

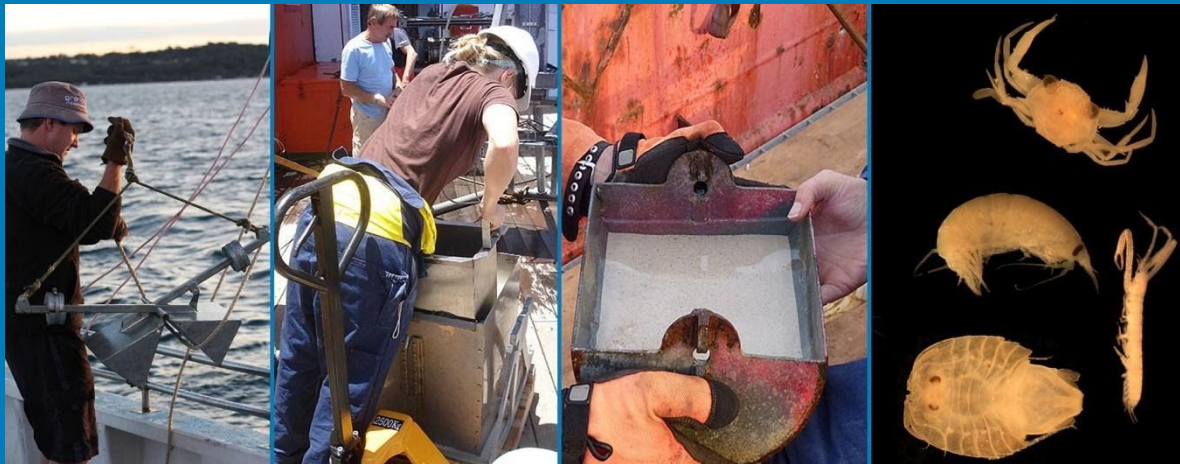


National Environmental Science Programme

9. Marine Sampling Field Manual for Grabs and Box Corers

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Chapter citation:

Przeslawski R, Berents P, Clark M, Dittmann S, Edgar G, Frid C, Hooper G, Hughes L, Ingleton T, Kennedy D, Nichol S, Smith J. 2020. Marine sampling field manual for grabs and box corers. In *Field Manuals for Marine Sampling to Monitor Australian Waters, Version 2*. Przeslawski R, Foster S (Eds). National Environmental Science Programme (NESP).

Platform Description

Grabs and box corers both use receptacles to collect sediment after they are dropped to the seafloor. While the scooping motion of grabs disrupts unconsolidated sediment to various degrees, box corers return largely undisturbed samples of the sediment strata (Eleftheriou 2013). Grabs and box corers target surface sediment and associated porewater and fauna. They are typically deployed over sandy or muddy substrates, although some grabs can also collect gravel or cobbles.

There is no single type of grab or box corer suitable for all environments, and selection of the most suitable type depends on the biological or physical target, substrate, depth, and vessel capabilities (Narayanaswamy et al. 2016). Acquired data can be quantitative (e.g. surface area, volumetric or mass specific) or semi-quantitative due to inconsistencies in sample volume and sediment disruption due to bow waves or other gear effects (Blomqvist 1991). For these reasons, this manual does not mandate specific gear types. There are numerous references to help facilitate decisions regarding grab and box corer equipment for a given marine survey (Riddle 1989, Eleftheriou and Moore 2013, Danovaro 2010, Narayanaswamy et al. 2016). Nevertheless, for monitoring purposes, it is preferable to maintain consistent gear through time and space, and we therefore recommend this where possible.

Scope

This field manual encompasses gear designed to sample unconsolidated sediment and organisms on the seafloor, including grabs, box corers, and push corers.

The samples collected by grabs and box corers can be used to derive a range of physical, chemical, and biological parameters (Eleftheriou 2013), and each of these parameters requires a particular method to process and analyse the sample (Danovaro 2010). In the interest of developing a standard protocol for marine monitoring that is readily accessible to multiple users among various disciplines, this field manual includes only a sub-set of these variables (Table 9.1). These variables were chosen because they can be used by multiple disciplines, are relatively easy to undertake, require minimal specialised equipment or chemicals, and are applicable to ecological indicators in marine monitoring (Hayes et al. 2015). Importantly, the protocol detailed here does not preclude other parameters from being investigated; rather it provides an achievable standard for acquiring fundamental data for monitoring that can be expanded as required to meet additional objectives on a given survey.

This field manual does not include methods for sediment contaminant monitoring, as this is comprehensively covered elsewhere (Simpson et al. 2005). As activities develop (e.g. deep-sea mining) the scope may be expanded in future field manual versions to encompass sediment contaminant monitoring.

Other equipment able to sample sediment is not included in this field manual due to difficulties deploying in deeper waters (e.g. suction samplers) or limited applicability to biological sampling (e.g. gravity, piston, vibro-cores) (Eleftheriou and Moore 2013). In addition, multicorers are not explicitly included because small sample volume may preclude the collection of representative biological communities without aggregation (Williams et al. 2018), although we note that multicorer samples can be aggregated and processed as described in this manual. Although they are able to quantify

infaunal activity, sedimentology, and biogeochemistry, sediment profile imaging (SPI) is also excluded from this field manual due to the vast differences in equipment requirements and data processing (i.e. imagery instead of sediment samples) (Aller et al. 2001, Germano et al. 2011).

Although larger grabs and box corers can sample larger macrofauna and megafauna, including epifauna, for the purposes of this field manual, we focus on smaller macrofauna, including infauna (e.g. Przeslawski et al 2018). Epifauna are targeted in the Sled and Trawl Field Manual. If researchers opt to use a grab or box corer to sample epifauna, we recommend combining Pre-Survey Preparations and Onboard Sample Acquisition from this Field Manual with Onboard Sample Processing from the Sled and Trawl Field Manual (Chapter 8). Meiofauna and microbes are not included in this field manual, and we refer researchers instead to Somerfield and Warwick (2013).

For the purposes of this manual, macrofauna refer to organisms larger than 500 μm . There are varying definitions of faunal size classifications, and these differences seem to reflect the environment under consideration. For example, deep-sea researchers often consider macrofauna to be anything larger than 300 μm (e.g. De Smet et al 2017) due to the prevalence of small body sizes in the deep-sea, while researchers in coastal or shelf waters are more likely to consider macrofauna as > 500 -1000 μm (e.g. Gray and Elliot 2009).

Table 9.1 List of potential measurements from grabs and corers, including whether they are included in this field manual.

	Parameter	Description	Included in field manual
Sedimentology	Sediment texture	A measure of the proportions of mud, sand and gravel size fractions within a sample	Y
	Mean grain size	A summary statistical measure of the size of sediment grains by using effective spherical diameter (ESD)	Y
	Kurtosis	A summary statistical measure of the range of grain size within a sample, ranging from platykurtic (wide range) to leptokurtic (narrow range)	N
	Skewness	A summary statistical measure of the size and direction of the tail in a sediment size frequency distribution, ranging from negative skewness (coarse-tailed) to positive skewness (fine-tailed)	N
	Carbonate	A measure of the proportion of a sample comprising calcium carbonate material	Y
	Mass physical properties	A measure of bulk or dry density, water content, porosity, or permeability	N
Biogeochemistry	Organic matter content	A measure of the total organic matter content, organic carbon, or organic phosphorus	Y
	Contaminants	Concentrations of various pollutants including heavy metals, PAHs, PCBs, etc	N
	Pigment	Quantification of chlorophyll-a, phaeophytin and other by-products of photosynthesis	Y
	Bioavailable organic matter	Quantification of carbohydrates, proteins and lipids	N
	Redox balance	Quantification of the Eh of sediments, providing an indication of anaerobic conditions and diagenesis	Y

Biology	Sediment respiration	Quantification of the release of CO ₂ from sediments over time	N
	Porewater chemistry	Chemical characterisation of water between sediment grains	N
	Microbes	Abundance, biomass, or composition of viruses, bacteria and other prokaryotes, protists	N
	Meiofauna	Abundance, biomass, or composition of metazoan meiofauna	N
	Macrofauna	Abundance, biomass, or composition of macrofauna	Y
	Mega fauna	Abundance, biomass, or composition of mega fauna	N

Grabs and Box Corers in Marine Monitoring

Grabs and box corers have been used successfully to monitor changes in benthic environments over time (Maurer et al. 1993, Ruso et al. 2007, Frid 2011, Clare et al. 2015), although the challenge of revisiting sites mean that multiple samples across a representative area of a given habitat type may be necessary to detect trends (Morrisey et al. 1992a,b, Rogers et al. 2008, Spencer et al. 2011). In addition, repeated sampling using grabs and corers in the same area may result in habitat disturbance and associated statistical artefacts (Skilleter 1996). Grabs and corers can also provide species inventories or biodiversity assessments which can then be applied to a monitoring program as baseline data or to inform the interpretation of imagery (Przeslawski et al. 2013). In this way, they are similar to sleds and trawls, but grabs and corers sample a much smaller spatial area (< 1 m², often considered a point location) rather than the hundreds of square metres often traversed by a sled. This characteristic needs to be considered in environments of low faunal abundance (e.g. some deep sea areas) or high heterogeneity.

In addition to their collection of fauna, grabs and corers are also useful to marine monitoring for their ability to characterise substrate and provide environmental baseline data. For example, EMODNet has harmonised data from disparate sediment samples to produce classification schemes at regional and national scales (Kaskela et al. 2019).

However, because they are a point location, grab and box corer sampling is a very effective method for quantifying heterogeneity at a range of spatial scales (i.e. within- and between-site heterogeneity), as long as the GPS position for each 'drop' is recorded and the sampling area is constrained for comparability of analysis (replicate samples are all collected within a standardised distance from the target position at every site).

Equipment

Equipment must be appropriately set-up to ensure as much consistency as possible among surveys and to facilitate gear replacement if necessary. The overarching goal of appropriately choosing and setting up equipment is to sample as much of the sediment as possible with minimal disruption, within the limitations of the given equipment. It is recommended that a survey include at least two gear types to sample sediments, one targeted for finer sediment (muds) and the other targeted for sands and coarser sediments (gravel). Researchers should ensure appropriate statistical tests are performed to test for potential confounding factors of gear type on biological variables, particularly regarding penetration depth and substrate type.

The key components for a grab include the following, all of which should be documented:

- Type of grab, including firing mechanism (e.g. Van Veen, Smith-McIntyre, Shipek);
- Weight of grab;
- Bucket dimensions (surface area sampled, shape, maximum volume);
- Maximum penetration into the substrate;
- Trap door to allow examination of sample volume upon recovery and to allow sediment sampling from the relative undisturbed centre. Most grab designs can have this feature, but not all manufacturers include it;
- Additional weights (by providing an option for extra attached weights to a grab or corer, equipment functionality can be optimised among more habitat types); and
- Standard electronics to be used (e.g. GPS, camera, USBL, vessel sounder).

The key components for a corer include the following, all of which should be documented:

- Type of corer (e.g. box, multicorer);
- Weight of corer;
- Surface area of sample;
- Maximum volume of sample;
- Additional weights (by providing an option for extra attached weights to a grab or corer, equipment functionality can be optimised among more habitat types); and
- Standard electronics to be used (e.g. GPS, camera, USBL, vessel sounder).

Grabs and box corers can also be fitted with other sampling platforms and sensors. A mounted video camera can add valuable information about the *in situ* appearance of the seabed that is sampled including opportunistic imagery of biota, as well as an indication of the performance of the gear (Blomqvist 1991). Similarly, conductivity-temperature-depth (CTD) meters and other sensors provide information about the surrounding environment, while a pinger (i.e. near-bottom echosounder) provides information to the operator about distance to the seafloor which can be very important for controlling the final operation near the seafloor (Narayanaswamy et al. 2016).

Pre-Survey Preparations

Identify a chief scientist who will be responsible for making onboard decisions related to samples, particularly regarding prioritisation of samples during onboard processing. This will be particularly helpful during busy periods with multiple back-to-back deployments. For 24-hour operations, a second-in-charge must also be identified to cover alternating shifts.

Confirm sampling design meets survey objectives, is achievable with planned equipment and time, and has been communicated to all key scientists and managers. See Chapter 2 for further details on sampling design.

Address fine-scale variation and the need for replication in survey sampling design. Although replication should be considered in sampling design among all platforms (Chapter 2), it is particularly important for grabs and box corers due to the large potential variation in biological and environmental variables across metres to centimetres that may preclude the detection of changes over time (Rogers et al. 2008). Each box core or grab deployment should be treated as a discrete sample (i.e. sub-dividing sample is not replication, nor is the aggregation of samples representative). In addition, the type and size of bedforms present should be considered in the assessment of replicates. For example, a grab may land on the crest of a sand wave, thereby returning a sample that is not representative of the variability of the seafloor at the sampling site. We recommend at least three replicate deployments be undertaken at each station (e.g. Long and Poiner 1994) to enable the quantification of fine-scale variation. When this is not be feasible (e.g. in deeper waters with long deployment times, priority to maximise spatial extent of sampling area), replicates should be collected from a sub-set of stations (e.g. Przeslawski et al. 2013) or appropriate geostatistical methods must be used to estimate grab-to-grab variance (Diggle and Ribeiro 2007).

The most appropriate grab or box corer must be identified to suit the vessel, environment, and scientific objectives (Rumohr 1999). Although this Field Manual does not require equipment that preserves the integrity of sediment samples (e.g. multicorer), the use of such equipment may be necessary if a marine survey has scientific aims complementary to the monitoring program (e.g. characterising infauna or geochemical variables through the vertical sediment profile (Eleftheriou 2013)). The results of some sedimentological and geochemical analyses are sensitive to the manner in which the original samples are collected, handled and stored. Ideally, marine sediment collection for the assessment of sedimentology and biogeochemistry should be carried out avoiding any unnecessary manipulation of the sample that could preclude identification of the surface layers. In order to concurrently acquire the fundamental data identified in this Field Manual (biology, sedimentology, biogeochemistry), the chosen grab or box corer should sample an area of the seafloor at least 0.1 m² and be able to penetrate 5-10 cm into the sediment (Rumohr 1999, Bale and Kenny 2005). To maintain consistency between sites and repeat surveys, only the top 2 cm should be sampled for sedimentology and biogeochemistry; if the sample is disturbed such that the top 2 cm cannot be identified, we recommend redeploying the gear.

Consideration must be given to the location of the grab or corer during deployment. For deep waters where the gear may be hundreds of metres away from the vessel during sample collection, an ultra-short baseline (USBL) is recommended to identify the true location (Narayanaswamy et al. 2016). If a USBL is unavailable, the angle and length of wire payed out should be recorded so that gear location can be trigonometrically estimated (Milroy 2016).

During the planning phases, taxonomists and museum curators must be engaged to ensure that all biological specimens are appropriately identified and lodged at national repositories (i.e. museums) if feasible. The appropriate taxonomic resolution at which specimens will be identified should also be determined. Species-level identification may be appropriate for voyages of discovery (Przeslawski et al. 2013) and impact assessments, while family level identifications may be acceptable measures of response to environmental gradients (Olsgard et al. 1998, Thompson et al. 2003, Wlodarska-Kowalczyk and Kedra 2007).

Similarly, contractors or collaborators for sedimentological and geochemical analyses must be engaged if in-house capability is not available, including cost and funding sources for such analyses.

Decide on sediment storage and biological specimen preservation or fixation methods. Sediment samples will need to be refrigerated (for sedimentology) or frozen (for biogeochemistry) while biological specimens will need to be preserved. Depending on the collaborating taxonomists and project objectives, larger or fragile biological specimens may be preserved separately (e.g. ophiuroids) or in a different preservative (e.g. buffered formalin to retain morphological integrity of soft-bodied animals). In addition, staining may be used to aide sorting, although this may hinder species-level identifications. Choice of fixatives, preservatives and stains must be done in consultation with taxonomists, molecular biologists, and biochemists who will be involved in using the samples. See Coggan et al. (2005) and Schiaparelli et al. (2016) for information about appropriate preservatives for a range of purposes (species identification and description, genetic analysis, biochemical analysis).

Ensure adequate work health and safety (WH&S) risk assessments are undertaken regarding correct use of sampling equipment and chemicals onboard (i.e. ethanol, formalin). This should include identifying appropriate storage locations for chemicals, as well as personal protective equipment, ventilation, and first aid kits.

Obtain appropriate permits that may apply to collect specimens. Ideally, all surveys using grabs or corers will have a permit for biological collection. If target samples are sediments for physical analyses (e.g. geology survey), biota will still be collected as part of the sample. Without appropriate permits, biological material simply gets discarded overboard. Permits must be considered not just for collecting activities, but also for sample transport to receiving institutions. For example, scleractinian corals are regulated under the Convention on International Trade in Endangered Species, and there may be restrictions on shipping these taxa to museums or other repositories (especially those overseas) without a permit. See Appendix A for a list of possible permits needed for sampling in Commonwealth waters.

Determine if specialists are needed for gear use. Many grabs and box corers require experience to safely prepare, deploy and retrieve. The details below are not targeted for any one particular equipment or system, and we recommend engaging an experienced crew who have previously deployed similar devices. Refer to point above regarding WH&S, and ensure all gear operators are thoroughly briefed.

Establish a standardized winching process suitable for the chosen gear, as this is critical to maintenance of sample quality. For example, most gear should involve a complete stop and slow lowering for the last few metres. This will reduce the bow wave and associated loss of surface material and reduce the likelihood of raising of the sampler before closure is completed (Rumohr 1999).

Design workspaces and workflows for sedimentology, biogeochemistry, and biological sub-sampling. Each collected sediment sample must be sub-sampled or whole samples assigned to a single discipline because each discipline requires particular methods and preservatives that may interfere with the other. For example, the decomposition of infaunal animals affects organic content and other biogeochemical parameters, but biological preservatives will interfere with many geochemical analyses (Bale and Kenny 2005).

Organise shipment of samples from vessel to repository. If only of a small size, refrigerated and frozen sediment samples may be more cost-effective to be transported as baggage with aircraft passengers in which case airline requirements should be considered. Samples in ethanol or

formalin are considered dangerous goods, and associated transport must be arranged. Planning for shipment of samples well in advance of the survey will expedite demobilisation and will ensure sample integrity.

Pre-survey checklist

Task	Description/comments
<input type="checkbox"/> Identify onboard chief scientist .	
<input type="checkbox"/> Confirm sampling design meets necessary criteria (e.g. replicates).	
<input type="checkbox"/> Identify most appropriate grab(s) or corer(s) to be used.	
<input type="checkbox"/> Engage taxonomists, curators and contractors. Cost activities.	
<input type="checkbox"/> Determine storage and preservation methods.	
<input type="checkbox"/> Complete WH&S risk assessment.	
<input type="checkbox"/> Decide method(s) for locating gear during deployment.	
<input type="checkbox"/> Obtain appropriate permits.	
<input type="checkbox"/> Document gear specification and establish winch protocols.	
<input type="checkbox"/> Determine if specialists needed for deployment.	
<input type="checkbox"/> Design workspaces and workflows.	
<input type="checkbox"/> Organise shipment of samples.	

Field Procedures

A visual summary of some the key steps to follow when deploying benthic sleds or bottom trawls is shown in Figure 9.1.

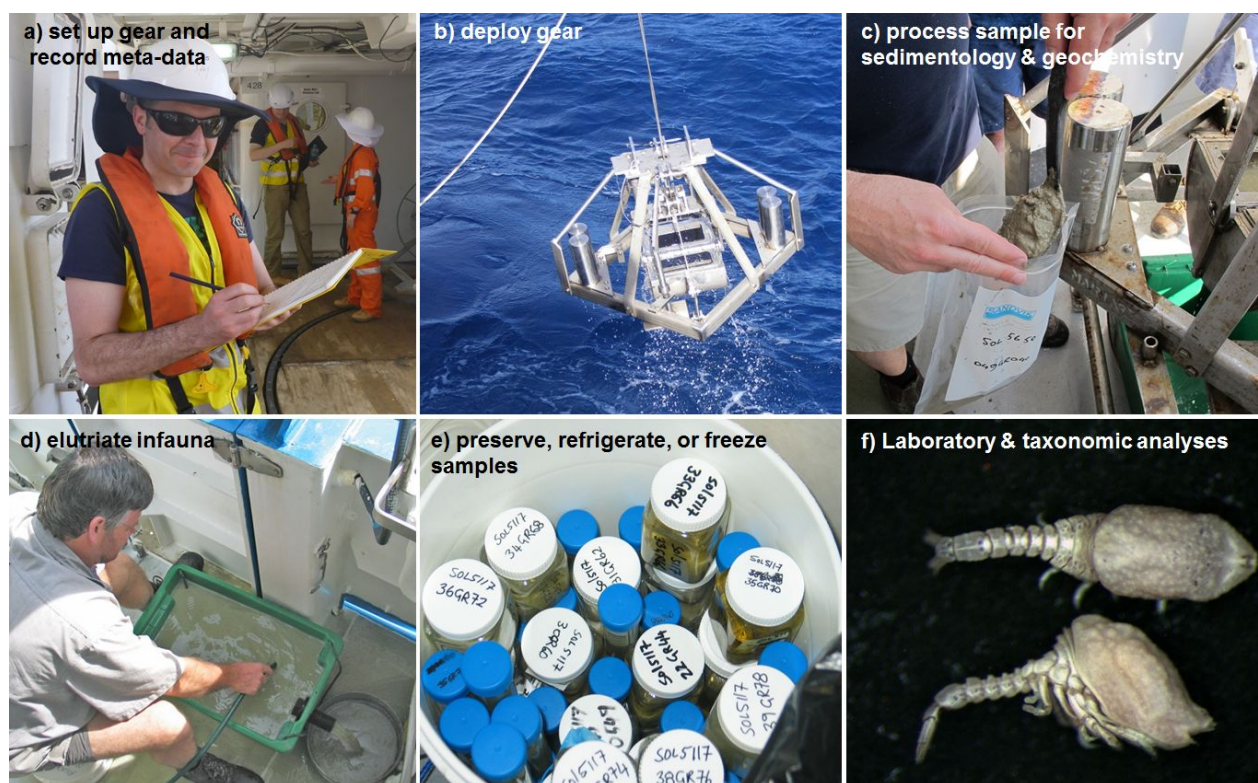


Figure 9.1 Images from key steps involved in the use of grabs or box cores for marine monitoring: a) Recording metadata during gear deployment, b) Retrieving a Smith-McIntyre grab, c) Transferring sample for sedimentological analysis from grab to storage bag, d) Elutriating sediment over a sieve, e) Preserving a bucket of infaunal samples in ethanol, f) Sorting cumaceans under the microscope from elutriated infaunal samples.

Onboard sample acquisition

1. Use multibeam data or underwater imagery to confirm appropriate areas to sample (soft vs hard substrate) and to identify the most appropriate equipment based on fine or coarse sediments.
2. Use USBL System to ensure accurate positioning (Schlacher et al. 2007, Williams et al. 2015) *[recommended, especially in deep waters]*
3. Document the specifications of all sampling gear to be used. This includes gear size and configuration (dimensions, weight) and deployment needs (wire length estimated, USBL methods), as well as sampling surface area, maximum volume, and maximum digging depth. This information must be included in survey metadata.
4. Record all metadata related to each sample station, specified in Table 9.2.

5. Deploy the grab or corer according to gear-specific protocols. Record GPS position, date, time and water depth when the sampler reaches the sea bed.
6. When the equipment is lifted from the water, follow gear-specific protocols for its safe return to deck and access to the sample. Special care may be needed in rough conditions to ensure the sample is not spilled or in situations when the grab or corer has not been triggered.
7. Assess the success of deployment and record the proportion of grab or corer filled (Table 9.2).
8. If there is significant damage to gear, gear closure failure, sample spillage or scant sample return, the sample should not be used in quantitative comparisons with other deployments. If possible, repeat a deployment at that location. Scant sample is defined as being at least 50% empty.
9. Record general observations, particularly conspicuous biota, general sediment description, and evidence of anoxic or reduced sediments (i.e. black/green colour, sulphur smell).
10. Photograph the entire sample with the station identification placard. If taking photos of individual biota from the sample (must be done after step 10), photograph them on the station identification placard. It may be worth considering including a basic scale bar on station identification placards for this purpose.
11. As soon as practical, begin onboard processing of the sample for sedimentology, biology, and biogeochemistry (next sections, Figure 9.2).
12. After all samples have been removed (next sections, Figure 9.2), thoroughly wash gear to prevent cross-contamination. Set up gear for next deployment or safely secure for long transits or if operations have ceased for the day.

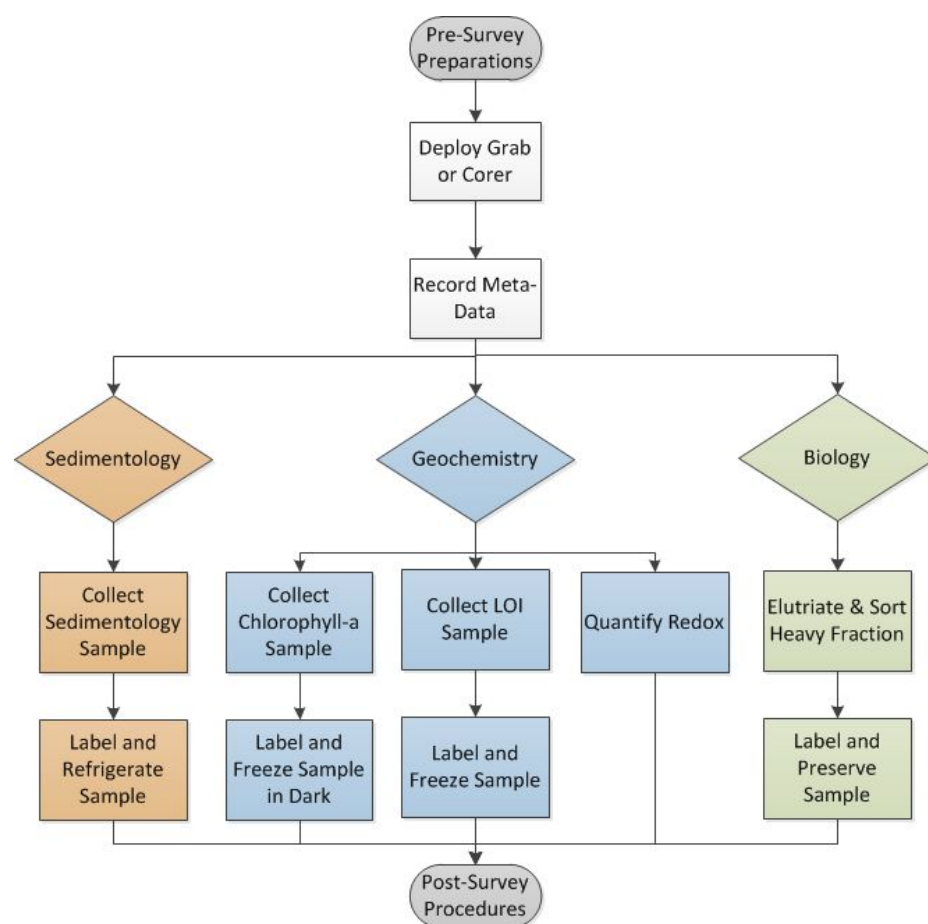


Figure 9.2 Workflow for onboard sample acquisition and processing from grabs and box cores.

Table 9.2 Sample field datasheet to record metadata from each grab or corer deployment. Waterproof paper and pen/pencil is required.

[illegible]

¹ Recording the length and angle of wire payed out during seafloor contact is required if the survey is in deep water with no USBL; otherwise this is just recommended

Onboard sample processing & storage

13. For most equipment, the sedimentology and geochemical sub-samples can be accessed while the sample remains in the grab or corer, thus minimising disturbance. The biological sub-sample can be processed after these sub-samples have been removed.
14. When processing biological samples, pass any excess water from the sampling gear over the sieve; for a box core this will likely need to be done with a siphon. Process the material retained on the sieve, refer to biological steps below.
15. Undertake geochemical, sedimentological, and biological processing steps below for each sediment sample collected.
16. After samples are processed, transcribe the metadata from Table 9.3 into digital format. This can be done in the evening or during other shipboard operations, but it should be done onboard because it provides an immediate back-up, allows for correction of obvious errors, and facilitates timely metadata release.
17. During demobilisation, ensure samples and drums are properly closed and implement shipping according to decision made during pre-survey planning.

Sedimentology (texture, colour and composition)

The following procedures are to be used to obtain sediment samples for quantification of commonly analysed metrics related to grain size and carbonate content (Nichol et al. 2013).

a) Using a spatula or spoon, scrape surface sediment, collect a maximum 300 g wet weight (~2-4 tablespoons) in a plastic zip-lock bag. If you're collecting a particle size sample for comparison with contaminants data or for integration in the national [seabed sediments collection](#), this must be taken from the top 2 cm of the sample. If you're collecting a particle size sample for comparison with infaunal data, then the particle size sample should be representative of the whole sample profile. If you are sub-sampling a grab sample for sedimentology, biogeochemistry, and biology, leave any visible living organisms for biological steps below, but retain shell material.

b) Describe the entire sediment sample using a visual assessment. First identify whether the dominant constituent is Mud, Sand or Gravel. Gravel is > 2 mm diameter, including any shell fragments, coral, rhodoliths or rocks. Sand is < 2 mm and > 0.063 mm diameter. Mud is < 0.063 mm diameter.

The following description will assist a visual and tactile assessment:

- **Sand** – Individual grains can be readily seen and felt. When wet, sand will form a cast that crumbles when touched.
- **Muddy sand** – Sand grains are visible but the sample contains enough mud (silt and clay) to make it somewhat cohesive. Will form a cast when moist that can bear careful handling without breaking.
- **Mixed sediments** – Mixture of sand and mud. Has a gritty feel, but smooth overall and slightly plastic. Will form a cast when moist that can bear firm handling without breaking.

- **Sandy mud** – Overall fine texture, slightly gritty to feel that can form a thin ribbon when rolled between fingers. Will form a cast when moist that can bear robust handling without breaking.
- **Mud** – Uniformly fine texture, sticky and with very slight gritty feel if silt is present. Will form a long flexible ribbon when rolled.

c) Assign a Simplified Folk Textural Class to the sample, based on the estimated mud, sand, and gravel proportions (e.g. Figure 9.3, Table 9.3).

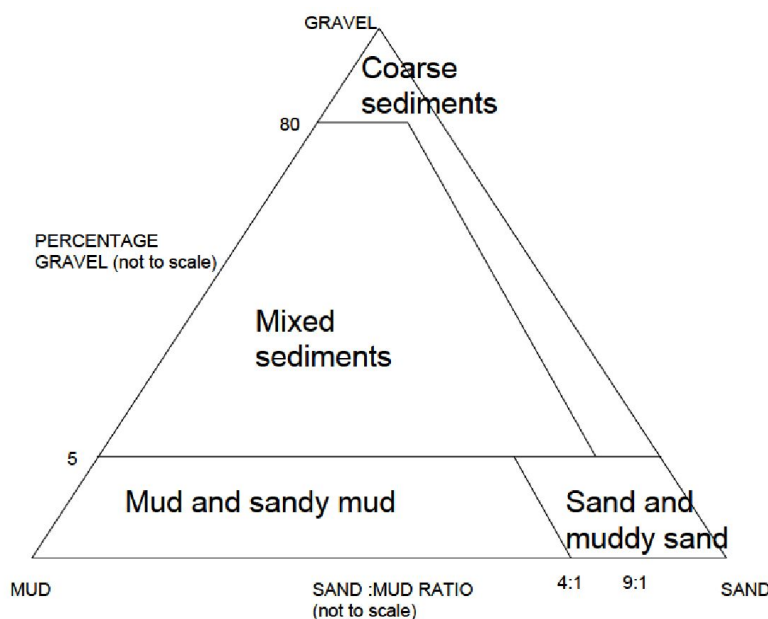


Figure 9.3: Simplified Folk Textural classes

Table 9.3: Simplified Modified Folk Textural classes for visual classification of seabed sediments

% Gravel	Sand : Mud Ratio	Simplified Folk Class
>80	>9:1	Coarse sediment
>5, <80	<9:1	Mixed sediments
<5	>4:1	Sand and muddy sand
<5	<4:1	Mud and sandy mud

d) Assign a colour to the whole sample using a Munsell colour chart, noting the Munsell code (colour, value, chroma) and colour name [*Recommended*].

e) Estimate whether the sample is comprised of dominantly (>50%) carbonate material, non-carbonate (i.e. lithics), or mixed.

f) Note the presence of other materials, such as whole shells, articulated bivalves, shell fragments, corals, wood or lithics and record the relative abundance as: Trace (just noticeable); Few (noticeable); Common (very noticeable); Abundant (little else noticeable).

Record the above properties with all available metadata (Table 9.2), as in the example below:

- Sand and muddy sand
- 7.5 YR 7/6 (reddish yellow)
- Carbonate dominant
- Trace of volcanic rock fragments

g) Photograph the sediment sample with a label, scale and Munsell colour chart [*Recommended*].

h) Double bag the sample. Label clearly on the surface of the bags, as well as on aluminium tags or waterproof paper placed between the bags. Refrigerate.

Biogeochemistry (chlorophyll-a, organic matter content, redox)

These geochemical analyses are based on the assumption that the sediment surface is relatively intact and the surface sediments can be identified. If this is not the case, it is recommended only organic matter content is assessed, with information on sediment mixing recorded in the comments section of the metadata sheets (Table 9.2). The following procedures are to be used to obtain geochemical samples for quantification of commonly analysed metrics related to chlorophyll-a (Danovaro 2010), organic matter content (Heiri et al. 2001, Wang et al. 2011), and redox (Danovaro 2010, Edgar et al. 2010). For all biogeochemical samples, record the geochemical samples taken on a station form with all available metadata (Table 9.2).

Chlorophyll-a & phaeophytin

- a) Using a spatula or spoon, scrape the surface sediment to a maximum depth of 2 cm. Collect ~ 100 g wet sediment (1-2 tablespoons).
- b) Remove any visible living or soft-bodied organisms for biological steps below, but retain shell gravel.
- c) Place a sub-sample of wet sediment into a 50 mL plastic vial for chl-a analysis. Chl-a degrades in sunlight so this step should be performed quickly and out of direct sunlight if possible.
- d) Wrap in foil and store frozen at -20°C in the dark until post-survey analysis of chl-a. Ensure sufficient head-space in the vial or bag to allow for the expansion of sample when frozen. Note that analysis should be performed within 4 weeks of collection, although use of ultra-cold freezers extends storage times.

Organic matter content

- e) Place another sub-sample of wet sediment into a 50 mL plastic vial or small zip-lock bag for post-survey analysis of total organic carbon.
- f) Homogenise this sample, and store frozen at -20°C until analysis of organic matter content, generally within 3 months of collection. If liquid nitrogen is available, samples should be snap frozen and stored in a dewar following appropriate protocols.

Redox

- g) Use a suitable redox probe consisting of a portable pH/Eh meter, redox electrode (with shaft >15 cm long, preferably as thin as possible, with Platinum indicator electrode) and a reference electrode (double junction silver/silver chloride).
- h) Use Zobell's solution as a reference to calibrate the redox electrode before each redox profile, recording the redox measurement of the solution. The solution (0.003M potassium

ferricyanide, 0.003M potassium ferrocyanide, and 0.1M potassium chloride) has an Eh value of +430 mV at 25°C.

- i) Carefully insert the redox electrode into the intact sediment surface as soon as possible after collection at depth intervals of 1 cm from the surface to 10-20 cm (depending on depth of sediment).
- j) Record the Eh readings (in mV) when the meter readings stabilise (for a minimum of 5 seconds) at each depth.

This method provides a rough indication of the levels of oxygen in the substrate. This information is crucial to assess the interstitial conditions of the sediment as affected by burrowing organisms or anthropogenic factors. Measured in millivolt, often reported as Eh (hydrogen standard electrode) the redox potential has a low-definition significance because of the multi-factors interacting in producing it, and as such is semi-quantitative. Generally positive values are associated with well-oxygenated sediments, whereas highly negative values (<-200 mV) are typical of suboxic or anoxic conditions (Danovaro 2010).

To convert to redox potential and ensure quantitative outputs for comparability between studies, measurements must be calibrated on the Zobell's measurement, i.e. add the difference to each reading in the profile (difference = std reading at 25°C – field Zobell's measurement) to standardise measurements for local conditions (e.g. temperature). This must then be standardised based on the standard hydrogen electrode which gives the potential.

Biology (infauna and macrofauna)

- a. After supernatant water has been passed through a sieve and sedimentology and geochemistry steps have been performed (< 5 tablespoons of sediment removed, see above), transfer the remaining sample from grab or corer to an elutriating bin. If additional survey objectives require data on sediment depth (see Pre-Survey Preparations), each sediment layer should be in a separate nally bin.
- b. Weigh the whole sample using an onboard scale. Record in metadata sheet (Table 9.3).
- c. Rinse the grab or corer thoroughly to avoid contaminating the next sample collected.
- d. Elutriate¹ the sample by running moderately flowing seawater into the elutriating bin and gently agitating the sediment to release light-bodied animals into the water. The water should flow from the bin through an outlet under which the sieve is placed (next step). To avoid damage to animals during elutriation, avoid directing water from the deck hose at the sieve, separate fragile visible animals, and remove rocks and shells (these can be saved as part of the heavy fraction if desired, Step 12). Elutriation should be performed until water runs clear, ideally the same amount of time among all sample sites. For coarse-grained sediments, this may only be ~5 minutes, but for deep-sea ooze this may be far longer due to stickiness of the sediment which makes elutriating a challenge.

Fine sediments may require two steps here: semi-elutriation which often retains clods of fine sediment on the sieve, followed by puddling in which a full sieve is immersed in seawater

¹ Elutriation is a process that uses water to float less dense animals away from the sediment. It is a useful technique to separate fauna such as crustaceans and polychaetes but not suitable for gastropods and bivalves.

and vertically agitated to further remove fine sediment (CEFAS 2002). This 2-stage option also accounts for shelled molluscs and other heavy-bodied organisms often missed by elutriation. Regardless, the main goal in this step is to ensure that all animals are separated intact from as much of the sediment as possible.

- e. Stacked sieving is an alternative to elutriation and can provide immediate data related to invertebrate size distribution and biomass (Edgar 1990), although sieving is not ideal in coarse-grained sediments where a large fraction is retained on the sieve and subsequently require much time to sort from organisms from the sediment. If a researcher elects this option, stack larger sieves (e.g. 1000 μm) on top of smaller ones (e.g. 500 μm), add small amounts of sample to the top sieve and gently flush through with seawater. Skip to Step 12.
- f. Retain macrofauna by allowing water to flow onto a 500 μm sieve. This size was chosen, as it has already been used in AMPs (Nichol et al. 2013, Przeslawski et al. 2013, Przeslawski et al. 2018) as well as successful international monitoring of soft sediment communities (Frid 2011). It is a compromise between the 1 mm recommended by other protocols (Rumohr 1999) and the time and effort needed to process specimens using 300 μm or smaller. If individual survey objectives require a finer mesh size (e.g. 100 μm or 300 μm) or comparison with datasets from larger mesh size (e.g. 1000 μm), layer the sieves and process samples separately so that the recommended standard of 500 μm is still followed and data are comparable.
- g. Sort the heavy fraction by hand and remove any live animals that do not float during elutriation (e.g. molluscs, hermit crabs, animals attached to rocks) (i.e. heavy fraction specimens) and place them in the sample container.
- h. Material retained on the sieve should be flushed off using seawater in a squirt bottle directed from the underside of the sieve into a funnel and sample container. It is important to minimize the amount of water used in this step to ensure adequate preservative concentration. If a large amount of seawater is used for flushing, the sample can be sieved and flushed again. Alternatively, a puddling bin can be used to concentrate the sample into one small area of the sieve for flushing into the sample container (CEFAS 2002).
- i. Preserve elutriated and heavy fraction specimens according to methods decided in 'Pre-survey Planning' in sample container. If there is a large volume of material, use multiple sample containers to ensure enough preservative in each container. Consider museum requirements for sample preservation, and also see Rees (2009) and Schiaparelli et al. (2016) for comprehensive description of fixatives and preservatives used for marine invertebrates. Larger organisms may be preserved separately (e.g. polychaetes may be relaxed in MgCl_2 and fixed in formalin).
- j. Place a solvent-hardy label in each sample container with sample and station number, date, location and vessel/collector. This information is essential for quality control in processing and archiving of specimens. It is not sufficient to label only the outside of the container, as

this can easily rub off. See Box 15.6 in Schiaparelli et al. (2016) for suitable label characteristics.

- k. Place the sample container in a large sealable container (i.e. lidded drum) double-lined with a durable plastic bag with other samples preserved using the same chemicals (e.g. ethanol). Label the drum with survey details and the type of chemical fixative/preservative inside. Since samples from the same grab may end up in different drums due to different preservatives, it is imperative to have a good record-keeping system.
- l. After placing samples within the inner bag of the drum, back fill between the bags with an appropriate amount of spill kit (e.g. vermiculite or absorbent kitty litter). In this way the contained specimens are compliant with handling (triple bagged) for road transport of Dangerous Goods. *[Recommended]*
- m. Store large drum onboard in an approved storage area for hazardous chemicals.

Post-Survey Procedures

Sample curation and submission for analysis

Sedimentology

Sedimentology samples can be transported as refrigerated freight in a fully sealed, rigid container (e.g. esky) to Geoscience Australia for laboratory measurement. Alternatively, researchers may transport samples to their own labs if performing analyses in-house or through laboratories accredited by the National Association of Testing Authorities (NATA). Regardless of whether the sample is analysed by GA or elsewhere, data should still be submitted to the national seabed sediments collection data repository on the AusSeabed Marine Data Discovery portal (<http://marine.ga.gov.au>) (Section 9.7.2). Analytical methods include wet sieve separation into mud, sand and gravel fractions, laser granulometry of mud and sand fractions, and acid digestion of carbonate content for the bulk or mud and coarse fractions. Other methods are also available for those with their own expertise and equipment (e.g. calcimeter method in Kennedy and Woods (2013)).

If lodging samples at GA for analysis, the following metadata are required prior to receipt of sediment samples:

- Survey metadata including: survey name, survey number, survey vessel, start and end date of survey, latitude and longitude of survey bounding area, name of chief investigator;
- Sample location for every sample listed in decimal degrees to at least five decimal places;
- Sample water depth for every sample listed;
- Sample ID follows a standard naming convention (see example attached);
- Sample bags are labelled clearly with the sample ID (as above); and
- Sample condition as when collected (i.e. wet, disaggregated, excess water drained).

Biogeochemistry

Geochemical analysis of sediment samples should be conducted by the organisation undertaking the survey. Alternatively, sample analysis should be outsourced to Geoscience Australia (Loss on Ignition analysis, as described below) or NATO-accredited commercial laboratories or collaborators (chl-*a* analysis).

Total organic matter content

Total organic matter content of marine sediments is determined by Loss on Ignition (LOI). Note that LOI is not the same as total organic carbon (TOC) (Schumacher 2002). Parameters such as temperature and combustion time vary among individual researchers, and there is no universally adopted standard. Here we choose parameters based on a compromise appropriate to a diverse range of environments (Heiri et al. 2001, Wang et al. 2011). We strongly recommend that researchers use these guidelines to ensure data from different surveys can be compared. The general recommended steps for LOI to contribute to a national standardised dataset are:

1. Record wet weight of the sample.
2. Homogenise the wet sample (1-2 g dry weight).
3. Place sample into a pre-weighed crucible.
4. Oven dry for at least 24 h at 105°; longer times may be needed in fine sediments.
5. Reweigh crucible and dry sediment.
6. Place crucible in muffle furnace and combust at 550°C for 4 h.
7. Weigh crucible and combusted sediment.

The water content is the difference between the wet and dry sediment weights and is expressed as a percentage of the initial sediment weight. The total organic matter content is obtained as the difference between the dry and combusted sediment weights and is expressed as a percentage of the sediment dry weight.

Chlorophyll-a & phaeophytin

Chlorophyll-a is the principal pigment in plants and is a biomass indicator of aquatic micro-algae which support food webs in the sea, and phaeopigments (e.g. phaeophytin) are the degraded non-photosynthetic products of chlorophyll (e.g. Bax et al. 2001). The ratio between them indicates the “freshness” of the organic matter. Note that samples can be freeze-dried first and this may increase extraction efficiency but also increases the risk of chlorophyll degradation over time. For the purposes of this field manual, we recommend using wet material; this will ensure comparability among datasets. The general steps for chl-a analysis are:

1. Place approx. 5 g wet sediment into centrifuge tube.
2. Add 10 mL acetone (90% saturated with MgCO_3).
3. Mix rigorously (with glass rod or vortex mixer).
4. Place in an ultrasonic bath for 30 minutes under dark conditions (Note: other methods can be used, e.g. shaker).
5. Centrifuge sample (>1500 g for 5 minutes) and decant extract.
6. Use a spectrophotometer to measure absorbance at 665 and 750 nm.
7. Acidify extract with 2 drops of 0.1 N HCl, mix and rest for 60 s.
8. Measure absorbance again at 665 and 750 nm.
9. Calculate the concentrations of corrected chl-a and phaeophytin using the equations of Lorenzen (1967).

Redox

Redox measurements are provided onboard with a probe and there are thus no post-survey procedures are required, other than to calculate redox potential and QC data.

Biology

1. All animals from a given grab or box core should be sorted into separate small containers based on phylum or class to facilitate taxonomic identifications (arthropod, annelid, mollusc, echinoderm, other). This can be done onboard if time permits, but consideration must be given to working under a microscope on a moving vessel. Sorting can usually be done by a non-expert, with only a few groups posing potential challenges (Figure 9.4). Containers should be filled with ethanol or formalin (as per Pre-Survey Preparations) and labelled appropriately with solvent-proof paper.
2. In order to test for potential bias due to differences in sorting efficiency among people, randomly selected samples should be re-sorted by a different person. Removal of 95% or more of the organisms during the sorting process is acceptable; otherwise, re-sorting may be necessary (Simpson et al. 2005) *[recommended when multiple people are involved in Step 1]*
3. Within each sorted phylum, identify organisms to a taxonomic resolution that enables data production in a timely manner, and then count individuals. Identifications can be done by the organisation that collected the samples, museum taxonomists, geneticists, or external

private consultants. Care must be taken to ensure consistent nomenclature is used for undescribed or unnamed species (e.g. defined operational taxonomic units, OTUs).

Figure 9.4 A brief description of taxa that can be challenging to identify but are often encountered when sorting organisms from elutriated sediment samples.

- a) **Scaphopods (molluscs).** These are curved shells with a larger and smaller hole on each end.
- b) **Aplacophorans (molluscs).** These are often confused with worms but are actually molluscs covered with spicules that can make them look furry.
- c) **Foraminiferans (protists).** Forams with tests (i.e. shells) can be mistaken for gastropod shells and can be particularly common in deep-sea sediments and beach sand. This field manual does not target forams so their inclusion in sample processing is not required (image from Wikimedia).
- d) **Crinoids (echinoderms).** The small animals or their dropped arms can superficially resemble polychaete worms.
- e) **Hermit crabs (crustaceans).** These can be mistakenly sorted as gastropods because the crab has retreated into its shell and is barely visible.
- f) **Ophiuroid arms (echinoderms).** These can often be confused with polychaetes, but you'll never see a head. There is no need to save ophiuroid arms unless the central disk is present.
- g) **Ostracods (crustaceans).** Ostracods can be mistaken for bivalves, but they are small shrimp-like animals encased in two shells. You can often see their legs protruding from the shell.



4. Lodge all specimens in an internationally recognised specimen collection (e.g. museum) for curation and public accessibility [*Recommended*].

5. If all specimens are unable to be lodged at a museum due to lack of resources or the need for destructive analyses (e.g. biochemical extractions), then a voucher collection should be produced (i.e. at least one animal per OTU). This voucher collection can be held temporarily by the agency undertaking the survey if there are other surveys planned in the region to aide in subsequent identification. Ultimately, this voucher collection should be lodged in an internationally recognised specimen collection (e.g. museum).

Data Release

Produce a technical or post-survey report documenting the purpose of the survey, survey design, sampling locations, sampling equipment specifications, and any challenges or limitations encountered. See Appendix C for a sample template. Provide links to this report in all associated metadata [*Recommended*].

Sedimentology

For samples submitted to GA for sedimentological analysis, sedimentology data will be publicly available in the national Marine Sediments collection (MARS, <https://portal.ga.gov.au/persona/marine>) following lab analysis and QC checks as part of GA's internal workflow processes. This database includes sediments from estuaries, coasts, shelf, and the deep-sea.

For samples from which sedimentological analysis were done elsewhere, please submit the data to marine@ga.gov.au, along with required metadata.

Biogeochemistry

Submit all geochemical sample metadata and analysis results to GA including:

- Reduced sediments (Y/N);
- Total organic matter content (%); and
- Chl a ($\mu\text{g g}^{-1}$ dry sediment).

The easiest way to do this is to add two columns to Table 9.3 for LOI and chl-a data and submit this to marine@ga.gov.au.

Biology

All biological data should be publicly released, unless circumstances require otherwise (e.g. confidentiality clause or embargo for commercial work). Even in situations when data cannot be shared, the metadata and deployment information should be made available (Steps 1-2 below). Poor scientific data management and lack of data sharing has been shown to hamper scientific progress (Stocks et al. 2016).

Traditional, data related to biological specimens have been delivered as presence-only taxonomic identifications. These are often managed by individual museum scientists or curators and subsequently harvested by the Atlas of Living Australia (ALA). Ala does not yet include absences or information related to sampling effort, thus reducing the applicability of such databases to monitoring purposes.

OBIS is using the data structure described in the project called OBIS-ENV-DATA that allows the linking of species data to other related information (e.g. environmental data, images, sampling effort) (De Pooter et al. 2017). It now has the capacity to store absence records and sampling effort, and is working to include this information in data downloads.

In the meantime, the steps listed below will ensure appropriate and timely release of both metadata and data:

1. Create a metadata record describing the data collection. Provide as much detail as possible on the collection/deployment (either directly in the metadata record itself, or in the form of attached field sheets as .csv, .txt or similar). This should include sampling locations and dates, equipment used, level of sorting applied, etc. All collection/deployment information must be QC-d before inclusion.
2. Publish metadata record(s) to the [Australian Ocean Data Network \(AODN\) catalogue](#) as soon as possible after metadata has gone through the QC process. This can be done in one of two ways:
 - If metadata from your agency is regularly harvested by the AODN, follow agency-specific protocols for metadata and data release.
 - Otherwise, metadata records can be created and submitted via the [AODN Data Submission Tool](#). Note that this tool requires user registration, but this is free and immediate.

This step provides immediate documentation of the methods and location of the collection of biological material. This stage may also include links to field reports or data sheets.

3. Complete the species identifications and associated abundance for targeted groups identified. This can take quite some time, depending on sample size and available resources. It is not unusual for taxonomic identifications to lag years behind survey completion, but this should not delay publication of initial metadata and deployment information. Care must be taken to ensure consistent nomenclature is used and documented for undescribed or unnamed species (e.g. defined Operational Taxonomic Units, OTUs). Ideally catalogues of OTUs are established such that subsequent surveys may use consistent OTU classification, thereby ensuring comparability of data between surveys.
4. QC the data. This includes checking for spelling errors, missing data, consistent nomenclature and use of OTUs, and confirmation that outliers are not data entry errors (e.g. 100 individuals really were collected, not just 10). Current taxonomic nomenclature can be checked using the World Register of Marine Species (WoRMS) 'match taxa' tool (<https://www.marinespecies.org/aphia.php?p=match>). This process provides accepted scientific name, the scientific authority and full taxonomic breakdown for each taxon.

5. Technical specialists should also consider whether the data has to be rationalised. This process is often required to remove potential 'ecological noise' which may adversely affect the statistical analysis of the data. Examples include non-target taxa (e.g. purely pelagic biota such as chaetognaths or ctenophores; or terrestrial biota such as dipterans in grab samples) or juveniles. Newly-settled juveniles are often ephemeral, with high abundance and post-settlement mortality rates, and are therefore not generally representative of prevailing benthic faunal communities (OSPAR Commission 2004).
6. Additional analysis of data may be required, including classifying trophic levels of taxa, and classifying or characterising habitat types for each sample (e.g. National Intertidal and Subtidal Benthic classification in Mount et al 2007; Combined Biotope Classification Scheme in Edmunds and Flynn 2018).
7. Attach or link the full data spreadsheet (including absences and abundances/biomass) to the metadata record previously created and published to the AODN. This will ensure public discoverability and accessibility of the complete data, including absences.

To then publish data to OBIS, inform OBIS Australia (OBISAU) using the contact details and information on <http://www.obis.org.au>.

OBISAU will download the data from AODN or any other site and apply the following procedures.

- OBISAU provides a taxa matching service using WoRMS web services and will validate the dataset as best as possible.
- The data is tested for any temporal or spatial outliers.
- Any observed parameters (biotic and abiotic) are matched where possible to vocabularies maintained by AODN and BODC.
- Metadata is authored from any existing metadata or publications.
- Finally the datasets is published via the OBIS Australia data node <http://ogc-act.csiro.au/ipt/>

OBISAU has the option to publish the data at the same time directly to GBIF, and it has developed a service to inform ALA that a new dataset is available to be harvested for inclusion into ALA.

Field Manual Maintenance

In accordance with the universal field manual maintenance protocol described in Chapter 1 of the Field Manual package, this manual was updated in 2020 as Version 2. Updates reflect user feedback and new developments. There is currently no long-term plan or support for future updates. See Chapter 1 (Introduction to field manual package) for further details.

The version control for Chapter 9 (field manual for grabs and box corers) is below:

Version Number	Description	Date
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0	Submitted for review (NESP Marine Hub, GA, external reviewers as listed Appendix A.	22 Dec 2017
1	Publicly released on www.nespmarine.edu	28 Feb 2018
2	Relevant updates addressing stakeholder feedback and new co-author contributions	July 2020

Acknowledgements

The authors are grateful to Dave Watts (OBISAU) and Seb Mancini (AODN) for providing updates to the data release section for Version 2, as well as the many colleagues and crew over the years who shared field experiences and insights with the co-authors, thereby shaping this manual. In particular, Matthew McArthur helped foster enthusiasm for small macrofauna and infauna in the lead author early in her career.

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