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A Multidisciplinary Approach for

GENERATING GLOBALLY CONSISTENT DATA

on Mesophotic, Deep-Pelagic, and Bathyal Biological Communities

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Image taken at 750 m depth on Atlantis Bank in the Southwest Indian Ocean. *NERC/IUCN Seamounts Project courtesy of A.D. Rogers*

ABSTRACT. Approaches to measuring marine biological parameters remain almost as diverse as the researchers who measure them. However, understanding the patterns of diversity in ocean life over different temporal and geographic scales requires consistent data and information on the potential environmental drivers. As a group of marine scientists from different disciplines, we suggest a formalized, consistent framework of 20 biological, chemical, physical, and socioeconomic parameters that we consider the most important for describing environmental and biological variability. We call our proposed framework the General Ocean Survey and Sampling Iterative Protocol (GOSSIP). We hope that this framework will establish a consistent approach to data collection, enabling further collaboration between marine scientists from different disciplines to advance knowledge of the ocean (deep-sea and mesophotic coral ecosystems).

INTRODUCTION

The marine realm encompasses an immense and complex interconnected matrix of diverse ecosystems. The least-known ocean regions occur below depths accessible to scuba diving and include mesophotic coral ecosystems (MCEs) and the deep sea. MCEs occur at 30 to >150 m in tropical or subtropical waters. These low-light environments support the deeper reaches of coral reefs and may be important for reef resilience, but their distribution beyond conventional scuba depths increases the challenge of surveying their biological diversity (Hinderstein et al., 2010). The deep sea, defined here as ocean environments deeper than 200 m, comprises more habitat by area or volume than any other on Earth (Snelgrove and Smith, 2002). The immense size and generally remote nature of the deep ocean limit sampling opportunities (Ramirez-Llodra et al., 2010). The knowledge gaps within mesophotic and deep-sea ecosystems present a tremendous opportunity for discovery (Mora et al., 2011) and for increased understanding of their functioning. However, the expanding array of sampling approaches creates a challenge in producing the standardized, comparable data needed to catalyze advances in knowledge (Clark et al., 2007). The present patchwork of studies, and the

diversity of techniques used, limit our capacity to examine broad-scale patterns and processes, to extrapolate between study locations, and ultimately to advance our understanding of Earth's largest environment (Rogers et al., 2015).

The immense diversity of life forms, from microscopic bacteria to large cetaceans, requires different sampling approaches depending on the size, abundance, and habitat of the target biota. For example, sampling for microbes requires small volumes of water, whereas sampling for megafauna may require extensive visual surveys across kilometers of water. These issues, in tandem with the high cost of accessing MCE and deep-ocean environments, point to the need to identify a common set of variables that are scientifically informative, robust, logistically tractable, and readily transferable among diverse environments. The Census of Marine Life started to address issues of how to integrate national or regional data sets, and promoted standardized sampling in the ocean. More recently, different initiatives, such as the Global Ocean Observing System (GOOS) and the Deep Ocean Observation System (DOOS) have begun to develop a strategy for identifying and prioritizing Essential Ocean Variables (EOVs; Lindstrom et al., 2012). The scientific community has widely accepted EOVs for physical parameters in

the ocean, including temperature, salinity, current velocity, and pressure. The GOOS Biochemistry Panel is presently defining EOVs for geochemical parameters and will present its findings after consultation with the user community. Although GOOS has suggested eight biological EOVs, the user community has not yet agreed on their adoption (but for a regional example, see Constable et al., 2016). The diversity of life, processes, and relevant variables that influence biological patterns in the ocean has impeded this decision by complicating the choice of favored parameters.

The authors of this article, along with many other marine researchers, already measure many of the parameters presented in the following protocol. Therefore, we do not presume to dictate a research method to the community but instead to present a formal framework to enable consistent data gathering. We hope that this standardized and multidisciplinary approach will galvanize long-term and multi-site research that can start to answer some of the most challenging, intractable, and complex questions about the marine environment, and some basic ones as well, such as: What are the environmental conditions of a location? What is the geographic range of species and habitats? What are the levels of connectivity between marine ecosystems? What are the drivers of marine biodiversity at different depths? In what ways do human activities impact the ocean environment (e.g., Rogers et al., 2015)? We propose a practical sampling plan to advance our understanding of ocean biodiversity based on finite resources. Although survey design and sampling equipment must be tailored to the specific objectives of any study, we suggest some key measurements and propose how to obtain such measurements in a robust, standardized, and affordable approach.

PRIMARY PARAMETERS AND WHY THEY ARE IMPORTANT

Many abiotic and biotic variables influence the distribution and diversity of marine life. These drivers can vary substantially in different habitats (e.g., open ocean, canyons, hydrothermal vents, and coral reefs) and often operate at very different spatial and temporal scales. Assessing the key environmental drivers of community composition and abundance requires the collection of environmental data simultaneously with biological surveys.

Figure 1 displays the General Ocean Survey and Sampling Iterative Protocol (GOSSIP) process as a flow diagram, and Table 1 summarizes the parameters and details of sampling. The variables we have highlighted are all important for determining composition or abundance of communities and the conditions of the area they inhabit. Measurement of these key parameters at all locations will allow direct comparisons among different sites to support evaluations of their general importance to overall community structure, and their roles in driving spatial and temporal differences.

Biological Parameters
PELAGIC BIOLOGY

The pelagic realm connects the surface and the ocean depths, partly through the largest daily migration of biomass on Earth. Our limited knowledge of the mesopelagic (200–1,000 m) creates a particularly "dark hole in our understanding of marine ecosystems and their services" (St. John et al., 2016), but a growing body of knowledge about animals throughout the water column demonstrates the arbitrary nature of depth divisions, and the need to view the water column as dynamic and transitional, without fixed boundaries (Sutton et al., 2017). The mesopelagic and deeper zones likely play an extremely important role in the global carbon budget (Irigoien et al., 2014). No single device can efficiently sample all sizes and body types of marine organisms. This creates a major difficulty in documenting life in the meso- and bathypelagic zones (hereafter deep pelagic). Nets with millimeter- to centimeter-scale meshes sample zooplankton and micronekton, optical samplers detect bioluminescence and provide images of organisms, and acoustic sampling provides proxy measures of community biomass over wider areas. Surface observations (e.g., from aircraft and/or ships) can record the presence of large mammals, but population estimates typically require a combination of many
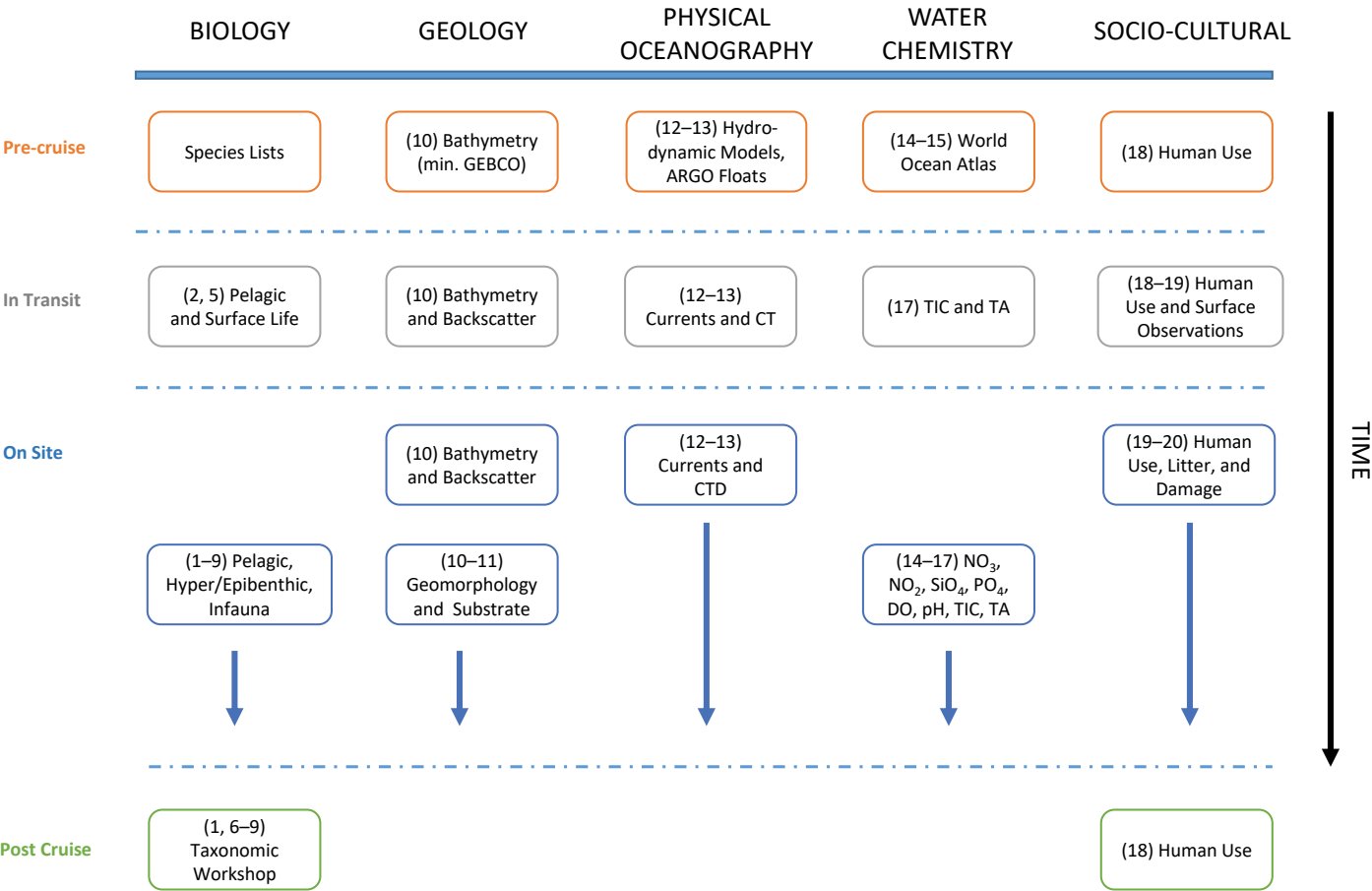

FIGURE 1. A flow diagram that reflects the timeline of when to conduct activities associated with parameters listed in Table 1. Activities are grouped by discipline, a summary of the data collection is provided, and numbers relate to parameter identification numbers in Table 1.

TABLE 1. Primary Components: Details on present and future methods.

Why it is Important and How it Relates to Other Biology Data Collected	Sampling Method/ Equipment of Choice	Any Standardization Already Determined (e.g., Mesh Size)	Post-Processing Methods	Potential New Technologies	Key Reference(s)
BIOLOGICAL PARAMETERS > PELAGIC					
(1) Size structure and species composition of mesozooplankton, pelagic micronekton, and pelagic nekton					
Key data for diversity metrics, community statistical analysis, ground-truthing active acoustics, and ecosystem modeling	Standardized trawls for estimation of biomass per unit volume Ideally, two net types would be used: (1) rectangular midwater trawl (RMT) with opening/closing capability and flowmeter for quantitative, discrete depth sampling; (2) large, high-speed rope trawl for sampling larger fishes and squids If only one possible, then the RMT would be priority *Other Options: In situ video and photo observation via remotely operated vehicle (ROV), submersible, and/or autonomous underwater vehicle (AUV)*	3 mm mesh size is standard for midwater trawls	Microscopic and genetic taxonomic identification Measuring (total or standard length) Weighing (wet weight and dry weight)	Machine learning and automated image recognition In situ holographic imaging Imaging acoustics	Pakhomov and Yamamura, 2010 Heino et al., 2011
(2) Acoustic sensing of water column biomass					
Provides large-scale, quasi-synoptic view of the distribution of animal life in the water column	Multi-frequency hydroacoustics (MFA)	"Standard" zooplankton and fisheries frequencies include 18, 38, 70, 120, 200, and 333 kHz; broadband can span this spectrum *Note: Before use, MFA need to be calibrated*	Echo-integration for biomass, inverse processing for species/ size composition, scattering-layer extraction for deep scattering layer (DSL) characterization	Imaging acoustics Moored acoustical arrays Deep-towed broad-band to overcome physics-based limitations of sampling with high frequencies at depth	Simmonds and MacLennan, 2005 Proud et al., 2015
(3) Size structure and abundance of gelatinous zooplankton					
Gelata are key ecosystem components as carbon cyclers and important biodiversity components	Standardized, quantitative ROV video transects *Other Options: Blue-water diving for shallower depths*	Transect methods of Monterey Bay Aquarium Research Institute	Video reanalysis and annotation; a searchable video annotation system is critical	Machine learning and automated image recognition In situ holographic imaging	Robison, 2004
(4) Microbial community					
Major primary producers, dictating much of the nutrients and energy	Niskin bottles on CTD rosette *Other Options: Other water collection gear*	1 L filtered through 0.2 µm polycarbonate filter	Use of universal primers for sequencing	Alternative technologies for sequencing	Sunagawa et al., 2015
(5) Census of associated biota					
Provides data on occurrence and distribution of large marine vertebrates and seabirds	Surface observation Passive acoustics	NOAA National Marine Fisheries Service has standardized visual transect methods		Airplane-borne light detection and ranging surveys (LIDAR)	Barlow et al., 2001 Haver et al., 2017
BIOLOGICAL PARAMETERS > BENTHIC					
(6) Deepwater hyperbenthos					
Prey species Unique zooplankton community Carbon cycling Larvae of benthos/nekton	Visual observation with fixed-focus HD video in tandem with a non-destructive physical sampling mechanism (e.g., pumping system or ROV/AUV towed net/continuous plankton recorder) *Other Options: Sledge mounted/ towed, nets, pumps, traps, visual systems, continuous plankton recorder; maximize filtration volume with net capacity and/or tow duration*	Nets: 0.5 mm mesh preferred, up to 1 mm if risk of clogging high; opening-closing mechanism essential in deep water; attached odometer and current meter necessary to assess volume filtered	Microscopic and genetic taxonomic identification	eDNA sampling and metagenomics Deep-sea video plankton recorders Holocams Machine learning and automated image recognition and integrated with standard classification schemes	Gallager et al., 2004

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Why it is Important and How it Relates to Other Biology Data Collected	Sampling Method/ Equipment of Choice	Any Standardization Already Determined (e.g., Mesh Size)	Post-Processing Methods	Potential New Technologies	Key Reference(s)
(7) Mesophotic hyperbenthos					
Prey species Unique zooplankton community Carbon cycling Larvae of benthos/nekton	Light traps, diver-operated mysid traps, diver-operated opening/ closing nets *Other Options: See deepwater hyperbenthos recommendations*	See deepwater hyperbenthos recommendations	Microscopic and genetic taxonomic identification	eDNA sampling and metagenomics Deep-sea video plankton recorders Holocams Machine learning and automated image recognition and integrated with standard classification schemes	Mees and Jones, 1997
(8) Epibenthos					
Distribution of unique or sensitive communities Prey and predator species Species of commercial importance (e.g., fish, lobster, shellfish)	Video recording using technical divers, submersibles, ROV can be used in most habitat types Non-destructive techniques in sensitive habitat areas Stereo-video recording for fish biomass measurements *Other Options: Drop/towed cameras, AUVs; direct sampling with submersibles/ROVs, trawls, sledges, and grabs/corers to allow specific identification; baited remote underwater video*	Straight line transects, minimum length 1 km where possible; crossing multiple substrate types will maximize biodiversity information Gear selection after multibeam and camera runs to ensure appropriate samples taken Refer to taxonomic standards (e.g., CATAMI)	Analysis of video and still camera images Subdivision of transect by distance or substrate type Microscopic and genetic taxonomic identification	eDNA sampling and metagenomics Seafloor observatories for temporal patterns Laser line scanning Machine learning and automated image recognition and integrated with standard classification schemes Digital library containing 3D models of specimens obtained via nano- and micro-computed tomography Regional standardized visual keys to species	Harvey et al., 2001 Howell et al., 2010 Clark et al., 2016 Althaus et al., 2015
(9) Infauna					
Key role in carbon and nitrogen cycling Community characteristics directly related to ecosystem functioning Food source for megabenthos	Multi- and megacorers operated from ship *Other Options: When working on rugged topography, the use of box corers and/or push corers is advised, the latter operated with the manipulator arms of submersibles and ROVs*	Sieve mesh size is taxon dependent; 300 µm for polychaetes, 45 µm for nematodes, and 125 µm for foraminifera, although 63 µm might be necessary in certain environments Sub-sectioning of cores for macrofaunal and metazoan meiofauna: 0–1, 1–3, 3–5, 5–10 cm; For protozoans: 0–0.5, 0.5–1, 1–1.5, 1.5–2, and each cm to 10 cm.	Microscopic and genetic taxonomic identification	Machine learning and automated image recognition and integrated with standard classification schemes Environmental DNA sampling and metagenomics Digital library containing 3D models of specimens obtained via nano- and micro-computed tomography	Danovaro, 2009

ENVIRONMENTAL DRIVERS

Why it is Important and How it Relates to Other Biology Data Collected	Sampling Method/ Equipment of Choice	Any Standardization Already Determined (e.g., Mesh Size)	Post-Processing Methods	Potential New Technologies	Key Reference(s)
(10) Bathymetry: Seafloor morphology (depth)					
Defines spatial environment and habitat Operational needs	Multibeam echosounder (MBES) *Other Options: Single-beam echosounder; Interferometric echosounder; For high-resolution surveys from AUV or ROV–Laser line-scan and photogrammetry*	Hydrographic standards (e.g., "Order 1a"), although those are more than what is needed for biological and habitat mapping, and difficult to achieve in deep water *Note: Before use, MBESs need to be calibrated, and during use, a correct sound velocity profile through the water column is needed*	MBES data processing is well established; several software packages are available (Caris HIPS & SIPS, Qimera, MB System) Processing includes georeferencing, data filtering, application of sound velocity, corrections for tide, gridding	Use of high-resolution MBES or side-scan sonar on AUVs and ROVs Spectrophotography and photogrammetry to reconstruct very-high-resolution bathymetry from photographs	Micallef et al., 2018

Why it is Important and How it Relates to Other Biology Data Collected	Sampling Method/ Equipment of Choice	Any Standardization Already Determined (e.g., Mesh Size)	Post-Processing Methods	Potential New Technologies	Key Reference(s)
(11) Seafloor composition (substrate type)					
Describes benthic habitat/ environment Geochemistry of pore water Operational needs	MBES backscatter to be ground truthed with one of the following: Sediment granulometry Photography/video Coring *Other Options: Side-scan sonar, interferometric echosounder, sediment granulometry*	Apart from sediment size, there are few standards, although the marine mapping community does recognize the need	Backscatter processing can now be carried out by a series of software packages (e.g., Fledermaus FMGT, Qimera); processing includes radiometric and geometric corrections to measured amplitudes and gridding of final data Photo/video interpretation Geochemistry: pore waters are generally extracted on ship and treated in similar ways to the water column chemistry below Particle size measurements on core sediments	Machine learning and automated image recognition In situ sensors are evolving to look at sediment geochemical processes, such as benthic chambers to measure respiration processes in the sediments and the exchange of chemicals across the sediment water interface	Glud, 2008 Lurton and Lamarche, 2015
(12) Current velocity					
Ambient environmental conditions Current shear Turbulence	Pelagic: Acoustic Doppler current profiler (ADCP; ship-mounted or lowered) Benthic: Acoustic Doppler velocimeter (ADV)/ADCP on a mooring or lander *Other Options: Argo profiling floats*			ADCPs on gliders or AUVs	Visbeck, 2002
(13) Temperature, salinity, pressure (derived density) (CTD)					
Ambient environmental conditions Density gradients required to calculate geostrophic currents, water mass	CTD profiler If available, a flow-through system should be used to measure the temperature and salinity of surface waters *Other Options: Temperature/ salinity, loggers on moorings or landers, expendable bathythermographs (XBT), Argo profiling floats*	Salinity verified against bottle samples with salinometer aboard	Pressure inversions and salinity spikes flagged	CTDs on gliders or AUVs	Thomson and Emery, 2014
(14) Nitrate/nitrite (NO_3, NO_2), silicate (SiO_4), and phosphate (PO_4)					
The main macronutrients that limit biological productivity Can also be an indicator of nutrient pollution (eutrophication)	Water collected by Niskin bottles and either preserved with mercuric chloride or frozen at −20°C in plastic *Other Options: Onboard analysis with an autoanalyzer*	Samples collected through the photic zone (e.g., surface, 5, 10, 15, 50, 75, 100, and 200 m); then additional depths to near seabed)	Lab analysis, standard approaches as defined by the International GEOTRACES project	In situ sensors available on market include the in situ ultraviolet spectrophotometer (ISUS) system, good for buoys and moorings; lab-on-chip systems coming online	Cutter et al., 2017
(15) Dissolved oxygen (DO)					
Key to understanding the state of the environment and vital for life	Water samples collected by Niskin bottles and from sediment cores, preserved with Winkler reagents; analysis to be done within 24 hours *Other Options: Oxygen optodes on CTD systems are available and give reasonable results*	Samples collected through the photic zone (e.g., surface, 5, 10, 15, 50, 75, 100, and 200 m); then additional depths to near seabed	Colorimetric determination using a benchtop spectrophotometer or titration	Optodes and new sensors being brought to market	Cutter et al., 2017

Why it is Important and How it Relates to Other Biology Data Collected	Sampling Method/ Equipment of Choice	Any Standardization Already Determined (e.g., Mesh Size)	Post-Processing Methods	Potential New Technologies	Key Reference(s)
(16) pH					
A key parameter to understand the carbon cycle, when combined with another of the commonly used parameters (TA, DIC, pCO_2) It is also the key measure for ocean acidification studies	Analyzed using onboard colorimetric or sensor technology on collected water samples *Other Options: Sensors are becoming more widely available*	Samples collected through the photic zone (e.g., surface, 5, 10, 15, 50, 75, 100, and 200 m); then additional depths to near seabed	Though a difficult measurement to make, pH is important in the context of ocean acidification and carbonate chemistry in coral habitats	New in situ optode sensors are being brought to market	Martz et al., 2010
(17) Dissolved inorganic carbon (DIC) and total alkalinity (TA)					
Indicators of the effects of climate change DIC determines the oceanic carbon inventory TA reveals buffering potential of the ocean against a changing pH	Water collected using Niskin bottles on a CTD rosette	Samples collected through the photic zone (e.g., surface, 5, 10, 15, 50, 75, 100, and 200 m); then additional depths to near seabed	DIC is measured colorimetrically Total alkalinity is determined by potentiometric titration	In situ sensor technologies in development	Johnson et al., 1985 Dickson et al., 2003 Hansell and Carlson, 2014
SOCIOCULTURAL PARAMETERS AND IMPACTS					
(18) Human use					
Quantify the anthropogenic impacts that may have altered the biological communities	Interview with stakeholders Databases of human activities (e.g., EMODnet) and approved exploration Surface observations Indirect impacts assessed from visual surveys	Semi-structured interviews to include personal understanding and use of the region		Satellite monitoring of vessels Flight initiation distance/minimum approach distance in fishes as indicator of fisheries pressure, recorded with stereo-video Fish body size proxies on mesophotic coral ecosystems (e.g., parrotfish body size structure highly correlates with fisheries exposure intensity)	Witt and Godley, 2007 Yasué et al., 2010
(19) Records of litter and anthropogenic damage					
Quantify the impacts that may have altered the biological communities	Surface observation: Benthic video recording or direct observation from in-water technical diving to 100 m Deeper depths: Photographs or video footage taken from submersibles, ROVs, or AUVs	Terminology should be defined as standard terms are currently limited	Analysis of video recordings	Machine learning and automated image recognition	Spengler and Costa, 2008
(20) Microplastic abundance and diversity					
Emerging but ubiquitous pollutant, with potential impacts for life	Surface: Neuston net or manta trawl Sediment: Corer (see infauna)	300 μm mesh for surface nets	Post-sampling contamination methods should be employed	Automated flow through techniques onboard and other automated processing techniques	Hidalgo-Ruz et al., 2012 Woodall et al., 2015

other methods, including tissue collections (Williams et al., 2014). However, in most locations, shipborne observers and passive acoustic monitoring provide sufficient details on abundance and diversity of mammals. Assessments of pelagic megafauna biomass, such as sharks and tuna, are historically derived from fisheries-dependent data, but have recently used midwater baited stereo-video systems (Letessier et al., 2015). Microbial assemblages are typically assessed by collecting water in Niskin bottles followed by filtering and sequencing with next-generation genetic tools (Gilbert et al., 2008).

The plethora of different sampling methods required to obtain a comprehensive assessment of biodiversity in the pelagic zone requires prioritizing the taxa. Growing databases of fisheries acoustic data (Proud et al., 2017) and net sample data (Sutton et al., 2017) suggest biogeographic structure in the deep pelagic. These biogeographic patterns are not the same as those observed in surface water (e.g., Longhurst, 2007), which is hardly surprising given ocean currents, the sinking of surface production, and the potential connectivity of mesopelagic populations. Nonetheless, temperature and wind stress can accurately predict depth and backscattering intensity (a proxy for biomass) of deep-scattering layers (Proud et al., 2017).

In addition to acoustic "remote-sensing" observations, an assessment of biological life requires collection of biological samples. In particular, a reasonably comprehensive evaluation requires information about:

- Microbial assemblage composition
- Size structure and species composition of (1) mesopelagic fishes, with an emphasis on myctophids because of their high proportional abundance in midwater assemblages and their role in carbon cycling (size range 1–10 cm); (2) siphonophores, because their morphology can bias acoustic estimates of fish biomass; and (3) mesozooplankton (including gelatinous taxa)

and cephalopods, because of their importance as prey for apex predators
- Pelagic megafaunal biodiversity
- Large mammal diversity and abundance

We prioritized these taxa to cover the very wide range of size classes of organisms and to represent multiple trophic levels and ecosystem functions. Obtaining these data requires a combination of net sampling, in situ and surface observations, acoustic surveys, and water sampling.

BENTHIC BIOLOGY

For the benthic component, we focus on the taxa in, on, and immediately above the seafloor. Below, we separate sampling of the hyperbenthos (animals living in the water immediately above the seabed), epibenthos (animals living on the seabed), and infauna (animals living within sediments). Benthic communities include size classes from small meiofaunal (32–300 µm), to macrofaunal (300 µm–2 cm), to megafaunal (>2 cm) organisms. Sampled taxa represent all size classes. In some cases, we suggest a typical taxon to study; however, relying on a single taxon identified using morphology alone is less frequently used in studies that investigate biodiversity patterns (e.g., Brandt et al., 2007).

Hyperbenthos. The hyperbenthos (sensu Mees and Jones, 1997) community links seafloor and pelagic ecosystems and occurs in a mixed layer of varying velocity and turbulence, known as the benthic boundary layer (BBL; Pepper et al., 2015). The organisms that inhabit the BBL can spend all or multiple periods of their lives in this zone. The hyperbenthic community composition differs significantly from that of the water column above it (Christiansen et al., 2010). These animals represent potential prey for benthic, pelagic, and demersal species, coupling pelagic and benthic food webs. They also contribute to the recycling of organic matter, and their larval dynamics influence the distribution and survival of adult populations.

Traditionally, hyperbenthic samplers span a range of volumes and designs (reviewed in Clark et al., 2016). In order of volume, sampling methods include water bottles, traps, pumping systems, and nets. On MCEs, light traps are also used to collect organisms (Luckhurst and Luckhurst, 1977; Andradi-Brown et al., 2017). The typically low plankton abundances in the deep sea (Christiansen et al., 1999) favor a high-volume system as the most reliable sampling method. However, because nets/sleds potentially cause environmental damage to the seabed, visual surveys using remotely operated vehicle (ROV)/sub-mounted video plankton recorder systems may be preferable (Gallager et al., 2004), though these approaches also require ground truthing via sampling.

For larger animals (>2 cm), high-definition video, set to a fixed shallow depth of field and run over a slow transect, offers volume coverage similar to nets in midwater tests (Robison et al., 2010), although again this requires checking with physical samples.

In the future, we expect that high-volume species identification and quantification methods, such as automated environmental DNA (eDNA) sampling and metabarcoding techniques (Bucklin et al., 2016), will prove particularly useful, augmented by automated image identification with high-volume video/holographic plankton recorders (Davies et al., 2015).

Epibenthos. Epibenthic organisms play an essential role in the provision of ecosystem services because they capture carbon, provide food sources, build three-dimensional habitats, and influence deepwater sediment structure through their effects on hydrodynamics, bioturbation, and movement across the seafloor (Thurber et al., 2014).

Assessments of the diversity of epibenthic communities traditionally used destructive sampling techniques (e.g., sledges and trawls); however, more recently, photographic platforms produce imagery that can be used to catalog the

diversity of fauna while minimizing damage to the seafloor. ROV, submersible, and autonomous underwater vehicle (AUV) surveys are now relatively commonplace tools, generally deployed along transects to document characteristics of the substratum as well as epibenthic animals. These surveys enable estimates of mega-epifaunal abundance and biomass, as well as assessment of variability in community distribution and composition (see chapters in Clark et al., 2016). Although images enable classification of the mega-epifauna into "morphospecies," species identification is often difficult, and physical specimens are frequently needed to adequately describe community structure (Howell et al., 2010). Although technical divers (MCEs only) or ROVs and submersibles can sample selectively, targeted and limited sampling by sledges, trawls, or corers can also provide physical specimens for identification. They can also sample the macrofaunal organisms that are too small to be seen in high-resolution photographs, and collect animals hidden from view in biogenic structures such as coral reef matrices.

Beyond sledge, trawls, and mobile video/image capture methods, additional tools for sampling include baited-remote underwater video (BRUV) for scavenging fishes and invertebrate megafauna, as well as grabs, corers, and ROV suction samplers for collecting macro- and meiofauna. Landers are increasingly used to document epibenthic organisms, especially when equipped with time-lapse cameras. Towed cameras can be used in most environments, whereas direct sampling gear cannot. Each gear type has its own selectivity characteristics, and hence results vary qualitatively and quantitatively, depending on habitat type and faunal composition (see chapters in Clark et al., 2016). Sampling design and gear type preference differ with habitat and topography.

Although many early MCE studies used deep-sea sampling methods, more recent efforts have shifted to diver surveys, prompted by advancements in

diving technology and safety (Turner et al., 2017). This shift has allowed the adoption of many shallow-water reef survey methodologies, enabling direct comparisons between adjacent MCEs and shallow reef communities. Divers can operate equipment close to the seabed, overcoming the challenges of sampling steep slopes associated with some survey techniques. Among other uses, stereo-video can assess fish biomass with the added benefit of allowing short survey times, while gaining accurate length estimates of individual fishes (Harvey et al., 2001; Andradi-Brown et al., 2016b). Divers can now also carry many other instruments normally deployed by deep-sea landers (e.g., temperature loggers, sediment corers, sediment traps), particularly with increasing miniaturization of sensors; they can also sample organisms directly.

Infauna. On a global basis, the sedimentary deposits that overlay the oceanic crust are on average 420 m thick (Olson et al., 2016). Typically, the most well-studied infauna are the macrofaunal polychaetes and meiofaunal nematodes and foraminifera that inhabit the upper oxygenated sediments. Through their activities, sediment-dwelling organisms create a unique mosaic of biogenic microenvironments that strongly influence carbon and nitrogen burial and remineralization rates, thus playing a key role in global biogeochemical cycles (Dunlop et al., 2016) and marine ecosystem functioning (Danovaro et al., 2008). The microfauna (i.e., protozoa) and microbes have traditionally been problematic to sample because of challenges in identification, but genetic techniques suggest massive undocumented diversity (Sinniger et al., 2016).

Most studies collect sedimentary infauna with corers, which obtain high-quality samples for quantitative analysis. The many types of corers each represent a compromise between sampled seabed area and magnitude of surface sediment disturbance within the sample obtained; thus, the choice of a

sampling device ultimately depends on the target benthic assemblage (reviewed in Clark et al., 2016). Most corers can be deployed from a surface ship, although some mini-corers are operated by the manipulator arms of submersibles and ROVs. Beyond the corers themselves, the methods and tools (sieve mesh size) used to process core samples post-collection influence their inter-comparability among studies.

Historically, the time-consuming nature of biodiversity assessments of sediment samples, especially in deep-sea settings where many species are new to science, created a practical need to focus on one group to serve as proxy for the whole infaunal community. Past studies justify such extrapolations by demonstrating similar distribution and diversity trends in foraminifera, nematodes, and macrofauna (dominated by polychaetes) from deepwater locations worldwide (Danovaro et al., 2008), although not in all cases (Ingels et al., 2014). Metabarcoding and other genetic tools are now commonly used in order to determine biodiversity (Aylagas et al., 2016). However, to identify taxa present in the sample, a comprehensive library of barcodes is required and this is still limited for many marine taxa.

Environmental Drivers
Despite limited understanding of the specific drivers of organism and assemblage distributions in the ocean, variables related to geology, physical oceanography, and environmental chemistry define the main abiotic factors that determine biological diversity, biomass, and abundance.

GEOLOGY
The geology of the seafloor forms one of the primary sets of boundary conditions defining benthic species' distributions. The combination of seafloor morphology and composition (i.e., grain size, geochemistry) provides the spatial environment within which communities reside. In addition, seafloor geology often records a history of environmental change

in that ecosystem, which may exhibit altered community development and biogeography over time. Given the importance of the seafloor as a boundary to the world ocean, it is striking that none of the widely accepted EOVs identify submarine geomorphology or seafloor composition as priority measurements. Beyond their environmental importance, of course, safe operations require good bathymetric maps of study areas prior to sampling.

The primary tools for recording seafloor depth and composition utilize acoustics (i.e., echosounders), whereas optical techniques (laser line scanners, video/photography) and physical sampling (cores, grabs, dredges) can provide more detailed observations (Table 1). When deciding on the optimal approach for a particular study, the appropriate scale defines primary considerations. The concept of "scale" consists of two parts: the grain of a data set (i.e., resolution, pixel size) and its extent (i.e., map coverage; Turner et al., 2001), and the two typically require trade-off. With the development of autonomous and robotic vehicles such as AUVs and ROVs, water depth beneath the ship no longer dictates the pixel resolution of acoustic maps, although bringing echosounders closer to the seabed reduces the area mapped (Wynn et al., 2014). As a result, most surveys now nest sampling, beginning with broad-scale, low-resolution shipboard surveys followed by zooming in with AUV, ROV, or physical sampling at locations of interest, and then adjusting the target pixel resolution, depending on terrain variability and ruggedness (Huvenne et al., 2018).

PHYSICAL OCEANOGRAPHY

The physical oceanographic processes that occur around and above the habitat of benthic and pelagic organisms exert a strong influence on these assemblages. These processes may include boundary currents, eddies, fronts, upwelling, wave and tidal motions, internal waves, and turbulence. They operate over spatial scales from hundreds of kilometers down to a few millimeters, and on vastly

different timescales, creating a significant challenge for any sampling scheme. In some instances, surface signatures of these processes can be sensed remotely using Earth-observing satellites; however, they often require in situ verification.

Routine CTD profiles quantify basic hydrographic variables (and derive density) to define water masses, which in turn play a major role in defining species' distribution patterns. Alternatively, autonomous ocean gliders and powered AUVs can collect background hydrographic data with minimal investment of valuable ship time, capturing spatial and temporal variation. These autonomous platforms can also carry a variety of biogeochemical, optical, and acoustic sensors (Wynn et al., 2014). Temperature and salinity sensors require regular calibration, particularly when investigating long-term environmental change for integrating into regional and global systems.

Acoustic Doppler current profilers (ADCPs) and single-point acoustic Doppler velocimeters (ADVs) are now standard instrumentation for current velocity measurements. A hull-mounted ADCP can measure near-surface currents (down to 1,000 m, depending on frequency), including while underway (noting that removal of tidal signals requires additional measurements). For deeper

measurements, an ADCP can be attached to a CTD rosette, although this method requires more complex data processing to yield absolute velocities (Visbeck, 2002).

The environment that most benthic and demersal organisms inhabit occurs

> " Although survey design and sampling equipment must be tailored to the specific objectives of any study, we suggest some key measurements and propose how to obtain such measurements in a robust, standardized, and affordable approach. "

entirely within the BBL. Here, large gradients in current velocity require higher-resolution observations in order to investigate biophysical interactions. In this environment, a bottom lander equipped with near-bottom ADVs and/or a high-frequency ADCP can measure current shear and estimate seabed shear stress accurately.

WATER AND SEDIMENT CHEMISTRY

Thorough interpretation of biological data requires a composite set of geochemical EOV data to assist the wider ocean observing community in understanding marine ecosystems more fully. The EOVs should cover both the water column and, where practical, the sediment, as fluxes of chemicals into and out of the sediments shape the biological communities in those environments (Glud, 2008). Table 1 suggests a subset of the EOVs proposed by GOOS and DOOS that can be collected from water sampled from Niskin bottles, and pore water sampled from sediment cores by rhizones. However, newly developed chemical

sensors will soon allow more frequent measurements and even continuous collection in situ. While oceanographers continue to develop a definitive guide for the collection and analysis of all EOVs, the GEOTRACES community is building upon the Joint Global Ocean Flux Study to produce a manual that covers many of the commonly measured EOVs (Cutter et al., 2017) and provides standard operating procedures for the variables highlighted in Table 1.

Sociocultural Parameters

Recognition of the social, cultural, and economic importance of the deeper ocean has recently increased, largely because of the exacerbated risks to these particularly sensitive ecosystems. These risks include both one-off human-mediated disasters (e.g., oil spills) and cumulative systemic effects of anthropogenic stressors on ecosystem services (e.g., fishing; Thurber et al., 2014). For example, deep-sea bottom-trawl fisheries cause significant and long-lasting damage to the seafloor and its associated fragile benthic communities (Clark et al., 2016), and in the tropics fishers increasingly depend on harvesting from uncharacterized MCE ecosystems (Andradi-Brown et al., 2016a). Biological parameters and environmental drivers already document many of these activities; however, some impacts of human activities require further recording/measurement. The most pressing issues include better understanding of impacts of climate change (e.g., ocean acidification), fishing pressure (e.g., spatial patterns of vessel operation), and seabed damage (e.g., fishing gear scars and energy/minerals industry footprints).

Comprehensive investigation of a region will require social scientists experienced in working with local communities and other marine stakeholders to evaluate the historical, cultural, economic, and institutional frameworks governing areas of interest. Ideally, this effort would synthesize the relevant literature and engage directly with these communities. This

engagement may create opportunities for collaboration and co-learning, such as in identification of areas for sampling, species classification, and management concerns. In order to realize these benefits of engagement, we envision a three-pronged approach: a pre-expedition scoping analysis of the relevant communities and human-ocean issues involved, a protocol for engaging with communities and local stakeholders in conjunction with the sampling expeditions, and a post-expedition follow-up for dissemination of results and opportunities for feedback, further research, and policy development.

DISCUSSION

We have here proposed a survey sampling framework by outlining key parameters that should be measured wherever possible, and listing methods and equipment to collect such data in a standardized scheme. We have avoided long and extensive lists of "nice to have" observations and prioritized "need to have" measurements instead. Such samples and data collection can be achieved as part of standard research surveys, even when they do not form key objectives for that particular survey. Given the complex selection procedure associated with a no "one size fits all" challenge, we detail some of the many caveats and limitations in Table 2. Furthermore, although the data collected as part of this protocol will not fit the needs of all researchers or research questions, the framework might provide an indication of additional parameters that could be collected, in some cases without much additional effort, to enable the increase of much-needed comparable data sets and thus more powerful ecosystem evaluations.

Some of the challenges of standardization and some guidance are offered in papers resulting from the DOOS, GOOS, and Census of Marine Life programs. Nevertheless, researchers and institutes tend to do things as they always have, resulting in sometimes significant methodological differences. While we readily acknowledge the primacy of designing

research to fit the requirements of program goals and objectives, societal needs demand large-scale regional syntheses and analyses. Such syntheses can improve understanding of underlying ecological patterns and functions to support ecosystem-based management, which is increasingly critical as human pressures on our ocean continue to increase.

Rapid development of statistical and analytical aspects of survey design and operation helps to meet the need to address scientific hypotheses on the one hand and management options on the other. Greater rigor in design of surveys and increased replication illustrate these advances. Nevertheless, limited resources for scientific research and attaining temporal and spatial coverage create a critical trade-off that remains a challenge in assessing the deep sea and MCEs. Research questions drive these options, and spatial scale represents a critical element in understanding the structure of ecosystems and how human activities might impact them. However, very few ocean research programs can afford seasonal sampling, highlighting the value of ensuring a consistent and standardized approach to survey design and sampling so that such replication over time and/or space is feasible. We have not addressed the required analysis and detailed sample processing in this summary paper because these facets depend on scientific questions and available resources. However, as taxonomic skills commonly limit studies, we propose post-cruise taxonomic workshops as an effective way to minimize this bottleneck while building capacity in the longer term.

Acknowledging the dynamic nature of scientific research, we believe the suggestions here will remain current for perhaps the next decade. Sampling requirements and protocols will invariably change over time, and aims of an operation, survey design, equipment, and analytical methods will evolve. Ultimately, society demands balancing sampling programs to meet objectives in a cost-effective way that maximizes the return on time and

TABLE 2. Details of selected limitations and caveats of the standardized GOSSIP framework.

Caveat/ Limitation	Ideal Situation	Problem 1	Problem 2	Problem 3	Possible Solution
ANALYSIS					
Taxonomy	Identify specimens to lowest level possible	Level of identification often different between gear types	Some taxa are likely to be new to science	If "morphospecies" groupings are used, then between-study comparisons are difficult, and there are challenges in combining morphological and genetic data (but see Glover et al., 2016)	Taxonomic workshop with recognized experts; produce a data paper with morphospecies detailed, if necessary
SAMPLING					
Lighting	Lighting sufficient to identify organisms	Fish avoid bright lights so biased assemblage results are likely			Test lighting options for optimum luminosity and direction, and use together with other data collection gear, if possible
Sampling Hyperbenthos	Quantitative near-seabed sample collection	Gear snags on seabed or is too far away from the seabed to collect samples	Stirs up the bottom when deployed		Use an altimeter on gear, and be prepared to try different gear options
Large Grain Size Sediment	Quantitative sediment core	In sediment of large grain size, push cores do not retain sediment	In stony areas, megacores are less likely to succeed		New coring devices to be developed
Sieve Size	Specimens collected and identified are representative of the infauna assemblage	Taxa collected result from mesh and sieve size used			Use standardized mesh and sieve sizes and record what they are; in some cases it might be necessary to use multiple sizes to ensure comparison is possible
Resolution Versus Area Mapped	Produce maps of sufficient resolution to achieve project aims and allow successful gear deployment	There is a trade-off between resolution and area if time is limited			Consider using AUVs to increase survey time; use a nested design approach
Transect Parameters	Sufficient transect length to capture full biodiversity of the depth gradient	There is a trade-off between the number of transects	The "ideal" transect length will differ depending on many environmental and biological parameters	Transect width is often governed by appropriate lighting	Current literature suggests 1 km, but this is likely to alter with sampling gear Undertake a power analysis to determine the power of the data collected
ENVIRONMENTAL					
Temporal Variability	Sampling frequency sufficient to account for diurnal, seasonal, and annual changes	Natural tidal and diurnal rhythm of biological movements (i.e., vertical migration of zooplankton)	Recruitment differs across seasons, so different measures of biodiversity are likely to be needed between seasons		Sample day and night when possible (e.g., zooplankton) Use within-site comparisons when possible and collaborate for longer-term comparisons
Moving Water Masses	Capture a full 3D picture of the water masses	One CTD cast may not be sufficient to capture water masses as they can be complex and move			Multiple CTD casts across the day (to capture tidal influence) and across a geographic area (to capture feature influence)
Habitat Heterogeneity	One taxon provides data that are representative for all	Patterns of abundance and distribution may be taxon specific	Changes in relative abundance can be important indicators, necessitating a multi-taxon approach		Standardize taxa sampled, but be aware of biases from previous literature (e.g., cold seeps)
Steep Topography	All biology and habitats observed	Selective sampling due to ability to access site with gear	ROVs optimized for uphill observation and sampling may miss observations if forced to travel downhill	Accuracy of bathymetry and backscatter can be reduced, making it harder to plan benthic sampling	Plan gear and transect direction with reference to local high-resolution multibeam sonar

expense in sampling offshore.

As a follow-up to this general framework paper, the authors intend to compile more detailed guidance and protocols for standardizing the collection of data for the key variables given here. This expanded treatment will draw on existing texts and reports, with updating based on the authors' experiences, which cover numerous multidisciplinary cruises and have led to many hundreds of papers. We hope to help support other scientists, managers, policymakers, and interested stakeholders to carry out, or at least to understand, best practice scientific techniques for generating globally comparable descriptions of mesophotic, deep-pelagic, and bathyal biological communities. ⟳

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