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In Situ Estuarine and Marine Toxicity Testing

A Review, Including Recommendations for Future Use in Ecological Risk Assessment

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EXECUTIVE SUMMARY

It is anticipated that *in situ* biological testing strategies will play an increasingly important role in aquatic and sediment risk characterization and management. The potential for biological effects in aquatic media has traditionally been assessed by collecting water or sediment samples from sites suspected of contamination and performing acute toxicity studies in the laboratory under strictly controlled conditions using standardized toxicity testing protocols. This approach, though well-established, often times does not accurately estimate true exposure or effects to aquatic communities, particularly when the source of contamination is ephemeral or when the exposure varies over time and space. Given the unstable nature of some contaminants that may degrade or volatilize, field testing provides a means to side-step the sampling and manipulation that may compromise the representativeness of laboratory tests (Anderson et al. 1996). Furthermore, laboratory tests may overestimate toxicity due to sediments leaching concentrations of toxicants into the static overlying water (Sasson-Brickson and Burton 1990, Anderson et al. 1998). For these and other reasons, laboratory testing approaches can fail to properly characterize environmental risk, resulting in inaccurate management decisions. Despite this, regulatory agencies still typically rely on these often disjointed, laboratory-based lines of evidence. In contrast, *in situ* toxicity and bioaccumulation tests provide continuous exposure with reduced sample manipulation and incorporate natural factors (i.e., tide, currents, temperature changes, light, sediment disturbance) that tend to influence bioavailability and potential for toxic effects. Therefore, the authors and others (e.g., Wharfe et al. 2007, and references therein) recommend that *in situ* bioassays be part of a weight-of-evidence approach in the risk assessment process.

In situ biological testing strategies have developed more in recent years, and one protocol has become a standardized method (ASTM 2003). One of the most difficult challenges that face biological assessments is the need to differentiate between anthropogenic-related effects and effects associated with natural stressors. This can be addressed by making certain that appropriate controls and reference sites are included in the experimental design, and tends to require a combination of laboratory and *in situ* experiments to help tease out the relative contributions of each stressor (Burton et al. 1996). In addition, placement of cages in different environmental compartments (i.e., water column, sediment–water interface, surficial sediment, and/or porewater) can provide an indication of the route of contaminant exposure (e.g., source). For example, *in situ* tests provide one line of evidence in an integrated approach that includes groundwater–surface water interactions (GSIs). Specific to *in situ* testing is the need to consider potential stress or exposure alterations due to caging of test organisms (i.e., physical stress, oxygen depletion, predation, sedimentation within the chamber). However, with the aid of other measurements, *in situ* bioassays can represent ecological responses more realistically than laboratory tests and therefore help better prioritize those sites that may need remediation, as well as prevent unnecessary and costly cleanup at Navy sites.

A Pellston Conference was convened in 2004 to advance the science of *in situ* testing to assess risks in aquatic systems. The current review builds on the Pellston Conference findings, but emphasizes approaches that adapt existing standard laboratory test methods, e.g., American Society for Testing and Materials (ASTM) and Environmental Protection Agency (EPA) approved test methods that are used in regulatory programs and recommended in California's new sediment quality objectives, that provide the opportunity to further test and/or confirm the effects of specific test conditions (e.g., range of salinities) under controlled conditions. In addition, we emphasize marine and estuarine testing to focus on conditions at most Navy Installation Restoration sites, as well as more specialized situations such as GSIs, which are often overlooked in traditional approaches.

Included is a discussion of the advantages and limitations of *in situ* testing strategies and summaries of the demonstrated benefits that come from such studies, as well as details on what others have done to address potential problems (i.e., reporting of false positives). Case studies that detail cage designs, testing strategies, and appropriateness of various species from different taxonomic groups are followed by recommendations on a strategy to assess the biological impact of potentially contaminated waste sites in the coastal environment.

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ACRONYMS

ASTM	American Society for Testing and Materials
BCF	Bioconcentration Factor
CoC's	Contaminants of Concern
DQO	Data Quality Objective
EC50	Median effects concentration (50%)
EPA	Environmental Protection Agency
GSI	Groundwater–surface water interaction
HDPE	High-Density Polyethylene
IR	Installation Restoration
LC50	Median lethal concentration (50%)
LEOT	Larval Exposure and Observation Tube
LOEC	Lowest Observable Effect Concentration
MFO	Mixed-Function Oxidase
NOEC	No Observable Effect Concentration
PAH	Polycyclic Aromatic Hydrocarbon
PCB	Polychlorinated Biphenyl
PCE	Tetrachloroethylene
PMT	Photomultiplier Tube
PSU	Practical Salinity Unit
PVC	Polyvinyl Chloride
RDX	Cyclotrimethylenetrinitramine (aka Royal Demolition Explosive)
SCUBA	Self-Contained Underwater Breathing Apparatus
SEAP	Sediment Ecosystem Assessment Protocol
SERDP	Strategic Environmental Research and Development Program
SQO	Sediment Quality Objective
SSC Pacific	Space and Naval Warfare Systems Center Pacific
SWI	Sediment–water Interface
TCE	Trichloroethylene

TIE	Toxicity Identification Evaluation
TNT	Trinitrotoluene
TSS	Total Suspended Solids
UV	Ultraviolet
USACE	United States Army Corps of Engineers
VOC	Volatile Organic Compound
WET	Whole Effluent Toxicity

1. INTRODUCTION

The potential for biological effects in aquatic systems has traditionally been assessed by collecting water or sediment samples from sites suspected of contamination and performing toxicity studies in the laboratory under strictly controlled conditions using standardized toxicity testing protocols. This approach is well-established, but does not sufficiently represent true exposure and effects to aquatic communities, particularly when the source of contamination is ephemeral or the exposure varies over time and with ambient conditions. Alteration of exposure due to sampling and manipulation of samples in preparation for laboratory testing is problematic (Anderson et al. 1996). Sample manipulation removes the natural stratification that affects exposure to test organisms and also may result in degradation, volatilization, or other alterations of contaminants that occur with exposure to air. In addition, laboratory tests may overestimate toxicity due to increasingly higher concentrations of toxicants in the static overlying water as toxicants desorb from the sediment (Sasson-Brickson and Burton 1990, Anderson et al. 1998). Therefore, *in situ* toxicity (and bioaccumulation) testing may be a preferable or complementary strategy in certain situations utilizing a weight of evidence approach (Baird et al. 2007).

One of the most difficult challenges in risk assessment is the need to differentiate between anthropogenic-related effects, test artifacts, and effects associated with natural stressors. For instance, exposure alterations due to caging of test organisms may include physical stress, oxygen depletion, predation, and sedimentation within the chamber. To a limited extent, these potential complications are addressed by making certain that appropriate controls and reference sites are included in the experimental design, as well as using the appropriate species and/or chamber design. An advantage in conducting both laboratory and *in situ* experiments is that, through comparison of results, experimental artifacts associated with each may be identified and minimized (Burton et al. 1996).

As with laboratory tests, *in situ* test protocols dictate which habitat conditions are to be represented. For instance, cages can be placed in different environmental compartments (i.e., water column, sediment–water interface, surficial sediment, porewater), with differing results that are dependent not only on the degree of contamination in each media, but also degrees and mechanisms for uptake to the organism. *In situ* biological tests also provide the opportunity for exposure to ephemeral exposure of contaminants such as those associated with groundwater–surface water interactions (GSI). Field exposures provide additional lines of evidence that can, ideally, integrate the predominant routes of exposures. The experimental design and interpretation of test results may require input from hydrologists, hydrogeologists, aquatic biologists or toxicologists, and environmental chemists, who should work together to develop a multiple line of evidence strategy including appropriate chemical analysis and fate and transport modeling (Burton and Greenberg 2002). Ultimately, with appropriate experimental design and interpretation, *in situ* bioassays can more accurately describe ecological impacts, better prioritize those sites that may need remediation, and help prevent unnecessary and costly cleanup at sediment sites.

Recently, *in situ* biological testing strategies have become better developed, and one method using caged bivalves is now an accepted standard (ASTM 2003). A Pellston Conference was convened in 2004 to advance the science of *in situ* testing to assess risks in aquatic systems. Contributors prepared four summary papers for the journal *Integrated Environmental Assessment and Management* (Crane et al. 2007, Liber et al. 2007, Baird et al. 2007, and Wharfe et al. 2007). Crane et al. (2007) emphasized the importance of realism in exposure conditions, as well as the ability to capture responses of native species, while emphasizing potential cost savings (e.g., for long-term exposures). Liber et al. (2007) provided a detailed analysis of artifacts that need to be taken into account when

conducting *in situ* tests, particularly with respect to exposure constraints. Baird et al. (2007) address ecological relevance and the pros and cons of site specific vs. standardized methods. Wharf et al. (2007) emphasize aspects of laboratory vs. field testing within a regulatory context, particularly by providing a feedback loop between the risk assessment process, remedial planning and, finally, monitoring effectiveness or remediation.

The present review builds on the Pellston Conference findings, but emphasizes approaches that adapt existing standard laboratory test methods, providing the opportunity to further test and/or confirm the effects of specific test conditions (e.g., range of salinities) under controlled conditions. In addition, we emphasize marine and estuarine testing to focus on conditions at most Navy Installation Restoration (IR) sites. We found relatively few examples of marine and estuarine *in situ* toxicity studies in the open literature (Table 1). It is acknowledged that freshwater and saltwater testing (both in the laboratory and in the field) share many qualities with regard to representativeness and realism. For instance, many standard freshwater and saltwater acute toxicity tests are conducted at 20 or 25 °C for 96 h. Yet protocols are always species-specific to some degree, often driven by requirements and the characteristics of specific life stages. Thus, assigning biological risks in aquatic environments is highly associated with the limited suite of standard tests that have been developed to represent respective habitats. The cadre of standard tests for effluents, development of aquatic life criteria, and sediment toxicity include accepted methods for unique sets of freshwater and saltwater species.

The intent of this review is to provide the reader with a background and quick reference to various considerations that need to be made when planning *in situ* toxicity or bioaccumulation studies, particularly with respect to marine and estuarine systems. The review first presents the generic advantages and limitations encountered in the development and execution of *in situ* tests. This is followed by presentation of findings from studies that have incorporated some of these tests, with reporting that includes proof-of-concept, challenges and, in some cases, options for improved testing. Modifications of standard laboratory toxicity tests using endpoints such as survival, growth, embryonic development, or post-exposure feeding rate are discussed. It is acknowledged that a plethora of additional endpoints have utility for use *in situ*, but these other endpoints are beyond the scope of this report. A short summary of several screening-level toxicity testing tools that can be used to detect toxic hot spots in preparation for more in depth studies, as well as some recommendations for further improvement of *in situ* bioassays, is also presented.

Table 1. Examples of successful demonstrations of marine and estuarine *in situ* toxicity and bioaccumulation testing strategies available in the open literature.

Organism Type	Species	Endpoint(s)	Exposure Duration (d)	Reference
Mussel	*Mytilus galloprovincialis*	Embryo-larval development	2 2 2	Anderson et al. (1998) Geffard et al. (2001) Katz and Rosen (2005)
Oyster	*Crassostrea gigas*	Embryo-larval development	2	Geffard et al. (2001)
Sea Urchin	*Paracentrotus lividus*[2]	Embryo-larval development and growth	3	Beiras et al. (2001)
Sea urchin[1]	*Strongylocentrotus purpuratus*	Embryo-larval development	3 to 4	Anderson et al. (1996, 2001)
Mussel, Clam	*Mytilus edulis, Mytilus galloprovincialis, Macoma nasuta, Macoma balthica*	Bioaccumulation, growth	28 to 90	ASTM (2003), Salazar and Salazar (2007)
Clam	*Mercenaria mercenaria*	Growth	7	Ringwood and Keppler (2002)
Amphipod	*Eohaustorius estuarius*	Survival	10	Anderson et al. (2004) Rosen et al. (2009)
Amphipod	*Corophium volutator*[2]	Survival	10	Kater et al. (2001)
Polychaete	*Hediste diversicolor*[2]	Survival, post exposure feeding	2 d + 1 hr feeding	Moreira et al. (2005)
Polychaete	*Neanthes arenaceodentata*	Survival, post exposure feeding	2 d + 1 hr feeding	Miller and Rosen (in prep) Rosen et al. (2009)
Mysid shrimp	*Americamysis bahia*	Survival	0.5 to 3 7	Clark et al. (1986, 1987) Comeleo et al. (1990) Comeleo et al. (1991)
Crab	*Cancer maenus*[2]	Survival, post exposure feeding	2 d + 30 min feeding	Moreira et al. (2006)
Fish	*Cyprinodon variegatus*	Survival	5	Clark et al. (1986, 1987)
Fish	*Atherinops affinis*	Bioaccumulation Embryo hatching success	28 unknown	Richter (2002) Jelinski and Anderson (1996)
Fish	*Menidia beryllina*	Embryo hatching success	unknown	Jelinski and Anderson (1996)

[1] Laboratory sediment–water interface tests using intact sediment cores.
[2] European species

2. ADVANTAGES OF *IN SITU* TOXICITY AND BIOACCUMULATION TESTS

In situ biological testing offers a number of advantages over traditional laboratory testing strategies (Martin and Black 1995, Shaw and Manning 1996, Burton et al. 1996, Chappie and Burton 1997, Anderson et al. 1998, Pereira et al. 1999, Sibley et al. 1999, Chappie and Burton 2000, Crane et al. 2000, Geffard et al. 2001, Kater et al. 2001, ASTM 2003, Phillips et al. 2004, Liber et al. 2007). Advantages of *in situ* tests include:

- Providing greater realism by exposing test organisms to true concentrations.
- Taking into account spatial and temporal variability of contaminant exposure.
- Better assessment of effects from volatile contaminants.
- Integrating multiple stressors, both natural and anthropogenic.
- Minimizing changes in sediment by reducing sampling and manipulation.
- Increasing ability to interpret toxicity when combined with laboratory studies.
- Site-specific placing to identify toxic sources.

2.1 INCREASED REALISM

Ecological risk assessments tend to compare water quality criteria or sediment quality guidelines derived from laboratory-generated 48- to 96-h LC50 values and 10-day tests using benthic organisms exposed to individual chemicals with the expected environmental concentrations. This strategy can either over- or under-estimate the true potential for effects, depending on the presence of multiple stressors, factors affecting bioavailability besides organic carbon or acid volatile sulfides, or for water, mean concentration and duration of contaminant exposure (Burton 1999). Laboratory tests in which chemical concentrations are held constant may overestimate the potential effects of field exposures when contaminant concentrations vary temporally (Clark et al. 1987). For instance, field exposures may have lower effective concentrations relative to laboratory exposures due to the diluting influence of continuous flow. On the other hand, laboratory studies may also underestimate toxicity if contaminant pulses are not captured during sampling. It can be expected that many laboratory tests overestimate sediment toxicity due to increasing concentrations of toxicants in the static overlying water as toxicants desorb from the sediment (Sasson-Brickson and Burton 1990, Anderson et al. 1998). *In situ* tests provide greater realism in that organisms are exposed directly to ambient conditions and indirectly via contaminated food (Shaw and Manning 1996). Results from *in situ* studies will provide much greater confidence in assessing true exposures and effects occurring at a particular site. This confidence is critical when costly decisions and implications to remediate or not is at stake.

Laboratory tests may also fail to incorporate realistic exposures to volatile and/or ephemeral compounds that are often associated with groundwater. Groundwater discharge has recently been recognized as an important pathway for diffuse pollution to coastal environments (Burnett et al. 2001, Bussman et al. 1999, Gallager et al. 1996). Since approximately one-third of Navy landfills have groundwater infiltrating the waste, water bodies (i.e., harbors, bays, estuaries, wetlands) adjacent to these sites are potentially exposed to elevated contaminant concentrations. A number of Navy waste sites with groundwater issues have been identified, some of which have a strong tidal influence (Chadwick et al. 2003). Daily fluctuations in tide have been shown to lead to appreciable volumes of groundwater extraction into the overlying water via the process of tidal pumping (Moore 1996). Although the more soluble groundwater contaminants such as the chlorinated volatile organic compounds can be attenuated as they near the sediment surface (Duncan et al. 2000), seepage

measurements and porewater sampling at coastal sites have indicated concentrations that could impact biological communities at the groundwater–surface water interface (Chadwick et al. 1999). Thus, elevated groundwater contaminant concentrations commonly observed at depth could impact ecologically significant groundwater–surface water transition zone communities.

2.2 INCORPORATION OF SPATIAL/TEMPORAL VARIABILITY

A key benefit expected from field exposures is that they allow for the integration of time-varying stressors. Contamination related to groundwater seepage, stormwater or pesticide runoff, or tidal inundation might be better represented by continuous exposure in the field compared to static laboratory exposures using grab samples (Ziegenfuss et al. 1990, Burton et al. 1996, Anderson et al. 1998, Phillips et al. 2004, Wharfe et al. 2007). Test organisms in the field are also exposed to temporal and spatial variations in food as well as predators that have the ability to enter the test chambers. This variability is more realistic (the same variability that indigenous organisms are exposed to), but also may pose a challenge to be aware of when performing *in situ* studies. Sibley et al. (1999) observed the increased potential for predators to enter the test chambers, but it was also noted that test organisms, food, and predators are continuously exposed in the field, resulting in more realistic conditions.

2.3 INTEGRATION OF MULTIPLE STRESSORS

The majority of research in ecotoxicology has focused on biological exposures that intentionally minimize ancillary non-toxicant stresses, but organisms in their natural environment are exposed to stress from multiple natural and anthropogenic sources (Burton 1995, Wharfe et al. 2007). The role of natural stresses on indigenous biota has been under-represented in most ecotoxicity studies. Complex site-specific conditions, such as suspended solids, light, dissolved oxygen, pH, salinity, and temperature, may alter contaminant toxicity and/or bioavailability (Chappie and Burton 2000). Examples of dynamic conditions that can enhance toxicity relative to that in a laboratory include events that cause resuspension of sediments resulting in oxidation of sulfide-bound metals and periods of low turbidity that increase ultraviolet (UV) light exposure, photoactivating contaminants such as polycyclic aromatic hydrocarbons (PAHs) (Landrum et al. 1987, Ankley et al. 1994, Burton 1995, Ireland et al. 1996, Monson et al. 1995, Spehar et al. 1999). These effects are typically accounted for only in field exposures, and may be vital for assessing ecosystem quality. At the same time, the possibility of enhanced toxicity due to confounding factors associated with laboratory studies (e.g., increased ammonia from storage, static conditions allowing unnatural buildup of contaminants in overlying water, and differences in pH, temp, and salinity) are avoided in the field.

2.4 REDUCED SAMPLE MANIPULATION

Laboratory toxicity tests generally require extensive handling of sediment via sampling and manipulations, such as sieving or mixing, as they are prepared for testing. These manipulations tend to disrupt the vertical distribution of contaminants and, therefore, alter the exposure relative to organisms in the field (Anderson et al. 1996, Burton et al. 1996, Sibley et al. 1999, Pereira et al. 1999, Burton et al. 2000). This makes extrapolation of laboratory results to natural populations difficult. In addition to alterations in contaminant distribution, variables such as pH, conductivity, ammonia, dissolved oxygen, acid-volatile sulfide content and redox potential within the sediment, porewater, and overlying water in laboratory exposures are often not representative of field conditions, which alter bioavailability and toxicity of contaminants in sediment to varying degrees (Pereira et al. 1999, Burton et al. 2000, Kater et al. 2001).

Sample manipulation may have particular consequences for some contaminants of concern due to their specific physical properties. Because many contaminants (e.g., those associated with groundwater infiltration) are extremely soluble in water and/or volatile in nature, they may dissipate during sampling and manipulation procedures as they are prepared for laboratory testing. *In situ* testing will avoid changes in contaminant concentrations or properties that might occur during sampling, transport, and manipulation (i.e., sieving) required for laboratory testing (Crane et al. 2000, Geffard et al. 2001).

2.5 MATRIX-SPECIFIC RISK IDENTIFICATION

By manipulating chamber design and/or placement of test chambers, *in situ* tests can also be used to synoptically characterize realistic exposures and effects (Martin and Black 1995, Burton et al. 1996; Figure 1). Simple experimental designs for field exposures can discriminate between any of the following: overlying water, bulk sediment, porewater, light, suspended solids, or predation. Placement at reference sites in addition to suspected polluted sites can tease out natural versus anthropogenic stressors, while placement along contamination gradients may be useful in providing exposure-response relationships (Chappie and Burton 1997).

More recently, simplistic toxicity identification evaluations (TIEs), which are useful for teasing out which contaminant classes in a given matrix are causing toxicity, have been incorporated into *in situ* evaluations (Burton and Nordstrom 2004a,b). Referred to as *in situ* TIEs, Burton and Nordstrom demonstrated a simple chamber made out of a modified 10-ml pipette tip that can house various sorption materials to selectively exclude specific contaminant classes as organisms are exposed to porewater that is slowly, yet continuously drawn through the device while in place. Using this approach, they were able to identify dominant chemical classes of potential concern at freshwater sites. One of the current limitations with the system is its restriction to relatively shallow waters (Burton and Nordstrom 2004b), but simple modifications should allow use in deeper waters (e.g., most marine sites).

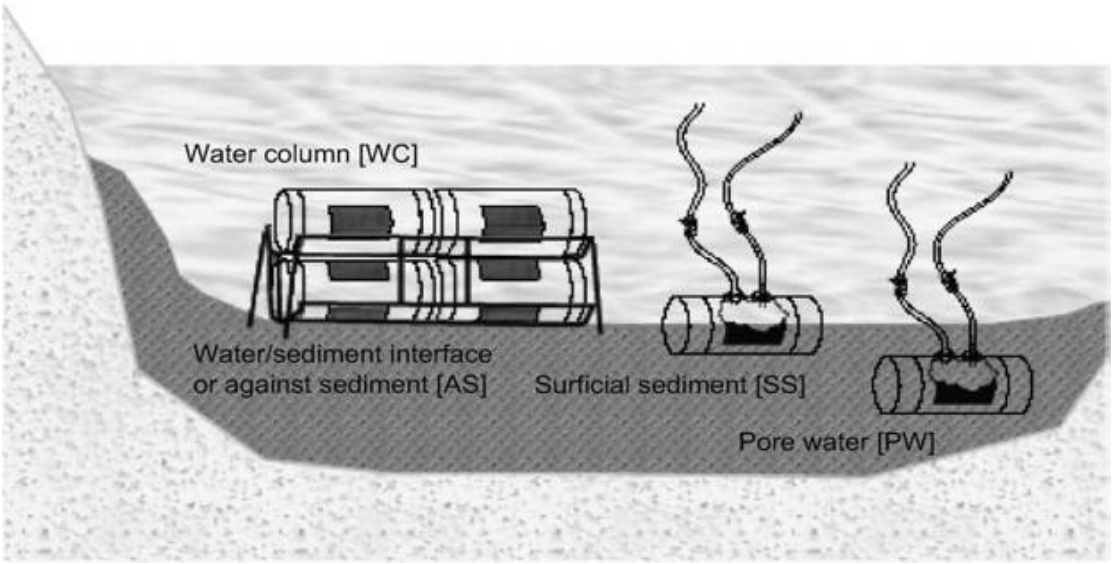


Figure 1. Example illustrating different options in placement location of *in situ* bioassay chambers for improved understanding of contaminant exposure pathways and conducting ecologically relevant exposures.

3. LIMITATIONS OF *IN SITU* TOXICITY AND BIOACCUMULATION TESTS

One of the key benefits of using toxicity testing is the ability to interpret the impacts of multiple stressors on aquatic communities. These multiple stressors, however, may not be related to contamination from anthropogenic inputs. Numerous studies have shown strong correlations between observed toxicity and various physical and chemical variables, but establishing cause-and-effect relationships requires TIEs and sediment-spiking experiments (Carr et al. 1996a,b). Understanding and minimizing factors that can cause false positive or negative conclusions will ultimately mitigate interpretation errors.

A number of potential confounding factors specific to *in situ* testing have been identified in the literature. It should be noted, however, that understanding these limitations and accounting for them in *in situ* toxicity studies prior to their application can substantially reduce the potential for misinterpreting the data. These factors include:

- No control of non-treatment natural factors (water quality parameters, indirect effects).
- Confounding effects of ammonia and/or sulfide.
- Groundwater-related effects.
- Issues associated with caging test organisms (e.g., reduced flow rates due to fouling).
- Issues associated with feeding.
- Transportation and acclimation challenges during cage deployment.
- Physical disturbance of test chambers.
- Predation and competition.
- Need for appropriate controls and reference sites.
- Spatial and temporal variations in the field require proper sampling design.
- Toxicity from caging materials.

3.1 NO CONTROL OVER NATURAL EXPOSURE FACTORS

Although field exposures reflect more realistic exposure conditions, the degree of control that one has over contaminant exposure factors may be less when compared with laboratory tests. The benefit of measuring biological response in an exposure more representative of that encountered by natural populations, however, is expected to outweigh this limitation. As with laboratory tests, toxicity observed *in situ* may be difficult to attribute to specific stressors due to the exposure to multiple stressors and other confounding factors, such as habitat quality (Norton et al. 1995). *In situ* conditions affecting contaminant bioavailability, and other factors such as organisms' behavior and rates of uptake can directly influence bioaccumulation and biological effects (DeWitt et al. 1988, ASTM 2003). Commonly measured endpoints such as mortality, growth, and feeding rate may be impacted by such factors (Maltby et al. 2002, Ringwood et al. 2002, ASTM 2003, Moreira et al. 2005).

In addition to toxicant stressors, parameters such as temperature, salinity, pH, dissolved oxygen, dissolved organic carbon and/or nutrient load that are easily monitored and/or controlled in the laboratory, tend to fluctuate in uncontrolled ways in the field, or differ among field exposure locations. Regression techniques have been used to successfully tease out the effects of temperature, pH, and dissolved oxygen concentration on growth of juvenile clams (*Mercenaria mercenaria*) (Ringwood et al. 2002). Similarly, Moreira et al. (2005) normalized exposure and feeding data for the estuarine polychaete (*Hediste diversicolor*) due to effects associated with salinity and temperature on

feeding rate. Pillard et al. (1999) found that of three common test species, *Cyprinidon variagatus* was particularly tolerant of a broad range of salinities (4–40 ppt) conditions while *Americamysis bahia* was less tolerant of salinities (~4 ppt). *Menidia beryllina* exhibited an intermediate range of tolerance.

3.2 CONFOUNDING EFFECTS OF AMMONIA AND/OR SULFIDE

Fluctuations in pH, salinity, and temperature may also have indirect effects by rendering naturally occurring constituents of interstitial water (e.g., ammonia, sulfide) more or less bioavailable (USEPA 1989, Greenstein et al. 1996, USEPA 1999, Wang and Chapman 1999). Total ammonia comprises toxic unionized ammonia (NH_3) and the less-toxic ammonium ion (NH_4^+). Unionized ammonia is a neutral molecule and, therefore, is able to diffuse across the epithelial membranes of aquatic organisms more readily than the charged ammonium ion (USEPA 1999). As pH increases under natural conditions, unionized ammonia concentrations dramatically increase, often contributing to or causing toxicity to the test organisms. Ammonia is also a common artificially induced confounding factor of concern in laboratory bioassays due to increases that may occur as a result of sample manipulation, storage, and static exposures. Increased temperature and decreased salinity also cause smaller, but potentially important, shifts towards the unionized form.

Sulfide also exists mainly in two forms, mediated largely by pH: unionized hydrogen sulfide (H_2S) and sulfide ions (e.g., HS^-), with the former being substantially more toxic (Wang and Chapman 1999). Unlike ammonia, sulfide becomes more toxic as pH decreases. While sulfide is more toxic than ammonia to most aquatic species (Wang and Chapman 1999), it is volatile and easily oxidized, and thus generally less problematic in laboratory toxicity tests. In contrast, elevated sulfide concentrations and associated anoxia (required for the anaerobic decomposition of organic matter that produces sulfides) are common in field exposures to marine and estuarine sediments and may play major roles in both lethal and sublethal stress. Selection of test species that are physiologically adapted and/or possess habits for reducing exposure to sulfides (e.g., tube building) confers certain advantages. Thresholds for sulfide sensitivity for various freshwater and marine test organisms are reported (Sims and Moore 1995). Measurements of ammonia and sulfide concentrations from test sites provide critical data for the interpretation of results.

3.3 GROUNDWATER-RELATED EFFECTS

Groundwater seep into sediments can carry contaminants as well as confounding factors. Characteristics that are not associated with anthropogenic inputs (i.e., fresh water, high and low pH, nutrients including ammonia, and hydrogen sulfide) may result in adverse effects on test organisms. When groundwater seeps into sediments, it can lower salinity, thus increasing osmotic stress on marine and estuarine organisms (Bussman et al. 1999). Upwelling groundwater that fills interstitial spaces in sediment is generally anoxic and characteristically has a low pH (Greenberg et al. 2002), which, in addition to direct adverse effects, can alter bioavailability of metals (Ho et al. 1999). Likewise, upwelling zones can result in a flux of ammonia (Greenberg et al. 2002). Nutrient loading into coastal waters via groundwater has been reported to result in increased growth of macroalgae and phytoplankton, reduction of seagrass beds, and reductions in local fauna. Nutrient constituents, habitat changes and the frequency of anoxic and associated hydrogen sulfide events characteristic of enriched waters are often responsible for these changes (Valiela et al. 1990). Other forms of sulfur, and high cation concentrations are also constituents of groundwater that can influence toxicity (Gustavson et al. 2000). Addressing the above non-contaminant factors requires appropriate water and sediment quality measurements at the test site and an understanding of their contribution to the observed effects for proper data interpretation.

3.4 ISSUES ASSOCIATED WITH CAGING

A common artifact of caging is the reduction of dissolved oxygen levels measured within *in situ* chambers, usually due to clogging of mesh screens (DeWitt 1996, Greenberg et al. 2002, Liber et al. 2007). Sibley et al. (1999) found declining dissolved oxygen inside test chambers (224-µm mesh) during tests with midges (*Chironomus tentans*) and oligochaetes (*Lumbriculus variegatus*) over the course of a 10-day study. To offset this, they suggested increasing the screened area of the chamber to maximize water exchange between the test chamber and the external environment. However, extensive fouling of cage mesh due to algal growth or accumulation of debris has been reported even with large mesh sizes (Jones and Sloan 1989, Comeleo 1991). Cage fouling is particularly problematic in embayments that are highly productive and in areas with restricted flow (ASTM 2003). Therefore, periodic cleaning of cages (e.g., brushing) during the exposure may be required (Szal et al. 1991, ASTM 2003) and should be considered when deployments are conducted in locations (e.g., deep water) where routine maintenance might be cost prohibitive. Addition of submersible battery-operated pumps to increase flow across mesh is another approach that has recently been explored to maintain water quality inside *in situ* cages (Rosen et al. 2009).

Reduction in water flow can also lead to the deposition of fine sediment or detritus within the enclosure. Test sediment within the chamber can be buried by deposited sediment that may either be cleaner or more contaminated (Dewitt et al. 1996). Sedimentation in the enclosure can, therefore, increase or decrease exposure to test organisms either by direct contact or through contributions from associated food sources. Caging may alter food availability, yielding different toxicity or bioaccumulation consequences relative to natural populations. The accumulation of fine particles in test chambers may also smother organisms or otherwise affect their behavior (Liber et al. 2007).

Another potential confounding factor associated with caging is the inability of the test organism to carry out various behaviors that ensure its survival. The inability to avoid predation (see Section 3.7 below) or to perform diurnal movements may result in an overestimation of actual field toxicity (Clark et al. 1987, Baughman et al. 1989, Simonin et al. 1993). Proper cage design for the species and use of species that are appropriate to the exposure routes being evaluated will help to prevent these (Liber et al. 2007).

3.5 ISSUES ASSOCIATED WITH FEEDING

As discussed above, food availability may be determined by the screen size enclosing the test organisms and whether or not a cap is used on the bottom of whole sediment cores used as test chambers. Small mesh or fouling of mesh may affect access to food (depending on the particular diet), which can alter both toxicity and bioaccumulation potential. Food supply will also vary among sites, potentially impacting survival or sublethal endpoints such as rate of growth of the test organisms (ASTM 2003). Specific examples include higher growth rates for clams (Peterson and Beal 1989) and mysid shrimp (Comeleo 1991) where food supplies are enhanced. Lack of control over food supply may also affect bioaccumulation of pollutants, which might be associated with factors such as the ingestion rate. Measurements of chlorophyll-*a*, particulate organic carbon, and suspended solids (depending on feeding strategy of the test organisms) are recommended to estimate food availability (ASTM 2003).

3.6 TRANSPORTATION, HANDLING, AND PHYSICAL STRESS

Stress from transport, confinement, or deployment of test organisms into the field may result in adverse effects unrelated to site conditions (Jones and Sloan 1989, Sasson-Brickson and Burton 1990, Simonin et al. 1993, Chappie and Burton 1997, Sibley et al. 1999, ASTM 2003). Therefore,

precautions must be taken to prevent such additional stress. Some simple measures include minimizing holding time in chambers before deployment, avoiding rough handling during transport, deployment, and recovery phases, and acclimating organisms to test conditions (i.e., temperature, salinity) beforehand (EPA 1994, Pereira et al. 1999, ASTM 2003). It has been shown that fish that have been acclimated to freshwater stream conditions before an episodic event (i.e., acidification induced by snowmelt) may tolerate the event better than non-acclimated fish (Simonin et al. 1993, Mount et al. 1990). Where these conditions change predictably, field exposures may be planned to fall within tolerance ranges of test species. In addition, it is useful to include handling controls that are treated in the same manner as those deployed in the field. They should be brought back to the laboratory to assess handling stress/mortality (Liber et al. 2007).

Optimizing transportation and deployment conditions for individual species needs to be considered. Chappie and Burton (1997) reported mortality of midges during transportation of test chambers to the field, while transportation of the test animals in test tubes reduced mortality. Factors that need to be considered with respect to transport to and from the site include water temperature and overall water quality, whether or not animals should be fed, whether they should be transported dry or wet, comparability in handling organisms deployed at reference sites versus test sites, and low stress deployment methods (Liber et al. 2007).

Selecting test organisms that are resistant to physical stress incurred while in the field is vital to detecting toxicity. Storm events, high flow conditions (Chappie and Burton 1997) and areas of high tidal surge (B. Phillips, pers. comm.) or high flow velocities are particularly challenging (Szal et al. 1991). Exposure sites or cage designs that result in better protection from physical stresses such as wind and waves may be required (Ziegenfuss et al. 1990, Pyle et al. 2001). Smaller mesh openings or careful placement of cages so that mesh openings are not facing currents may also be necessary (ASTM 2002). Floating or suspended debris and high suspended solids are also associated with adverse effects. Sasson-Brickson and Burton (1990) and Sibley et al. (1999) both reported storm activity, and the subsequent elevated turbidity and presence of dissolved constituents, having contributed to mortality.

3.7 PREDATION AND COMPETITION

The presence of indigenous organisms in test chambers can make interpretation of *in situ* studies difficult. Predation on test organisms, as well as competition for food and space, may lead to an incorrect conclusion that observed effects (i.e., mortality, growth inhibition) were due to sediment-associated contaminants (Sibley et al. 1999, DeWitt et al. 1996, Reynoldson et al. 1994). When predators cannot be excluded, it is important to record the presence of indigenous organisms at the end of the test to assist with data interpretation (Sibley et al. 1999, Crane et al. 2000). Modifications to the cage design may also prove worthwhile. Chappie and Burton (1997) found that changing the mesh size from 149 μm to 74 μm in field experiments with midges reduced the number of indigenous organisms entering the chambers. Similarly, Pereira et al. (1999) found decreased predation by reducing mesh to 70 μm for studies with *Ceriodaphnia dubia* and *Daphnia magna*. Optimal mesh size depends on species selection as well as conditions at the site, and should be adapted to reduce false positive results. While predation may be related to maladaptive behavior of the test organisms (i.e., contribute to contaminant-related risk), it is generally best to exclude predators because their presence and effect on test species can be expected to be highly variable from station to station. Marking test organisms (e.g., with paint) before the start of the test makes reliably distinguishing test animals from indigenous organisms feasible (Crane et al. 2000, Chappie and Burton 1997).

A number of strategies have been attempted to pre-treat sediments in order to remove endemic organisms. These include sieving, autoclaving, freezing, antibiotics, mercuric chloride, and gamma irradiation of sediments (ASTM 2000, Reynoldson et al. 1994, Burton et al. 1992, Day et al. 1992, Powlson and Jenkinson 1976). These methods should be used with caution, as they tend to disrupt the chemical equilibrium of the sample. For example, sieving sediment may remove finer grained sediment (< 63 μm), drastically reducing organic contaminant concentrations because fine-grained sediment has a higher surface area and generally a higher sorptive capacity for organic contaminants (Day et al. 1992). Manipulation of the sediment by these processes has also been shown to reduce survival in tests, and may be due to changes in the physical structure of the sediments (Sibley et al. 1999), increased bioavailability of contaminants due to changes in chemical equilibrium, or reduction in food supply following sterilization procedures.

Since one of the primary reasons for conducting tests *in situ* is to provide a more realistic exposure to test organisms, manipulation of sediment by one of the above methods may not be desired. Other studies have addressed predatory effects by using testing strategies that result in little disturbance of the test sediment, followed by a comparison of indigenous organism densities in test chambers at the end of the tests to the desired endpoint (i.e., survival, growth) of the test species (Sibley et al. 1999, Crane et al. 2000). In addition, increasing the number of test replicates may help reduce variability due to predation (B. Phillips, pers. comm.).

3.8 NEED FOR APPROPRIATE CONTROLS AND REFERENCE SITES

Laboratory and field controls, as well as field reference tests are required for *in situ* tests (ASTM 2002). Laboratory controls are used to assess the acceptability of a test by providing evidence of test organism health. Field deployment controls, treated in a similar manner as field-deployed organisms but brought back to the laboratory for further observation, can help to assess handling effects. Finally, reference sites tested near the site of concern help assess conditions exclusive of the contaminants of concern. Inclusion of a full-term field control with clean sediment may also be appropriate.

Artifacts associated with caging (i.e., predation and competition, reduction in water flow and dissolved oxygen, sedimentation of select grain sizes) require careful consideration for additional control treatments and experimental designs to estimate the presence/effects of these (DeWitt et al. 1996). Conducting tests with a series of different cage or mesh sizes, for example, might determine whether or not differences are constant across all sizes. Variations in physico-chemical characteristics also need to be considered. For example, temperature can affect metabolic rate and toxicant uptake kinetics, affecting test sensitivity (Anderson et al. 1994). Conducting exposures in the laboratory may help remove one or more of these variables to determine their impact on organism response. High spatial and temporal site variability suggests the need for multiple replicates and possibly several reference sites.

4. SPECIES AND EXPOSURE METHOD CONSIDERATIONS

A number of species, life stages, and experimental approaches are available for use in field toxicity studies. Species and test method selection, however, need to be considered on a case-by-case basis. Some considerations by Mac et al. (1990), ASTM (2002), ASTM (2003), Liber et al. (2007) include:

- Indigenous species present or representative of species that occur at the site versus surrogate species
- Age of test organisms at initiation
- Exposure duration
- Availability through culture or field collection
- Demonstrated sensitivity to the chemicals of concern
- Tolerance to site conditions
- Tolerance to caging
- Relevance for the ecological compartment of concern (i.e., bulk sediment, porewater, surface water)
- Accumulation potential (for bioaccumulation studies)
- Costs

4.1 INDIGENOUS VERSUS SURROGATE SPECIES

In some cases, it may be preferable to use indigenous organisms (Salazar and Salazar 2000, Baird et al. 2007). This might be particularly important if the objective of the study is to ensure adequate protection for those species that play a critical role within the food web at the site (keystone species); species that occur in very high numbers (dominant species); species that have high social, political, or cultural importance (flagship species); or those of particularly high economic importance (Baird et al. 2007). In addition, use of local indigenous species collected from a nearby uncontaminated site can potentially reduce transportation and acclimation stress and provide results directly applicable to the community of concern (Chappie and Burton 2000). Standard test species, however, are often just as relevant if an understanding of whether or not toxicity at the site is a primary concern (Pereira et al. 2000, Baird et al. 2007). Many *in situ* studies reported in the literature are simply modifications of laboratory protocols, using the same standardized surrogate species. Use of standard test organisms and methods allow the researcher to take advantage of the wealth of published toxicity or bioaccumulation data. Standard test species are typically commercially available or cultured, and unlike resident species, may not need to be acclimated to any contamination that may be present at the site (e.g., newly hatched organisms or embryo-larval tests).

Other factors being equal, it is generally best to test species that are endemic to the site under study. For instance, *Eohaustorius estuarius* and *Rhepoxynius abronius* are probably better suited to testing at west coast sites, while *Ampelisca abdita* or *Leptocheirus plumulosus* are probably better suited for testing at east coast sites. Other factors, however, such as contaminant sensitivity, should also be considered. In addition, the introduction of non-native species may be legally limited under various federal, state, and tribal regulations. Under no circumstances should invasive species not already present be deployed at a site.

4.2 TEST ORGANISM AVAILABILITY

A number of standardized test organisms are either easy to culture or are available from commercial suppliers, allowing year round use (Table 2). The advantage of cultured organisms is a

reliable supply of known age, size, and condition (Liber et al. 2007). *Americamysis bahia, Menidia sp., Atherinops affinis, Neanthes arenaceodentata, Leptocheirus plumulosus,* and *Mytilus sp.* are available from commercial vendors that maintain live cultures. Other common test organisms including *Eohaustorius estuarius, Rhepoxynius abronius, Macoma sp., Holmesimysis costata,* and *Strongylocentrotus purpuratus* are currently restricted to field collection. Because gravid adults are required for some of the early life stage tests that use field collected organisms (e.g., bivalve or echinoderm embryogenesis tests), condition and spawning season of the test species should be considered. Table 2 provides information relevant to test organism availability for a number of common marine and estuarine toxicity and bioaccumulation test species.

4.3 SENSITIVITY TO CONTAMINANTS

It is important to consider contaminant sensitivity of the test organism/endpoint in determining which tests to use in field studies. Table 3 lists LC50 or EC50 data for a number of species and endpoints that might be considered for *in situ* toxicity tests. No single organism or endpoint is sensitive to all chemicals or appropriate for every situation. Hence, study objectives along with prior knowledge regarding contaminants of concern should be considered when selecting toxicity tests and species. In some cases, using test species that differ in sensitivities may facilitate discrimination of risks associated with organic vs. metal contaminants, and/or confounding factors such as ammonia, low oxygen, and hydrogen sulfide. In other cases, the contaminant class associated with the greatest risks will be known, and discrimination of risk drivers within a toxicant class may be a study object.

Ranking the relative sensitivities of several standard toxicity tests to common metal and organic contaminants provides a basis for some useful generalizations. Relative sensitivities can, in turn, contribute to the basis for selection of the most useful test to adapt for *in situ* applications. Table 4 and Table 5 list the sensitivity rank (quotient of effect concentration relative to the most sensitive effect concentration) for some common test species used in laboratory tests for metals and organics, respectively. Highly resistant species (e.g., *Artemia salina*) were not presented, given that field testing with insensitive species should generally be avoided. Each toxicant was tested with a different number of species. While only a few common test species are listed in the tables, the range of rankings is considered to be inherent to the mode(s) of toxicity, as long as the standard taxonomic groups (i.e., echinoderms, bivalves, crustaceans and fishes) are represented. Table 4 presents ranked sensitivity (as mean, min, max) for copper, zinc and cadmium. It was apparent from these rankings that copper and zinc were different from cadmium. For example, bivalve and echinoderm embryo-larval tests are particularly sensitive to copper and zinc, while cadmium affects mysid survival at lower concentrations relative to embryo-larval tests (Table 3). Based on available data, the dinoflagellate *Lingulodinium* (used in the QwikLite test) and the amphipod *Ampelisca* are moderately sensitive to all three metals. Mysids are relatively insensitive to copper and zinc, while fish (e.g., *Menidia*) are relatively insensitive to copper, zinc, and cadmium.

Crustacea (e.g., amphipods and mysids) are ranked as most acutely sensitive to the organophosphate pesticide diazinon, while fish are most sensitive to the organochlorine pesticide endosulfan (Table 4). Mysids are also relatively sensitive to polychlorinated biphenyls (CEPA 2003). For some chemicals testing of one member of a taxonomic group (class or family) will not provide sufficient representation of sensitive species. For example, the amphipod *R. abronius* is substantially more sensitive to cadmium than *E. estuarius.*

Table 2. Culture status and availability of marine and estuarine organisms commonly used in toxicity and bioaccumulation testing.

Species	Type	Life Stage	Lab Culture?	Commercially available?	Time Available
Mytilus spp.	Mussel	Embryo, Adult	N	Y	All year[1]
Crassostrea gigas	Oyster	Embryo	N	Y	Jun-Sept
		Adult	N	Y	All year
Macoma balthica	Clam	Adult	N	Y	All year
Macoma nasuta		Adult	N	Y	All year
Stronglocentrotus purpuratus	Sea urchin	Embryo, Egg	N	Y	Oct-May
Dendraster excentricus	Sand Dollar	Embryo	N	Y	Jun-Oct[2]
Haliotis rufescens	Abalone	Embryo	Y	Y	All year
Eohaustorius estuarius	Amphipod	Adult	N	Y	All year
Rhepoxynius abronius			N	Y	All year
Leptocheirus plumulosus			Y	Y	All year
Ampelisca abdita			Y	Y	All year
Neanthes arenaceodentata	Polychaete	Juvenile, Adult	Y	Y	All year
Nereis virens	Polychaete	Adult	N	Y	All year
Americamysis bahia	Mysid	Juvenile	Y	Y	All year
Holmesimysis costata			N	Y	All year[3]
Menidia beryllina	Fish	Larva, Juvenile	Y	Y	All year
Atherinops affinis			Y	Y	All year
Cyprinodon variegatus			Y	Y	All year
Lingulodinium polyedrum	Dinoflagellate	Adult	Y	N	All year
Ceratorcorys horrida			Y	N	All year
Pyrocystis lunula			Y	Y	All year
Vibrio fischeri Microtox	Bacterium	Adult	Y	Y	All year
Brachionus plicatilis	Rotifer	Juvenile	Y	Y	All year
Artemia salina	Brine Shrimp	Juvenile	Y	Y	All year

[1]Sometimes spawning difficulties in summer months
[2]April-October in Puget Sound
[3]Can be difficult to find when kelp not abundant

Table 3. Aqueous toxicity metrics (LC50s/EC50s) for common estuarine and marine test organisms and endpoints of potential use in screening-level and/or *in situ* toxicity and bioaccumulation studies. Bold values indicate among the most sensitive endpoints.

Species	Type	Exp. Duration	Endpoint	EC50/LC50 (mg/L)								
				Copper	Cadmium	Zinc	Diazinon	Endosulfan	Fluoranthene	PCB†	NH_3	H_2S
Mytilus edulis	Mussel	48 h 96 h	ED AS	**0.0058 A**	1.2 A 1.62 E	**0.175 A** 2.5E		0.212 B			**0.120 C**	**0.19 D**
Mytilus galloprovincialis	Mussel	48 h	ED AS	**0.010 F**	3.890 G							>50 H
Crassostrea gigas	Oyster	48 h	ED	**0.017 F** **0.0053 A**	**0.611 A**	**0.119 A**					0.13 I	
Macoma sp.	Clam	96 h	AS									6.0 J
Mulinia lateralis	Clam	72 h	S	0.04 BQ		0.500 BQ			>188 BM			
Stronglocentrotus purpuratus	Sea urchin	72-96 h	ED	**0.016 K**	0.510 L	**0.023 L**		0.227 L		100-500 BN	**0.057 I**	**0.18 D**
Stronglocentrotus purpuratus	Sea urchin	40 min	F	**0.026 K**	18.4 L	**0.029 N**	>12.0 O	0.081 L			1.150 O	
Arbacia punctulata	Sea urchin	48 h	ED	**0.021 F;** **0.014 S**	7.38 P	**0.073 P**	>9.6 Q					
Arbacia punctulata	Sea urchin	40 min	F	**0.012 S**	20.1 P	**0.112 P**				1000 BO		
Haliotis rufescens	Abalone	48 h	ED	**0.071 F;** **0.009 T**		**0.068 T**		0.252 U			**0.082 C**	
Eohaustorius estuarius	Amphipod	96 h	S	3.7 V	9.330 W				**0.066 Y**		2.49 Z	3.32^ D
Rhepoxynius abronius	Amphipod	96 h	S		**0.920 AA**		**0.009 AB**		**0.024 AC** **0.014 Y**		1.590 Z	1.6^ D
Leptocheirus plumulosus	Amphipod	96 h	S	0.461 BR	1.060 AD				**0.069 Y**			
Ampelisca abdita	Amphipod	96 h	S	**0.026 F;** **0.034 V**	**0.330 AD**	0.390 AE	**0.010 AB**		**0.067 BM**		0.830 Z	
Neanthes arenaceodentata	Polychaete	96 h	JS	**0.077 AF**	5.60 AG	1.40 AG		0.73 AH	0.50 BM			5.5* AJ

Table 3 (cont.) Aqueous toxicity metrics (LC50s/EC50s) for common estuarine and marine test organisms and endpoints of potential use in screening-level and/or *in situ* toxicity and bioaccumulation studies. Bold values indicate among the most sensitive endpoints.

				EC50/LC50 (mg/L)								
Species	**Type**	**Exp. Duration**	**Endpoint**	**Copper**	**Cadmium**	**Zinc**	**Diazinon**	**Endosulfan**	**Fluoranthene**	**PCB†**	**NH_3**	**H_2S**
Americamysis bahia	Mysid	96 h	JS	0.141 Q; 0.181 AL	**0.063 Q; 0.03 AM**	0.498 Q,AM	**0.005 Q**		**0.031 BM**	57 BP	1.02 BL	
Holmesimysis costata	Mysid	96 h	JS	**0.017 AO**		**0.097 AO**					0.839 C	
Menidia beryllina	Fish	96 h	LS	0.111 F; 0.136 Q	**0.8 Q**	3.9 Q	1.17 Q	**0.002 AP**	0.620 BM		1.117 BL	
Menidia menidia	Fish	96 h	LS	0.066-.217							1.050 BL	
Atherinops affinis	Fish	96 h	LS	0.238 AQ		0.880 AS		**0.001 AP**			0.560 C	
Cyprinodon variegatus	Fish	96 h	LS	0.368 BI	0.555 BJ, BK			**0.003 AT**	>20 BM		2.717 BL	
Lingulodinium polyedrum	Dinoflagellate	24 h	B	**0.023 AU;** 0.090 AV	0.782 AU 0.843 AV	0.430 AU 0.349 AV					**0.068 AV**	
Ceratorcorys horrida	Dinoflagellate	24 h	B	0.166 AV	1.17 AV	0.394 AV					**0.142 AV**	
Pyrocystis noctiluca	Dinoflagellate	24 h	B	0.185 AV	1.13 AV	0.345 AV					0.405 AV	
Pyrocystis lunula	Dinoflagellate	24 h	B	0.125 BR							0.706 BR	
Vibrio fischeri Microtox	Bacterium	15 min	B	0.397 AV; 1.3 AM	102 AM; 56.7 AV	12.0 AM; 10.5 AV	1.71-365 AW				1.7 AM	
Brachionus plicatilus	Rotifer	24 h	S	**0.063 BA, BE**	39.1 BA	>4.8 BA					3.1 BF	
Phaeodactylum tricornutum	Diatom	72 h	PG	0.470-0.635 BB; 0.050 BC	22.4 BG							
Artemia salina	Brine Shrimp	24 h	S	0.44 AX; 0.800 BD	3.1 AZ	4.46 AZ		>0.1, <1.0 AY			14.6 AM	

Table 3 (cont.) Aqueous toxicity metrics (LC50s/EC50s) for common estuarine and marine test organisms and endpoints of potential use in screening-level and/or *in situ* toxicity and bioaccumulation studies. Bold values indicate among the most sensitive endpoints.

Footnotes

*LOEC

^48 hour exposure

†Arochlor 1254

Endpoints

E=Embryo Development
AS=Adult Survival
S=Survival
F=Fertilization
LS=Larval Survival
JS=Juvenile Survival
B=Bioluminescence
PG=Population Growth

References

A=Martin et al. (1981)
B=Dinnel (1991)
C=Phillips et al. (2005)
D=Knezovich et al. (1996)
E=Ahsanullah (1976)
F=USEPA (1995a)
G=Phillips et al.(2002)
H=Abel (1976)
I=Greenstein et al. (1996)
J=Caldwell (1975)
K=USEPA (1995b)
L=Dinnel et al. (1989)
M=Phiilips et al. (1998)
N=Schiff et al. (2002)
O=Bay et al. (2003)
P=Carr et al. (1996)
Q=Bay et al. (1993)
S=Nacci et el. (1986a)
T=Hunt & Anderson (1989)
U=Martin et al. (1986)
V=McPherson & Chapman (2000)
W=DeWitt et al. (1989)
X=Swartz et al. (1994)
Y=Boese et al.(1997)
Z=Kohn et al. (1994)
AA=Swartz et al. (1985)
AB=Werner & Nagel (1997)
AC=Swartz et al. (1990)
AD=ASTM (1994)
AE=USEPA (1987)
AF=Pesch & Morgan (1978)
AG=Reish & Gerlinger (1984)
AH= USEPA (1980)
AI=Rossi & Neff (1978)
AJ=Dillon et al (1993)
AK=Miller et al. (1990)
AL=Lussier et al. (1985)
AM=Touissant et al. (1995)
AN=Cripe (1994)
AO=Martin et al. (1989)
AP=Hemmer et al. (1992)
AQ=Anderson et al. (1991)
AS=Katz et al. (2006)
AT=Schimmel (1981)
AU=Lapota et al. (2007)
AV=Rosen et al. (2008)
AW=Kaiser and Plabrica (1991)
AX=Asavagatmanee (1990)
AY=Knauf & Schulze (1973)
AZ=Govindarajan et al. (1993)
BA=Snell et al. (1991)
BB=Sun et al. (1990)
BC= Florence & Stauber (1986)
BD=Govindarajan et al. (1993)
BE= Moffat & Snell (1995)
BF= Ostrensky & Wasielesky (1992)
BG=Torres et al. (1997)
BH=Cardin (1985)
BI=Huges et al. (1989)
BJ=Hutchinson et al. (1994)
BK=Hall et al. (1994)
BL=USEPA (1989)
BM = Spehar et al (1999)
BN = SAIC (1993)
BO= Adams & Slaughter-Williams (1988)
BP= Ho et al. (1997)
BQ = Ho and Zubkoff (1982)
BR = Burton et al. (2008)

Table 4. Metal sensitivity associated with common toxicity tests (LC50 relative to most sensitive species per metal[1], i.e., if most sensitive then value = 1)

Species	Mean	Min	Max[2]	Across Metals Response
Strongylocentrotus purpuratus	9.7	1.0	25.2	Sensitive
Crassostrea gigas	12.4	1.0	31.0	Sensitive
Americamysis bahia	16.7	1.0	27.0	Sensitive
Mytilus edulis	26.2	1.1	70.0	Variable
Menidia beryllina	78.7	26.0	170.0	Moderate
Arbacia punctulata (e-l)	93.7	2.5	369.0	Variable

[1]Mean based on responses to Cu, Zn, Cd (i.e, n=3)

[2] Max was for Cd in all cases except for *A.bahia*, where the max was for Cu.

Table 5. Organic contaminant sensitivity associated with common toxicity tests (value relative to most sensitive per compound[1])

Species	Mean	Min	Max[2]	n	Across Contaminant Response
Americamysis bahia	1.6	1	3.1	6	DDT excluded; ratio = 280
Ampelisca abdita	1.2	1	1.4	FLU, DIAZ	Sensitive
Rhepoxynius abronius	2.1	1.8	2.4	FLU, DIAZ	Sensitive
Eohaustorius estuarius	3.0			DDT	Sensitive
Menidia beryllina	143.0	2.0	400.0	5	Variable
Strongylocentrotus purpuratus	660.1	5.3	2400.0	4	Very variable

[1]Response to Fluoranthene, Phenol, Arochlor 1254, Diazinon, Endosulfan, DDT.

Note: *Menidia* is sensitive to Endosulfan, and insensitive to Fluoranthene, Diazinon and DDT

A study by Reish (1988) examined the relative toxicity of six metals (arsenic, cadmium, chromium, copper, mercury, and zinc) to crustaceans, polychaetes, pelecypods, and fishes, and concluded that no one species or group of test organisms was the most sensitive to all of the metals. For amphipods exposed to PAHs, Rust et al. (2004) found that *A. abdita* and *L. plumulosus* were less efficient at metabolizing PAHs than other amphipod species, and hence may be the species of choice for studies requiring site-specific PAH bioaccumulation rates.

Sensitivity to potential confounding factors such as ammonia, sulfide, and physical stressors (discussed previously) also needs to be taken into account. Use of sea urchin or bivalve embryo-larval development tests in porewater from organically rich sediments may make interpretation of toxicity data difficult due to these test's relatively high sensitivity to both ammonia and sulfide. Some dinoflagellates species used in the QwikLite assay are also highly sensitive to ammonia (Rosen et al. 2008). Infaunal amphipods and polychaetes tend to have a higher tolerance for high exposure to ammonia and/or sulfides. *E. estuarius* is more tolerant of elevated ammonia (Kohn et al. 1994) and hydrogen sulfide (Knezovich et al. 1996) compared to other commonly used amphipod species, but is comparably sensitive to most anthropogenic contaminants relative to other amphipods (USEPA 1994b, Schlekat et al. 1995, CEPA 2003). Some amphipod species, such as *R. abronius,* also tend to be more sensitive to fine-grained sediments, and are not recommended for use at sites where fines are >90% (DeWitt et al. 1988; Table 6).

One limitation in our current representation of sensitivity is that most tests, particularly with hydrogen sulfide and hypoxic exposures, have largely been conducted in the absence of sediment. Thompson et al. (1991) found that lethality to adult *L. pictens* occurred above 0.31 µM in water-only exposures, and above 32.9 µM porewater concentrations in sediment exposures.

4.4 TOLERANCE TO SITE CONDITIONS

As described earlier, field exposures can result in a number of abiotic stressors not observed in the laboratory. Selection of species and life stages that are tolerant to a range of physicochemical characteristics such as temperature, salinity, and grain-size may be preferable in estuaries and/or during seasons where these conditions may vary. Table 6 lists the tolerances of some of these characteristics for potential *in situ* test organisms and life stages.

Organisms that are particularly fragile (e.g., sea urchin embryos) or do not tolerate handling well (e.g., *Corophium volutator*) may not be appropriate for some types of exposures (e.g., long distances to test site, areas of high surge). Also of note is that some endpoints, such as embryo-larval development, may take longer to achieve when exposure is outside the standard temperature range. For instance, Burton et al. (2008) reported 72-h exposure times required to achieve the normal D-shape characteristic of normally developing mussel embryos, instead of the usual 48 h. They also observed increased sensitivity at lower temperature and salinity combinations, which could have been due to the increased time the larvae spent at critical stages of cell differentiation as well as being physiologically challenged (Figure 2). A salinity of 10 psu (practical salinity unit) resulted in substantially lower survival of the amphipod *Eohaustorius estuarius* when exposed to a median dose of copper as compared to higher salinities of 20 and 30 psu (Burton et al. 2008).

It should also be recognized that acclimation of indigenous communities to low-level exposures may increase tolerance to short-term concentration spikes. Furthermore, the on-site community may exhibit different tolerance through both physiological and genetic adaptation. Therefore, it is feasible that positive "effects" observed in field-deployed test organisms may not be exhibited by individuals/populations that inhabit the site (Liber et al. 2007).

Table 6. Tolerance range of several parameters for commonly used marine and estuarine toxicity and bioaccumulation test organisms and life stages.

Species	Type	Life Stage	Salinity (psu)	Temp. (°C)	Max. Grain Size (% fines)	Tolerance to Physical Stress (Low/High)
Mytilus edulis	Mussel	Embryo-larval Adult	20-35 A 5-33 C	10-20+* B to ~25 C		
Mytilus galloprovincialis	Mussel	Embryo-larval Adult	20-35 A 5-33 C	10-20+* B to ~25 C		High C
Crassostrea gigas	Oyster	Adult	25-35 C	4-24 C		
Crassostrea virginica	Oyster	Embryo-larval Adult	20-35 A 5-32 C	 0-36 C		
Macoma sp.	Clam	Adult	5-30 C	2 to 23 C		
Mercenaria mercenaria	Clam	Juvenile; Adult	0-35 C	12-35 C		
Stronglocentrotus purpuratus	Sea urchin	Embryo-larval	>30 D			Low
Eohaustorius estuarius	Amphipod	Adult	2-34 E,F	5-21+ B,G	>90 E	High E
Rhepoxynius abronius	Amphipod	Adult	>25 H	0-20+ G	<80 G; <90 E	High E
Leptocheirus plumulosus	Amphipod	Adult	1.5-32 E		>90 E	
Ampelisca abdita	Amphipod	Adult	>20 E		>90 E	
Corphium volutator	Amphipod	Adult				Low H
Hediste diversicolor	Polychaete	Adult	5-35 I	5-30 I		
Neanthes arenaceodentata	Polychaete	Juvenile; Adult	>20 J		>90 J	
Americamysis bahia	Mysid	Juvenile	5-30 K	10-31 L,M		
Holmesimysis costata	Mysid	Juvenile	>29 D			
Menidia beryllina	Fish	Larva	<5 to >32 K			
Menidia menidia	Fish	Larva	<5 to >32 K			
Atherinops affinis	Fish	Larva; Juvenile	5 to >35 N,O	10.1-31.7 P		
Cyprinodon variegatus	Fish	Larva; Juvenile	0 to >35 K	0-40 K		
Lingulodinium polyedrum	Dino-flagellate	Adult				Low Q
Pyrocystis lunula	Dino-flagellate	Adult	>30 R	15-25 B		High
Brachionus plicatilus	Rotifer	Juvenile	1-60 S	10-32 S		

*Lower temperatures can delay rate of embryo-larval development, but viable with additional exposure time.

Table 6. (cont.) Tolerance range of several parameters for commonly used marine and estuarine toxicity and bioaccumulation test organisms and life stages.

A = Geffard et al. (2001

B = Burton et al. (2008)

C = ASTM (2003)

D = USACE and USEPA (1998)

E = USEPA (1994b)

F = Anderson et al. (2004)

G = ASTM (2000a)

H = Kater et al. (2001)

I = Moreira et al. (2005)

J = Dillon et al. (1993)

K = USEPA (2002)

L = McKenney (1994)

M = Mueller et al. (1992)

N = USEPA (1995b)

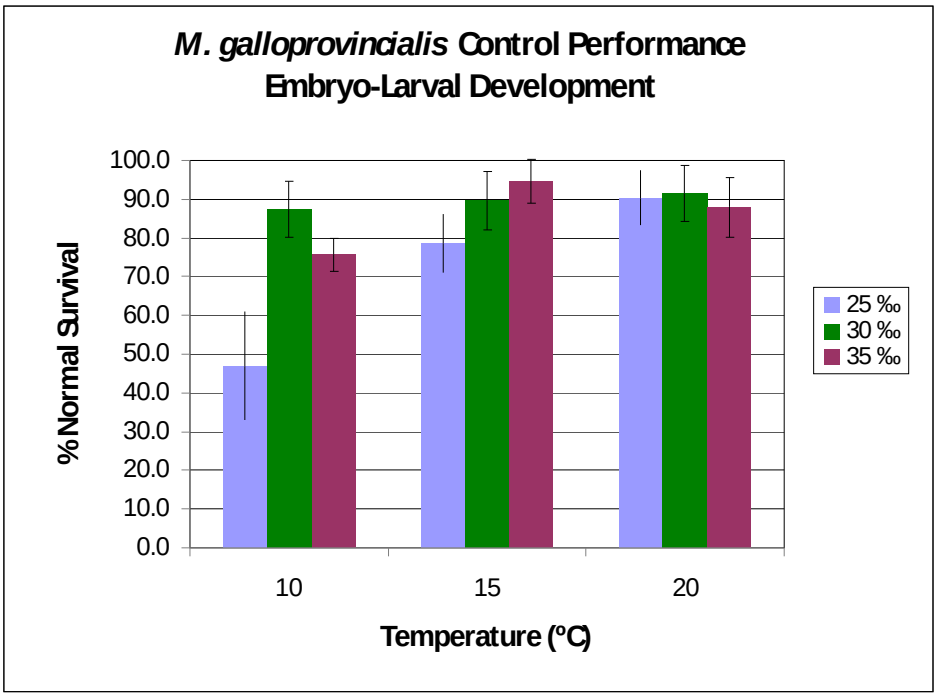
O = Anderson et al. (1995)

P = Emmet et al. (1991)

Q = Rosen et al. (2008)

R = ASTM (2005)

S = Clesceri et al. (1998)



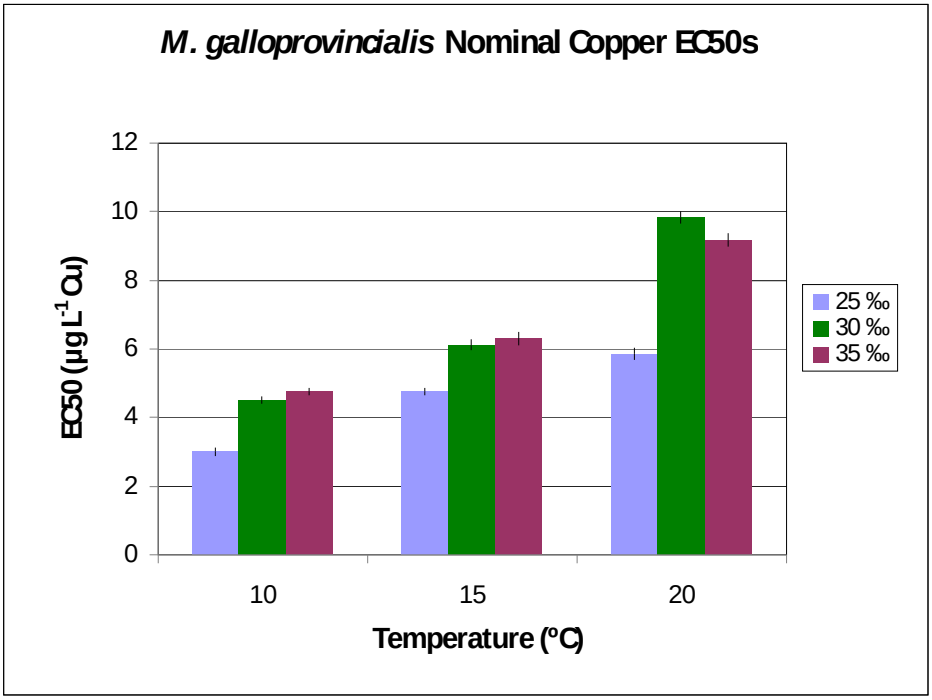


Figure 2. Mean control performance (±1 s.d.)(top figure) and EC50 values (±95% C.L.) (bottom figure) for mussel (*Mytilus galloprovincialis*) embryo-larval development following 48–72 h exposures in clean or copper-spiked seawater under varying salinity and temperature combinations (from Burton et al. 2008).

4.5 ECOLOGICAL RELEVANCE

While exposure of epibenthic organisms to porewater, for example, may have proven utility, it is generally preferable to evaluate toxicity using ecologically relevant species and life stages (i.e., those that normally occupy the matrix being evaluated). This often helps reduce incidence of issues associated with confounding factors (e.g., water column organisms are often not adapted to conditions in sediments) and allows for more realistic assessments of contaminant exposure.

4.6 ACCUMULATION POTENTIAL

One of the primary characteristics of a good bioaccumulation test organism is a low susceptibility to the contaminant(s) of concern (USEPA/USACE 1998). Some commonly used bioaccumulation test organisms, however, have been reported to metabolize contaminants to a high degree. This appears to be particularly true for polycyclic aromatic hydrocarbons. Rust et al. (2004) reported much higher rates of benzo[a]pyrene metabolism for the polychaete *Nereis virens* compared to other polychaete species, bivalves (e.g., *Macoma nasuta, Macoma balthica*) and amphipods. Appropriate species should be selected following a thorough review of species-specific bioaccumulation potential relative to targeted chemicals of concern. Consideration of exposure pathways and expected exposures for organisms at the site of interest is also important (i.e., *Macoma* may both deposit and filter feed while *Nereis* is a deposit feeder only). Also, it is important to point out that bivalves can close or only partially open their shells for long periods of time, thereby reducing their exposure.

Tissue volume required also requires consideration. Larger organisms such as *N. virens* and *M. nasuta* provide more tissue than other organisms (e.g., *Neanthes arenaceodentata*), making them potentially more suitable for bioaccumulation endpoints, depending on the analytical needs of the study.

4.7 APPROPRIATE SIZE FOR CAGING OR TISSUE ANALYSIS

Liber et al. (2007) noted that the effect of chamber size on toxicological effects has not been sufficiently investigated. They cite two studies that do address chamber size, one where small microcosms underestimated effects on phytoplankton (Perez et al. 1991), and another where the rate of contaminant exposure dissipation was faster in small enclosures (Solomon et al.1989). Where organisms used in standard laboratory tests are employed, it would be reasonable to assume that maximum densities established to avoid crowding and/or adverse water quality conditions would be adequate for field studies. However, some investigators have found increased variability may require larger counts per chamber (and hence, larger chambers) or increased replication. For bioaccumulation tests, minimum tissue mass for chemical analysis may dictate size requirements for cages.

4.8 COSTS

Costs are somewhat difficult to determine because project-specific requirements will affect the level of effort to a large degree. A range of costs for a typical commercial laboratory are provided in Table 7. Normally, rapid or screening toxicity tests will cost less than full-scale laboratory or field tests. This tends to be the case for luminescence-based tests such as QwikLite and Microtox®, as fewer labor hours and laboratory overhead are typically required to carry out these tests. Rapid tests often utilize test organisms that require very little culture or handling (Toussaint et al. 1995, Rosen et al. 2008). Test duration, however, does not necessarily correspond with costs. Short-term tests such as sea urchin fertilization (40-min exposure) and bivalve embryo-larval development (48-hr

exposure) require substantial preparation time as well as subsequent microscope work, and therefore, may require nearly as much effort as some longer term tests (Table 7). Other considerations that may affect costs include whether or not the laboratory cultures the test organisms in-house, and in the case of bioaccumulation testing, the desired detection limits for the contaminants of concern and associated tissue mass requirements. Contaminants with lower detection limits generally cost more due to increased tissue requirements.

In situ toxicity and bioaccumulation testing may or may not be more expensive than equivalent testing performed in the laboratory, and will vary depending on a variety of factors. Typically, sampling crews and vessels are required for collecting grab samples regardless of whether exposures are conducted in the laboratory or field. Retrieval of deployed chambers can increase costs, but the lack of laboratory overhead by conducting the testing in the field may minimize this difference. Accessibility to the field site and site conditions such as water depth and currents may influence costs. Field deployments may require the use of SCUBA divers, which can increase costs.

Table 7. Costs (as of July 2009) from a commercial bioassay laboratory for conducting various marine and estuarine toxicity and bioaccumulation tests.

Matrix	Species	Common name	Test Type	Cost per Test
Surface water/effluent	*Microtox (Vibrio fischeri)**	Bacteria	Luminescence	$400
	Atherinops affinis	Topsmelt	Acute Survival-Definitive	$700
			Chronic Survival & Growth-Definitive	$1,500
	Americamysis bahia	Mysid	Acute Survival-Definitive	$700
			Chronic Survival & Growth-Definitive	$1,600
	Mytilus spp.	Mussel	Chronic Embryo-larval Development	$1,500
	Strongylocentrotus purpuratus	Sea urchin	Chronic Embryo-larval Development	$1,500
			Chronic Fertilization	$1,000
	Brachionus spp.	Rotifer	Acute Survival-Definitive	$1,000
Sediment-water interface	*Strongylocentrotus purpuratus*	Sea urchin	Chronic Embryo-larval Development	$1,500
	Mytilus spp.	Mussel	Chronic Embryo-larval Development	$1,500
	Americamysis bahia	Mysid	10-day Survival	$1,300
Whole Sediment	*Ampelisca abdita*	Amphipod	10-day Survival	$1,300
	Eohaustorius estuarius	Amphipod	10-day Survival	$1,300
	Rhepoxynius abronius	Amphipod	10-day Survival	$1,300
	Leptocheirus plumulosus	Amphipod	10-day Survival	$1,300
	Neanthes arenaceodentata	Polychaete worm	20-28 day Survival/Growth	$1,500
Pore Water	*Microtox (Vibrio fischerii)*	Bacteria	Luminescence	$400
	Strongylocentrotus purpuratus	Sea urchin	Chronic Embryo-larval Development	$1,500
Bioaccumulation	*Macoma spp.*	Clam	28-day Bioaccumulation	$2,200-$2,600
	Nereis virens	Worm	28-day Bioaccumulation	$2,000-$2,400

*Note: QwikLite testing using bioluminescent dinoflagellates are expected to be comparable in cost to Microtox.

5. CAGE MATERIALS AND DESIGN FEATURES

5.1 CAGE MATERIALS

A typical *in situ* test chamber consists of a polycarbonate, polyvinyl chloride (PVC), or acrylic core body, with end caps to retain test organisms, and mesh cutouts made from Nitex nylon or fluorocarbon on at least two sides to allow for adequate water flow (Figure 3). Pyrex® glass has also been used in studies of photoinduced toxic effects because Pyrex attenuates relatively little UV light (Monson et al. 1995). Glue used to seal mesh screens to the test chambers can be toxic in itself, and therefore, should be evaluated for toxicity before use. Pereira et al. (1999) found that white thermal glue composed of 50% ethylene-vinyl-acetate copolymer, 45% synthetic hydrocarbon, 5% polyethylene wax (Elis-Taiwan, Tawian, catalog number: TN122/WS) did not cause significant toxicity to *Ceriodaphnia dubia* in 96-h exposures, while silicone glue and yellow thermal glue did. Other researchers have found clear silicone adhesives acceptable following a 48-h minimum soak in overlying water before use (Chappie and Burton 1997, ASTM 2002). Anderson et al. (1998) used acrylic glue in their cages (which were made of plexiglass), but recommended allowing adequate time for curing, followed by extensive leaching in flowing water. Glue type used may depend on cage materials (i.e., acrylic, PVC). In addition, new lots of plastic products used in cage construction should be washed and tested for toxicity before use (ASTM 2002).

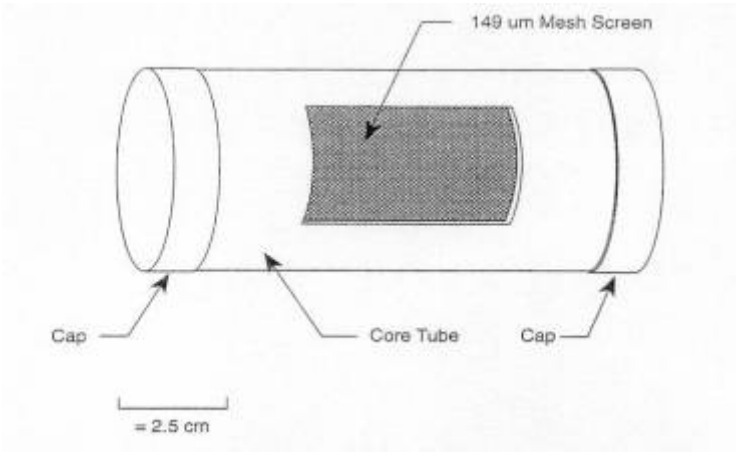


Figure 3. Typical *in situ* toxicity exposure chamber design. Reprinted from Chappie and Burton (1997).

5.2 CAGE DESIGN FEATURES

Except for one method for caged bivalves (ASTM 2003), standardized protocols do not exist for *in situ* toxicity or bioaccumulation testing. Therefore, testing strategies, including cage designs, tend to vary from one study to another. A list of desirable cage features provided by Comeleo (1991) include those that:

- Are durable.
- Require minimal maintenance during the exposure period.
- Maintain a water exchange rate that does not reduce dissolved oxygen levels and provides enough food for the test organisms.
- Keep predators out.

- Allow easy removal and enumeration of surviving organisms at test termination.

Various ports can be added to the chambers to allow for test organism addition or sampling for chemical analyses while in the field (Figure 4). Inlet and outlet ports can be used to direct water flow (Figure 5). In order to keep cages in place, they may be secured with line attached to stakes that have been driven into the sediment or are simply pushed into the sediment (Figure 6). Cage size depends on species-specific requirements, but typical cages for toxicity tests are less than 13 cm long with an outer diameter of 7 cm (Greenberg et al. 2002). Bioaccumulation studies tend to require larger cages due to the heavy tissue requirements for such studies, but are based on the same general premise. Some of the larger cage designs are modifications of those used in the aquaculture industry (Martin and Black 1995).

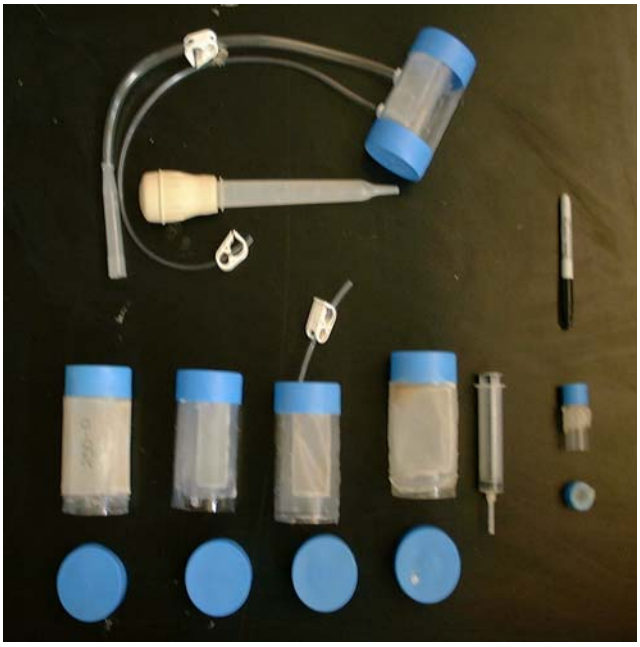


Figure 4. *In situ* chambers used by G.A. Burton lab (University of Michigan).

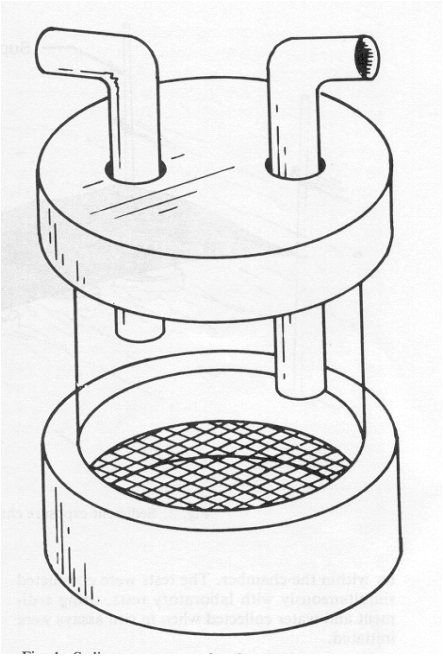


Figure 5. *In situ* chamber with inlet and outlet. Reprinted from Sasson-Brickson and Burton (1991).

Figure 6. Deployed *in situ* toxicity test chambers. Printed with permission from G.A. Burton, Jr. (University of Michigan).

Microcosm exposures have been found to be a useful means to test the utility of chambers that hold promise for *in situ* testing (Figure 7). Testing exposure chambers in microcosms may be particularly useful in identifying potential problems associated with water exchange. Liber et al.

(2007) note that inhibited water exchange is probably the most serious artifact associated with *in situ* experiments. Where natural flow is impeded, concentrations of food, nutrients, dissolved oxygen, and ammonia may not represent conditions outside of the test chamber, conditions that can bias organism growth and survival. Rosen and Lotufo (in prep) deployed mussel embryos in mesh-capped vials within microcosm tanks containing solid phase "chunks" of Composition B (a military formulation containing TNT and RDX) under static and continuous flow through conditions. The consistency between "grab" sample concentrations from the tanks and concentrations in the test vials suggests that use of the mesh-capped vials could have utility in true "*in situ*" exposures.

Figure 7. Laboratory microcosm exposures used as a proof of concept for marine and estuarine *in situ* exposure development (Photo by G. Rosen, SSC Pacific).

6. CASE STUDIES

In the previous sections, we described many of the advantages and limitations of *in situ* testing for the purposes of aquatic risk assessments. In general, it can be concluded that each has its place in a myriad of "weight-of-evidence" approaches. Specific experimental designs should provide a balance of evidence that will best meet the Data Quality Objectives of the study. Crane et al. (2007) provide decision trees for the types of field testing that may be most appropriate, both with and without native species. Liber et al. (2007) provides lists of specific considerations and confounding factors associated with each step in the process of selecting the most appropriate exposure design to meet study objectives. Much of the current state of knowledge with regard to *in situ* testing has been gained through trial and error, and a major objective in most studies to date has been first and foremost, survivability and statistical resolution with respect to controls. Given that there has been no umbrella program for the development and refinement of *in situ* testing, it is important to document findings from the many tests that have been conducted to date. This effort will provide some clarity by identifying patterns of responses associated with certain repeated or similar test designs and also in presenting alternatives that may hold particular promise for certain types of study objectives. This section includes the following subsections:

- *In Situ* Tests with Standard Test Organisms (6.1)
- Transplanted Bivalves (6.2)
- Sediment–water Interface (SWI) exposures (6.3)
- Porewater Testing (6.4)

Subsections have headings that refine taxonomic groupings. The final section provides a brief discussion of the more salient findings from the review along with some recommendations to improve test protocols and to develop objectives to advance *in situ* testing methods that will make aquatic risk assessments more accurate and efficient.

6.1 *IN SITU* TESTS WITH STANDARD TEST ORGANISMS

The decision as to whether to use standard test organisms or indigenous fauna for *in situ* biological testing depends on the objectives of the study, as was discussed in the previous section. Below are selected case studies from several taxonomic groups (fish, bivalves, echinoderms, crustaceans, and polychaetes). Most of the case studies involve standardized, or commonly used, laboratory-based test organisms and/or toxicity endpoints. Where possible, the emphasis is on estuarine and marine studies, but some freshwater studies have been included because the similarities in tools used and problems encountered make the findings relevant to both freshwater and saltwater testing.

6.1.1 Fish – toxicity

Caged fish have been widely used in field exposures using a range of endpoints, including mortality, embryo development, biochemical responses, and bioaccumulation (Jelinski and Anderson 1996, Chappie and Burton 1997, and references therein). Many fish species have an intimate association with sediment, and water-column species have also been shown to be very sensitive to sediment contamination (Burton et al. 2000). The vast majority of *in situ* studies with fish have been conducted in the water column of freshwater environments, using early life stages of standard test species such as the fathead minnow (*Pimephales promelas*). Below are brief summaries of experimental approaches and results from several studies, focusing on estuarine and marine species, when available.

Clark et al. (1986) used 1-month-old laboratory-reared sheepshead minnows (*Cyprinodon variegatus*) in 5-day caged experiments to assess the toxicity of fenthion following aerial application to control adult salt marsh mosquitoes at an estuarine site in Florida. Cages were constructed by removing the bottom 2 cm and top 3.5 cm of wide-mouthed polypropylene jars (8 cm I.D.), and attaching to the end of a 14-cm high by 8-cm outside-diameter cylinder of nylon mesh (600 µm) with silicone adhesive. The cages were connected with nylon cords to submerged bricks so that they floated with the top side exposed to the water surface. Fish were added to the chambers in the field randomly from laboratory-loaded incubation cups. Each cage contained 10 fish, which were not fed during the deployment. No fish mortality was observed on either of two deployments. Fenthion concentrations, however, were substantially below LC50 values for sheepshead minnows. It should be noted that sheepshead minnows may also be good candidate organisms for assessments at the sediment–water interface because of their tendency to graze at the sediment surface (Rowe 2002, personal observations, G. Rosen).

Rice et al. (1994) measured sublethal effects in caged speckled sanddabs (*Citharichthys stigmaeus*), the most common fish in Moss Landing Harbor, CA. The endpoint desired was mixed-function oxidase (MFO) activity. MFOs are microsomal enzymes that catalyze the metabolism of endogenous steroids and hydrocarbons, including PAHs and polychlorinated biphenyls (PCBs). Activity of these enzymes increases upon exposure to hydrocarbons. It has been suggested that fish with elevated MFO activities have reduced fertilization success. Wild caught sanddabs were held for 2 to 3 weeks before deployment in the field. Fish were fed frozen brine shrimp and chopped frozen squid during this acclimation period, during which 1% mortality was observed. Fish were then loaded into cages in groups of 40. Cages, consisting of a PVC frame covered with 13-mm plastic mesh attached with cable ties, were deployed at four sites and secured to the bottom by attaching to rebar stakes that were previously driven into the sediment. The exposure period was 14 days. Non-caged sanddabs were also assayed for MFO activity. No significant correlation was observed between MFO activity and sediment concentrations in non-caged sanddabs, while a very good relationship was observed with the caged organisms. The authors suggest that natural populations avoided more contaminated areas, while caged organisms reduce the uncertainty about actual exposure conditions.

Larval survival was the primary endpoint in several studies conducted with striped bass (*Morone saxatilis*) to assess ecological health of spawning areas in the Chesapeake Bay area for anadromous fish (Hall et al. 1987, 1988, 1992, 1993). Hatchery-reared 24- to 48-h-old prolarvae were used in a series of 96-h exposures throughout the spawning season. Controls were housed in *in situ* chambers held in a 945-l circular tank, filled with purified groundwater with a salinity of 2 ‰ after addition of synthetic sea salts. Control tanks received a 50% water renewal daily. Five hundred prolarvae were exposed in each chamber, and chambers were deployed in replicates at each site. Chemical analyses were performed on composited aqueous samples and Datasonde units (HydroLab Corp.) were suspended from the frame of the *in situ* raft to collect water quality parameters. Significant mortalities were observed at the test sites, while control survival was high in all experiments. Acidic conditions and trace metals (Al, Cd, Cu, and Zn) were suspected to contribute to the observed toxicity at most sites, while some sites were likely impacted by sudden drops in temperature.

Pyle et al. (2001) deployed larval (< 24 h old) fathead minnows for 7 days to determine effects on survival and growth at sites surrounding a uranium mine in northern Saskatchewan, Canada. Exposures were conducted in lakes in water along the shoreline at depths of 1.0 to 1.5 m. Near-shore sites were chosen to conduct tests because they are easily accessible for deployment and monitoring, they are where fathead minnows would most likely be found, and physical stress due to wind and waves is minimized. Larval exposure and observation tubes (LEOT) were made of 10-cm sections of

PVC plastic tubes with an inner diameter of 7 cm. Each end was capped with 400-µm Nitex mesh. In order to protect the LEOT from debris and other sources of potential damage, they were tied inside clean plastic buckets (with the bases removed) that were secured to the lake sediment with wooden stakes.

Szal et al. (1991) used larval (8–14 days old) fathead minnow survival to assess the toxic effects of chlorinated wastewater both *in situ* and in laboratory exposures. Caged organisms were exposed for 24 h, and deployment was synchronized with the compositing of effluent samples for the laboratory studies. Laboratory experiments were 48-h, static exposures. Cages were made from two containers, one overlapping the other. The inner container held the minnows, and was constructed from a plexiglass tube with a 6.3-cm diameter, and 0.5-mm Nitex screen on both ends. The outer container protected the fish from possible flow-induced stress. Toxicity observed in the field was primarily attributed to chlorine, as unchlorinated effluent was not toxic in laboratory exposures. Variation among sites in chlorine toxicity may have been due to interactive affects among chlorine, ammonia, and stress due to low dissolved oxygen.

6.1.2 Fish – Bioaccumulation

Large tissue quantities render fish generally suitable for tissue residue analyses. Because bottom-dwelling fish accumulate sediment-related contaminants in their tissues, natural populations are often used as sentinels of environmental health. The migratory behavior of fish, spatial variability of sediment contaminant loading, and possible avoidance behaviors of non-migratory fish, however, make it difficult to make conclusions about their true exposure to natural populations (Rice et al. 1994). Even non-migratory fish can avoid contaminated sediment, due to its tendency to be patchy on relatively small scales. Exposure of transplanted caged fish for known periods in specific locations eliminates this uncertainty. The case studies summarized below are in freshwater, but in many cases are useful in designing studies using estuarine or marine fish.

Mac et al. (1990) described a bioassay for bioaccumulation of contaminants with adult fathead minnows (*P. promelas*) and earthworms, validating laboratory exposure with field tests. Minnows weighing between 2 and 4 g were used, providing adequate tissue for the required chemical analyses. Fish were kept in aluminum cages (100 x 52 x 32 cm) that were divided into four compartments similar in size to aquaria used for conducting simultaneous laboratory exposures. Dividers and walls of the apparatus were made of 7-mm mesh plastic netting that was attached to the frame with aluminum pop rivets. Access to the inside was through a hinged lid. Ten fish were loaded into each compartment just before deployment. Cages were placed by divers on the sediment surface and at mid-water depth to distinguish between uptake through the sediment and the water column. Exposures lasted 10 days, followed by a 2-day laboratory depuration process to eliminate sediment from the gut. Fish were not fed during the exposure, but weight loss was not expected to be an issue in this relatively short test. The authors noted that weight loss could be an issue with non-infaunal feeders in 28-day tests. Fathead minnows were selected over yellow perch (*Perca flavescens*) because they tended to bioaccumulate more (PCBs were the contaminants of concern), which could be important in sediments with low levels of contamination. Suggested explanations for the higher accumulation included behavioral differences between the two species (as minnows have a more intimate association with the sediment–water interface, resulting in more resuspension of sediment) as well as higher lipid contents in the minnows. Reference sediment exposures are important for providing assurance of test organism health, as well as allowing assessment of growth and lipid content changes that can affect tissue residues.

Jones and Sloan (1989) developed an *in situ* chamber for assessing bioaccumulation of hydrophobic organic contaminants by fish in the water column of relatively large streams. These chambers could hold large quantities of larval fish (i.e., 350 juvenile fathead minnows), providing ample tissue required for numerous subsamplings for determination of uptake kinetics and estimation of steady-state residue levels. The large quantity of fish also reduced variation among replicate samples. Cages were constructed from 50-l Nalgene carboys, with 1-mm flexible fiberglass mesh covering about one-third of the total surface area. The bottom portion of the vessel had a 20-l capacity so that fish would not have to be out of the water at any time during the deployment, subsampling, or recovery phases. A pour spout at the bottom was particularly useful for frequent subsampling of fish during the 28-day test periods, while minimizing stress on the fish. Extensive testing of the exposure chambers took place at both contaminated (high PCB concentrations) and uncontaminated (control) sites. A total of 250 to 350 juvenile (0.3 to 0.75 g each) fathead minnows (*P. promelas*) were loaded into each chamber, with three chambers deployed at each site. Cages were deployed 20 to 30 m from shore, 1 m below the water surface. Overall, survival of fish was 95%, with no significant difference among test sites. Fish weight and lipid content of fish did not drop during the exposure period, allowing the fish to accumulate contaminant residues comparable to levels found in resident fish.

Rice and White (1987) used 5- to 10-cm-long fathead minnows (*Pimephales promelas*) and 8-mm fingernail clams (*Sphaerium striatinum*) in caged experiments to monitor bioavailability of PCBs before and after dredging of the Shgiawassee River in south-central Michigan, and compared uptake to water column concentrations. Clam cages were fashioned from heavy wire screen, forming 10-cm-deep rectangular enclosures that were filled with Lake Michigan sand and about 200 clams. Cages were held in place by tying them to metal pipes driven into the sand. Approximately 100 fish were placed in triangular shaped boxes made of the same heavy wire screen. In some instances, the fish cages were compartmentalized to compare PCB uptake in fish that were in contact with the sediment with those exposed to only waterborne PCBs. Uptake of PCBs increased after dredging, but only on a localized scale. The fish were very sensitive indicators of changes in availability of the PCBs, even more than 6 miles downstream from the dredge site. Clam uptake seemed to reflect local conditions at the SWI, and was likely influenced by near-surface sediment and detritus. Unlike the fish, the clams were not a sensitive indicator of conditions more than 1 mile downstream. Differences in route of exposure explained the differences. Uptake rates and bioconcentration factors (BCF) for fathead minnows and fingernail clams were similar to those obtained in laboratory studies.

Martin and Black (1995) designed chambers for assessing exposure and effects to fish *in situ*. By altering the position of the cage floor, fish could be allowed contact or prevented from contact with the sediment, allowing exposure to the water column or the sediment. Cages were constructed entirely of plastic components (PVC frame and HDPE mesh) only to eliminate any potential for metal contamination associated with the cage materials. The sediment exposed cage design had dimensions of 4.7 ft in height and 4.2 ft in diameter, with a volume of 65.1 ft^3. The water-only exposure design was slightly smaller. These cages were designed to be able to stock up to 300 6- to 7-in. juvenile channel catfish, and have the potential to house demand feeders. The cages were tested for durability in shallow water environments, but can be modified for deeper water freshwater or marine deployments. No results were reported.

6.1.3 Amphipods – Toxicity and Bioaccumulation

Richter (2002) evaluated survival of the free-burrowing estuarine amphipods, *Eohaustorius estuarius* and *Rhepoxynius abronius* in field exposures in San Diego Bay. Volatile organic compounds (VOCs) had previously been detected in surficial sediment samples, and were attributed to groundwater migration from a nearby Navy waste site via seepage meter and porewater measurements (Chadwick et al. 1999). Both of these amphipods are routinely used in laboratory sediment tests for monitoring and research purposes on the west coast of North America. Amphipods were deployed in acrylic cages with 500-µm mesh screen covering cutouts on the sides and bottom (Figure 8). Three cages, each containing 30 amphipods, were embedded into the top 5 cm of sediment at five reference stations and five test sites for 10 days. No effects were attributable to VOCs, but survival as a whole was reduced, even at reference sites. Elevated levels of nonpolar organics were measured in sediment and porewater samples. Also noted was the presence of polychaetes and other potential predators inside the cages upon recovery of test chambers. It was suggested that smaller mesh size may be required to prevent entry of predators. Pretreatment measures such as freezing sediment within the cage were also evaluated. Shading cages during the exposure appeared to help reduce fouling of mesh windows.

Figure 8. Amphipod test chamber (without cover) used by Richter (2002).

Anderson et al. (1998, 2004) also recommended *E. estuarius* for *in situ* sediment assessments due to its wide salinity, temperature, and grain-size tolerances, and demonstrated sensitivity to contaminants (USEPA 1994b). Their chamber was constructed of a polycarbonate core fitted with 500-µm mesh screen that retained the amphipods but allowed adequate flow and dissolved oxygen saturation in the overlying water (Figure 9). The chamber had no bottom. Following deployment, pre-counted amphipods were released through a 20-ml syringe embedded into the cap. Control *in situ* chambers contained control sediment, and were deployed at both a reference and previously characterized contaminated site alongside chambers containing field sediment. Control survival was high (at least 84%) in the field, compared to 96% survival in concurrent laboratory controls, demonstrating the suitability of the approach, even in the presence of large temperature and salinity fluctuations that were observed in the field. Overall, *in situ* toxicity was greater than toxicity observed in both undisturbed and homogenized samples tested in the laboratory. The authors suggested that salinity and temperature shifts in the field may have been partially responsible for the observed lower survival *in situ*, and indicated that laboratory experiments with this species under variable environmental conditions are warranted.

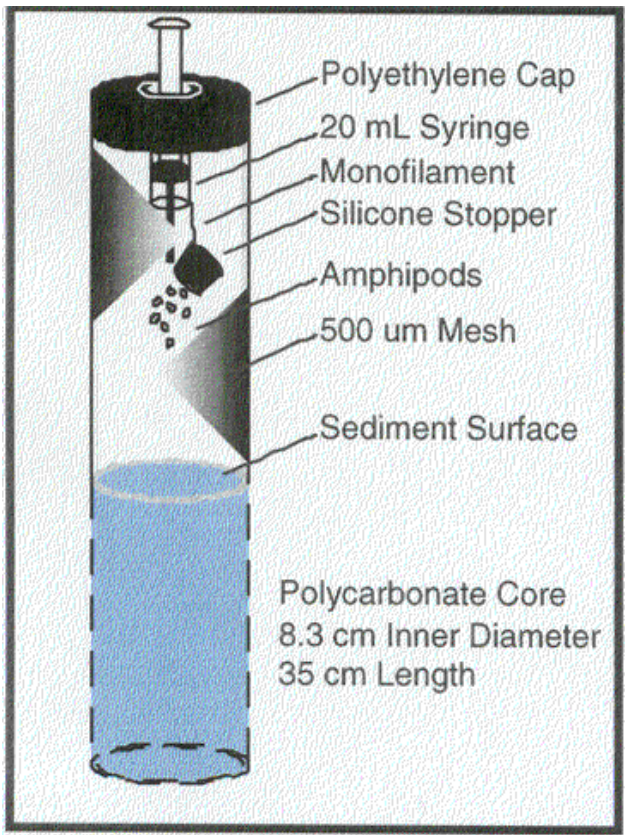


Figure 9. Amphipod *in situ* chamber used by Anderson et al. (2004). Diagram courtesy of B. Phillips, Marine Pollutions Studies Laboratory.

Kater et al. (2001) used an amphipod chamber similar to that of Anderson et al. (1998, 2004), with the exceptions that the core was made from PVC pipe and employed a coarser mesh (1 mm) for water exchange above the sediment. An aluminum field chamber held five chambers at the proper sediment depth and vertical orientation. The installation of the frame and chambers was accomplished with SCUBA diver support. Like the Anderson et al. (1998, 2004) chambers, these had no bottom, and amphipods were introduced through a syringe shortly after placement of the chambers at the test site. Exposures were for 10 days. Generally higher toxicity was reported *in situ* than in laboratory experiments with *Corophium volutator*, a marine amphipod commonly used for toxicity testing in Europe. They did not believe, however, that the increased toxicity in the field was due to caging or sediment homogenization, as these effects were tested both *in situ* and in the laboratory. Rather, they suggested that harbor activity, storms, and temperature fluctuations *in situ* may have contributed to the differences observed compared to the controlled laboratory experiments. They advised that *in situ* studies using this species be conducted at only certain times of the year.

DeWitt et al. (1999) tested cadmium-spiked sediments in the laboratory and *in situ*, and concluded that estuarine amphipod (*Chaetocorophinium* cf. *lucasi*) sensitivity in the laboratory was equal to or greater than *in situ.* Unspiked control treatments i*n situ* and in the laboratory performed similarly (>90% survival), even though conditions at the intertidal study site were physically volatile (i.e., extreme changes in temperature, wave action), while laboratory exposures were strictly controlled with respect to physical parameters.

Rosen and Lotufo (in preparation) exposed *Eohaustorius estuarius* and *Leptocheirus plumulosus* for 10 days in aquaria consisting of bedded sediment and Composition B explosives. The amphipods were contained in chambers used by Burton et al. (2005) and shown in Figure 7, with the aquaria being held under different flow rates. Multiple other species and differing placement locations of the Composition B fragments were included in the study in order to simulate a variety of potential real-world conditions. *E. estuarius* recovery from control chambers was high, but some reduced recovery of *L. plumulosus* was noted. Bioconcentration factors for mussels, amphipods, and polychaetes were reported to be similar to those previously observed in static laboratory exposures. This study concluded that a number of marine species commonly used in ecological risk assessments in laboratory exposures also showed potential for caging, and therefore, useful in field deployments.

Lavoie et al. (2001) used the freshwater amphipod *Hyallela azteca,* as well as the cladoceran *Daphnia magna,* in caged exposures as part of an integrated assessment for evaluating stormwater impacts. The study included chemical analysis of sediment and water, habitat, benthic invertebrate indices, fecal coliforms/*E. coli* presence, and toxicity testing *in situ* and in the laboratory. Abiotic conditions (e.g., scouring from high flow, increased total suspended solids [TSS]) were thought to be contributors of toxicity rather than stormwater chemical inputs.

6.1.4 Mysids

The feasibility of employing mysid shrimp (*Americamysis bahia*, formerly *Mysidopsis bahia*) (Figure 10) in caged studies has been evaluated (Clark et al. 1986, 1987, Comeleo et al. 1990, Comeleo 1991). In one effort, survival, growth, and fecundity were measured following 7-day exposures in the field at clean and polluted sites off the New England coast (Comeleo 1991). Field studies were complemented with laboratory tests using grab samples of receiving water collected at each site. Cages were constructed out of polycarbonate core liners with a 2.75-in. diameter, and had a volume of 200 ml. Attached to each end were removable covers affixed with 500-µm Nitex mesh screen for water exchange. Cages were deployed in a plexiglass triangular frame that supported 10 replicate cages. The frame was held 1 m below the surface with trawl floats and cinder block anchors. Step-by-step instructions for assembly of these cages are available (Mueller et al. 1992).

Figure 10. *Americamysis bahia* (mysid shrimp).

Notable results were very high survival at control sites for both field and laboratory tests, suggesting that mysids were amenable to caging. Variability in growth and fecundity may have been related to patchiness of zooplankton concentrations observed; however, food availability was not insufficient at any site tested due to the relatively large mesh size. One problem encountered with the exposure was fouling of the cages by diatoms. This resulted in required cleaning of cage mesh with a brush every 48 h. It was suggested that these studies be conducted during the less productive summer season, which also ensures high enough temperatures for this subtropical species. Temperatures below 10 °C and salinities below 20‰ were not tolerated by *A. bahia*.

Clark et al. (1987) used caged *A. bahia*, as well as three other estuarine species, to evaluate the acute, lethal effects of an organophosphate insecticide (fenthion) during prespray, spray, and post-spray periods. Mysids were contained in conical, double-layered nylon mesh cages (363-µm mesh on the inner layer, 1,000-µm outer layer) or in floating cylindrical cages with 450-µm nylon mesh. Exposures at four field sites ranged from short term (12 h or less) of rapidly decreasing fenthion concentrations to extend intervals (more than 72 h) with slowly increasing or decreasing fenthion concentrations. Laboratory-derived LC50s provided a reliable benchmark for predicting acute (lethal) effects of fenthion on caged animals in the field when exposures persisted for 24 h or more but overestimated the toxicity for exposures of less than 24 h. Laboratory pulse-exposure tests with

rapidly changing concentrations for 12 h were predictive of the nonlethal and lethal effects observed for short-term field exposures.

The mysid *Holmesimysis costata* is indigenous to the eastern Pacific and is an EPA recommended species for the testing of west coast effluents (USEPA 1995b). It is more sensitive to a number of contaminants as compared to other crustaceans (Anderson et al. 1994). Its use *in situ* was not found in the literature, but several factors render it potentially difficult species to use for field studies (Chris Stransky, personal communication). *H. costata* requires field collection, and availability at any given time depends on the presence of surface kelp canopy and availability of gravid adults. They are normally gravid year round, but they do show some seasonality effects, with greatest fecundity found during the spring and summer months (Turpen et al. 1994) After collection, gravid adults must be separated and held for several days while they produce offspring suitable for testing purposes. Attempts to keep laboratory cultures long-term have been unsuccessful both at a number of specialty culture facilities. In addition, control performance issues have also been noted among all known west coast labs that have used this species. Another west coast mysid *Mysidopsis intii* that can be cultured in the laboratory (Langdon et al. 1996) and appears to be reliable in short-term toxicity testing (Harmon and Langdon 1996) may have utility in west coast *in situ* exposures.

6.1.5 Bivalve and Echinoderm Larvae

Bivalve and echinoderm embryo-larval development tests are among the most sensitive of the early life stage tests, particularly to metals (Table 3) and are widely used in the laboratory for the assessment of whole effluent, water column, or sediment toxicity, (USEPA 1995, Carr et al. 1996a,b, Anderson et al. 1998, His et al. 1999, Beiras et al. 2001). The preferred endpoint for these tests is normal larval development, which is reported as the proportion of embryos achieving the desired developmental stage (e.g., pluteus for echinoderms, hinged D-shape for bivalves), generally requiring an exposure period of 48 to 96 h (Figure 11). Only recently have there been reports of using these methods in field exposures (Anderson et al. 1998, Beiras et al. 2001, Geffard et al. 2001), all of which indicate feasibility of conducting these tests *in situ*. Proportionately more reports of field studies appear to be available for bivalve larvae, probably due to their general tolerance to a wide range of salinities and temperatures, lack of feeding requirements, simple and inexpensive test set up, short exposure time, and sensitivity to a wide range of pollutants. Drawbacks include sensitivity to natural factors (i.e., ammonia, wave action) and the lack of spawnable adults during some parts of the year for some species.

Figure 11. Larval stages of mussel (above) and echinoderm (below).

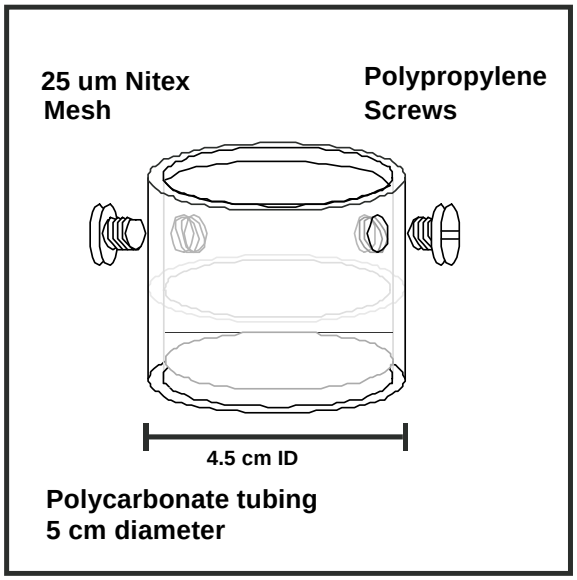


Figure 12. Drum cage used to house bivalve or echinoderm larvae in the field. Drawing courtesy of B. Phillips, Marine Pollution Studies Laboratory.

Caged mussel, oyster, and sea urchin embryos have been used to assess toxicity in receiving waters (Beiras et al. 2001, Geffard et al. 2001, Katz et al. 2006) and at the sediment–water interface (Anderson et al. 1998). Anderson et al. (1998) experimented with these tests both in the field and in the laboratory. Embryos were deployed in drum-shaped cages made of polycarbonate tubing (5-cm diameter) affixed with 25-µm Nitex mesh on both sides, resulting in a design that was 44% screened surface (Figure 12). Polypropylene screws on two sides of the chamber allowed for easy cleaning as well as addition and removal of test organisms. Ten chambers pre-loaded with embryos were then placed in polypropylene mesh bags and deployed at the sediment–water interface by attaching the bags to PVC stakes. Simultaneous laboratory comparisons using sediment–water interface exposures (see SWI exposures below) were made with water collected from the field site.

In addition to laboratory comparisons, travel control chambers were also taken to the site and then tested in the laboratory. High salinity fluctuations (15 to 22‰) and temperature ranges (12.4 to 30.2 °C) were cited as possible causes of low success in initial trials with *Mytilus* larvae. Subsequent trials were successful, however, with less toxicity generally observed in field exposures. It was suggested that greater dilution of fluxed chemicals from the sediment was likely in field exposures compared to static laboratory exposures. Laboratory tests also indicated lower pH values, which may have increased the bioavailability of contaminants, particularly metals. Finally, sampling for intact sediment cores to be used in the laboratory took place over a wider spatial area than the *in situ* exposures, possibly resulting in somewhat altered exposures between the two test types.

A few problems associated with the *Mytilus* embryo exposures conducted by Anderson et al. (1998) were reported. First, initial trials revealed apparent toxicity associated with the acrylic glue used to secure the mesh screen to the core tubing. Curing for 1 week at 40 °C followed by extensive leaching for several days with flowing seawater resolved the problem. The small mesh and cage size also required alternative cleaning measures, which included leaching in flowing seawater for two days after cleaning. Finally, it was suggested that some experiments where surge and wave action was high could have been responsible for the lack of embryo development.

Phillips et al. (2004) also used the drum cage design in field evaluations of the effects of exposure of pesticides in an agricultural watershed to freshwater daphnids (*Ceriodaphnia dubia*). Results were characterized by high survival in field exposures at upstream sites and complete mortality at stations downstream of the chemical inputs. Water quality inside the drums was satisfactory for the test species, while pesticide concentrations exceeded published thresholds. They noted that the drums probably reduced water flow and allowed particles to settle inside them. Toxicity identification evaluations (TIEs) confirmed, however, that removal of the particles still resulted in concentrations of organophosphates above those required for mortality. Therefore, toxicity was not attributed to the presence of the particles.

Katz et al. (2006) used the drums developed by Anderson et al. (1998) in a “floating” bioassay in an effort to quantify acute and chronic effects of stormwater as it mixed with receiving water in San Diego Bay, CA. Water was pumped onto a research vessel that housed the drums with *Mytilus* larvae, as well as fish and mysid shrimp, in flow-through 400-ml polypropylene beakers. The vessel was anchored within a few feet of the stormwater discharge pipe. Although copper was elevated above ambient water quality criteria (which are driven by *Mytilus* embryo toxicity thresholds), no toxicity was observed to the embryos or the other organisms over an exposure of up to 4 days. This contrasted with toxicity that was observed in grab samples taken from the end of the discharge pipe and tested in the laboratory. It was suggested that the more realistic exposure results were more relevant to the protection of organisms residing in San Diego Bay.

In another study, Geffard et al. (2001) reported that *Crassostrea gigas* (Pacific oyster) and *Mytilus galloprovincialis* (Mediterranean mussel) embryos were good *in situ* test organisms due to their high sensitivity to pollutants and the fact that they are euryhaline, tolerating salinities as low as 20‰. Field demonstrations used 1-l chambers made of low-density polyethylene with 30-µm mesh on both ends. Mesh was made of polyamide gauze and was applied by heat-soldering, as a number of glues tested were found to be toxic. Water retention time in the containers was estimated at 15 minutes by conducting a dye study. The authors concluded that the mussels are suitable for year round biomonitoring, but the oyster is limited seasonally by the need for warm water (i.e., at least 20 °C) for spawning inducement and embryonic development. Other research confirms the wide range of temperature and salinity tolerances for the mussels and oysters, with the mussels only being negatively affected at temperatures around 30 °C (His et al. 1989). Longer periods (i.e., 4 days), however, may be required for completion of embryogenesis at reduced temperatures in mussels. Feeding of test organisms was deemed a non-issue, as the long-term survival of bivalve larvae is well beyond the short exposure period (Bayne 1965, Masson 1977).

Laboratory testing with bivalve embryos typically takes place in 20- to 30-ml glass vials (USEPA 1995). Simple modifications to the screw caps that come with these vials (e.g., replacement of the solid top with 25- to 30-µm Pecap mesh) results in a suitable, inexpensive, and compact *in situ* chamber that allows easy laboratory enumeration of the developed larvae upon recovery from the field site. The lack of any requirement to transfer larvae to a secondary chamber for microscope counting also minimizes the risk for losing larvae, which is important for survival determinations. Rosen and Lotufo (in prep) have successfully demonstrated the use of this approach in exposures at the sediment–water interface in microcosm exposures designed to mimic field exposure.

Beiras et al. (2001) found sea urchin (*Paracentrotus lividus*) embryos to be good indicators of pollution in field monitoring studies in the marine environment. Fertilized eggs were placed in 50-ml cages with 20-µm filtered seawater collected from the study site. Cages were subsequently tied to weighted ropes and placed at a depth of 2 m by divers at low tide. After 72 h, proportions of four-arm pluteus larvae were calculated, as well as larval length. The study differentiated between well-known polluted and unpolluted sites. Temperature and other natural factors concerned the authors, though exact details were not provided as to why.

Spawning seasons for individual species direct the ability to use these tests. Approximate spawning periods are provided in Table 2; however, these dates vary to some extent depending on collection location.

6.2 TRANSPLANTED BIVALVES

In situ bioassays that use transplanted bivalves (Figure 13) combine the experimental control of laboratory testing and the environmental realism of field testing (Green et al. 1985, Salazar and Salazar 1995). Transplanted bivalves are good for biomonitoring studies because they readily accumulate a wide variety of contaminants. Metals (Mueller et al. 1992, Malley 1996, Beckvar et al. 2000), chlorinated hydrocarbons including DDT and PCBs (Green et al. 1986), polycyclic aromatic hydrocarbons (Salazar and Salazar 2007), and volatile chlorinated hydrocarbons such as trichloroethylene (TCE) and tetrachloroethylene (PCE) (Saisho et al. 1994) have been measured in bivalve tissues.

In addition to bioaccumulation, other endpoints ranging from survival to sublethal effects such as growth or biochemical responses (biomarkers) can be used to characterize both exposure and associated biological effects. Salazar and Salazar (1995, 2000, 2007) have used transplanted mussels to assess contaminant exposure via tissue residues and compared the results to mussel growth. Such measurements are difficult with resident organisms due to the uncertainty of the exposure period and lack of experimental control. By taking measurements repetitively, temporal and spatial variability can be determined. For example, the source of contamination (i.e., sediment, surface water) may be determined by comparing tissue residues of transplanted animals at various depths.

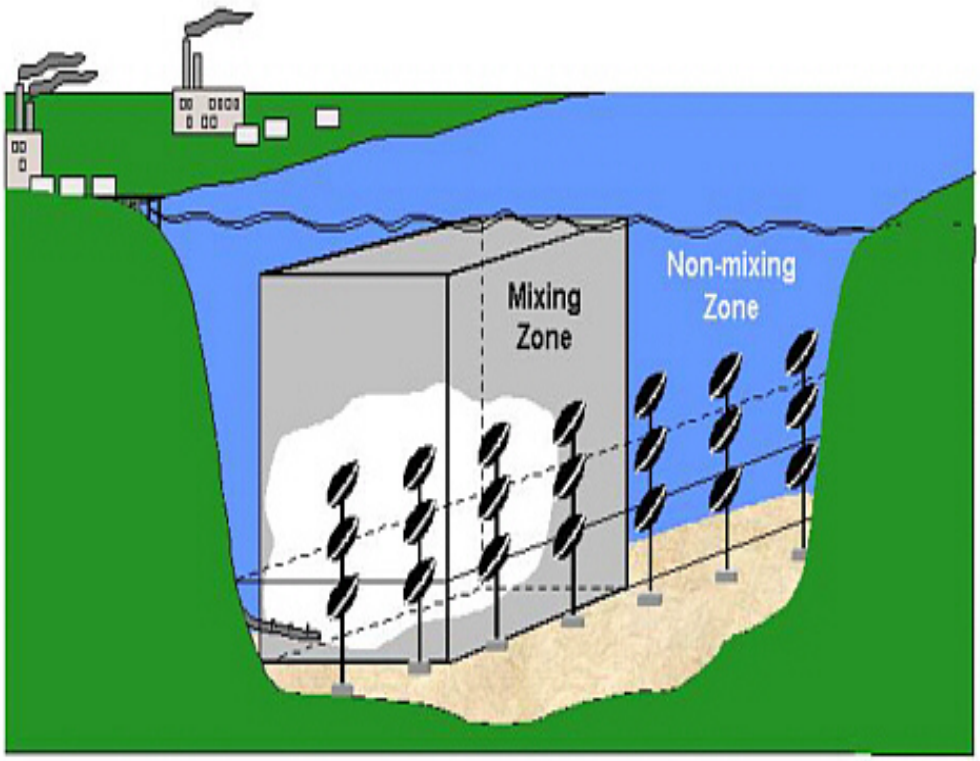


Figure 13. Illustration of transplanted bivalves as a means of evaluating exposure and effects over space and time. Illustration from www.appliedbiomonitoring.com.

Advantages attributed to bivalves for *in situ* exposures include that they are sedentary, easy to handle, cage, and measure. They also concentrate bioavailable contaminants at levels orders of magnitude higher than in water or sediment and are very tolerant of caging and poor water quality. Bioaccumulation is a more direct link between exposure and bioeffects than chemical measurements in water or sediment. The growth endpoint is a sensitive sublethal response that is easily quantified. Extensive guidance for *in situ* use of transplanted bivalves is available (ASTM 2003, Salazar and Salazar 2007). Salazar (1989) has also documented that mussels survive better in the field and microcosms than they do under laboratory test conditions. In the case of tributyltin (TBT) exposures in San Diego Bay, he found that despite higher toxicant concentrations and longer exposure periods, higher stress in laboratory tests resulted in higher mortality. He noted that unnatural diet and unrealistic test water are common to laboratory tests, while suspended particulates, sediment type, colloids, and dissolved organics affect bioavailability in both the laboratory and the field and are nevertheless rarely measured. He also cites studies conducted by Clave et al. (1986), Stang et al. (1989) and Zirino et al. (1978) who report variability in exposures up to a factor of 20 associated with tidal cycles in the study area, emphasizing the problem of adequate characterization of exposure conditions in the field.

Because juvenile clams and mussels tend to grow faster relative to older specimens, statistically significant growth of bivalves can be measured in *in situ* exposures of as little as 7 to 10 days (Ringwood and Keppler 2002, Bartsch et al. 2003). Standard caged bivalve exposures, however, typically last for 30 to 90 days due to the time required for some hydrophobic organic contaminants to reach steady-state in bivalve tissue (ASTM 2003).

There are some potential problems associated with the use of bivalves in bioaccumulation bioassays. One problem is that accumulation, growth, and animal health depend on filtering rate, which may be reduced in certain sediment types or water conditions that are not due to contamination (Mac et al. 1990, Salazar and Salazar 2000). In addition, different species have different ventilation rates, affecting bioaccumulation potential (Malley 1996). Another consideration when using these approaches for routine monitoring is that some desired species are difficult to culture, forcing collection of specimens from existing stocks and potentially placing pressure on natural populations (Malley 1996). Mac et al. (1990) also suggested that the general lack of predation by fish on hard shell clams, particularly larger ones, reduces their ecological significance in the aquatic food chain.

One of the characteristics of a good bioaccumulation test organism is a low capacity to metabolize the contaminant(s) of concern (USEPA and USACE 1998). Some commonly used bioaccumulation test organisms, however, have been reported to metabolize PAHs, for example, to a high degree. Rust et al. (2004), for example, reported much higher rates of benzo[a]pyrene metabolism for the large polychaete *Nereis virens* compared to other polychaete species, bivalves (e.g., *Macoma nasuta*, *Macoma balthica*) and amphipods.

Only one study involving transplanted bivalves and groundwater–surface water interactions was identified. Boneillo and Gobler (2001) observed reduced growth rates in clams deployed near groundwater plumes compared to clams located at intermediate distances. The authors suggested that groundwater seepage may have affected not only clam growth, but also upper trophic levels in North Sea Harbor, NY. The reason for this suggestion was unclear. In the laboratory, Saisho et al. (1994) measured bioaccumulation of several VOCs in mussels (*Mytilus edulis*) and killifish (O*ryzias latipes*). Trichloroethylene (TCE) bioconcentration factors (BCF) were 4.52 and 2.71 for the mussel and fish, respectively. Tetrachloroethylene (PCE) bioaccumulation was relatively high with BCFs of 25.7 and 13.42. This suggests that these VOCs are of greater trophic risk, depending on the route of exposure, in mussels. As discussed earlier, however, bioaccumulation of the VOCs is not expected to

be significant due to relatively low bioaccumulation potential (low log K_{ow}s) and the volatile nature of these compounds.

6.2.1 Polychaetes

Polychaetes such as *Neanthes arenaceodentata* (Figure 14) may be appropriate for *in situ* surficial sediment exposures due to their sensitivity to anthropogenic contaminants but lack of sensitivity to nontreatment factors such as sediment grain size, ammonia, hypoxia, and hydrogen sulfide (Dillon et al. 1993, and references therein). Standardized laboratory toxicity tests with this species typically use survival and growth as measures of toxicity (USACE and USEPA, 1994). The utility of a growth endpoint in field exposures, however, may be problematic due to the differences in food quality among different sediments and the fact that feeding specified rations to field organisms (as is done in laboratory testing) might be logistically challenging.

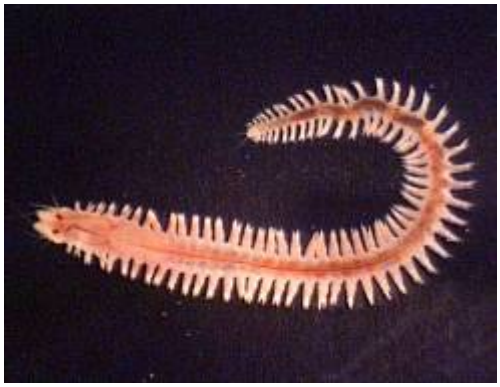


Figure 14. The marine polychaete *Neanthes arenaceodentata.*

Another sublethal endpoint, post-exposure feeding, might be more suitable for short-term *in situ* use of marine polychaetes. Moreira et al. (2005) reported success with such exposures with the polychaete *Hediste (Nereis) diversicolor*. The study involved observations of feeding rate on *Artemia* (brine shrimp) nauplii for 1 hr following 48 h *in situ* exposures in surficial sediment, and indicated significant effects on feeding rate in contaminated sediment-exposed worms compared to worms exposed to reference sediments. Feeding rate was also substantially more sensitive than survival in laboratory exposures to copper. Because temperature and salinity affected feeding rate on *H. diversicolor*, regression equations were developed to derive "adjusted" feeding rates that factor in these parameters for better interpretation of resulting data.

Post-exposure feeding rate is also currently being examined with *N. arenaceodentata*, which would provide a North American relevant species alternative to *H. diversicolor*. Initial results from spiking studies and field deployments modeled after those by Moreira et al. (2005) suggest that this species is amenable to *in situ* exposure and that the endpoint is considerably more sensitive than lethality (Miller and Rosen, in preparation). Janssen et al. (in review) also reported successful use of *N. arenaceodentata* in the field, with high recoveries after 14 days of exposure for PCB uptake evaluations in the presence of activated carbon amended sediments.

The sediment burrowing polychaete *Dinophilus gyrociliatus* has also been used in laboratory-based surface water, porewater, and effluent investigations (Carr et al. 1986, Carr et al. 1989, Nipper et al. 2001). This test utilizes 1- to 2-day-old juveniles that are 0.1 mm in size, requiring the aid of a microscope, but also allows testing with small sample volumes (10 ml per replicate). Test endpoints typically evaluated are survival and/or reproduction in exposures of 4 to 10 days. These organisms tolerate a fairly wide salinity and temperature range (Carr et al. 1986, and references therein), but their use in field studies has not been reported.

6.2.2 Other Invertebrates

Crane et al. (2000) designed an *in situ* system for testing sediment without overlying water interference, by installing PVC pipes into sediment long enough to come above water surface. Water

inside the pipe was removed and replaced with laboratory dilution water used for culturing test organisms. The midge *Chironomous riparius* was successfully tested with this exposure. Test organisms were labeled with a droplet of paint to differentiate from indigenous organisms. Retrieval of *in situ* systems was achieved either by rocking back and forth and removing complete with a sediment core (firm sediments), or by placing a gloved hand underneath the core to retain the sediment core in place (soft sediments). Indigenous predators posed a problem by reducing survival of chironomid larvae, and the authors stressed the importance of recording the presence of predators to aid in data interpretation.

Pereira et al. (1999) developed and extensively validated an *in situ* chamber and methodology for standard freshwater test organisms (i.e., zooplankton species such as *Ceriodaphnia dubia* and *Daphnia magna*). Exposures were conducted in a river system adjacent to an abandoned mine that has resulted in heavy metal contamination and acidic conditions. Water column and solid phase field exposures were compared with simultaneously conducted controlled studies in the laboratory. *In situ* chambers were constructed from 50-ml polypropylene jars with caps, with three 20-mm cutouts covered with 50-μm nylon mesh. Mesh covered two sides of the jar and the cap. Chambers rested on the sediment surface cap-side down to allow exposure to the sediment.

Greenberg et al. (2002) conducted *in situ* toxicity and bioaccumulation tests with four freshwater invertebrate species in a river where chlorobenzene contamination was known to be present via groundwater upwelling. Survival data did not correlate well with porewater chemistry, but when evaluating the chemical and toxicity data in combination with hydrologic data (which revealed upwelling or downwelling conditions at each site), the exposure-effects relationships became clear.

6.3 SEDIMENT–WATER INTERFACE (SWI) EXPOSURES

Sediment–water interface (SWI) tests are laboratory-based toxicity bioassays that offer some of the advantages of *in situ* bioassays (i.e., increased realism). As with *in situ* toxicity testing, organisms can be exposed at the interface via an intact, unmanipulated sediment sample (Anderson et al. 1996, 2001) (Figure 15). Samples collected by coring are more effective at maintaining sediment integrity than more destructive measures used for grab sampling (Burton 1995). Although some of the realism of true field studies is lost with this exposure, much of the control that is obtained by conducting tests in the laboratory is maintained. In addition, like field studies, this exposure is useful in assessing effects due to contaminants fluxed out of the sediment. An optimal experimental design might include both *in situ* and SWI laboratory exposures, thus allowing for a weight-of-evidence scenario. For example, the laboratory studies might aid in characterizing sediment contaminants as toxicants by eliminating one or more stressors (i.e., contaminant exposure due to tidal pumping, dissolved oxygen reduction, temperature fluctuations), providing an opportunity to make better interpretations of the field data. Sediment–water interface tests also eliminate or reduce the potential for confounding effects associated with porewater testing, and are often more ecologically relevant exposures compared to porewater testing, particularly to early life stages of many epifaunal and water column organisms (CEPA, 2003).

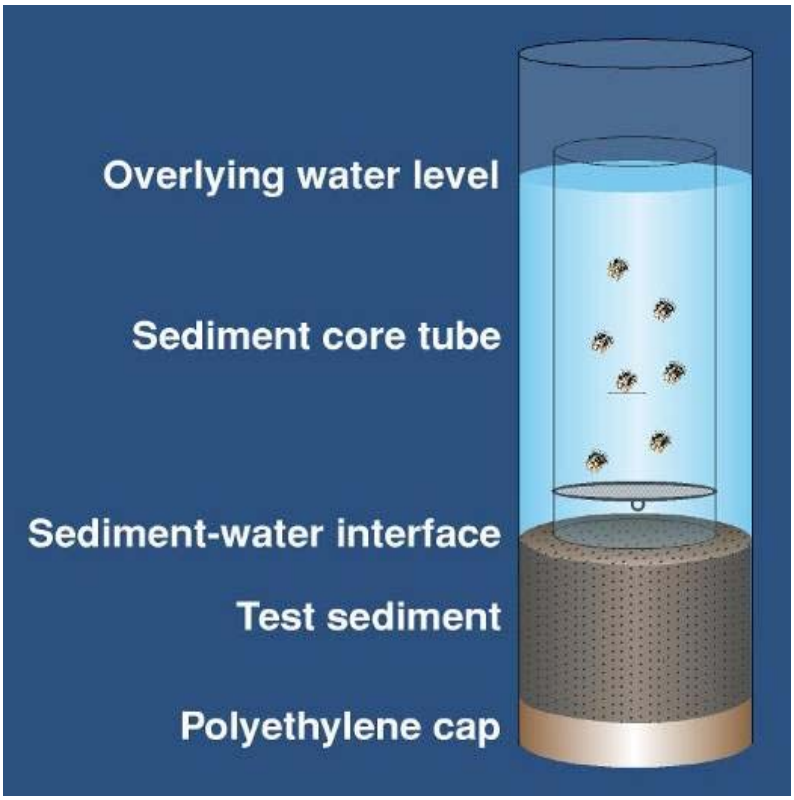


Figure 15. Sediment–water interface exposure system, based on method developed by Anderson et al. (1996).

The SWI exposure system consists of a 7.3-cm (I.D.) polycarbonate core tube cut to a length of 20 cm. A polyethylene cap may be fitted onto the bottom to contain the sediment. A screen tube with an I.D. of 5 cm is fitted with 37-µm (or other appropriate size) mesh on one end, and holds test organisms about 1 cm from the bottom of the tube. A hole is drilled just under the mesh to prevent air from being trapped under the screen. The SWI exposure was originally designed for use with embryos of sea urchins and mussels. This has been expanded to include mysid shrimp and other small invertebrates (Bryn Phillips, personal communication). Mysids are epibenthic dwellers that typically prefer to remain stationary, passively capturing plankton in a current. Hence, SWI provides more realistic exposures than water-only tests, but still lack flow conditions that generally cannot be achieved in a laboratory setting. *In situ* SWI exposures have been conducted with fish embryos (*Atherinops affinis* and *Menidia beryllina*), and they demonstrated successful hatching success in a range of salinities and temperatures (Jelinski and Anderson 1996).

Bivalve and echinoderms embryos are also used in an alternative laboratory exposure scenario to estimate effects associated with contaminated sediments (PSWQA 1995). In these tests, 18 g (wet weight) of sediment is mixed with 900 ml of seawater, and embryos are added after a 4-h settling period. Larvae are then poured off with the overlying water and subsampled for normal development and survival assessment, as compared to controls. The guidance acknowledges that the survival endpoint has error associated with it due to the nature of the test. Modifications to this protocol, however, may render it appropriate for *in situ* testing.

6.4 POREWATER TESTING

Because porewater, or interstitial water, is a major route of exposure to some sediment-dwelling organisms (Whiteman et al. 1996) and often contains the most bioavailable fraction of contaminants (Swartz et al. 1990, Skalski et al. 1990), porewater toxicity testing has been used to provide additional evidence in ecological risk assessments (Carr and Nipper 2003). Theoretically, conducting such tests *in situ* should be preferable to laboratory tests due to chemical alterations caused by the porewater extraction process. Porewater pH can increase as much as 1 to 2 units by the time the sample is removed from the field to a test container due to sampling and mixing procedures that allow the pH to equilibrate with atmospheric carbon dioxide concentrations (Ho et al. 1999). This pH change can have dramatic consequences on the bioavailability and toxicity of some contaminants. Oxidation of anoxic or suboxic samples due to gas exchange between the porewater and the atmosphere, atmospheric loss of volatile compounds, and sorption of hydrophobic organic compounds to filters, centrifuge tubes, or even *in situ* porewater samplers also can alter porewater chemistry (Burton 1996, Burton et al. 2000, Carr et al. 2001, Carr and Nipper 2003). It has been suggested that some constituents such as dissolved inorganic carbon, ammonia, sulfide, and sulfate might not be affected during the extraction process if the processing occurs in an inert atmosphere (i.e., argon, nitrogen, helium, carbon dioxide) which should prevent the oxidation of reduced chemicals (Burton 1992).

The fact that most porewaters are anoxic in the field presents challenges for conducting bioassays with organisms that require oxygen. Many porewater tests utilize organisms and life stages that are pelagic or epibenthic (CEPA 2003), which reduces the ecological relevance of such tests. Another criticism of porewater tests is that they reduce or eliminate the sediment ingestion route, which can be a major route of contaminant exposure (CEPA 2003). Field porewater exposures should ideally utilize organisms that are infaunal, including certain amphipods and polychaetes. It is key to measure physico-chemical characteristics and chemical concentrations of porewater, as opposed to relying on bulk sediment concentrations, in order to more accurately interpret porewater toxicity data in laboratory studies (CEPA, 2003), and this is equally true of potential *in situ* porewater studies.

Only very limited *in situ* porewater toxicity testing attempts are reported in the literature. An equilibration requirement, the need for on-site acclimation of test organisms, and difficulties associated with deployment and retrieval of porewater chambers are potential limitations of conducting such toxicity tests in the field (Carr et al. 2001). Fisher (1991) used peepers (dialysis chambers used to collect interstitial water samples *in situ*) as exposure chambers for *in situ* toxicity testing with the water flea *Daphnia magna*. Skalski et al. (1990) modified the dialysis chamber design to better accommodate test organisms by using plastic chambers covered with polymer mesh screens ranging in size from 0.15 to 0.50 mm (Figure 16). Chambers were used for 7-day chronic exposures with *Pimephales promelas* (fathead minnow) larvae and 48-h acute tests with *Daphnia magna* (water flea). Porewater exchange within test chambers was reported to reach equilibrium in 24 to 48 h. High mortality was observed in fine-grained sediments with high organic matter, where anoxic conditions or unionized ammonia existed, confounding results. Good survival was noted, however, in sandy sediments where dissolved oxygen levels remained high. These observations suggest the influence of abiotic factors and indicate *in situ* porewater toxicity testing may not be inappropriate for species not adapted to such conditions.

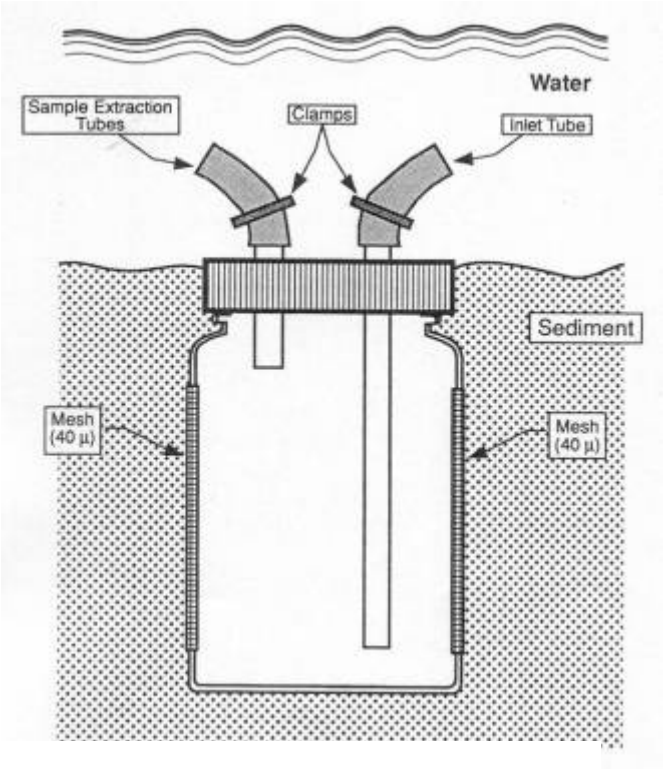


Figure 16. Porewater toxicity test chamber used by Skalski et al. (1990).

To our knowledge, no marine *in situ* porewater toxicity studies have been reported to date, but could be worth investigation. Typical organisms used in lab-based porewater tests include polychaete larvae, sand dollar embryos, bivalve larvae, and sea urchin sperm, embryos, or larvae (Luoma and Ho 1993). Such species are ideal due to minimal test volume and short exposure period requirements, as well as sensitivity to pollutants. Porewater tests with these species have become quite routine for TIEs due to the numerous inherent challenges working with complex solid-phase matrices. As stated above, however, ecological relevance and sensitivity to the effects of ammonia, hydrogen sulfide, and low dissolved oxygen needs to be considered when selecting organisms for field use.

7. SCREENING TOXICITY TESTS

Screening-level toxicity tests are typically conducted in a laboratory or on-site and can be useful for quickly mapping the extent of contamination at contaminated sites, allowing for the majority of resources to be focused on the locations of most interest within a site. These types of tests are typically rapid and can be conducted at relatively low cost and in high numbers. A quick turnaround time is useful for decision making with respect to subsequent steps in the assessment of ecological risk at a site. In addition, rapid tests can quickly alert dischargers or monitoring agencies of potentially toxic conditions (Dutka et al. 1983, Codina et al. 1993). Because screening toxicity tests are often field portable, they share a place with other *in situ*-based toxicity testing strategies in a weight-of-evidence based approach. Presented below are short discussions of a subset of available rapid toxicity tests that were considered as part of a current project (SERDP #ER-1550) to rapidly and accurately characterize toxicity at sediment sites. These tests can be conducted on surface waters or interstitial waters collected *ex situ*, or *in situ* using devices such as the Trident Probe (SSC San Diego 2003).

7.1 SEA URCHIN FERTILIZATION TESTS

Standardized toxicity testing using echinoderms (Bay et al. 1993, USEPA 1995b) has focused on embryo-larval development or fertilization success endpoints for sea urchins (e.g., *Strongylocentrotus purpuratus, Arbacia punctulata, and Lytechinus pictus*) and sand dollars (e.g., *Dendraster excentricus*). Embryo-larval development tests have been discussed above in detail (see Section 6.1.5). The fertilization success endpoint refers to the percentage of eggs that develop fertilization membranes following 20 minutes of exposure to sperm that have been previously exposed to test samples (also for 20 minutes). These life stages are ecologically relevant because of their tendency to be negatively buoyant, and therefore, are likely to be associated with surficial sediment (Anderson et al. 1996). These short-term exposures tend to be as sensitive as similar short-term chronic methods for other marine invertebrates and fish (Bay et al. 1993), but appear to be somewhat less sensitive than embryo-larval development tests for many contaminants (Bay et al. 1993, Losso et al. 2004). Table 3 shows the sensitivity of this endpoint relative to other common test methods.

These tests are characterized by small sample volumes (5-10 ml per replicate), short exposure period (< 1 h), sensitivity to a variety of anthropogenic contaminants, and high ecological relevance, when the route of exposure is expected to be the water column or surficial sediments. Although short in duration, however, extensive preparation time and microscope work is required, thereby increasing costs and time to obtain results.

7.2 MICROTOX®

The Microtox assay measures the effects of contaminants on light production of bioluminescent bacteria, *Vibrio fischeri* (formerly referred to as *Photobacterium phosphoreum*). Light is emitted as a result of a metabolic pathway that is intrinsically linked to cellular respiration, so disruption of normal cellular metabolism causes a decrease in light production. The inhibition of luminescence represents inhibition of electron transport systems, basic processes found in all organisms (Ringwood et al. 1997). The test can be used on water, elutriates, and solid-phase samples. Solid-phase samples, however, are reportedly influenced by the sediment grain size, with lower light output corresponding with higher percent silt-clay (Ringwood et al. 1997). Dose response data for single chemicals

indicate that Microtox is moderate to low in sensitivity to common contaminants compared to other test methods (Table 3). Another criticism of the Microtox test stems from concerns with respect to the ecological relevance of basing decisions of risk on toxicity to a marine bacterium.

7.3 QWIKLITE

QwikLite is a 24 h rapid toxicity test involving the use of marine bioluminescent dinoflagellates (Figure 17; Lapota et al. 2007, Rosen et al. 2008). A reduction in bioluminescence relative to a control is the endpoint. This Navy-developed test is similar to Microtox in that a photomultiplier tube (PMT) can been used to quantify light output, but QwikLite uses a higher level organism. Unlike bacteria used for Microtox, bioluminescent dinoflagellates emit light only upon mechanical stimulation. Therefore, a mechanical stirrer is used to agitate the contents of exposure units for a specific period of time using a continuous voltage in prototype versions of the test. Because dinoflagellates are a large component of phytoplankton communities in the ocean, bays, and estuaries, and form the basis of the food chain, they have high ecological relevance. The test requires small sample volumes (< 3 ml/replicate) and requires little labor to set up. Exposures are typically 24 h, but can be shorter or longer if desired. Dinoflagellate cultures require little maintenance. Some species (e.g., *Pyrocystis sp.*) are also fairly tolerant of handling and traveling. *Lingulodinium polyedrum* is particularly sensitive to metals, but is also less tolerant of physical stress and is susceptible to confounding effects associated with ammonia (Rosen et al. 2008). Therefore, alternate species such as *Ceratocorys horrida* and *Pyrocystis sp.* are more likely appropriate for toxicity screening of porewater samples.

Figure 17. QwikLite 200 (Assure Controls, Inc.) rapid toxicity test system. Unicellular bioluminescent dinoflagellates (*Pyrocystis lunula*; lower right) are added to cuvettes containing test solution (upper right), and read using the test unit (left).

Recently, a commercial unit known as the QwikLite 200 has been developed by Assure Controls, Inc. The unit uses a photodiode to quantify light output instead of a PMT, and also uses a controlled flow of air instead of a stirrer to induce light emission. The commercial unit is marketed using the species *Pyrocystis lunula* due to its ease of culture, heartiness with respect to handling, and sensitivity to contaminants.

The QwikLite 200 was recently demonstrated as a contaminated sediment mapping tool at a Navy estuarine site located adjacent to a former landfill (Rosen et al. 2009). Porewater (collected from the top 12 in. of sediment) from 20 stations was sampled over a period of 2 days using a Trident Probe, which samples porewater *in situ* (SSC San Diego 2003). Testing was conducted on-site with the QwikLite instrument on the day that samples were collected. Results indicated significant light reduction at previously characterized sites with elevated contaminant concentrations, while reference sites resulted in no negative affects.

7.4 TOXKITS

The algae *Phaeodactylum tricornutum* (72-h exposure), the rotifer *Brachionus plicatilis* (24-h exposure), and the crustacean *Artemia franciscana* (24-h exposure), are commercially available as ToxKits. They are ecologically relevant species representing key trophic levels and have served as surrogates for other organisms (Janssen et al., 2000). The assays are easy to conduct and use dehydrated organisms to initiate, thus alleviating the need for culturing or shipping of live organisms. A 2005 project in Italy (G.A. Burton, unpublished data) showed that rehydrated ToxKit organisms were as sensitive as laboratory species when compared with traditional standard laboratory assays and with *in situ* exposures. The ToxKits have the advantage of not requiring culture facilities, require little equipment and training, and can be easily and quickly conducted under a wide range of environmental conditions.

Comparisons of published data for single chemicals indicate that these organisms are generally less sensitive than many of the standardized tests, but they are also less at risk to influence from confounding factors such as ammonia. We found the rotifer test with *B. plicatilis* to meet the majority of our test criteria with respect to ease of use, ecological relevance, availability, contaminant sensitivity, sensitivity to potentially confounding factors, degree of method development, and costs.

7.5 MODIFICATION OF OTHER SHORT-TERM TESTS

Screening toxicity tests should be rapid, cost effective, require small sample volumes, and ideally be field-portable. Some standard laboratory-based toxicity tests can be modified for use as screening-level tests. Early efforts in SERDP Project ER-1550 compared the above mentioned screening tests with modifications of several standard test species including mysid shrimp (*A. bahia*) juveniles, mussel (*M. galloprovincialis*) embryos, and adult amphipods (*E. estuarius, L. plumulosus*) as well as more innovative tests (e.g., post-exposure feeding rate using the polychaete *Neanthes arenaceodentata*) on porewater samples collected from San Diego Bay using the Trident Probe (Burton et al. 2008). All tests were conducted in sample volumes of 10 ml per replicate or less. This volume is consistent with sea urchin fertilization, mussel embryo, rotifer, and QwikLite standard tests methods, but required a reduction in the typical chamber size and test volume for mysids and amphipods. Amphipods and mysids, however, have been tested successfully in small volumes in TIEs using porewaters (USEPA 1996, Ho et al. 1997, Anderson et al. 2007). All tests were held static, and only mysids were fed. Overall, control performance in all tests was acceptable, suggesting that all of the incorporated tests were amenable to small volume exposures with porewater. A variety of responses were observed to the different porewaters, with the historically most contaminated

stations being toxic to most endpoints. Amphipods tended to be the least sensitive, followed by the polychaete and rotifer. QwikLite and mussel embryos were the most sensitive, with ammonia sometimes confounding results, depending on species used.

8. CONCLUSIONS AND RECOMMENDATIONS

This review illustrated the advantages and limitations associated with *in situ* bioassays, and presented a number of case studies from the current peer-reviewed literature where a number of different exposure approaches and relevant standard test organisms and endpoints were successfully demonstrated *in situ.* Overall, the literature suggests that field exposures using such methods in marine settings are indeed viable. Because caged organisms are continuously exposed to all variables (natural and anthropogenic), a more realistic assessment of biological effects can be made compared to traditional laboratory tests held under highly controlled conditions using grab samples collected from one or multiple moments in time. The incorporation of *in situ* bioassays in the ecological risk assessment process, therefore, can provide a highly valuable line of evidence required for making accurate management decisions. When performed correctly, inclusion of *in situ* bioassays in a weight-of-evidence approach can be particularly valuable for linking exposure with potential for biological effects. This is especially critical when contaminant exposure is ephemeral (e.g., associated with tidal influences, or stormwater pulse exposure), where the contaminants of concern are volatile in nature, or where sediment or porewater manipulation might affect toxicity results.

One of the most challenging issues facing *in situ* studies is the need to differentiate between anthropogenic-related and natural- or cage-induced effects. Because a number of naturally varying factors (i.e., pH, temperature, food, particulate matter, turbidity, salinity, dissolved oxygen, ammonia, sulfide, UV exposure) can affect contaminant uptake and toxicity, it is highly recommended that appropriately tolerant test species be used, and that physico-chemical characteristics be monitored, preferably continuously. An integrated approach involving concurrent characterization of both exposure and effects is recommended. An integrated approach might include deployment of organisms and toxicity endpoints relevant to the matrix being evaluated (e.g., water column, sediment–water interface, surficial sediment, or porewater), while making concurrent physico-chemical measurements (e.g., using field-deployable collection devices), and measuring water quality representative of that inside the field chambers. This integrated approach is currently being explored in SERDP Project ER-1550, “Sediment Ecosystem Assessment Protocol (SEAP)”, where *in situ* bioassays are being paired with proven physico-chemical assessment tools (e.g., Trident probes for rapid detection of groundwater upwelling in surficial sediments and porewater sampling; and UltraSeep seepage meters for quantifying groundwater discharge rates and upwelling contaminant concentrations (SSC San Diego 2003), passive samplers (e.g., solid phase micro-extraction; diffusive gradient in thin film); and water quality monitoring, in addition to the more traditional laboratory-based assessment strategies.

An initial prototype instrument, the Sediment Ecotoxicity Assessment Ring (SEA Ring) is the result of this state of the science review, and other objectives associated with SERDP ER-1550 (Figure 18). The design builds on the most promising of the strategies presented in this report, resulting in a multi-compartment, deep water deployable, integrated unit for accurately assessing exposure and effects in marine and estuarine systems. Exposure can be compartmentalized into water column, sediment–water interface, or surficial sediment exposures depending on variations of the inner chamber design. The unit houses water quality sensors capable of measuring a variety of important physical parameters. Unlike other approaches, the datasondes continuously record water quality representative of conditions *inside* the test chambers. This is critical, as possible fouling of mesh screens and reduced flow due to small mesh sizes reported in many *in situ* studies can affect water quality, which needs to be properly documented in order to accurately assess results. To date, the SEA Rings have been deployed successfully at two sites (Naval Station San Diego in San Diego

Bay, CA; and Naval Air Station Pensacola, in Pensacola, FL). The two sites varied considerably in depth, tides, temperature, salinity, sediment characteristics, and contaminants of concern. The results of these studies will be detailed in peer-reviewed journal articles in the near future.

For each SEA Ring deployment, different suites of organisms were selected based on geographic location and appropriateness based on test species performance criteria identified in the SERDP project (see Table 8). The table is a semi-quantitative ranking of a number of factors that were deemed important for identification of appropriate test types. This is by no means an exhaustive list of all marine and estuarine toxicity tests available for use. Use of standardized test organisms and endpoints, however, was an objective and may be preferable due to the vast amount of toxicity or bioaccumulation data available, ability to compare results with other studies, and regulatory acceptance. In general, use of test species indigenous to the area is preferred over surrogate species if possible. This ensures efforts aimed at protection of an ecologically relevant species and decreases the likelihood of release a non-native species that could negatively impact the local ecosystem.

Figure 18. Prototype Sediment Ecotoxicity Assessment Ring (SEA Ring) developed in SERDP Project #ER-1550. Photo by Roy Fransham, SSC Pacific.

The preceding examples indicate that a variety of standard fish and invertebrate test species including topsmelt, amphipods, mysids, cladocerans, bivalves, and echinoderms are amenable to *in situ* exposures. For the current SERDP project, invertebrates were focused on and are prioritized in Table 8. However, some standard fish species were also identified as potentially useful in field deployments. Larval or juvenile sheepshead minnows, for instance, have a high association with surficial sediments, easily available, and are tolerant of physical stress (see Section 6.1.1).

Amphipods are generally easy to work with and are sensitive to a variety of anthropogenic contaminants. The west coast amphipods *Eohaustorius estuarius* and *Rhepoxynius abronius* are EPA-approved species for which much laboratory data already exist. *Leptocheirus plumulosus* is a fine selection for *in situ* studies on the Gulf and east coasts, and is readily available through laboratory cultures. Future *in situ* work with these species will require efforts to minimize predation and competition inside the test chambers. Possible approaches that do not involve manipulation of sediment (i.e., freezing) include reducing mesh size while maintaining water quality. Increasing screen surface area with smaller mesh can reduce issues associated with flow. Addition of battery operated pumps to increase flow across the mesh surface is currently being explored as another means of maintaining water quality in organic rich, low oxygen environments in SERDP ER-1550, with apparent success. Predation has also been addressed by others by marking test organisms to differentiate between indigenous and caged animals, increasing replication to reduce variability due to predation in some chambers, or simply quantified predator presence for better data interpretation.

We are also encouraged by initial results from post-exposure feeding rate studies using the marine polychaete *Neanthes arenaceodenta*. This sediment dwelling species has been an integral part of marine sediment assessment studies in the past, and is recommended for use in California's new sediment quality objectives (SQOs), Bay et al. 2007), but the growth endpoint is too timely and complicated for efficient use *in situ.* Slight modification of successful post-exposure feeding rate studies with other polychaete species (Moreira et al. 2005) suggests that use of this endpoint with *N. arenaceodentata* is sensitive and can be employed in exposures of as little as 48 h.

Mysid shrimp appear to be good candidates for *in situ* water column toxicity exposures. Sediment–water interface studies provide useful additional information with respect to sediment associated tests, and the mysid's habit of aligning against currents to obtain food makes them particularly good candidates for field studies. *A. bahia* has been used extensively in laboratory testing and is known to be particularly sensitive to organic contaminants, and with metals is generally somewhat less sensitive than the echinoderm or bivalve embryo-larval test. Hence, field studies that use both tests would provide the opportunity for an initial screen with regard to the principal contaminants(s) associated with toxicity.

Embryo-larval development tests can be conducted with mussels, oysters, sea urchins, sand dollars, or abalone, all of which are used routinely for whole effluent toxicity testing. Mussels (i.e., *Mytilus galloprovincialis*) tend to be more tolerant of salinity and temperature fluctuations and handling stress as compared to some of the others (particularly echinoderms). Mussels are also generally available for spawning throughout the year, while other species can be seasonal. Rate of larval development can be affected by temperature, which needs to be considered when conducting these tests in the field. General sensitivity of these tests to ammonia may also be of concern depending on site-specific conditions.

Caging artifacts should be minimized through laboratory and field experiments to determine optimal cage design based on species-specific requirements. Many of the issues associated with caging effects were discussed earlier in this review. Appropriate field controls should also be included to be evaluated in the laboratory during field deployments. This will provide an assessment of the organism health and effects due to exposure vessels and/or transport to and from the field.

A final consideration concerns the limited experience of *in situ* testing relative to laboratory testing. Along with the often intentional site-specificity of experimental designs for field exposures, the absence of an historical framework and the minimal database of results from *in situ* testing will, in some cases, reduce certainty associated with study findings. As protocols to establish acute and chronic effects are established for *in situ* testing, it will also be important to bring forward specialized testing that has traditionally been conducted in the laboratory. For instance, laboratory methods to identify contaminant classes that causes toxicity (toxicity identification evaluations, TIEs) can and should be adapted for field applications. This has recently been initiated (Burton and Nordstrom 2004a,b), and should be pursued further. Another group of specialized tests that should have a place in field-based toxicity testing is the rapid screening tests that have been briefly noted above. It is hoped that the examples provided will add clarity, allow opportunities to illustrate key features and/or problems, and they may collectively be used to identify patterns of results associated with certain test designs that hold particular promise for a variety of study objectives.

In situ bioassays should be considered a supplement to laboratory testing. They can be very informative when used in combination with the latter, especially when multiple stressors are involved (Burton et al. 1996). *In situ* tests provide more realistic exposures and minimize the numerous

confounding factors related to collecting samples and processing them for laboratory testing. An integrated approach incorporating laboratory and *in situ* testing as well as community surveys should reduce uncertainties associated with the assessment of contaminant or naturally induced effects, and provide for improved decision making with respect to management decisions. The increased development and use of passive samplers as surrogate measures of contaminant bioavailability, and the need for validation of such tools, also requires the development and refinement of *in situ* toxicity and bioaccumulation testing tools.

Results from *in situ* studies will provide much greater confidence in assessing true exposures and effects occurring at a particular site. This confidence is critical when costly decisions and implications to remediate or not is at stake.

Table 8. Semi-quantitative ranking of candidate test species and endpoints for use in laboratory (L) or field (F) deployments as part of the Sediment Ecosystem Assessment Protocol (SEAP).

Test Endpoint	**Lab or Field?**	**Developmental Status**	**Availability**	**Robustness/Relevance**	**Test volume**	**Exposure Duration**	**Salinity Tolerance**	**Temperature Tolerance**	**Contaminant Sensitivity**	**Confounding Effects**	**Costs**	**Total**
Mysid (*A. bahia*) Survival	L, F	3	3	3	3	2	3	3	3	3	2	28
Rotifer (*B. plicatilis*) Survival	L, F	3	3	**2**	3	3	3	3	2	3	1	26
Amphipod (*E. estuarius*) Survival	L, F	3	3	3	3	1	3	2	2	3	2	25
Amphipod (*L. plumulosus*) Survival	L, F	3	3	3	3	1	3	2	2	3	2	25
Mussel (*Mytilus sp.*) Embryo Development	L, F	3	2	3	3	2	2	2	3	2	2	24
Polychaete (*N. arenaceodentata*) Feeding Rate	L, F	**2**	3	3	3	3	2	2	2	**2**	2	24
Dinoflagellate (*P. lunula*) Luminescence	L,F	**2**	3	**2**	3	3	2	2	**3**	3	1	24
Oyster (*C. gigas*) Embryo Development	L, F	3	1	3	3	2	2	2	3	2	2	23
Amphipod (*R. abroniu*s) Survival	L, F	3	3	3	**3**	1	2	2	2	2	2	23
Amphipod (*A. abdita*) Survival	L, F	3	3	3	**3**	1	2	2	2	2	2	23
Polychaete (*N. arenaceodentata*) Survival, Growth	L, F	3	3	3	3	1	2	2	2	3	1	23
Dinoflagellate (*C. horrida*) Luminescence	L,F	**2**	3	**2**	3	3	2	2	**3**	2	1	23
Sea urchin (*S. purpuratus*) Fertilization Success	L	3	2	2	3	3	1	1	2	3	2	22
Sea urchin (*S. purpuratus*) Embryo Development	L, F	3	2	3	3	2	1	1	3	1	2	21
Bacterium (*V. fischeri*) Luminescence	L	3	3	1	3	3	1	1	1	2	1	19

1 = Low Ranking (poor), 3 = High Ranking (good)
Bold = lack of knowledge

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