

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 semi-permeable 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.

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?

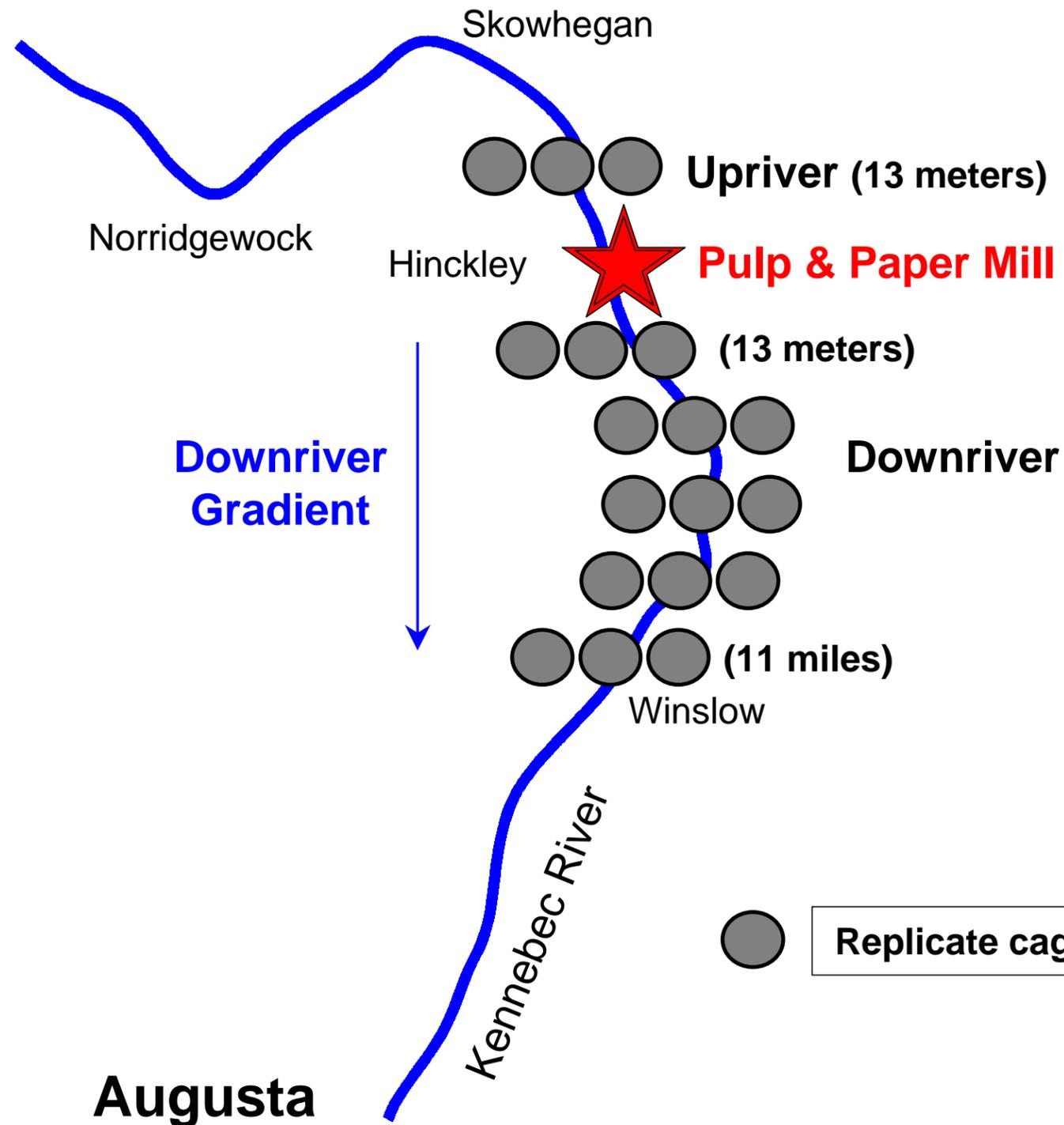
Objectives of caged mussel pilot study

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

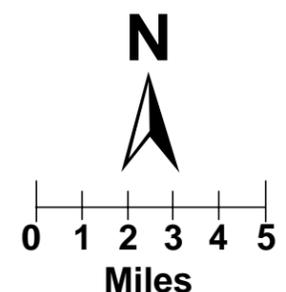
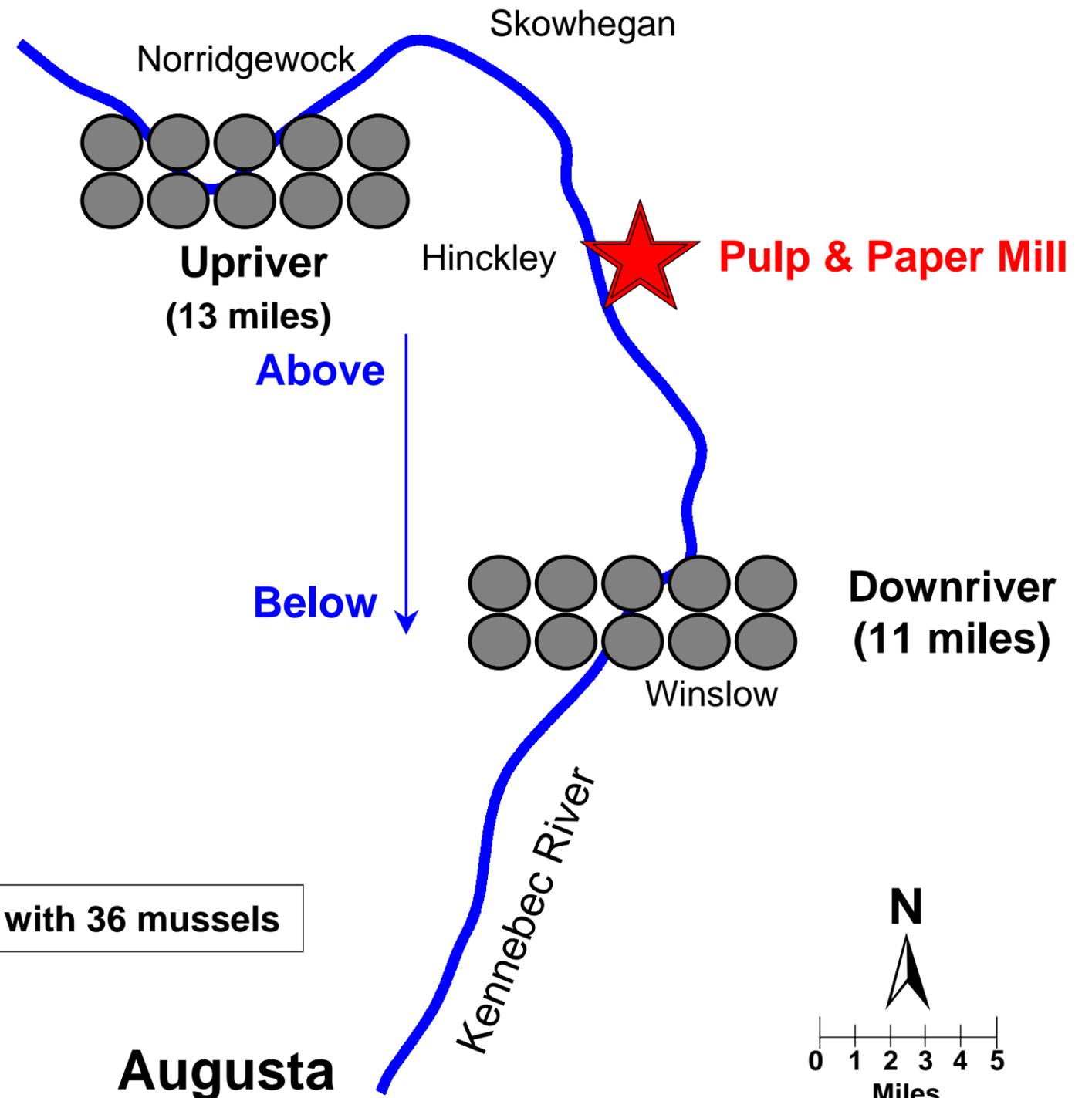
Site Map

- *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*

Proposed Gradient Design



Actual "Above/Below" Test



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





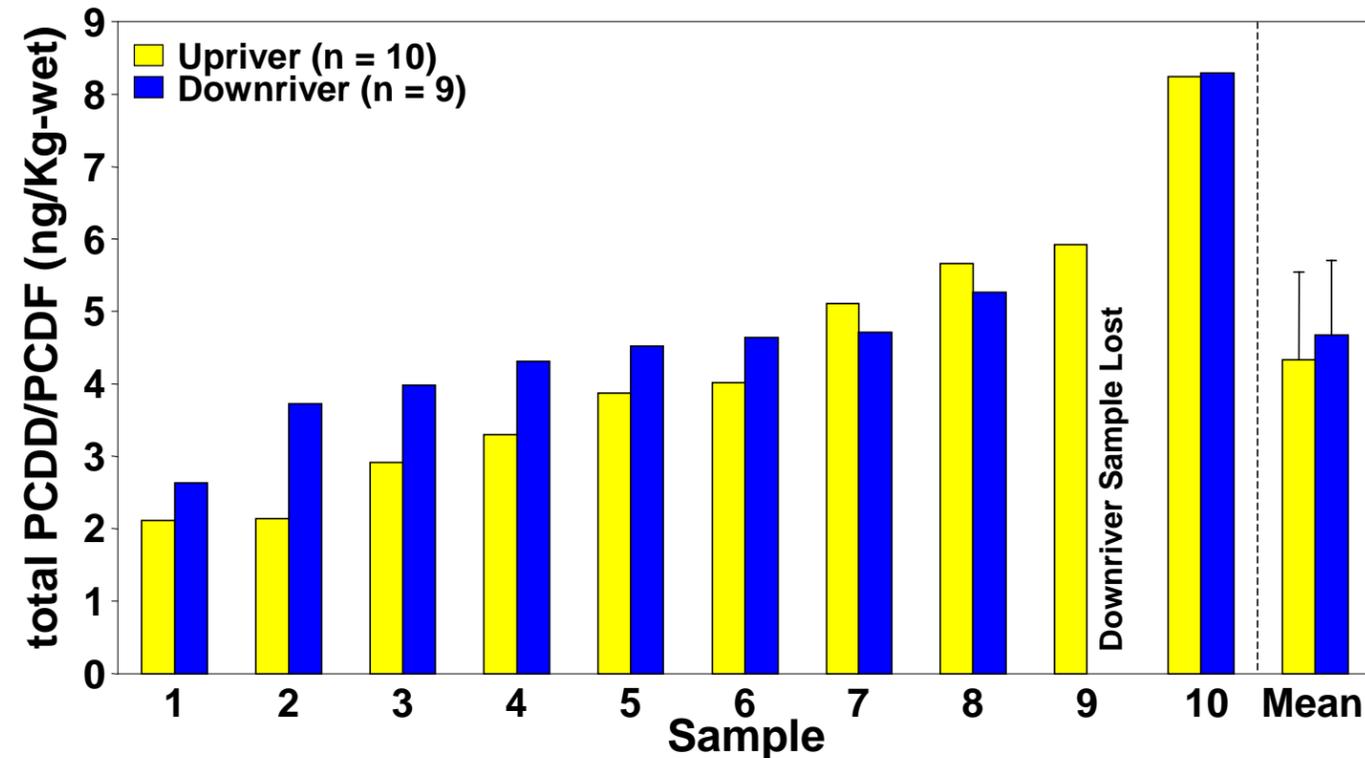
Measuring Distributing Deploying

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



Upriver & Downriver T-PCDD/PCDF

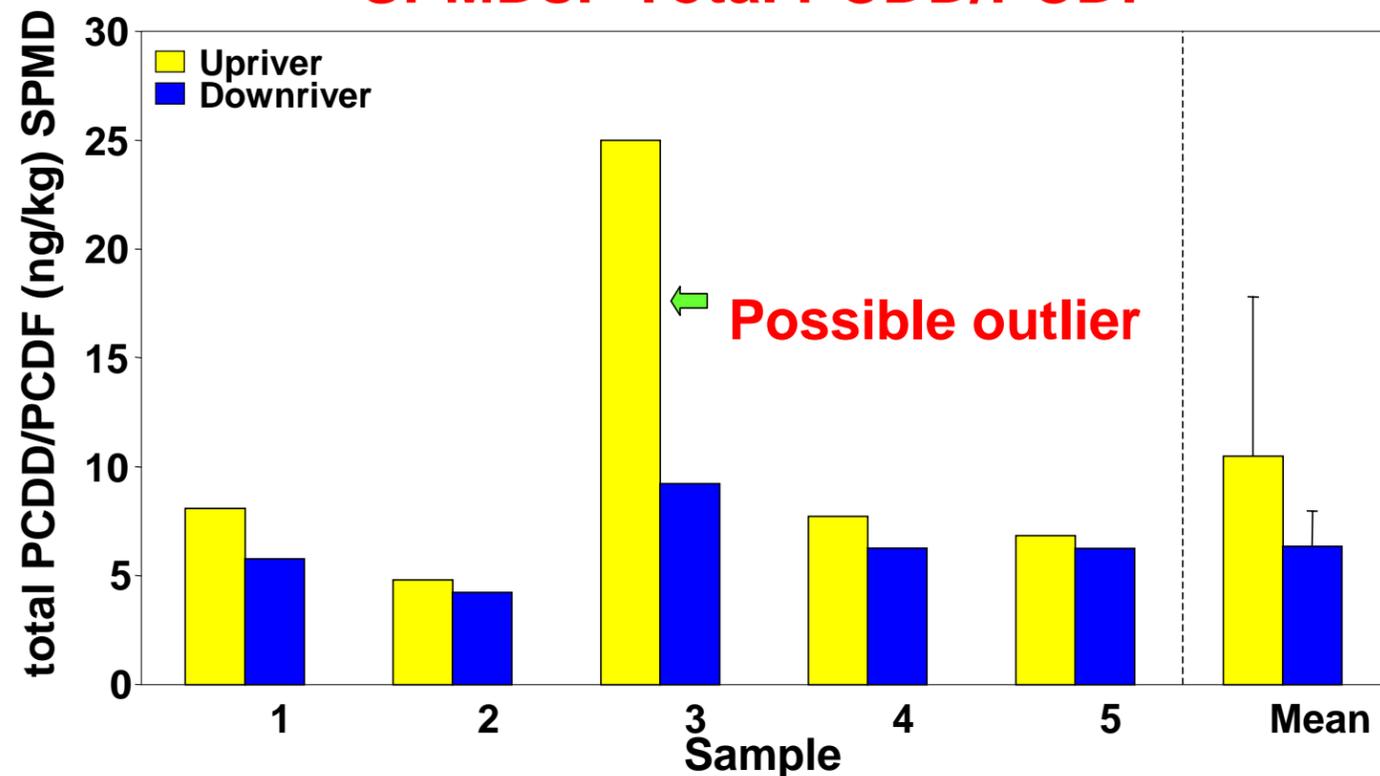
Mussels: Total PCDD/PCDF



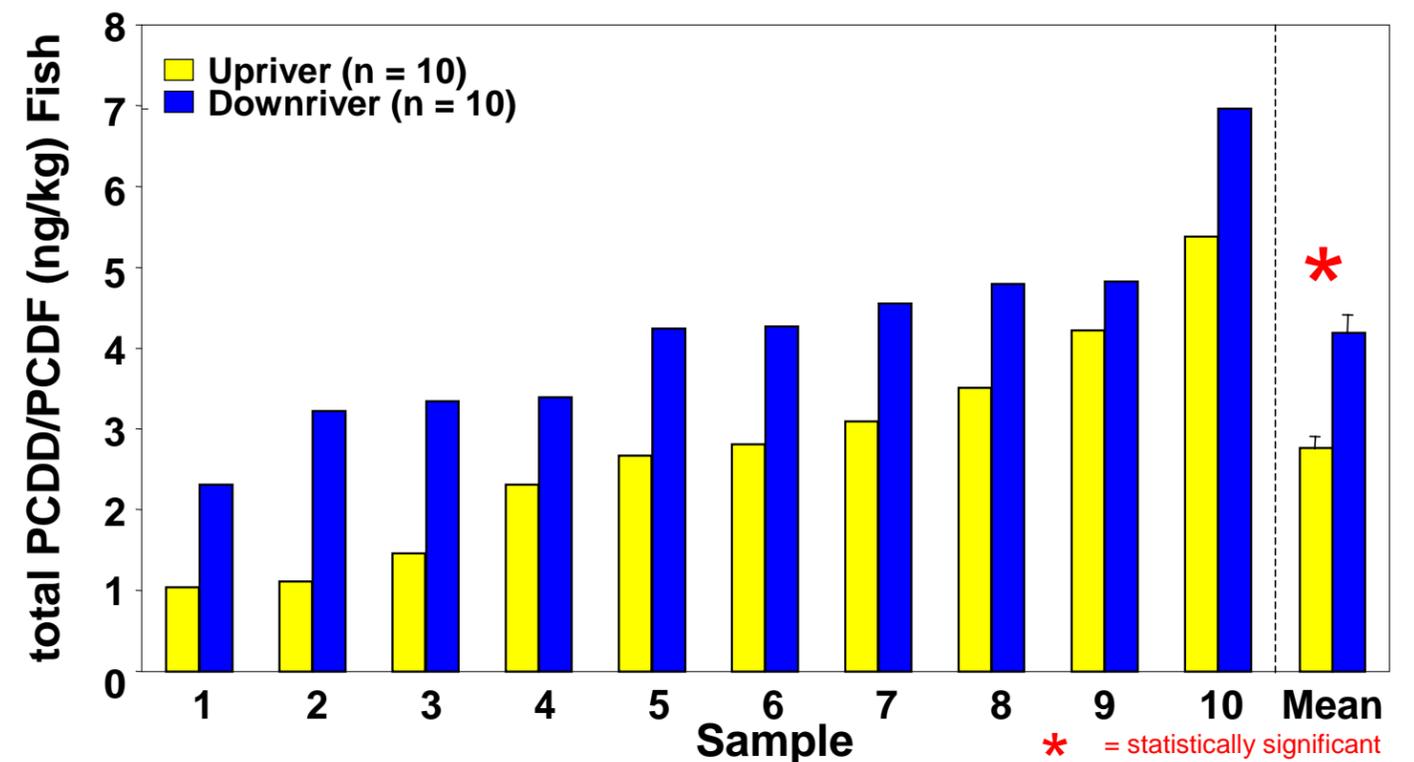
Summary

- 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.

SPMDs: Total PCDD/PCDF



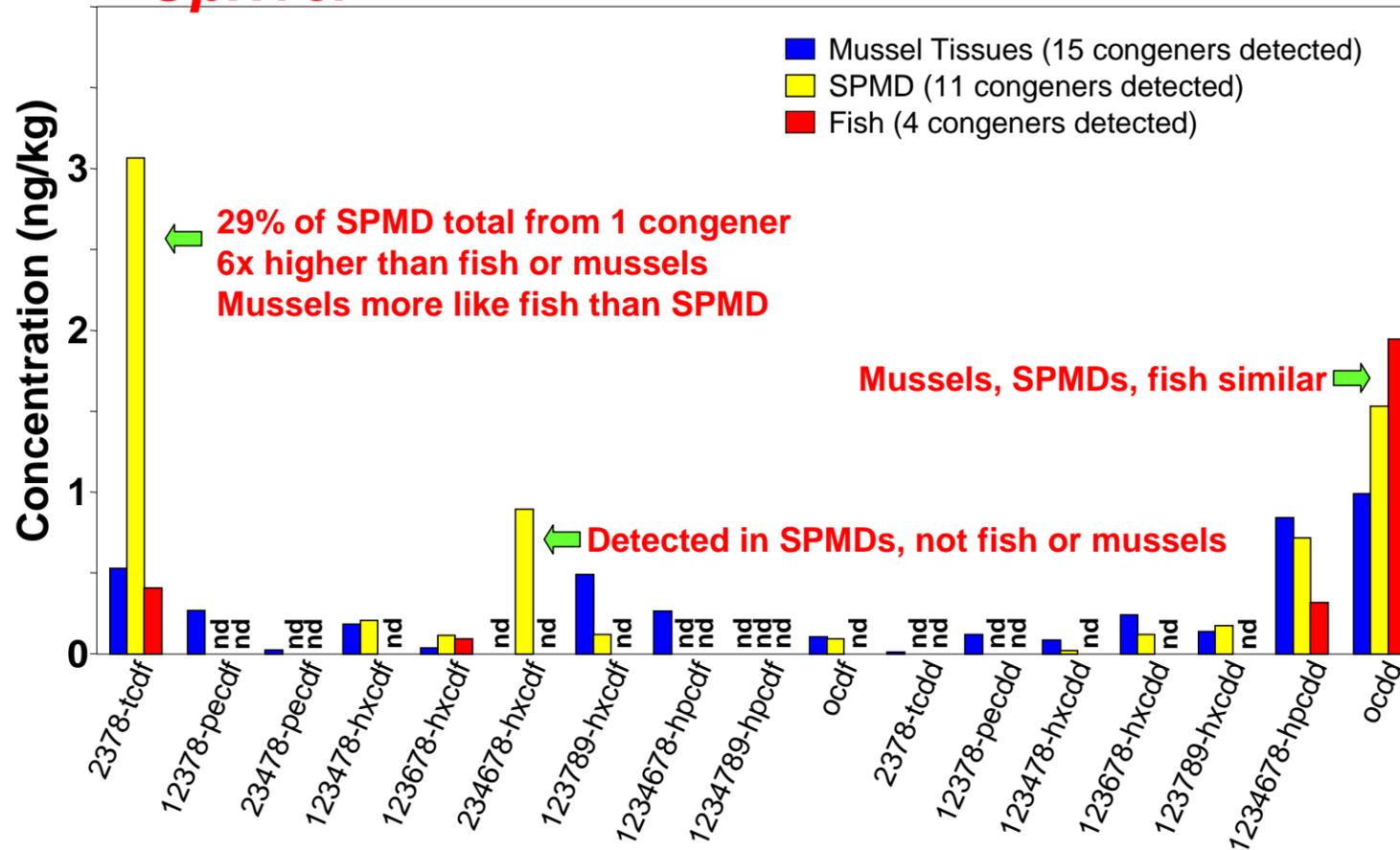
Fish: Total PCDD/PCDF



* = statistically significant

Congeners: Mussels, Fish, SPMDs

Upriver



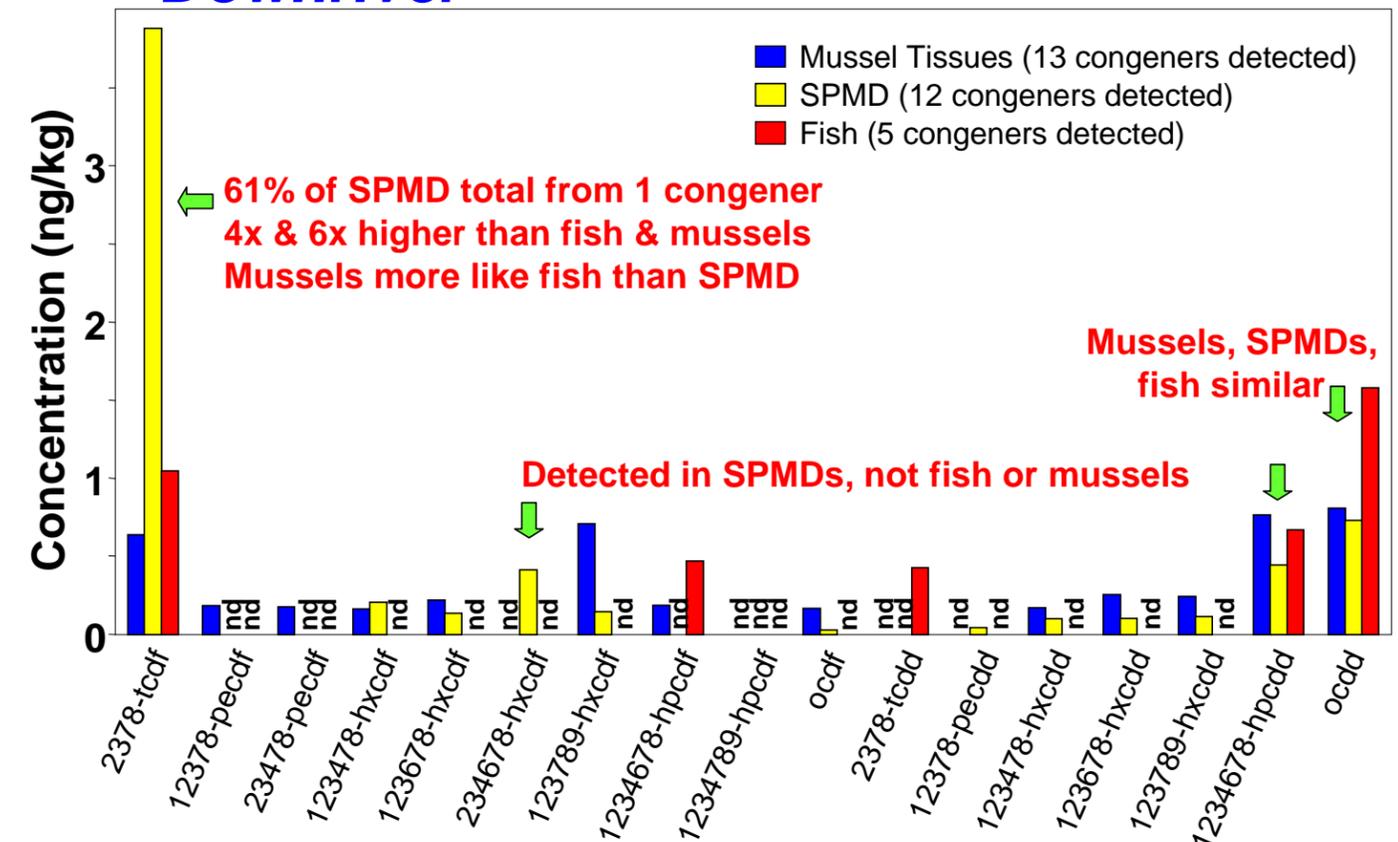
Upriver: 13 miles from mill

- 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

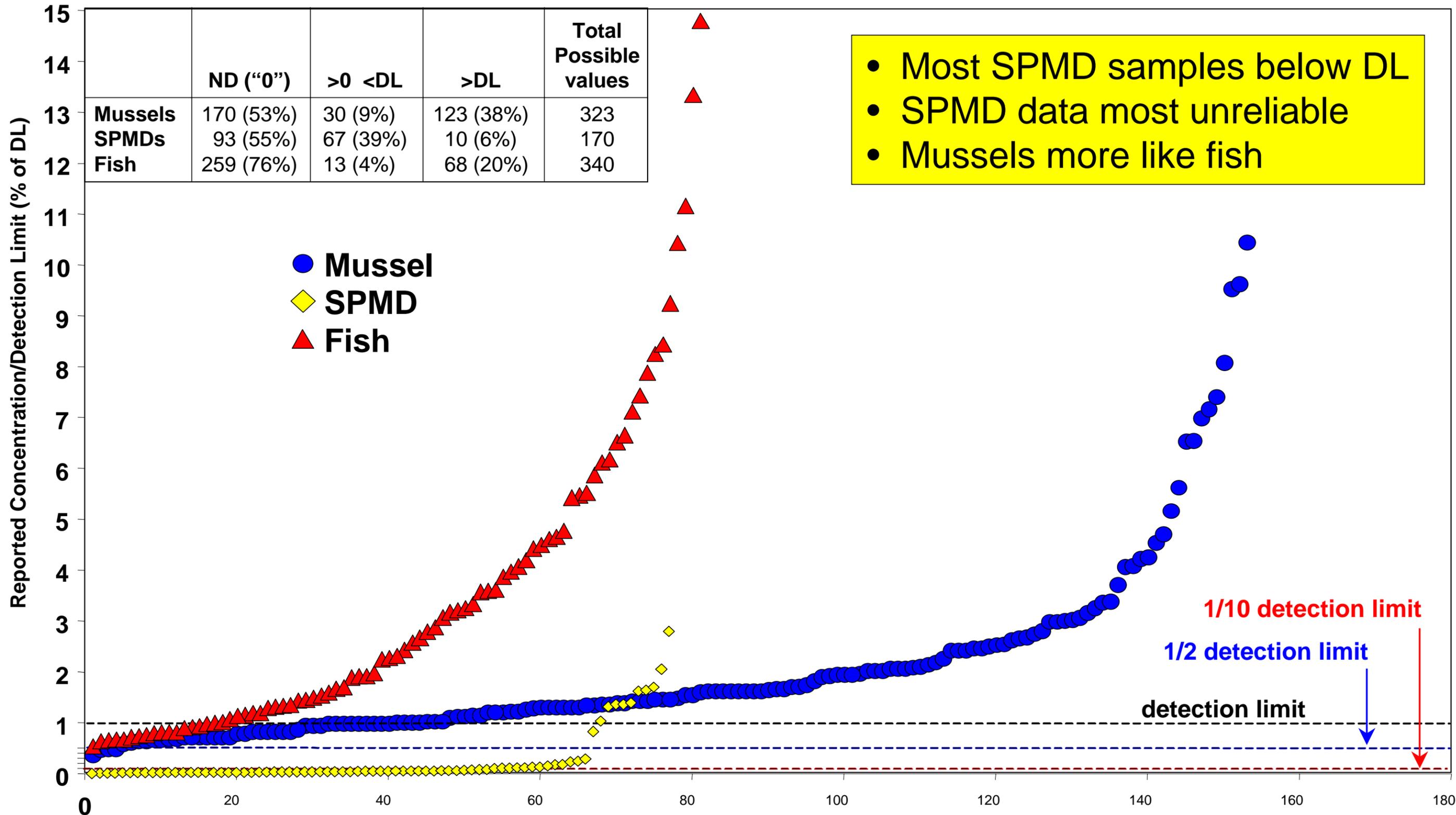
Downriver: 11 miles from mill

- 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



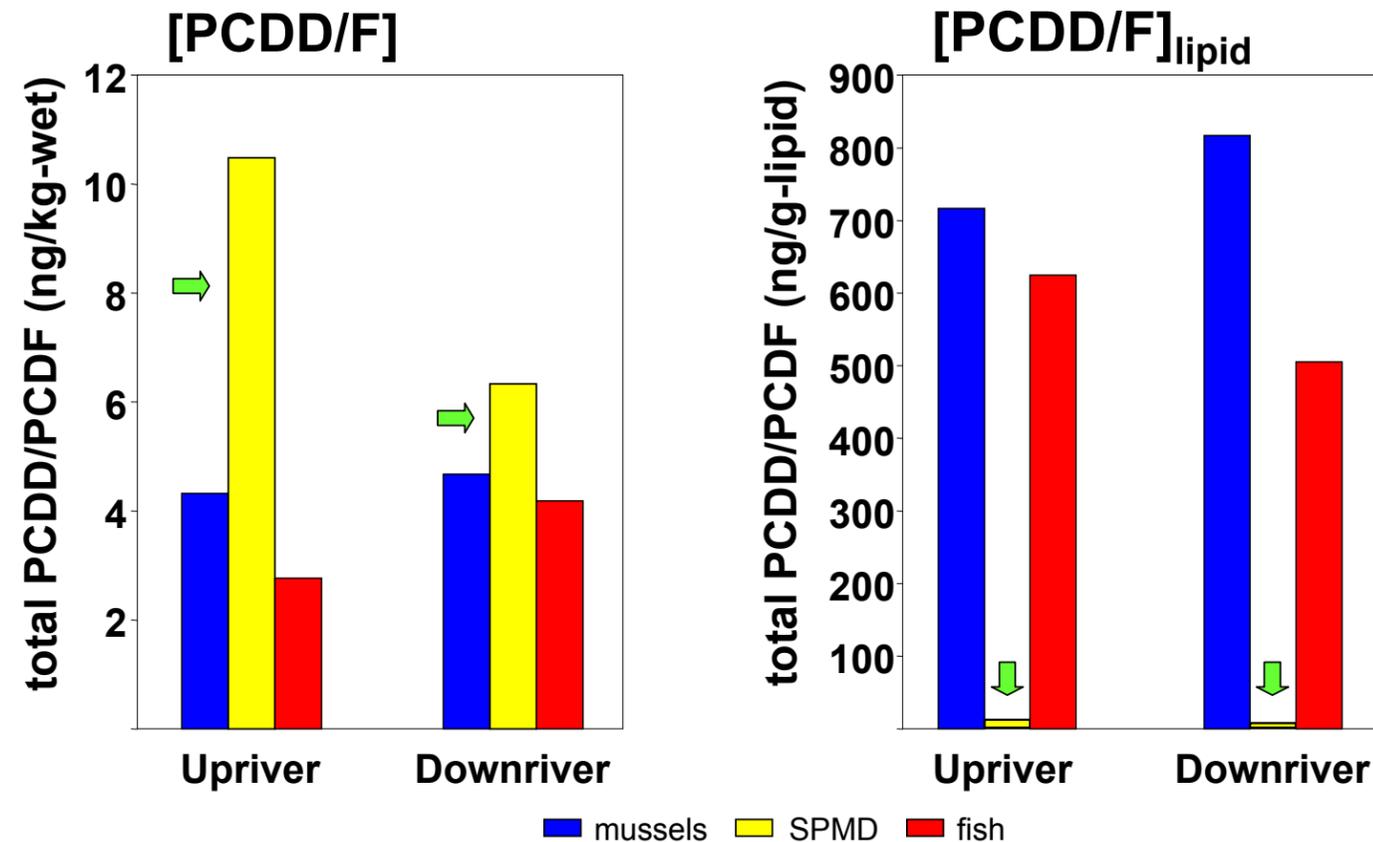
SPMD, Fish & Mussels: % Detection Limit



- 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.

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
- Cleaning up 100% lipids enhances measurement artifacts
- Results are difficult to interpret
- 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

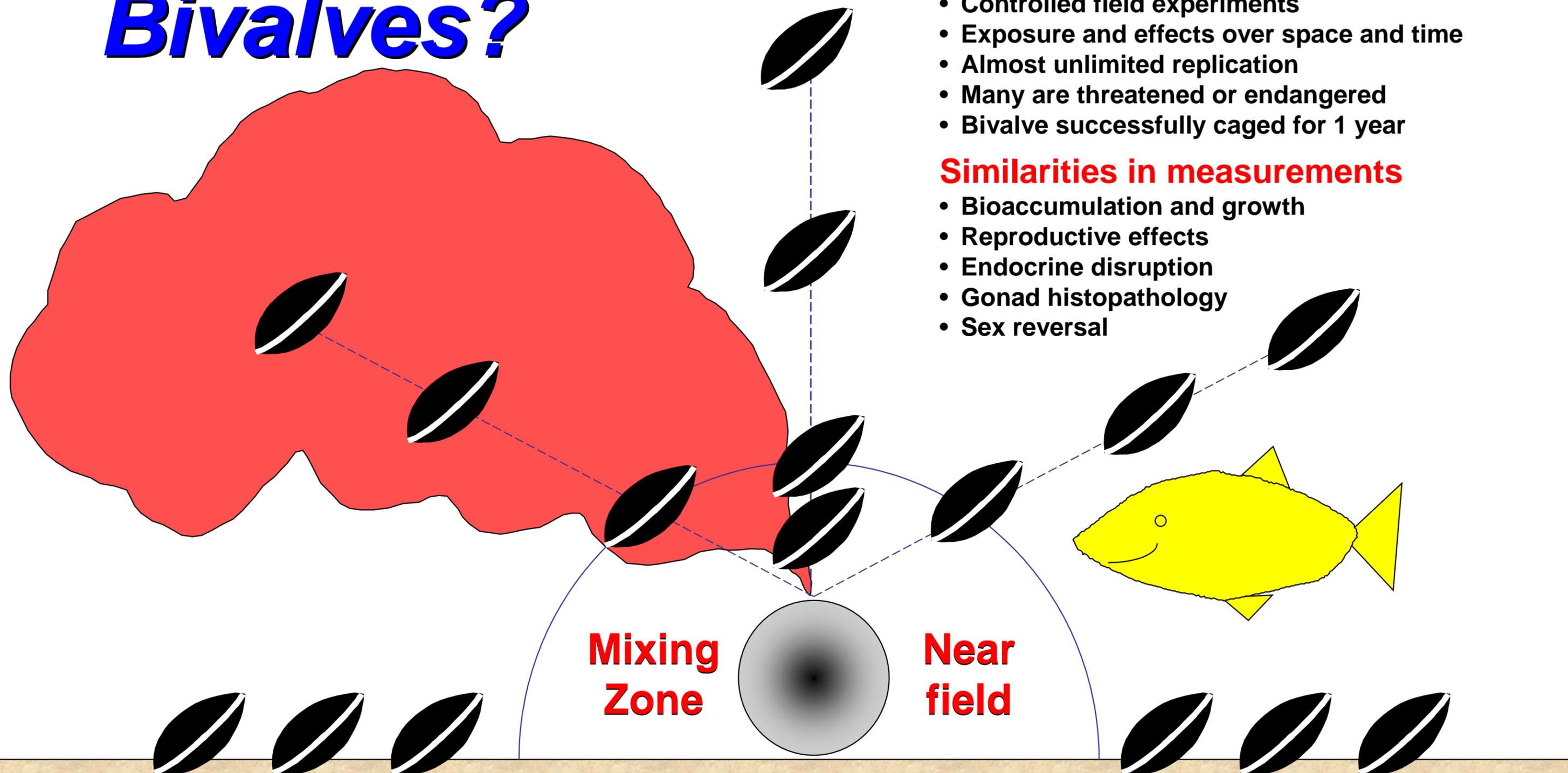
Why Caged Bivalves?

Advantages of bivalves over wild fish

- Bivalves do not move
- Exposure position and duration are known
- Controlled field experiments
- Exposure and effects over space and time
- Almost unlimited replication
- Many are threatened or endangered
- Bivalve successfully caged for 1 year

Similarities in measurements

- Bioaccumulation and growth
- Reproductive effects
- Endocrine disruption
- Gonad histopathology
- Sex reversal



ASTM Protocols for In-situ Bioassays

- *Monitoring mobile fish is not an experiment*
- *Monitoring the benthos is not an experiment*
- *Toxicity testing is an experiment but environmentally unrealistic*

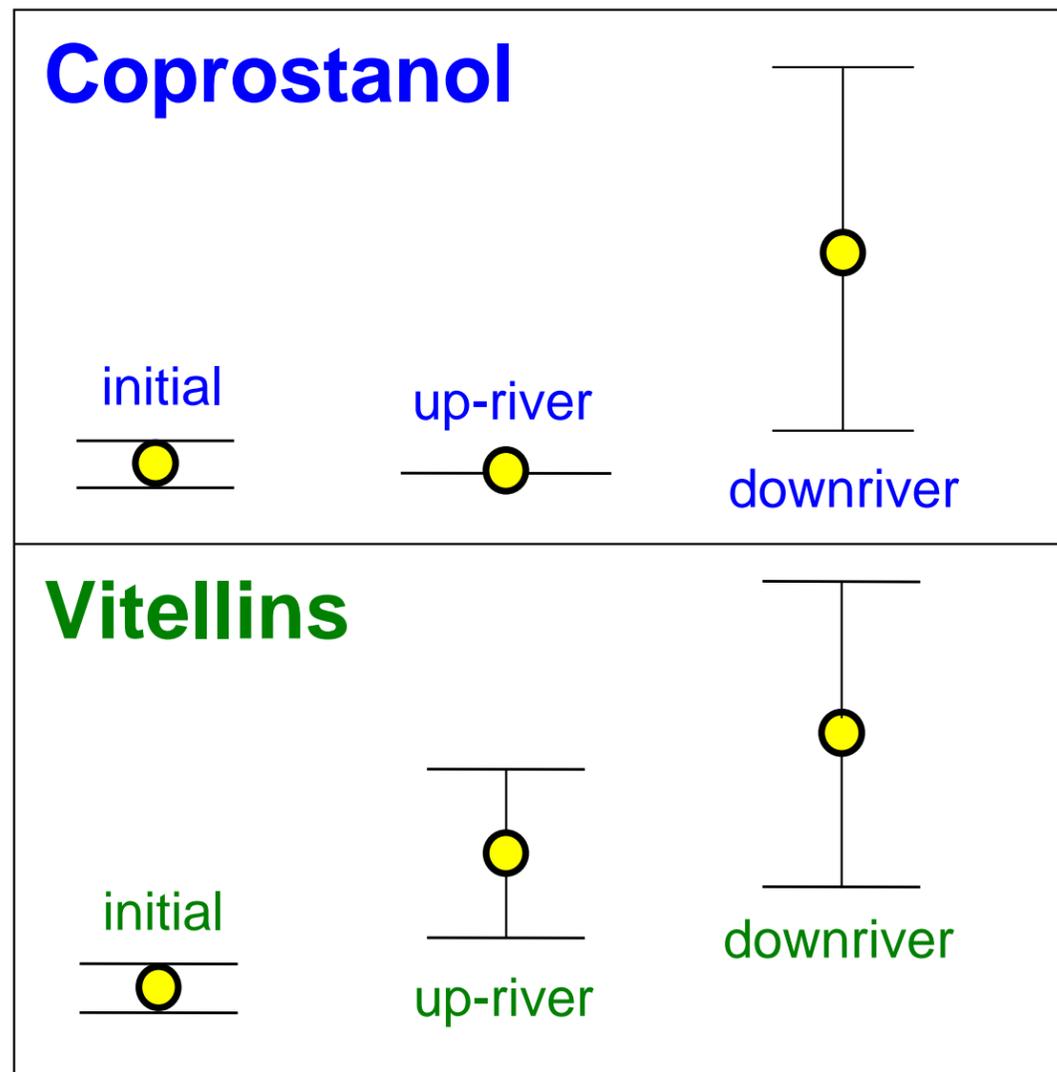
Other Advantages of using mussels:

Synoptic measurement of exposure & effects

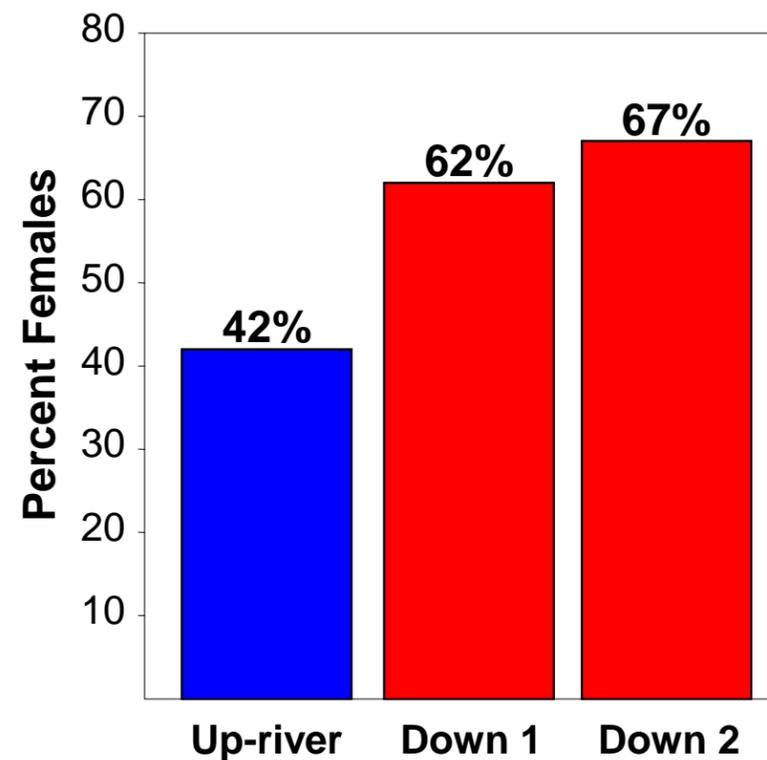
Other similarities with fish monitoring

Measurements of bioaccumulation, growth, reproduction

Biomarkers on the St. Lawrence River

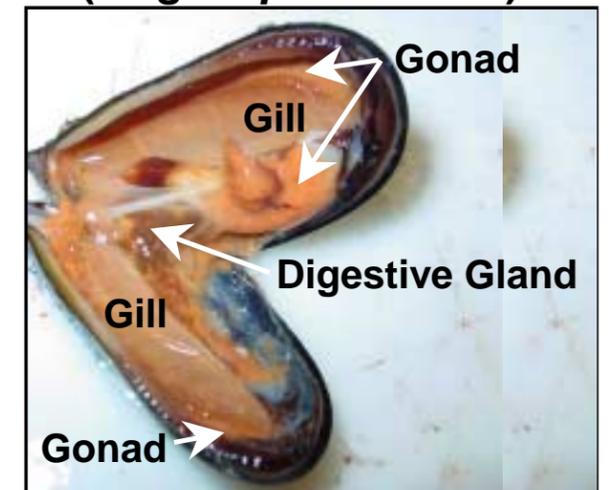


Sex Reversal on the St. Lawrence



- 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

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



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

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

Listen to the animals

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

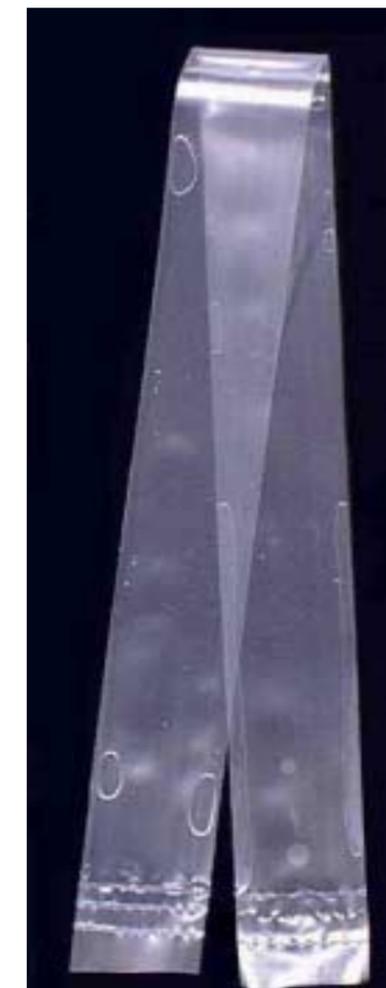
BIVALVES

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



Freshwater Mussel
Elliptio complanata

**Semi-Permeable
Membrane Device
(SPMD)**



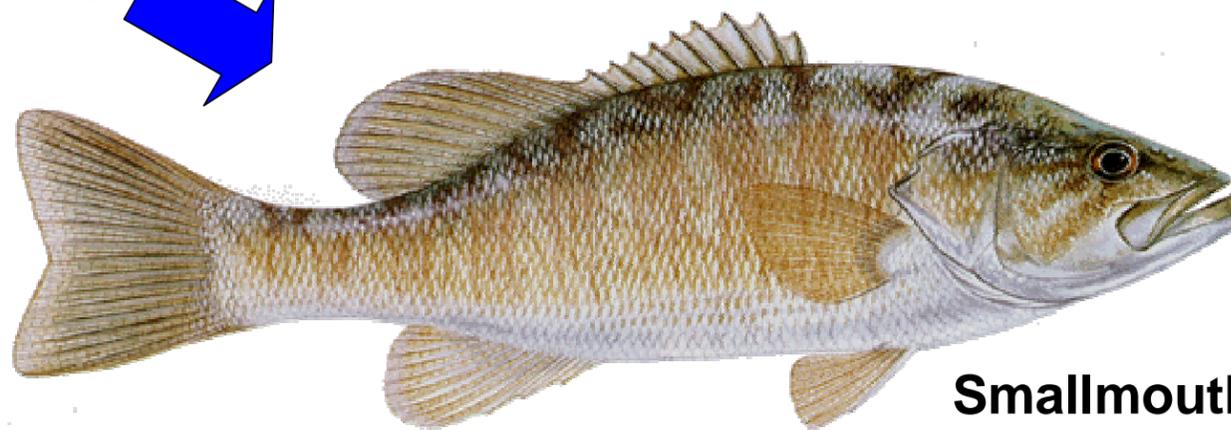
FAT BAGS

- Integrate only waterborne chemicals
- Easy to collect, cage & measure
- 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

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**

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
- 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

Cairns & Mount, 1990

Salazar & Salazar, 2000

Summary & Conclusions

Summary

- **Mussels detected more congeners than either fish for lipid bags**
- **Most of the lipid bag data were below the detection limit**
- **Mussel data was 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