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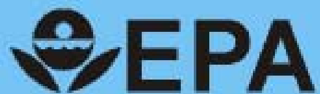

National Coastal Assessment

Field Operations Manual

Environmental Monitoring
and Assessment Program

EPA/620/R-01/003
June 2001

National Coastal Assessment Field Operations Manual

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NOTICE

The U.S. Environmental Protection Agency through its Office of Research and Development collaborated in the research described here under interagency agreement #DW14938557 to U. S. Geological Survey, National Wetlands Research Center. It has been subjected to Agency review and approved for publication. Mention of trade names does not constitute endorsement or recommendation for use.

This field manual represents the evolution of past EMAP-Virginian Province manuals. As such, much of this document was left as originally written for the Coastal 2000 program. As a result, the reader will encounter the terms "*Coastal 2000, C2000, CM*," etc.; in most cases those terms now imply National Coastal Assessment (NCA). I would also like to acknowledge the contribution past "EMAPers" have made to those documents. Contributors have included Dan Reifsteck, Ray Valente, Jill Schoenherr, Darryl Keith, Steve Schimmel, Kellie Merrell, Rebecca Fischman, Mike Daly, Don Cobb, and Kelly Byron.

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FOREWORD

The core elements of this field manual were prepared by Charles Strobel, EPA - Atlantic Ecology Division, as a guidance document for the state teams responsible for field collections of samples and data for EMAP's Coastal 2000 Monitoring in the northeastern (NE) region of the U.S. (Delaware to Maine). Although originally written specifically for NE field teams, the methods described in the manual reflect standard procedures developed by EMAP-Estuaries during 10 years of coastal monitoring activities conducted in all coastal regions of the contiguous U.S. This manual has been modified to reflect a more national focus.

The overall objective is to put a practical field methods guide into the hands of the participating field teams that allows a reasonable degree of flexibility to individual states, while at the same time, provides adequate structure to ensure the uniform collection of comparable field data on a national basis. This manual is intended as a "living document;" additional sections can, and should, be appended as needed.

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SECTION 1
INTRODUCTION

As a regulatory agency, the U.S. Environmental Protection Agency (EPA) is charged with the mission to set environmental policy, obtain funds for research and development, and evaluate the efficacy of environmental regulations in preserving the Nation's natural resources. EPA's National Coastal Assessment (NCA), also referred to as *Coastal 2000, C2000*, *CM* in this document, is a five-year effort led by EPA's Office of Research and Development to evaluate the assessment methods it has developed to advance the science of ecosystem condition monitoring. C2000 represents the current state of evolution of EPA's Environmental Monitoring and Assessment Program (EMAP). EMAP was originally designed to provide a quantitative assessment of the regional extent of environmental problems by measuring status and change in selected indicators of ecological condition. EMAP provides a strategy to identify and bound the extent, magnitude, and location of environmental degradation and improvement on a regional scale.

Beginning in the year 2000, C2000 will attempt to assess the condition of the Nation's estuarine waters through statistically valid subsampling. Whereas the original EMAP effort was conducted primarily by EPA and contract staff, C2000 is being implemented in partnership with the 24 coastal states. This partnership recognizes that each of these entities plays an important role in estuarine monitoring. Wherever possible, existing state monitoring programs are being incorporated into the C2000 design. This provides for the maximum utilization of a limited budget, and the flexibility of allowing states to often maintain historical sampling designs. Many of these state programs have been in existence for many years, providing a basis for possible C2000 trends analyses. Each state will conduct the survey and assess the condition of their coastal resources independently. These estimates will then be aggregated to assess the condition at EPA Regional, biogeographical, and National levels. Through this partnership EPA hopes to build infrastructure within the coastal states to improve, and make more inter-comparable, the multitude of estuarine monitoring programs throughout the country.

As stated above, C2000 is being implemented in cooperation with the coastal states. Most of the field sampling, and some of the sample analysis, will be conducted by state agencies through cooperative agreements with EPA. Analyses which state agencies choose not to perform will be conducted through a national-level contract with qualified laboratories. A common suite of "core" indicators will be measured using comparable methods:

- sediment contaminant concentrations
- sediment toxicity (*Ampelisca abdita*)
- benthic species composition
- sediment characteristics (grain size, organic carbon content, percent water)
- water column dissolved nutrients
- chlorophyll *a* concentrations,
- total suspended solids concentration,

C surface and bottom dissolved oxygen, salinity, temperature, and pH
C water clarity
C contaminant levels in fish and shellfish
C external pathological condition of fish
C fish community structure

C2000 is designed as multi-year program. In the northeast portion of the United States (Delaware to Maine), estuarine waters will be sampled over a two-year span (2000-2001). Approximately 30 to 40 stations will be sampled per state each year. Tentatively, the following two or three years will be dedicated to other ecosystems, such as coastal waters and/or salt marshes, with the hope of returning to estuaries in years five and six.

Each major region will be coordinated through a central EPA location:

EPA's Atlantic Ecology Division (AED), Narragansett, RI - Northeast States
EPA's Gulf Ecology Division (GED), Gulf Breeze, FL - Gulf and Southeast States and Puerto Rico
EPA's Western Ecology Division (WED), Newport, OR - West Coast, Alaska, and Hawaii
EPA's Mid Ecology Division (MED), Duluth, MI - Great Lakes

The purpose of this manual is to document suggested field data and sample collection procedures for C2000-NE. These protocols have been developed by EMAP over the past 10 years. They will be identical to, or at least comparable with, those used in other regions of the country. Individual states may prefer to use other methods, especially if they are currently being used in existing programs. This is acceptable providing that comparability can be demonstrated to the appropriate Regional Field Coordinator and the QA Officer.

SECTION 2
OVERVIEW OF FIELD SAMPLING ACTIVITIES

2.1 Sampling Period

The sampling period for C2000 is based on the historical index period established for EMAP-Estuaries efforts. This is based on the time frame in which the benthic biota are most active and hypoxia is most prevalent. The established index period is July through September. Some deviation from this period may be acceptable if the criteria for defining the index period are met.

2.2 Sampling Design

The EMAP-Estuaries sampling design on which C2000 is based combines the strengths of systematic and random sampling with our understanding of estuarine systems. It provides a design that will allow probability-based estimates of the status of the Nation's estuarine systems, the variability associated with that status, its spatial and temporal components, and the temporal trends associated with changes in these systems. The Coastal 2000 sampling design is based on a single, annual sampling season of each station during the Index Period. The design differs from previous EMAP designs in that existing monitoring programs were incorporated where appropriate. "Biased" programs, such as those designed to evaluate the effects of a treatment plant, would NOT be appropriate for inclusion. Working with the states, the C2000 design team was able to identify a large number of sites that are currently being monitored and meet the criteria for being unbiased in their location. Many were randomly located in the original monitoring design.

The objective of the sampling design is to provide a statistically defensible strategy for collecting information about selected indicators of ecological condition and their variability. The design is flexible to allow alternative future uses.

2.3 Indicators of Ecosystem Health

The primary goal of C2000 is to provide an assessment of overall ecosystem condition. To accomplish this goal, a number of "indicators" of ecosystem health will be monitored. The core set of indicators agreed upon by all entities involved in this project is listed in Table 1, with the goal being to collect data on all of these indicators at all sampling stations.

Table 1. List of core ecological indicators being measured by C2000

Water Quality Indicators

Hydrographic Profile
- dissolved oxygen
- salinity
- pH
- temperature
- depth
- light attenuation (PAR, transmittance)
- secchi depth

Water Quality Samples
- dissolved nutrients (ortho-phosphates, nitrites, nitrates, ammonia)
- chlorophyll a
- total suspended solids (TSS)

Sediment Quality

Composited Surficial Sediment
- sediment contaminants (organics and metals)
- sediment TOC
- sediment toxicity (amphipod)
- percent silt/clay

Biota

Fish/Shellfish
- community structure (species; abundance; total length, up to 30 individuals)
- tissue contaminants (organics and metals)
- external pathology (fish)

Benthos
- community structure (standard grab - 0.04m^2)

Habitat

- SAV (presence/absence)
- basic habitat type (e.g., open water, tidal flat, marina, harbor, inlet, tidal river/stream, seagrass bed, sandy/muddy bottom, rocky bottom, shelly bottom, coral reef, etc.)
- marine debris (presence/absence)

2.4 Site Reconnaissance

Prior to the start of field activities, a thorough reconnaissance of the area to be sampled should be performed whenever field crews will be working in new areas. This includes determining the locations of boat ramps, hotels, and dry ice suppliers; visiting any stations that may fall in water too shallow for boats; and attempting to identify any potential problems that the field crews may face during the Index Period. Needless to say, reconnaissance may not be needed in areas with which state field crews are familiar.

2.5 Station Location

The randomly selected sampling locations for each state (or specific study area) will be provided to the field crews as coordinates of latitude/longitude in degrees-minutes, expressed to the nearest 0.01 minute (*i.e.*, 00E 00.00'). The crews will use GPS (preferably DGPS) to locate the site. Three different locations will be provided for each station. These are identified as “A”, “B”, and “C”. The primary site is the “A” location; “B” and “C” are backups. If the primary site is not accessible, or the bottom is too rocky to obtain a sediment sample, then the crew may move to the “B” site. If that site is also unsampleable, then they should move to the “C” site. If all three are unsampleable, then the site is not sampled. If one of the sites can be sampled for only some of the indicators, then that sampling should be conducted. It is important that the crew note on the datasheet at which of these locations (A, B, or C) the samples were collected.

Crews will attempt to navigate to the location to within 0.02 nm (± 37 meters) of the given coordinates. This reflects the accuracy expected from a properly functioning GPS unit of the caliber that will used for the study. The crew will record the actual coordinates of the vessel **after anchorage**, NOT the initial intended coordinates, on the field data sheet.

2.6 Sampling

In order to collect data as efficiently as possible and reduce the potential for sample contamination, the samples should be collected in the order shown in Figure 1. Details about each sampling procedure can be found in following chapters. A complete list of needed supplies and equipment can be found in Appendix A. As stated in the Introduction, variations from these methods must be approved, in advance of sampling, by the Field Coordinator and QA Officer. An overview of Quality Control/Quality Assurance (QC/QA) protocols for each sampling technique can be found after the description of each procedure. A more detailed account of QC/QA proposals can be found in the C2000 Quality Assurance Plan.

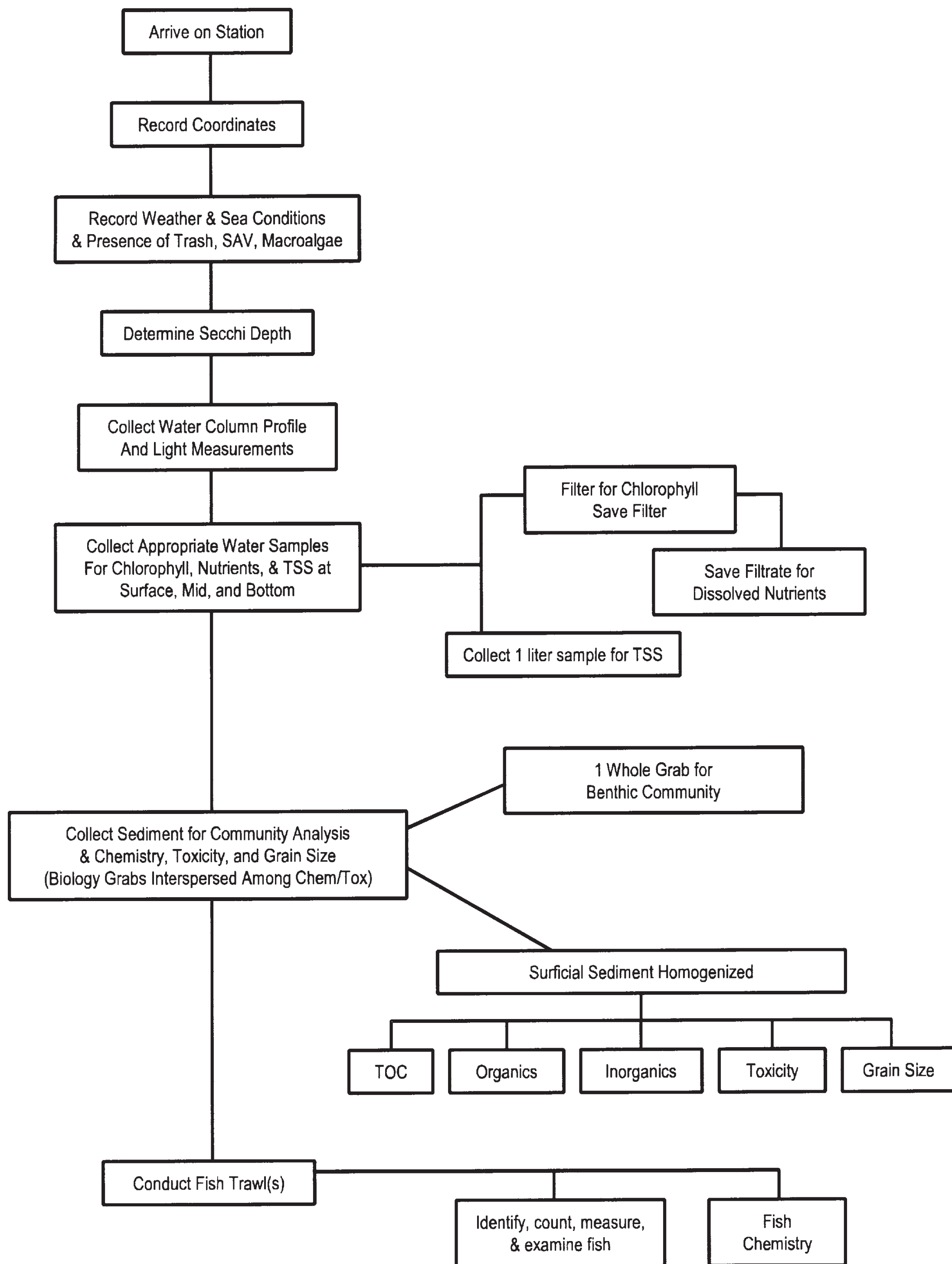


Figure 1. Flow chart of sampling activities conducted at C2000-NE stations.

SECTION 3
FIELD DATA BASE MANAGEMENT

Management of data in the field is of paramount importance. Without proper data management the quality of the data generated is questionable. Field data management consists of two categories; written data sheets and electronic data.

In general all data will be recorded on hard-copy datasheets while on-station, and entered into a computer back on shore. The use of bar code readers will facilitate the entry of sample numbers and eliminate transcription errors. EPA can provide datasheets and electronic forms for data entry if desired. EPA can also assist in the procurement of bar codes. C2000 datasheets are included in Appendix C. Although their use is not required, it is highly recommended.

It is the responsibility of the chief scientist to guarantee the quality of the data. At the end of each day it is his/her responsibility to review the data collected that day and "sign-off" on it.

3.1 Sample Tracking Procedures

A variety of water, sediment, and biological samples and data are collected during the C2000 sampling effort. These include physical samples (*i.e*., sediment and nutrient samples) and non-physical samples (*i.e*., Hydrolab or YSI cast data). It is vital that all of these samples and data be tracked from collection to the receipt of analytical results. To accomplish this purpose, all samples collected are assigned unique sample identification numbers (SAMPLE IDs) composed of the station number and a sample-type code. These numbers are used to track samples from collection to inclusion in the final National database.

3.2 Station and Sample Numbers

Because Coastal 2000 is a large-scale national monitoring program being implemented by many agencies with data feeding into a centralized database, it is critical that all stations and samples be assigned unique identifiers. All information sent to the national database must be associated with a station using the C2000 convention. Station names will include the state in which the station is located, the year of sampling, and a number. The format adopted is as follows:

SSYY-XXXX

Where,

SS is the state where the station is located,
YY is the last two digits of the year (00 for year 2000), and
XXXX is a four-digit incremental identifier, beginning at 0001.

So, for example, the first 35 stations in Massachusetts, Texas, and California, sampled in year 2000 would be identified as:

MA00-0001 to MA00-0035
TX00-0001 to TX00-0035
CA00-0001 to CA00-0035

Note that where one state entity will be sampling in another state's waters, the station number is based on the "location" not the organization conducting the sampling. This is likely to occur when one state will be responsible for an entire water body even though portions fall within the neighboring state's jurisdiction.

Sample numbers will be made up of the station code with a sample type identifier attached to the end. Sample number formats are illustrated in Table 2. All sample information sent to the national database must use this format.

3.3 Use of Bar Codes

The use of bar codes to label samples is highly recommended. Ten years of experience with EMAP has demonstrated their utility. Bar codes are preprinted for every sample that might be collected during the sampling season, with side-by-side duplicates for each sample, by a professional service. Each label contains both the bar code itself, and the printed sample number. Labels are waterproof and do not come off when frozen or immersed in formalin.

When a sample is collected, one of the duplicate labels is placed on the sample and the other on the datasheet. When the data are transcribed from the datasheet into the computer, the sample numbers need not be typed in. They can just be scanned with a bar code reader. This virtually eliminates transcription error.

Bar codes also make shipping of samples easier. As a sample is placed in the shipping container, the sample ID is scanned into the computer. A packing list can then be printed out for inclusion with the shipment.

3.4 Electronic Data Entry

All information recorded on the datasheets must be entered into a computer for eventual transfer to the national database. This should be done as soon as possible, while the sampling event is "fresh" in the crew's mind. It is the responsibility of the chief scientist to ensure the accuracy of the electronic data file.

One method to facilitate this process is to use form-filler software. This software can be used to design and print hard-copy datasheets, and to create an identical electronic form. Having the computer screen as an exact copy of the field forms facilitates data entry. QA is easier as the completed electronic form can be printed out for side-by-side comparison with the original field form.

EPA can provide both the field and electronic datasheets to any participant desiring them. The participant would only need to purchase the appropriate commercial software to access the electronic forms.

Table 2. Sample numbers assigned to each sample type. Sample number consists of the station number (state/year - number, *e.g.*, RI00-0001 for Rhode Island, year 2000, station 1) followed by a sample type code. The list below uses station RI00-0001 as an example to illustrate the appropriate sample coding. For QA samples, the state identifier is replaced with "QA" and the station designator is sequential rather than being associated with a given station. The link is made in the database. While collection of specific QA samples is helpful, EPA does not require all participants to collect these samples.

Sample Type	Type Code	Example Sample Number	Bar-Coded?
CTD Cast	CTD	RI00-0001-CTD	**N**
Light measurement (PAR) profile	PAR	RI00-0001-PAR	**N**
Surface chlorophyll	SCL	RI00-0001-SCL	Y
Surface suspended solids	SSS	RI00-0001-SSS	Y
Surface dissolved nutrients	SN	RI00-0001-SN	Y
Mid-depth chlorophyll	MCL	RI00-0001-MCL	Y
Mid-depth suspended solids	MSS	RI00-0001-MSS	Y
Mid-depth dissolved nutrients	MN	RI00-0001-MN	Y
Bottom chlorophyll	BCL	RI00-0001-BCL	Y
Bottom suspended solids	BSS	RI00-0001-BSS	Y
Bottom dissolved nutrients	BN	RI00-0001-BN	Y
Benthic infauna(1)	BI1	RI00-0001-BI1	Y
Benthic infauna(2)	BI2	RI00-0001-BI2	Y
Benthic infauna(3)	BI3	RI00-0001-BI3	Y
Sediment Toxicity	ST	RI00-0001-ST	Y
Sediment grain size	SG	RI00-0001-SG	Y
Sediment Organics	SO	RI00-0001-SO	Y
Sediment Metals	SM	RI00-0001-SM	Y
Sediment TOC	OC	RI00-0001-OC	Y
Standard fish trawl	STRL	RI00-0001-STRL	**N**
Non-standard fish trawl	TRL	RI00-0001-TRL	**N**
Fish chem. species 1 composite	FC1	RI00-0001-FC1	Y
Fish chem. species 1 individuals	FC1-1 to FC1-9	RI00-0001-FC1-1 To RI00-0001-FC1-9	Y
Fish chem. species 2 composite	FC2	RI00-0001-FC2	Y
Fish chem. species 2 individuals	FC2-1 to FC2-9	RI00-0001-FC2-1 To RI00-0001-FC2-9	Y
"Other" sample type 1	OTH-1	RI00-0001-OTH-1	Y
"Other" sample type 2	OTH-2	RI00-0001-OTH-2	Y
Quality Assurance Samples (full range of sample numbers given for C2000-Northeast)			
Fish pathology QA	PATH	QA00-0001-PATH To QA00-0500-PATH	Y
Chlorophyll QA	CL	QA00-0001-CL To QA00-0300-CL	Y
Dissolved nutrients QA	N	QA00-0001-N To QA00-0300-N	Y
TSS QA	SS	QA00-0001-SS To QA00-0300-SS	Y

HYDROGRAPHIC PROFILE
Coastal 2000 - Gulf Region

Station Name ____________

QC CHECK TIME: (HH:MM) QC'd BY:

PARAMETER	TEMP (°C)	SAL	pH 7	pH 10	DO (%)	DEPTH
STANDARD:			7.0	10.0	100.0	0.0
MEASURED:						
CALIBRATE:						

DATE: (MMDDYY)	TIME: (HH:MM)	INSTRUMENT #:

DOWN CAST

DEPTH (m)	TEMP (°C)	Ph	DO (mg/l)	SAL (o/oo)	LIGHT (AMB)	LIGHT (UW)	SECCHI DEPTH(m)

UP CAST

DEPTH (m)	TEMP (°C)	Ph	DO (mg/l)	SAL (o/oo)	LIGHT (AMB)	LIGHT (UW)

PLACE COMMENTS ON BACK

RECORDER:____________ COMPUTER ENTRY BY:____________

SECTION 4
WATER QUALITY MEASUREMENTS

One of the activities to be performed at every station is the collection of water quality information (salinity, temperature, pH, and dissolved oxygen [DO]). At each station a vertical profile through the water column will be obtained using a profiling instrument. Multiparameter water quality probes are available from several manufacturers (e.g., Hydrolab®, Yellow Springs Instruments, YSI®, and SeaBird®); each manufacturer offers various models with varying capabilities. The following instructions are specific for crews using the Hydrolab H20 probe in conjunction with the Scout 2 deck display; these procedures will require some modification when different, but similar instrumentation is used. Appendix D details the calibration and use of the Hydrolab DataSonde3/Surveyor 4, the array used extensively by NE crews which requires the use of a PC and the software package PROCOMM to setup or calibrate.

4.1 Hydrolab® H20/Scout 2 - Hydrographic Profiling Instrumentation

The Hydrolab H20 probe/stirrer used with the Scout 2 Display Unit is powered by an internal battery pack of 10 conventional, alkaline "AA" batteries . This combination provides a self-contained, hand-held water quality data system that displays real-time measurements. The terms "Hydrolab", "sonde", or "unit" may be used instead of H20/Scout 2 when referring to the instrumentation.

4.1.1 Setup and Calibration

The following is a brief description of the calibration procedures for the Hydrolab H20/Scout 2 pairing. Refer to the manufacturer's manual for detailed discussion of the equipment, including maintenance and repair information. Calibration should be performed every morning prior to the start of the day's sampling and documented in the "QC CHECK" block appearing near the top of the Hydrographic Profile field data form (see attached form). It is recommended that a "field calibration kit" consisting of the following supplies and items be assembled and stored in a good quality, plastic tackle box:

- calibration cup with removable cover
- DO membranes
- backup probes and spare parts (e.g., pH and DO probes, o-rings, etc.)
- standard seawater (known salinity, 15-22 ppt)
- pH buffers (7 and 10)
- laboratory thermometer
- deionized (DI) water
- ring stand with large clamp
- squeeze bottle (rinsing)
- calibration forms
- appropriate size leak-proof, screw-cap containers (e.g., Nalgene) for the additional calibration solutions
- basic tools (assorted screwdrivers, allen wrenches)

A properly maintained Hydrolab that is used on a regular basis, should remain fairly stable and free from marked drift or variation in its measurements from one day to the next. Daily QC checks of the Hydrolab provide the user with immediate feedback on the instrument's general reliability and a written performance record for each of the parameters. Based on years of experience with Hydrolab units, EMAP considers the following ranges acceptable for day-to-day instrument drift:

DO	± 0.5mg/l (or ± 8% saturation)
pH	± 0.3 units
salinity	± 1.0 ppt
temperature	± 1.0EC
depth	± 0.5m

These acceptability ranges take into consideration real-world factors to which a unit is typically exposed during the rigors of a day in the field (e.g., pounding seas, temperature extremes, rough rides in a truck, etc.). Usually, a well maintained Hydrolab will remain within tighter agreement than the ranges listed. Although the above criteria represent acceptable instrument drift, on the morning of each field day, the unit still should be calibrated to correct for drift as much as possible (see QAPP for additional discussion).

Performance that falls outside of the acceptability range or sluggish equilibration response, signals that the unit needs maintenance focused on the deficient functions (e.g., battery replacement, DO membrane replacement, pH reference solution renewal, cleaning electrodes, etc.). After determining and correcting the problem, the Hydrolab should readily accept new calibration values and quickly equilibrate. If performance remains substandard, the unit should be removed from service until it is repaired. It is highly advisable to have a backup unit available.

1. General Setup

Attach the probe to the deck unit using data cable; make sure the connections are locked or secured. Power up the unit by pressing the on/off switch; display window should illuminate; bring up primary operating screen. Remove the storage cup from the probes and replace with the calibration cup. Hold or use ring-stand clamp to secure the H20 in an inverted (probes up) position during calibration procedures. The preferred sequence of calibration is temperature, salinity, pH, DO, and depth; regardless, salinity should be calibrated prior to DO.

2. Salinity Calibration and Temperature Check

Rinse probes with about 10 mls of salinity standard; dump and refill the calibration cup with salinity standard to a level that completely covers the white conductivity cell. Place thermometer into the calibration solution and allow to equilibrate; record the thermometer reading to the nearest whole degree in the "STANDARD" box under the TEMP column; record the temperature value appearing on the deck display as the "MEASURED" value for TEMP; the

measured temperature should be within ±1EC of the standard. NOTE: the temperature function for the H20 is set at the factory and cannot be corrected in the field; it must be sent back to Hydrolab for adjustments. There is no field calibration procedure for temperature, but rather a QC check to verify proper function.

Continue with the salinity cal check. First record the “true” value of the salinity standard in the STANDARD box under SAL; then, record the salinity reading from the display screen in the MEASURED box. If MEASURED differs from STANDARD, calibrate the function.

To calibrate (see Hydrolab Operating Manual for detailed discussion): on the deck display, depress the “Calibrate” key to bring up the menu of variables (abbreviations or symbols); scroll across the menu by keying the right or left direction arrows to “S” for salinity; initiate the calibration. The screen will display the sensors current reading; use directional (R/L) arrows to scroll to the numerical character that needs to be adjusted, then use the up/down arrows to increase or decrease the value to represent the true value of the standard. Press “‘Enter” to calibrate, then select Y (yes) to accept the new calibration value for salinity. Return to Main Screen to verify the calibration; the displayed value for salinity should be within ± 0.5 ppt of the standard.

Carefully pour the salinity standard solution from the calibration cup back into the field supply bottle. It is permissible to reuse the salinity standard for several days as long as precautions are taken to ensure that the solution is not diluted or allowed to evaporate. Leave the calibration cup on the instrument and rinse the probes and cup with a few squirts of DI water; dump and shake to remove residual, then proceed to the pH calibration.

3. pH Calibration

Two buffer solutions are required to set up the pH calibration; pH 7 and pH 10 standards are recommended. First, pour pH 7 buffer into calibration cup to a level that completely covers the reference pH cannister. Bring up Main Screen and allow the pH reading to equilibrate (approximately 20-30 secs); record the pH reading on the QC Check form in the MEASURED box under pH 7; then proceed to calibrate in similar manner as described for salinity, only select the pH variable from menu. Key in Calibration mode; scroll with arrows; adjust value to standard (7.00); enter new value and accept by selecting “y” (yes); record 7.0 in the CALIBRATE box. Decant pH 7 buffer and rinse probes with DI water. Repeat process with pH 10 to complete the two-point calibration slope.

4. Dissolved Oxygen (DO) Calibration

Remove the plastic guard from the DO probe to fully expose the membrane and fill the calibration cup with DI water to a level just to, but not over the DO membrane (even with the O-ring); gently wipe any water droplets from the membrane using a tissue. Place the cap over the calibration cup, then allow 2-3 minutes for the environment around the DO membrane to become saturated with moisture (this system of calibration is commonly referred to as water saturated-air

calibration). After the system has equilibrated, switch to Alternate Screen to the display that reads out "% Saturation." The standard value is 100%; record the instrument's measured value in the MEASURED box on QC Check form. Any value $ 95% is good indication that the DO function is performing well. To calibrate DO, press the "Calibration" key; scroll over to "%" and select; a screen will appear with that displays standard pressure at 760 mm (normal for sea level); press the Enter key and Y to accept calibration. Return to Alternate Screen to verify that DO calibration was successful - 100 ± 2%. Record the instrument reading in the CALIBRATE box.

5 Depth Sensor

The depth sensor on the H20 is a transducer that responds to water pressure. For the daily QC Check on depth, when the unit is out of the water, the transducer experiences no significant pressure and the depth sensor should register 0.0 meters; therefore, on the QC Check block, the standard value for depth is 0.0 m. For QC Check, refer to the main screen and record the displayed depth measurement in the MEASURED box. If the reading is not 0.0, calibrate by pressing the "Calibration" key, then scroll over to "D" and press Enter. Adjust the depth reading to 0.0 by using the up/down arrows; Enter the new calibration value and accept by selecting "Y"(yes) key followed again with Enter. Record the instrument's depth reading in the CALIBRATE box.

At the conclusion of calibration, be sure to turn off the power (to conserve battery life). Remove the calibration cup and replace with the storage cup. Be sure that it contains a small amount of water (approximately 100-200 ml); the probes must be maintained in a wet environment.

Field Verification for Depth

To further verify the accuracy of the depth function, while in the field, lower the sonde to a known depth (e.g., 2 meters via marked data cable) and compare to the instrument's reading for depth. If noticeably different (e.g., 0.2 m), re-calibrate by performing the calibration procedures above, except use the "known" depth as the STANDARD.

NOTE: When conducting water column profiles, the preferred determination for depth is to use an accurately marked cable line. However, in conditions of strong tides and currents, due to the scope on the cable, it may be necessary to utilize the depth sensor to estimate depth.

4.1.2 Field Deployment and Water Column Measurements

After successfully completing the daily QC Checks/Calibration, the Hydrolab is ready for use. A five gallon bucket is a convenient storage/transport container.

Once aboard the vessel, the unit can be readied for use by removing the protective cup and attaching the stirrer; however, the exposed probes must be held in water. The bucket can be filled to a level that covers the stirrer and probes, or, a storage well can be constructed from 6-inch PVC piping. Cap one end of a 24-in section of pipe with a plug and affix the resultant well

to the vessel railing (or other suitable point of attachment) with line or tape. Insert the sonde into the well and fill with site water; the instrument can then remain intact and ready to go for the duration of a sampling day.

A station of nominal depth (<10 m) field measurements for the water column profile will be taken at near surface (probes approximately 0.5 m below surface), at 1-meter intervals down the water column, and at 0.5 m off bottom. On the down cast, at each interval, the parameters of interest are recorded on the Hydrographic Profile data form; a replicate set of measurements is recorded at each interval coming back up the water column. See QAPP for discussion on sampling interval for deep stations (>10 m).

Remember to always turn the power off after completing the water column profile; a unit left on with the stirrer attached will quickly drain the batteries.

4.2 Light Attenuation

C2000-NE crews will also obtain a vertical profile of light for the purpose of calculation of the light attenuation coefficient at each station. This can be accomplished using either a PAR (photosynthetically active radiation) meter or a transmissometer. This profile can be obtained in conjunction with the CTD profile or separately, depending upon the equipment available. PAR sensors require no field calibration, however, they should be returned to the manufacturer prior to each field season for annual calibration.

To obtain a PAR profile using an independent datalogger such as the LI-COR LI-1400:

1. Connect a deck sensor and an underwater sensor to the LI-1400. Make sure the correct calibration factors are entered for each probe. These are supplied by the manufacturer.

2. Place the deck sensor on the boat in a location where it will is not shaded.

3. Lower the underwater sensor on the SUNNY (or at least unshaded) side of the boat to a depth of about 10 cm (represents "surface").

4. Once readings stabilize, record the values from both sensors (FE/m^2/s), along with the water depth of the underwater sensor, on the datasheet. Log the values in the datalogger.

5. Lower the underwater sensor to 0.5 meters, allow the values to stabilize, and record the values from both sensors, along with the water depth of the underwater surface.

6. Repeat at the following schedule:
   Shallow sites (# 2 m) - every 0.5 m interval;

   Nominal depths (>2<10 m) - 0.5 m (near-surface) and every 1-m interval to near-bottom (0.5 m off-bottom);

Deep sites (>10 m) - 0.5 m (near-surface) and every 1-m interval to 10 m, then at 5-m intervals, thereafter, to near-bottom (0.5 m off-bottom).

7. If the bottom is impacted with the meter, allow 2-3 minutes for the disturbed conditions to settle before taking the reading.

8. If the light measurements become negative before reaching the bottom, terminate the profile at that depth.

9. Repeat the process on the upcast.

4.3 Secchi Depth

The Secchi disk is used to give a measurement of the transparency of the water column, also called the secchi depth. This measurement is made at every station and is recorded on the CTD datasheet. A 20 cm black and white Secchi disk is held by a non-stretch line that is marked in two tenths of a meter intervals. To determine the Secchi depth:

1. Slowly lower the Secchi disk on the shady side of the boat until it is no longer visible and note the depth using the markings on the line (interpolate between markings to the nearest 0.1 meter). If the disk hits the bottom, meaning the Secchi depth is greater than the water depth, note this on the datasheet.

2. Slowly raise the Secchi disk until it just becomes visible and note the depth.

3. Perform steps 1 and 2 three times, noting both readings. Record the average of the readings.

QUALITY CONTROL FOR SECCHI DISK

1. If the range of measurements for the three sets of depth readings is greater than 0.5 m, the entire process should be performed again.

2. No sunglasses or any other devices should be used to shade the eyes while this procedure is being performed.

3. The Secchi depth should be determined from the shady side of the boat during daylight hours.

SECTION 5
WATER COLUMN NUTRIENTS

Water samples will be collected at each site and analyzed for:

Chlorophyll *a*
Dissolved ammonia, nitrites, nitrates, orthophosphates, and
Total Suspended Solids.

Samples should be collected at three depths; surface, mid-water, and bottom, depending upon the depth of the water:

Shallow sites (< 2 m) - 0.5 m (near-surface) and 0.5 m off-bottom[1]

Standard sites (>2 m) - 0.5 m (near-surface), mid-depth, and 0.5 m off-bottom;

[1] Unless he depth is so shallow that near-surface and near-bottom overlap; then sample at mid-depth only.

Water samples should be obtained, either using a pumped system or a water sampling bottle such as a Niskin or 5 *Go-Flo®* bottle, and transferred to a rinsed (3x with water from the sampling bottle) one gallon HDPE container.

5.1 Chlorophyll *a* and Phaeophytin

Chlorophyll samples must be filtered no more than 4 hours after collection. Any further delay is strongly discouraged due to the possible lysis of phytoplankton cells. Samples that cannot be filtered immediately after collection must be held at 4EC until filtered. Filtering can be accomplished by either of two methods. The first requires the use of a vacuum pump, either electric or hand operated. The second uses positive pressure. The method used must be noted on the datasheet.

5.1.1 Vacuum filtration

Immediately concentrate the algae by filtering onto two, separate 47 mm GF/F filter pads; Process a sufficient amount of sample (*i.e*. 100-1,500 ml) to produce a green color on the filter; the volumes of replicate samples should be the same. Record the volume filtered on the datasheet. The filtrate should be saved for dissolved nutrient analyses (Section 4.2). To avoid cell damage and loss of contents during filtering, do not exceed a vacuum of 15 psi or a filtration duration of greater than 5 minutes. Add 1 ml of saturated $MgCO_3$ solution (10 mg/L) during the last few seconds of filtering AFTER THE NUTRIENT FILTRATE HAS BEEN REMOVED. This buffers the sample to reduce the possibility of degradation. Carefully remove the filters using forceps (never touch the filter with your fingers), fold in half, and wrap in clean aluminum foil or small disposable petri dish. Mark both the **volume filtered** and the sample number (SCL,

MCL, BCL: surface, mid, or bottom chlorophyll) on the foil envelope or petri dish. Place both filters in a whirl pak or small disposable petri dish and affix the appropriate bar code or hand-write the sample number. Place the package on dry ice.

Note that filter funnels should be rinsed with DI water prior to filtration. In addition, graduated cylinders should be rinsed with site water.

If too much sample is filtered (*i.e.*, there is a thick layer of material on the filter pad), it should be discarded and the filtration repeated with a smaller volume. Too much material may result in some "oozing out" when the pad is folded.

5.1.2 Positive pressure filtration

The alternative method is to use positive pressure to push a sample through the filter. A disposable, graduated 50-cc polypropylene syringe fitted with a stainless steel or polypropylene filtering assembly is used to filter the site water through 25 mm GF/F filters; the volume of water filtered must be documented. If conditions allow (based on the suspended solids load), up to 200 ml of site water should be filtered for each chlorophyll sample; for a 50-cc syringe, that equates to 4 fills. To refill when the syringe barrel empties, carefully detach the filter assembly and fill the syringe to the mark, replace the filter and continue with the filtration until the desired volume has been processed. The filtrate from this process is saved for the analysis of dissolved nutrients (see Section 4.2). After filtering the sample, add 1 ml of saturated $MgCO_3$ solution (10 mg/L) to the syringe (AFTER THE NUTRIENT FILTRATE HAS BEEN REMOVED) and pass this through the filter pad. This buffers the sample to reduce the possibility of degradation. Using tweezers, carefully remove the filter from its holder and fold once on the pigment side, then place it in foil as described in 4.1.1. Record the volume of water filtered on both the foil and on the field form. Mark the sample number on the foil pack, then place the foil pack in a whirlpack and label the whirlpack with the appropriate bar code. Place on dry ice. Repeat the filtering process for the second sample and store filter in the same whirlpack containing the first sample. The samples must remain frozen until time of analysis. Discard the used syringe. Rinse the filtering assembly with deionized water and store in a clean compartment between sampling stations (a small tackle box makes a good carrying kit for supplies and equipment used in this activity).

5.2 Dissolved Nutrients

Approximately 40 ml of filtrate from the above chlorophyll filtration will be collected into a prelabeled, clean 60-ml Nalgene screw-capped bottle and stored on dry ice. Before placing sample in the freezer, affix the appropriate bar code to the bottle and record the approximate salinity (±2 ppt) on the container. This is a convenience for the analyst who will perform the nutrient analysis. Depending on the analytical instrumentation used, matrix matching of solutions (*e.g.*, standards or wash solutions) may be required for certain of the analytes. The salinity value can be obtained from the water column data or by refractometer reading of the actual water sample taken by sampling bottle. The nutrient samples should remain frozen until time of analysis.

5.3 Total Suspended Solids

Approximately 1 liter of unfiltered seawater from the sampling bottle is poured into a 1-L polypropylene bottle and stored at 4EC to await laboratory analysis.

5.4 Quality Control

Field duplicates: A field duplicate is a sample taken at the same location and depth as a regular sample and processed for chlorophyll, nutrients, and total suspended solids. The duplicate and sample should be taken in quick succession. A field duplicate should be collected once for every 10 samples. The data from field duplicates indicates sampling precision.

Although some filtering may be done on shore, many times it will be necessary to filter while on the boat. Working with liquids on a rocking boat presents many opportunities for contamination, and therefore, special care must be taken. The following guidelines will help prevent accidents while working with the water samples:

1. After every station empty the overflow bottle and all reservoirs.

2. Rinse the filtering apparatus with DI water before putting in a new filter.

3. Only handle filters with tweezers.

4. All filters should be inspected and damaged filters should be discarded.

SECTION 6
SEDIMENT COLLECTIONS

6.1 Sediment Collections

Sediments are collected for a variety of analyses. One grab sample is collected for benthic species composition and abundance; the entire content of the grab will be sieved to collect benthos. Additional sediment grabs are collected for chemical analyses, grain size determination, and for use in acute toxicity tests. For these analyses, only surficial sediment (top 2-3 cm) will be collected. The surficial sediment from multiple grabs will be composited to obtain an adequate volume (approximately 4-5 liters) of sample for the analyses; the number of grabs needed may vary based on the sediment characteristics. While a biology grab is being processed (sieved), grabs should be collected for chemistry/toxicity.

A 1/25 (0.04) m^2, stainless steel, Young-modified Van Veen Grab sampler is used to collect sediments. The sampler is constructed entirely of stainless steel and has been Kynar®-coated (similar to Teflon) and is therefore appropriate for collecting sediment samples for both biological and chemical analyses. The top of the sampler is hinged so the top layer of sediment can be easily removed for chemical and toxicity analyses. This gear is relatively easy to operate and requires little specialized training.

Other gear is also acceptable, following approval by the C2000 Field Coordinator. The gear size must be identified on the appropriate datasheet.

Listed below is the protocol for obtaining sediment samples.

1. The sampler must be thoroughly washed with Alconox prior to use at a station, then rinsed with ambient seawater to ensure no sediments remain from the previous station.

2. Attach the sampler to the end of the winch cable with a shackle and **tighten the pin**. Attach a pinger to the grab.

3. Cock the grab.

4. Lower the grab sampler through the water column such that travel through the last 5 meters is no faster than about 1 m/sec. This minimizes the effects of bow wave disturbance to surficial sediments.

5. Retrieve the sampler and lower it into its cradle on-board. Open the hinged top and determine whether the sample is successful or not. A successful grab is one having relatively level, intact sediment over the entire area of the grab, and a sediment depth at the center of at least 7 centimeters (see Figure 2). Grabs containing no sediments, partially filled grabs, or grabs with shelly substrates or grossly slumped surfaces are

unacceptable. Grabs completely filled to the top, where the sediment is in direct contact with the hinged top, are also unacceptable. It may take several attempts using different amounts of weight to obtain the first acceptable sample. The more weight added, the deeper the bite of the grab. In very soft mud, pads may be needed to prevent the sampler from sinking in the mud. If pads are used, the rate of descent near the bottom should be slowed even further to reduce the bow wave.

6. Carefully drain overlying water from the grab. If the grab is used for benthic community analysis, the water must be drained into the container that will receive the sediment to ensure no organisms are lost.

7. Enter notes on the condition of the sample (smell, texture, presence of organisms on the surface, etc.) on the benthic infauna data sheet.

8. Process the grab sample for either benthic community analysis or chemistry/toxicity testing as described in Figure 3 and in Sections 6.2 and 6.3.

9. Repeat steps 4-8 until all samples are collected. To minimize the chance of sampling the exact same location twice, the boat engines can be turned periodically to change the drift of the boat, or additional anchor line can be let out.

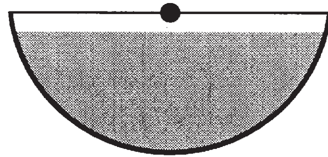
6.2 Field Processing of Samples for Benthic Community Assessment

Grab samples to be used in the assessment of macrobenthic communities are processed in the following manner:

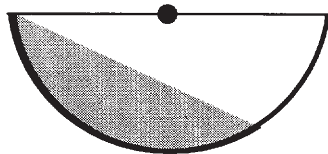
1. Assign a sample number to the sample; affix the bar coded labels to the sample jar and datasheet.

2. Measure the depth of the sediment at the middle of the sampler and record the value on the data sheet. The depth should be >7 cm. Record descriptive information about the grab, such as the presence or absence of a surface floc, color and smell of surface sediments, and visible fauna in the computer.

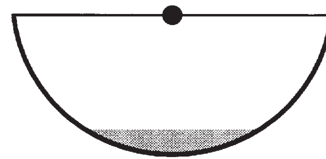
3. Dump the sediment into a basin and then into a 0.5 mm mesh sieve. Place the sieve into a table (sieve box) containing water from the sampling station. Agitate the tray in the sieve box thus washing away sediments and leaving organisms, detritus, sand particles, and pebbles larger than 0.5 mm. This method minimizes mechanical damage to fauna that is common when forceful jets of water are used to break up sediments. A gentle flow of water over the sample is acceptable. Extreme care must be taken to assure that no sample is lost over the side of the sieve.



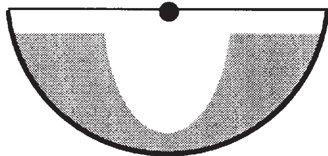
Acceptable grab
At least 7 cm deep with even surface



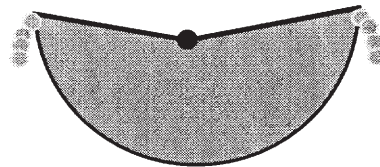
Unacceptable grab
Sloping surface



Unacceptable grab
Insufficient volume



Unacceptable grab
Wash-out



Unacceptable grab
Overfilled

Figure 2. Illustration of acceptable and unacceptable grabs for benthic community analysis. An acceptable grab is at least 7 cm in depth (using a $0.04m^2$ Van Veen sampler), but not oozing out of the top of the grab, and has a relatively level surface.

4. Drain the water from the sieve box and gently rinse the contents of the tray to one edge. Using either your fingers or a spoon, GENTLY scoop up the bulk of the sample and place it in the plastic screw-top bottle labeled in Step 1 (which should be placed in the sieve or a bucket in case some of the sample spills over). Rinse the outside of the sample jar into the sieve, then, using a funnel, rinse the contents into the jar. The jar should be filled no higher than the 700 ml mark. If the quantity of sample exceeds 700 ml, place the remainder of the sample in a second, unlabeled container. Using a waterproof marker, write the sample number on the second container and tape the two together. Note on the datasheet that the sample consists of more than one container.

5. Carefully inspect the sieve to ensure that all organisms are removed. Use fine forceps (if necessary) to transfer fauna from the sieve to the bottle containing the proper sample number.

6. Ten percent buffered formalin is used to fix and preserve samples. A 100 % buffered, stained stock formalin solution should be mixed according to the recipe in Table 3. 100 ml of the formalin should be added to each sample jar, and a teaspoon-full of borax added to assure saturation of the buffer. FILL THE JAR TO THE RIM WITH SEAWATER TO ELIMINATE ANY AIR SPACE. This eliminates the problem of organisms sticking to the cap because of sloshing during shipment. Gently invert the bottle to mix the contents and place in the dark. If the sample occupies more than one container, tape all the sample bottles containing material from that grab together.

7. Prior to sieving the next sample, use copious amounts of forceful water and a stiff brush to clean the sieve, thereby minimizing cross-contamination of samples.

Table 3. Directions for mixing stock solutions of formalin.

Chemical	Volume Desired	Total Quantity
100% formalin stock (stained and buffered)		
Rose Bengal stain	8 R	1/4 teaspoon
Borax	8 R	8 heaping tablespoons
100% formalin	8 R	two gallons

6.3 Field Processing of Sediments for Chemistry and Toxicity Testing

In addition to the grab collected for benthic community analyses, additional grabs are collected for chemical analyses and toxicity testing. The top 2 cm of these grabs are removed, homogenized, and split for chemistry and toxicity testing. Because of contamination concerns these samples are removed and processed in the order described below:

1. As each grab is retrieved, carefully examine it to determine acceptability. The grab is considered acceptable as long as the surface layer is intact. The grab need not be greater than 7 cm in depth for chemistry samples, but the other criteria illustrated in Figure 2 apply. Carefully drain off, or siphon, any overlying water, and remove and discard large, non-living surface items such as rocks or pieces of wood.

   NOTE: Great care must be taken to avoid contamination of this sample from atmospheric contaminants. The boat engine should be turned off or the boat maneuvered to assure the exhaust is down wind.

2. A clean stainless steel or Teflon spoon is used to remove sediments from grab samples for these analyses. All items must be washed with Alconox and rinsed with ambient seawater before use.

3. Remove the top two cm of sediment using the stainless steel or Teflon spoon. Place the sediment removed in a stainless pot and place the pot in a cooler on ice (NOT dry ice). The sample must be stored at 4EC, NOT FROZEN.

4. Repeat this procedure, compositing the sediment in the same stainless pot until a sufficient quantity of sediment has been collected for all samples (approximately 4 L). Stir sediment homogenate after every addition to the composite to ensure adequate mixing. Keep the container covered and in the cooler between grabs.

5. Homogenize the sediment by stirring with a Teflon paddle or stainless steel spoon for 10 minutes.

6. **ORGANICS -** Using a stainless steel spoon, carefully place 250 cc of sediment in a 500 ml glass bottle for chemical analysis. **Do not overfill container**; adequate head space must be maintained to allow for sample expansion if frozen at later date. CARE MUST BETAKEN TO ASSURE THAT THE INSIDE OF THE BOTTLE, BOTTLE CAP, AND THE SAMPLE ARE NOT CONTAMINATED. Record the sample number, wrap the jar in "bubble wrap" to protect it from breakage, and place the sample on ice (NOT dry ice). To reduce the possibility of breakage, the sample should be stored at 4EC, NOT FROZEN.

7. **METALS** - Using a stainless steel spoon, place approximately 100cc of sediment into a pre-cleaned plastic (HDPE) sampling jar. Record the sample number and keep on ice at 4EC.

8. **Total Organic Carbon** - Using a stainless steel spoon, place approximately 100cc of sediment into a pre-cleaned glass sampling jar. Record the sample number and keep on ice at 4EC.

9. **SEDIMENT GRAIN SIZE** - Using a stainless steel spoon, place approximately 100cc of sediment into a clean plastic (HDPE) sampling jar. Record the sample number and keep on ice at 4EC. Store this sample on ice (NOT dry ice). The sample must be stored at 4EC, NOT FROZEN.

10. **SEDIMENT TOXICITY** - Using the stainless steel spoon, fill approximately 75-85% of the 1 gallon plastic container for toxicity testing with sediment (minimum volume required is 3000 ml). Record the sample number on the bottle, and place the sample on ice (NOT dry ice). The sample must be stored at 4EC, NOT FROZEN.

6.4 Quality Control/Quality Assurance

6.4.1 Chemistry samples

There are a number of steps that can be taken to ensure the integrity of the samples collected.

1. The interior surfaces of the grab sampler (including the underside of the hinged top) must be washed with a laboratory-grade detergent and thoroughly rinsed prior to use to assure that no sediment remains from the previous station.

2. Prior to use, all Teflon and stainless steel supplies which are to come into contact with samples must also be properly cleaned. Once washed, crews must take precautions to assure that they do not become contaminated (*e.g.*, by laying the stainless steel spoon on the deck).

3. As soon as any of the stainless spoons or bowls begin to rust they should be discarded. Equipment made from high-quality stainless steel will reduce the rate at which equipment needs to be replaced.

4. ASSURE THAT THE PROPER LABELS (*e.g.*, BAR CODES) ARE AFFIXED TO ALL SAMPLES.

5. Excess seawater should be carefully drained from the surface of the grab by “cracking” the sampler slightly or siphoning off the water.

6. All grabs used in the composite must meet the criteria for an acceptable grab. It is especially important to make sure that the surface sediments did not wash out of the sampler.

7. Care should be taken to assure that the sediment saved for chemical and toxicological analyses is collected only from the top two cm of the grab.

8. Care must be taken to assure that the chemistry samples do not become contaminated. This requires great care in extracting the sample, homogenizing it, and placing it in the proper container. Because of the potential for contamination, the chemistry samples should be the first ones removed from the homogenate. If it is raining when the sample is collected, all activities should be conducted under a tarp to prevent contamination of the sample by rain water.

9. Great care must be taken to avoid atmospheric contamination from engine exhaust. The boat engine must be turned off or the boat maneuvered to assure the engine exhaust is down wind of the sample.

10. Exposure of the sample to the atmosphere should be minimized. Whenever possible the sample should be covered because contamination from the atmosphere, even without the engines running, can be significant.

11. Samples should be placed in a cooler on ice as soon as they are collected and recorded.

12. The grab must be suspended off the deck at all times to avoid contamination.

13. If the vessel is unable to anchor, the position relative to station should be monitored carefully during benthic colletion.

6.4.2 Benthic biology

Field crews must assure that all grabs processed are acceptable according to the criteria described above, and that no organisms are lost during any step, including transferring the sample to the sieve, and during sieving. Also, samples must be properly identified and preserved to assure they are received by the processing laboratory in acceptable condition.

6.4.3 GRAIN SIZE

Samples collected for grain size analysis require no special QA steps other than carefully following the directions discussed earlier and assuring proper storage. Note that grain size samples must NOT be frozen.

6.4.5 TOXICITY

Since sediment toxicity samples are collected from the same homogenate used for sediment chemistry, the steps outlined above should be followed. In addition, because of the possibility of failure of a toxicity test, it is important that a full 3 L of sediment be collected for analysis at each station. This will provide a sufficient volume of sediment for re-testing if

necessary. NOTE: Toxicity sample must not be frozen.

6.5 Safety Considerations

All sediment grab samplers are dangerous pieces of equipment. Once the device is cocked, it could accidentally trip at any time. The operators must be careful not to place hands or fingers in a position where they could be damaged (or amputated) in the event that the device trips prematurely.

The sampler is a heavy piece of equipment (especially when full). At all times, the operators must take care when deploying or retrieving this gear; under adverse weather conditions, extreme caution is mandated.

SECTION 7
FISH TRAWLS

After all required sediments are collected, one or more trawls are made to collect fish for species composition, relative abundance, chemical analysis, and pathological examination. Many states already have their own trawling protocols. Described below are the protocols for Coastal 2000. Existing state protocols and gear may be substituted following discussions with the C2000 Field Coordinator.

7.1 Gear and General Protocols

A fish trawl is a funnel-shaped net that filters fish from the near bottom waters. Fish are herded by ground wire and doors into the mouth of the funnel where fish are captured. The basic components of a trawl net are described briefly below. The actual specifications of the net used by AED for EMAP are found in Appendix B. The following discussion describes the use of a net with 13.5-m footrope, that used by the NE field teams; however, a smaller trawl with a 5-m (16-ft) footrope is employed for the shallow conditions routinely encountered in other regions (e.g., the gulf and southeast estuaries).

The doors of the net provide spreading power to the net. Water pressure against the doors force them to spread the wings of the trawl. The wings are the beginning of the webbing and form the mouth of the funnel on two sides of the net. The wings are bordered on top and bottom by a headrope and a footrope, respectively. For a single warp rig, each end of the headrope, or top line, is attached directly to the upper ring on the back of the doors. Each end of the footrope, or bottom line, is attached to the bottom ring of the doors. For strength and weight, a sweep is attached to the footrope. At the bosom, or top of the curve of the mouth, the wings attach to the body of the net. The top portion of the body has an overhanging panel, or square, which prevents fish from escaping over the top panel of the trawl. Continuing back toward the terminus of the net are the first and second bellies which are normally symmetrical top and bottom. The bellies contribute most of the body of the net, and therefore make up most of the taper. The cod-end is the rear portion of the trawl net which serves as a collecting bag for all that is captured by the trawl.

Fish are collected using a high rise sampling trawl with a 13.5-meter footrope with a chain sweep. Tow duration is 10 minutes with a towing speed of 2-3 knots against the prevailing current. Speed over the bottom should be 1-3 knot. Fish are sorted and enumerated, examined for evidence of gross pathological conditions, and selected specimens retained and properly processed for tissue chemical analysis. Subsampling of fish is conducted as necessary. The outline below describes the specific protocol to be followed during trawling operations. The procedures include: net deployment, vessel operation while under tow, net retrieval, and processing.

Types of trawls can be defined as follows:

STANDARD TRAWL - This trawl is the "quantitative" trawl performed at all stations for community structure and abundance determination. Only one standard trawl should be performed at EVERY station. Any fish sample type can be taken from a standard trawl. Fish are identified, measured, and examined for pathological conditions.

NON-STANDARD TRAWL - At selected stations non-standard trawls may need to be performed following the completion of a standard trawl only to obtain a sufficient number of fish for *tissue chemistry*.

The type of fish samples that will be collected are as follows:

Pathology Fish - These are fish observed by the field crew to have a gross external pathology (lump, growth, ulcer, fin rot, gill erosion, and/or gill discoloration). ALL species are examined for external pathology, therefore, pathology fish may be of any species collected. Pathology fish are collected only during the standard trawl. Any fish found with one of these conditions is preserved in Dietrich's fixative for confirmation by a specialist. These fish are Pathology fish.

Taxonomy QA fish - Fish that cannot be identified in the field are to be sent back to the appropriate agency for identification by an expert taxonomist.

7.2 Trawl Preparation

(Portions of these instructions are specific to 24-26' EPA boats. Modifications may be necessary depending upon the vessel used for trawling)

1. Inspect the trawl net for holes, including cod-end liners, and mend/replace as necessary prior to departure from the dock. Inspect all hardware for wear and replace as needed. All connections should be made securely and tightened with a wrench. Do NOT rely on hand tightening shackles, bolts, or other fasteners.

2. Lead the winch wire from the drum through the turning block on the mast assembly and through the snatch block at the end of the boom.

3. Attach the bridle to the winch wire with a shackle. Wind both legs of the bridle onto the main winch drum, while maintaining tension on the wire. All bridle connections should be tightened with a wrench.

4. Arrange the net on the deck with the cod-end aft and the head rope on top. Close the end of the cod-end by using a cod-end knot. Check to make sure there is no escapement possibility through the cod end rings. The line should pass through the rings at the back of the cod end and around the net just in front of these rings. Coil the float line from the

cod-end to the float, and position it on the net for easy access.

5. Attach the legs of the net to the trawl doors. The top leg of the net is the extension of the headrope and must be secured to the top aft ring of the door. The bottom leg is the extension of the sweep and must be secured to the bottom aft ring of the door. One bridle wire should be attached to each door at the towing point of the chain harness. Shackles should be used for all connections.

7.3 Net Deployment

(Portions of these instructions are specific to 24-26' EPA boats. Modifications may be necessary depending upon the vessel used for trawling)

1. After all preparation steps have been completed, the chief scientist or captain should check all resources available (chart, navigational aids, land marks etc.) to determine that there are no under water hazards. Determine the direction of current flow and survey the probable trawl track for potential hazards, such as other vessels, deployed commercial fishing gear (nets, pots, etc.), shallow water, or unsuitable substrate. In addition, depth, weather, and sea conditions should also be evaluated prior to each trawl. The decision as to whether or not to collect a sample is the responsibility of the chief scientist or captain.

2. With the starboard engine in neutral, the boom should be positioned out over the starboard gunnel with a enough incline for the doors to clear the rail. Lead the bridles through the snatch block on the boom, raise the doors with the winch, and bring them to rest on the gunnel (starboard door forward, port door aft). Circle the boat slowly to starboard. When the starboard side is down current, deploy the float and safety line attached to the cod-end. Flake the net into the water from the cod-end to the wings. Check to make sure that the legs of the net are not twisted before continuing deployment. Pay out wire until the doors are well behind the engines. Swing the boom to the centerline then lower the boom, releasing tension on the snatch block (the wire should now be on the goalpost assembly). Head slowly into the current (*e.g.*, 1 knot) and continue to pay out wire until appropriate warp length is obtained (consult Table 4 for the proper amount of wire to be released based on water depth). Great care should be taken to prevent fouling of the propeller with the net. Care should also be taken to maintain tension on the tow warp to avoid fouling the net on bottom. The starboard engine can be engaged when the gear is clear of the props and the doors spread.

7.4 Trawling

1. As soon as the required warp length is reached, the winch operator should inform the captain that the net is ready for towing. The captain then visually resurveys the trawl track, records the time, initiates the trawl clock, records the start coordinates, and begins the tow. An attempt should be made to trawl along a uniform depth contour.

2. Boat speed should be 2-3 knots. Speed over bottom, as measured by GPS position, should be between 1 and 3 knots. If it becomes apparent that these conditions will not be met, the net should be retrieved and a different trawl direction tried.

Table 4. Amount of Winch Wire to be Used for Trawling

Water depth (ft)	Ratio of line to water depth	Line out (including the 125' bridle)
10 (3 m)	7:1	Bridle only (38m)
20 (6 m)	7:1	Bridle+20' (44m)
30 (9 m)	7:1	Bridle+60' (56m)
40 (12 m)	6:1	Bridle+120' (75m)
50 (15 m)	5.5:1	Bridle+155' (85m)
60 (18 m)	5:1	Bridle+180' (92m)
70 (21 m)	4.6:1	Bridle+202' (100m)
80 (24 m)	4.2:1	Bridle+216' (104m)
90 (27 m)	3.8:1	Bridle+222 (106m)

3. During the trawl tow, the captain should monitor the depth finder for potential obstructions or sudden changes in depth. If a hazard is identified or a hang up occurs, the net should be retrieved and another tow attempted approximately 100 m from the initial trawl track. If three unsuccessful attempts are made, or 1.5 hours effort is expended, trawling operations should be aborted. If a successful 10 minute trawl cannot be accomplished, fish can still be collected from a shorter trawl for chemistry.

4. The duration of all standard trawls should be 10 minutes from the time the pay-out of warp is completed until the time hauling begins.

7.5 Net Retrieval

(Portions of these instructions are specific to 24-26' EPA boats. Modifications may be necessary depending upon the vessel used for trawling)

1. After approximately 10 minutes of trawling, record the end coordinates then haul back the wire until approximately 10 meters of the bridle is still out.

2. Put the starboard engine in neutral. Throttle back and raise the boom so the wire clears the goal post assembly. Turn the boat slightly to starboard and move the boom over the starboard side (the boom should be controlled by the vangs during this process).

3. Take in wire until the doors are at the block. Haul the cod end in by hand or use the capstan head to assist. Retrieve the float line and float.

7.6 Safety Considerations

Operation of the trawl can be a dangerous operation. In addition to the dangers of using the winch and capstan, improper towing procedures could capsize the boat. The net should always be towed off the stern, with the winch cable passing through the towing bracket. Towing off the side of the boat can capsize it. Care must also be taken when pulling the net in over the side. If the net is full, the total weight may be too great to use the mast and boom.

When deploying the net, the crew must be careful not to entangle themselves or other gear in the net, bridle, or winch cable. This could result in serious personal injury or damage to equipment.

All trawling operations must be conducted in a manner consistent with maintaining the safety of the crew. The captain will determine when weather or sea conditions are unsafe for trawling.

In the event of net hang-ups on bottom obstructions, the captain must consider the safety of the crew before attempting to free the gear. A means to sever the tow line should be

immediately available to the crew during all trawl operations. SEVERING THE LINE SHOULD ONLY BE PERFORMED AS A LAST RESORT AND WHEN THE LINE IS SLACK!!! SEVERING IT WHILE UNDER TENSION COULD RESULT IN WHIPLASH OF THE LINE AND SEVERE PERSONAL INJURY.

Before deploying the trawl, the captain should ensure that other vessels do not present a safety hazard during the tow. Whenever possible, the captain shall contact nearby vessels by marine radio to make them aware of the trawling operation. In addition, the marine radio should be monitored by the crew prior to and during trawl operations. Appropriate day shapes must be flown.

7.7 Criteria for Voiding Tows

A standard tow will be considered void if one or more of the following conditions occur:

1. A tow cannot be completed because of hang down, boat malfunction, vessel traffic, or major disruption of gear.

2. Boat speed or speed over bottom is outside the prescribed, acceptable range.

3. The cod-end is not tied shut.

4. If the tow continues for more than two minutes beyond the ten-minute tow duration, or is discontinued less than eight minutes following the start.

5. The net is filled with mud or debris.

6. A portion of the catch is lost prior to processing.

7. The tow wire, bridle, headrope, footrope, or up and down lines parted.

8. The net is torn (>30 bars in the tapered portion, >20 bars in the extension or cod end, or multiple tears that, in the opinion of the chief scientist, may have significantly altered the efficiency of the net).

7.8 Endangered Species

All species considered to be rare, threatened, or endangered should be processed immediately and released alive. At the discretion of the chief scientist, photographs may be taken to document the catch.

7.9 Sample Processing

Once a catch is brought on deck, fish are identified to species, measured, counted, examined for external pathology, and processed for chemical analysis.

7.9.1 General Processing

1. After all fish have been sorted, process fish for pathological examination as described below. Sampling for pathology and Chemistry are performed concurrently with the collection of composition and abundance data. Only fish, lobster, and blue crab are recorded. Other invertebrates and trash are noted in the datasheet then discarded.

2. Measure, with a measuring board, the fork length to the nearest millimeter, of individuals of each species. If there are fewer than 30 individuals of a species, all individuals should be measured. If it is estimated that more than 30 individuals of a species were caught, a subsampling procedure should be used to measure between 30-50 individuals. Subsampling will be accomplished by randomly selecting fish from the buckets. All data are entered onto data sheets and later into the computer.

   NOTE -

   Dog fish - stretched total length
   Skates - total length
   Rays - wing tip to wing tip, and total length
   Unforked - total length without extraneous filaments
   Blue crab - carapace width
   Lobster - Carapace length

3. Enter data on the fish data sheets. Common names are preferred.

4. All fish not measured for length (*i.e.,* those subsampled) are counted, either by direct count or weight-counts. When extremely large catches of schooling fish such as bay anchovy or other clupeids are made, abundance may be estimated by weight-counts. At least 100 individuals should be weighed in a batch, and 2 batches should be weighed to determine mean weight per individual. All remaining fish should be weighed, and the total number of fish estimated and recorded on the data sheet. If two or more obvious size classes are present in a sample (*e.g.,* young-of-year and adults), the size classes should be treated as separate species for the purpose of counting.

5. After all processing has been completed, the chief scientist should review the trawl data sheet for discrepancies and inaccuracies. When any questions have been resolved, he/she signs the data sheets as being reviewed and the remaining portion of the catch can be returned to the water. When significant mortality occurs and the trawl site is in a highly visible area, the captain may elect to retain the catch until more discrete disposal can be accomplished. Under no circumstances should the crew give fish away to the general public.

7.9.2 Processing of Fish for Gross External Pathological Analysis

I. Gross examination of fishes:

All individuals collected from a standard trawl will be identified and counted, and the first 30 individuals of each species will be measured. All individuals measured (*i.e*. the first 30) that exceed 75 mm in length will be examined for evidence of gross external pathology (lumps, growths, ulcers, fin rot, gill erosion, and gill discoloration). The examination is intended to be a rapid scan of the surface of individuals to be completed while other fish measures are being completed (*i.e*., identifying, enumerating, measuring). This scan should take no longer than 10-15 seconds per fish. Fish determined to show evidence of a pathology are assigned a sample number and processed appropriately (see below). The type of pathology will be noted on the data sheet. These are **PATHOLOGY FISH**. **Only fish collected in "standard" trawls are saved for pathology.**

II. Selection, killing and fixation for transfer:

Proper fixation of specimens is critical to the ultimate quality of the data obtained. Fish should be examined and fixed while still alive or shortly after death (within one hour of collection). Specimens should not be frozen or kept on ice at any time.

A. All specimens with gross lesions or other suspect conditions, as identified in Section I above, will be processed and coded individually. All these fish will be transferred as indicated below (Section III) to EPA's Gulf Ecology Division (GED) for subsequent examination.

1. Carefully cut the entire length of the abdominal cavity open using scissors or a sharp knife. Gently insert the instrument into the abdomen near the anus and make an incision to the operculum. Cut with a lifting motion so that the incision is made from the inside outward, taking care not to injure the visceral organs. Remove the lateral musculature from one side of the animal's visceral cavity to facilitate the fixation of the internal organs. Remove the opercula, and immerse in fixative (see step 4).

2. If the total length of the fish exceeds 15 cm, only a portion of the fish will be saved for laboratory analysis. Carefully cut, through the entire thickness of the fish, from the top of the operculum back along the spine, until a position behind the visceral cavity is reached, and then a 90E change in direction towards the anus. The head and viscera are then saved. Remove both opercula, and musculature covering the visceral cavity on one side. The head and thorax can be separated at the esophagus if needed. Any abnormalities found on the remaining portion of the fish (which is to be discarded) are excised along with the surrounding tissue, and saved with the head and visceral cavity. For fishes smaller than 15 cm, the entire fish is saved. (See Figure 3).

3. If an external growth is present, slice through the lesion with one clean cut using a sharp razor blade.

4. Place the sample (whole fish or head, visceral cavity and abnormalities excised) in an "onion bag" or a plastic zip lock bag with multiple perforations. Assign an appropriate sample number to each fish, affix the bar code to a fish tag, and attach the tag to the fish. Record this number on the data sheet, along with all other pertinent information on that fish. Place the bag in a tight sealing plastic container with sufficient fixative to completely cover the specimen. Specimens should be fixed in Dietrich's fixative for one or two days.

Dietrich's Fixative (to make ~5 gals.)
37-40% Formaldehyde or 100% formalin --------------- 1500 ml
Glacial Acetic Acid --- 300 ml
95% Ethanol -- 4500 ml
Distilled water --- 9000 ml

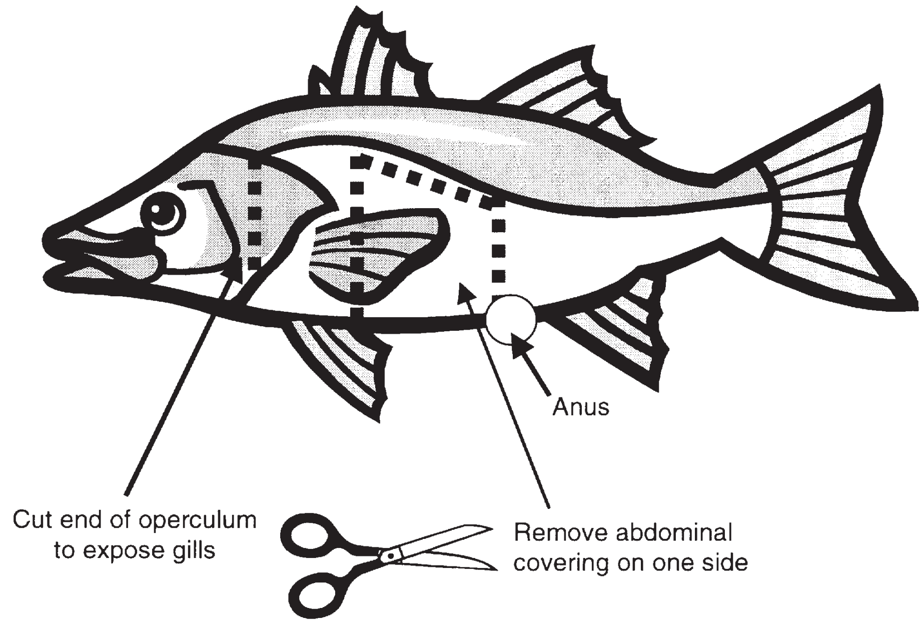
5. Carefully record pertinent information relating to each individual sample on the data sheet.

III. Shipping of preserved specimens:

Fish should soak in Dietrichs Fixative for at least two days prior to shipment. To ship, wrap the fish in cheesecloth dampened with Dietrichs. Place the wrapped fish in several layers of airtight plastic bags and pack in cardboard boxes or coolers. No specific temperature criteria apply.

7.9.3 Tissue Chemistry

Fish less than 15 cm in length



Fish greater than 15 cm in length

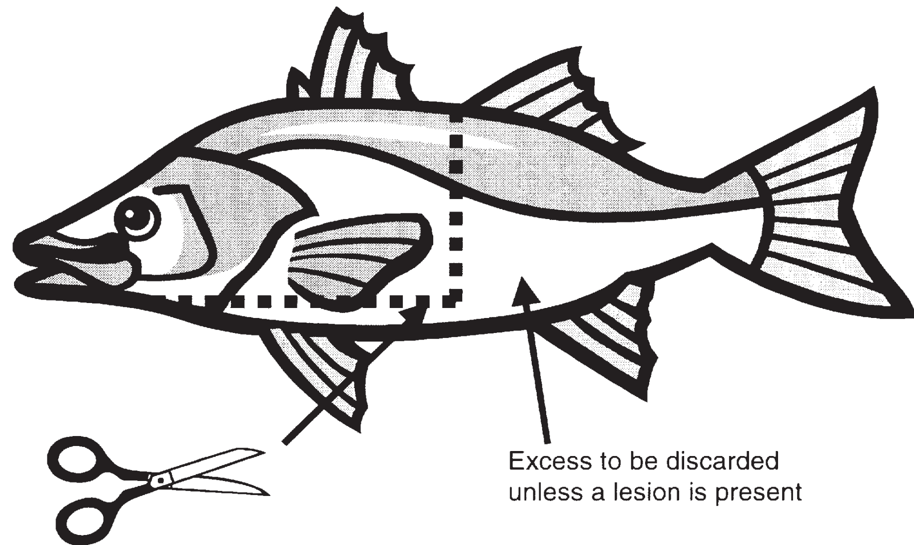


Figure 3. Description of how to expose interior organs for proper preservation of Pathology Fish.

1. For the two most abundant taxa designated as target species, measure and retain nine individuals within the desired size range for chemical analysis. Individuals are randomly selected from all those collected until nine of the appropriate size have been selected. If no individuals in the primary size range were collected, those that are closest to the preferred size range are selected.

NOTE:

a. Even if a tow is voided for species composition and abundance, fish collected can still be processed for chemistry.

b. Target species for tissue analysis are designated on a regional or state-by-state basis. If no target species are caught from a site but other species are taken, a surrogate target can be selected from the best available by-catch; attributes that define a good target include: demersal, high tropic level, representative of area, and adequate size or age class.

2. Record on the datasheet the size, species, sample number (see Step 3), and any other appropriate notes.

3. Place one bar code on the data sheet. Place the twin bar code on a plastic tag and affix to the fish by placing the twist-tie through the mouth and out the operculum.

4. Wrap individual fish in aluminum foil (with the tag exposed), place all fish of that species in a single zip-lock bag, affix the "composite" bar code, and place it in a cooler on DRY ICE.

5. All samples should be placed immediately on DRY ICE for freezing. When adding new samples to the cooler containing the dry ice, samples should be rearranged to assure that these samples are in contact with the dry ice so they will freeze rapidly. One option would be to use one cooler for freezing fish, and a second for storing them. This is dependent on the equipment carried on the boat, and therefore, the amount of space available. If freezing on-board is not practical, fish must be stored on ice until the crew reaches the dock. The time before freezing should be minimized.

6. Repeat trawling (standardized methods not required) for up to 1½ hours if needed to obtain at least five individuals of at least one target species. Fish collected in these trawls are processed for chemistry only.

7.10 Quality Assurance

In order for the net to "fish" properly, the proper amount of winch cable must be let out. Consult Table 4 for the proper scope. Care must also be taken to assure that fish are not lost from the net during retrieval.

It is important that the tow time and speed be as close to the desired values as possible. Any deviations should be noted on the data sheet.

It is important not to contaminate fish which are saved for chemical analysis. Every effort should be made to keep them from coming in contact with very dirty surfaces. It is especially important to ensure that no cuts are made into the flesh.

Table 5. Listing of Northeast Region Target Species for Chemical Analysis (sizes are the target sizes for fish saved for chemical analyses).

SPECIES	SIZE RANGE (mm)
Catfish Species	
Channel Catfish	200-300
White Catfish	200-300
Scup	70-115
Summer Flounder	350-450
Weakfish	300-400
Winter Flounder	100-200
Blue Crab	120-170
Lobster	

7.11 Contingency Plans

Considering the wide variety of environments to be sampled by C2000, it is likely that towing a net will be impossible at some stations. If, due to repeated snags, a successful trawl cannot be performed within 1.5 hours of starting, no further attempts should be made. This is noted on the data sheet.

In the event that a "standard" trawl cannot be obtained because of space limitations, the crew can still use either alternative gear to collect fish and shellfish for chemistry. This may include purchasing lobster or crab from local fishermen. The preferred method would be to accompany the fishermen during the collection to ensure the crabs or lobster are collected in the proximity of the station. **It is important that the crew is sure the lobsters or crabs were**

collected in the vicinity of the station.

7.12 Collection Permits

Many states require scientific collection permits for the collection of fish using trawls. Permits issued for C2000 activities must be carried on each boat. A permit must be presented to any appropriate state official that requests to see it.

SECTION 8
PACKAGING AND SHIPPING SAMPLES

After samples are collected, following proper packaging and shipping procedures are critical steps in assuring the integrity of the samples. Failure to follow these procedures could result in the loss of valuable data. Each sample type requires different handling as described below. Packaging and shipping are to be performed within several days of sample collection.

Samples may be sent either to an approved state lab or one of the Coastal 2000 "national labs." This determination is made well before the start of the sampling season. The protocols described below are specific to samples being sent to the national labs; however, it is recommended that samples being sent to local labs be processed similarly.

As samples are packaged for final shipment, the sample number of each sample must be recorded. This can be done on a hard-copy shipment form, or electronically entered into a computer. Upon completion of packaging, a unique tracking number (bar code label) is affixed to the SIDE of the cooler, and this number is also recorded on the shipment form. The number must be placed on the side, not top, so as not to interfere with the carrier's tracking system (which also may use bar codes). In addition to the carrier's air bill, a mailing label should also be affixed to the cooler as an additional precaution against loss. A packing list must accompany the shipment. This can be a photocopy of the completed shipment form, or a printout from the computer. Samples that are "hand-carried" require the same paperwork (less the air bill) and tracking as those shipped by commercial carrier. Since coolers need to be shipped back to the crew, a return air bill should also be included in the cooler.

All samples, except those preserved in formalin or Dietrichs, are shipped overnight. **Shipping should only take place on Mondays through Wednesdays, otherwise samples will arrive at the analytical laboratory on the weekend when there may be no one available to accept them.**

The C2000 Field Coordinator **must** be informed each time a shipment is sent out. The information needed includes the name of the analytical laboratory, the shipment ID number, the carrier's air bill number (this is especially important), and a list of the samples included in the shipment.

8.1 Proper Packaging Methods

Proper packaging of samples is critical in assuring they arrive at the receiving laboratory in good condition. Improper packaging can result in damaged or lost samples. This is costly in terms of time and money. There are several important aspects of proper packaging: assembly of the shipping box (if required), the amount of blue or dry ice needed, and proper packaging of the contents.

Each team should be supplied with several sizes of coolers. The appropriate size should be selected to minimize "dead" space.

Each team carries coolers with dry ice and blue ice to keep samples frozen or cool prior to shipment. Blue ice blocks can be frozen by placing them under the dry ice, or in a freezer for crews operating out of a home base. For dry ice, a general rule of thumb is at least 5-10 pounds, with another pound for every pound of sample (ASSUMING THE SAMPLES ARE ALREADY FROZEN). The amount of blue ice needed to keep samples cool is approximately one pound per pound of sample. This should guarantee samples arrive frozen or cool (depending on the ice type) even if the shipment is delayed a day. Frozen samples must always be shipped on dry ice, and refrigerated samples must always be shipped on blue ice packs.

Because of the need to ship fish and crabs frozen, relatively large amounts of dry ice will be needed.

In addition, it is recommended that the sample be sandwiched between refrigerant, *i.e*. dry ice should be packed both above and below the sample. **It is also important that the cooler contain a minimum of air space.** Therefore, packing material should be inserted above the top ice layer to fill the cooler.

A third consideration for all sample types (not just cooled or frozen samples) is proper packaging within the shipping cooler. While packing a shipment cooler, one should assume that the cooler will be improperly handled. All samples should be protected and sufficient packing material included to eliminate any possible movement of the samples within the cooler. All material that could possibly leak, such as water or sediment samples, should be sealed with sealing tape and packaged in zip lock bags. All glass sample bottles should be bubble wrapped and sealed in a zip lock bag. Any whirl paks should also be taped to ensure the metal tabs do not puncture adjacent bags, and placed in a zip lock bag.

Proper storage and shipment conditions are summarized in Table 6. Federal Express no longer requires a Dangerous Materials waybill for all shipments of formalin in concentrations of 10% or less. Federal Express does, however, require a class 9 placard, UN number, packing description and an emergency phone number for all shipments of dry ice.

Table 6. Sample holding and shipping conditions

SAMPLE TYPE	HOLDING CONDITIONS	SHIPPING CONDITIONS
Sediment Biota	Preserved in Formalin	Once per Week
Sediment Grain Size	Refrigerated	* Ship Weekly
Sediment Organics	Refrigerated	* Ship Weekly
Sediment Metals	Refrigerated	* Ship Weekly
Sediment TOC	Refrigerated	* Ship Weekly
Sediment Toxicity	Refrigerated	* Ship Weekly
Chlorophyll Filter	Frozen on Dry Ice	* Ship Weekly
Total Suspended Solids	Refrigerated	* Ship Weekly
Dissolved Nutrients	Frozen on Dry Ice	* Ship Weekly
Fish Chemistry	Frozen on Dry Ice	* Ship Weekly
Pathology QA	Wrapped in Cheesecloth	Once per Week

* Crews should attempt to ship as frequently as logistically possible. Samples must be shipped at least once per week.

8.2 Benthic Biology Samples

Samples for benthic community analyses are preserved in formalin in the field. These samples are in plastic containers with tight fitting screw-top lids. As these samples are preserved, there is no need to keep them cool. Shipment boxes should not weigh more than 50 pounds. The lid of each jar should be checked to assure that it is tight, and the lid taped with sealing tape. The bar code label of each container is then read and the samples placed in an insulated shipping box. The insulation is for protection rather than thermal regulation. As described above, a computer printout of the sample numbers included in this shipment is enclosed in the box.

The box is then sealed and an appropriate shipping label affixed. Be sure to pack all bottles upright, and to fill gaps with packing material. Overnight delivery is not required.

This shipment contains formalin; however, since the final concentration is 10% or less, Federal Express no longer requires a Dangerous Goods Airbill and Shipper Certification form.

8.3 Sediment Chemistry Samples

Following collection, sediment samples for TOC, organics, and metals chemical characterization should be refrigerated rather than frozen because freezing greatly increases the likelihood of breakage of the glass container. It is also recommended that samples be shipped

cool, but not frozen, for the same reason.

Sediment chemistry samples should be shipped at least weekly, preferably early in the week. Sample bottles should be wrapped in bubble wrap to protect them from breakage, and sealed in a plastic zip lock bag. They should then be placed in an insulated cooler with an appropriate amount of blue ice.

Sediment chemistry samples must be shipped Next Day Service to the appropriate lab.

8.4 Sediment Toxicity Samples

Sediment samples collected for sediment toxicity testing must be kept refrigerated (4EC), NOT FROZEN. Sample must be shipped at least weekly. Of all the samples to be analyzed, the sediment toxicity samples are the most “time-critical” due to the nature of the testing and the relatively short permitted holding time (<28 days). Containers are then placed upright, along with an appropriate amount of blue ice, in an insulated box or cooler. Sediment toxicity samples are shipped Next Day Service to the appropriate lab.

8.5 Grain Size Samples

Samples for grain size analysis are collected along with each sample collected for benthic biology and sediment chemistry/toxicity analyses. Samples for grain size analysis should be kept cool (4EC), but not frozen. If these samples are contained in Whirl Packs sealed with metal wraps, tape should be placed around the ends of these wraps at the time of collection to prevent the metal tips from piercing one of the other bags. Samples should be shipped weekly. Whirl Packs should be placed in a ziplock bag and packed into an insulated box or cooler with an appropriate amount of frozen blue ice to keep the samples cool. Place a thin layer of paper between the blue ice and the Whirl Paks to keep them from freezing.

Samples should be shipped Next Day Service to the appropriate lab.

8.6 Chlorophyll, Nutrient, and Total Suspended Solids Samples

Samples should be shipped at least weekly. Chlorophyll and nutrient samples should be frozen and shipped on dry ice. TSS samples are shipped on blue ice.

Samples should be shipped Next Day Service to the appropriate lab.

8.7 Fish Chemistry

Samples should be shipped at least weekly. Samples must be frozen and shipped on dry ice.

Samples should be shipped Next Day Service to the appropriate lab.

8.8 Pathology QA Samples

These samples are preserved in Dietrich's fixative. Fish must be well preserved in Dietrichs Fixative prior to shipment. The fish should be removed from the bucket of fixative, wrapped in multiple layers of Dietrichs-soaked cheesecloth, then placed in multiple layers of airtight plastic bags. Samples then should be packaged into cardboard boxes or coolers and shipped to the appropriate lab. Overnight service is not required.

8.9 Instructions for FEDEX Shipping with Dry Ice

A. Use Regular Airbill

1. Sender's Section: Fill in the Date and Your Name.

2. Confirm the recipient's name, shipping address and phone number

3. Payment Section: Confirm that Bill Sender Box (#1) is checked

4. Services Section: Check Priority Overnight Box /your packaging, leave Freight Service and Instructions sections blank
Delivery and Special Handling Section: Check Dry Ice Box and Fill in the total weight of dry ice for the shipment.

5. Complete section 6 of the Airbill.

   Note: print the weight of the dry ice in Kg, not the total box weight!

B. Proper Labeling of Shipping Boxes containing Dry Ice

1. Stick Number **9** placard label (available from FEDEX) so that clearly visible on side of box

2. On the same side of the box the following information must be printed:

   Dry ice, 9, UN 1845, ___box X ___ Kg , 904 III
   Dangerous goods - Shipper's declaration not required

   Note: print the weight of the dry ice in Kg, not the box! Up to 2.2 kg permitted (5 lbs).

3. Be sure that each box has an Address Label with the correct address of the receiving facility.

APPENDIX A

List of Suggested Supplies and Equipment

This list provides a complete (more or less) listing of recommended supplies and equipment for Coastal 2000 sampling in the northeast states. It is intended as an aid in planning efforts.

General

Boat set up appropriately for sampling
Appropriate safety gear
Navigation equipment - GPS or Differential GPS (preferred), depth finder
Vehicle to move people around as needed
Vehicle to store gear in, including shipping coolers (only needed for crews traveling away from a base location)
Communications equipment between shore and boat (*e.g.*, cellular telephones, VHF...)
Computer for data entry (laboratory or portable)
Bar code reader (recommended)
Bar codes for all samples - EPA can coordinate
Datasheets - EPA can provide templates
Pre-labeled station datasheet packages
Data entry software (*e.g.*, JetForm's Formflow Filler for EPA datasheets)
Shipping containers (*e.g.*, coolers)
Shipping labels
FEDEX (or other carrier) airbills
Shipping Bar codes
"Blue ice"
Coolers for storing samples (both frozen and chilled)
Field notebooks
Water-resistant paper for datasheets (*e.g.*, Rite-in-the-rain)
Waterproof pens for writing on datasheets
Clipboards
Insulated gloves for handling dry ice
Bubble wrap for shipping
Shipping scale for weighing packages
"Packing list enclosed" envelopes
"This side up" labels
Class 9 placards for dry ice shipments
Strapping tape for shipping
Duct tape for everything else
Buckets and/or hose and washdown pump
Meter stick
Waterproof markers
Paper towels
Kimwipes

Latex or other gloves for handling contaminated sediments or formalin
Scissors
Suggest pingers for overboard gear in case it is lost

Water Quality Monitoring

Profiling instrument to measure depth, temperature, salinity, pH, and DO (e.g., Hydrolab DataSonde4, YSI 6000, etc., with appropriate deck unit and cabling as needed)
Back-up/QA instrumentation:
  Additional DO meter (unless doing Winklers)
  Thermometer
  Refractometer
pH standards
Salinity standards
Spare DO membranes & electrolyte
Spare parts for profiler
Batteries
Light (PAR) or transmissometer with appropriate deck unit or datasheets and cables
Secchi disk with marked line
Water sampling bottle for nutrients
Filtration apparatus for Chlorophyll
  a) 2- 47mm filter holders
    vacuum manifold
    4 liter overflow bottle
    12vdc vacuum pump or hand pump or
  b) stainless steel, 25 mm filter holder
    standard luerlock syringe
47 or 25 mm GF/F filter pads (2 per sample, up to 6 per station)
Clean 60cc nalgene bottle for nutrients (3 per station)
1-L Nalgene for TSS samples (3 per station)
Storage containers for filters and other supplies
$MgCO_3$
Filter forceps
Graduated cylinders, 250, 100, 50, 10 ml
DI water for rinsing
Squirt bottles
Aluminum foil for wrapping samples (suggest pre-cut squares from Thomas Scientific)
Whirlpaks or ziplock bags for foil-wrapped filter pads
Dry ice for freezing samples

Sediment sampling

$0.04m^2$ Young-modified Van Veen grab sampler (or other)
Grab stand
Weights for grab (several)
Pads for grab (several)
0.5 mm stainless steel sieve
Sieve box
“Tub” or bucket for dumping sediment into
High-quality stainless mixing pot, with lid, for sediments (2)
Stainless spoons (several)
500 cc glass jars for organics (Ichem pre-cleaned)
250 cc HDPE jar for metals (Ichem pre-cleaned)
125 cc glass jar for TOC (Ichem pre-cleaned)
125 cc HDPE jar for grain size
4-L HDPE jar for toxicity (pre-cleaned)
1-L Nalgene for benthic infauna (3 per station plus spares)
Electrical tape for sealing lids of benthic containers
Formaldehyde (formalin)
Rose Bengal stain
Borax (can get at supermarket)
Centimeter ruler
Wide mouth funnel
Squirt bottle
Alconox
Scrubbing brushes
Fine forceps for picking worms from screen

Fish Sampling

13.5 m otter trawl (several)
Doors for otter trawl
Bridles
Timer
Fish measuring board
Heavy duty aluminum foil
Waterproof tags with tie strings
Taxonomy keys
Heavy duty dissecting scissors
Scalpel or sharp razor blade
Cheesecloth

5-gallon bucket for Dietrich's fixative
LARGE ziplock bags for fish composites
Onion bags
Dietrich's fixative
- Formalin
- Glacial Acetic acid
- 95% ethanol

APPENDIX B

Trawl Net Specifications

C2000-NE (EMAP) Trawl Net Specifications

- 3:1 trawl net of 3" webbing
- Headrope length = 13.5 meters
- Sweep = 16.5 meters
- Hanging line and headrope of ½” poly dacron with thimbles spliced into ends
- Up and down lines of ½” poly dacron spliced into the headrope and hanging line
- Webbing of 3" #21 twisted polyethylene (= European #312 twisted stranded) reinforced along the mouth frame with gussets
- Headrope flotation of 4 small (5", 760 grams buoyancy) plastic floats
- Codend of 1½” #24 nylon, 64 wide by 65 deep
- Sweep of 3/16" chain with 12 feet of ¼” chain along the mouth
- Bridle is 125', ¼” stainless steel wire

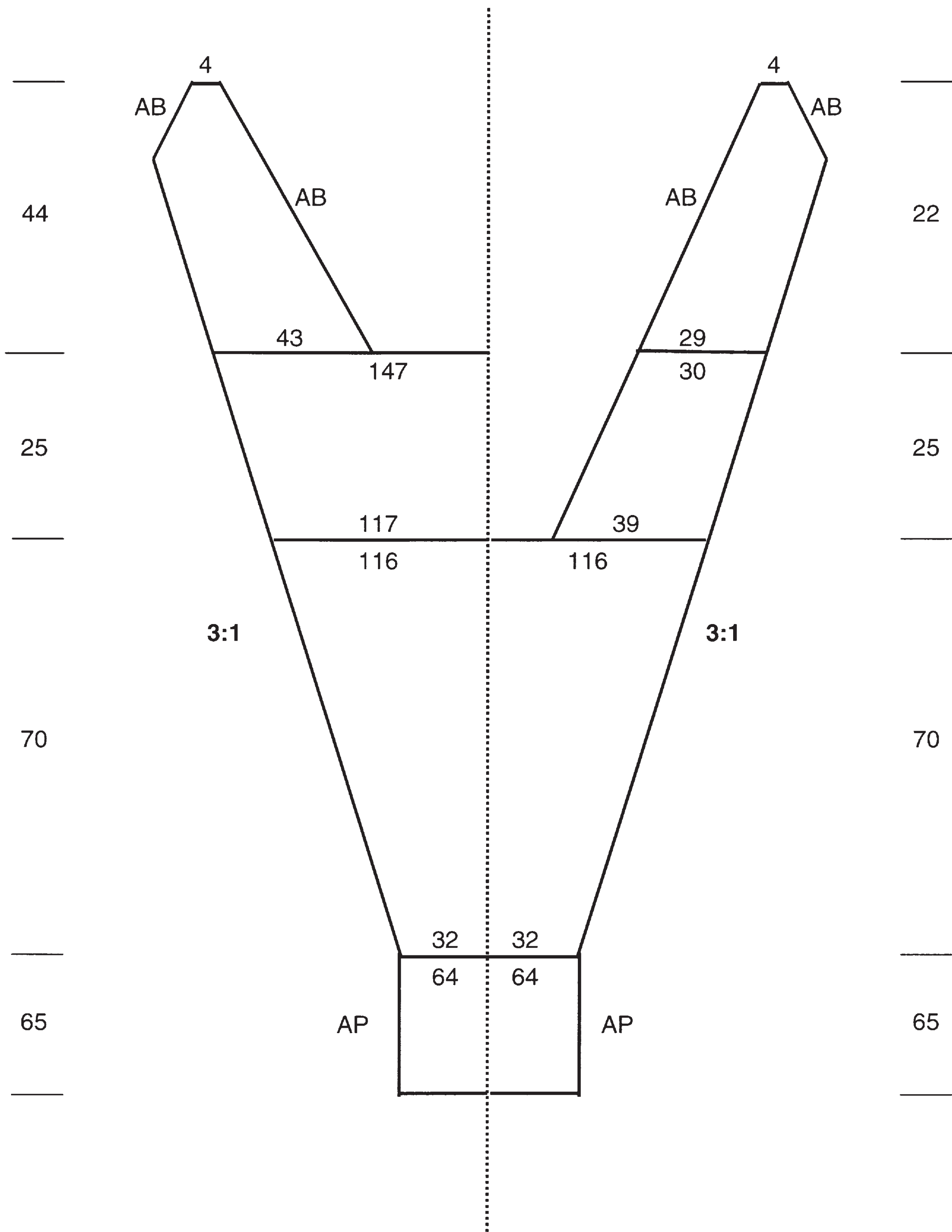


Figure B1. Diagram of EMAP trawl net. Headrope is 13.5 meters, sweep is 16.5 meters.

APPENDIX C

Coastal 2000 Field Data Forms

Gulf Region Field Data Sheets

The following field data sheets have been designed by GED for the Gulf region Coastal 2000 effort. Their use is recommended but not required. GED can provide electronic templates for these forms. Included are the following data sheets (sample forms are attached):

Station Form

Hydrographic Profile

Water Sample Collection

Benthic Infauna Collection

Sediment Sample Collection

Fish trawl

Fish Data

STATION FORM
Coastal 2000 - Gulf Region

STAGING AREA ____________________

STATION DEPTH (ft.): ________________

STATION NAME
____

DAY	DATE: (MM/DD/YY)	ARRIVAL TIME:(HH:MM)
	TIME ZONE: ◯ Eastern / ◯ Central	DEPART TIME:(HH:MM)
GPS	LAT: (00°00.00)	LON: (00°00.00)
	Site Within 0.02 NM: ◯ Yes or ◯ No	If NO, explain in 'NOTES'
CREW	CAPTAIN:	VISITOR 1:
	CREW 1:	VISITOR 2:
	CREW 2:	VISITOR 3:
WEATHER	CONDITIONS AT STATION:	

HABITAT TYPE	☐ **Tidal River**	☐ **Coral Reef**	☐ **Other**
	☐ **Open Water**	☐ **Marsh**	
	☐ **Bayou/Inlet**	☐ **Oyster Bed**	
	☐ **Inter-Tidal**	☐ **Marina**	
	☐ **Rocky/Shell Bottom**	☐ **Grass Bed**	
SAV	Present ? ◯ Yes or ◯ No		

MARINE DEBRIS	Present?	Type		
	◯ Yes or ◯ No	☐ **GLASS**	☐ **WOOD**	☐ **CANS**
		☐ **PLASTIC**		☐ **Other. . .**

PLACE COMMENTS ON BACK OF FORM

RECORDED BY:____________________ COMPUTER ENTRY BY:______________________

HYDROGRAPHIC PROFILE
Coastal 2000 - Gulf Region

Station Name
____

QC CHECK | TIME: (HH:MM) | QC'd BY:

PARAMETER	TEMP (°C)	SAL	pH 7	pH 10	DO (%)	DEPTH
STANDARD:			7.0	10.0	100.0	0.0
MEASURED:						
CALIBRATE:						

DATE: (MMDDYY)	TIME: (HH:MM)	INSTRUMENT #:

	DEPTH (m)	TEMP (°C)	Ph	DO (mg/l)	SAL (o/oo)	LIGHT (AMB)	LIGHT (UW)	SECCHI DEPTH(m)
D								
O								
W								
N								
C								
A								
S								
T								

	DEPTH (m)	TEMP (°C)	Ph	DO (mg/l)	SAL (o/oo)	LIGHT (AMB)	LIGHT (UW)
U							
P							
C							
A							
S							
T							

PLACE COMMENTS ON BACK

RECORDER:________________ COMPUTER ENTRY BY:________________

WATER SAMPLE COLLECTION
Coastal 2000 - Gulf Region

STATION NAME
____

DATE:______________ **FILTRATION METHOD:** ◯ **SYRINGE or** ◯ **VACUUM**
(MMDDYY)

SURFACE (0.5m)		
	NUTRIENT *(SN)**	☐ (Filtered Sample - 60 ml)
	CHLOROPHYLL *(SCL)*	☐ (25 mm Filter) **Volume Filtered:** __________ **(ml)**
	TSS *(SSS)*	☐ (Unfiltered Sample - 1 Liter)

MID-DEPTH		**N/A (<2 m)** ☐
	NUTRIENT *(MN)*	☐ (Filtered Sample - 60 ml)
	CHLOROPHYLL *(MCL)*	☐ (25 mm Filter) **Volume Filtered:** __________ **(ml)**
	TSS *(MSS)*	☐ (Unfiltered Sample - 1 Liter)

BOTTOM (0.5 off bottom)		
	NUTRIENT *(BN)*	☐ (Filtered Sample - 60 ml)
	CHLOROPHYLL *(BCL)*	☐ (25 mm Filter) **Volume Filtered:** __________ **(ml)**
	TSS *(BSS)*	☐ (Unfiltered Sample - 1 Liter)

*** Sample label to be placed on container is listed in Italics**

RECORDED BY:___________________________________ COMPUTER ENTRY BY: ______________________

BENTHIC INFAUNA COLLECTION
Coastal 2000 - Gulf Region

STATION NAME
______

DATE: (MMDDYY)__________ GRAB TYPE: ◯ Van Veen or ◯ Corer

SAMPLE NO. 1 *(BI1)**	**TIME: (HH:MM):**	**DEPTH (cm):**
	DESCRIPTION:	
	NO. OF JARS USED:	
SAMPLE NO. 2 *(BI2)**	**TIME (HH:MM):**	**DEPTH (cm):**
	DESCRIPTION:	
	NO. OF JARS USED:	
SAMPLE NO. 3 *(BI3)**	**TIME (HH:MM):**	**DEPTH (cm):**
	DESCRIPTION:	
	NO. OF JARS USED:	

***Sample label to be placed on container is listed in Italics**

RECORDED BY:__________________ COMPUTER ENTRY BY: __________________

SEDIMENT SAMPLE COLLECTION
Coastal 2000 - Gulf Region

Station Name ______________

DATE: (MMDDYY) ____________________

SEDIMENT CHEMISTRY	I. ☐	Organics *(SO)**	(Glass Jar)
	II. ☐	Metals *(SM)*	(Nalgene 125 ml)
TOXICITY *(ST)*	☐		(1 gal - Nalgene)
GRAIN SIZE *(SG)*	☐		(250 ml - Nalgene)
SEDIMENT TOC *(TOC)*	☐		(60 ml)

*** Sample label to be placed on container is listed in italics**

RECORDED BY:____________________ COMPUTER ENTRY BY:____________________

SEDIMENT SAMPLES ONLY

FISH TRAWL #
Coastal 2000 - Gulf Region

Net Width (ft): ___________

Station Name
____

TRAWL INFO	DATE (mm/dd/yy):
	HELMSMAN:
	LINE OUT (m):
TRAWL START	LAT (00°00.00'):
	LON (00°00.00'):
	HEADING IN DEGREES MAGNETIC:
	START TIME (HH:MM):
TRAWL END	LAT (00°00.00'):
	LON (00°00.00'):
	END TIME (HH:MM):
TRAWL DETAIL	TRAWL TAKEN: ○ Yes or ○ No
	IF NO, EXPLAIN:
	TRAWL SUCCESSFUL: ○ Yes or ○ No
	IF NO, EXPLAIN:
	ANYTHING CAUGHT: ○ Yes or ○ No

RECORDER:________________________________ COMPUTER ENTRY BY: ______________________

FISH DATA
Coastal 2000 - Gulf Region

Station Name ____________

DATE: ____________

COMMON NAME: ____________

GENUS SPECIES NAME ____________

Fish Number	Fish Length	Samples		Pathology Type	TRAWL INFO:
		Chemistry	Pathology		NUMBER
1					
2					TYPE
3					○ STANDARD
4					○ NON-STANDARD
5					○ OTHER. . .
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					PATHOLOGY OBSERVATIONS
18					
19					G - GILL ABN
20					U - ULCERS
21					L - LUMPS/BUMPS
22					S - SKELETAL ABN
23					E - EYE ABN
24					
25					
26					
27					
28					
29					TOTAL COUNT:
30					

RECORDER: ____________ ENTRY BY: ____________

APPENDIX D

Water Quality Measurements by Hydrolab® DataSonde 3/ Surveyor 4

(Routinely Utilized in Northeast Region)

WATER QUALITY MEASUREMENTS BY HYDROLAB® DATASONDE 3/ SURVEYOR 4

This section describes the procedures for the calibration and operation of the Hydrolab DataSonde 3 datalogger (referred to as either "Hydrolab" or "DataSonde") attached to a Surveyor 4 deck unit. This is the equipment used by AED field crews to perform water column profiles for the parameters of dissolved oxygen, salinity, temperature, pH, and depth. The protocols following are excerpted from past EMAP-Virginian Province field manuals. Similar procedures should be followed by field crews using other instruments.

Included in this section is the operation of a YSI model 58 DO meter. Protocols require a duplicate measurement, using a different instrument or method, at each station for the purpose of Quality Assurance. Winkler titrations are recommended for crews experienced with this procedure. If this wet chemistry method is not practical, a duplicate surface measurement can be made with a separate properly-calibrated DO meter. For the data to be acceptable, both values must agree to within 0.5 mg/L.

D.1 Hydrolab Datasonde3® unit

Obtaining a vertical profile of the water column using a Hydrolab Datasonde3® or similar unit is one of the first activities performed at every station. A Hydrolab is a sophisticated instrument designed to collect high-quality data for salinity, temperature, dissolved oxygen (DO) concentration, pH, and water depth. At each station the instrument will be used as a CTD (instrument that measures Conductivity, Temperature, and Depth - in this case, also measures pH and DO) to obtain a vertical profile of water column conditions. Training of all personnel expected to operate this instrument is necessary to assure reliable operation and acceptable data.

Below are general instructions for calibrating and deploying these units.

D.1.1 Setup and Calibration

The following is a brief summary of the calibration of the Hydrolab. The manual should be referred to for detailed instructions and should be read prior to calibration. During calibration, the Datasonde unit should be attached to the gel pack battery to conserve the unit's internal battery supply. Calibration should be performed every morning prior to the start of sampling.

1. To calibrate the Hydrolab Datasonde units, the software package "Procomm" will be used. Attach the DataSonde unit to the computer with the data cable, making sure the computer is reading from the correct port (com 2 on AED "Rocky" laptops) and at a baud rate of 9600bps. The first time you calibrate the Hydrolab, you will have to select the parameters that C2000 will be using and remove any other parameters (in order to save memory and battery power). Once you are in Procomm, lines of data will be displayed (if this is not the case check the battery or refer to the manual). Pull up the menu by depressing the space bar. To select the parameters hit 'P'.

2. You can now add the following parameters by hitting the letter in parentheses for each parameter and then choosing E for (E)nable. The parameters that need to be added are:

   (p)H
   (S)alinity
   D(O)
   (%) Sat
   (D)epth/Level
   (B)attery

3. Then remove the following parameters by choosing (D)isable:
   Specific (C)onductance/Resistivity
   (R)edox

D.1.2 Calibration of the salinity sensor

The salinity sensor will be calibrated against a sample of seawater that has a known salinity [from a high quality laboratory salinometer calibrated with IAPSO Standard Seawater (a.k.a. "Copenhagen" water)]. The Hydrolab will always be equipped with the salt water cell block. Rinse the sensor & calibration cup 3 times with a small amount of the salinity standard (shaking vigorously with the calibration cap in place). Fill the calibration cup to within a centimeter of the cup's edge and make sure there are no bubbles in the conductivity cell block. From the Calibrate menu, choose (S)alinity and enter the standard value in parts per thousand.

D.1.3 Calibration of the pH sensor

Rinse the sensors and calibration cup thoroughly with deionized water prior to and following filling the cup with the standard pH buffers. Fill the calibration cup with the pH 7 standard buffer. Wait until the reading stabilizes to hit the space key, access the calibrate menu and enter the pH value. Now finish calibrating the pH sensor using the pH 10 standard. It is important that there is not a lot of drift in the measurement before entering the calibration standard value. If you cannot get the readings to stabilize, it is time to clean the sensors and check the battery power.

D.1.4 Calibration of the Dissolved Oxygen sensor

The calibration of the dissolved oxygen sensor is highly sensitive to the maintenance of the sensor itself. To make the calibration process go more smoothly, it is important to examine the DO membrane and make sure it is has not dried out, become damaged or dirty, that there are no bubbles in the electrolyte, and that **you have waited at least 12 hours (preferably 24 hours) after changing a membrane to calibrate.**

With the unit turned upside down, fill the calibration cup with ambient room temperature tap water (or DI) to the O-ring line on the DO sensor, making sure all of the sensors have been well rinsed prior to this. Tightly put the calibration cap on and shake the unit to aerate the water.

Remove the cap on the calibration cup and, using the corner of a kimwipe, remove all water droplets from the membrane surface. Put the calibration cap on (upside down). Wait for the readings to stabilize, and then depress the space key to access the calibration menu. Enter 760mm for the barometric pressure (if a barometer is available the exact pressure can be entered, but the range at sea level has only a minimal effect on the calculated DO reading), and then enter the DO percent saturation (100% for the standard membrane).

D.2 Obtaining Hydrolab Profile

At each station, the general procedures for collection of data are as follows:

1 Connect the Hydrolab to the end of the winch cable with a shackle and **TIGHTEN THE PIN**. Make sure a “pinger” is attached to the unit. A 50 pound weight should be hanging approximately 0.5 meter below the unit, and one float (sufficient buoyancy to float the Hydrolab without the weight) attached to the top. This will prevent the unit from impacting the bottom.

2. Remove the protective cover from the probes and connect the stirrer.

3. Connect the unit to the Surveyor 4 deck unit and initialize logging.

4. Connect the stirrer to the upper bulkhead connector.

5. Lower the unit over the side and allow it to equilibrate at the surface for at least two minutes after the unit begins logging.

6. While the unit is equilibrating, lower a YSI probe (see Section 4.3) with stirrer over the side to the same depth as the Hydrolab. Record the reading from the YSI on the CTD datasheet. This serves as a Quality Control check on the operation of the Hydrolab. A surface salinity and temperature should also be obtained with a refractometer and the YSI meter or thermometer, respectively.

   Make sure that the Hydrolab surface readings agree with those from the QC check (*e.g.*, the DO readings must agree to within 0.5 mg/L). If they agree, continue with the cast. If they do not agree, recalibrate the YSI and obtain another surface reading. If they then agree, continue with the cast. If they do not, try another Hydrolab.

7. Lower the Hydrolab according to the following schedule:

   Shallow sites (# 2 m) - every 0.5 m interval;
   Nominal depths (>2<10 m) - 0.5 m (near-surface) and every 1-m interval to near-bottom (0.5 m off-bottom);
   Deep sites (>10 m) - 0.5 m (near-surface) and every 1-m interval to 10 m, then at 5-m intervals, thereafter, to near-bottom (0.5 m off-bottom).

Allow the unit to stabilize at each stop during descent. Save the data from each depth on the Surveyor unit and record the values on the Hydrographic Data Sheet. Once the weight hits the bottom the unit will float 0.5 meter above.

8. Repeat the process on the upcast.

9. Connect the Surveyor to the computer and download the data (this can be done back at the dock). The file should be saved as "XX00xxxxctd.csv" where XX00xxxx is the station number (*e.g.*, MA000001ctd.csv).

D.3 YSI Model 58 Dissolved Oxygen Meter and Probe

The YSI will be used to take oxygen measurements at the surface as a Quality Control check on the Hydrolab. The following information details the maintenance and operation of the YSI Model 58 Dissolved Oxygen Meter.

D.3.1 Initial Setup of The YSI

1. The YSI Model 58 has two separate sets of batteries, one for the oxygen meter and the other for the stirrer. Both sets consist of 4 D-size Alkaline batteries. These are accessed by removing the four screws on the back panel then carefully pulling the meter back away. The upper battery holder is for the oxygen meter, the lower holder for the stirrer batteries. Note that the stirrer batteries will probably require more frequent replacement, whereas the meter batteries will most likely last throughout the entire field season. Observe correct polarity whenever changing batteries in either holder.

2. When the YSI meter batteries are low, the LOWBAT warning will show **continuously** on the display (the LOWBAT warning may flash momentarily as the meter knob is turned off, but this is normal). The initial appearance of LOWBAT indicates about 50 hours of meter battery life. The normal life for the meter batteries is about 1000 hours.

   To check the YSI stirrer batteries, turn and hold the STIRRER knob to the BATT CHK position. If the LOWBAT warning shows **continuously** on the display then the stirrer batteries should be changed. The initial appearance of the LOWBAT warning in the BATT CHK position indicates 5 hours or less of stirrer battery life. The normal life for the stirrer batteries is about 100 hours.

3. While the meter is still open, observe the position of the sliding switch in the upper right hand corner of the meter. This switch sets the meter sensitivity for the type of membrane on the oxygen probe. The switch should be in the middle position, set for a 1 mil ("standard") membrane.

4. Close the meter housing and gently tighten the corner screws. DO NOT OVER TIGHTEN these screws, as they are easily stripped. As you close the meter, work the

rubber gasket so that the outer edge overlaps both halves of the housing.

D.3.2 Changing the YSI Probe Membrane

The procedure for changing the YSI probe membrane is similar to that for the Hydrolab membrane. However there are some differences, so it's important to be familiar with both procedures. The YSI membrane should be changed weekly, or sooner if the probe is difficult to calibrate or is slow to respond. Visual inspection is the best indication of when to change the membrane: if the membrane is fouled, wrinkled, cut, has bubbles underneath it, or the gold cathode is tarnished...then it's time. Try to schedule membrane replacement at the end of a field day, or the night before. This allows the membrane more time to "relax" and equilibrate.

1. Prepare the electrolyte by dissolving the KCl crystals in the dropper bottle with distilled water. Fill the bottle to the top.

2. Unscrew the sensor guard, and remove the O-ring and membrane. Rinse the sensor with distilled water and then with electrolyte. Gently wipe the gold cathode ring with a kimwipe or paper towel.

3. Fill the sensor with electrolyte. If you're right-handed, grasp the sensor in your left hand with the pressure compensating vent to the right. Successively fill the sensor body with electrolyte, then pump the diaphragm with the ERASER end of a pencil or with some similar soft, blunt tool. Continue filling and pumping until no more air bubbles appear. Tap the sensor with the pencil to free any bubbles trapped on the sides.

4. Remove a membrane from the "standard membrane" package (DO NOT use the Hydrolab membranes - they are different). Secure the membrane under your left thumb. Add a few more drops of electrolyte to the sensor to form a meniscus over the gold cathode.

5. With the thumb and forefinger of your other hand, grasp the free end of the membrane.

6. Using a continuous motion, stretch the membrane UP, OVER, and DOWN the other side of the sensor. Stretching forms the membrane to the contour of the probe.

7. Secure the end of the membrane under the forefinger of the hand holding the probe.

8. Set the O-ring on the membrane above the probe, and using your thumb and index finger, roll the O-ring down over the probe until it is seated. Try not to touch the membrane surface while doing this. Gently tug at the exposed corners to remove all wrinkles, then trim away the excess membrane below the O-ring and replace the sensor guard. Inspect the membrane to make sure there are no bubbles, wrinkles, or cuts.

9. The probe should be stored in the open-ended plastic bottle provided for that purpose. Moisten the sponge or paper towel in the end of the storage bottle to prevent the

membrane from drying out. The membrane needs to relax for a minimum of 12 hours following installation.

D.3.3 Calibration of the YSI Oxygen Meter

The YSI should be calibrated before sampling at EACH station, and the meter and attached probe should be turned on for at least 10 minutes prior to calibration or sampling. In practice this means turning the meter on at the beginning of the day and leaving it on (with the possible exception of very long transit periods between stations). On field days when the probe is not being used leave the meter in the % switch positions (or, in the case of the model 57, in the 0-10 MG/L position).

1. Calibration will be done in the probe storage/calibration chamber. Confirm that a moist piece of towel or sponge is present in the bottle. Remove any water droplets from the membrane surface by drying with the corner of a paper towel.

2. Set the function switch to ZERO, and when the display reading has stabilized, readjust display to read 0.00.

3. Reset the function switch to % mode. When the display reading has stabilized, unlock the O2 CALIB control locking ring and adjust the display to read 100%. Relock the locking ring to prevent inadvertent changes. Avoid exposing the calibrated probe to large thermal changes, such as from direct sunlight or lying on a hot deck.

D.3.4 Operation of the YSI Oxygen Meter

In general the YSI will be used to confirm the proper operation of the CTD.

1. Calibrate the YSI (See above; Section 4.3.3).

2. Remove the storage/calibration chamber and the sensor guard, and CAREFULLY screw the probe into the stirrer. The probe membrane should NOT touch the stirrer blades. Membrane damage occurs most often when the probe is being inserted or removed from the stirrer. If a measurement isn't to be taken immediately, wrap the stirrer-probe unit in a moist towel and set it out of the sun.

3. Set the function switch to 0.01 MG/L mode.

4. To perform a surface YSI check place the probe next to the CTD DO probe with the stirrer ON. Set the YSI salinity from refractometer reading. Record temperature from a thermometer, and DO from the YSI on the "CTD CAST DATA SHEET".

5. If using the Hydrolab to obtain a bottom water dissolved oxygen concentration:

    A. Collect a bottom water sample in the *GO-FLO* bottle.

B. Draw out a small sample from the bottle and measure the salinity using the refractometer. Set the SALINITY switch to this value, and record the salinity on the “CTD CAST DATA SHEET”.

C. Prop open the Go-Flo bottle. A Hydrolab sensor guard without the weight works well for this.

D. Insert the stirrer-probe unit into the GO-FLO bottle and turn the stirrer ON.

E. When the meter reading has stabilized, record the oxygen value on the “CTD CAST DATA SHEET”.

F. Remove the probe, turn the stirrer OFF, rinse the probe with freshwater, replace the storage bottle, and store the unit out of sunlight.