CMarZ OE Cruise Protocol Hand Book
CMarZ / OE cruise on the R/V Ron Brown
Ver. 2 (10 March 2006)

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Section 1. MOCNESS

I. MOCNESS deployment protocols

This is a brief overview description of the cruise plan taken from circular one. Most specific details of the sampling plan and sample work up follow. Suggestion for additions or modifications to this plan are solicted and welcome

Area of proposed operations: A transect extending from the Northern Sargasso Sea to 14 N east of the Leeward Islands will be sampled during a 21 day period, with comprehensive surface-to-bottom sampling at five major stations (Figure 1). Intensive sampling will occur in the northern Sargasso, Southern Sargasso, and North equatorial current. Additional sampling will take place as time permits as the ship moves between primary stations.

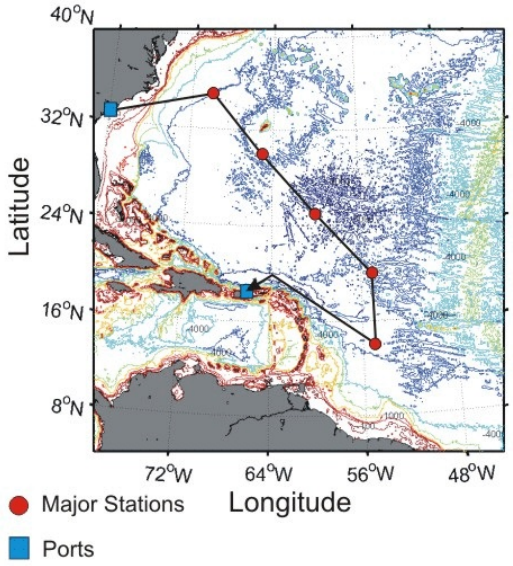

Figure 1. Proposed Cruise Track and Station Locations.

Station Characterization: A multi-beam echosounder will be used to map the seafloor at the primary stations. Characterization of the bottom topography and benthic environment will allow examination of species diversity and abundance in the deepest samples in relation to benthic properties. Also, real-time multibeam images are needed to allow near-bottom sampling, with the net system sampling as close to the seafloor as possible. Sampling to within 100 m of the bottom in ~5000 m of water is anticipated.

Zooplankton sampling: Zooplankton and micronekton will be quantitatively sampled throughout the water column using a 10-m MOCNESS (Multiple Opening-Closing Net and Environmental Sensing System, which will allow rapid capture of zooplankton from great depths (~5000 m) and ensure their good condition for molecular analysis. The MOC-10 will carry five nets. Net 0 will have 3 mm mesh and nets 1 to 4 will have 335 um mesh. Another 3 mm mesh net may be used as Net 5 to stabilize the trawl as it is brought to the surface. It is intended for sampling primarily below 1000 m (Figure 2). Above 1000 m, vertically-stratified sampling will be done using a 1-m MOCNESS using 9 nets with 335 um mesh (Figure 2). In addition, in the upper water column a ½-m MOCNESS using 9 nets with 0.65 um mesh will be used to collect foraminifera and other micro-zooplankton (Figure 2). Deeper tows with the fine mesh may be undertaken.

A draft scheme for sampling at each station has been developed that will enable replicate tows with each MOCNESS to be made during an approximately 48 hr period (Figure 3). In addition,

time has been allocated for two blue water dives. Not shown on this scheme is time for a CTD/Rossette cast or opportunistic sampling with ring nets.

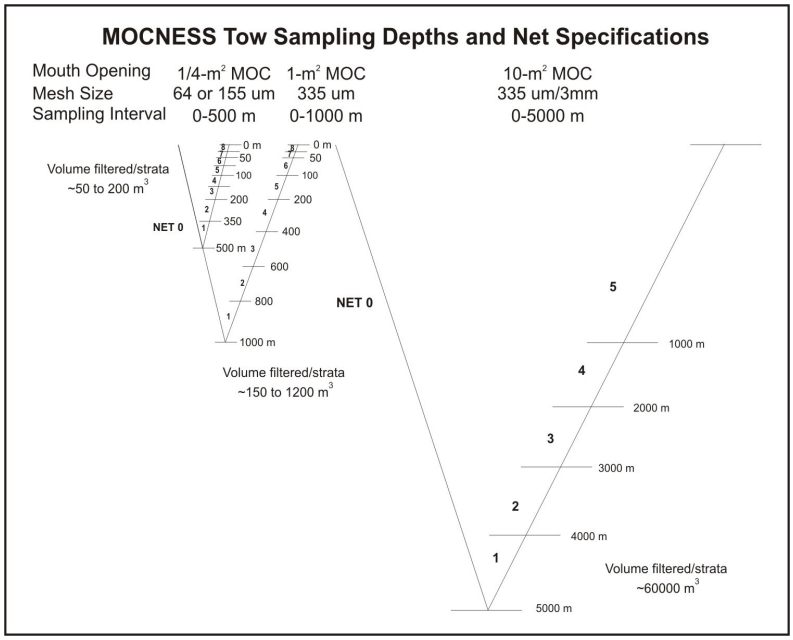

Figure 2. Proposed MOCNESS sampling depth strata.

Blue-water SCUBA diving for gelatinous zooplankton: Living or intact specimens of gelatinous zooplankton will be collected by blue-water diving techniques. A group of (usually) 4 divers work from a small inflatable boat launched from the ship. They are connected by 10 m long tether lines to a central line hanging down from the inflatable and manned by a afety-diver who watches over the others. Each diver can then move about within a 10 m radius to locate, observe, and collect free-swimming gelatinous animals. The technique is only semi-quantitative, but allows collection of live and undamaged specimens, as well as in-situ photos and videos of behavior. Organisms are collected in simple wide-mouth jars and returned to the ship for further study. The same technique is used at night, with the addition of underwater flashlights or headlamps. During this cruise while on station, we expect dives to take place daily and sometimes at night, depending on weather conditions and other activities.

II. Sample-handling protocols

Samples collected with the MOCNESSes will be processed using a standard protocol (Figure 4).

On Deck: Wash nets with seawater hoses, moving organisms still in net carefully into cod-end buckets. Put cod-ends into numbered white buckets or pour cod-end contents carefully into the buckets. The buckets should be moved expeditiously into the walk-in cold room for preliminary analysis.

Specimen removal: In the walk-in cold room, three teams, each consisting of one PICKER and one RECORDER, should be standing by during net recovery. Three teams are needed for each recovery, to immediately remove large specimens of 1) gelatinous forms, 2) fish, and 3) macrozooplankton/nekton.

The PICKER may remove specimens of the designated taxonomic group(s) from the buckets. The specimens should be placed individually in sequentially-numbered jars, shell vials, or cryovials. The RECORDER should write down all specimen information on the data sheets provided, linking the vial number to specimen and collection data. The identified specimen may

either be divided or dissected as desired, with the specimen and/or tissue immediately preserved (in alcohol, frozen nitrogen, or formalin as appropriate and previously agreed-upon) or taken for photographic imaging prior to preservation. The RECORDER should write down the disposition of the specimens.

All specimens removed will be recorded in a CMarZ Specimen Log so the actual taxonomic composition and species count for each sample can be reconstructed.

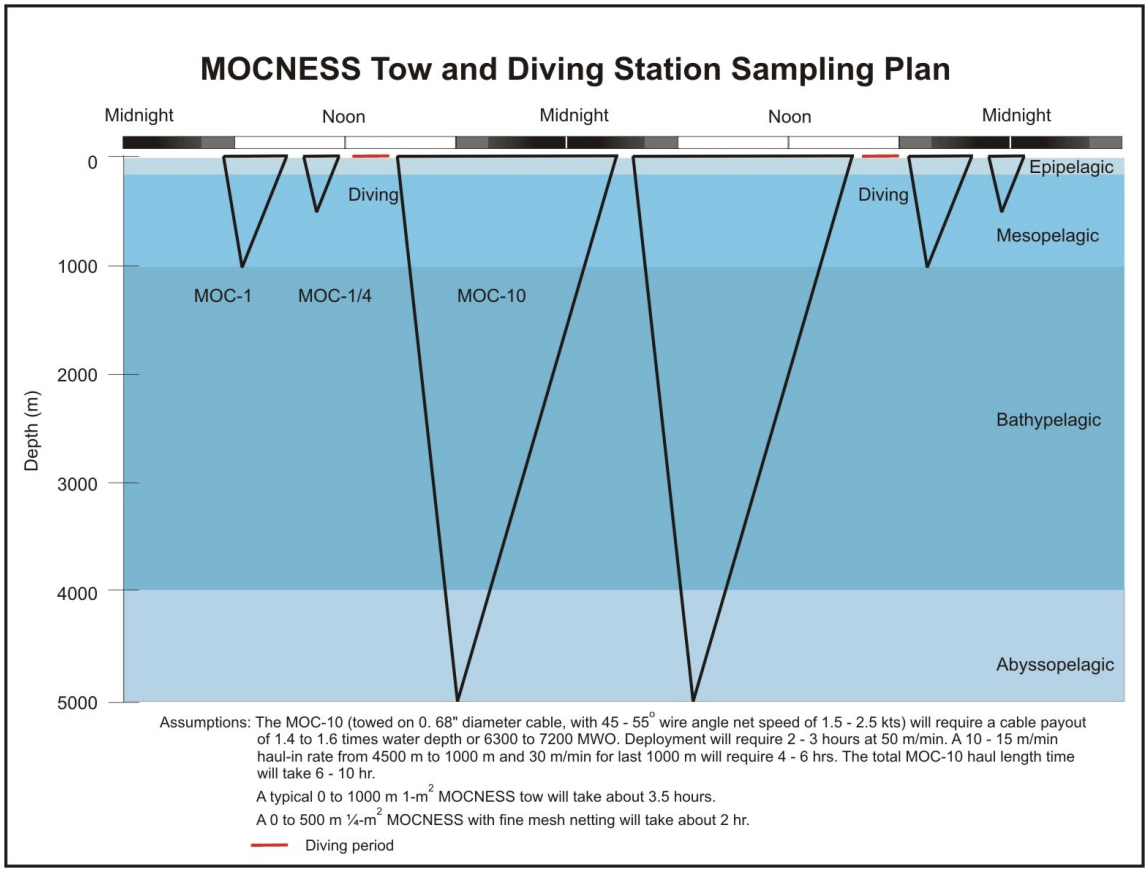

Figure 3. Draft Station net towing and diving sampling plan.

Sample splitting and preservation: Within 5 minutes of arrival, the bucket (with most large gelatinous forms, fish, and macrozooplankton/nekton removed) should be passed to the SPLITTERS. The samples will be split in the following way:

1/2 split: (A) removed, sieved, preserved in alcohol

1/2 split again to

1/4 split (B) to formalin for later taxonomic analysis

1/4 split (C) preserved in alcohol

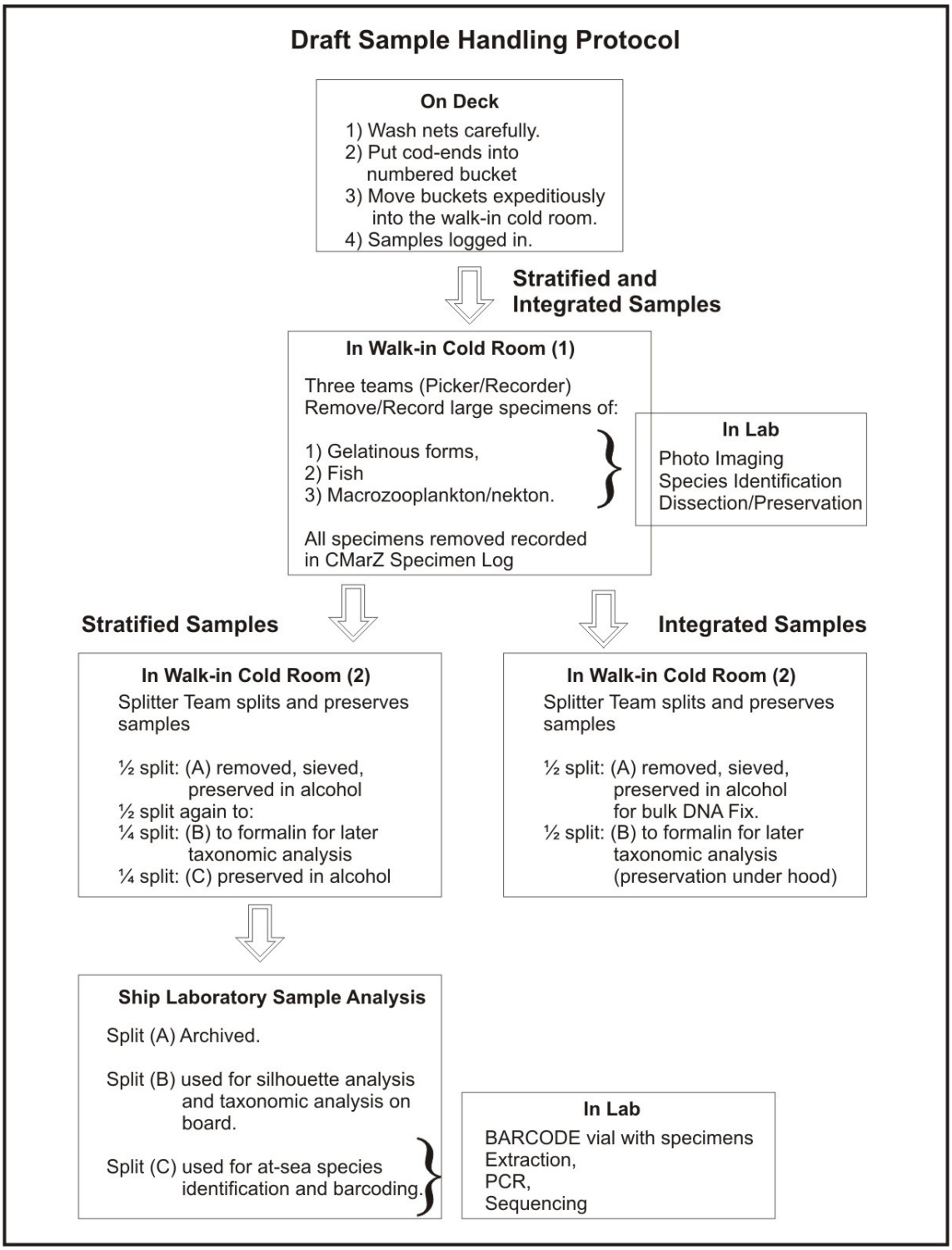

Figure 4. Sampling Handling Protocol.

If specimens in any split are moribund at the time of preservation, this should be marked clearly on the outside of the jar. Glass and plastic ware used with formalin should be clearly labeled with red tape and kept separate from alcohol glass and plastic ware.

After 24 hr, alcohol should be changed on A and C splits.

Note: This protocol may need to be modified for the 1.4-m MOCNESS tows and some aspects of the protocol for the 10-m and 1-m MOCNESSs may also need modification. Comments welcome.

III. Sample analysis

Split (A) will be retained untouched for post-cruise future studies, including biomass estimates (e.g., displacement volume), species counts, DNA sequencing, and other quantitative analyses.

Split (B) will be used for silhouette analysis and taxonomic analysis on board, and then retained for future taxonomic analysis, including new species descriptions, specimen vouchering, etc.

Split (C) will be used for at-sea species identification and removal of identified species for barcoding. It is essential that this split be preserved in alcohol rapidly to ensure that specimens are alive and kicking when they are preserved.

Taxonomic analysis: The taxonomic experts on board should examine split (C) and remove 10 individuals of each species to a vial, with an external label ARCODE and an internal label with specimen and collection information. This vial will be used for DNA barcoding, with the remaining individuals retained as specimen vouchers. Additional specimens can be removed and placed in separate vials for further morphological or taxonomic examination expert. All vial(s) should be numbered and the numbers and relevant information should by recorded in a CMarZ Specimen Log*.

The sample (C) splits will be passed among the taxonomic experts, until each jar has been examined by all available taxonomists to the extent desired. A SAMPLE LIST will be available to allow people to check off jars as they examine them.

Molecular analysis: Up to 1500 DNA sequences may be determined during the cruise (based on the duration of the cruise, time required for analysis, and supplies available).

Up to 10 identified individuals of each species will be removed to a separate BARCODE vial and made available for DNA sequencing at sea. DNA will be extracted from up to 3 specimens for each species. Unused DNA from each specimen will be appropriately labeled and stored in liquid nitrogen as DNA vouchers. The remaining individuals in the BARCODE vial will be retained as specimen vouchers.

Barcoding protocol: Extraction, PCR, Sequencing (Additional details are in the Barcoding Protocol document).

Specimen tracking: All steps in molecular analysis are recorded in a CMarZ Specimen Log, including designations for the Extraction, PCR, and Sequence reactions. Standard labeling of BARCODE specimen and DNA vouchers will be used.

IV. Sample Tracking

CMarZ Specimen Log is an ACCESS database available for distribution to all cruise participants.

V. Sample and data disposition and ownership

Cruise participants wishing to retain specimens of portions of samples for their own future research uses should indicate this on the CMarZ Specimen Log under the pecimen Disposition field. Expected use and location of the specimen or sample fraction should be indicated. All specimens must remain available to any cruise participants in the future.

Data ownership: DNA barcode data: all will be submitted to the BARCODE section of GenBank. No individual cruise participant will wn the DNA sequence data.

Section 2. Live Net tows

Ring net surface tows or vertical/oblique tows to depths to be determined.

Reeve net Vertical tows to depths to be determined.

What happens to live specimens? How are they shared? Who do they belong to?

Section 3. Water samples / CTD

Need description of water collection depths and how the water samples are going to be handled.

Section 4. Diving

Living or intact specimens of gelatinous zooplankton will be collected by blue-water diving techniques. A group of (usually) 4 divers work from a small inflatable boat launched from the ship. They are connected by 10 m long tether lines to a central line hanging down from the inflatable and manned by a afety-diver who watches over the others. Each diver can then move about within a 10 m radius to locate, observe, and collect free-swimming gelatinous animals. The technique is only semi-quantitative, but allows collection of live and undamaged specimens, as well as in-situ photos and videos of behavior. Organisms are collected in simple wide-mouth jars and returned to the ship for further study. The same technique is used at night, with the addition of underwater flashlights or headlamps. During this cruise while on station, we expect dives to take place daily and sometimes at night, depending on weather conditions and other activities.

Larry Needs to provide more details about this. What happens to live specimens? How are they shared?

Section 5. Training

STUDENTS will SHADOW taxonomic experts individual agreements and sign-up sheets. Better than group workshops / discussions, except as individually desired and arranged, partly due to watch and work schedules.