Using Caged Bivalves to Characterize Exposure & Effects over Space & Time: *Controlled Field Experiments*

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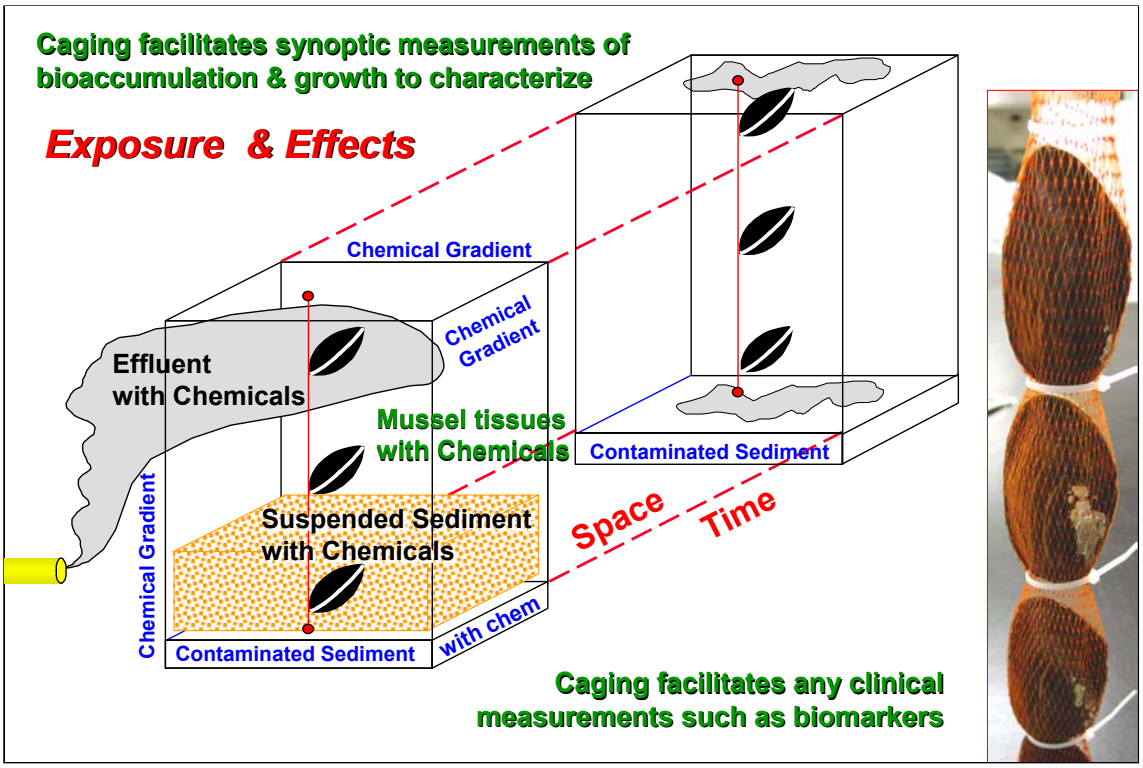
- We have been measuring bioaccumulation and growth in caged bivalves for over 30 years.
- We have learned a great deal about characterizing exposure and effects under environmentally realistic conditions in the field and how to interpret the results.
- Recently, our methods have been adopted by the American Society for Testing and Materials (ASTM 2122-01).
- Today, we will summarize the lessons we have learned about various aspects of this work and how we have worked together with Environment Canada Scientists at the St. Lawrence Center over the past 4 years with transplant studies of a municipal effluent in Montreal.
- Studies conducted in 1998, 1999, and 2001 focused on organic chemicals, freshwater bivalve biomarkers, indicators of endocrine disruption and induction of sex reversal in the field. The 2002 study is focusing on dietary pathways of exposure for effluent-associated metals such as copper and silver.

Why In-situ Tests?

"The shift from aquaria to microcosms to field studies is not concerned with toxicity; it is concerned with the real variable in hazard assessment, the exposure assessment."

Parrish et al., 1988

- It is important to understand how history, methods development, and paradigm shifts have influenced the state-of-the-science today.
- Parrish et al (1998) describe the current shift toward field studies as the most important variable in the risk assessment paradigm; i.e., characterizing exposure.
- It is generally believed that field exposures are more environmentally realistic than laboratory exposures and that there is less uncertainty in the exposure data.
- Perhaps most important, although not explicitly stated, is that characterizing exposure may be the most crucial element of the risk assessment process. In other words, if exposure is not properly characterized, the effects measurements and the rest of the assessment may not be useful.
- At one recent Pellston Workshop on sediment assessment, bioaccumulation was a major topic of concern and more and more monitoring and assessment programs are including tissue chemistry endpoints.
- At another recent Pellston Workshop on dietary exposure pathways it was emphasized that this pathway is often neglected in standard laboratory toxicity tests.
- It should be made very clear however, that exposure assessments are not restricted to sediments and the same concerns are important relative to monitoring and assessing the water column.



- Mussel monitoring is integrated at several different levels as shown in the conceptual diagram above. Monitoring is integrated in terms of measuring exposure and effects in the same animal at the same time by measuring bioaccumulation (or biomarkers) and growth. Exposure measurements could be integrated by measuring bioaccumulation and biomarkers. Effects measurements are traditionally integrated by measuring various growth metrics such as whole animal wet weight, shell length, tissue weight and shell weight. Effects measurements could be integrated further by measuring reproduction through tissue morphology or weight or biomarkers such as vitellin. Another form of integration is monitoring all of the metrics mentioned above over space and time. In the diagram above the two cubes could be viewed as samples in space or time. In terms of spatial integration the lower cube could be viewed as one location with samples at three depths and the upper cube as another location with three depths. In terms of temporal integration the lower cube could be viewed as one sampling event at several locations and the upper cube as another sampling event at several locations. This provides a characterization of exposure and effects over space and time under environmentally realistic, site-specific conditions.

- We routinely sample space and time with bioaccumulation and growth to integrate exposure and effects. This slide shows PAHs measured in water, sediment, suspended sediment, and mussel tissues in a weight-of-evidence approach.

- Mussels are strategically placed along suspected chemical gradients.
- These mussels filter the water and as they do, they concentrate and integrate the chemicals they are exposed to in their tissues. Therefore, a single tissue measurement provides more representative information than 100s of water or sediment samples.
- Growth measurements also integrate bioeffects.
- The photograph shows compartmentalized cages that facilitate repetitive measurements on individual mussels *(Elliptio complanata)*.

Rationale

Why Bivalves?

Integrate & concentrate chemicals

Why Transplants?

Experimental control, environmental realism

Why Growth?

Associated with population effects

Why Bioaccumulation?

Most direct method of confirming exposure

Why Biomarkers & Sex?

Characterize & understand processes

First, we will provide a brief description of the rationale for what we do.

Why Bivalves?

- Because they are good model organisms and can serve as surrogate sentinels
- They integrate & concentrate chemicals in their tissues,
- They are important members of many ecosystems and reasonable surrogates for other species,
- Are sedentary and do not move, and they are relatively easy to collect, cage, and measure,
- There is a large database of tissue chemistry data from Mussel Watch monitoring and associated effects from laboratory and field studies.

Why Transplants?

- Because transplant studies provide experimental control associated with traditional laboratory tests and environmental realism associated with traditional field monitoring.
- Our in-situ transplant studies with caged bivalves are controlled experiments conducted in the field.
- Some of you may remember the gas attack on the Tokyo subway a few years ago. When the police arrived at the perpetrator's compound, this is how they looked: flack jackets, helmets, gas masks, weapons, and carrying canaries. We have often used the analogy of a canary in a coal mine to describe our work but we thought this approach went out with old mining techniques. This photo of the policeman provides an example of taking the experiment into the field instead of attempting to duplicate field conditions in the laboratory.

Why Growth?

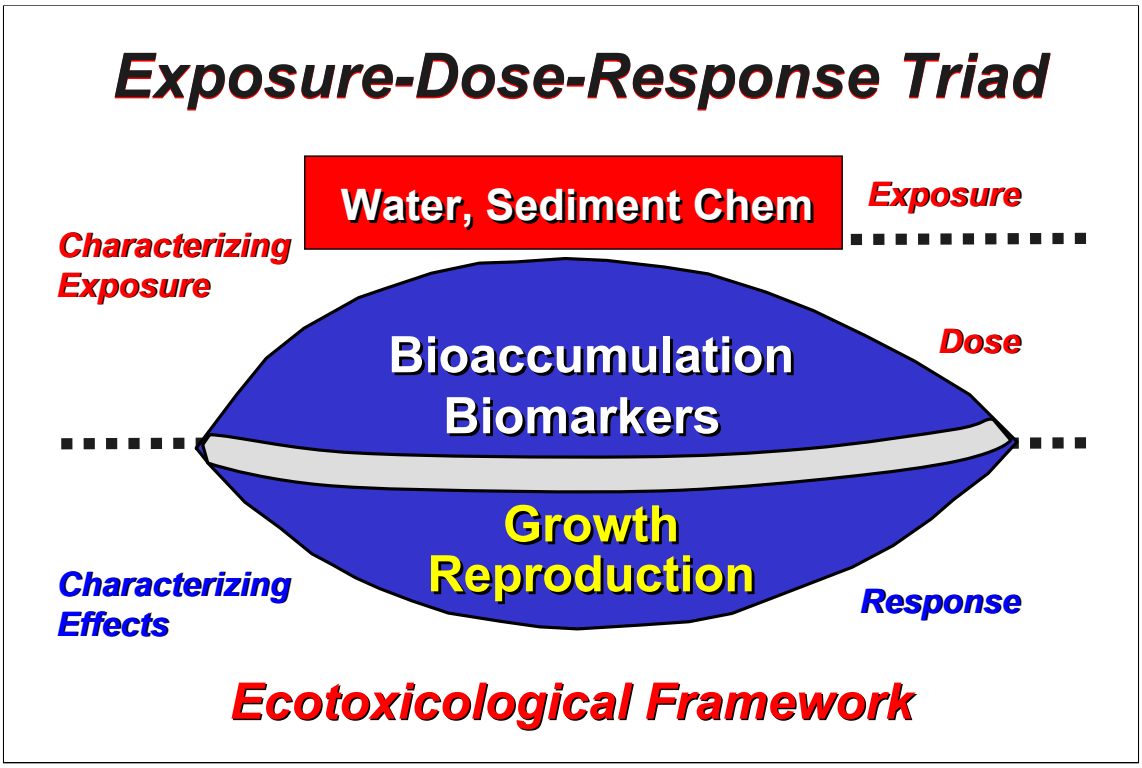
- It is relatively easy to measure and has been Associated with population effects. It is also relatively easy to understand as shown at the pictures showing mussels at the beginning and end of a test

Why Bioaccumulation?

- Most direct method of confirming that exposure has occurred

Why Biomarkers & Sex?

- Because they help characterize & understand processes of bioaccumulation and growth. The pictures to the right show removing gonads and staining to confirm the sex of individual mussels.



- After a number of years of monitoring and assessment with caged bivalves we developed the **Exposure-Dose-Response Triad**

- It includes using bivalves to characterize exposure and characterize effects as in the **Ecological Risk Assessment** paradigm

- **Exposure** is characterized by measuring chemicals in water and sediment.

- The **dose** is characterized by measuring bioaccumulation and biomarkers.

- The **response** is characterized by measuring growth and reproduction.

- This is our **ecotoxicological framework** and caged bivalves can be an integral part of this risk assessment based monitoring and assessment strategy.

- Clearly, ecological effects must also be assessed by measuring responses at various levels of biological organization such as benthic community structure.

Paracelsus circa 1525:
The dose makes the poison

Tissue

Sediment

Water

…and the dose that matters, is in the tissues

In about 1525 Paracelsus was the first to suggest that

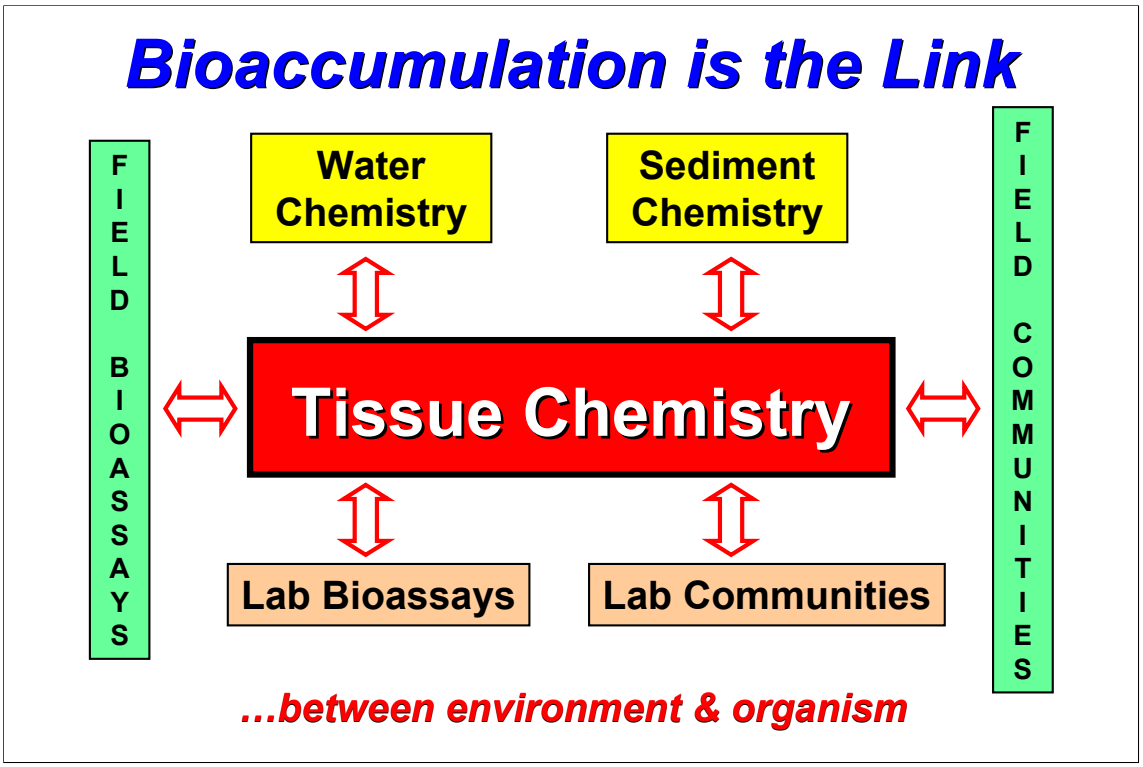
The dose makes the poison

…and the dose that really matters, is in the tissues

This is where the potentially harmful chemical reactions interactions occur, at ***internal receptors***

Not in the water, and ***Not*** in the sediment.

The picture to the far right shows Christian Blaise removing tissues from the freshwater mussel *Elliptio complanata*



Bioaccumulation is the link between environment and organism

- It can also be used as the link between other 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 are further divided into those measured in the lab and those measured in the field.

- ***It is the universal link and a "common currency" for comparing exposure and effects relationships.***

Asking the Right Questions

Exposure

1. **Are contaminants entering the system?**
2. **Are contaminants bioavailable?**

Effects

3. **Is there a measurable response?**
4. **Are contaminants causing this response?**

AETE 1999, Borgmann 2000

Traditional approaches (e.g., Sediment Quality Triad) successfully address questions 1 & 3 but do not directly address 2 & 4

- Canada's Aquatic Effects Technology Evaluation Program and a series of papers by Uwe Borgman (of Environment Canada) and his colleagues state that exposure assessments should address the following two questions:

1) Are contaminants entering the system?

2) Are contaminants bioavailable?

- Effects assessments should address the following two questions:

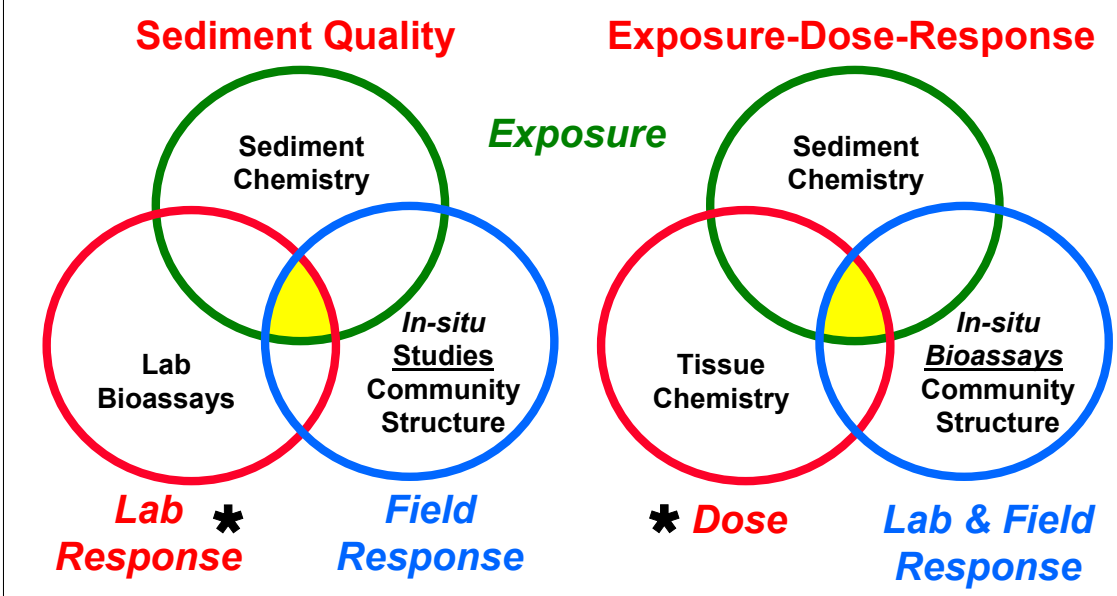
3) Is there a measurable response?

4) Are contaminants causing this response?

- Borgmann and his colleagues have concluded that traditional approaches such as the sediment quality triad successfully address questions 1 and 3 but do not directly address questions 3 and 4.

- A growing number of scientists agree with that assessment and is one of the reasons why we have developed the exposure-dose-response triad; i.e., to place more emphasis on bioaccumulation and directly address questions 2 and 4.

Comparing the Triads: Uptake or Effects?



- There are several major differences between the sediment quality triad as commonly practiced but the most important differences hinge on whether emphasis should be placed on uptake or effects. While most scientists would agree that we are ultimately interested in effects, as mentioned earlier, the shift in emphasis toward mesocosms and in-situ testing is to better characterize and understand the processes involved in exposure so that toxicity can be expressed less ambiguously.

- In the diagrams above the asterisk denotes the leg of the triads that deal with a lab response versus a dose. It also shows that the sediment quality triad focuses on responses measured in the lab and in the field and not the importance of tissue chemistry as a link between measurements. There is also a difference between so-called in-situ studies of benthic community structure versus in-situ bioassays and benthic community structure. Assessments of community structure are purely observational and do not include experimentation. We conduct controlled experiments in the field using ASTM-standardized protocols that could play an important role in any monitoring and assessment program and various tiers of assessment.

- This approach also facilitates establishing dose-response relationships in the field under environmentally realistic conditions.

Why Bioaccumulation

"Without observations linking levels (of pollutants) in the water or sediment with tissue concentrations and then with effects on organisms and populations and, ultimately, with the well being of the ecosystem as a whole, an adequate assessment of pollution is impossible."

GESAMP 1980

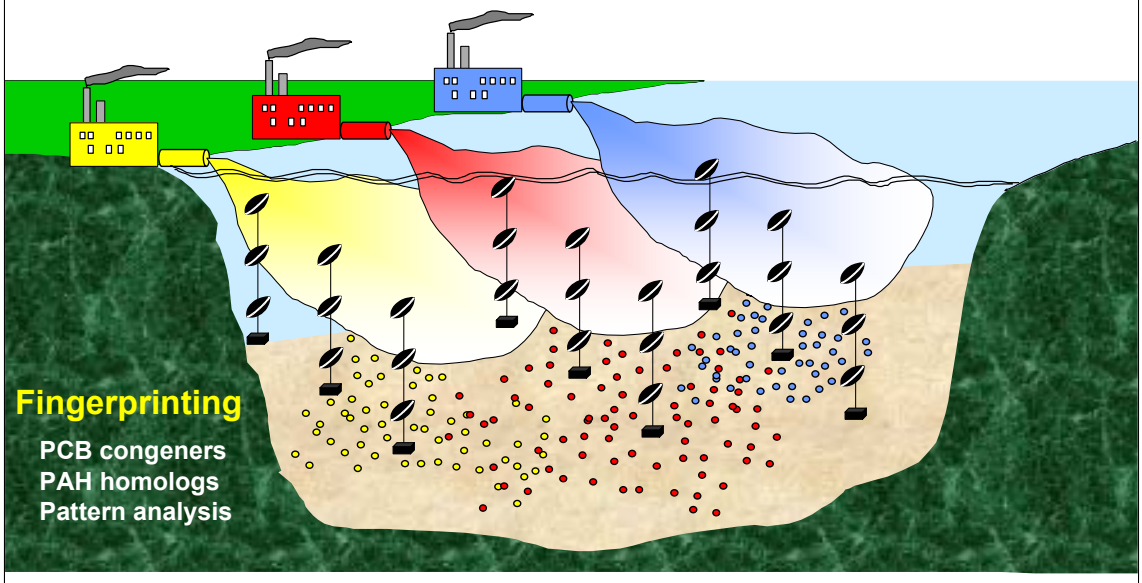
- This approach is not new. The Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP) was established in 1967 by a number of United Nations Agencies. Its purpose was to provide advice to the agencies and, through them their Member Governments monitoring and assessment strategies.

- As early as 1980 they recognized the importance of bioaccumulation and emphasized that :

"Without observations linking levels (of pollutants) in the water or sediment with tissue concentrations and then with effects on organisms and populations and, ultimately, with the well being of the ecosystem as a whole, an adequate assessment of pollution is impossible."

- It is somewhat surprising that it has taken over two decades for many scientists and organizations to understand the importance of bioaccumulation in monitoring and assessment. It is even more surprising that many still do not have an appreciation of this importance or applicability in their programs.

Identifying Chemical Sources & Plume Mapping



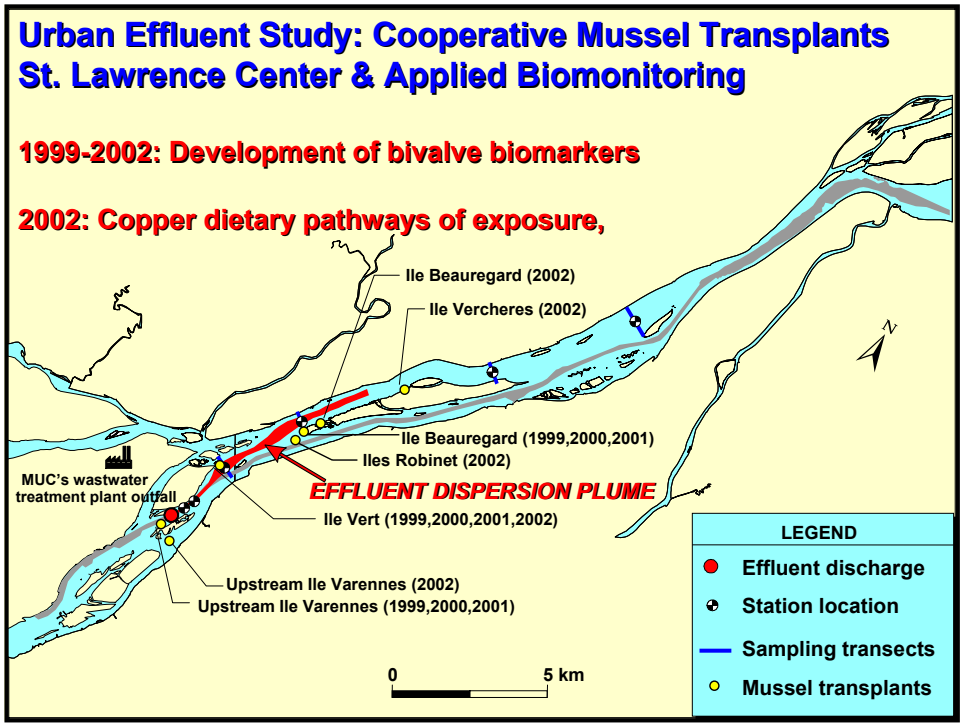
Using caged bivalves & chemical fingerprinting

- In addition to characterizing exposure and effects over space and time, and under site-specific conditions, we can use these controlled field experiments to identify chemical sources, map effluent plumes as well as contaminated sediments.

- The figure above shows three chemical sources, three chemical plumes and chemicals in sediment associated with those sources. The figure also shows how the strategic placement of caged bivalves along suspected chemical gradients in three dimensional space can be used to map those plumes as well as contaminants in sediment.

- This process has been aided tremendously with the development of chemical fingerprinting methods such as congener-specific analysis for PCBs, alkylated homolog analysis for PAHs, and pattern recognition analysis.

- Collectively, the discriminating power of caged mussel monitoring in specific locations with well-defined exposure periods ***AND*** sophisticated chemical analyses become an even more powerful tool in environmental monitoring and assessment.

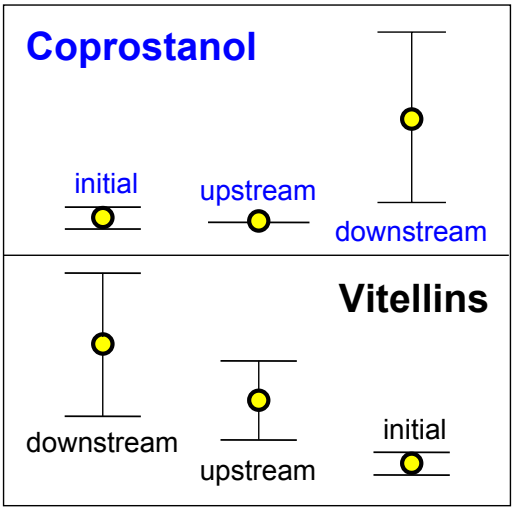


• We have been working with Environment Canada over the past 4 years on various aspects of urban effluents. Environment Canada has been conducting a variety of studies including mapping, modeling, bivalve biomarkers, and pathways of exposure using a City of Montreal effluent.

• The map above identifies the various stations where we have caged mussels in cooperative studies with Environment Canada scientists in 1999, 2000, 2001, and 2002.

Summary of Urban Effluent Results

- **Correlations between mussel tissue chemistry, biomarkers & growth**
- **Identified coprostanol as a possible inducer of endocrine disruption**
- **Experimentally induced feminization in controlled field experiments**



• Christian Blaise and Francois Gagne (shown in picture) of the St. Lawrence Center of Environment Canada have been instrumental in the development of a suite of biomarkers.

• Data shown in the picture above demonstrate the relationship between the concentrations of coprostanol and vitellins at initial (To), upstream, and downstream sites.

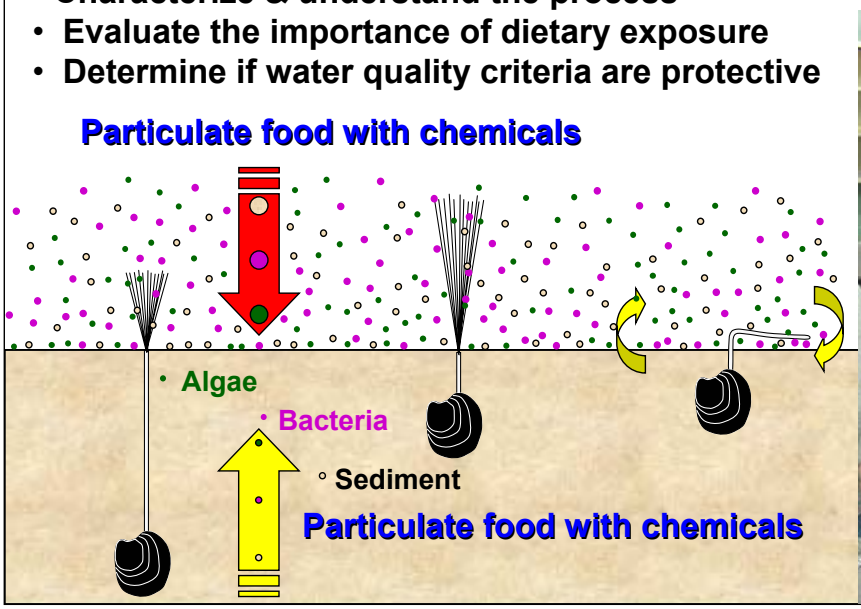
• The data suggest that in addition to acting as a tracer of sewage effluent, coprostanol could be acting as an endocrine disrupter.

• This is important because it shows that bivalves could be used as indicators of endocrine disruption in some of the ways that fish have been used over the years.

• Bivalves have the additional advantage of not moving to confound the interpretation of where exposure has occurred.

Current Studies on Cu Exposure Pathways

- Characterize & understand the process
- Evaluate the importance of dietary exposure
- Determine if water quality criteria are protective



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

- Christian Gagnon of the Environment Canada St. Lawrence Center, shown in the picture to the right, has done pioneering work on metal pathways of exposure and the importance of site-specific variability and is coordinating the copper dietary exposure work during 2002.

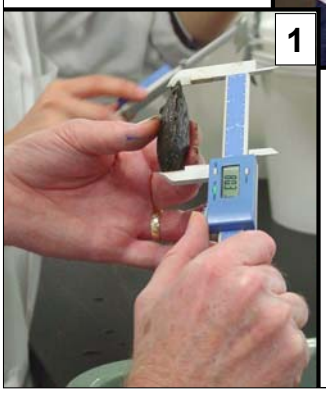
- For overlying water, filter-feeding bivalves uptake chemicals directly from the water column (i.e., the dissolved pathway) and indirectly from suspended particulate matter (i.e., the particulate pathway).

- 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. However these properties can vary on a site-specific basis.

- For sediment, deposit feeding bivalves ingest sediment directly and chemicals sorbed to sediment become biologically available during the digestive process, where the pH in the gut is about 5.

- The ability of bivalves to utilize multiple pathways of exposure and to transplant them to evaluate site-specific effects makes them good surrogate test animals.

Measuring & pre-sorting into 1-mm increments



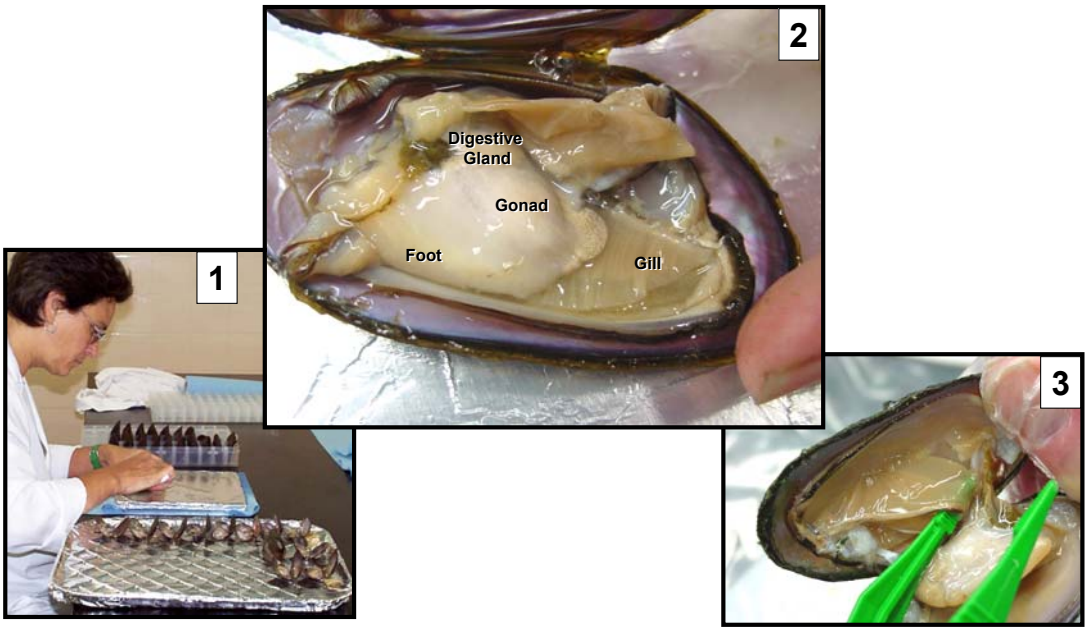
Final length & weight measurements

• One of the reasons why our transplant studies have been more discriminating than others is that we spend more time minimizing the size range of mussel used in each test. We pre-sort mussels into 1-mm increments by measuring with plastic digital calipers (Figure 1) and placing them into plastic buckets (Figure 2) in a particular size category.

• When all the mussels have been pre-sorted, they are counted to determine the range where we have the maximum number of animals in the minimum size range and with the most even distribution in each size category. This is the range that is used for each test.

• When that range is determined, each mussel is re-measured with a more accurate digital calipers and weighed on a portable analytical balance (Figure 3). These data are recorded digitally on a portable computer (Figure 3) by using appropriate hardware and software and the data are also recorded manually on data sheets in case of computer failure and as a form of quality assurance and quality control.

Removing Tissue for Chemical Analysis



• Whole soft tissues (after removal as shown in Figure 1) and shells are also weighed at the beginning of the test to estimate initial weights and to provide another growth metric for comparative purposes with tissues and shells at the end of the test and then preserved for chemical analysis for comparison with tissue concentrations measured in mussels at the end of the test.

• In most tests only whole soft tissues are used for these exposure and effects measurements. In the dietary copper experiments, tissues were separated into gill, digestive gland, gonad, and foot + mantle (Figure 2). Individual tissues were also weighed in representative samples at the beginning of the test and all samples at the end of the test to estimate changes in each individual tissue. Pooled samples of each tissue were also preserved for chemical analysis to compare concentrations at the beginning and end of the test.

• Ultra-clean trace metal techniques were used in these experiments. Although a stainless steel knife was used to sever the adductor muscle, plastic forceps (Figure 3) and ceramic knives were used to remove and dissect individual tissues.

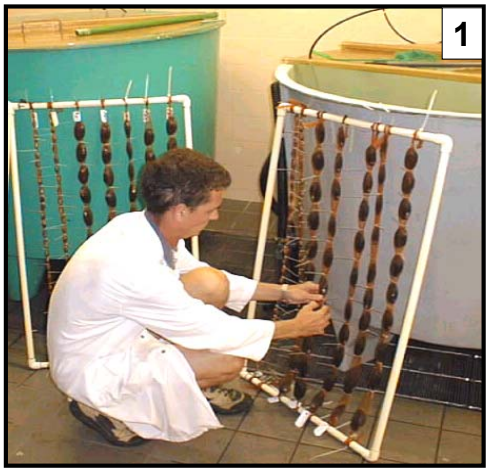
Distributing

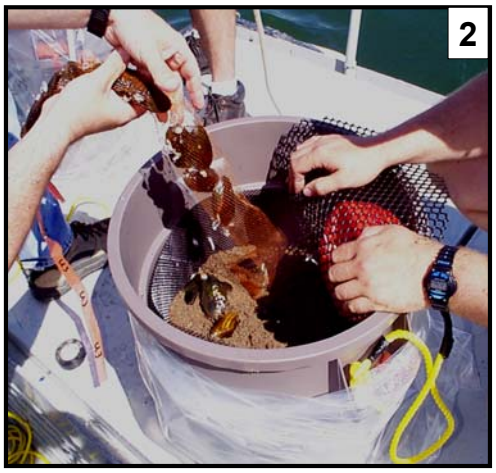
• After weighing and measuring whole animals at the beginning of the test the animals are distributed to mesh bags by size until each bucket is completed. This ensures an even distribution across cages. As shown in the pictures above, one person weighs and measures, another uses plastic cable ties to separate each individual so that repetitive measurements can be made on the same individuals and a third person records the data manually on data sheets and watches the computer to ensure that each measurement is recorded in the appropriate row and column on the spreadsheet.

• When large numbers of animals are used (2000-4000) two teams of three each are used to make the measurements. When less than 2000 animals are required only one team is necessary to complete the measurements and remove the tissues in one day.

• Figure 1 shows a transplant study in Puget Sound, Washington using the marine mussel *Mytilus trossulus* (second team not shown). Figure 2 shows a transplant study in Guelph, Ontario, Canada using the freshwater unionid mussel *Elliptio complanata*. Figure 3 shows a transplant study in Winnipeg, Ontario, Canada using the freshwater unionid mussel *Pyganodon grandis*.

Attaching & Adding Mussels to Cages

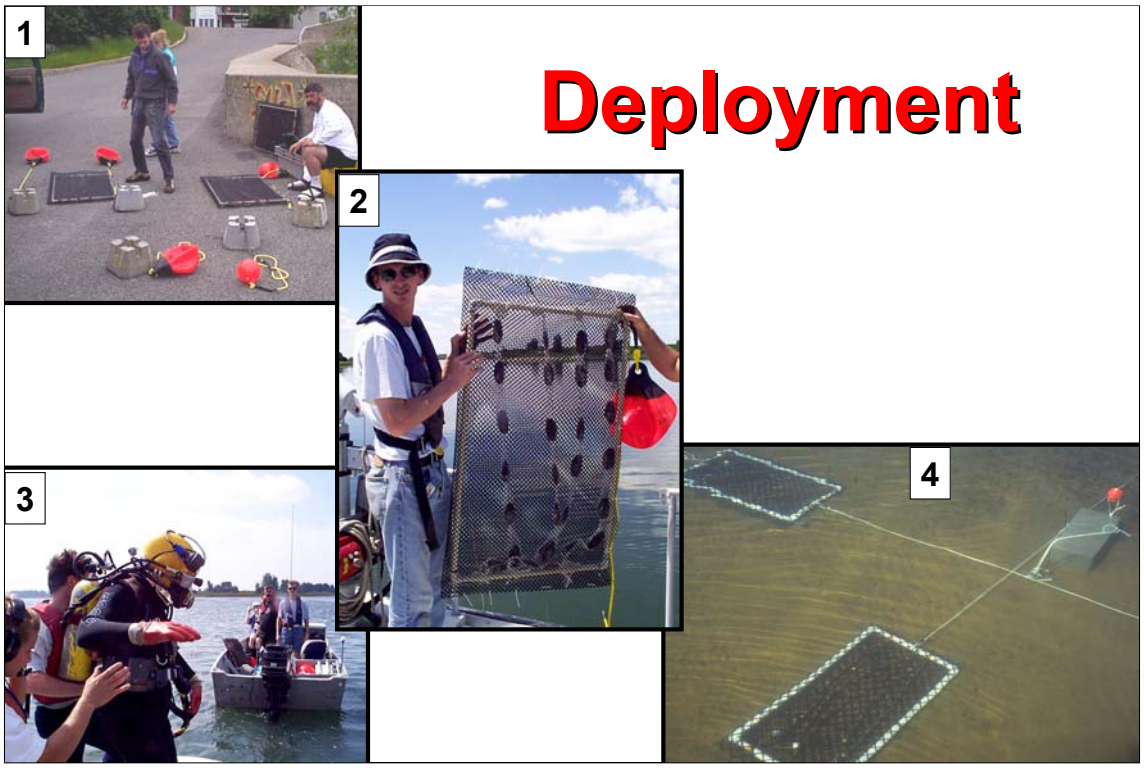




• Figure 1 shows how bags with *Elliptio complanata* are attached to PVC frames in preparation for deployment. The flow through tanks from the Environment Canada St. Lawrence Center shown in the background (Figure 1) were used to hold the cages of mussels overnight before deployment the next day.

• Figure 2 shows a new cage developed cooperatively between the St. Lawrence Center and Applied Biomonitoring scientists. This benthic cage or corral was developed to hold mussels in sediment for 1 year exposures downstream of a City of Montreal effluent. Both cage designs were used in this 1-year study (2001-2002) and the benthic cage proved more successful in maintaining high survival and recruiting new sediment into the cage. *Elliptio* migrated from the clean sand initially added to the cage into the fine sediment collected in the cage over the 1-year exposure period.

• The benthic cage was analogous to an aquaculture net pen with a solid outer enclosure. Mussels were confined by an internal plastic mesh cage and sediment was retained by a fixed plastic trash can. Newspaper was used to retain sediment. There were no individual compartments.



• Figure 1 shows floats, anchors, and cages with *Elliptio complanata* enclosed in plastic predator mesh being prepared for deployment in the St. Lawrence River in Montreal. Figure 2 is a close-up of the cage, with individual compartments for each mussel about to go over the side of the boat. Figure 3 shows the diver used to check the position of each cage on the bottom and re-position if necessary.

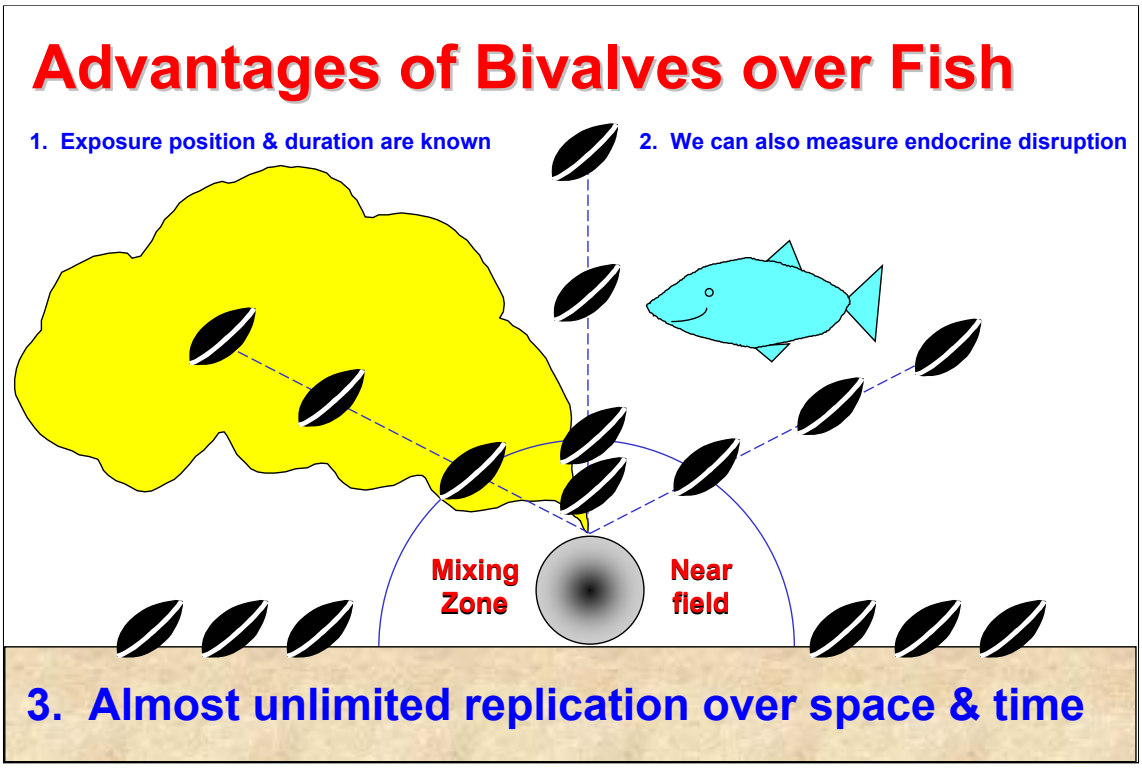
• In 1999, 2000, and 2001 all 60-day deployments used an anchor and float to maintain position of each cage in the water column approximately 1 meter off the bottom. Only in the 1 year study were the both standard cages (shown above) and the benthic cages shown previously used.

• Figure 4 shows another application of the standard cage positioned on the bottom in immediate contact with the sediment. This was a transplant study with *Corbicula fluminea* at a Superfund site in Sault Ste. Marie, Michigan. These studies were conducted in 1997 and 2000 and the purpose was to monitor the effective of remediation of these contaminated sediments where the remedy was natural attenuation by transport and covering with clean sediment.

Retrieval

• The pictures above show the retrieval of the benthic cages in August, 2002. Figure 1 shows the benthic cage being placed in a plastic tray to retain sediment being washed away. Figure 2 shows how *Elliptio complanata* initially placed in clean sand, migrated to the surface where fine sediment had accumulated over the 1-year exposure period.

• Mussel survival was much higher in the benthic cages (86%) than in the standard cages (13%), and the benthic cage demonstrated clear advantages in terms of trapping sediment and exposing *Elliptio complanata* to those sediments. This preliminary sex-reversal test conducted by the St. Lawrence Center laboratory has also shown that feminization in freshwater mussels can be experimentally induced and has confirmed the working hypothesis of this study.



• The figure above demonstrates pictorially the advantages of using fish over bivalves for environmental monitoring and assessment. The biggest problem with fish is that they move and the position and duration of exposure is uncertain. There is far less certainty associated with caged bivalves where position and duration of exposure are controlled.

• It is extremely difficult to map the effluent plumes and define the mixing zone using natural fish populations because they are so mobile. It is not just that large numbers of fish tissue samples that have been analyzed with undetectable concentrations but the uncertainties associated with not knowing where the fish have been and how to characterize their exposure.

• Environment Canada scientists at the St. Lawrence Center in Montreal are developing bivalve biomarkers to provide an alternative to fish biomonitoring. Collectively, the combined experience and expertise of the St. Lawrence Center in bivalve biomarkers and the experience and expertise of Applied Biomonitoring have made substantial progress in characterizing exposure and effects associated with the City of Montreal municipal effluent.

Many Canadian Freshwater Mussel Species are Threatened & Endangered

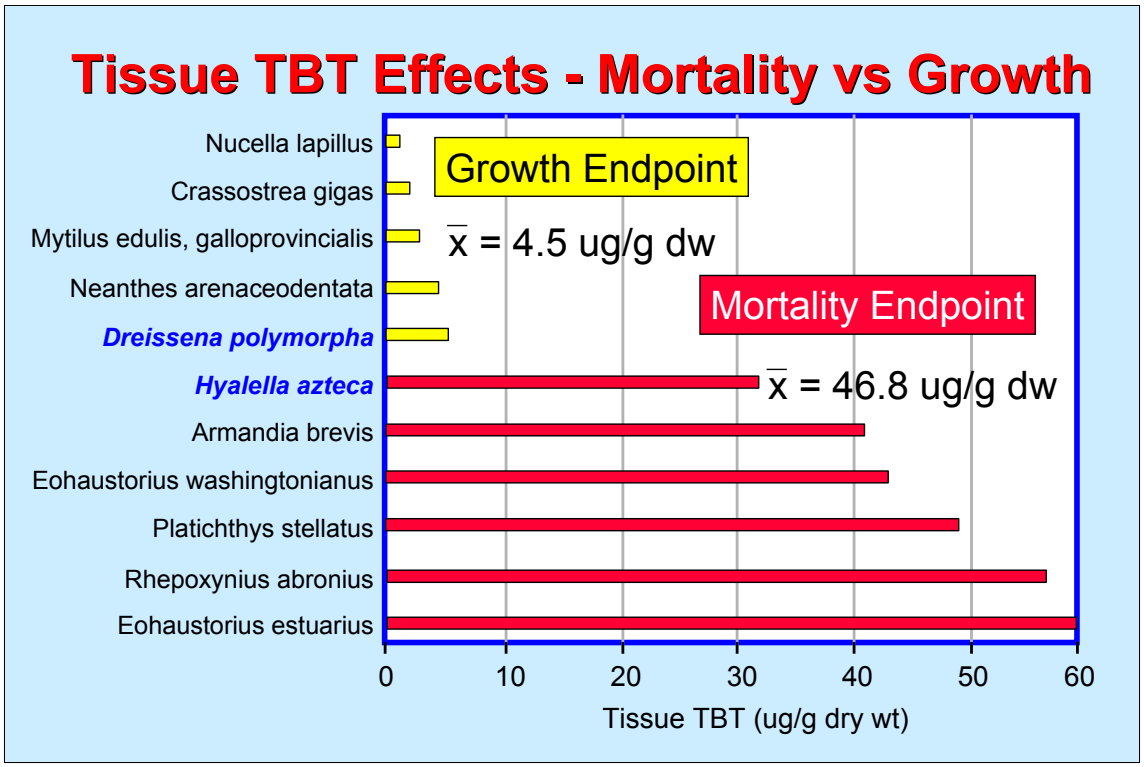
Elliptio complanata

Freshwater mussels In the U.S.

- **The most imperiled animal group**
- **70% are threatened or endangered**
- **20% presumed extinct**
- **10% may be extinct this century**

Like the canary in the coal mine, freshwater mussels are indicators of environmental quality

Another reason for using caged freshwater bivalves for environmental monitoring and assessment is their conservation status. Many U.S. and Canadian freshwater mussel species are threatened and endangered. This makes it even more important to monitor these bivalves as part of environmental monitoring and assessment programs. In the US, freshwater mussels have been shown to be the most imperiled animal group; not fish, not birds, mammals, or insects. Approximately 70% are threatened or endangered and 20% are presumed extinct. It has been predicted that 10% may become extinct in this century. Freshwater mussels are particularly sensitive to anthropogenic stresses because of their reliance on a fish host for reproduction. Their numbers have declined due to dredging, filling, diking and other activities of municipal populations that have caused a loss of habitat for either the mussels or their fish hosts. The most successful species are those who have multiple fish hosts and the least successful those who only have one. Like the canary in a coal mine, freshwater mussels are indicators of environmental quality and their declining numbers suggest that there is a significant environmental problem associated with loss of habitat or the introduction of chemical stressors or both. Freshwater mussels are also threatened by the introduction of exotic species such as zebra mussels. These multiple stressors and their associated effects make these studies even more important.

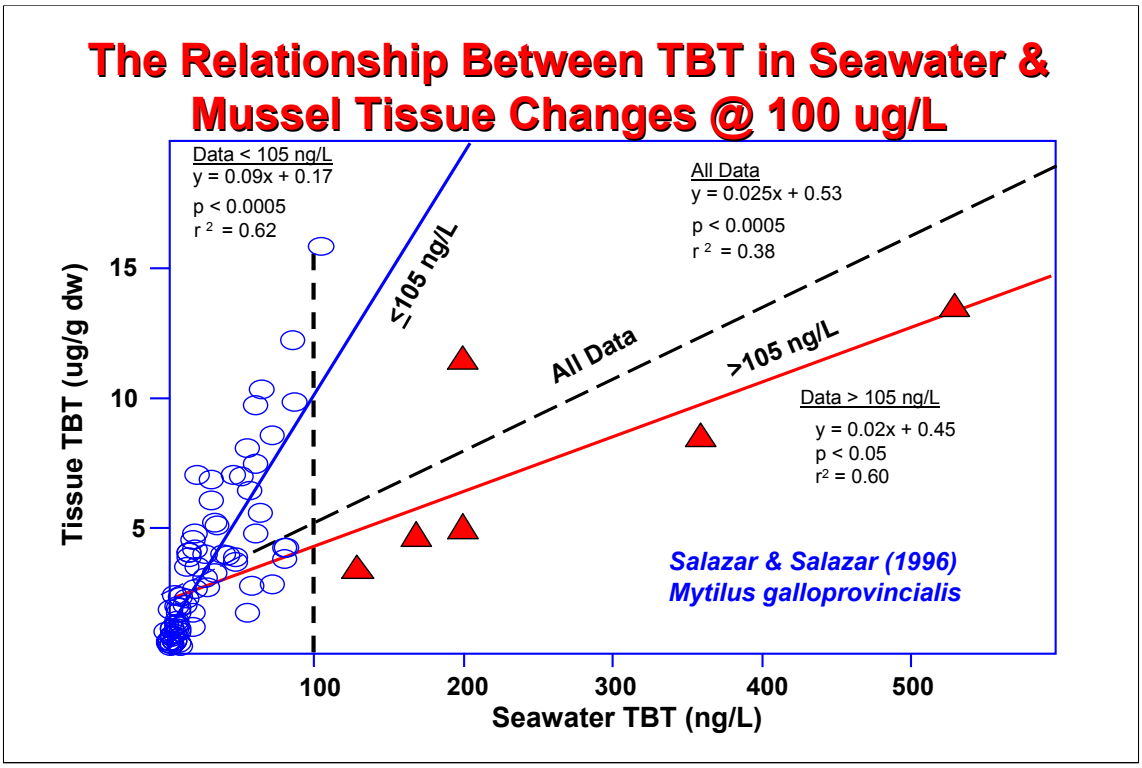


There are a number of reasons for using Tissue Residue Effects (Critical Body Residues)

- Better predictions regarding the fate and effects of chemicals in aquatic ecosystems
- Better estimates of bioaccumulation in organisms and food chains
- The ability to relate tissue chemistry with various acute and chronic effects in laboratory toxicity tests, benthic community studies, and mussel watch monitoring.
- Directly assess questions regarding the bioavailability of chemicals
- Considerations regarding the kinetics of accumulation
- Uptake from food and water are explicitly considered
- Toxic potencies are expressed in a less ambiguous manner than through water or sediment concentrations and this facilitates investigating modes of toxic action
- The effects of metabolism are considered
- Mixture toxicity may be more accurately assessed
- Experimental verification is applicable from both laboratory and field studies

The graph above shows that TBT threshold effects levels are similar to those predicted for mussels in other studies but also for TBT threshold effects levels in other species and that the numbers for marine and freshwater were surprisingly similar. Also, the average critical body residue is very similar to what we predicted from our in-situ bioassays with caged bivalves.

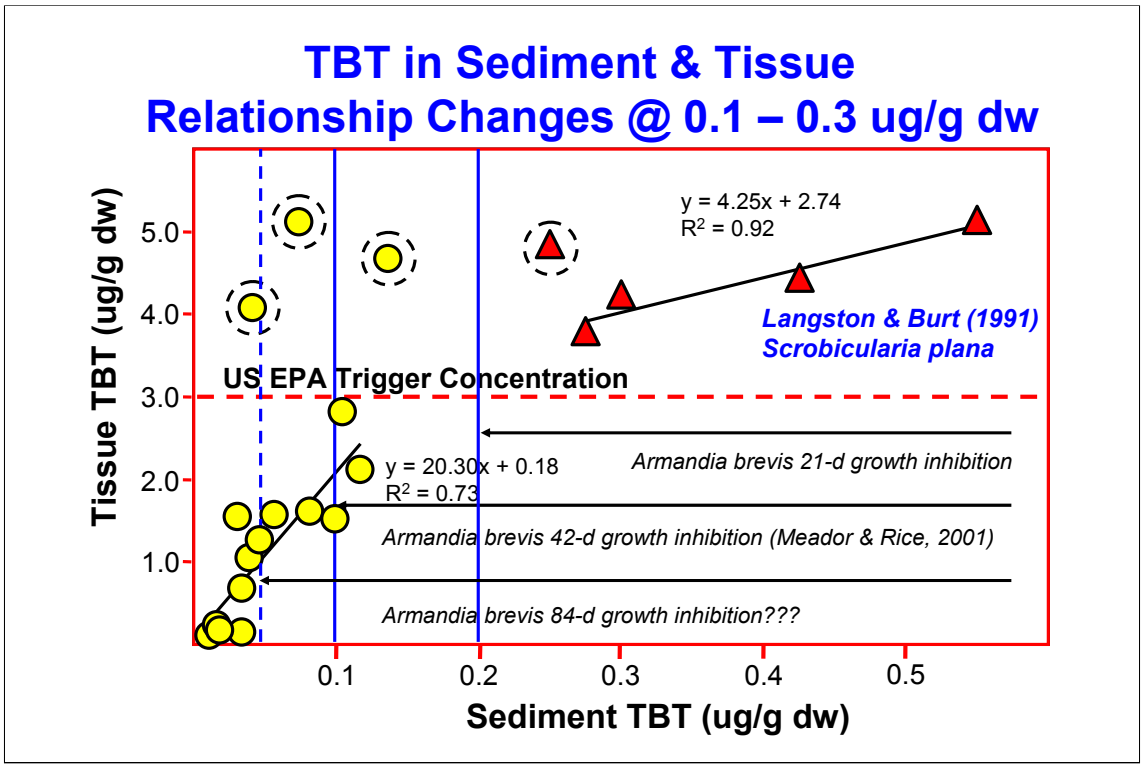
Interestingly, the average critical body residue for the growth endpoint in these species is about 4.5 ug/g dry weight and the average critical body residue for the mortality endpoint is about 46.8 ug/g dry weight. This order of magnitude difference is identical to that predicted by critical body residue theory.



• We were initially surprised to find that the threshold water TBT concentration for probable effects on mussel growth (100 ng/L) was very close to the water concentration where the relationship between TBT in water and TBT in mussel tissues began to change as shown in the figure above; Salazar and Salazar 1996). The significance of the regression for data greater than 105 ng/L improves from $r^2 = 0.60$ to $r^2 = 0.99$ if the apparent outlier is removed from the dataset. The figure also shows how the significance of the relationship can be misleading when all the data are pooled together rather than looking for more discriminatory relationships. Important information can be lost by pooling (Hurlbert 1984).

• It is encouraging that we were able to establish these relationships based on repetitive controlled experiments in the field. The relationships were established with multiple linear regression analyses to identify where the significance of the relationship began to deteriorate.

• This relationship is important because it helps establish a mechanistic link between bioaccumulation and associated biological effects. The is also important because it is part of characterizing and understanding processes controlling uptake of chemicals and bioeffects. Finally, in the absence of other data it shows how this relationship could be used to predict where effects might begin to occur, even in the absence of effects measurements. While this approach might not be very accurate, it would at least allow first-order approximations of effects levels for ecological risk assessment purposes and screening of tissue burdens and helping establish bioaccumulation trigger levels for additional testing in subsequent tiers of monitoring and assessment.

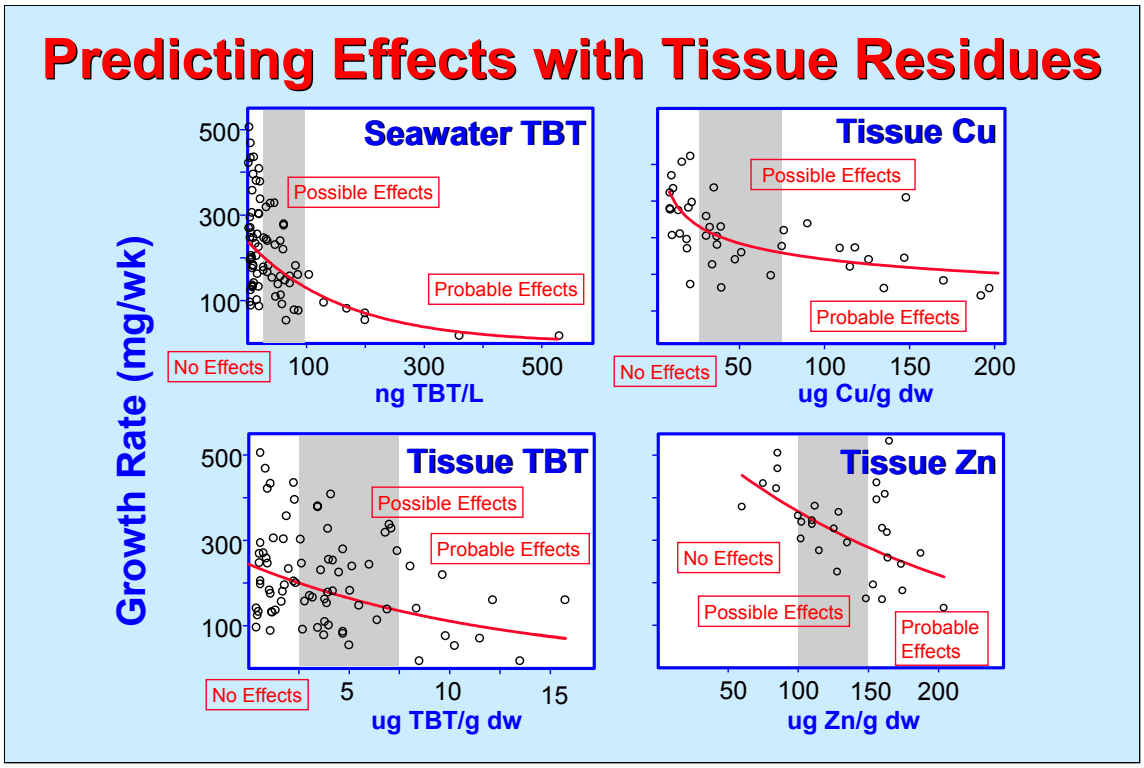


• Even more surprising was the fact that we were able to establish a similar relationship for TBT-contaminated sediment by re-plotting and re-analyzing TBT data for sediment and tissues of the deposit-feeding bivalve *Scrobicularia plana* (Langston and Burt 1991). When their data was re-plotted on an arithmetic scale, it shows the concentration of TBT in sediment where the relationship with uptake begins to change; i.e., between 0.1 and 0.3 ug/g dw as shown in the figure above..

• It should be emphasized that these are field data for natural bivalve populations collected along with seawater and sediment chemistry data. The data points that are circled represent three or four sites that had high seawater concentrations of TBT and could be considered as outliers for purposes of establishing the relationship between sediment and tissue concentrations of TBT.

• Similar sediment concentrations have been associated with adverse effects on growth in the marine polychaete worm *Armandia brevis* (Meador and Rice 2001). While these two datasets represent the best available data for these relationships, it is encouraging that the results are similar and suggest that the change in relationship could be used to predict potential effects on at least two marine species.

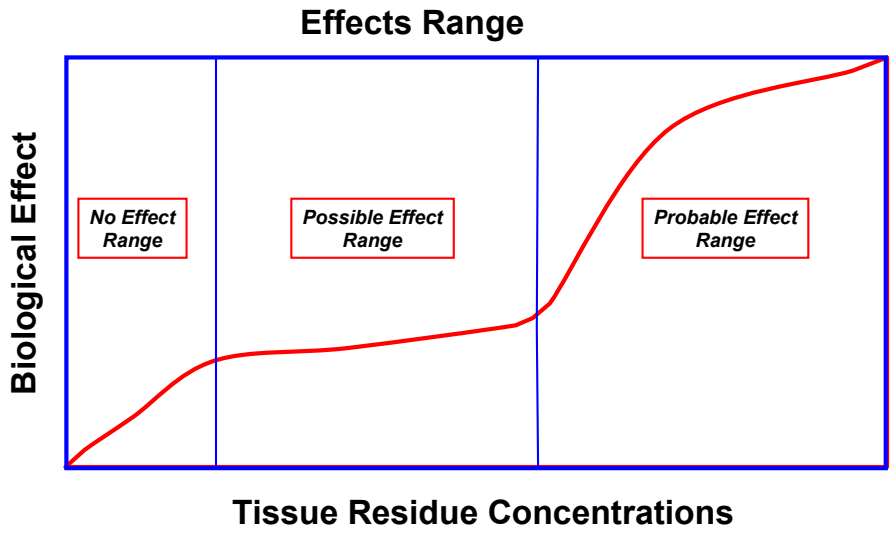
• The figure also depicts how Meador & Rice (2001) demonstrated that threshold concentrations for effects decreased by 50% with a doubling of the exposure time from 21 to 42 days. While it should not be expected that threshold concentrations would continue to decrease with each incremental increase in exposure time, we have speculated how the threshold concentration would decrease by another 50% if the exposure time was increased from 42 to 84 days. This is relevant because most of our caged bivalve studies have exposure periods between 60 and 90 days and this was is recommended in the ASTM Standard Guide.



We were among the first to establish threshold effects level for water and tissue concentrations of TBT (Salazar and Salazar 1991) using a series of caged mussel transplants in San Diego Bay. These estimates were later refined (Salazar and Salazar 1996b, 1998). Using the TBT data we were able to characterize and understand the processes associated with exposure, dose and response and developed the exposure-dose-response triad (Salazar and Salazar 1998). Using the same template we were able to use data for other chemicals such as copper and zinc to establish tissue threshold effects levels for those metals (Salazar and Chadwick 1991, Salazar 1997). These predicted effects levels are shown in the figure above.

- The graph in the upper left shows the prediction of threshold effects levels of Tributyltin (TBT) in seawater.
- Critical threshold concentrations were predicted through multiple regression analyses to determine the inflection point of the curves. Each graph shows probable, possible, and no effects levels. For seawater the threshold for effects on mussel growth is between 25 and 75 ng/L.
- For TBT in mussel tissues the threshold for effects on mussel growth is between 2.5 and 7.5 ug/g dry weight.
- For copper in mussel tissues the threshold for effects on mussel growth is between 25 and 75 ug/g dry weight.
- For zinc in mussel tissues the threshold for effects on mussel growth is between 100 and 140 ug/g dry weight.
- Each of these threshold effects levels is similar to that predicted for effects in other studies.

Using the Effects-Range Paradigm for Tissue Guidelines

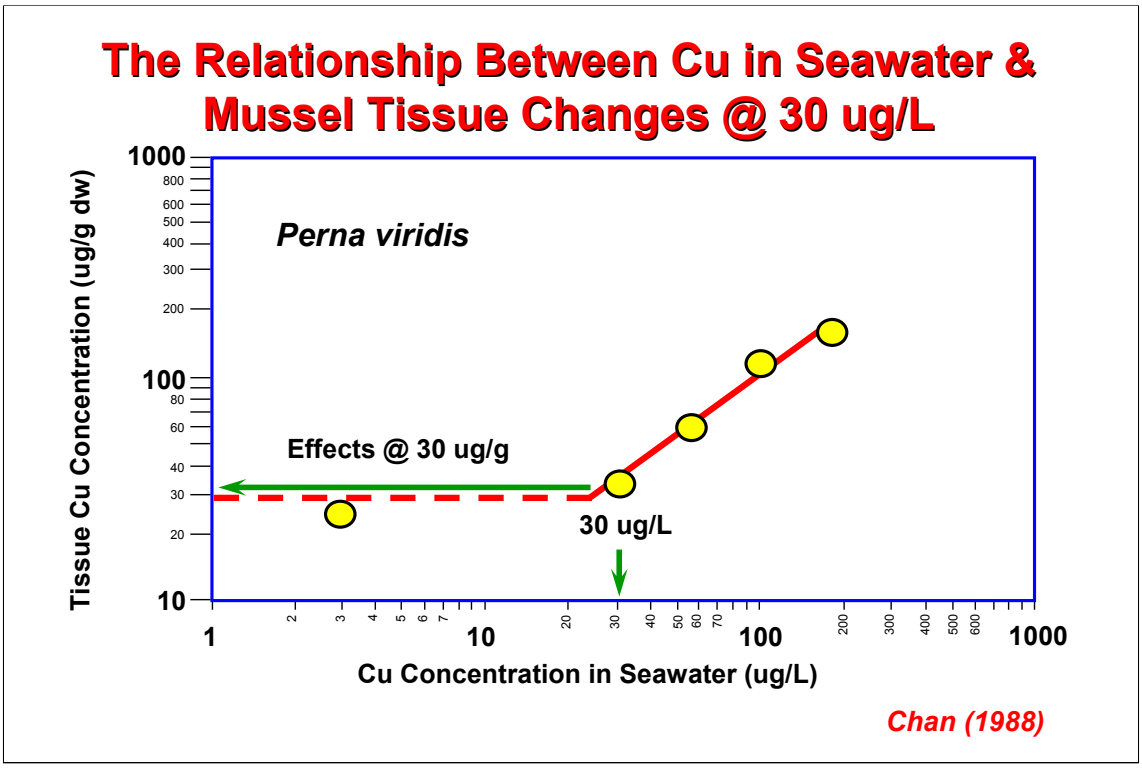


Adapted from Long & Morgan (1990)

• Long and Morgan (1990) initially developed sediment quality guidelines by employing a preponderance of evidence assembled from a variety of approaches and from data gathered from many geographic areas. They acknowledged that sediment chemistry data provide neither a measure of adverse biological effects or an estimate of the potential for effects. They used a 3-step approach for their evaluation: 1) assemble and review data where estimates of sediment concentrations are linked with adverse biological effects; 2) determine ranges in concentrations of chemicals in which effects are likely to occur; and 3) evaluate other data relative to these effects ranges.

• Although tissue residues have been used more routinely to determine the potential for bioaccumulation of chemicals from sediments and dredged materials, they also provide a representative measure of "effective exposure dose" that may be more meaningful than chemical measurements in water or sediment. It represents an integrated measure of actual exposure over time to a chemical of concern. This exposure is related to the dose expected at water and sediment quality criteria and guidelines and the potential for toxic effects.

• Similar effects ranges could be established using the paradigm initially used by Long and Morgan and modifications currently being used by those to develop more sophisticated sediment quality guidelines as shown in the figure above. Given the ability to measure tissue residues in water and sediment exposures, it is possible to establish tissue residue guidelines based on residue-toxicity relationships. These relationships can provide a basis for criteria without bias associated with bioavailability of chemicals from water or sediment. This is particularly true when in-situ measurements provide the residue-toxicity link as with the caged bivalve approach. .



• A number of investigators have suggested the following with respect to the environmental significance of bioaccumulation:

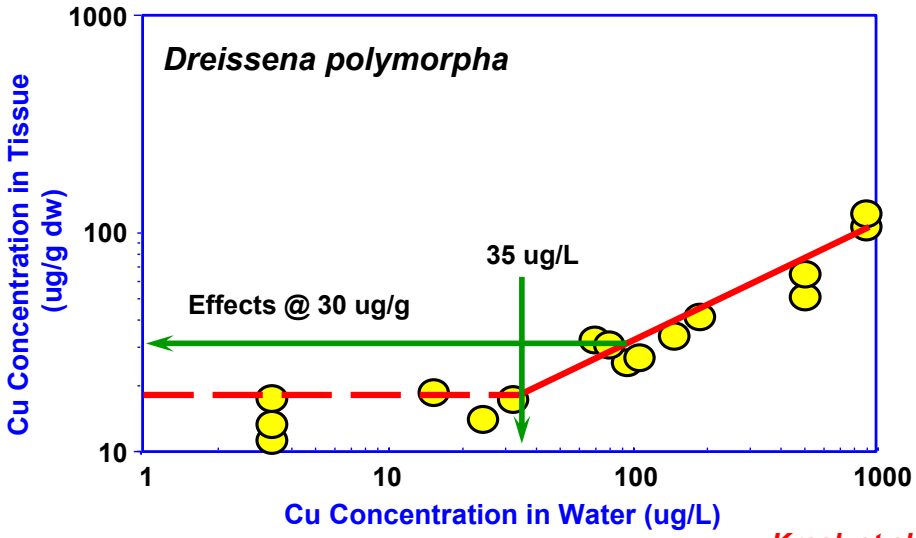
1) bioaccumulation is not an effects endpoint and has little environmental significance;

2) many organisms, including invertebrates such as bivalves cannot be used to as effective monitoring tools because they have the ability to regulate essential metals such as copper;
3) it is more important to measure effects endpoints rather than exposure endpoints.

• While it is true that bioaccumulation in itself is not an effect, effects can be predicted based on the way organisms change the way they accumulate at threshold exposure concentrations in water, sediment, and tissues. This concept is demonstrated in the figure above and the following figure where two different species of bivalves (one marine and one freshwater species) change the way they accumulate copper at about 30 ug/L.

• The figure above demonstrates this change in relationship for the marine mussel *Perna viridis* (Chan 1988). Although there is only one data point between 1 and 30 ug/L, it does appear as though the relationship changes dramatically at about 30 ug/L and above. This change in relationship has been demonstrated in many similar experiments with bivalves and other species of freshwater and marine bivalves for several different metals.

The Relationship Between Cu in Freshwater & Mussel Tissue Changes @ 35 mg/L



Kraak et al. (1993)

• The figure above demonstrates a similar change in the relationship for the freshwater zebra mussel *Dreissena polymorpha* Kraak et al 1993). The relationship changes near 35 ug/L and this is associated with a critical body residue of about 30 ug/g dry weight. In both laboratory examples an effects endpoint was measured to demonstrate that effects were actually occurring. The point to be made is that the effect could have been predicted based only on where the relationship between exposure and uptake changes. While we do not advocate this approach over measuring exposure and effects endpoints simultaneously, it demonstrates how the process can be characterized and better understood to make these kinds of predictions in the absence of effects endpoints.

• There are many more data points in the *D. polymorpha* experiment, and the data more clearly demonstrate the change from regulation of copper by the mussel to one of increased uptake. The mechanistic explanation for this phenomenon is that the regulatory system is overwhelmed by increasing concentrations to the point where the animals can no longer regulate copper. The data in Figures 2 and 3 also suggest that these processes are similar in freshwater and marine mussels. More data will be provided later to demonstrate that based on all the available information that the processes and critical effects thresholds are similar

• It is also interesting to note that based on our extensive field studies with caged bivalves in San Diego we had predicted over 10 years ago that effects could begin to occur at tissue copper concentrations above 25 ug/g dry weight. All of these results are encouraging and consistent with critical body residue theory. It is also encouraging that we could establish these dose-response relationships in the field under environmentally realistic conditions.

Tissue Cu Effects on Marine Bivalves

SPECIES	EC	NOEC	EXPOSURE	ENDPOINT
Cerastoderma edule	250	4	Field	Survival
Cerastoderma edule	25		Field	Condition Index
Chama isotoma		20.3	Field transplant	Survival
Chama isotoma		20.3	Field transplant	Condition Index
Crassostrea gigas		178.2	Field	Normal shells
Crassostrea gigas	1219	592	Field	Thick shells with holes
Crassostrea virginica	100		Lab - 14d	None - controls
Crassostrea virginica	500		Lab - 14d	Normal O2 use
Crassostrea virginica	800		Lab - 14d	Increased O2 use
Crassostrea virginica	175	20	Field	Growth
Crassostrea virginica	607		Lab - 20wk	6% Mortality
Macoma balthica	60	20	Field	Reproduction
Macoma balthica	137.5		Field	Condition Index
Macoma balthica	125	20	Lab	Survival
Macoma balthica	58		Field 11d	Moderate Mortality
Macoma balthica	144		Field 11d	High mortality
Macoma balthica	304		Field 11d	Low Mortality
Macoma balthica	314		Field	Survival & disappearance
Meretrix casta	1005		Lab	Mortality; ED50
Mya arenaria	123		lab	6wk - LC100
Mytilus californianus	15.6	13	Field	Scope for Growth
Mytilus edulis	100	50	lab	Survival
Mytilus edulis	100		Lab	Change in bioaccumulation
Mytilus edulis	23.9	18.45	Lab - 6 mo - J (Field Collected)	Growth - no effect
Mytilus edulis	21	20.5	Lab - 6 mo - A (Field Collected)	Growth - no effect
Mytilus edulis		53	Lab	Histopathology - no effects
Mytilus edulis	75		Lab Reared- 4 mo	Growth - reduced
Mytilus edulis		96.2	Lab Reared - 12 mo	Growth - no effect
Mytilus edulis	100		Lab Reared - 18 mo	Histopathology - effects on
Mytilus edulis	888	100	Lab - 21 mo	Survival - decreased
Mytilus edulis	21	7	Lab	Inhibited glycine uptake
Mytilus edulis		75	lab	10-d survival
Mytilus edulis	100	50	lab	Survival
Mytilus edulis	100	50	field	Survival
Mytilus edulis	20		Field transplant	Growth (length)
Mytilus edulis	20		Field transplant	Growth (weight)
Mytilus edulis	40		Field transplant	Survival
Mytilus edulis	40		Field transplant	Condition index
Mytilus edulis		14	Lab - 30d HNEC	No Mortality
Mytilus edulis	40	14	Lab	Enzyme effects
Mytilus edulis	40		Lab - 44d	Mortality 100%
Mytilus edulis	45.1	14	Lab - 30d	Lysosomes in cytoplasm
Mytilus edulis	45.1		Lab - 76d LEC	Mortality 12%
Mytilus edulis	7.5		Lab - 6d	Lysosomal destabilization
Mytilus edulis	37.5		Field	Condition Index

SPECIES	EC	NOEC	EXPOSURE	ENDPOINT
Mytilus edulis	337		Lab	Mortality; ED50 after 7 to 8 days
Mytilus edulis	337		Lab	Behavior LOED; total valve closure
Mytilus edulis	100	10	Lab	Survival
Mytilus edulis	124	6.75	Lab	Filtration rate, SFG
Mytilus edulis	59	7	Lab 18d	Pathology
Mytilus edulis	12.1	7.2	Microcosm - LEC	Cellular tubes dilated
Mytilus edulis	16.5	17	Field - LEC	Reduced Gametogenesis
Mytilus edulis	60		Lab	Mortality ED100
Mytilus edulis	60		Lab	Mortality ED100
Mytilus edulis	60		Lab	Mortality ED100
Mytilus edulis	75		Lab	Mortality ED100
Mytilus edulis	115		Lab	Mortality ED100
Mytilus edulis	180		Lab	Mortality ED100
Mytilus edulis	280		Lab	Mortality ED100
Mytilus edulis	400		Lab	Mortality ED100
Mytilus edulis	59		Lab	Lethal
Mytilus edulis	73.2	35.6	Lab	SFG, lysosomal latency
Mytilus edulis	53	7.2	Microcosm - 15 wk	None - lipofuscin normal
Mytilus edulis	17	14.3	Field	Low - Lysosome
Mytilus edulis	25	10	Lab/Field	Gametogenesis
Mytilus edulis	1.3	0.4	Lab - 23d	50% survival 267 ppb
Mytilus edulis	2		Lab - 23d	90-95% survival 26 ppb
Mytilus edulis	2		Lab - 23d	70-75% survival 134 ppb
Mytilus edulis	37.5		lab	6d - LC50
Mytilus edulis	7.3		Microcosm	Low B(a)P Phase
Mytilus edulis	27		Microcosm	Low B(a)P Phase
Mytilus edulis	27		Microcosm	Low Epoxide Phase
Mytilus edulis	27		Microcosm	High Epoxide Phase
Mytilus edulis	100		Lab	Histopathology
Mytilus edulis	27		Mesocosm	Metallothionein, protein
Mytilus edulis	24	12		Growth effects begin to occur
Mytilus edulis		7.3	Microcosm	SFG, predicted mortality
Mytilus edulis		16.3	Microcosm	SFG, predicted mortality
Mytilus edulis	16.5	14.3	Field	Scope for Growth
Mytilus edulis	17	16.6	Field	Scope for Growth
Mytilus edulis	26.8		Microcosm	SFG, predicted mortality
Mytilus edulis	59		Microcosm	SFG, predicted mortality
Mytilus galloprovincialis	75	25	Field transplant	Growth rate
Mytilus galloprovincialis	50	20	Lab - 3d	Reduced lysosomal stability
Mytilus galloprovincialis	23.2	4.4	Lab - 15d	Pathology
Mytilus trossulus	100	10	Field transplant	Condition index, proteins
Perna canaliculus	8.1		Field	Scope for Growth
Perna viridis		30	Lab	Survival
Prototthaca staminea	50		Lab	Enzyme, protein induction
Scrobicularia plana	100		lab	Survival - LC50

EC_{tissue}	$NOEC_{tissue}$	
129	41	**MEANS for all bivalves* (ug/g dw)**
80	24	**MEANS without oysters* (ug/g dw)**

*excluding highlighted entries

- We use copper as an example of establishing tissue residue guidelines because we could find more data linking adverse effects with measured tissue residues than for any other chemical. In the two tables that follow we present available data for marine and freshwater bivalves.

- The table above summarizes tissue copper concentrations and associated effects, or at least potential effects, in marine bivalves. These data were not screened or grouped by specific acute or chronic endpoints but pooled to give an overall approximation. When all bivalve data are included in the calculated means, the predicted effects concentration (EC_{tissue}) and no-effects concentration ($NOEC_{tissue}$) are 129 and 41 ug/g dw. If the oyster data are excluded, these means decrease to 80 and 24 ug/g dw. Oysters are known hyper-accumulators of copper, and the variance is significantly reduced without these data. NOAA (1989) routinely separates their use of copper data for mussels and oysters given these observed differences.

- Even more interesting is that the mean threshold concentrations without oysters are virtually identical to those we predicted over 10 years ago using results from our San Diego Bay caged bivalve studies with the marine mussel *Mytilus galloprovincialis:* EC_{tissue} = 75 ug/g dw, $NOEC_{tissue}$ = 25 ug/g dw. Given that the relationships in this table were established using over 40 different studies with a variety of effects endpoints, exposure conditions, and measurement techniques, the utility of the average threshold concentrations appears to be reasonably robust. It also shows that the caged bivalve method could establish reliable dose-response data using caged mussels and associated measurements of bioaccumulation and growth. The method becomes even more powerful with the addition of bivalve biomarkers and reproductive endpoints.

- These threshold concentrations are also consistent with threshold copper concentrations associated with reproductive effects in natural populations of *Macoma* based on a 23-year time series in San Francisco Bay (Hornberger et al 2000).

Tissue Cu Effects on Freshwater Bivalves

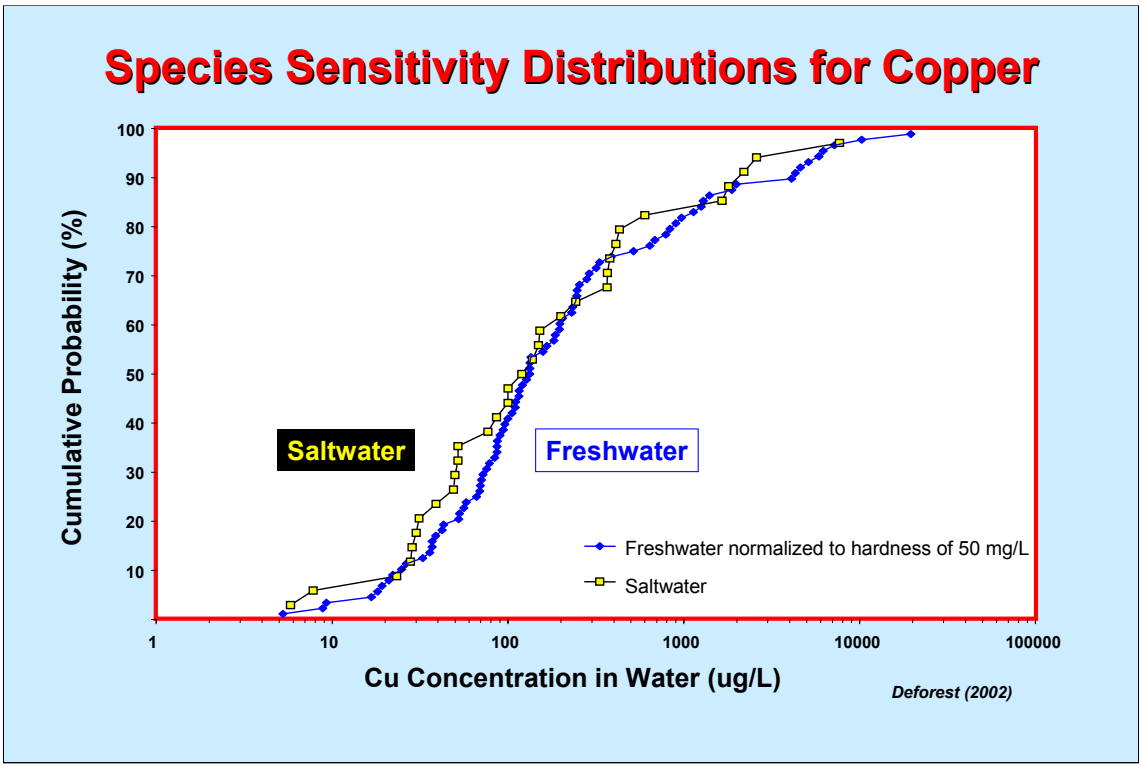
EC_{tissue}	$NOEC_{tissue}$	Species	Exposure	Endpoint
100	50	*Corbicula fluminea*	Artificial stream	Growth: weight, shell length
120	65	*Corbicula fluminea*	Artificial stream	Growth: weight, shell length
-	46.8	*Corbicula fluminea*	Artificial stream	Growth: shell length
40	20	*Dreissena polymorpha*	Lab	Filtration rate
93.5	-	*Quadrula quadrula*	Transplant	Mortality
8.1	2.7	*Dreissena polymorpha*	Lab	Regulation breakdown
6.5	2.7	*Unio pictorum*	Lab	Regulation breakdown
70	15	*Dreissena polymorpha*	Lab	Mortality
100		*Elliptio complanata*	Field	Mortality
83		*Dreissena polymorpha*	Field	Tissue energy reduction
20.8	14.3	*Dreissena polymorpha*	Field	Scope for growth
64.3	**28.9**	**MEANS for all bivalves (µg/g dw)**		

- Unfortunately, as shown in the figure above, there are far fewer data available for freshwater bivalves. Nevertheless, the threshold EC_{tissue} and $NOEC_{tissue}$ copper concentrations are 64.3 and 28.9 ug/g dw, respectively. Again, these values are very close to those for marine bivalves and to those we predicted using caged bivalves in San Diego Bay.

- These values include data for five different species and endpoints that ranged from mortality to filtration rate. The data were not screened. The data strongly suggest that similar processes are occurring between most bivalve species.

- The only exception identified so far are marine oysters which are known hyper-accumulators of copper.

- The freshwater data are important because they show that it may be possible to pool freshwater with marine data to reduce the uncertainty in the risk assessment based monitoring and assessment. Furthermore, it may be necessary to use more euryhaline species or even freshwater species in some areas of regional monitoring.

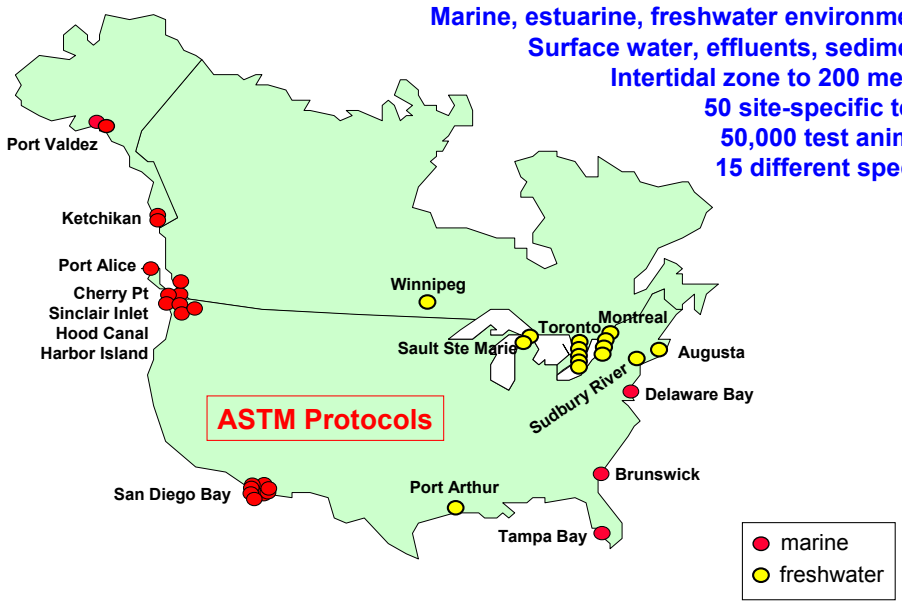


• Just as most of the tissue chemistry data for copper thresholds for effects suggest a striking similarity between marine and freshwater bivalves, Deforest (personal communication) has shown that the species sensitivity distributions for copper are also identical for freshwater and saltwater organisms with respect to water exposures using species sensitivity distributions as shown in the figure above. This approach is also the basis of a probabilistic risk assessment-based approach that should be used instead of hazard quotient approaches to reduce uncertainty in the assessment.

• Leung et al. (2001) provide examples where it might be appropriate to predict toxicity in saltwater (all species) from freshwater data based on species sensitivity distributions for water exposures. For metals, they demonstrate that the species sensitivity distributions for cadmium are virtually identical while the saltwater values for nickel are lower than those for freshwater species. Brix et al. (2001) used species sensitivity distributions from water exposures and a probabilistic risk assessment methodology based on Parkhurst et al. (1995), to assess acute and chronic copper risks to freshwater aquatic life. Just as Brix et al. (2001) evaluated different taxonomic groups for relative sensitivity, we examined relative sensitivity among different bivalves and then used an approach similar to that of Long and Morgan (1990) to establish ranges of effects for tissue burdens of copper in bivalves.

• While it could be argued that the toxic concentrations in the more sensitive larval forms of bivalves that play a significant role in driving these criteria not directly linked to the tissue burdens and effects measured for establishing critical body residues, the similarity is encouraging. Just as McCarty and Mackay (1993) suggested that whole body tissue residues are a reasonable surrogate for the critical concentrations at receptors of concern and have some relationship with exposure concentrations in water, there should be some relationship between the species sensitivity distributions and tissue burdens. We interpret the species sensitivity distributions as additional evidence that supports the tissue residue effects approach at the level of species sensitivity distributions and suggest that this approach should be used instead of the hazard quotient approach.

Our Caged Bivalve Studies



• We have conducted over 50 caged bivalve studies with over 50,000 bivalves and representing over 15 different species of marine, estuarine, and freshwater bivalves. The net result of all these studies was the development of an ASTM Standard Guide on the use of caged bivalves for environmental monitoring and assessment, an exposure-dose-response model for developing monitoring and assessment strategies, and an appreciation of the significance and utility of tissue residue effects thresholds.

• These studies have been conducted in marine, estuarine, and freshwater environments from the intertidal zone and depths of less than 1 foot to over 200 meters. Collectively, these studies show the versatility and applicability of the ASTM protocols developed over the past 30 years in a variety of environments and for a variety of species.

• It also shows that the methods are robust. In recent studies survival of over 90% was demonstrated at depths of over 200 meters in Port Valdez for 90-d exposures, survival of 86% in the St. Lawrence River for a 1-yr exposure period, and 100% survival in less than 1 foot of water in a small tributary to the Speed River in Ontario in a 120-d exposure.

• With the discriminating power of the caged bivalve methodology to distinguish differences in exposure and effects at sites separated by as little as 3 meters vertical distance in several different studies and the discriminating power of chemical fingerprinting to identify sources and map effluent plumes in water and sediment the combined approach is a potentially powerful tool in environmental monitoring and assessment.

Controlled Field Experiments

Using caged bivalves

- **Characterize exposure & effects**
- **Over space & time**
- **Under site-specific conditions**
- **Support eco-risk assessments**
- **Tissue residue effects relationships**

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Merci beaucoup!

Refinement, Validation, & Application

- We have shown the numerous advantages of using caged bivalves in controlled field experiments to characterize exposure and effects over space and time and under site-specific conditions in the field associated with our **refinements** to the caged mussel methodology. Moreover, the addition of chemical fingerprinting and tissue residue effects approaches have helped **refine** this approach to an even higher level not anticipated, appreciated, or understood by many.

- We are currently planning additional controlled experiments with scientists from the St. Lawrence Center to continue the development and **validation** of these combined approaches. This will lead to the **application** of bivalve biomarkers and dietary pathways of exposure relative to a City of Montreal effluent and associated environments during 2003 and in the coming years.

- It is hoped that these continuing projects will help advance the science and provide greater insight into characterizing and understanding processes involving the fate and effects of chemicals associated with the effluent. Similar plans are also underway for sediment evaluations.

- We wish to thank all of our collaborators at the St. Lawrence Center, in particular Christian Blaise, Francois Gagne, Christian Gagnon, and Sylvain Trottier.