Monitoring Endocrine Disruption Using Caged Bivalves

Michael H. Salazar & Sandra M. Salazar
Applied Biomonitoring

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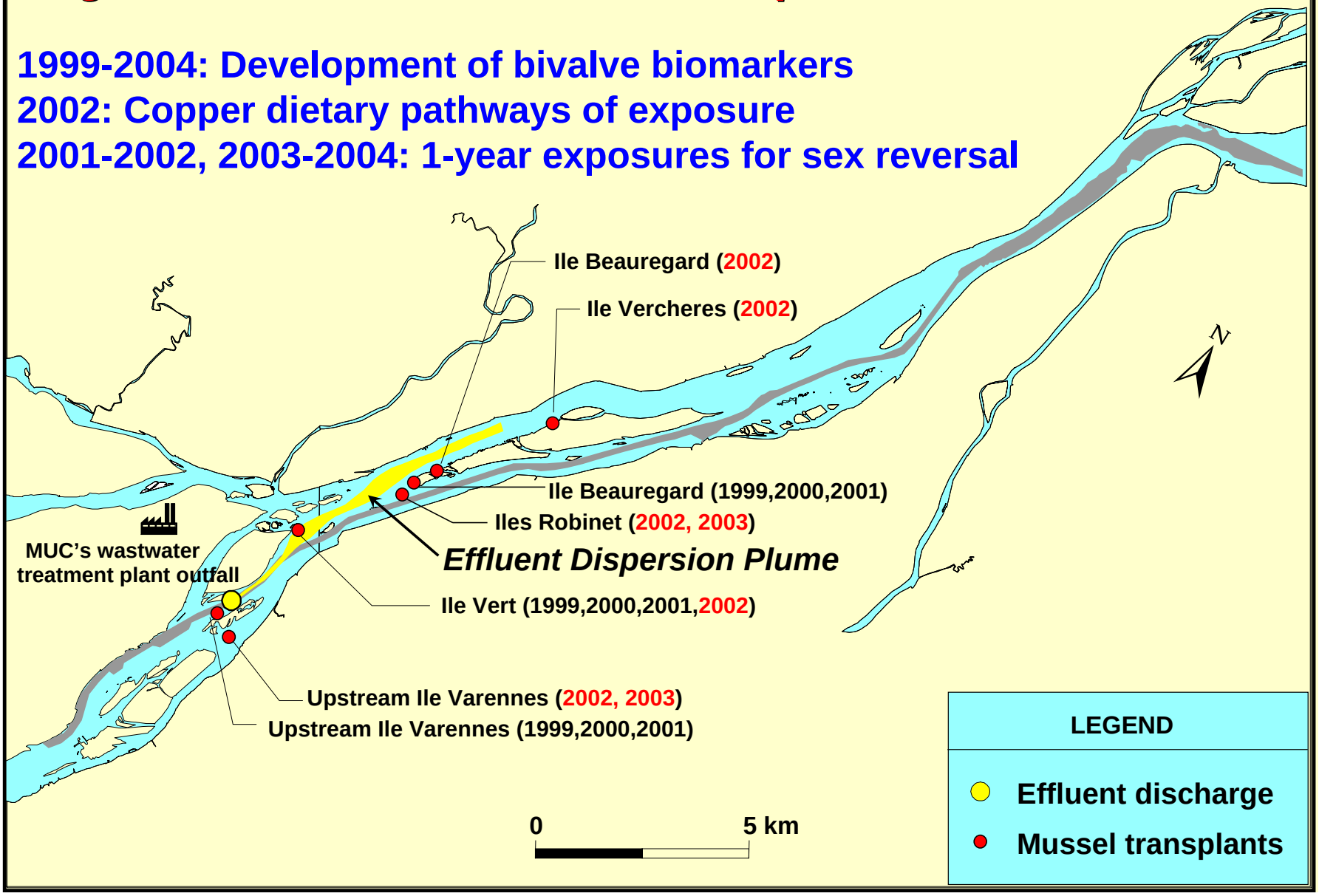
Abstract

Collectively, the results from the caged mussel studies using *Elliptio complanata* on the St. Lawrence River in Montreal and the Kennebec River in Maine, show that the vitellin (Vn) biomarker is a sensitive effects endpoint, suggesting endocrine disruption (ED) and effects on reproduction. Furthermore, specific estrogenic effects (increased vitellin & feminization) appear to be related to unidentified chemicals in both municipal and pulp and paper mill effluents. Similar studies with *Mya arenaria* (a marine clam) show androgenic effects (decreased vitellin & masculinization) may be related to TBT exposure. Although relatively little is known about the endocrine system in invertebrates, synthesis of vitellin, the major protein found in oocytes of invertebrates synthesized from vitellogenin, has been shown to be regulated by estrogens in freshwater and marine bivalves. This process appears to be susceptible to endocrine disruption in a manner similar to that of fish. Increased levels of these vitellin proteins have been reported in the marine clams, mussels, and oysters. Feminization and masculinization have been shown in several of these species, depending on the chemicals of exposure. Results from a 1-year benthic cage study suggest that prolonged exposure to these chemicals can results in sex reversal. Due to their high filtration rate, an ability to accumulate and bioconcentrate chemicals, and their sedentary life style, bivalves may be at particular risk to ED chemicals. This increases their utility as sentinels for monitoring purposes. The caged bivalve methodology provides the advantage of knowing the bivalves' chemical, biochemical, and biological properties at the beginning of the test as well as conducting field experiments at locations of interest, even though they may not normally be found at those locations. Results also suggest that ED chemicals can be added to the list of potential stressors on native unionid populations by affecting reproduction and the sex ratios of existing populations.

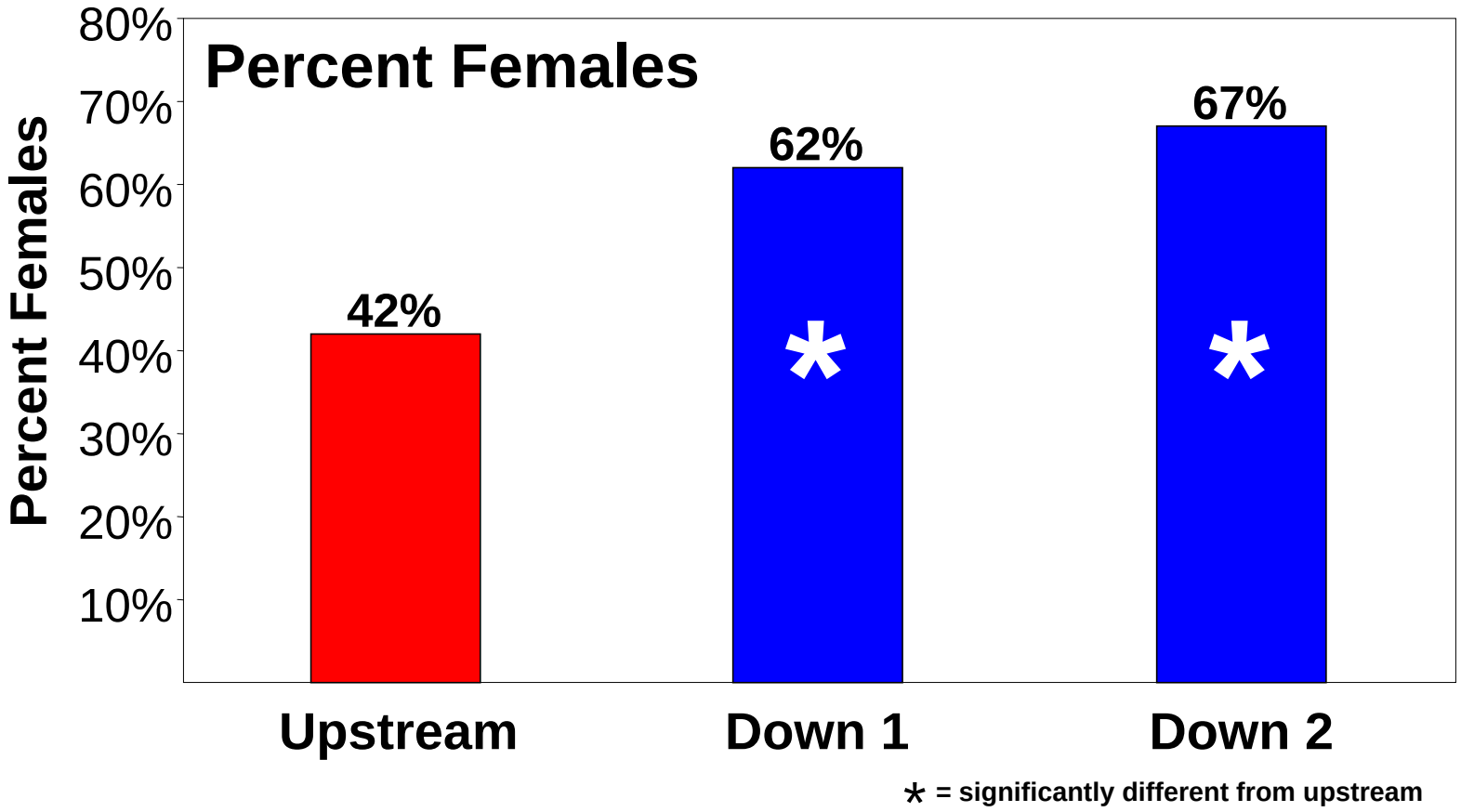
Urban Effluent Study on the St. Lawrence River: Caged Freshwater Mussel Field Experiments

1999-2004: Development of bivalve biomarkers
2002: Copper dietary pathways of exposure
2001-2002, 2003-2004: 1-year exposures for sex reversal



Endocrine Disruption Mode of Action – Percent Feminization

Caged *Elliptio complanata* field experiments on the St. Lawrence River

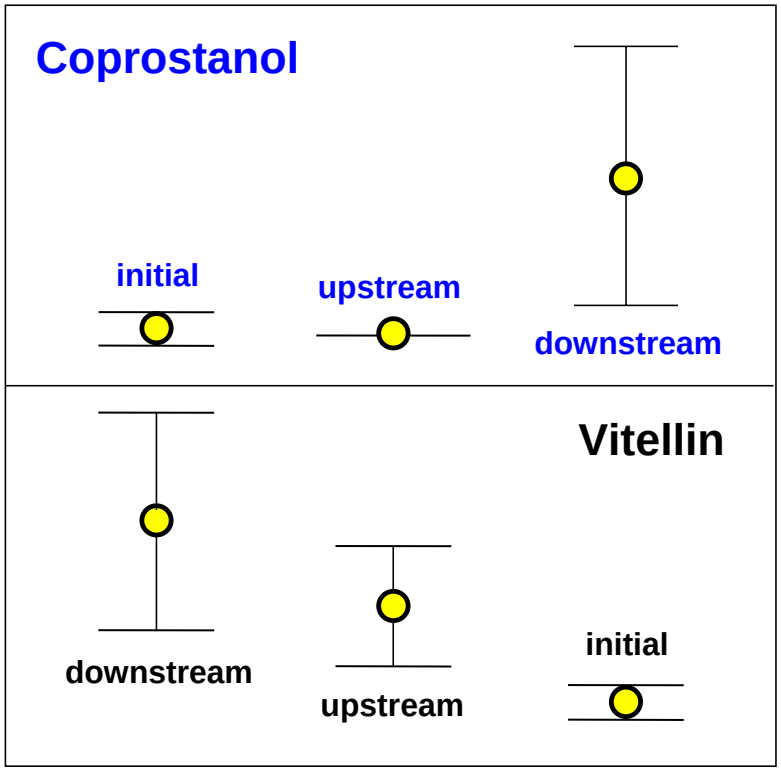


- There was a higher percentage of females in the downstream than in the upstream stations.
- The cages provided a method for long-term sediment exposures and experimental induction of sex changes.
- These results confirmed preliminary observations by Environment Canada of a higher percentage of females than males in the natural populations downstream from the City of Montreal municipal effluent.

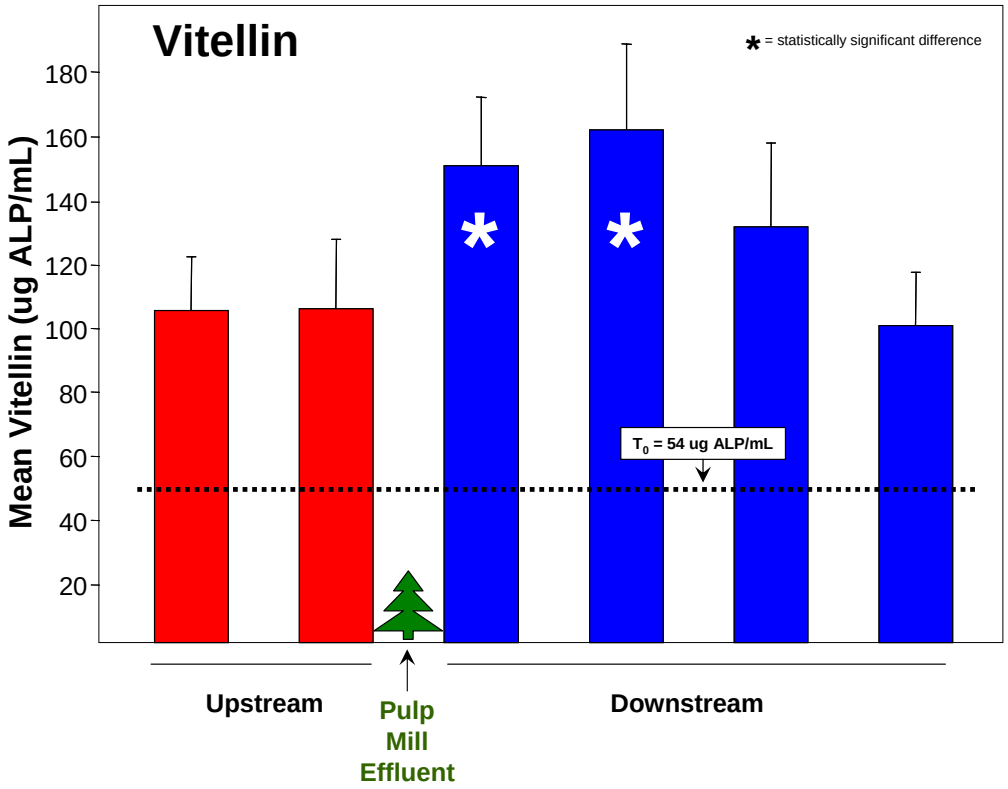
Endocrine Disruption Mode of Action – Feminization

Caged *Elliptio complanata* field experiments on two rivers

St. Lawrence River, Montreal



Kennebec River, Maine



Summary of Urban Effluent Studies on the St. Lawrence River

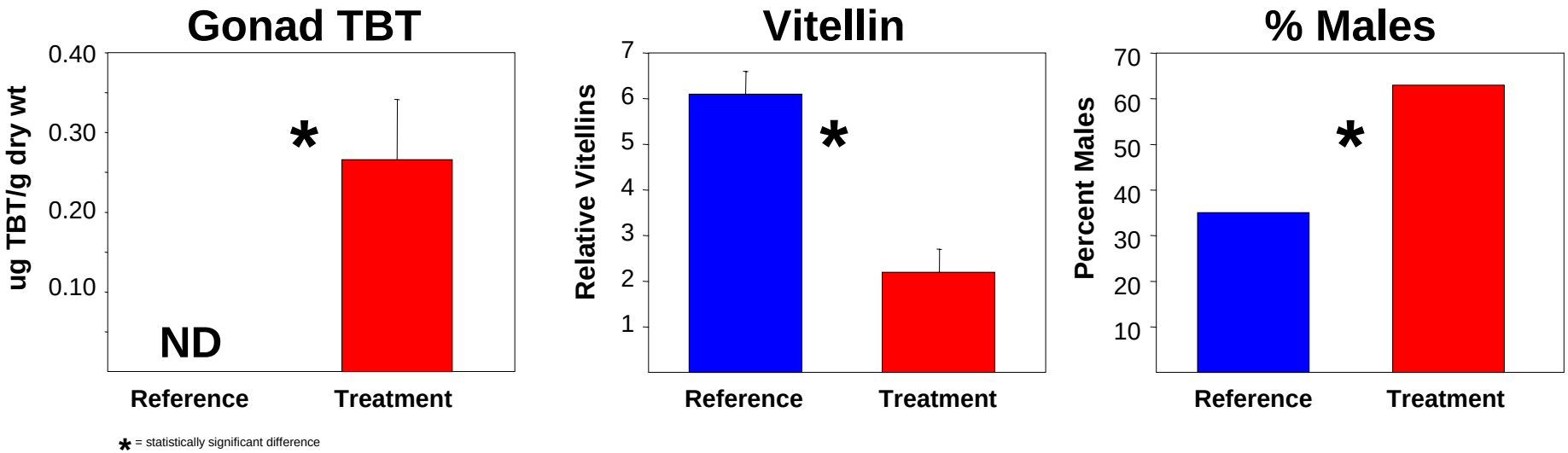
- Correlations between mussel tissue chemistry, biomarkers & growth
- Identified coprostanol as a possible inducer of endocrine disruption
- Experimentally induced feminization in controlled 1 year field experiments

Summary of Pulp Mill Effluent Studies on the Kennebec River

- Tissue chemistry results suggest mill not discharging dioxins & furans
- Growth rate & vitellin results suggest possible adverse effects associated with mill
- Vitellin results suggest effects not associated with dioxins & furans

Endocrine Disruption Mode of Action – Masculinization

Indigenous *Mya arenaria* populations in Saguenay Fjord
Effects observed at tissue concentrations comparable to those associated with imposex in dogwhelks

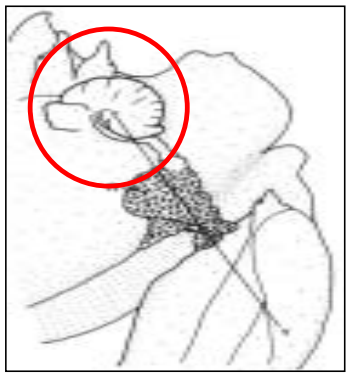


Why Endocrine Disruption?

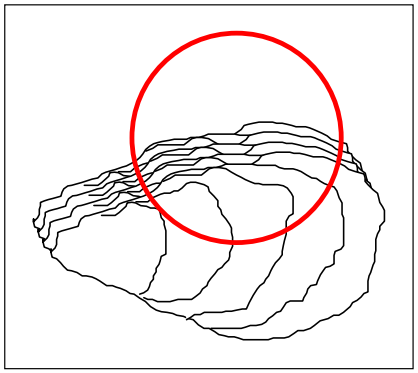
- Emerging importance
- Need monitoring/assessment tools
- Difficult to measure with other methods

Applications

- Contaminated sediment
- Effluents
- Nonpoint sources

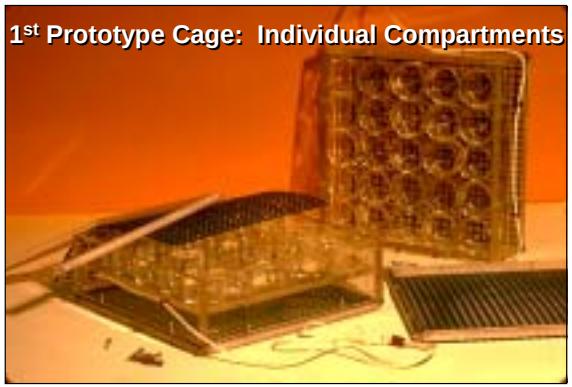


Imposex in dogwhelks



Shell thickening in oysters

Initial Development of a Compartmentalized Cage



Beginning of Test

End of Test

From aquaria to mesocosms to the field: It all started in the lab as a toxicity test

Between 1971 and 1973 we developed and applied a laboratory bioassay using byssal thread production as an effects endpoint. This work was based on initial studies conducted by Don Reish. Between 1973 and 1977 we developed a prototype cage using glass crystallizing dishes to facilitate counting byssal threads as we had done in the lab. The next step in the development process, between 1985 and 1986, was the use of plastic ice cube trays drilled to maximize water circulation for the mussels while they were held in mesocosm tanks. After demonstrating that the caged juvenile mussels deployed at the seawater intake grew almost 4 times faster than those in the mesocosm tanks, we focused on field transplant studies. We began by using plastic cutlery trays for these field transplant studies. These cages held more test animals and provided more room for growth. The picture above (bottom, center) shows 10 – 12 mm mussels at the beginning of the test and mussels (35 – 45 mm) from the control site measured 12 weeks later. Growth at the most contaminated site was not significant, and after 12 weeks, mussels looked similar to those at the beginning of the test. Measurement endpoints were bioaccumulation and growth.

The Shift from Rigid to Flexible Cages

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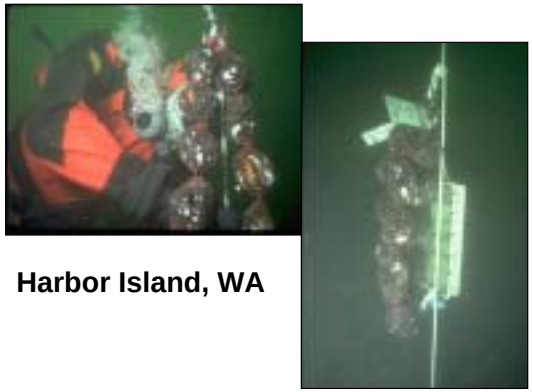
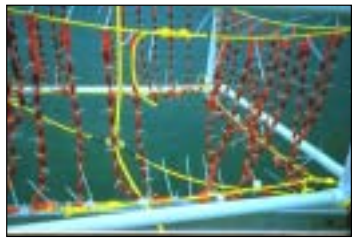


Harbor Island, WA

San Diego Bay, CA

Delaware Bay, DE

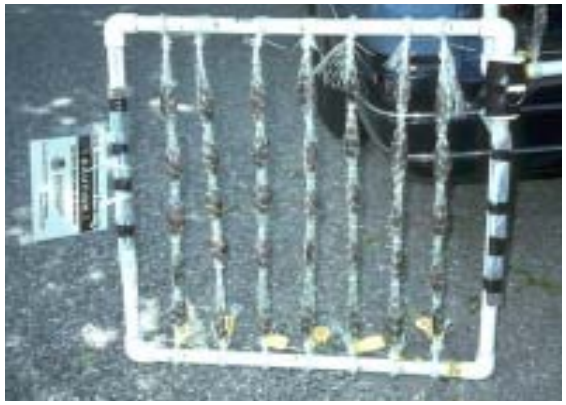


Sinclair Inlet, WA

From rigid cages with individual compartments to flexible cages with individual compartments

In 1991 we conducted a mussel transplant in Puget Sound at the Harbor Island Superfund Site. It was the first combination of a rigid cage with individual compartments and a flexible mesh cage with 10 mussels per compartment. Juveniles in the compartmentalized cages were used primarily for growth measurements and the adults in the flexible mesh bags for bioaccumulation. A similar approach was used in San Diego Bay in 1993, although in this case extra mussels were used for evaluating various bivalve biomarkers. It quickly became apparent that the flexible mesh cages offered the most versatility in terms of holding a wider size range of test animals and maintaining individual compartments for repetitive measurements of each individual. In addition, the mesh is inexpensive, disposable, and does not have to be cleaned. The first flexible mesh cage was used for monitoring tissue chemistry and growth in Sinclair Inlet, in the vicinity of the Puget Sound Naval Shipyard. All of these studies could be considered water column studies, even though the Harbor Island mussels were caged 1 meter above the bottom. We were able to demonstrate a statistically significant relationship between chemicals in mussel tissues, in sediment, and mussel growth rates.

Freshwater Bivalves on Sediment



1995 Sudbury River, MA

Nyanza Superfund Site

In 1996 we conducted our first freshwater caged bivalve study at the Nyanza Superfund site on the Sudbury River, MA to evaluate the bioavailability of mercury. Freshwater mussels (*Elliptio complanata*) were transplanted at the Nyanza Superfund site on the Sudbury River, MA to evaluate the bioavailability of mercury. Results showed that methylmercury was biologically available all along the reach of the Sudbury River being tested. Although measured MeHg concentrations decreased downriver from the Superfund Site, growth rate measurements showed that when the concentrations were normalized to account for growth dilution there was no statistically significant difference with distance from the contaminated site. This study helped confirm the importance of measuring growth to help interpret tissue chemistry results.

Cannelton Tannery Facility

1997, 2000 Tannery Bay, MI

Caged clam (*Corbicula fluminea*) studies in were conducted in 1997 and 2000 at the Cannelton Superfund site in Sault Ste. Marie, MI to evaluate the effectiveness of remedial activities. Clams were transplanted to 10 sites in Tannery Bay, an area used for disposal of tannery waste products, and two reference sites on the St. Mary's River. Tissue chemistry results showed that chromium bioavailability had decreased over time in most areas. A statistically significant relationship was found between chromium in sediment and chromium in mussel tissues. Methylmercury was not biologically available.

2001, 2002 Guelph, Ontario, Canada

Guelph Industrial Site

Applied Biomonitoring was contracted by GR Craig & Associates to help design and conduct freshwater monitoring studies in 2001 and 2002 to characterize polychlorinated biphenyl (PCB) exposure in the Speed River, Guelph, Ontario. The study required caging bivalves in small channels with less than 1 foot of water during the summer. This requirement was just as challenging as deploying caged mussels at a depth of 200 meters in Port Valdez. The "frameless" cage design was used to place mussels within the concave structure of these small streams. The logistics of this study were very complicated and required continued coordination with the Ministry of Environment (MOE) and local freshwater bivalve experts. *Lasmigona costata* and *Elliptio complanata* were used as test species. Other obstacles included high summer temperatures, low oxygen at a treatment site, and the presence of natural predators and other mammals that disturbed the sediment in the study area.

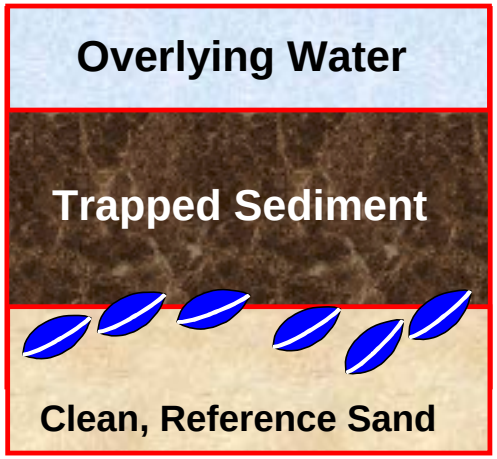
Freshwater Bivalves in Sediment

Beginning of Test

End of Test

St. Lawrence River, 2001- 2002

Mussel survival was significantly higher in the benthic cages (83%) than in the standard cages (9%). The number of females was significantly higher in downstream mussels (62% and 67%) than in upstream mussels (42%). The benthic cage trapped suspended sediment, and the mussels migrated up into these surficial sediments (Figure 4). This preliminary sex-reversal test has shown that feminization in freshwater mussels can be experimentally induced in a controlled field experiment with a long-term, 1-year exposure period. The benthic cage was successful in achieving high mussel survival, directly exposing the mussels to both contaminated water and contaminated sediment, and demonstrating that sex changes could occur in the field downstream of a municipal effluent and have effects on mussel reproduction.



Why Develop a Benthic Cage for Bivalves?

- **To validate laboratory studies**
  Characterizing exposure may be the most critical element in ecological risk assessment because an inappropriate interpretation of exposure can diminish the significance of characterizing effects. Recognition of this concern has caused a shift from laboratory toxicity tests to mesocosms and field studies where environmentally realistic exposures are easier to achieve. It is also helpful to characterize effects under environmentally realistic conditions in the field. These are both critical elements to ecological risk assessment.

- **To assess long-term exposures and associated effects**
  Currently, the most subtle effects can only be manifested after long-term exposures under environmentally realistic conditions. Field observations by Environment Canada scientists had shown a higher percentage of females downstream of a City of Montreal municipal effluent than upstream. Environment Canada wanted to determine if sex reversal could be experimentally induced in the field under environmentally realistic conditions. For this reason, it was necessary to include the mussel's entire reproductive cycle to induce these effects and thus a long-term exposure period of one year.

- **To characterize benthic exposure pathways**
  Several studies conducted by Applied Biomonitoring have shown a statistically significant relationship between chemicals found in bivalves suspended above the bottom or on bottom sediment, chemicals in sediment, and various effects endpoints. Nevertheless, other scientists have questioned whether or not the pathways of exposure, bioaccumulation, and associated biological effects would be the same in bivalves just above or on bottom sediment as in those living in bottom sediment.

- **To supplement laboratory bioaccumulation tests**
  Questions have also been raised with respect to the ability of the standard 28-day *Macoma* bioaccumulation test conducted under laboratory conditions adequately represents "real-world" conditions in marine benthic communities. These questions are primarily attributable to the relatively short-term exposure, the ability of *Macoma* and other bivalves to remain closed for extended periods of time, and potentially unrealistic laboratory exposures. Using the benthic cage in marine environments would help validate the results of laboratory bioaccumulation tests. A comparable 28-day laboratory test has been developed for freshwater using the standard freshwater bivalve test organism *Corbicula fluminea*.

Summary

The test was successful

- Survival after a 1-year exposure period was high
- Increased feminization was experimentally induced
- All cages were retrieved in good condition
- Contaminated sediment was trapped in the cage
- Mussels migrated to the contaminated sediment

Conclusions

The benthic cage was a success

- More robust than mesh cages for long-term tests
- Useful for many different bivalve species
- Supplement to laboratory bioaccumulation tests
- Can be used to study exposure pathways
- Particularly useful in highly depositional areas

Applications

1-yr bivalve exposures in a benthic cage are useful tools

- Caging bivalves facilitates characterizing exposure & effects & any clinical measurements
- Controlled field experiments combine experimental control & environmental realism
- Long-term exposures to evaluate subtle phenomena such as feminization are possible
- Ability to experimentally induce phenomena observed in the field
- Validation of laboratory testing

Recent Developments

- 1998 to 2003 – Development of bivalve biomarkers, including vitellin like proteins and sex steroids
- 2001 – ASTM Standard Guide for In situ Field Bioassays
- 2002 – Development of a benthic cage for long term field exposures
- 2002 – Experimental induction of sex reversal in freshwater mussels caged for 1 year downstream of a municipal effluent
- 2003 – 100% mortality in mussels caged for 90 days in another municipal effluent where standard toxicity tests showed no acute or chronic toxicity
- 2003 – Increased vitellin production downstream of a pulp & paper mill effluent
- 2003 – Increased vitellin production downstream of tertiary-treated effluent