AN *IN-SITU* BENTHIC CAGE TO CHARACTERIZE LONG-TERM ORGANOCHLORINE EXPOSURE AND ESTROGENIC EFFECTS

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Introduction

Environment Canada scientists at the St. Lawrence Center have been studying the effluent from the Montreal Urban Center (MUC) wastewater treatment plant outfall for several years and during the past four years have conducted cooperative mussel transplant studies with Applied Biomonitoring. The purpose of these studies was to combine the traditional approach of measuring bioaccumulation and growth of transplanted bivalves with bivalve biomarkers. As part of this monitoring program, higher proportions of female *Elliptio complanata* were observed downstream of the effluent than upstream, and a test was designed to determine if sex reversal could be experimentally induced in controlled field experiments to complement the bivalve biomarker monitoring and measurements of increased vitellin production in downstream mussels. However, some scientists had expressed concern that mussels in the traditional mesh cage and suspended above the bottom substrate would not survive a one-year deployment in the St. Lawrence River because of the extremely low water temperatures. Under natural conditions and uncaged, mussels can avoid exposure to these low water temperatures, which may be outside of their tolerance limits, by burying themselves in sediment. The benthic cage allows mussels to bury themselves in sediment in a more natural way, and helps avoid potential problems associated with cages that are placed on or above the bottom.

One might ask the question, "Why develop a benthic cage for bivalve testing?" A benthic cage can be used to provide more realistic characterizations of exposure and effects for several different applications, including: *1) Validating 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[1]. Recognizing 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. *2) Assessment of 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 had shown a higher percentage of female mussels in the St. Lawrence River downstream of a City of Montreal municipal effluent than upstream. Environment Canada scientists wanted to determine if sex reversal could be experimentally induced in the field under environmentally realistic conditions. Therefore, a long-term exposure period of 1 year, which included the mussel's entire reproductive cycle, was used to induce these effects.

Characterizing 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.

Supplementing laboratory bioaccumulation tests. Questions have also been raised with respect to the ability of the standard 28-day marine bioaccumulation test, using the deposit-feeding *Macoma* exposed 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 exposure conditions in the laboratory. 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*, a filter feeder.

Bivalves have been shown to be an appropriate test organism using a variety of criteria. Environment Canada scientists from the St. Lawrence Center chose to develop bivalve biomarkers and conduct sex reversal tests because bivalves have many internal systems, such as an endocrine systems, which are similar to fish and because bivalves are easier to collect, cage, and measure. Previous studies have shown that mussels downstream of the MUC had higher concentrations of vitellin than upstream mussels and long-term exposures of fish to estrogenic chemicals has led to increased number of females. Applied Biomonitoring scientists previously developed an American Society for Testing and Materials (ASTM) Standard Guide for caging bivalves[2] and has extensive experience and expertise in using bioaccumulation and growth to characterize exposure and effects in marine, estuarine, and freshwater bivalves. Caged bivalves have also been accepted by Environment Canada as an alternative to the adult fish survey for Environmental Effects Monitoring (EEM) at pulp and paper mills in Canada. Other scientists have used benthic cages[3] and it was felt that development of such a cage would be relatively simple.

Materials and Methods

The overall design of the benthic cage (Figure 1) includes an inner mesh chamber, held in place with plastic cable ties, that holds the clean sand and test mussels at the beginning of the test. The bottom of the inner mesh chamber was lined with biodegradable newspaper to help retain the clean sand during deployment. Prior to placement in the benthic cage, all mussels were measured for length and weight at the beginning of the test; a subset of the mussels were sexed. Mussels were evenly distributed on top of the clean sand in the benthic chamber before deployment. Pingers were attached to cages prior to deployment. Divers helped support the cage as it was lowered to the bottom by a winch. The divers buried the benthic cage up to about 5 cm of the top and secured it to the river bottom with stainless steel stakes. In addition to testing the benthic cage, the standard cage utilizing mesh bags and a PVC frame was also deployed to compare its suitability for long-term exposures in highly depositional environments on the St. Lawrence River. The standard cages were held upright in the water column with floats and secured to the river bottom with cinder block anchors.

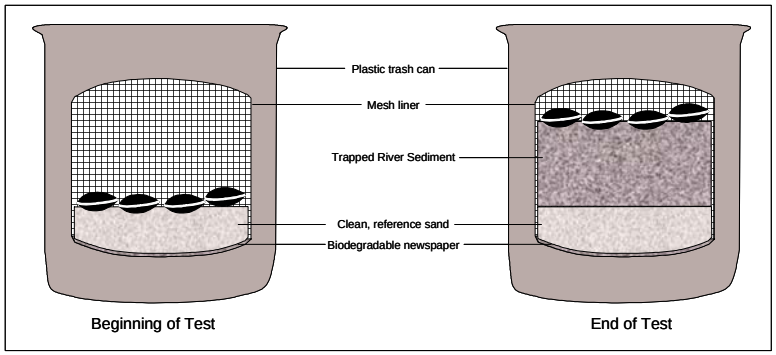

Figure 1. Benthic cage.

Acoustic receivers were used to locate the pingers attached to the cages. A boat winch and divers were again used to raise the benthic cage out of the water. Once on board, the top mesh was detached and the mussels were removed from the sediment. In addition to the clean sand originally placed in the benthic cage, there was a 4 to 6" layer of natural sediment (fine, dark mud) in each cage. It was easy to distinguish the clean sand from trapped sediment because of differences in color and texture. Nearly all mussels had migrated to the surface of the trapped sediment after initially being placed on top of the fine, clean sand (Figure 1). The number of surviving mussels was recorded during the removal process. After removal, the mussels were placed in ice chests until they were returned to the laboratory and held in flow-through laboratory tanks. After overnight holding in flow-through laboratory tanks, the number of survivors in each cage was confirmed and several growth measurements made, including whole animal wet weight, shell length, tissue weight, and shell weight. Tissues were removed for weighing, chemical analysis, and biochemical analysis. Gonads were separated for sex determination and biomarker analysis.

Results and Discussion

The test was considered successful since survival after a 1-year exposure period was high and feminization was experimentally induced. All cages were retrieved in good condition and contaminated sediment was trapped in the cage. Survival of freshwater mussels (*Elliptio complanata*) was significantly higher in the benthic tubs than in the epibenthic cages (Figure 2). There was a significantly higher percentage of females at the downstream stations (62% and 67%) than at the upstream station (42%). The cages provided a method for characterizing long-term water and 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 MUC. The benthic cage is a potentially powerful tool in ecological and human health risk assessment of organochlorines and other chemicals. Caging bivalves facilitates characterizing exposure and effects and any clinical measurements. These long-term exposures to evaluate subtle phenomena such as feminization associated with

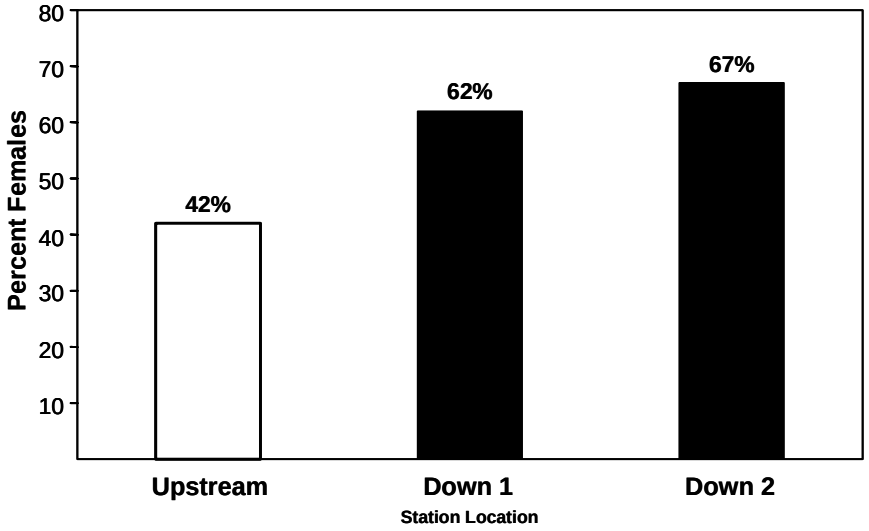

Figure 2. Percent females in benthic cages after 1-year deployment.

organochlorines are possible on a more routine basis. It facilitates controlled field experiments that combine experimental control and environmental realism and the ability to experimentally induce phenomena observed in the field. It can also be used to validate laboratory testing results.

The benthic cage is another useful tool in the environmental monitoring and assessment toolbox. When used in combination with the more traditional cages used to transplant bivalves along suspected chemical gradients in the water column, it provides the ability to conduct a more thorough assessment of exposure and effects in water column and sediment. It can also be used to validate laboratory sediment studies, assess long-term exposure and effects, characterize benthic exposure pathways, and supplement laboratory bioaccumulation tests. Additional experiments are planned to verify the feminization results and test the cage in different natural environments.

References

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