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Sediment sampling, sample preparation and general analysis

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2.1 Sampling design for sediment quality assessments

Sampling design should be considered as a major component of the study design and broader framework applied for a sediment quality assessment program (see Chapter 1, Fig. 1.1) (ANZECC/ARMCANZ, 2000a; USEPA, 2001, 2006). First, the study type needs to be determined, and this will lead to a definition of the study scope (spatial boundaries, scale and duration), and the design of the required sampling program to achieve the data requirements. In many cases, sediment investigations are descriptive studies, simply designed to investigate the spatial and temporal distribution of contaminants, for 'state of the environment' reporting, for compliance monitoring, or to guide management actions such as dredging. In rarer instances, the objective may be to examine contaminant transport and depositional processes. In most instances, the assessment objectives are likely to be driven by regulatory requirements and evaluation of the potential impacts on ecosystem or human health.

Quality assurance (QA) needs to be addressed in any assessment exercise. This should include a consideration of the desired sampling and data quality objectives. Standard operating procedures should exist for methods that may be replicated and require audit or review to check compliance against the QA plans. The assessment of data quality objectives will usually require statistical evaluation to ensure that the right type, quantity and quality of environmental data are collected. There are several documents that provide guidance on these matters (for example, ANZECC/ARMCANZ, 2000a; USEPA, 2001, 2006).

Specific details on the design of a sampling program for an ecological assessment are provided in Chapter 7. Factors such as the depth of sediment collected, in-field processing and the degree of replication required may differ for different assessments and environments.

The design of a sampling program for sediments needs to consider:

- the number and location of sampling sites and their selection;
- spatial variability;
- sampling frequency;
- precision and accuracy;

- measurement parameters; and
- cost effectiveness.

The selection of sites and sampling program design must take into account the fact that sediments are quite heterogeneous, both chemically and physically, with contaminant distribution being very dependent on grain size. In general, contaminants that accumulate via adsorption to particles will be associated with the finest high surface area particles. Sandy and other coarse-grained sediment particles will generally have low contaminant content and will generally pose a low threat to benthic organisms.

The frequency of sampling undertaken in monitoring studies may also be dictated by the rate of sedimentation, or by changes in industries or their practices (for example, discharge conditions, 'footprint'). Sedimentation rates in waterbodies typically vary from 1–2 mm/y to 1–2 cm/y, although in tropical areas with large seasonal variability in river flows, the sediment accumulation in off-river areas can be much larger. Except in the latter cases, recent sedimentation is therefore unlikely to be seen at depths below 5 cm, so this should be noted when deciding the depth of core sections to be selected for analysis. Licensing conditions for industrial discharges are frequently reviewed, and water and sediment quality are affected both by changes to discharges and by events such as storms or spillage. For monitoring of the impacts of single industries, targeted sampling may be most appropriate.

Sampling program design may depend on the distribution of biological activity in sediments, which can be quite variable. Biota use the sediment variously as a refuge, a habitat and a food source. In the case of burrowers, for example, the acceptability of the sediment particle size for burrowing might determine their distribution, while for microorganisms the availability of organic matter or nutrient sources might be critical. Most biological activity occurs in the upper 10 cm, although some organisms can burrow to greater depths.

The depths to which sediments are sampled should be relevant to the monitoring objective. It may be appropriate, in any monitoring survey, to establish the nature of the depth profile of contaminants at the sites under consideration. For example, if the objective is to look at changes over time in the concentrations of contaminants in the top 10 cm of sediment where the sedimentation rate is less than 1 cm/y, measurements every 2–3 years or longer are likely to be more useful than annual measurements; or an alternative could be to sample only the uppermost (0–2 cm) sediment layer each year.

The size of the study area will greatly influence the type of sampling design and site selection methods that are appropriate. More detail on sampling design, including sample numbers and sampling frequency, is provided in ANZECC/ARMCANZ (2000a).

2.2 Patterns of sampling

2.2.1 Random sampling

Random sampling involves the selection of sites randomly in order to provide an unbiased assessment of the condition of the sediments within a waterbody. However, random sampling designs are quite likely to omit sites that could be important in identifying relationships between variables; for example, in estimating a benthic response or contaminant concentration in relation to a known contaminant discharge point. Also, a random selection of stations may not include a sufficient number of such key sites, because many randomly selected sites will be well away from the contaminant source. Simple random sampling is therefore not recommended.

To overcome these problems with random sampling designs, forms of systematic and stratified random designs are usually preferred; sampling each stratum of interest, for

example. Chapter 7 provides further information on these and other sampling designs. Spatial heterogeneity (both horizontal and vertical) must be taken into consideration. Sampling should involve replicate samples to determine localised heterogeneity (cluster sampling). Vertical homogeneity can be readily assessed from core samples and they are preferable to surface grab samples for this reason. Sediment deposition in a waterbody will not occur uniformly but will be dictated by complex patterns of flow. Scouring of bottom sediments is common in the channels of fast flowing rivers, for example, while deposition will occur in low-flow regions.

2.2.2 Stratified random sampling

In stratified random sampling, the system is divided into parts or strata, not necessarily of equal size, in which the variable of interest is as uniform as possible. The number of samples to be taken within in each stratum will depend on the size of the stratum and the variance within it. Strata can vary spatially or temporally. For example, the strata in a sediment may be defined on the basis of grain size (sandy versus silty). Seasonal effects on parameters are less likely in sediments, although there may be effects on the benthic ecology. If so, sampling may need to focus on the seasons in which greatest variability is expected (ANZECC/ARMCANZ, 2000a).

2.2.3 Systematic sampling

Systematic sampling involves sampling at even intervals in space or time. Spatially it usually entails sampling along transects or grids, while temporally it might involve a regular monitoring schedule, such as monthly sampling. Care must be taken to avoid biases that might occur by sampling at a fixed time or location.

Existing information is helpful in forming the sampling program design (ANZECC/ARMCANZ, 2000a). The term 'targeted sampling' is used to describe sites selected based on prior knowledge of other factors, such as contaminant sources, substrate types, water depths, tidal influences, and human activities. Targeted sampling designs can often be implemented quickly and offer much more flexibility than statistically-based (random) sampling designs. Data from targeted sampling cannot, however, be easily used to make predictions of contamination at other sites (stratified random sampling is better for this purpose).

2.2.4 Cluster sampling

Replication can be achieved through cluster sampling; that is, collection of several pseudo-replicated samples in close proximity, as discussed further in relation to ecological sampling in Chapter 7 Section 7.3.1.

2.3 Collection of sediments

For the assessment of sediment quality, surface sediments are most commonly collected, but deeper sediments may need to be collected for evaluating the risks of activities such as dredging. Generally, the upper 10 cm of sediments will be occupied by 'epifaunal' (or 'epibenthic') and 'infaunal' organisms. Epibenthic species (those living at the sediment surface or just above it), such as shrimps and some amphipods, might only be exposed to contaminants in surficial sediments (0–1 cm), while others (such as bivalves, polychaetes) that are infaunal irrigators may be exposed primarily to contaminants at several centimetres depth. Determining contaminant concentrations in both the 0–2 cm and 2–10 cm depth sediments should provide sufficient information to assess major contaminant exposure pathways for most organisms.

A large range of devices is available for collecting sediments, and reviews of their uses and suitability for different collection conditions are available (Mudroch and Azcue, 1995; USEPA, 2001). The most important requirement for sediment collection devices is that they minimise changes to the integrity of the collected sediment, because substantial disruption of the sediment's structure will distort its chemical and physical characteristics. An example of possible disruption includes mixing of previously redox-stratified chemical substrates with layers of differing particle size and composition, thereby influencing the bioavailability of contaminants and the potential toxicity of the sediment (Simpson and Batley, 2003). Experience with the use of any sediment sampling apparatus is required to minimise such disruption.

The quantities of sediments that should be collected will depend on the analyses and tests to be undertaken. Generally, 1 kg of sediment from each site should be sufficient for analyses of most contaminants (for example, 500 g for elutriate tests, 250 g for organics, 50 g for metals and metalloids, 50 g for acid volatile sulfide (AVS), 50–200 g for analysis of particle size and other physical properties). All samples should be stored using equipment and techniques appropriate for the desired analysis (for example, glass jars for organics; sediments frozen for AVS) (see Chapter 3). In addition, 2–3 kg may be required for bioaccumulation or toxicity tests (for example, 0.1–1 kg/species; 4 × 200 g replicates), and these samples should be stored cold (not frozen).

Sample containers and sampling devices should be cleaned thoroughly before use by soaking plastic containers and devices in 10% nitric acid (for metals) or rinsing glass containers or devices with acetone for organics (ASTM, 2008; USEPA, 2001). Obviously, nitric acid is inappropriate where nitrogen forms are being analysed. Before sample retrieval, and between each sampling event, the outside of the sampling device should be rinsed clean with water from the sampling station. For some assessments, more rigorous between-sample cleaning of the sampler might be required (for example, washing with detergent to remove oil films, then further rinsing to remove excess detergent).

When sampling sediments of unknown composition, ASTM (2008) recommends that samplers and containers be subject to: (i) a soap and water wash, (ii) a distilled water rinse, (iii) an acetone or ethanol rinse, and (iv) a site-water rinse. Where the test sediments are expected to be very contaminated, reference sites should be sampled first, to minimise cross-contamination.

It is important to check the integrity of the collected sample before accepting it for subsequent physical, biological, chemical or toxicity testing. Grab samples are acceptable if the surface layer appears to be undisturbed (sediment–water interface is intact and relatively flat, with no sign of channelling or loss of fine materials), and if the volume of sediment is sufficient. For both grab and core sampling, several replicate samplings of a site may be required to obtain the desired quantity of material.

2.3.1 Collection of surface sediments

The surface layer provides information on the most recently deposited sediment materials and should be used to determine the horizontal variation in sediment properties and the distribution of contaminants. Knowledge of the bathymetry and distribution of physically different sediment types and habitats (for example, seagrass areas) may aid selection of sampling sites.

Grab samplers should generally be used to collect surface sediments, because of their ease of handling and operation and their versatility for collecting a range of sediment substrates. The Birge-Ekman sampler is suitable for sampling soft sediments in shallow

quiescent water, and small or lightweight designs may be operated by handline while wading or from a boat. The Van Veen grab sampler is more versatile for collecting sediments with a range of sediment properties, and is generally operated by winch from a boat. Importantly, the grab sampler should protect the sample from disturbance, minimise washout of fine-grained sediments and allow easy access to the surface layer by lifting of movable cover flaps. Both the Birge-Ekman and Van Veen samplers permit relatively non-disruptive sampling. During deployment of a grab sampler, the speed of descent should be controlled, with no 'free fall', so that a bow wave is not created that mixes or disperses the surface layer upon impact. Birge-Ekman samplers are not recommended for use in strong currents or high waves and may be less stable during sediment penetration. A discussion of these and other grab samplers (for example, Ponar, Petersen, Shipek, Smith-McIntyre) is available elsewhere (Mudroch and Azcue, 1995; USEPA, 2001). Grab samplers are preferred for the collection of all submerged surface sediment samples. If sediments are collected from areas exposed at low tide, a shovel or other hand implement may be appropriate.

2.3.2 Collection of sediments at depth

Sediments from depths greater than 15 cm below the sediment–water interface may be collected to determine the spatial (vertical and horizontal) variation in sediment properties and the distribution of contaminants. This is necessary when defining volumes of contaminated sediment for dredging or for investigating historical contamination. Three-dimensional mapping (for example, kriging) of contaminants may be used to identify zones of more highly contaminated materials (ASTM, 2010).

Core samplers should be used when assessments require: (i) accurate resolution of the depth of surficial sediments, (ii) detailed vertical profiles of sediment properties, contaminants or sedimentation history, and (iii) where it is important to maintain an oxygen-free environment (USEPA, 2001).

Hand corers can be used to collect sediment from <1 m sediment depth, by wading in shallow waters, or by divers. Vibrocorers yield excellent sample integrity and are recommended for the collection of deep cores (up to 6 m), or where sediment consists of very compacted or large-grained material (for example, gravel). Box corers (<1 m depth) are particularly useful for (i) collecting large volumes of sediment from a given depth (they allow sediment for all tests to be collected in one sample), and (ii) for collecting sediments for pore-water extraction and characterisation. For routine monitoring, the Phleger, Alpine, and Kajak-Brinkhurst corers may be more suitable. A discussion of the operation of these and other core samplers (for example, Alpine, Box, Gravity, Kajak-Brinkhurst, Phleger, Piston) is available elsewhere (Mudroch and Azcue, 1995; USEPA, 2001).

Hand corers are typically 60 cm long × 5–10 cm diameter and made from Perspex® or polycarbonate, desirably with a bevelled leading edge. For clay sediments, corers of, for example, >7 cm diameter are preferable, to reduce the friction of the clay within the core tube. This allows the sediment to pass more freely and results in less-compressed core samples (giving more accurate depth information). After immersion in the sediment, the tubes are capped with tight-fitting polyethylene (or other appropriately non-contaminating) caps, and as soon as they have been withdrawn from the sediment the bottoms are similarly capped. In water of less than a few metres depth, PVC core tubes up to 4 m in length can be immersed from a boat, and sectioned on shore to recover only the desired sample depth (<1 m). Perspex® corer tube designs with extendable aluminium pole sections can also be constructed for use from a boat in shallow waters, but they should be designed so that the cores can be easily extruded immediately following collection (USEPA, 2001).

Where measurement of fluxes of contaminants from sediments is an objective, 40 cm × 15 cm diameter Perspex® corer–reactors are ideal (Jung *et al.*, 2003). Here the corer, containing collected sediment, becomes a laboratory reactor with the addition of site water and a reactor head comprising stirrer, gas bubbler and sampling ports (see Chapter 3 Section 3.8.2).

Core samples are generally acceptable if the core was inserted vertically in the sediment and minimal disturbance and loss of sediment occurs during retrieval. To prevent mixing of sediment inside the core, care should be taken to keep the core upright and stationary during transport to the water surface and before sectioning. The entire space over the sediment in the core tube should be filled with site water, and both ends of the core tube capped and taped to prevent mixing of the sediment inside. If sediment oxidation is a concern (for example, changes in metal bioavailability or volatile substances), then the headspace of the core tube should be purged with an inert gas such as nitrogen or argon. Repeated sampling of a site (that is, several cores) may be necessary to obtain the desired quantity of material from a given depth. Records should be made of vertical stratification (for example, via photographs and a geological log that identifies profiles and records strata of interest).

2.3.3 Collection of suspended sediments

Suspended sediments are often collected from surface waters to determine the concentrations of particulate-associated contaminants as distinct from dissolved contaminants. Depending on their particle size, these sediments may remain in suspension, be transported depending on flow conditions, or settle to the bottom sediments. Methods used in collecting suspended contaminants depend largely on the questions being addressed. The commonly used methods have been reviewed in several publications (Ongley, 1996; Perks, 2014), and include:

- grab sampling,
- pump samplers,
- integrating samplers, and
- sediment traps.

For most sediment quality assessments, grab sampling will suffice and the sediment will be isolated by either filtration or centrifugation, with the rest of the sample usually being used for dissolved contaminant analysis. The sample size will need to be decided, to ensure that the volume of sediment is sufficient to allow the analytes of concern to be reliably quantified. If grain size is to be considered, larger sample volumes will be needed.

Sediment traps are a common means of collecting settling particles, providing a useful insight into the contaminant status of newly deposited sediments that are not easily assessed by sampling the surface layers of bottom sediments. Gardner (1980, 2000) has comprehensively evaluated the optimal design considerations for sediment traps and discussed how they are best used.

2.4 Field records

Records of field measurements and observations are important for any assessment of sediment quality. Field records should include site identifier (name or number), site location (recorded by GPS), time and date of sample collection, sample identifier (number or name) and replicate number. Depth should also be recorded if cores are sectioned in the field. Measurements should include (i) water quality parameters (pH, redox potential, dissolved

oxygen, temperature, conductivity/salinity, turbidity and water depth) in the water column 5–20 cm above the sampling site, and (ii) pH and redox potential of the surface sediments and depth sediments if sectioned from cores in the field. Observations should include (i) water column depth and conditions during sampling (tides, waves, clarity), (ii) sediment properties (gravel, sand, silty-sand, silt), the occurrence of debris (wood, shells, other debris) and plants (for example, seagrass), the sediment depth sampled, and the overlying water depth. Collected cores should be photographed, visually examined and any changes in strata with depth recorded (texture and consistency, colour, presence of biota or debris, evidence of oil). If the sediment colour changes from light brown to grey to black down the sediment core, the depth and thickness of these layers should be recorded; they may be evidence of differing redox conditions. Chain-of-custody forms should be prepared that identify each sample collected and the analyses to be conducted on the sample.

2.5 Field processing, transport and storage

Any form of disturbance to the sediments, whether through the act of sampling, field processing or transportation, will affect the bioavailability of the contaminants (Thomson *et al*., 1980; Bull and Williams, 2002; Langezaal *et al*., 2003; Simpson and Batley, 2003; Simpson *et al*., 2004). Although disturbances to the sediments cannot be eliminated, it is important that they are minimised. Following collection, sediment samples should be stored cold (on ice) to reduce loss of volatiles and decrease bacterial activity. Field processing, or manipulation, of sediments may result in changes in the speciation, and bioavailability, of substances by disruption of the equilibrium in the pore-water/sediment system. For example, sediment mixing or sub-sampling may cause intrusion of air into sediments and the oxidation of sensitive substances, changes in the oxidation states of previously redox-stratified sediment components ($Fe(II)/Fe(OH)_3/FeS$) and the subsequent reactions of these new phases, and changes to the availability of organic compounds by disruption of their equilibrium with organic carbon in the pore-water/sediment system. To minimise sediment oxidation the headspace above the sample should be purged with an inert gas such as nitrogen or argon, or the sample should be stored in an inert gas atmosphere. Filling containers completely will minimise the immediate interaction of samples with air.

Sediment samples to be analysed for metals or inorganic contaminants should be stored in plastic materials. High-density polyethylene (HDPE) or polytetrafluoroethylene (PTFE) containers are most suitable, although well sealed plastic bags may also be suitable (samples are generally double or triple bagged to avoid losses or contamination if one bag opens). Samples for organic contaminant analysis should be stored in borosilicate glass containers with PTFE lid liners (preferably brown glass for photo-reactive compounds such as PAHs). These materials will minimise leaching, dissolution and sorption (ASTM, 2008). Sub-samples should be collected away from the sides of the collection apparatus to avoid potential contamination. All utensils (for example, spoons, scoops, spatulas) that come in direct contact with sediment samples during handling and processing should be made of non-contaminating materials (for example, HDPE or PTFE for samples for metals analyses, and high-quality stainless steel for samples for organics analyses). All equipment and containers used to sub-sample and store sediments should be cleaned using appropriate techniques (ASTM, 2008).

Before sub-sampling from a grab sampler, the overlying water should be removed by slow siphoning using a clean tube near one side of the sampler. For sediment cores, the choice of depth horizon(s) and the techniques for sectioning of the core will depend on the study objectives as well as the nature of the substrate. Sectioning can be undertaken either

by splitting the core tube longitudinally or by extrusion through applying upward pressure on the sediment from the base (for example, using a Perspex® piston designed to fit snugly in the core tube). The exposed sediment should be immediately cut into sections of the desired thickness using a stainless steel or plastic (HDPE or PTFE) cutter. The outer layer of sediment that has been in contact with the cutting blade and the core tube (1–2 mm) may need to be removed and discarded to eliminate possible contamination before the sample is transferred to the storage container. Further discussion of techniques for sectioning cores is available elsewhere (Environment Canada, 1994; Mudroch and Azcue, 1995; USEPA, 2001). Depending on the tests to be made on the collected sediment, oxygen-free conditions may be necessary when the sediment within the core is extruded and processed (Simpson and Batley, 2003).

Sub-sampling is not easily done in the field, in which case cores should be chilled (on ice) or frozen, depending on the measurements to be undertaken. In either case, cores should be stored vertically, and undue agitation should be avoided during transportation because that will particularly mix the surface layers in unfrozen cores. Freezing has been found to rupture bacterial cells and release accumulated elements, such as selenium, into pore waters (Jung and Batley, 2004). Freezing of sediments is recommended if they are to be analysed for either AVS or total contaminants.

Maximum holding times and storage methods are governed by sediment type, contaminant characteristics, the expected use of the collected sediments or sediment components (for example, pore waters), and the tests to be undertaken on the sediments. The general recommendation is to store sediments and pore water in the dark at 4°C (Carr and Nipper, 2003; Geffard *et al*., 2004). Specific preservation requirements for metals, ammonia, cyanide and sulfide in pore-water analysis are given in Chapter 3. Samples for analyses of total metals can be held indefinitely, but changes to metal speciation will begin occurring within days of collection, as also will partitioning of contaminants between sediments and pore waters (Carr and Chapman, 1995; DeFoe and Ankley, 1998; Cole *et al*., 2000; Simpson and Batley, 2003). Changes in bacterial activity will cause changes to the concentrations of ammonia, sulfide, Fe(II) and biologically-active sediment components, particularly in pore waters. It is generally recommended that if pore waters are of interest, they should be extracted immediately after collection and subjected to appropriate preservation procedures, with suitable storage containers for each analyte (may need separate containers). Most extractable organics (for example, phthalates, organochlorine pesticides, polychlorinated biphenyls (PCBs), PAHs, hydrocarbons and dioxins) should be extracted from sediments within 14 days of sample collection, while 7 days should be the maximum storage length before extraction of samples for analyses of organic contaminants that are susceptible to losses due to volatility or microbial degradation. Storage containers for samples for analyses of organics (sediments or water extracts) should be glass, and plastic lids should be PTFE-lined to minimise adsorptive losses.

Sediments for use in toxicity tests should be tested as soon as possible after collection, but this interval will often be determined by the time required for chemical analyses. It is suggested that sediments should be stored for no longer than 8 weeks before toxicity testing (USEPA/USACE, 1998; Geffard *et al*., 2004). Longer storage times may be appropriate, depending on properties of the sediments and the concentrations and types of contaminants. Extended storage of sediments may result in: (i) losses of labile or volatile contaminants (for example, ammonia, volatile organics, AVS) or (ii) changes to the redox properties of the sediments because of increased or decreased bacterial activity (Simpson and Batley, 2003). Either of these processes may result in major changes to the concentrations or bioavailability of the

sediment contaminants. For sediments that are stored for long periods (for example, greater than 8 weeks), re-analysis of some contaminant concentrations may be required before toxicity testing.

2.6 Sediment manipulations prior to testing

Sediments are often manipulated in the field or laboratory before chemical or toxicity testing. Manipulation may involve sieving to remove large particles and debris, or the separation of indigenous biota, or homogenisation so that a large sample can be used for several chemical and biological tests (see, for example, Bufflap and Allen, 1995; Carr and Chapman, 1995; Sarda and Burton, 1995; Burgess and McKinney, 1997; Sijm *et al*., 1997; Chapman *et al*., 2002, Simpson and Batley, 2003; Fisher *et al*., 2004). Most manipulations of sediments will alter the properties of the sediments and affect contaminant bioavailability, and the effects of these on the test data need to be evaluated. All procedures used to prepare sediment samples for analyses and tests should aim to minimise disturbances and should be fully documented in reports.

It is desirable to undertake some assessment of how sample manipulation may affect the concentrations (for example, via loss of volatiles), bioavailability (for example, via changes to AVS; partitioning in pore waters) and toxicity of contaminants in the collected sediments. In freshly collected whole-sediment samples that have been minimally manipulated, later interpretation of bioavailability and toxicity test data will be aided by preliminary measurements of pH, redox potential, total organic carbon (TOC), AVS, iron and particle size distribution, and analyses of total and weakly-extractable contaminants and pore-water contaminants. If sediment samples undergo major manipulation (for example, sieving) or are stored for long periods before testing (for example, longer than 4 weeks), reanalysis is desirable for those parameters likely to be affected by these manipulations (for example, pH, AVS, pore waters, volatile organic compounds).

2.6.1 Sieving

Sieving of sediments causes major changes to sample integrity and possible losses of particular components (for example, volatile organics). Valid reasons for sieving sediments include:

- to remove coarse material (debris, rock, shells, wood >2 mm in diameter) that may interfere in analyses (only the <2 mm sediment fraction is used when comparing contaminant concentrations against guideline values);
- to obtain information on the distribution of contaminants in different sediment size fractions; or
- to remove indigenous organisms from the sediments before performing toxicity tests.

For toxicity tests, it is preferable that none of the test sediment samples be sieved. However, if test procedures require sieved sediment, then all of the test samples, including control and reference sediments, should be sieved. In some cases, indigenous organisms may be handpicked from the samples. Indigenous organisms may confound results of toxicity tests by being similar to test organisms or by preying on the test organisms. To remove indigenous organisms, the most appropriate procedure is press sieving, whereby the sediments are pressed through a chemically-inert (non-metal) sieve, using either a gloved hand or a plastic or Teflon®-coated spatula. Generally, 2 mm sieves should be sufficient for removal of most problematic macrofauna. As an alternative to sieving, organisms may be handpicked (using tweezers or forceps) from sediments that have been spread out in

a shallow tray. A record should be made of what is removed or retained on the sieve (for example, organisms, shells, gravel and other debris). Sediment samples that are to be used for toxicity testing should not be washed through sieves using water, because that will remove contaminants and alter bioavailability, and further processing is likely to be required to remove the excess water. Samples that are to be used for both chemical analysis and toxicity tests should be sieved together, homogenised, and then split for their respective analyses. Sieving (or handpicking) may need to be carried out in an oxygen-free atmosphere to minimise oxidation of sediment components.

Wet sieving of sediments is recommended when information is required on contaminant partitioning between different particle size fractions. Samples should be thoroughly homogenised before wet sieving is undertaken. Deionised water or clean seawater should be used to wash the sediment through a chemically-inert sieve material with the aid of a chemically-inert spatula. Sieves and spatulas should be high quality stainless steel when organics are the main contaminants of interest, or non-adsorbing plastics (for example, nylon, polyethylene, polypropylene, Teflon®) when metals are to be analysed. Generally the silt fraction of the sediments (approximately <63 μm) is considered to be the most important with regard to contaminant partitioning, especially for metals. Some redistribution of contaminants to the finer sediment fraction may be expected due to solubilisation of larger particles. If there is a concern about losses and redistributions of contaminants during sieving, the recommendation is to undertake only total contaminant analyses, with wet sieving used to determine the grain size distribution only on a separate sub-sample.

2.7 Sediment heterogeneity

As discussed earlier, sediments, unlike water, can be remarkably heterogeneous. Vertical stratification of contaminants frequently occurs in sediments because of varying historical inputs and natural layering of sediments from differing origins which have different contaminant-binding properties. Spatial heterogeneity both in grain size and in contaminant distribution has also been shown to involve micro-niches with high concentrations of contaminants, organic matter and microbial activity (Shuttleworth *et al.*, 1999; Robertson *et al.*, 2009; Stockdale *et al.*, 2009). These processes are superimposed on the normal layering of biogeochemical processes within sediments (for example, Fig. 2.1; see also Chapter 1 Fig. 1.3). It is well documented that organisms engage in bioturbation (burrowing) and bioirrigation (introducing overlying water into burrows) and that these affect the migration of sediment contaminants (Forster, 1996; Petersen *et al.*, 1998; Ciarelli *et al.*, 1999; Rasmussen *et al.*, 2000; Ciutat and Boudou, 2003; Simpson and Batley, 2003). The different feeding and burrowing behaviours of organisms affect how they sort particles, enrich or deplete organic matter, inject oxygen into localised sediments (Pischedda *et al.*, 2008; Volkenborn *et al.*, 2010) and alter contaminant fluxes from sediments (see Chapter 1 Fig. 1.2). Some of these complex issues may challenge our more simplistic view of sediment chemistry which underpins the sediment quality guideline values and their application, where sediments are considered as homogeneous and in some instances are homogenised before investigation. To resolve heterogeneity issues associated with defining contaminant concentrations within an area (for example, mean or 95% upper confidence limits), a systematic and statistical evaluation of potential outliers may be required (as discussed in Chapter 3 Section 3.4.1).

The effects of localised heterogeneity with respect to organic contaminants was illustrated by Guerrero *et al.* (2003) in a study of pyrene bioaccumulation by clams with various types of artificial sediments and a natural one. Variations in the sediment–water partition coefficient (K_d) of the particles defined the window of bioavailability for pyrene when it was

adsorbed on the surface of ingested sediments. However, the natural sediment tested did not fit easily into the partitioning interpretation. The main reason could be that K_d is really only meaningful for a single defined surface. In natural sediments there is a large range of components, each with a different partition coefficient, so that the averaged value that is measured experimentally needs to be interpreted carefully. This could be particularly true if the test organism is a selective feeder that may ingest components of the sediment with quite specific K_d values that differ considerably from those of the 'bulk' phase.

The microbial degradation of labile organic matter in bulk sediments determines the redox potential (Eh) and the pH observed at various depths in bulk sediment, and is responsible for a variety of secondary reactions involving metals (for example, desorption, release to pore water, fixation as sulfides). These redox reactions lead to vertical zonation of pH, Eh and various chemical species in sediments. Since the flux of labile organic matter to the sediments is usually much faster than the diffusive flux of oxygen across the sediment–water interface, it is commonly observed that oxygen drops to zero within a few millimetres of the sediment–water interface in productive sediments. The sub-oxic depth, dominated by Fe(III)-reduction, can be present at reasonable depths even in productive sediments. Sediments can be anoxic well above the depth to which most benthic animals burrow.

In addition, during the microbial degradation of organic matter in the sediments, reduced forms of the electron acceptors are produced and released to the pore waters. Some of these solutes, such as sulfide, are toxic to most benthic animals. The natural concentrations of sulfide found in pore water are not toxic to most invertebrates (Wang and Chapman, 1999). It is important that such dynamics are understood and the chemical changes that occur when such sediments are disrupted are appreciated when designing and conducting laboratory toxicity tests on field-collected sediments and on artificially-prepared test sediments. Lee *et al*. (2000), for example, documented how disruption of anaerobic sediment spiked with four metals shifted the exposure from ingestion of particulates (the primary route *in situ*) to pore-water exposure.

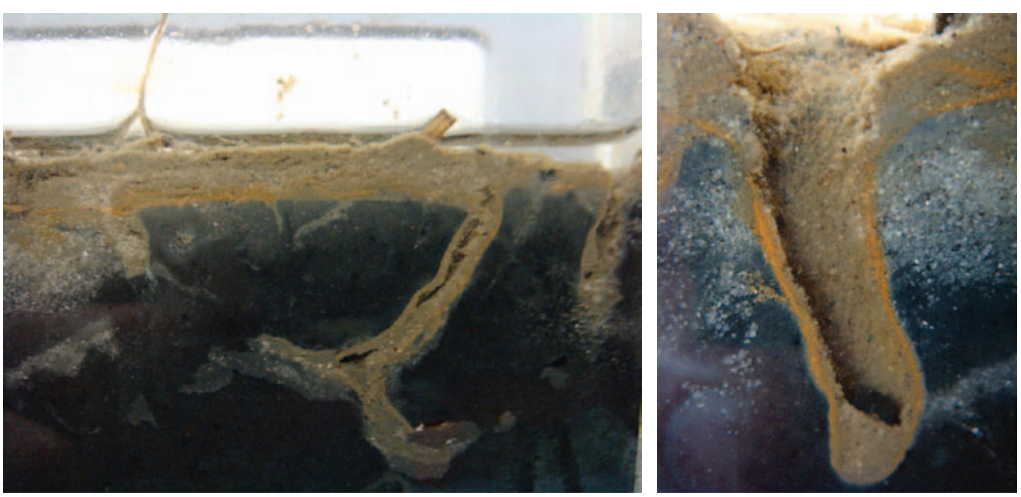

Figure 2.1. Photographs of cross-sections through the sediment–water interface and amphipod burrows showing the oxic (brown; here 5–10 mm depth at surface, 1–2 mm at burrow walls), sub-oxic (light to dark grey transition; here 5–10 mm depth below oxic surface strata, 2–5 mm beyond oxic strata from burrow walls) and anoxic strata (dark grey to black transition, beyond sub-oxic strata) (photos by David Robertson (2007), provided by Peter Teasdale, Griffith University).

2.8 Quality assurance/quality control (QA/QC) procedures for sediment collection and manipulation

Field replicates, field duplicates and field blanks are important components of all sediment assessment programs.

- Field replicates are two separate samples collected from the same location (same site and position) and analysed by the same laboratory to identify variations in sediments (for example, particle size, TOC and contaminant concentrations).
- Field duplicates are prepared from a single sample that is split in two and placed into two identical sample containers to assess variation in the sub-sampling and analytical methods. These differ from inter-laboratory comparisons, where duplicates are sent to different laboratories to identify possible variation due to analysis techniques (verification of primary laboratory).
- Field blanks (also used as sample handling blanks) are used to estimate the amount of contamination introduced during collection, transport and storage of the samples. For example, fine-grained clean sand or silt (with known contaminant concentrations) can be placed first in the sampling equipment, then transferred to the sample containers and transported to the laboratory and submitted as a sample.

These samples should be collected and handled in exactly the same way as the sediment samples and should be treated as blind samples so as to minimise bias in the analysis. For very volatile chemicals, spiking in the field may be a useful way of estimating losses occurring during transport and before testing. Chain-of-custody forms should accompany all samples from the time of collection through to reporting of analysis results.

2.9 Spiking of sediments with contaminants

The spiking of sediments with particular contaminants is undertaken: (i) to check recoveries of analytes (for QA/QC); (ii) to understand the partitioning of contaminants between sediments and water in different sediment matrices; (iii) to understand the transformation rates (degradation, volatilisation) of contaminants in different sediment matrices; and (iv) to quantify the effects of known concentrations of contaminants in toxicity tests (Chapter 4) or manipulated ecology experiments (for example, field-based recolonisation experiments, Chapter 7). Appendix B describes preparation of contaminant-spiked sediments.

Because sediment spiking will involve major manipulation of sediment properties, it is necessary to assess how the procedures will influence the sediment properties and contaminant bioavailability, in particular the partitioning between the dissolved and particulate phases (Northcott and Jones, 2000; Simpson *et al.*, 2004; Simpson and Batley, 2007). Thus, full descriptions of spiking procedures should be reported, with careful consideration of the necessary time required to achieve equilibrium with the sediments. It is recommended that the sediment parameters moisture content, pH, redox potential, organic carbon, AVS, particulate iron and manganese, and pore-water constituents (ammonia, sulfide, iron, metals) be measured before and after spiking so that losses or recoveries of added chemicals and the bioavailability of the added chemicals (or other chemicals present) can be adequately assessed. Failure to report these parameters will make it difficult to interpret results and assess contaminant exposure pathways and organism sensitivities (King *et al.*, 2006; Roman *et al.*, 2007; Simpson *et al.*, 2011; Besser *et al.*, 2013; Vandegehuchte *et al.*, 2013). Control sediment should undergo the same treatment as spiked sediments.

The most appropriate spiking technique will depend on the properties of the spiked contaminant (different metals and organic chemicals are influenced by different factors). For metals, generally the most important factor is an adequate period for equilibration. Oxidation of sediments during the disturbance introduced by the spiking process results in Fe(II) oxidation (of Fe(II) existing in pore waters or displaced from particles by added metals) with the release of H^+. An equilibration period of weeks to months may be required for the original redox potential to re-establish, and for pore-water metal concentrations to diminish as insoluble metal sulfides are formed. Detection of unrealistically low pH values or unnaturally high pore-water metal concentrations indicates that equilibrium has not yet been re-established (Simpson *et al*., 2004; Hutchins *et al*., 2009; Brumbaugh *et al*., 2013; Vandegehuchte *et al*., 2013). Dilution of a 'superspike' (a very high spiked concentration; also referred to as indirect spiking) has generally been found to result in the desired level of metal partitioning (low pore-water metals) being achieved more quickly (Hutchins *et al*., 2009; Brumbaugh *et al*., 2013).

For organics, equilibration times are often shorter than for metals (for example, days rather than weeks) (Landrum *et al*., 1992; Northcott and Jones, 2000). Here, a range of factors become very important considerations: namely, the hydrophobicity or solubility (often indicated from the K_{OW}, the octanol–water partition coefficient), choice of organic carrier solvent for spiking (solvent persistence, volatility, toxicity), and losses of chemical through non-target adsorption, degradation (UV light), transformation (hydrolysis) and volatilisation, along with the organic carbon content of the sediment (Ankley *et al*., 1994; Northcott and Jones, 2000; Fuchsman and Barber, 2000; OECD, 2007, 2010). Passive sampling techniques can be particularly useful for assessing the concentrations, bioavailability and equilibration of spiked organics (Sormunen *et al*., 2010; Section 2.12).

Generally, larger debris and indigenous organisms should be removed from sediments before spiking. Wet 'slurry' spiking techniques are recommended over dry spiking, as this will facilitate sediment–water interactions (Landrum *et al*., 1992; Northcott and Jones, 2000; USEPA, 2001; Simpson *et al*., 2004). Modification of sediment organic carbon will strongly affect the partitioning (and bioavailability) of organic chemicals (Di Toro *et al*., 2000; USEPA, 2012) and of metals (Besser *et al*., 2003; Strom *et al*., 2011); and modification of sediment sulfide concentrations (for example, AVS) will affect the partitioning of metals (Gonzalez, 1996; Leonard *et al*., 1999; Simpson *et al*., 2011).

Formulated control sediments have frequently been used for sediment-spiking studies, and procedures for their preparation are given elsewhere (Environment Canada, 1995; USEPA, 2001; OECD, 2007, 2010). The sources of materials used in the formulation can influence the contaminant binding phases (for example, organic carbon, AVS, percentage of silt) present in the formulated sediments, and should be carefully considered. The materials should be readily available, homogeneous, and have low contaminant concentrations and properties that do not change substantially upon storage or during use (that is, their concentrations of AVS or dissolved ammonia should be stable). The formulation should support the life-cycles (survival, growth and reproduction) of a wide range of benthic organisms.

2.10 General sediment quality parameters

There are several indirect stressors that modify sediment chemistry, thereby affecting contaminant bioavailability. Measurements should be made of pH, redox potential, moisture content, particle size distribution, TOC, AVS, particulate iron and manganese, pore-water

constituents (iron, manganese, ammonia, sulfide) and contaminants of concern in the various sediment fractions (total, weakly-extractable, pore water). The pH and redox potential measurements should be made in the field, on surface and depth sediments (the latter only if sectioned in field). It may be useful to repeat some measurements (for example, pH, redox potential, pore-water iron, AVS) before and after sample manipulation. In estuaries, pore-water salinity should be measured because it often differs significantly from the salinity of overlying waters (Chapman and Wang, 2001).

Important measurements of overlying water quality include pH, redox potential, dissolved oxygen, temperature, electrical conductivity/salinity, and turbidity at 5–20 cm above the sampling site. Measurements of total water depths, the amplitude of tides, and light penetration near the sediment–water interface will also aid data interpretation.

2.10.1 Sediment pH and redox potential

Sediment pore-water pH is the master variable controlling the speciation and bioavailability of metals. The water-quality guideline values for ammonia, cyanide and sulfide (all of which ionise as a function of pH) are pH-dependent (ANZECC/ARMCANZ, 2000b). The oxidation–reduction (redox) potential (Eh) is a useful indication (within limitations) (Teasdale *et al.*, 1998) of the biogeochemical condition of sediments, especially whether various substances are likely to be found in an oxidised or reduced state. Redox potential is an important control on sediment metal chemistry, particularly iron and manganese oxidation states and metal-sulfide chemistry of Ag, Cd, Cu, Fe, Hg, Mn, Ni, Pb and Zn. The oxic zone is typically a few millimetres below the sediment–water interface in productive sediments, underlain by a sub-oxic and then an anoxic area. The presence of acid volatile sulfides (FeS, MnS) is a buffer against metal release to pore waters because of their ability to exchange with soluble metals; this reaction will have a direct impact on bioavailability if the preferred uptake route is by exposure to pore waters. Although iron- and sulfate-reducing zones may overlap considerably, Fe(III)-reducing conditions often dominate in the Eh range from 0 to 200 mV and sulfate-reducing (sulfide-forming) conditions in the Eh range from –50 to –150 mV (Schüring *et al.*, 2000). Boundaries are difficult to define and are also dependent on pH (Stumm and Sulzberger, 1992). The pH- and redox-dependent equilibrium between Fe(II) in pore waters and the formation of Fe(III) hydroxide phases is very important in controlling concentrations of pore-water metal contaminants (Vink, 2002; Simpson and Batley, 2003). Both pH and redox potential measurements of pore waters provide useful process-related information on the nature of the sediments under investigation.

The measurement of sediment pH and redox potential are really measurements of the pore water rather than the sediment, and therefore need to be performed on wet sediment. Electrodes are available for both, but the act of insertion into the sediment may disturb the redox and pH profiles, so it is important to allow time for the equilibrium to re-establish before taking a measurement. The use of 'spear tip' pH (combination) electrodes that allow greater penetration into the sediments and less disturbance is the preferred approach for sediment pH measurements. Potentiometric measurements of redox potential should be made using a millivolt reader and, generally, a platinum electrode with combination Ag/AgCl or calomel reference electrodes (ASTM, 2014; APHA/AWWA/WEF, 2012). The millivolt reading should be reported as Eh versus the normal hydrogen electrode (NHE, also referred to as the standard hydrogen electrode (SHE)), and can be calculated from the measurement as follows:

$$Eh = E_{obs} + E_{ref}$$

where Eh = measured redox potential (mV) reported versus NHE, E_{obs} = observed redox potential for electrode pair used, and E_{ref} = redox potential of the reference electrode versus NHE.

The NHE is fragile and impractical to use directly. The combination platinum/reference electrode is usually calibrated against a redox standard solution to determine Eh, and then E_{obs} is calculated using the known E_{ref} for the standard. A range of redox standards are available: Light's solution (E *v.* NHE = 675 mV, at 25°C), Zobell's solution (428 mV), quinhydrone solutions (285 mV at pH 7, 462 mV at pH 4), and triiodide/iodide (420 mV at 25°C). As the redox potential will depend on the type of reference electrode used, the offset introduced must be compensated for. If, for example, a potential of 86 mV was obtained for the quinhydrone pH 7 redox buffer (at 25°C) (typical of E_{ref} *v.* Ag/AgCl), then use E_{ref} (= Eh – E_{obs} = 285 mV – 86 mV = 199 mV) as the offset. Each of these redox buffer solutions has a different storage life, ranging from hours to months. The use of Zobell's solution is recommended, but it is toxic and subject to oxidation (store in the dark). Inaccuracy, instability and poor reproducibility are common when measuring redox potential, resulting from disturbance of the sediment sample during insertion of the electrode. Details on preferred calibration solutions and how to clean indicator electrodes are provided by Teasdale *et al.* (1998). Acceptable error ranges for pH and redox potential measurements for sediments will be of the order ± 0.1 pH units and 20–40 mV respectively.

2.10.2 Water content

It is usual for measurements on sediments to be related to dry weight, but it is frequently preferable to undertake contaminant analyses on a wet sediment, because drying can alter the chemistry and in some instances result in losses of analyte. To convert these data to the preferred dry weight units it is therefore necessary to measure the water content.

Water content (often called moisture content) of wet sediment is determined gravimetrically by measuring the mass of water lost following drying at 110°C (Mudroch *et al.*, 1997). Before taking a sub-sample for moisture analysis, the sediment should be well mixed so that the water is evenly distributed throughout. The combined determination of sediment water content, density and porosity can be made according to the methods described in Mudroch *et al.* (1997). Sediment density and porosity are often useful parameters for describing sediment characteristics.

2.10.3 Particle size

Sediment particles (grains) generally range from sand, through silty sand and sandy-silt, to clays, although shells and other detritus may also be a significant proportion of many sediments (Table 2.1). The surface areas of these materials vary over orders of magnitude, and therefore so do the number of binding sites for metal and organic contaminants. Particle size often defines whether sediment is a good habitat for biota (for example, suitability for easy burrowing, or burrows not collapsing). Some species show preferences for sediments of particular particle sizes while others can happily survive in a range of particle sizes. Particle size will also influence benthic community structure. Fine sediments (for example, <63 μm) are typically those that are most heavily contaminated (greater surface area and more binding sites). A contaminant at a given bulk concentration in a sandy sediment will generally be more toxic than the same concentration in a silty sediment, because the partitioning to pore water will be greater. Because particle size influences both chemical and biological characteristics, it can be used to normalise chemical concentrations and account for some of the variability found in toxicity testing results and ecological

Table 2.1. Grain size classification of sediments[a]

Grain Size	Classification
<0.06 µm	Fine clay
0.06–0.63 µm	Medium clay
0.63–2 µm	Coarse clay
2–6.3 µm	Fine silt
6.3–20 µm	Medium silt
20–63 µm	Coarse silt
63 µm–2 mm	Sand
>2 mm	Gravel, coarse material, rocks, detritus

[a] Mudroch *et al.* (1997).

datasets (for example, biological assemblages). Particles <63 µm are more common in the gut of sediment-ingesting biota (Tessier *et al.*, 1984).

Sediment particle size analysis can be made by wet sieving, hydrometer or pipette methods or by laser particle size analysis (Mudroch *et al.*, 1997). For most assessments, it will be sufficient to determine just the fraction of sediment that is <63 µm (a standard sieve size), which will include the silt and clay fractions. Wet sieving is the recommended method, where deionised water (or clean seawater) is used to wash sediment through the sieve (see Section 2.6.1). The retained sediment is collected quantitatively and weighed following drying; this is done for each sediment size fraction if using multiple sieve sizes.

2.10.4 Total organic carbon

The forms of carbon in sediments may include elemental (for example, charcoal, coal, soot), inorganic (for example, carbonate minerals, shell debris) and organic (for example, wood debris, decomposed plants and animals, ash, and also hydrocarbon contributions from oils, tars and plastics) (Schumacher, 2002). In terms of analyses, Total Carbon = Inorganic Carbon + Organic Carbon. The total organic carbon (TOC) content of sediment is the sum of particulate organic carbon (POC) and dissolved organic carbon (DOC). Decaying detrital POC is distributed amongst mineral and amorphous particles in sediments. Inorganic particles are sites of bacterial activity and binding sites for both metal and organic contaminants. The binding of hydrophobic organic contaminants to different particulate phases will have quite different partition coefficients. In the ANZECC/ARMCANZ (2000b) guidelines, all organic contaminants are normalised to the TOC concentration of the sediment (that is, normalised to 1% TOC).

The TOC is the total amount of oxidisable organic material, and it will generally be measured using high temperature (for example, 1000–1500°C) dry combustion techniques where the combustion releases CO_2 which is quantified by titrimetric, gravimetric, manometric, spectrophotometric or gas chromatographic techniques, for example using instruments such as analysers of CHN (carbon, hydrogen, nitrogen) or TOC (Mudroch *et al.*, 1997; Schumacher, 2002). Chemical oxidation techniques (for example, dichromate oxidation) are not recommended because some organic compounds may not be analysed by these techniques. Inorganic carbon (for example, carbonates and bicarbonates) can be a significant proportion of the total carbon in some sediments. Therefore, analyses of TOC use samples that have been dried at 75–110°C following the removal of inorganic carbon

(by heating the sample with dilute acid until effervescence due to carbonates ceases). The methods generally have a limit of determination (LOD) of 100 mg/kg.

Another, less accurate, method of estimating sediment organic carbon content measures the 'loss-on-ignition' when a known mass of dried sediment is heated at ~400°C for 24 h. This is then followed by gravimetric analysis. However, a range of ignition temperatures are possible (temperatures from 350°C to 500°C have been used) which means that not just organic carbon but also other volatiles can be consumed or driven off; inorganic carbonates can also be lost at temperatures >440°C (Schumacher, 2002). Therefore, when the loss-on-ignition technique has been used, the report should make it clear that the measurement is an estimate of sediment organic matter, not TOC.

Black carbon (pyrogenic carbon or soot) has been shown to be important for binding hydrophobic organic contaminants (for example, PAHs) in sediments (Gustafsson *et al.*, 1997; USEPA, 2012). Black carbon is produced from the incomplete combustion of fossil fuels and vegetation. Examples of black carbon include charcoal (often remaining after bushfires) and coal dust (found near power stations or at coal ports), and these are often found in freshwater and estuarine sediments in Australia.

Hydrophobic organic contaminants are generally much more strongly associated with ('partitioned to') black carbon than other forms of natural organic matter. The differentiation of black carbon from other forms of carbon is usually made on the basis of the temperature of combustion. An oxidation temperature of 375°C has generally been found to provide a reasonable distinction between non-black carbon which is fully combusted below that temperature while black carbon remains and is not fully combusted until over 450°C (Gustafsson *et al.*, 2001). Black carbon measurements are recommended for assessments of sediments containing high concentrations of hydrophobic contaminants; they will assist in estimating partition coefficients used for predicting PAH bioaccumulation.

It is recommended that high temperature dry combustion techniques (for example, using CHN or TOC analysers) be used for analyses of POC where measurements are to be used to normalise organic contaminant concentrations to sediment TOC (as recommended in the ANZECC/ARMCANZ (2000b) guidelines). Loss-on-ignition measurements are useful when additional information is sought on relative differences in sediment organic carbon concentrations (for example, considerations for metal partitioning).

Note that the term 'blue carbon' refers to atmospherically-derived carbon (largely as carbon dioxide) that is sequestered by mangroves, salt marshes and seagrasses in sediments via leaves and roots (McLeod *et al.*, 2011).

2.11 Collection of pore water from sediments

Sediment pore water (or interstitial water) is defined as the water occupying the spaces between sediment particles. Typically pore water will occupy 30–80% of the volume of sediment, the volume being greater for fine-grained (silty) sediments than for sandy sediments. Water currents driven by surface water movements (for example, currents, tides, wind) or groundwater upwelling will influence pore-water composition and stability. In most depositional sediments, pore waters will be relatively static and it is expected that thermodynamic equilibrium will exist between contaminant concentrations in the pore water and in surrounding sediments. Sediment characteristics (for example, pH, organic carbon, sulfides, mineralogy and particle size) will greatly affect the partitioning of contaminants between the particles and pore waters (Di Toro *et al.*, 1991; Chapman *et al.*, 1998; Simpson and Batley, 2007).

Because many benthic organisms are in direct contact with sediment pore waters, this component of sediments is potentially a major exposure pathway. Accurate measurement of contaminant concentrations in sediment pore waters is therefore useful for assessing the potential bioavailability of contaminants. Pore waters are often isolated from the sediment matrix for toxicity testing with organisms that are sensitive to dissolved contaminants. The use of pore waters for toxicity assessment and toxicity identification evaluation (TIE) of sediment is discussed in Chapter 4.

Pore-water sampling, chemical and toxicity assessments are usually only undertaken on sediments for which total contaminant concentrations are above sediment quality guideline values (ANZECC/ARMCANZ, 2000b). Generally, pore-water assessments will not be necessary in sediments made up of coarse particles (sand, gravel) that have little binding capacity for sediment contaminants, nor in compacted clays that have little pore water with which organisms can interact. Where only chemical assessment is required, a range of techniques may be considered, including direct pore-water extraction and analysis, or passive sampling methods (which may be equilibrium techniques or kinetic techniques), or equilibrium partition calculations to predict pore-water concentrations based on the particulate concentrations.

Extraction of pore waters should be completed as soon as possible after sample collection. As already noted, sediments should not be frozen before pore-water analyses because that may potentially mobilise metals or metalloids through the rupturing of biological membranes (for example, cells of algae) (Section 2.5). Pore-water extractions from sediments should be conducted in an inert atmosphere, for example in a nitrogen-filled glove bag (or at least with minimal atmospheric contact), so that reduced species are minimally exposed to oxygen. Significant chemical changes can occur even when pore waters are stored for periods as short as 24 h (for example, Hulbert and Brindle, 1975; Sarda and Burton, 1995; Carr and Nipper, 2003; Simpson and Batley, 2003). Air exposure will result in the rapid oxidative precipitation of dissolved Fe(II) as Fe(III) hydroxide and slower oxidation of dissolved Mn(II) and sulfide. Following isolation from sediments, the pore waters should be stored so that oxidative changes, adsorption to containers or volatilisation are minimised (Carignan, 1984). Containers should be filled, with no headspace, to minimise changes in dissolved oxygen and contaminant bioavailability. Pore-water samples for chemical analyses should be preserved immediately, if appropriate (for example, acidification for metal analyses, frozen or preserved for pesticide or phenol analyses), or cooled to 4°C as soon as possible. Pore-water samples to be used for toxicity tests should be cooled to 4°C immediately after isolation and used in tests as soon possible. Storage containers should be appropriate to minimise adsorption or leaching of chemicals.

2.11.1 Pore-water sampling by centrifugation or squeezing techniques

A large variety of methods have been used for the isolation of pore waters from sediments (Carr and Nipper, 2003; Chapman *et al*., 2002). It is important to recognise that all methods have been shown to alter pore-water chemistry and affect the bioavailability and toxicity of metal contaminants (for example, Bufflap and Allen, 1995; Sarda and Burton, 1995; Chapman *et al*., 2002, Simpson and Batley, 2003). Pore waters will generally contain very low concentrations of dissolved oxygen and often have high concentrations of easily oxidisable species (for example, Fe(II)), and it is almost impossible to maintain these properties once pore waters are isolated from sediment (Simpson and Batley, 2003). Several good reviews are available that discuss pore-water sampling, precautions and artefacts (Carr and Nipper, 2003; Chapman *et al*., 2002).

Centrifugation or squeezing (*ex situ* extractions) will generally be the most useful methods for extracting pore waters for chemical analyses or toxicity testing. Centrifugation is the preferred laboratory method as it is a relatively simple procedure that allows rapid collection of large volumes. If exposure to oxygen is a concern, the sediments can be handled in a nitrogen-filled glove box or glove bag. With a bigger glove box, centrifugation can be undertaken in the box, although exposure can be minimised in the laboratory if centrifuge tubes are capped after purging with nitrogen in the glove bag. The centrifuge speed (and rotor radius) must be sufficient to create a relative centrifugal force (RCF) of 2000–5000 × g, and the centrifugation time needs to be chosen to achieve effective compression of the sediment and settling of particles to the sediment surface. For metal/metalloid analyses, it is desirable to use low-adsorption plastic containers (for example, Teflon®, HDPE, polycarbonate) and rapid extraction (for example, within 5–10 min), and to filter the isolated pore waters as soon as possible after separating and then preserve them to minimise changes in dissolved concentrations (Simpson and Batley, 2003). For analyses of organic contaminants, the solids should be removed by centrifugation (2000–5000 × g) using glass centrifuge bottles (for example, Corex, Corning®), and refrigeration (for example, 4°C) during centrifugation is desirable (to minimise adsorption and volatilisation). Longer centrifugation times can be used (for example, 20–90 min), and storage at 4°C in glass bottles with minimum headspace. For analyses of organic contaminants, pore waters should not be filtered following centrifugation as this can result in unacceptable losses. Dissolved organic carbon (DOC) analyses may also be made on these samples.

When pore waters are to be used for toxicity tests, filtration should generally be avoided because studies have shown that filtered samples generally have lower toxicity than unfiltered samples (Carr and Nipper, 2003). This is to be expected, because filtration procedures generally remove a larger proportion of fine or colloidal solids than do centrifugation or squeezing techniques. For accurately characterising sediment toxicity, colloidal material and fine particles present in the pore water may be of importance.

2.11.2 Pore-water peepers

For passive sampling, the most common early form of sampler is the peeper, in which solutes from adjacent pore waters diffuse across a membrane into compartments containing water (Hesslein, 1976; Carignan, 1984; Carignan *et al*., 1985; Teasdale *et al*., 2003; Brumbaugh *et al*., 2013). Pore-water peepers are equilibrium dialysis samplers in which each chamber is filled with deoxygenated deionised water and covered with a fixed membrane (Teasdale *et al*., 1995; Brumbaugh *et al*., 2013) (Fig. 2.2). They can be single- or multi-chambered. The chambers are kept small to minimise disturbance to the redox gradients that influence the pore-water equilibrium and concentrations of dissolved metals in the surrounding pore water. When iron staining ($Fe(OH)_3$ precipitate) is observed in peepers, this may indicate that diffusion of dissolved oxygen from the overlying water into the pore water is being facilitated by the container's internal wall being in contact with both water and sediment.

Single-chamber peepers can be particularly useful for providing information on pore-water exposure during bioaccumulation and toxicity tests on sediment in the laboratory or in the field (Brumbaugh *et al*., 2013). For this purpose, the peepers are positioned so they are completely submerged below the sediment–water interface (with only a small nylon cable tie protruding above the sediment surface). Single-chamber peepers are smaller than multi-chamber designs and cause less disturbance to localised redox conditions in pore waters near the membrane (Teasdale *et al*., 1995; Doig and Liber, 2000). The peepers consist of laboratory low-density polyethylene (LDPE) snap-cap vials, with a hole punched in the

Figure 2.2. Photographs of single chamber (mini-peeper) (a, b) and multi-chamber (c) pore-water peepers (mini-peeper photos provided by William Brumbaugh, USGS).

cap and a polyethersulfone (PES) filter membrane inserted below the cap as the vial is sealed. To make 'mini'-peepers for use in laboratory sediment studies, use 2.5 mL vials with 0.45 μm pore-size and 25 mm diameter membrane (Fig. 2.2). Larger peepers for field deployment use 25 mL vials and a 47 mm diameter PES membrane.

Typically, multi-chambered devices (useful for obtaining depth profiles) can be made from a polymethylmethacrylate (acrylic) block ($8 \times 30 \times 1$ cm^3), into which are machined horizontal chambers 6.5 cm wide and around 4.2 mL in volume along its 30-cm length at intervals of 1 cm. The chambers are covered by a polysulfone membrane (on both sides), held in place by a thin acrylic cover sheet that is screwed into the block with nylon screws. The sheet has windows cut into it, to expose the chambers and their membrane windows. In use, these peepers are partially immersed in the sediments so that approximately one-quarter of their length is exposed in the overlying water, and they are left in place for at least 5 days (depending on the size of the chambers). When they are retrieved, the external surface is washed quickly to remove sediment particles and the membrane over each chamber is pierced with a micropipette and the water transferred to a suitable container and acidified for subsequent analysis. Chambers should be sampled in order from the bottom to the top so that anoxic samples have less time to be exposed to oxygen in the air.

The equilibration time of peepers and other *in situ* devices depends on their design (that is, the ratio of volume to surface area) (Teasdale *et al*., 1995; Davison *et al*., 2000). Thus a multi-chambered peeper of the type shown in Fig. 2.2 will equilibrate faster if it has windows on both sides rather than only on one side. (Much faster equilibration (for example, often 10 times faster than a peeper) is possible using diffusive equilibrium in thin films (DET), discussed in the next section.)

Multi-chamber peepers are suitable for obtaining low-resolution vertical distributions of pore-water contaminants in sediments (Carignan *et al*., 1985; Bufflap and Allen, 1995; Teasdale *et al*., 1995). The measurements in both the overlying water and at 1-cm intervals in the sediment pore waters yield a useful depth profile that reflects the effects of localised conditions of redox potential and pH on the partitioning of metals to pore waters (Teasdale *et al*., 2003). The concentration differences at the sediment–water interface can be used to calculate fluxes of metals to the overlying water.

One disadvantage of using peepers is that they take a relatively long time to reach equilibrium (several days to weeks) which increases opportunities for interference (natural or human). As with many trace metal techniques, there is considerable potential for error. Contamination from sediment particles is a major concern, so peeper users must be trained so they can obtain reproducible results. On recovery of the peeper from the sediment, anoxic pore waters will oxidise reasonably rapidly and so the chambers should be sampled without delay to avoid metals being removed as iron oxyhydroxide precipitates. Peepers generally produce insufficient volumes of pore water for toxicity testing purposes.

As with all passive samplers, considerable care is required to avoid inadvertent contamination of the device, and it is important to use preparation techniques suitable for sampling trace metals (for example, laminar flow cabinet, high purity deionised water, ultra-pure acids, acid-washing techniques, metal-free equipment for example, such as Teflon® or HDPE) when preparing peepers and handling samples.

2.12 Passive samplers

2.12.1 Diffusive equilibrium/gradients in thin films (DET/DGT) samplers for metals

The technique of diffusive equilibrium in thin films (DET) uses a thin hydrogel. Equilibration is reached much more rapidly than using peeper designs, allowing pore-water measurements to be made at higher resolution (Davison and Zhang, 1994; Harper *et al*., 1997). Complementary to DET is the technique of diffusive gradients in thin films (DGT), which uses a kinetic regime passive sampler that can provide *in situ* measurement of inorganic analyte concentrations in pore waters (or overlying waters) and fluxes from sediment pore waters (Zhang *et al*., 1995). In a DGT® device (registered trademark omitted hereafter), dissolved analyte species diffuse through a thin hydrogel layer (as used in DET) and become trapped in a gel typically impregnated with a chelating resin that selectively accumulates the metal of interest (or metalloid, using a specialised adsorbent). Analysis of DGT-accumulated metal has shown it consists of free metal ions, metal ions present as simple inorganic complexes, and labile organic complexes that dissociate over the time it takes to diffuse into the device (Zhang and Davison, 2000; van Leeuwen *et al*., 2005). DGT devices with different binding layers have been developed for measuring Cd, Co, Cr, Cu, Fe, Mn, Hg, Ni, Pb and Zn (Chelex-100 binding phase), Al, As(III, V), Hg, Mo(VI), Sb(V), S^{2-}, Se(IV, VI), V(V), W(VI) and U (using other binding phases) (Peijnenburg *et al*., 2014). The

major advantages of DET and DGT samplers over peepers are the short deployment times required (typically 8–48 h) and the greater resolution of pore-water depth profiles.

The DET/DGT devices (Fig. 2.3) can be purchased from DGT® Research, which also provides a detailed guide for the preparation and use of DGT samplers (DGT Research, 2015). The most common DGT device contains a layer of chelating resin (Chelex-100) separated from the test phase (for example, water, sediment or soil) by a polyacrylamide diffusion gel layer and a 0.45 μm membrane filter (Fig. 2.3). The resin strongly binds labile trace metal species that diffuse through the diffusive layer, creating a linear concentration gradient in the diffusive gel layer (Harper *et al*., 1998). When inserted into sediment, the accumulation of metals tends to locally deplete trace metal concentrations in the solution near the DGT probe, but resupply from the sediment solid phase can partially counterbalance this depletion. This is a 'kinetic regime' device, and so a linear relationship is assumed to exist between the accumulation of analytes in the DGT sampler and the deployment time.

In other words, during application of DGT, the removal of metals from sediment pore waters causes the concentration to decline immediately adjacent to the device. The DGT device causes a localised decline in pore-water metals that disturbs the dynamic equilibrium (partitioning) between pore water and sediment-bound metals and induces the release of metals into solution, the extent of which will depend on the rate of metal resupply (lability) from the sediment solid phase to the pore water (Harper *et al*., 1998). Release of metals from sediment particles to pore water (the DGT-induced metal flux) is likely to be more rapid for sediments that contain reactive forms of metals than for sediments that contain more inert forms of metals. Hence, differences in the DGT-induced metal fluxes (or the calculated pore-water concentration when assuming linear accumulation–time relationships) can provide useful information on the bioavailability of the metals in sediments (Roulier *et al*., 2008; Dabrin *et al*., 2012; Simpson *et al*., 2012; Amato *et al*., 2014). Peijnenburg *et al*. (2014) discuss recognised advantages and limitations of DGT measurements in sediments.

As for all trace metal sampling and analyses, it is important to avoid inadvertent contamination of the DGT device both before (for example, during acid-washing), during and

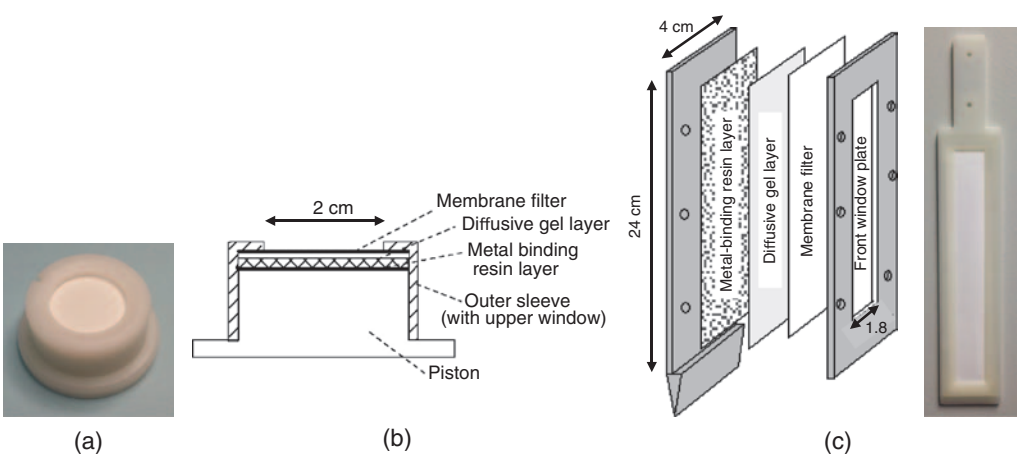

Figure 2.3. Examples of DGT samplers: (a) piston type (b, in cross-section); (c) planar type (exploded view). The concentration of metal measured by DGT (C_{DGT}, μg/L) can be calculated as $C_{DGT} = M\Delta g/DtA$, where Δg is the thickness of the diffusive gel and membrane (typically ≈0.09 cm, but this can be varied), D is the diffusion coefficient of free metal in the gel, t is the deployment time and A is the exposure area (A = 3.14 cm^2). (Modified from DTG Research, 2015.)

following deployment. When deploying and retrieving from sediments, (i) care should be taken to avoid adversely disturbing the sediment (for example, prevent the introduction of oxygen to sub-oxic or anoxic pore waters), (ii) the position of the sediment–water interface on the planar DGT sampler should be carefully recorded at the time of probe collection, (iii) observations such as of bubbles within the DGT sampler, torn membranes, or other anomalies should be recorded and the validity of these DGT results reconsidered.

After recovery from the sediment, the DGT device is disassembled, the resin layer is cut into appropriate slices (for sediment, sediment–water interface, overlying water) and the slices are immersed in a known volume of 1 M HNO_3 for at least 24 h to release the accumulated metals, before subsequent analysis. The mass of metal (M, in μg) accumulated in the resin gel is calculated from

$$M = C_e (V_{HNO3} + V_{gel}) / f_e,$$

where C_e represents the metal concentration in the eluent (μg/mL), V_{HNO3} is the volume of HNO_3 for the elution (1 mL), V_{gel} is the resin gel volume and f_e is the elution factor which is specific for each metal (typically 0.8).

The resin gel volume (V_{gel}, in mL) is calculated from

$$V_{gel} = l \cdot L \cdot \Delta r,$$

where l represents the width of the resin gel (typically 1.73 cm for the sediment probes available from DGT Research), L is the length of the resin gel (in cm) measured experimentally for each slice, and Δr is the thickness of the resin gel (typically 0.025 cm).

The flux of metal to the DGT device ($F_{DGT,}$ μg/m^2/h) is calculated from

$$F_{DGT} = 10,000 \cdot M / (t \cdot A),$$

where M is the amount of metal accumulated in the resin layer (μg), t represents the deployment time (h) and A is the surface area exposed to the overlying water (cm^2, equal to l · L).

The time-averaged metal concentration at the interface between the diffusive gel layer and the overlying water (C_{DGT}, μg/L) is calculated from

$$C_{DGT} = F_{DGT} \cdot \Delta g / (D \cdot 1000),$$

where F_{DGT} is the flux of metal to the DGT device (μg/m^2/h), Δg is the thickness of the diffusive gel layer plus the membrane filter (for example, 0.064 cm), and D is the diffusion coefficient of the specific analyte in the DGT gel at the deployment temperature (for example, 0.0189 cm^2/h for copper at 19°C).

2.12.2 Passive samplers for organic contaminants

A variety of passive sampling methods are now available for *in situ* characterisation of pore-water concentrations of hydrophobic organic contaminants (HOCs) (and some quite polar compounds), and detailed reviews outlining advantages and limitations should be consulted to determine which is most appropriate for a specific assessment (USEPA 2012; Perron *et al.*, 2013a,b; Lydy *et al.*, 2014). These methods include semi-permeable membrane devices (SPMDs) that comprise low-density polyethylene (LDPE) tubing containing a high molecular weight synthetic lipid (triolein); polyethylene devices that consist of flat strips of LDPE but lack the triolein fluid used in SPMDs; solid phase micro-extraction (SPME) devices comprising fused silica fibres that are coated with a layer of absorbing polymer (for example, polydimethylsiloxane, PDMS); and polyoxymethylene devices which are similar to the polyethylene devices but comprise a harder polymer with greater sorption capacity

than PDMS. Standardised and commercially-available SPME methods are available for PAHs (ASTM, 2013).

Many of the considerations that apply to passive sampling devices for metals also apply to organic contaminants. For example, kinetic mode passive sampling devices may potentially deplete the concentration of the organic chemical in the pore water (that is, equilibrium is not maintained), resulting in the accumulated concentration representing a flux that is dependent on the sediment properties and the contaminant resupply rate (lability) from the sediments. The rate of exchange of hydrophobic organic contaminants from sediments to the sampler will depend on the characteristics of those contaminants, the sediment properties (particularly the concentration and form and diffusion properties of the particulate organic carbon), and the characteristics of the passive sampling device. Two main configurations of passive sampling device exist; they involve thin films or membranes cut into sheets or strips (including liquid or solids phases that accumulate hydrophobic organic contaminants), or coatings applied to fibres (in SPMEs for example) or surfaces (Lydy *et al.*, 2014), but the thicknesses (for example, of sheets) and dimensions can be modified for a range of purposes. The calibration method, the efficiency at which accumulation occurs, the choice of deployment time and the level of detection achievable vary considerably between the different devices and configurations. Some passive sampling devices can be deployed in either kinetic or equilibrium modes.

Quality assurance in relation to sample integrity, replication and repeatability is also very important. Ghosh *et al.* (2014) provide guidance for selecting, calibrating and implementing passive sampling devices for sediments. Lydy *et al.* (2014) provide a range of examples of the use of passive sampling devices for assessing bioaccumulation. Perron *et al.* (2013a) observed the following when several passive sampling devices were compared for monitoring estuarine waters:

- concentrations of PAHs were approximately three times greater using polyoxymethylene than when using polyethylene devices;
- concentrations of PCBs were approximately three times greater using polyethylene than when using polyoxymethylene; and
- SPMEs had inadequate detection limits for either PAHs or PCBs.

Such studies highlight the care required when using passive sampling devices for routine monitoring purposes. Even greater care is required when interpreting results for sediments or for emerging contaminants, such as polybrominated diphenyl ethers (PBDEs) and triclosan (Perron *et al.*, 2013b).

2.13 Preparation of sediment elutriates

Sediment elutriates are commonly used for assessing the effects of dredging operations on water quality (USEPA/USACE, 1998; NAGD, 2009). Elutriate tests are used to approximate the concentrations of contaminants that might be released from sediments that are disturbed or undergo unconfined disposal within waterbodies. The data analysis should consider the initial dilution, which in a sea-dumping context (for dredged material) is defined as mixing which occurs within 4 h of disposal. This dilution will depend on several factors, such as depth, layering in the water column, and current velocities and directions. Within the ocean disposal framework, typically a dilution factor of 100 is applied before comparison of the elutriate concentration with water quality guidelines (NAGD, 2009), but this factor can be refined using hydrodynamic modelling. Elutriates are often also prepared

and used for analyses where it is not possible to obtain sufficient pore water from a sediment to enable detection of particular analytes, for example, tributyltin or trace organic contaminants such as PCBs and some pesticides. Biological tests on elutriates in place of pore waters are not recommended, owing to the considerable changes in composition that occur following preparation and storage without preservation (as is necessary for toxicity testing) (see Chapter 4 Section 4.3.1) Elutriate manipulations are also applicable to any situation where the re-suspension of sediment-bound toxicants is of concern, such as during bioturbation and storms that might disturb sediments and affect water quality.

Elutriate procedures should suit the intended study. However, the general method for elutriate preparation involves combining water and sediment in a ratio of 4 parts water to 1 part sediment (by volume) and shaking the mixture end-over-end for 1 h (USEPA/USACE, 1998). After the 1-h mixing period, the mixture is allowed to settle for 1 h. The supernatant is then siphoned off and centrifuged to remove particulates before chemical analysis. As already noted (Section 2.11.1), filtration should be avoided when using elutriate waters for toxicity tests. Re-centrifuging elutriates may be a better alternative than filtration. If filtration is necessary, filters should be pre-treated (cleaned, soaked) and the first 10 mL of elutriate to pass through the filter should be discarded (Environment Canada, 1994). The dissolved or colloidal contaminant retained (adsorbed) by the filter may require analysis. Elutriates should be analysed or used in biological tests as soon as possible after preparation. If the elutriate needs to be stored, the storage period should be no longer than 24 h and storage should be at 4°C with minimum headspace in the storage container (Geffard *et al*., 2004).

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