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Hello Everyone,

Thank you for expressing interest in our caged bivalve methodology. We believe that ATW 2000 in Newfoundland was a landmark conference in terms of an increased number of papers expressing significant concerns about the adult fish survey currently required for environmental effects monitoring (EEM) in Canada. There was also an increased number of papers advocating the use of bivalve biomonitoring for EEM and other purposes. We also taught our caged bivalve methodology course for the fourth time (twice @ SETAC and twice @ ATW). Recently, caged bivalves and mesocosms have been included in the June 2000 Consultation Document "Proposed Additional Amendments Being Considered by Environment Canada in Respect of the Pulp and Paper Effluent Regulations and the Port Alberni Pulp and Paper Effluent Regulations." We are also excited about our collaboration with Christian Blaise and Francois Gagne of Environment Canada in Montreal. Over the past two years we have transplanted freshwater mussels (Elliptio complanata) upstream and

downstream of a municipal effluent in Montreal. We have provided the transplant methodology and growth measurements and they have provided a suite of biomarkers including one for estrogenicity and another for metallothioneins in the mussel gills. Metals and organics were also measured in mussel tissues. While we have refined the caged bivalve methodology to a level where many have not even considered, the addition of this biomarker suite raises the approach to another level and shows promise for cost-effective meaurements that can help establish links with fish as part of routine EEM monitoring. Four papers were presented on this work at ATW and can be located by searching by author or topic in the attached abstract book. We routinely enter the most interesting abstracts into our electronic database which is currently over 10,000 citations but this time we entered all 120 platform papers and thought we would share them with you. As previously mentioned, we believe that this was a landmark conference and that most of you will find useful information by browsing the abstracts and searching for keywords or authors. The attached file is in WordPerfect but you should also be able to read it in Microsoft Word. You may also want to enter some of these citations into your personal database. You can find more information regarding the proposed pulp and paper effluent regulations by visiting the following website:
http://www.ec.gc.ca/p&p/Consult Doc-Eng.pdf . A paper has also recently been accepted for publication in Comparative Biochemistry and Physiology titled "Evaluation of estrogenic effects of municipal effluents to the freshwater mussel Elliptio complanata" (Gagne, Blaise, Salazar, Salazar, Hansen). More information on these results can be obtained by contacting Francois directly at the following email address: : francois.gagne@ec.gc.ca

The attached paper (PDF format) is a humorous yet critical evaluation of pickle jars as a monitoring tool in EEM: "A pickle jar is a pickle jar and a fish is a moving target but a fish is not a fat bag and bivalves don't eat pickles". The basic premise is that all containers are really just pickle jars bivalves are particularly sensitive flow rates and suspended particulate matter that may be reduced in any test chamber, whether it is flow-through or whether it is conducted on site. Our results with mesocosms in San Diego Bay in 1986 showed that mussels caged at the seawater intake grew about 4 times faster than those held in the mesocosm control tanks. While some of the initial mesocosm development work validated test results by comparing benthic populations in the tanks and in the field, none of recent fish mesocosm tests have attempted a similar validation. While it is possible that fish may not be as sensitive to water flow and suspended particulate matter as bivalves, there should still be some type of field validation for each test since receiving waters differ significantly on a site-by-site basis. It is inappropriate to assume that any test conditions represent the real world unless they are tested and compared. Even if the fish are not as sensitive to suspended particulate matter and water flow, some measurements should be made of the mesocosm water and the receiving waters to determine if they are comparable. Our mesocom and lab results suggest that there is a tendency to over-estimate toxicity and under-estimate bioaccumulation when bivalves are stressed by rough handling and overcrowding in test containers. In concept, field bioassays using caged bivalves and fish mesocosms bridge the gap between the lab and the field by combining elements of experimental control and environmental realism. The mesocosms have more experimental control and the caged bivalves have more environmental realism. We validated our mussel growth rates in cages by comparing them to adjacent wild mussels. In one recent bivalve mesocosm test, mussels lost about 40% of their lipids during the 89-day exposure period. This suggests that the mussels spawned during the test and test conditions were not sufficient for the mussels to restore their energy reserves. We have encountered a similar loss in lipids when mussels were close to spawning and were stressed by mesocosm holding for approximately 10 days. However, when the mussels were transplanted to field, they restored their lipid reserves in less than 30 days. The main problem with the adult fish survey is that they are moving targets and exposure to pulp mill effluent is always undefined. For this reason, it is not easy to interpret monitoring results. We provide a relative ranking of the adult fish survey with bivalves (caged, resident, mesocosm) using the original criteria suggested by various EEM committees and believe that on balance, caged bivalves are potentially superior to the other methods.

There are certain attributes that allow the caged bivalve methodology to map effluent plumes and identify potential sources of contamination by measuring chemicals in their tissues. Tissue chemistry can therefore be used to establish links with other monitoring methods such as waer and sediment chemistry, field bioassays and benthic community structure and lab studies using bioassays and community structure. We also discuss the exposure-dose-response triad that we presented several years ago and explain why this approach is more consistent with the ecological risk assessment paradigm than other approaches such as the sediment quality triad. Finally, we describe in this paper a proposed monitoring framework based on comparing tissue residues in bivalves and fish from previous lab and field studies where body burdens have been associated with adverse effects and use this as a guideline comparable to water quality criteria and sediment quality guidelines to compare with field monitoring results. We believe that it is a mistake to focus on effects-based monitoring because if exposure has not been adequately characterized, the results from effects measurements are not very useful. This is the crux of ecological risk assessment: characterization of exposure and characterization of effects.

We hope that you find some of this information useful and would appreciate your comments regardless of whether or not you agree with what we have presented.

APPLIED BIOMONITORING

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HAVE MUSSELS, WILL TRAVEL

pickle jar extended abstract.pdf **Abstract Book.wpd**

Presented: 27th Annual Aquatic Toxicity Workshop
October 1 - 4, 2000; St. John's Newfoundland (Extended Abstract Submitted for Proceedings)

A Pickle Jar is a Pickle Jar and a Fish is a Moving Target but a Fish is not a Fat Bag and Bivalves Don't Eat Pickles. Michael H. Salazar and Sandra M. Salazar, Applied Biomonitoring, Kirkland, WA.

Introduction

Bivalves have been used to estimate chemical bioavailability and biological effects in laboratory and field exposures for over 30 years because they are good surrogate test organisms. Bivalve tests have been conducted with static and flow-through systems in the laboratory, mesocosms in the field, and using *in-situ* cages. We have conducted tests under all of these exposure conditions and have learned a great deal about the advantages and pitfalls of each approach. It appears that any test chamber may adversely affect their health because bivalves are sensitive to levels of both suspended particulate matter as a food source and adequate flow to deliver that food. This observation has important implications for environmental effects monitoring (EEM) at pulp and paper mills since fish mesocosms and caged bivalves have been proposed as alternatives to the adult fish survey. The shift from pickle jars to mesocosms to field studies is not really about measuring effects. It is about providing environmentally realistic test exposures. This paper will discuss our work on characterizing exposure and effects in bivalves and compare results from various exposure systems. For purposes of this discussion, a "pickle jar" will refer to any enclosed test chamber.

Overestimating Toxicity

The primary effect of laboratory-induced stress is reduced growth, and this is usually attributable to nutritive stress. Bivalves in laboratory tanks seldom, if ever, achieve the growth rates measured in natural populations or in field-deployed mussels. One example of this comparison from our work is shown in Figure 1. Mussels of a similar initial size were transplanted to six field stations and held in the flow-though laboratory tanks. Lab mussels in flow-through tanks had significantly lower whole-animal wet-weights, tissue weights, and shell lengths after a 12-week exposure. This particular site was selected for the trailer with flow-through tanks because it had the lowest concentrations of chemicals in seawater and the highest growth rates ever measured in field-transplanted mussels. Our studies also suggest that laboratory exposures tend to

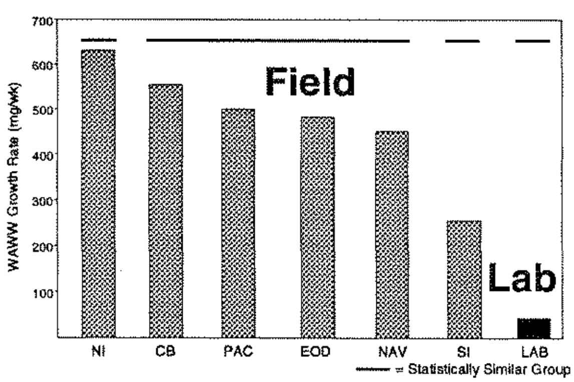

Figure 1. Lab & field mussel growth.

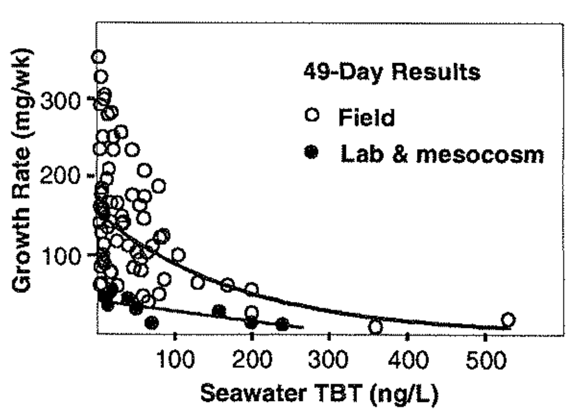

Figure 2. Growth in lab, mesocosm, & field.

overestimate the concentration at which effects begin to occur. A comparison of mussel growth rates measured in the lab and in mesocosms with mussel growth rates measured from *in-situ* exposures at various concentrations of tributyltin (TBT) is provided in Figure 2. Growth rates increased exponentially for mussels caged *in-situ* mussels, but linearly for mussels held in the lab and in mesocosms. Over-estimating toxicity in the lab and in mesocosms has significant ramifications for effects monitoring and in the development of national water quality criteria in the US and Canada.

Bridging the Lab-Field Gap

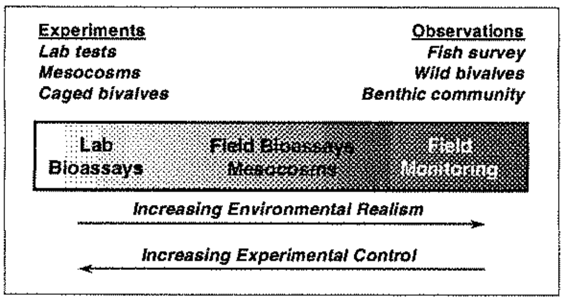

Figure 3. Bridging the lab-field gap.

Fish mesocosms and caged bivalves are being proposed as alternatives to the adult fish survey for EEM, at least in part, because they potentially combine elements of experimental control characteristic of laboratory exposures with elements of environmental realism from traditional field monitoring (Figure 3). Lab tests, mesocosms, and caged bivalves all facilitate conducting experiments rather than just making observations. Fish surveys, wild bivalve surveys, and benthic community studies are more observational rather than experimental approaches. There is a continuum of increasing environmental realism and a continuum of experimental control and each method must be validated based on other observations. For example, we found that weekly measurements of field- transplanted mussels reduced growth rates and switched to bi-weekly measurements and found no significant difference in growth rate when compared with growth rates of adjacent wild mussels.

Mesocosms versus Caged Bivalves

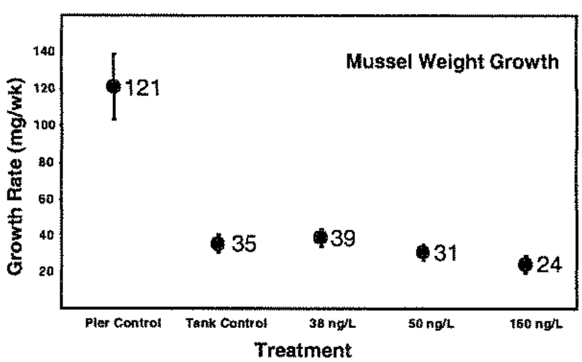

Figure 4. Pier control versus mesocosm tanks.

We have compared mussel growth in mesocosm control and treatment tanks with growth in caged mussels placed adjacent to the seawater intake (Figure 4). It was difficult to identify differences among growth rates for mussels in the mesocosm tanks, even though field studies indicated there should be effects. The pier control mussels grew significantly faster than the tank control mussels. The mesocosm system included a high-volume pump to maximize flow rates and potential food sources, but a large percentage of the particulate material became trapped in the pipes and was not available to test animals. It is important to validate results by using field controls to test for potential differences in exposure and effects in all test systems.

Need for Field Validation

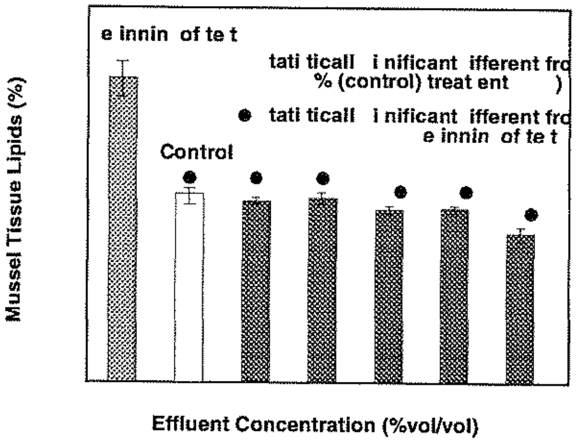

Figure 5. Mesocosm-induced stress.

Field validation for mesocosm systems can help explain observed results. In a recent mesocosm study to assess pulp mill effluents, control mussels had about 40% less lipids than mussels prior to placement in mesocosm tanks (Figure 5). As with our mesocosm tests, there was not much difference in end-of-test lipid content among mesocosm treatments. This made it difficult to identify all potentially significant effects associated with exposure to mill effluents. Significant declines in lipids and increase in percent water are indicators of stress, and the use of stressed mussels most likely lead to an overestimation of toxicity and an underestimation of bioaccumulation potential. This adds a great deal of uncertainty to the test results. As suggested by the authors, the 40% loss of lipids during the test could have been associated with spawning. However, if the mussels spawned, it is likely that mill-related chemicals accumulated during the test were eliminated with the spawning event(s). It is unclear if and when the mussels spawned during the 89-day exposure period. The very low lipid content suggests that they either spawned immediately before ending the test, or they spawned earlier and, due to insufficient food in the mesocosm tanks, could not fully recover by the end of the study. As with most other mesocosm studies conducted for EEM, field validation was not conducted. Without determining the effects of containment, overcrowding, flow rates, and other variables on test animals, it is inappropriate to draw conclusions regarding the ability of this on-site system to provide environmentally realistic test conditions, use results from this test to justify future use of this approach, or use these data for regulatory purposes.

Moving Targets

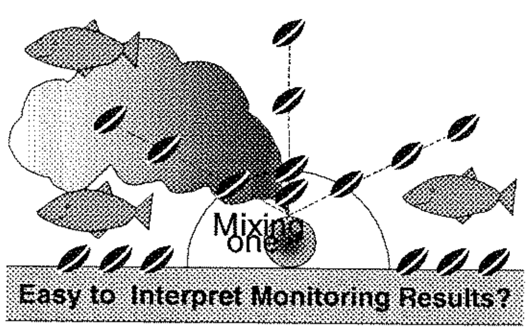

Figure 6. Fish are moving targets.

Fish are moving targets. This makes it difficult to collect them, to satisfy monitoring requirements, and to interpret results. Some of the fundamental concerns are:1) has exposure occurred? (2) if so, was it at the site of interest? (3) how long was the exposure? and (4) has the fish been exposed to other chemicals? The use of caged bivalves for effects monitoring eliminates these concerns because of the experimental control inherent in the approach (Figure 6). Caged bivalves can be strategically placed along suspected chemical gradients and results validated by measuring natural populations in the vicinity.

One significant area that has not been adequately addressed in terms of laboratory toxicity tests, ecological risk assessments, or EEM is the importance of threatened and endangered species. In the US, freshwater bivalves are the most imperiled animal group. Freshwater bivalve populations in the US are the most diverse in the world, but their densities are decreasing due to a number of factors such as dredging, filling, diking, decline of fish hosts, and chemical contamination. Freshwater bivalves are particularly susceptible to environmental stresses. Not only can chemicals directly affect freshwater bivalves, but indirectly because they rely on fish as a host for the glochidial stage. Therefore, a decline in fish species may also affect the survival of individual bivalves and ultimately bivalve communities and populations. It has been suggested that the adult fish survey may have more of an impact on adult fish populations than the mill effluent. The potential impact of adult fish surveys impacting threatened and endangered freshwater bivalves in Canada has not been addressed.

Table 1 provides a comparison of caged bivalves, resident bivalves, mesocosm bivalves, and the adult fish survey for EEM applications. This comparison uses many of the criteria developed by the EEM program. It includes the number of animals available for testing, time necessary to collect those animals, time to conduct the test, cost of the test, the ability to verify exposure, mapping ability, associations with sediment, scientific defensibility, efficiency, flexibility, and programmatic applications. We believe that in each category, caged bivalves are equal to or superior to the other approaches. This is evident in the most important category, "ease of interpretation." In ecological risk assessments, characterization of exposure and characterization of effects are the two most important monitoring elements. They may also be the most important to EEM. If exposure is unclear, measured effects are of limited value.

Table 1. Comparison of the adult fish survey with bivalves: caged, resident, and in mesocosm

	Caged Bivalves	Fish	esident Bivalves	Mesocosm Bivalves
Number Animals	3000	50	300	3000
Exposure	verified	no	?	verified
Experiment	yes	no	no	yes
Mapping	yes	no	?	no
Sediment	yes	?	Yes	?
Defensible	yes	?	?	?
Efficient	yes	?	yes	no
Flexible	yes	?	no	?
Program	yes	?	?	?
Collection Time	hours	weeks	hours/days	hours
Cost	low	high	low	high
Ease of Interpretation	yes	no	no	yes?

Surveys of natural bivalve populations are probably most similar to the adult fish survey in terms of environmental realism, number of test animals normally used, lack of experimental control, and questionable exposure. Exposure is questionable because there are no direct measurements of exposure and it is not clear if the effects endpoints represent the last day, the last week, or the last month. This is even more important when the organic enrichment associated with some effluents can increase growth rates. Therefore, it would be misleading to assume that faster growth rates are necessarily associated with healthier test animals. There is also a high degree of uncertainty with estimates of age and shell growth. Given the extreme variation in size in most wild bivalve studies and the imprecision in the measurements, it is unlikely that they will ever approach the discriminating power of the caged bivalve methodology. This is probably why national and regional mussel watch programs have not been more successful.

Underestimating Exposure

One important reason for using bivalves is that they integrate chemical exposure by accumulating and concentrating chemicals in their tissues. This includes both dissolved and particulate pathways (i.e., water and food). Holding bivalves in cages in-situ does not appear to alter exposure pathways or growth. However, holding bivalves in test systems consisting of tanks and pipes may not adequately simulate all dietary pathways, as our mesocosm work has shown in some environments. Dietary pathways have been shown to be important for fish in some pulp and paper environments. Caging fish may also affect dietary exposure pathway, particularly if bottom feeding fish do not consume contaminated food or if they are fed uncontaminated food. Fish may be less sensitive to water flow and suspended particulate matter. However, if fish mesocosms alter dietary exposure pathways by excluding contaminated food, they may overestimate toxicity and underestimate exposure as our bivalve mesocosms did. A field control is needed to validate exposure and effects. Fat bags (semi-permeable membrane devices) are not good surrogates for fish because fat bags do not adequately represent total chemical exposure. Fat bags only estimate water pathways of exposure and do not accumulate chemicals bound to suspended particulate matter. Measuring tissue concentrations of chemicals in organisms remains the most environmentally realistic method for characterizing chemical exposure, and several studies have shown that bivalves can be used as a reasonable surrogate for fish.

Caged Bivalves for Biomonitoring, Mapping, and Source Identification

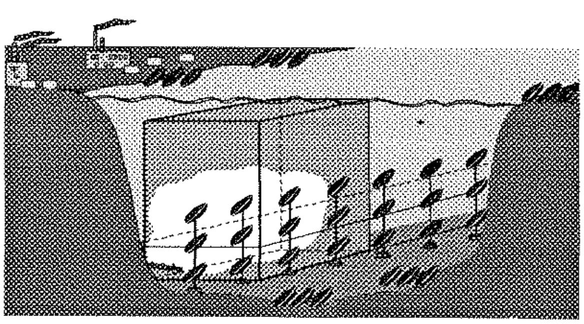

Figure 7. Exposure & effects over space & time.

Caged bivalves can be used for source identification and to delineate the extent of chemical contamination in 3-dimensional space and time (Figure 7). They can be used to quantify exposure and effects over space and time by measuring chemicals accumulated in their tissues and associated biological effects such as growth and reproduction. Results can validated by measuring natural populations in the vicinity.

Bioaccumulation is the link between environment and organism and should be emphasized more in EEM. It is not sufficient just to know if chemicals are present by taking discrete water samples that only evaluate an instant in time. Biological integrators like mussels are necessary to evaluate whether these chemicals are biologically available and likely to cause effects. Bivalves are particularly well suited to quantifying exposure because of their ability to integrate chemicals of concern or chemical tracers in their tissues as they filter the water for food. Figure 8 shows how tissue chemistry can be used as a focal point to establish links between external exposure from water and sediment in field studies using caged bivalves and from studies of traditional benthic community structure. Laboratory approaches can include both bioassays and mesocosm studies of benthic community structure.

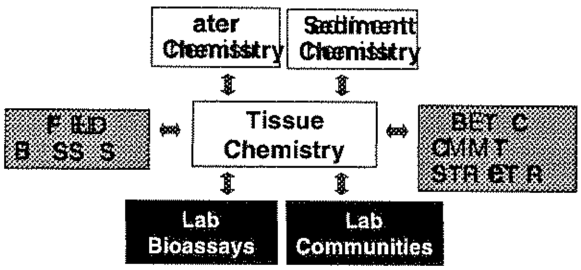

Figure 8. Tissue chemistry links.

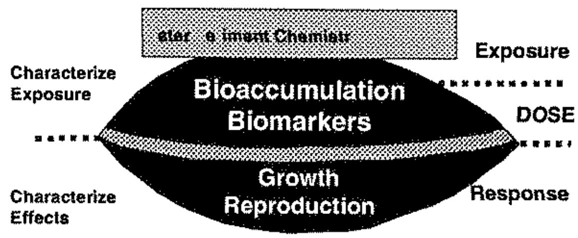

Figure 9. Exposure-dose-response triad.

Recently, we have developed and exposure-dose-response (EDR) triad (Figure 9) that is more consistent with the ecological risk assessment paradigm of characterizing exposure and effects than other approaches. Our approach was refined using elements of the sediment quality triad, the exposure-uptake-effects triad, and developments in ecological risk assessment. Recently, several investigators have questioned the use of the sediment quality triad. At this Aquatic Toxicity Workshop, it was suggested to add bioaccumulation as a necessary component, thus creating a quad. We prefer the EDR triad because it emphasizes the importance of bioaccumulation as a focal point, and a link between other monitoring approaches (Figures 8, 9). We need to change the way we think about environmental assessments to focus on quantifying exposure first and then focus on the effects. This is a major shortcoming of emphasizing effects-based approaches in EEM. If exposure is not adequately characterized, the effects measurements are less powerful indicators. EEM should consider a risk assessment-based approach, not an effects-based approach.

Once relationships have been established between water and sediment chemistry, tissue chemistry, and effects, it will be possible to establish effects thresholds based on body burdens. Figure 10 shows how body burdens can be used to establish numerical criteria where effects are expected. 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

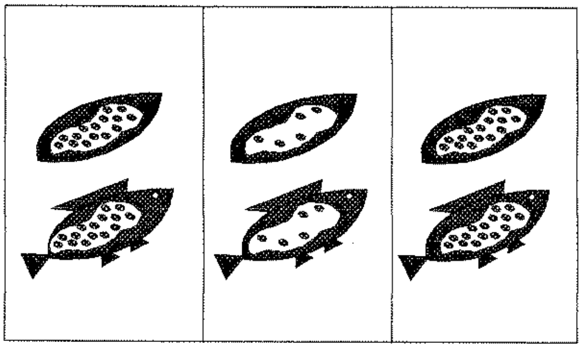

Figure 10. Proposed monitoring framework.

effects from biomonitoring data. In the first column, a threshold effects level is established from tissue concentrations of a given chemical (indicated by the dots) associated with effects as measured in laboratory and field studies using fish and bivalves. The relative concentration of the chemical is indicated by the relative number of dots. In the second column, biomonitoring of tissue burdens indicates no expected effects because the tissue concentrations are below the predicted thresholds, as shown by a fewer number of dots. In the third column, biomonitoring of tissue burdens indicates expected effects because the tissue concentrations are above predicted thresholds, as shown by an equivalent number of dots. This approach uses the bioaccumulation link between exposure and effects to establish effects thresholds and could be used as part of EEM as a regulatory framework.

Integration & Synthesis

We have refined and standardized the caged bivalve methodology for various species and habitats. The application of this methodology has been enhanced by cooperative studies with our colleagues from Environment Canada in Montreal who have added a suite of biomarkers. These biomarkers can be used to help explain the relationship between exposure, dose, and response and to help establish relationships between bivalves and fish. The EDR triad has been developed as a framework for applying these results. During this development process, pitfalls associated with other approaches have been identified and a method established for validating results from these other approaches through testable hypotheses. A pickle jar will always be a pickle jar and may not simulate natural conditions very well, even if it is a flow-through pickle jar and located on site. The use of field controls can help validate results from those exposures. Fish will always be moving targets, but can also be used as part of an EDR triad and as part of an integrated approach that includes controlled experiments in the field. Fat bags do not adequately simulate natural chemical exposures to fish, particularly when dietary exposure is important. Bivalves don't eat pickles but they do eat suspended particulate matter and it is important to include and quantify food in all pickle jars. At ATW '98, Peter Campbell gave an inspirational plenary presentation on ecotoxicological challenges for the next century that included the following messages: 1) toxicity test results are tenuous; 2) get out of the lab and into the field; 3) don't just whine about limitations of field work, do something about it; and 4) don't just observe in the field, do field experiments. This is what we have done and we believe that this approach is appropriate for EEM applications.

Salazar, M. H. and S. M. Salazar. Using Caged Bivalves to Monitor Canadian Effluents. Abstract: Caged bivalves have been proposed for monitoring industrial and municipal effluents in Canada, and Environment Canada is considering approving this approach as an alternative to the adult fish survey. Caged bivalves have a long history of use throughout the world, including Canada. Over the past three years we conducted caged bivalve studies at the Port Alice pulp mill on Vancouver Island and near municipal effluent plumes in Winnipeg and Montreal. Others have used our methods in both freshwater and marine environments in Canada for monitoring pulp and paper mill effluents. Scientists in Finland have been using caged bivalves to monitor their effluents since the mid-1980s. The Port Alice study showed that caged bivalves separated by only two meters vertical distance could identify the fine structure of exposure and associated biological effects. The Winnipeg study showed that caged bivalves at field sites survived considerably better than those in the laboratory and that previous laboratory studies have probably over-estimated the effects of ammonia under real-world conditions. The Montreal study showed that caged bivalves were a good indicator of exposure, and Environment Canada scientists demonstrated a relationship between mussel tissue chemistry and a suite of biomarkers. Collectively, these studies have validated the operational utility of caged bivalve monitoring. A monitoring framework will be presented to demonstrate how mussel tissue chemistry can be used to establish links with measurement endpoints in other species like fish.

Salazar, MH. and S. M. Salazar. A Pickle Jar is a Pickle Jar and a Fish is a Moving Target but a Fish is not a Fat Bag and Bivalves Don't Eat Pickles. Abstract: A pickle jar will always be a pickle jar regardless of whether tubes are added and the test system is called flow-through, a mesocosm, or even if the test is conducted on-site. The preponderance of evidence in the literature, our previous work in San Diego Bay, and recent studies in Canada have shown that bivalves are particularly sensitive to water flow and suspended particulate matter used as food, and that bivalves are generally stressed by the pickle jar test system, even under site-specific, flow-through conditions. This has important implications for EEM monitoring at pulp and paper mills since this approach has recently been approved as an alternative to the adult fish survey. Similarly, proponents of lipid bags often suggest that lipid bags are superior to organisms such as bivalves because the relationship between exposure and uptake is more linear. Unfortunately, these relationships may not always be linear in the real-world and lipid bags may not accurately represent exposure, particularly when diet is an important exposure pathway. Examples will be given to demonstrate that the use of organisms for controlled biomonitoring in the field can provide important information for EEM monitoring at pulp and paper mills that cannot be provided with these other methods, or even the adult fish survey using moving targets.