used for deployment or recovery operations.

LINE TENSION

Be aware of expected line tension. An abrupt decrease in tension when the rosette approaches the bottom means it has landed on the bottom.

350 lbs – Tension reading with no load
870 lbs - Rosette in the air before deployment
570 lbs - Rosette in the water at the surface
1200 lbs - Rosette at a depth of 1200 meters
900 lbs - Rosette with full water bottles during retrieval

Standard Operating Procedure for the Quantech Fixed Wavelength Fluorometer

The Quantech Life Sciences Fluorometer has the ability to measure fluorescence at two emission wavelengths (340 and 450nm) during excitation by UV light at 280 nm.

The unit should be equipped with 2 sets of filters (one for use; one spare). There should be one narrow band excitation wavelength filter at 280nm (280NB), one narrow band emission filter at 340nm (340NB), and one narrow band emission filter at 450nm (450NB) in each set; there should also be one blocking filter.

Filter Placement

The positioning of the filters are as follows:

- When facing the instrument for operation with the cover open, the 280nm excitation filter fits into the filter slot at the 6 o'clock position.
- The emission filters (either 340nm or 450nm) fit into the filter slot at the 9 o'clock position.
- The blocking filter fits into the filter slot at the 12 o'clock position; it and the 280nm filters should remain in place (not be moved).

Startup

- At least 1 hour before the first sample is to be analyzed, the instrument is turned on (switch at the back of the unit near the power cord). The instrument will go through a start up sequence and display a 15 minute countdown.
- After 15 minutes has elapsed, the display will show ← Menu →. Push the <Right Arrow> once until the display reads ← Advanced Functions →. Push the <Enter> key. <Date and Time> will appear. Press the <Right Arrow> key until <UV Lamp Options> is displayed. Press <Enter>. The display will read <UV Lamp Installed?> with <No> underneath. Press the <Up/Yes> key to turn the UV lamp on and press <Enter>. You now must wait **20 minutes** for the UV bulb to warm up. ← Menu → should once again be displayed.
- After at least 20 minutes, press the <Right Arrow> to select ← Advanced functions →(Press <Enter>). Press the <Right Arrow> until <Manually Set Gain and PMT Voltages>. Press <Enter>. The display will show <Turn off Auto Gain?> Press the <UP/Yes Arrow>and press <Enter>. Press the <Up/Yes Arrow> until Gain= 100 is displayed and press <Enter>. .

- ← Menu → will be displayed. Press the <Right Arrow> to select ←Advanced Functions→ (Press <Enter>) and <Right Arrow>until <View Diagnostic Information> is on the display. When ←Raw PMT…. Hardware→ id displayed, press the <Left Arrow> key to select <Raw fluorescence>, and press the <Right Arrow> to select <UV Lamp>. Press the <UP/Yes> key to select the gain at 100. The raw fluorescence value should start fluctuating.

Drawing Samples for Analysis

Note: SAMPLES FOR THE FLUORESCENCE INTENSITY RATIO SHOULD ONLY BE TAKEN FROM BOTTLES FIRED IN AREAS OF ELEVATED FLUORESCENCE IN THE CTD TRACE.

- A 20 ml sample is acidified with 20μL of 10% HCl in a 20 mL scintillation vial equipped with a foil or Teflon lined cap and stored at 4^{o}C.
- Samples are drawn from the same cups as used for the LISST with the following exception – pour the contents of sample cup #2 into sample cup #1 (after the LISST sample from cup #2 has been successfully analyzed). This creates an integrated sample from the one niskin bottle/sample depth.
- Draw duplicates (labeled “sampleID”-1 and “sampleID”-2) from the integrated cup. Acidify the sample. (NB – It is easier to add the acid to the vial first and then the sample to ensure adequate mixing).
- Place approximately 3.5 mL of sample into a methacrylate disposable cuvette and allow the samples to approach room temperature; a cold sample will effect the fluorescence measurement. Refrigerate the remaining sample for storage, or discard if not to be archived.

Sample Analysis

- Place the cuvette into the holder of the Quantech fluorometer. Make sure the 340nm emission filter is in the proper filter holder position (9 o’clock). Close the lid.
- Wait a moment (count to five) and glance at the display. As the reading is not stable, glance at the reading and record the first number you see in the back of the fluorometry notebook under a column labelled “340”. Repeat this procedure twice more for a total of three (3) times (for example, sample ID BM550107-2 had 340 nm readings of 55, 63, 44).
- Open the cover of the fluorometer and replace the 340nm filter with the 450nm filter. Close the cover, count to five, and read the display three times in the same way as for the 340nm wavelength (for example: sample ID BM550107-2 had 450 nm readings 27, 23, 33).

- Average the three 340nm readings (eg, 55+63+44=162 / 3= 54) and the three 450 readings (eg, 27+23+33=83 / 3= 27.7)
- Obtain the fluorescence intensity ratio (FIR) by dividing the 340 nm by the 450 nm average (eg, 54 / 27.7 = 1.95)
- Repeat this procedure for the next samples.

Reporting Data

The FIR data are to be included with the LISST particle data in the daily report. FIR should be recorded as a number on the LISST particle graph above the corresponding LISST sample in the histogram.

LISST-100X Particle Size Analyzer Standard Operation Procedure

General Description

The Sequoia Laser In-situ Scattering and Transmissometry (LISST-100X) instrument uses the technique of laser diffraction to determine *particle size-distribution* (PSD) and/or *volume distribution*. It consists of an optical system that produces a collimated laser beam, a detector array, electronics for signal pre-amplification and processing, a data storage and scheduling computer, and a battery system.

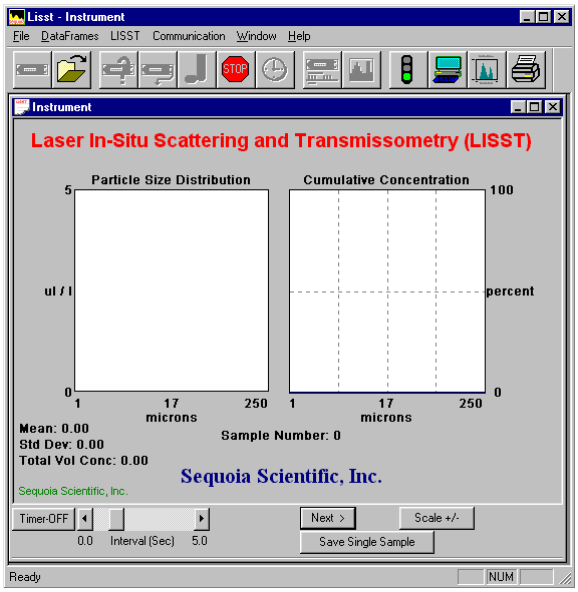
List of Materials

- User's Manual with software disk
- LISST-100X instrument
- Bench top instrument stands
- Communication cable
- External power cable
- Small volume horizontal test chamber
- Allen wrench set
- Pre-moistened alcohol wipes
- De-ionized water

Bench-top standard operation procedures

1. Remove the instrument stands and set them up on a flat bench top working surface.
2. Remove the LISST-100X from the case and set it on the stands.
3. Check the optical windows. Clean the Transmit window and the Receive window by wiping them with a pre-moistened wipe, and then by rinsing them with de-ionized water.
4. Remove the small volume horizontal test chamber from the case. Attach the flexible tubing and tubing stop clamp.
5. Slip the chamber between the optical windows of the instrument such that the optic can be submerged for calibration and testing (see Appendix F of the User's manual for more detail of chamber installation)
6. Check and adjust the chamber and the spacer to make sure that the chamber attachment to the Transmit and the Receiver windows are sealed properly without leaking.
7. Fill the small chamber with clean de-ionized water for calibration
8. Remove the Communication cable for the case. Connect the 5-pin underwater connector of the cable to the LISST-100X, and the 9-pin DB-9 of the cable to a lap-top personal computer.
9. Install software for communication and processing program, including the calibration files specific for the instrument. Create a shortcut of the LISST-100X program on desktop for the ease of operation.
10. Start the LISST-SOP software by clicking on the LISST.exe file.

11. Select *Wake up LISST* from the *LISST* menu. A dialogue box will appear counting down the maximum time (138 seconds) required for the instrument to wake up.
12. Acquire background scattering profiles by selecting *Collect Background Scatter Data* from the LISST menu or by pressing the  button on the toolbar. The background scattering measurement is critical to good instrument performance and will also check the overall health of the instrument. It will verify that all of the systems are functioning and that the optics in proper alignment. The current background will be acquired and displayed relative to the factory background scattering for the instrument (specific to each unit).
13. Press the *BEGIN Collection* button to acquire 20 samples and the display the average background scattering after the collection. If the values are acceptable and the values can be saved to a file, enter the filename in the box and press the *Accept and Save* button. If there is a problem, an error message will be displayed. Take corrective actions and make sure the current laser power is close to the Factory laser power.
14. Choose Open Real-Time Session from the File menu or press the  button to open the Real-Time session. Choose a background file to use when processing the data. Choose an output PSD file. A display similar to this will appear,



The left hand figure displays a bar chart showing the volume concentration in each of the 32 log spaced size classes. The right hand plot will be the Cumulative Concentration. To view the samples as a movie press the *Timer Off* button. The slider bar next to the button adjusts the refresh rate. The *First*, *Prev*, *Next*, and *Last* buttons allow you to step through the measurements one frame at a time. When the last frame is reached the movie display will stop. The slider bar controls the data acquisition time. The Scale +/- Button adjusts the Particle Size Distribution scale.

15. After finishing with the instrument, press *LISST* to sleep from the *LISST* menu to put it back into its low power sleep mode.
16. Choose *Convert binary PSD to ASCII* file under the File menu, save file as .xls file for data processing and manipulation in Excel.

Vessel side in-water continuous monitoring standard operation procedures

1. Connect the LISST-100X to the PC and start the LISST-SOP program.
2. Open the Operating Modes window by choosing Operating Modes from the LISST menu or by pressing the  button on the toolbar. Select the *Operating Mode Tab* at the top of the window. Choose Fixed Sample Rate. Select Start Conditions tab to configure when the instrument will begin sampling among five options: Depth, Time, External Mechanical Switch, External Digital Input, and Time Delay. Select the mode by clicking on the button next to the its label. Select the correct parameters as required.
3. Choose the Stop Conditions Tab to select the conditions when sampling should stop. The available stop conditions are: Depth, Time. External Mechanical Switch, External Digital Input, Fixed number of samples, and Maximum Memory or Low Battery.
4. Select *Apply* or *OK* buttons to configure the instrument with the current settings. If the *Apply* button is pressed the program will return to the current window. Returning to the *Instrument Status* window will display a summary of the current settings. If the *OK* button is pressed, when the configuration is complete the user will be prompted to open the *Terminal* window to start the program. To start the program and have it start looking for the Start conditions press the *Run* button on the *Terminal* window.
5. Once the program is confirmed to be running and waiting for the correct start conditions, the LISST-SOP program can be closed and the communications cable can be disconnected.
6. Replace the connector cap before deployment.
7. Deploy the configured LISST in water beside the vessel to carry out in situ monitoring of *in situ* particle size distribution in the water column.
8. When the start conditions are met and the instrument will start sampling. The green LED on the connector endcap will illuminate each time a sample is acquired. The instrument will continue collecting data until the pre-set stop conditions are met.
9. Stop a running program by pressing the Stop button on the Terminal window or the 

button.
10. Press the Instrument Query Button  to display the instrument status including the number of samples saved.

11. Select *Offload* from the *LISST* menu or choose the  button from the toolbar. A list of files will appear. Choose the files to offload by clicking on them while hold down the CTRL key. The data is offloaded at 115K baud.
12. To process the data file choose *Open Raw Data file* or press the  button on the toolbar. Select the instrument serial number, raw data file to open, and background scatter file to use when processing the data. Press the *Process File* button to convert the raw file into processed size distributions.
13. Processed data files are stored as binary files with a PSD extension. The PSD files can be opened and viewed on the screen. To open a processed Particle Size Distribution file choose Open Particle Distribution File or press the button on the toolbar. The steps are the same as bench top operation.

STANDARD PROTOCOL FOR SHIPBOARD ROTOXKIT M TESTING DURING MC252 OIL SPILL RESPONSE

22 June 2010

NOTE: As of the date of this revision, inclusion of a reference toxicant has not been added to the protocol. As soon as SDS solutions are prepared in an analytical laboratory, the solutions will be sent to each vessel and this protocol will be updated to include use of the reference toxicant.

PREPARATION OF STANDARD SEAWATER (35 ppt) AND HATCHING MEDIUM

1. Add 800mL Deionized (DI) water to a 1000mL volumetric flask or graduated cylinder.
2. Add the contents of vial #1 (NaCl) and shake to dissolve.
3. Add the contents of salt media solutions in vials #2 through #7 (in sequential order) located in the Rotoxkit M in numerical order.
4. Add DI water so the total volume equals1000mL and shake to homogenize the solution.
5. To prepare the Hatching Medium, dilute the standard seawater solution to 20ppt by mixing 5.7mL of standard seawater to 4.3mL DI water for a total of 10mL hatching medium. If large volume testing is required,, increase the scale to make 100mL at a time.

HATCHING THE ROTIFERS

6. Label the multi-well test plate with date of cyst initiation and appropriate ID number.
7. Empty One (1) rotifer cyst vial[1] into hatching well.
8. Add 2.5 mL of Hatching Medium (20ppt seawater) to the hatching well, using a pipette to rinse cysts away from edges. If using hatching medium to rinse the cyst vial, add 2.0 mL hatching medium to the hatching well.
9. Cover plate with parafilm, replace lid and place in incubator (25°C) for 28 hours under continuous illumination. If an insufficient number of rotifers have hatched, allow rotifers to incubate for a longer time period. Continue to check on rotifers each hour until optimal hatch rate has occurred. Do not exceed 30 hours incubation.

PERFORMING TOXICITY TESTING

10. Check hatching success to ensure adequate numbers of rotifers are available for testing.
11. Add 0.7mL of standard seawater (35 ppt) to the rinsing trough of the first row and use the microscope and pipette to transfer approximately 50 rotifers to the rinsing trough of row X of the test plate. This is the control.

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[1] Up to two vials of cysts may be used to ensure optimal hatching rate.

12. For rows 1-5, transfer 0.7 ml of the water sample taken at the first depth of the station to the rinsing trough for row 1. The 0.7 ml of the sample taken at the second depth should be added to row 2, and so on. Each row contains a sample taken at a specific depth. Rows 1 through 5 will be used to test up to five water samples collected from the rosette sampler. The chief scientist will determine which samples will be tested, by using fluorometry readings accompanying the CTD cast to identify the samples with highest likely oil concentrations. Samples will not be diluted during initial screening. Use 1.0 mL pipette to transfer 0.3 mL of the sample water to each of six wells in a given treatment row.
13. Allow the rotifers to acclimate in the rinsing trough for 1-2 hours. While this acclimation is occurring, proceed with steps 14-17 below.
14. Use a 1.0 mL pipette to transfer 0.3mL of standard seawater (35 ppt) to each of six wells in the first row (Row X). Row X will serve as the control and each well will serve as one replicate of n=5 rotifers.
15. This same procedure is followed to add each toxicant sample to the respective row (1-5).
16. All water samples used in the Rotoxkit M will be archived in a refrigerator until the test has concluded. In the event that mortality exceeds 50% in any given treatment, the test will be repeated by warming the archived sample to room temperature, and repeating the testing using the prescribed dilution series.
17. Record the treatment sample numbers assigned to each row.
18. Once the 1-2 hour acclimation (Step 13 above) is complete, transfer exactly 5 rotifers to each well. Once transfers are complete, recount each well in that row to confirm a count of 5 rotifers.
19. Once rotifers have been added to all wells, remove and dispose of all excess rotifers in hatching and rinsing troughs by pipette to avoid spill over during the exposure period.
20. Cover the plate with the parafilm, replace lid, and wrap in aluminum foil to shield from light since the exposure is conducted in darkness.
21. Place in incubator at 25°C for 24 hours.

READING AND RECORDING RESULTS

22. At T=24 hour exposure, remove the test plate from the incubator, remove aluminum foil and place under dissection scope for counting.
23. Count the number of live rotifers in each well. If rotifer is not moving, gently prod the organism with a micropipette to determine if it is alive. A rotifer is considered dead if it does not exhibit any signs of movement for 5 seconds after gentle prodding.
24. Record number of dead and alive rotifers in each well.
    Tally up each row and add together to get a row total, which should be 30 (5 rotifers x 6 wells). Mortality data is reported on a per well basis and on a cumulative (total row) basis.
25. Data findings are reported to the Chief Scientist.