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.

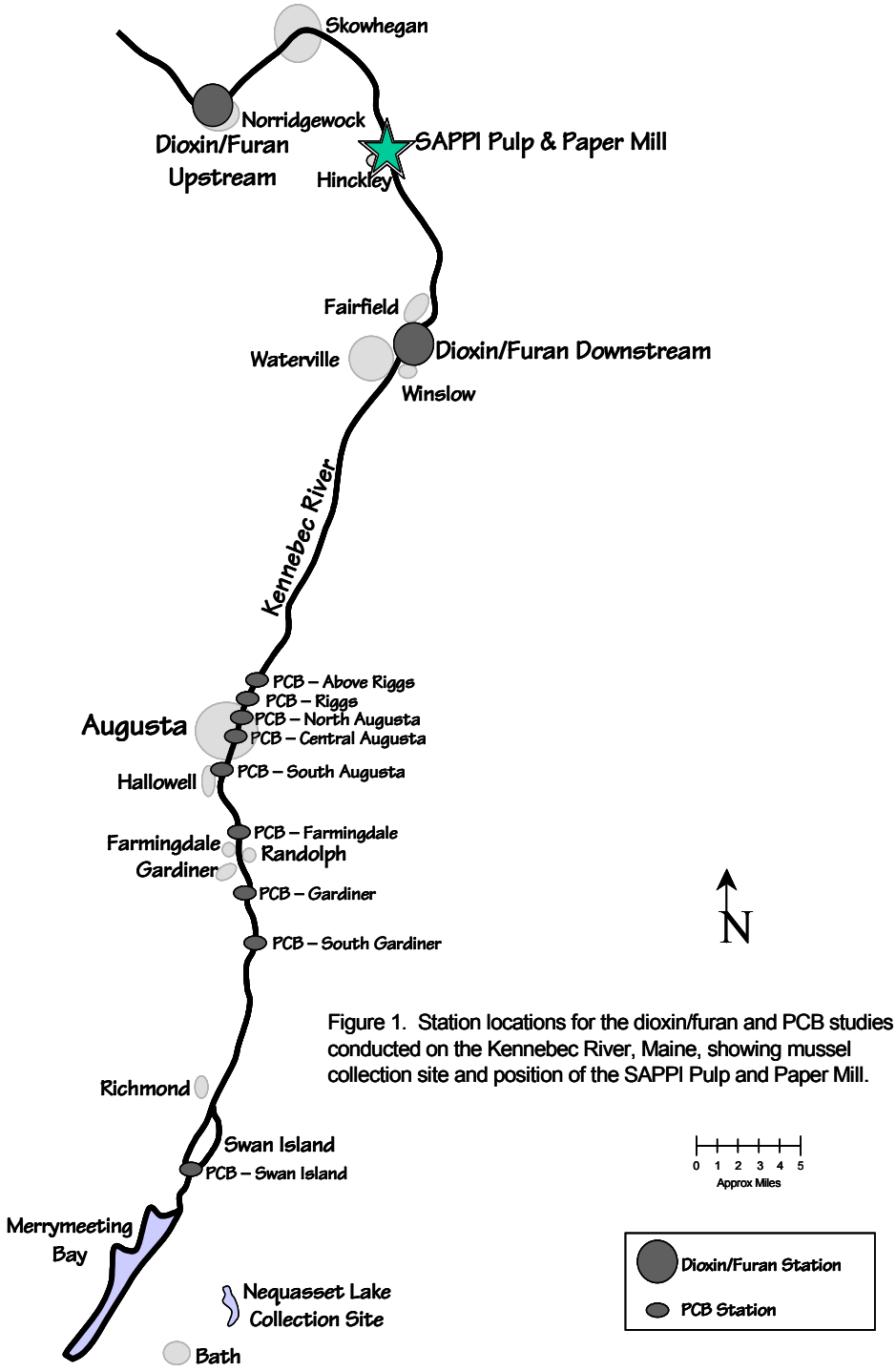

Figure 1. Station locations for the dioxin/furan and PCB studies conducted on the Kennebec River, Maine, showing mussel collection site and position of the SAPPI Pulp and Paper Mill.

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.

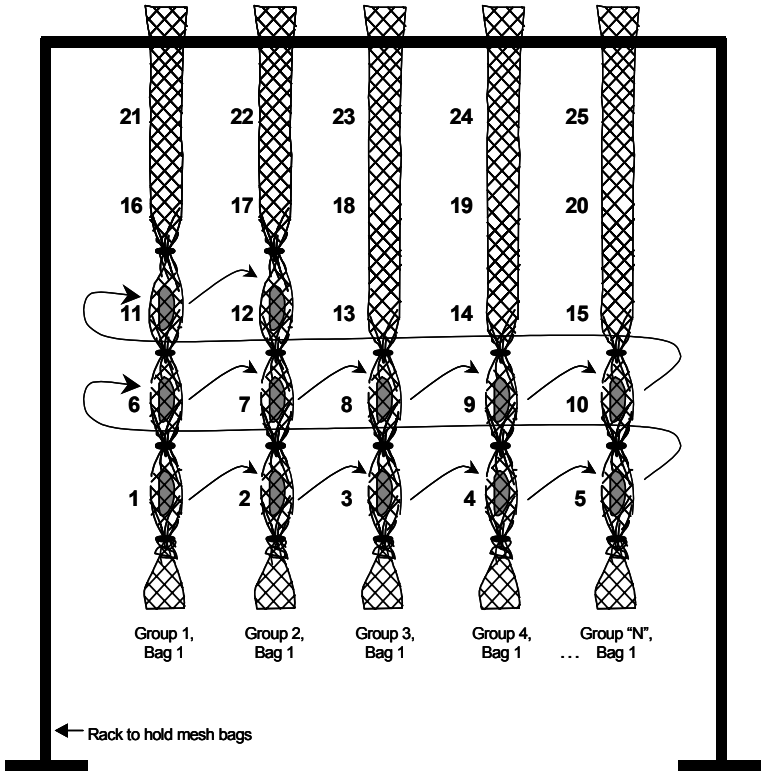

Figure 2. Mussel distribution process.

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).

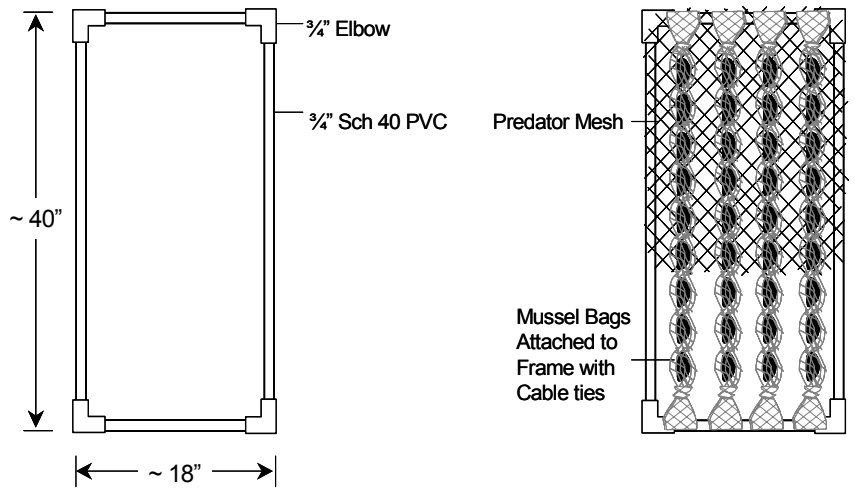

Figure 3. Cage design, attachment of mussel bags to frame, and predator mesh.

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.