FINAL REPORT
Kennebec River Caged Mussel Pilot Study

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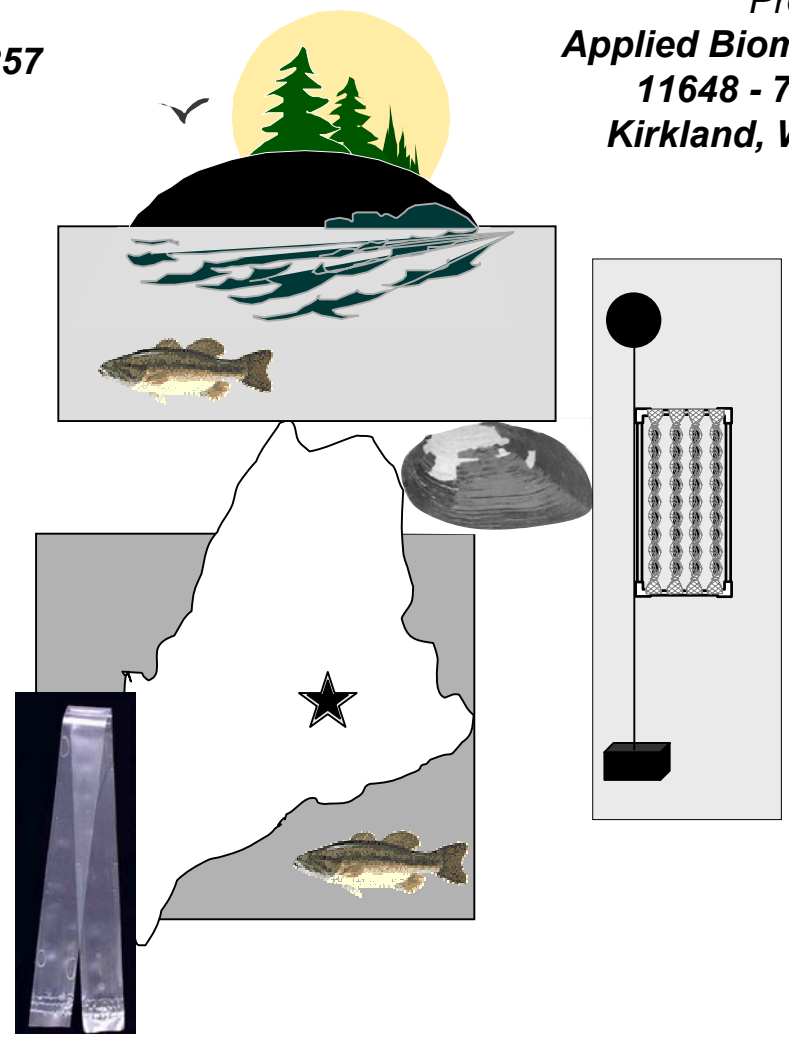

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1.0 EXECUTIVE SUMMARY

A pilot study was conducted during the summer of 2000 on the Kennebec River, Maine to evaluate the ability of caged freshwater mussels to monitor and assess the bioavailability of dioxins (polychlorinated dibenzo-dioxins, PCDDs), furans (polychlorinated dibenzo-furans, PCDFs), and polychlorinated biphenyls (PCBs). The caged mussel study had two purposes: (1) to determine whether this approach would be a reasonable surrogate for resident fish used in upstream versus downstream comparisons of chemical exposures associated with pulp and paper mills, and (2) to identify hotspots of polychlorinated biphenyls (PCB) contamination in the lower Kennebec River. Because of the limitations of fish sampling and dams on the river, the nearest upstream location (Norridgewock) where fish could be collected was approximately 13 miles from the mill, and the nearest downstream location (Fairfield) was approximately 11 miles from the mill. Caged mussels were deployed at these locations.

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 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. The Friends of Merrymeeting Bay (FOMB) have expressed concerns regarding hotspots of PCBs as well as dioxin/furan contamination on the Kennebec River associated with elevated exposures and possible adverse biological effects. FOMB and others have identified problems with monitoring indigenous fish populations for upstream/downstream comparisons at mill sites, including uncertainty associated with mobility, accumulation from other sources, previous mill discharges sequestered in sediments, and the inability to collect fish near the mill discharge. As with the PCB monitoring, FOMB supported 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 focused on upstream/downstream locations where mussels could be compared directly with fish data.

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. 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. A secondary objective is to document the status and trends in 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.

In 2000, DEP continued to develop an appropriate "above/below" fish test, but as dioxin and furan concentrations decline, there are 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. Although concentrations of dioxins and furans measured in fish tissues were higher below than above pulp mill discharges in 1999, questions have been raised 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 semi-permeable membrane devices (SPMDs) as potential surrogates for monitoring dioxins and furans in fish tissues.

Caged freshwater bivalves have been used to monitor dioxins and furans associated with pulp and paper mill effluents in Finland and for similar chemicals 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 Environmental Effects Monitoring (EEM) at 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. 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.

For both studies, freshwater mussels (*Elliptio complanata*) were collected from Nequasset Lake, a relatively clean lake within the Kennebec watershed in Woolwich, Maine, caging individuals of a minimum size range, and transplanting them to upstream and downstream (dioxin/furan) and gradient (PCB study) location on the Kennebec River. *Elliptio* were deployed for 53 days. After retrieval, the soft tissues of mussels were measured for PCBs or dioxins and furans, percent lipids, and percent moisture. Survival and growth of caged mussels indicated they were all in adequate health to accumulate ambient dioxins, furans, and PCBs if present. Mean concentrations of total PCBs in mussels increased from below detection at the beginning of the test to 2.7 to 188 ug/kg-dw at the lower Kennebec River stations at the end of the test. Most of the total PCB concentrations measured in mussel tissues were between 20 and 60 ug/kg-dw (~4 to 12 ug/kg-ww). The three highest values were above the fish tissue action level (FTAL) for screening evaluations of 11 ug/kg-ww for cancer endpoints. No measurements were above the FTALs of 43 ug/kg-ww for non-cancerous endpoints. The highest concentration of total PCBs (188 ug/kg-dw, ~37.6 ug/kg-ww) was measured in mussel tissues from midstream just below the Augusta Sewage Treatment plant at South Augusta and in the vicinity of a midstream outfall pipe. The second highest concentration of total PCBs (125 ug/kg-dw, ~25 ug/kg-ww) was measured in

mussels deployed on the west side of the Kennebec River, just below the former Williams gravel/asphalt facility (now Ferraiolo) in Farmingdale.

Mean concentrations of total PCDD/PCDF 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. These concentrations are both above the FTALs for screening evaluations of 1.5 ng/kg-ww for cancer endpoints and 1.9 ng/kg-ww for non-cancerous endpoints. The concentrations of dioxins and furans measured in mussel tissues are approximately four orders of magnitude lower than most of the PCB concentrations measured in mussel tissues. The units of the dioxin measurements (ng/kg-ww = parts per trillion) are three orders of magnitude lower than the PCB units (ug/kg-dw = parts per billion).

There was no statistically significant difference between upstream and downstream total PCDD/PCDF concentrations at the end of the test. More individual dioxin/furan congeners were measured in mussel tissues from both upstream and downstream locations than in SPMDs or fish tissues. Given that the downstream site was located 11 miles away from the mill, this result was encouraging. However, concentrations of the most predominant dioxins and furans in mussel tissues were not significantly higher downstream than upstream. In fact, the predominant dioxins (123478-HpCDD and OCDD) were higher upstream than downstream.

The concentrations of total dioxins and furans in fish tissues were 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 more efficient accumulators of dioxins and furans than mussels or SPMDs, and the existing fish monitoring approach is appropriate. However, on a lipid-normalized basis, concentrations of total dioxins/furans in fish collected at upstream and downstream stations are not significantly different. As with the data for mussels and SPMDs, the lipid-normalized concentrations for fish are higher upstream than downstream, but not significantly different. These data reinforce the significance of the important questions mentioned earlier regarding where the fish were exposed to dioxins and furans, whether they accumulated dioxins and furans from sediment or food that was contaminated from previous, rather than recent mill discharges, or how long ago exposure and accumulation occurred.

These questions, as well as concerns regarding upstream and downstream comparisons, may be addressed, at least in part, by using a weight of evidence approach and a more careful scrutiny of the total concentrations of dioxins and furans measured in each test matrix (mussels, SPMDs, fish), the lipid normalized concentrations, and the individual congener analysis. A more direct approach would be to repeat the caged mussel pilot study with more stations closer to the mill. Downstream mussels accumulated 13 congeners, SPMDs, 12, and fish only 5. Upstream mussels accumulated 15 congeners, SPMDs 11, and fish only 4. Although the fish appeared to be the most suitable monitoring tool based on 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. The data further suggest that the upstream and downstream locations are inappropriate since the upstream station appears to be contaminated by another source upstream of Norridgewock. The downstream station was

too far away to be sure that the fish are being exposed to dioxins and furans from the SAPPI mill in Hinckley. While the experimental design in the caged mussel pilot study was 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. 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.

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, furans and PCB congeners) and mussel growth rates.

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 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 and from hotspots of PCB contamination on the Kennebec River.

2.0 INTRODUCTION

A caged mussel study was conducted in the Kennebec River, Maine during the summer of 2000 to determine the applicability of this approach for monitoring PCBs, dioxins, and furans. This study was conducted under the auspices of and funded by the Maine Department of Environmental Protection (DEP), and was consistent with their environmental monitoring strategy for dioxins and furans. However, the study never would have been conducted without the development, encouragement, and assistance from the Friends of Merrymeeting Bay (FOMB), a regional environmental organization. DEP began a standardized biological monitoring program in 1983 (Davies et al. 1999) acknowledging that the best way to assess water and sediment quality is through integrated biomonitoring, as opposed to only chemical monitoring of water, sediment, and tissue. By placing emphasis on tissue chemistry and associated biological effects, it is possible to more directly determine the degree of ecological impact caused by chemical exposure. Traditional measures of water and sediment quality provide only an indirect way to assess effects because such approaches do not measure biological responses or account for the interaction of physical, chemical, or biological factors. FOMB have expressed concerns regarding hotspots of PCBs as well as dioxin/furan contamination on the Kennebec River associated with elevated exposures and possible adverse biological effects. FOMB have identified problems with monitoring indigenous fish populations. Problems with using natural fish populations for upstream/downstream comparisons for mill sites include uncertain exposures associated with the following: mobility, accumulation from other sources, previous mill discharges sequestered in sediments, and the inability to collect fish near the mill discharge. FOMB supported 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 focused on upstream/downstream locations where mussels and SPMDs could be compared directly with fish data.

This report summarizes the tissue chemistry and effects data collected in 2000 to assess the bioavailability of dioxins and furans associated with the South African Paper and Pulp Industries, Ltd. (SAPPI) pulp and paper mill near Hinckley and characterization of PCBs along a suspect reach of the Kennebec River.

2.1 Study Objectives

The objective of the dioxin study was to determine if caged bivalves are a viable alternative to resident fish in assessing bioavailable dioxins and furans. This would be accomplished by determining whether these bivalves accumulated significantly higher concentrations of dioxins at the downstream station when compared to the station upstream of the SAPPI pulp and paper mill. The downstream station was the closest site where fish could be collected because it was only just above here that a dam prevented the fish from access to upstream habitat. The objective of the PCB study was to help identify contaminated areas and their potential sources along one suspect reach of the lower Kennebec River.

3.0 METHODS & MATERIALS

American Society for Testing and Materials (ASTM) standardized protocols were followed for collection, transport, caging, and measurement of freshwater mussels. Complete details of transplant methodology used in this study are described in ASTM Standard Guide for Conducting In-situ Field Bioassays with Marine, Estuarine and Freshwater Bivalves (ASTM 2001).

Bioaccumulation in mussel tissues was used to estimate exposure to and bioavailability of dioxins, furans, and PCBs. This was accomplished by comparing end-of-test (EOT) concentrations in mussel tissues to concentrations in mussel tissues before deployment. Growth based on changes in whole-animal wet-weight (WAWW), shell length, tissue wet weight, and shell weight was measured to 1) to calibrate bioaccumulation (i.e., to determine if chemical dilution due to tissue increase or chemical magnification due to tissue loss has occurred), 2) to determine the health of the mussels, and 3) establish acceptability of test results. Measurements of mussel WAWW and shell length before and after deployment, and of mussel soft tissue weights at the end of the test, aid in interpreting contaminant accumulations and potential effects. Percent lipids and percent water will be used to corroborate effects, and tissue chemistry used to estimate exposure.

3.1 Study Design

The primary purpose of the dioxin/furan study was to determine whether measurable and biologically available concentrations of these chemicals are leaving the pulp and paper mill by comparing upstream and downstream locations. The primary purpose of the PCB study was to determine whether measurable and biologically available concentrations of PCBs are present in selected portions of the Kennebec River. For both studies, freshwater mussels (*Elliptio complanata*) were collected from Nequassett Lake, a relatively clean lake within the Kennebec watershed in Woolwich, Maine, caging individuals of a minimum size range, and transplanting them to upstream and downstream (dioxin/furan) and gradient (PCB study) location on the Kennebec River (Figure 1). *Elliptio* were deployed for 53 days. After retrieval, the soft tissues of mussels were measured for PCBs or dioxins and furans, percent lipids, and percent moisture. Table 1 summarizes the study designs.

The decision to use *E. complanata* as the test species and Nequassett as the transplant location was made with assistance from local agency personnel and experts; representatives of the Maine Department of Environmental Protection (DEP), the Maine Department of Inland Fisheries and Wildlife (DIFW), Friends of Merrymeeting Bay (FOMB), and the Bath Water District.

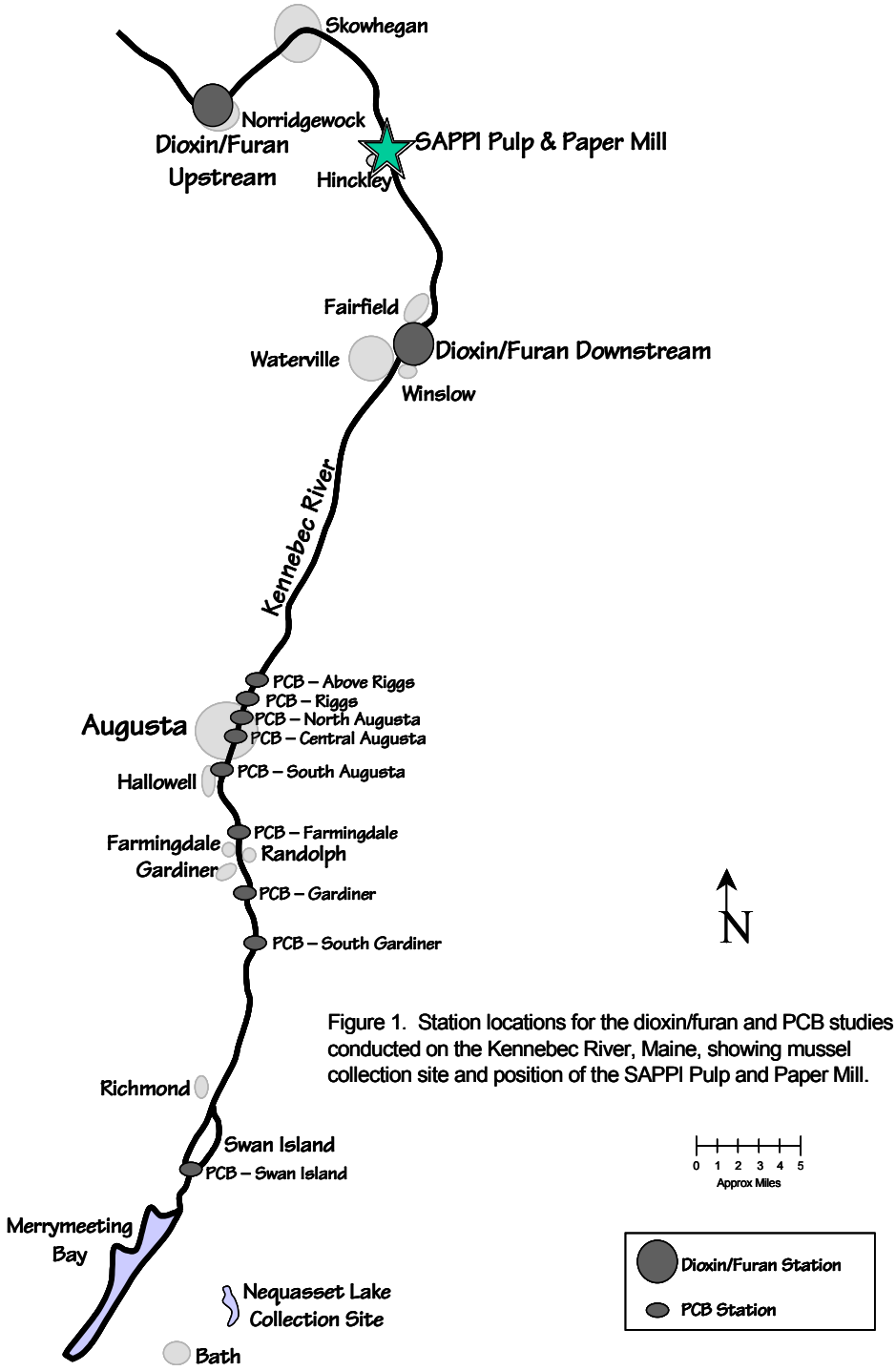

Figure 1. Station locations for the dioxin/furan and PCB studies conducted on the Kennebec River, Maine, showing mussel collection site and position of the SAPPI Pulp and Paper Mill.

Table 1. Summary of Dioxin/Furan and PCB Study Designs

Dioxin/Furan Study Design
• 2 Stations: Upstream, Norridgewock near Varney Road (approximately 13 miles upstream from the mill) Downstream, Fairfield (approximately 11 miles downstream from the mill)
• Caged mussels suspended mid water column
• 53-d exposure period
• Exposure endpoints: dioxins and furans
• Effects endpoints: growth (changes in WAWW, shell length & tissue weight), percent lipids, percent water

Number of stations	2
Number of cages (40" x ~ 18") per station	10
Number of mussels per cage	36
Number of mussels per mesh bag	9
Number of mesh bags/cage	4
Total number of mussels deployed	720
Number of mussels required for T_0 measurements & chemistry	180
Total number of mussels required	900

PCB Study Design
• 9 Stations: Above Riggs, Riggs, North Augusta, Central Augusta, South Augusta, Farmingdale, Gardiner, South Gardiner, Swan Island
• Caged mussels suspended mid water column
• 53-d exposure period
• Exposure endpoints: PCBs
• Effects endpoints: growth (changes in WAWW, shell length & tissue weight), percent lipids, percent water

Number of stations	9
Number of cages (24" x ~ 18") per station	3
Number of mussels per cage	20
Number of mussels per mesh bag	5
Number of mesh bags/cage	4
Total number of mussels deployed	540
Number of mussels required for T_0 measurements & chemistry	see above
Total number of mussels required	540

3.2 Test Duration and Schedule

The caged mussel study was conducted from August to September 2000. A 53-day deployment period was used. The in-situ mussel study was conducted according to the following schedule:

- August 2, 2000: *Elliptio* collected from Nequasset Lake, presorted into 1-mm size groups. Distributed dioxin/furan *Elliptio* to mesh bags. Mesh bags attached to PVC frames, unit wrapped with predator mesh. Dioxin/Furan cages placed in Nequasset Lake for overnight holding.
- August 3, 2000: *Elliptio* deployed at all dioxin/furan field stations during the morning. Distributed PCB *Elliptio* to mesh bags, mesh bags attached to PVC frames, unit wrapped with predator mesh. *Elliptio* deployed at all PCB field stations during the afternoon.

- September 26, 2000: Retrieved all *Elliptio* cages from upstream and downstream dioxin/furan stations. Mussels measured and shucked; tissues frozen for chemical analysis.
- September 27, 2000: Retrieved all *Elliptio* cages from all PCB stations. Mussels measured and shucked; tissues frozen for chemical analysis.

3.3 Mussel Processing Locations

The beginning-of-test(BOT) mussel sorting, measurements, and distribution took place approximately 3.5 miles East of Bath in Woolwich, at the Bath Water District treatment plant adjacent to Nequasset Lake. Since the lake is only about 50 meters from the treatment plant, it was a short distance to carry the bags of collected mussels to the measurement facility at the beginning of the test and return unused mussels at the end of the initial measurement sequence. BOT tissue removal and storage for future chemical analyses occurred at the DEP laboratory in Augusta, ME. The end-of-test (EOT) mussel measurements, tissue removal and storage for chemical analysis occurred at the DEP laboratory in Augusta, ME.

3.4 Mussel Collection

Mussels in the 40- to 60-mm shell length size range were collected from Nequasset Lake, an area believed to be relatively free of contamination and high in *Elliptio complanata* density. Ed Friedman and Steve Pelletier (FOMB) and Slade Moore (DIFW) used SCUBA to collect the mussels by hand. Divers followed several transects parallel to shore and collected every 10^{th} individual, while using gauges to limit size range. Each bucket of mussels collected by the divers was returned to the shore where the species of each individual and the number of individuals were confirmed by Beth Swartz (DIFW). The number of mussels removed from their natural habitat was limited by keeping a running tally of the number collected. During the collection process, approximately 50 mussels were randomly selected and assessed for reproductive status. None of the mussels contained glochidia suggesting all *Elliptio* were in a non-reproductive state when the test began. All collection and measurement efforts were overseen by Slade Moore and Beth Swartz.

3.5 Mussel Sorting and Distribution

Shell length (longest axis, generally from the anterior end near the beak to the leading posterior end, as determined with vernier calipers) was used to sort and select mussels to be used in the study. The final size range for *Elliptio*, 58 to 67.2 mm shell length, was based on obtaining the maximum number of mussels in the minimum size range.

Elliptio were presorted into 1-mm size groups prior to distribution to mesh bags. Mussels were held in tubs without water or ice prior to sorting. During sorting they were kept in buckets to minimize exposure to air and drying out. They were held without water until after the presort to eliminate the potential of oxygen depletion in the holding water. Once sorted into smaller groups, water was added to the buckets containing the mussels. All unused mussels were returned to Nequasset Lake by divers and placed in the approximate location of their collection. This helped ensure that the unused mussels could reposition themselves in the sediments without excessive stress.

Mussels were distributed in two phases, the dioxin/furan cages were prepared on the first day and the PCB cages on the second day, to facilitate deployment (i.e., dioxin/furan cages deployed on one day; PCB cages on the following day). So that both the dioxin/furan and PCB studies utilized mussels of similar sizes, each 1-mm size group was divided into two portions: 60% for the dioxin/furan study and 40% for the PCB study.

Prior to distributing mussels to the mesh bags (Figure 2), the mussel lengths were remeasured (to nearest 0.1 mm) and weighed (to nearest 0.01 g) for the first time using ASTM (2001) procedures. The whole-animal wet-weights and shell lengths were recorded by hand on data sheets and electronically by a computer connected to the electronic balance. Only live mussels that were fully closed, or those that closed immediately upon light physical stimulation were used.

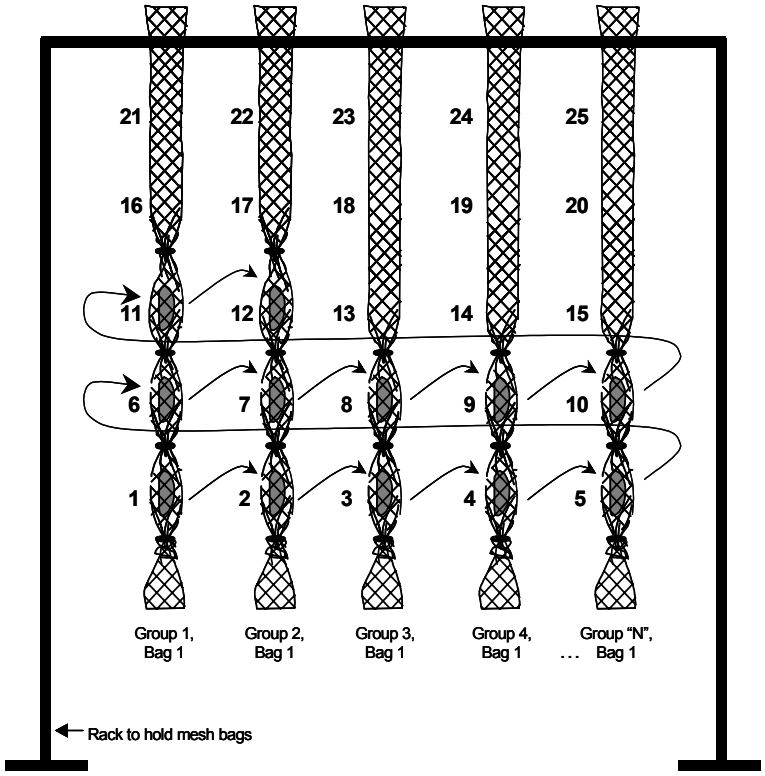

Figure 2. Mussel distribution process.

In addition to placing mussels into mesh bags for deployment, a subgroup of mussels from the same size class deployed in the field were retained in a separate compartmentalized tray. These mussels were used for BOT tissue weights, shell weights, and tissue chemistry. These mussels were treated in exactly the same way as those being deployed in the field, i.e., they were selected from the same size groups as the mussels deployed in the field and they were measured for length and whole-animal wet-weight at the same time and in the same order as the mussels to be deployed in the field. An Analysis of Variance (ANOVA) confirmed no statistical difference in size distribution among cages or stations (including mussels used for the BOT measurements). The mussels used in dioxin/furan study were tested separately from those used in the PCB study because distribution to mesh bags were done on separate days . No significant differences were found for either the dioxin/furan or PCB mussels when comparisons were made by cage or station:

	Dioxin/Furan	*PCBs*
WAWW by cage	p = 0.3979	0.7692
WAWW by station	p = 0.9865	0.7888
Length by cage	p = 1.0000	1.0000
Length by station	p = 0.9638	1.0000

3.6 Mesh Bags and PVC Cages

Tubular plastic mesh bags (approximately 4" in diameter and 6' long; 0.25" mesh size) made from material used in bivalve (e.g., mussels, oysters, clams) aquaculture were used to hold the mussels. A plastic tag showing Station Number and Bag Number was attached to each bag. Mussels were placed in the mesh bags sequentially. Nylon cable ties were used to separate individuals so they had a more even exposure to environmental conditions (Figure 2), keep track of position, and prevent mussels from shifting position in the bag. Four bags were prepared for each cage. Each bag prepared for the dioxin/furan study contained nine individuals because more mussels were required for chemical analysis. Each bag prepared for the PCB study contained five *Elliptio*.

Cages (approximately 18" x 40" for the dioxin/furan study and approximately 18" x 24" for the PCB study) were constructed from 3/4" Schedule 40 polyvinyl chloride (PVC) pipe. The loose ends of the mesh bags were tied to the PVC frame, the knot was secured with nylon cable ties approximately 6" in length. Once the mussel bags were attached to the PVC cage, the unit was wrapped with heavy duty plastic mesh (approximately 1" mesh size) to provide security, discourage predators, and protect the mussels during transport, deployment, and retrieval (Figure 3).

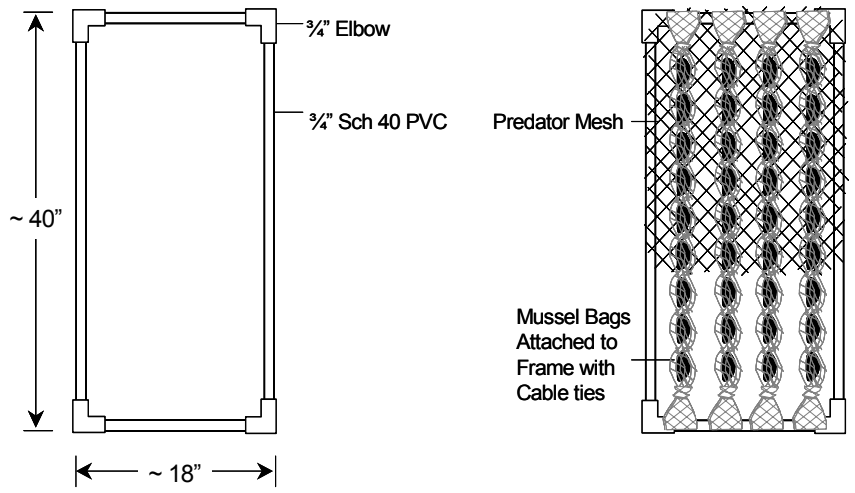

Figure 3. Cage design, attachment of mussel bags to frame, and predator mesh.

3.7 Baseline Tissue Weight, Shell Weight and Tissue Chemistry

By random assignment, five groups of mussels, each consisting of 36 individuals, were put into separate compartmentalized trays rather than mesh bags, and used to determine baseline tissue weights, shell weights, and tissue chemistry. In addition to making WAWW and shell length measurements on these individuals, their tissues were removed and weighed; the empty shells were also weighed. Because weighing tissues and shells is a destructive process and could not be made on individuals deployed in the field, the tissue and shell weight measurements made on these baseline individuals were used to estimate tissue and shell weights for mussels deployed in the field. Tissues from all 36 individuals in each group were composited for chemical analysis. Each composite baseline tissue sample was analyzed for dioxins, furans, PCBs, and percent lipids.

3.8 Overnight Holding

Caged mussels were held in Nequasset Lake for up to 16 hours at the beginning of the test (i.e., end of the first day after collection, after filling a series of bags, and until deployed). Surface water from this lake was used during the BOT and EOT measurement activities, as required. After retrieval from deployment stations on the Kennebec River, caged mussels were returned directly to the DEP lab in Augusta for final growth measurements, removal of mussel tissues for chemical analysis, and storage of those samples until shipment for analysis. There was no overnight holding at the end of the test.

3.9 Station Locations and Deployment

The Kennebec River originates at Moosehead Lake and flows southward to discharge into the Atlantic Ocean at Phippsburg and Georgetown, Maine. The dioxin/furan study focused

on discharges from the S.D. Warren/SAPPI pulp and paper mill, located in Hinckley, ME, approximately 7 miles south of Skowhegan. For the dioxin/furan study, mussels were deployed at two stations (Figure 1; Table 2). One station was upstream of the paper mill near Varney Road in Norridgewock, approximately 13 miles from the mill. The second station was approximately 11 miles downstream from the mill near Fairfield. Ten cages of 36 mussels each were deployed at each of these stations. Cages were deployed so they floated approximately 5 to 10 feet below the surface.

Table 2. Kennebec River 2000 – Station Locations
(* = cage with temperature probe)

Station	Latitude	Longitude	Station	Latitude	Longitude
Dioxin/Furan Study (Deployed 8/3/2000)					
Upstream (Temperature Probe #58)			Downstream (Temperature Probe #59)		
Cage 1	44°43.810'	69°46.423'	Cage 3	44°34.871'	69°35.823'
Cage 2	44°43.814'	69°46.421'	Cage 6	44°34.867'	69°35.831'
Cage 4	44°43.818'	69°46.422'	Cage 9	44°34.870'	69°35.835'
Cage 8	44°43.824'	69°46.409'	Cage 11	44°34.870'	69°35.835'
Cage 10	44°43.826'	69°46.401'	Cage 13	44°34.869'	69°35.849'
Cage 14	44°43.830'	69°46.391'	Cage 17	44°34.867'	69°35.851'
Cage 15	44°43.836'	69°46.387'	Cage 18	44°34.865'	69°35.847'
Cage 19	44°43.841'	69°46.380'	Cage 20	44°34.862'	69°35.846'
Cage 22	44°43.846'	69°46.379'	Cage 21	44°34.861'	69°35.860'
Cage 25	44°43.850'	69°46.368'	Cage 24	44°34.858'	69°35.861'
PCB Study (Deployed 8/4/2000)					
Station 1: Above Riggs (Temperature Probe #19)			Station 6: Farmingdale (Temperature Probe #54)		
Cage 8	44°20.623	69°45.510	Cage 3	44°15.652	69°46.380
Cage 11*	44°20.616	69°45.504	Cage 14*	45°15.617	69°46.287
Cage 15	44°20.609	69°45.479	Cage 23	46°15.593	69°46.185
Station 2: Riggs (Temperature Probe #50)			Station 7: Gardiner (Temperature Probe #55)		
Cage 2	44°20.248	69°45.804	Cage 10	44°12.211	69°45.691
Cage 26*	44°20.231	69°45.787	Cage 17*	44°12.193	69°45.760
Cage 29	44°20.226	69°45.774	Cage 25	44°12.188	69°45.803
Station 3: North Augusta (Temperature Probe #51)			Station 8: S. Gardiner (Temperature Probe #56)		
Cage 6	44°19.050	69°46.343	Cage 5	44°10.578	69°45.191
Cage 21*	44°19.035	69°46.325	Cage 22*	44°10.582	69°45.227
Cage 30	44°19.023	69°46.313	Cage 24	44°10.581	69°45.264
Station 4: Central Augusta (Temperature Probe #52)			Station 9: Swan Island (Temperature Probe #57)		
Cage 12	44°18.865	69°46.403	Cage 1	44°01.821	69°48.355
Cage 13*	44°18.862	69°46.374	Cage 7*	44°01.530	69°48.927
Cage 19	44°18.766	69°46.385	Cage 20	44°02.184	69°49.219
Station 5: South Augusta (Temperature Probe #53)					
Cage 9	44°17.924	69°46.698			
Cage 18*	44°17.902	69°46.661			
Cage 27	44°17.911	69°46.643			

The PCB study focused on an approximate 25-mile stretch of the lower Kennebec River from north of Augusta to Bowdoinham, with most stations in the Augusta area where PCB concentrations in fish tissue had been found as high as 800 ppb (Ed Friedman, personal communication). For the PCB study, mussels were deployed at 9 stations (Figure 1; Table 2). Three cages of 20 mussels each were deployed at each station at approximately the same water depth. One cage was situated in the center of the river, one placed closer to eastern shore, and the remaining cage placed closer to the western shore. Cages were deployed so that they floated 10 to 15 feet above the bottom.

Precise station locations were determined by DEP and FOMB. Station positions were identified and recorded on site using GPS (Table 2). Surface buoys were used to identify the deployment locations. Buoys were labeled with pertinent agency names and phone numbers.

Mussel cages were deployed from boats provided by DEP and Maine Department of Marine Resources. The attachment of weights, lines, and buoys occurred just prior to deployment. Two whole cinder blocks were used as anchors. FOMB, State agency, and Applied Biomonitoring staff deployed all caged mussels. The distribution of cages across stations (cages were randomly assigned to stations) is shown in Table 2.

3.10 End-of-Test Retrieval and Measurements

Retrieval and measurements were made on three consecutive days. Mussels from the dioxin/furan upstream stations were retrieved and measured on September 25, and mussels from the downstream stations were retrieved and measured on September 26. Mussels from all PCB stations were retrieved and measured on September 27.

During transportation from field stations and while holding at the DEP laboratory in Augusta, the caged mussels were placed on tarps to avoid exposure to chemicals on the ground and covered with additional tarps to minimize exposure to sun and wind. The mesh bags were removed from the PVC cages and placed in small buckets containing water from the holding site. Mussels were allowed to equilibrate (i.e., replace any air between shells with water) for a minimum of 10 minutes before making growth measurements.

End-of-test measurements were made using live mussels only according to procedures in ASTM (2001). The number of survivors per cage was recorded. Mussels with broken shells or those that did not close upon light physical stimulation were considered dead. Mussels were placed into compartmentalized trays to keep their order during measurements. The trays containing mussels to be measured were placed in water so that the mussels were completely submerged. Mussels were then measured for change in size: individuals were measured for WAWW, shell length, shell weight, and soft-tissue weight. For each cage, tissues from all surviving mussels were pooled by cage and analyzed for selected chemicals, percent lipids, and percent solids. DEP was responsible for delivery of tissues to the Senator George J. Mitchell Center Laboratory. Appropriate chain-of-custody forms were completed and accompanied the tissue samples.

3.11 Collection and Preparation of Mussel Tissues for Chemical Analysis

Tissues were removed according to ASTM (2001). All shucking knives used in tissue removal were stainless steel. Cutting boards and plastic trays were covered with aluminum foil prior to cleaning. The knives, foil-covered cutting boards, holding trays, and weigh boats were "chemically" cleaned at the start of the shucking process by (1) washing with a soap-free biological cleaning solution, (2) rinsing with hot tap water, (3) rinsing with distilled water, and (4) a final rinse with hexane. Decontamination was overseen by Barry Mower (DEP). Gloves were not worn during the shucking process to reduce the potential for injury as handling and shucking wet mussels causes the latex gloves to become slippery. Shuckers washed their hands with the same soap-free biological cleaning solution before shucking mussels. All knives and foil-covered surfaces were thoroughly cleaned before proceeding to another sample. If the foil was ripped, it was replaced prior to cleaning.

The mussels were not kept in water once the growth measurements were made. The order of mussels was maintained during the shucking and weighing process. To facilitate maintaining order, the mussels were placed into compartmentalized trays prior to shucking.

Once detached, the tissues were kept in their original shell, using the shell as a "holding dish" to prevent contact with other surfaces until tissues were weighed. Shucked mussels were placed in order on a foil-lined tray. All mussels from one cage were shucked before making tissue and weight measurements. Caution was used to minimize contact of tissue with surfaces other than the interior of the specimen's original shell.

Once all mussels in a given cage were shucked, the individual tissues were weighed and placed in a chemically-clean sample jar. Composite tissue samples were prepared by pooling tissues from all living mussels within a particular cage. The tissues were transferred from the weigh pan to a certified chemically-clean sample jar by gently sliding them off the foil. All sample jars were provided by the analytical laboratory. The sample jar was capped. Sample labels were affixed to the outside of the jar. Tamper-proof tape was applied over the cap and side of jar prior to placing the sample in the freezer.

Shells were weighed after the tissues were removed and weighed. Tissue and shell weights were recorded for each individual mussel to allow pairing with WAWW, shell length, and other growth metrics. The tissue and shell weights were recorded electronically to an Excel spreadsheet and by hand to a hard copy. The aluminum foil weigh boat and cutting board cover were then discarded. All shucking equipment was decontaminated before processing mussels from another cage.

Tissue samples were frozen at -20°C within one hour of collection, and were kept at this temperature (or below) until sample analysis.

3.12 Mussel Tissue Chemistry

Tissues were analyzed for dioxins, furans, PCBs, lipids, and percent water. All analyses were conducted at the Senator George J. Mitchell Center Laboratory. All dioxin/furan analyses were conducted according to EPA Method 1613B. All PCB analyses were conducted according to "*Standard Operating Procedure: Draft Method. Polychlorinated*

Biphenyls in Solid Matrices by Capillary Gas Chromatography - Electron Capture Detector And/or Mass Spectrometry (Revision 7, 6/29/2000). The detection limits (DLs) reported are actually practical quantitation limits (PQLs), or the concentrations of the lowest standards used to calibrate the instrument. The PQLs represent the bottom point of the calibration curve. Although values that are below the DL (or PQL) were intended primarily for information only because they are estimates based on the standard curve, these values were included in all calculations.

Mussel tissues for the dioxin/furan study were analyzed for percent lipids but were not analyzed for percent solids because the entire sample was used to achieve detection near the practical quantitation limit. Although there was sufficient tissue from the PCB samples for solids determination, these tissues were not analyzed for percent lipids because the microwave method for sample preparation does not accommodate the analytical measurement of lipids (T. Anderson, personal communication).

3.13 Water Temperature Measurements

Water temperature was recorded at 15-minute intervals during the entire test with *in situ* temperature monitors (Onset® Tidbit). One temperature monitoring device was deployed at each dioxin/furan and PCB station by attaching it directly to one of the cages deployed at the station.

3.14 Data Analysis

3.14.1 Bioaccumulation Data

Two types of comparisons were made on the mussel tissue chemistry data:

- Station comparisons
- Beginning-of-test versus end-of-test comparisons to determine if significant accumulation occurred

The following conventions were used for all tissue chemistry data:

- A zero ("0") was used for all concentrations reported as <DL.
- All data, including zeros, were used when calculating means and 95% confidence intervals by congener.

For the dioxin/furan study, a t-test was used to test for significant differences in accumulation between upstream and downstream. If the data did not meet the requirement of equal standard deviations, a t-test with the Welsh's correction was used. If the data failed to meet the normality requirement, the Mann-Whitney non-parametric test was used.

For the PCB study, a one-way Analysis of Variance (ANOVA) and a multiple range test were used to test for differences among stations. If the data failed to meet the assumptions of normality and common variances as determined by the Kolmogorov/Smirnov test and Bartlett's test, respectively, the nonparametric Kruskal Wallis test was conducted. All tests were conducted at the 95% confidence level ($\alpha = 0.05$).

3.14.2 Survival & Mussel Health Metrics

Percent survival was calculated as initial number deployed minus number dead divided by number deployed. Dead mussels were defined as those with empty shells. Lost cages were not included in calculating mean station survival. No statistical comparisons were conducted on survival by station because of survival at all stations was similar and very high.

Growth was measured to calibrate bioaccumulation (i.e., to determine if chemical dilution due to tissue increase or chemical magnification due to tissue loss has occurred) and to determine the health of the mussels after the exposure period. Four growth metrics were used: shell length, WAWW, wet tissue weight, and shell length. Percent lipids and percent solids were also used as an indication of mussel health.

Descriptive summary statistics (i.e., mean, minimum, maximum, and percent change) were calculated for all growth metrics. Using these data, the end-of-test growth metrics were compared to beginning of test to determine if there was measurable growth during the deployment periods. Particular attention was given to changes in tissue weight, as this metric is critical for evaluating and interpreting the tissue chemistry data. A cursory examination of these metrics showed very small changes in any of the growth metrics, most of which are probably within measurement error.

An ANOVA followed by a multiple range test were used to test the following general null hypothesis:

- There is no significant difference in mussel whole-animal wet-weights, shell length, tissue weight, or shell weight between stations

If the data failed to meet the assumptions of normality and common variances as determined by the Kolmogorov/Smirnov test and Bartlett's test, respectively, the nonparametric Kruskal Wallis test was conducted. All tests were conducted at the 95% confidence level ($\alpha = 0.05$).

3.14.3 Water Temperature

Maximum, minimum, mean, and the range in water temperatures were calculated for the entire exposure period for each station. Water temperature profiles based on all the data collected during the field deployment were made for each station and used to identify overall water temperature trends. To facilitate comparing water temperatures across stations, averages, minimum, maximum, and ranges in daily water temperature were calculated (i.e., from 1201 am until midnight). Statistical comparisons were made on the daily average water temperature data only. Comparisons were made between upstream and downstream dioxin stations and among the PCB stations.

3.15 Data Quality Review & Acceptability

Tissue chemistry results were reviewed for acceptability by identifying any potential outliers using Grubbs extreme studentized deviate test. One potential outlier was identified:

Sample Number DN-17 from the downstream station contained 1234678-heptachloro dibenzo-dioxin (HpCDD) at a concentration that was significantly higher than all other replicates from this location. Concentrations of all other congeners for this sample were similar to concentrations measured in the other replicate samples. It is unclear whether the reported concentration is an analytical error or a true representation of 1234678-HpCDD concentrations present in the immediate vicinity of mussels assigned to cage DN-17. The data were analyzed with this outlier because there was insufficient evidence to conclude that it was a an outlier and additional comparisons with and without were not necessary.

The ASTM standard guide (ASTM 2001) suggests that two criteria be used to determine bioaccumulation data acceptability: 1) There should be no significant loss in tissue weight during the exposure period; and 2) If survivors have not lost significant tissue mass, a survival criterion of >45% may be acceptable to interpret the bioaccumulation data. The lowest survival in any cage was 95%; lowest mean survival at any station was 97.5%. There were no significant losses in tissue weight, so all the *Elliptio* effects data were considered acceptable for data analysis.

4.0 RESULTS

4.1 Survival

Mean *Elliptio* survival at the dioxin/furan upstream and downstream stations was 99.7% (Table 3). Only one cage was lost at the dioxin/furan stations (Cage #3; downstream). Survival by cage was 100% for all cages except two; one individual died in each of Cages 22 (upstream) and 17 (downstream).

Mean *Elliptio* survival at the PCB stations ranged from 97.5 to 100% (Table 3). Two cages were lost at the PCB stations; one from Station 8 and one from Station 9. Survival by cage was 100% for all cages except six; one individual died in one cage from each of Stations 2, 3, 6, 7, 8, and 9.

Table 3. Percent Survival by Cage and Station

Dioxin/Furan Study

	Station	% Survival	Station	% Survival
	Upstream		***Downstream***	
	Cage 1	100	Cage 3	lost
	Cage 2	100	Cage 6	100
	Cage 4	100	Cage 9	100
	Cage 8	100	Cage 11	100
	Cage 10	100	Cage 13	100
	Cage 14	100	Cage 17	97.2
	Cage 15	100	Cage 18	100
	Cage 19	100	Cage 20	100
	Cage 22	97.2	Cage 21	100
	Cage 25	100	Cage 24	100
Station Mean:		***99.7***		***99.7***

PCB Study

Station	% Survival	Station	% Survival	Station	% Survival
Station 1: Above Riggs		***Station 4: Central Augusta***		***Station 7: Gardiner***	
Cage 8	100	Cage 12	100	Cage 10	95
Cage 11*	100	Cage 13*	100	Cage 17*	100
Cage 15	100	Cage 19	100	Cage 25	100
Station Mean:	***100***		***100***		***98.3***
Station 2: Riggs		***Station 5: South Augusta***		***Station 8: S. Gardiner***	
Cage 2	95	Cage 9	100	Cage 5	95
Cage 26*	100	Cage 18*	100	Cage 22*	100
Cage 29	100	Cage 27	100	Cage 24	lost
Station Mean:	***98.3***		***100***		***97.5***
Station 3: North Augusta		***Station 6: Farmingdale***		***Station 9: Swan Island***	
Cage 6	100	Cage 3	100	Cage 1	95
Cage 21*	95	Cage 14*	100	Cage 7*	100
Cage 30	100	Cage 23	95	Cage 20	lost
Station Mean:	***98.3***		***98.3***		***97.5***

The very high survival measured for each of the cages indicates that the caging process, suspension in the river, and exposure to high currents and changing water levels did not have an adverse effect on the mussels.

4.2 Bioaccumulation

4.2.1 Dioxin/Furans in Mussel Tissues

Mussels at all upstream and downstream stations accumulated dioxins and furans at concentrations that were significantly elevated above the mean concentration at the beginning of the test. All individual dioxin and furan congeners in the five BOT mussel tissue samples were below the detection limit (BOT concentration <DL). Mean and total polychlorinated dibenzo-dioxin/ polychlorinated dibenzo-furan (PCDD/PCDF) concentrations in mussel tissues were calculated by substituting "zero" for non-detects, by convention. Based on station means, mussel tissues from the upstream station contained 15 of the 17 congeners, and mussel tissues from the downstream station contained 13 of the 17 congeners (Tables 4 and 5, Figure 4). 2378-tetrachloro dibenzo-dioxin (TCDD), the most toxic dioxin congener, was only detected in one mussel tissue sample, from the upstream location. 2378-TCDD was not detected in any downstream tissue sample. For the dioxins, mussel tissues from both the upstream and downstream stations contained predominantly octachloro dibenzo-dioxin (OCDD; 0.900 and 0.807 ng/kg-ww, respectively) and 1234678-HpCDD (0.841 and 0.764 ng/kg-ww). The combined percentages of OCDD and 1234678-HpCDD of total dioxins at the upstream and downstream stations were 76% and 70%, respectively (42 and 34% of total PCDD/PCDF). These two congeners made up the vast majority of dioxins, but both were higher upstream than downstream.

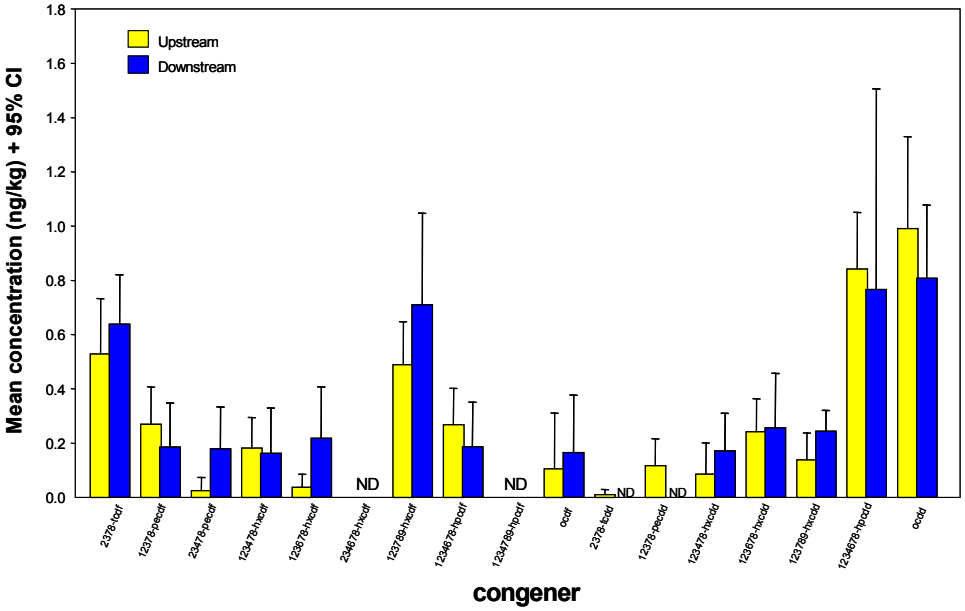

Figure 4. Mean concentration (ng/kg-wet + 95% CI) of individual dioxin/furan congeners measured in mussel tissues from upstream and downstream stations. ND = not detected. BOT concentration <DL.

Table 4. UPSTREAM: A. Dioxin/furan congeners (ng/kg-ww) in mussel tissue samples. B. Calculated Toxicity Equivalent Concentrations (TEQs). "0" substituted for non-detects, DL = detection limit; *bold, italicized*, shaded = concentration equal to or above DL; other reported concentrations estimated because below DL; TEF = toxicity equivalent factor[1]

A. Measured Concentrations (ng/kg-ww)

		UP-04	UP-08	UP-15	UP-10	UP-01	UP-25	UP-19	UP-02	UP-22	UP-14	Mean	95% CI
Compound	***DL***												
2378-TCDF	0.11	***0.33***	***0.19***	***0.28***	***0.36***	***0.47***	***0.62***	***1.06***	***0.52***	***0.31***	***1.15***	0.529	0.204
12378-PeCDF	0.25	0	0	***0.25***	***0.31***	0.21	0	***0.42***	***0.36***	***0.54***	***0.61***	0.270	0.138
23478-PeCDF	0.25	0	0	0	0	0	0	0	0	0	***0.25***	0.025	0.049
123478-HxCDF	0.25	0	0	0	0.21	0.2	0	0.18	***0.33***	***0.41***	***0.49***	0.182	0.114
123678-HxCDF	0.25	0	0	0	0	0.17	0	0.2	0	0	0	0.037	0.049
234678-HxCDF	0.25	0	0	0	0	0	0	0	0	0	0	0.000	
123789-HxCDF	0.25	***0.51***	***0.37***	***0.28***	0.17	***0.26***	***0.41***	***0.49***	***1.02***	***0.75***	***0.63***	0.489	0.159
1234678-HpCDF	0.5	0	0	***0.51***	0.25	0	***0.61***	0.19	0.33	0.42	0.36	0.267	0.136
1234789-HpCDF	0.5	0	0	0	0	0	0	0	0	0	0	0.000	
OCDF	0.5	0	0	0	0	0	0	0	0	1.05	0	0.105	0.206
2378-TCDD	0.1	0	0	0	0	0	0	0	0	0	0.1	0.010	0.020
12378-PeCDD	0.25	0	0	0.18	***0.25***	0	0	0	0	***0.35***	***0.39***	0.117	0.100
123478-HxCDD	0.25	0	0	0	0	0	0	***0.35***	0	0	***0.51***	0.086	0.115
123678-HxCDD	0.25	***0.26***	***0.41***	0.18	0	0	***0.36***	0.21	***0.51***	0	***0.48***	0.241	0.122
123789-HxCDD	0.25	0	0	0	0	0	***0.34***	***0.26***	0.15	0.22	***0.41***	0.138	0.099
1234678-HpCDD	0.5	0.35	***0.69***	***0.51***	***1.06***	***0.51***	***0.83***	***1.14***	***0.75***	***1.22***	***1.35***	0.841	0.210
OCDD	0.5	***0.66***	0.48	***0.72***	***0.69***	***2.05***	***0.84***	***0.61***	***1.69***	***0.65***	***1.51***	0.990	0.339
Total PCDD/PCDF		**2.11**	**2.14**	**2.91**	**3.3**	**3.87**	**4.01**	**5.11**	**5.66**	**5.92**	**8.24**	**4.33**	

B: Calculated TEQs

		UP-04	UP-08	UP-15	UP-10	UP-01	UP-25	UP-19	UP-02	UP-22	UP-14
Compound	***TEF***[1]										
2378-TCDF	0.050	0.0165	0.0095	0.014	0.018	0.0235	0.031	0.053	0.026	0.0155	0.0575
12378-PeCDF	0.050	0	0	0.0125	0.0155	0.0105	0	0.021	0.018	0.027	0.0305
23478-PeCDF	0.500	0	0	0	0	0	0	0	0	0	0.125
123478-HxCDF	0.100	0	0	0	0.021	0.02	0	0.018	0.033	0.041	0.049
123678-HxCDF	0.100	0	0	0	0	0.017	0	0.02	0	0	0
234678-HxCDF	0.100	0	0	0	0	0	0	0	0	0	0
123789-HxCDF	0.100	0.051	0.037	0.028	0.017	0.026	0.041	0.049	0.102	0.075	0.063
1234678-HpCDF	0.010	0	0	0.0051	0.0025	0	0.0061	0.0019	0.0033	0.0042	0.0036
1234789-HpCDF	0.010	0	0	0	0	0	0	0	0	0	0
OCDF	0.0001	0	0	0	0	0	0	0	0	0.000105	0
2378-TCDD	1.000	0	0	0	0	0	0	0	0	0	0.1
12378-PeCDD	1.000	0	0	0.18	0.25	0	0	0	0	0.35	0.39
123478-HxCDD	0.500	0	0	0	0	0	0	0.175	0	0	0.255
123678-HxCDD	0.010	0.0026	0.0041	0.0018	0	0	0.0036	0.0021	0.0051	0	0.0048
123789-HxCDD	0.010	0	0	0	0	0	0.0034	0.0026	0.0015	0.0022	0.0041
1234678-HpCDD	0.001	0.00035	0.00069	0.00051	0.00106	0.00051	0.00083	0.00114	0.00075	0.00122	0.00135
OCDD	0.0001	0.000066	0.000048	0.000072	0.000069	0.000205	0.000084	0.000061	0.000169	0.000065	0.000151
Total TEQ		**0.071**	**0.051**	**0.242**	**0.325**	**0.098**	**0.086**	**0.344**	**0.190**	**0.516**	**1.084**

[1]from Van den Berg et al. (1998)

Table 5. DOWNSTREAM: A. Dioxin/furan congeners (ng/kg-ww) in mussel tissue samples. B. Calculated Toxicity Equivalent Concentrations (TEQs). "0" substituted for non-detects, DL = detection limit; *bold, italicized, shaded* = concentration equal to or above DL; other reported concentrations estimated because below DL; outlined cell (□) = possible outlier; TEF = toxicity equivalent factor[1]

A. Measured Concentrations (ng/kg-ww)

		DN-13	DN-24	DN-20	DN-11	DN-09	DN-06	DN-21	DN-18	DN-17	Mean	95% CI
Compound	***DL***											
2378-TCDF	0.11	***0.77***	***0.72***	***0.57***	***0.79***	***0.41***	***0.18***	***0.35***	***1.05***	***0.89***	0.637	0.183
12378-PeCDF	0.25	0	***0.41***	0	0.24	0	***0.69***	0	***0.32***	0	0.184	0.163
23478-PeCDF	0.25	0	0	0	***0.37***	0	0	0.18	***0.61***	***0.44***	0.178	0.155
123478-HxCDF	0.25	0	0	0	0	0	0	***0.29***	***0.52***	***0.64***	0.161	0.168
123678-HxCDF	0.25	0	0	0	***0.52***	0	0	***0.25***	***0.41***	***0.77***	0.217	0.189
234678-HxCDF	0.25	0	0	0	0	0	0	0	0	0	0.000	
123789-HxCDF	0.25	***1.14***	***0.85***	***1.06***	***0.33***	0.21	***1.64***	***0.66***	***0.49***	0	0.709	0.338
1234678-HpCDF	0.5	0	0	0	0.25	***0.61***	***0.57***	0	0	0.24	0.186	0.165
1234789-HpCDF	0.5	0	0	0	0	0	0	0	0	0	0.000	
OCDF	0.5	0	0	***0.81***	0	0	0	***0.66***	0	0	0.163	0.213
2378-TCDD	0.1	0	0	0	0	0	0	0	0	0	0.000	
12378-PeCDD	0.25	0	0	0	0	0	0	0	0	0	0.000	
123478-HxCDD	0.25	0	***0.52***	0	***0.33***	***0.25***	0	0	0	***0.43***	0.170	0.140
123678-HxCDD	0.25	0	***0.62***	0	0	***0.55***	***0.67***	***0.46***	0	0	0.256	0.201
123789-HxCDD	0.25	***0.37***	0.18	***0.25***	***0.25***	***0.29***	***0.25***	***0.41***	0	0.18	0.242	0.078
1234678-HpCDD	0.5	0	0	***0.51***	***0.57***	***0.68***	0.31	0.48	***0.62***	***3.71***	0.764	0.740
OCDD	0.5	0.35	0.42	***0.78***	***0.66***	***1.52***	0.33	***0.97***	***1.24***	***0.99***	0.807	0.270
Total PCDD/PCDF		**2.63**	**3.72**	**3.98**	**4.31**	**4.52**	**4.64**	**4.71**	**5.26**	**8.29**	**4.67**	

B: Calculated TEQs

		DN-13	DN-24	DN-20	DN-11	DN-09	DN-06	DN-21	DN-18	DN-17
Compound	***TEF***[1]									
2378-TCDF	0.050	0.0385	0.036	0.0285	0.0395	0.0205	0.009	0.0175	0.0525	0.0445
12378-PeCDF	0.050	0	0.0205	0	0.012	0	0.0345	0	0.016	0
23478-PeCDF	0.500	0	0	0	0.185	0	0	0.09	0.305	0.22
123478-HxCDF	0.100	0	0	0	0	0	0	0.029	0.052	0.064
123678-HxCDF	0.100	0	0	0	0.052	0	0	0.025	0.041	0.077
234678-HxCDF	0.100	0	0	0	0	0	0	0	0	0
123789-HxCDF	0.100	0.114	0.085	0.106	0.033	0.021	0.164	0.066	0.049	0
1234678-HpCDF	0.010	0	0	0	0.0025	0.0061	0.0057	0	0	0.0024
1234789-HpCDF	0.010	0	0	0	0	0	0	0	0	0
OCDF	0.0001	0	0	0.000081	0	0	0	0.000066	0	0
2378-TCDD	1.000	0	0	0	0	0	0	0	0	0
12378-PeCDD	1.000	0	0	0	0	0	0	0	0	0
123478-HxCDD	0.500	0	0.26	0	0.165	0.125	0	0	0	0.215
123678-HxCDD	0.010	0	0.0062	0	0	0.0055	0.0067	0.0046	0	0
123789-HxCDD	0.010	0.0037	0.0018	0.0025	0.0025	0.0029	0.0025	0.0041	0	0.0018
1234678-HpCDD	0.001	0	0	0.00051	0.00057	0.00068	0.00031	0.00048	0.00062	0.00371
OCDD	0.0001	0.000035	0.000042	0.000078	0.000066	0.000152	0.000033	0.000097	0.000124	0.000099
Total TEQ		**0.156**	**0.410**	**0.138**	**0.492**	**0.182**	**0.223**	**0.237**	**0.516**	**0.629**

[1]from Van den Berg et al. (1998)

For the furans, mussel tissue from both the upstream and downstream stations contained predominantly 2378-tetrachloro dibenzo-furan (TCDF; 0.529 and 0.637 ng/kg-ww, respectively) and 123789-hexachloro dibenzo-furan (HxCDF; 0.489 and 0.709 ng/kg-ww). The combined percentages of 2378-TCDF and 123789-HxCDF of total furans at the upstream and downstream stations were 54% and 55%, respectively (24 and 29% of total PCDD/PCDF). Although concentrations of 123789-HxCDF were higher downstream than upstream, the differences were not statistically significant. These two congeners made up the vast majority of furans, with both higher downstream stream than the upstream location. Two congeners, 234678-HxCDF and 1234789-HpCDF, were not detected in any mussel tissue sample (Figure 4).

Using all data, a mean total PCDD/PCDF concentration of 4.67 ng/kg ww (Figure 5) was measured in mussel tissues from the nine cages deployed at the downstream site (one cage was lost). A mean concentration of 4.33 ng/kg ww was measured in mussel tissues from the 10 upstream cages. Although the mean concentration of total PCDD/PCDF at the downstream station was slightly higher than at the upstream station, the difference was not statistically significant ($p = 0.6732$). Total PCDD/PCDF concentrations in the upstream samples ranged from 2.11 to 8.24 ng/kg ww, a factor of 3.9. Total PCDD/PCDF concentrations in the downstream samples ranged from 2.63 to 8.29 ng/kg ww, a factor of 3.2. The total PCDD/PCDF concentrations measured in each tissue sample and the mean concentration (±95% CI) are shown in Figure 5 in increasing concentration, with the lowest upstream value paired with the lowest downstream value, etc., to allow comparisons on a cage basis. The total PCDD/PCDF concentrations measured in mussels deployed downstream were higher in seven of the nine samples. As seen from the relative height of the error bars for the mean concentrations, variability in the measurements precluded detecting a statistically significant difference between upstream and downstream. The mean total PCDD/PCDF concentration for the downstream station becomes 4.31 ng/kg ww upon elimination of the one suspect outlier from the data set. This concentration is essentially the same as that measured in upstream mussels, 4.33 ng/kg ww. If the cages were closer to the sources, it would have been easier to detect a difference, if the plant was discharging chemicals.

The upstream and downstream dioxin/furan data were also compared on a lipid-normalized basis. Lipid normalization did not appear to improve the ability to detect differences between the two stations, suggesting similar lipid content (see Section 4.3.1). Lipid-normalized total PCDD/PCDF concentrations at the upstream station ranged from 361 to 1088 ng total PCDD-PCDF/g-lipid wet, with a mean of 716 ng total PCDD-PCDF/g-lipid wet. At the downstream station, concentrations ranged from 431 to 1714 ng total PCDD-PCDF/g-lipid, with a mean of 816 ng total PCDD-PCDF/g-lipid wet (Figure 6). The total PCDD/PCDF concentrations measured in mussels deployed downstream were higher in six of the nine samples. Lipid-normalization appears to have had a large effect on the difference between upstream and downstream for Sample Pair No. 10. This large a difference between upstream and downstream was not found in the non-lipid normalized data. As with the non lipid normalized data, variability precluded detecting a statistically significant difference among upstream and downstream samples.

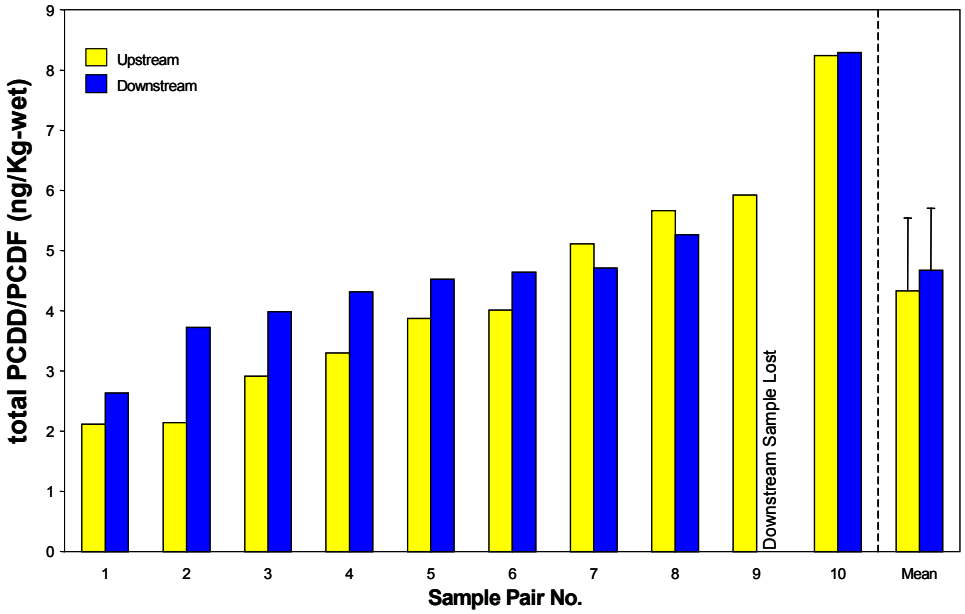

Figure 5. Total PCDD/PCDF concentration (ng/kg-wet) measured in each mussel tissue sample from upstream and downstream dioxin stations. Station mean (+95% CI) is also provided.

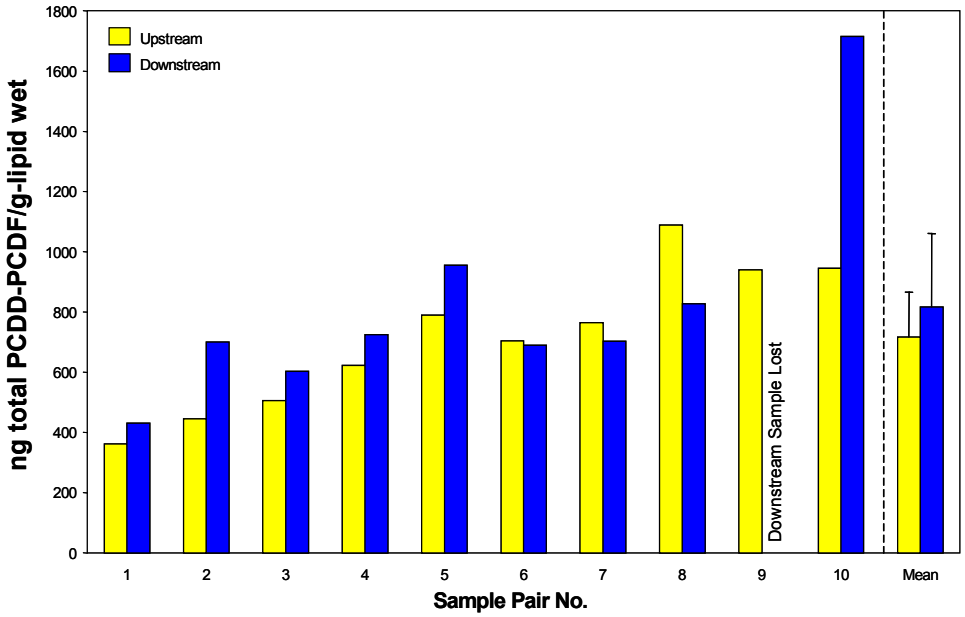

Figure 6. Lipid-normalized total PCDD/PCDF concentration (ng total PCDD-PCDF/g-lipid wet) measured in each mussel tissue sample from upstream and downstream dioxin stations. Station mean (+95% CI) is also provided.

Toxicity equivalent concentrations (TEQs) were calculated for mussel tissue burdens of PCDDs and PCDFs (Tables 4 and 5) by using toxicity equivalence factors (TEFs) provided by the World Health Organization (WHO; Vanden Berg et al. 1998). The TEQs were calculated for each sample using the detected concentrations; as with the calculation of total PCDD/PCDF, a "0" was used for concentrations reported as <DL. TEQs at the upstream station ranged from 0.051 to 1.084 (mean = 0.301). TEQs at the downstream station ranged from 0.138 to 0.629 (mean = 0.331). There was no statistically significant difference in TEQs between the upstream and downstream stations (p = 0.8004).

4.2.2 Dioxin/Furans in SPMDs and Fish

Semipermeable membrane devices (SPMDs) were deployed with the mussels at the upstream and downstream locations. The concentrations of the dioxin and furan compounds measured in each device are summarized in Figure 7, Table 6. The detection limits for the SPMDs were at least one order of magnitude higher than for the mussel tissues making nearly all of the SPMD data estimates. Two values (Sample 70-S, OCDF and 1234678-HpCDD) were identified as outliers using the Grubbs extreme studentized deviate test. However, as with the mussel tissue chemistry data, all values were used because of the uncertainty in the reason for the outlier.

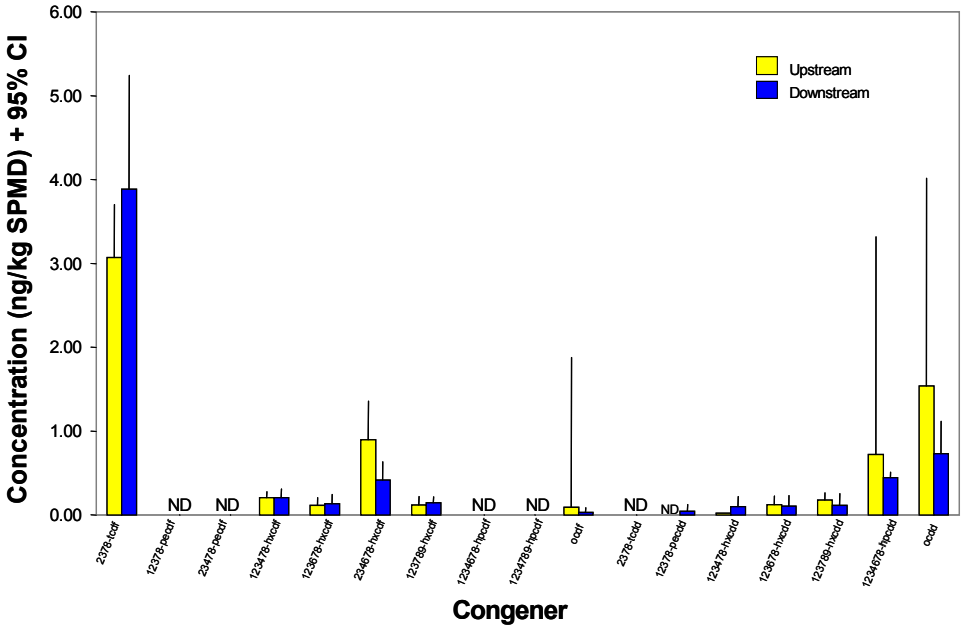

Figure 7. Mean concentration (ng/kg +95% CI) of individual congeners measured in SPMDs from upstream and downstream dioxin stations. ND = not detected.

Table 6. Dioxin/furan congeners (ng/kg) in SPMDs.
"0" substituted for non-detects, DL = detection limit;
***bold, italicized, shaded* = concentration equal to or above DL;**
other reported concentrations estimated because below DL;
outlined cell (□) = possible outlier.

Upstream	DL	68-S	69-S	70-S	71-S	72-S	Mean	95% CI
Compound								
2378-TCDF	2.31	***3.14***	1.92	***3.22***	***3.14***	***3.92***	3.07	0.63
12378-PeCDF	8.46	0.00	0.00	0.00	0.00	0.00	0.00	-
23478-PeCDF	10.77	0.00	0.00	0.00	0.00	0.00	0.00	-
123478-HxCDF	8.83	0.18	0.10	0.21	0.20	0.33	0.20	0.07
123678-HxCDF	6.74	0.16	0.00	0.21	0.19	0.00	0.11	0.09
234678-HxCDF	10.04	0.57	0.49	1.74	0.59	1.07	0.89	0.46
123789-HxCDF	3.29	0.18	0.00	0.17	0.00	0.24	0.12	0.10
1234678-HpCDF	4.91	0.00	0.00	0.00	0.00	0.00	0.00	-
1234789-HpCDF	8.30	0.00	0.00	0.00	0.00	0.00	0.00	-
OCDF	36.07	0.37	0.00	4.63	0.00	0.00	0.09	1.78
2378-TCDD	4.71	0.00	0.00	0.00	0.00	0.00	0.00	-
12378-PeCDD	8.21	0.00	0.00	0.00	0.00	0.00	0.00	-
123478-HxCDD	8.88	0.00	0.10	0.00	0.00	0.00	0.02	-
123678-HxCDD	6.05	0.22	0.13	0.00	0.25	0.00	0.12	0.10
123789-HxCDD	13.19	0.18	0.25	0.00	0.22	0.23	0.17	0.09
1234678-HpCDD	3.57	0.64	0.38	***7.32***	0.82	1.04	0.72	2.60
OCDD	30.16	2.43	1.42	7.48	2.28	0.00	1.53	2.48
Total PCDD/PCDF		**8.07**	**4.79**	**24.98**	**7.71**	**6.83**	**10.47**	

Downstream	DL	76-S	77-S	78-S	79-S	80-S	Mean	95% CI
2378-TCDF	2.31	***2.38***	***3.04***	***6.45***	***3.74***	***3.80***	3.88	1.36
12378-PeCDF	8.46	0.00	0.00	0.00	0.00	0.00	0.00	-
23478-PeCDF	10.77	0.00	0.00	0.00	0.00	0.00	0.00	-
123478-HxCDF	8.83	0.23	0.22	0.29	0.00	0.28	0.20	0.10
123678-HxCDF	6.74	0.18	0.00	0.25	0.24	0.00	0.13	0.11
234678-HxCDF	10.04	0.46	0.41	0.00	0.67	0.53	0.41	0.22
123789-HxCDF	3.29	0.18	0.18	0.17	0.00	0.19	0.14	0.07
1234678-HpCDF	4.91	0.00	0.00	0.00	0.00	0.00	0.00	-
1234789-HpCDF	8.30	0.00	0.00	0.00	0.00	0.00	0.00	-
OCDF	36.07	0.14	0.00	0.00	0.00	0.00	0.03	0.06
2378-TCDD	4.71	0.00	0.00	0.00	0.00	0.00	0.00	-
12378-PeCDD	8.21	0.21	0.00	0.00	0.00	0.00	0.04	0.08
123478-HxCDD	8.88	0.00	0.00	0.20	0.00	0.28	0.10	0.12
123678-HxCDD	6.05	0.24	0.00	0.28	0.00	0.00	0.10	0.13
123789-HxCDD	13.19	0.24	0.00	0.33	0.00	0.00	0.11	0.14
1234678-HpCDD	3.57	0.41	0.37	0.47	0.56	0.41	0.44	0.06
OCDD	30.16	1.10	0.00	0.77	1.05	0.74	0.73	0.38
Total PCDF/PCDD		**5.76**	**4.21**	**9.20**	**6.26**	**6.23**	**6.33**	

SPMDs at both the upstream and downstream locations accumulated primarily 2378-TCDF at concentrations ranging from 1.92 to 3.92 ng/kg (mean = 3.07 ng/kg) at the upstream station, and 2.38 to 6.45 ng/kg (mean = 3.88 ng/kg) at the downstream station (Figure 7, Table 6). The SPMDs from both the upstream and downstream stations accumulated primarily 2378-TCDF (3.07 and 3.88 ng/kg, respectively). Other congeners accumulated were OCDD (1.53 and 0.73 ng/kg), 1234678-HpCDD (0.72 and 0.44 ng/kg), and 123789-HxCDF (0.12 and 0.14 ng/kg). 2378-TCDF made up 29 and 61% of the total PCDD/PCDF for the upstream and downstream stations, respectively. For OCDD, the upstream and downstream percentages were 15% and 12%; 7% and 7% for 1234678-HpCDD; and 1% and 2% for 1234678-HpCDD.

Total PCDD/PCDF concentrations (Figure 8) were higher at the upstream station (mean = 10.47 ng/kg) than at the downstream station (mean = 6.33 ng/kg). There was no statistically significant difference in the concentration of total PCDD/PCDF accumulated by the SPMDS between the upstream and downstream stations ($p = 0.332$). The outliers are probably responsible for the elevated mean at the upstream location. Although the measured concentrations for all congeners except 2378-TCDF were well below the detection limit and their validity is uncertain, all reported values were used in calculating total concentrations for the SPMDs.

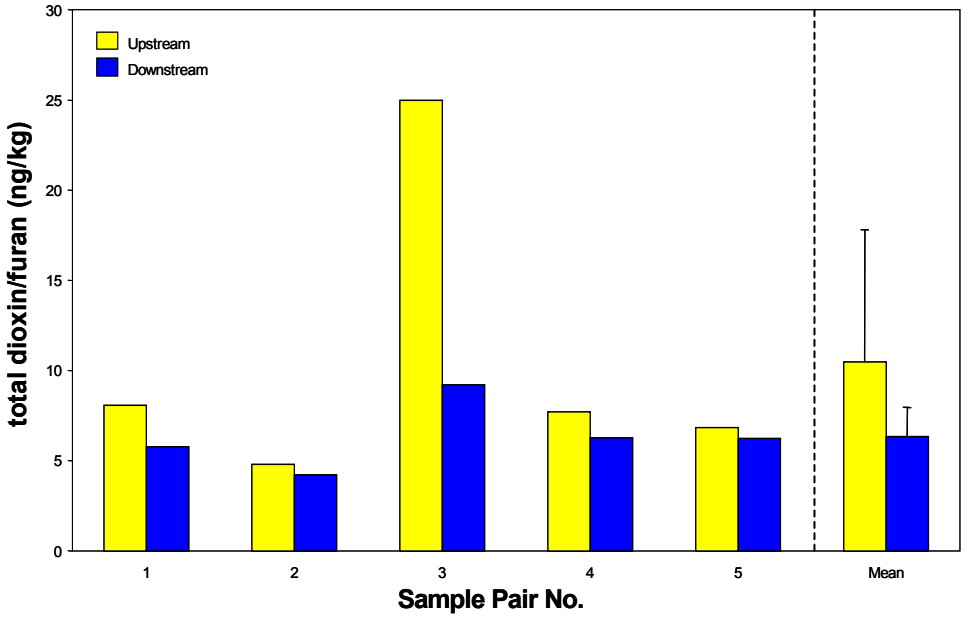

Figure 8. Total PCDD/PCDF concentration (ng/kg) measured in individual SPMD samples from upstream and downstream dioxin stations. Station mean (+95% CI) is also provided.

Mean concentrations of total PCDD/PCDF in fish (i.e., smallmouth bass) collected from the upstream and downstream stations were 2.76 and 4.19 ng/kg, respectively (Table 7). Fish at the downstream station had significantly higher concentrations of total PCDD/PCDF ($p = 0.027$). However, when the data were lipid normalized, mean upstream concentrations (624.5 ug total PCDD-PCDF/g-lipid) were higher than the downstream concentration (505.1 ug total PCDD-PCDF/g-lipid), but there was no statistically significant difference between them ($p = 0.198$).

Table 7. Dioxin/furan congeners (ng/kg) in Fish.
"0" substituted for non-detects, DL = detection limit;
***bold, italicized* = concentration equal to or above DL;**
other reported concentrations estimated because below DL.

Upstream	DL	SMB-1	SMB-2	SMB-3	SMB-4	SMB-5	SMB-6	SMB-7	SMB-8	SMB-9	SMB-10	Mean
Compound												
2378-TCDF	0.11	***0.19***	***0.15***	***0.51***	***0.49***	***0.6***	***0.31***	***0.44***	***0.25***	***0.91***	***0.22***	0.41
12378-PeCDF	0.25	0	0	0	0	0	0	0	0	0	0	0.00
23478-PeCDF	0.25	0	0	0	0	0	0	0	0	0	0	0.00
123478-HxCDF	0.25	0	0	0	0	0	0	0	0	0	0	0.00
123678-HxCDF	0.25	0	0	***0.25***	0.21	0	0	0.19	0	***0.29***	0	0.09
234678-HxCDF	0.25	0	0	0	0	0	0	0	0	0	0	0.00
123789-HxCDF	0.25	0	0	0	0	0	0	0	0	0	0	0.00
1234678-HpCDF	0.5	0	0	0	0	0	0	0	0	0	0	0.00
1234789-HpCDF	0.5	0	0	0	0	0	0	0	0	0	0	0.00
OCDF	0.5	0	0	0	0	0	0	0	0	0	0	0.00
2378-TCDD	0.1	0	0	0	0	0	0	0	0	0	0	0.00
12378-PeCDD	0.25	0	0	0	0	0	0	0	0	0	0	0.00
123478-HxCDD	0.25	0	0	0	0	0	0	0	0	0	0	0.00
123678-HxCDD	0.25	0	0	0	0	0	0	0	0	0	0	0.00
123789-HxCDD	0.25	0	0	0	0	0	0	0	0	0	0	0.00
1234678-HpCDD	0.5	0	0	***0.52***	0.47	0.28	0	0.42	***0.51***	***0.61***	0.33	0.31
OCDD	0.5	***0.85***	***0.96***	***1.81***	***2.34***	***3.34***	***1.15***	***1.62***	***1.55***	***3.57***	***2.26***	1.95
Total PCDD/PCDF		**1.04**	**1.11**	**3.09**	**3.51**	**4.22**	**1.46**	**2.67**	**2.31**	**5.38**	**2.81**	2.76

Downstream	DL	SMB-1	SMB-2	SMB-3	SMB-4	SMB-5	SMB-6	SMB-7	SMB-8	SMB-9	SMB-10	Mean
2378-TCDF	0.11	***1.02***	***1.15***	***1.63***	***1.47***	***0.87***	***0.93***	***0.82***	***0.72***	***1.23***	***0.61***	1.05
12378-PeCDF	0.25	0	0	0	0	0	0	0	0	0	0	0.00
23478-PeCDF	0.25	0	0	0	0	0	0	0	0	0	0	0.00
123478-HxCDF	0.25	0	0	0	0	0	0	0	0	0	0	0.00
123678-HxCDF	0.25	0	0	0	0	0	0	0	0	0	0	0.00
234678-HxCDF	0.25	0	0	0	0	0	0	0	0	0	0	0.00
123789-HxCDF	0.25	0	0	0	0	0	0	0	0	0	0	0.00
1234678-HpCDF	0.5	0.39	0.48	***0.67***	0.46	0.34	0.37	***0.61***	0.42	***0.59***	0.35	0.47
1234789-HpCDF	0.5	0	0	0	0	0	0	0	0	0	0	0.00
OCDF	0.5	0	0	0	0	0	0	0	0	0	0	0.00
2378-TCDD	0.1	***0.41***	***0.55***	***0.62***	***0.36***	***0.29***	***0.48***	***0.59***	***0.32***	***0.39***	***0.26***	0.43
12378-PeCDD	0.25	0	0	0	0	0	0	0	0	0	0	0.00
123478-HxCDD	0.25	0	0	0	0	0	0	0	0	0	0	0.00
123678-HxCDD	0.25	0	0	0	0	0	0	0	0	0	0	0.00
123789-HxCDD	0.25	0	0	0	0	0	0	0	0	0	0	0.00
1234678-HpCDD	0.5	***0.55***	***0.73***	***0.97***	***0.81***	***0.66***	***0.78***	***0.69***	0.41	***0.76***	0.35	0.67
OCDD	0.5	***0.97***	***1.64***	***3.07***	***1.17***	***1.23***	***1.68***	***2.11***	***1.35***	***1.82***	***0.74***	1.58
Total PCDF/PCDD		**3.34**	**4.55**	**6.96**	**4.27**	**3.39**	**4.24**	**4.82**	**3.22**	**4.79**	**2.31**	4.189

The mussel tissue chemistry data were compared to the SPMD and fish data with respect to absolute concentrations and type of compounds accumulated (Figure 9). Although the SPMDs accumulated significantly higher concentrations of 2378-TCDF, they really only accumulated this one congener. The concentrations of the remaining 16 congeners are estimated as the measured concentrations are significantly below the detection limit. The 2378-TCDF congener has the lowest molecular weight of all congeners, and it is likely that the SPMD has a limitation on the amount of higher molecular weight congeners it can actively transport though the fixed pore size of the plastic membrane. The congener profile for the mussel tissues probably better reflects the proportions of bioavailable congeners, even though the concentrations accumulated are in the low ng/kg-ww range, because mussels feed on particulate matter and dioxins and furans are mostly bound to organic particulates in water. A primary limitation of the SPMDs is that only the dissolved fraction can be adsorbed through the plastic membrane device, and dioxins and furans are poorly soluble in water. Figure 9 and Table 8 show that the fish accumulated far fewer congeners than either mussels or SPMDs. At both the upstream and downstream stations, fish accumulated predominantly OCDD and 2378-TCDF.

Table 8. Comparison of the number of congeners detected (above detection limit) by mussels, SPMDs, and fish upstream and downstream of the SAPPI pulp mill at Hinckley.

	Upstream	Downstream
Mussels	15	13
SPMDs	11	12
Fish	4	5

Figure 10 shows that the SPMDs accumulated different concentrations of total PCDD/PCDF than either mussels or fish. This is particularly striking at the upstream station where mean total PCDD/PCDFs in the SPMDs were approximately 2.5 times higher than in mussels and about 3.5 times higher than in fish. Figure 10 shows the similarity between mussels and fish, with mussels accumulating slightly higher total PCDD/PCDFs than fish, suggesting that the mussels were better surrogates for fish than SPMDs. Mean total PCDD/PCDFs were much closer among mussels, SPMDs, and fish at the downstream station, but again, concentrations in mussels were more similar to those measured in fish.

There is a greater difference in mean total PCDD/PCDF concentrations accumulated by SPMDs if the mussel and fish data are lipid normalized (Figure 10). Lipid-normalized total PCDD/PCDF concentrations measured in mussels and fish are approximately an order of magnitude higher than those measured in SPMDs at both the upstream and downstream stations. Furthermore, the concentrations measured in mussels and fish are within about 10% at the upstream station, and 40% at the downstream station.

Figure 11 further illustrates the uncertainty in the SPMD data when compared to the mussel tissue chemistry. The pair of bars on the left (A) shows the percent of data reported above the detection limit for mussels and SPMDs. Nearly 40% of the congeners in mussel tissues were present at concentrations exceeding the detection limit, while less than 10% for the SPMDs. The bars on right (C) show that some results for both the mussel tissues (<10%)

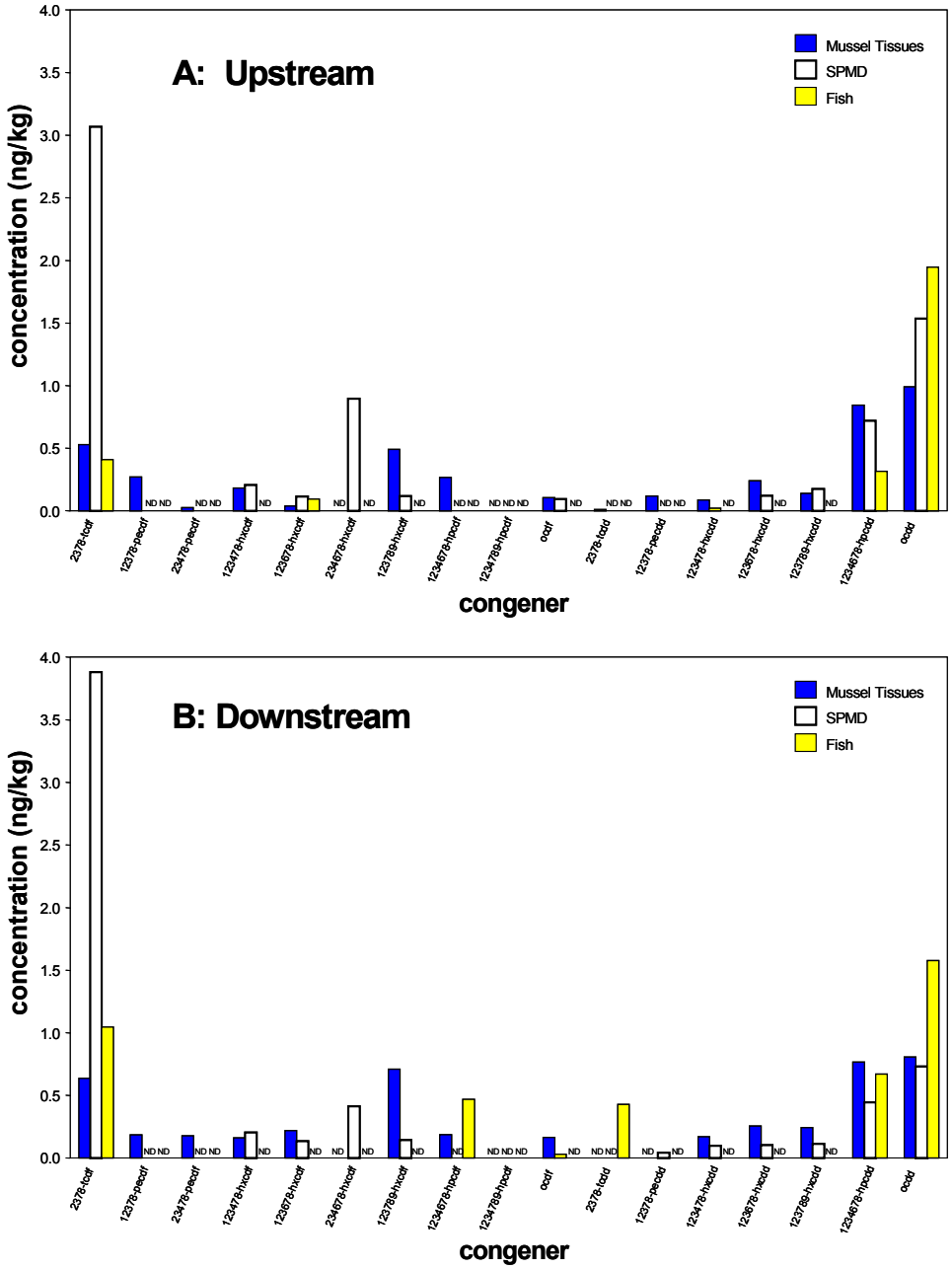

Figure 9. Mean concentration (ng/kg) of individual congeners measured in mussel tissues, SPMDs, and fish tissues.

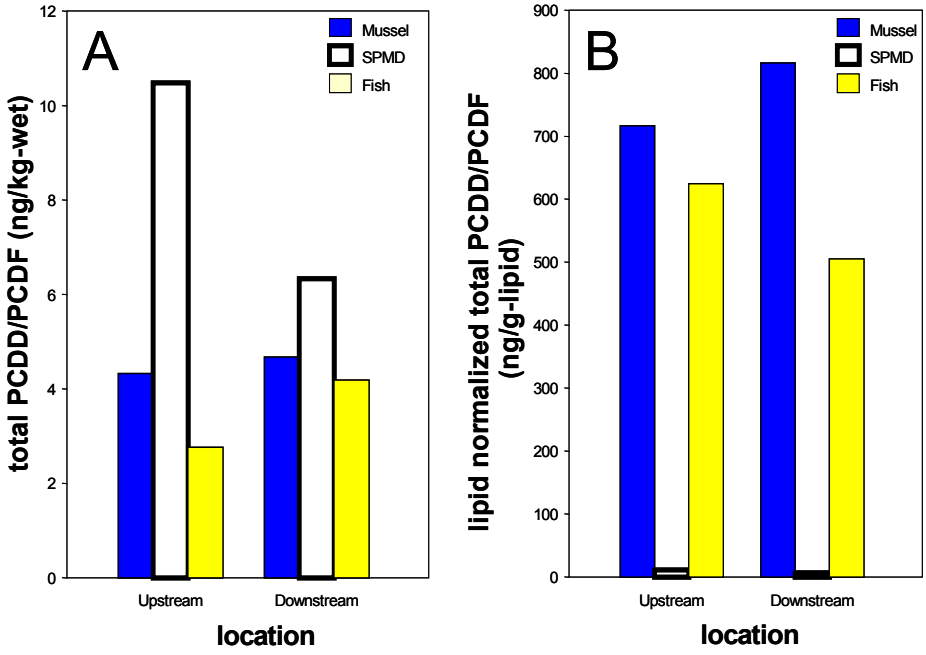

Figure 10. Mean concentration of total PCDD/PCDF measured in mussel tissues, SPMDs, and fish from the upstream and downstream stations.

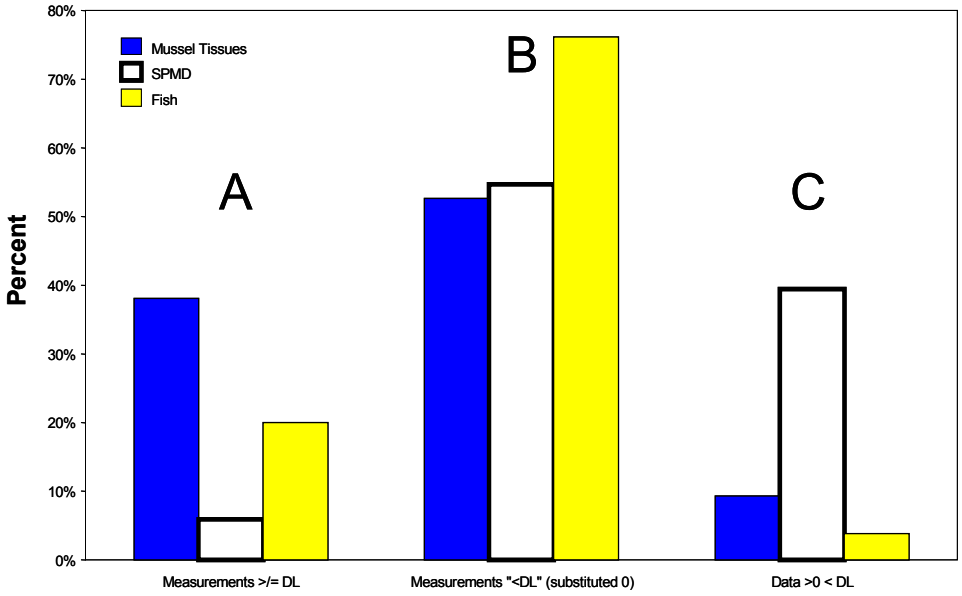

Figure 11. Comparison of mussel tissues, SPMDs, and fish data. A = percentage of reported measurements that were equal to or greater than the detection limit; B = percentage of reported measurements that were "0" or <DL; C = percentage of reported measurements that were greater than 0 but less than the detection limit (i.e., estimated values).

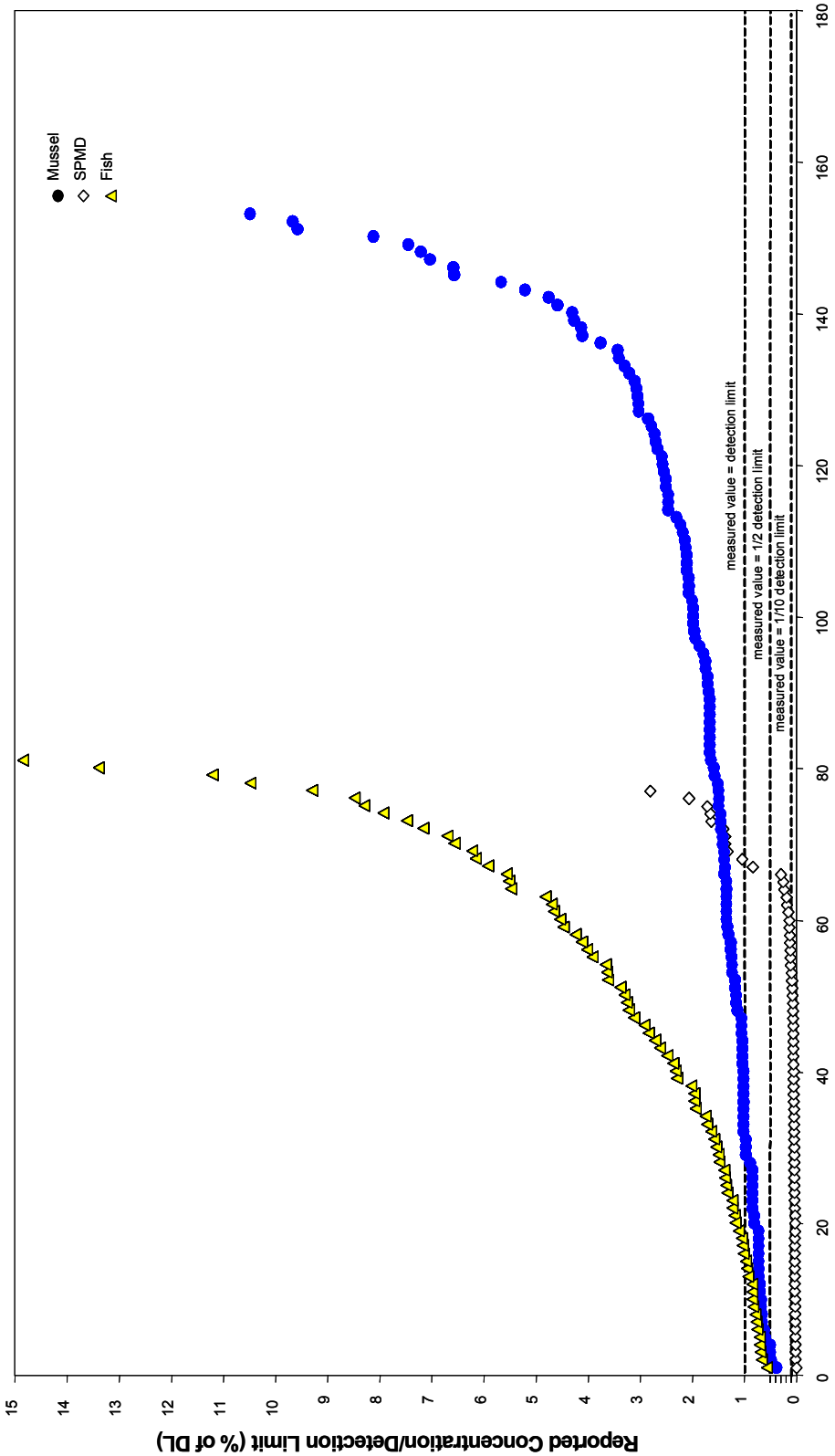

Figure 12. Percent of detection limit for each reported concentration (calculated as reported concentration divided by detection limit) for each congener measured in mussels, SPMDs, and fish.

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 less than the detection limit. This suggests that the mussels were more efficient at sequestering dioxins and furans associated with the particulate material. Approximately 50% of the congeners were undetected in both the mussel tissues and SPMDs, as shown in the center bars (B). 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 lipid bag data (Figure 12). Only 10 of the measured values (12%) for SPMDs are above the detection limit, and only one value within 50% of the detection limit. While the rest of the values were between 0.4 and 29% of the detection limit. These values 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.

4.2.3 PCBs in Mussel Tissues

Mussel tissues were analyzed for a set of PCB congeners and total PCBs (Table 9). These 20 specific PCB congeners were selected by DEQ as part of a total PCB analysis and not for a TEQ evaluation. Therefore, no TEFs are available for the specific congeners selected. For comparative purposes, the NOAA Status and Trends Mussel Watch program measures 18 different PCB congeners, 13 of which were included in the DEQ list. The National Marine Fisheries Service (NMFS) measures 8 dioxin-like PCBs, all of which have TEQs, and seven PCB non-dioxin like congeners which do not. The purpose of the NMFS approach is to estimate total PCBs from this selected list, and include estimates of dioxin-like toxicity from TEQs. The reported concentrations for a large portion of the individual congeners were below the detection limit, making the reported concentrations estimated values. The total PCB values provided by the laboratory are based on the sum of all the peaks that match the detection requirements in each homolog window, and is more likely a true representation of PCB exposure. The sum of the individual congeners does not equal the reported total PCB concentration because data for all congers were not reported by the laboratory.

The concentrations of all individual PCB congeners and total PCBs were below the detection limit in the five beginning-of-test mussel tissue samples. Using the convention of replacing non-detects with a "0", the total PCB concentration in these mussel tissues was “zero.” Based on these data, mussels at all Kennebec River stations accumulated PCBs at concentrations that were significantly elevated above the mean beginning of the test concentration. Concentrations of total PCBs accumulated by mussels ranged from 2.7 to 188 ug/kg-dw (Figure 13, Table 10). There was no west to east gradient in accumulated PCBs for mussels at any station except Central Augusta, where mussels accumulated the least amount of PCBs, ranging from 3.0 to 6.1 ug/kg-dw. The highest concentration, 188 ug/kg-dw, was measured in mussels from the mid location at South Augusta. Mussels at the west location at Farmingdale also accumulated relatively high concentrations of PCBs, 125 ug/kg-dw. The highest mean total PCB concentrations were found at South Augusta and Farmingdale, 78.7 and 74.0 ug/kg-dw, respectively (Table 10). Mussels deployed at Above Riggs, Central Augusta, and South Gardiner had the most consistent total PCB concentrations among sampling locations. Variability among "replicates" was lowest for these stations. For each of the other stations, there was a wide range in total PCBs accumulated; the highest variability was found at South Augusta.

Table 9. Concentrations (ug/kg dry weight) of selected PCB congeners and total PCBs measured in mussel tissues. "0" substituted for non-detects; DL = detection limit; *bold, italicized, shaded* = concentration equal to or above DL; other reported concentrations estimated because below DL.

		Above Riggs			*Riggs*			*North Augusta*			*Central Augusta*		
Compounds (IUPAC #)	**DL**	**West**	**Mid**	**East**	**West**	**Mid**	**East**	**West**	**Mid**	**East**	**West**	**Mid**	**East**
2,4′-Dichlorobiphenyl (8)	1.0	0.639	0.360	0.480	0.561	0.400	0.761	0	0	0	0	0	0
2,2′,5-Trichlorobiphenyl (18)	1.0	0	0	0.360	0	0	0	0	0.505	0.291	0.453	0.313	0.169
2,4,4′-Trichlorobiphenyl (28)	1.0	0	0	0	0	0	0	0	0.269	0	0	0	0
2,4,5-Trichlorobiphenyl (29)	1.0	0	0.880	0	0.641	0.760	0.361	0	***24.020***	0	0	0	0
2,2′,3,5′-Tetrachlorobiphenyl (44)	1.0	0	0	0	0	0	0	0	0	0	0	0	0
2,2′,4,6-Tetrachlorobiphenyl (50)	1.0	0	0	0	0	0	0	0	0	0	0	0	0
2,2′,5,5′-Tetrachlorobiphenyl (52)	1.0	0	0	0	0	0	0	0	0	0	0	0	0
2,3′,4,4′-Tetrachlorobiphenyl (66)	1.0	0	0	0	0	0	0	0	0	0	0.113	0.267	0
2,2′,3,4,5′-Pentachlorobiphenyl (87)	2.0	0	0	0	0	0	0	0	0	0	0	0	0
2,2′,4,5,5′-Pentachlorobiphenyl (101)	2.0	0	0	0	0	0	0	0	0	0	0	0	0
2,2′,4,6,6′-Pentachlorobiphenyl (104)	2.0	0	0	0.480	0	0.320	0	0	0	0	0	0	0
2,2′,3,3′,4,4′-Hexachlorobiphenyl (128)	2.0	0	0	0	0	0	0	0	0	0	0	0	0
2,2′,3,4,4′,5′-Hexachlorobiphenyl (138)	2.0	0.599	0	0.480	0	1.001	0.801	0.123	0	0	0	0	0
2,2′,4,4′,5,5′-Hexachlorobiphenyl (153)	2.0	1.159	0.640	0	0	0	0.921	0.163	0	0	0	0	0
2,2′,4,4′,5,6′-Hexachlorobiphenyl (154)	2.0	0	0	0	0	0	0	0	0	0	0	0	0.626
2,2′,3,4′,5,5′,6-Heptachlorobiphenyl (187)	2.0	0.559	0	0	0	0	0.120	0	0	0	0	0	0
2,2′,3,4′,5,6,6′-Heptachlorobiphenyl (188)	2.0	***3.357***	1.680	0.480	***7.813***	***5.643***	0	0	0	0	0	0	0
2,2′,3,3′,4,4′,5,6-Octachlorobiphenyl (195)	3.0	0.200	0.160	0	0	0	0.521	0	0	0	0	0	0
2,2′,3,3′,4,5′,6,6′-Octachlorobiphenyl (200)	3.0	0	0	0	0	0	0	0	0	0	0	0	0
2,2′,3,3′,4,4′,5,5′,6,6′-Decachlorobiphenyl (209)	5.0	0	0	0	0	0	0	0	0	0	0	0	0
Total PCBs		**29.5**	**25.8**	**18.4**	**45.8**	**26.9**	**18.4**	**3.9**	**54.8**	**2.7**	**3.0**	**4.3**	**6.1**

		North Augusta			*Farmingdale*			*Gardiner*			*South Gardiner**		*Swan Island**	
Compounds (IUPAC #)	DL	**West**	**Mid**	**East**	**West**	**Mid**	**East**	**West**	**Mid**	**East**	**Mid**	**East**	**Mid**	**East**
2,4′-Dichlorobiphenyl (8)	1.0	0	***1.398***	0.400	0.759	***3.078***	***1.202***	***1.082***	0.440	***1.478***	0.518	0.719	0.399	***1.361***
2,2′,5-Trichlorobiphenyl (18)	1.0	0	***5.353***	0.280	0	0	0	0	0	0	0	0	0	0
2,4,4′-Trichlorobiphenyl (28)	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0
2,4,5-Trichlorobiphenyl (29)	1.0	***1.439***	***2.037***	0	***1.438***	***1.159***	0	0	0.400	0	***2.073***	0.958	0.599	2.922
2,2′,3,5′-Tetrachlorobiphenyl (44)	1.0	0	***2.557***	0	0	0.120	0	0	0	0.320	0	0	0	0.280
2,2′,4,6-Tetrachlorobiphenyl (50)	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0
2,2′,5,5′-Tetrachlorobiphenyl (52)	1.0	0	0	0.600	0	0	0	0	0	0	0	0	0	0
2,3′,4,4′-Tetrachlorobiphenyl (66)	1.0	0	0.959	0	0	0	0	0	0	0	0	0	0	0
2,2′,3,4,5′-Pentachlorobiphenyl (87)	2.0	0	0	0.200	0.200	0.160	0	0	0	0	0.159	0.200	0	0
2,2′,4,5,5′-Pentachlorobiphenyl (101)	2.0	0	0.360	0	0	0	0.120	0	0	0	0	0	0	0
2,2′,4,6,6′-Pentachlorobiphenyl (104)	2.0	0	0.160	0	0	0	0	0	0	0.120	0	0.200	0	0
2,2′,3,3′,4,4′-Hexachlorobiphenyl (128)	2.0	0	0	0	0	0	0	0	0	0	0	0	0	0
2,2′,3,4,4′,5′-Hexachlorobiphenyl (138)	2.0	0	0.439	1.280	1.239	1.279	***6.891***	1.883	0	0	0	1.158	0.878	***4.763***
2,2′,4,4′,5,5′-Hexachlorobiphenyl (153)	2.0	0.719	0.320	***4.401***	0	0	***2.243***	0	0	0.839	0.120	0	0.359	0
2,2′,4,4′,5,6′-Hexachlorobiphenyl (154)	2.0	1.199	0	0	0	0	0.401	0	0	0	0.439	0.280	0.439	0.320
2,2′,3,4′,5,5′,6-Heptachlorobiphenyl (187)	2.0	***7.193***	***55.248***	0.520	0	***2.079***	1.162	1.803	0	***6.633***	0.120	0.359	0.758	4.282
2,2′,3,4′,5,6,6′-Heptachlorobiphenyl (188)	2.0	0	***4.314***	1.080	0	0.160	1.402	0.481	0.240	1.638	1.675	0.240	0.399	0.360
2,2′,3,3′,4,4′,5,6-Octachlorobiphenyl (195)	3.0	0	0.200	2.160	0	0	0	0.200	0.120	0.240	0	0.240	0	0.160
2,2′,3,3′,4,5′,6,6′-Octachlorobiphenyl (200)	3.0	0	0	0	0	0	0	0	0	0	0	0	0	0
2,2′,3,3′,4,4′,5,5′,6,6′-Decachlorobiphenyl (209)	5.0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total PCBs		**31.5**	**188.0**	**16.5**	**125.0**	**35.9**	**61.2**	**24.8**	**6.6**	**50.3**	**26.9**	**20.1**	**16.7**	**64.2**

*Cage lost at the West Station

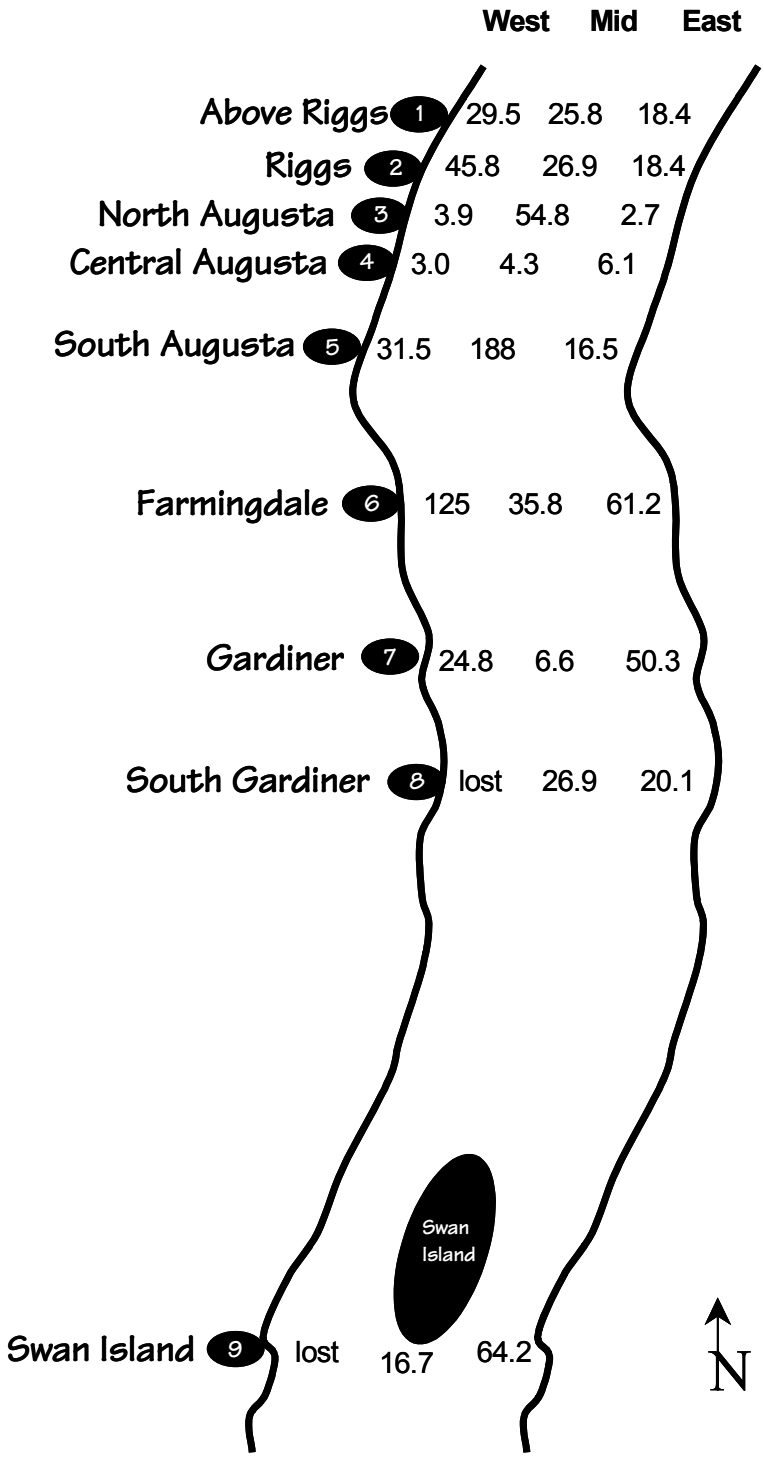

Figure 13. Concentration of total PCBs (ug/kg-dw) measured in mussels deployed in the Kennebec River by station.

Table 10. Concentration of total PCBs (ug/kg-dw) by station and position.

	West	Mid	East	Mean	95% CI
Above Riggs	29.5	25.8	18.4	24.6	6.40
Riggs	45.8	26.9	18.4	30.4	15.87
North Augusta	3.9	54.8	2.7	20.4	33.68
Central Augusta	3.0	4.3	6.1	4.5	1.75
South Augusta	31.5	***188***	16.5	78.7	107.48
Farmingdale	***125***	35.9	61.2	74.0	51.96
Gardiner	24.8	6.6	50.3	27.2	24.83
S. Gardiner	lost	26.9	20.1	23.5	6.66
Swan Island	lost	16.7	64.2	40.5	46.55

4.3 Health Metrics: Mussel Growth, Percent Lipids & Percent Water

4.3.1 Dioxin/Furan Mussels

Elliptio deployed at the dioxin/furan upstream and downstream stations had very slight increases in shell length and WAWW during the study, with changes in WAWW less than 4% and changes in length less than 1% (Table 11). The most significant changes occurred in tissue weight. When compared to the beginning of test estimate of tissue weight, *Elliptio* tissue weights at both upstream and downstream increased by approximately 15%. However, there is less accuracy is estimating changes in tissue weight between beginning and end of test because of the variability in mussel shape and form for the size range used, i.e., mussels of a common length have different heights and widths and different sizes of tissues to accommodate the internal space. The data suggest that none of the mussels lost a significant amount of tissue weight, and therefore, were in good health so that the tissue chemistry data could be used with confidence.

4.3.1.1 Shell Length

At the start of the test, individual shell lengths for mussels deployed at the dioxin/furan stations ranged from 58.0 to 67.2 mm, a range of 9.2 mm (Table 11). Mean BOT shell length for upstream and downstream mussels was 62.1 mm (Table 11). There were no statistically significant differences in mean shell lengths among individual cages ($p = 1.000$) or between the upstream and downstream stations ($p = 0.964$) at the beginning of the test. Shell lengths increased by approximately 0.4 and 0.3% at the upstream and downstream stations, respectively (Table 11). EOT shell lengths were significantly larger than BOT for both the upstream and downstream stations (Table 12). However, there was no significant difference between upstream and downstream in shell length at the end of the test (Table 12). Mean length growth rates for the upstream and downstream stations were 0.03 mm/wk (Table 11). There was no significant difference between upstream and downstream in length growth rate (Table 12).

Table 11. Summary growth metrics for mussels deployed at dioxin stations.

	Upstream	Downstream	T_0	All Data
Percent Survival	99.7%	99.7%	na	99.7%
% Change Weight	3.8%	2.7%	na	3.3%
% Change Length	0.4%	0.3%	na	0.4%
Estimated % Change Tissue Weight	15.9%	15.1%	na	15.5%
Initial Length (mm)				
Mean	62.1	62.1	62.2	62.1
Min	58.0	58.0	58.0	58.0
Max	67.2	67.0	66.8	67.2
Std. Dev.	2.53	2.51	2.53	2.52
N	360	360	180	900
EOT Length (mm)				
Mean	62.4	62.3	na	62.4
Min	57.8	58.0	na	57.8
Max	67.5	67.3	na	67.5
Std. Dev.	2.49	2.49	na	2.49
N	359	323	na	682
Length Growth Rate (mm/wk)				
Mean	0.03	0.03	na	0.03
Min	-0.06	-0.10	na	-0.10
Max	0.20	0.20	na	0.20
Std. Dev.	0.04	0.04	na	0.04
N	359	323	na	682
Initial Weight (g-wet)				
Mean	20.39	20.05	20.09	20.20
Min	13.19	13.55	14.31	13.19
Max	32.72	31.50	33.40	33.40
Std. Dev.	3.74	3.32	3.47	3.53
N	360	360	180	900
EOT WAWW (g-wet)				
Mean	21.13	20.53	na	20.85
Min	13.64	14.13	na	13.64
Max	33.50	31.29	na	33.50
Std. Dev.	3.74	3.29	na	3.54
N	359.00	323.00	na	682
WAWW Growth Rate (mg/wk)				
Mean	97.53	68.92	na	83.98
Min	-129.46	-130.78	na	-130.78
Max	458.39	298.55	na	458.39
Std. Dev.	62.87	49.87	na	58.81
N	359	323	na	682
EOT Wet Tissue Weight (g-wet)				
Mean	5.54	5.43	4.73	5.49
Min	3.14	2.75	3.32	2.75
Max	9.66	9.79	7.72	9.79
Std. Dev.	0.93	0.88	0.77	0.91
N	359	323	180	682
EOT Shell Weight (g-wet)				
Mean	9.48	9.25	9.45	9.37
Min	3.25	4.85	5.33	3.25
Max	17.22	16.81	19.44	17.22
Std. Dev.	2.05	1.83	2.04	1.95
N	359	323	180	682
Percent Lipids (wet)				
Mean	0.59	0.59	0.63	0.59
Min	0.48	0.47	0.62	0.48
Max	0.87	0.67	0.66	0.77
Std. Dev.	0.11	0.08	0.02	0.10
N	10	9	5	19

Table 12. Summary of statistical results (p values) for mussel growth metrics: Dioxin/Furan (* = statistically significant; na = not applicable)

	Length	Length GR	WAWW	WAWW GR	Tissue Weight	Shell Weight	Percent Lipids
Upstream: EOT vs BOT	< 0.0001*	na	< 0.0001*	na	< 0.001*	0.3001	0.4246
Downstream: EOT vs BOT	< 0.0001*	na	< 0.0001*	na	< 0.001*	0.3001	0.4246
EOT: Upstream vs Downstream	**0.8554**	**0.2917**	**0.0261***	**<0.0001***	**0.1937**	**0.1324**	**0.9675**

4.3.1.2 Whole-Animal Wet-Weight (WAWW)

At the start of the test, individual WAWWs for mussels deployed at the dioxin/furan stations ranged from 13.19 to 33.40 g, a range of 20.21 g (Table 11). Mean BOT WAWW for upstream and downstream mussels was 20.39 and 20.05 g, respectively (Table 11; Figure 14). There were no statistically significant differences in mean WAWWs among individual cages (p = 0.9865) or between the upstream and downstream stations (p = 0.3979) at the beginning of the test. WAWWs increased by approximately 3.8 and 2.7% at the upstream and downstream stations, respectively (Table 11). EOT WAWWs were significantly larger than BOT for both the upstream and downstream stations (Table 12). EOT WAWWs were significantly higher at the upstream station when compared to the downstream station (Table 12). Mean WAWW growth rates for the upstream and downstream stations were 97.53 and 68.92 mg/wk (Table 11), with the WAWW growth rates at the upstream station significantly higher than at the downstream station (Table 12; Figure 14).

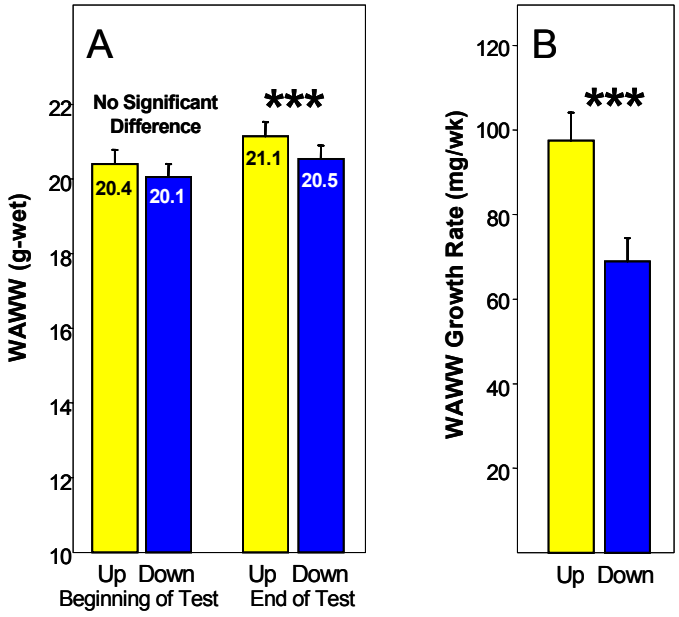

Figure 14. (A) Whole animal wet weight (WAWW), and (B) WAWW growth rates at dioxin/furan upstream and downstream stations. *** = statistically significant difference between upstream and downstream stations.

4.3.1.3 Tissue Weight

Mean wet tissue weight at the start of the test was estimated at 4.73 g-wet (Table 11) based on the tissue weights from the 180 baseline BOT measurements. There was no significant difference ($p = 0.5984$) in tissue weights among the five BOT replicates. Based on this estimate, tissue weights increased by approximately 15.9 and 15.1% at the upstream and downstream stations, respectively (Table 11), and EOT tissue weights were significantly larger than BOT for both the upstream and downstream stations (Table 11; Figure 15). Mean EOT tissue weights for the upstream and downstream stations were 5.54 and 5.43 g-wet, respectively, and there was no significant difference between upstream and downstream stations in EOT tissue weights (Table 12).

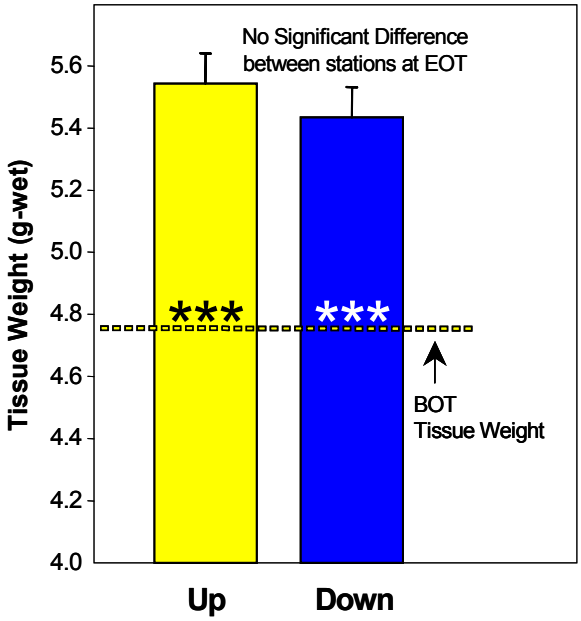

Figure 15. End-of-test (EOT) tissue weight at dioxin/furan upstream and downstream stations. EOT values also compared to beginning of test (BOT). *** = statistically significant difference between EOT and BOT tissue weight.

4.3.1.4 Shell Weight

Mean wet shell weight at the start of the test was estimated at 9.45 g-wet (Table 11) based on the shell weights from the 180 baseline BOT measurements. Mean EOT shell weights for the upstream and downstream stations were 9.18 and 9.25 g-wet, respectively (Table 11). There was no significant difference between EOT shell weights for either station when compared to the BOT estimate. Similarly, there was no significant difference between upstream and downstream stations in EOT shell weights (Table 12).

4.3.1.5 Percent Lipids and Water

Mean percent lipids at the start of the test was estimated at 0.63% (Table 11) based on the analytical results for the five composite tissue samples (Table 11). EOT percent lipids were slightly lower at 0.59% at both the upstream and downstream stations. There was no statistically significant difference between initial percent lipids and EOT at either the upstream or downstream stations (Table 12). Similarly, there was no significant difference between upstream and downstream stations in EOT percent lipids (Table 12).

Percent water was not measured in mussel tissues analyzed for dioxins and furans.

4.3.2 PCB Mussels

Elliptio deployed at the PCB stations had very minor increases in shell length and WAWW during the study. All changes in WAWW were less than 7.5%, and all changes in length were less than 1% (Table 13). The most significant changes occurred in tissue weight. When compared to the beginning of test estimate of tissue weight, mean change in tissue weights was 17%. However, there is less accuracy in estimating changes in tissue weight between beginning and end of test because of the variability in mussel shape and form for the size range used, i.e., mussels of a common length have different heights and widths and different sizes of tissues to accommodate the internal space. The data suggest that none of the mussels lost a significant amount of tissue weight, and therefore, were in good health so that the tissue chemistry data could be used with confidence.

4.3.2.1 Shell Length

At the start of the test, individual shell lengths for mussels deployed at the PCB stations ranged from 57.6 to 67.6 mm, a range of 10 mm (Table 13). Mean BOT shell length by station ranged from 62.4 to 62.6 mm (Table 13). There were no statistically significant differences in mean shell lengths among individual cages ($p = 1.000$) or between stations ($p = 1.000$) at the beginning of the test. EOT shell lengths were significantly larger than BOT at all stations except Stations 5, 6, and 9, (Table 14). The greatest increase in shell lengths occurred at Stations 7 and 8 (0.65% and 0.75%, respectively). There was very minor increase in shell length at the other stations (Table 13). However, there was no significant difference in shell length among stations at the end of the test (Table 14). Mean length growth rates ranged from -0.006 to 0.061 mm/wk (Table 13) and were significantly higher at Stations 7 and 8 than at any other station (Table 14).

Table 13. Summary growth metrics for mussels deployed at PCB stations in the Kennebec River.

PCB Station	1	2	3	4	5	6	7	8	9	T_0	All Data
Percent Survival	100%	98.3%	98.3%	100%	100%	98.3%	98.3%	97.5%	97.5%	na	98.8%
% Change Weight	5.11%	4.89%	4.71%	5.03%	3.04%	3.54%	7.06%	7.31%	4.32%	na	4.9%
% Change Length	0.12%	0.12%	0.19%	0.19%	0.01%	-0.05%	0.65%	0.75%	-0.08%	na	0.2%
Estimated % Change Tissue Weight	8.2%	17.3%	14.6%	11.0%	5.9%	17.2%	30.1%	30.9%	23.1%	na	16.8%
Initial Length (mm)											
Mean	62.5	62.5	62.5	62.5	62.4	62.5	62.4	62.4	62.6	62.2	62.5
Min	58.7	58.0	58.5	58.2	58.1	58.0	58.1	58.2	58.1	58.0	58.0
Max	66.6	66.7	66.9	66.8	66.6	66.8	66.7	66.5	66.9	66.8	66.9
Std. Dev	2.35	2.28	2.38	2.37	2.33	2.32	2.41	2.36	2.32	2.53	2.3
N	60	60	60	60	60	60	60	60	60	180	540
EOT Length (mm)											
Mean	62.6	62.5	62.6	62.6	62.4	62.5	62.8	63.0	62.6	na	62.6
Min	58.7	58.0	58.5	58.4	57.6	58.0	58.4	58.2	58.0	na	57.6
Max	66.9	67.1	67.6	67.1	66.6	66.8	67.5	66.8	67.2	na	67.6
Std. Dev	2.40	2.29	2.40	2.40	2.37	2.36	2.37	2.26	2.38	na	2.35
N	60	59	59	60	60	59	59	39	39	na	494
Length Growth Rate (mm/wk)											
Mean	0.010	0.010	0.015	0.016	0.001	-0.004	0.053	0.061	-0.006	na	0.016
Min	-0.040	-0.073	-0.055	-0.095	-0.063	-0.058	-0.145	-0.020	-0.062	na	-0.145
Max	0.082	0.124	0.098	0.112	0.090	0.135	0.213	0.186	0.071	na	0.213
Std. Dev	0.022	0.033	0.034	0.033	0.030	0.036	0.054	0.051	0.032	na	0.042
N	59	59	59	60	60	59	59	39	39	na	493
Initial Weight (g-wet)											
Mean	20.29	20.67	19.61	20.41	20.47	20.41	19.98	20.06	20.70	20.09	20.29
Min	14.92	14.43	12.21	11.81	14.82	14.50	13.87	13.44	14.51	14.31	11.81
Max	29.11	31.75	29.48	32.95	28.71	31.06	29.52	30.47	29.86	33.40	32.95
Std. Dev	3.16	3.52	3.35	4.18	3.28	3.50	3.32	3.45	3.89	3.47	3.52
N	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	180	540
EOT WAWW (g-wet)											
Mean	21.30	21.60	20.45	21.40	21.07	21.16	21.35	21.96	21.83	na	21.30
Min	15.77	15.62	13.01	12.62	15.13	15.18	15.01	14.90	15.07	na	12.62
Max	30.65	33.27	29.66	33.66	28.81	31.40	31.29	31.88	30.37	na	33.66
Std. Dev	3.15	3.50	3.36	4.25	3.27	3.45	3.24	3.61	3.73	na	3.50
N	60	59	59	60	60	59	59	39	39	na	494
WAWW Growth Rate (mg/wk)											
Mean	133.20	128.32	117.82	131.40	79.85	91.62	178.29	193.75	115.98	na	127.92
Min	31.70	-19.82	-19.82	2.64	-26.42	-5.28	72.66	77.94	-97.75	na	-97.75
Max	232.50	364.60	302.51	359.31	212.68	232.50	379.13	332.89	256.27	na	379.13
Std. Dev	42.83	62.40	60.93	59.39	43.32	50.95	72.04	50.30	63.35	na	65.54
N	60	59	59	60	60	59	59	39	39	na	494
EOT Wet Tissue Weight (g-wet)											
Mean	5.16	5.65	5.33	5.31	5.07	5.62	6.15	6.28	6.00	4.73	5.57
Min	3.31	4.23	2.99	3.60	3.50	3.61	4.39	4.81	4.08	3.32	2.99
Max	7.17	9.99	7.96	8.07	6.94	7.75	9.31	9.84	8.28	7.72	9.99
Std. Dev	0.71	0.95	0.96	0.93	0.77	0.90	1.05	1.05	0.99	0.77	1.00
N	60	59	59	60	60	59	59	39	39	180	494
EOT Shell Weight (g-wet)											
Mean	9.71	9.75	9.21	9.81	9.73	9.90	9.70	10.00	10.10	9.45	9.75
Min	6.98	6.32	5.29	5.23	5.76	6.16	6.55	6.11	6.39	5.33	5.23
Max	16.15	14.87	13.97	16.78	14.33	16.10	14.52	14.32	15.20	19.44	16.78
Std. Dev	1.72	1.86	1.85	2.10	1.87	2.14	1.68	1.78	2.13	2.04	1.90
N	60	59	59	60	60	59	59	39	39	180	494
Percent Water											
Mean	58.7	58.0	67.8	70.8	66.5	61.5	58.4	50.0	61.3	na	61.9
Min	53.0	55.3	64.5	68.9	64.1	59.3	55.7	44.7	60.8	na	44.7
Max	61.8	60.3	72.0	74.0	68.8	62.7	63.7	55.3	61.8	na	74.0
Std. Dev	4.91	2.55	3.84	2.78	2.38	1.94	4.51	7.47	0.69	na	6.54
N	3	3	3	3	3	3	3	2	2	na	25

Table 14. Summary of statistical results (p values) for mussel growth metrics: PCBs (* = statistically significant; na = not applicable)

	Length	Length GR	WAWW	WAWW GR	Tissue Weight	Shell Weight
EOT vs BOT						
Station 1 (Above Riggs)	0.0012*	na	<0.0001*	na	<0.01*	0.3525
Station 2 (Riggs)	0.0240*	na	<0.0001*	na	<0.01*	0.3525
Station 3 (North Augusta)	0.0009*	na	<0.0001*	na	<0.01*	0.3525
Station 4 (Central Augusta)	0.0006*	na	<0.0001*	na	<0.01*	0.3525
Station 5 (South Augusta)	0.8841	na	<0.0001*	na	>0.05	0.3525
Station 6 (Farmingdale)	0.43087	na	<0.0001*	na	<0.01*	0.3525
Station 7 (Gardiner)	<0.0001*	na	<0.0001*	na	<0.01*	0.3525
Station 8 (S. Gardiner)	<0.0001*	na	<0.0001*	na	<0.01*	0.3525
Station 9 (Swan Island)	0.2341	na	<0.0001*	na	<0.01*	0.3525
EOT: Comparison of All Stations	0.9710	<0.0001*	0.5854	<0.0001	0.0001*	0.5180

4.3.2.2 Whole-Animal Wet Weight

At the start of the test, individual WAWWs for mussels deployed at the PCB stations ranged from 11.81 to 32.95 g, a range of 21.14 g (Table 13). Mean BOT WAWW by station ranged from 19.61 to 20.70 g (Table 13; Figure 16). There were no statistically significant differences in mean WAWWs among individual cages ($p = 0.7692$) or between stations ($p = 0.7888$) at the beginning of the test. WAWWs increased between 3% (at Station 5) and 7.3% (at Station 8) (Table 13). EOT WAWWs ranged from 20.45 to 21.96 g and were significantly larger than BOT at all stations (Table 14). There was no significant difference in EOT WAWWs among stations (Table 14). Mean WAWW growth rates by station ranged from 79.85 to 193.75 mg/wk (Table 13), and there were several statistically significant differences among stations (Table 14; Figure 16). Underlined stations, in order from lowest to highest, are statistically similar:

South Augusta | Farmingdale | Swan Island | North Augusta | Riggs | Central Augusta | Above Riggs | Gardiner | S. Gardiner

4.3.2.3 Tissue Weight

Mean wet tissue weight at the start of the test was estimated at 4.73 g-wet (Table 13) based on the tissue weights from the 180 baseline BOT measurements. There was no significant difference ($p = 0.5984$) in tissue weights among the five BOT replicates. Based on this estimate, tissue weights increased from 5.9 to 30.9% (Table 13), and EOT tissue weights were significantly larger than BOT at all stations except Station 5 (Table 14; Figure 17). Mean EOT tissue weights by station ranged from 5.07 to 6.28 g-wet (Table 13), and there were several statistically significant differences among stations (Table 13; Figure 17). Underlined stations, in order from lowest to highest, are statistically similar:

South Augusta | Above Riggs | Central Augusta | North Augusta | Riggs | Farmingdale | Swan Island | Gardiner | S. Gardiner

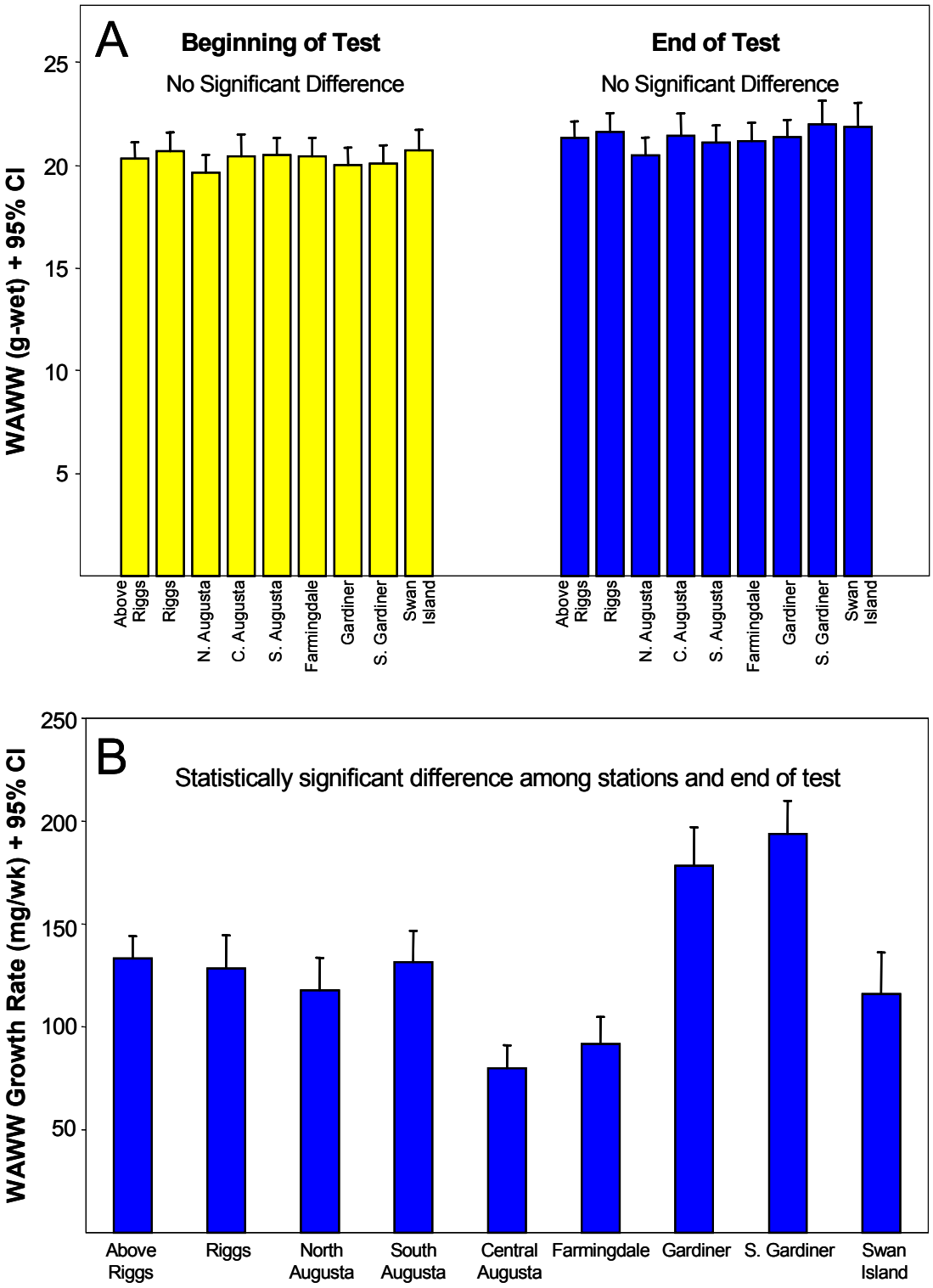

Figure 16. (A) Whole animal wet weight (WAWW), and (B) WAWW growth rates at PCB stations.

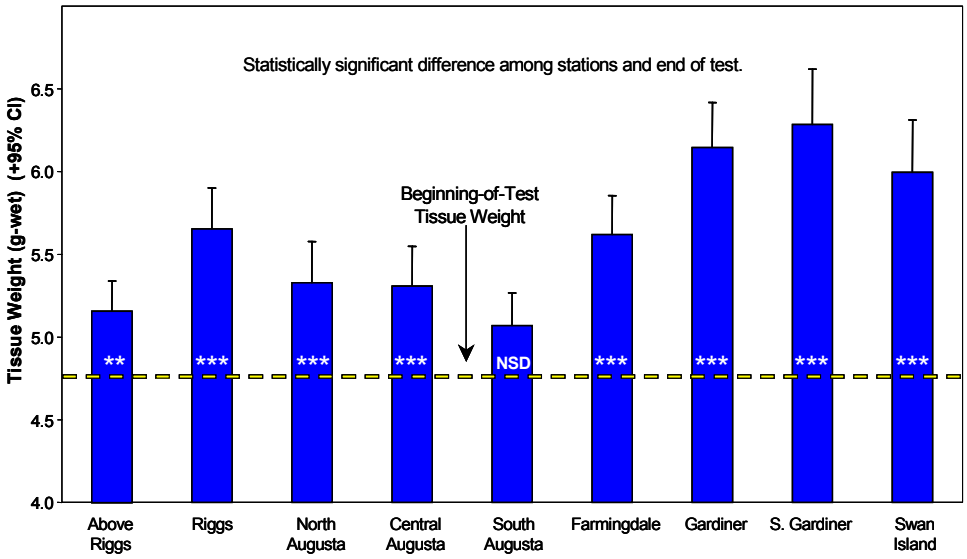

Figure 17. End-of-test (EOT) tissue weight at PCB stations. EOT values also compared to beginning of test (BOT). *** = statistically significant difference between EOT and BOT tissue weight.

4.3.2.4 Shell Weight

Mean wet shell weight at the start of the test was estimated at 9.45 g-wet (Table 13) based on the shell weights from the 180 baseline BOT measurements. Mean EOT shell weights by station ranged from 9.21 to 10.10 g-wet (Table 13). There was no significant difference between EOT shell weight when compared to the BOT estimate for any station. Similarly, there was no significant difference among stations in EOT shell weights (Table 14).

4.3.2.5 Percent Lipids and Water

Mean percent lipids at the start of the test was estimated at 0.63% (Table 10) based on the analytical results for the five composite tissue samples (Table 10). EOT percent lipids were not measured in the PCB mussels.

Percent water in mussel tissues ranged from 44.7 to 74% (Table 13). Mussels at Station 8 (S. Gardiner) had the lowest mean percent water (50%) while mussels at Station 4 (Central Augusta) had the highest mean percent water content (70.8%). Percent water was not measured in the BOT tissue samples.

4.3.3 Water Temperature

4.3.3.1 Dioxin Stations

Water temperatures at the upstream dioxin station ranged from 24.4°C on August 3, 2000, to a low of 16.2°C on September 26, 2000, and at the downstream station from a high of 27.97°C on August 3, 2000 to a low of 16.7°C on September 26, 2000 (Table 15; Figure 18).

Table 15. Summary of water temperatures.

	Min	Max	Mean
Dioxin Stations			
Upstream (Norridgewock)	16.19	24.42	20.3
Downstream (Fairfield)	16.67	27.97	21.1
PCB Stations			
Station 1 (Above Riggs)	16.24	24.62	21.1
Station 2 (Riggs)	16.37	25.49	21.1
Station 3 (North Augusta)	16.43	24.47	21.2
Station 4 (Central Augusta)	16.21	24.96	21.1
Station 5 (South Augusta)	16.02	24.38	21.1
Station 6 (Farmingdale)	16.01	24.73	21.2
Station 7 (Gardiner)	16.49	24.42	21.3
Station 8 (S. Gardiner)	16.35	24.40	21.3
Station 9 (Swan Island)	16.08	25.71	21.5

In general, water temperatures seemed higher at the downstream station, and generally decreased from August to the end of September, with daily fluctuations on the order of 2°C at the downstream station and only 1°C at the upstream station. Multiple measurements at short intervals can be autocorrelated and interfere with appropriate statistical analyses. Therefore, daily average temperatures were calculated for upstream and downstream (Figure 19). Statistical analyses of these daily averages showed that the daily average water temperatures at the upstream station were significantly higher than at the downstream station ($p = 0.0084$).

4.3.4.2 PCB Stations

Minimum water temperatures at the PCB stations ranged from 16.0°C at Farmingdale to 16.5°C at Gardiner. Maximum water temperatures ranged from 24.4°C at South Augusta to 25.7°C at Swan Island. Mean water temperatures were very consistent among stations, and ranged from 21.1°C at several stations to 21.5°C at Swan Island. There appeared to be a general increase in water temperature from north to south (Table 15, Figure 20). The most daily variability occurred at Farmingdale and Swan Island, where daily ranges were often close to 2°C. Daily average water temperatures generally decreased at all stations from about 24°C at the beginning of the test to about 16°C at the end of the test (Figure 21).

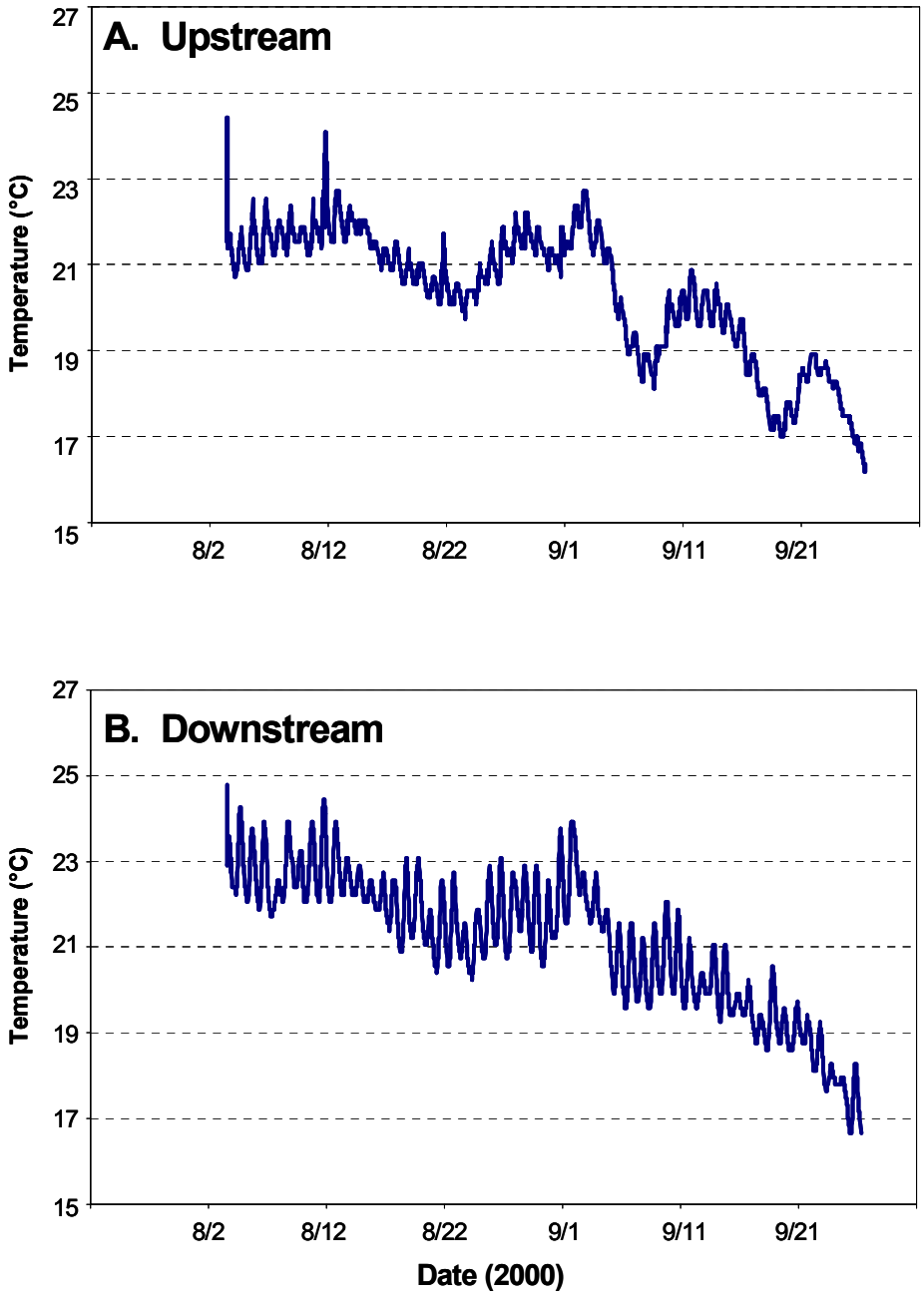

Figure 18. Water temperature profiles at (A) upstream and (B) downstream dioxin stations.

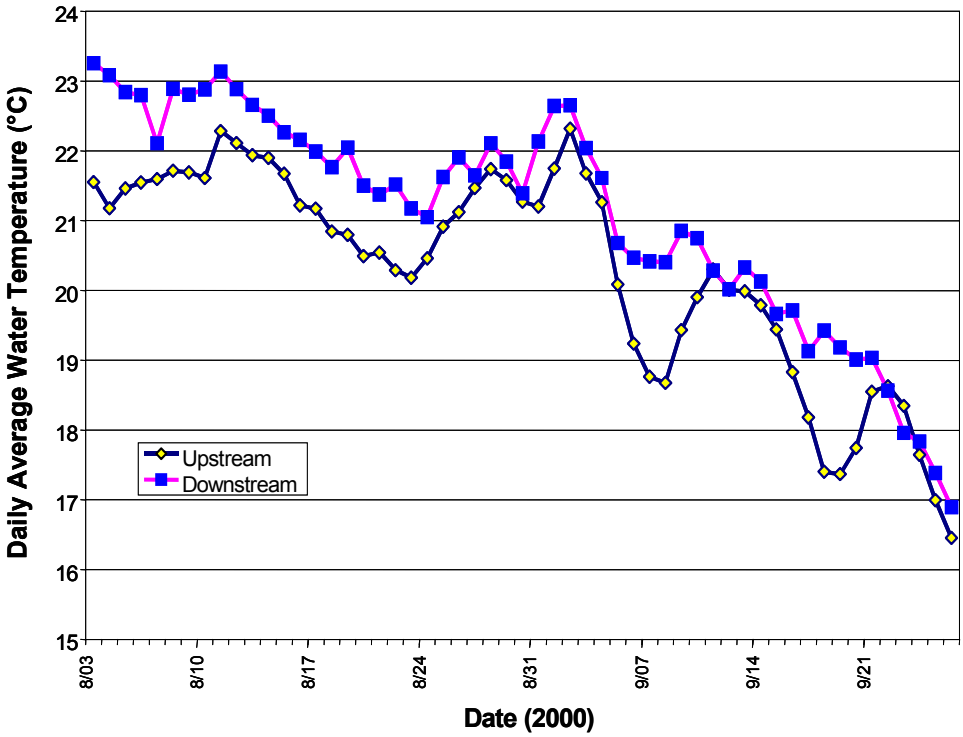

Figure 19. Daily average water temperatures at upstream and downstream dioxin stations.

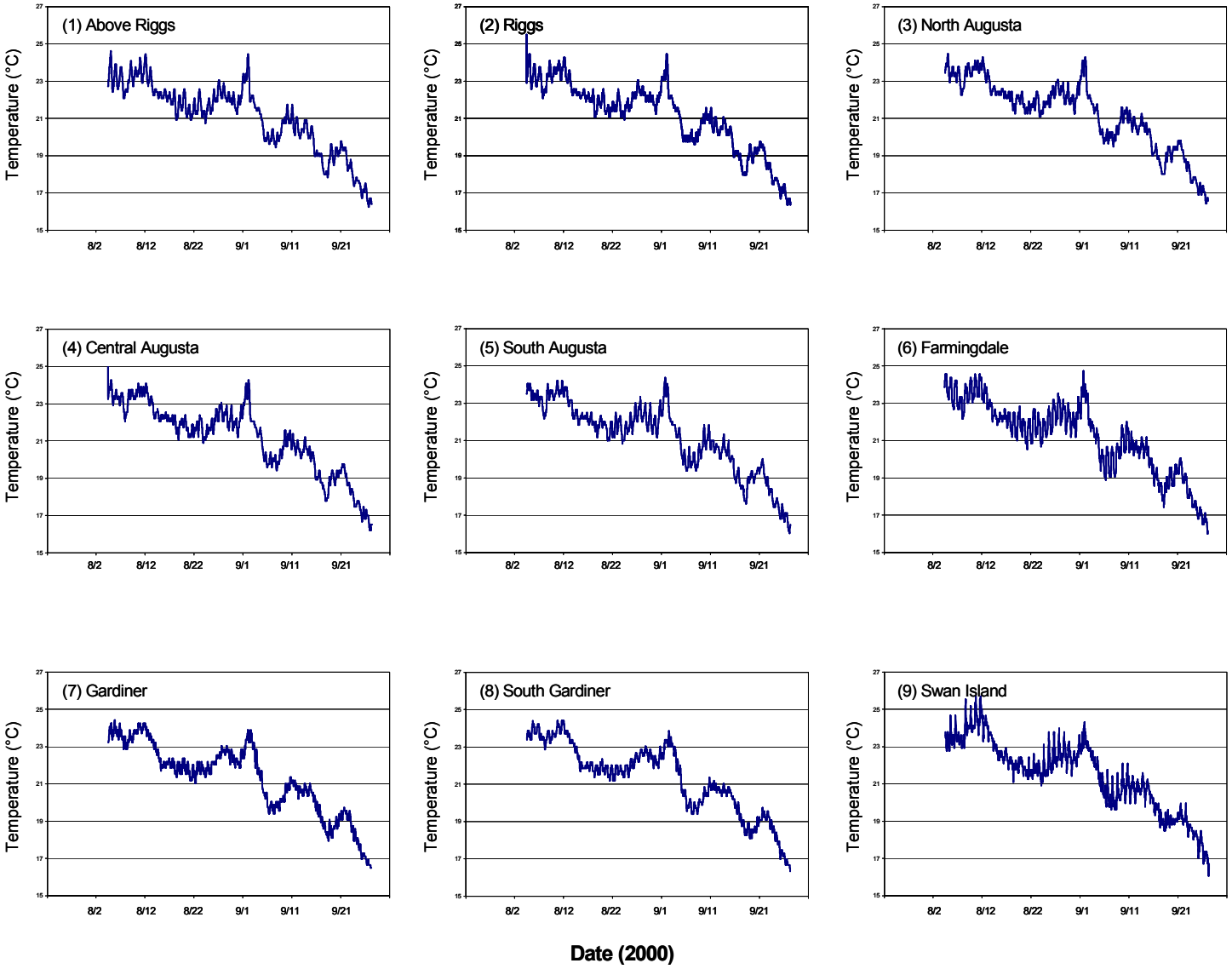

Figure 20. Water temperature profiles for Kennebec PCB stations.

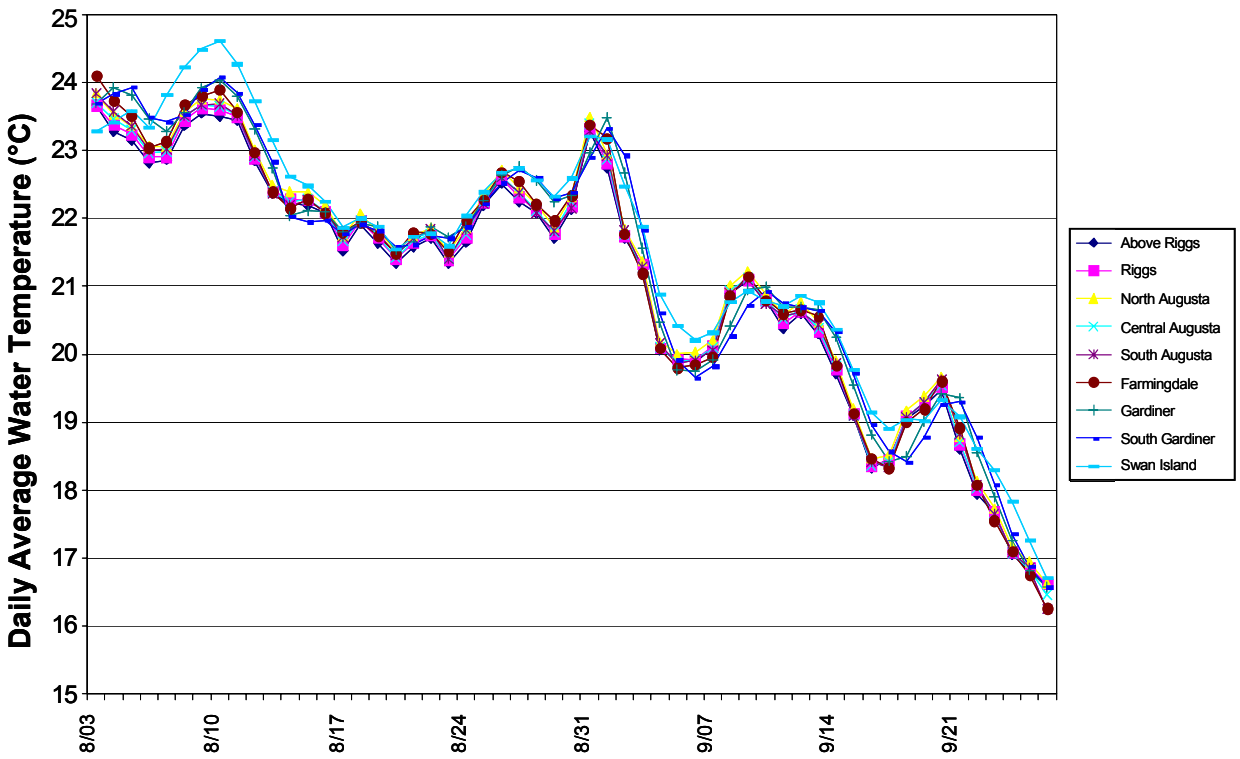

Figure 21. Daily average water temperature profiles at Kennebec PCB stations.

5.0 DISCUSSION

The primary criterion used by DEP for deciding whether or not caged mussels would be a useful monitoring tool for dioxins and furans was to demonstrate a significant difference in dioxin/furan accumulation between upstream and downstream stations. Stations were selected based on areas where fish were previously collected and showed differences in dioxin/furan accumulation, and could also be collected in the current study for comparisons with mussel tissue chemistry. Stations selected by DEP for the caged mussel pilot study were positioned 11 to 13 miles from the suspected source because this was the nearest location where fish could be collected, and because previous fish monitoring had demonstrated significant differences in dioxins and furans when upstream and downstream stations were compared. Accumulation of dioxins and furans in fish tissues could represent exposure to contaminated sediment or food resulting from previous mill discharges and not current discharges. Fish may not be reliable indicators for station selection because of their mobility. Accumulation of dioxins and furans in fish reflects an integration of all exposure conditions encountered during their movement and migration in the river, not just the immediate area selected for upstream and downstream stations.

The decision-making criterion used by DEP was inappropriate for the experimental design used because it is uncertain whether there was really a difference in bioavailable dioxins and furans between the two stations. In addition, the design limited the relative position of the mussel cages and did not provide a true test of the methodology. The approach selected by DEP in this study may have been appropriate for direct comparisons with fish tissue chemistry, but it was inappropriate for a valid test of the caged mussel methodology. In order to evaluate the ability to detect differences between upstream and downstream stations, the downstream station should have been positioned as close as possible to the suspected source in order maximize potential downstream exposures. Similarly, the upstream station should have been as close as possible to the suspected source to eliminate the possibility of contamination from additional upstream sources.

The data require a much more intense analysis and interpretation before reaching conclusions on the utility of caged mussels as a monitoring tool in Maine. More information is provided regarding the utility of the caged mussel approach by evaluating accumulation of individual and lipid-normalized congeners, in addition to the total PCDD/PCDFs. Using only total PCDD/PCDFs in data interpretation (Mower 2001) results in a loss of information, in particular the details associated with bioavailable congeners and potential pathways of exposure. Although statistically significant differences were not found between upstream and downstream stations in the caged mussel dioxin/furan study, the mussels were as effective as fish in accumulating bioavailable dioxins and furans. The mussels accumulated a wider range of congeners than the fish, suggesting uptake from various exposure pathways. DEP also evaluated the utility of SPMDs during the 2000 monitoring study. The data support results from other studies that accumulation of organic chemicals in SPMDs primarily represents aqueous exposures from the water column. As the majority of dioxins, furans, and PCBs available to fish and other aquatic life are probably bound to particles, just measuring the aqueous fraction provides a partial estimate of bioavailability.

The mussels demonstrated their effectiveness as biomonitoring tools because they accumulated many dioxin and furan congeners both upstream and downstream of the mill,

showed some differences in upstream and downstream exposures, and identified hotspots of PCB contamination. It is promising that any dioxins and furans were even detected because these stations were situated 13 and 11 miles from the mill, respectively. Although the mussels at the downstream station had a higher mean total PCDD/PCDF concentration, the difference was not statistically significantly. Surprisingly, the data suggest that there is a dioxin/furan source further upstream on the Kennebec River that has affected exposure in the vicinity of the "upstream station." This is important because it suggests that DEP may require additional monitoring further upstream to identify the sources of these contaminants. If available, dioxins and furans originating from the SAPPI pulp and paper mill may have become too dilute at the downstream station to be statistically and environmentally different than concentrations measured at the upstream station. The downstream station was probably too far from the source to answer this question, and the upstream station was apparently impacted by other sources.

The caged mussel methodology demonstrated its utility by identifying concentrations of PCB as high as 125 ppb and 188 ppb along the stretch of the Kennebec where PCB contamination was suspected, meeting objectives established by FOMB for the PCB study. Concentrations in most other mussel tissues were in the range of 20 to 60 ppb. Since PCBs are in the same class of chemicals (i.e., organochlorines) as dioxins and furans, it would be expected that mussels would accumulate dioxins and furans at proportionately similar concentrations if they were deployed at similar distances away from the sources and the sources were proportional.

5.1 Mussel Tissue Chemistry

5.1.1 Dioxins and Furans

Mussels accumulated many individual dioxin and furan congeners although 10% of reported congeners were present at concentrations slightly below the method detection limit. Most of the total dioxin/furan concentrations in mussel tissues from both upstream and downstream stations were between 2 and 8 ng/kg-wet. This is not surprising since the mussel cages were placed approximately 13 and 11 miles upstream and downstream from the mill, respectively. These results are promising if the test were designed to evaluate the limits of detection at various distances along the suspected chemical gradient of dioxins and furans away from the mill. Assuming an exponential rate of dilution, it might be speculated that concentrations of dioxins and furans in the vicinity of the mill would be one or two orders of magnitude higher if the mill were truly the source of the dioxins and furans measured in this study.

The caged mussels did not meet the primary criterion by the DEP in that there must be a significant difference in dioxin/furan accumulation between the upstream and downstream stations for the mussels to be considered useful. Distance and dilution have likely caused the high variability in the replicates and are attributable to site selection by DEP. One purpose of using caged mussels was to reduce the variability observed in fish studies. Unfortunately, the distance from the suspected source was so great that exposure concentrations were extremely low and near the limit of detection, thus, introducing the variability that was supposed to be avoided. These uncertainties alone provide additional evidence that the caged mussel pilot study should be repeated.

However, the data show that mussels accumulated chemicals that were present, and that there may not be significant differences in exposure at the two locations where mussels were deployed. There may be an additional source of dioxins and furans upstream, and the mussels were sensitive and successful at accumulating these compounds. The mussels, unlike fish, were deployed at a fixed location for a specific duration, and their tissue chemistry reflected site-specific exposure conditions. Although DEP may find statistically significant differences between upstream and downstream concentrations of dioxins and furans in fish, the reasons for those differences are unclear. By looking at accumulation of individual dioxin/furan congeners by mussels, it is clear that they are exposed to and accumulating nearly all congeners. It is the chemical structure and molecular weights of these specific congeners that affect bioavailability, bioaccumulation, and toxicity. The data from this study are consistent with results from other studies suggesting that the mussels are accumulating dioxins and furans from aqueous, particulate, and dietary exposure pathways.

With respect to the utility of using caged mussels as a monitoring tool, the methodology demonstrated its effectiveness by identifying elevated concentrations of dioxins and furans both upstream and downstream of the SAPPI mill at Hinckley, as well as PCBs in the lower Kennebec. The elevated concentrations of the most predominant furan congeners (2378-TCDF and 123789-HxCDF) downstream suggest the SAPPI mill could be a source of these congeners. Although the concentrations of these congeners in the upstream mussels is slightly lower, the concentrations are significantly elevated above T_0, suggesting there is another source of furans upstream of Norridgewock where the mussels were deployed. This is important because it has affected, at least in part, the ability to detect a statistically significant difference between upstream and downstream of the mill. Similarly, concentrations of the most predominant dioxins (1234678-HpCDD and OCDD) were higher upstream than downstream and provide additional evidence of another source of dioxins north of Norridgewock.

5.1.2 PCBs

The objective of the PCB caged mussel pilot study was to help identify specific contaminated areas, or hotspots, along one suspect reach of the Kennebec River. It is important to note the difference between identifying hotspots and monitoring point sources such as pulp mill effluents requires different sampling strategies. Because of the single point source from the pulp mill at Hinckley, caged mussels (and SPMDs) were deployed at one upstream and one downstream station. A more diffuse monitoring design was appropriate for the PCB study because multiple hotspots of PCB contamination were expected. As in the dioxin/furan study, the mussels had high survival, positive growth rates, and accumulated PCBs.

Most of the total PCB concentrations in mussel tissues were between 20 to 60 ug/kg and well above the fish tissue action level (FTAL) of 11 ug/kg. The highest concentrations were more than an order of magnitude above the FTAL. The highest concentration of total PCBs (188 ug/kg) was measured in mussel tissues from midstream just below the Augusta Sewage Treatment plant at South Augusta and in the vicinity of a midstream outfall pipe (Ed Friedman, personal communication). The second highest concentration of total PCBs (125 ug/kg) was measured in mussels deployed on the west side of the Kennebec River, just

below the former Williams gravel/asphalt facility (now Ferraiolo) in Farmingdale. This facility contains a large unlined pit of leaky oil and water, leaky asphalt pipe valves, and a number of 3-phase motors (Ed Friedman, personal communication). New transformers are also on-site, but the disposition of the old transformers is unclear. There is an aquifer under this site and a stream that flows through the site which discharges to the Kennebec (Dennis Kinney, personal communication). This facility has been operating at least since the 1940's and is a potential source of PCBs. This information on two potentially significant sources of PCB contamination has been provided to DEP by FOMB and they are both continuing to investigate.

As part of this mussel study, a limited analysis of congener-specific PCBs was conducted because a suite of only 20 congeners were quantified and reported by DEP. None of the reported PCBs have dioxin-like TEQs, which provide a means of estimating potential toxic effects. Certain PCBs are extremely toxic in chronic exposures. The most toxic PCBs are those that closely mimic the potency and mechanism of toxicity of 2,3,7,8-TCDD (one of the most toxic compounds known). These PCBs can cause toxic symptoms similar to those caused by dioxin exposure, including developmental abnormalities, disruption of the endocrine system, impairment of immune function, and cancer promotion.

DEP representatives could not explain the reasons for selecting the 20 congeners measured as part of this caged mussel pilot study and those commonly measured as part of their regular monitoring program. In the future, DEP reports should include the rationale for selecting the particular PCB congeners reported and their potential environmental significance. It seems inefficient to go to the time and expense of congener-specific analysis and not quantify PCB congeners of potentially more environmental significance.

The identification of hotspots of contamination is one of the stated purposes of several mussel monitoring programs that use both indigenous and caged bivalve species (Ontario Ministry of Environment, California Mussel Watch, NOAA mussel watch). Although it could be argued that the use of fish is not appropriate for upstream and downstream comparisons for dioxin/furan monitoring, there is general agreement with respect to the inappropriateness of fish for monitoring isolated and discrete pockets of contamination over small spatial scales on the Kennebec River. Monitoring chemicals in fish tissues is more appropriate for consumption advisories. One of the most obvious advantages of caged mussels (and SPMDs) over fish is the ability to place them along suspected chemical gradients or in the vicinity of suspected sources. The ability to monitor and assess small-scale, microgeographic exposures and effects with caged and indigenous mussels in freshwater and marine environments has been well-documented (Green et al. 1985, Salazar and Salazar 1995). Another reason for deciding to use caged mussels for PCB monitoring in the Kennebec River is that chemical analysis of discrete water samples is generally assumed too variable to be environmentally significant, fish are too mobile, and there are no significant amounts of surficial sediment to collect and measure in this area of the Kennebec. Therefore, caged mussels, SPMDs, or caged fish were the most viable approaches; caged mussels have the longest history of application for these types of assessments.

5.2 Mussel Survival and Growth

The main purpose of measuring survival and growth in this caged mussel pilot study was to demonstrate that the test mussels were in sufficiently good health to accumulate the chemicals of concern; i.e., dioxins, furans, and PCBs. Given the high survival and significant increase in tissue weight, it is concluded that the mussels would have accumulated chemicals at concentrations representative of exposure conditions on the Kennebec. Changes in whole-animal wet-weight and shell weight were not expected due to large size and slow growth rates associated with this species. Nevertheless, for the dioxin/furan study, changes in whole-animal wet-weight were higher at the upstream stations than the downstream stations, but these differences were likely related to differences in physical-chemical factors rather than dioxins and furans because the concentrations of these chemicals were higher upstream than downstream. Although the upstream tissue weights at the end of the test were higher than those at the downstream station, these differences were not statistically significant. As suggested previously, if there were correlations between mussel growth rates and tissue burdens of dioxins and furans, they would be more meaningful if they could have been established along a chemical gradient rather than one discrete location at a distance of 11 to 13 miles from the mill.

For the PCB study, both growth rates and tissue weights were lowest at South Augusta when compared to other stations and highest at Gardiner and South Gardiner. Mussels at all stations except South Augusta had significant increases in tissue weights. The combined low growth rates, low tissue weights, and high PCB concentrations measured in mussel tissues at the mid-river location suggest a correlation between high tissue burdens and decreased mussel growth. A similar relationship between low growth, low tissue weight, and high PCB concentration in mussel tissues was found at Farmingdale. Low growth rates at South Augusta and Farmingdale do not appear to be related to water temperature.

5.3 Water Temperature

Temperature did not appear to be a significant factor that influenced survival, bioaccumulation or growth in this caged mussel pilot study, although there was a significant difference in daily average water temperature between the upstream and downstream dioxin stations. The downstream station was significantly higher that the upstream station, although the means were extremely close. Mean temperatures were even closer among the nine PCB stations.

5.4 Caged Mussels as a Monitoring and Assessment Tool

The data from this study and from hundreds of studies conducted worldwide suggest that caged mussels are a useful and meaningful monitoring tool. The most important concept to remember is that there are no perfect monitoring and assessment tools, and each has its own advantages and disadvantages. The most successful monitoring program integrates elements such as those represented by fish, caged bivalves, and SPMDs. Based on a weight of evidence evaluation of the data from this study, it is concluded that caged mussels are a potentially useful tool for monitoring dioxins, furans, and PCBs in the state of Maine. This is the opposite conclusion to that reached by DEP in their 2000 dioxin monitoring report (Mower 2001).

"Of all the test types (large and small bass, large sucker filets and whole fish, sucker liver composites, freshwater mussels, and SPMDs) tested in 2000, only the fish and livers were able to detect significant differences between stations above and below some bleached kraft pulp and paper mills. Freshwater mussels and SPMDs did not detect any differences. SPMDs were tested again in 2001 with an enhanced sample design that may lead to improved capability to detect differences. Freshwater mussels did not appear to be a useful monitoring device, perhaps because they are at a lower trophic level than fish. MSDs were generally lower for bass than for suckers or livers. Neither liver nor mussel studies were repeated, but studies with fish were repeated in 2001."

While it is true that the total PCDDs/PCDFs were significantly higher in smallmouth bass downstream than upstream, they were lower downstream on a lipid-normalized basis. It is probably most appropriate to compare the concentrations among mussel tissues, SPMDs, and fish using the lipid-normalized data. The DEP interpretation is also opposite the interpretation reached in this report. The possible reason for this discrepancy may be the method used for calculating the mean total dioxin/furan concentrations. DEP only used measured values to calculate the mean whereas "zero" is substituted for undetected values in this report. This latter approach is the one most commonly used in fish and mussel monitoring programs conducted at national, state, or regional levels. Some monitoring programs use half the detection limit, but no other studies could be found where non-detects are completely rejected and not included in calculating the mean. Algorithms are available for estimating the values that might be replaced for non-detects.

Using fish collected at locations upstream and downstream of pulp mills to characterize exposure conditions is complicated by several major factors. Three issues have been identified with respect to using fish to monitor dioxins and furans to satisfy the upstream versus downstream requirement: 1) fish of different ages in same species may contain different concentrations of dioxins and furans, 2) different fish species may bioaccumulate dioxins and furans at different rates and may attain different body burdens at steady state or different stages of reproduction with different lipid levels, and 3) fish are mobile and the source of the accumulated chemicals cannot be guaranteed (Shoven et al. 2001). The Natural Resources Council of Maine (NRCM) explicitly states that a fish monitoring program as currently conducted is not adequate for quantifying differences in dioxin and furan exposures at upstream and downstream locations, primarily for the reasons cited above. The NRCM further suggests that the uncertainty in the fish tissue chemistry data will not be resolved and will lead to future debate regarding the environmental significance of these data. The NRCM concludes that DEP has not yet developed an appropriate fish monitoring program for compliance with the 1997 law (Bennett 2001).

5.4.1 Comparison of Caged Mussels, SPMDs and Fish

The main advantage of using caged mussels as a monitoring and assessment tool is their ability to accurately quantify chemical exposure and associated biological effects over space and time and under environmentally realistic conditions. Another advantage is that they can be strategically placed along suspected chemical gradients to confirm the source of chemical exposure, allowing comparisons to be made, such as those required by regulations regarding pulp and paper mill emissions in the state of Maine. Unfortunately, a gradient design was not tested in this pilot study. The stated purpose of the DEP was to

evaluate mussels and SPMDs relative to fish, and not to explore the various advantages of caged mussel biomonitoring. Caged mussel monitoring can also monitor chemical exposure over time and establish the status and trends of dioxin, furan, and PCB contamination in a historical context.

Mussels accumulated more dioxin and furan congeners than either fish or SPMDs. It was surprising to find that fish from the upstream station only accumulated five dioxin/furan congeners and those from the downstream station only accumulated four. In this respect, it could be argued that mussels were actually a better monitoring tool than fish. In terms of total PCDD/PCDFs, the mussels were much more similar to fish than the SPMDs. SPMDs accumulated approximately 2.5 times higher concentrations of dioxins and furans than mussels and 3.8 times higher than fish at the upstream station. At the downstream station, SPMDs only accumulated about 50% more dioxins and furans than either fish or mussels. While it could be argued that this is evidence that SPMDs are superior accumulators, the data from living organisms such as mussels and fish are probably more environmental realistic and relevant. In addition, other studies have shown that SPMDs primarily accumulate lower molecular weight compounds. This interpretation is also consistent with the congener data presented in this study. At the upstream dioxin station, for example, SPMDs accumulated a concentration of 2378-TCDF that was 5.8 and 7.5 times higher than mussels or fish, respectively. The measured concentration of 2378-TCDF in the upstream SPMDs represents 68% of all furan congeners measured. At the downstream dioxin station, SPMDs accumulated a concentration of 2378-TCDF that was 6.1 and 3.7 times higher than mussels or fish, respectively.

Although it may appear that the SPMDs are efficient at accumulating dioxins and furans, it is important to accurately interpret the data in light of the method detection limits. The only congener that was measured at concentrations equal to or greater than the detection limit was 2378-TCDF. Only 12% of all reported concentrations were equal to or greater than the detection limit, with the other 88% reported concentrations less than 1/10 of the detection limit. The general rule of thumb in interpreting "estimated" data is to put more weight on values that are within 50% of the detection limit, and values less than this are considered extremely unreliable. For all practical purposes, the SPMDs only accumulated one congener. If this congener was absent and the others dominated, it is unclear if the SPMDs would accumulate anything.

There are many different monitoring tools, and each tool has appropriate applications and uses, and advantages and disadvantages (Table 16). SPMDs are potentially useful as screening tools for assessing soluble components in the water column. Caged bivalves are useful for characterizing exposure conditions and quantifying bioavailable chemicals. Resident fish are useful for developing fish consumption advisories and monitoring compliance. They are less useful for the upstream/downstream comparisons required by state law. SPMDs and caged bivalves produce complementary data sets, however, they do not appear to be directly comparable on a congener-specific basis (Peven et al. 1996). An integrated monitoring approach using the most appropriate tools provides information on both the bioavailable (bivalves) and water column (SPMD) concentrations of the analytes of interest. The data from the caged mussel pilot study suggests that SPMDs tend to preferentially accumulate the lower molecular weight dioxins and furans while mussels may tend to preferentially accumulate the higher molecular weight congeners.

Table 16. Advantages and disadvantages of caged bivalves, SPMDs and fish as monitoring and assessment tools.

	Advantages	Disadvantages
Caged bivalve transplants	Experimental control Environmental realism Characterization of exposure Characterization of effects Status & trends monitoring Large bioaccumulation database Aqueous & particulate pathways Link between lab & field testing Integration of bioavailability Integration of effects Little or no metabolism of chemicals Large toxicity database	Natural factors can affect responses Effects of caging & transplanting Loss of cages (theft, vandalism, nature) Cost & time of collection Cost & time of measurements May not be most sensitive species Preferential accumulation of some groups No direct assessment of community Not found in all areas Only conduct tests when not reproducing Potential effects on indigenous populations Potential introduction of exotic species
SPMD transplants	Experimental control Characterization of exposure Status & trends monitoring Aqueous exposure Link between lab & field testing Integration of exposure Minimal effects of natural factors Commercially available Minimal setup time Minimal labor Easy to transport long distances	Contamination during caging & transplanting Loss of cages (theft, vandalism, nature) Little environmental relevance No measurements of effects Preferential accumulation of some groups Effects of fouling and current speed Only aqueous exposures Relatively small database
Natural fish populations	Environmental realism Characterization of exposure Characterization of effects Status & trends monitoring Large database Aqueous & dietary pathways Link between lab & field testing Integration of bioavailability Integration of effects Commercial & recreational importance Direct human health implications	Uncertain exposure due to mobility Often difficult to collect in sufficient number Difficult to collect similar size ranges Dietary exposure may represent previous inputs from mill, not current effluent Can only collect 11 miles from this mill Effects of reproduction on sampling Effects of sampling on populations Time consuming and expensive to collect Different species in different rivers Metabolism of some chemicals

5.4.2 Risk Assessment-based Monitoring

There is increasing support for using more integrated approaches in environmental assessment programs (Chapman 1996, Hall 1996). However, this integration should be based on approaches best suited to answer the questions posed by the monitoring model. The risk assessment framework provides a very focused approach to environmental assessment and monitoring of chlorinated hydrocarbons because it includes characterizations of both exposure and effects (Carey et al. 1998). Measuring exposure and effects in natural populations and caged organisms provides a realistic approach to evaluate the success of environmental regulations and resulting mill process changes. However, the issues are complex and appropriate field monitoring methodologies are still being refined. The following have been identified as necessary improvements: 1) the capability to detect effects and establish causal relationships; 2) integration of chemical, biochemical, population-, and ecosystem level measurements; and 3) better sampling designs to account for temporal and spatial variability (Carey et al. 1998).

The recently proposed exposure-dose-response (EDR) triad (Salazar and Salazar 1995, 1998) facilitates those characterizations. With the EDR triad approach, exposure is characterized through the chemical analysis of environmental media (i.e., water and sediment) and biological tissues. Effects are characterized through bioassays and community structure studies, both of which are conducted in the lab and in the field. Using caged bivalves facilitates the field bioassay element of the EDR triad. Through synoptic measurements of bioaccumulation and growth, uncertainties associated with exposure and effects can be reduced. The methods for using field bioassays with caged bivalves have been refined to facilitate synoptic bioaccumulation and growth (ASTM 2001, Salazar and Salazar 1995). Growth is the recommended effects endpoint; in bivalves it is easily measured and understood. Growth represents an integration of all internal biological processes and can be quantified as a dose-response. Bivalve growth data can be readily extrapolated to potential population effects.

Bivalves are commonly used as biological indicators of exposure because of their ability to concentrate and integrate chemicals from water and sediment in their tissues (Metcalfe and Charlton 1990, Phillips and Rainbow 1993) and the utility of caged bivalve transplants in monitoring (de Kock and Kramer 1994). Field bioassays with caged bivalves combine the advantages of experimental control from standard laboratory bioassays with the environmental realism from traditional field monitoring. Strategic placement of caged bivalves along chemical gradients facilitates more environmentally representative descriptions of chemical exposure over space and time than water or sediment samples. The integrating power of bivalve filtration helps to normalize the variability associated with quantifying pulp and paper mill effluents and their receiving waters. These factors include intermittent and variable discharges, variability in the direction and velocity of water currents, and natural factors such as storm events, episodic sedimentation, and runoff. All of these factors affect chemical exposure and associated biological effects and have been addressed previously (Beck 1996, Whitfield and Wade 1996). A single chemical analysis of bivalve tissue provides an integrated record of bioavailable chemicals that cannot be defined with thousands of water or sediment samples. Chemicals in bivalve tissues, which can be referred to as the "dose," provide a direct link between chemical exposure and associated biological effects. It also provides a way to compare the results of bioassays and population or community responses in the field.

In the late 1980s, Swedish scientists were among the first to document that fish collected in the vicinity of bleached kraft pulp mill discharges exhibited chronic sublethal effects such as altered growth rates, carbohydrate metabolism, maturation, recruitment, mortality, and community structure (Servos et al. 1996). It is interesting to note that caged bivalves were already being used on a regular basis for monitoring exposure to chemicals associated with freshwater discharges for several years in Canada (Richman 1997) and Finland (Herve et al. 1996) before this discovery. This is one of the first examples of the dichotomy that still exists today with respect to using bivalves to characterize exposure by measuring bioaccumulation of chemicals of concern in their tissues and using fish to characterize effects by measuring various internal health parameters and community structure. Following those early reports of effects on fish in Sweden, similar effects were reported at a number of pulp mills in the Canada and the US (Servos et al. 1996). Collectively, the potential ramifications of these reported effects on fish led to a series of meetings, increased monitoring and regulations to reduce the discharge of dioxins and furans throughout the

world. However, the complexity of these process changes, effluent discharges, and receiving environments have made it difficult to establish a causal relationship between reductions in dioxins and furans and improved fish condition. Many recent studies have shown that altered fish physiology and biochemical composition still occurs, even after elimination of dioxins and furans. These results suggest that it is the natural constituents in wood that are responsible for acute and chronic toxicity as well as biochemical and physiological effects. It has been suggested that low molecular weight PAHs may be causing the observed effects in fish (Hodson 1996). DEP is attempting to structure their dioxin/furan monitoring program against this complex history of exposure and effects monitoring at pulp and paper mills.

Although caged bivalves have been used to monitor organochlorines in countries around the world such as Argentina (Colombo et al. 1997), Australia (Haynes et al. 1995), Brazil (Furley and Oliveira Filho 2000), France (Hayer and Pihan 1996a,b), Germany (Huhnerfuss et al. 1995), Hong Kong (Kannan et al. 1989), Japan (Miyata et al. 1987), New Zealand (Burggraff et al. 1996), Russia (Stepanovaa et al. 2000), and Sweden (Bergkvist et al. 1998), the discussion will focus on studies with the most extensive freshwater monitoring over the longest period of time. In Finland, the emphasis has been on pulp and paper mill monitoring, whereas in Canada monitoring has been used for a variety of sources, and in the US a variety of approaches have been used in marine and freshwater environments.

5.4.2.1 Monitoring in Canada

It is important to remember this distinction in a risk-based monitoring strategy between using fish or bivalves as indicators of exposure, indicators of effects, or both. Caged bivalve monitoring for pulp and paper mills in Canada began in with measuring effects such as growth in oysters (Quayle 1964). Subsequent studies with caged marine mussels deployed adjacent to a pulp and paper mill outfall in Canada measured effects on growth and reproduction (Wu and Levings 1980). These results were correlated with a previous study showing reduced densities of natural mussel populations near the outfall (Levings and McDaniel, 1976).

Canadian scientists were among the first to develop a generic monitoring approach for and justify the use of *Elliptio complanata* as a useful monitoring tool (Curry 1977). Subsequently, several studies have been conducted using *Elliptio complanata* for that purpose (Kauss and Hamdy 1985, Koenig and Metcalfe 1990), as well as comparing accumulation in tissues of mussels and leeches (Metcalfe and Hayton 1989).

Although all caged bivalve monitoring in freshwater in Canada between 1980 and the present was not necessarily associated with pulp and paper mills, organochlorines have been measured in *Elliptio complanata* using the in-situ transplant method for over 20 years as part of a regional monitoring program developed by the Ontario Ministry of the Environment (Hayton and Hollinger 1989a,b, Hayton et al. 1990, Anderson et al. 1991, Richman 1992 1997, Ontario Ministry of the Environment 1996 1999). All of these studies have focused on characterizing exposure by measuring concentrations of organochlorines, such as dioxins and furans, in freshwater mussel tissues.

Initial draft plans for environmental effects monitoring (EEM) at pulp and paper mills in Canada included caged bivalves and measurements of tissue chemistry and growth (Parker et al 1991), but this approach was not required in the first cycles of EEM. Additional studies advocated using caged bivalves to characterize exposure and effects associated with pulp and paper mill effluents and provided the rationale for this approach (Salazar and Salazar 1997). The first integrated monitoring study at a pulp and paper mill with caged bivalves was conducted at Port Alice, Vancouver Island, in 1997 (Applied Biomonitoring 2000). In this study, mussel growth metrics were measured as effects endpoints, as in the Kennebec River study. Several resin acids and plant sterols were also measured in mussel tissues as exposure endpoints. However, in contrast to the Kennebec River study, the Port Alice study used a gradient design, and a significant inverse relationship was established between campesterol in mussel tissues and mussel growth rates. Both of these endpoints were also correlated with distance from the mill. A similar study was conducted in Pictou Harbor by Environment Canada using the same methods (Andrews and Parker 1999). As a result of these two pilot studies and the acceptance of the caged bivalve protocols by ASTM, Environment Canada accepted caged bivalves as an alternate method for the required adult fish survey at all pulp and paper mills in Canada as part of environmental effects monitoring. The caged bivalve methodology is an integrated, risk-based approach that allows simultaneous collection of exposure and effects information.

Adult fish surveys have been required as part of environmental effects monitoring in Canada since 1994 (McMaster et al. 2002). However, only effects endpoints related to fish health are measured as part of this program. Extensive development on fish survey methods has occurred (Munkittrick et al. 2000), and these methods are currently being used throughout Canada. The major shortcoming of measuring only effects endpoints in fish or other species is that there is no confirmation that exposure has occurred or where it has occurred. One reason that exposure endpoints such as bioaccumulation of dioxins and furans in Canadian fish has not been used in association with pulp and paper mill effluents is that, even though effects continue to occur, mill discharges of dioxins and furans are essentially undetectable (reference). It has been suggested that some low molecular weight PAH compound is causing the observed effects in fish (Hodson 1996).

Many people do not understand that invertebrates such as freshwater mussels have endocrine systems that are subject to the same disruption as in fish (deFur et al. 1999). In addition to studying endocrine disruption in fish, Environment Canada is also developing bivalve biomarkers as a complementary monitoring tool (Blaise et al. 2002, Gagne et al. 2000 2001a,b,c). Applied Biomonitoring has participated in these cooperative studies with Environment Canada by helping them sort, distribute, cage, and transplant *Elliptio complanata* upstream and downstream of a municipal effluent in Montreal during 1999, 2000, 2001, and will help in another study planned for May 2002. Other Canadian studies have documented physiological and biochemical changes associated with exposure to organic chemicals (Day et al. 1990).

5.4.2.2 Monitoring in Finland

Caged bivalves and indigenous fish populations have been used in Finland to monitor exposure and effects from freshwater pulp and paper mill effluents since approximately 1985 (Heinonen et al. 1986, Herve 1991, Herve et al. 1988 1996 Koistenen et al 1997, Makela

et al. 1992, Pellinen 1994) and some studies have compared accumulation of organochlorines in mussels and SPMDs (Herve et al. 1995). However, most of the bivalve monitoring has been for exposure and most of the fish monitoring for effects. The freshwater unionid mussel (*Anodonta piscinalis*) has proven useful for this type of monitoring because of its ability to survive even under adverse conditions and its high uptake rates of lipophilic persistent pollutants. Altogether, 20 freshwater sites downstream of the pulp and paper industry are included as part of the National Monitoring Program of harmful substances. In studies where bioaccumulation of chlorinated hydrocarbons in caged mussels and natural fish populations have been compared, results have been variable depending on the specific compound being measured as well as the site (Rantio et al. 1996). Most effects monitoring in fish has paralleled the development of endocrine disruption endpoints similar to those developed and routinely measured in Canada (McMaster 2002).

5.4.2.3 Monitoring in the US

Some of the earliest and most innovative caged bivalve monitoring approaches were developed in the state of California and provided important information on the fate and effects of organochlorines associated with an ocean outfall (Green et al. 1986, Young 1982, Young and Heesen 1974, 1977, Young et al. 1976, 1977, 1978, 1988, 1991). One of those findings was a demonstration that contaminated sediments were the primary source of DDT and PCBs and not the water column exposure. This is extremely important relative to DEP being able to make the distinction between exposures associated with current mill discharges versus previously contaminated sediments.

The State of California has been using caged and indigenous marine mussels to monitor chemical exposure since 1977 and is the longest running mussel watch program in the world (Martin and Severeid 1984). Freshwater clams (*Corbicula fluminea*) were added to the monitoring program in the early1990s. The National Oceanic and Atmospheric Administration (NOAA) has been monitoring chemicals in marine mussel and oyster tissues since 1986 (O'Connor et al. 1994). Freshwater mussels (*Dreissena polymorpha*) were added to the program in the late 1990s. The San Francisco Estuary Institute administers a regional monitoring program that includes caged and indigenous freshwater and marine bivalves and has been collecting data since the mid 1990s (Gunther et al. 1999). The USGS conducts regular surveys of chemicals in natural populations of the freshwater clam (*Corbicula fluminea*) tissues at a number of locations throughout the US (Schmitt and Dethloff 2000). All of the above are exposure-based monitoring approaches and do not include effects measurements.

There are also several individual chemical monitoring studies conducted in the US for marine, estuarine, and freshwater environments (Brown et al. 1994). Some of these have been associated with monitoring marine outfalls in Massachusetts (Hall et al. 1995, Massachusetts Water Resources Authority 1993, 1994) and others with freshwater non-point sources (Pereira et al. 1996, Petreas et al. 1992).

In 1994, caged bivalve monitoring was required at a pulp and paper mill in southeast Alaska as part of their NPDES permit (EPA 1994). This resulted in measurements of dioxins and furans in the marine mussel (*Mytilus trossulus*) as well as five different growth metrics in 1996 and 1997 (EVS Consultants 1996 1997).

In addition to these integrated studies combining exposure and effects measurements, there is an increasing trend toward measuring bivalve biomarkers and histopathological changes in marine, estuarine, and freshwater environments associated with organochlorines (Cooper et al. 1989, Cristini and Cooper 1988). Some of these studies have been conducted by scientists at the University of Maine (Butler et al. 2001, Garling and Van Beneden 2001, Harring et al. 2001). USGS also has a program for monitoring freshwater bivalves in the vicinity of pulp and paper mill effluents (Kernaghan et al. 2001). The rarely used estuarine clam *Rangia cuneata* has also been used to monitor organochlorines (Harrel and McConnell 1995, Lunsford and Blem 1982).

Several studies have also compared the utility of caged bivalves and SPMDs for organochlorine accumulation in freshwater (Hayward et al. 1996, Prest et al. 1992) and marine environments (Hofelt and Shea 1997, Peven et al. 1996, Prest et al. 1995, Richardson et al. 2001). Some have even compared mussels, SPMDs, and fish (Bowker et al. 1995)

In addition to new trends in using freshwater bivalve biomarkers to assess potential exposure and effects, there is an increasing trend toward monitoring toxicity in adult, juvenile, and glochidial stages of freshwater unionid bivalves such as *Elliptio complanata* (Keller and Lydy 1997). This is important for DEP because some of these studies are demonstrating the sensitivity of freshwater bivalve toxicity testing. In several cases bivalves have been shown to be among the most sensitive test species and are driving the US EPA water quality criteria for some chemicals. Monitoring freshwater bivalves is also important because they are the most threatened and endangered species in North America (Naimo 1995).

5.4.2.4 Synthesis

A rationale has been presented for a risk assessment-based monitoring approach and examples given based on two of the longest running caged mussel monitoring programs that have focused on the measurement of organochlorines in mussel tissues associated with pulp and paper mills and other industries discharging them. A dichotomy has also been identified between using caged mussels and natural fish populations as indicators of exposure and indicators of effects. Measuring bioaccumulation and growth in caged mussels combines exposure and effects measurements as well as the advantage of experimental control of position, exposure period, and animal size range. Like fish, caged mussels also include the element of environmental realism. The caged mussel methodology has been placed in the context of an exposure-dose-response triad which integrates a variety of monitoring elements. This triad could include caged mussels, SPMDs, and fish. This combination could be used in a weight-of-evidence approach consistent with ecological risk assessment.

In a broader context, caged mussels could also become an integral part of the Surface Water Ambient Toxics (SWAT) Program. This program was developed to document the status and trends of toxic chemicals in Maine's surface waters and to assess the effects of these chemicals on human and ecological health. Caged mussels could fill a needed gap in this monitoring program that currently only includes effects monitoring (Davies et al. 1999). In the context of ecological risk assessment, the missing element is characterization of exposure. The current SWAT approach is based on characterization of effects. The

problem with this approach is exemplified in any program that focuses on either exposure or effects; i.e., without the weight of evidence from risk assessment-based monitoring, there is greater uncertainty in the results. An important element in Maine biomonitoring is monitoring benthic community structure, but there is no link to help establish causality. Even without caged bivalves, this element could be improved by measuring bioaccumulation in indigenous bivalves. The SWAT program includes an innovative experimental field approach similar to caged bivalves by using rock-filled baskets, riffle bags, and cones, but no characterization of exposure is included (Davies et al. 1999).

The caged bivalve methodology is consistent with the DEP strategy of assessing water and sediment quality through integrated biomonitoring. Equal emphasis, however, should be placed on developing a program that is more risk assessment based and includes the measurement of biological effects and tissue chemistry. Controlled field experiments with approaches such as caged mussels and riffle bags provide an experimental element to complement the observational monitoring currently emphasized by DEP. The risk assessment-based approach helps characterize and understand processes controlling bioaccumulation and associated biological effects. Routine monitoring without these elements essential to ecological risk assessment cannot establish causality. The opposite dichotomy occurs in the Gulfwatch chemical monitoring program established by the Gulf of Maine council for mussel watch monitoring using the marine mussel *Mytilus edulis* (Environmental Quality Monitoring Committee 1998). This program measures only chemical exposure and not associated biological effects, although caged mussels have been proposed to facilitate the addition of growth and health endpoints as measured in the Kennebec River caged mussel pilot study with *Elliptio complanata*.

Finally, to place the monitoring issues in a perspective of a smaller scale, it is appropriate to consider the dedication in the DEP Biomonitoring Retrospective (Davies et al. 1999): "*This work is dedicated to the smallest creatures, existing at the edges of our awareness. Through them we glimpse intricate realities other than our own, and we are reminded to stay humble."* Similarly, those intricate realities of nature cannot possibly be fully appreciated with characterizing exposure and effects in a risk assessment-based monitoring program such as the one conducted here. It is not the purpose of this report to suggest that one biological indicator is necessarily superior to another, but rather that an integrated risk assessment-based strategy is the most appropriate. This integrated program could include caged mussels, SPMDs, and natural fish populations.

6.0 CONCLUSIONS

1. The weight of evidence from the caged mussel pilot study and similar studies conducted all over the world suggest that the use of caged mussels is a useful monitoring tool.

2. There are no perfect monitoring and assessment tools and each has its own advantages and disadvantages.

3. A truly integrated monitoring and assessment program should include the elements represented by fish, caged bivalves, and SPMDs.

4. The caged mussel pilot study was conducted to compare dioxin and furan accumulation between fish, caged mussels, and SPMDs at stations upstream and downstream from a pulp and paper mill. This was not a test of the caged mussel methodology, but rather a very specific application dictated by the ability to collect fish at particular locations. Upstream and downstream stations were 13 and 11 miles, respectively, from the outfall. This did not evaluate one of the major advantages of the transplant methodology, i.e., transplanting bivalves and SPMDs along suspected chemical gradients.

5. Bivalves primarily provide estimates from aqueous, particulate, and dietary exposure pathways and better represent bioaccumulation from all those pathways while SPMDs primarily represent aqueous exposures from the water column and are better suited for comparison with water. As the majority of dioxins, furans, and PCBs available to fish and other aquatic life are bound to particulates and other materials, just measuring the aqueous fraction gives a biased estimate of bioavailability.

6. Although statistically significant differences were not found between upstream and downstream in the dioxin/furan study, the mussels were as or more effective than fish in accumulating bioavailable dioxins and furans as demonstrated by the wider range of congeners accumulated than the fish.

7.0 RECOMMENDATIONS

1. The caged mussel (and SPMD) pilot study should be repeated by transplanting mussels (and SPMDs) along a suspected chemical gradient beginning at a point close to the mill.

2. DEP should establish more specific performance criteria for comparing the utility of caged bivalves, fish, and SPMDs.

3. DEP should explicitly state the reasons for emphasizing certain dioxin and furan congeners in their evaluations and the reasons for including or excluding specific PCB congeners in their chemical analyses.

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