CHAPTER C

Collection of Interstitial Water

Sediment interstitial water, or pore water, is defined as the water occupying the spaces between sediment particles. Interstitial water might occupy about 50% (or more) of the volume of a depositional (silt-clay) sediment. The interstitial water is in contact with sediment surfaces for relatively long periods of time and therefore, might become contaminated due to partitioning of the contaminants from the surrounding sediments. In addition, interstitial waters might reflect ground water – surface water transition zones in upwelling or downwelling areas. In these areas their chemistry might be more reflective of ground or surface waters at the site. Therefore, flow, residence time and other physicochemical factors (e.g., pH, temperature, redox potential, organic carbon, sulfides, carbonates, mineralogy) might have varying roles in determining whether interstitial waters are contaminated.

In many depositional sediments, interstitial waters are relatively static, and therefore contaminants in the interstitial water and in the solid phase are expected to be at thermodynamic equilibrium. This makes interstitial waters useful for assessing contaminant levels and associated toxicity. Interstitial water is often isolated to provide either a matrix for toxicity testing and/or to provide an indication of the concentration and/or partitioning of contaminants within the sediment matrix.

6.1 General Procedures

Interstitial water sampling has become especially important in regulatory programs because interstitial water toxicity tests yield additional information not currently provided by solid-phase, elutriate, or sediment extract tests (Carr and Chapman, 1992; SETAC, 2001). Furthermore, interstitial water toxicity tests have proven to be useful in sediment toxicity identification evaluation (TIE) studies (e.g., Burgess et al., 1996; Carr, 1998; Burton, 2001) as test procedures and sample manipulation techniques are generally cheaper, faster, and easier to conduct than solid-phase tests (SETAC, 2001). Thus, the collection of interstitial water has become increasingly important in sediment quality monitoring and remediation programs.

Interstitial water sampling is most suitable for sediment types ranging from sandy to uncompacted silt-clays (Sarda and Burton, 1995; SETAC, 2001). Such sampling is not typically performed on sediments with coarse particle size (such as gravel) or on hard, compacted clays, as the potential for interstitial water contamination in these sediment types is relatively low.

As with all sampling discussed in this manual, the principle aim is to use procedures that minimize changes to the *in situ* condition of the water. It should be recognized that most sediment collection and processing methods have been shown to alter interstitial water chemistry (e.g., Schults et al., 1992; Bufflap and Allen, 1995; Sarda and Burton, 1995), thereby potentially altering contaminant bioavailability and toxicity.

Laboratory-based methods (e.g., centrifugation, pressurization, or suction) are commonly used as alternatives to *in-situ* interstitial water collection (see Section 6.2). While these methods have been shown to alter interstitial water chemistry, they're sometimes necessary or preferred, especially when larger sample volumes are required (e.g., for toxicity testing).

As both *in-situ* and laboratory-based or *ex-situ* (e.g., methods might be appropriate for many study objectives, *it is critical that the same procedures are used for all stations sampled in a study, or program, so that appropriate sample comparisons can be made.* Furthermore, the *sediment depth at which interstitial water is sampled (either using in-situ or ex-situ extraction methods) should match the depth of interest in the study* (SETAC, 2001). For example, samples for dredging remediation should be sampled to the depth to be disturbed by dredging activity, whereas samples for a status and trends survey should be collected at the biologically active depth (often < 15 cm). Figure 6-1 summarizes the major considerations for selecting *in-situ* or *ex-situ* procedures in a given study.

The two major issues of concern regarding interstitial water sample integrity are: 1) the ability of the sampling device to maintain physicochemical conditions in the natural state by minimizing adsorption/leaching of chemicals to/from the device, and 2) the ability to maintain the sample in the redox state existing at the site. Precautions required to reduce the likelihood of sample artifacts will vary with each study as indicated in the following sections.

6.2 In-situ Collection

In situ methods might be superior to *ex-situ* methods for collecting interstitial water, as they are less subject to sampling/extraction related artifacts and therefore, might be more likely to maintain the chemical integrity of the sample (Sarda and Burton, 1995; ASTM 2000a; SETAC, 2001). However, *in situ* methods have generally produced relatively small volumes of interstitial water, and often

limited to wadeable or diver-accessible water depths. These logistical constraints have limited their use and applicability in sediment monitoring studies.

The principal methods for *in situ* collection of interstitial water involve either deployed "peepers" (Bufflap and Allen, 1995; Brumbaugh et al., 1994; Adams, 1991; Carignan and Lean, 1991; Carignan et al., 1985; Bottomley and Bayly, 1984) or suction techniques (Watson and Frickers, 1990; Knezovich and Harrison, 1988; Howes et al., 1985). A summary of these methods is provided in Table 6-1. Both methods have a high likelihood of maintaining in situ conditions. In cases where in situ deployment is impractical, peepers or suction devices can be placed in relatively undisturbed sediments collected by core or grab samplers (see Chapter 3).

Recommendation Box #1 In-situ interstitial water collection

- Use peepers for sampling interstitial waters, rather than (or in addition to) grab or core sediment extractions if site conditions, volume requirements, and logistical considerations allow.
- Reduce potential for oxygenation of samples by proper deployment and retrieval procedures.
- Allow adequate equilibration of peepers prior to sampling.
- Minimize handling and processing of fieldcollected interstitial waters.
- Field collected interstitial water samples should be stored in containers, without headspace at 4° C in the dark, until analyzed/tested. Samples for certain chemical analyses (e.g., pesticides, phenols), should be frozen or preserved immediately.



Figure 6-1. Considerations for selecting the appropriate type of interstitial water sampling method.



Photo and illustration on this page, courtesy of Allen Burton

Peepers deployed in the field



General peeper design with in-situ sample extraction

6.2.1 Peeper Methods

Peepers are small chambers with membrane or mesh walls containing either distilled water or clean water of the appropriate salinity or hardness. Samples are collected by burying the devices in sediments and allowing surrounding interstitial waters to infiltrate. In principle, dissolved solutes will diffuse through the porous wall into the peeper and the contained water will reach equilibrium with the ambient interstitial water. The design concept for sediment peepers originated as modifications of the dialysis bag technique used by Mayer (1976) and Hesslein (1976), and has been modified successfully for use in laboratory sediment toxicity tests (Doig and Liber, 2000). The initial designs consisted of either a flat base plate or a cylindrical dialysis probe (Bottomley and Bayly, 1984) with compartments covered by dialysis membranes and a manifold for collection of multiple samples at various depths in the sediment profile (Figure 6-2). Further modifications to these designs have incorporated sampling ports, large sample compartments, and various types of membranes with different pore sizes. These modifications are usually required based on specific project objectives regarding sample volumes and contaminants of interest.

Device	Sediment Depth (cm)	Sample Volume (L ³)	Advantages	Disadvantages
Peeper	0.2 - 10	≤ 0.5	Most accurate method, reduced artifacts, no lab processing; relatively free of effects from temperature, oxidation, and pressure; inexpensive and easy to construct; some selectivity possible depending on nature of sample via specific membranes; wide range of membrane/mesh pore sizes, and/or internal solutes or substrates available.	Requires deployment by hand, thus requiring diving in > 0.6 m depth water; requires hours to days for equilibration (varies with site and chamber); methods not standardized and used infrequently; some membranes such as dialysis/cellulose are subject to biofouling; must deoxygenate chamber and materials to prevent oxidation effects; some construction materials yield chemical artifacts; some chambers only allow small sample volumes; care must be used on collection to prevent sample oxidation.
In situ Suction	0.2 - 30	≤ 0.25	Reduced artifacts, gradient definition; rapid collection, no lab processing; closed system which prevents contamination; methods include airstone, syringes, probes, and core-type samplers.	Requires custom, non-standard collection devices; small volumes; limited to softer sediments; core airstone method; difficult in some sediments and in deeper water (>1 m); method might require diving for deployment in deep waters; methods used infrequently and by limited number of laboratories.

Table 6-1. In-situ interstitial water collection methods (Sarda and Burton, 1995; SETAC, 2001).

Note: Incorporation of filtration into any collection method might result in loss of metal and organic compounds.

Various peeper devices have been recently used effectively to collect interstitial water. For example, a simplified design using a 1 µm polycarbonate membrane over the opening of a polyethylene vial was successful in capturing elevated levels of copper and zinc (Brumbaugh et al., 1994). Other



Figure 6-2. Front view and components of peeper sampling devices (top: plate device; bottom: cylindrical probe)

designs have been used to collect nonpolar organic compounds in a variety of aquatic systems (Bennett et al., 1996; Axelman et al., 1999) and in overlying water (Huckins et al., 1990).

Peepers have also been used to expose organisms to sediments *in situ* (Burton et al., 2001). Burton et al. (1999) successfully introduced organisms to aerobic sediments using peepers. However, anoxic sediments are not amenable to *in situ* organism exposure.

Different materials might be advisable in constructing peepers depending on the contaminants of concern. For example, for many contaminants, peepers constructed from acrylic material appear to yield interstitial water samples with minimal chemical artifacts (Burton et al., 2001). Some polymer materials might be inappropriate for studies of certain nonpolar organic compounds. Cellulose membranes are also unsuitable, as they decompose too quickly. Plastic samplers can contaminate anoxic sediments with diffusible oxygen (Carignan et al., 1994).

In preparation for interstitial water collection, peeper chambers should be filled with deoxygenated water, which can be prepared by nitrogen purging for 24 hours prior to insertion. If sediment oxidation is a concern, the peepers should be transported to the deployment site in a sealed oxygen-free water bath to avoid potential changes to the sediment-water equilibrium caused by dissolved oxygen interactions. However, during peeper equilibration periods, anoxic conditions are likely to be quickly reestablished. In addition, when samples are collected and processed, exposure to oxygen should be minimized.

Following initial placement, the equilibration time for peepers may range from hours to a month, but a deployment period of one to two weeks is most often used (Adams, 1991; Call et al., 1999; Steward and Malley, 1999). Equilibration time is a function of sediment type, study objectives, contaminants of concern, and temperature (e.g., Skalski and Burton, 1991; Carr et al., 1989; Howes et al., 1985; Simon et al., 1985; Mayer, 1976). Membrane pore size also affects equilibration time, with larger pore sizes being used to achieve reduced equilibration times (Sarda and Burton, 1995). For example, using a peeper with a 149-µm pore size, Adams (1991) reported equilibration of conductivity within hours of peeper insertion into the sediment. Thus, it appears that equilibration time is a function of the type of contaminant, sediment type, peeper volume, and mesh pore size.

Peepers with large-pored membranes, while shortening equilibration time, also allow particulates to enter the chamber. The larger solids tend to settle to the bottom of the peeper chamber, and caution should be used to avoid collecting the solids when retrieving the water sample from the chamber. Colloidal particles will remain suspended in the sample and thereby present an artifact, but the concentration of such particles is typically lower than that found in laboratory- centrifuged samples (Chin and Gschwend, 1991).

In several studies, analysis of interstitial water from replicate peepers has demonstrated from low to high heterogeneity in water quality characteristics (Frazier et al., 1996; Sarda and Burton, 1995). The potential for high variability in interstitial water chemical characteristics should be taken into account when developing the sampling design.

6.2.2 Suction Methods

There are a variety of suction devices for collecting interstitial water. A typical suction device consists of a syringe or tube of varying length, with one or more ports located at the desired sampling positions (ASTM, 2000a). The device is inserted into the sediment to the desired depth and a manual, spring-operated, or vacuum gas suction is applied to directly retrieve the water sample. A

variation on this approach employs a peeper-like porous cup or perforated tube with filters. The unit is inserted into the sediment for a period of time, allowing interstitial water to infiltrate the chamber before suction is applied. The samples are then retrieved by suction. Another variation that has been used successfully employs an airstone embedded into the sediment which forces interstitial water upward where it can be collected via syringe or tube. All of these suction methods generally yield smaller quantities of interstitial water than peepers and chemical (toxicological) artifacts are more likely due to greater potential exposure of interstitial water to oxygen (ASTM, 2000a).

6.2.3 Processing of Field-Collected Interstitial Water Sample

Following sample retrieval, interstitial water might need to be recovered and stabilized quickly to prevent oxidative changes or volatilization (Carignan, 1984). Containers should be filled, with no headspace to minimize changes in dissolved oxygen and contaminant bioavailability. Procedures for stabilization are dependent on the analyses to be performed. When non-volatile compounds are the target analytes, acidification is often stipulated, while organic carbon and methane may be stabilized with saturated mercury chloride (Mudroch and MacKnight, 1994).

Samples to be analyzed for toxicity, are normally cooled to 4° C as soon as possible for transport to the laboratory. EPA methods for toxicity testing of surface waters and effluents (USEPA 1991) recommend that samples not be frozen in storage or transport. However, recent information suggests that freezing of interstitial water may not affect toxicity in some cases (Ho et al., 1997; Carr and Chapman, 1995; SETAC, 2001). Unless a demonstration of acceptability is made for the sites of interest, interstitial water samples should not be frozen prior to biological testing. Samples for chemical analyses should be preserved immediately, if appropriate, or cooled to 4° C as soon as possible.

6.3 Ex-situ Extraction of Interstitial Water

Ex-situ interstitial water collection methods are often necessary when relatively large volumes of interstitial water are required (such as for toxicity testing), when *in-situ* collection is not viable or when a brief sampling time is critical. While these extraction methods can be done in the field or in the laboratory, extraction in the laboratory, just prior to analysis or testing, is preferable so that the sample is maintained as close to its original state as much as possible during transport and storage (SETAC, 2001). Guidance in this chapter reflects recommendations presented in several recent publications including proceedings from two workshops devoted entirely to interstitial water extraction methods, water handling, and use in toxicity applications: (1) a dredged materials management program workshop on interstitial water extraction methods and sample storage in relation to tributyltin analysis (Hoffman, 1998) and (2) a Pellston workshop on interstitial water toxicity testing including interstitial water extraction methods and applications (SETAC, 2001). Figure 6-3 summarizes many of the issues associated with laboratory isolation of interstitial water discussed in this section.

6.3.1 General Procedures

Centrifugation and squeezing are the two most common techniques for collecting interstitial water, and are generally preferred when large volumes are required. Other methods include pressurization (e.g., vacuum filtration) devices, which can be used to recover small volumes of interstitial water.

Regardless of the method used, interstitial water should be preserved immediately for chemical analyses, if appropriate, or analyzed as soon as possible after sample collection if unpreserved (such as for toxicity testing; Hoffman, 1998; SETAC, 2001). Significant chemical changes can occur even



Figure 6-3. Summary of recommended procedures and considerations for laboratory isolation of interstitial water*

*Note: Emphasis should be placed on minimizing the duration of all sample manipulations whenever possible

when interstitial water is stored for periods as short as 24 h (Hulbert and Brindle, 1975; Watson et al., 1985; Kemble et al., 1999; Sarda and Burton, 1995; SETAC, 2001).

If sediments are anoxic, as most depositional sediments are, sample processing, including mixing of interstitial water that has separated from the sediment, should be conducted in an inert atmosphere or with minimal atmospheric contact. Exposure to air can result in oxidation of contaminants, thereby altering bioavailability (Bray et al., 1973; Lyons et al., 1979; Howes et al., 1985). Air exposure can also result in loss of volatile sulfides, which might increase the availability of sulfide-bound metals (Allen et al., 1993; Bufflap and Allen, 1995). In addition, iron and manganese oxyhydroxides are quickly formed upon exposure to air. These compounds readily complex with trace metals, thus

altering metals-related toxicity (Bray et al., 1973; Troup et al., 1974; Burton, 1991; Bufflap and Allen, 1995). Maintaining anoxic processing conditions is not necessary when study objectives are concerned with exposures to aerobic sediments, or if target contaminants are unaffected by oxidation in short-term toxicity or bioaccumulation testing.

Interstitial water filtration should be avoided (SETAC, 2001). Numerous studies have shown that filters reduce toxicity and contaminant concentrations by retaining contaminant-associated particles and also by contaminant sorption onto the filter matrix (Bray et al., 1973; Troup et al., 1974; Sasson-Brickson and Burton, 1991; Schults et al., 1992). If filtration is stipulated by a test method, treated filters (e.g., pre-soaked in distilled, deionized water, or combusted at 400° C overnight for glass fiber filters) should be used, and an unfiltered sample should also be tested for toxicity and contaminant concentrations. The characteristics of filters and the filtering apparatus should also be carefully considered, as different filters have different sorptive capacities for different contaminants.



6.3.2 Centrifugation

Centrifugation is the generally preferred laboratory method for collection of interstitial water (SETAC, 2001). It is a relatively simple procedure that allows rapid collection of large volumes of interstitial water. It also facilitates the maintenance of anoxic conditions (if required). However, centrifugation, like other *ex-situ* procedures might yield chemical and/or toxicological artifacts due to

the extraction procedures themselves, which might alter the natural equilibrium between interstitial water and sediment.

Prior to centrifugation, the sediment sample is homogenized (see Section 4.3) and partitioned among centrifuge bottles. If the homogenized sample is stored prior to centrifugation, interstitial water might accumulate on the surface of the sediment. This overlying water should be mixed into the sediment before subsampling for centrifugation. Samples are then partitioned among centrifuge bottles. In general, approximately 50% of sediment moisture content can be extracted as interstitial water. If interstitial water volume requirements are lower, smaller sediment subsamples may be used.

For more information about centrifugation:

Interstitial waters have been isolated over a range of centrifugal forces and durations (Landrum et al., 1987; Giesy et al., 1988; Schults et al., 1992; Burgess et al., 1993; Ankley et al., 1990; Schubauer-Berigan and Ankley, 1991; Ankley and Schubauer-Berigan, 1994). For toxicity testing of interstitial waters, some sources recommend that sediments be centrifuged at 10,000 x g for a 30 min period (ASTM, 2000a; Environment Canada, 1994). Such high speed centrifugation is often necessary to remove most colloids and dispersible clays (Adams, 1991; Chin and Gschwend, 1991; Brownawell and Farrington, 1986; Ankley and Schubauer-Berigan, 1994), which can introduce interferences to chemical or toxicological analysis. However, such high speed centrifuges are not commonly available. Furthermore, many materials (glass, plastic) are not able to withstand high centrifugation speeds. Finally, it should be noted that toxicity is typically reduced with high speed centrifugation due to the removal of particle-associated contaminants (Sasson-Brickson and Burton 1991; Schults et al., 1992; Ankley and Schubauer-Berigan, 1994; Bufflap and Allen, 1995).

Based on research to date, both slower and faster centrifugation speeds (and associated differences in colloid/suspended solids removal) may be appropriate depending on the study objectives. For many programs that are interested in characterizing site toxicity, high speed centrifugation may not be appropriate because one is interested in toxicity potential of the interstitial water in its entirety (i.e., including colloidal material). However, if one is interested in comparing interstitial water contaminant concentrations to specific sediment quality values, or model exposure compartments for example (EPRI, 2000), then high speed centrifugation might be necessary. As our knowledge is still limited in this area, it is perhaps most important to note that centrifugation speed often has a dramatic effect on observed sample toxicity and chemical characteristics. Therefore, in any sediment monitoring study, *one centrifugation protocol (including speed and time) should be identified and used throughout for all samples*.

Centrifugation has been performed at various temperatures. ASTM (2000a) recommends that the centrifugation temperature reflect the *in situ* sediment temperature to ensure that the equilibrium between the particulate and interstitial water is not altered. Alternatively, a temperature of 4° C may be preferred to minimize temperature-mediated chemical and biological processes (Environment Canada, 1994).

When centrifuging coarse sand, it might be desirable to use a modified centrifuge bottle to aid interstitial water recovery (USEPA/ACOE, 1998). The modified bottle is equipped with an internal filter that can recover 75% of the interstitial water, as compared to 25 - 30% recovery from squeezing (Saager *et al.*, 1990).

As discussed in Section 4.2, all containers have limitations with regards to adsorption or leaching of chemicals, ease of use, and reliability. For example, polytetrafluororthylene (PTF) bottles have been used successfully up to 2500 x g when filled to 80% of capacity, but collapse at 3000 g (Burgess et al., 1993). Polycarbonate bottles have been used successfully for tributytin analyses in interstitial water (Hoffman, 1998). If small volumes of water are required for testing, higher speed centrifugation can be performed with glass tubes (up to 10,000 g, Word et al., 1987). Larger glass tubes, however, can not be centrifuged at such high speeds. If metal toxicity is not a concern, then high speed centrifugation in larger stainless steel centrifuge tubes is suitable. If test samples are contaminated with photoreactive compounds such as PAHs, exposure of the sample to light should be minimized to limit degradation or alteration of potentially toxic compounds. This can be accomplished by using reduced lighting.

6.3.3 Sediment Squeezing

Isolation of interstitial water by squeezing has been performed using a variety of procedures and devices (Reeburgh, 1967; Kalil and Goldhaker, 1973; Jahnke, 1988; Carr et al., 1989; Long et al., 1990; Watson and Frickers, 1990; Adams, 1991; Carr and Chapman, 1995; Carr, 1998). Inexpensive low pressure mechanical squeezers can be constructed, and may provide specialized capacities such as collection of interstitial water profiles from core samples (Bender, *et al*, 1987). In all cases, the interstitial water is passed through a filter that is a part of the squeezing apparatus.

Squeezing has been shown to produce a number of artifacts due to shifts in equilibrium from pressure, temperature, and gradient changes (e.g., Froelich et al., 1979; Kriukov and Manheim, 1982; Bollinger et al., 1992; Schults, 1992). Squeezing can affect the electrolyte concentration in the interstitial water particularly with a decrease in chemical concentrations near the end of the squeezing process. However, others reported that squeezing did not produce artifacts in interstitial water toxicity studies (Carr and Chapman, 1995; Carr, 1998; SETAC, 2001). It is therefore recommended that if squeezing is performed, moderate pressures be applied along with electrolyte (conductivity) monitoring during extraction (Kriukov and Manheim, 1982). Squeezing should also be performed at *in situ* ambient temperatures, as significant alterations to interstitial water composition can occur when squeezing is conducted at temperatures different from ambient conditions (e.g., Mangelsdorf et al., 1969; Bischoff et al., 1970; Sayles et al., 1973).

Other sources of interstitial water alteration during squeezing are: contamination from overlying water; internal mixing of interstitial water during extrusion; and solid-solution reactions as interstitial water is expressed through the overlying sediment. As interstitial waters are displaced into upper sediment zones, they come in contact with solids with which they are not in equilibrium. This intermixing causes solid-solution reactions to occur. Most interstitial water chemical species are rapidly transformed, as observed with ammonia and trace metals (Rosenfield, 1979; Santschi et al., 1997). Bollinger et al. (1992) found elevated levels of several ions and dissolved organic carbon in squeezed samples as compared to samples collected by *in situ* peepers. The magnitude of the artifact will depend on the pollutant sediment characteristics and redox potential.

6.3.4 Pressurized and Vacuum Devices

Other methods for extraction of interstitial water from sediment samples can include vacuum filtration (Jenne and Zachara, 1987; Knezovich and Harrison, 1987; Winger and Lasier, 1991), gas pressurization (Reeburgh, 1967), and displacement (Adams, 1991). These methods typically recover only small volumes of interstitial water and are not commonly used.



Photo courtesy of Allen Burton

Sediment squeezing apparatus for extracting interstitial water

Use of a hand vacuum with an aquarium stone is an effective vacuum filtration method (Winger and Lasier, 1991; Sarda and Burton, 1995). The procedure typically involves attaching the air stone to a 50 mL syringe via plastic tubing, inserting it into the sediment to the desired depth, and then applying suction. This method can recover relatively large volumes of interstitial water; Santschi et al. (1997) used this procedure to extract up to 1,500 mL from 4 L of sediment. Sarda and Burton (1995) found that ammonia concentrations in water obtained by this procedure were similar to those collected by *in situ* peepers. Drawbacks to this method include loss of equilibrium between the interstitial water and the solids, filter clogging, and oxidation (Brinkman et al., 1982).