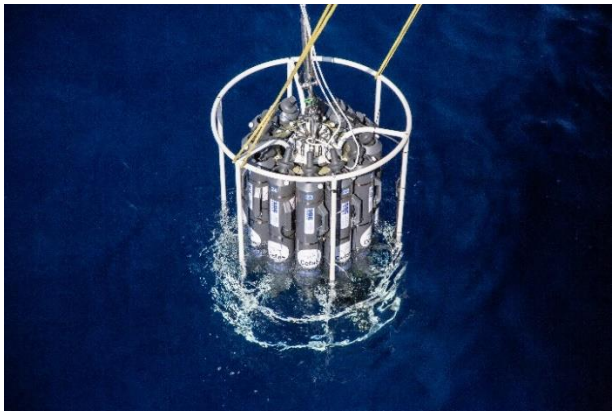
General Ocean Survey and Sampling Iterative Protocol (GOSSIP)

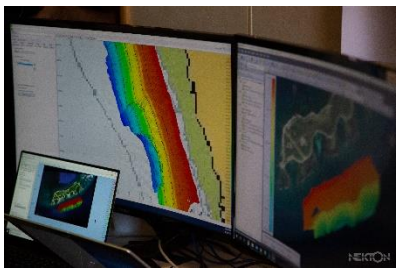
Marine Operations Guide



In 2018 we introduced 20 sampling parameters, that comprised the General Ocean Survey and Sampling Iterative Protocol (GOSSIP), with the aim of proposing a standardised strategy to investigate deep sea life. We hoped that standardising the data collection for deep ocean biological, environmental, chemical, physical, and socioecological processes would enable novel collaborations across scientific disciplines and sectors and facilitate effective knowledge exchange and dissemination.

Many of these parameters have been explored and discussed in detail in the past so this technical guide has been crafted to briefly share a brief overview of each (including what it consists of and why it is important, data collection and gear recommendations, data storage and formatting guidelines, data analysis tools and some top tips). This guide is intended to support initial investigations of project design and provides a reference list of where further, more detailed information can be sought.

Our aim is to create a live document that can be updated continuously allowing us to keep up with advances in sampling gear and data processing standards and be useful for the scientific community. Based on your recommendations and suggestions this document will be updated to include new standards based on new literature and practice.



If you would like to contribute to the guide or have any suggestions or comments, then please contact Nico Fassbender at nico@nektonmission.org.

Additional Resources

Where possible, we provide example data collection sheets and metadata table-header sheets (in the form of Excel, Word or PDF files) which can be found here and on the webpage where this guide is available.

At the end of this guide on p.55, we provide additional metadata formatting guidelines in the form of a short table with some tips to accompany the supplementary material that can be downloaded from the google drive.

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(1) Size structure and species composition of mesozooplankton, pelagic micronekton, and pelagic nekton

To date there is still little knowledge about the mesopelagic zone (200m-1000m) even though it has been hypothesised that it plays a major role when considering seascape biodiversity patterns. With its large spatial range and its inhabitants ranging from a few millimeters to several meters, a range of sampling gear is needed to effectively sample the species living within. Surveying the mesopelagic provides key data for diversity and community studies and the gathered information can be used to ground-truth acoustic gear data and within ecosystem modelling.

Sampling, data collection and storage equipment

The mesopelagic zone is sampled by deploying nets and trawls from the main survey vessel to estimate species diversity, species density, and quantify biomass per unit volume. If possible, different net types would be used, including a multinet, single net and a rectangular midwater trawl (RMT). The RMT possesses either a single or multiple nets which can be remotely controlled to open and close at various depths, depending on the sampling depth intervals chosen and the selected wire time. One oblique tow is normally the most common. Additionally, a flowmeter attached to the nets, coupled with a deck unit with inline data transfer, are mandatory to allow quantitative depth sampling. The net should possess a mesh size that is tailored to the targeted organisms, i.e. mesoplankton nets have a size of 300 µm. When sampling in eutrophic conditions mesh size should be adjusted to prevent clogging of the net. Containers (e.g., centrifuge tubes) of various sizes, depending on the preservation process and sorting capacity on board, are needed to store samples. Depending on the post processing needs samples will be stored in either Formalin (10%) or Ethanol.

Secondly, a high-speed rope trawl can be deployed to sample larger fish and squid in the water column. This method surveys the larger inhabitants of the pelagic that are outside of the scope of multinet plankton sampling deployments. It should be noted that this method presupposed extensive post processing capabilities due to the large amount of biomass caught. It should be carefully considered if the space, time, and effort to process the samples onboard the vessel are available. Otherwise, the additional gear types and sampling possibilities mentioned below offer less destructive and laborious ways to sample pelagic nekton.

Additional gear types include video and photo surveys via remotely operated vehicles (ROV), submersibles, autonomous underwater vehicles (AUV) or even technical divers (for depths <150m). Midwater transects can be conducted across a range of preselected depths and are a great less-destructive alternative to traditional trawls. Combining these with sonar will provide additional information on species target depth.

Ideal data and collection format

Upon its return to the surface, the individual nets should be washed with seawater from the outside to flush all collected material into the cod end. The cod end should then be promptly emptied into a tray or a bucket (for larger samples) and photographed as a whole/parent sample. After, larger samples should be separated into splits and the individual sub-splits should be preserved depending on further processing needs (either in seawater, 10% Formalin or 70% Ethanol). As some organisms are usually weakly represented, a threshold on the number of preserved organisms, depending on the collected samples, should be proposed, to allow for greatest possible diversity. Where bony fish are caught, fin clips can be

taken and should be immediately stored in 98% Ethanol for later DNA analysis and identification. Where video surveys were done, they can be analysed as outlined in detail in the section "(8) Epibenthos" using the SeaGIS program EventMeasure.

Data analysis

Post processing methods include sorting the collected samples in the laboratory with the help of a stereomicroscope and a light microscope to classify the organisms into their taxonomic group. Samples should be identified to the lowest level possible using the help of species identification guides (where available) or DNA sequencing (where accessible). To obtain quantifiable data, individuals should be manually separated, and total or standard length should be measured where possible (depending on the surveyed species). This can be done by taking photos of the collected specimens and using image analysis software that comes with microscope cameras. Alternatively ImageJ can be used. A black background should be used for transparent organisms such as gelatinous zooplankton and decapod larvae. The samples should be weighed (wet weight and dry weight respectively). Taxa weight and wet weight should be done at sea as preservation will alter this measurement, dry weight can be done later and measured at ither taxa level or species level. The total volume sampled can be calculated from flowmeter reads.

With ever increasing post-processing capabilities, new technologies that help with data analysis include machine learning and automated image recognition to identify specimens. Combining data between trawl types needs to be carefully thought through due to the differences in net sizes and water masses sampled.

Top tips

- If sampling capabilities are limited and it is only possible to deploy one system, the RMT should be prioritized.
- Potential new technologies include in-situ holographic imaging and imaging acoustics – these could be applied when available to streamline data collection and analysis.
- Time of deployment should be carefully considered when planning the surveys as local up- or downwelling processes vary throughout the day and might reduce comparability of samples.
- Unwantedly collected biota such as seagrass or macroalgae should be rinsed with seawater so that all plankton and larvae are retained.
- Sturdy scales meant for sea going conditions are mandatory.

Key references

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(2) Acoustic sampling of watercolumn biomass

Scientific echosounders can provide continuous data along survey transects on the distribution and abundance of pelagic fauna in the mm to m size range (i.e mesozooplankton to fish). Data are typically visualised as an 'echogram' where depth (or range) is on the Y axis, distance (or time) is on the X, and echo intensity is represented on a colour scale. Sound spanning the tens to hundreds of kHz bandwidth propagates effectively over thousands to tens of m respectively in seawater: sampling from surface vessels underway can provide data from very large volumes of water in short periods of time, enabling biomass in large areas to be evaluated and distribution to be related, for example, to bathymetric features that acoustic surveys can also resolve. Acoustic sampling does not suffer from net avoidance that can bias net-based surveys. However, although the ability to identify and differentiate species and/or size classes on the basis of acoustic data alone is ever-increasing, echosounding is a kind of 'remote sensing', and sampling with nets and/or video to 'ground truth' acoustic sampling is desirable or essential.

Sampling, data collection and storage equipment

As a very general rule of thumb, targets in the water column (be they bubbles, or mesozooplankton or fish) scatter sound most effectively when the wavelength approximates the target size. Antarctic krill, for example (length c. 4 cm) return stronger echoes at 120 kHz than at 38 kHz. Use of multiple frequencies enables inferences to be made on target identity and on size distributions of known targets. Returned echo intensity can also vary as a function of target orientation (the dorsal aspect of a krill returns a stronger echo than the nose or tail) and tissue density, so acoustic-only identification of targets is not infallible. It is imperative for quantitative analysis of echosounder data that echosounders be properly calibrated for the environmental conditions they are used in: this is achieved by recording echo intensity from calibration spheres, and adjusting system gains until observations match theoretical expectations.

Conventional 'fisheries' echosounders operate over the frequency range 10 to 350 kHz (common frequencies include 18, 38, 70, 120, 200 and 333 kHz; this spacing is adopted to avoid interference across multiples). Echosounders typically sample conical beams (7 degrees is a common beam width, and it is desirable that each single frequency samples the same volume of water), with physics dictating that lower frequency transducers are larger (hence heavier) than higher frequency transducers for the same beamwidth. This has implications for the frequencies that can practically be deployed: although a 7-degree 18 kHz transducer can be accommodated in a tens-of-meters research vessel, it could not easily be deployed on a pole mount from an RIB. In the past c. 15 years progress has been made from multiple single frequency echosounders to 'broadband', whereby chirps of sound spanning multiple kHz are used. This brings advantages in terms of size/species identification and range resolution, but also brings a huge overhead in terms of data storage and processing.

Prominent manufacturers of scientific echosounders include Simrad (Norway) and BioSonics (USA). Both produce a variety of instruments in configurations for use from research vessels or deployment as self-contained units on moorings or autonomous vehicles. In order to facilitate observations at high frequencies in deep water self-contained echosounders can be towed at depth or used in cast mode on station. Numerous data-analysis packages are available for acoustic data, including Echoview (Myriax, Australia) and LSSS (Norway). As well as these commercial options, user-written packages including in Matlab and R are gaining traction. There is an increasing capability for software to read data from a variety of instruments, and moves to adopt common data formats (e.g. HAC). Analysis steps may include scaling echo intensity by target strength (TS; the proportion of sound reflected by an individual of a given species

and size) to provide biomass or abundance, or extraction of echogram 'features' such as fish schools or Deep Scattering Layers to enable behavioral/ecological investigation.

Ideal data and collection format

The previous sections have focused on 'fisheries' echosounders that typically sample a single beam. Multibeam sonars, that same a fan of beams, can be used to build up 3D views of the water column along the transect and to sample entire fish schools / krill swarms. Acoustic Doppler current profilers (ADCPs) can also be used to make inferences on the distribution of 'biologicals', but calibrating echo intensity is not straightforward so ADCPs should not be used for routine biomass estimation.

Data analysis

Covered above

Top tips

- Ensure that echosounders are properly calibrated for the area they are to be used (water temperature and salinity)
- Log raw data: this may be costly in terms of storage, but will enable replay/reanalysis of valuable data
- Be aware of the behavior of the animals you are interested to survey. Many organisms ascend to the near surface at night, for example, so surveys using echosounders in ships' hulls may be blind to the target species at night.

Key references

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(3) Size structure and abundance of gelatinous zooplankton

The species diversity and assemblage structure of gelatinous zooplankton in pelagic waters is diverse and complex, yet very little is known about its dynamics. Gelata are not only key ecosystem components as carbon cyclers, but they also serve as an important link that connects primary production to higher trophic levels. Their delicate bodies make them extremely difficult to study, and thus careful consideration and preparation is needed to effectively sample them, especially at depth.

Sampling, data collection and storage equipment

As most gelatinous specimens are damaged when collected as part of net and trawl surveys, in-situ observations and video surveys are increasingly being utilized to study gelatinous taxa effectively and with minimal invasiveness. Where nets are used, net mesh size, tow speed and hauling speed should be standardized (see Hosia et al., 2017 for a comparison of net and video survey results). Hereby, a trawl system or a multinet are commonly used, with methodological considerations similar to macronekton surveys as described in section 1. ROV, submersible or (technical) SCUBA video transects can be used to quantify gelatinous assemblages. Equipment considerations are similar to other video surveys, where stereo video systems should be used to not only count but also size the observed specimens. For water depths shallower than 500 m, action cameras (i.e. Paralenz or GoPro with deep-water housing) are a cheap yet highly reliable option. If deeper waters are surveyed, specialised deep ocean cameras from DeepSea Power & Light (DSPL) or Sony (Sony's SNC-VB770 using the Sony SEL24F18 lens and, for example, an NXM housing) offer a robust solution. MicroSD cards with sufficient storage space (e.g. SanDisk) can be bought from most tech-vendors, and some of the DSPL cameras even come with built-in memory of up to 1TB. Torches and light systems should be added as appropriate, based on the targeted depths. The collected data must be backed up onto additional harddrives and where possible backup servers on the main vessel daily, ideally after each deployment. The company LaCie offers a range of harddrive sizes and are made to last in non-office conditions.

Ideal data and collection format

Overall sampling effort and equipment use may lead to differences amongst surveys therefore standardization is key to acquire comparable results. The transect methods outlined by Raskoff et al. (2003), developed in cooperation with the Monterey Bay Aquarium research institute, should be followed to ensure quantifiable and comparable data is collected during dives. Additionally, where possible and feasible, data can be collected as part of other video survey deployments such as pelagic surveys (BRUVS) or benthic fish surveys (ROV, submersible, diver). We suggest that transects should follow predetermined lengths, similar in effort to those described in the Epibenthos section of this guide.

Video lights are needed to illuminate dark, deeper water, yet the type of light and its impact on animal behaviour should be considered. A combination of visible light and infrared light could be an option to observe light patterns that are displayed by many gelatinous taxa. Additionally, blue/yellow filters can be used to study bioluminescence.

Where possible, water samples should be taken to understand the prevalent environmental conditions at the time of sampling. These can consist of CTD deployments and chlorophyll a samples. Additionally, where surface waters are sampled, wind data can be assessed online (i.e. https://www.wunderground.com/)/. Sampling should be planned and carried out adhering to strict time-

windows, as plankton communities will undergo diurnal migration, with distinct communities present during daytime and nighttime.

Data analysis

Video footage should be analysed in video annotation software (e.g. ImageJ / EventMeasure / TransectMeasure). A searchable video annotation system can be helpful to cross-compare identified individuals and return to certain observations with ease. A species list should be created as soon as possible before starting the actual quantifiable analysis process, so that as many ID's as possible can be agreed on which reduces time needed to reanalyze videos. Taxonomic experts should be consulted with initial species list drafts as soon as possible. A database of collected images coupled with expert species identification should be built and shared as the analysis is performed. This will immensely reduce post-processing time and speed up analysis. Where available, machine learning and automated image annotation software can be used to speed up the process. In future, in-situ holographic imaging could allow cutting edge data to be collected and shared with a wide audience.

Top tips

- Be consistent in terms of duration, time of day and length of transect across all sites. Local conditions may vary significantly across temporal scales, even throughout the day.
- Collection of live specimens can be a great add-on when using ROVS and submersibles which would allow non-destructive sampling.
- Expert identification must be sought to get species identifications at the lowest possible taxonomic level. This will allow building a comprehensive image database.

Key references

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(4) Microbial community

Microbes are some of the most important organisms living in the ocean, creating half of the oxygen on our planet and acting as major primary producers, yet little is known about their deep-sea communities. They are crucial in ocean biochemical cycling, but we need understand more about them and their vital role in ocean processes and ultimately their contribution to planetary health

Sampling, data collection and storage equipment

The way microbes are sampled depends on the targeted microbial communities – either free swimming in the water column or settled as colonial mats on the seafloor.

Microbes suspended in the water column can be sampled by deploying a CTD Rosette. The rosette consists of Niskin bottles (typically 12 to 36 with a capacity between 1.2 to 30 l) and a CTD sensor, both fitted to a frame and lowered through the water column from a winch of the main survey vessel (CTD sampling is described under parameter 13). The Niskin bottles are deployed with their top and bottom ends cocked open to allow water flow-through. They are fitted with controlled releases that can be triggered from a computer connected to the rosette system. At each target depth, a signal is given from the computer that triggers the releases and closes the Niskin bottles, trapping the water inside them. Once returned to the surface, the drain pin allows easily emptying the bottle and extracting the desired sampling water volume (the vent screw located near the top of the bottle hereby controls the flow speed). Water samples can also be collected from other water collection gear. Examples are miniature rosettes fitted to submersibles, ROVs, deep ocean landers or wires. Where remotely triggering the bottles' releases is impossible, on deep ocean landers for instance, burn-wires can be used that trigger the bottles to close after a certain amount of time has passed. Low-cost sampling solutions that require little extra equipment are possible for collected surface and subsurface water, through vessel deployed buckets, diver held containers or vessel water in-takes.

Once water has been collected, microbial communities are extracted through filtration. To decrease time requirement a peristaltic pump is often employed to create a vacuum filtration. Volume of water filtered, and size of filter pore size differ and choice depends on research question and note this choice does influence the microbial community that is later documented

If the targeted microbe communities are present on or within the sediment, corers and grabbers should be used to gather samples (see Indian for more detail). The sample will then have to be cross-sectioned (normally in 5 mm slices).

Ideal data and collection format

Most samples will be processed using genetic sequenced or investigated using scanning electron microscopes (either on board the ship in a wet/dry lab or post-cruise on land. Typically, the 16S gene is used for genetic analysis but this is changing as next and third-generation sequencing becomes more plausible. Specimens can then not only be identified but also quantified and further analysed as needed.

Data analysis

The M2B3 reporting standard proposed by ten Hoopen et al. (2015) should be followed wherever possible. Proposing a set of recommended descriptors, it ensures data collection and dissemination are streamlined, simplified and intercomparable results are collected.

Top tips

- Positive and negative control are vital during sampling
- Sterilsation between sample sites is necessary for a robust study
- Careful planning of sampling and processed is needed to ensure effective use of resources

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(5) Census of associated pelagic biota

Pelagic biota can be surveyed using visual, auditory, and video sampling methodologies, with the aim to collect data on the occurrence, distribution, and diversity of large marine vertebrates and seabirds. The pelagic realm and its soundscapes are increasingly affected by anthropogenic impacts such as noise and illegal fishing, which has been linked to changes in the behaviour and detrimental effects to the health of marine mammals. For researchers, pelagic waters pose some unique challenges compared to coastal waters and certain adaptations to sampling gear will have to be considered.

Sampling, data collection and storage equipment

Pelagic biota can be surveyed by shipborne observations in the form of visual transect surveys, deploying passive acoustic monitoring devices, or by using specialised video systems, depending on the target species. Shipborne observers can use binoculars or cameras (i.e. Nikon) or drones (i.e. DJI) to survey large marine mammals and seabirds. Species identification and abundance information can be recorded by trained observers or supervised observers with the help of a photographic record of the encounter and regional experts. For example, data on the position of a pod of dolphins can be recorded, estimated group size and presence of calves can be accompanied by images that allow for species identification to be verified later. Additionally, the direction of travel can be recorded and behavioural state (logging at the surface, feeding, travelling, milling). Where species identification is not possible, either through direct observation or during subsequent inspection of images, the species is given the highest taxonomic hierarchy possible (for example, unidentified delphinid, unidentified balaenopterid) to provide a description of species diversity.

Wherever possible, drones will provide much more robust data viewing of the animal in question from above rather than solely from horizontal, ship-based observations. Aerial photographs can be especially useful for identification of species such as Bryde's whales, where rostral ridges on the top of the head will confirm species identity. In addition, drone imagery when collected according to safe protocols can help to infer behaviour, group structure and age-class.

Marine mammals can also be surveyed using passive acoustic monitoring in the form of towed, static (bottom mounted) and drifting hydrophone arrays. Hydrophones (whether towed, static, or drifting) can be programmed to record continuously at the frequency of the elements used (high, medium and low). Recordings can then be analysed using standard software (www.pamguard.org) in combination with information on species distributions and visual data to make inference on species identity. Information on man-made and other sounds, such as fish, will also provide an overview of the soundscape of the region surveyed. Acoustic data can be collected by towing a hydrophone behind a vessel, or entirely passively by deploying a static bottom mounted sound trap for a set period of hours or days or using drifting hydrophone with tracking devices that allow retrieval.

Photoidentification images can help to identify individuals within species, populations, and groups. Matching these images to existing catalogues can provide direct evidence of individual movements and / or site fidelity. Understanding individual identity and size- age-class within a pod can help in understanding behaviour. Any opportunistic collection of tissue (preferably skin and blubber) from either dead cetaceans (strandings) or live animals (through biopies) will enable downstream genomic analyses (species identity, genetic diversity, population connectivity) and dietary analyses (stable isotopes and fatty acids). Lastly,

pelagic baited remote underwater video systems (pelagic-BRUVs) can be used to survey the abundance, diversity, and biomass of pelagic predators. This consists of a single or stereo video camera frame with an attached bait container that is lowered into the water column on a line (to target depth). While bait is most often used, auditory and/or visual attractants are also effective. In shallow coastal environments, pelagic-BRUVs can be anchored in a set position, however in the open ocean they are left free floating and are tracked on the surface via AIS (automatic identification system) or satellite beacons. Overall, visual or auditory assessments are often sufficient, but tissue samples are needed to quantify population estimates and connectivity.

Video files from observations or pelagic-BRUVs can take up an incredible amount of space and data must be backed up to multiple harddrives and, where possible, servers on the main vessel, daily. Field harddrives are commonly used (e.g. LaCie) and advancements in MicroSD card storage space allows capturing data for hours at a time without swapping cards during deployments (e.g. SanDisk). Sound files can take up around 300mb / hour, so a whole day of recording can easily add up to 8GB. They should be backed up and stored in a similar way to the video data.

Ideal data and collection format

- NOAA National Marine Fisheries Service has standardized visual transect methods.
- Collected BRUV data can be uploaded to online open access data repositories such as OBIS and GBIF, Specify 7, and Global Archive.
- Collected marine mammal data can be uploaded to Happywhale and Whale mAPP.

Data analysis

Analysis consists of data processing and actual scientific analysis. These steps vary between the different methodologies and following, a quick overview was given for each method:

Visual observation data

- Data is normally collected by hand in forms of recording sheets filled in as animals are being observed. Alternatively, captured drone footage or camera footage will have to be viewed post sighting, processed and analysed on a computer.
- Species can be identified visually from characteristic features and behaviour. For identifying individuals, different species have different visual cues as to their identity. For example, sperm and humpback whales are generally identified through markings of the tail flukes and / or fin, with noticeable nicks, injuries or very obvious markings. Dolphins, beaked whales and baleen whales, such as blues and Bryde's whales can be identified using their dorsal fin shapes and scratches or colouration on their flanks.

Hydrophone data

- Hydrophones record sound files in a number of file formats. For example, .sud file format must be converted to .wav before being able to analyse the files. Other systems write straight to .wav.
- The program Audacity can be used to edit and pre-process files, amplifying low gain sounds and cutting them as necessary.
- Analysis of sound files can then be done in the freely available software PAMGuard using packages such as 'Multi-Hypothesis Tracking Click Train Detector' and the 'Whistle and Moan Detector' (www.pamguard.org). Ideally, acoustic data will be accompanied by verified visual

data to help identify the acoustic encounter to species level, particularly in areas of the ocean where there are few species-specific classifiers, i.e. where the vocal repertoire for that species or population is not well characterised.

Pelagic-BRUVs data

- Currently, collected videos must be manually processed with the first step being the identification of the observed specimens and the creation of a species list.
- Secondly, the relative abundance of the different species needs to be measured. MaxN (maximum number of one species at any given time within the video) is the most commonly used metric as it prevents recounting already counted individuals. However, alternative metrics may need to be considered for the drifting pelagic-BRUVs that cover a large area on a single deployment.
    - This can be done within the SeaGIS software package EventMeasure. When used with the CAL software and hardware the frames can be calibrated and size and distance measurements can also be made.
- In the future machine learning solutions for the identification and counting of fishes will streamline this process.

Top tips

- Work with local scientists to get info on local species, specific local behaviour, access to pre-existing databases (especially important to add to local marine mammal photo ID work).
- Getting enough distance to the main vessel is crucially important to reduce background noise when acoustic sampling.
- Ensure you have local permits and ethics clearance for all research.
- When deploying a hydrophone from a smaller vessel, take a Handheld GPS / depth measurement device / snorkeling gear to check water depth when deploying the hydrophone and make sure it doesn't get dragged along the bottom.
- Never steer in front of a pod of marine mammals, always observe from the side or behind.
- For drone work, stay behind the pod of marine mammals and never fly in front.
- Bring plenty of water, umbrellas, snacks when deploying / retrieving pelagic BRUVs - it's one of the hardest jobs and days can be very long with multiple deployments.
- Use local, oily, crushed fish as bait. 1kg is sufficient, but make sure it's mashed up to ensure good plume dispersal.
- Synchronise the times among the cameras, sensors and tracking beacons to optimize integration of different data streams.
- Always collect ancillary environmental data to aid interpretation of observations and keep records of incidental sightings.
- Always check and clean your O-rings!

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(6 & 7) Hyperbenthos

This community (*sensu* Mees and Jones 1997) predominantly consists of demersal/benthopelagic plankton found within the benthic boundary layer (BBL). This includes both holoplankters (e.g. mysids, amphipods, chaetognaths, copepods) and meroplankters (e.g. invertebrate and fish larvae) and consequently contributes to the bentho-pelagic coupling of the wider ecosystem as prey species for larger fauna, recyclers of organic matter, and a source of propagules influential to adult population dynamics. The hyperbenthos can be significantly different from other planktonic communities (Christiansen et al 2010; Frutos et al. 2017).

Sampling, data collection and storage equipment

Sampling of the hyperbenthos can be done using many gear types including (in order of small to large volume): water-bottles, traps, visual survey, pumping systems, nets/sleds. Note the low density of deep-water plankton recommends high volume sampling systems.

The bottom substrate and topography will affect the efficiency of the equipment being used.

- SOFT SEDIMENT: nets/epibenthic sledges with a mesh size of 300–500 µm, and an open/closing design is preferred. Larger meshes may be suitable if there is a high risk of clogging. The Rothlisberg & Pearcy (1976, R-P) type Sledge is often used due to its compact and light design, but many models are available (see Christiansen 2016; Brandt et al., 2013). Care should be given for choosing equipment suited to the ship's capacity to launch and recover a device that could weigh between 80-1200kg when empty. Note that these highly efficient net/sledge sampling gears are also highly destructive (see below for alternatives). Soft sediment can be hand-sampled in shallower to mesophotic waters by SCUBA divers.
- HARD/ROUGH SEDIMENT: Pumping systems, usually static, can be used over long periods (e.g. 24hrs) to maximize sampling volume. Static traps are often utilized on reefs, with light traps being useful at photic/mesophotic/rariphotic depths. Alternatively, ROV/Sub/AUV mounted opening/closing systems (e.g. SyPRID, Billings et al 2017) could be used for higher volume sampling.
- NON-DESTRUCTIVE AND EMERGING TECHNIQUES: High resolution video has been successful for observing larger hyperbenthos (>2cm, Robinson et al 2010) and could be modified for more detailed sampling. In-line digital holographic systems are also being pioneered for passive plankton observation (e.g. Graham et al 2010) and any such visual samplers could be coupled with automated identification, a technique which is improving in accuracy (e.g. Luo et al 2018, Allken et al 2019). ROV/Sub/AUV/SCUBA mounted sampling systems are non-destructive alternatives for physical sampling. Environmental DNA sampling has potential depending on the study focus and the availability of libraries of relevant genetic sequences to compare to (Garlapati et al 2019). Current clashes between wire times and time constraints mean that more autonomous sampling gear will need to be developed in the future (see Brandt et al., 2016).

Once acquired, samples should be processed in a cold (e.g. 0-4°C) environment to prevent thermal shock. The presence of any sediment/bottom contact indicators should be recorded. Samples will need sorting and sieving with graduated mesh sizes (depending on target fauna size) in order to keep small fragile animals as intact as possible (Riehl et al., 2014).

Sample processing methods depend on the research reasons for sampling, but usually involve some form of enumeration and morphological ID of fauna.

Present-day preservation methods should not preclude molecular work in the future, so biological samples should be stored in high grade ethanol (95%-100%) or RNAlater (if separated and cleaned thoroughly) then frozen (-20° or -80°). Buffered Formalin (4-10%) or lower grade ethanol (e.g. 70%) can be used if no genetic processing is anticipated and sometimes it is necessary for further anatomical studies, for example for polychaetes (Musia et al., 2016). Preservation liquid should be clearly marked on storage containers along with sample metadata.

Ethanol should be changed at least once after 12 to 24hrs. Formalin should be changed to lower grade ethanol or IMS after 24hrs.

Ideal data and collection format

Together with species IDs and enumerations, aim to gather information on water volume sampled, altimetry of sampling, and size fractions of samples, along with positional data, and any associated metadata useful for analysis (e.g. any associated CTD data). Fixative medium, number of changes, and freezer temperature is also useful to record for future information.

Data analysis

Data analysis techniques are dependent on the aim of the research being undertaken. Sample processing and analysis is much the same as with shallow water plankton analysis. For more information see Harris et al (2000).

- Samples are likely to be semi-quantitative at best depending on the sampling device
- Analysis is likely to utilise multivariate community-based techniques

Top tips

- Longer net/sled tows mean trade-offs in sample preservation
- Pump filtration volume needs to be weighed against battery power and duration, and preservation of animals must be prioritised
- The collection of biogeochemical parameters in parallel to hyperbenthic sampling is advantageous (e.g. with lander deployments)
- Positional accuracy is important, especially altimetry data to ensure you are targeting the BBL.
- Inevitably some species will be missed due to evasion ability, mesh size bias, and positioning of gear. The bottom few centimetres are often missed to avoid sediment disturbance and benthic species capture.
- There will be variable communities over time with the meroplanktonic community in particular likely to be seasonal.
- Sieve with gentle rinsing or agitation within a basin of water, do not use pressure which could damage organisms.
- Light traps are likely to be affected by turbidity and can only be effectively used in low flow
- Flushing of sieves and cod end nets should be with filtered seawater to avoid surface contamination.

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(8) Epibenthos

Epibenthos refers to the flora and fauna living on the ocean floor. Epibenthic communities are shaped by environmental drivers such as currents, wave action, depth, light and oxygen and can display great variation and uniqueness across scales, especially due to anthropogenic or climatic disturbances. These species provide a plethora of ecosystem services such as capturing and sequestering carbon, providing habitat and shelter, and acting as a food source. They harbor many commercially important species including fish, lobster and shellfish and their health is of crucial importance to the success of countless artisanal fisheries in coastal communities.

Sampling, data collection and storage equipment

The main method to survey epibenthic communities are underwater stereo video surveys in the form of photographic or video surveys and physical specimen collections. Hereby, mesophotic depths can be reached by technical divers, with deeper environments surveyed by submersibles or remotely operated vehicles (ROV). Other options include drop cameras or towed cameras, autonomous underwater vehicles (AUV) or baited/unbaited remote underwater video systems (RUV/BRUV/landers). For collections, grabs, corers, trawls and sledges can be deployed in depths below technical dive limits.

Submersibles can vary in their depth rating and size (number of passengers). The main commercial manufacturers are Triton. ROV's can come in a variety of sizes and depth ratings, from small, handheld miniROVs to large, commercial deep sea ROVs weighing several tons and carrying a plethora of survey equipment, including CTD's and corers. Manufacturers are Kystdesign and Ocean Modules.

Where camera systems are used, they normally consist of two cameras (e.g. GoPro or Paralenz for shallower depths or DeepSea Power and Light for rariphotic and beyond) that capture high definition stereo video footage of the targeted benthic and fish communities. The cameras are either pre-calibrated using calibration equipment (e.g. SeaGIS calibration cube) or fitted with lasers to allow measuring observed organisms during post processing. The camera systems should record in high-definition resolution (at least 1080p, preferably 2.7k) and with a high frame rate (>60fps), ensuring high quality footage is captured and high-quality frames can be extracted. Maximising image quality will ease post-processing and make video analysis a lot more flexible. Large (256GB+ MicroSD cards with fast riding speeds, e.g. SanDisk extreme) are crucial to ensure cameras can record for the full dive, and sufficient storage in forms of servers and harddrives is needed to ensure data can be saved and backed up upon returning to the vessel.

Multiple camera systems can be mounted on the submersibles and ROVs at a time, capturing downward, forward, and sideward facing footage, thus allowing it to be prepared for all types of topographies encountered during a dive. Normally, one pair of stereo cameras will be carried by divers where the fish assemblage is captured with the cameras facing forward on the first pass of the transect, with the benthos captured on the way back. In shallow waters (up to ~30m) red filters can be used to minimize post processing and colour corrections. As colours can get lost extremely quickly with increasing depth, lights should be used for surveys below 30m (i.e. manufacturer Deep Sea Power and Light). Turning these on and off for a few seconds at the start and end points of individual transects will also make video analysis a lot easier.

Ideal data and collection format

- Belt transects following a fixed, predetermined depth contour parallel to shore, repeated at multiple depths (60m, 120m, 250m, etc.). Transects should be broken into fixed length sections, but ideally covering 1 km distance where possible (ROV, submersible etc), and ideally at least 250 m (typically broken into 50 m transects) for diver-based surveys. Transects should ideally be segregated by substrate types to allow quantification or different habitats, and multiple substrates at the survey depth should be sampled to maximize biodiversity information.
- Short transects (<50m) or quadrats (~4x4m) filmed with single or stereo cameras to conduct structure from motion for benthic habitat complexity.
- Gear selection (Divers or ROV or Sub) after multibeam and dropcam runs to ensure appropriate samples can be taken and selected gear type matches local conditions (e.g. BRUV is not suitable for walls or steep slopes). For mesophotic, reference to freely available bathymetry (e.g. Allen Coral Atlas), or collected data as part of on-site multibeam surveys, may be useful.
- Refer to taxonomic standards (e.g., CATAMI) or when describing morphotypes refer to ON signs.
- Camera recording solution and frame rate don't have to be maximised to collect the best data. Consider a tradeoff between resolution and frame rate to maximise recording time. Adjust this according to local conditions.

The appropriate gear type and the transect heading should be determined after multibeam bathymetry surveys (deep sea) or preliminary dives (mesophotic) were conducted on site. The direction will often be governed by prevailing current conditions. Transects should run in replicates of at least three for each depth interval surveyed. While vertical transects are great additional data sources to document changes between depth contours and fill knowledge gaps, complete replicate sampling at a range of depths should be prioritized.

Data analysis

- Analysis of video and still camera images
    - Create species files of observed individuals when first scanning footage. Attempt to do this as soon as possible after deployments and get divers / pilots who observed organisms in situ to assist with ID's whilst knowledge is still fresh.
    - SeaGIS software (https://www.seagis.com.au/) can be used to analyze stereo videos of both fish and benthic organisms. CAL and EventMeasure are used to sync cameras, extract frames and survey fish assemblage. TransectMeasure can be used to create quadrats for analysis of abundance, counts and size of benthic organisms.
    - Agisoft metashape (https://www.agisoft.com/) can be used to analyze benthic transects and quadrats and create 3D photogrammetry models assessing habitat complexity and rugosity on finer scales than multibeam bathymetry surveys.
    - Images of captured organisms should be shared with taxonomic experts throughout video analysis. This is especially important when creating first species list drafts and prevents ID's changing over and over again by continuously updating ID lists.

- Subdivision of transect by distance or substrate type
    - Time stamps or USBL georeferencing can be used to subdivide or measure transects and combine video data outputs with bathymetry data for further modelling.

- Microscopic and genetic taxonomic identification
    - Collected samples can be identified via microscopic examinations and DNA sequencing and information can be used to confirm IDs from video transects.

Top tips

- Modern action cameras like GoPro and Parelenz are comparably cheap and have great depth ratings. They can be a cost-effective alternative to expensive deep ocean camera systems. Be aware that cheap action cameras may use rolling shutters, this means footage collected at lower framerates (typically 60 frames/second) will be unsuitable for stereo-video analysis.
- Remember genus or even family level is often as specific as you can be for image analysis.
- The best way to sync cameras is using a flashlight on deck after turning them on to record. Additionally, a handheld torch or lights fitted to the underwater vehicle should be flashed to signal the start and stop between transects and also for camera synchronization for analysis.
- Keep survey sheets and ensure co-pilots or divers fill them in either during or immediately after each dive.
- Where possible conduct a try dive during sea trials with each type of equipment / deployment to ensure the recording parameters work in the local conditions. This is the best time to try out different frame rates and resolutions.
- Create 'wishlists' for species or morphotypes to be targeted during collection dives, based on observations from video transects.
- Create a species list as soon as possible! A fresh memory of seeing specimens in-situ can be very helpful.

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(9) Infauna

On a global basis, the sedimentary deposits that overlay the oceanic crust are on average several hundreds of meters thick. Typically, the most well-studied infauna (animals living within sediments) are the macrofaunal polychaetes and meiofaunal nematodes and foraminifera that inhabit the upper oxygenated sediments. Through their activities, sediment-dwelling organisms create a unique mosaic of biogenic microenvironments that strongly influence carbon and nitrogen burial and remineralization rates, thus playing a key role in global biogeochemical cycles and marine ecosystem functioning.

Sampling, data collection and storage equipment

Sampling Introduction

Corers and grabs represent the main tools for collection of marine sedimentary infauna. Corers are generally preferred in marine biological surveys since they can obtain high-quality samples suitable for quantitative analysis. Grabs on the other hand can be deployed from smaller vessels and during rougher weather conditions, although it should be noted that it is not possible to collect and process distinct sediment layers.

There are many types of corers including push corers, box corers and multi- and megacorers (that can collect 4-20 samples in parallel, each representing a compromise between sampled seabed area and magnitude of surface sediment disturbance within the sample obtained; thus, the choice of a sampling device ultimately depends on the target benthic assemblage (reviewed in Narayanaswamy et al. 2016). Most corers used for sampling infauna are efficient when deployed in soft and relatively coarse sediment habitats without cobbles or pebbles. However, collecting sediment over rough topography such as steep banks or on top of and around seamounts that often contain rocky outcrops and have strong current flows might be problematic. In those environments, multicorers and box corers that cover smaller sampling areas might be better at collecting samples than large area multi- and megacorers, although the removal of some meiofaunal organisms living close or on the sediment-water interface due to the bow-wave effect must be considered when interpreting the data. Furthermore, box corers are more suitable to collect macro-infauna while multi/mega-corers should be used to target meiofauna. Multicorers have been deployed around seamounts, but a decameter thick sediment layer must be present to allow successful collections. Another option is the use of push corers using the manipulator arms on subsea vehicles such as submersibles and ROVs. This allows for more targeted sampling of replicates with defined distances, wider than with a multi- and megacorer and/or of sediment pockets in rough topography. The maximum number of cores collected per deployment hereby depends on the maximum load of the vehicle used.

Beyond the corers themselves, the methods, and tools (sieve mesh size) used to process core samples post-collection influence their inter-comparability among studies.

Data collection

Pre-sampling

- Consumables and tools: Preservation methods depend not only on the taxa but more and more on the following analysis. While soft bodied taxa like sea cucumbers or meiofauna are often fixed in 4% formosaline solution, samples for genetic work would be fixed in ethanol or RNA later, and samples for genomics and geochemical analysis would be frozen at -80C.
- Quantitative samples are necessary for community structure (species composition).

- Multiple corers are better in fine sedimented habitats as they provide less disturbed samples. Coarse sediments might necessitate the use of box corers.
- Due to the patchy distribution of infaunal organisms, especially in deep-sea sediments, replicate samples are necessary to increase precision.
- Replicate samples should preferably come from different deployments to avoid pseudo replication.
- Have tools ready for post-processing: sieves in required sizes, fixatives in right concentration and temperature, sample vials, slicers, cutter, etc. to subsample sediment layers. Labelled buckets to hold sediment layer samples until they are processed.
- Have sampling data sheet ready to collect metadata (see example).
- Consider what samples would be needed to collect environmental data – for grain size analysis from a box corer, have a tube ready for subsampling.

Post- sampling

- The choice of sieve mesh size is taxon and maybe depth dependent (e.g. 300 µm polychaetes, 45 µm nematodes (32 µm deep sea), 125 µm foraminifera (63 µm deep sea)).
- Sub-sectioning of cores is taxon dependent (e.g. macrofaunal and metazoan meiofauna: 0-1, 1-3, 3-5, 5-10 cm, foraminifera: 0-0.5, 0.5-1, 1-1.5, 1.5-2, and each cm to 10 cm).
- Treatment of samples in ambient temperature or cooler rooms required – arrange accordingly.
- Disturbed samples can still be useful for creating species inventories and non-quantitative or semi-quantitative samples might be sufficient. Data system in hand to log each taken sample/subsample.
- Have a protocol in hand what type of sampling is done first, e.g. cores for pore water analysis, live sorting, experiments, … then for biodiversity assessments, grain size, microplastic.

Data analysis

- As typical ecological data possibly using multivariate approaches using software such as PRIMER (Clarke et al. 2015) and / or ecology packages in R (e.g. ape4 and vegan) (Dray et al. 2007; Oksanen et al. 2013). Detailed data analysis considerations can be found in O'Hara et al. 2016.
- Provide a taxon abundance per core and sediment layer data matrix.

Top tips

- Visually inspect your sediment cores once they arrive on deck and look for any signs of sediment disturbance caused during sampling. Record any signs of biological activity (e.g. burrow mounds) as this will affect interpretation of your data. Ideally take top and side-view photographs of your core samples prior to slicing. This can provide important qualitative information (e.g. change in sediment colouration indicates change in sedimentary processes operating in your area).
- Water on the top of each core should be syringed/siphoned off and added to the topmost sediment horizon sample. Meiofaunal organisms living close to or on the sediment-water interface, are particularly prone to resuspension caused by the gear itself (e.g. bow wave effect), by stormy conditions or by manual handling of the core.
- Measure the depth of the sediment core
- Have all sampling tools ready prior to retrieving the corer on deck, including the labels and fixative proof pens.

- The removal of meiofauna through the bow-wave effect in a box corer is a general point, not only valid for rough terrain – make sure to account for this

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(10) Bathymetry

Bathymetry can be defined as “seafloor topography”, i.e. the spatial distribution of seafloor depth. It is typically one of the first parameters to be measured when a new area is under investigation. Apart from its scientific value to understanding the geology, sediment distribution, current regime, and general environment of biological communities, bathymetry is also of high importance for the planning of sampling activities, and, particularly in shallow waters, for general safety of operations at sea.

Sampling, data collection and storage equipment

Nowadays, the main method to measure bathymetry is through the use of multibeam echosounders (MBES). These determine seafloor depth using the two-way travel time of the acoustic pulse between the vessel and the seabed, which depends on sound velocity in the water column Using knowledge of the speed of sound as it travels through water, the two-way travel time can be converted into a range value.

A MBES system consists of a transmit acoustic array (Tx), which sends out an acoustic signal in a narrow fan across the ship’s track, plus a receiver array (Rx), which measures the return signal in a series of narrow fans perpendicular to the transmitted pulse. The intersection of the Tx and Rx results in a beam, from within which, a depth point (or sounding) can be derived. Additionally, the intensity of returned signal (“the backscatter) is collected and can be used to determine the relative seabed hardness. Harder substrate types (e.g. bedrock) result in higher intensity of the return signal than softer substrate types (e.g. mud). By continually transmitting and receiving pulses (pinging), a swath of seafloor can be mapped as the ship progresses.

The main commercial MBES manufacturers are Kongsberg (Simrad), Teledyne Reson and R2Sonic; each of which produce a range of different MBES for various operational environments. The need for different systems is mainly due to frequency: higher frequency signals (~200-700kHz, 0.5m-600m) provide better (vertical) resolution but are attenuated quicker in the water column, low-frequency systems (~12kHz) are necessary to reach full ocean depth. Alternatively, high-frequency (~400kHz) systems are now increasingly deployed from deep-submergence vehicles (ROVs or AUVs), providing high-resolution mapping capabilities at depth (Huvenne et al., 2018). However, the trade-off is still that high-frequency systems map a narrower swath and will cover a smaller surface area in each time span.

In addition to the actual MBES system, measurements of vessel position (GPS for ships & surface vehicles, USBL or inertial navigation for underwater vehicles) and attitude (pitch, roll, heave, yaw, measured with a Motion Reference Unit) are necessary and important to give position and correct for ship movements that affect the recorded data. The recently developed Norbit system conveniently combines these sensors into one unit, which helps a lot with installation on small boats of opportunity. Furthermore, as sound velocity can change through the water column as a function of salinity, temperature and pressure, a correct sound velocity profile needs to be obtained regularly, either from a sound velocity profiler, or derived from CTD data. The speed of sound not only affects the direct conversion of the two-way travel time of the acoustic returns, its distribution through the water column also determines the degree of refraction of the acoustic signal (“ray”). The corrected ray path must be accounted for to obtain an accurate position of the seafloor depth measurements. All this information is integrated with the raw measurements in real time, using software packages such as SIS, HYPACK, PDS2000, Qinsy or EVIA.

MBES systems record large volumes of data (10-200MB per hour) as a function of seafloor depth (which determines the ping rate) and the number of beams (~256 or 512). Increasingly, MBES systems also provide the opportunity to record "water column data": the amplitude and position of reflections within the water column. These can provide a 3D insight into phenomena such as the deep scattering layer, the presence of fish schools or gas bubble streams. Recording water column data, however, can multiply the required disk space by an order of magnitude.

Finally, bathymetry data can also be collected with alternative methods, including interferometric systems, bathymetric side-scan sonars (e.g. EdgeTech, GeoSwath, Klein) or Synthetic Aperture Sonars (e.g. Kongsberg), all of which work on similar acoustic principles. The most basic method consists of the single beam echosounder, which simply provides the depth under the vessel. Interpolation of the depth values across the area provides a preliminary bathymetric map. On the other hand, newer visual methods can be used in specific cases: structure-from-motion photogrammetry can result in ultra-high-resolution maps (cm2) of small areas, while laser line scanners and bathymetric LIDAR surveys can be used in cases where water turbidity is minimal and depths are relatively shallow (<50m)

Ideal data and collection format

MBES data consist of the measurements of all the parameters discussed above: two-way-travel-time and amplitude for individual beams, attitude, motion and navigation information, sound velocity profiles. All data are tied together by time, and the multiple files and data streams are generally packaged into proprietary formats by the MBES data acquisition software packages. The most well-known format are the .all files produced by the Kongsberg MBES systems, but formats including .pds, .xtf, .db, .s7k... are also common.

Data analysis

Analysis of MBES data consists of data integration and processing, prior to gridding the data for further analysis. Data processing involves post-processing the navigation and attitude data, using satellite corrections, for more accurate results. The post-processed navigation and motion data, sound velocity profiles, and tidal corrections are applied to the raw sounding data and merged to produce depths relative to a known datum (e.g. Lowest Astronomical Tide). Next, the correctly positioned depth measurements are 'cleaned': erroneous soundings are removed. The outliers could result from material in the water column, interference with other sonars, "noisy" environments. Part of this task can be done automatically, using filters, but in most cases a manual review is necessary to identify if potential outliers could represent real data (e.g. from a wreck or unexpected structure). The gridded data is then finalised and prepared for export. Common export formats include .bag (bathymetric attributed grid), .xyz, ASCII format and GeoTIFF for viewing exports. Typical software packages for MBES data processing are CARIS Hips & Sips (industry standard), QPS Qimera, Evia, Globe (IFREMER) and MBsystem (freeware).

The data is ready to be brought into a mapping software, such as GMT, Geographic Information Systems (GIS – e.g. ArcGIS, SAGA, QGIS) or 3D visualisation packages (e.g. QPS Fledermaus). These softwares allow for further analysis and to derive information such as terrain slope, aspect or hillshade. The manual or automated delineation of seabed features (Micallef et al., 2012), along with the integration of backscatter data, will help the interpretation of geological or sedimentological processes and seafloor habitats. The combination of bathymetry data with other spatially distributed environmental parameters and biological data sampled at discrete points enables the creation of full coverage habitat maps (Brown et al., 2011).

Top tips

- Consider the aim of the bathymetry mapping: what size features would you need to be able to identify (this determines the resolution), which area would you need to cover (extent), how deep is the area on average, and how much time/money is available?
- Every vessel has an optimal speed for MBES surveying, a good trade-off between data collected and data quality. Maximum speed is often not optimal. Avoid creating bubbles!
- Sometimes reducing the swath width by a little bit improves the MBES data a lot: it avoids noise in the outer beams and allows for a higher ping rate, increasing data density.

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(11) Seafloor Composition (substratum type)

Seafloor composition, or spatial distribution of substratum type, is a descriptive parameter that summarises the physico-chemical characteristics of the material that makes up the seabed. It is generally presented as a categorical variable, using classification systems based on sediment grain size such as the Folk or Wentworth scale, or on the origin of the seabed material (e.g. biogenic vs. terrigenous substrata). Substratum type is an important aspect of the benthic environment, both for infauna and epifauna, and is an indirect indicator for bottom currents and seafloor disturbance regime (e.g. strong currents = coarser sediments).

Sampling, data collection and storage equipment

To fully characterise the physico-chemical characteristics of the seabed, physical samples and/or in-situ measurements are needed. If the seabed is hard, consolidated or rocky, this will require sampling with a rock drill (e.g. the RD2 from the British Geological Survey or MeBo from the MARUM centre in Bremen, Germany), an ROV, or a rock dredge. The rock samples will be dried, labelled, and stored in boxes in a dry, ventilated area.

When the seabed consists of non-consolidated material, sampling is carried out with grabs or corers. Grabs (e.g. Van Veen or Hammon grab) are simpler instruments, easier to operate and generally more robust when sampling coarse sediments such as gravel and boulders, but they are not fully sealed and hence may lose part of the finer sediment fractions through wash-out during recovery. For sandy and muddy sediments, corers are a better choice. These can range from boxcores over mega/multicores to full-on gravity or piston cores. The latter ones are most useful for geological studies, as they sample the seabed down to several metres. However, their heavy impact generally results in disturbance of the top centimetres of the core. Boxcores can sample down to 50cm into the seabed if conditions are good and provide a relatively large volume of seabed material that can be sub-sampled for various analyses, or can be sieved, for example, to identify the infauna community. Although boxcores are sealed on recovery, a certain amount of disturbance of the sediment-water interface does take place, and the finest fractions can be washed out. Mega/multicores and ROV pushcores are the best option to obtain a good quality sample of the sediment water interface. They can also sample several 10s of centimeters of the seabed but recover less sediment volume compared to boxcores.

Cores (gravity, piston, mega/multicores, ROV pushcores) can either be stored complete by closing them with endcaps, sealing them with electrical tape and storing them in a refrigerated environment (~4-6°C). Alternatively, they can be subsampled in various ways, depending on the analysis needed. A common approach, particularly for mega/multicores is to extrude the cores bit by bit and to slice them in specific intervals (e.g. every centimeter), for example storing the slices in zip lock plastic bags for grainsize analysis. Other analysis types (e.g. organic matter, lipid content, …) may require other protocols and storage requirements, such as wrapping in aluminium foil or freezing at -80°C. A full description of core sample protocols for biological studies is given in Clark et al. (2016).

Using the above sampling techniques, however, the seafloor composition will only be known at discrete points across the study area. To obtain a wider coverage, alternative methods based on proxies can be used, to extrapolate the physico-chemical characteristics measured in the samples over the wider terrain. First of all, video or photographic observations from ROV, AUV or towed cameras can be used to identify classes such as rock, gravel, boulders, sand, mud and biogenic substrata over a larger area.

Depending on the camera quality and resolution, distinction between sand and mud may be difficult. It is not possible to obtain any further, more detailed classifications (e.g. sandy mud vs. muddy sand) using such visual approaches.

To obtain full spatial coverage of the study area, seafloor acoustic reflectivity maps can be used, as recorded by multibeam echosounder (MBES), or by using sidescan sonar, interferometric sonar, or equivalent methods. These systems all measure the amplitude of the return acoustic signal also referred to as 'backscatter', which is affected by a combination of seafloor composition, seafloor roughness and angle of incidence of the incoming acoustic signal. Rocky and 'hard' substrata will create a strong acoustic return, while soft sediments tend to absorb a large amount of the acoustic energy, causing a reduced backscatter signal. Unfortunately, none of the systems currently on the market provide calibrated, absolute backscatter values, hence every survey must be interpreted in a relative sense (Lamarche & Lurton, 2018). This means ground-truthing, through actual samples or imagery, is always needed to obtain an accurate measurement of substratum type.

Ideal data and collection format

Sediment grain size (or particle size), as represented by particle size distribution curve. Characteristic values such as mean, median, standard deviation, skewness, kurtosis, and Folk classification can be calculated with the software gradistat.

Other sediment parameters: mineral composition (microscopy), percentage organic carbon, total carbon, lipid content, pigments, depending on what research questions need to be addressed.

Data analysis

Once dried, rock samples can be analysed in various ways depending on the research question. They can be cut into thin slices for mineral analysis, ground into powders for X-Ray Diffraction (XRD) analysis of elemental composition or dissolved for chemical studies.

Core subsamples for grain size analysis are generally quite small (~cm^3 for sandy & muddy sediments), and can be analysed with laser diffraction techniques (e.g. using a Malvern Mastersizer or a Coulter Counter instrument), X-ray sedimentation instruments (e.g. Sedigraph), or with traditional dry and wet sieving and pipette methods. The chemical composition of seabed sediments, and of the interstitial pore waters is analysed as explained by Glasby, 1973.

Substratum identification from video & photography follows similar protocols as epibenthic megafauna analysis (see tech guide on parameter 8). Data processing of acoustic backscatter data is typically carried out with standard MBES processing packages such as CARIS Hips & Sips or MB system, or with specific packages such as QPS' FMGT or Sonarwiz. The resulting maps are imported into a GIS and interpreted, either through manual delineation of areas with homogeneous seafloor backscatter, or through novel automated methods that make use of spatial statistics and machine learning (Diesing et al., 2014)

Top tips

- When capping and storing whole cores for later splitting and analysis, take extra care to mark the top and bottom of the core. Generally, arrows pointing towards the top of the core are drawn on the liners, but even better is using different colours of electrical tape to seal the top and bottom end caps.

- The different methods of sediment grain size analysis are based on different physical characteristics of the sediment grains and therefore are not entirely comparable. It may be important to keep this in mind when comparing datasets analysed using different methods.

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(12) Current velocity

To understand the oceanographic processes that are important in driving habitat zonation and formation across study sites, current velocity is commonly measured using an Acoustic Doppler current profiler (ADCP) or a single-point acoustic Doppler velocimeter (ADV) system. These profilers are not only useful to discover physical processes, but also to collect information on ambient conditions that can be directly used when planning gear deployments and calibrating survey equipment.

Sampling, data collection and storage equipment

An Acoustic Doppler system (see Teledyne marine for example) detects the current speed via sound, transmitting and detecting a pulse which can be used to visualise oceanographic processes. The emitted sound waves ricochet off particles suspended in the water and depending on which direction the particle reflecting the sound is travelling, the sound returns to the profiler with a slightly different frequency. In general, if the object moves away from the profiler, the soundwave returns at a slightly lowered frequency and if the object is moving towards the profiler, the soundwave returns with a slightly higher frequency. The profiler uses this information to calculate how fast the particle reflecting the sound wave, and thus how fast the water column around it is moving. By measuring the time that passed between sending out the signal and receiving it, the profiler can display currents at various depth intervals.

ADCPs can be deployed alone, in which case they either require a battery and internal data logger and need to be submerged for an extended period of time before being recovered and the data extracted. However, the most common forms are ship-mounted systems (hull mounted or lowered through a moon-pool), which are linked to an onboard computer and the vessel's GPS system. The collected data needs to be stored and analysed on an external computer with dedicated software as the systems themselves do not come with external read-outs. Alternatively, smaller ADCP systems can be attached to CTD rosettes and lowered via a winch to various depths. To acquire the highest resolution results in complex near-bottom environments, a bottom lander can be equipped with an ADV and/or a high frequency ADCP which can measure current shear and seabed shear stress more accurately than ship-mounted systems.

Ideal data and collection format

The collected data depend on the frequency setting of the profiler and can be tailored to measure various factors coupled with current velocity, including upwelling, wave motion, boundary currents, eddies, fronts, tidal motions, turbulence, and internal waves. Frequency can be adjusted in many systems and typically range from 40kHz to around 150kHz. The outputs vary depending on the type of survey and can show cross sections of currents including current direction (East, North, vertical), depth and lateral distance.

Data analysis

The measurements made by the ADCP are commonly displayed as velocity vectors (Beam 1, 2, 3, 4), which will then need to be converted to East, North and Vertical format. Secondly, Echo intensity, Attitude data (heading, pitch, roll, orientation), physical parameters (temperature and pressure), physical position (bottom track velocity, range to bottom) are collected. Teledyne Marine has various data post processing protocols.

Top tips

- You need to carefully consider how precise your measurements need to be as high frequency sounds yield more precise data, but do not travel as far as lower frequency pulses.
- Clear, tropical waters are not your friend! They carry less particles and soundwaves may not hit enough particles to produce accurate data.
- Bubbles in turbulent waters and schools of marine life can create errors due to erroneous pulse returns.

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(13) Temperature, salinity, pressure (derived density) (CTD)

A CTD is a set of sensors that measure temperature, salinity, and pressure, gathering important information about the ambient physical environmental conditions across a study area/through a water column. It furthermore provides density gradients that are required to calculate geostrophic currents/water mass and sound velocity. Water mass data is crucial when studying species distribution patterns and comparing biological observations to oceanographic processes, allowing scientists to model ambient conditions across spatiotemporal scales.

Sampling, data collection and storage equipment

CTD systems can come in a variety of forms, depending on the targeted depth and parameters surveyed. If available, a flow-through system (occasionally called Ferrybox) can be deployed to continuously measure many parameters of surface waters. Other options to sample deeper waters include the deployment of CTDs as part of Niskin bottle rosettes or attaching the loggers to moorings, landers, submersibles, ROVs, AUVs, gliders, expendable bathythermographs (XBT) and Argo profiling floats. Even minimized systems (smaller and lighter units) can nowadays be carried by technical divers, either attached to their tanks or underwater scooters. Depending on the make of the sensor and its purpose, it either comes with inbuilt batteries or with a connector that allows the plugin of an external power source.

The actual sensors attached to the CTD system can vary depending on the specific targets/objectives of the cruise/survey/voyage. These can measure additional physical, chemical and biological parameters, including but not limiting to: turbidity, conductivity, redux, pH, oxygen, temperature, pressure, and chlorophyll fluorescence spectrum. The vertical resolution depends on the target depth and can be less than/equal to 1m depth intervals. The systems continuously sample the water flowing through and can measure the desired parameters. These are either transmitted to a survey computer based on the main vessel (for systems lowered via a winch and suitable cable) or saved internally on a storage unit inside the sensor for systems mounted onto other survey gear.

Ideal data and collection format

For remotely deployed systems, the measured data can be recorded inside the CTD unit on a data logger and can be read out post deployment when back on deck. Units connected to a water bottle rosette normally possess data transmitter outputs and come with no internal memory - they are attached to a deck box and a survey computer on deck via a conductor sea cable and allow real-time profiling of the collected data during the deployment. The scientists on board can not only view the data in real time (usually displayed as live updated plots) but can also remotely control the system and trigger the water sampling bottles to shut at different depths, collecting precise samples (more on this under section 5 - Microbial community).

On a typical SeaBird system data is recorded as *.hex and *.con files which must be kept together. Traditionally on these systems the optical or electrochemical sensors output raw voltages these are recorded in the hex file and need to be converted to useable/readable formats using the accompanying con (configuration) file. This con file contains all sensor calibrations, system setups and parameter channels specific to each cast. The hex file should also start with a header, this is generally user input Metadata at the beginning of each cast, additional physical logsheets should be made to confirm when processing. Data processing applies many calculations to the raw voltage data to provide .cnv (converted) files. Fixed procedures and a secure system need to be in place to prevent data loss and allow long term

availability and re-use of the data. More often further processing is required to give the most reliable or true data, this could include filtering out noise, correcting for Cell Thermal Mass (CTM) and deriving new/corrected variables.

Data analysis

A CTD collects an incredible amount of data which needs to be visualised and statistically analysed post deployment. A practical reference guide was written by Thomson and Emery, 2014 which includes information on data acquisition, recording, processing of collected data including statistical analysis and error handling and methods to spatio-temporally analyse the collected data. Depending on the system, different analysis software can be used (for example Seabird CTD data is analysed using SBE SeaSoft).

Top tips

- The deployment orientation of the CTD (horizontal vs vertical) governs the way the sensors are connected. Double check this deploying your system.
- Plumbing is crucial for successful deployments – make sure the plumbing is arranged in a way that allows all air to escae before lowering it into the water column.
- Noise or salinity spiking might come from incorrectly attached TC ducts.
- Y fitting valve should be removed on horizontal deployments. Instead, the pump-exhaust should be the highest point on the system.
- Always provide sensors a clear flow path, irrespective of deployment orientation.

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(14) Nutrients: Nitrate/nitrite (NO3, NO2), silicate (SiO4), and phosphate (PO4)

Nutrients are essential elements needed for biological growth, in most open ocean areas nutrient concentrations are limiting to growth, but in coastal areas nutrients may be in excess of the biological requirements and lead to blooms in phytoplankton. These blooms can have an impact through the increase in anoxia due to degradation of the biological material by bacteria, or may stimulate the growth of harmful algae, harmful either by further increasing oxygen consumption, or through the production of toxic compounds such as demoic acid.
The main macro-nutrients are phosphate, nitrate, and silicate. For nitrate and phosphate, sources include runoff from agriculture, sewage inputs and other diverse smaller but important anthropogenic sources, for silicate the main sources are non-anthropogenic and are a result of weathering processes.

Sampling, data collection and storage equipment

Water samples are collected using rosette or Niskin bottle deployments and should be stored in acid washed plastic vials that have been rinsed with the seawater to be analysed. The samples are either preserved by freezing where possible, or by using 100ul of mercuric chloride per litre of seawater. Freezing is preferable but where freezers are not available mercuric chloride can be used, this compound is extremely toxic and should be handled with care.

Ideal data and collection format

There are a number of commercially available nutrient analysis systems on the market, and some of these are widely used. There is a full review and intercomparison of these on the Alliance of coastal technologies website, http://www.act-us.info/evaluations.php#Nutrient
A new suite of lab on chip based sensors are now being commercialised through a new company Clearwater Sensors. nitrate, silicate and phosphate, based on developments over many years at the National Oceanography Centre in the UK (Beaton et al., 2012; Clinton-Bailey et al. 2017)

Data analysis

The standard methods for the analysis of nutrients in seawater can be found in the GO-SHIP Repeat Hydrography Nutrient Manual, 2019, Becker et al, 2019. The precise and accurate determination of dissolved inorganic nutrients in seawater; Continuous Flow Analysis methods and laboratory practices.
The optimal method for the analysis of dissolved macronutrients is the on-board analysis, using a gas segmented continuous flow analyser; if available samples do not need to be frozen and can be stored for up to 24 hours in a refrigerator. If there is no means of analysing on board the samples should be preserved as outlined above. There are now certified reference materials for nutrients and full details of theses is available on the IOCCP website (www.ioccp.org).

Top tips

- Sample containers can be reused if proper cleaning procedures are followed between stations – this would largely reduce waste on longer cruises.
- Containers can be rinsed with ultrapure water (distilled deionized water), followed by a rinse with 10% Hydrochloric Acid, and again with ultrapure water.

- When retrieving seawater samples from the niskin bottles, rinse the clean sample containers and caps or tubes three times before transferring samples.
- If analysis will be delayed for >2h, samples should be stored in a dark and cool place (fridge).
- Cigarette smoke can contaminate samples – smokers and people who recently smoked a cigarette should stay well away from the sample processing areas.

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(15) Dissolved oxygen (DO)

Dissolved oxygen (DO) is a primary requirement for life in the oceans, with the exception of some anaerobic organisms, all life on the planet require oxygen. The oxygen in the surface oceans is maintained at a certain concentration based on equilibration with the atmosphere, controlled by temperature, pressure and water salinity, and the in-situ production and consumption by marine organisms. As on land, oxygen is produced by marine phytoplankton using carbon dioxide in the presence of sunlight through photosynthesis, plants consume oxygen in the dark, but on balance produce more than they consume. This photosynthetic production can lead to oversaturation of oxygen in the water column and thus provide oxygen to the overlying atmosphere. Oxygen is reduced in the water column by processes such as animal consumption and through the consumption by bacteria during organic matter degradation. In highly productive areas of the ocean this consumption can result in large areas of low oxygen concentrations in the worlds oceans.

Sampling, data collection and storage equipment

The method for dissolved oxygen is called the Winkler method and has been used since 1888, been modified by Strickland and Parsons (1968). Oxygen sampling is difficult to do and takes a reasonable level of training. Purpose designed bottles (125ml) are used, these Winkler bottles are flushed with at least three full bottle volumes of the sample to be analysed, using a flexible tube that samples directly into the bottle with no entrainment of bubbles. Once filled the sample is preserved by adding 1ml of 3M manganous chloride and 1 ml of 4M sodium iodide, the bottle is stoppered carefully so as not to add any bubbles of gas and shaken. The solution forms a brown precipitate. The samples can be stored for up to 48 hours in a cool place. The precipitate is dissolved with an aliquot of sulfuric acid and analysed titrimetrically using starch as an indicator and sodium thiosulfate as the titrant. The sample is titrated until it is colourless.

Ideal data and collection format

Full methodology can be found in Strickland and Parson (1968) and amended and updated by Hood et al. (2010).

In situ sensor technology.

There are several commercial in situ oxygen sensors that are accurate and precise if they are calibrated regularly. There are systems that can be integrated into most CTD packages which whilst they may not be as accurate as collected samples offer good results for most applications. In a recently published article by Bittig and colleagues (Bittig et al., 2018) the most used commercial sensors from Aanderaa and Seabird are investigated with the aim of improving the data that is obtained from these widely used sensors.

Top tips

- Always collect gas samples (oxygen, DIC, He) first from any sampling system such as niskins, this reduces contamination with background air.

- Ensure that the Manganous chloride is not allowed to contaminate tubing, bottles, benches, where samples for trace metals may be taken, this is a major source of contamination for manganese.

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(16) pH

pH is a measure of the acidity or alkalinity status of the water column. Most of the world's oceans are basic in nature, however with increasing concentrations of CO_2 in the atmosphere the pH is decreasing due to the dissolution of CO_2 into the water and forming carbonic acid. Most animals seem to have a wide tolerance for pH, but there are concerns about those organisms that form calcareous shells; and there is increasing evidence that these organisms are exhibiting the effects of increasing pH by a thinning of the carbonate shells. This has important repercussions as the shell weight impacts how fast the organisms fall when they die as this is an important part of the export of carbon, and by proxy carbon dioxide from the upper water column. There is a coordinating action called the Global Ocean acidification observing network GOA-ON (www.goa-on.org), with a wealth of resources around ocean acidification.

Sampling, data collection and storage equipment

pH was traditionally not a commonly measured component of the marine system but with the increasing concerns around ocean acidification it is becoming more widely studied. The generally accepted methods of collection are to sample seawater directly after oxygen samples have been collected and sealing the sample in a gas tight glass bottle, with no headspace to limit gas exchange and changes in pH.

The samples are analysed as soon as possible by one of two analytical methods: potentiometric or spectrophotometric. Both methods give good results; potentiometric methods are simpler and fast but are not as accurate as the more expensive and complex spectrophotometric techniques. pH calibration requires a set of standards; in freshwater studies this is a simple process, however with the complex chemical nature of seawater, and a range of interfering elements in natural waters a series of standards specifically for pH determination in seawater has been produced by Professor Andrew Dickson at Scripps Institute of oceanography using 2-amino-2-methyl-1,3-propanediol (Tris) in artificial seawater**. It is advised that this is the only standard used** (Rerolle et al, 2012).

Ideal data and collection format

There are two commercially available pH sensors on the market for use in marine systems; the SAMI-pH sensor from Sunburst (Seidel et al, 2008) and the SATLANTIC SEAFET pH sensor (Martz et al, 2010, Bresnahan et al, 2014). There are several promising sensors in development and there is a review available as a technology roadmap on the IOCCP sensors and technology webpage. (www.ioccp.org). A new sensor for pH measurements has been brough to market by Clearwater Sensors (https://www.clearwatersensors.com/ph-sensor/) based on many years of development work at the National Oceanography Centre in the UK (Rerolle et al., 2018).

Data analysis

For instruction on how to best analyse and process the collected samples, please refer to the HELCOM Guidelines for sampling and determination of pH in seawater: http://www.helcom.fi/Lists/Publications/Guidelines%20for%20sampling%20and%20determination%20of%20pH.pdf

Additionally, the book "Methods of seawater analysis" by Koroleff & Grasshoff (1976) provides a detailed overview.

Top tips

- Laboratories carrying out pH analyses should adhere to EN ISO/IEC 17025 standards.

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(17) Dissolved inorganic carbon and alkalinity

The carbonate system and seawater alkalinity are the most important components to study in seawater to monitor the increasing impact that rising CO_2 has on the oceans and is intricately linked ocean acidification and its impacts on marine life and the health of the oceans. Dissolved inorganic carbon in seawater comprises dissolved carbon dioxide (CO_2 *aq*), carbonic acid (H_2CO_3), bicarbonate ions (HCO_3^-) and carbonate ions (CO_3^{2-}). A full review is available freely online (Dickson, 2010).

The carbonate system in seawater is complex but in simplified terms can be considered as the system below:
When carbon dioxide dissolves in seawater it forms carbonic acid per the following equation:
$CO_2 + H_2O \rightleftharpoons H_2CO_3$
This in turn dissociates to give free hydrogen and bicarbonate: **$H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$**
The free hydrogen reacts with carbonate in the seawater from the dissolution of calcium carbonate to form more bicarbonate: **$H^+ + CO_3^{2-} \rightleftharpoons HCO_3^-$**
So, when CO_2 is added to seawater the net effect is to increase dissolved CO_2, increase H^+ ions and HCO_3^- and to decrease CO_3^{2-}. These equations show why increasing carbon dioxide in the atmosphere leads to a decrease in the pH of sweater (an increase in H^+ ions).
Alkalinity is the acid buffering capacity of the seawater, and total alkalinity (TA) is the ability of seawater to buffer a pH change due to the addition of an acid.
$TA= 2[CO_3^{2-}] + [HCO_3^-] + 2[H_2BO_3^{-2}] + 3[BO_3^{-3}] + [OH^-] + [\text{organic and inorganic H+ acceptors}] – [H^+]$
In seawater Carbonate Alkalinity (CA) comprising HCO_3^- and CO_3^{2-} represents the majority of proton acceptors so usually are close to TA. To constrain the CO_2 system in seawater ideally, we would measure ΣCO_2, P_{CO2}, pH and CA. In effect a good estimate can be made by measuring any two of the 4 components, traditionally pH and alkalinity were used but a better pair is the ΣCO_2 and P_{CO2}.

Sampling, data collection and storage equipment

In general the key aspect of sampling for carbonate system parameters is to minimise mixing of the sample with air. A flexible tube should be used to transfer a sample to a glass bottle with a ground glass stopper. A small headspace should be left in the bottle to allow for expansion of the water and the sample should be preserved with mercuric chloride and stored in a cool dry place.

Ideal data and collection format

The IOCCP (www.ioccp.org) web resource provides an excellent overview of the currently commercially available systems for analysing the components of the carbonate system. Some of these systems can be operated in an underway method and some can be deployed on moorings and left, however there are still real issues around instrument sensitivity and accuracy when sensors are deployed for long periods and yet there is no in situ sensing system able to address all the components of the carbonate system in seawater.

Data analysis

The analysis of samples for the 4 components of the carbonate system in seawater is generally technically difficult, expensive, and challenging. Most of the analyses require returning the samples to the laboratory and the use of costly equipment with specialised technical staff. To that end the document produced by Dickson (2010) provides an excellent overview of the techniques available and their challenges.

Top tips

- Samples for carbon system parameters should be the first ones collected when using niskin samplers and other water collection systems, this reduces mixing with the background air.

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(18) Human use

This parameter consists of understanding and quantifying the anthropogenic impacts on ecological communities in our oceans. A socioecological approach that integrates relevant literature, local scientists, and practitioners is key to put the observed environmental data into a broader human context that allows for robust conclusions about human-ocean interactions. Quantitative data across spatiotemporal scales, especially in the high seas, is urgently needed to understand the distribution and intensity of human use and its impact on marine communities.

Sampling, data collection and storage equipment

Human use impacts around a given study area can best be quantified across larger regional and temporal scales by completing a literature review for relevant studies ahead of the research cruise. Meeting formally with local stakeholders, such as scientists, management or governing bodies, and Indigenous and local knowledge holders, and utilizing online databases (e.g. EMODnet), scientific literature, and workshop or meeting reports, and even archival sources (for historical trends), can be useful sources of qualitative and quantitative data. The local ministries and fishing authorities will often have relevant quantitative data of the study areas, such as stock assessments and catch records. Additionally, local research bases situated within protected areas should be contacted if the study falls within such an area. Where no "external" anthropogenic impacts such as major commercial fishing or tourism are otherwise recorded [TT1] , data on local subsistence or other small-scale fishing, and oceanic events (such as oil spills, die-offs, illegal dumping, anomalous occurrences of species, etc.) may be recorded by the teams on site (e.g., Seychelles Island Foundation research base on Aldabra, Seychelles). Where conversations with local partners are undertaken, data should be collected in the form of interviews (structured or semi-structured) which focus on human uses in and around the specific region or site to develop a deeper and more integrated understanding of the local anthropogenic engagements with and impacts on ocean communities over time. Data and information are most relevant when local partners are recognised, valued, and co-create the research questions

Surface observations and indirect impacts assessed from visual surveys (see also parameter 19 - Records of marine litter) can provide additional data, however it is important to note that their potential to quantify human use may be limited and these data offer only a snapshot view across temporal scales. Animal behaviour (e.g., flight distance), abundance, distribution, and size (e.g., of commercially important species) can often give important clues to fishing pressure in the area. Standard stereo video survey data collected as part of benthic and pelagic surveys (see also parameter 8 - Epibenthos) can be used to obtain size measurements and quantify behavioural parameters.

A post-expedition follow-up with stakeholders and managing authorities should be considered to effectively distribute the gathered knowledge and share lessons learned which can help with interpretation and implementation of the findings in marine spatial planning or marine management and in the design of further studies.

Ideal data and collection format

The data collected depends on the survey method and can vary. Information acquired from literature and interviews, or personal correspondence should be coded and standardised and where possible quantified. Spatial patterns of vessel operation and fishing data such as landing numbers, vessels dispatched, and fishing methods used can all be obtained as raw quantitative data. Increasingly, human use data can be gathered from satellite monitoring of vessels and/or observing vessel activity via AIS.

Ecological data and environmental data on the other hand is usually collected from stereo video surveys and in .mp4 format. The files can be analysed for human use whilst conducting the ecological analysis in dedicated programs.

Data analysis

Literature and interviews

- Decide on terminology / usage data you want to collect when assessing relevant literature to structure interviews after.
- Vice versa consider personal observations collected during interviews to find parameters for literature and data search.

Satellite and socioecological data

- Data from Vessel Monitoring Systems (VMS) data can be used to investigate activity patterns across spatial and temporal scales of larger vessels (Witt & Godley, 2007).

Stereo Video data

- Species behaviour - Flight initiation distance or minimum approach distance can be an indicator for fishing pressure (Pereira et al., 2020). Can be measured from stereo video parameters using, for example, the software EventMeasure, which can measure distance from the camera and thus calculate flight distance.
- Body size – the body size of certain fish species (e.g., parrot fish) can be a direct indicator for fishing pressure as size has been found to correlate highly with fishing intensity (Vallès et al., 2015). Again, EventMeasure can be used to size fish and additional data can be obtained from FishBase (max size, growth rate,...)

Top tips

- Be flexible – incorporate your interview results into your literature search and vice versa.
- Listen to, understand, and document the perspectives of local stakeholders - they will know their local seas and what impacts they face better than visiting scientists. Failing to engage locals can result in distrust, missed dimensions or causal variables, and shallower understandings of local oceanic patterns and conditions over time.
- Share data – whilst local NGOs might provide data on subsistence and artisanal fisheries and other local human uses, they may lack funds to monitor other phenomena, such as illegal activity through satellite data for larger MPA's. Help each other out.
- Respectful collaborations provided more valuable data than one-time interactions. Take time to connected with partners and integrate them into the research.

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(19) Records of litter and anthropogenic damage

Marine litter is found in every habitat of the marine realm, from debris floating on the ocean surface to litter spread across the seafloor. Similarly, anthropogenic damage can be observed impacting benthic communities, for vertebrates and sea birds across the globe. This parameter aims to quantify marine litter present at a study site and the impacts on marine communities.

Sampling, data collection and storage equipment

Data collection consists in most cases of visual transect dives and surveys, where sometimes data can be collected passively as part of a variety of benthic, pelagic, and sea surface surveys and observations. Video surveys conducted by technical divers, submersibles, ROVs and AUVs can collect this data as part of their primary transect surveys, allowing us to quantify results during post-processing. For benthic surveys, litter can be recorded during video analysis where the debris type (plastic, fishing gear, glass, etc.) and size can be noted.

When recording anthropogenic damage to marine organisms and environments, the surveyed environment and type of damage will determine the nature of the collected data. If structural damage to benthic organisms is surveyed, the likely source should be noted, such as damage from anchor chains or destructive fishing practices (where known). For pelagic surveys (i.e. pelagic BRUVs), the type and size of floating litter captured by the cameras can be noted. Also, where observed organisms are directly interacting with litter (i.e. though colonisation or entanglement) or impaired from human activities such (e.g. propeller injuries), the type and severity of impact should be noted. The goal is to capture images and note observations that allow the creation of an evidence base and document change.

Where surface observations and surveys are taking place, floating litter surveys can be coupled with marine mammal and seabird transects. The type of litter can be recorded, but care should be taken when sizing debris as there is no way to check measurements from aerial photos or size items accurately and consistently when they are far away and only seen through binoculars or cameras.

Ideal data and collection format

Together with descriptions of the type of litter or damage, size class information should be collected wherever possible. NOAA has created guidelines on how to monitor and assess marine debris (Lippiat et al., 2013). Importantly, terminology should be clearly defined, as standard terms for marine litter are currently limited and thus comparability of results between studies is often difficult.

The online databases LITTERBASE (https://litterbase.awi.de/), MBARI (Schlining et al., 2013), and JAMSTEC (https://www.godac.jamstec.go.jp/catalog/dsdebris/e/) contain global data on marine litter, including seafloor litter. They compile and pool data from publications, dating back as far as the 1980s. They contain not only analyses of litter, but also maps of litter distribution and impact.

The Project Aware recording sheet contains the most common marine litter types for shallower depths (SCUBA orientated) and could be used for shallow and mesophotic nearshore surveys. Plastic, metal, glass, rubber, wood, cloth and paper materials are listed, and the sheet can be found on the Project Aware website. The data card and marine debris identification guide can be found on https://www.diveagainstdebris.org/DiveAgainstDebrisToolkit.

Data analysis

Data analysis depends on the data format. Video analysis programs such as ImageJ and SeaGIS can be used to annotate the collected videos and photos. Statistical analysis can then be carried out using a range of softwares such as R or GIS.

From video data:

- For example, the material and damage identification terms (such as 'plastic', 'bleached',etc.) can be included in annotation software such as TransectMeasure which is commonly used to analyse benthic video footage. Thus, impacts can be identified and most importantly counted and quantified across transects and environments.

During observation surveys:

- A separate datasheet (example from NOAA here) can be added to the survey in which marine debris can be recorded. Additionally, injuries or entanglements of marine mammals can be noted and imagery can be analysed during post-processing.

Machine learning and automated image annotation are future targets to scale up data analysis and reduce time spent to analyse and annotate footage by hand.

Top tips

- Standardise the terms used in the annotation software before starting your video analysis so that it includes set debris and damage annotations.
- Check with local scientists on what types of marine debris and anthropogenic damage are commonly encountered to easier identify what you recorded and match these observations with pre-existing datasets.
- Where marine mammals are observed, check with records of previous entanglements / injuries to build on existing data.

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https://marinedebris.noaa.gov/research/marine-debris-monitoring-and-assessment-project

https://marinedebris.noaa.gov/sites/default/files/2018%20NOAA_Form_75-103_Marine_Debris_Visual%20Survey_fillable_0.pdf

(20) Microplastic abundance and diversity

Microplastics (small fragments of plastic debris generally accepted as less than 5mm long) were first discovered in plankton samples from the 1960s. They have since been documented in every oceanic and coastal habitat on our planet. Identified as a major concern for future ocean health, we propose a standardised method to identify and quantify these fragments to assess, monitor, and compare their impacts across spatial and temporal scales.

Sampling, data collection and storage equipment

Microplastics can be sampled using a variety of gear types, depending on the targeted environment, but consideration of sample sizes that permit reliable and robust quantification are important. Nets such as manta, neuston or multinets, and cores are some of the most common ways to collect samples from water and sediment respectively. It's important to note that all methods can be hampered by a range of limitations (adverse weather, mesh size, contamination), and alternative methodologies such as submersible pumps, seawater intakes on research vessels and novel sensor systems (such as FerryBox) are increasingly used, but also have challenges in their implementation.

Surface and shallow water nets can be deployed either by hand or with the help of a small winch off the back of the main vessel and little training and equipment is required to effectively run deployments. A team of three should be sufficient, with two people lowering the net and securing it in place whilst the third person acts as a communicator with the bridge/helmsperson and takes survey notes on a sampling sheet. Deployment length, depth (if applicable), time of day and vessel speed should be standardised across all sites and deployments replicated to allow for comparison between sites. The nets should be fitted with a flowmeter which has been found to more reliably quantify results (rather than the vessel log or GPS track), as the total amount of water flow through the net can be recorded. A simple record sheet with deployment time in/out and flowmeter flow in/out at the start and end of each replicate should be filled in by the team. Multinets require a dedicated gear and a trained technician, however they allow to collect samples throughout the water column and down to great depths. With all net types, organic matter can clog nets, and may be a problem when sampling in areas that are highly biologically active. Nets used for microplastic studies typically have a mesh size of 300µm, but some have used mesh size as small as 10µm. See Michida et al., (2019) for more detailed descriptions of surface water microplastic sampling procedures.

Marine sediment is known to be a sink for microplastics and therefore a sediment core can be used to quantify microplastics with higher density than surface water aggregations or with settling properties due to biofouling. All types of corers can be used, including standard push corers, mega- or multi- corers. Grabs are less useful as they collect disturbed samples that do not permit quantification of distinct sediment layers. While mega- and multi-corers require dedicated deployments and relatively calm conditions, push corers require additional platforms such as ROVs, submersibles, or divers, to collect the samples.

Bulk water sampling is now most often used when targeting smaller fragment sizes (<300µm). Generally, this type of sampling uses pumping systems deployed either via attachment to other equipment (CTD rosette or subsurface seawater intakes), or directly from a vessel. The sampling pump consists of a water pump (motorized or manual), a filter and a flow meter.

Strict anti-contamination procedures must be followed to reduce the abundance of accidental fibers from sample collection through to analysis (Woodall et al., 2015). Due to the necessity of anti-contamination

measures, in-depth processing often has to be delayed until reaching the shore where there is a dedicated lab available. Instead, samples should be sealed, clearly labelled, and stored away safely. To quantify the amount of microplastics sampled, the total weight, abundance, total of the longest length measurements and total surface area should be recorded using a stereomicroscope and scales, as presented by Rivers et al., (2019). Enumeration alone does not adequately quantify the extent of microplastics in marine environments. Abundance alone does not capture variation in size classes recorded. Reporting both size (surface area / length), and count measurements (abundance), allows for a robust analysis and comparison between studies.

Ideal data and collection format

Plastic abundance, total weight, total of the longest measurements and total surface area should all be considered when reporting microplastic pollution. Microplastic equipment deployments can be combined with collecting of samples for example, neuston tows can combine microplastic and zooplankton measurements, and sediment cores can be used to collect microplastics and infaunal metrics. A high level of quality assurance and quality control, especially contamination mitigation, is paramount for all types of sampling. Field control measures during sample collection, procedural control during pre-treatment (removal of biological material), positive and negative controls should be used and corrections for contamination must be enforced for all collection steps.

Data analysis

Plastic type can be determined following the steps outlined by Pimple et al., 2020. Concentrations will initially be determined per m^3 for samples taken from nets and per kg dry weight or cm2 for sediment samples.

Top tips

- Contamination can skew results so utmost care should be taken during all steps and all anti-contamination procedures should be clearly documented in outputs with results.
- Where possible, get advice from an experienced microplastic scientist to support sample processing and anti-contamination procedures.
- Bathymetry surveys and visual transects can help in determining soft sediment areas for sediment coring deployments.
- Calm conditions are ideal to sample microplastics to reduce vertical skew induced by wind and wave action.

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Appendix 1

Deployment Number	Continuous and similar format for all deployments. For example: Deployment 001.
Gear	Defined abbreviations. For example, Red Submersible (RS), CTD, SCUBA, etc.
Date	One format for all deployments (i.e., DD/MM/YYY). Check all deployment sheets and sampling sheets at the end of every day to make sure all formats are the same.
Time	One format for all deployments (i.e., 24h as HH:MM). Time stamps should be recorded for time in (time of gear in the water) and time out (time of recovery). Additionally, transect start and stop time, time on/off bottom can be added for dives.
Location	This can be split into various sub-categories. Firstly, Location (i.e., name of island or seamount). Secondly, Site (North 1, East 3, etc.). Lastly, the exact coordinates should be noted where gear was deployed and recovered. The coordinate formats must be the same across all gear deployments, and again this should be checked at the end of each day to ensure consistency.
Unique ID	This is important for biological samples and can be seen as a parent ID for each sample taken. For example, a collected soft coral would be SEY1_0001. If the coral has epifauna on it or a subsample is chipped off, their Parent ID (SEY1_0001) will be noted, followed by their own unique ID in continuous order (i.e., SEY1_0002.)
Storage	Note which storage container was used, i.e., 50ml container or 2ml vial. Note the preservation method (RNA later, 70% ethanol, etc.). Add additional columns for transit storage location and post-expedition storage location to track and trace samples that might be shipped to partner organizations or stored off-site. Also add person in charge of shipping and the recipient on the data sheet, ideally with contact email addresses.
Data Sheet Photos and Scans	Take a photo of each data sheet every evening and scan it. Make sure to save these copies saved in separate folders, ideally backed up on separate harddrives/servers to prevent data-loss at all costs. Have the file names saved on the metadata sheet (i.e., YS_D010_ALP_E1_Scan_Dive_Log – translates to Yellow Submersible, Dive 10, Alphonse Island, East 1, Scan of Dive log sheet.
Event Log Notes	During deployments (or immediately after when SCUBA diving), note down any events, comments or anything of interest that occurred during the dive. For example, gear malfunction, current changes, etc.