First Circular to Cruise Participants
CMarZ Western North Atlantic Cruise
January 16, 2006

Project Title: **Zooplankton biodiversity in tropical / subtropical Atlantic Ocean waters: a CoML Census of Marine Zooplankton (CMarZ) partnership project.**

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Cruise: **on R/V Ron Brown, 10 to 30 April 2006, Charleston, SC to San Juan, Puerto Rico**

Cruise Background and Work Plan

Introduction

The ocean ecosystem is home to at least 7000 holozooplankton species (Table 1). They are distributed throughout the world's oceans in biogeographic provinces that were described nearly a century ago (e.g. McGowan 1971, 1974; Longhurst, 1998). These patterns are based almost exclusively on species that inhabit the upper 500 to 1000 m of the water column, i.e., the epipelagic and mesopelagic zones. Below these depths – in the bathypelagic and abyssopelagic zones - far less is known about zooplankton species diversity, distribution, and abundance. This lack of knowledge is even more notable since the bathypelagic zone accounts for 60% of the ocean's volume, making it the largest marine habitat on earth (Table 2).

Comprehensive understanding of zooplankton biodiversity has eluded oceanographers because of the fragility, rarity, small size, and/or systematic complexity of many taxa. For many zooplankton groups, there are long-standing and unresolved questions of species identification, systematic relationships, genetic diversity and structure, and biogeography. The global geographic scale of the investigations required to address these issues, as well as the three-dimensional complexity of the world ocean, make complete knowledge of marine zooplankton diversity challenging. With increasing depths, the need for fundamental knowledge of pelagic biodiversity becomes even more marked.

This CMarZ project will address the second grand challenge of the Census of Marine Life (CoML), "What lives in the ocean now?" We seek to explore under-sampled deep-ocean pelagic environments and to document zooplankton species diversity across the 15 phyla comprising the holozooplankton. CoML has estimated that as many as 5,000 fish species may be undiscovered, and CoML discoveries include a new cetacean (Wada et al., 2003) and gigantic cephalopod species (Veccione et al., 2001). However, most of the new discoveries are likely to come at the other end of the size spectrum. The Census of Marine Zooplankton (CMarZ), a CoML ocean realm field project, has estimated that at least 1600 new zooplankton species will be discovered by 2010 (CMarZ, 2004; see Table 1).

Consistent with Ocean Exploration mission goals, our project is designed not only to expand the boundaries of researchers' knowledge, but also to provide graduate-level training in taxonomic

identification of zooplankton groups, engage undergraduate students in biodiversity research, and showcase the beauty of marine life through web-based imagery. The education and outreach goals of the project will be achieved in large part through partnership with CoML and CMarZ.

Scientific Rationale

Deep-dwelling zooplankton: The mesopelagic layer connects the zone of primary production, the photic zone or epipelagic, with the deep ocean sinks of biologically important elements. Globally, the mesopelagic zone is the source for the return of essential elements back to the photic zone. This critical function is mediated by the planktonic food web, but our

Phylum	**Group**	**# described species**	**# new species**
Phylum	Group	# sp known	# sp new
Foraminifera	Foraminifera	49	100-300
Actinopoda	Acantharea	150	
	Polycystinea (rads)	350	
Cercozoa	Phaeodarea (rads)	350	
Ciliophora	Aloricate ciliata	150	Many
	Tintinnida	300	
Cnidaria	Hydromedusae	842	many
	Siphonophora	160	~100
	Cubomedusae	18	
	Scyphomedusae	161	
Ctenophora	Ctenophora	90	50-150
Rotifera	Rotifera	50?	
Platyhelminthes	Platyhelminthes	3?	
Nematomorpha	Nectonema	5	?
Nemertea	Nemertina	99	35+
Annelida	Polychaeta	110	25+
Mollusca	Heteropoda	29	
	Pteropoda	109	
	Nudibranchs	6	
	Cephalopoda	370	
Arthropoda	Cladocera	8	~5
	Ostracoda	169	200-400
	Isopoda	20	
	Copepoda	2000	1000-2000
	Mysidacea	700	
	Amphipoda	400	
	Euphausiacea	86	10-20
	Decapoda	50	
Chaetognatha	Chaetognatha	93	25-200
Chordata	Appendicularia	64	30+
	Pyrosoma	8	10
	Doliolida	17	10
	Salpidae	45	5-10
	TOTAL	7061	>1605

Table 1. Numbers of known (i.e., described) species and estimated numbers of undescribed (new) species for groups of holozooplankton.

understanding of species composition and biodiversity of mesopelagic food webs is limited. At greater depths, the bathypelagic and abyssopelagic are even less well-known. Coarse-resolution sampling in the deep ocean from the late 1800s (the early days of ocean exploration) to the mid-1900s used mechanically activated closing or opening/closing nets (Wiebe and Benfield, 2003). During this era, deep-sea zooplankton species were described and their depth distributions were characterized (Banse, 1964). With the advent of multiple opening-closing net systems in the mid-1900s, high-resolution sampling of the bathypelagic and abyssopelagic zones became possible (Table 3). More recently, observations and collections made from submersibles, ROVs, and free-vehicles have shaped our understanding of the deep sea (Smith et al, 1979).

Table 2. Atlantic Ocean pelagic habitat volume based on hypsometry, as presented by Menard and Smith (1966). The ocean pelagic habitat has been divided vertically into five zones: epipelagic, mesopelagic, bathypelagic, abyssopelagic, and hadopelagic (Hedgepeth, 1957). The latter zone occupies a small fraction of the ocean volume and is present in the Atlantic Ocean's Puerto Rico Trench (8400 m).

Habitat Zone	**Volume 10^6 km^3**	**Percent volume in depth zones**
Epipelagic (0-200 m)	17311.6	4.8
Mesopelagic (200-1000 m)	64382.4	17.7
bathypelagic (1000 - 4000 m)	213140.0	58.6
abyssopelagic (4000 - 7000 m)	68859	18.9
Hadopelagic (>7000 m)	10.0	0.003

A number of studies have reported that copepods dominate the deep sea in terms of numbers of species, and that a number of these are many new or undescribed species. Of 67 copepod species found deeper than 2000 m in the Guaymas Basin, about half were undescribed (Wiebe et al., 1988). Wishner (1980) identified over 100 species in deep-sea near-bottom tows, including many that were undescribed. Despite these evident prospects for species discovery, taxonomic work on deep-sea zooplankton moves at a snail's pace. Few studies have sought to integrate morphological and molecular systematic approaches to the study of deep-sea zooplankton.

Integrated morphological / molecular approaches to species identification: Traditional morphologically-based analysis of zooplankton samples can be enhanced by the parallel use of molecular genetic characters, forming a basis for accurate assessment of zooplankton species diversity. For example, de Vargas (personal comm.) used molecular genetic analysis to show that the global biodiversity of planktonic foraminifera is 5 – 10 times higher than previous morphologically-based estimates. Some genetic species showing strong adaptation to different depth zones, water-masses, and/or latitudes (Darling et al., 1999). This effort will result in a public archive of DNA sequences linked to voucher specimens (or voucher DNA) for the zooplankton species that are being identified or newly described, counted, and analyzed as part of this effort. The DNA sequence archive will be available on the CMarZ website and GenBank database, and will be useful for identifying species, characterizing morphological and/or molecular diversity, searching for cryptic species, and/or contributing to the description of new species.

Spatial patterns of biodiversity in relation to the environment: The proposed effort will characterize pelagic and benthic environments (using CTD / rosette, multi-beam sonar), census the inhabitants (MOCNESS, mid-water trawl), and discover new species (expert taxonomic analysis and DNA sequencing). Our results will provide new understanding of species diversity, distribution, and abundance in relation to environmental conditions for bathy- and abyssopelagic zones.

Accurate palaeoproxies: For researchers using microfossils as palaeoproxies and routine tools, accurate assessment of species diversity is key to valid results. Previous results by de Vargas and colleagues suggest that numerous cryptic species exist among foraminifera and other skeleton-bearing pelagic protests (see de Vargas et al., 1999), with distinct species in different depth zones. If this depth-speciation hypothesis is borne out, the implication will be that the same morphological "species" extracted from a different zone of a given deep-sea sediment core are in fact distinct species, and cannot be used as similar palaeoproxies.

Project Priorities

The priority for the proposed effort is a taxonomically comprehensive survey of zooplankton biodiversity in the deep zones of the Atlantic Ocean, using both morphological and molecular systematic approaches to understanding species diversity, and describing species distribution and abundance in relation to water column and benthic environmental characters. We will address the overarching question for the CMarZ project: "What are the patterns of zooplankton biodiversity throughout the world ocean, and how are they generated and maintained?" (CMarZ, 2004). In particular, the hypotheses to be tested are:

Hypothesis 1. Zooplankton in the bathypelagic zone exhibit significant spatial heterogeneity in species diversity and abundance along the subtropical / tropical transect.

Hypothesis 2. Spatial patterns of species diversity and abundance of abyssopelagic zooplankton can be correlated to benthic habitat characteristics.

The goals and objectives of the proposed effort include:

Goal 1. Comprehensive sampling and expert taxonomic analysis of zooplankton collected from selected stations in mesopelagic, bathypelagic, and abyssopelagic waters of the tropical / subtropical Atlantic Ocean.

Goal 2. Discovery and description, including morphological and molecular systematic analysis, of new zooplankton species in deep zones of the Atlantic Ocean.

Goal 3. Contribution to OE / CoML outreach and education goals, including: graduate training in taxonomic identification of zooplankton; undergraduate and high school students and educators involvement in biodiversity research; and popular appreciation for marine biodiversity.

Approach and Methodology

Area of proposed operations

A transect extending from the Northern Sargasso Sea to the equator east of Brazil will be sampled during a 24 to 28 day period, with comprehensive surface-to-bottom sampling at seven major stations (Fig. 1). Intensive sampling will occur in the northern Sargasso, Southern Sargasso, and North equatorial current (Table 2). Additional sampling will take place as time permits as the ship moves between primary stations.

Zooplankton sampling

Figure 2. The 10 m^2 MOCNESS being deployed from R/V Iselin on cruise 9307.

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. Based on previous experience by Wiebe et al. (1988), we anticipate sampling to within 100 m of the bottom in up to 4000 m of water.

Zooplankton and micronekton will be quantitatively sampled throughout the water column using a 10-m MOCNESS (Multiple Opening-Closing Net and Environmental Sensing System; Wiebe et al., 1985) trawl (Fig. 2), which will allow rapid capture of zooplankton from great depths (5000 m) and ensure their good condition for molecular analysis. The MOC-10 is a multiple opening/closing net, carrying 5 separate nets that can be controlled from the surface to open at chosen depths. The mesh size of the nets is normally 3.0 mm, so the nets are able to catch macrozooplankton as well as micronekton, but here we propose to construct nets of 0.335 um mesh with the appropriate mesh area to mouth opening ratio R to conduct the sampling. The MOC-10 can be launched and recovered through a stern A-frame or by a crane.

The trawl is deployed with the first net open, which fishes obliquely down to the deepest depth desired. It is closed at that point, and subsequent nets are opened at desired depths as the trawl comes up obliquely. Thus, one MOC-10 net will sample from the surface to the bottom and the other nets will sample 500 to 1000 m intervals from the bottom up to a depth of 1000 m. The trawl telemeters data continuously to the ship, including depth, temperature, salinity, horizontal speed, and volume filtered. This allows on-the-fly adjustment of sampling depths or times, and completion of a continuous series of stratified hauls in a relatively short time. All data are recorded electronically for subsequent analysis.

Above 1000 m, vertically-stratified sampling will be done using a 1-m MOCNESS. In addition, a ¼-m MOCNESS with 0.65 um mesh will be used to collect foraminifera and other micro-zooplankton. Cod-ends to insulate the zooplankton from temperature changes as they are being retrieved on bathy- and abyssopelagic tows will be used (Childress et al., 1978). The use of the large trawl below 1000 m will enable large volumes of water to be sampled (tens of thousands of meters cubed) to compensate for the very low abundance of species expected to occur at bathy- and abyssopelagic depths (Table 2).

The smaller 1-m^2 and 1/4 – m^2 MOCNESS will provided adequate sample sizes in the upper 1000 m. Samples will be processed immediately upon capture with live sample sorting and preservation in formalin for taxonomic analysis, and alcohol for molecular analysis. Species identification will proceed at sea prior to DNA extraction, PCR amplification of target genes, and sequencing for as many species as possible. Ancillary observations will include biological, physical, and chemical properties of the water column.

Although gelatinous organisms like medusae, ctenophores, siphonophores, molluscs, and tunicates are fragile and not ideally sampled with a trawl net, there is no practical alternative for sampling them over the depth and geographic ranges we intend to sample. While there is usually some damage to the specimens from net sampling, in most cases there are identifiable remains, and certainly sufficient tissue for bar-coding or other genetic analyses. Only some of the larger lobate ctenophores are likely to be entirely lost in trawl sampling.
Trawl samples from the nets will be rough-sorted immediately on retrieval to pick out specimens of particular interest and take sub-samples for genetic studies. Live biomass of the catch at each depth will be measured by displacement volume and preliminary counts of larger species made prior to preservation. Representative specimens of most species will be digitally photographed immediately, with the image files logged to the preserved specimen. This procedure is important

to provide an approximate picture of the fauna immediately, valuable for preliminary data reports and to guide further sampling. Detailed sorting and identification of preserved trawl samples can take months. The bulk of the catch will be preserved in 5% buffered formalin in seawater; samples for genetic study will be either flash-frozen in liquid nitrogen,, preserved in 95% ethanol, or on dry-fixative samplers (Whatman FTA cards). Large fish will be injected with 5% formalin.

Table 3. Summary of zooplankton and micronekton sampling in the bathypelagic and abyssopelagic zones in the Atlantic, Pacific, and Indian Oceans. Investigations limited to the upper 1000 m are not included here.

Location	Depth (m)	Sampling Device	Reference
Atlantic Ocean			
Atlantic & California,	>4,000	Underwater camera	Hartman & Emory (1956)
Sargasso, off Bermuda	To 2,000		Deevey (1974)
Bermuda	To 1,170		Kornicker et al. (1976)
Carribean	To 4,000	Open/closing net	Michel & Foyo (1976)
Cape Verde I.	To 1,250		Boxshall (1977)
Greenland Sea Gyre	To 3,000		Richter (1995)
Northeast Atlantic	To 4,500	RMT 1+8 net/trawl	Angel & Baker (1982)
Madeira, Atlantic	To 5,440	RMT 1+8 net/trawl	Roe (1987)
North Atlantic	3,400-4,700	1 m2 MOCNESS	Beckmann (1988)
Northeast Atlantic	To 4,000	1 m2 MOCNESS	Koppelmann & Weikert (1992)
NW Levantine/SE Aegean	> 1,000		Pancucci et al. (1992)
Worldwide	> 1,000	Various	Park (1994)
NW Atlantic	To 2,000	Rect. Midwater Trawl	Mauchline (1995)
Cap-Ferret Canyon, France	To 2,000		Maycas et al. (1999)
Northeast Atlantic	To 4,250	1 m2 MOCNESS	Koppelmann & Weikert (1999)
MedOff Malta	1,800-3,700	Horizontal hauls	Lapernat & Razouls (2001)
Levantine Sea	To 4,270	MOCNESS	Weikert et al. (2001)
Eastern Med. Sea	To 4,000	1 m2 MOCNESS	Koppelmann & Weikert (2003)
Pacific Ocean			
S. California basins	To 1,200		Hartman & Emery (1956)
Kuril Trench, Pacific	To 6,000	Nets, trawls	Zenkevich & Birstein (1956)
Pacific (CalCOFI)	> 1,500		Brinton (1962)
Pacific Ocean	To 1,200	IKMT	Gueredrat (1973)
Eastern North Pacific	3,800-5,700	Free-vehicle, nets, traps	Smith et al. (1979)
Oregon coast, Pacific	2,000-4,300	Rope & Otter trawl	Stein (1985)
Guaymas Basin, Pacific	To 2,000	1 m2 MOCNESS	Wiebe et al. (1988)
Guaymas Basin	1,900-2,000	1 m2 MOCNESS	Copley & Wiebe (1990).
Kuril region, Pacific	To 6,000	Submersible	Shushkina et al. (1991)
Kuril-Kamchatka, Pacific	To 6,000	Net, IKMT, Mir sub	Shushkina et al. (1991)
Eastern North Pacific	4,200 and 5,200	Acoustics, trawl, etc	Smith et al. (1992)
North Pacific	To 6,000	Mir Sub	Vinogradov & Chin… (1994)
North Pacific	To 5,945	Submersible	Vinogradov & Shushkina (1994)
Endeavour Ridge, Pacific	To 2,500	1.4m2 multiple net	Burd & Thomson (1995)
Endeavour Ridge, Pacific	To 3,000	1.4m2 multiple net	Burd & Thomson (2000)
Western North Pacific	To 5,000	NORPAC, VMPS	Yamaguchi et al. (2002)
Western subarctic Pacific	To 2,000	Closing net	Nishibe & Ikeda (2004)

Indian Ocean			
Arabian Sea	>1,050	50 µm net	Boettger-Schnack (1997)
Arabian Sea	To 4,000	1 m2 MOCNESS	Fabian et al. (2005)
Arabian Sea	To 4,430	1 m2 MOCNESS	Koppelmann & Weikert (1997)
Arabian Sea	To 4,050	1 m2 MOCNESS	Koppelmann et al. (2003)
Arabian Sea / Eastern Med.	To 1,850	50 µm net	Boettger-Schnack (1994)

Sampling of foraminifera

Specimens of the skeletonized protists will be recovered from epi- and mesopelagic samples using simple decantation processes. The taxa of interest will be manually sorted under a dissecting microscope immediately after collection. The isolated cells will be cleaned in filtered sea-water using micro-brushes, and put into individual tubes containing 100 µl of GITC buffer. This buffer has been developed in the de Vargas laboratory, and allows extracting the nucleic acids from the organisms while preserving their micro-shell. The material will be stored at -20˚C before further analyses. Total DNA will be extracted according to protocols developed by de Vargas et al. (personal comm.).

Blue-water SCUBA diving for gelatinous zooplankton

Collection of living or intact specimens of gelatinous zooplankton is difficult with nets or trawls because the organisms are usually damaged and sometimes destroyed, as noted above. During the last 30 years, the technique of blue-water diving to make observations and collections of these fragile animals by SCUBA has been developed (Hamner 1975). 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 'safety-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. Blue water diving can be carried out safely in sea conditions up to about 2 m swells, and wind speeds up to about 20 knots. During this cruise, we would expect to dive once daily and sometimes at night, depending on weather conditions and other activities. Further description of blue water diving is in the WHOI Diving Safety Manual and the NOAA Diving Manual. Diving operations would be supervised by L. Madin, one of the originators of the technique.

Blue-water diving will also provide opportunities to photograph fragile organisms. Still photographs and video footage will be taken and used for research (species identification) and education (CMarZ website) purposes. Madin's photographs have already helped familiarize many people with the beauty of plankton.

Ship-board analysis of zooplankton samples

Traditional morphological analysis will be the primary means of processing new and existing collections from the cruise (see Harris et al., 2000). As sample collections are made and analyzed, a reference specimen collection will be assembled for all species or groups. These reference collections will be listed in an appropriate, internet-accessible database, and can be used for quality control of the analysis, and as a resource for expert confirmation of problem species. Taxonomic analysis of samples will be the most labor-intensive part of the proposed effort. Greater efficiency in sample analysis will be sought by integration and coordination among taxonomic experts, students, and technicians both during the cruise and afterward through the CMarZ Virtual Taxonomic Network, which is being developed to link practitioners and provide a portal for the identification and engagement of taxonomic expertise.

The proposed cruise will include participation from a number of CMarZ Steering Group members, who together can cover nearly the entire taxonomic spectrum represented among the holozooplankton. Researchers who focus on other organisms, including cephalopods and fish, have been invited to join the cruise, and/or will be engaged to study specimens sent to them following the cruise. The cruise participants will be assembled into "expert groups" (composed of CMarZ Steering Group researchers and their accompanying postdocs, technicians, and students) for each of the component taxa in the zooplankton assemblage. Initial sample analysis by cruise participants will involve sorting specimens by taxonomic group, with specimens distributed to the designated expert group for more detailed analysis. The goals of the on-board analysis will be the confirmed identification of known species, as well as the isolation of putative unknown or undescribed species, by a qualified taxonomic expert. Specimens that cannot be identified confidently will be sorted into vials for further analysis by othe/ appropriate taxonomic experts, who will be identified and engaged through the CMarZ Taxonomic Network (see below and www.CMarZ.org). Specimens with confirmed identifications will be prepared for molecular analysis while on board, with appropriate vouchering of material, related individuals, and/or DNA consistent with approved protocols of the Consortium for the Barcode of Life (see below http://barcoding.si.edu).

At the conclusion of the cruise, a species list for all identified specimens will be produced, which will be posted to the CMarZ website and database, consistent with protocols and policies of OBIS (Ocean Biogeographical Information System; see Zhang and Grassle, 2003). A list will also be posted of putative new or undescribed species, for which further analysis and eventual description will be sought through the CMarZ Virtual Taxonomic Network and other CoML field projects. Sample jars with pre-sorted or tentatively-identified specimens that require additional analysis will be available for participants to take with them and/or send to colleagues.

Molecular analysis of zooplankton samples

Molecular systematic analysis of zooplankton will involve DNA sequencing of a target gene region for each taxonomic group. Initial results from the Census of Marine Zooplankton (CMarZ) indicate that species of the 16 phyla represented in the holozooplankton will be identifiable using DNA sequences for a target gene region, although the genes selected for species-level diagnostic DNA sequences will vary among taxa.

For crustaceans and most other invertebrates, mitochondrial cytochrome oxidase I (mtCOI) clearly discriminate even the most closely related species and resolves taxonomically-significant geographic variation (Bucklin et al., 2003). DNA sequences for each species will be based on at least 3 specimens or three different sequences from purified DNA. For widely distributed and/or polymorphic species, DNA sequences from specimens collected during the proposed cruise will be compared with those from other regions of the species' range. Consistent with the approach used for the earlier ZooGene project (www.ZooGene.org), mtCOI sequences will be used to provide characters for uniform standards of species' identification; evaluation of the taxonomic significance of geographic variation within widespread species; identification of cryptic species; accurate estimation of species' diversity; and design of rapid molecularly-based species' identification protocols (see Bucklin, 2000; Bucklin et al., 2001, 2002, 2003; Hill et al., 2001).

For gelatinous zooplankton, fragments of identified specimens to be used for molecular analysis will be excised upon collection. Portions of the same individuals will be preserved for standard taxonomic analysis and identification. Tissue samples for molecular analysis will be preserved in alcohol or flash-frozen in liquid nitrogen. The target gene for each taxon may vary, with mtCOI appropriate for some groups, while mitochondrial 16S rRNA is now becoming standard for others (L. Madin, pers. comm.). The nuclear intervening transcribed sequence (ITS) regions may also be useful for some taxa (see Ortman et al., 2004).

For foraminifera, the exceptional rates of DNA substitution within the nuclear ribosomal genes (18S and 28S rRNA) and internal transcribed spacer regions (ITS-1 and -2) make them suitable for species identification (de Vargas et al., 1999, 2002). DNA sequencing will be done for a region of 28S rDNA (5' end), which has been shown to be an excellent marker for species level differences.

DNA sequencing will be done at sea, using an older model capillary machine, such as an ABI PRISM® 310 Genetic Analyzer, an automated single-capillary genetic analyzer designed for a wide range of sequencing and fragment analysis applications. We will have a used DNA sequencer for ship-board use. DNA samples and identified specimens will be returned to the Center for Marine Molecular Analysis (COMMA) for additional DNA sequencing.

DNA will also be purified from unsorted zooplankton and microplankton samples. Recent work by CMarZ researchers has demonstrated that zooplankton samples may be rapidly analyzed by PCR amplification and cloning of DNA purified from bulk samples. The PCR amplifications can be done using consensus, group-specific, or species-specific PCR primers, to facilitate DNA sequencing and species identification. The resultant sequences are compared to DNA sequences from identified species. Molecular analyses of bulk zooplankton samples are one means toward rapid, molecularly-based analysis of zooplankton samples. The DNA sequences may also be used as a basis for species identification on DNA microarrays (i.e., DNA chips).

Silhouette Analysis

Taxonomic/size composition analysis will be accomplished using silhouette photography (Davis and Wiebe, 1985), yielding size specific abundance of different taxa (e.g., large and small

copepods, chaetognaths, euphausiids, etc.). Size and abundance will be used to extrapolate sample biomass using empirical relationships between size and taxon-specific biomass.

Data analysis and hypothesis testing

Multivariate analysis will be used to examine possible correlations between species diversity and individual species distribution and abundance, and all measurable environmental parameters. Spatial heterogeneity in species diversity of the samples, abundances of the individual species, and their relationship to hydrographic conditions and benthic habitat characteristics will be statistically evaluated using the multivariate statistical package, Plymouth Routines In Multivariate Ecological Research (PRIMER, Version 5.2.9, PRIMER-E Ltd.). The Biotic-Environmental (BIO-ENV) routine in PRIMER will be used to assess the relationships between physical variables and species abundances, and to determine which factors produce a similarity matrix that most closely matches the community matrix (Clarke and Ainsworth, 1993, Clarke and Warwick, 1994). PRIMER is a user-friendly and powerful statistical package for oceanographic studies (see e.g., Manning and Bucklin, 2005).

Education and graduate training

During the cruise, a workshop on "Taxonomic Identification of Zooplankton" will treat each holozooplankton group in turn, in order to provide a comprehensive view for the post-doctoral fellows, technicians, and students. Approaches to species identification will be taught, as well as lectures on morphology, ecology, and evolution of the component taxa. Students and staff will be taught the molecular protocols associated with molecular analysis of zooplankton, from DNA purification and vouchering, PCR amplification, and DNA sequencing. Interested participants will also be shown how to design simple protocols for DNA-based species identification, including multiplexed species-specific PCR (see Bucklin, 2000; Hill et al., 2001).

One or more bunks on the cruise will be made available for "Teacher-at-sea" opportunities. We will announce the availability of the cruise opportunity through the National Marine Educators Association (NMEA) and other professional groups. Participating teachers will need to prepare a plan for dissemination of the information and course materials beyond their own students. Teachers will work with the CoML Education & Outreach Office to prepare and follow-up on the cruise.

Data storage and serving

This project, being part of the COML CMarZ program, will utilize the existing and developing data management system and web site (http://www.cmarz.org/) that will provide the basic common services, logistical support, and data management required by the CMarZ project investigators as they conduct field research, analyze the data from individual efforts and integrate results, and integrate all of these with education and outreach activities. The Web site server is located in Woods Hole and employs the expertise developed and used by the U.S. GLOBEC Program and based on the Joint Global Ocean Flux Study (JGOFS) data management and data server software (Groman and Wiebe, 1998). The system is a distributed, object-based data

system, with the data sets residing with the responsible scientists when possible. Core data from the CMarZ activities are accessible to all the scientific investigators in a timely manner. The data include measurements of physical, chemical, and biological parameters from discrete samples and continuous probe profiling instrumentation. The data are made available via the Web, and accessible by any standard browser, such as Internet Explorer, Netscape, and FireFox. Data quality control is the responsibility of the contributing investigator(s), although initial quality control checks are done by the data management office prior to placing any data on-line. All on-line requests for data are made through the CMarZ central "data server", even though the data files can be stored on other computer systems. Before data will be served, the necessary metadata file(s) are created to ensure that these data can be readily used by other researchers. In addition, standard metadata, Directory Interchange Format (DIF), records will be prepared and submitted to the Global Change Master Directory (an FGDC clearinghouse) to ensure the widest possible distribution of the data sets.

Web-based forms provide data selection and data projection capabilities, common to SQL type queries. The data selection capability permits people to view, plot, and download data that are contained within specified geographic boundaries and satisfies any other retrieval conditions. The data projection capability permits people to select for viewing and downloading only those parameters they want without having to deal with other data stored in the same files. The web-based system also supports data joins between two data sets that share a common data attribute, such as station number. Data contributors are encouraged to follow a data field name thesaurus so that field names are used consistently and without ambiguity.

To ensure the rapid and extensive dissemination of data and results, following the cruise, the chief scientist (assisted by other participants) will prepare an initial assessment of the field activities and acquired data (a cruise report) so that the investigators will have a common record of navigational data, and general information and environmental data based on shipboard, satellite imagery, and other remote sensing observations. The cruise report and other forms of information will be made available on-line to foster information exchange and further analysis. There will be pre- and post-cruise activities that will facilitate the logistical aspects of the research. CMarZ sponsored workshops will facilitate integration of the field survey data. Data and synthesis products will be provided for education and outreach.

In summary, the approach to data management and data retrieval is flexible, extensible, and has been proven through use by existing large interdisciplinary projects (i.e., U.S. GLOBEC, U.S. JGOFS). Our personnel have extensive knowledge and experience in enabling web sharing of user contributed data, and it is our intention to thoroughly integrate into the COML CMarZ data access and data sharing approach with appropriate links to OBIS.