5

Bioaccumulation

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5.1 Introduction

Bioaccumulation refers to the accumulation of contaminants in the tissues of organisms through any route, including respiration, ingestion, or direct contact with contaminated sediment or water (USEPA, 2000a; Rainbow, 2007; ASTM, 2010). Organisms living in or on sediments are able to bioaccumulate contaminants from both pore waters and overlying waters and via ingestion of sediment particles and food. Organisms suitable for use as 'biomonitors' have the capacity to concentrate those portions of contaminants that are in a bioavailable form.

Metals and metalloids bioaccumulate particularly in organisms at lower trophic levels, such as some polychaetes and molluscs (Taylor and Maher, 2003; Waring *et al.*, 2006), and trophic transfer may be observed; for example, transfer of metals from phytoplankton to filter-feeding molluscs and herbivorous gastropods or barnacles and then to carnivorous gastropods and polychaetes (Wallace *et al.*, 1998; Zhang and Wang, 2006; Rainbow and Smith, 2010). Generally, biomagnification (increase in concentration through three or more trophic levels) does not occur with metals (Goodyear and McNeill, 1999; Cardwell *et al.*, 2013) but can occur for the metalloid selenium and mercury (Bowles *et al.*, 2001; Barwick and Maher, 2003).

Most non-ionic hydrophobic organic chemicals (HOCs), such as PCBs and PAHs, are readily taken up by organisms and accumulate in tissues (Moore *et al.*, 2005; ASTM, 2010). Synthetic organic chemicals such as PCBs are highly resistant to metabolic degradation and so can accumulate to high concentrations (Fiedler *et al.*, 1994). Some organic chemicals, for example PAHs, are readily taken up by many organisms but are rapidly metabolised (Maher and Aislabie, 1992). Many non-ionic organic chemicals are lipophilic so can also biomagnify through food chains (Kelly *et al.*, 2007).

A comparison of bioaccumulated concentrations in sedentary organisms from different sites can assist in assessing the risk posed by contaminants and sources. The organisms can be either field-sampled or caged. Phillips (1980) has identified three categories of factors that may contribute to changes in tissue contaminant concentrations through time:

- variation in contaminant delivery to the environment;
- changes in ambient factors affecting metabolism, such as salinity and temperature; and
- the organisms' physiology, especially aspects relating to reproductive cycles and changes in mass.

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These factors rarely operate in isolation, and the interactions between them complicate interpretation of their combined effects.

5.1.1 Bioaccumulation as a line of evidence

The focus of this chapter is on the use of bioaccumulation data as a line of evidence to indicate that organisms have been exposed to bioavailable contaminants. That evidence is valuable support for other lines of evidence, such as measures of high concentrations of sediment contaminants (Chapter 3), or toxicity (Chapter 4), or biomarker responses (Chapter 6). Attempting to quantify bioaccumulation to provide a ranking is somewhat arbitrary, the intent being to derive a ranking of accumulated tissue concentrations from values measured to represent ranges for unimpacted, potentially impacted and impacted. A ranking scheme recommended for Australia and New Zealand (Chapter 1, Section 1.4) has three classes: (i) Not significantly different from control; (ii) Significantly different ($P < 0.05$) and ≤3 × control; and (iii) Significantly different ($P < 0.05$) and >3 × control. These rankings are, however, arbitrary and meaningless unless the baseline contaminant concentrations and potential toxicity are taken into account. Species or types of organisms will have different natural baseline concentrations of essential metals such as copper and zinc, and changes in concentrations relative to baseline will have different effects in different organisms. Selenium is an example of an element with a narrow concentration range: there is little difference between it being essential and being toxic, and small increases can have deleterious effects (Janz *et al.*, 2010). In the case of HOCs, the baseline concentrations should be zero.

As the following pages will show, detailed investigations can improve the value of bioaccumulation assessments by providing an overview of sources of contaminant variability in organisms, and knowledge of the range of concentrations that may found in clean natural background situations. Bioaccumulation beyond that range then may be an indication of contaminated sediment.

5.2 Use of bioaccumulation data

5.2.1 Measures of bioaccumulation

Data for use in a weight-of-evidence (WOE) assessment are best obtained from measurements of contaminant concentrations either in field-collected native organisms or in field-transplanted (caged) organisms (which may be sourced from aquaculture). Organisms can be exposed to collected sediments in the laboratory, but diffusion of contaminants from pore water can result in elevated contaminant concentrations in the overlying water which are not representative of a field situation, even when frequent changes of overlying water are made.

The simplest assessment involves measuring contaminant bioaccumulation from a particular sediment (at a test location or in a laboratory test) and comparing that to the bioaccumulation of the same contaminant from at least three reference sediments, to establish whether a statistically significant difference exists.

Alternatively, if background concentrations of contaminants within organisms are known, these can be used to determine the bioaccumulation ratio. For example, in Australia, an extensive database has been established of metal concentrations in the oyster *Saccostrea glomerata* that inhabits the NSW–Queensland coast (Scanes and Roach, 1999; Robinson *et al.*, 2005).

Bioaccumulation can be modelled (USEPA, 2000b). However, in the context of a weight-of-evidence assessment, modelling is generally considered inappropriate beyond use in

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screening to determine which contaminants should be included in the assessment. Simple equilibrium partitioning models have been useful for predicting the bioaccumulation of HOCs from sediments (for example, Di Toro and McGrath, 2000). Biodynamic models that consider the uptake and efflux rates of metals from water and dietary routes have been used for predicting bioaccumulation of some metals (Luoma and Rainbow, 2005). For accurate predictions, a strong knowledge of geochemical and biological influences on metal bioavailability is needed, including the effects of feeding strategies on exposure (Simpson, 2005; Baumann and Fisher, 2011a,b; Höss *et al.*, 2011; Camusso *et al.*, 2012; Yu *et al.*, 2012; Proulx and Hare, 2014; Campana *et al.*, 2015).

5.2.2 Prediction of effects

Once bioaccumulation has been established, the significance of the accumulated concentrations both to ecosystem health and to human health may need to be determined. Where bioaccumulated concentrations of a contaminant exceed maximum residue limits in organisms for human consumption (FSANZ, 2013), the first management action might be to ban the collection of the affected species, at the same time evaluating remediation options to remove the contaminant source by approaches such as dredging or capping. Assessing the risk to ecosystem health is more problematic. High concentrations of a bioaccumulated contaminant are not necessarily linked to toxicity in an organism, and more detailed investigations will be needed to assess if toxicity is occurring. These investigations would involve other lines of evidence such as toxicity testing (Chapter 4) or the use of biomarkers (Chapter 6) to determine the extent to which the organism's biological functions are altered or impaired as a result of the bioaccumulation.

Organic contaminants

For HOCs, bioaccumulation can be directly related to toxicity and used as a valuable tool for assessing the effects of mixtures of HOCs and for developing guidelines (Meador *et al.*, 2011; Burgess *et al.*, 2013) (see also Chapter 3, Section 3.6). The bioaccumulation of HOCs is dependent on many factors, including exposure medium, uptake rate, metabolic capability, lipid content, and feeding strategy (Meador *et al.*, 1995; Moore *et al.*, 2005; Meador, 2006).

Two factors, lipid and organic carbon content, control to a large extent the partitioning behaviour of non-ionic organic chemicals between sediment, pore water and tissue (Ankley *et al.*, 1992; USEPA, 2000b). These two factors, along with the octanol:water partition coefficient (K_{OW}), have been used in simple equilibrium partitioning (EqP) models to predict the partitioning and bioaccumulation behaviour of PAHs in sediments (Di Toro *et al.*, 1991, 2000; Meylan *et al.*, 1999; Di Toro and McGrath, 2000).

Biota-to-sediment accumulation factors (BSAFs) and theoretical bioaccumulation potential (TBP) are screening tools based on equilibrium partitioning models which are useful for estimating the bioaccumulation of persistent non-ionic organic chemicals by benthic organisms exposed to contaminated sediments (USEPA/USACE, 1998; USEPA, 2000a; Moore *et al.*, 2005). For organics, BSAFs are typically derived using a sediment concentration (C_S) normalised to organic carbon (f_{OC}) and a tissue concentration normalised to its lipid content (f_L) (Moore *et al.*, 2005). The TBP is the expected concentration in an exposed organism's tissues and is the simplest and most easily understood model for estimating bioaccumulation, but it is also subject to a large degree of uncertainty (USEPA/USACE, 1998; USEPA, 2000a). It is related to BSAF by the equation:

$$TBP = BSAF \times (C_S / f_{OC}) \times f_L.$$

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For many HOCs, equilibrium partitioning theory provides useful relationships between water concentrations, bioaccumulation and toxic effects to some benthic organisms, although the approaches may under- or over-estimate bioaccumulation (Di Toro and McGrath, 2000; McGrath and Di Toro, 2009). The quality of predictions of TBP is therefore dependent on the choice of BSAF, and a BSAF–lipid database is available (USACE, 2014). The models do not consider the kinetics of processes that determine contaminant bioavailability from sediments, nor contaminant retention, metabolic degradation, or elimination from organisms. Equilibrium partitioning approaches do not adequately consider sediment ingestion by marine invertebrates as a major exposure pathway (Meador *et al.*, 1995; Kaag *et al.*, 1997; Baumard *et al.*, 1999). For deposit-feeding bivalves and worms particularly, sediment ingestion is a major uptake route (Kaag *et al.*, 1997; Mackay and Fraser, 2000; Weston and Maruya, 2002; Meador, 2006; Maruya *et al.*, 2012; Burgess *et al.*, 2013). The equilibrium partitioning approach usually does not take into account the different forms of organic carbon present in sediments. Desorption kinetics of HOCs vary greatly depending on organic and sediment characteristics (Cornelissen *et al.*, 1997; Hendriks *et al.*, 2001; Kraaij *et al.*, 2003). McGroddy *et al.* (1995) found only a fraction (0.01–0.4, or 1–40%) of sediment-associated PAHs appears to be involved in equilibrium partitioning with the pore water. Thus, adsorption and desorption kinetics, which are not considered by equilibrium partitioning approaches for estimating bioaccumulation, may greatly affect partitioning and bioaccumulation.

As described in Chapter 3, Section 3.6, relationships between sediment concentrations, tissue concentrations and toxic effects have been used to develop mechanistic guidelines for many HOCs (Di Toro and McGrath, 2000; McGrath and Di Toro, 2009). An environmental residue-effects database (ERED) has also been developed for studies where both tissue contaminant concentrations and biological effects have been measured (USACE/USEPA 2015). The toxico-kinetics and toxico-dynamics of bioaccumulated chemicals also play a large role in determining if and when effects may occur, and to what magnitude (McCarty *et al.*, 2011; Ashauer and Brown, 2013).

Metal contaminants

Most of the metals taken up by an organism do not bioaccumulate, but instead are processed internally and excreted (Wallace *et al.*, 2003), with only a fraction of the metals remaining in forms that contribute to toxicity within the organism (Vijver *et al.*, 2004; Luoma and Rainbow, 2005; Rainbow, 2007). For most metals, this means that bioaccumulation data cannot be used to predict the risk of toxicity to an organism (Adams *et al.*, 2011). Oysters, for example, can accumulate high concentrations of copper and zinc, but still function (Pan and Wang, 2012), and it has been demonstrated that oysters (and other bivalves) are able to sequester metals into sub-cellular non-toxic forms with only very small amounts of metals remaining in metal-sensitive cell components (Wang *et al.*, 2011).

Biota-to-sediment accumulation factors (BSAFs) were never intended for use with metals (Moore *et al.*, 2005). Literature generally indicates that BSAF data for metals show extreme variability. Hence it is inappropriate to use BSAFs as criteria for identifying and classifying metals as hazards.

To use metal bioaccumulation data for the assessment of toxic effects, it is necessary to determine the tissue metal concentration that will cause adverse effects. The critical body residue (CBR) approach has been used to model dose–response relationships in aquatic organisms (USACE/USEPA, 2002). The strengths of the CBR approach are that

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bioavailability, exposure to food, and accumulation/depuration rate kinetics are explicitly addressed. The major uncertainty of the approach is in determining a dose or response that is protective of ecological health (McGeer *et al*., 2003). Metals bind at a range of sites within organism tissues that have different functions, and impairment of function is the potential cause of toxicity within an organism (Wallace *et al*., 2003; Vijver *et al*., 2004; Rainbow, 2007). Uncertainties in using the CBR approach have been discussed by Moore *et al*. (2005) and Adams *et al*. (2011).

Organisms' body concentrations of metals may provide useful predictions of possible effects only if strong and clear relationships exist between bioaccumulation and biological effects (Borgmann, 2000; Borgmann *et al*., 2004; Simpson and King, 2005; Rainbow, 2007; Adams *et al*., 2011). Borgmann *et al*. (1991, 1998, 2001) found that chronic toxicity of Cd, Tl and Ni to the freshwater amphipod *Hyalella azteca* was a function of the total amount of metal accumulated, and not the total metal concentration in water or sediment. These relationships were used to calculate lethal body concentrations (LBCs) and internal effect concentrations (IECs). Taylor and Maher (2010, 2012a,b,c, 2013, 2014b) have shown that there are clear significant relationships between metal accumulation (Cd, Pb and Se) in the bivalves *Anadara trapezia* and *Tellina deltoidalis* and sub-lethal effects (antioxidant capacity, lipid peroxidation and lysosomal destabilisation). Marasinghe Wadige *et al*. (2014a,b) have shown similar relationships for the freshwater bivalve *Hyridella australis*. These data can also be used to define metal concentrations at which harmful effects can occur. For metals that are sequestered in non-toxic forms or are regulated over the concentration range of interest, the use of body concentrations to predict effects is not appropriate (Borgmann, 2000; Rainbow, 2002; Rainbow *et al*., 2004). For predicting toxic effects of metals in sediments for many species, it may be more useful to understand the processes that affect the rate of uptake of contaminants, than to know the net bioaccumulation of contaminants (Rainbow, 2002, 2007; Simpson, 2005; Casado-Martinez *et al*., 2010).

5.3 Choice of biomonitor organism

Bivalve molluscs and oligochaetes are among the most well-established biomonitors of contaminants in marine and freshwater environments (Phillips and Rainbow, 1994; USEPA, 2000a; Robinson *et al*., 2005; OECD, 2007). Although used less intensively for biomonitoring, organisms such as amphipods, chironomids, gastropods, polychaetes, sponges, nematodes and others also have the required attributes to be effective biomonitors (Phillips, 1977; Phillips and Rainbow, 1994; Langston and Spence, 1995; Taylor and Maher, 2003, 2006; Meador, 2006; Waring *et al*., 2006; de Mestre *et al*., 2012; Ding *et al*., 2012; Yu *et al*., 2012).

Phillips (1990) has suggested that for organisms to be effective biomonitors they must be:

- sedentary and therefore representative of the study site;
- hardy and tolerant of high concentrations of contaminants;
- widespread and abundant in the environment being studied;
- easy to identify and collect, with sufficient tissue for analysis of contaminant concentrations; and
- able to accumulate higher tissue concentrations of contaminants in contaminated environments than in uncontaminated environments.

Lee (1998), when discussing methods for the use of marine or estuarine benthic organisms for assessing bioaccumulation in sediments, suggested the following criteria:

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- sediment ingester;
- infaunal (preferably non-tubicolous);
- hardy;
- easily collected or cultured;
- sufficient biomass for analysis;
- high bioaccumulation potential;
- feeding behaviour that is understood; and
- suitable for mechanistic/kinetic studies.

There is some commonality between the Phillips (1990) and Lee (1998) criteria. For metal bioaccumulation studies, an organism that ingests sediment is not always required. Organisms that live in sediment and feed near the sediment surface and cause bioturbation will take in metals in the pore waters and bacteria and algae they ingest. Thus these organisms can be effective biomonitors, their bodies having metal concentrations that reflect sediment contamination (Taylor and Maher, 2012a,b,c; Marasinghe Wadige *et al*., 2014a,b). For HOCs that are strongly bound to sediments and probably not found in pore waters, or that are readily transferred to biota, a sediment-ingesting organism is required. If bioaccumulation of contaminants from suspended sediments is to be assessed, for example from a dredging event, a filter-feeding organism that lives in the water column or attaches to a solid substrate, such as an oyster, may be a suitable test species (Edge *et al*., 2014; Schmitz *et al*., 2015).

Organisms to be used for laboratory studies of contaminant bioaccumulation should be able to tolerate a range of conditions, such as of salinity and temperature. Additionally, species should be sufficiently tolerant of contaminants that they can survive relatively long exposure times, and have a low potential for metabolising contaminants (USEPA, 2000b). To be used as a biomonitor, an organism needs to accumulate contaminants, preferably in proportion to the bioavailable concentrations in sediments. This can be established through exposure to laboratory-spiked sediments (Taylor and Maher, 2010) or by measurements of organisms *in situ* along sediment contamination gradients (McCarthy, 1990; Luoma and Rainbow, 2005). The bivalves *Anadara trapezia*, *Tellina deltoidalis* and *Hyridella australis*, for example, have clear relationships of metal uptake relative to the prevailing sediment metal concentrations (King *et al*., 2010; Campana *et al*., 2013; Taylor and Maher, 2013, 2014b; Marasinghe Wadige *et al*., 2014a,b). For HOCs there are numerous studies demonstrating strong relationships between sediment contaminant concentrations (pore-water or sediment concentrations normalised to organic carbon) and bioaccumulation (Meador, 2006; You *et al*., 2011; Ding *et al*., 2012).

When using organisms transplanted in the field or in the laboratory, it is recommended that bioaccumulation studies be conducted for at least 28 days (ASTM, 2010, 2013), as this time is believed to be sufficient for most infaunal benthic species and contaminants to reach steady-state tissue concentrations. This, however, is not always the case. Burt *et al*. (2007) found that 60–90 days were required for *A. trapezia* to reach steady-state tissue metal concentrations. Many species can regulate metals or metabolise organic contaminants, for example PAHs (Maher and Aislabie, 1992), and may give a misleading indication of the bioaccumulation potential of an ecosystem. It is essential, therefore, that bioaccumulation studies include one or more species with very low ability to regulate metals or metabolise organic contaminants.

There is general agreement that metals accumulated over long periods are concentrated in the muscle tissues or stored as granules, and shorter-term accumulations are

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concentrated either in the tissues that process metals – for example, gills, hepatopancreas, digestive tissues, liver – or in the gut via ingested food and sediments (Phillips, 1990). For hydrophobic organics, accumulation is mainly in fatty tissues, so accumulated concentrations are usually normalised to lipid content.

Organisms that have most commonly been used internationally as biomonitors of contaminants in sediments (in the field) and to study bioaccumulation (in the laboratory or field) are shown in Table 5.1. However, a far greater range of species could potentially be used to evaluate bioaccumulation, provided the chosen species can be demonstrated to be an effective biomonitor; for example those shown in Table 5.2 for Australia. The ecology of all species chosen for bioaccumulation measurements needs to be fully understood so as to obtain interpretable results. Polychaetes, for example, have a variety of habitats within sediments and can be filter feeders, omnivores or carnivores (Waring and Maher, 2005; Waring *et al.*, 2005). Not all of the organisms listed in Table 5.2 have been used to measure bioavailable contaminants in sediments, although organisms such as the oyster *S. glomerata* are

Table 5.1. Common organisms that have been used internationally as biomonitors of contaminants in sediments

Species	Food source[a]	Use[b]	Organs[c]	References
Estuarine–Marine				
Polychaetes		M, O	W	ASTM, 2010; Lee *et al.*, 1993, 2001; Millward *et al.*, 2005; Morales-Caselles *et al.*, 2008; Casado-Martinez *et al.*, 2013; Ramos-Gomez *et al.*, 2011
Neanthes arenaceodentata	Om			
Capitella capitata	Non-selective DF, coprophagous			
Hediste (Nereis) diversicolor	Om, capable of F			
Arenicola marina	Surface DF			
Nereis virens	Om			
Bivalves		M, O	W	ASTM, 2010; Riba *et al.*, 2004; Cheggour *et al.*, 2005; Hendozko *et al.*, 2010; Tankoua *et al.*, 2011; Hylleberg and Gallucci, 1975; Lee *et al.*, 1993
Scrobicularia plana	DF			
Macoma balthica	DF, F			
Macoma nasuta	DF			
Freshwater				
Oligochaetes		M, O	W	ASTM, 2010; Higgins *et al.*, 2007; Phipps *et al.*, 1993; Mendez-Fernandez *et al.*, 2013; Mackenbach *et al.*, 2012; Ankley *et al.*, 1992
Tubifex tubifex	Selective DF			
Lumbriculus variegatus	D			
Amphipods and Midges *Hyalella azteca* *Chironomus tentans*	Selective DF	M, O	W	Borgmann, 2000; Ingersoll *et al.*, 1995, 1998; Landrum *et al.*, 2004

[a] Om = ominivores, F = filter feeders, D = detritivores, DF = deposit feeders.
[b] M = metals/metalloids, O = organics.
[c] W = whole organism.

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Table 5.2. Common organisms that have been used in Australia as biomonitors of contaminants in sediments

Species	Food source[a]	Use[b]	Organs[c]	References
Marine				
Anadara trapezia (bivalve – Sydney cockle)	Om	M	W	Jolley *et al.*, 2004; Burt *et al.*, 2007; Taylor and Maher, 2012a,b,c
Mytilus edulis (bivalve – mussel)	F	M, O	W	Talbot, 1989; Haynes *et al.*, 1995
Tellina deltoidalis (bivalve – deposit feeder)	DF	M	W	Campana *et al.*, 2013; King *et al.*, 2005; Taylor and Maher, 2010, 2013, 2014a,b
Trichomya hirsuta (bivalve – hairy mussel)	F	M	W	Lopez *et al.*, 2014
Saccostrea glomerata (Sydney rock oyster)	F	M, O	W	Hardiman and Pearson, 1995; Scanes, 1996; Scanes and Roach, 1999; Spooner *et al.*, 2003; Robinson *et al.*, 2005; Edge *et al.*, 2014
Mugil cephalus (fish – sea mullet)	D	M	Mu, L, K, G, Go, S, H	Kirby *et al.*, 2001a,b; Waltham *et al.*, 2013
Fish (assorted)	H, D, Om, C	M	Mu, R	Eustace, 1974; Plaskett and Potter, 1979; Marks *et al.*, 1980; Roach *et al.*, 2008
Polychaetes	F, Om, D	M	W	Waring *et al.*, 2006
Suberites sp. and *Mycale* sp. (sponges)	F	M	W	de Mestre *et al.*, 2012
Freshwater				
Hyridella depressa (bivalve – mussel)	F	M	W	Jeffree *et al.*, 1993; Byrne and Vesk, 1996; Adams *et al.*, 1997; Adams and Shorey, 1998
Hyridella australis (bivalve – mussel)	F	O	W	Ryan *et al.*, 1972; Marasinghe Wadige *et al.*, 2014a,b
Velesunio ambiguous (bivalve – mussel)	F	M	W	Jones and Walker, 1979; Millington and Walker, 1983; Jeffree *et al.*, 1993
Velesunio angasi (bivalve – mussel)	F	M	W	Jeffree and Brown, 1992; Ryan *et al.*, 2008; Bollhöfer *et al.*, 2011
Alathyria condola (bivalve – mussel)	F	CY	W	Negri and Jones, 1995
Westralunio carteri (bivalve – mussel)	F	O	W	Storey and Edward, 1989

[a] Om = omnivores, F = filter feeders, D = detritivores, DF = deposit feeders, H = herbivores, C = carnivores.
[b] M = metals/metalloids, O = organics, CY = cytotoxins.
[c] W = whole organism, Mu = muscle, L = liver, K = kidney, G = gills, Go = gonads, S = stomach, H = heart, R = reproductive organs.

known to be useful for measuring bioavailable contaminants in suspended sediments (Edge *et al.*, 2014; Schmitz *et al.*, 2015). As physical suspension, bioturbation and uptake by micro-organisms and algae result in multiple pathways of metal exposure, most organisms listed in

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Tables 5.1 and 5.2 will be appropriate for assessing the bioaccumulation of metals. For assessing organics that are strongly bound to sediment particles and not likely to be easily re-mobilised or taken up by microrganisms, and/or for algae, it is best to use organisms that ingest sediments, for example polychaetes or oligochaetes.

5.3.1 Contaminant-specific considerations

Metals

Bioaccumulation of metals in molluscs and other organisms is influenced by physiological factors including age, growth, gender, reproductive condition and genetics. Sampling strategies should aim to minimise the effects of these factors in bioaccumulation studies (for example, comparing organisms of similar age and gender). Sediment properties, redox potential, pH, salinity and temperature are factors that affect metal bioavailability (Brown and Depledge, 1998). These factors also influence the physiological activity of organisms through their effects on metabolic rates (Frazier, 1976). It is necessary to understand the effects of all of these factors on metal accumulation in organisms because they may lead to variations in tissue metal concentrations that if not accounted for will make results difficult to interpret.

It has been established that the tissue metal concentrations may vary with mass and size of molluscs (Taylor and Maher, 2003). Temporal fluctuations in mass and metal body burdens may also occur (Robinson *et al.*, 2005; Taylor and Maher, 2006). For some organisms, tissue metal and metalloid concentrations can remain relatively constant – selenium for example – suggesting that the organisms have reached equilibrium with their environment (Taylor and Maher, 2012c).

Many studies have found a trend of decreasing tissue metal concentrations with increased tissue mass (Boyden, 1977; Lobel *et al.*, 1991; Langston and Spence, 1995) although this does not always occur (Cubadda *et al.*, 2001). This 'dilution effect', where growth dilutes the metal content, appears to be a common phenomenon in molluscs. It is also postulated that where mass is independent of tissue metal concentration, there may be some form of regulation of uptake and excretion (Phillips and Rainbow, 1994). An examination of data comparing mass and metal concentration shows that a minimum of three orders of magnitude in mass is required before a significant relationship between mass and tissue metal concentration is evident in bivalves.

Age may also influence metal concentrations: older organisms show less variability, probably because of high metabolic activity in juveniles. Robinson *et al.* (2005) found there was less variability between individual oysters' metal concentrations once they entered adulthood, and suggested that this is due to biochemical changes occurring during rapid faster growth in juveniles. For testing, collection of mature individuals would reduce the within-sample variability, but size is not necessarily a good indicator of age and therefore often it is not possible to sample mature individuals.

Genetic differences may cause an overlap in the distributions of tissue metal concentrations in contaminated and uncontaminated locations. In other words, individuals from a contaminated environment may accumulate lower metal concentrations than individuals from uncontaminated environments (Taylor and Maher, 2003). It appears, for example, that some molluscs may take up metals at a lower rate, or they may have enhanced regulatory mechanisms (Lobel *et al.*, 1982, 1991).

Gastropods from contaminated sites had a higher degree of individual variability in metal concentrations than those from an uncontaminated site in studies by Taylor and Maher (2003). Positive skewness in the distributions of metal concentrations occurs in populations of gastropod and bivalve molluscs from both contaminated and uncontaminated environments

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(Taylor and Maher, 2003; Robinson *et al.*, 2005). Although some individuals from contaminated environments accumulate lower concentrations of metals than individuals in an uncontaminated environment, the majority do not. At the individual level there can be a large range in tissue metal concentrations, while at the population level the distributions are separate (Taylor and Maher, 2003). Some individuals may either be taking up less metal or have enhanced excretory mechanisms, and others are accumulating excessive quantities of metals, but the majority of the population falls somewhere in the middle. These results (Taylor and Maher, 2003; Robinson *et al.*, 2005) could not be explained on the basis of either mass or gender or habitat differences. Skewness seems to be a common factor in sample distributions of natural populations because of the natural variation between individuals. Collecting greater numbers of controls/reference organisms may help inform the test laboratory about skewness and improve the power of the study in determining if differences between populations are significant. In summary, molluscs will reflect the levels of biologically available metals of their respective environments, and, in the contaminated environment, they are net accumulators. Variances usually increase as mean metal concentrations increase.

Significant differences found in metal concentrations due to gender, where they exist, have been thought to be associated with spawning (Lobel *et al.*, 1991) because metal concentrations vary as mass fluctuates when oocytes are produced and shed.

Collecting organisms that are at different stages of spawning may also contribute to differences between individuals and hence overall variability (Simpson, 1979; Cossa *et al.*, 1979). The use of triploid oysters, which do not reproduce, has been shown to considerably reduce variability in observed metal concentrations (Robinson *et al.*, 2005).

Organics

For non-ionic HOCs, the lipid content of an organism is an important factor in its contaminant uptake and storage. Lipid content can vary considerably within a single species, based on life stage, gender, sexual maturity and season, and this will affect the bioaccumulation of organic contaminants (Moore *et al.*, 2005).

Organic contaminant concentrations are likely to vary with mass and size of molluscs (USEPA, 2000). Greater mass and size are often a reflection of age and it would be expected that older organisms would have had longer exposure times and exhibit greater contaminant concentrations. Gender can also be expected to have significant effects on organic contaminant concentrations. For example, during spawning there are fluctuations in the mass of an organism, especially its gonads (Meador *et al.*, 1995). Collection of organisms that are at different stages of spawning would contribute to differences observed between individuals and hence overall variability (Bruner *et al.*, 1994, Meador *et al.*, 1995).

Genetic differences may also accentuate the individual variability in organic contaminant concentrations which occurs in natural populations and skews the natural distributions of contaminant concentrations.

Organic contaminant content can fluctuate through time, related to an organism's feeding behaviour and reproductive cycle. Many organisms feed more extensively and grow more rapidly during warmer periods (for example in summer) and thus lipid content may be greater at these times than at other times when lipid reserves are used. As well, lipid content can increase as gonadal tissues grow. On spawning, this gonadal material will be lost (Bruner *et al.*, 1994).

Selection of sample size

When comparing locations, it is important to select a large enough sample size to gain a true estimation of the mean bioaccumulated contaminant concentrations and to allow

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concentration differences to be detected. Inherent variability existing in organism populations may obscure trends and lead to incorrect conclusions. Sample size calculations are relatively easy to perform (Zar, 1984), but the concentration differences required to establish social or environmental significance are a management decision. Three simple procedures can be used to calculate sample size:

- sub-sampling the dataset using a random number table to determine the number of replicates required to obtain a value within 10% of the population mean (Taylor and Maher, 2003);
- using the means and standard deviations of contaminant concentrations and Student's *t* tables as outlined by Zar (1984) (Taylor and Maher, 2003);
- using bootstrap analysis of metal concentration data to obtain the 95% confidence interval for discrete sample sizes (Robinson *et al*., 2005).

For oysters, for example *Saccostrea glomerata*, and gastropods, for example *Austrocochlea constricta* and *Bembicium auratum*, although the inherent variability is large, only 10 samples need be analysed to obtain an estimate of the mean concentration within ±10% of the population mean (Taylor and Maher, 2003; Robinson *et al*., 2005). This sample number should allow changes of 30% from the mean contaminant concentration to be detected.

Many studies have used pooled samples (that is, they combine multiple individual samples to provide a composite) to reduce variability and reduce analysis costs. The disadvantage is that information on contaminant variability is lost. If subtle changes in bioaccumulation are occurring they are often seen as increases in contaminant variability rather than increases in means. As well, skewing of the means of contaminant concentration can occur if a few samples contain high concentrations of contaminants, especially metals. This cannot be detected if pooled samples are used.

As a consequence of the above, when collecting organisms, attention should be given to the following factors to minimise variability and aid in interpretation of results.

- Mass. Organisms of similar mass and size should be selected from all locations. A regression analysis should be used to establish that tissue contaminant concentrations are independent of mass (or size). If mass or size dependence is established, then concentrations should be normalised to a chosen mass.
- Gender. Most studies have found that gender *per se* is not a large contributor to metal concentration variability; but known periods of spawning should be avoided because contaminants may be lost during this time. For HOCs, gender differences may be associated with lipid content differences and collection should be standardised to female or male organisms.
- Genetics. Inherent variability appears to be a 'universal characteristic' of contaminant concentration distributions particularly in molluscs; thus sufficient replicate samples should be collected to account for this variability. All measured contaminant concentrations should be used in statistical analyses because high contaminant concentrations at uncontaminated locations are not outliers.
- Accumulation of chemicals of interest at contaminated and uncontaminated locations. Preferably, organisms should accumulate contaminants in direct proportion to the contamination in sediments. If organisms are to be used as biomonitors, it must be established that these organisms accumulate higher tissue concentrations of contaminants in contaminated environments than in uncontaminated environments.
- Temporal variation. For comparison purposes, organisms from contaminated and uncontaminated locations need to be collected after similar exposure periods and at similar times.

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- Sample size. Sample size needs to be large enough to produce a true estimation of the mean contaminant concentrations and to allow concentration differences to be detected. Sample size calculations are relatively easy to perform (Zar, 1984), but concentration differences required to reveal social or environmental significance are a management decision. Where there is insufficient mass of organisms to provide a large enough sample, pooling of samples is an option, noting the limitations discussed earlier.

5.3.2 Choice of tissues or sub-cellular fractions to be measured

Metals

Bioaccumulated contaminants will be distributed within the test organism, depending on the major route of uptake, the site and mechanism of storage, and the mechanism of excretion. The distribution is very significant for assessments because it is now recognised that, for example, metals bound at different sites have different functions, and impairment of function is the potential cause of toxicity within an organism. Thus, organisms in the field that have been accumulating metals for a long period may be storing most of the metal in a detoxified form. Toxic effects are elicited when a critical dose of a chemical is reached in one or more sensitive compartments of the organism, or toxicity may take effect if the metabolically available concentration exceeds a threshold concentration, or if the metal influx rate exceeds the combined rates of detoxification and excretion (Rainbow and Luoma, 2011).

Most dietary-derived metals are processed in the gut and then accumulated internally and stored in the hepatopancreas or other tissues as granules; they may be excreted via the liver. Metals taken up directly from the water column are concentrated in the gills, hepatopancreas and mantle. Excretion may occur by a range of processes: via direct egestion, excretion through the gills, or via the liver (Luoma and Rainbow, 2008). While for most applications the data from the bioaccumulation line of evidence will be based on whole body concentrations, more detailed examination of tissue distribution can add value.

In bivalves, the distribution of a metal at the sub-cellular level can be measured in the gill and hepatopancreas to determine metabolically active and detoxified metal fractions, and used to interpret biological effects (Taylor and Maher, 2010). Biological effects are related to the threshold concentrations of metabolically-available metals and not to total accumulated metal concentration (Rainbow, 2002; Vijver *et al*., 2004; Simpson and King, 2005). A scheme for separating operationally defined sub-cellular fractions in molluscs is given in Fig. 5.1. Typically, information can be gathered on five major sub-cellular fractions of metals within organisms:

- metal-rich granules (MRG);
- nuclei and cellular debris;
- organelles (ORG), including mitochondria, microsomes and lysosomes;
- heat-denaturable proteins (HDP), also referred to as heat-sensitive proteins; and
- metallothionein-like proteins (MTLP), also referred to as heat-stable proteins.

The biologically active metal fraction (BAM), which combines ORG and HDP, is the target of attack of metals in cells, and a biologically detoxified metal fraction (BDM), which combines MRG and MTLP, is considered to alleviate toxicity.

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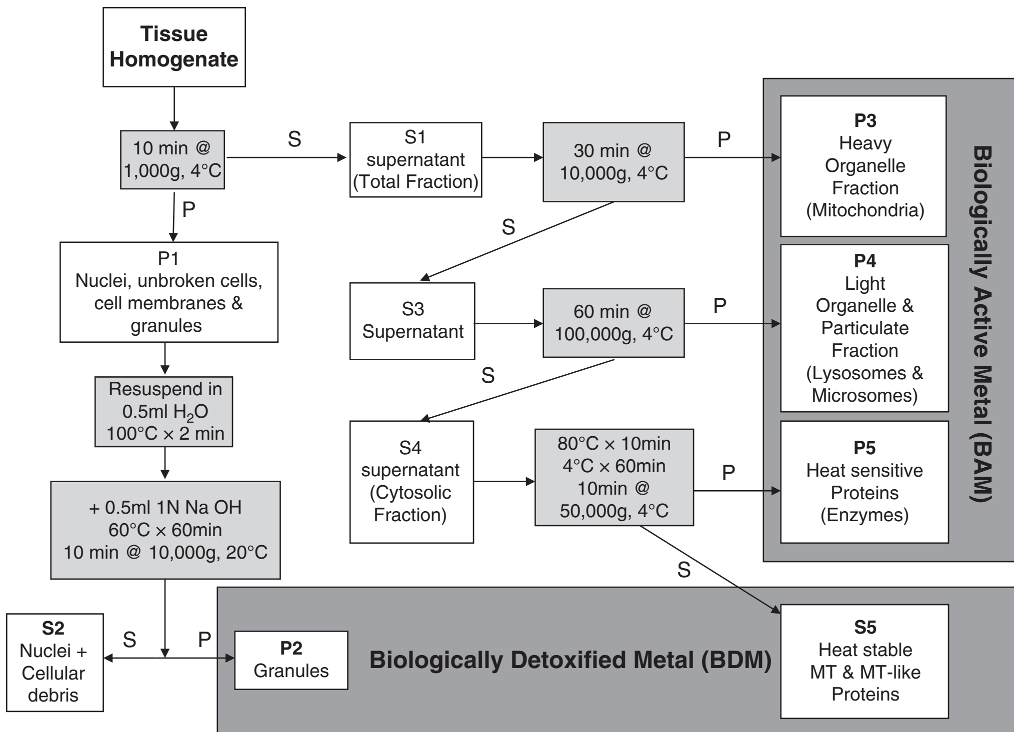

Figure 5.1. Procedure for sub-cellular fractionation of bivalve tissues by differential centrifugation. The shaded boxes show details of the centrifugation and digestion or heating steps used to obtain the specific fractions. The final fractions – four pellets P2, P3, P4 and P5 and two supernatants S2 and S5 – are grouped as: biologically detoxified metals (BDM) P2 and S5; and biologically active metals (BAM) P3, P4 and P5; or as S2 which contains metal associated with dissolved tissues (Taylor and Maher, 2012b). MT = metallothionein.

Thus consideration should be given to:

- selection of tissues; for general monitoring purposes analysis of whole tissues is sufficient. If a deeper understanding of the pathway of contaminant uptake is required, individual tissues need to be analysed to ascertain the route of uptake. Metals such as cadmium and zinc found accumulated in gill tissues, for example, are generally attributed to water, while metals/metalloids such as Se, Hg and Pb found accumulated in digestive tissues are from food;
- need for sub-cellular fractionation; if the focus is on relating bioaccumulated contaminants to effects, then measuring contaminants (for example metals in sub-cellular fractions) provides additional information for understanding the mechanism of toxicity. This information can also be used to explore the transfer of contaminants from prey to predator organisms if food web interactions or ecosystem questions relating to contaminants are being explored.

Organics

For bioaccumulated organic contaminants, a major limitation is analytical detection. A requirement is that the tissue mass be sufficient for chemical analyses (Exponent, 1998).

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The analysis of the concentrations in specific tissues will therefore be restricted to larger organisms such as fish. Only where the concern is for human health effects would tissues be sub-sampled to reflect the concentrations of contaminants in the parts that are consumed. Additionally, specific organs might be sampled where the mechanism of bioaccumulation is a concern (Bruner *et al*., 1994; Meador *et al*., 1995). For a weight-of-evidence assessment, because HOCs associate with lipid tissue, it is usual to use only the whole organism for analysis. Bioaccumulated concentrations are usually expressed on a wet weight basis or normalised to lipid content.

Methods used for lipid analysis have been summarised by Schlechtriem *et al.* (2012).

5.4 Choice of approach

5.4.1 Field collection versus transplantation studies

There are three approaches that can be used to assess contaminant bioaccumulation in field or laboratory experiments:

(i) passive biomonitoring: the measurement of contaminant concentrations in indigenous organisms;
(ii) field transplantion (active biomonitoring): where organisms are transplanted into contaminated environments and their contaminant concentrations are measured after a specified time;
(iii) laboratory transplantion (active biomonitoring): where organisms are transplanted into microcosms containing contaminated sediments in the laboratory and their accumulated concentrations are measured after a specified time.

Each approach has advantages and disadvantages. Passive biomonitoring requires organisms to be present at the sites of interest in sufficient numbers for statistical analyses. Contaminant concentrations will reflect exposure over the lifetime of the organism and will account for population adaptation to contaminated environments, so may exhibit fewer effects. A disadvantage is that organisms will be genetically different, introducing some inherent variability into contaminant concentrations.

Field transplantation allows genetically similar organisms to be put into depauperate environments and for the uptake of contaminants to be measured in absolute terms, and rates of uptake calculated. Organisms are also subject to real environmental variability (temperature, pH, dissolved oxygen, redox potential and food) that may influence the uptake of contaminants. A limitation is that organisms will not have adapted to contaminated environments and uptake, and effects may be greater than for indigenous organisms. Another limitation is the lack of security when deploying caged organisms in populous areas.

Laboratory transplantation also allows the uptake of contaminants to be measured in genetically similar organisms, in absolute terms, and rates of uptake to be calculated. The major limitations are that organisms will not be subject to real environmental variability and will experience potentially artificially elevated contaminant exposure via the overlying water, and so the study may over- or under-estimate uptake. As well, sediment properties influencing contaminant variability (for example pH, dissolved oxygen, redox potential) may be affected by removing sediments from their natural environment, so sufficient time for their re-equilibration (7–10 days) is required. The main advantages

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are the security that the laboratory setting gives, and the opportunity to control extraneous variables.

5.4.2 Study design and statistical analysis

The three approaches are illustrated in a study using the bivalve *Anadara trapezia* to measure the bioaccumulation of metals from contaminated sediments. This study focused on the estuarine Lake Macquarie, New South Wales (NSW), Australia. Similar protocols would be employed for the determination of HOCs in bivalves or other biota.

Lake Macquarie, NSW, Australia – an example

Industrial development around Lake Macquarie (Fig. 5.2) is extensive and consists of a decommissioned lead–zinc smelter, a fertiliser plant, a steel foundry, collieries, sewage treatment works and two coal-fired power stations. The lake supports a large recreational fishery and is also of ecological significance, providing breeding and nursery grounds for many commercial fish species. In comparison to other NSW estuaries, Lake Macquarie has significantly higher concentrations of metals, particularly lead and the metalloid selenium, in its sediment (Roy and Crawford, 1984; Peters *et al*., 1999b; Kirby *et al*., 2001b). The lead–zinc smelter to the north of the lake was in operation between 1897and 2003, and is a known source of Zn, Se, Cd and Pb (AWACS, 1995). There is a clear contamination gradient for Cu, Zn, Cd and Pb in the northern part of the lake (Burt *et al*., 2007). Metal concentrations above background levels in the southern reaches and selenium hot spots near power stations indicate that the coal-fired power stations are also contributing selenium and metals to the lake (Peters *et al*., 1999a).

The locations in Lake Macquarie from which indigenous organisms were collected and which were used for field and laboratory sediment transplant experiments are indicated in Fig. 5.2. Lead concentrations in sediments at these locations are shown in Fig. 5.3, together with values for three reference locations. The Sydney cockle, *Anadara trapezia*, was chosen for assessing bioaccumulation and effects because it is a sediment-dwelling organism native to Lake Macquarie and has been shown to accumulate metals in areas where sediments are contaminated with metals (Furner, 1979; Batley, 1987; Burt *et al*., 2007).

Field sampling (passive biomonitoring)

Experimental design principles

A typical field study will collect organisms from several locations to assess and rank bioaccumulation. If only one location is of interest, this location will need to be assessed relative to at least three reference locations. Replication within locations ('sites') is required to enable statistical analysis. Thus studies will need to be designed to enable a two-factor nested analysis of variance ('factors': location and site). Locations are the general areas of sampling; sites are specific areas at least 100 m apart, randomly chosen within locations for sampling. Typically 10–20 organisms are collected by hand for analysis at each site.

Study sites, results and analysis

Maher *et al*. (1998, unpublished data) collected *A. trapezia* from four locations in the northern part of the lake (Fig. 5.2) and three reference locations in nearby estuaries (Bagnalls Beach, Tea Gardens and Smiths Lake). Lead concentrations in whole tissues were significantly different among locations within the lake, with mean concentrations

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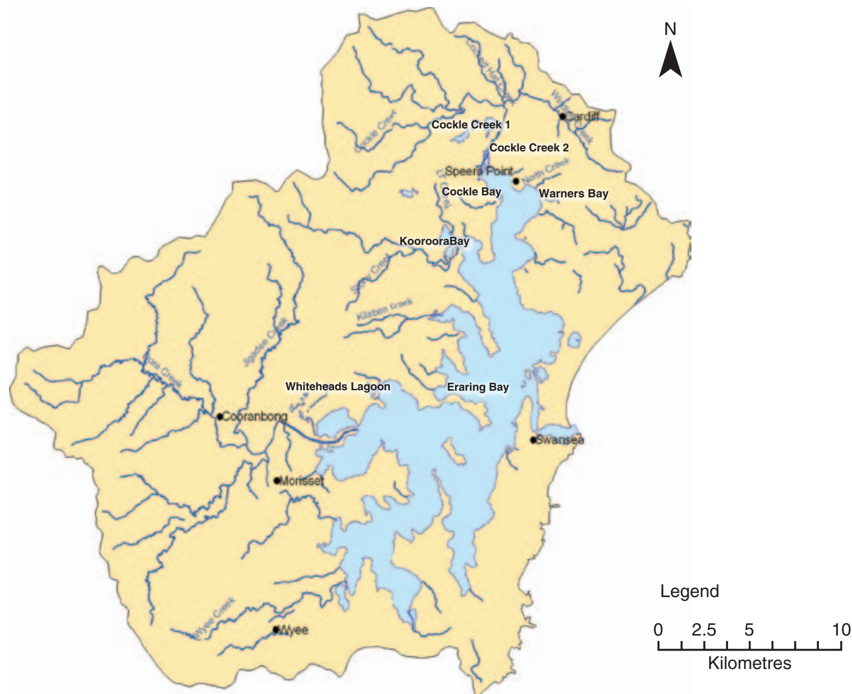

Figure 5.2. Map of Lake Macquarie NSW. The seven study locations were Cockle Creek 1, Cockle Creek 2, Cockle Bay, Warners Bay, Kooroora Bay, Eraring Bay and Whiteheads Lagoon. The lake is connected to the Pacific Ocean at Swansea.

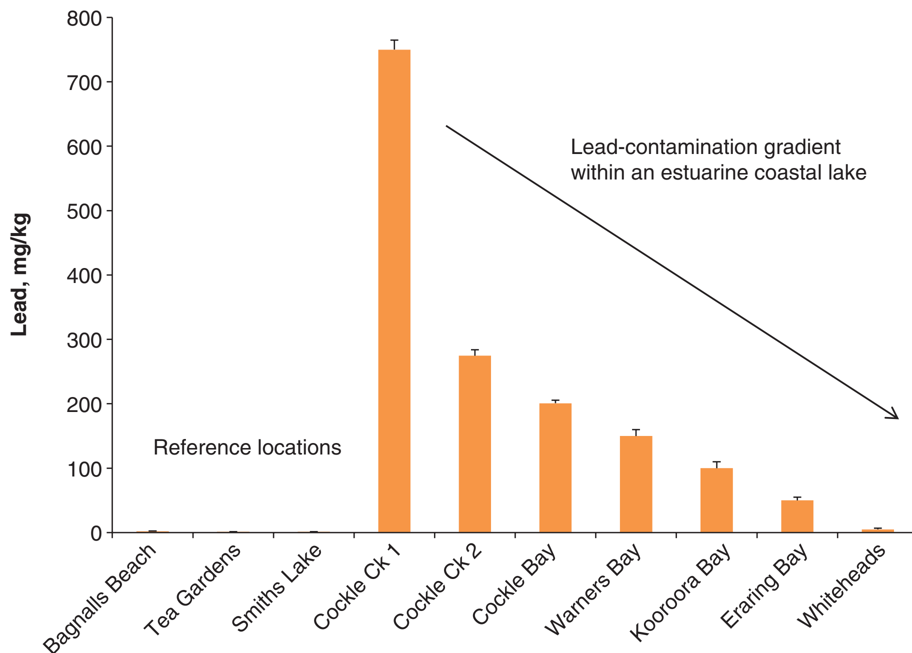

Figure 5.3. Concentrations of lead in sediments of Lake Macquarie and reference locations.

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decreasing in the order Cockle Bay > Warners Bay > Kooroora Bay > Eraring Bay, and greater than at the three reference locations (Fig. 5.4). The bioconcentration factors were 33, 12, 9 and 3 for cockles collected from the respective sampling locations.

Field transplantation

Experimental design principles

Studies with caged organisms in the field need to be designed to allow a four-factor nested analysis of variance (factors: time, location, site and cage). Locations are the general areas of sampling; sites are specific areas within locations, at least 100 m apart and randomly chosen for cage deployment. A typical design might consist of three sampling times (30, 60 and 90 days), three or four locations (including control), two sites nested in each location and two cages nested in each site. The design can be simplified by choosing one time – typically 60 days for *A. trapezia*, and 28 days for other bivalves. If a time component is included, at least two randomly chosen cages need to be retrieved at each time from each site, nested within the locations. Organisms to be caged are collected from a reference location and transported in a portable cooler box containing sediment and water from the collection site and an aquarium air pump to aerate overlying water during transportation and maintain ambient temperature. Typically 10–20 organisms are placed in each cage.

Choice and deployment of cages

Previous studies (Cain and Luoma, 1990; Martinčić *et al.*, 1992; Couillard *et al.*, 1995; Dewitt *et al.*, 1999) have concluded that cages *per se* have no treatment effects. The cages

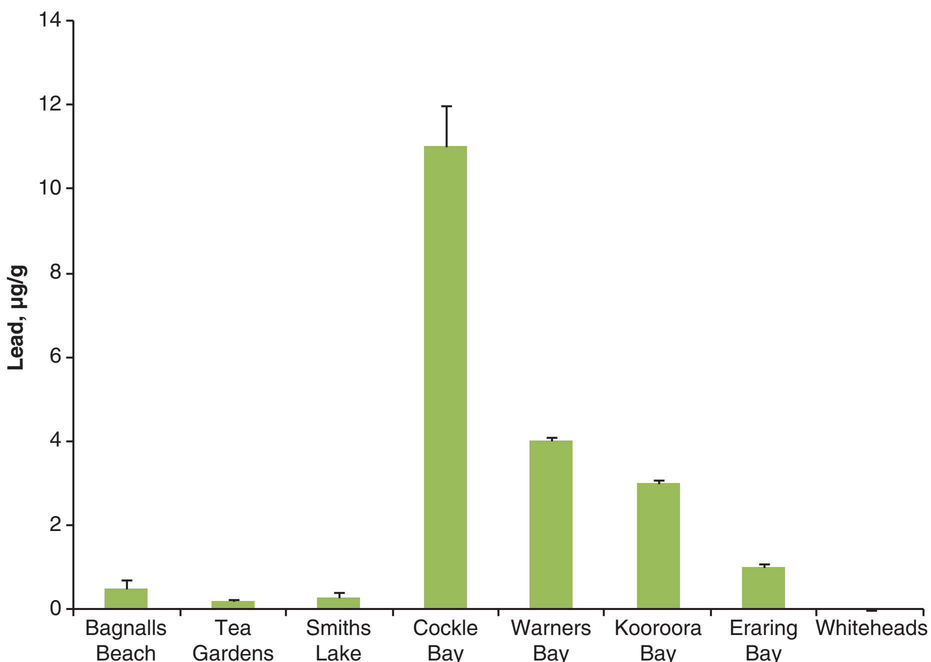

Figure 5.4. Concentrations of lead in tissues of indigenous *Anadara trapezia* from Lake Macquarie and reference locations.

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may need to be deployed by Scuba divers, depending on the water depth at the sites of interest. In design, the cages should:

(i) be large enough to hold the number of organisms required, with adequate access to sediments;
(ii) allow flow-through of water;
(iii) be of non-contaminating materials; typically, plastic oyster cages have been used (see Fig. 5.5); and
(iv) be secured to avoid predation and escape of organisms; oyster trays with netting have been used for this purpose (Fig. 5.5).

A consideration, based on the question to be answered, is whether cages are to be buried in sediments or placed on the sediment surface. If cages are buried in sediments, organisms will be exposed to metals through ingestion (sediment particles and food), dermal absorption and metals released by bioturbation. If cages are placed on sediments, organisms will only be exposed to metal through ingestion of food and metal fluxes.

The organisms chosen will also influence how cages are deployed. Most oysters and mussels are filter feeders and do not have to be in sediments. Other organisms, such as *A. trapezia* and *Ostrea angasi* (mud oyster), live in sediments and need to be able to bury themselves to function. *Tellina deltoidalis* is a deposit feeder and feeds from the sediment, so also requires sufficient sediment to burrow and feed.

A period of 60–90 days is sufficient for *A. trapezia* to reach equilibrium with their environment (Burt *et al*., 2007), but this equilibration period needs to be determined for each species of organism chosen. Individual cages and organisms needed to be marked (for example using adhesive numbers) to allow an assessment of the condition of the organisms (mass:volume ratio) at the end of the deployment period.

Figure 5.5. Field transplantation cage, about 60 cm × 40 cm × 10 cm.

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To avoid vandalism, cages need to be fully submerged to at least a depth of 1 m with no visible markers. To retrieve the cages, the locations need to be accurately known. A simple method of achieving this in shallow waters near the shore is to attach two ropes to known points on the shore and use these to triangulate the cage positions. In deeper waters, a GPS device may be needed to locate cages.

Study locations, results and analysis

Burt *et al.* (2007) and Taylor and Maher (2011 unpublished) transplanted *A. trapezia* to seven locations in Lake Macquarie (Fig. 5.6). Lead concentrations in whole tissues reached a maximum after 2 months and were significantly different among locations. Mean lead concentrations decreased in the order Cockle Creek 1 > Cockle Creek 2 = Cockle Bay > Warners Bay > Kooroora Bay > Eraring Bay > Whiteheads Lagoon and were significantly higher than the reference locations Bagnalls Beach, Tea Gardens and Smiths Lake (Fig. 5.6). The bioconcentration factors were 67, 41, 42, 18, 9, 6 and 3 at the respective locations, and were similar to those of indigenous *A. trapezia*.

Laboratory transplantation – microcosm exposure

Experimental design principles

A typical laboratory study will compare the bioaccumulation of metals by organisms in laboratory microcosms using sediments collected from a gradient of contamination (minimum three sites). Again, if only one location is of interest, this location will need to be assessed relative to at least three reference locations. Replication within locations is required to enable statistical analysis. Studies need to be designed to enable either a one-way (factors: location and replicates) or a two-way analysis of variance (factors: location, time and replicates).

Sediment, seawater and organism collection

Sediments are collected with a stainless-steel spade and press-sieved through a 2 mm stainless-steel mesh to remove rocks, large pieces of organic material and organisms. The sediments are

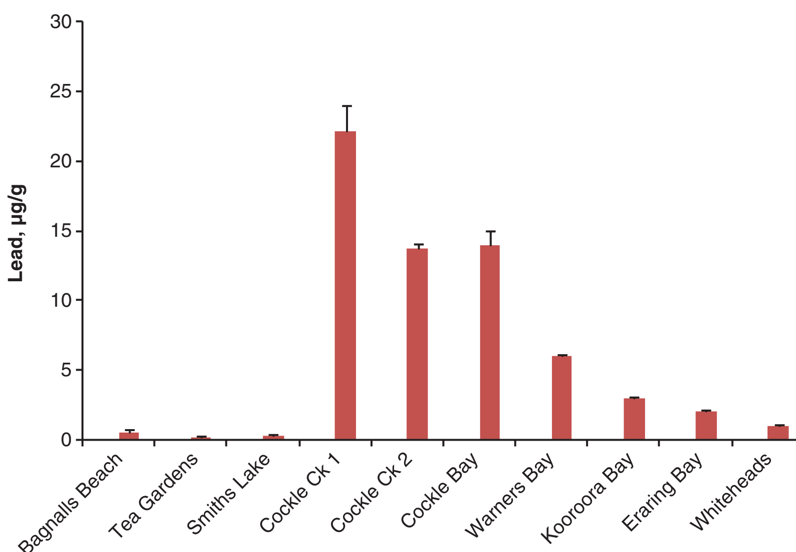

Figure 5.6. Concentrations of lead in tissues of *Anadara trapezia* field-transplanted in Lake Macquarie and from reference locations.

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placed in a series of plastic buckets with lids and sealed with tape for transport. Sediments can be stored at 4°C for up to 2 months. Before use in experiments, the sediments are sub-sampled for analysis of metal concentrations, to determine metal exposures. Moisture content, salinity, pH and sediment grain size are also measured to establish the physico-chemical properties of both the control and the test sediments. Properties of control and test sediments should be closely matched to ensure the results for contaminant dose and effects are not influenced by physico-chemical factors, rather than the contaminants of interest.

Seawater is collected from uncontaminated coastal waters and adjusted with deionised water to match the salinity of locations where test organisms are being collected: usually 30 ± 2‰.

Test organisms are collected from reference locations and placed in portable cooler boxes containing sediment and water from those sites, with aquarium air pumps to aerate overlying water during transportation and maintain ambient temperature. The organisms are maintained in aquaria, with control sediments 10 cm deep and water of the same salinity as the collection site, for up to 2 weeks to acclimatise before experimentation. Overlying waters are aerated using in-line control valves on air hoses to achieve ≥85% oxygen saturation without disturbing sediments. Water temperature is maintained at 22 ± 1°C and the photoperiod is 14-h light : 10-h dark. If ambient water temperatures at the time of collection are 10 ± 5°C cooler or warmer than 22 ± 1°C, the water temperature of the holding tanks is adjusted gradually by 2 ± 0.5°C per day until the experimental temperature is reached. A 3-day feeding/half water-change cycle is maintained during the acclimatisation period using a suitable supplementary food such as the unicellular green algae *Nannochloropsis* preparation (Nanno 3600, Instant Algae®, USA).

Laboratory microcosms

Typical microcosms are 10–12 L glass or polystyrene containers or aquaria containing 1000 g of wet sediment (with a minimum of 20% <63 µm fraction) and 8–10 L of seawater (Fig. 5.7). Overlying waters are aerated using in-line control valves on air hoses to achieve ≥85% oxygen saturation without disturbing sediments. As in the holding tanks, water temperature is maintained at 22 ± 1°C and the photoperiod is 14-h light : 10-h dark (3.5 µmol photons/s/m^2).

Study, results and analyses

Taylor (2009) used microcosms to expose *A. trapezia* to sediments collected from three locations in Lake Macquarie, representing a sediment lead gradient (Cockle Creek sites 1 and 2 and Cockle Bay; Fig. 5.3). Three replicate microcosms of each treatment and of the control sediment were set up, each containing 15 *A. trapezia*. Organisms were exposed to the sediments for a total of 60 days.

Anadara trapezia accumulated lead from the three exposure treatments compared to the control organisms (Fig. 5.8), indicating that lead in the sediment was bioavailable and could be accumulated by this organism. After 60 days, tissue lead concentrations were significantly different between locations. Regression analysis showed significant positive correlations between lead concentrations in *A. trapezia* whole tissue and sediments from Lake Macquarie. The bioconcentration factors were 139, 50 and 35, which was higher than in transplanted and indigenous *A. trapezia* from the same locations in Lake Macquarie.

Comparison of approaches

Comparison of the assessment procedures shows that the collection of indigenous organisms and use of transplantation gave similar results for the bioaccumulation of lead in *A. trapezia*. The laboratory accumulation experiment gave results similar to those from the

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other approaches, except for the sediment with the highest lead concentration. Although the laboratory results did not exactly replicate the field results, they did provide a consistent ranking of locations in terms of potential bioaccumulation of lead.

5.5 Sample collection, preparation and analysis

Sediment-dwelling organisms

Species that live buried in sediment, such as polychaetes and some bivalve molluscs, are obtained by sieving. The top 10–20 cm layer of sediment is collected, using a stainless-steel

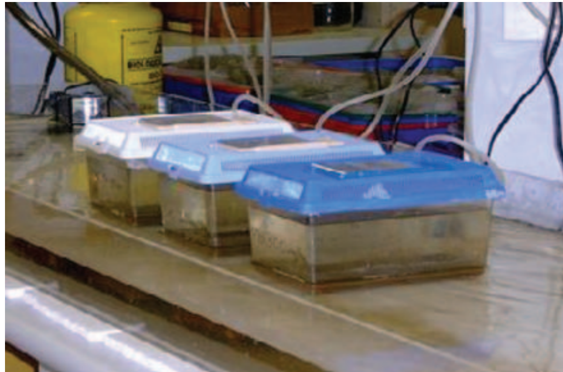

Figure 5.7. Laboratory microcosm exposure set-up.

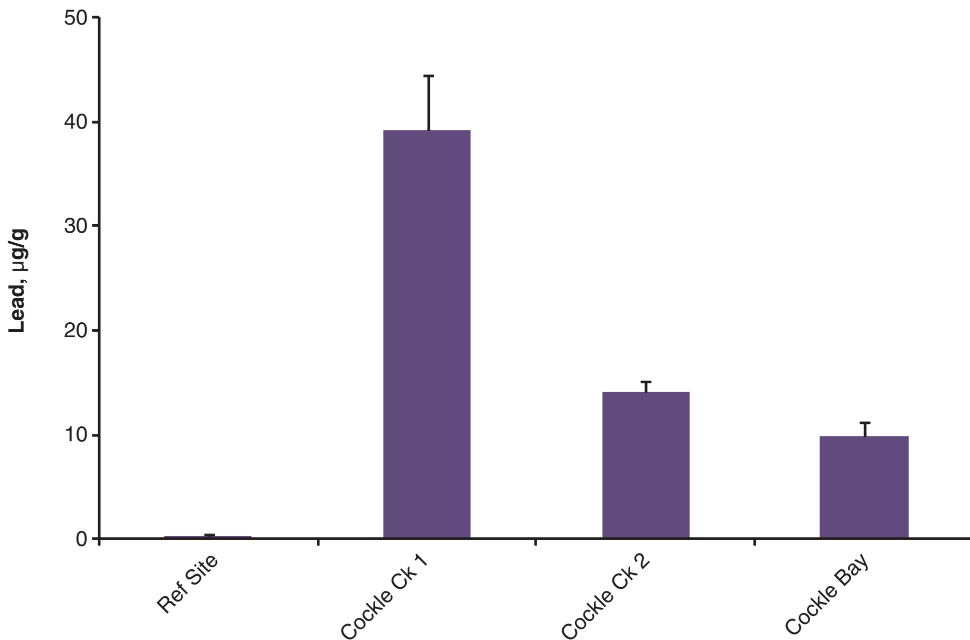

Figure 5.8. Concentrations of lead in tissues of *Anadara trapezia* in the laboratory microcosm study.

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shovel, and either a 2 mm- or 1 cm-mesh stainless-steel sieve is used to separate the organisms (depending on organism size).

Organisms that live at the sediment surface can be collected by hand, wearing plastic gloves and locating the bivalves by gently sweeping the fingers through the surface sediments. Extraneous material adhering to mollusc shells is removed by gently scrubbing the shell with a nylon brush and rinsing it in collection water.

Live specimens are transported in a portable cooler box containing sediment and water from the collection site. An aquarium air pump is used to aerate overlying water during transportation and to maintain ambient temperature. For organisms used in laboratory-uptake studies, the field-collected organisms are held in aquaria for up to 2 weeks to acclimatise before experimentation.

Before being analysed for contaminants accumulated at their indigenous location in the field, all organisms normally should be depurated for at least 24 h in clean aerated water from the location where they were collected. If organisms are not depurated, contaminants in gut contents that have not been taken into the organisms' tissues (that is, have not bioaccumulated) will be included in analyses. Organisms are only not depurated when an estimate of total contaminants consumed by predators (and humans) is required.

After depuration, organisms such as polychaetes are frozen and stored at –20°C until analysis. For molluscs, soft tissue is removed from shells and either whole organisms or individual tissues are frozen and stored at –20°C until analysis.

Fish

For sediment assessments, only fish species that are bottom feeders are used. These species are directly affected by sediment particles, pore waters and ingested benthic biota. Fish are collected using nets or electrofishing, and need to be killed by passing a needle into the brain ('pithing'). They are stored individually in sealed plastic bags before freezing on-site using dry ice.

Fish are dissected using stainless-steel dissecting implements. Selected tissues are removed, placed in plastic vials and frozen at –20°C until analysed.

Individual versus composite samples

As mentioned previously, many studies use pooled tissue samples from several individual samples (whole organisms or individual tissues) to reduce variability and reduce analysis costs. The disadvantage is that information on contaminant variability is lost. If subtle changes in bioaccumulation are occurring, these are often seen as increases in contaminant variability rather than increases in means.

Metals

For analysis of metals, the tissue and sediment samples are normally freeze-dried and ground. Sub-samples are typically digested with concentrated acids (for example, nitric acid or *aqua regia*) using microwave heating (Baldwin *et al*., 1994) and the digests are analysed by inductively coupled plasma mass spectrometry (Maher *et al*., 2001). The recovery of metals from relevant certified reference materials (CRMs) can guide the choice of acid mixture.

Organics

Unlike metals, for organics there are particular procedures for extraction, concentration and analysis specific to the organic contaminant of interest, and measurement of lipid content is also often useful. It is outside the scope of this document to provide details of

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these methods; guidance should be sought from qualified analysts with experience in analysing the organic contaminant of interest.

Quality assurance/quality control

Digestion, extraction and measurement processes are all subject to errors, including contamination, degradation, matrix effects and calibration errors. Certified reference materials are available to assist in quantifying full procedural errors (see Chapter 3, Section 3.4). In the absence of CRMs, inter-laboratory studies also provide a means of assessing the quality of results. For a full discussion of quality assurance/quality control procedures consult the *Australian guidelines for water quality monitoring and reporting* (ANZECC/ARMCANZ, 2000).

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