Using Caged Bivalves to Characterize Exposure & Effects over Space & Time

Michael H. Salazar & Sandra M. Salazar
Applied Biomonitoring
Kirkland, WA

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There are no perfect biomonitoring tools.

We are here today to discuss caged bivalves as an alternative, complementary approach in DEP biomonitoring programs.

Provide a different perspective in considering possible tools in the environmental monitoring toolbox and reducing uncertainty in the existing approach.

Using these complementary tools to fill data gaps in existing biomonitoring programs and increase the scientific value of the existing data.

Purpose

To discuss

- Rationale & methods for bivalve biomonitoring
- Need to include caged bivalves in the DEP strategy
- Using appropriate tools to answer appropriate questions

To make recommendations for

- Conducting a caged bivalve pilot study in Maine
- Establishing links with other biomonitoring results
- Using a weight-of-evidence approach

Rationale & methods

Based on other studies.

Complementary approach to answer most difficult questions: PCBs, dioxin

What are the most important questions

Recommendations

Proposals for using caged mussels for pilot studies to evaluate the feasibility and scientific value of in situ monitoring with caged bivalves for problematic areas with dioxin and PCBs where other methods have not been able to answer the most important regulatory questions: 1) Upstream and downstream of a pulp and paper mill to test the null hypothesis of no significant differences in dioxin tissue chemistry; and 2) A diffuse gradient grid in a suspected PCB hotspot on the Kennebec River near Augusta to identify the extent of bioavailable PCBs.

The caged bivalve methodology is consistent with the DEP biomonitoring philosophy although the emphasis on using tissue chemistry to establish links with other monitoring elements is somewhat different, but in a way that should be complementary and provide information that cannot be provided using current methods.

The suggested approach is also consistent with a weight-of-evidence strategy currently being used by DEP and current regulations that do not allow elevated tissue concentrations of dioxins downstream of pulp and paper mills.

Caged Bivalve Monitoring

Consistent with DEP strategy

- The best way to assess water and sediment quality is through integrated biomonitoring
- Emphasis should be placed on biological effects and associated tissue chemicals
- Controlled field experiments can reduce uncertainty associated with traditional approaches

Caged bivalve monitoring is consistent with DEP monitoring strategy.

This is the slide we commonly use to discuss the caged bivalve monitoring strategy so all we did was change the subtitle. As you will see, our approaches have a virtually identical monitoring philosophy. The major difference is our emphasis on tissue chemistry. In the Pacific Northwest where there is more emphasis on laboratory toxicity testing as part of environmental monitoring programs we generally spend a great deal of time attempting to explain why field biomonitoring is necessary and identify the pitfalls in relying on laboratory toxicity testing. Like DEP, we are extremely skeptical about using water and sediment quality criteria as guidelines for regulatory decisions.

DEP has an integrated biomonitoring strategy but we are placing more emphasis on tissue chemistry to establish programmatic and measurement endpoint links. Rock bags for example look very similar to caged mussels and the philosophical approach is very similar. We believe that it makes more sense to listen to the animals and measure sublethal endpoints under environmentally realistic conditions to establish regulatory criteria as DEP has done with their biocriteria. Biomonitoring with caged bivalve can help refine the existing approach and answer longstanding questions regarding dioxins and PCBs as an example.

Byssal Thread Field Bioassay

The concept of using compartmentalized cages to monitor individual organisms began as a laboratory test using byssal thread production in mussels as an effects endpoint. The number of byssal threads produced is a sublethal indicator of stress. As shown in the photograph, glass crystallizing dishes were used to facilitate separating individual mussels and counting the byssal threads before they were broken. A Plexiglas box was designed to hold 50 glass crystallizing dishes with mussels for use as a field bioassay.

1. Following the success of the laboratory studies the opportunity arose to develop a comparable field bioassay using byssal thread production in mussels in San Diego Bay, CA.

2. The method was tested over a 2-year period at 8 sites.

3. The exposure period was shortened to 4 days to make the test comparable to the standard 96-hour laboratory exposures.

4. Although we were able to rank sites in terms of environmental stress, there was insufficient chemical monitoring of water, sediment, and tissues to explain the results. It was not until some 15 years later that we were able to explain some of these stresses with more intensive chemical and biological monitoring data.

This is our “canary in a coal mine”.

Mussels are used as aquatic sentinels to characterize exposure and effects over space and time.

Some of you may remember the gas attack in a Tokyo subway a few years ago. This photograph, which appeared in our newspaper the next day, showed how the police were armed and dressed when they invaded the perpetrator’s compound. They had guns, gas masks, helmets, flak jackets, and canaries in cages. We have often used the analogy of using caged mussels as canaries in a coal mine, but thought that the use of canaries as sentinel organisms vanished with the modernization of coal mines. It is interesting that even with all of the advanced technologies of the 21st century, canaries are still being used. This makes an important statement for bringing the experiment into the field rather than attempting to duplicate nature in the laboratory. In our view, this is the primary rationale for biomonitoring. We have focused on caged bivalves for that monitoring and DEP has relied on benthic community structure.

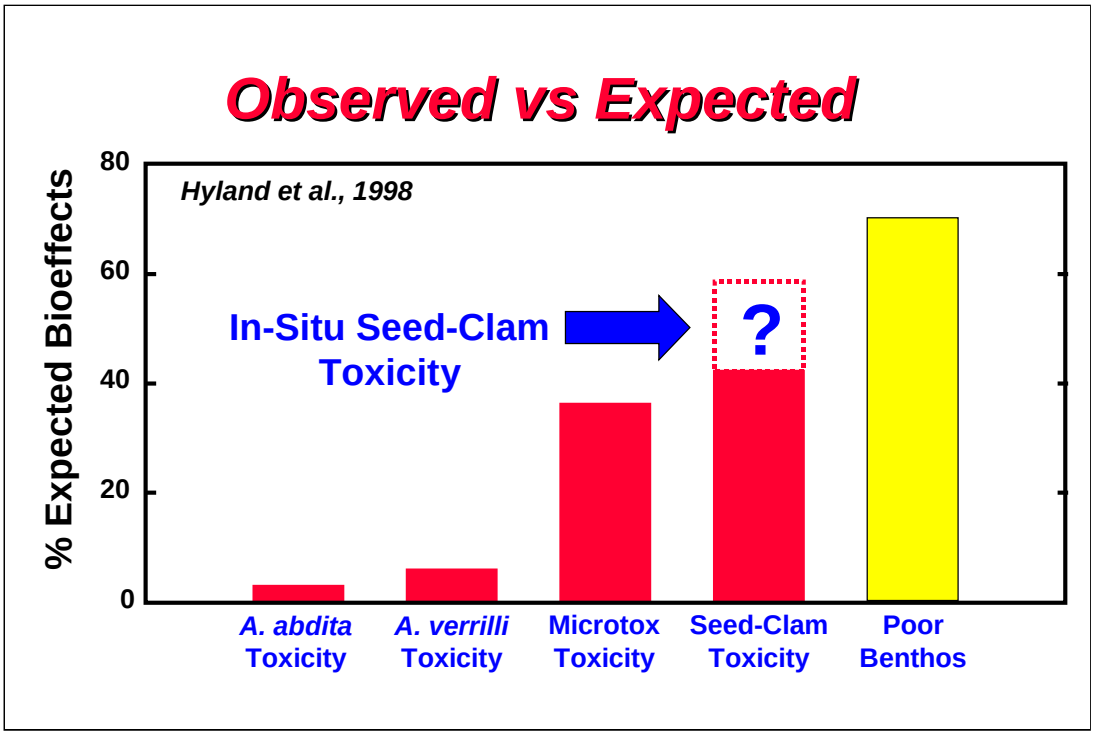
Relative Sensitivity of Bivalves

	Bivalve Species	Species Compared	Exposure	Endpoint	Sensitivity
FRESHWATER	*Anodonta grandis* (Giant Floater)	Daphnia, Fathead Minnow, Rainbow Trout	Municipal Effluent	LC-50	Equal
	Anodonta imbecilis (Paper Pondshell)	Daphnia	Pulp & Paper Mill Effluent	10-d vs 7-d mortality	More
	Anodonta imbecilis (Paper Pondshell)	Daphnia, Midge, Fathead Minnow	Metals	7-d mortality	Equal
	Musculium trans. (Fingernail Clam)	17 different species	Ammonia	20-d mortality	More than 16
MARINE	*Mercenaria mercenaria*	2 Amphipods, Microtox	Sediment	7-d growth, 10-d mortality	More
	Caged *Mercenaria* more sensitive than lab *Mercenaria*				
	Mulinia lateralis	Amphipod	Sediment	7-d growth, 10-d mortality	More
	Mytilus galloprovincialis	Amphipod	In-situ water column	84-d growth, 10-d mortality	More, [tissue TBT]

It is often suggested that bivalves can only be used for chemical biomonitoring because they are insensitive to chemicals. However, as seen in the table, a number of studies have shown that bivalves are just as sensitive or more sensitive than commonly used laboratory test organisms. In some cases, sensitivity may be more related to duration of exposure and measurement endpoints.

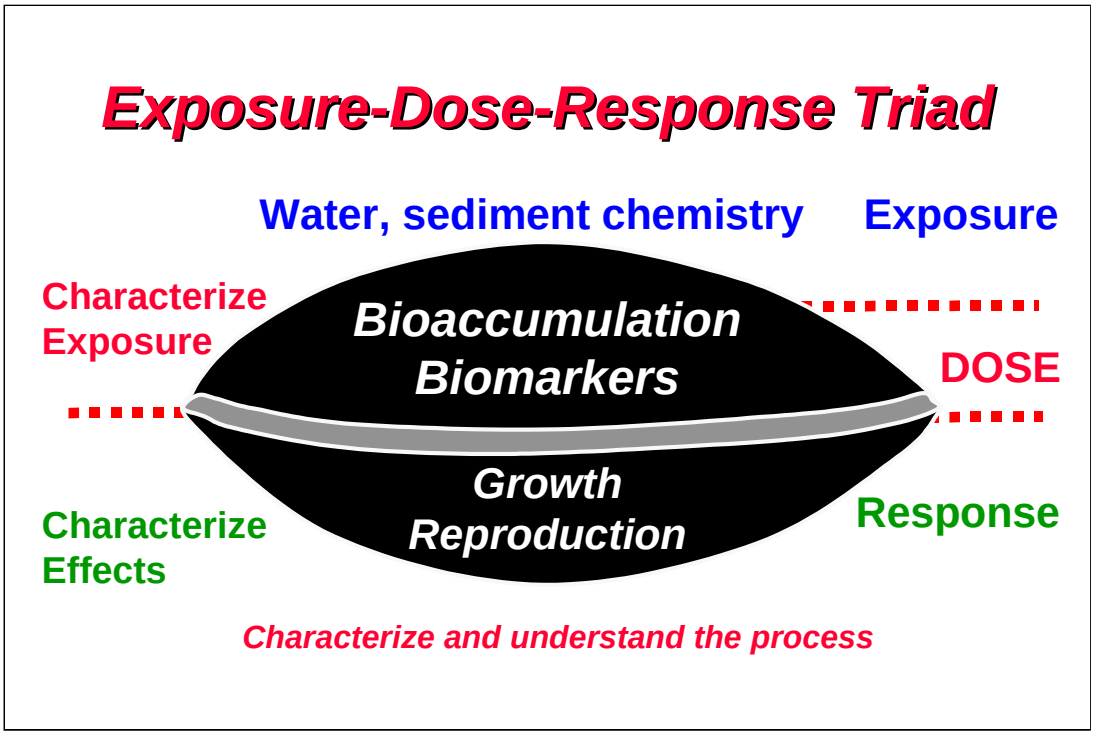
On the freshwater side perhaps the most interesting results come from the EPA ambient water quality criterion document for ammonia where fingernail clams were more sensitive to ammonia than 17 of the most sensitive species.

In our work, based on tissue chemistry, mussels were more than an order of magnitude more sensitive than amphipods. We believe that the difference in sensitivity was associated with measurement endpoints and exposure duration. Mussels were exposed for 84 days in the field and the endpoint was growth. Amphipods were exposed for 10 days in the lab and the measurement endpoint was survival. This points out another important advantage of caged bivalves, i.e., they can be held for extended periods with little or no maintenance and sublethal endpoints like growth are relatively easy to measure.



To further demonstrate bivalve sensitivity, we have included a bargraph comparing the percent agreement between expected and observed bioeffects based on benthic infaunal condition vs. results of four different sediment bioassays included in in an EMAP study in the Carolinian Province. The graph shows that measures of benthic condition detected bioeffects in a higher percentage of samples where bioeffects were expected, based on sediment chemistry, than did any of the individual sediment toxicity bioassays. The concordance between observed and expected toxicity hits based on the amphipod assays was very low (<3%).

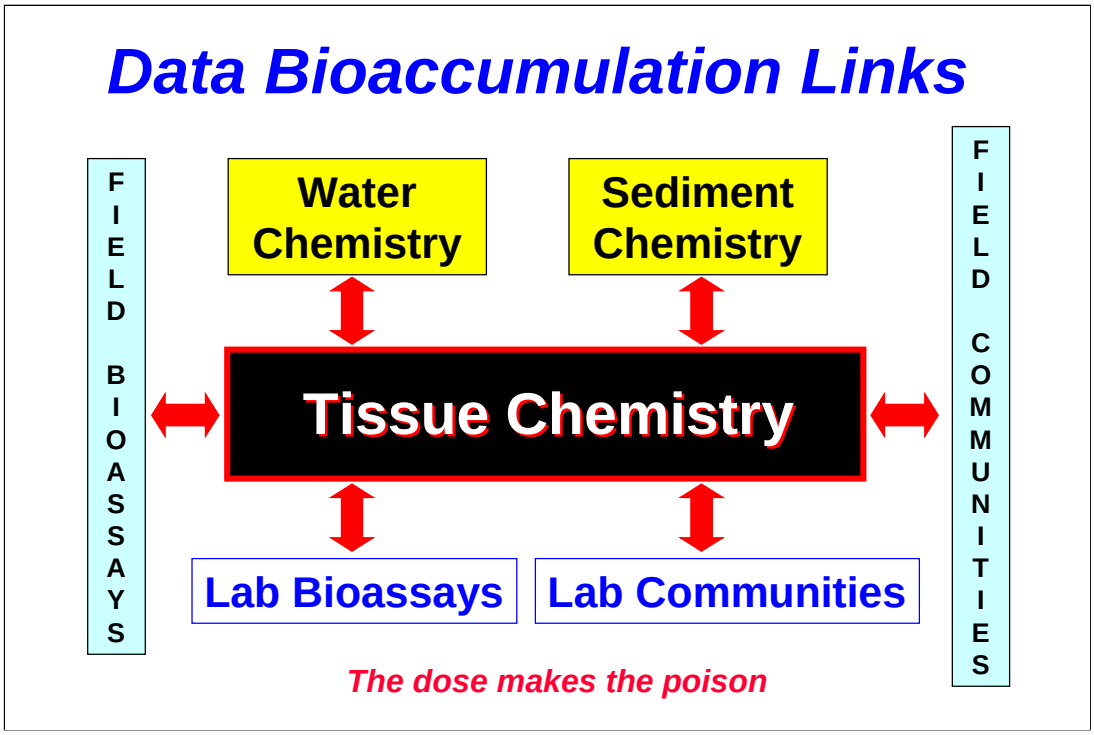
Most interesting from our perspective is that of the laboratory bioassays, juvenile clam growth was the most sensitive indicator or the best predictor of effects on benthic community structure. Growth of clams caged in-situ was the best overall predictor of effects on the benthos. The "?" is used because all tests were not concurrent, but the authors believe that the graph is an accurate representation of relative test sensitivity.



Based on our years of experience in caged mussel monitoring, we developed the exposure-dose-response triad as a practical monitoring framework that was more consistent with the US EPA's risk assessment framework of characterizing exposure and characterizing effects than with the sediment quality triad.

We believe that in the context of the EPA framework environmental monitoring, programs must include both external chemical exposure (water and sediment chemistry) AND the internal chemical exposure or dose (bioaccumulation and biomarkers) to adequately characterize "exposure." Effects can then be characterized by synoptic measurements of associated biological responses (growth and reproduction).

In our view, DEP already has a version of the TRIAD. It consists of the following: 1) Fish and shellfish tissue chemistry; 2) Sediment analysis; and 3) Biomonitoring. Since DEP is already monitoring tissue chemistry of bivalves, could easily add effects measurements to supplement the program. Tissue residue effects are the wave of the future. Both COE & EPA have tissue residue effects databases and regulatory criteria have been predicted in the next decade. DEP has also recognized the importance of monitoring filter-feeders because of their ability to concentrate and integrate chemical exposure in their tissues. Bivalves fit that category.



This diagram shows the elements of the Exposure-Dose-Response triad in its generic form, and how tissue chemistry can be used to form links between various monitoring elements for predictive purposes.

Links for characterizing exposure are established by combining measurements of the 2 external exposure elements (water & sediment chemistry) with the dose element (tissue chemistry).

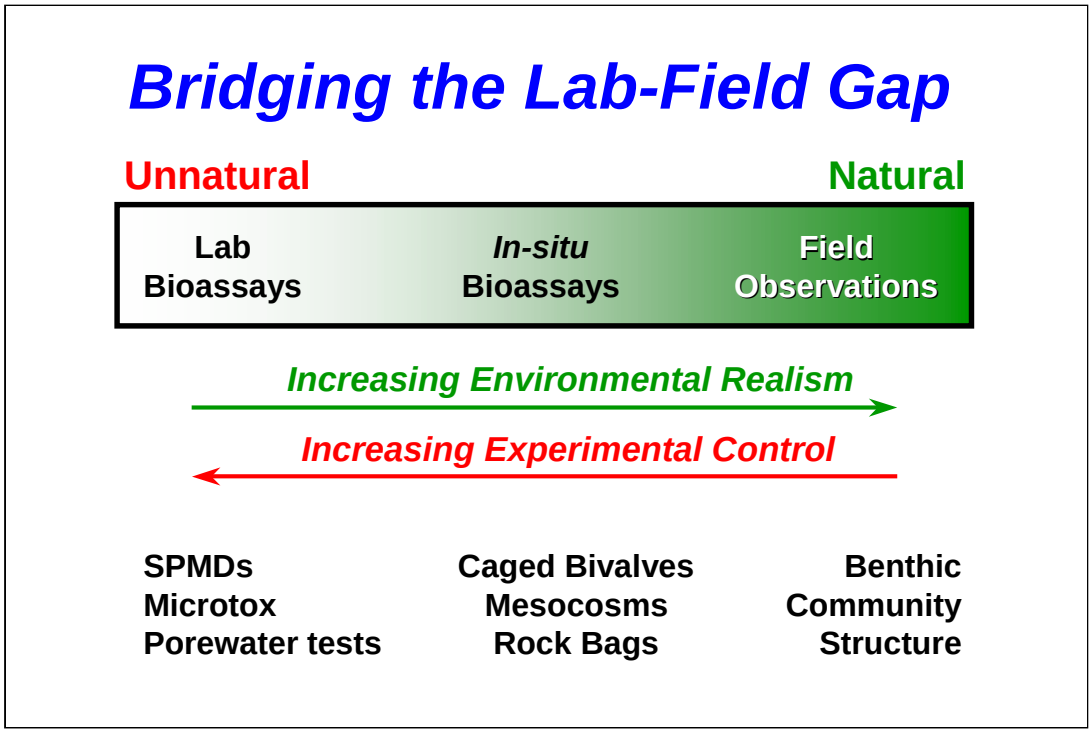
Links for characterizing effects are established by combining the dose element (tissue chemistry) with response element (single species bioassay and community endpoints). These bioassay and community endpoints can be further divided into those measured in the lab and those measured in the field.

Tissue chemistry is the common link in all of these approaches.

This is important because the “dose” makes the poison.

Weighing Tissues & Storing for Analysis

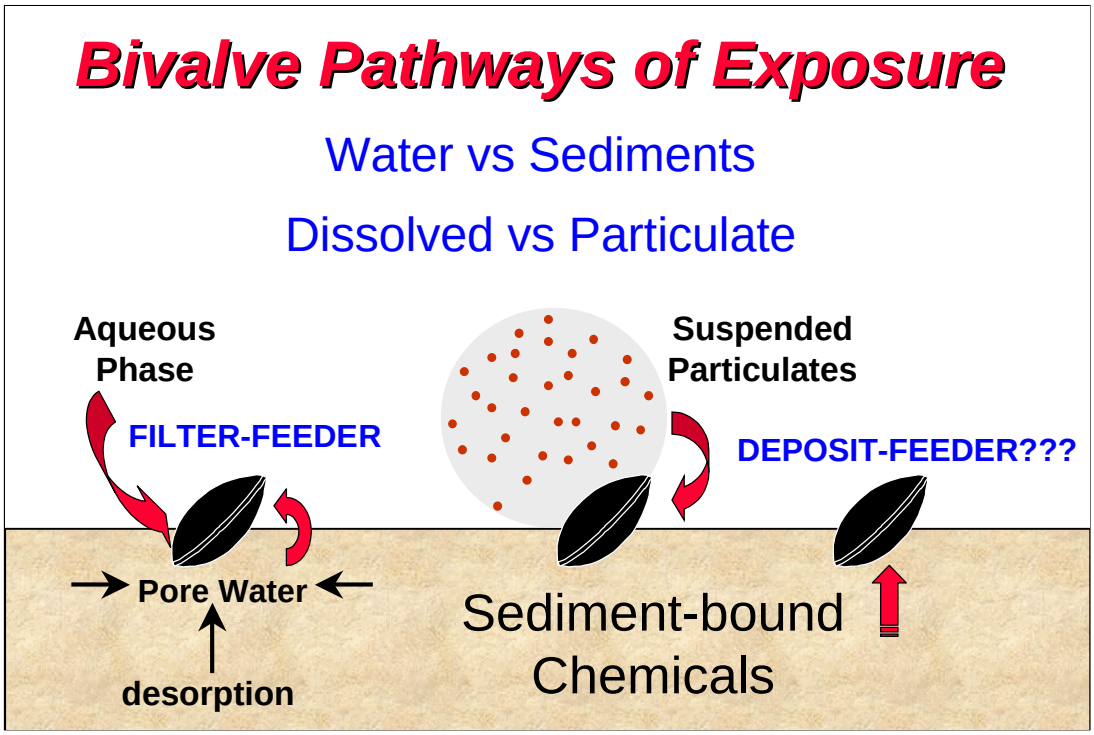
This photograph shows the process of weighing mussel tissues as they are removed from their shells. The measurement process takes less than a minute per mussel. Valuable information is gained regarding the health of the mussels by using tissue weight as an indicator of effects. These growth metrics can also be used to calibrate bioaccumulation by normalizing for the possible effects of growth on bioaccumulation. For example, in a gradient design, mussels caged at greater distances from chemical sources generally exhibit higher growth rates. If the amount of tissue added per unit time is greater than the amount of chemicals accumulated during that time, this could result in growth dilution that biases the interpretation of biologically available chemicals. By tracking individual tissue weights, changes in tissue weight can be used to help explain the amount of chemicals accumulated independently of growth. Changes in tissue weight and shell weight are estimated by measuring a surrogate number of animals at the beginning of the test. This is used in a weight of evidence approach in addition to changes in whole animal wet weight, length, percent, lipids, and percent water. Therefore, chemical analysis of bivalve tissues represents a characterization of exposure and measuring tissue weights represents a characterization of effects. This approach is consistent with the EPA ecological risk assessment paradigm.



We describe our caged bivalve approach as bridging the gap between traditional field monitoring and laboratory bioassays by including the experimental control of the lab with the environmental realism of the field

Caged bivalves are not unique with regard to bridging this gap; mesocosms and rock bags both have similar elements. The important point to make here is that we are all interested in predicting effects in the real-world and not in a “pickle jar.”

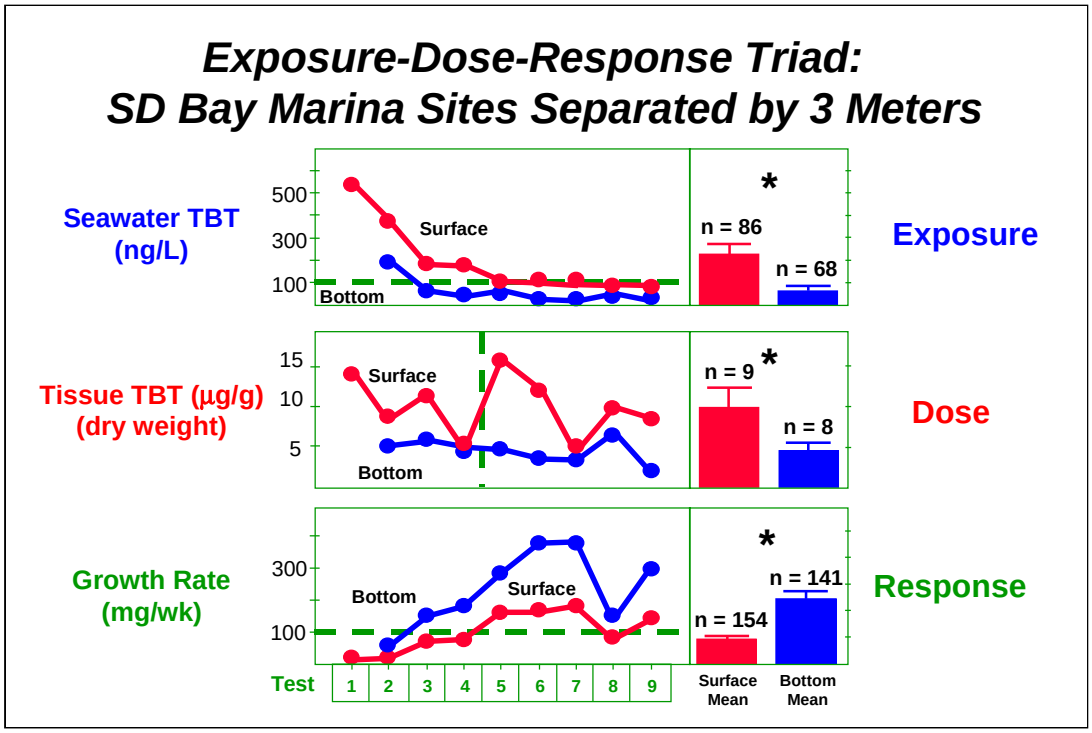
Benthic community structure is probably the most direct measurement of real-world effects, but traditionally these approaches and metrics have had a high degree of variability and uncertainty. Conversely, we consider approaches such as SPMDs, Microtox, and porewater bioassays to be so unnatural as to promote even greater uncertainty in the results.



We also recommend using bivalves for in situ monitoring because they integrate multiple pathways of exposure, which may not occur in other species.

For overlying water, filter-feeding bivalves uptake chemicals directly from the water column (i.e., the dissolved pathway) and indirectly from suspended particulate matter (i.e., the particulate pathway). It should be emphasized, however, that chemicals in overlying water could originate in the sediment. These chemicals become biologically available as particles are suspended from contaminated bottom sediment and as chemicals desorb from bottom sediment either in the water column or in the bivalve gut.

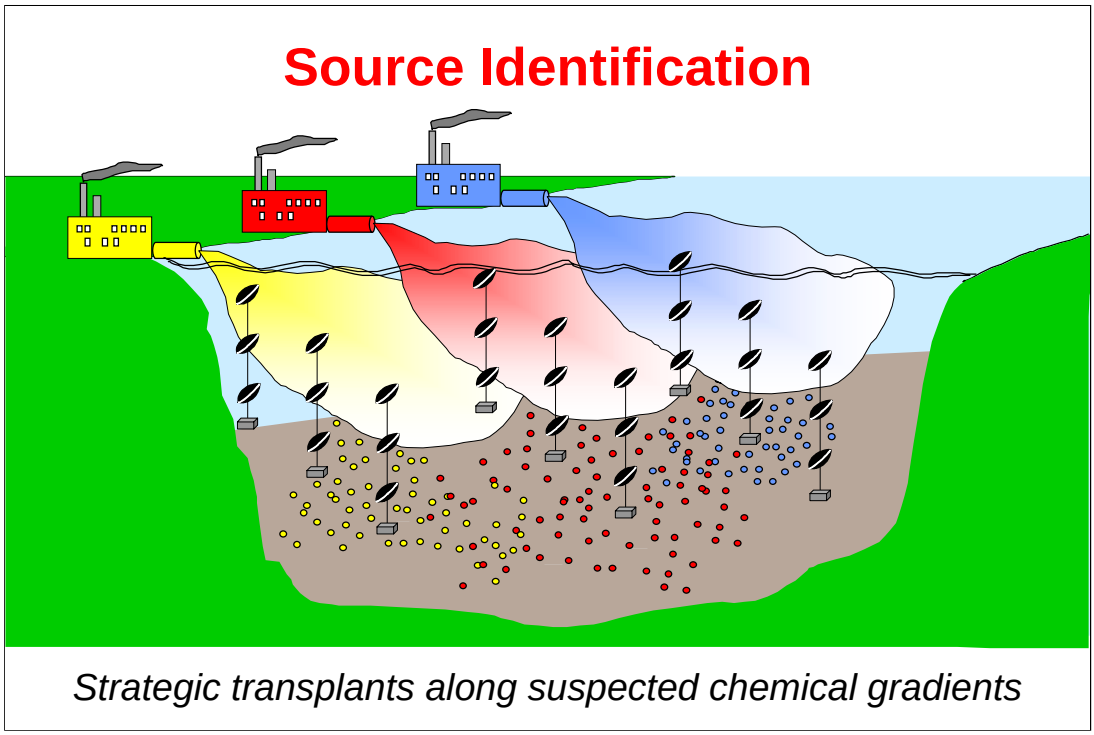
For sediment, deposit feeding bivalves ingest sediment directly and chemicals sorbed to sediment which become biologically available during the digestive process, where the pH in the gut is about 5. The ??? Are used to describe the deposit feeder because all known deposit feeders are actually facultative deposit feeders that can rapidly switch back and forth between filter- and deposit feeding with changing environmental conditions as with tidal fluctuations. The ability of bivalves to utilize multiple pathways of exposure makes them good surrogate test animals.



Caged bivalves can be used as part of a monitoring program to establish status and trends of exposure, dose, and response. Other monitoring programs, such as NOAA's Mussel Watch, the California Mussel Watch, and Gulfwatch, use indigenous bivalve populations.

The most important finding from studies with TBT, as summarized in these graphs, is that we were able to identify statistically significant differences in exposure, dose, and response with caged mussels, even though the sites were separated by only 3 meters vertical distance. The studies were conducted between 1987 and 1990 at two sites in the most TBT-contaminated marina in San Diego Bay. These graphs show overall decreases in TBT water concentrations, TBT tissue concentrations, and increases in mussel growth rate.

Subsequently, we have shown similar differences with PAHs in Port Valdez and campesterol at the Port Alice pulp mill. For both of these studies, the sites were separated by only 2 meters vertical distance, which clearly demonstrates the discriminating power of the methodology and the ability to identify the fine structure in bioavailable chemicals and associated effects.



Strategic transplants along suspected chemical gradients

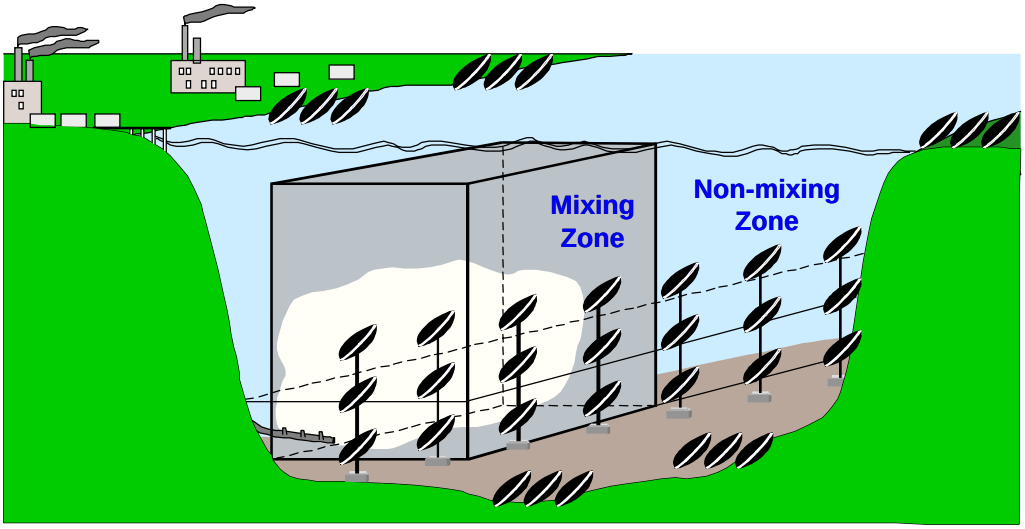
Caged mussels have been used in a number of studies to identify chemical sources and quantify the relative contribution of different effluents. The approach involves transplanting caged mussels along suspected chemical gradients (as shown in the diagram above) over 3-dimensional space and time and confirming that exposure has occurred by measuring chemicals accumulated in mussel tissues. Bivalves like mussels are particularly well-suited for this approach because of their ability to concentrate and integrate chemicals in their tissues. Caged bivalve studies offer the additional advantages of a known exposure period and the ability to place relatively large numbers of test animals in areas of concern. This is generally not possible with natural bivalve populations.

The experimental control provided by this approach (# and size range of test animals, exposure period, and position) is particularly useful for projects where it is necessary to differentiate similar or identical chemicals and the potential sources are relatively close together. In all three tests using our methodology where multiple depths were assessed in the experimental design, statistically significant differences in bioaccumulation and growth were found among the depths. In each of these tests, the caged mussels were separated by only 2 or 3 meters vertical distance. While it is true that all three locations had a stratified water column, the method was successful in detecting differences.

There are few other monitoring tools available capable of characterizing exposure and effects over space and time under site-specific conditions and still identify the fine structure of these chemical gradients.

DEP has already identified one future priority as development of periphyton indicators of nutrient, aesthetic, and biological impacts. This could also be development of caged bivalve indicators. DEP has also recognized the difficulties inherent in attempting to establish true reference or control sites and using upstream/downstream comparisons, particularly with mobile species like fish. Using caged bivalves for these comparisons has the following advantages: 1) Stationary position; 2) Pooled tissue samples to reduce variability; and 3) Controlled exposure period and level of replication.

Mapping Mixing Zones

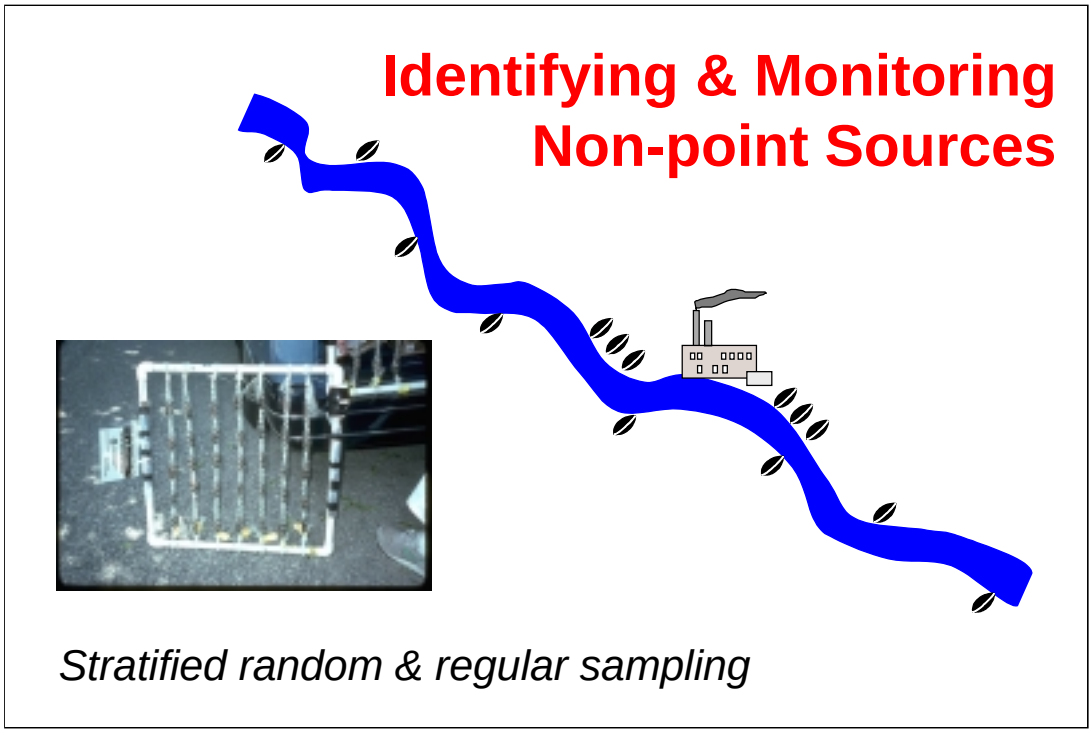


Quantifying exposure & effects over space & time

Caged mussels can be strategically placed along suspected chemical gradients to identify potential sources of metals and organic chemicals. This diagram shows how caged mussels were used at the Port Valdez Ballast Water Treatment Facility diffuser at a depth of 70 meters. As demonstrated in several other studies using this approach, gradients could be established from effluents, bottom sediments, and non-point source runoff. The diagram also shows how natural populations of benthic mussels are not always positioned in the appropriate location to adequately assess these various sources.

Using caged bivalves to characterize exposure and effects over space and time can also help define mixing zones more realistically than other traditional methods such as laboratory toxicity tests or benthic community studies because the integrated tissue chemistry measurements can be used in dispersion models. Using caged bivalves was the most accurate way to identify biologically available chemicals from Port Valdez Ballast Water Treatment Facility effluent.

DEP has also identified as another future priority expanded reliance on spatial data integration & analysis. The caged bivalve approach can help meet that requirement.



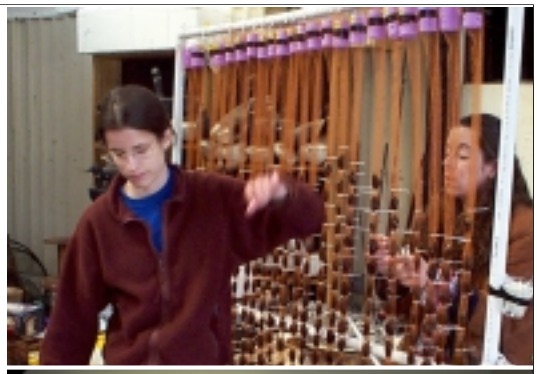
The graphic above shows an integrated experimental design that combines both stratified random and regular (upstream & downstream) monitoring to evaluate point and non-point chemical sources. In this example, three cages of mussels are placed at upstream and downstream locations relative to the potential chemical sources such as a pulp and paper mill. In addition, other sites may be selected randomly to evaluate potential non-point sources along the study area.

The photograph shows a mussel cage from a study at the Nyanza Superfund site on the Sudbury River in Massachusetts to evaluate the biological availability of methylmercury. The photo shows the use of compartmentalized cages, temperature monitors, and the freshwater mussel *Elliptio complanata*. In this particular study, cages were placed directly on bottom sediment. In two studies on the St. Lawrence River in Montreal, *Elliptio* were placed one meter above the bottom to evaluate input from the sediment and the water column.

Another DEP future priority is expanded emphasis on the assessment of non-point source biological impacts. Caged bivalves can help meet that requirement as well.

- **Measurement setup**

PVC rack holding mesh bags, PC, balance, calipers



- **Measurement teams**

2 teams of 3 each:

Recorder, Stuffer and Cable-tie installer

The above pictures show the measurement setup and the measurement teams. The top picture shows the PVC rack holding the mesh bags, PC, balance, and calipers. All measurements were made in a hotel room in B.C.

The bottom picture shows 2 measurement teams of 3 each. The 2 people standing in the foreground are measuring weights and lengths, and dropping mussels in the mesh bags. The 2 people seated in the middle are recording data manually and checking the electronic database to ensure that the data are in the appropriate columns. The two people in the background (1 standing, 1 bending over), are the cable tie-installers who install plastic cable ties to separate the individual mussels. They must insure that there is sufficient space for the individual mussels to grow and that the cable ties are loose enough to allow movement.

Caged Bivalve Monitoring

Pulp & Paper Mill Effluents

FRESHWATER

- 1984-2000: Kymijoki River, Finland
- 1985: Kaministikwia River, Canada
- 1986: Rainy River, Canada
- 1992: San Joaquin River, USA
- 1994: Ton River, France
- 1995: Pond #22, New Zealand
- 1997-1998: Pointe Claire, PQ, Canada

MARINE

- 1995-96: Ward Cove, USA
- 1997: Vancouver Island, BC, Canada
- 1998: Pictou Harbor, NB, Canada

Applied Biomonitoring Methodology

Most caged bivalve monitoring associated with pulp and paper mills has been conducted in Finland & Canada

Much of the information is in the grey literature

Most of the studies have been in freshwater

Most studies have only measured bioaccumulation as an indicator of exposure

We are aware of only three studies conducted in the marine environment. We conducted two of these studies, and the third used our methodology. In each of these three studies, bioaccumulation and growth was measured. Only one freshwater study, the most recent short-term study utilized our methods.

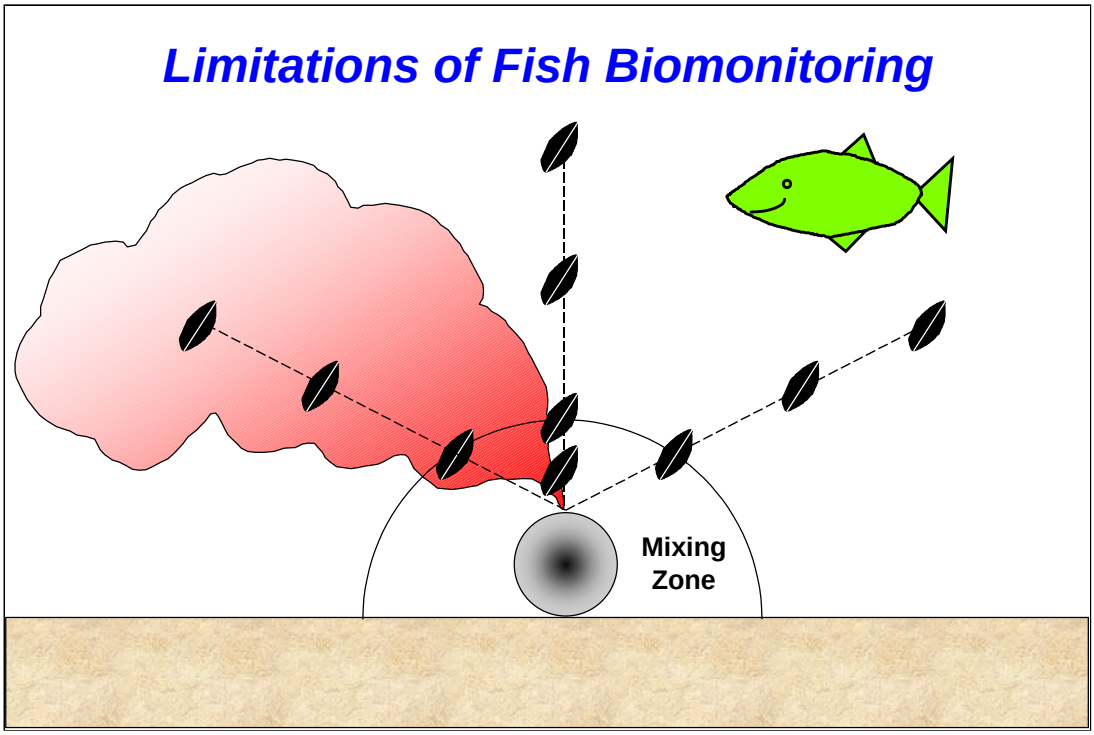
Limitations of SPMDs

Environmentally unrealistic

- Only organics, no metals
- Only aqueous fraction not particulate or dietary
- Emphasis on low MWs
- No effects endpoints
- Extremely small database, little predictive power
- Numerous extrapolations
- Slime fouling retards equilibrium partitioning
- Fragile bags

Limitations of lipid bags include but are not limited to the following:

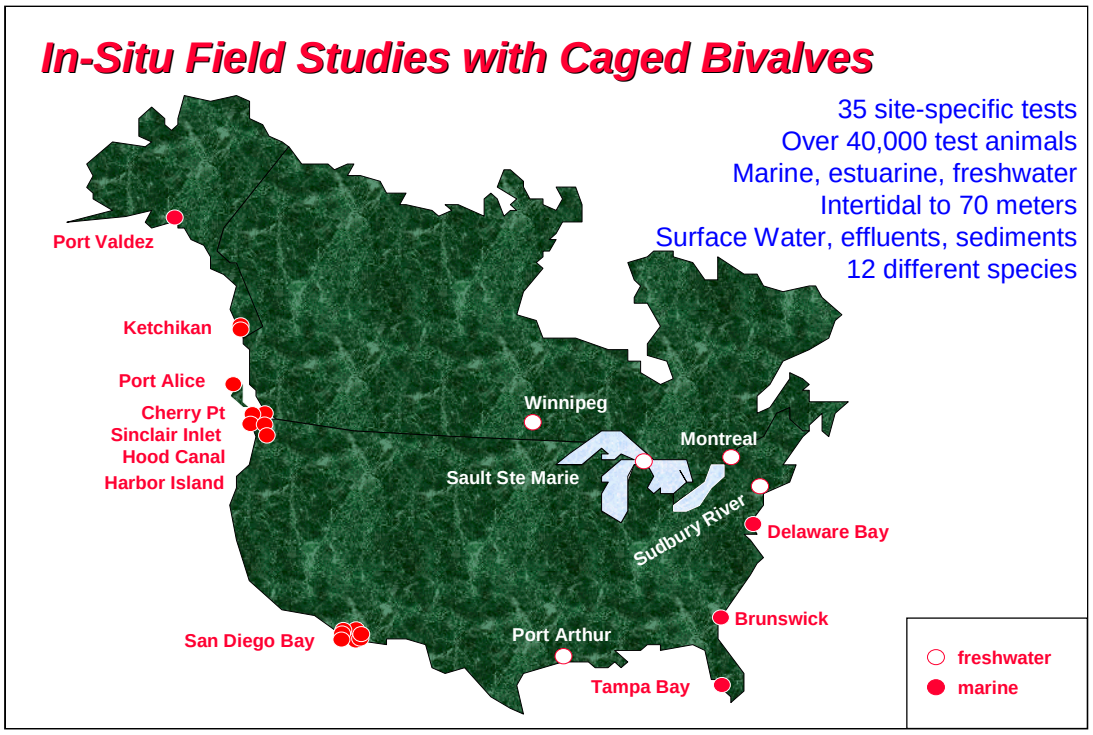
1) The exposures are environmentally unrealistic because no organism accumulates exactly like a bag of fat
2) SPMDs only reflect exposure to the aqueous fraction and not particulate or dietary exposure
3) Emphasis is on the low molecular weight organic compounds because this is what the SPMDs preferentially accumulate
4) There are no effects endpoints associated with the SMPDs so it is difficult to relate the measured concentrations to any organism
5) There is an extremely small database compared to the database for accumulation for bivalves and other organisms
6) There are numerous extrapolations and assumptions associated with the measurements;
7) Slime layers and fouling can significantly retard accumulation within the bags;
8) The bags are extremely fragile and tend to tear without extreme care in handling;
9) The lipids only accumulate organic chemicals and not metals; and
10) The bags only allow dissolved chemicals to pass and yet the particulate pathway of exposure is extremely significant for many species, including bivalves.



Measuring the accumulation of effluent-associated chemicals in mussel tissues provides a more direct method of mapping and monitoring effluents and defining mixing zones because tissue chemistry reflects only biologically available chemicals. This cannot be accomplished by analyzing thousands of samples because the results do not clearly distinguish either: a) bioavailable chemicals or b) integrated estimates of chemical exposure.

This graphic shows why the use of fish in characterizing exposure, characterizing effects, or identifying chemical sources is problematic. One of the major limitations of monitoring exposure and effects in a mobile species like a fish is that the position and duration of exposure generally has a high degree of uncertainty.

We have often said that the best way to measure water quality is not to measure the water but to measure the chemicals in bivalve tissues. As anomalous as it might sound, particularly for those concerned about effects on fish, the best way to measure exposure and effects in fish might be to measure those parameters in a surrogate species like a bivalve.



This is a map showing where we have conducted caged bivalve studies.

The white circles are freshwater transplants and the red circles are marine.

FRESHWATER:

5 freshwater studies using

6 different species,

Over 10,000 individuals

From Canada to Texas, Michigan to Massachusetts

This year we will be returning to Michigan and Montreal for follow-up studies

MARINE:

30 marine studies using

5 different species (experience with 3 others)

Over 30,000 individuals

From British Columbia to San Diego Bay on the West Coast

From Delaware Bay to Tampa Bay on the East Coast

This year we are conducting two studies in Puget Sound.

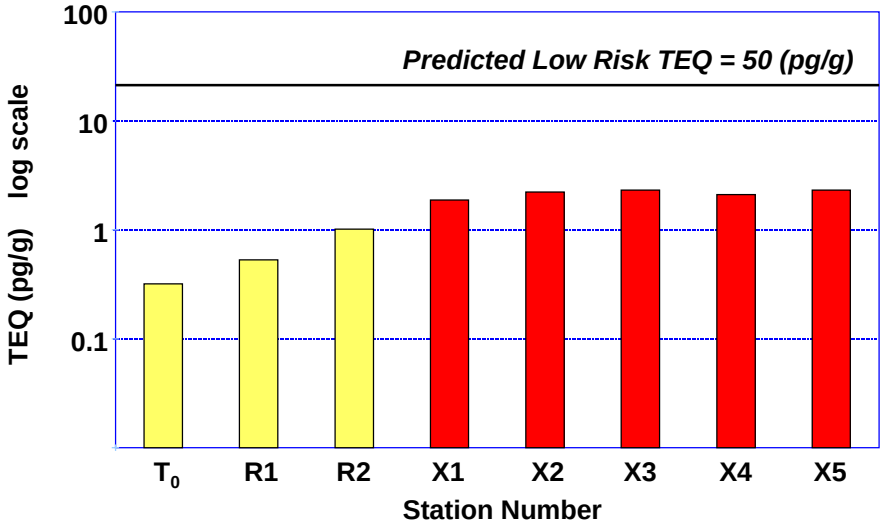
Deploying Cages from Floats: 2,4,6 Meters

The above picture shows one of 6 floats that were used for a caged mussel study on Vancouver Island, BC to assess pulp mill effluents. Here, the deployment team is fastening the line with the mussel cages to the float. Cages were deployed at depths of 2, 4, and 6 meters below the surface. Previous monitoring by the mill had shown that this is where the effluent plume was expected.

A gradient in decreasing mussel growth with proximity to the diffuser was correlated with a number of physical-chemical factors such as spent sulphite liquor, dissolved oxygen, and temperature as well as campesterol (a plant sterol) in mussel tissues.

Since a number of these factors co-varied, more work will be necessary to confirm meaningful relationships and establish the causative factors for reduced mussel growth near the diffuser.

Mussel Bioaccumulation: Toxic Equivalence Concentration (TEQ) For Dioxins & Furans

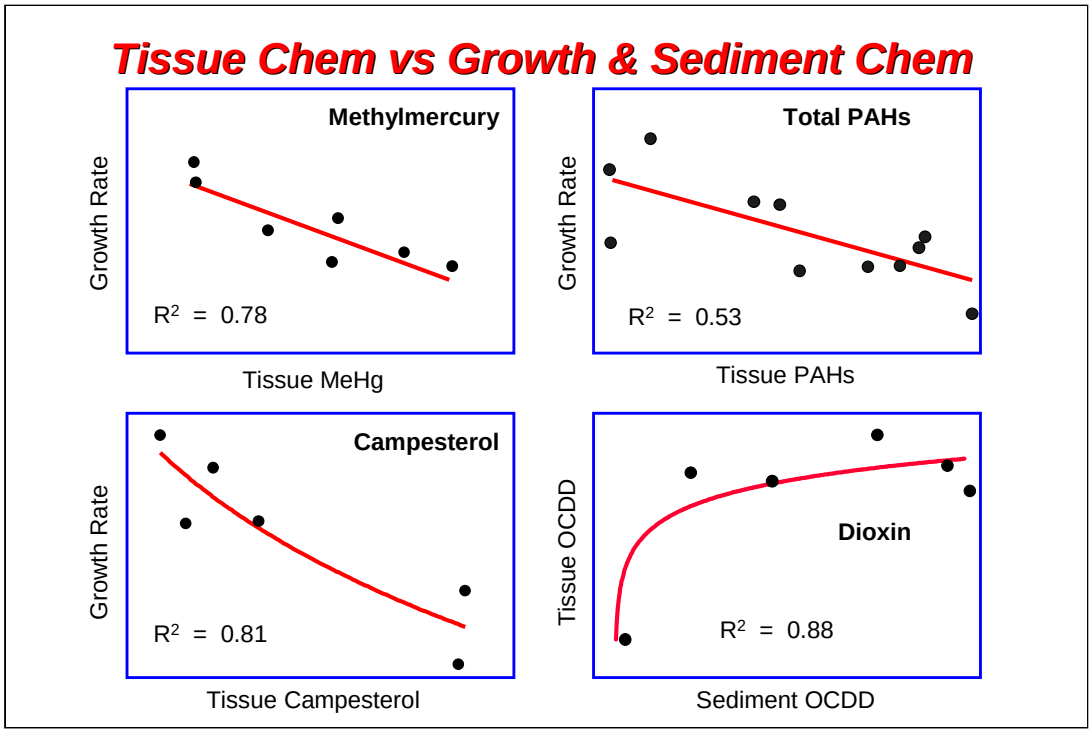


Caged bivalves can be used for both ecological and human health risk assessments. Although mill sites in this study showed statistically significant elevations in dioxins/furans, it does not appear that these tissue burdens are environmentally significant.

Based on toxic equivalency concentration (TEQ) of 50 pg/g, all mill site concentrations are about an order of magnitude below the predicted effects level on a tissue concentration basis. However, it should be recognized that recent re-evaluations by EPA suggest that effects could be occurring at concentrations that are at least an order of magnitude lower.

Tissue burdens are being used more often to predict effects because they reduce the uncertainties associated with water & sediment concentrations.

Some years from now regulations will probably also shift toward a tissue residue basis.



These graphs show relationships we established between exposure, dose, and response for methylmercury, Total PAHs, campesterol, and dioxin during our caged bivalve studies in the Sudbury River (Massachusetts), Delaware Bay (Delaware), Port Alice (Vancouver Island, British Columbia, Canada), and Ward Cove (Ketchikan, Alaska).

There is less confidence in these relationships as predictive tools since they were established during one-time studies that may not have accounted for natural variability.

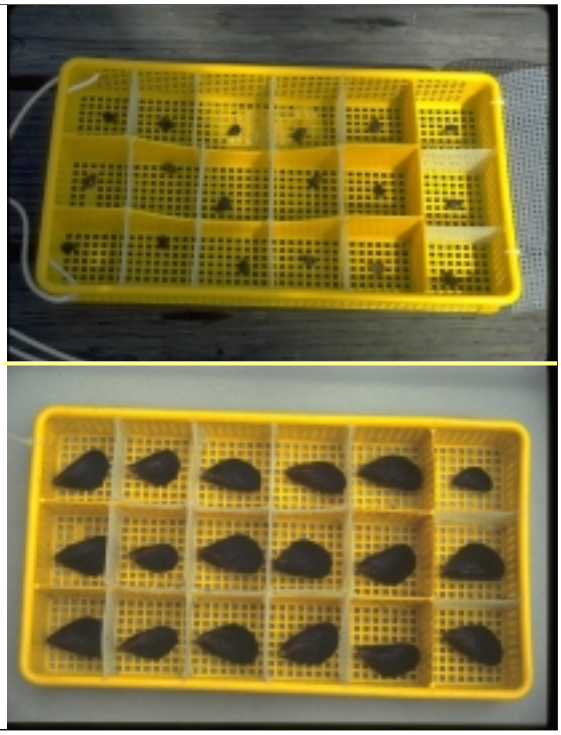
Nevertheless, the data can be used as first order approximations, to develop testable hypotheses, and to provide interim guidance on monitoring needs.

TBT Studies in SD Bay

- **Minimized size range @ beginning: 10-12 mm**

At maximum seawater [TBT] almost no growth or difference between beginning and end-of-test lengths and weights

- **Maximum growth at control site after 12 wks**

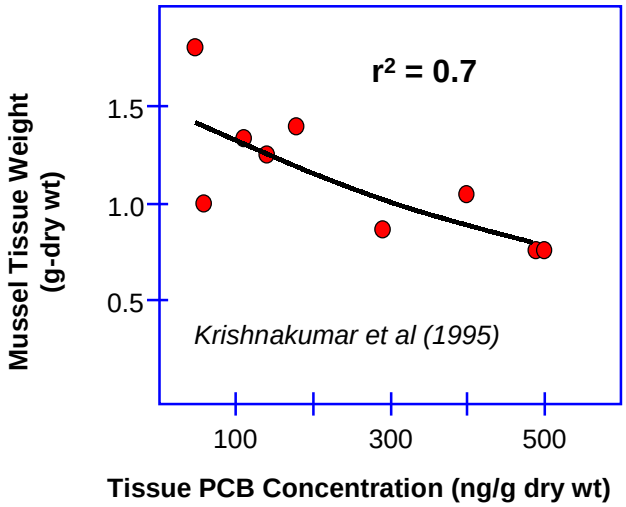


The top picture shows mussels between 10-12 mm at the beginning of the test. To assure an even distribution among cages, we always pre-sorted the mussels into 1-mm increments and then filled each tray with the same size group until each was used up.

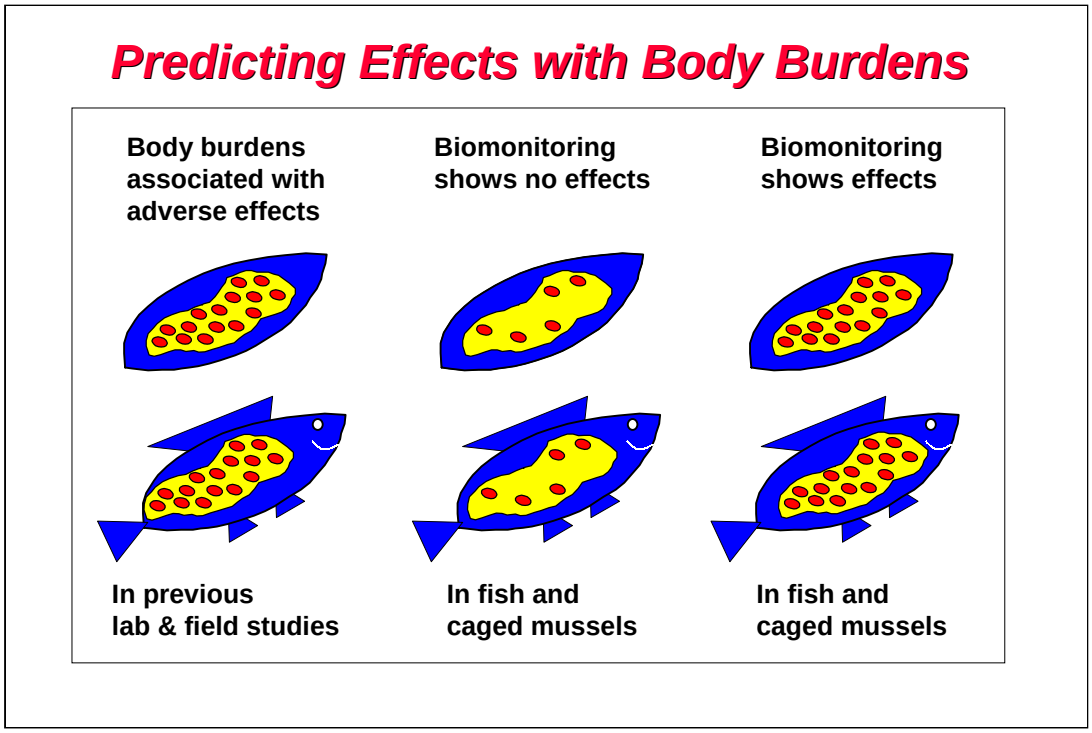
The bottom picture shows the maximum growth rates measured at the “control” or “reference” site. We have subsequently determined that it is virtually impossible to select a true control site and have emphasized the importance of gradient studies and regression analysis to characterize exposure and effects.

Mussels at the most contaminated site (i.e, 530 ng TBT/L) had the lowest growth rates ever measured. They appeared the same at the end of the test because growth was undetectable by observation (top picture).

Effects of Tissue PCB on Mussel Growth



The graph above provides another example of using tissue chemistry to predict associated biological effects. This work was actually conducted by other investigators and used a variety of mussel metrics and biomarkers to characterize exposure and effects. It should be made clear however that this graph does not appear in their paper. The reason is that many investigators do not think of dose-response in terms of tissue chemistry and effects. We plotted this graph using their data for tissue chemistry and tissue weights at each location associated with a particular tissue concentration of PCBs. The point is that we can use these data to predict effects based on tissue chemistry from field data. The slides that follow show how we can use these relationships to establish a link between tissue chemistry in bivalves and adverse effects in fish.



This graphic is intended to show a practical approach to utilizing relationships established between tissue chemistry and adverse biological effects in previous studies to develop a threshold effects level for predicting effects from biomonitoring data.

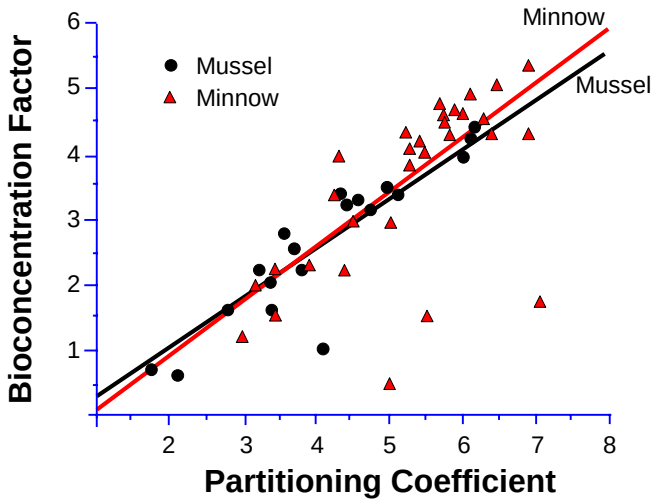
This somewhat complicated graphic is best viewed in three vertical sections from left to right. In the first column, a threshold effects level is established from tissue concentrations of a given chemical (indicated by the red dots) associated with effects in previous laboratory and field studies using fish and bivalves. The relative concentration is indicated by the relative number of red dots.

In the second column, biomonitoring of tissue burdens indicates no expected effects because the tissue concentrations are below the predicted thresholds. This is shown by a fewer number of red dots.

In the third column, biomonitoring of tissue burdens indicates expected effects because the tissue concentrations are above predicted thresholds. This is shown by an equivalent number of red dots.

This is the bioaccumulation link between exposure and effects

Rationale for Predicting Effects

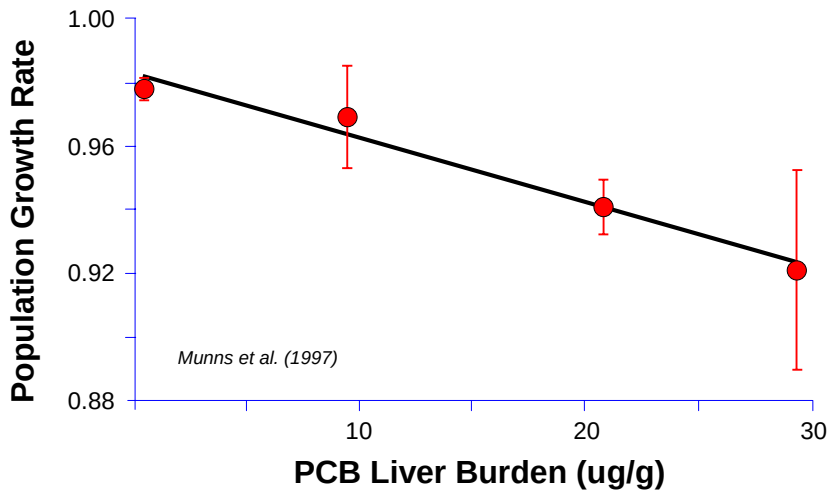


Geyer et al. 1982; Veith et al. 1979; Knezovich (1994).

This graph shows the relationship between K_{ow} and BCF for mussels (*Mytilus edulis*) and fathead minnows (*Pimephales promelas*) for various organic compounds (from Geyer et al. 1982; Veith et al. 1979). Graph from Knezovich (1994).

It supports the tissue residue approach for interpreting environmental data across species by showing that the octanol water partitioning coefficient (Kow) is directly proportional to the bioconcentration factor (BCF) in mussels and fish.

Rationale for Predicting Fish Effects



This graph shows population-level effects on mummichogs as a function of PCB liver burden (from Munns et al. 1997). They predicted population level effects based on fish tissue chemistry.

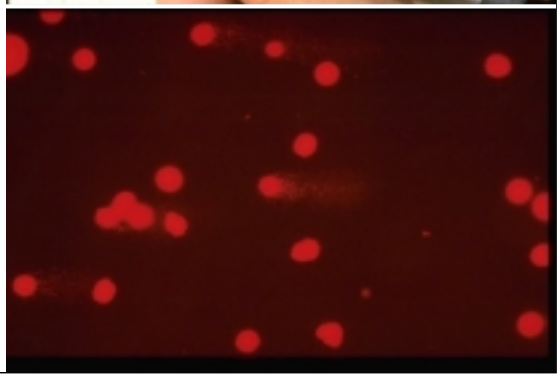
Theoretically then, if we can predict population level effects from individual fish tissue chemistry, and we can predict fish tissue chemistry from mussel tissue chemistry, we can predict fish population effects from mussel tissue chemistry.

Therefore, since it is easier to measure mussels associated with specific sites, it may be more convenient to measure mussel tissue chemistry to predict effects in fish.

Bivalve Biomarkers

- **Extracting hemolymph from adductor muscle**

- **Comet assay for DNA strand breaks**



In its simplest form, caging mussels could be viewed as merely an exposure system that facilitates making clinical measurements on individual mussels. These pictures represent two of the more successful biomarkers measured as part of our caged bivalve studies.

The top picture shows removing hemolymph from the posterior adductor muscle to measure lysosomal enzyme responses. This method has been refined at the NMFS lab in Seattle and the Plymouth lab in the U.K.

The bottom picture shows the comet assay to assess genotoxicity by measuring DNA strand breaks. The number of “tails” and the length of the “tails” provide an estimation of exposure and possibly effects. This analysis has also been used to demonstrate phototoxicity of PAHs in San Diego Bay.

We are currently working with Environment Canada scientists in Montreal to establish relationships between our bioaccumulation and growth measurements with their multiple biomarker approach for bivalves. One of those bivalve biomarkers is for vitellogenin which may provide an additional link with vitellogenin measurements in fish.

Summary & Conclusions

Need for combined exposure & effects endpoints, more emphasis on the following:

- Understanding processes
- Bioaccumulation links
- Controlled biomonitoring

Develop an integrated monitoring approach with the strategic use of caged & indigenous bivalves

Bivalve biomonitoring can help DEP achieve its short- and long-term goals.

Take-Home Message

"…glimpse intricate realities…"

Complementary tool in the toolbox

BIOMONITORING RETROSPECTIVE:

15-yr Summary for Maine Rivers & Streams

"…glimpse intricate realities…" of nature.

Glimpse: snapshot or incomplete picture

Intricate: Complicated ecosystem, difficult to understand

Realities: real-world exposures & real-world effects

Caged bivalves can help increase the resolution of that glimpse, reduce the complexity of the ecosystem into smaller and more easily understood components, and maintain the environmental realism of the current DEP biomonitoring approach.