USING CAGED FRESHWATER MUSSELS TO MONITOR DIOXINS & FURANS IN THE KENNEBEC RIVER

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Abstract During the summer of 2000, a 53 day pilot study was conducted in the Kennebec River, Maine to determine whether caged freshwater mussels (*Elliptio complanata*) would be a reasonable surrogate for resident fish to assess upriver and downriver exposures of dioxins and furans associated with pulp and paper mill effluents. Caged mussels were deployed 13 miles upriver and 11 miles downriver from a pulp and paper mill. Mussels were deployed at these locations because they were the closest areas where fish could be collected due to the limitations of fish sampling and dams on the river. Mean total dioxin/furan concentrations in mussel tissues increased from below detection before deployment to 4.33 and 4.67 ng/kg ww (parts per trillion) at the upriver and downriver stations after deployment. There was no statistically significant difference between upriver and downriver total dioxin/furan concentrations. More individual dioxin/furan congeners were measured in mussel tissues from both upriver and downriver locations than in either semipermeable membrane devices (SPMDs) or fish tissues collected during the same time period. Advantages and disadvantages of caged mussels, natural fish populations, and SPMDs will be discussed along with the benefits of a gradient sampling design relative to using only upriver and downriver comparisons where the fish could be caught.

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. In 2001 our methods were accepted by the American Society for Testing and Materials (ASTM) and appeared as a Standard Guide. In the same year Environment Canada approved caged bivalve monitoring, based largely on our work and presentations at several meetings and short courses conducted in the US and Canada. These same protocols will appear on-line in early 2003 in the Standard Methods for the Examination of Water and Wastewater published by the American Public Health Association.

It is important to note that we have built these methodologies based on a number of caged bivalve studies conducted over the last 30 years and refined the methods to a level of sophistication many scientists had not even considered. This history is important because of the acrimonious nature of the Kennebec River study and the biased view of some state of Maine DEP representatives and their technical advisory group. This work did not appear at a scientific meeting until 2003 because DEP told Applied Biomonitoring in 2000, after the work was completed, that they would not need the report that we were initially contracted to write. It was their belief that they had the experience and expertise to properly interpret the data even though nobody on their staff had ever conducted a caged mussel study. Furthermore, the 2000 Dioxin Monitoring report, which concluded that fish and SPMDs had the most potential for dioxin monitoring and that the caged mussels did not provide useful information, was written before all the data were available.

This poster, which was presented at the 2002 Aquatic Toxicity Workshop and the 2002 Annual SETAC Meeting, will also be presented at the 2003 International Pulp and Paper Conference in Seattle. It summarizes the lessons learned from this study, the data available for fish, SPMDs, and caged mussels and invites unbiased observers to draw their own conclusions regarding which methods have the most promise regarding future monitoring at pulp and paper mills in Maine, and which methods provided the most useful information. We invite all of you to make those decisions on the available data, not unsupported rhetoric.

The main purpose for pursuing this work is an attempt to convince the state of Maine DEP that the 2000 study was not conducted properly and that it should be re-done by placing SPMDs and caged mussels closer to the mill in a gradient design as originally proposed. While the single-station, upstream-downstream comparisons did provide a direct comparison with fish, it did not test the ability of either SPMDs or caged mussels to detect higher concentrations of dioxins and furans closer to the mill. Most scientists outside of DEP and their Technical Advisory Group have agreed that the test was not a true test of either SPMDs or caged mussels.

State of Maine Background

Most stringent dioxin/furan monitoring regulations in US

- DEP responsible for developing the monitoring program
- Assess ecological/human health effects
- Measure chemical exposure in fish tissues
- Status & trends for compliance, need for more stringent regulation
- Are mills discharging dioxins/furans into water?
- 1997 law prohibiting discharge requires compliance by December 31, 2002

In practice

- Environmental exposures cannot be higher upriver than downriver
- Estimated by concentrations in fish tissues or some surrogate
- Becoming more difficult to detect differences in fish
- DEP interested in developing surrogates to replace or complement fish?

Objectives of caged mussel pilot study

- Are caged mussels a reasonable surrogate for fish?
- Are mills are in compliance with regulations?
- Are mills discharging dioxins (based on "above/below" test)?

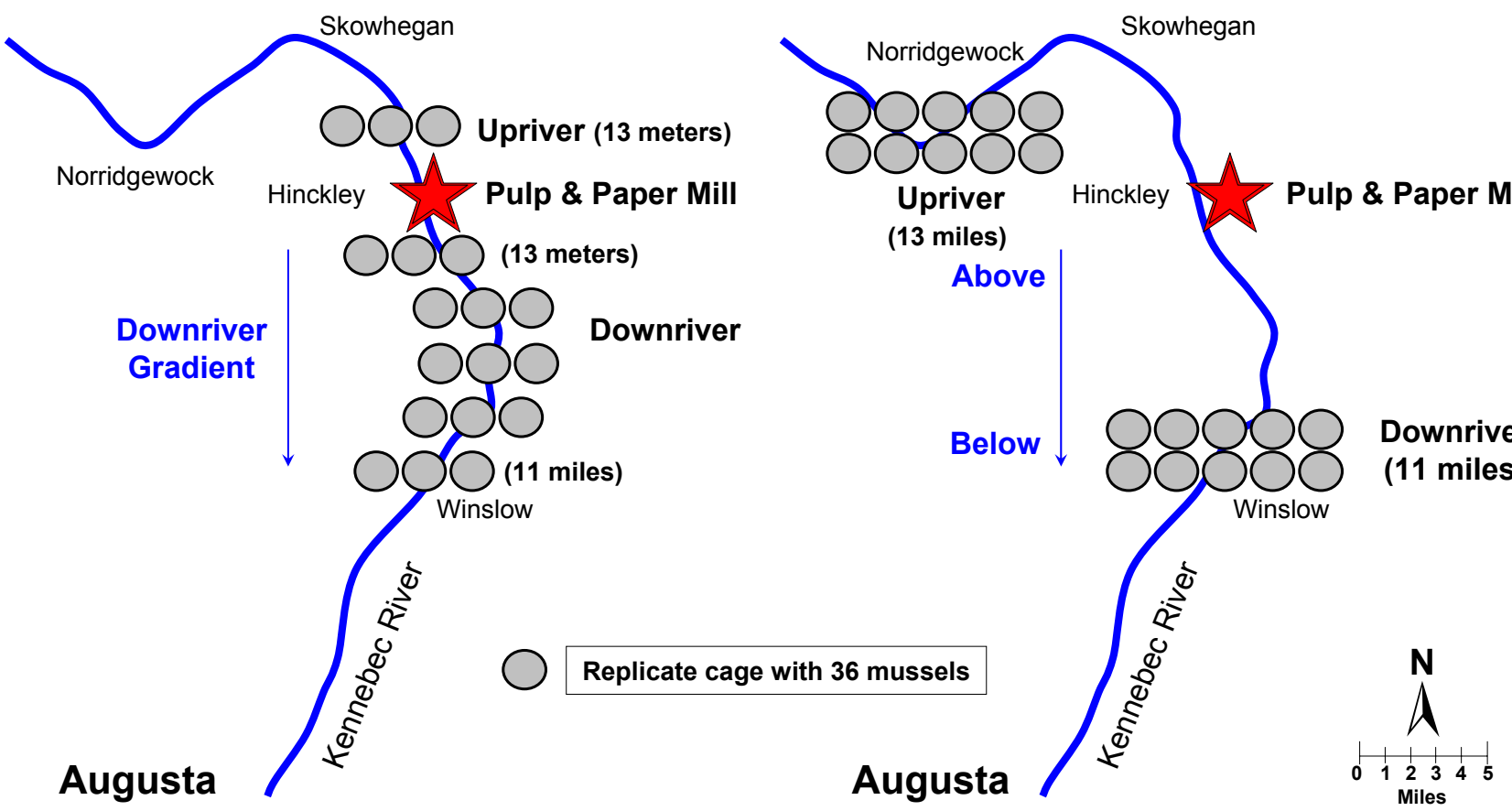
The State of Maine Department of Environmental Protection (DEP) has expressed concern regarding the ability to detect statistically significant differences in chemical exposure when comparing ***upstream*** and ***downstream*** locations from pulp and paper mills due to declining tissue concentrations of dioxins and furans in fish. These comparisons are important because environmental regulations do not allow significant differences in upstream versus downstream exposures associated with those effluents. Academic and public environmental groups and mill representatives have all expressed concerns about using the fish test for this purpose and the fish test has limited support outside DEP. Many have identified problems with monitoring indigenous fish populations for upstream/downstream comparisons at mill sites, including uncertainty associated with mobility, accumulation from other sources, accumulation from previous mill discharges sequestered in sediments, and the inability to collect fish near the mill discharge. **One environmental group, Friends of Merrymeeting Bay (FOMB), supported and advocated the caged mussel pilot study anticipating that concerns regarding fish monitoring could be eliminated by using a surrogate, such as caged mussels, that could be deployed closer to the mill discharge where fish could not be collected.**

DEP is responsible for developing a monitoring program to assess the nature and extent of dioxin and furan contamination in the waters and fisheries of the state but many have suggested that they have yet to develop an appropriate test. Maine has adopted the most stringent environmental regulations for dioxins in the US, and the primary objective of the dioxin/furan monitoring program is to assess potential ecological and human health effects by measuring chemical exposure in fish tissues. Interestingly, Environment Canada has adopted the opposite approach and focused on measuring effects in fish or suitable surrogates. Caged mussels and mesocosms have been accepted as alternatives to the adult fish survey in required Environmental Effects Monitoring (EEM) at pulp and paper mills in Canada. A secondary objective of dioxin monitoring in Maine is to document the status and trends of dioxin/furan exposures, evaluate progress in reducing environmental concentrations by compliance with existing regulations, and the need for even more stringent regulations. The third, and most specific objective is to ***determine if kraft pulp mills are discharging dioxins or furans into the rivers of Maine.*** A state law enacted in 1997 prohibits such discharges and requires compliance by December 31, 2002. In practice, environmental exposures of dioxins and furans estimated by measuring concentrations in fish tissues or some surrogate, cannot be higher downstream of a pulp mill discharge than upstream. This is commonly referred to as the "above/below" test.

Unfortunately, using stations 11 miles downstream and 13 miles upstream from the mill to represent "above/below" exposures to dioxins and furans may not accurately represent current mill exposures.

Site Map

- *DEP did not allow caged mussels close to the mill*
- *A gradient design would have been more like a lab test*
- *This was not a true test of the caged mussel methodology*



Freshwater mussels (*Elliptio complanata*) were collected from Nequasset Lake, a relatively clean lake within the Kennebec watershed in Woolwich, Maine. Individuals of a minimum size range were caged and transplanted 13 miles upstream and 11 miles downstream from a pulp and paper mill in the Kennebec River (Figure 1). The mill is located in Hinckley, approximately 30 miles north of Augusta, Maine. **DEP insisted on using only one upstream and one downstream station**, i.e., locations closest to the mill where fish could be collected, so that mussel data could be directly compared with fish data. **They did not allow us to place caged mussels any closer to the mill than 13 miles upstream and 11 miles downstream, even though there were extra cages that could have been used.** This precluded a thorough evaluation of the caged mussel methodology.

Ten cages with 36 mussels each were deployed for 53 days at these two locations in accordance with the upstream/downstream test paradigm. Figure 1 also shows our proposed experimental design with only three cages upstream and three cages placed at each of five downstream stations in a gradient design. **This was our recommended approach and the one recommended in the ASTM Standard Guide for conducting in-situ bioassays with caged marine, estuarine, and freshwater mussels.** After retrieval, the whole soft tissues of mussels were analyzed for dioxins and furans, percent lipids, and percent moisture. Percent lipids were measured as another indicator of animal health and to normalize the measurements on a lipid basis. Percent moisture was another indicator of animal health and used to normalize the data on a dry weight basis. Percent survival and multiple growth metrics were used as the primary indicators of animal health.

One of the reasons why representatives of Friends of Merrymeeting Bay and Applied Biomonitoring are still concerned with the experimental design and the results is that these issues were not fully discussed before the test began. The original proposal was made via a presentation to DEP on June 6, 2000. The decision to fund this project and conduct the study was not made until later that summer. It was not until Friends of Merrymeeting Bay and Applied Biomonitoring representatives arrived on the scene did they learn that DEP intended "only" to place mussels at two sites; one upriver and one downriver. Not only did DEP forbid any of the 20 cages to be transplanted closer than 11 or 13 miles away from the mill, they would not allow any of the extra cages prepared for a concurrent Kennebec River PCB study to be deployed near the mill effluent discharge. This is perplexing in view of the fact that placement of the cages closer to the effluent discharge could have provided more information, is consistent with all other studies we have conducted using a gradient design, is a recommended approach in the ASTM Standard Guide, and is consistent with a recent evaluation of Maine's dioxin monitoring program conducted by industry. **It is important to note that both industry and Friends of Merrymeeting Bay, who are typically in opposition on many environmental issues, are in agreement that there is a problem with the above/below fish test, and that the experimental design of the 2000 caged mussel study did not use the most appropriate methods with respect to the gradient design recommended by Applied Biomonitoring.**

Experimental Design

Pilot study conducted between August – September 2000

- 2 stations (13 miles upriver, 11 miles downriver)
- 10 cages/station
- 36 mussels/cage
- 720 mussels deployed
- 180 for beginning-of-test measurements (5 reps of 36 mussels)
- Total of 900 mussels used
- 53-day exposure period
- Test species *Elliptio complanata*
- 9 mm size range (58-67 mm)

After collection from Lake Nequasset

Sorting into 1-mm sizes

The flawed experimental design used in the 2000 caged mussel pilot study used only two stations, 1 station 13 miles upriver and another 11 miles downriver. DEP insisted that this was necessary to make a direct comparison with the above/below fish test. Applied Biomonitoring scientists have conducted over 50 caged mussel studies across the US and Canada and have never placed 10 cages at a single site before. We could have made the comparison with the fish test without using all 10 replicate cages at each site. Again, DEP was so focused on the ability to detect a statistically significant difference at the fish sites that they apparently did not even consider the fact that this was a pilot study and that one of the purposes should have been to test the utility of the caged bivalve methodology as a surrogate in a variety of applications and not just find a statistically significant difference. This bias has also interfered with an objective interpretation by DEP of the fish data. **DEP has assumed that since a statistically significant difference was found between dioxin-furan concentrations between upstream and downstream sites that this is a real difference in dioxin-furan exposure attributable to current discharges as the current regulations require.** Many believe that this difference could be attributable to past discharges and remobilization of sediment-associated chemicals. Furthermore, the distances from the source are so great (13 and 11 miles from the mill) that it is difficult to conclude that current discharges from the mill are truly the source of chemicals measured in fish. **In the final analysis, the most direct method for demonstrating that the current source of these chemicals is the mill is by transplanting mussels and lipid bags closer to the mill.**

In 2000, DEP continued development of an appropriate “above/below” fish test, but as dioxin and furan concentrations decline, there were concerns that the existing monitoring approach may not be sufficiently sensitive to detect statistically or environmentally significant differences in exposure to properly evaluate compliance with the 1997 state law. **Many believe that limitations of the fish test may preclude a scientifically or legally defensible use of the fish test in its current form.** Instead of considering methods such as the caged mussels or SPMDs as surrogates for fish, it might be more appropriate to consider the use of fish in addition to surrogate tests in a weight-of-evidence approach. **Although concentrations of dioxins and furans measured in fish tissues (not lipid normalized) were higher below than above pulp mill discharges in 2000, questions remain about the suitability of fish as effective monitors.** These questions are related to: 1) The mobility of fish and where exposure to dioxins and furans actually occurred, 2) Whether fish accumulated dioxins and furans from sediment or food that was contaminated from previous, rather than recent mill discharges and 3) When exposure and accumulation in collected fish occurred. In response to some of these questions, DEP modified the 2000 fish monitoring program to include measuring dioxins and furans in tissues of caged mussels and in lipids of SPMDs as potential surrogates for monitoring dioxins and furans in fish tissues. **On a lipid normalized basis the dioxins and furans measured in fish tissues were higher above than below the pulp mill in 2000.**

Measuring
Distributing
Deploying

1. Measuring & distributing
2. Attaching mussels
3. Preparing transportation
4. Deploying cages
5. Transporting cages
6. Soaking cages

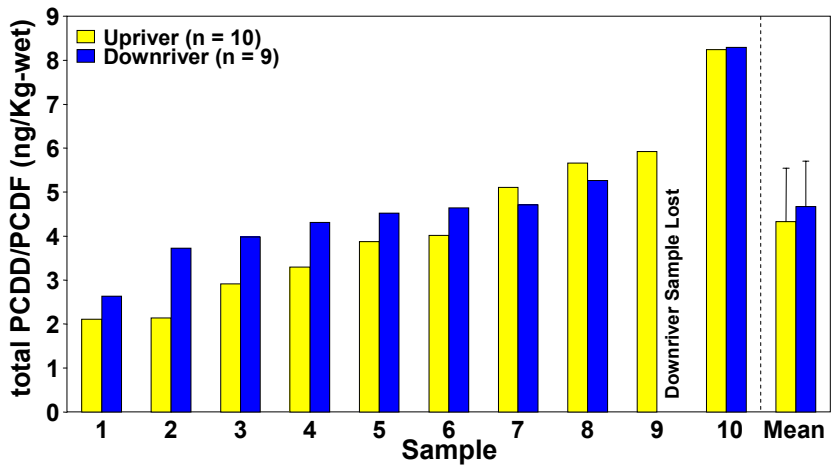
Caged freshwater bivalves have been used to monitor dioxins and furans associated with pulp and paper mill effluents in Finland and for similar chemicals such as PCBs in Canada for approximately 20 years. Environment Canada has recently adopted caged bivalve monitoring as an alternative to the required adult fish survey in their EEM program for pulp and paper mills in Canada. Standardized protocols have been adopted by the American Society for Testing and Materials (ASTM) for conducting caged bivalve studies, and a standard guide appeared for the first time in the 2001 ASTM Annual Book of Standards. A revised version will also appear in the upcoming Standard Methods for the Examination of Water and Wastewater. Caged bivalves are a potentially powerful tool because of their ability to quantify exposure and effects over space and time. In situ studies with caged bivalves could complement and help establish links between various elements of the existing DEP monitoring program through the use of tissue chemistry and mussel growth measurements. This approach could also help reduce uncertainty in the current approach and answer questions within government, industry, and the public regarding chemical exposure and biological effects associated with pulp mill effluents. It is also consistent with the ecological risk assessment process of characterizing exposure through bioaccumulation and characterizing effects through mussel growth rates. As mentioned previously, Environment Canada has focused on characterizing effects in its EEM program while the State of Maine has focused on characterizing exposure. The ecological risk assessment paradigm suggests equal emphasis on exposure and effects in a more balanced approach.

The protocols included in the ASTM Standard Guide include a level of sophistication and refinement for measuring, distributing, and deploying mussels that many scientists did not even consider a few years ago. It is this refinement that has reduced variability in the data and facilitated distinguishing differences in chemical exposure and associated biological effects among stations used in previous studies.

One of the reasons for deciding to conduct the Kennebec River caged mussel pilot study was to reduce variability in the tissue chemistry measurements, which was generally higher with fish. It was intended by using mussels, that the variability in tissue chemistry would be reduced because the size range, exposure, and genetic history of the test mussels could be controlled by pre-screening for size and age. This is not possible to do with natural fish populations because of the difficulty in collecting sufficient numbers in each size category. There was higher variability in the mussel data than expected. There are several possible explanations for this. 1) The measured concentrations were so low, and so near the limits of detection that there were several possible sources of error in the measurements. 2) At distances of 13 miles upstream and 11 miles downstream, the concentrations of bioavailable dioxins and furans were probably too low and too near the limits of detection, which introduced other errors in the measurements. 3) A recent QA/QC review conducted by industry has revealed several problems with the way the samples were analyzed and problems with the lab conducting the tests have been identified previously.

Upriver & Downriver total PCDD/PCDF

A. Mussels: Total PCDD/PCDF



Summary

- Only fish showed statistically significant differences up and downriver, suggesting they are the most suitable monitoring tool.
- Congener analysis and the lipid normalized data suggest fish are not.
- When lipid normalized, fish concentrations higher up than downriver.
- Location and duration of exposure are unknown because fish move.
- SPMDs also showed higher concentrations up than downriver.
- SPMD data were more variable and include a possible outlier.
- Mussels had higher concentrations of total PCDD/PCDF downriver than upriver on both a lipid normalized and non-lipid normalized basis.

B. SPMDs: Total PCDD/PCDF

C. Fish: Total PCDD/PCDF

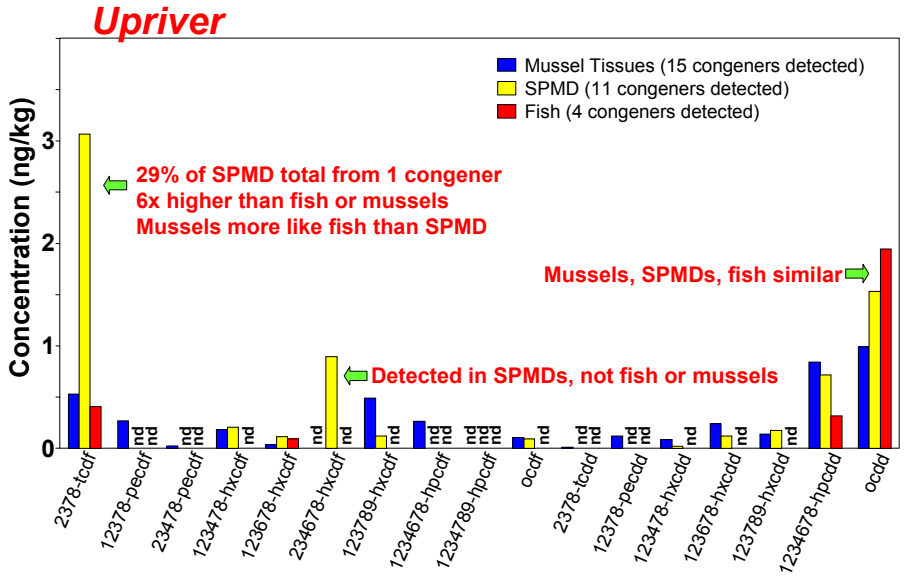
Mussels caged downstream of the mill accumulated higher concentrations of dioxins and furans than fish. The only reason that fish showed a statistically significant difference is that the upstream concentrations were much lower. Both mussels and SPMDs suggest higher concentrations upstream and so did fish, on a lipid normalized basis. Survival and growth of caged mussels indicated they were all in adequate health to accumulate dioxins and furans if present. This is also an important difference between caged mussels, fish, and SPMDs; i.e., health indices can be used to help demonstrate a successful test. Since there are no health measurements with SPMDs, it is difficult to determine whether they are functioning properly. Some have speculated that elevated temperatures near the mill effluent could complicate data interpretation with respect to accumulated dioxins and furans. While this could add a complication, quantifying mussel survival and growth rates can be used to help determine their ability to accumulate chemicals as we have done here. Conversely, if temperature is a complicating factor for SPMDs, this is difficult to quantify. Nevertheless, DEP has finally acknowledged that SPMDs can be affected by algal films that clog the pores of the semi-permeable bags. The problem with using wild fish is that there is considerable uncertainty in determining whether measured health parameters represent the last week or the last month of exposure. Since caged mussels are measured at the beginning and the end of the test, there is much greater certainty that accumulated chemicals were taken up during the exposure period and that health parameters measured at the end represent differences between the beginning and the end of the test. This represents a controlled experiment and not just uncontrolled monitoring.

Mean concentrations of total dioxins/furans in mussels increased from below detection at the beginning of the test to **4.33** and **4.67 ng/kg-ww** at the upstream and downstream stations, respectively, at the end of the test. Concentrations were higher downstream than upstream in most samples. However, the difference was not statistically significant between upstream and downstream total PCDD/PCDF concentrations at the end of the test. Conversely, the concentration of dioxins/furans in SPMDs was higher upstream than downstream in every sample. Again the difference was not statistically significant. This is the first piece of evidence to suggest that the SPMDs did not show as much promise as the caged mussels, even though DEP concluded SPMDs were more promising. The elevated concentrations in the SPMD sample 3 upriver is identified as a possible outlier that could have contributed to the greater variability in the SPMD data, but identifies another problem with the SPMD data. The mean concentration of dioxins/furans in SPMDs was **10.47** upstream and **6.33** ng/kg ww downstream.

The concentration of total dioxins/furans in fish tissues was significantly higher 11 miles downstream (**4.19 ng/kg-ww**) than 13 miles upstream (**2.76 ng/kg-ww**) of the mill. These data suggest that fish are better able to detect differences in dioxin and furan exposure than mussels or SPMDs, and the existing fish monitoring approach is appropriate. However, given that the stations were so far away from the mill it is not clear that these measurements represent current mill discharges. There are other important points to make from these data: 1) The mussel data was similar to the fish in showing higher concentrations of dioxins and furans downstream; 2) The fish data were more similar to the mussel data than the SPMD data; and 3) The SPMD data were the most variable.

Congeners: Mussels, Fish, SPMDs

Upriver



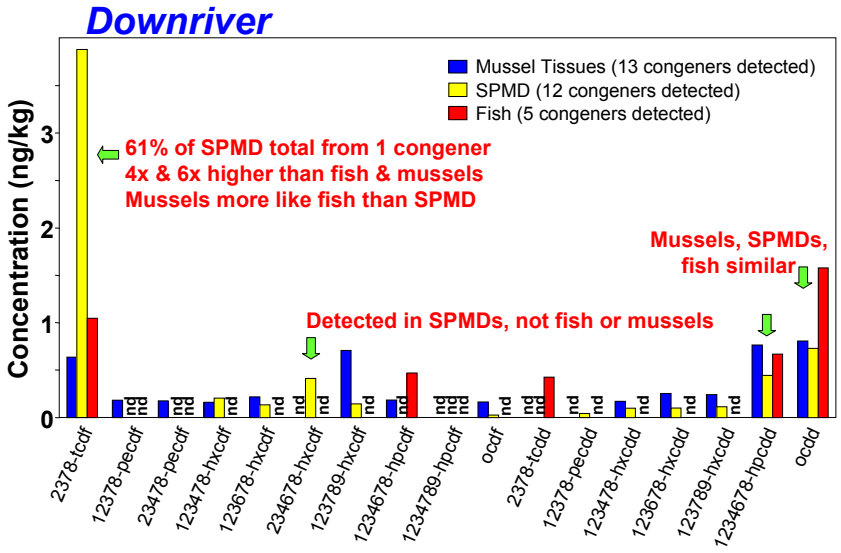
Upriver: 13 miles from mill

- Mussels detected more congeners than SPMDs or fish
- 29% of total PCDD/PCDF in SPMDs from 1 congener
- Concentrations for this congener 6x higher than fish or mussels
- Mussels more like fish than SPMDs for this congener and most others
- When lipid normalized, fish concentrations higher up than downriver
- Most concentrations were below the detection limit
- This suggests a problem with the “above/below” sampling design
- It also suggests a problem with the analytical methodology
- Possible problem with lipids obscuring detection of some congeners

Downriver: 11 miles from mill

- Mussels detected more congeners than SPMDs or fish
- 61% of total PCDD/PCDF in SPMDs from 1 congener
- Concentrations for this congener 4x and 6x higher than fish or mussels
- Mussels more like fish than SPMDs for this congener and most others
- When lipid normalized, concentrations in fish higher up than downriver
- Most concentrations were below the detection limit
- This suggests a problem with the “above/below” sampling design
- It also suggests a problem with the analytical methodology
- Possible problem with lipids obscuring detection of some congeners

Downriver



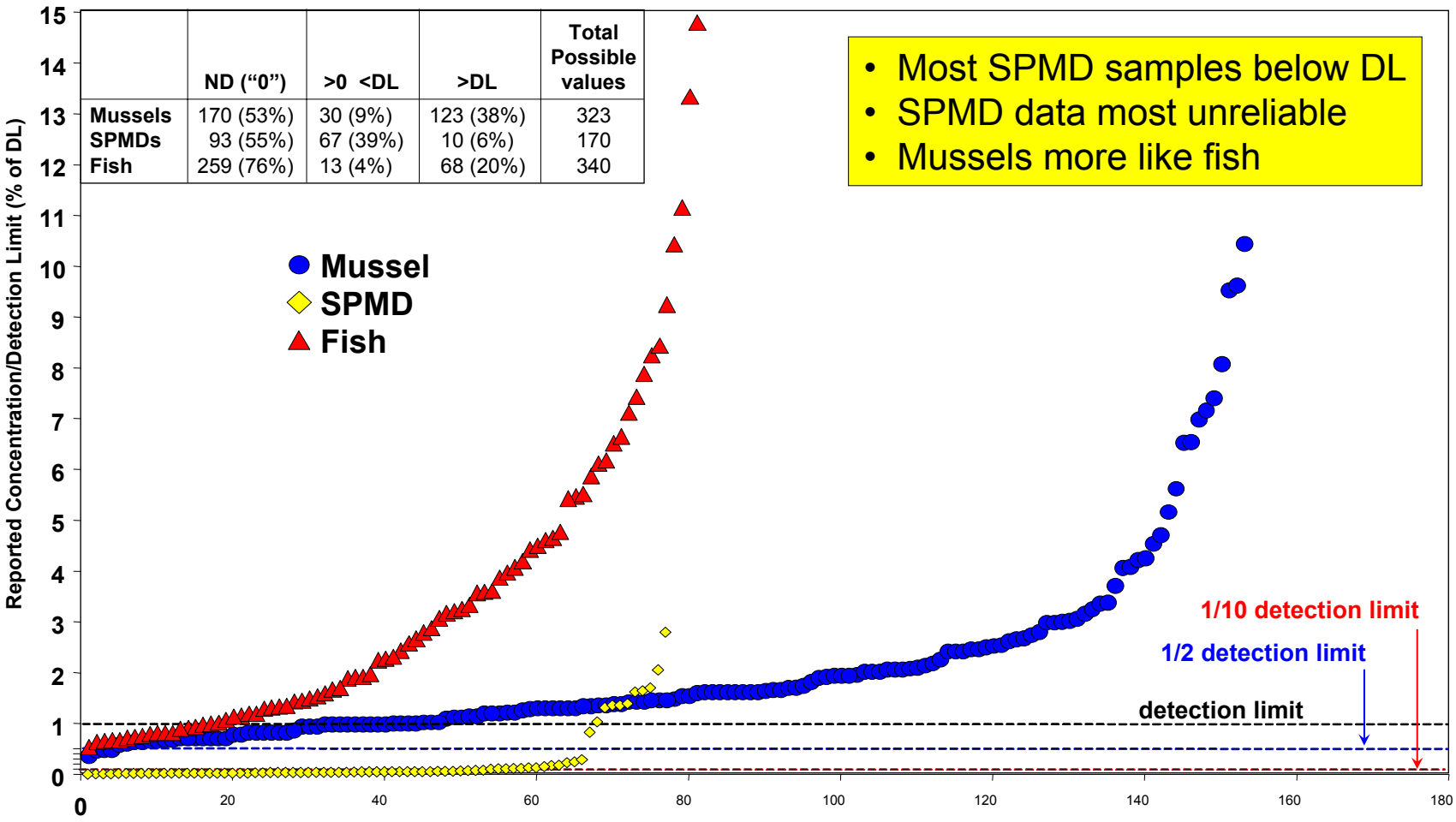
Additional evidence for mussels showing more promise than SPMDs or fish as a dioxin monitoring tool is the fact that more individual dioxin/furan congeners were measured in mussel tissues from both upstream (15 congeners) and downstream (13 congeners) locations than in SPMDs (11 and 12 congeners) or fish tissues (4 and 5 congeners).

The congener-specific data also highlights the real problem with using SPMDs, and that they do not represent fish, or total dioxin/furan exposure as well as mussels. Upriver, 29% and downriver 61% of the total dioxins/furans in SPMDs came from 1 congener; 2,3,7,8 tcdf.

We believe these results are encouraging with respect to using caged mussels as a surrogate for fish, particularly since the downstream station was located 11 miles from the mill and mussels still accumulated dioxins and furans. The gradient design could have proven the existence of dioxins and furans closer to the mill if they were really being discharged by the mill. We also believe that these data support the working hypothesis that caged bivalves are a potentially more useful monitoring tool than either fish or SPMDs.

SPMD, Fish & Mussels: % Detection Limit

	ND ("0")	>0 <DL	>DL	Total Possible values
Mussels	170 (53%)	30 (9%)	123 (38%)	323
SPMDs	93 (55%)	67 (39%)	10 (6%)	170
Fish	259 (76%)	13 (4%)	68 (20%)	340



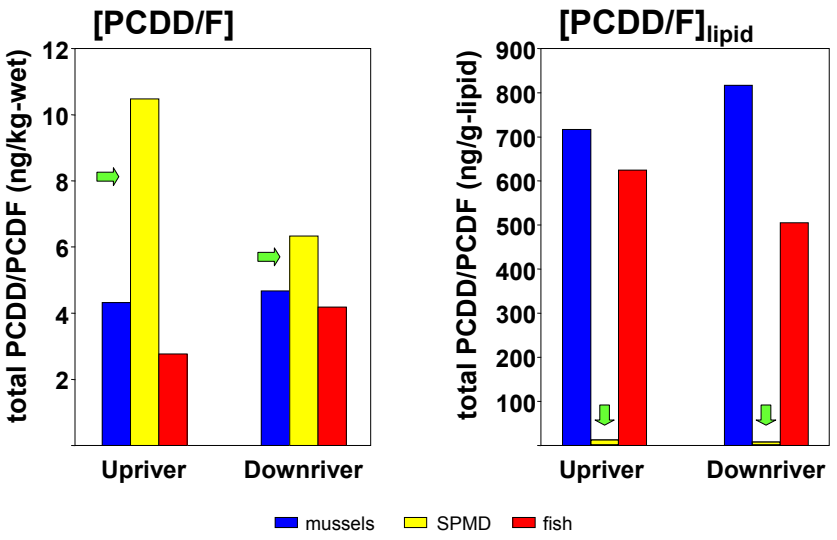
- Most SPMD samples below DL
- SPMD data most unreliable
- Mussels more like fish

Percent of values >0 compared to detection limits (DL) for caged mussels, SPMDs, and fish. For non-detected (ND) values, a "0" was used to represent reported concentration. Total possible values = number of samples analyzed x 17 congeners. Graph does not show percent of values that were non-detects.

There was also much greater uncertainty in the SPMD data when compared to the mussel and fish tissue chemistry data. Nearly 40% of the congeners in mussel tissues were present at concentrations exceeding the detection limit, compared to approximately 20% for fish, and less than 10% for the SPMDs. This is based on results of congener-specific analyses that yielded 153 values for mussel tissues (30 + 123), 81 values for fish tissues (13 + 68), and 77 values for SPMDs (67 + 10). Some results for both the mussel tissues (<10%) and SPMDs (<40%) were reported at concentrations greater than zero, but less than the detection limit. **For the SPMDs, these concentrations were generally at least one order of magnitude lower than the detection limit.** Plots of the ratio of measured concentrations of the individual congeners divided by the method detection limit for each congener for mussels, SPMDs, and fish show the greater uncertainty in the SPMD data. Only 10 of the measured values (6%) for SPMDs are above the detection limit, only one value within 50% of the detection limit, and the rest of the values were between 0.4% and 29% of the detection limit. These reported concentrations were estimated from the calibration curve of the analytical instrument, but have the greatest uncertainty because they are so far away from the instrument detection limit. **These data suggest that the extremely low measured concentrations and the large number of non-detects from samples collected 13 miles upstream and 11 miles downstream are not reliable indicators of dioxin/furan exposure, and that there may have been analytical problems associated with these data.** A recent quality assurance/quality control (QA/QC) review has suggested a methodological problem at the laboratory conducting the analyses, and when extra fish samples were analyzed, the concentrations were significantly higher. The original data were questioned when the reported concentrations were significantly lower than the previous year and there were no process changes at many of the mills that were being monitored.

These data also support the working hypothesis that caged bivalves are a potentially more useful monitoring tool than either fish or SPMDs.

Problems with Lipid Bags



General

- Environmentally unrealistic exposures and regulations
- Only reflect dissolved fraction and not dietary exposure
- Over-trap low molecular weight compounds
- No effects endpoints or environmental significance
- Small database compared to bivalves and fish
- Numerous extrapolations and assumptions
- Only accumulate organic chemicals and not metals
- Results are difficult to interpret
- Difficult to "clean up" lipids
- Deployment issues:
  - Long handling and boat times
  - Potential contamination from a variety of sources
  - Slime layers and fouling inhibit accumulation
  - Current velocity and other factors affect accumulation

Specific

- SPMDs showed higher concentrations up- than downriver, on both a lipid-normalized and non-lipid-normalized basis.
- Based largely on apparent over-trapping of a single low-molecular-weight congener, SPMD results were very different than either fish or mussels.
- Collectively, these data and the congener-specific data suggest that mussels are a more effective surrogate for fish.

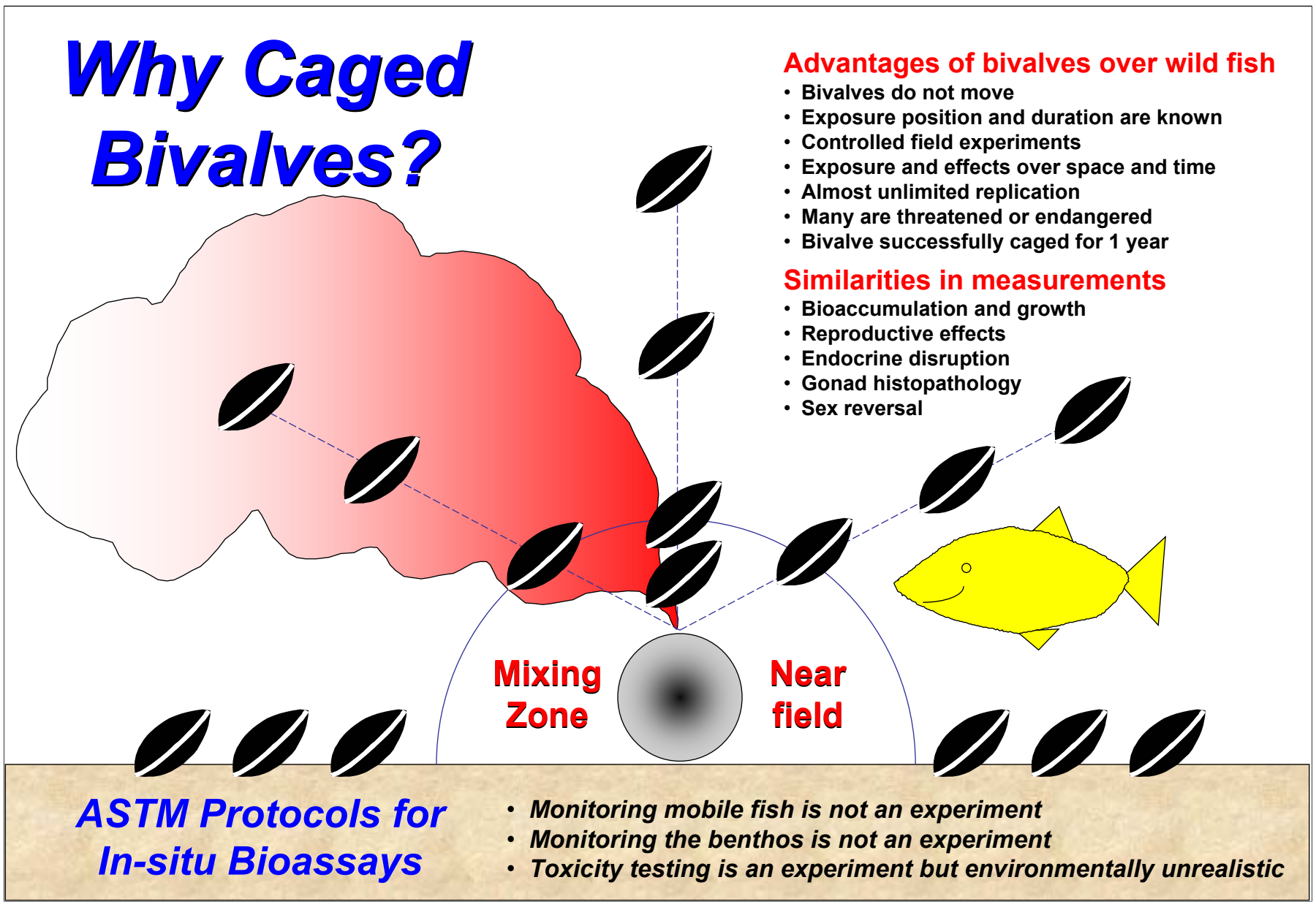
Listen to the animals, not the fat bags
Animals are more than bags of fat
Toxicological interpretation requires animals

When the state of Maine implemented the new regulations regarding dioxin exposure, it probably did not envision a bag of fat serving as a surrogate for a living, breathing organism. The net result is that measurements provided by SPMDs are environmentally unrealistic in that they only represent the concentration of chemicals in water. Hydrophobic chemicals like dioxins and furans quickly sorb to suspended particulate matter and the food of mussels and fish. SPMDs do not characterize this exposure, and many recent studies demonstrate that food is often the most important exposure pathway for these types of chemicals. DEP representatives have speculated that the reason for the lower concentrations of dioxins and furans in mussels compared to fish is that they are at a lower trophic level. While this could be true, mussels have the ability to filter large volumes of water and ingest substantial quantities of particulate material containing dioxins and furans. **In this context of characterizing ambient concentrations of bioavailable dioxins and furans in the water column, mussels are more efficient than either fish or SPMDs.** Mussels ingest both aqueous and particulate forms of dioxins and furans, SPMDs only characterize aqueous forms. Fish characterize aqueous and particulate forms like mussels, but can accumulate higher concentrations from their food in the form of prey organisms. **The advantage of the caged mussel methodology that has not been tested by DEP is the ability to place the test animals much closer to the mill to quantify that potentially higher exposure. Furthermore, while DEP has also suggested that SPMDs show more promise, they represent no trophic level and no dietary exposure. DEP has never directly addressed any of these issues. As mentioned previously, the statistically significant difference in dioxins and furans measured in fish tissues is not attributable to fish accumulating higher concentrations than mussels downstream (fish and mussels were about the same) but the relatively low concentrations measured upstream. Both the mussel and SPMD data suggest that higher concentrations of dioxins and furans should have been measured in the upstream fish.**

The other characteristic that makes SPMDs questionable as an environmentally realistic monitoring tool is that they tend to over-trap the lower molecular weight chemicals due to the pore size of the bags. As shown from the 2000 data, the concentrations of dioxins and furans and the congener pattern was much similar between mussels and fish than with SPMDs. The SPMDs even showed a higher concentration of dioxins and furans at the upstream site. Nevertheless, DEP still concluded that SPMDs showed more promise than mussels as a monitoring tool. Another reason given for using caged mussels is that this approach would fit better into DEPs other monitoring programs than SPMDs. 1) Effects endpoints can be used to add environmental significance to dioxin and other monitoring. 2) There is a relatively small database for SPMDs when compared to mussel and fish monitoring. 3. There are numerous extrapolations and assumptions in SPMD monitoring. 4) SPMDs accumulate only organic chemicals and not metals so they are less applicable to other DEP monitoring programs. 5) Results form SPMDs will always be difficult to interpret. 6) All samples need to be "cleaned up" to remove excess lipids that interfere with instrument detection and since the lipid bags only include lipids this makes them even more difficult to handle and measure.

There are also a number of practical problems associated with lipid bag deployments including long handling and boat times, potential contamination from a variety of sources including air and boat exhaust, slime layers and fouling that inhibit accumulation, and current velocity and other factors affect accumulation. Although graduate students from the University of Maine have identified many of these problems at several meetings, DEP and senior advisors and the University continue to suggest that SPMDs show more promise than caged mussels as a monitoring tool.

The DEP continues to use unsupported statements to suggest that bass and SPMDs show more promise than caged mussels but the data do not support this position. If rigorous peer review by unbiased scientists were to prevail, there is no question that they would recommend that the 2000 study be repeated with caged mussels, SPMDs, and fish, to fully explore the advantages and disadvantages of each. It does not appear that DEP is interested in rigorous peer review or an open debate on the scientific issues associated with dioxin monitoring.



Having identified the numerous advantages of using caged bivalves over SPMDs, it is important to also identify the numerous advantages of bivalves over fish. The most important reason is that bivalves do not move and it is relatively easy to conduct controlled field experiments with caged bivalves. Furthermore, precise locations and exposure durations are clearly identifiable, which allows a characterization of exposure and effects over space and time in a risk-assessment based approach. There can be virtually unlimited replication using animals of a similar size, age, and previous history. **Perhaps more importantly, many species of freshwater bivalves are threatened or endangered and it would be helpful to know about exposure and effects on related species used in various monitoring programs.**

In addition to the similarities in the dioxin and furan patterns between caged mussels and fish, there are also similarities in the number of effects measurements that can be made. Measuring bioaccumulation and growth in mussels and fish are well established. However, working with scientists at Environment Canada's St. Lawrence Center, we have been able to show effects on reproduction, endocrine disruption, gonad histopathology, and even sex reversal in a 1-year exposure on the St. Lawrence River last year.

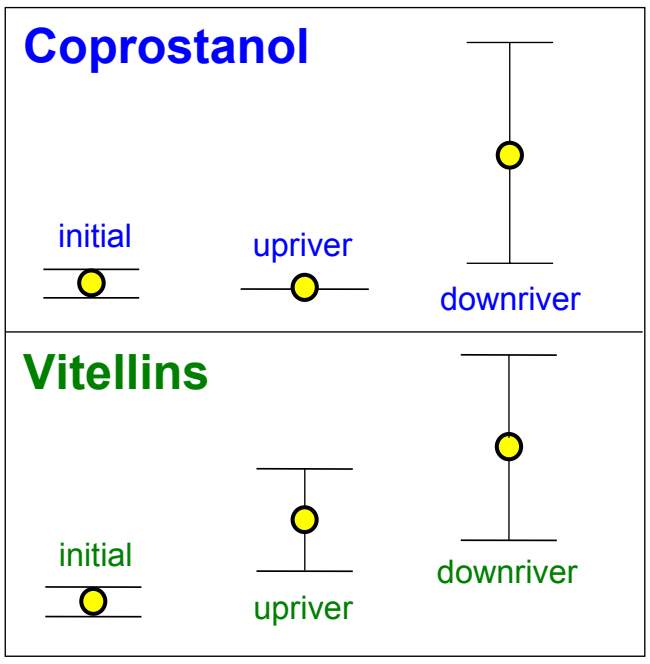
Collectively, all of these attributes make caged mussels a potentially powerful tool in many DEP monitoring programs and it is difficult to understand their unwillingness to conduct an appropriate test. It almost appears as though DEP did not want the caged mussel test to succeed. Furthermore, there are ASTM protocols for conducting these test that have been through a rigorous peer review process to be accepted.

In summary, monitoring mobile fish is not an experiment. Monitoring the benthos is not an experiment. Toxicity testing is an experiment, but it is not environmentally realistic. Given the widespread use of indigenous and caged bivalves around the world for the past 30 years, the DEP position cannot be supported, particularly with the 2000 monitoring results where all the chemistry results are questionable.

Other Advantages of Using Mussels:

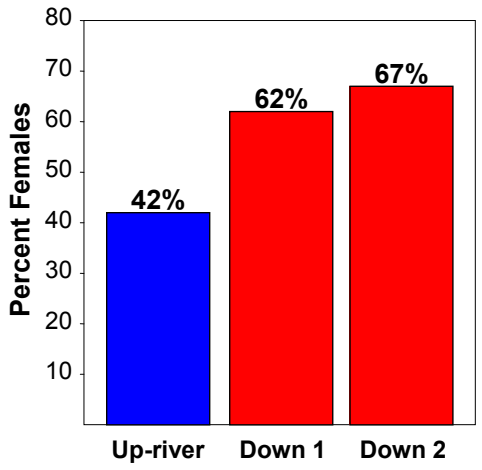
Synoptic measurement of exposure & effects
Other similarities with fish monitoring
Measurements of bioaccumulation, growth, reproduction

Biomarkers on the St. Lawrence River



- Coprostanol used as an indicator of effluent exposure
- Vitellins used as an indicator of effluent effects
- Vitellins similar to vitellogenin in fish

Sex Reversal on the St. Lawrence



- Higher % females down-river from municipal effluent
- Experimentally induced in caged *Elliptio complanata*
- Successfully caged for 1 year to induce effects
- Similar to sex reversal demonstrated in fish

- Recent studies suggest histopathological effects
- Experimentally induced in caged *M. galloprovincialis*
- Similar to histopathological effects in fish

Histopathology in San Diego Bay (*M. galloprovincialis*)

The graphics above summarize some of the other numerous advantages of using caged mussels. These include synoptic measurements of exposure and effects, other similarities with fish monitoring endpoints, and measurements of bioaccumulation, growth, and reproduction.

The left panel represents biochemical markers developed by Environment Canada scientists in cooperation with Applied Biomonitoring in a series of studies conducted between 1999-2002 on the St. Lawrence River. These graphs show the relationship between coprostanol and the relative amount of vitellins in caged mussel tissues. Although these data do not clearly establish a cause-and-effect relationship, they show that the amount of vitellins (related to reproductive capacity) is directly related to the amount of coprostanol from the City of Montreal effluent. In this example, coprostanol was used as an indicator of effluent exposure and vitellins as an indicator of effects. Vitellins are the mussel counterpart to fish vitellogenin.

The center panel shows experimentally-induced induction of sex reversal in *Elliptio complanata* deployed downstream of the same effluent. This experiment was conducted because preliminary surveys conducted by Environment Canada showed a higher percentage of females downriver from the effluent in the natural population. To test this hypothesis *Elliptio complanata* were caged for 1 year at two downriver sites and one up-river site. Although the test animals were virtually identical at the beginning of the test, there were significantly more females downriver at the end of the test. This change is similar to sex reversal in fish which has been associated with chemicals such as dioxins, furans, and PCBs. Many of these biochemical markers are similar to those being developed at the University of Maine by other scientists and it is surprising that they have not actively supported the caged mussel work. It seems as though the influence of the chemistry department and DEP have influenced the entire review process.

The right panel shows the ability to measure histopathological effects in mussel gonads. Work similar to this has also been conducted by the University of Maine in conjunction with EPA. In this example, marine mussels exposed to high concentrations of copper in San Diego Bay had a high percentage of abnormal gonads. Again, these are only gross correlations and do not establish cause-and-effect, but it appears as though the effect could be quantified. It was also experimentally induced, and the histopathological effects are similar to those induced in fish by chemicals such as dioxins, furans, and PCBs.

Collectively, these studies demonstrate all of the effects endpoints that can be measured using indigenous or caged bivalves as part of an integrated, risk assessment based monitoring program.

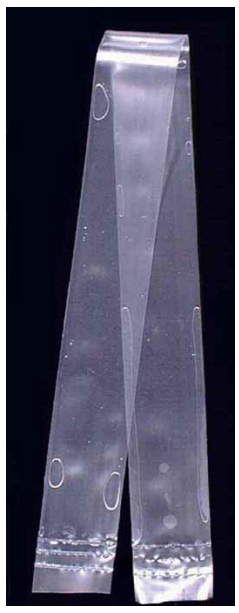
Listen to the Animals

Unless they move and you don't know where they've been

BIVALVES
- Integrate dietary & waterborne chemicals
- Easy to collect, cage, measure & analyze
- Measure exposure & effects
- They don't move

Freshwater Mussel
Elliptio complanata

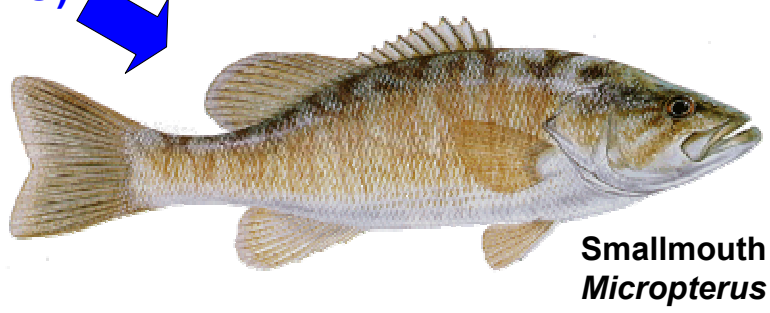
Semi-Permeable Membrane Device (SPMD)



FAT BAGS
- Integrate only waterborne chemicals
- Easy to collect, cage & analyze
- Measure only exposure to organics
- They don't move, live, or breathe

Fully- Permeable Living Organism (FPLO)

FISH
- Integrate dietary & waterborne chemicals
- Difficult to collect, cage & measure
- Measure exposure & effects
- They move



Smallmouth Bass
Micropterus dolomieu

The purpose of this somewhat tongue-in-cheek graphic is to highlight the similarities and differences between caged mussels, SPMDs, and fish. The first, and most important point is that it is easier to interpret environmental data from living, breathing organisms rather than a bag of fat. The take-home message is, listen to the animals. The only caveat is, listen to the animals unless they move and you don't know where they've been. Bivalves integrate dietary and waterborne chemicals, they are relatively easy to collect, cage, measure, and analyze, and methods have been developed and refined for measuring both exposure and effects. To repeat for emphasis, mussels do not move.

The semi-permeable membrane device (SPMD) gets its name from the fact that the plastic material from which the bag is made is only semi-permeable. In other words, the pore size is such that only molecules below a certain size can enter the bag and be sequestered in the lipids. The larger molecules, which are generally the most persistent, and often have the most long-lasting effects, do not enter the bag. Like mussels, they are easy to collect, cage, and analyze, but they only characterize exposure to organic chemicals such as dioxins and furans. While this would be acceptable for above-below monitoring, it could not be readily incorporated into other DEP monitoring programs. Mussels could be used in all DEP monitoring programs to supplement traditional monitoring methods such as fish, benthic community structure, and laboratory toxicity tests. Unlike fish and mussels, SPMDs do not move, live, or breathe or accumulate chemicals from water and food like mussels and fish. Data from the 2000 monitoring study show that the dioxin furan patterns in fish were much more similar to mussels than to SPMDs.

Like mussels, fish integrate both dietary and waterborne chemicals and there are a number of exposure and effects endpoints than can be quantified. Unlike mussels, they are relatively difficult to collect, cage, and measure. Most importantly however, fish can move and the exposure is always uncertain. Being restricted to measurements of dioxins and furans in tissues of animals caught at 13 and 11 miles upriver and downriver from the mill, respectively, is not a very powerful monitoring tool. Both mussels and SPMDs provide the capability of characterizing exposure along suspected chemical gradients, but this feature was never tested by DEP.

Finally, we have jokingly referred to both mussels and fish as FPLOs; i.e., fully permeable living organisms, because they do not have the same small size restrictions as SPMDs and because they are living organisms that can be used to measure both chemical exposure and associated biological effects. This should be the core of any integrated risk assessment based monitoring program.

Maine DEP Conclusions - 2000 Monitoring Report

"Since the development of the Above/Below test began in 1997, over 78 tests have been conducted for different dioxins, species, tissue types, and other surrogates in an attempt to develop a test powerful enough to accurately measure any differences above and below a mill. Bass and semi-permeable membrane devices show the most promise and will be tested again in the 2001 program."

"Freshwater mussels did not appear to be a useful monitoring device, perhaps because they are at a lower trophic level than fish."

We do not believe these conclusions are scientifically defensible
We believe DEP is biased toward fish and SPMDs
We support development of an independent peer review panel

Monitoring with caged bivalves have been used for over 30 years

- There are fewer uncertainties in the mussel data than SPMD or fish data
- Bivalves can be used as indicators of exposure and effects
- Freshwater bivalves need more study because many are threatened and endangered:
  - **The most imperiled animal group**
  - **70% are threatened or endangered**
  - **20% presumed extinct**
  - **10% may be extinct this century**

Endangered
Northern Riffleshell

With respect to comparing the results of the two surrogate tests evaluated as part of this study, the following conclusions reached by DEP in their 2000 Dioxin Monitoring Report are not scientifically defensible.

"Since the development of the Above/Below test began in 1997, over 78 tests have been conducted for different dioxins, species, tissue types, and other surrogates in an attempt to develop a test powerful enough to accurately measure any differences above and below a mill. Bass and semi-permeable membrane devices show the most promise and will be tested again in the 2001 program."

"Freshwater mussels did not appear to be a useful monitoring device, perhaps because they are at a lower trophic level than fish."

There are no data or statements in the DEP report that support these conclusions. The concentrations of dioxins/furans in fish were significantly higher downstream than upstream, the difference was very small, and on a lipid-normalized basis the dioxin/furan concentrations were higher in fish upstream than downstream. Most SPMD samples were below the detection limit. The SPMD data were the most unreliable because reported concentrations were estimated at a fraction of the detection limit. The response of the mussels was more like fish than the responses of the SPMDs. In addition, approximately 29% and 61% of the total dioxins/furans in SPMDs at upstream and downstream sites was attributable to a single congener (2,3,7,8-tcdf). In almost every study where SPMDs have been compared with mussels, SPMDs have been shown to "over-trap" the lower molecular weight organic compounds. Concentrations of this furan congener were about six times higher than fish or mussels and suggest that the majority of the dioxins/furans from SPMDs did not represent fish or any other living organism and that it was an artifact of the surrogate sampling procedures. In other words, the SPMDs were good accumulators of the compound which was least environmentally relevant to fish or mussels. For comparative purposes, 2,3,7,8-tcdf is approximately 1/20 as toxic as 2,3,7,8-tcdd, according to the 2002 WHO guidelines based on Van den Berg et al. 1998. Caged bivalves are better monitoring tools because they do not move and do not metabolize dioxins and furans.

There are too many uncertainties in the results from accumulation of dioxins and furans in caged mussels, SPMDs, and fish tissues to unconditionally accept the results and make important programmatic decisions regarding the utility of these three methods. Another pilot study is suggested that directly tests the utility of the caged mussel methodology (and SPMDs) using a gradient design downstream from the mill and placing cages as close as possible to the effluent discharge. The weight of evidence from bivalve biomonitoring studies conducted on chlorinated hydrocarbons such as dioxins furans, and PCBs throughout the world suggest that caged bivalves can be an effective monitoring tool for pulp and paper mill effluents in the State of Maine. This is not to say that bivalves should be the only monitoring tool. Most experts have agreed that there is no perfect monitoring tool and that a weight of evidence approach should be used to make the most meaningful assessments. It seems reasonable to assume that a triad approach using caged mussels, SPMDs, and fish would provide DEP with the best possible data to make informed decisions with respect to potential exposure from dioxins and furans from pulp and paper mills on the Kennebec River. We have previously suggested that the best way to measure water quality is to not measure chemicals in water but measure them in mussel tissues because they provide a more integrated picture of exposure. As anomalous as it may seem, the best way to quantify exposure in fish may be to measure chemicals in caged mussels rather than fish. Mussels are potentially better indicators of dioxin/furan exposures because they do not move and because they can be placed closer to the mill.

Concerns with DEP Conclusions

DEP did not ask the right questions regarding exposure & effects

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 such as the Sediment Quality Triad successfully address questions 1 & 3, but do not directly address questions 2 & 4.

DEP did not conduct the appropriate tests

The DEP monitoring study was not a true test of the caged mussel methodology

- The study design was dictated by the ability to collect fish at particular locations.
- Stations 13 and 11 miles from the outfall are not representative.
- This approach did not evaluate one of the major advantages of the transplant methodology, i.e., transplanting bivalves along suspected chemical gradients.

Weight of evidence suggests bivalves a useful monitoring tool

- No instrument has yet been devised that will measure toxicity
- Chemical concentration can be measured with an instrument, but only living material can be used to measure toxicity

Cairns & Mount, 1990

- No instrument has yet been devised that will accurately measure bioavailability from all exposure pathways
- Chemical concentration can be measured with an instrument, but only living material accurately reflects bioavailability

Salazar & Salazar, 2000

The primary concerns with DEP conclusions with respect to 2000 monitoring with caged mussels, SPMDs, and fish are the following: 1) DEP did not ask the right questions regarding exposure and effects; 2) DEP did not conduct the appropriate tests; and 3) DEP did not adequately consider the weight of evidence in evaluating the utility of bivalves as a monitoring tool.

Environment Canada's 1999 AETE report, evaluated by Borgmann in 2000 asks the following 4 questions:

1) Are contaminants entering the system; 2) Are contaminants bioavailable; 3) Is there a measurable response, and 4) are contaminants causing this response. While it is not necessary to ask all of these questions for the above-below monitoring, DEP should have an idea with respect to characterizing and understanding exposures in order to properly evaluate existing data and design a useful monitoring program. To place this concept in the appropriate perspective, Borgmann suggests that traditional approaches such as the sediment quality triad (sediment chemistry, benthic community structure, and laboratory toxicity tests) successfully address questions 1 and 3 but do not directly address questions 2 and 4. The reason for this is that the sediment quality triad does not include bioaccumulation measurements. Synoptic measurements of exposure and effects provide a method for establishing cause-and-effects relationships. **Controlled field experiments provide a method for reducing the uncertainty in traditional measurements such as fish monitoring.**

DEP did not conduct the appropriate tests to adequately assess the caged mussel methodology. Caged mussels were only placed at two sites where fish could be caught. While this may provide the most direct comparison with fish monitoring, it does not test the most important aspect of caged bivalve monitoring; i.e., the ability to place mussels at various distances from the mill. Furthermore, stations located 13 and 11 miles respectively from the mill cannot be considered representative control and treatment sites because they are so far apart. A "control" site should be the same as the "treatment" site in all physical/chemical aspects other than the chemical of concern. This is not possible at those distances. Applied Biomonitoring suggested a gradient approach, a gradient approach was also recommended by a recent industry evaluation of the 2000 monitoring report and a gradient design is what is recommended in the standardized ASTM protocols. **DEPs refusal to follow commonly accepted procedures for the caged mussel approach and their insistence on relying on fish and SPMDs is one of the reasons why even after all this time and all this money they still do not have a reliable test method.**

The weight of evidence from this and other studies throughout the world over the last 30 years suggests that caged mussels provide a versatile and reliable monitoring tool. There is no analytical instrument for direct measurements of toxicity. Chemical concentration can be measured with an instrument, but only living material can be used to measure toxicity (Cairns & Mount 1990). Similarly, no instrument has yet been devised that will accurately measure bioavailability from all exposure pathways. Chemical concentration can be measured with an instrument, but only living material accurately reflects bioavailability (Salazar & Salazar 2000).

Summary & Conclusions

Summary

- Mussels detected more congeners than either fish or lipid bags
- Most of the lipid bag data were below the detection limit
- Mussel data were more comparable to fish than SPMD data

Conclusions

- Surrogate mussels may be a better indicator of exposure than fish
- Surrogate mussels have a greater potential for the above/below test because they do not move
- DEP was biased in their interpretation of the fish and lipid bag data

Recommendations

- Conduct another study using gradient design with stations close to mill
- Have samples analyzed by another lab to avoid bias and poor methodology
- Develop an independent peer review panel to review all the results

Mussels are better indicators than fish because they do not move and because they can be placed closer to the mill

This integrated pilot study compared three approaches as alternative monitoring tools for assessing the fate and effects of dioxins and furans associated with a pulp mill effluent. While water samples have been used to characterize aqueous chemical exposures for over 50 years, new elements used here include the use of caged mussels to integrate chemical exposure and associated biological effects. **Caged mussels have been used for approximately 30 years, but recent refinements have increased the sensitivity of this approach to a new level, and these methods have only recently been adopted by the ASTM. SPMDs represent the newest of these methodologies and applications of this approach are still being refined.** This study is unique not only in terms of comparing these three monitoring methods, but applying them in areas where they have not been commonly measured in Maine, using state-of-the-art chemical analyses with low detection limits, and using extensive experience and expertise to interpret the results of congener analysis (i.e., dioxins and furans) and mussel growth rates.

Collectively, the congener-specific data which showed detection of more congeners in mussels than SPMDs and fish, results that showed higher concentrations in mussels downstream than upstream (on both a lipid-normalized and non-lipid-normalized basis), and the larger number of samples above the detection limit suggests that mussels were better dioxin/furan indicators than SPMDs or fish. The most important question to be asked may be whether or not the fish data are believable, and represent current mill discharges, particularly given their ability to move and accumulate dioxins and furans through other exposure pathways. Just because the fish test satisfied the requirements of the above/below test and implicated the mill does not mean that these data represent current mill current mill discharges or current exposures from those discharges. Demonstrating a statistically significant difference appeared to be one of the most important considerations for DEP in evaluating the suitability of caged mussels as a surrogate test. These questions, as well as concerns regarding upstream and downstream comparisons, can be addressed, at least in part, by using a weight of evidence approach and not relying on fish alone.

Although the fish appeared to be the most suitable monitoring tool based on the ability to detect statistically significant differences between upstream and downstream concentrations of total dioxins and furans, the congener analysis and the lipid-normalized data suggest that they are not. On a congener basis the data suggest that mussels and SPMDs are more representative of all dioxin and furan exposures. Further, on a lipid-normalized basis there was no statistically significant difference between upstream and downstream locations in the fish data. More importantly, the concentrations were higher upstream than downstream. The caged mussel and SPMD data further suggest that the using the fish test at these upstream and downstream locations is inappropriate since the upstream station appears to be contaminated by another source somewhere above of the mill. The downstream station was too far away to know whether fish are being exposed to current dioxin and furan discharges from the mill, other sources, or previous discharges from the mill. While the experimental design in the caged mussel pilot study may have been appropriate for comparing dioxin and furan exposures with those in fish and SPMDs, it was not appropriate for addressing the upstream/downstream issues concerning these potential fish surrogates. That would be a gradient design as used in most effluent monitoring studies. **A combination of caged mussels and SPMDs should have been placed as close to the pulp mill discharge as possible for a more accurate evaluation of their ability to detect upstream/downstream differences. A more direct approach would be to repeat the caged mussel pilot study with more stations closer to the mill in a gradient design as originally proposed.**