Recommendations for plankton measurements on the GO-SHIP program with relevance to other sea-going expeditions.

SCOR Working Group 154
Submitted to SCOR on January 23rd, 2020

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We acknowledge reviews of the published draft document by Bernadette Sloyan, Rik Wanninkhof, Ed Urban, Jason Graf, Klas Ove Moller, Peer Fietzek, Rudy Kloser, and Richard Feely. Their comments helped improve this protocol significantly. The most substantial issues addressed after these reviews are bio-acoustics and measurements contributing to constrain the biological pump associated with calcifiers.

Table of Contents

1.0 INTRODUCTION 3

1.1 MOTIVATION - WHY IS IT CRITICAL THAT WE MEASURE BIOLOGICAL VARIABLES 3
1.2 OBJECTIVES 5
1.3 ORGANIZATION OF THIS REPORT 6

2.0 SAMPLING MODES AND CONFIGURATIONS: AN OVERVIEW 7

2.1 DISCRETE WATER SAMPLING 7
2.1.1 Discrete Samples from CTD 7
2.1.2 Discrete samples from flow-through system - 7
2.2 INSTRUMENTATION SYSTEMS 8
2.2.1 Rosette instrumentation 8
2.2.2 Flow-through instrumentation: 8
2.2.3 Ship mounted instrumentation: 8

3.0. DISCRETE SAMPLE ANALYSIS 8

3.1 HPLC PIGMENTS 8
3.2 ELEMENTAL ANALYSIS 9
3.3 GENETICS 9
3.4 IMAGING AND ENUMERATION OF INDIVIDUAL ORGANISMS / PARTICLES IN COLLECTED DISCREET WATER SAMPLES 10
3.4.1 Flow cytometry 10
3.4.2 Shore-based microscopy 10

4.0 SHIP-MOUNTED SYSTEMS 10

4.1 BIO-ACOUSTIC SENSORS 10
4.1.2 Quantitative echo-sounders 11
4.1.3 Acoustic Doppler Current Profilers – ADCPs 11
4.2 PHOTOSYNTHETICALLY ACTIVE RADIATION (PAR) RADIOMETERS 12

5. MEASUREMENTS FROM ROSETTE-MOUNTED SENSORS 12

5.1 BIO-ACOUSTICS 12
5.2 UNDERWATER IMAGING 12
5.3 BIO-OPTICAL SENSORS 12
5.3.1 Beam Transmissometers 12
5.3.2 Fluorometers 13
5.3.3 Optical scattering Sensors 13

6. MEASUREMENTS FROM INLINE FLOW-THROUGH SENSORS 13

6.1 BIO-OPTICAL MEASUREMENTS: 13
6.2 IMAGING: 13
6.3 FLOW CYTOMETRY: 13

7. COST AND BENEFITS OF PROPOSED MEASUREMENTS 14

8. DATA MANAGEMENT AND REPOSITORIES 14

9. RECOMMENDED SAMPLING PLAN 17

10. DETAILED DEVELOPMENT OF IMPLEMENTATION PLAN: 18

11. REFERENCES 19

12. APPENDICES 22

12.1 APPENDIX I: CASE STUDY IN HOLISTIC SAMPLING 22

12.2 APPENDIX II: A SAMPLE OF SCIENCE QUESTIONS THESE DATA WILL HELP ANSWER: 22
12.3 APPENDIX III: RECOMMENDATION DOCUMENTS FOR THE SPECIFIC MEASUREMENTS AND SENSORS ENDORSED. 23
12.3.1 HPLC recommendations *23*
12.3.2 Elemental analysis recommendations *27*
12.3.3 Genetics recommendations *32*
12.3.4 Flow cytometry (FCM) recommendations *37*
12.3.5 Automated flow cytometery (Cytosense) recommendations *40*
12.3.6 Automated imaging flow cytometery (IFCB) recommendations *43*
12.3.7 Automated imaging of particles and zooplankton (UVP) recommendations *46*
12.3.8 Acoustic backscattering (ADCP) recommendations *50*
12.3.9 Quantitative echosounding recommendations *52*
12.3.10 Photosynthetically available radiation (PAR) recommendations *56*
12.3.11 Backscattering recommendations *58*
12.3.12 Chlorophyll fluorescence recommendations *60*
12.3.13 Beam attenuation recommendations *63*
12.3.14 Angular scattering (LISST – particle size distribution) recommendations *65*
12.3.15 Spectral attenuation and absorption (AC-s) recommendations *67*

1.0 Introduction

Tracking how ocean life is responding to increased human use and climate change will empower the global community to predict, mitigate, and manage our ocean. In this document we demonstrate the existence of mature technologies to measure 'biology' as a combination of biomass and diversity indicators across the plankton size spectrum. These are now ready to deploy within the GO-SHIP constraints.

1.1 Motivation - why is it critical that we measure biological variables

Oceanographic programs become transformative when they are integrated as systematic multidisciplinary observing programs, and enacted at global scales (Karsenti et al., 2012; Appendix I). GO-SHIP has the opportunity to lead the implementation of this long-term vision through sustained global observation of ocean physics, chemistry and biology as well as linkages among these.

The measurements of biological Essential Ocean Variables (EOVs[1]; Lindstrom et al., 2012; Miloslavich et al., 2018) to characterize life in the ocean—including its composition, abundance, and changes in distribution—are fundamental to our understanding of marine ecosystems. Biomass and diversity of bacteria, phytoplankton and zooplankton are all EOVs and Essential Biodiversity Variables (EBVs[2], Mueller-Karger et al., 2018) and phyto- and zoo-plankton are represented as a single Essential Climate Variable (ECVs[3], WMO, 2016,). The abundance of many fish species, sea birds, and marine mammals is critically linked to fluctuations in the abundance and diversity of

[1] https://www.goosocean.org/index.php?option=com_content&view=article&id=14&Itemid=114
[2] https://geobon.org/ebvs/what-are-ebvs/
[3] https://public.wmo.int/en/programmes/global-climate-observing-system/essential-climate-variables

smaller planktonic organisms. Similarly, plankton, forming the foundation of aquatic food webs, mediate the cycles of many chemical elements in the ocean that are critical for life, including iron, oxygen, nitrogen, phosphorus, and carbon. Many societally relevant ocean challenges and scientific questions at scales from local to global, and from coastal to the open ocean, remain unaddressed because of the lack of biological observations and monitoring.

In part, because the ocean is physically variable across multiple scales, oceanic plankton remain undersampled in terms of their diversity, abundance, biomass, productivity, and variability. High-resolution information (both temporal and spatial) regarding EOVs on phytoplankton and zooplankton diversity and distribution is critical to develop scientific understanding, constrain ecosystem and biogeochemical models through their initialization and validation, and use this information in practical applications of benefit to society. There is a need to establish a baseline of plankton distributions and phenology (seasonal timing in phenotype and abundance) in different regions of the ocean, given critical gaps in our understanding of mechanisms controlling phytoplankton and zooplankton. There are incompatible top-down and bottom-up arguments to describe the same phenomena (e.g., the North Atlantic spring bloom). Some of the greatest uncertainties in prediction of future climate are associated with the response of the biosphere to present and future environmental change, and the subsequent biotic interactions and responses. For a large number of questions (including the impact of primary productivity and the biological pump on atmospheric CO_2 drawdown) we do not even know the sign of the feedback.

A variety of models are used to forecast the success and recruitment of organisms such as fish, the efficiency of food webs in cycling elements, the transfer of energy across trophic levels, and understanding and forecasting of water quality and other changes that affect rates and composition of biological stocks. These models are critical tools to evaluate multi-scale processes such as the availability and quality of food material for fish and other organisms, potential expansion of hypoxic areas in the ocean, the rate of ocean acidification, the modulation of air-sea exchange of gases (e.g., oxygen, carbon dioxide), and the amount of organic matter sinking to the deep ocean, where life is critically food-limited. However, significant volumes of in-situ data are necessary to build, constrain and evaluate models.

Large investments have been made in long-term ocean measurement infrastructure (e.g., Global Ocean Observing System (GOOS) and many others, see Sloyan et al., 2019). These include the development of long-term ecological monitoring stations, coordination for repeated observations on ship lines, sustained moorings, deployment of autonomous vehicles, and various other remote data-collection technologies such as cabled observatories and satellite sensors. Many of these are deployed over large geographical domains. Important developments in technologies to measure physical and chemical EOVs (e.g., salinity, temperature, oxygen, pH, nutrients, currents) have resulted in these parameters making up the vast majority of the observations collected from sampling platforms today. This is especially important under a changing climate, where a new generation of oceanographic programs, and recent demands for societal deliverables,

drive an urgent need to provide interlinked measurements among physics, geochemistry and biology to address scientific or practical problems relevant to life in the sea including societal needs (productivity, fisheries, water quality and other environmental impacts or planning, etc.). Further biological information is critically needed.

To understand biology in the ocean, we need to move to a more holistic description of planktonic communities, resolving their biomass and diversity across their eight orders of magnitude in size, from viruses to zooplankton. With new instruments and sensors, we have the capacity to include measurements of biological EOVs, EBVs and ECVs, on existing platforms, such that such data can be collected globally, across a wide range of scales. Broadening global programs to include biological traits will fill critical gaps in our knowledge of ecosystem function and dynamics and improve our ability to forecast the response of the system for policy making and management of coastal and marine systems. This is a key recommendation for ocean observing systems in general by many scientific and applied programs (e.g., Lindstrom et al., 2012). The possibility of comprehensive *in situ* multidisciplinary measurements would support many other programs, including, for example, ocean color radiometry, other remote sensing technologies, and all global observing systems (e.g., BGC-Argo: Roemmich et al., 2019, CPR surveys: Batten et al., 2019). Such observations will assist in better characterizing and explaining synoptic changes of life in the ocean.

Here we outline a program of measurements of biological variables that provides the context to study links between ocean physics, chemistry, and, in particular, ecosystem function and health. The program proposes the implementation of readily available technologies and methods that can be deployed today at a modest cost, to complement the Global Ocean Ship-Based Hydrographic Investigation Program (GO-SHIP), which plans and executes large-scale oceanographic surveys from the coast to the interior of the ocean in different ocean basins, repeating oceanographic transects on a regular schedule.

GO-SHIP has specific existing constraints in terms of steaming speed, number of stations, water budget, depth of CTD profiling, and available channels on the CTD, which we took into account in our recommendations. For example, we do not mention rosette sensors that are not rated to 6000 m as that cannot be accommodated in GO-SHIP at this time. While our recommendations are likely to be useful for other ship-based expeditions, a wider set of measurements may be possible elsewhere and should be considered (e.g., Lombard et al., 2019).

1.2 Objectives

The purpose of this report is to provide the necessary materials to incorporate routine biologically relevant measurements on GO-SHIP, which is likely to inform other operational and routine oceanographic expedition programs. We provide an inventory of validated plankton-related measurements and commercial sensors that could be implemented / installed on board research vessels, describe the associated effort involved (personnel time, costs), and present the existing data-dissemination infrastructure where such data are currently disseminated. In addition, we provide links to model parameter(s) in ecosystem models and/or biogeochemical models that the biological measurement can

help constrain. Overall, we aim to provide justification for some of these measurements to become Level 1 (core measurements, see: https://www.go-ship.org/DatReq.html) on GO-SHIP. Specifically, we:

- Provide justification for the need to make a limited number of biologically relevant EOVS measurements.
- Provide a description of existing technology and associated protocols.
- Provide water volumes necessary for different analyses.
- Provide a detailed cost analysis including personnel time.
- Provide an implementation strategy.

We limit ourselves to technologies that are commercially available, which are documented in publications and protocols from groups other than their inventors or manufacturers. This is an important indicator for technology readiness level (TRL, https://www.nasa.gov/pdf/458490main_TRL_Definitions.pdf). There are many technologies not documented here, that are working toward a higher TRL, but have not yet achieved it. We recommend that there is a process in place to update the measurement recommended as novel sensing techniques mature.

1.3 Organization of this report

This document recognizes and assesses six categories of measurements: flow-cytometry, imaging systems, genetics, HPLC & elemental analysis, bio-optics, and bio-acoustics. For each category we provide (in appended documents), the relevant technology (e.g., instrument name/company that makes it), water analysis, associated effort, key references and existing protocols, data repositories, and identified experts.

We consider the pros and cons of two modes of sample and information collection:

- sampling and analysis of water samples, and
- collection of data with automated sensors.

Both are possible on GO-SHIP cruises in various configurations. Among the deployment strategies we consider are:

1. Analysis of water collected with CTD-rosette, hull-mounted flow-through systems, or an over-the-side bucket.
2. Ship-mounted sensors (bio-acoustics and PAR).
3. Sensors mounted on a CTD-rosette.
4. Sensors measuring properties of water pumped from ocean to the R/V (‘in-line’ or ‘flow-through’ system).

All the sensors and water sample analyses recommended here are provided in detailed appendices, including salient references and protocols. We have contributed to and borrowed from a recent review by Lombard et al. (2019), which advocated a holistic approach to plankton sampling and detailed a strategy to get there in a much more general observational context than here.

2.0 Sampling Modes and Configurations: an Overview

2.1 Discrete water sampling: Water collected from either rosette or flow-through system can be collected for analysis (primarily on shore), including high-performance liquid chromatography (HPLC), particulate organic and inorganic carbon (POC, PIC), genetic sequencing, molecular cell counts, and flow cytometry (FCM) including classic FCM for picoplankton (including picophytoplankton, viruses, bacteria) and nanoplankton (phytoplanktonic nanoeukaryotes and heterotrophic nanoflagellates). Analysis of discrete samples requires dedicated personnel to execute on-board filtration of (sometimes significant volumes of) sea water (250 mL to 4 L, depending on the analysis).

2.1.1 Discrete Samples from CTD - Where possible, the use of the GO-SHIP dedicated rosette and standard protocol would require no additional wire time or platform configuration. However, the volume of water available may be insufficient for many of the analyses of interest. Another possibility, if time permits, is to use the GO-SHIP dedicated rosette for an additional shallow cast (e.g., 0 – 500 m or 1000 m depth), providing sufficient water for the measurements suggested here. The additional station time (> 1 hr per station) may prove challenging to schedule. A solution could be to have a biology-dedicated rosette on board for use to 500 m or 1000 m depth to save time between casts which may require a second winch, not available on all vessels.

2.1.2 Discrete samples from flow-through system - Samples from a clean seawater system (see below) can be analyzed for the same properties as those from the rosette. This method, while limited to sampling waters from a water intake near the ocean surface, has the advantage of providing as much water as needed and sampling while underway to cover larger distances, potentially at high spatial resolution. Because it allows quasi-continuous sampling, this approach is particularly useful in resolving scales of spatial and temporal variability near the ocean surface.

To sample plankton-relevant EOVs, these systems need to:

1. Be cleaned before every transect (requires flushing bleach through the system).
2. Use diaphragm or peristaltic pump rather than impeller pumps as the latter break up cells (Cetinić et al., 2016).
3. Minimize residence time within the system (e.g., by sampling near intake) to < five min.
4. Flow rate should be on the order of at least 5L/min (note that if other instrumentation exist on the flow-through system (e.g. for dissolved gasses) they should be taken into account in flow calculations).
5. For each measurement conducted, a few samples from the flow-through system should be compared with samples from the rosette surface bottle to evaluate possible contamination and/or bias.

2.2 Instrumentation systems

2.2.1 Rosette instrumentation. Instrumentation on the rosette can provide proxy measures for phytoplankton concentration (e.g., chlorophyll fluorescence (F_chl) or fine particle density (transmissometer (660nm), backscattering), zooplankton and particle biomass proxy and vertical flux estimates, as well as zooplankton diversity (images via the Underwater Vision Profiler 5 (UVP5-HD)).

2.2.2 Flow-through instrumentation: Nano and micro phytoplankton and microzoopankton biomass and diversity can be estimated using an Imaging FlowCytobot (IFCB). The IFCB provides highly resolved taxonomic information of phytoplankton in the 6 -130 μm range. Pico- and nanophytoplankton abundances can be estimated using a Cytosense automated flow cytometer. It resolves the conventional clusters determined by flow cytometry (*Prochlorococcus*, *Synechococcus*, pico- and nano-eukaryotes and cryptophytes). The image-in-flow device offers a plus to identify some of the particles analyzed. Proxies of total phytoplankton biomass include F_chl or an inline spectrophotometer [AC-S (filtered/unfiltered mode)]. The later also provide a proxy for 6 pigment groups, POC and a size index.

2.2.3 Ship mounted instrumentation:

2.2.3.1. Downwelling irradiance in air is measured with a Photosynthetically Available Radiation (PAR) radiometer, and

2.2.3.2. Bio-acoustic sensors on the hull, an ADCP (required measurement on GO-SHIP) and a wide band/multi frequency quantitative eco-sounder (calibrated) which provide a measure of zooplankton and fish vertical distributions, semi-quantitatively. Issues of potential interference between the eco-sounder and ADCP will need to be resolved prior to using the eco-sounder.

3.0. Discrete sample analysis

The ultimate aim of water sample analyses of plankton EOVs is an assessment of holistic plankton ecosystem structure and function. Quantitative estimates of plankton biomass and community composition, picoplankton and heterotrophic bacteria, picoeukaryotes, as well as their genetic composition and metabolic function, all are fundamental to characterize life in the ocean. All of the analyses described below have recommendations documented in the appendix III. Discrete sampling as described below is limited by the volume sampled to relatively small organisms, biased towards micro-plankton and smaller.

All sensors on Rosette and flow-through system that are used to derive biogeochemical proxies require the sampling of water (e.g., for POC and pigments) to help evaluate uncertainties and relationships between sensor measurement and biological proxies. Similarly, if net tows are possible, acoustic proxies of zooplankton can be validated.

3.1 HPLC pigments

Information regarding phytoplankton diversity can be gained from High Performance Liquid Chromatography (HPLC) analysis of pigments present in bulk samples. Chlorophyll *a* is typically used as a proxy of phytoplankton biomass, since it is present in

all phytoplankton (although in its *divinyl* form in prochlorophytes). Accessory pigments vary with phytoplankton community composition, and some pigments can be used as biomarkers of specific taxa, however, chlorophyll per cell or per carbon varies with phytoplankton taxa and physiology, and the indices must be used with caution. Several pigment-based approaches have been proposed that allow estimation of the relative contribution to chlorophyll *a* of different phytoplankton taxa (CHEMTAX algorithm Mackey et al., 1996) or taxonomic groupings or size classes (Uitz et al., 2006 and references therein). Pigment-based methods have the advantage that they cover the whole phytoplankton assemblage in a single analysis and provide a quantitative assessment of phytoplankton community composition at the level of class or higher (Bax et al., 2001). By combined validation with flow cytometry and microscopy the uncertainties of these methods that are linked to variability in accessory pigmentation within a given taxon or induced by environmental factors can be reduced.

3.2 Elemental analysis

Total suspended biomass in the upper open ocean is dominated by plankton-derived particles. The analysis of organic carbon, phosphorous, nitrogen and inorganic carbon associated with bulk particulate samples retained on a filter provides essential descriptors of the dynamics of such particles. The associated methods have been tested and refined for decades (e.g., Hurd and Spencer, 1991; Cutter et al., 2017).

3.3 Genetics

Analyzing the genetic sequences contained within filtered samples has revolutionized our understanding of planktonic diversity and function (see review by Pedrós-Alió et al., 2018). High throughput sequencing (HTS) provides relatively cost-effective and fast sequencing of DNA and RNA and more genetic information is being extracted as techniques evolve and mature. Sequencing of DNA (the genes of marine organisms) gives information on which organisms are present in a sample (Rusch et al., 2007). RNA (the transcripts of genes, produced when a specific gene is active) can give information on the activity of key processes (e.g., nutrient uptake) (Carradec et al., 2018).

“Barcoding” targets specific sequences that are common across a very wide range of plankton taxa, identifying a specific organism through minute genetic differences in the common sequence. Initially developed for individual bacteria, it has been progressively applied to the whole marine ecosystems, encompassing plankton, nekton and even benthic organisms. “Metabarcoding” is the application of species identification through barcoding at the scale of a whole filtered water sample (Bucklin et al., 2016). “Metagenomics” similarly allows the study of all genes present on a given filter to infer the metabolic and functional capacities of microbial communities (Rusch et al., 2007; Sunagawa et al., 2015), with the possibility to extract the taxonomic (16S/18S rRNA gene fragments — what it is referred to as 16S/18S mTags; Logares et al., 2014) or functional genes of interest (Farrant et al, 2016) and giving access to the reconstruction of full genomes of thousands of microorganisms (e.g., Delmont et al., 2018; Tully et al., 2018). "Environmental" DNA (eDNA) analyses are executed on DNA released by metazoan animals into seawater. New techniques allow estimation of the eDNA diversity within samples with unprecedented taxonomic resolution, and as they become relatively

affordable and automated, eDNA has the potential to revolutionize diversity analysis in global surveys for marine animals (Deiner et al., 2017; Ortega et al., 2019).

Because all these techniques are evolving very quickly, extra samples are often stored on filters at -80°C for future analysis. Preservation of samples is thus critical to enable application of future techniques on stored samples (Pesant et al., 2015).

Finally, genetic sequences only yield their valuable information through statistical comparison with known sequences, a process known as bioinformatics. The development of bioinformatics techniques is complex and ongoing, and should be integrated with preexisting knowledge. Full integration of genetic information with the assessment of whole organisms, through techniques such as isolation and imaging, will help avoid the biases of pure sequencing and in-silico approaches (Pedrós-Alió et al., 2018).

3.4 Imaging and enumeration of individual organisms / particles in collected discreet water samples

3.4.1 Flow cytometry

Flow cytometry provides a means to enumerate and characterize fluorescence and light-scattering properties of microbes in suspension in a liquid sample. Individual particles are measured in flow as they pass through a focused light source (usually one or several laser beams) and combinations of fluorescence and scattering signals can be analyzed to distinguish Prochlorococcus, Synechococcus, eukaryotic picophytoplankton, and nanophytoplankton. With the addition of a nucleic acid stain to samples at the time of analysis, it is also possible routinely to enumerate heterotrophic prokaryotes. More specialized analyses with stains can also be used for viruses and microzooplankton, or cell viability.

3.4.2 Shore-based microscopy

If net tows are possible, as has been done on some GO-SHIP cruises, samples from these should be subsampled for microscopy. These can then be used for comparison with UVP (which suffers from avoidance by strong swimmers as well as bio-acoustical data (e.g. choice of acoustic model based on prevalent organisms).

4.0 Ship-mounted systems

4.1 Bio-acoustic Sensors

Acoustic methods can reveal much about the spatial distribution and temporal dynamics of zooplankton. For example, echosounders led to the discoveries of the diel vertical migration of plankton and micronekton (Johnson, 1948) and their ubiquitous and dense, but previously hidden, aggregations (Cheriton et al., 2007). The ability of acoustic tools to simultaneously assess animals ranging in size from sub-millimeter to meter scale allows ecological processes in the plankton to be examined when appropriate frequencies are chosen. However, this ability also highlights a key challenge—separating animal types and accurately assessing the biomass of each. While these approaches have long been used for fish stock assessment and management of many species (MacLennan and Simmonds, 1992), dramatic differences in plankton body size, species composition,

elastic properties of the animals and orientation markedly influence the acoustic reflectivity or target strength, coupled with the complexity of the community, make separation of taxa and assessment of biomass difficult. Acoustic measurements have inherent uncertainties.

Many of the greatest insights on zooplankton resulted from creative integration of multiple, complementary sampling devices including acoustics with nets, optics, imaging and animal tagging to take advantage of the different strengths and fill in the gaps of each approach (reviewed in Benoit-Bird and Lawson, 2016). Multi-sensor fusion efforts have the potential for wider application through the use of autonomous platforms, which resolves the limited range issue of high-frequency acoustics.

Hull mounted bio-acoustic sensors come in two types:

4.1.2 Quantitative echo-sounders

Multi-frequency quantitative echo-sounders are calibrated sensors designed to ensonify a significant part of the water column to obtain quantitative information about the organisms through acoustic backscatter. The more independent information about the organisms in the water (e.g., from imaging systems and nets), the better the inversion can be carried out to estimate biomass. Many academic R/Vs already have such systems installed on them. Interference with ADCPs is an issue for these instruments and efforts (tuning) should be applied to avoid such interference, and in some cases it will require turning them off (as ADCPs are core GO-SHIP measurements). In the past on some GO SHIP voyages all other acoustic systems were turned off not it interfere with the ADCP. This represents a great loss of data that is becoming increasingly important in understanding the biological changes in the ocean. Acoustic systems work at many frequencies and cross talk can come about due to either a frequency or timing issue. Modern real time and post processing data processing can remove known artefacts (transmit spikes) even when produced at the same frequency and produce functional data. Therefor with appropriate tests and tools many acoustic systems can operate independently without functionally compromising the ADCP current measurement. In addition, recently some echosounders have been able to also measure ADCP like signals (https://www.kongsberg.com/maritime/products/mapping-systems/fishery-research/scientific-echo-sounders/ec150-3c?OpenDocument) making it possible to avoid interference issues.

4.1.3 Acoustic Doppler Current Profilers – ADCPs

Single-frequency ADCPs are primarily designed to obtain water velocity via the Doppler shift of sound scattered off particles in the water column. Considering the theoretical energy loss along the acoustic beam, and characteristics of the individual ADCP devices, the volume backscatter (S_v) in decibels (dB) can be estimated from the backscatter intensity records. Although S_v signals are not exclusively linked to plankton further analyses (e.g., the diurnal variation in vertical maxima in S_v) have been very useful in investigating plankton migration. Through calibration of S_v with in-situ plankton observations (e.g., imaging), ADCPs can be used to obtain quantitative estimates of biomass and be related across vessels and over time. ADCPs are mandated on all GO-

SHIP cruises. Data can be recorded with seconds temporal resolution of seconds and covering a depth range from near the surface to more than 1500 m depth and are available along the entire cruise track.

4.2 Photosynthetically Active Radiation (PAR) Radiometers

Sensors measuring the downwelling photosynthetically active radiation (PAR), are cheap, robust, require minimal cleaning and have been used regularly as part of a weather station on top of vessels. This measurement is a critical input to phytoplankton productivity and photo-physiology.

5. Measurements from Rosette-mounted sensors

All GO-SHIP cruises use CTD + rosettes to collect water samples. There are a variety of sensors that could be accommodated on rosettes that could be used to study plankton described below. However, given limitation on number of available ports on the CTD rosette, it is critical to assess how many can be supported in each case and whether self-logging of data is optional (and include the data download time in the work flow)

5.1 Bio-acoustics

ADCPs are deployed on rosettes for in-situ estimates of velocity and turbulence using, typically, higher-frequency systems than hull-mounted ones. As discussed above, the strength of the returned signal and the mean vertical velocities are diagnostic for biomass and swimming patterns of plankton. Quantitative echosounder, use multi frequency or wide band acoustics to constrain the distributions of zooplankton and fish.

5.2 Underwater Imaging

The Underwater Vision Profiler 5HD (UVP, Picheral et al., 2010) operates a 4 MPix camera imaging a field of view of approximately 1 liter of water. The UVP size spectrum includes marine snow aggregates > 100µm and images plankton > 500 µm. It is integrated on a CTD-Rosette system as a standard sensor delivering images indexed to the different environmental data collected at a rate of 20 images s^{-1}. The UVP is self-powered, with rechargeable batteries, logs data internally, and provides real time output (if a CTD port is available) proportional to the total particle concentration.

5.3 Bio-optical sensors

Like acoustic sensors, optical measurements are best used with complementary sampling approaches of biological EOVs. Measurements of the optical characteristics of water in situ have been used for decades (e.g., Gardner et al., 2018) to characterize bulk properties associated with micrometer-size particles in general and phytoplankton in particular (near-forward scattering extends this range to a few 100s µm).

5.3.1 Beam Transmissometers

Measurements of beam transmission near 660 nm have been conducted with commercial sensors since the 1980s to provide a rapid assessment of water quality and the amount of particles in the water column. The measurements are simple to perform but may require significant effort in ocean waters where the concentration of particles is very low (these

waters are often used to provide a blank for the instrument (e.g., Gardner et al., 2006). Transmissometer measurements can provide a proxy for POC.

5.3.2 Fluorometers

Single and multiple excitation-emission fluorometers can provide information on other pigments beyond chlorophyll a (Proctor and Roesler, 2010). Some studies have been used to provide estimates of phytoplankton functional groups. These measurements are best combined with biological EOVs that provide biomass and diversity observations, as fluorescence observations are hard to interpret quantitatively because of a number of factors, including the physiology and diversity of the phytoplankton community.

5.3.3 Optical scattering Sensors

Backscattering sensors have been used since the 1990s as proxies of particulate materials. They can provide estimates of particulate concentration at great depth (e.g., Poteau et al., 2017). If measured at multiple wavelengths, not affected by particulate absorption, they can provide a size proxy for micron-sized particles (e.g., Slade and Boss, 2015).

These techniques are useful as proxies of particulate organic carbon in general (Cetenić et al., 2012) and phytoplankton carbon in particular (Graff et al., 2015).

6. Measurements from inline flow-through sensors

6.1 Bio-optical measurements: All the technologies mentioned in section 5.3 above (fluorometers, backscatter sensors and transmissometers, as well as hyperspectral spectrophotometers) are readily used with most inline flow-through seawater systems. If the flow-through systems and sensors are well-maintained (see section 2.1.2), they can provide high-resolution measurements of relevant surface properties. Bio-optical measurements require discrete observations of abundance (e.g. elemental analysis, pigments) to develop proxy relationships (and check that published ones are applicable locally). Additional measurements of diversity and productivity of groups of organisms can significantly enhance their utility..

6.2 Imaging:

The Imaging Flow Cytobot (IFCB) is an integrated flow cell and imaging system that provides clear, in-focus images of eukaryotes and larger organic particles as they are pumped past the sensor (Sosik and Olson, 2007). IFCB images give biodiversity information as well as organism-specific size, shape, fluorescence and scattering, and this instruments are usually configured to sample automatically 5 ml every 25 minutes from the uncontaminated seawater flow. This sensor has been routinely integrated to flow-through systems, often with no dedicated technician on board.

6.3 Flow Cytometry:

The Cytosense (Cytobuoy) is an automated flow cytometer specially designed to analyze aquatic microbes from ~0.1 µm to 4 mm in length and up to 1.3 mm in width (Dubelaar et al., 1989). It can record forward and sideward scatter light intensities, and several wavelengths of fluorescence, as well as detecting curvature and polarized light. The

Cytosense records the entire optical profiles as the particles flow through the laser beam, generating an optical fingerprint of the particles (from cells to colonies). An image-in-flow device also provides the ability to take pictures of some targeted cells. The Cytosense does not require any pre-filtration. The Cytometer is fully automated and designed to perform sampling and analysis several times per hour (typically every 20 to 30 min).

7. Cost and benefits of proposed measurements

In Table 1 we summarize the salient logistic considerations and resource requirements associated with the measurements we advocate for GO-SHIP cruises. All the measurements proposed here have a history of being done on research vessels and several have already been deployed on GO-SHIP cruises. More detailed and specific information regarding each can be found in the recommendation documents in Appendix II. A holistic sampling effort would ideally include all the measurements in the list. However, each cruise will have different logistic constraints, so we include a consideration of the key constraints below.

The assignment of "information content" is intended to reflect the breadth of biological information provided by a single measurement. For example, POC or 1-channel optics give information on concentration alone, while genetics and imaging yield information about diversity and community composition.

8. Data management and repositories

There is a need to have a carful data management plan that takes into account:

1. Use FAIR practices (data needs to be findable, accessible, interoperable and reusable). Hence appropriate metadata should be created, community standards are followed and data is easily available.
2. Current practices used by the wider international community.
3. Easy way to link between data that is currently deposited in a different databases.
4. Longevity of database – choose to have the data in databases that are likely to be arounds for years to come.
5. Curated samples should be kept in appropriate facilty and access to them should follow agreed upon protocols.

It is recommended that the Bio-GO-SHIP community identify the data products and repositories which will be hosting which type of data (ie, flow cytometry, eDNA, etc) to assist groups like the GOOS Observations Coordination Group develop data flow mapping as well as advertise data and metadata availability.

Table 1: Information content generated under GO-SHIP sampling configuration, price, sampling mode (and hence density of sampling) and spatial scale (vertical or horizontal depending on sampling mode), average time for sample collection effort, and post-cruise data processing (not analysis), and curation effort. All personnel needs some training which is more extensive if it is desired that instrument be repaired at sea. Instrumentation was selected based on minimal need for intervention.

Sample	**Information content**	**Approximate Price per sample**	**Water source (R- rosette, I-inline, H-hull) and sample size**	**Spatial scale Verical/horizontal**	**Sampling effort (technician time)**	**QA/QC + submission to database**
POC	low	$20	R/I 1 L	Bottles / 100 km	2 hr for a full rosette	One day per cruise.
PIC	low	$20	R/I 1 L	Bottles / 100 km	2 hr for a full rosette	One day per cruise.
HPLC	medium	$80	R/I 1 L	Bottles / 100 km	2 hr for a full rosette	One day per cruise.
FCM	medium	$20	R/I 10 mL	Bottles / 100 km	0.2 hr for a full rosette	One day per cruise.
Genetics	high	$100	R/I 4-10 L	Bottles / 100 km	2 hr for a full rosette	
Sensor		**Sensor Price**	**Deployed on**		**Sampling effort (technician time)**	**Analysis effort**
Single channel optics	low	~4K per channel	R/I	2m/ 300m	5 min per day	2 days per cruise
ADCP	low	Already on GO-SHIP	H/R	10m/ 10m	5 min per day	1 week per cruise
Hyper-spectral optics	medium	~40K	I	300m horizontal scale	2-30min per day	2 days per cruise

Quantitative eco sounder	medium	~400K – already on some GO-SHIP	H	0.3m vertical bin for hull mounted	5 min per day	1 week per cruise
LISST	medium	~35K	I	300m	2-30min per day	2 days per cruise
FCM(cytosense)	medium	~130K	I	10km	30min battery charging and data download	Size distribution - 2 days per cruise. Images – currently 2 months.
Imaging (UVP)	high	~130K	R	0.1m	2-30min per day	Size distribution - 2 days per cruise. Images – currently 2 months.
Imaging (IFCB)	high	~130K	I	10km	5 min per day	Size distribution - 2 days per cruise. Images – currently 2 months.

9. Recommended Sampling Plan

Note, the measurements below have not been ordered based on priority in recognition that priority will depend on available water, available instrumentation, shore-based infrastructure and resources. Table 1 could be used as a guide for prioritizing.

1. **Scenario 1 – Lowest impact on GO-SHIP operations** (i.e., no extra time needed)
   Two technicians (instrument operations and maintenance and water sampling)
   a. Flow-through (same for all scenarios)
      i. Instrumentation
         1. IFCB
         2. LISST
         3. ACS Filtered/Unfiltered
         4. Cytosense

      ii. Water – replication for technical replicates quality control 1/day.
         1. HPLC
         2. Genetics
         3. Elemental analysis
         4. FCM
   b. Rosette
      i. Instrumentation
         1. Transmission (1 CTD port that can be shared with Y cable with the Fchl-backscatter sensor to power sensor and obtain data).
         2. Combination Chl Fluorometer and backscatter sensor
         3. UVP (It is recommended (but not necessary) that an additional data channel to monitor the UVP is working well).
         4. ADCP
      ii. Rosette – residual water as available
         1. FCM
         2. HPLC
         3. Genetics
         4. Elemental analysis
   c. Ship-board sensors
      i. ADCP
      ii. PAR
      iii. Quantitative Echosounder (if no ADCP interference)

2. **Scenario 2 – Medium impact on GO-SHIP** (1.5-2.5 h extra time needed)
   Same as above + biology-dedicated cast to 1500 m – Minimum 6 depths including within mixed layer, chlorophyll maximum, particle max (~10 m below chl max or 200 m), 500m, 1000 m, and 1500 m.
      1. FCM
      2. HPLC (to 500 m max)

    3. Genetics
    4. POC
3. **Scenario 3 – Scenario 2 + Biologically dedicated Rosette** for greater efficiency and less time impact on GO-SHIP operations.

4. **Scenario 4 – Scenario 2 or 3 to 6000 m or bottom** if shallower, with Bathypelagic sampling including near-bottom. 10 samples.
    a. FCM at all depths (10 mL)
    b. HPLC at 6 shallower depths (as determined in Scenario 2)
    c. POC at all depths (2 – 4 L)
    d. Genetics at all depths (2 – 10L)

Scenarios 2-4 could take place at every GO-SHIP station or at regular interval. Additionally, as was done on some GO-SHIP cruises, Bongo net tows could take place to provide samples for visual and genetic information on higher trophic level and to calibrate the bio-acoustics.

10. Detailed development of Implementation Plan:

"samples and sensors – a marriage made in P-OBS"

- Include biological expertise on the GO-SHIP Committees (Global and National), including advisory groups to answer pressing questions.
- Link to and communicate with potential local biological user groups to obtain buy-in, interest and build capacity.
- Sensors that are not part of routine sampling should be housed at a local experts lab when not on GO-SHIP cruise so they are used, serviced, and ready to be deployed on upcoming GO-SHIP cruises.
- Curation and taxonomic expertise should be assembled and consulted to insure data is fully utilized.
- Stepwise implementation – start with a limited amount of instruments, cruises and samples and increase as skill improve – ensures data collected are high quality, relevant best-practice protocols are in place and that data users are invested.
- Data management infrastructure is critically important. Data archives exist for all the measurements proposed but in several different repositories. Make sure all are linked and appropriate terms and units are used in all and that procedures have been reviewed by data specialists.

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12. Appendices

12.1 Appendix I: Case Study in Holistic Sampling

As a case study, we present the holistic sampling (Karsenti et al., 2012) during the TARA Ocean expedition. Most of the technologies and sampling we are advocating here have been deployed during the 3 years of the Tara Ocean Expedition (including many additional measurements, Pesant et al., 2015). Sampling was done using a CTD rosette as well as a flow-through system both of which included bio-optical sensors and imaging systems as well as a suite of physical and chemical sensors). What makes it a good case study is that all of the instruments and sampling were done by 5-6 scientists/technician following the same protocols for month-long legs on a 36m schooner turned R/V (doing much of the usually shore-based analysis on-board). Given that research vessels are much larger and can carry many more personnel, the Tara experience provides a realistic bound for the total personnel needed as well as costs. In terms of impact and science produced the yield per $ invested has been very high (see https://www.embl.de/tara/tara-oceans-science/publications/).

12.2 Appendix II: A sample of science questions these data will help answer:

- How does biological diversity, composition and biomass vary along sampling lines in relation to environmental parameters?
- How much biological variability is there as a function of time and space exists in the bathy- and meso-pelagic region and how coupled is it to the surface ecology?
- To what degree can community composition be predicted from environmental forcing?
- To what degree do prey-predator, parasitism, mutualism and other ecological processes determine community composition?
- How do the processes of ocean deoxygenation, acidification and warming restructure the composition and diversity of organisms in the ocean and their function?

12.3 Appendix III: Recommendation documents for the specific measurements and sensors endorsed.

12.3.1 HPLC recommendations

HPLC recommendations for plankton-related measurements onboard research vessels and the GO-SHIP program. Contribution from the HPLC/POC subgroup.

Recommendations for measurements appropriate for GO-SHIP based on the following criteria

Application to key science needs and questions to support the measurement of biological EOVs
Measurements of HPLC-determined pigments provide information on phytoplankton biomass and diversity, both recognized as Essential Ocean Variables (EOVs). They also represent fundamental reference measurements required for the calibration of data derived from optical sensors (chlorophyll fluorometer) and for the validation of Ocean Color Radiometry products.
Broad accessibility of the infrastructure, ease of use and availability of necessary resources
No specific instrumentation is required for HPLC pigment measurements. Seawater samples are collected onboard and shipped to on-land dedicated facilities for analyses in the laboratory.
Minimal interruption to current GO-SHIP standard operations
If water is available, only the seawater collection from the CTD-rosette (< 30 min for 12 bottles). HPLC pigments is GO-SHIP Level-3 data.

For HPLC pigment water analysis

Sample name
HPLC pigments: The concentration of phytoplankton pigments determined from High Performance Liquid Chromatography (HPLC) analysis
Amount of water per whole-water samples or amount filtered per sample
The volume of seawater to be collected varies with expected particle load, i.e., 1–4L for HPLC pigments.
Type of sampling (whether sample could be taken from in-line water flow and/or rosette. For the latter, depths of interest)
Seawater sampling can be performed either from a surface flow-through system or from a CTD-rosette device equipped with Niskin bottles, the latter enabling the acquisition of depth-resolved profiles. When the CTD-rosette device is used, seawater samples are typically collected at 10-12 preselected depths, located between the surface to 200 m.

Price per sample
$50-150 for HPLC analysis (does not include supply costs)
Price per lab instrument
~$55 k
Maintenance cost per lab instrument
~$8.5-11.5k / year
Personnel time to run a sample
Seawater filtration is not highly technical but requires constant care and surveillance. In particular, the water collection under bloom situations should be performed quickly or ideally through sub-sampling (after agitation) from a large carboy filled from a whole CTD-bottle. Depending on sample particle load, filtration may last 1 to 2 hours.
Necessary infrastructure onboard (e.g., freezers, liquid N2, Filtration rack, filters, Milli-Q system, type of pump for in-line) and relevant cost (if not standard onboard equipment)
Sampling for HPLC pigments requires: -PET bottles for seawater sampling, e.g., Nalgene $30/bottle -A filtration rack equipped with polysulfone or polycarbonate funnels, e.g., Pall Science Lab ~$220 / funnel -A water-jet pump for gentle, low-vacuum filtration of seawater samples (e.g., Water Jet Aspirator pump from Cole Parmer ~$2000) -Whatman 25-mm GF/F glass-fiber filters (pore size 0.7 µm) - $0.85 / filter -Nunc cryogenic tubes - $0.75 / tube, or tissue capsules such as the BioPlas HistoPrep - €0.75 / capsule, or aluminum foils for storing frozen samples -A tank (e.g., CryoLab aluminum 50L Dewar $1450) filled with liquid nitrogen for flash-freezing of HPLC pigment samples -A 80°C-freezer for storage until shipping; samples can also be stored in liquid nitrogen if enough is available onboard
Shipping needs (e.g., temperature samples need to be maintained at and facilities available for sample analysis)
Frozen HPLC pigment samples are shipped in dry shippers (~1300$ / dry shipper) pre-filled with liquid nitrogen back to the facility for analysis. There are several national analytical HPLC analysis laboratories where samples may be shipped, e.g SAPIGH platform at the Institut de la Mer, Villefranche-sur-Mer France; NASA facility at Ocean Ecology Laboratory at NASA Goddard Space Flight Center, Greenbelt, Maryland, USA; DHI Institute for Water and Environment, Denmark; The Australian Commonwealth Scientific and Industrial Research Organisation, CSIRO. The cost of HPLC analysis per sample varies greatly depending on the chosen facility, from $50 to $150. HPLC necessitates an experienced operator for laboratory analysis and chromatogram interpretation.
Relevant/necessary ancillary measurements (beyond GPS):
Temperature Salinity

Conductivity Radiometer data Nutrients DOC / POC / CDOM Bio-optics Flow cytometry Imaging (IFCB, UVP)
Ecosystem/biogeochemical model parameter constrained by this measurement
HPLC analysis permits the identification and quantification of phytoplankton pigments, i.e., chlorophyll *a* and accessory pigments. Chlorophyll *a* is the main pigment involved in photosynthesis and is found in all phototrophic organisms. The concentration of chlorophyll *a* (Chla) is thus the most widely used proxy for phytoplankton biomass. In contrast, accessory pigments yield quantitative information on the composition phytoplankton communities over the whole size range they cover. Chla is the key parameter used as input to biogeochemical and ocean-color based bio-optical models to produce large-scale estimates of phytoplankton biomass and primary production. These may be combined with pigment-based community composition information to provide phytoplankton group-specific biomass or production estimates. Units: mgChl_a m^-3, mg Pigment_x m^-3 Constrains phytoplankton concentration, phytoplankton size classes, phytoplankton functional types, together with other measurements (light, MLD, temperature) constrains primary production. Together with POC, constrains chl_a/Phyto_C ratio and growth rate.
Existing protocols and relevant publications
Bidigare et al. (2003) present a very detailed sampling and analysis protocol that is consistent with the SCOR recommendations. The recommended analytical protocol is Wright et al. (1991) or, alternatively, Goericke and Repeta (1993) or Van Heukelem and Thomas (2001). The latter has been optimized by Ras et al. (2008) to increase the sensitivity in the analysis of ultra-oligotrophic waters. <u>References:</u> Bidigare R. R., L. Van Heukelem and C. C. Trees, 2003. HPLC phytoplankton pigments: sampling, laboratory methods, and quality assurance procedures, Chap 2, In: J L Mueller and G S Fargion and C R McClain (Eds), *Ocean optics protocols for satellite ocean color sensor validation, Rev 5, Vol 5: Biogeochemical and bio-optical measurements and data analysis protocols*. NASA Goddard Space Flight Center, Greenbelt, MD, pp. 5-14. Goericke R., Repeta D. J.,1993. Chlorophyll-a and Chlorophyll-B and Divinyl Chlorophyll-a and Chlorophyll-B in the Open Subtropical North-Atlantic Ocean. *Marine Ecology-Progress Series*, 101, 307-313.

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Obvious synergies with current measurements performed on board GO-SHIP cruises and other measurements proposed here

GO-SHIP data on physical forcing and nutrient stocks would help in interpretation of environmental conditions prevailing in the establishment of phytoplankton communities.

Standardized vocabulary for the data and the associated metadata, for managing the large and diverse data sets collected around the world (oceanographic fleets, automated observation systems deployed in situ), and available among various data centers aiming at preserving and making reusable marine observations (from physics to chemistry and biology)

An example of standardized vocabulary from SeaDataNet:
http://seadatanet.maris2.nl/v_bodc_vocab_v2/search.asp?lib=p01&screen=0

Label: chl-a_water>GF/F_HPLC
Preferred label: Concentration of chlorophyll-a {chl-a CAS 479-61-8} per unit volume of the water body [particulate >GF/F phase] by filtration, **acetone** extraction and high performance liquid chromatography (HPLC)
Definition: The amount (mass or moles) of the specified pigment determined by HPLC assay of a sample collected by dissolution in **acetone** of the residue collected by GF/F filtration of a known volume of any water body. The quoted value either results from a single determination or the average of replicate determinations.

Label: chl-a_water>GF/F_HPLC**meth**
Preferred label: Concentration of chlorophyll-a {chl-a CAS 479-61-8} per unit volume of the water body [particulate >GF/F phase] by filtration, **methanol** extraction and high performance liquid chromatography (HPLC)
Definition: The amount (mass or moles) of the specified pigment determined by HPLC assay of a sample collected by dissolution in **methanol** of the residue collected by GF/F filtration of a known volume of any water body.

Data dissemination infrastructure and likely data latency. If further shore-based analysis is necessary (e.g., image classification and validation), the likely personnel time associated

An example for HPLC pigment data dissemination is the MAREDAT database, published in Earth System Science Data (ESSD) (Peloquin et al. 2013a) and archived (+ DPI) at PANGAEA (Peloquin et al. 2013b). HPLC pigment data from one of the GO-SHIP voyages have been archived on PANGAEA (Raes et al. 2017, https://doi.org/10.1594/PANGAEA.884052). Another option is publishing a reference (data description) paper in ESSD (Organelli et al. 2017) and archiving data in SEANOE, as was recently done for the first global BGC-Argo dataset (Organelli et al. 2016, https://www.seanoe.org/data/00360/47142/). References: Organelli E., Barbieux M., Claustre H., Schmechtig C., Poteau A., Bricaud A., Boss E., Briggs N., Dall'Olmo G., D'Ortenzio F., Leymarie E., Mangin A., Obolensky G., Penkerc'h C., Prieur L., Roesler C., Serra R., Uitz J., Xing X., 2017. Two databases derived from BGC-Argo float measurements for biogeochemical and bio-optical applications at the global scale. *Earth System Science Data,* doi:10.5194/essd-9-861-2017 Organelli E., Barbieux M., Claustre H., Schmechtig C., Poteau A., Bricaud A., Uitz J., D'ortenzio F., Dall'olmo G., 2016. A global bio-optical database derived from Biogeochemical Argo float measurements within the layer of interest for field and remote ocean color applications. *SEANOE*, doi:10.17882/47142. Peloquin J. et al., 2013a. The MAREDAT global database of high performance liquid chromatography marine pigment measurements. *Earth System Science Data*, doi:10.5194/essd-5-109-2013. Peloquin J. et al., 2013b. The MAREDAT global database of high performance liquid chromatography marine pigment measurements - Gridded data product (NetCDF) - Contribution to the MAREDAT World Ocean Atlas of Plankton Functional Types. PANGAEA, doi:10.1594/PANGAEA.793246. Raes E. J., Clementson L., Bodrossy L., Strutton P., Waite A., 2017. Pigment and primary productivity on the P15S GO-SHIP transect: From the ice edge to the equator along 170°W. *PANGAEA*, doi:10.1594/PANGAEA.884052.
A list of relevant experts who can be called upon to help and know they have been identified
For onboard work: Joséphine Ras (jras@obs-vlfr.fr) Céline Dimier (celine.dimier@obs-vlfr.fr)

12.3.2 Elemental analysis recommendations

Elemental analysis recommendations for plankton-related measurements for samples collected onboard research vessels and the GO-SHIP program. Contribution from the HPLC/POC subgroup.

Recommendations for measurements appropriate for GO-SHIP based on the following criteria

Application to key science needs and questions to support the measurement of biological EOVs
Measurements of POC provide information on total particulate organic carbon and constrain phytoplankton carbon biomass, both of which are Essential Ocean Variables (EOVs). They also represent fundamental reference measurements required for the calibration of data derived from optical sensors (backscattering, transmissometer) and for the validation of Ocean Color Radiometry products. PIC allows quantifying the associated inorganic carbon biomass of calcifying organisms that are key species in ballasting and exporting organic material.
Broad accessibility of the infrastructure, ease of use and availability of necessary resources
No specific instrumentation is required onboard for POC, PN and PIC measurements. Seawater samples are collected onboard and shipped to on-land dedicated facilities for analyses in the laboratory.
Minimal interruption to current GO-SHIP standard operations
If water is available, only the seawater collection from the CTD-rosette (< 30 min for 12 bottles).

For POC, PC and PIC water analysis

Sample name
The stock of particulate organic carbon (POC), particulate nitrogen (PN) and particulate inorganic carbon (PIC) determined from CHN elemental analyzer.
Amount of water per whole-water samples or amount filtered per sample
The volume of seawater to be collected varies with expected particle load, on the order of 1–4L. Two seawater samples have to be collected, one for POC/PN, one for PIC.
Type of sampling (whether sample could be taken from in-line water flow and/or rosette. For the latter, depths of interest)
Seawater sampling can be performed either from a surface flow-through system or from a CTD-rosette device equipped with Niskin bottles, the latter enabling the acquisition of depth-resolved profiles. When the CTD-rosette device is used, seawater samples are typically collected at preselected depths, located between the surface to 1000 m.
Price per sample
$9-18 for CHN analysis (does not include supply costs)
Price per lab instrument
~$45k for a CHN elemental analyzer
Maintenance cost per lab instrument
~$1.4-3k / year
Personnel time to run a sample
Filtration for POC, PN and PIC is not highly technical, but requires constant care and surveillance. In particular the water collection under bloom situations should be performed quickly (e.g., after sampling for gases) or ideally through sub-sampling

(after agitation) from a large carboy that would have been filled from a whole CTD-bottle. Depending on particle load, filtration may require 1 to 2 hours.
Necessary infrastructure onboard (e.g., freezers, liquid N2, Filtration rack, filters, Milli-Q system, type of pump for in-line) and relevant cost (if not standard onboard equipment)
Sampling for POC, PN and PIC requires: -PET bottles for seawater sampling, e.g., Nalgene $30/bottle -A filtration rack equipped with polysulfone funnels (to prevent from POC contamination), e.g., Pall Science Lab ~$220 / funnel -A water-jet pump for gentle, low-vacuum filtration of seawater samples (e.g., Water Jet Aspirator pump from Cole Parmer ~$2000) -Whatman 25-mm GF/F glass-fiber filters (pore size 0.7 µm) prepared for filtration, i.e., pre-combusted in an oven or pre-washed with dichloromethane depending on chosen protocol (see below) -Pre-combusted scintillation vials, petri dishes, or aluminum foil, depending on preferred storing and shipping option (see below) -An oven and a dessicator to dry the filters / or liquid nitrogen, a -20°-C freezer or a -80°C-freezer for storage until shipping to analytical facility, depending on preferred storage and shipping option (see below)
Shipping needs (e.g., temperature at which samples need to be maintained, facilities available for sample analysis)
There are two major storing and shipping options. After filtration: -The filters may be placed in pre-combusted scintillation vials or petri dishes, oven-dried at 50°C overnight, and stored in a dry place or in a desiccator until shipping and analysis in the lab. -The filters may be wrapped in pre-combusted aluminium foils and stored in either -20°C, -80°C or liquid nitrogen, and shipped in a dry shipper (~$1300 / dry shipper). The cost of POC analysis is around $10 per sample when analyses are conducted at the Laboratoire d'Océanologie et Geosciences (LOG), Wimereux, France, or the Institut de la Mer de Villefranche (IMEV), Villefranche-sur-Mer, France. In the US, UCSB, OSU, and UMaine, among many other laboratory, perform HCN analysis.
Ancillary measurements relevant/needed (beyond GPS)
Temperature Salinity Conductivity Radiometer data Nutrients HPLC pigments Bio-optics Flow cytometry Imaging (IFCB, UVP)
Ecosystem/biogeochemical model parameter constrained by this measurement

POC analysis permits the quantification of carbon biomass. Combined with chlorophyll a measurements it provides a constraint on the phytoplankton chlorophyll-to-carbon ratio, an indicator of phytoplankton physiology.

POC is a key parameter used as input to biogeochemical and ocean-color based bio-optical models to produce large-scale estimates of phytoplankton biomass and primary production.

PIC allows quantifying the biomass of calcifying organisms that are key players in ballasting and exporting organic material.

POC and PN data are useful for the initialization and validation of biogeochemical models. PIC is additionally useful in models of the biological pump.

Units: mgC m^{-3}, mgN m^{-3}
Constrains POC, PON, PIC, proxy for Phyto_C and together with other measurements constrains Chla/Phyto_c and growth rate.

Existing protocols and relevant publications

The reference JGOFS protocol for CHN POC/PN analysis (UNESCO 1994, Chapter 15) is detailed in Knap et al. (1996). Yet one may prefer to use the treatment with dichloromethane instead of pre-combustion of the GF/F filters, as described in Claustre et al. (1999), in order to prevent changes in the porosity of the GF/F filters that may be induced by combustion.
In addition to the reference JGOFS protocol, Gardner et al. (2003) recommend that onboard filtration of seawater samples for POC analysis is accompanied by the collection of blank filters to account for contamination by adsorbed dissolved organic carbon (DOC) which may lead to substantial overestimation of POC.
Several methods have been proposed with no consensus (Gardner et al. 2003 and references therein; Behrenfeld and Boss, 2006; Cetinić et al. 2012; Novak et al. 2018). Here we recommend that filtered seawater, resulting from the collection of the seawater filter samples, is taken and re-filtered through new 25-mm precombusted GF/F filters (similar to those used for samples). A sufficient amount of DOC blank filters must be collected so an average of all DOC blanks can be determined and subtracted from all samples (regardless of the sampling depth).

References:

Behrenfeld M. J., Boss E., 2006. Beam attenuation and chlorophyll concentration as alternative optical indices of phytoplankton biomass. *Journal of Marine Research*, 64(3), 431–451.
Claustre H., Morel A., Babin M., Caillau C, Marie D., Marty J.-C., Vaulot D., 1999. Variability in particle attenuation and stimulated fluorescence in the tropical and equatorial Pacific: Scales, patterns and some biogeochemical implications. *Journal of Geophysical Research-Oceans*, 104**,** 3401–3422.
Cetinić I., Perry M. J., Briggs N. T., Kallin E., D'Asaro E. A., Lee C. M., 2012. Particulate organic carbon and inherent optical properties during 2008 North Atlantic

Bloom Experiment. *Journal of Geophysical Research-Oceans*, 117, C06028, doi:10.1029/2011JC007771.
Gardner W. D., Richardson M. J., Carlson C. A., Hansell D., Mishonov A. V., 2003. Determining true particulate organic carbon: Bottles, pumps and methodologies. *Deep Sea Res., Part II*, 50(3–4), 655–674, doi:10.1016/S0967-0645(02)00589-1.
Knap A., Michaels A., Close A., Ducklow H., Dickson A., 1996. Protocols for the Joint Global Ocean Flux studies (JGOFS) core measurements, JGOFS Rep. 19, JGOFS Core Proj. Off., Bergen, Norway. *Reprint of Intergovernmental Oceanographic Commission Manuals and Guides*, no. 29, 170 pp., UNESCO, Paris, 1994.
Novak M. G., Cetinić I., Chaves J. E., Mannino A., 2018. The adsorption of dissolved organic carbon onto glass fiber filters and its effect on the measurement of particulate organic carbon: A laboratory and modeling exercise. *Limnol. Oceanogr. Methods,* 16: 356–366. doi:10.1002/lom3.10248.

Two major protocols are available for PIC analysis:
Garcia et al. (2011) recommend that one of the two collected filters is saturated with hydrochloric acid fumes to remove inorganic carbon. Both filters (acidified and un-acidified) are analyzed using a CHN elemental analyzer. PIC is computed as the difference between PC (non-acidified filter) and POC (acidified filter).
In the Poulton et al. (2006) protocol, a 0.45-mm cellulose nitrate filter is used to collect seawater for PIC analysis. The filter is then rinsed with a potassium tetraborate solution, acidified with nitric acid and analyzed by Atomic Mass Spectrometry.

References:
Garcia C. A. E., Garcia V. M. T., Dogliotti A. I., Ferreira A., Romero S. I., Mannino A., Souza M. S., Mata M. M., 2011. Environmental conditions and bio-optical signature of a coccolithophorid bloom in the Patagonian shelf. *Journal of Geophysical Research*, 116, C03025, doi:10.1029/2010JC006595.
Poulton A. J., Sanders R., Holligan P. M., Stinchcombe M. C., Adey T. R., Brown L., Chamberlain K., 2006. Phytoplankton mineralization in the tropical and subtropical Atlantic Ocean. *Global Biogeochemical Cycles*, 20, GB4002, doi:10.1029/2006GB002712

Obvious synergies with current measurements performed on board GO-SHIP cruises and other measurements proposed here

GO-SHIP data on physical forcing and nutrient stocks would help in interpretation of environmental conditions prevailing in the establishment of phytoplankton communities.

Standardized vocabulary for the data and the associated metadata, for managing into the large and diverse data sets collected around the world (oceanographic fleets, automated observation systems deployed in situ), and available among various data centers aiming at preserving and making reusable marine observations (from physics to chemistry and biology)

An example of standardized vocabulary from SeaDataNet:
http://seadatanet.maris2.nl/v_bodc_vocab_v2/search.asp?lib=p01&screen=0

Label: POC_>GF/F_Acid_ElemAnal Preferred label: Concentration of organic carbon {organic_C CAS 7440-44-0} {POC} per unit volume of the water body [particulate >GF/F phase] by filtration, acidification and elemental analysis Definition: Particulates collected on a GF/F filter were acid fumed then analyzed using a carbon/nitrogen elemental analyser.
Data dissemination infrastructure and likely data latency. If further shore-based analysis is necessary (e.g., image classification and validation), the likely personnel time associated
An example conducted for HPLC pigment data dissemination is the MAREDAT database, published in ESSD and archived (+ DOI) on PANGAEA (Peloquin et al., 2013). Another option is publishing a reference paper in ESSD, but archiving the data in SEANOE, as was recently done for the first global BGC-Argo dataset (Organelli et al., 2016): https://www.seanoe.org/data/00360/47142/
A list of relevant experts who can be called upon to help and know they have been identified
For onboard work: Ivona Cetinic (ivona.cetinic@nasa.gov) Toby Westberry (westbert@science.oregonstate.edu) Wilford Gardner (wgardner@ocean.tamu.edu)

12.3.3 Genetics recommendations

Genetics recommendations for plankton-related measurements onboard research vessels and the GO-SHIP program. Contribution from the Genetics subgroup.

Recommendations for measurements appropriate for GO-SHIP based on the following criteria

Application to key science needs and questions to support the measurement of biological EOVS
Collection of microbial plankton cells to further analyze the genes (DNA) of microbial communities provides high-resolution information at three different levels: 1) taxonomy of the protists, prokaryotes and viruses; 2) patterns and diversity estimations of these communities; and 3) metabolic and functional capacities of microbial communities including the reconstruction of uncultured and abundant microbial genomes.
Broad accessibility of the infrastructure, ease of use and availability of necessary resources
The genetic analyses of planktonic communities rely on three steps (see details below): 1) Microbial Biomass Sampling. Seawater collection to concentrate microbial plankton cells to be done on board. 2) DNA extraction to be done on-land and accessible in most biology labs. 3) PCR amplification of 16S/18S rRNA gene sequencing and/or whole DNA sequencing to be performed on-land generally through external services.

Minimal interruption to current GO-SHIP standard operations
Genetic samples are simple to collect and require only small volumes from Niskin bottles or in-line water flow systems (2-12L).

Sample name:
Microbial Plankton Biomass Collection for genetic analyses
Type of sampling:
In-line water flow always and if possible rosette sampling at three different depths (Surface, Deep Chlorophyll Maximum, and Mesopelagic (500-1000m approx.)
Price per sample:
$25
Price per lab instrument:
$9200 Filtration Masterflex Peristaltic Pump with Pump Head (Cole-Parmer; HV-77963-10): $4100 2x Filter Holder 142 mm, stainless steel (YY3014236, Millipore): $4,452 3x Compactable Water sample containers (20L): $150 Masterflex L/S Silicone Tube (Cole-Parmer; 96410-73): $498
Amount filtered per sample
Usually about 12L sample for genetic analyses including amplicon 16S/18S rRNA gene Tags and metagenomics. Minimum 2L for amplicon 16S/18S rRNA gene iTAGs.
Mode of operation (automated vs. discrete samples)
Discrete samples from in-line High Volume Peristaltic pump and/or rosette.
Maintenance cost per lab instrument:
$2500
Personnel time to run a sample:
1h maximum
Necessary infrastructure onboard: (e.g., freezers, liquid N2, Filtration rack, filters, MiliQ system, type of pump for in-line) and relevant cost (if not standard onboard equipment).
Freezer (-20ºC) MiliQ System or Distilled water system On board in-line High-Volume Peristaltic pump / Continuous Water Sampling Filtration Masterflex Peristaltic Pump with Pump Head (HV-77963-10) 2x Filter Holder 142 mm, stainless steel (YY3014236) Masterflex L/S Silicone Tube (96410-73) Regular Silicone Tube 3x Compactable Water sample containers (20L)
Necessary consumables onboard:
Isopore Polycarbonate Membrane Filters 0.2µm GTTP14250

Isopore Polycarbonate Membrane Filters 3.0µm TSTP14250 Corning® 15 mL centrifuge tubes CLS430791-500EA
Human resources needed
One person to run the filtration, cleaning before and after sampling. Estimated time is 1h to set up the whole filtration system and prepare the material 15 min / sample.
Output
Filters with concentrated plankton biomass for genetic analyses. Archiving DNA (>25 nanograms) as genetic resource for future analyses
Ancillary measurements needed/relevant (beyond GPS).
Temperature Salinity Conductivity Radiometer data Chlorophyll *a* and other pigments (if possible) Nutrients DOC / POC / CDOM Cell counts by flow cytometry (if possible)
Ecosystem/biogeochemical model parameter constrained by this measurement:
Biodiversity of pico- and nano-plankton if amplicons 16S/18S rRNA gene are performed. Biodiversity and functional analyses if metagenomics (whole genome sequencing) is done.
Existing protocols and relevant publications:
Ten selected Papers related with Protocols of Microbial Plankton collection and genetic analyses of global expeditions such as ICOMM, GOS, *Tara* Oceans and Malaspina Expeditions or GO-SHIP transect. (Note: An extent list and papers associated can be found in the Genetic folder). 1. **Stéphane Pesant** , Fabrice Not, Marc Picheral, Stefanie Kandels-Lewis,Noan Le Bescot, Gabriel Gorsky, Daniele Ludicone, Eric Karsenti, Sabrina Speich,Romain Troublé, Céline Dimier, Sarah Searson & Tara Oceans Consortium Coordinators; **Silvia G. Acinas,** Peer Bork, **Emmanuel Boss**, Chris Bowler, **Colomban De Vargas**, Michael Follows, Gabriel Gorsky, Nigel Grimsley, Pascal Hingamp, Daniele Iudicone, OlivierJaillon, Stefanie Kandels-Lewis, Lee Karp-Boss, Eric Karsenti, Uros Krzic, Fabrice Not, Hiroyuki Ogata, **Stéphane Pesant,** Jeroen Raes, Emmanuel G. Reynaud, Christian Sardet, Mike Sieracki, Sabrina Speich, Lars Stemmann, **Matthew B. Sullivan**, **Shinichi Sunagawa**, Didier Velayoudon, Jean Weissenbach, Patrick Wincker. **2015**. *Open science resources for the discovery and analysis of Tara Oceans data*. **Scientific Data**. 2:150023. doi: 10.1038/sdata.2015.23. 2. Adriana Alberti, Julie Poulain, Stefan Engelen, Karine Labadie, Sarah Romac, **Isabel Ferrera**, Guillaume Albini, Jean-Marc Aury, Caroline Belser, Alexis Bertrand, Corinne Cruaud, Corinne Da Silva, Carole Dossat, Frédéric Gavory, Shahinaz Gas, Julie Guy, Maud Haquelle, E'krame Jacoby, Olivier Jaillon, Arnaud Lemainque, Eric Pelletier, Gaëlle Samson, Mark Wessner, Genoscope

Technical Team, **Silvia G. Acinas**, Marta Royo-Llonch, Francisco M. Cornejo-Castillo, Ramiro Logares, Beatriz Fernández-Gómez, Guy Cochrane, Clara Amid, Petra Ten Hoopen, **Colomban De Vargas,** Nigel Grimsley, Elodie Desgranges, Hiroyuki Ogata, Nicole Poulton, Michael E. Sieracki, Ramunas Stepanauskas, **Matthew B. Sullivan**, Jennifer R. Brum, Melissa B. Duhaime, Bonnie T. Poulos, Bonnie L. Hurwitz, *Tara* Oceans Consortium Coordinators, **Stéphane Pesant**, Eric Karsenti, Patrick Wincker. **2017**. *Marine plankton from viruses to metazoans: nucleotide sequences from the* Tara *Oceans expedition* (2009-2013). **Scientific Data**. 2017 Aug 1;4:170093. doi: 10.1038/sdata.2017.93.

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4. Zinger L, Amaral-Zettler LA, Fuhrman JA, Horner-Devine MC, Huse SM, et al. **2011**. *Global Patterns of Bacterial Beta-Diversity in Seafloor and Seawater Ecosystems*. PLoS ONE 6(9): e24570. doi:10.1371/journal.pone.0024570.
5. **Colomban de Vargas**, Stéphane Audic, Nicolas Henry, Johan Decelle, Frédéric Mahé, Ramiro Logares, Enrique Lara, Cédric Berney,Noan Le Bescot, Ian Probert,Margaux Carmichael,Julie Poulain, Sarah Romac, Sébastien Colin, Jean-Marc Aury, Lucie Bittner,Samuel Chaffron, Micah Dunthorn, Stefan Engelen, Olga Flegontova, Lionel Guidi, Aleš Horák, Olivier Jaillon, Gipsi Lima-Mendez, Julius Lukeš,Shruti Malviya,Raphael Morard, Matthieu Mulot, Eleonora Scalco, Raffaele Siano,Flora Vincent, Adriana Zingone, Céline Dimier,Marc Picheral,Sarah Searson,Stefanie Kandels-Lewis,**Tara Oceans Coordinators**, **Silvia G. Acinas**, Peer Bork, Chris Bowler, Gabriel Gorsky, Nigel Grimsley,Pascal Hingamp, Daniele Iudicone, Fabrice Not,Hiroyuki Ogata, **Stephane Pesant**, Jeroen Raes, Michael E. Sieracki,Sabrina Speich,Lars Stemmann, **Shinichi Sunagawa**, Jean Weissenbach,Patrick Wincker, Eric Karsenti. **2015**. *Eukaryotic plankton diversity in the sunlit global ocean.* **Science**. 348(6237):1261605. doi: 10.1126/science.1261605.
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Biogeography of deep-sea pelagic prokaryotes. ISME J. doi: 10.1038/ismej.2015.137. Epub 2015 Aug 7.

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10. Ruiz-González C, Logares R, **Sebastián M,** Mestre M, Rodríguez-Martínez R, Galí M, Sala MM, **Acinas SG,** Duarte CM, **Gasol JM**. **2019.** *Higher contribution of globally rare bacterial taxa reflects environmental transitions across the surface ocean*. Mol Ecol. Jan 21. doi: 10.1111/mec.15026.

Obvious synergies with current measurements performed on board GO-SHIP cruises and other measurements proposed here.

Bio-optics
Bio-acoustics
Imaging, Flow Cytometry
HPLC

Vocabulary for the data and the associated metadata, for managing the large and diverse datasets collected around the world (oceanographic fleets, automated observation systems deployed in situ), and available among various data centers aiming at preserving and making re-useable marine observations (from physics to chemistry and biology).

Plankton Biomass and Biodiversity, DNA Sequencing, archiving DNA

Data dissemination infrastructure and likely data latency. If further shore-based analysis is necessary (e.g., image classification and validation), the likely personnel time associated.

Public databases such as SRA, ENA, PANGEA, etc.
Likely data Latency: 6-12 months
Shored-based analysis: DNA extraction, sequencing and analysis. One lab and one bioinformatics person needed.

A list of relevant experts who can be called upon to help and know they have been identified.

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Shinichi Sunagawa (microbial bioinformatics and genomics)
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Laurence Garczarek (picoplankton diversity)
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Matthew Sullivan (virus diversity and genomics)
- The Ohio State University (US)
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12.3.4 Flow cytometry (FCM) recommendations

Flow cytometry recommendations for plankton-related measurements onboard research vessels and the GO-SHIP program. Contribution from the Flow cytometry subgroup.

Recommendations for measurements appropriate for GO-SHIP based on the following criteria

Application to key science needs and questions to support the measurement of biological EOVs
Flow cytometry provides a means to enumerate and characterize fluorescence and light scattering properties of microbes. Individual particles are measured in flow as they pass through a focused light source (usually one or several laser beams) and combinations of fluorescence and scattering signals can be analyzed to distinguish *Prochlorococcus*, *Synechococcus*, and eukaryotic picophytoplankton, and nanophytoplankton. With the addition of a nucleic acid stain to samples at the time of analysis, it is also possible to routinely enumerate heterotrophic prokaryotes. More specialized analyses with stains can also be used for viruses and microzooplankton, or cell viability (NADS protocol).
Broad accessibility of the infrastructure, ease of use and availability of necessary resources
Flow cytometry is in routine use in aquatic plankton research and facilities that analyze samples as a fee-based service are available.
Minimal interruption to current GO-SHIP standard operations
Flow cytometry samples are simple to collect and require only small volumes of seawater collected from rosette bottles.

Instrument name and description
A sensitive flow cytometer configured for analysis of plankton is required in a shore-based facility. Many, but not all benchtop flow cytometers available on the market are sensitive enough to be used in marine microbial ecology. Cell sorters can also be used. Examples include the BD Influx Mariner operated at the J. J. MacIsaac Facility for Aquatic Cytometry at the Bigelow Laboratory for Ocean Sciences and the PRECYM flow cytometry platform of the Mediterranean Institute of Oceanology.
Volume of water analyzed
For post-cruise flow cytometry, typically 2-milliliter of whole seawater samples are preserved with a fixative and must be kept frozen until analysis.
Type of sampling (whether sample could be taken from in-line water flow and/or rosette. For the latter, depths of interest)
Rosette samples from euphotic zone depths are of highest scientific interest, through deeper samples would also be useful for characterizing heterotrophic plankton. Samples from in-line flow-through water can also be collected and analyzed if higher spatial/temporal resolution is desired for surface waters.
Price per sample
~$20 for pico/nano-phytoplankton, ~$20 USD for heterotrophic prokaryotes, ~$26 for heterotrophic protists
Price per lab instrument
N/A (use of existing facility with fee-per-sample service recommended). The price per lab instrument is dependent on the instrument configuration (number of lasers, of photodetectors, etc.).
Maintenance cost per lab instrument
N/A (use of existing facility with fee-per-sample service recommended).
Personnel time to run a sample
N/A (use of existing facility with fee-per-sample service recommended).
Necessary infrastructure onboard (e.g., freezers, liquid N2, Filtration rack, filters, Milli-Q system, type of pump for in-line) and relevant cost (if not standard onboard equipment)
Preserved samples in cryovials require storage in a liquid nitrogen dewar or -80 °C freezer. Ideally preservative is added in a fume hood. Samples must be kept frozen.
Shipping needs (e.g., temperature that samples need to be maintained at, facilities available for sample analysis)
Samples must be kept frozen and should be express shipped to the analysis facility either in a cryogenic dry shipper or in styrofoam packed with dry ice.
Ancillary measurements needed/relevant (beyond GPS)
No special ancillary measurements are required. Chl fluorescence measurements acquired during CTD-rosette casts are useful to guide sampling (e.g., targeting depths with elevated chlorophyll concentration)
Ecosystem/biogeochemical model parameter constrained by this measurement

Many biogeochemical models in current use include multiple taxa or functional types of phytoplankton, bacteria, and zooplankton. Flow cytometry provides direct information about concentrations and size distributions of these organisms.
Existing protocols and relevant publications
Sample preservation: Marie, D., F. Rigaut-Jalabert, and D. Vaulot. 2014. An improved protocol for flow cytometry analysis of phytoplankton cultures and natural samples. Cytometry Part A 85: 962-968. 10.1002/cyto.a.22517
Obvious synergies with current measurements performed on board GO-SHIP cruises and other measurements proposed here
Automated imaging of nano- and microplankton (such as with Imaging FlowCytobot or automated analysis with the Cytosense flow cytometers provide highly complementary information about larger size classes of phytoplankton and microzooplankton. Imaging provides some taxonomy to the flow cytometry analysis.
Standardized vocabulary for the data and the associated metadata, for managing into the large and diverse data sets collected around the world (oceanographic fleets, automated observation systems deployed in situ), and available among various data centers aiming at preserving and making reusable marine observations (from physics to chemistry and biology)
There are well-established standards for flow cytometry data files (FCS standard file format, e.g., Spidlen et al. 2010). Flow cytometry analysis products are routinely handled by existing repositories and data systems such as BCO-DMO, SeaDataNet, and SeaBASS. Several groups (such as for example the European JERICO Next group, Bengt et al., 2017) are developing novel methods for automated in situ observations of phytoplankton diversity and for the standardization required to compare results from various studies. Spidlen, J., Moore, W., Parks, D., Goldberg, M., Bray, C., Bierre, P., … Brinkman, R. R. (2010). Data File Standard for Flow Cytometry, version FCS 3.1. *Cytometry. Part A : The journal of the International Society for Analytical Cytology*, *77*(1), 97–100. doi:10.1002/cyto.a.20825 Bengt K., Felipe A., Veronique C., Arnaud L., Guillaume W., Jukka S et al. (2017). JERICO-NEXT. Novel methods for automated in situ observations of phytoplankton diversity. D3.1. JERICO-NEXT-WP3-D3.1, 4 Oct. 2017. http://archimer.ifremer.fr/doc/00422/53393/
Data dissemination infrastructure and likely data latency. If further shore-based analysis is necessary (e.g., image classification and validation), the likely personnel time associated
Shore-based analysis of preserved samples will likely require several months for scheduling and completion.
A list of relevant experts who can be called upon to help and know they have been identified
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Dominique Marie - marie@sb-roscoff.fr

12.3.5 Automated flow cytometery (Cytosense) recommendations

Automated flow cytometery recommendations for plankton-related measurements onboard research vessels and the GO-SHIP program. Contribution from the Flow cytometry subgroup.

Recommendations for measurements appropriate for GO-SHIP based on the following criteria

Application to key science needs and questions to support the measurement of biological EOVs
Autonomous flow cytometry enables automated counting of pico- to micro-phytoplankton (cells cm^{-3}). Light scattering and several fluorescence emissions are recorded (pulse shapes along the particles). Image-in-flow performed at the single cell level is an option to take pictures of the cells while flowing in the instrument. Light scattering can be used as a proxy of the size, and the fluorescence intensities are in relation to the pigment content available within each particle (cell, colony).
Broad accessibility of the infrastructure, ease of use and availability of necessary resources
Automated flow cytometry, performed with the Cytobuoy flow cytometers is widely accessible. Protocols developed and shared by the community exist, including for data quality. The quantitative output (absolute counts) requires absolute calibration. The protocols are presented in the best practices of the H2020 JERICO-NEXT (D2.2) European Program.
Minimal interruption to current GO-SHIP standard operations
Added to flow-through system.

Instrument name and description
CytoSense pulse recording of laser-based fluorescence and scattering uses a wide field-of-view FOV in a very wide flow cell of 1000x1200 µm cross section and by its zero dead time electronics it detects and analyses all particles flowing through, up to ca. 20K particles/sec, surrounded by a particle free sheath fluid. For particles smaller than 5 µm, the scan data converges to normal flow cytometry data, that is correlated multiparameter data for discrimination of the various picoplankton. The laser-based scattering and fluorescence, with high numerical aperture optics yields the high sensitivity required for picoplankton, down to 0.1µm mineral particles or 0.3µm cells. The scanning principle is "endless" by electronic design allowing the full capturing of elongated and filamentous particles, normally too long for high-resolution image frames. With particles increasing over 5µm (laser sheet thickness), the morphological information in the scans also increases allowing higher resolution clustering and recognition. The emission of scattered light and fluorescence is highly independent of the orientation of the particles around their length axis, therefore a robust, highly linear direct measure of particle size and biomass. Processing rate can be of > 10,000 particles sec. Pulse scans consist of up to 8 different light scatter and/or fluorescence

detector courses digitized in real-time at 4MHz while the particle traverses one or two sharply focused excitation laser beams at velocity of 2 m/s.
Instrument manufacturer
CytoBuoy b.v., Woerden, Netherlands
Type of sampling (whether sample could be taken from in-line water flow and/or rosette. For the latter, depths of interest)
Rosette samples from euphotic zone depths are of highest scientific interest, through deeper samples would also be useful for characterizing heterotrophic plankton. Samples from in-line water flow can also be collected and analyzed if higher spatial/temporal resolution is desired for surface waters.
Price per sample
€1 to <5 per sample, depending on the sampling frequency.
Price per instrument
€90 - 150 K depending on configuration (number of lasers & detectors, submersible, autonomy etc). An automated device (BST) for extra sheath cleaning and bead solution loader for calibration and control of the cytometer is an option.
Maintenance cost per lab instrument
About €7K/year are needed for maintenance (by Cytobuoy) and consumables (tubes, filters, calibration beads).
Personnel time to run a sample
The Cytosense flow cytometers are autonomous and automated. They do not need any personnel time to run a sample when operated in flow-through mode. However, supervision is recommended, and can be performed remotely if Internet is available.
Necessary infrastructure onboard (e.g., freezers, liquid N2, Filtration rack, filters, Milli-Q system, type of pump for in-line) and relevant cost (if not standard onboard equipment)
Power supply Bench or equivalent to install the flow cytometer. Seawater supply (through a FerryBox already installed or another water supply from the ship, like the one used for the thermosalinograph).
Shipping needs (e.g., temperature at which samples need to be maintained, facilities available for sample analysis)
N/A
Ancillary measurements needed/relevant (beyond GPS)
T, S, Nutrients, Chl *a*, PAR, primary production, HPLC pigment analyses, Diversity (microscopy)
Ecosystem/biogeochemical model parameter constrained by this measurement
Phytoplankton functional group abundances, size classes, high resolution distribution, contribution to total fluorescence per functional group.

Existing protocols and relevant publications

Existing protocols are presented in the best practices of the H2020 JERICO-NEXT (D2.2) European Program.

Relevant publications:

L. Haraguchi, H. H. Jakobsen, N. Lundholm, J. Carstensen, Monitoring natural phytoplankton communities: a comparison between traditional methods and pulse-shape recording flow cytometry, Aquat. Microb. Ecol. 80 (2017) 77-92

H. Tan, T. Oishi, A. Tanaka, R. Doerffer, Y. Tan, Chlorophyll-a specific volume scattering function of phytoplankton, Optics EXPRESS 25 (2017) A564-A573

L. Duforêt-Gaurier, W. Moutier, N. Guiselin, M. Thyssen, G. Dubelaar, X. Mériaux, L. Courcot, D. Dessailly, H. Loisel, Determination of backscattering cross section of individual particles from cytometric measurements: a new methodology, Opt. Express. 23 (2015) 31510-31533.

S. Fontana, O.L. Petchey, F. Pomati, Individual-level trait diversity concepts and indices to comprehensively describe community change in multidimensional trait space, Funct. Ecol. 30 (2015) 808–818.

M. Thyssen, S. Alvain, A. Lefèbvre, D. Dessailly, M. Rijkeboer, N. Guiselin, V. Creach, L.-F. Artigas, High-resolution analysis of a North Sea phytoplankton community structure based on in situ flow cytometry observations and potential implication for remote sensing, Biogeosciences. 12 (2015) 4051–4066.

M.N. McFarland, J. Rines, J. Sullivan, P. Donaghay, Impact of phytoplankton size and physiology on particulate optical properties determined with scanning flow cytometry, Mar. Ecol. Prog. Ser. 531 (2015) 43–61.

S. Bonato, U. Christaki, A. Lefebvre, F. Lizon, M. Thyssen, L.F. Artigas, High spatial variability of phytoplankton assessed by flow cytometry, in a dynamic productive coastal area, in spring: The eastern English Channel, Estuar. Coast. Shelf Sci. 154 (2015) 214–223.

M. Dugenne, M. Thyssen, N. Garcia, N. Mayot, G. Bernard, Monitoring of a Potential Harmful Algal Species in the Berre Lagoon by Automated In Situ Flow Cytometry, In Marine Productivity: Perturbations and Resilience of Socio-ecosystems, Springer International Publishing, (2015) 117–127.

Hofstraat JW, Vreeze MEJ, Zeijl van WJM, Peperzak L, Peeters JCH, Balfoort HW, 1991. Flow Cytometric Discrimination Of Phytoplankton Size Classes By Fluorescence Emission And Excitation Properties. J. of Fluorescence 1, p249-265

Peeters J.C.H.., G.B.J. Dubelaar, J. Ringelberg and J.W.M. Visser, 1989 The Optical Plankton Analyser (O.P.A.): A Flow Cytometer For Plankton Analysis, I: Design Considerations. Cytometry 10: 522-528,1989 Dubelaar G.B.J., A..C. Groenewegen, W. Stokdijk, G.J. van den Engh and J.W.M. Visser, 1989. The Optical Plankton Analyser (O.P.A.): A Flow Cytometer For Plankton Analysis, II: Specifications. Cytometry 10: 529-539,1989
Obvious synergies with current measurements performed on board GO-SHIP cruises and other measurements proposed here
Synergies already exist with various measurements (T, S, dissolved O2, pCO2, nutrients). Possible synergies could occur with imaging and genomics.
Standardized vocabulary for the data and the associated metadata, for managing into the large and diverse data sets collected around the world (oceanographic fleets, automated observation systems deployed in situ), and available among various data centers aiming at preserving and making reusable marine observations (from physics to chemistry and biology)
A standardized vocabulary for the data and the associated metadata (still under development) is available at http://vocab.nerc.ac.uk/collection/F02/current/
Data dissemination infrastructure and likely data latency. If further shore-based analysis is necessary (e.g., image classification and validation), the likely personnel time associated
Typically, it takes several weeks to analyze the data produced for a whole cruise (mostly with a manual analysis). However, automated data analysis software is now available (Easyclus and Rclustool) and should reduce that time.
A list of relevant experts who can be called upon to help and know they have been identified
● Melilotus Thyssen, melilotus.thyssen@mio.osupytheas.fr ● Gérald Grégori, gerald.gregori@mio.osupytheas.fr ● Machteld Rijkeboer, machteld.rijkeboer@rws.nl

12.3.6 Automated imaging flow cytometery (IFCB) recommendations

Automated imaging flow cytometry recommendations for plankton-related measurements onboard research vessels and the GO-SHIP program. Contribution from the Imaging subgroup.

Recommendations for measurements appropriate for GO-SHIP based on the following criteria

Application to key science needs and questions to support the measurement of biological EOVS
The Imaging FlowCytobot (IFCB), generates images of protists to give biodiversity information as well as organism-specific size, shape, fluorescence and scattering .
Broad accessibility of the infrastructure, ease of use and availability of necessary resources
The instrument is commercially available, and easily incorporated into an on-board flow-through surface seawater system.

Minimal interruption to current GO-SHIP standard operations
No interruption, instrument is integrated into the seawater system; however, that system needs to be cleaned regularly (see cleaning protocols). Ideally, the surface seawater should be pumped with a diaphragm pump (not impeller pump) to minimize damage to fragile organisms.

Instrument name and description
Imaging FlowCytobot (IFCB): The IFCB is a imaging-in-flow cytometer, measuring individual particle fluorescence and light scattering, and capturing a high-resolution (~1 µm) image of each cell or chain in the size range ~5-150 µm width. Controlled flow and illumination conditions ensure a very high rate of images containing in focus, single targets aligned in the flow such that the largest cross-section is imaged. Images can be collected at up to ~15 Hz, depending on particle concentrations encountered. IFCB is typically operated with a light scattering trigger and configured to sample automatically 5 ml every 20 minutes from the uncontaminated seawater flow. IFCB can also be used to analyze discrete samples from Niskin bottles.
Volume of water analyzed
IFCB is typically operated with a light-scattering trigger and will be configured to sample automatically 5 ml every 20 minutes from the uncontaminated seawater flow.
Type of sampling (whether sample could be taken from in-line water flow and/or rosette. For the latter, depths of interest)
IFCB can be configured to sample automatically from the in-line water flow. Samples from the rosette can also be analyzed, but this would require an analyst to collect samples and run them on the instrument. Depths of interest are typically restricted to the euphotic zone, though protozoa and detritus from deeper samples can also be analyzed.
Price per sample
N/A
Price per instrument
Instrument price: ~ €90k
Maintenance and calibration of instrument
Calibration: Main calibration issues are (1) ensuring sample volume is properly quantified (a function design criteria set during manufacture; user verification is good practice, but experience suggests this does not need to be repeated unless there are hardware changes in the instrument); and (2) determination of image scaling (micrometers per pixel; user determined with particles of interest).
Personnel time to run a sample
The IFCB needs to be set up by an experienced scientist or technician and it is useful, but not necessary, to have such a person on board to ensure data quality. Image analysis requires experienced analyst for the processing and interpretation of images. https://github.com/hsosik/ifcb-analysis/wiki

Necessary infrastructure onboard: (e.g., freezers, liquid N2, Filtration rack, filters, MilliQ system, type of pump for in-line) and relevant cost (if not standard onboard equipment)

Clean flow-through system, electronic data storage backup, on the order of 200-500 Mb per day (though complete raw datasets can be stored internally on IFCB for a year or longer).

Shipping needs (e.g., temperature at which samples need to be maintained, facilities available for sample analysis)

The instrument can be shipped directly to the ship.

Ancillary measurements (beyond GPS)

Flow cytometry (FCM) is an ideal complement to the IFCB since the largest cells imaged by the FCM overlap with the smallest imaged by the IFCB. In addition, the range of organisms / particles imaged with the UVP starts at the largest IFCB size, so these three instruments complement each other ideally.

Ecosystem/biogeochemical model parameter constrained by this measurement

Links to BGC modeling: Plankton cell size distributions can inform models on the physical structure of organic matter in ecosystems, and their contribution to the biological pump.

Links to Ecosystem modeling: Organism imaging is changing how we manage taxonomic analyses – allowing larger-scale analysis of species distributions than has previously been possible. The phytoplankton concentrations and diversity provided by the IFCB could better inform ecosystem models.

Parameter	**Units**
Images of phytoplankton & other fluorescent particles (~10-200 μm)	unitless
Biovolume (individual image target)	μm^{-3}
Concentration	Cells mL^{-1}
Biovolume concentration	μm^{-3} mL^{-1}
Concentration by taxon	Cells mL^{-1}
Biovolume concentration by taxon	μm^{-3} mL^{-1}
Concentration by size class	Cells mL^{-1} μm^{-1}
Biovolume concentration by size class	μm^{-2} mL^{-1}

Constrains species/group composition, size distribution of phytoplankton (5>D>200um). Proxy for phytoplankton biomass (biovolume concentration relates to

Chl_a and POC). PSD can be combined with flow-cytometry to extend the PSD of phytoplankton.
Existing protocols and relevant publications
Olson, R. J., and H. M. Sosik. 2007. A submersible imaging-in-flow instrument to analyze nano- and microplankton: Imaging FlowCytobot. Limnol. Oceanogr. Methods 5: 195-203. Sosik, H. M., and R. J. Olson. 2007. Automated taxonomic classification of phytoplankton sampled with imaging-in-flow cytometry. Limnol. Oceanogr. Methods 5: 204-216. Sosik, H. M., J. Futrelle, E. F. Brownlee, E. Peacock, T. Crockford, and R. J. Olson. 2016. hsosik/ifcb-analysis: IFCB-Analysis software system, initial formal release at v2 feature stage [Data set]. Zenodo. http://doi.org/10.5281/zenodo.153978 Peacock, E.E., E. T. Crockford, and H.M. Sosik. 2018. IFCB at sea user guide. https://docs.google.com/document/d/14IfQBriV2AZs1akefM8JYirSAApnVFbDG2XQ74klIOI/
Obvious synergies with current measurements performed on board GO-SHIP cruises and other measurements proposed here
CTD, ADCP, carbonate chemistry.
Standardized vocabulary for the data and the associated metadata, for managing into the large and diverse data sets collected around the world (oceanographic fleets, automated observation systems deployed in situ), and available among various data centers aiming at preserving and making reusable marine observations (from physics to chemistry and biology)
Data dissemination infrastructure and likely data latency. If further shore-based analysis is necessary (e.g., image classification and validation), the likely personnel time associated
Data can be stored indefinitely, and analysis can therefore be executed as personnel become available. Particle size distributions are generated automatically. However, image classification for phytyoplankton biodiversity and concentrations requires experienced analyst for the checking and quality control of images.
A list of relevant experts who can be called upon to help and know they have been identified
Heidi M. Sosik (hsosik@whoi.edu) Lee Karp-Boss (lee.karp-boss@maine.edu)

12.3.7 Automated imaging of particles and zooplankton (UVP) recommendations

Automated imaging particles and zooplankton recommendations for plankton-related measurements onboard research vessels and the GO-SHIP program. Contribution from the Imaging subgroup.

Recommendations for measurements appropriate for GO-SHIP based on the following criteria

Application to key science needs and questions to support the measurement of biological EOVS

Marine imaging (also known as marine visual imaging (Durden et al., 2016) or marine optical imaging (Schoening et al., 2017)) has become a major tool for oceanographers. The underwater vision profiler, or UVP, generates images of organisms from the size of protists to small zooplankton give biodiversity information, as well as organism-specific size, shape and optical density. Images of non-living particles generate size, shape and optical density data for organic particles which are useful for mapping particle dynamics, transport, and sedimentation (Guidi et al., 2009).
Broad accessibility of the infrastructure, ease of use and availability of necessary resources
The instrument is mounted within the center of a 24-bottle rosette without removing any bottle or in its own frame outside a standard 12-bottle rosette.
Minimal interruption to current GO-SHIP standard operations
No interruption, instrument is integrated into the rosette system.

Instrument name
Underwater Vision Profiler (UVP): A submersible profiling imaging system which can count and size particles ~60 μm – ~6 mm in situ. The UVP needs monitoring by a trained scientist or technician on board. The data must be downloaded regularly (usually daily) during a research voyage in order to prevent memory saturation and to control data quality.
Amount of water per whole-water samples or amount filtered per sample
The volume of seawater to be imaged varies with instrument configuration, but is on the order of 1L.
Type of sampling (whether sample could be taken from in-line water flow and/or rosette. For the latter, depths of interest)
The instrument is designed to be associated with CTD instruments, such that it can be spatially and temporally aligned with other measurements, performing continuous vertical profiles. The UVP be deployed to 6,000 m, acquiring and processing images at up to 25 Hz and providing a theoretical 0.04 cm vertical resolution at 1m/s. Data are usually integrated over 5 m vertical bins.
Price per sample
N/A
Price per instrument
Instrument price: Deep (6000 m) €90k
Maintenance cost per instrument
The initial field calibration is done when the instrument is built by the factory. Because UVPs are imaging particle counters, it is recommended to calibrate the instruments yearly against a reference instrument. On deck, regular light checks are recommended to verify their integrity. On deck, black measurements also permit evaluation of instrument noise.
Personnel time to run a sample

The UVP requires attention one hour daily to perform light check and data download, to fill metadata, to initiate automatic initial process to check data quality and backup. Usual underwater instrument maintenance should also be done as for any instrument (i.e., connector cleaning). The daily battery charge does not require the continuous presence of an operator.
Particle size distributions and image analysis are generated automatically by the provided software. Image classification can be performed on board or on land using the *Ecotaxa* application. The precision of the classification relies on the expertise of an operator even if the initial sorting is done by non-specialized personnel. A trained operator can classify up to 35,000 images per day.

Necessary infrastructure onboard: (e.g., freezers, liquid N2, Filtration rack, filters, MilliQ system, type of pump for in-line) and relevant cost (if not standard onboard equipment)

The instrument is usually deployed in a standard sampling rosette, adding no ship time or specific infrastructure needs. Data are saved in the provided computer memory and back-up drive.

Shipping needs (e.g., temperature at which samples need to be maintained, facilities available for sample analysis)

The instrument can be shipped directly to the ship as with any electronic instrument. It is important to note that the instrument contains a Lithium battery which must be shipped as dangerous goods, and/or brought on board at a convenient port of origin. In the latter case, a trained technician will need to open its housing to set the battery.

Ancillary measurements (beyond GPS)

The Imaging Flow Cytobot (IFCB) is an ideal complement to the UVP since the largest cells imaged by the IFCB overlap with the smallest imaged by the UVP, giving a full size distribution from 5 microns to > 5 mm. When combined with flow cytometry (FCM), the size range is expanded down to < 1 micron. The lower particle size class data from the UVP overlaps with transmisometer / back scattering instruments, providing an in-situ quality check.

Ecosystem/biogeochemical model parameter constrained by this measurement

Links to BGC modeling: Particle size distributions (UVP, LISST) can inform models on the physical structure of organic matter in ecosystems, particularly the presence of large particles with the capacity to sink quickly, contributing to the biological pump.

Links to Ecosystem modeling: Organism imaging is changing how we manage taxonomic analyses – allowing larger-scale analysis of species distributions than has previously been possible. The zooplankton concentrations and diversity provided by the UVP could better inform ecosystem models.

Parameter	**Units**
Images of zooplankton, particles and other organisms (~60-6,000 μm)	unitless

Biovolume (individual image target)	μm^{-3}	
Concentration	Particles L^{-1} or organisms L^{-1}	
Biovolume concentration	μm^{-3} mL^{-1}	
Concentration by taxon	Cells L^{-1}	
Biovolume concentration by taxon	μm^{-3} L^{-1}	
Concentration by size class	Cells L^{-1} μm^{-1}	
Biovolume concentration by size class	μm^{-2} L^{-1}	

Constrains POC of large/sinking particles, constrains functional type grazers and their biodiversity, constrains large phytoplankton chains/colonies, provide a proxy for sinking flux. Size distribution of particles > 50um and organisms > 0.5mm.

Existing protocols and relevant publications

Picheral, M., et al., 2010: the core UVP technology and configuration
Pascal and Santiago, 2009: Zooplankton concentration estimation from images
Leon and Montero 2006: Calibration of zooplankton biomass
Guidi et al., 2009: Estimation of sedimentary flux from size distributions

Obvious synergies with current measurements performed on board GO-SHIP cruises and other measurements proposed here

UVP particle size data can be used to estimate the strength of the biological pump at high resolution. The UVP provides information on biologically generated particles and organisms at the same scale and frequency as current measurements of temperature, salinity, oxygen and nitrate sensors. This means that new insights on biological fluxes can be nested in their physical context, and contribute to a global understanding of ocean processes.

Standardized vocabulary for the data and the associated metadata, for managing into the large and diverse data sets collected around the world (oceanographic fleets, automated observation systems deployed in situ), and available among various data centers aiming at preserving and making reusable marine observations (from physics to chemistry and biology)

The dedicated *Ecopart* application maintained at Institut de la Mer de Villefranche sur Mer allows investigators to save, display and export particle and image data for the scientific community or for the public. Data availability is set by the data owners for each of the saved projects. International discussions regarding long-term image storage and access are ongoing.

Ecotaxa is a web-based application for the classification of images of organisms using a reference taxonomy (www.unieuk.org/). Images, associated metadata and descriptive variables are loaded for each organism and permit identification (automatic classification). The registered users can then validate (check and perform

a finer sorting) on the web by visualizing the classified images using an efficient interface. All operations are recorded, to allow close control of tasks. www.ecotaxa.obs-vlfr.fr
Data dissemination infrastructure and likely data latency. If further shore-based analysis is necessary (e.g., image classification and validation), the likely personnel time associated
Data can be stored indefinitely, and analysis can therefore be executed as personnel become available. Particle size distributions are generated automatically. However, image analysis for zooplankton biodiversity and concentrations requires an experienced analyst for checking and quality control of images. Depending on the number of casts, a large basin-wide transect can take on the order of 1-2 months of post-processing for the highest levels of quality control.
A list of relevant experts who can be called upon to help and know they have been identified
Marc Picheral marc.picheral@obs-vlfr.fr Lars Stemmann stemmann@obs-vlfr.fr Lionel Guidi lguidi@obs-vlfr.fr Rainer Kiko rkiko@geomar.de Andreas Rogge andreas.rogge@awi.de

12.3.8 Acoustic backscattering (ADCP) recommendations

Acoustic backscattering recommendations for plankton-related measurements onboard research vessels and the GO-SHIP program. Contribution from the Bio-acoustic subgroup.

Application to key science needs and questions to support the measurement of biological EOVs
Acoustic backscattering, depending on frequency, is a proxy for particles spanning from mm to m. As such it is related to zooplankton abundance and fish distribution EOVs.
Broad accessibility of the infrastructure, ease of use and availability of necessary resources
Widely accessible (already required on GO-SHIP). Protocols exist including for data quality. For quantitative output requires absolute calibration.
Minimal interruption to current GO-SHIP standard operations
Already GO-SHIP core measurement.

Instrument name and description
Acoustic Doppler Current Profile . Sensor measures the Doppler shift of sound scattered back to the sensor from particles in the water. Volume backscattering strength (denoted as Sv) is indicative of the density of particles in the water column. Mean vertical velocity is often indicative of migration speed of organisms.
Instrument manufacturer

RD Instruments, Nortek.
Amount of water per sample
Hundreds of liters.
Sampling
Hull mounted and/or CTD rosette.
Price per sample
N/A
Price per instrument
No additional cost. Already a core measurement for GO-SHIP.
Maintenance cost per instrument
N/A
Personnel time to run a sample
N/A
Necessary infrastructure onboard:
N/A
Shipping needs (e.g., temperature at which samples need to be maintained, facilities available for sample analysis)
N/A
Ancillary measurements (beyond GPS, e.g., Temperature, light level etc.)
Deployed in conjunction with a hull-mounted quantitative echosounder with similar frequency Sv can be better constrained. CTD to provide sound speed profile and absorption coefficient.
Ecosystem/biogeochemical model parameter constrained by this measurement
Depending on sound frequency used, the biomass and behavior of different types of zooplankton and fish can be constrained.
Existing protocols and relevant publications
Obvious synergies with current measurements performed on board GO-SHIP cruises and other measurements proposed here
Synergies exist with imaging and genomics sampling proposed here.
Standardized vocabulary for the data and the associated metadata, for managing into the large and diverse data sets collected around the world (oceanographic fleets, automated observation systems deployed in situ), and available among various data centers aiming at preserving and making reusable marine observations (from physics to chemistry and biology)
Data dissemination infrastructure and likely data latency. If further shore-based analysis is necessary (e.g., image classification and validation), the likely personnel time associated
Data processing and QA/QC for a whole cruise will take about a week. Necessary to have access to raw data.

A list of relevant experts who can be called upon to help and know they have been identified
For all aspects of measurement: Ryan Downie (Ryan.Downie@csiro.au) Peter Gaube (pgaube@apl.washington.edu) Rudy Kloser (Rudy.Kloser@csiro.au) Wu-Jung Lee (wjlee@apl.washington.edu)

12.3.9 Quantitative echosounding recommendations

Quantitative echosounding recommendations for plankton-related measurements onboard research vessels and the GO-SHIP program. Contribution from the Bio-acoustic subgroup.

Recommendations for measurements appropriate for GO-SHIP based on the following criteria

Application to key science needs and questions to support the measurement of biological EOVs
Acoustic backscattering, depending on frequency, is a proxy for particles spanning from mm to m. As such it is related to the zooplankton abundance and fish distribution and abundance Essential Ocean Variables (EOVs). Acoustic backscatter is seen as a key element in developing an ecosystem EOVs for biological parameters such as zooplankton and nekton. To do this effectively requires the integration of observations with models given the complexity of the task (e.g., Handegard et al. 2013). Recent developments have significantly advanced the incorporation of acoustic data into ecosystem models and proposing that acoustic backscatter become an EOVs (Lehodey, 2019). These data could inform our knowledge of the status and trend of global open ocean ecosystems address two key science questions being, 1) understanding potential risks and rewards of the open ocean mesopelagics to be a global food resource and 2) the role of mesopelagics in active carbon sequestration (St John et al., 2016, Boyd et al., 2019). Constable, Andrew J., Daniel P. Costa, Oscar Schofield, Louise Newman, Edward R. Urban Jr, Elizabeth A. Fulton, Jessica Melbourne-Thomas et al. "Developing priority variables ("ecosystem Essential Ocean Variables"—eEOVs) for observing dynamics and change in Southern Ocean ecosystems." *Journal of Marine Systems* 161 (2016): 26-41. Handegard, N. O., Buisson, L. D., Brehmer, P., Chalmers, S. J., De Robertis, A., Huse, G., ... & Stenseth, N. C. (2013). Towards an acoustic-based coupled observation and modelling system for monitoring and predicting ecosystem dynamics of the open ocean. *Fish and Fisheries*, *14*(4), 605-615. Lehodey, P. (2019). Report of the 3rd MESOPP Workshop: Designing the needs for the implementation of a global coupled acoustic-based observation-modelling system. Third MESOPP Workshop 9-11 Oct 2018, Falmouth, USA, MESOPP-19-0001: 32 pp. www.mesopp.eu/documents/

St John, M. A., Borja, A., Chust, G., Heath, M., Grigorov, I., Mariani, P., ... & Santos, R. S. (2016). A dark hole in our understanding of marine ecosystems and their services: perspectives from the mesopelagic community. *Frontiers in Marine Science*, *3*, 31.
Broad accessibility of the infrastructure, ease of use and availability of necessary resources
Accessible (already exist on some vessels used in GO-SHIP, used in OOI). Protocols exist including for data quality. For quantitative output requires absolute calibration.
Minimal interruption to current GO-SHIP standard operations
There is an issue of potential interference with ADCP on board GO-SHIP. ADCP is a core measurement and the quantitative eco-sounder will need to be tuned such that it does not interfere. Note that recently some echosounders have been able to also measure ADCP like signals (https://www.kongsberg.com/maritime/products/mapping-systems/fishery-research/scientific-echo-sounders/ec150-3c?OpenDocument) making it possible to avoid interference issues.

Instrument name and description
Echosounder Sensor measures the sound scattered back to the sensor from particles in the water. Volume backscattering strength (Sv) is indicative of the density of particles in the water column when the type of particle is known or can be inferred. If split-beam system is used, target strength (TS), corresponding to the size of organisms, can be measured. Temporal changes in acoustics scattering layers are often indicative of organism behavior.
Instrument manufacturer
Simrad, Biosonics, ASL Environmental Sciences, HTI, Nortek
Amount of water per sample
hundreds of liters
Sampling
Hull-mounted
Price per sample
N/A
Price per instrument
Varies from $40,000 (one transducer/bandwidth)-300,000 (full wideband system). Most research vessels already have scientific echosounders (e.g., Simrad EK60/80 echosounders)
Maintenance cost per instrument
Calibration cost: ~$5,000 recommended once a year.
Personnel time to run a sample

N/A
Necessary infrastructure onboard:
Vessel equipped with a digital calibratable scientific echosounder at any or all of the following frequencies 12, 18, 38, 70, 120, 200, 333 kHz, or similar with broadband capacity.
Shipping needs (e.g., temperature at which samples need to be maintained, facilities available for sample analysis)
N/A
Ancillary measurements (beyond GPS)
CTD to provide sound speed profile, pressure and temperature, which can be used to calculate the acoustical absorption coefficient. Ground truth if possible (nets, imaging).
Ecosystem/biogeochemical model parameter constrained by this measurement
The biomass and behavior of different types of zooplankton, jellies, cephalopods and fish can be constrained and is dependent on frequencies employed. Ecosystem models and data assimilation methods have been developed and are continuing to be developed internationally to incorporate bioacoustics data (e.g., Lehodey et al. 2014, MESOPP.org.eu). Lehodey, P., Conchon, A., Senina, I., Domokos, R., Calmettes, B., Jouanno, J., ... & Kloser, R. (2014). Optimization of a micronekton model with acoustic data. *ICES Journal of Marine Science*, *72*(5), 1399-1412.
Existing protocols and relevant publications
There exists a rich literature on the use of scientific echosounders to measure zooplankton[KR(H1] to fish in the upper ~1,000m of the ocean (Simmonds and MacLennan [WJL2] 2005, Kloser et al. 2009, Handegard et al., 2013). Methods and protocols have been developed to collect, store and process the data (Ryan et al. 2015, Wall et al. 2016, Harris et al., 2018, Macaulay and Peña 2018[WJL3] , Lee and Staneva, 2019[WJL4]). These data have been used to make some global inferences of biological structures and biomass (e.g., Proud et al., 2018a, proud et al., 2018b). Handegard, N. O., Buisson, L. D., Brehmer, P., Chalmers, S. J., De Robertis, A., Huse, G., ... & Stenseth, N. C. (2013). Towards an acoustic-based coupled observation and modelling system for monitoring and predicting ecosystem dynamics of the open ocean. *Fish and Fisheries*, *14*(4), 605-615. Haris, K., Kloser, R., and Ryan, T. 2018. IMOS SOOP-BA NetCDF Conventions (Version2.2). Integrated Marine Observing System: CSIRO Report No. EP185001. 42pp Kloser, R. J., Ryan, T. E., Young, J. W., & Lewis, M. E. (2009). Acoustic observations of micronekton fish on the scale of an ocean basin: potential and challenges. *ICES Journal of Marine Science*, *66*(6), 998-1006. Lee, W.-J., and Staneva, V. (**2019**). "Echopype: Enhancing the Interoperability and Scalability of Ocean Sonar Data Processing for Biological Information," Sci. Comput. with Python 2019, Austin, Texas.

Macaulay, G. and Peña, H. (Eds.). 2018. The SONAR-netCDF4 convention for sonar data, Version 1.0. ICES Cooperative Research Report No. 341. 33 pp. https://doi.org/10.17895/ices.pub.4392

Proud, R., Cox, M. J., Le Guen, C., & Brierley, A. S. (2018). Fine-scale depth structure of pelagic communities throughout the global ocean based on acoustic sound scattering layers. *Marine Ecology Progress Series*, *598*, 35-48.

Proud, R., Handegard, N. O., Kloser, R. J., Cox, M. J., & Brierley, A. S. (2018). From siphonophores to deep scattering layers: uncertainty ranges for the estimation of global mesopelagic fish biomass. *ICES Journal of Marine science*, *76*(3), 718-733.

Ryan, T. E., Downie, R. A., Kloser, R. J., & Keith, G. (2015). Reducing bias due to noise and attenuation in open-ocean echo integration data. *ICES Journal of Marine Science*, *72*(8), 2482-2493.
Simmonds, E. J., MacLennan, D. N. (2005). *Fisheries acoustics : theory and practice*, Blackwell Science, 437 pages.
Wall, C. C., Jech, J. M., & McLean, S. J. (2016). Increasing the accessibility of acoustic data through global access and imagery. *ICES Journal of Marine Science*, *73*(8), 2093-2103.

Obvious synergies with current measurements performed on board GO-SHIP cruises and other measurements proposed here

Synergies exist with CTD, in-situ imaging and genomics sampling proposed here.

Standardized vocabulary for the data and the associated metadata, for managing into the large and diverse data sets collected around the world (oceanographic fleets, automated observation systems deployed in situ), and available among various data centers aiming at preserving and making reusable marine observations (from physics to chemistry and biology)

There are well established and internationally accepted processing methods for bioacoustics data with established data storage and metadata protocols[WJL1] .

ICES. 2016. A metadata convention for processed acoustic data from active acoustic systems. Series of ICES Survey Protocols SISP 4-TG-AcMeta. 48 pp.

Macaulay, G. and Peña, H. (Eds.). 2018. The SONAR-netCDF4 convention for sonar data, Version 1.0. ICES Cooperative Research Report No. 341. 33 pp. https://doi.org/10.17895/ices.pub.4392
(e.g., http://imos.org.au/facilities/shipsofopportunity/bioacoustic/ , mesopp.org.au, https://www.ngdc.noaa.gov/mgg/wcd/, http://imos.org.au/fileadmin/user_upload/shared/SOOP/BASOOP/IMOS_SOOP-BA_NetCDF_Conventions_Version_2.2.pdf)

Data dissemination infrastructure and likely data latency. If further shore-based analysis is necessary (e.g., image classification and validation), the likely personnel time associated

Data processing and QA/QC will depend on the purpose, but with current automated routines it is possible to have Level 1 (raw data) and Level 2 (automated processing)

products without the significant improvement of shore-based personnel. This level of calibrated data are available for further analysis to suit user requirements. Existing data center infrastructure should be capable of housing and disseminating the data. Processing time for a whole cruise is on the order of a week.
A list of relevant experts who can be called upon to help and know they have been identified
For all aspects of measurement: Ryan Downie (Ryan.Downie@csiro.au) Peter Gaube (pgaube@apl.washington.edu) Rudy Kloser (Rudy.Kloser@csiro.au) Wu-Jung Lee (wjlee@apl.washington.edu) Mei Sato (m.sato@oceans.ubc.ca)

12.3.10 Photosynthetically available radiation (PAR) recommendations

PAR recommendations for plankton-related measurements onboard research vessels and the GO-SHIP program. Contribution from the Bio-optics subgroup.

Recommendations for measurements appropriate for GO-SHIP based on the following criteria

Application to key science needs and questions to support the measurement of biological EOVS
PAR provides a measure of the solar energy available for photosynthesis. Related to phytoplankton EOVS.
Broad accessibility of the infrastructure, ease of use and availability of necessary resources
Widely accessible (used on BGC-Argo and OOI). Protocols exist including for data quality.
Minimal interruption to current GO-SHIP standard operations
No interruption, instrument is integrated into CTD rosette to measure and/or installed on the R/V to measure above surface PAR.

Instrument name and description
PAR sensor. Sensor provide a quantitative measure of solar energy available for photosynthesis. A spherical (scalar) sensor is recommended for in-water measurement (though may not be available for depth > 2,000m) in which case a cosine collector is an OK substitute (to avoid needing to take on/off rosette each time a deep cast takes place). For above water, one with cosine-collector suffices. Unnecessary If spectral measurements of radiation are performed in sufficient bands.
Instrument manufacturer
Biospherical, Sea-Bird, LIcor, Trios
Amount of water per sample

N/A
Sampling
On CTD and on R/V. Typical frequency is ~1Hz. Both analogue and digital outputs are possible.
Price per sample
N/A
Price per instrument
~ $1,000
Maintenance cost per instrument
Yearly calibration (~$200).
Personnel time to run a sample
Daily cleaning recommended (DIW rinse, tap with isopropyl alcohol. Mild detergent if dirty) – 5min.
Necessary infrastructure onboard:
Above water: room to install sensor with minimal shading of sun. A gimbal, a power source and datalogger (possibly part of the weather station). sensors stays oriented upward. On CTD: Attach to top of rosette to minimize shading. On CTD needs a port to provide power and receive data.
Shipping needs (e.g., temperature at which samples need to be maintained, facilities available for sample analysis)
N/A
Ancillary measurements (beyond GPS, e.g., Temperature, light level etc.)
N/A
Ecosystem/biogeochemical model parameter constrained by this measurement
Solar forcing for net primary production.
Existing protocols and relevant publications
Ruddick, K.G.; Voss, K.; Banks, A.C.; Boss, E.; Castagna, A.; Frouin, R.; Hieronymi, M.; Jamet, C.; Johnson, B.C.; Kuusk, J.; Lee, Z.; Ondrusek, M.; Vabson, V.; Vendt, R. 2019. A Review of Protocols for Fiducial Reference Measurements of Downwelling Irradiance for the Validation of Satellite Remote Sensing Data over Water. Remote Sens., 11, 1742. QARTOD manual - https://cdn.ioos.noaa.gov/media/2017/12/QARTODOceanOptics_v1.1_Final.pdf
Obvious synergies with current measurements performed on board GO-SHIP cruises and other measurements proposed here
Synergies exist with HPLC pigment and all bio-optical parameters as phytoplankton adapt to light in their distribution in the water column and pigmentation. Light strongly affects vertical migration of organisms. Other synergies: Support validation PAR product obtained from space.

Standardized vocabulary for the data and the associated metadata, for managing into the large and diverse data sets collected around the world (oceanographic fleets, automated observation systems deployed in situ), and available among various data centers aiming at preserving and making reusable marine observations (from physics to chemistry and biology)
See: http://www.oceanopticsbook.info/
Data dissemination infrastructure and likely data latency. If further shore-based analysis is necessary (e.g., image classification and validation), the likely personnel time associated
SeaBASS and PANGAEA have PAR data within them. Data processing and QA/QC will take on the order of a day.
A list of relevant experts who can be called upon to help and know they have been identified
For all aspects of measurement: Emanuele Organelli (emanuele.organelli@obs-vlfr.fr) Robert Frouin (rfrouin@ucsd.edu)

12.3.11 Backscattering recommendations

Backscattering recommendations for plankton-related measurements onboard research vessels and the GO-SHIP program. Contribution from the Bio-optics subgroup.

Recommendations for measurements appropriate for GO-SHIP based on the following criteria

Application to key science needs and questions to support the measurement of biological EOVs
Backscattering is a proxy for both POC and phytoplankton carbon (in the surface ocean). As such, it is related to the phytoplankton EOV.
Broad accessibility of the infrastructure, ease of use and availability of necessary resources
Widely accessible (e.g., on all OOI moorings). Protocols exist including for data quality. Has been used on GO-SHIP cruise associated with SOCCOM.
Minimal interruption to current GO-SHIP standard operations
No interruption, instrument is integrated into the rosette system and/or flow-through system. Requires a data/power port on the CTD.

Instrument name and description
Backscattering sensor. Sensor measures light backscattered around a specific angle in the back direction typically at several wavelengths. Having a 700 nm measurement is recommended for comparison with data collected with BGC-Argo.
Instrument manufacturer
Seabird Scientific

Amount of water per sample
O(2ml)
Sampling
CTD Rosette - Attached to Rosette near the bottom facing undisturbed water. Make sure it is far from instruments that have light sources that may be scattered into sensing volume (e.g., UVP) and is pointed to the side or downward and not facing any surface that may scatter light to it.. Flow-through - Sensor is deployed in a dedicated waterproof sampling box. Typical frequency is 1Hz.
Price per sample
N/A
Price per instrument
Depends on number of channels (that is, can be combined with fluorometer(s) and other backscattering channels). About $4,000 per channel. If used in flow-through mode, requires a custom-made box (see Boss et al., 2019 below), which costs ~ $3,000 (custom made to reject reflected light from sides of box).
Maintenance cost per instrument
Calibration cost: $800, recommended once per year (for slope parameter).
Personnel time to run a sample
Daily cleaning recommended (DIW rinse, tap with isopropyl alcohol. Mild detergent if dirty) - 5min. Twice in a cruise, profile the sensor with black tape to evaluate the value of the dark current on the sensor.
Necessary infrastructure onboard:
Port on CTD for data and power. Could share a port with another sensor (Y-cable or a combined sensor). Dependent on model output is analogue, digital or both.
Shipping needs (e.g., temperature at which samples need to be maintained, facilities available for sample analysis)
N/A
Ancillary measurements (beyond GPS, e.g., Temperature, light level etc.)
Temperature and salinity - for removal of signal from salts in the computation of particulate backscattering. POC samples for proxy calibration
Ecosystem/biogeochemical model parameter constrained by this measurement
A proxy for POC and Phyto_C in the surface ocean.
Existing protocols and relevant publications
Sullivan, J., M. Twardowski, J. Zaneveld, and C. Moore, **"**Measuring optical backscattering in water,**"** in Light Scattering Reviews 7, A. A. Kokhanovsky, ed. (Springer, 2013), pp. 189–224.

Schmechtig Catherine, Poteau Antoine, Claustre Hervé, D'Ortenzio Fabrizio, Dall'Olmo Giorgio, Boss Emmanuel (2018). Processing Bio-Argo particle backscattering at the DAC level. Argo data management. https://doi.org/10.13155/39459 QARTOD manual - https://cdn.ioos.noaa.gov/media/2017/12/QARTODOceanOptics_v1.1_Final.pdf Talley et al., 2019. SOCCOM BGC floats: shipboard calibration data requirements. https://drive.google.com/file/d/1ERefhCuV-bjxUvwBu13dJgtQmQo_Iv5R/view Boss et al., 2019. Best practices for the collection and processing of ship-based underway flow-through optical data. https://ioccg.org/what-we-do/ioccg-publications/ocean-optics-protocols-satellite-ocean-colour-sensor-validation/
Obvious synergies with current measurements performed on board GO-SHIP cruises and other measurements proposed here
Backscattering, chlorophyll and beam-attenuation covary near the surface. Their ratio are indicative of particle composition. Backscattering is related to POC and HPLC and spikes in backscattering are related to rare large particles. Total cross-section of particles from imaging and sizing system are related to particulate backscattering. With chlorophyll fluorescence (Fchl) could be used to analyze phytoplankton photo-physiology.
Standardized vocabulary for the data and the associated metadata, for managing into the large and diverse data sets collected around the world (oceanographic fleets, automated observation systems deployed in situ), and available among various data centers aiming at preserving and making reusable marine observations (from physics to chemistry and biology)
See: http://www.oceanopticsbook.info/
Data dissemination infrastructure and likely data latency. If further shore-based analysis is necessary (e.g., image classification and validation), the likely personnel time associated
SeaBASS, NODC, PANGAEA all have backscattering data within them. Data processing and QA/QC will take on the order of a day.
A list of relevant experts who can be called upon to help and know they have been identified
For all aspects of measurement: Giorgio Dall'Olmo (gdal@pml.ac.uk) Michael Twardowski (mtwardowski@fau.edu) Nathan Briggs (nathan.briggs@noc.ac.uk)

12.3.12 Chlorophyll fluorescence recommendations

Chlorophyll fluorescence recommendations for plankton-related measurements onboard research vessels and the GO-SHIP program. Contribution from the Bio-optics subgroup.

Recommendations for measurements appropriate for GO-SHIP based on the following criteria

Application to key science needs and questions to support the measurement of biological EOVS
Chlorophyll fluorescence (Fchl) is a proxy for phytoplankton chlorophyll_a concentration. As such it is related to the phytoplankton EOVS.
Broad accessibility of the infrastructure, ease of use and availability of necessary resources
Widely accessible (e.g., on all OOI moorings) protocols exist including for data quality. Has been used on GO-SHIP cruises associated with SOCCOM.
Minimal interruption to current GO-SHIP standard operations
No interruption, instrument is integrated into the rosette system and/or flow-through system. Requires a data/power port on the CTD.

Instrument name and description
Chlorophyll fluorescence sensor. Sensor emits an excitation wavelength in the blue and measures the red light which emanates from the illuminated water volume.
Instrument manufacturer
Seabird Scientific (most common on BGC-Argo floats). Sensors by Seapoint, Chelsea, and other manufacturer exists.
Amount of water per sample
O(2ml)
Sampling
CTD Rosette - Attached to rosette near the bottom facing undisturbed water. Make sure it is far from instruments that have light sources that may be scattered into sensing volume (e.g., UVP). Flow-through - Sensor is deployed in a dedicated waterproof, black sampling box. Typical frequency is 1 Hz.
Price per sample
N/A
Price per instrument
About $4,000. Varies with manufacturer.
Maintenance cost per instrument
Calibration cost: $800. Can be calibrated with samples collected on board.
Personnel time to run a sample
Daily cleaning recommended (DIW rinse, tap with isopropyl alcohol. Mild detergent if dirty) – 5min.
Necessary infrastructure onboard:
Port on CTD for data and power. Could share a port with another sensor (Y-cable or a combined sensor). Dependent on make/model output is analogue, digital or both.
Shipping needs (e.g., temperature at which samples need to be maintained, facilities available for sample analysis)

N/A
Ancillary measurements (beyond GPS)
HPLC samples for calibration
Ecosystem/biogeochemical model parameter constrained by this measurement
A proxy for chlorophyll a, a pigment shared by all phytoplankton. Note: measurements near the surface and during the day suffers from depression of fluorescence per chlorophyll (termed non-photochemical quenching). Method to correct for this exists (applied to BGC-Argo).
Existing protocols and relevant publications
Roesler, C., J. Uitz, H. Claustre, E. Boss, X. Xing, E. Organelli, N. Briggs, A. Bricaud, C. Schmechtig, A. Poteau, F. D'Ortenzio, J. Ras, S. Drapeau, N. Haëntjens and M. Barbieux, 2017. Recommendations for obtaining unbiased chlorophyll estimates from in situ chlorophyll fluorometers: A global analysis of WET Labs ECO sensors. Limnology and Oceanography, Methods, DOI: 10.1002/lom3.10185. Schmechtig C., Claustre H., Poteau A., D'Ortenzio F. (2018). Bio-Argo quality control manual for the Chlorophyll-A concentration. https://doi.org/10.13155/35385 QARTOD manual - https://cdn.ioos.noaa.gov/media/2017/12/QARTODOceanOptics_v1.1_Final.pdf Talley et al., 2019. SOCCOM BGC floats: shipboard calibration data requirements. https://drive.google.com/file/d/1ERefhCuV-bjxUvwBu13dJgtQmQo_Iv5R/view Boss et al., 2019. Best practices for the collection and processing of ship-based underway flow-through optical data. https://ioccg.org/what-we-do/ioccg-publications/ocean-optics-protocols-satellite-ocean-colour-sensor-validation/
Obvious synergies with current measurements performed on board GO-SHIP cruises and other measurements proposed here
Fchl, backscattering, and beam-attenuation covary near the surface. Their ratios are indicative of particle composition. Fchl is related to the total chlorophyll-a concentration determined by HPLC. Spikes in backscattering are related to rare large particles containing chlorophyll. Total cross-section of phytoplankton from imaging and sizing system are related to Fchl.
Standardized vocabulary for the data and the associated metadata, for managing into the large and diverse data sets collected around the world (oceanographic fleets, automated observation systems deployed in situ), and available among various data centers aiming at preserving and making reusable marine observations (from physics to chemistry and biology)
See: http://www.oceanopticsbook.info/
Data dissemination infrastructure and likely data latency. If further shore-based analysis is necessary (e.g., image classification and validation), the likely personnel time associated
SeaBASS, NODC, PANGAEA all have Fchl data within them. Data processing and QA/QC will take on the order of a day.

A list of relevant experts who can be called upon to help and know they have been identified
For all aspects of measurement: Collin Roesler (croesler@bowdoin.edu) Xiaogang Xing (xing@sio.org.cn) Nathan Briggs (nathan.briggs@noc.ac.uk)

12.3.13 Beam attenuation recommendations

Beam attenuation recommendations for plankton-related measurements onboard research vessels and the GO-SHIP program. Contribution from the Bio-optics subgroup.

Recommendations for measurements appropriate for GO-SHIP based on the following criteria

Application to key science needs and questions to support the measurement of biological EOVs
Beam attenuation is a proxy for POC and total suspended mass. As such it is related to the phytoplankton abundance EOV.
Broad accessibility of the infrastructure, ease of use and availability of necessary resources
Widely accessible (optical parameter with longest history of commercial instruments) protocols exist including for data quality. Already GO-SHIP level 2 data.
Minimal interruption to current GO-SHIP standard operations
No interruption, instrument is integrated into the rosette system and/or flow-through system. Requires a data/power port on the CTD.

Instrument name and description
Transmissometer, beam-c, or beam-attenuation sensor. Sensor measures light transmitted through the water typically at 650 nm wavelength. Length is typically 25 cm long and acceptance angle (AA) of receiver is 1.2degrees (C-star sensor). There exist spectral beam transmissometers (SBE ac-s, AA=0.93 degrees, 25cm) and laser based systems (Sequoia LISST 100X, 670nm, AA=0.02 degrees, path-length 5cm).
Instrument manufacturer
Seabird Scientific (C-star, and ac-s sensor), Sequoia Sci. (LISST 100X and 200 sensors).
Amount of water per sample
O(50ml) for 25 cm path length.
Sampling
CTD Rosette - Attached to Rosette near the bottom. Can be pumped through. Flow-through - Sensor is deployed with flow sleeves or flow chambers. Typical frequency is ~1Hz.
Price per sample
N/A

Price per instrument
About $7,000 for single wavelength and deep sensor. $40,000 for ac-s and $38,000 for LISST (the last two provide significantly more information).
Maintenance cost per instrument
Calibration cost: $1000. Could be done by experience user except temperature compensation table (ac-s) and alignment (LISST).
Personnel time to run a sample
Daily cleaning recommended (DIW rinse, tap with isopropyl alcohol, mild detergent if dirty) - 5min. Once per week (and before and after cruise) determine dark current by measuring signal with interrupted beam (c-star). Run DIW through for absolute calibration (necessary for ac-s on a near-daily frequency).
Necessary infrastructure onboard:
Port on CTD for data and power (c-star and LISST). Could share a port with another sensor (Y-cable or a combined sensor). Dependent on model, output is analogue, digital or both.
Shipping needs (e.g., temperature at which samples need to be maintained, facilities available for sample analysis)
N/A
Ancillary measurements (beyond GPS)
Temperature and salinity - for removal of signal from salts if measurements are done with ac-s. PM or POC samples for proxy calibration. One must be cautious with POC below a few hundred meters because POC becomes very small.
Ecosystem/biogeochemical model parameter constrained by this measurement
A proxy for POC in the surface ocean.
Existing protocols and relevant publications
IOCCG Protocol Series (2019). Beam Transmission and Attenuation Coefficients: Instruments, Characterization, Field Measurements and Data Analysis Protocols. Boss, E., Twardowski, M., McKee, D., Cetinić, I. and Slade, W. IOCCG Ocean Optics and Biogeochemistry Protocols for Satellite Ocean Colour Sensor Validation, Volume 2.0, edited by A. Neeley and I. Cetinić, IOCCG, Dartmouth, NS, Canada. http://dx.doi.org/10.25607/OBP-458 Sampling and Sample-handling Protocols for GEOTRACES Cruises (Cookbook, version 3.0, 2017). http://www.geotraces.org/images/Cookbook.pdf QARTOD manual - https://cdn.ioos.noaa.gov/media/2017/12/QARTODOceanOptics_v1.1_Final.pdf Boss et al., 2019. Best practices for the collection and processing of ship-based underway flow-through optical data. https://ioccg.org/what-we-do/ioccg-publications/ocean-optics-protocols-satellite-ocean-colour-sensor-validation/
Obvious synergies with current measurements performed on board GO-SHIP cruises and other measurements proposed here

Beam-attenuation, particulate backscattering, and chlorophyll covary near the ocean's surface. Their ratios are indicative of particle composition. Beam attenuation is related to POC and spikes in attenuation are related to rare large particles. Total cross-section of particles from imaging and sizing system are related to the beam attenuation.
Standardized vocabulary for the data and the associated metadata, for managing the large and diverse data sets collected around the world (oceanographic fleets, automated observation systems deployed in situ), and available among various data centers aiming at preserving and making reusable marine observations (from physics to chemistry and biology)
See: http://www.oceanopticsbook.info/
Data dissemination infrastructure and likely data latency. If further shore-based analysis is necessary (e.g., image classification and validation), the likely personnel time associated
SeaBASS, NODC, PANGAEA all have backscattering data within them. Data processing and QA/QC will take on the order of a day.
A list of relevant experts who can be called upon to help and know they have been identified
For all aspects of measurement: Wilf Gardner (wgardner@ocean.tamu.edu) Wayne Slade (wayne.slade@gmail.com) Ivona Cetinic (ivona.cetinic@nasa.gov)

12.3.14 Angular scattering (LISST – particle size distribution) recommendations

Angular scattering recommendations for plankton-related measurements onboard research vessels and the GO-SHIP program. Contribution from the Bio-optics subgroup.

Recommendations for measurements appropriate for GO-SHIP based on the following criteria

Application to key science needs and questions to support the measurement of biological EOVs
Near-forward scattering is a proxy of the particulate size distribution of particles from 2-200 μm. As such it is related to the phytoplankton abundance EOV.
Broad accessibility of the infrastructure, ease of use and availability of necessary resources
Widely accessible. protocols exist including for data quality. Has been deployed on GO-SHIP lines.
Minimal interruption to current GO-SHIP standard operations
No interruption, instrument is integrated into the rosette system and/or flow-through system. Can self-log and power or be logged and powered externally.

Instrument name and description
LISST - laser in situ scattering and transmittance sensor.
Instrument manufacturer

Sequoia Scientific
Amount of water per sample
~10 ml.
Sampling
Flow-through - Sensor is deployed with a flow chambers. Typical frequency is ~1 Hz which could be changed to maximize S/N.
Price per sample
N/A
Price per instrument
About $35,000.
Maintenance cost per instrument
Calibration cost: $1000.
Personnel time to run a sample
Daily cleaning recommended (DIW rinse, tap with isopropyl alcohol, mild detergent if dirty) - 5min. Once a week (and before and after cruise) determine zscat using filtered seawater.
Necessary infrastructure onboard:
Power/data for inline.
Shipping needs (e.g., temperature at which samples need to be maintained, facilities available for sample analysis)
N/A
Ancillary measurements (beyond GPS)
N/A
Ecosystem/biogeochemical model parameter constrained by this measurement
In-situ particles size distribution provides information on particle concentration, aggregates and settling.
Existing protocols and relevant publications
Boss, E., N. Haentjens, T. K. Westberry, L. Karp-Boss, and W. Slade, 2018. Validation of the particle size distribution obtained with the laser in-situ scattering and transmission (LISST) meter in flow-through mode. Optics Express, 26(9), 11125-11136. https://doi.org/10.1364/OE.26.011125 QARTOD manual - https://cdn.ioos.noaa.gov/media/2017/12/QARTODOceanOptics_v1.1_Final.pdf Boss et al., 2019. Best practices for the collection and processing of ship-based underway flow-through optical data. https://ioccg.org/what-we-do/ioccg-publications/ocean-optics-protocols-satellite-ocean-colour-sensor-validation/
Obvious synergies with current measurements performed on board GO-SHIP cruises and other measurements proposed here
Imaging systems such as the IFCB and UVP.

Standardized vocabulary for the data and the associated metadata, for managing the large and diverse data sets collected around the world (oceanographic fleets, automated observation systems deployed in situ), and available among various data centers aiming at preserving and making reusable marine observations (from physics to chemistry and biology)
See: http://www.oceanopticsbook.info/
Data dissemination infrastructure and likely data latency. If further shore-based analysis is necessary (e.g., image classification and validation), the likely personnel time associated
SeaBASS and PANGAEA have LISST data within them. Data processing and QA/QC will take on the order of a day.
A list of relevant experts who can be called upon to help and know they have been identified
For all aspects of measurement: Wayne Slade (wayne.slade@gmail.com) Benedetto Barone (benedetto.barone@gmail.com) Andrew McDonnell (amcdonnell@alaska.edu)

12.3.15 Spectral attenuation and absorption (AC-s) recommendations

Spectral attenuation and absorption recommendations for plankton-related measurements onboard research vessels and the GO-SHIP program. Contribution from the Bio-optics subgroup.

Recommendations for measurements appropriate for GO-SHIP based on the following criteria

Application to key science needs and questions to support the measurement of biological EOVs
The particulate spectral beam attenuation coefficient is a proxy for POC and total suspended mass. As such it is related to the phytoplankton abundance EOV. It also provides a size index of micron-sized particles (<20um). The particulate absorption coefficient provides a proxy for several phytoplankton pigments including chlorophyll_a. As such it is related to the phytoplankton abundance EOV.
Broad accessibility of the infrastructure, ease of use and availability of necessary resources
Accessible (deployed on OOI moorings). Protocols exist including for data quality. Recommended for deployment in flow-through mode (not CTD).
Minimal interruption to current GO-SHIP standard operations
No interruption, instrument is integrated into the flow-through system. There are requirements from the flow-through system that are detailed in the report referenced below.

Instrument name and description
Transmissometer, beam-c, or beam-attenuation sensor.

Sensor measures light transmitted through the water typically at wavelengths ranging from the blue (~400nm) to the NIR(~750nm). Path-length is typically 25cm long and acceptance angle (AA) of receiver is 0.93 degrees. Absorption sensor. Sensor measures light transmitted through the water typically at wavelengths ranging from the blue (~400 nm) to the NIR, (~750 nm) and uses a special flow cell to collect scattered light into the detector. Length is typically 25cm long.
Instrument manufacturer
Seabird Scientific.
Amount of water per sample
50ml of water per sample for 25 cm path-length.
Sampling
Flow-through - Sensor is deployed with flow sleeves. Typical frequency is ~4 Hz.
Price per sample
N/A
Price per instrument
~ $40,000 For this method an automated valve(e.g., Sequoia Sci. Flow Control, https://www.sequoiasci.com/product/flowcontrol-lab/, ~$10,000), a vortex debubbler (can be purchased through SBE or built by user) and a filter housing with (0.2-um pore size)cartridge filters (~$100 per filter, replaced about once a week) are necessary. Details in Boss et al., 2019, cited below.
Maintenance cost per instrument
Calibration & rebuild cost: $1500. Needs to be done yearly. Lamps have a lifetime of 2-3 mo of continuous operation. Calibration should be performed yearly.
Personnel time to run a sample
Daily cleaning recommended (DIW rinse, tap with isopropyl alcohol. Mild detergent if dirty) – 5min. Run DIW through for absolute calibration if dissolved measurements are of interest (CDOM). Optical flow-through system cleaning takes <0.5hrs daily.
Necessary infrastructure onboard:
Clean pipes and an intake pump that is gentle to particles (see IOCCG flow-through protocol).
Shipping needs (e.g., temperature at which samples need to be maintained, facilities available for sample analysis)
N/A
Ancillary measurements (beyond GPS)
Temperature and salinity - for removal of signal from saltwater. POC samples for proxy calibration.
Ecosystem/biogeochemical model parameter constrained by this measurement

Beam attenuation: A proxy for POC in the surface ocean and a useful size parameter. Absorption: A proxy for several phytoplankton pigments or pigments group in the surface ocean.
Existing protocols and relevant publications
IOCCG Protocol Series (2019). Beam Transmission and Attenuation Coefficients: Instruments, Characterization, Field Measurements and Data Analysis Protocols. Boss, E., Twardowski, M., McKee, D., Cetinić, I. and Slade, W. IOCCG Ocean Optics and Biogeochemistry Protocols for Satellite Ocean Colour Sensor Validation, Volume 2.0, edited by A. Neeley and I. Cetinić, IOCCG, Dartmouth, NS, Canada. http://dx.doi.org/10.25607/OBP-458 IOCCG Protocol Series (2018). Inherent Optical Property Measurements and Protocols: Absorption Coefficient, Neeley, A. R. and Mannino, A. (eds.), IOCCG Ocean Optics and Biogeochemistry Protocols for Satellite Ocean Colour Sensor Validation, Volume 1.0, IOCCG, Dartmouth, NS, Canada. http://dx.doi.org/10.25607/OBP-119 QARTOD manual - https://cdn.ioos.noaa.gov/media/2017/12/QARTODOceanOptics_v1.1_Final.pdf Boss et al., 2019. Best practices for the collection and processing of ship-based underway flow-through optical data. https://ioccg.org/what-we-do/ioccg-publications/ocean-optics-protocols-satellite-ocean-colour-sensor-validation/
Obvious synergies with current measurements performed on board GO-SHIP cruises and other measurements proposed here
Beam-attenuation, particulate backscattering, and chlorophyll (whichever method is used for proxy) all covary to some degree near the ocean's surface. Their ratio are indicative of particle composition. Beam attenuation is related to POC and spikes in attenuation are related to rare large particles. Total cross-section of particles from imaging and sizing system are related to the beam attenuation. Particulate absorption, Beam-attenuation, particulate backscattering, and chlorophyll (whichever proxy is used) all covary to some degree near the ocean surface. Their ratios are indicative of particle composition. Particulate absorption is related to phytoplankton pigments. Total cross-section of phytoplankton from imaging and sizing systems are related to the particulate absorption.
Standardized vocabulary for the data and the associated metadata, for managing the large and diverse data sets collected around the world (oceanographic fleets, automated observation systems deployed in situ), and available among various data centers aiming at preserving and making reusable marine observations (from physics to chemistry and biology)
See: http://www.oceanopticsbook.info/
Data dissemination infrastructure and likely data latency. If further shore-based analysis is necessary (e.g., image classification and validation), the likely personnel time associated
SeaBASS and PANGAEA have spectral particulate data within them.

Data processing and QA/QC will take place on the order of a day.
A list of relevant experts who can be called upon to help and know they have been identified
For all aspects of attenuation measurement: Emmanuel Boss (emmanuel.boss@maine.edu) Wayne Slade (wayne.slade@gmail.com) Giorgio Dall'Olmo (gdal@pml.ac.uk) For all aspects of absorption measurement: Collin Roesler (croesler@bowdoin.edu) Alison Chase (alison.p.chase@maine.edu) Yangyang Liu (yangyang.liu@awi.de)