FINAL REPORT
2003 Androscoggin River Caged Mussel Study

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International Paper
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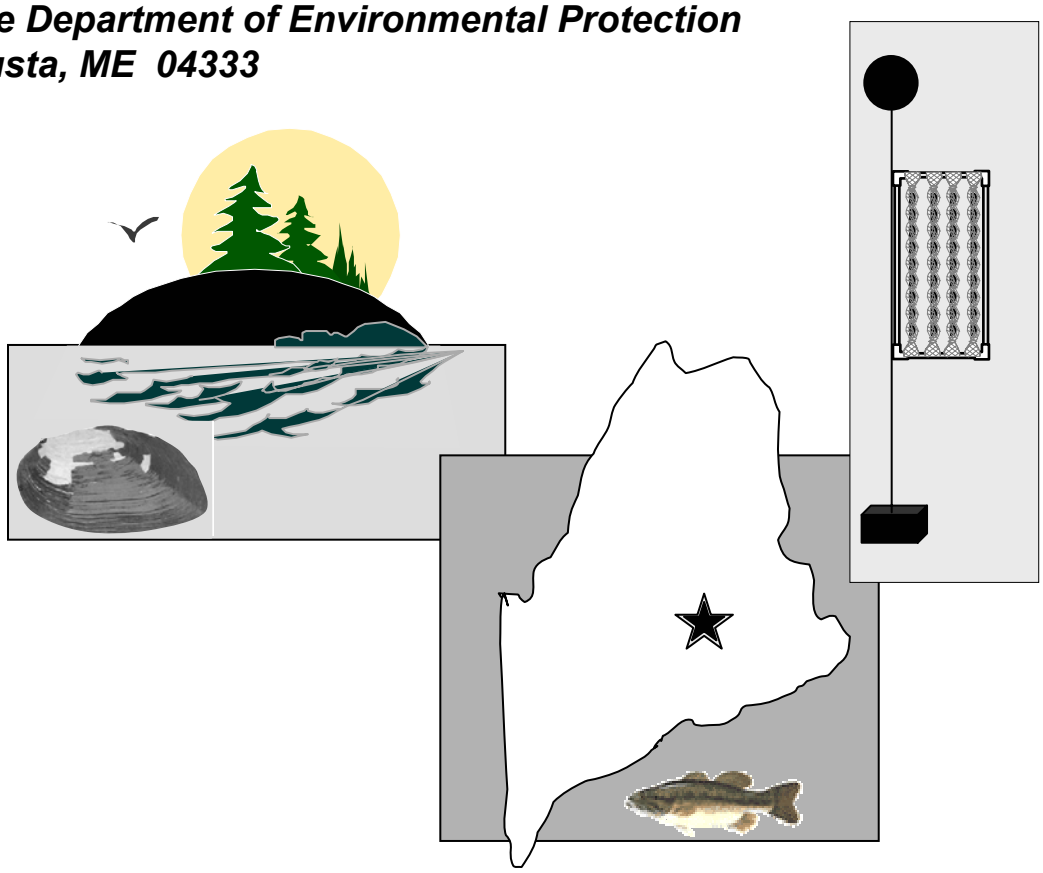

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TABLE OF CONTENTS

List of Figures

List of Tables

1.0 EXECUTIVE SUMMARY

A caged mussel study was conducted in the Androscoggin River, Maine during the summer of 2003 to determine the feasibility and scientific value of using transplanted mussels to monitor the effluent from International Paper's (IP) kraft mill at Jay, Maine. The study was designed to test whether caged mussels are a viable surrogate for fish in monitoring the effluent discharged by kraft mills. Results suggest that caged mussels are a viable option and can provide more detailed information over fine spatial scales that cannot be provided by collecting fish in the impoundments above and below the mill. They also suggest that neither 2,3,7,8-TCDD or 2,3,7,8-TCDF are currently being discharged.

Caged freshwater mussels (*Elliptio complanata*) were deployed in the Androscoggin River at 12 stations over a distance of approximately 6 miles. Four stations were positioned above the mill discharge, three stations within the mixing zone, and five stations below the Jay Dam. A total of 720 freshwater mussels were used. After 67 days in the river, survival and growth data show that the mussels at all stations were in good health so that the tissue chemistry data can be used with confidence. Average mussel survival was 99%. Increases in shell lengths and whole-animal wet-weights (WAWW) were small, but statistically significant at all stations. Percent increase in shell length was generally less than 1% while percent changes in WAWW were less than 6%. Of all growth metrics, tissue weights had the greatest increases, based on comparing the end-of-test tissue weights with the estimated tissue weight determined from the beginning-of-test mussels used for tissue chemistry analysis. Tissue weights increased by up to 43%.

Mussels accumulated a limited number of congeners at all stations in the low to sub-parts-per-trillion range. A total of eight congeners were detected, two dioxins and six furans (1,2,3,4,6,7,8-HpCDD, OCDD, 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF, and OCDF). Three congeners were detected at every station: HpCDD, OCDD, and 2,3,7,8-TCDF. Mussels furthest below the mill accumulated the most congeners at the highest concentrations, and had the highest dioxin toxic equivalents (TEQs). Mussels directly downstream of the discharge and within the impoundment accumulated the fewest number of congeners, and had among the lowest concentrations of total PCDD-Fs and the lowest TEQs. Total PCDD-F concentrations were driven by the presence of OCDD and TEQs by the presence of 2,3,7,8-TCDF. There was no significant gradient, either increasing or decreasing, away from the mill for total PCDD-F, total TEQ, or 2,3,7,8-TCDF (the most toxic congener measured in this study). The tissue chemistry data suggest that the two most toxic dioxin-furan congeners on which the regulations are based (i.e., 2,3,7,8-TCDD and 2,3,7,8-TCDF) are not being discharged by the Jay Mill.

The caged mussels survived, grew, and demonstrated the ability to accumulate dioxins and furans in their tissues if these compounds were present in the water column. Within the impoundment, concentrations of 2,3,7,8-TCDF in mussel tissues were significantly higher above the diffuser than below. Concentrations of 2,3,7,8-TCDF in mussel deployed immediately below the mill's diffuser were among the lowest measured in this study. The lowest concentrations were measured in mussels deployed furthest upstream from the diffuser, above the Riley Dam. The highest concentrations were measured in mussels deployed at the most downstream locations, just above and below the Livermore Dam. 2,3,7,8-TCDD was not found in any of the mussel tissue samples.

2.0 INTRODUCTION & STUDY OBJECTIVES

A caged mussel study was conducted in the Androscoggin River, Maine during the summer of 2003 to determine the feasibility and scientific value of using transplanted mussels to monitor dioxins and furans in the effluent from International Paper's (IP) kraft mill at Jay, Maine. Caged mussels were used instead of assessing natural fish populations because of the uncertainties associated with fish exposures due to mobility, accumulation of dioxins and furans from other sources, previous mill discharges sequestered in sediments, and the inability to collect fish near the mill discharge. A caged mussel pilot study conducted on the Kennebec River in 2000 demonstrated that concerns regarding fish monitoring could be eliminated by using a surrogate, such as caged mussels, because they could be deployed closer to the mill discharge where fish could not be collected. The study was designed to address three important questions relative to the state dioxin monitoring program and comparisons between above and below the mill discharge:

- Is there a difference between "above" and "below" dioxin-furans exposures as measured by uptake in mussel tissues?
- Do the mussel tissue chemistry data suggest a chemical gradient above or below the mill indicating a potential chemical source?
- Do dams affect the distribution patterns, chemical gradients, and availability of dioxins-furans to mussels?

This report summarizes the results of the 2003 study.

3.0 METHODS

American Society for Testing and Materials (ASTM) standardized protocols were followed for collection, transport, caging, and measurement of freshwater mussels. Complete details of the transplant methodology used in this study are described in the ASTM Standard Guide for Conducting In-situ Field Bioassays with Marine, Estuarine and Freshwater Bivalves (ASTM 2001). Table 1 summarizes the key elements of this study. Accumulation of dioxin and furan congeners in mussel tissues was used to estimate the presence of these chemicals in mill effluent. End-of-test (EOT) concentrations in mussel tissues were compared to concentrations in mussel tissues before deployment to determine relative accumulation during the deployment period. Growth, based on changes in whole-animal wet-weight (WAWW), shell length, tissue wet weight, and shell weight, was measured to 1) to characterize the health of the mussels and determine if adverse effects are occurring as a result of exposure to site-related conditions, 2) to calibrate bioaccumulation (i.e., to determine if chemical dilution due to tissue increase or chemical magnification due to tissue loss has occurred), and 3) to evaluate whether the caged mussels met standardized performance criteria for a successful test.

Freshwater mussels (*Elliptio complanata*) were collected from Lake Nequasset, Woolwich, Maine, sorted by size, and placed into cages. The caged mussels were transplanted to 12 stations in the Androscoggin River beginning above the Riley Dam and ending below the Livermore Falls Dam (Figure 1; Table 2), and retrieved after 67 days. Mussel tissues were removed for chemical analysis of dioxins, furans, percent lipids, and percent moisture.

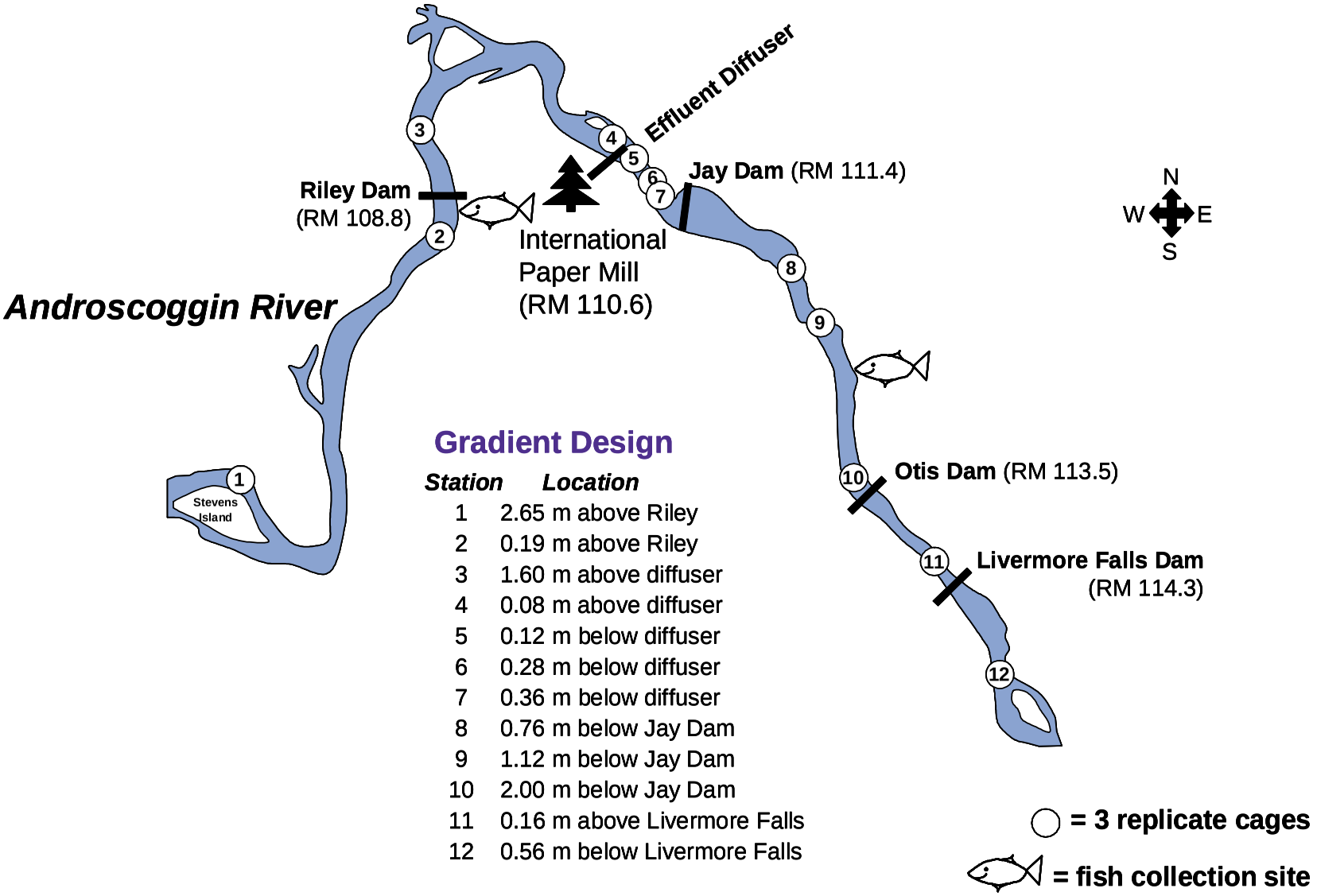

Figure 1. Station locations and overall experimental design.

Table 1. Summary of caged mussel study experimental design

Study Design	
Stations	12 on the Androscoggin River, beginning upstream of Riley Dam (RM 108.8) and ending downstream of the Livermore Falls Dam (RM 114.3)
Size range of mussels at start of test	58 to 62.99 mm
Number of cages per station	3
Number of mussels per cage	20 (4 bags with 5 mussels/bag)
Number of mussels deployed	720 (12 stations x 3 cages x 20 mussels/cage)
Number of mussels for T_0 tissue chemistry	60 (3 replicates x 20 mussels/replicate)
Total number of mussels required	780
Deployment configuration	Gradient Design
Deployment period	67 days
Exposure endpoints	Dioxins and Furans
Effects endpoints	growth (Δ WAWW & length; EOT tissue & shell weight), % lipids, % water
Test Schedule	
July 27, 2003	Mussel collection, sorting, and distribution to mesh bags. Mesh bags attached to PVC frames, predator mesh applied. Cages and T_0 mussels held overnight in Nequasset Lake.
July 28, 2003	Cages deployed at all stations. T_0 mussels shucked and tissue samples frozen.
October 4, 2003	Cages retrieved; all surviving mussels measured and shucked; tissues frozen for chemical analysis
Processing Locations	
BOT sorting, measurements & distribution	Bath Water District Treatment Plant
EOT measurements	Biology Laboratory, IP Jay Mill
Containment & Deployment System	
Flexible compartmentalized mesh	Mesh bags (approximately 4" in diameter and 5' long; 0.25" mesh size) made from plastic netting were used to hold the mussels during the deployment period. Each bag contained five individuals.
Cages	approximately 18" x 24"; constructed from 3/4" Schedule 40 PVC
Deployment array	25-lb cinder blocks used as anchors; polypropylene line, surface float

Four stations were positioned above the mill discharge located at river mile (RM) 110.85, three stations were within the mixing zone (i.e., below the diffuser and above the Jay Dam) along a suspected chemical gradient in the impoundment which receives effluent from the pulp and paper mill, and five stations below the Jay Dam. Three cages were deployed at each station, with 9 of 36 placed within the mixing zone and in the impoundment. Four impoundments were created by dams within the study area: Riley Dam (RM 108.8), Jay Dam (RM 111.4), Otis Dam (RM 113.5), and Livermore Falls Dam (RM 114.3). Precise station locations were identified by IP personnel with consultation with Applied Biomonitoring, FOMB, and DEP. Station 1 was furthest upstream on the river and Station 12 furthest downstream. Cages were deployed at each station left to right across the river facing upstream.

Table 2. Station locations for the Androscoggin River 2003 caged mussel study. Diffuser located at RM 110.85. Location of dams also shown.

Station	River Mile (RM)	Distance (mi) from Diffuser	Distance (mi) from Nearest Structure	Cage Number	Depth (Ft)	North Latitude (deg/min)	West Longitude (deg/min)
1	105.91	4.94 above	2.89 above Riley	8	14.9	44°28.632	70°16.307
				21*	14.4	44°28.701	70°16.304
				32	16.8	44°28.707	70°16.287
2	108.56	2.29 above	0.24 above Riley	1	15	44°29.968	70°15.012
				28*	15	44°29.973	70°15.004
				38	15	44°29.982	70°15.068
Riley Dam	***108.8***						
3	109.22	1.63 above	1.63 above diffuser	10	9.7	44°30.463	70°15.148
				16*	7.4	44°30.482	70°15.165
				26	5.6	44°30.472	70°15.189
4	110.74	0.11 above	0.11 above diffuser	2*	10	44°30.448	70°13.982
				17*	10	44°30.448	70°13.948
				34	10	44°30.458	70°13.922
5	110.97	0.12 below	0.12 below diffuser	11	16	44°30.347	70°13.690
				13*	16	44°30.363	70°13.677
				24	16	44°30.375	70°13.660
6	111.13	0.28 below	0.28 below diffuser	6*	11	44°30.235	70°13.587
				23*	11	44°30.245	70°13.568
				30*	11	44°30.258	70°13.555
7	111.21	0.36 below	0.36 below diffuser	4*	17.9	44°30.172	70°13.521
				18*	13.1	44°30.179	70°13.515
				37*	18.3	44°30.189	70°13.498
Jay Dam	***111.4***						
8	112.10	1.25 below	0.70 below Jay Dam	5	16	44°29.823	70°12.567
				36	12.8	44°29.818	70°12.587
				15*	10.2	44°29.814	70°12.605
9	112.46	1.61 below	1.06 below Jay Dam	19	10	44°29.550	70°12.382
				22*	10	44°29.553	70°12.375
				27[1]	10	44°29.567	70°12.360
10	113.37	2.52 below	1.97 below Jay Dam	29	20	44°28.758	70°12.163
				33*	15	44°28.793	70°12.132
				39	12	44°28.822	70°12.127
Otis Dam	***113.5***						
11	114.12	3.27 below	0.18 above LMF Dam	14	10	44°28.405	70°11.605
				35	10	44°28.393	70°11.578
				25*	10	44°28.395	70°11.568
Livermore Falls Dam	***114.3***						
12	114.80	3.95 below	0.50 below LMF Dam	7	11	44°27.843	70°11.143
				9*	11	44°27.847	70°11.128
				31	11	44°27.847	70°11.113

*Cage with temperature monitor
[1]Cage not retrieved at end of test

Tissue removal and weights were conducted according to ASTM (2001). All shucking knives used in tissue removal were stainless steel. Cutting boards, plastic trays, and weigh boats were covered with aluminum foil prior to cleaning. All shucking implements were decontaminated by (1) washing with a soap-free biological cleaning solution, (2) rinsing with hot tap water, (3) rinsing with distilled water, (4) rinsing with acetone, and (5) a final rinsing with hexane. Decontamination was supervised by Barry Mower (Maine Department of Environmental Protection; MDEP). Composite tissue samples, consisting of all tissues from surviving mussels from a given cage, were frozen at -20°C prior to shipment to Columbia Analytical for analysis of dioxins, furans, percent lipids, and percent solids. MDEP was responsible for shipment and delivery of tissues to the Columbia Analytical Services, Houston, Texas. All dioxin-furan analyses were conducted according to USEPA Method 1613B.

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 station by attaching it directly to one of the three cages deployed there.

A practical, step-wise approach to data analysis was used for this study. First, the tissue chemistry and growth data were summarized by station, and descriptive statistics such as mean, standard deviation, and 95% confidence interval (CI) were calculated. Bar graphs, with 95% CIs, were used to identify stations with the highest and lowest means as well as possible gradients. Comparative statistics (i.e., t-test or Analysis of Variance (ANOVA), depending on the hypothesis) were used to help confirm general differences identified by examining the graphs. Several parameters were regressed against distance from the diffuser to determine whether the mill was a potential source of chemicals measured in mussel tissues or growth affects could be associated with exposure to the effluent. These regressions, and the subsequent statistical analyses, only represent a first order approximation because mean values were used. Means were used rather than the individual data points to minimize the effects of variability in the data.

For the growth metrics, each individual mussel was considered a replicate. If all mussels survived, the level of replication at each station was 60 (i.e., 20 mussels/cage x 3 cages) for shell length, WAWW, tissue weight and shell weight. For the bioaccumulation portion of the study, the level of replication at each station was three, because one composite sample was prepared from each of three cages for each station.

All statistical analyses were conducted using GraphPad InStat software (version 3.05, Win 95/NT; GraphPad Software, San Diego, California, www.graphpad.com). InStat automatically assesses data for normality and common variances, and recommends alternative approaches if the data failed to meet the assumptions. For the ANOVAs, data that failed to meet these assumptions were analyzed with the nonparametric Kruskal Wallis test. For the t-tests, a Welch correction was applied to data that failed to these assumptions. All tests were conducted at the 95% confidence level ($\alpha = 0.05$).

4.0 RESULTS

Results will focus on tissue chemistry and the dioxin-furan congeners generally believed to be most relevant to kraft mill processes, 2,3,7,8-TCDD and 2,3,7,8-TCDF, and possibly 1,2,3,7,8-PeCDD and 1,2,3,7,8-PeCDF. This results section identifies eight of the 17 measured congeners that were accumulated by mussels, and compares differences in accumulation across stations.

4.1 Tissue Chemistry

The relative contribution of each congener by station, on a concentration basis and on a percentage basis of both total PCDD-F concentration and total TEQ, provide the best overview of the results. They can be used for above-below comparisons, to show chemical gradients, or lack thereof, potential sources, and the influence of the dams.

4.1.1 Dioxin-Furan Congeners

Mussels accumulated a limited number of congeners at all stations in the low to sub-parts-per-trillion range (Table 3; Figure 2). At the beginning of the test, the T_0 tissue samples only contained detectable concentrations of OCDD (Table 3), and the mean concentration was approximately 1.0 ng/kg-ww. At the end of the test a total of eight congeners were detected in mussel tissues: two dioxins (1,2,3,4,6,7,8-HpCDD, OCDD) and six furans (2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF, and OCDF). Two of the four congeners commonly associated with kraft mill effluents were accumulated by mussels deployed on the Androscoggin River: 2,3,7,8-TCDF, and 1,2,3,7,8-PeCDF. Mussels did not accumulate detectable levels of either 2,3,7,8-TCDD or 1,2,3,7,8-PeCDD.

Three congeners were accumulated by mussels at each station: HpCDD, OCDD, and 2,3,7,8-TCDF. Mussels at Stations 11 and 12, furthest below the mill, accumulated the most congeners at the highest concentrations, and had the highest dioxin toxic equivalents (TEQs). Mussels at Station 11 (3.3 miles below diffuser), below Otis Dam, accumulated seven congeners and mussels at Station 12 (4 miles below diffuser), below Livermore Falls Dam, accumulated eight congeners. Mussels directly downstream of the discharge and within the impoundment (i.e., Stations 5, 6, and 7 at 0.12, 0.28, and 0.36 miles below diffuser) accumulated the fewest number of congeners, and had among the lowest concentrations of total PCDD-Fs and the lowest TEQs.

Total PCDD-F concentrations (Figures 2, 3) were driven by the presence of OCDD, which ranged from a low of 80% at Station 4 (0.11 miles above diffuser) to 90% at Station 8 (1.25 miles below diffuser). Conversely, 2,3,7,8-TCDF ranged from a low of 1.3% at Station 8 to 15% at Station 4. Collectively, these two congeners accounted for 88 to 96% of the total PCDD-F concentration.

Table 3. Androscoggin 2003 - Mean congener concentration (ng/kg-wet) by Station

	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6	Station 7	Station 8	Station 9	Station 10	Station 11	Station 12	T_0
2,3,7,8-TCDD	0	0	0	0	0	0	0	0	0	0	0	0	0
1,2,3,7,8-PeCDD	0	0	0	0	0	0	0	0	0	0	0	0	0
1,2,3,4,7,8-HxCDD	0	0	0	0	0	0	0	0	0	0	0	0	0
1,2,3,6,7,8-HxCDD	0	0	0	0	0	0	0	0	0	0	0	0	0
1,2,3,7,8,9-HxCDD	0	0	0	0	0	0	0	0	0	0	0	0	0
1,2,3,4,6,7,8-HpCDD	0.07	0.11	0.23	0.04	0.08	0.09	0.10	0.59	0.08	0.07	0.23	0.16	0
OCDD	1.05	1.45	2.75	0.74	0.97	1.28	1.11	9.60	0.99	1.06	4.09	4.31	1.03
2,3,7,8-TCDF	0.08	0.08	0.19	0.14	0.12	0.13	0.12	0.14	0.15	0.13	0.25	0.33	0
1,2,3,7,8-PeCDF	0	0	0	0	0	0	0	0	0	0	0	0.03	0
2,3,4,7,8-PeCDF	0	0	0	0	0	0	0	0	0.02	0	0.02	0.03	0
1,2,3,4,7,8-HxCDF	0.01	0.02	0.04	0	0	0.02	0	0.05	0.02	0.02	0.04	0.03	0
1,2,3,6,7,8-HxCDF	0	0	0	0	0	0	0	0	0	0	0	0	0
2,3,4,6,7,8-HxCDF	0	0	0	0	0	0	0	0	0	0	0	0	0
1,2,3,7,8,9-HxCDF	0	0	0	0	0	0	0	0	0	0	0	0	0
1,2,3,4,6,7,8-HpCDF	0	0	0.04	0	0	0	0	0.03	0.02	0	0.04	0.02	0
1,2,3,4,7,8,9-HpCDF	0	0	0.00	0	0	0	0	0	0	0	0	0	0
OCDF	0	0	0.08	0	0	0.08	0.03	0.24	0	0	0.16	0.17	0
Total	1.21	1.66	3.32	0.92	1.17	1.60	1.36	10.65	1.27	1.28	4.83	5.08	1.03
Lipid Normalized Congener (ng congener/g-lipid ww)													
2,3,7,8-TCDD	0	0	0	0	0	0	0	0	0	0	0	0	0
1,2,3,7,8-PeCDD	0	0	0	0	0	0	0	0	0	0	0	0	0
1,2,3,4,7,8-HxCDD	0	0	0	0	0	0	0	0	0	0	0	0	0
1,2,3,6,7,8-HxCDD	0	0	0	0	0	0	0	0	0	0	0	0	0
1,2,3,7,8,9-HxCDD	0	0	0	0	0	0	0	0	0	0	0	0	0
1,2,3,4,6,7,8-HpCDD	1668	825	1014	269	948	1393	1477	6263	406	309	787	344	0
OCDD	26019	12716	12477	5183	13170	17866	17677	101991	4554	4753	12964	8888	304
2,3,7,8-TCDF	2230	997	1003	1075	1382	1650	1856	1064	721	569	917	649	0
1,2,3,7,8-PeCDF	0	0	0	0	0	0	0	0	0	0	0	57	0
2,3,4,7,8-PeCDF	0	0	0	0	0	0	0	0	81	0	40	65	0
1,2,3,4,7,8-HxCDF	129	87	173	0	0	408	0	363	60	112	95	54	0
1,2,3,6,7,8-HxCDF	0	0	0	0	0	0	0	0	0	0	0	0	0
2,3,4,6,7,8-HxCDF	0	0	0	0	0	0	0	0	0	0	0	0	0
1,2,3,7,8,9-HxCDF	0	0	0	0	0	0	0	0	0	0	0	0	0
1,2,3,4,6,7,8-HpCDF	0	0	103	0	0	0	0	264	106	0	171	49	0
1,2,3,4,7,8,9-HpCDF	0	0	0	0	0	0	0	0	0	0	0	0	0
OCDF	0	0	201	0	0	1256	253	2708	0	0	658	409	0
Total	30046	14625	14971	6528	15500	22573	21263	112654	5927	5743	15633	10516	304

4.1.2 TEQs

Mean TEQs, based on the relative toxicity of each congener were calculated for each station and ranged from 0.0101 at Station 1 (4.9 miles above diffuser) to 0.0572 at Station 12 (Figure 4). There was no gradient, either increasing or decreasing, away from the mill for total TEQ, which is consistent with the trends found for 2,3,7,8-TCDF. The TEQ calculations were driven by the presence of 2,3,7,8-TCDF for all stations except Station 2, even though 2,3,7,8-TCDF contributed a very small amount to the total PCDD-F concentration. 2,3,7,8-TCDF accounted for 54% of the total TEQ at Station 8 and 97% of the total at Station 4 (Figure 5). In contrast, OCDD, which contributed the most to the Total PCDD-F concentration, accounted for 0.4% of the total TEQ at Station 9 (1.6 miles below diffuser) and 3.8% at Station 8. These data show that 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF, which were detected far less frequently than OCDD, made a much greater contribution to the total TEQ.

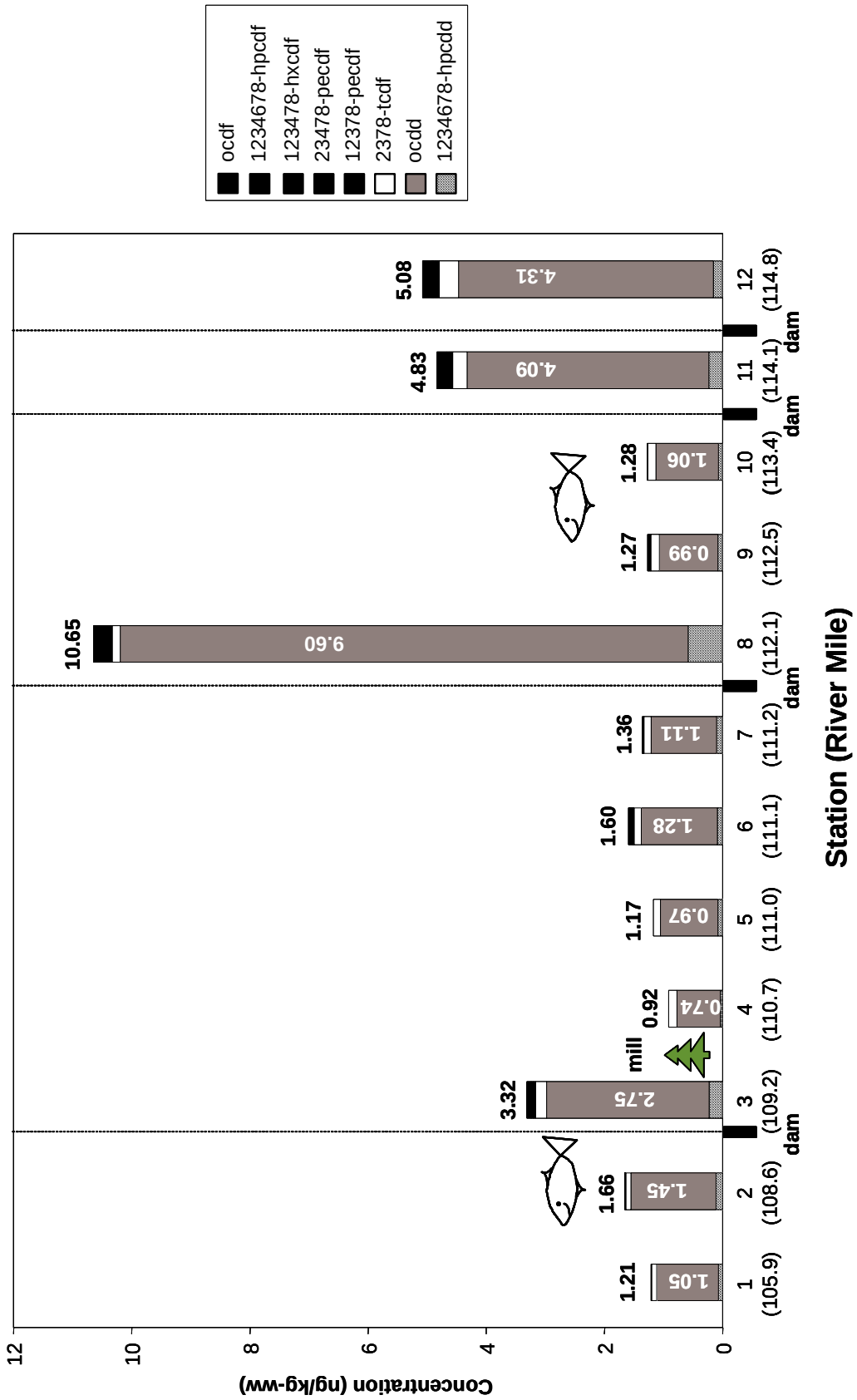

Figure 2. Congener concentration by station.

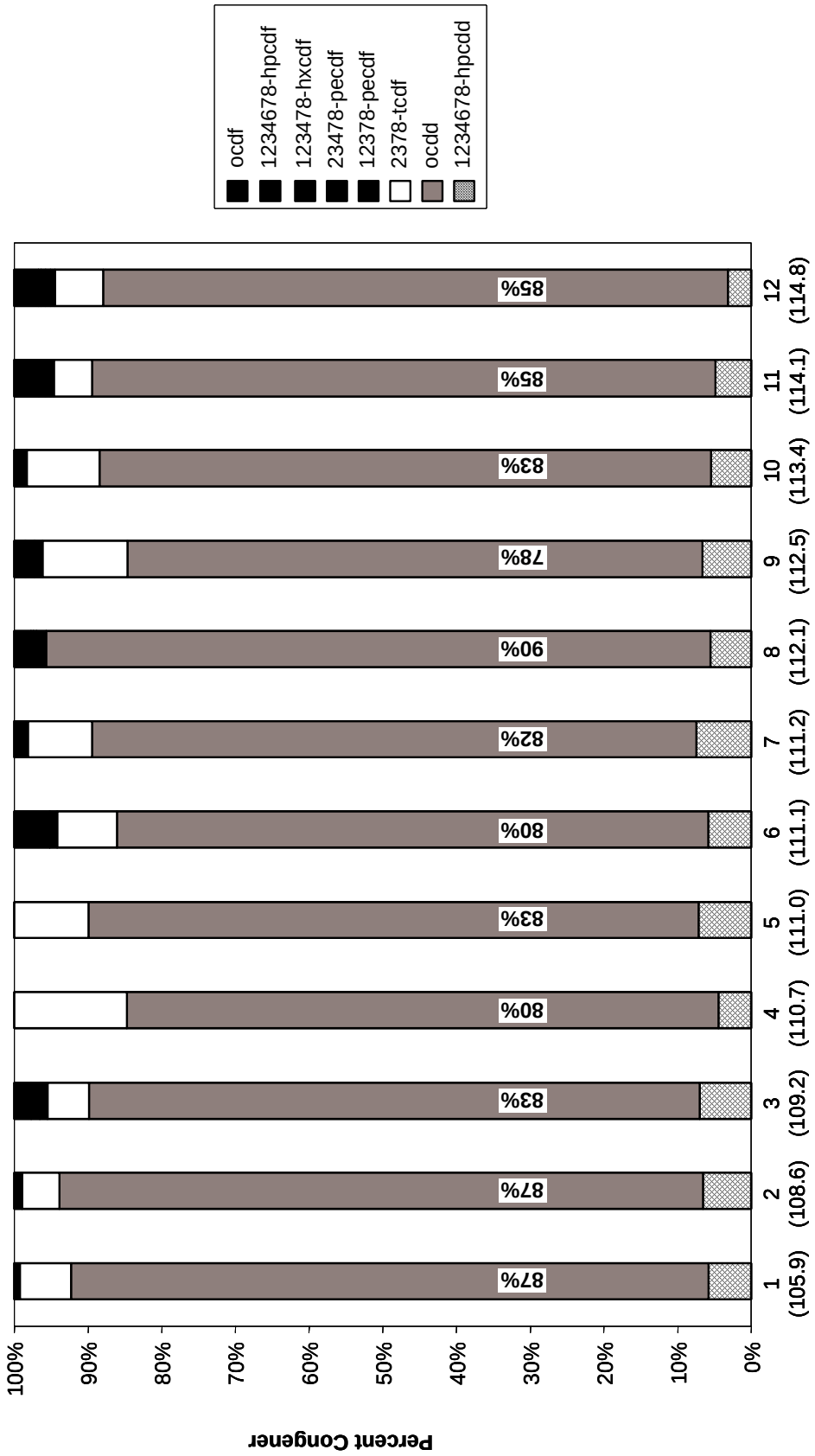

Figure 3. Percent contribution of congener to total PCDD-F concentration.

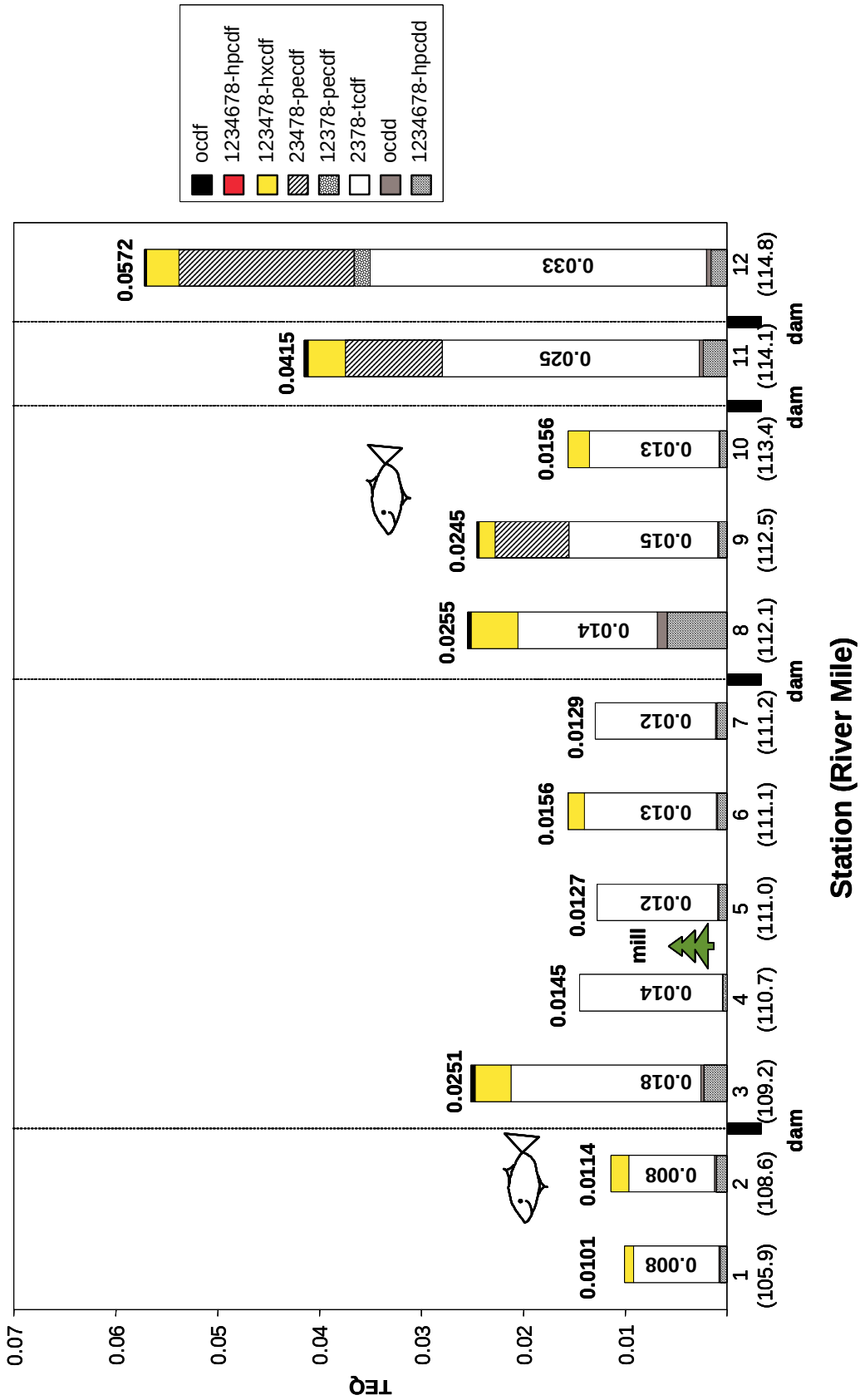

Figure 4. Mean TEQ composition and total by station.

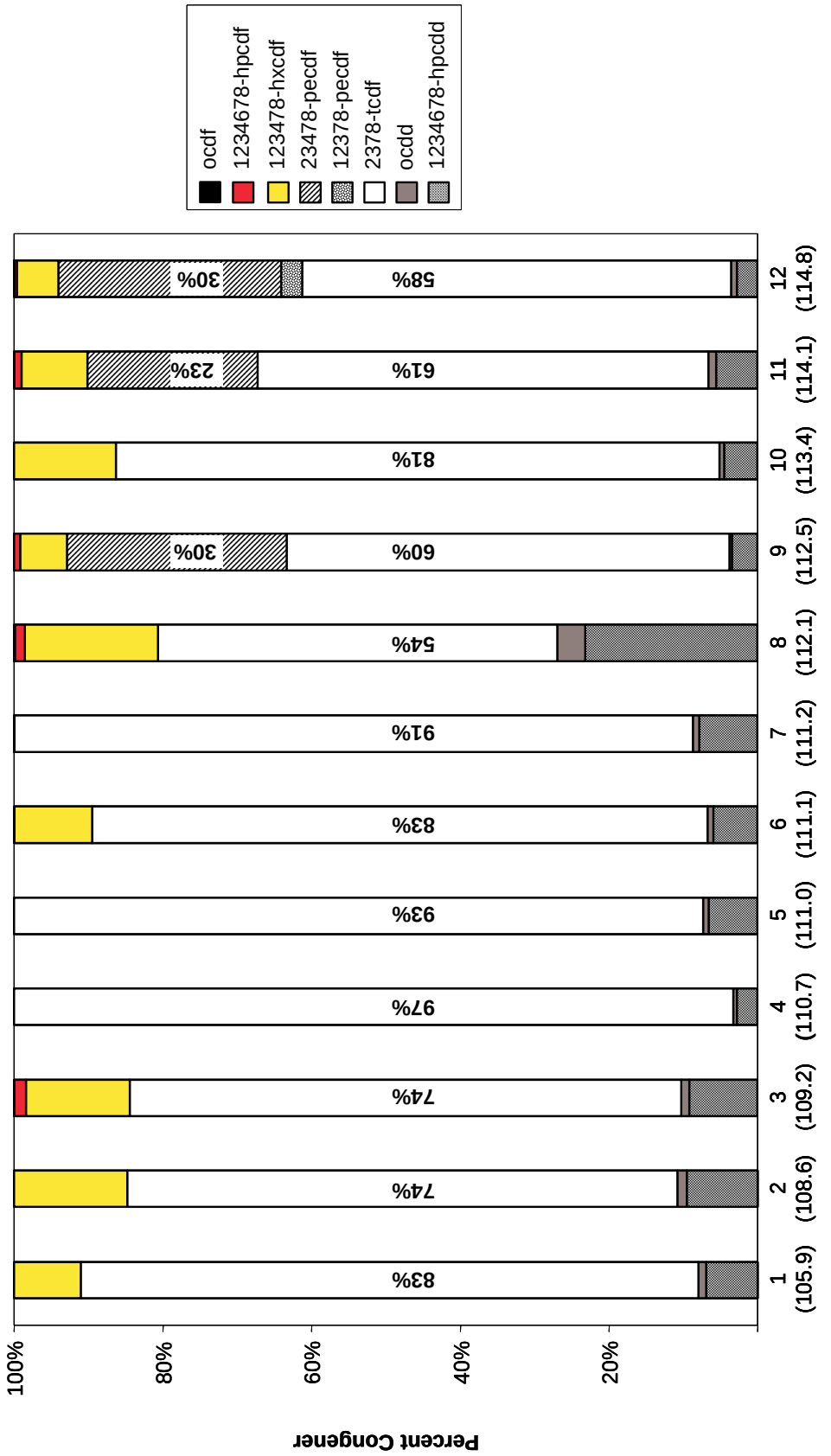

Figure 5. TEQ percent contribution to total.

4.1.3 2,3,7,8-TCDD and 2,3,7,8-TCDF

2,3,7,8-TCDD was not detected in any mussel tissue samples.

2,3,7,8-TCDF was accumulated by mussels at all stations (Table 3) with the highest concentrations, 0.25 and 0.33 ng/kg-ww, measured in mussels deployed at Stations 11 and 12. Concentrations of 2,3,7,8-TCDF immediately downstream from the diffuser were among the lowest measured in mussel tissues, approximately 0.12 ng/kg-ww. These data suggest that the mill is not a source of 2,3,7,8-TCDF. The relatively higher concentrations of this congener at Stations 3, 11, and 12 suggest that there are other potential sources of 2,3,7,8-TCDF in the Androscoggin River.

4.1.4 1,2,3,7,8-PeCDD and 1,2,3,7,8-PeCDF

1,2,3,7,8-PeCDD was not detected in any mussel tissue samples.

Only mussels at Station 12 accumulated 1,2,3,7,8-PeCDF, a congener potentially associated with pulp and paper mill operations. Two of the three replicate tissue samples for Station 12 had detectable concentrations of this congener, ranging from 0.041 to 0.055 ng/kg-ww. The presence of this congener in mussels deployed 3.95 miles below the diffuser but no accumulation by mussels immediately downstream of the diffuser is evidence that the mill is probably not discharging these dioxin or furans into the Androscoggin River.

4.1.5 OCDD

Mussels at most stations accumulated little if any additional OCDD. Although concentrations ranged from 0.97 ng/kg-ww at Station 5 to 9.60 ng/kg at Station 8, concentrations of OCDD were generally about 1 ng/kg-ww (Table 3), which is similar to the concentration measured in the T_0 tissue samples. Elevated concentrations were also measured in mussels deployed at Stations 11 and 12.

4.1.6 Total PCDD-Fs

The concentration of Total PCDD-Fs were highest in mussels deployed at Station 8 (10.65 ng/kg-ww; Table 3). The lowest concentration was measured in mussels deployed at Station 4 (0.92 ng/kg-ww; Table 3). The concentration of Total PCDD-Fs in mussels deployed in the mixing zone (i.e., Stations 5, 6, and 7) were among the lowest measured in the entire study (1.17, 1.60, and 1.36 ng/kg-ww, respectively).

4.1.7 Above-Below Comparisons

Several above-below comparisons were made in an attempt to determine whether dioxin-furan concentrations were higher below the mill than above. Only stations within the impoundment that received mill effluent were used in these comparisons. As summarized in Table 4, concentrations of 2,3,7,8-TCDF in mussels deployed below the diffuser and within the impoundment were less than measured in mussels deployed above the diffuser, regardless of whether the above and below stations were compared individually or on a pooled basis. Similar results were found for Total TEQ. The lipid-normalized values showed no significant difference for any of the same five comparisons.

Table 4. Results of above-below statistical comparisons

Above Station(s)	Below Station(s)	p value	Result
Non-normalized 2,3,7,8-TCDF			
Pooled 3, 4	Pooled 5, 6, 7	0.022	Above significantly higher than below
3	Pooled 5, 6, 7	0.005	Above significantly higher than below
4	Pooled 5, 6, 7	0.356	No significant difference
3	5	0.041	Above significantly higher than below
4	5	0.435	No significant difference
Total TEQ			
Pooled 3, 4	Pooled 5, 6, 7	0.053	Not quite significant; Above higher than below
3	Pooled 5, 6, 7	0.003	Above significantly higher than below
4	Pooled 5, 6, 7	0.781	No significant difference
3	5	0.036	Above significantly higher than below
4	5	0.570	No significant difference

4.2 Survival

Survival by cage ranged from 95 to 100%. Mean *Elliptio* survival by station ranged from 96.7 to 100%; mean survival for all stations was 98.8% (Table 5). Of the 720 mussels deployed (20 mussels/cage x 36 cages), 692 mussels survived, 8 mussels died, and one cage of 20 mussels was not retrieved.

4.3 Mussel Growth Metrics

Elliptio deployed on the Androscoggin River had very small increases in shell length and small increases in WAWW during the 66-day exposure period. Percent increase in shell length was generally less than 1% while percent changes in WAWW were less than 5.6% (Table 5). Of all growth metrics, tissue weights had the greatest increases, based on comparing the end-of-test tissue weights with the estimated tissue weight determined from the T_0 tissue chemistry individuals. Tissue weights increased by up to 42.6%. 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 can be used with confidence.

4.3.1 Shell Length

There was no statistically significant difference in mean shell lengths among individual cages or among stations at the beginning of the test. Mean shell length increased at some stations during the 67-day exposure period. When compared to the beginning-of-test measurements, there was a significant increase in shell length only at Stations 1, 2, 3, 4, 10, and 11 ($p < 0.0001$). The average increase in shell length across all stations was approximately 0.22 mm. There was no statistically significant difference in shell length among stations at the end of the test.

Table 5. Mussel growth metrics

	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6	Station 7	Station 8	Station 9	Station 10	Station 11	Station 12	T_0	All Data
Percent Survival	100.0%	98.3%	100.0%	98.3%	100.0%	98.3%	100.0%	98.3%	97.5%	98.3%	96.7%	100.0%	na	98.8%
% Change Shell Length	0.46%	1.02%	0.53%	0.92%	0.05%	0.11%	0.15%	0.04%	0.02%	0.47%	0.24%	0.19%	na	0.36%
% Change Weight	3.2%	4.7%	4.4%	5.6%	3.1%	2.1%	2.2%	3.2%	2.8%	3.0%	3.1%	4.5%	na	3.5%
Est % Change in Tissue Weight	23.9%	17.0%	22.3%	24.6%	26.1%	28.7%	26.1%	42.6%	21.4%	28.1%	25.1%	34.5%	na	26.9%
Est % Change in Shell Weight	4.8%	1.5%	4.0%	7.6%	1.1%	3.1%	6.7%	1.2%	-2.9%	5.0%	-3.7%	-0.8%	na	2.5%
Initial Length (mm)														
mean	60.46	60.69	60.63	60.55	60.75	60.64	60.71	60.78	60.63	60.44	60.59	60.60	60.65	60.62
min	58.07	58.00	58.02	58.03	58.04	58.00	58.01	58.03	58.12	58.05	58.07	58.00	58.10	58.00
max	62.89	62.94	62.97	62.93	62.88	62.78	62.86	62.92	62.92	62.85	62.84	62.83	62.99	62.99
stdev	1.44	1.56	1.38	1.51	1.51	1.41	1.45	1.37	1.41	1.37	1.53	1.49	1.49	1.45
count	60	60	60	60	60	60	60	60	60	60	60	60	60	780
95% CI	0.363	0.394	0.350	0.383	0.382	0.358	0.368	0.347	0.356	0.347	0.386	0.377	0.377	0.377
EOT Length (mm)														
mean	60.73	61.28	60.95	61.13	60.79	60.68	60.80	60.80	60.57	60.73	60.77	60.71	na	60.84
min	58.22	58.24	58.05	58.53	57.63	57.83	57.80	57.93	58.00	58.05	58.19	57.46	na	57.46
max	63.30	64.70	63.39	63.90	63.16	63.72	63.36	63.09	63.26	63.66	63.82	63.93	na	64.70
stdev	1.41	1.71	1.42	1.46	1.46	1.41	1.39	1.39	1.48	1.49	1.56	1.57	na	1.48
count	60	59	60	59	60	59	60	59	39	59	58	60	na	692
95% CI	0.36	0.44	0.36	0.37	0.37	0.36	0.35	0.36	0.47	0.38	0.40	0.40	na	0.11
Length Growth Rate (mm/wk)														
mean	0.029	0.065	0.033	0.059	0.003	0.007	0.009	0.002	0.001	0.030	0.015	0.012	na	0.023
min	-0.101	-0.019	-0.102	-0.088	-0.072	-0.099	-0.133	-0.078	-0.055	-0.036	-0.114	-0.068	na	-0.133
max	0.154	0.206	0.136	0.188	0.056	0.221	0.076	0.159	0.084	0.127	0.103	0.167	na	0.221
stdev	0.048	0.047	0.052	0.054	0.029	0.048	0.044	0.048	0.034	0.040	0.043	0.053	na	0.050
count	60	59	60	59	60	59	60	59	39	59	58	60	na	692
95% CI	0.012	0.012	0.013	0.029	0.007	0.012	0.011	0.012	0.011	0.010	0.011	0.013	na	0.004
Initial WAWW (g-wet)														
mean	19.25	18.90	19.26	19.10	18.73	19.14	19.54	19.03	18.57	18.92	18.37	18.95	19.14	18.99
min	13.94	13.65	13.67	13.11	13.69	14.11	12.80	12.91	13.80	14.01	13.89	13.19	12.95	12.80
max	24.88	23.59	26.77	27.78	24.60	27.40	25.99	27.34	27.97	26.22	24.74	27.38	26.94	27.97
stdev	2.37	2.41	2.89	3.08	2.56	2.82	3.13	3.06	2.97	2.66	2.30	2.68	2.98	2.77
count	60	60	60	60	60	60	60	60	60	60	60	60	60	780
95% CI	0.60	0.61	0.73	0.78	0.65	0.71	0.79	0.77	0.75	0.67	0.58	0.68	0.75	0.75

EOT WAWW (g-wet)														
mean	19.85	19.76	20.07	20.06	19.30	19.61	19.93	19.62	18.71	19.49	18.95	19.77	na	19.62
min	15.06	14.86	14.71	14.86	13.98	14.86	13.15	13.95	14.01	14.21	15.02	14.91	na	13.15
max	25.14	23.92	26.81	29.86	24.52	26.93	25.61	29.13	24.79	28.13	24.84	29.75	na	29.86
stdev	2.3	2.3	2.8	3.0	2.5	2.8	3.0	3.1	2.6	2.8	2.3	2.7	na	2.7
count	60	59	60	59	60	59	60	59	39	59	58	60	na	692
95% CI	0.59	0.58	0.71	0.75	0.64	0.72	0.76	0.79	0.82	0.72	0.60	0.69	na	0.20
WAWW Growth Rate (mg/wk)														
mean	63	88	84	106	59	42	40	60	50	59	59	86	na	67
min	-38	-31	-22	-74	-60	-63	-92	-95	-55	-48	-49	-67	na	-95
max	180	186	197	226	147	210	199	187	171	200	188	248	na	248
stdev	47	53	40	56	43	53	55	48	46	48	46	60	na	53
count	60	59	60	59	60	59	60	59	39	59	58	60	na	692
95% CI	11.8	13.6	10.0	14.3	10.9	13.6	14.0	12.1	14.5	12.2	11.9	15.3	na	4.0
Tissue Weight (g-wet)														
mean	5.80	5.48	5.73	5.84	5.91	6.03	5.91	6.68	5.69	6.00	5.86	6.30	4.69	5.94
min	4.14	2.99	4.15	3.16	3.95	4.42	3.97	4.49	4.31	4.16	4.55	4.15	3.15	2.99
max	8.02	7.00	8.46	10.09	7.37	9.26	8.84	15.25	7.68	10.40	8.43	11.07	7.62	15.25
stdev	0.78	0.69	0.83	1.01	0.75	0.96	1.02	1.94	0.82	1.12	0.77	1.15	0.80	1.08
count	60	59	60	59	60	59	60	59	39	59	58	60	60	692
95% CI	0.20	0.18	0.21	0.26	0.19	0.24	0.26	0.50	0.26	0.28	0.20	0.29	0.20	0.08
Shell Weight (g-wet)														
mean	9.16	8.88	9.09	8.83	9.40	9.01	9.32	8.84	8.49	9.18	8.42	8.67	8.74	8.96
min	6.40	6.07	6.08	5.93	6.09	6.20	5.36	6.14	5.54	6.30	6.04	5.55	4.85	5.36
max	13.00	12.08	13.30	12.50	14.16	13.28	12.96	12.37	13.36	13.40	11.94	12.56	15.23	14.16
stdev	1.45	1.46	1.58	1.50	1.79	1.59	1.62	1.66	1.64	1.55	1.32	1.40	1.72	1.56
count	60	59	60	60	59	59	60	59	39	59	58	60	60	692
95% CI	0.37	0.37	0.40	0.38	0.46	0.41	0.41	0.42	0.51	0.40	0.34	0.35	0.43	0.12
Percent Lipids														
mean	0.004	0.496	0.022	0.495	0.510	0.030	0.009	0.018	0.022	0.024	0.033	0.064	0.359	0.147
min	0.003	0.004	0.013	0.007	0.004	0.004	0.004	0.007	0.018	0.015	0.017	0.034	0.231	0.003
max	0.007	1.474	0.038	1.464	1.470	0.077	0.012	0.036	0.026	0.029	0.047	0.108	0.465	1.474
stdev	0.002	0.847	0.014	0.839	0.832	0.041	0.004	0.016	0.006	0.008	0.015	0.039	0.119	0.411
count	3	3	3	3	3	3	3	3	2	3	3	3	3	35
95% CI	0.003	0.958	0.015	0.949	0.941	0.046	0.005	0.018	0.000	0.009	0.017	0.044	0.134	0.136
Percent Moisture														
mean	86.38	85.98	86.87	86.30	87.02	86.71	87.42	86.51	87.59	87.12	86.36	87.05	36.31	86.751
min	85.77	85.87	86.02	85.90	86.79	86.24	87.09	85.04	86.71	86.69	85.74	86.58	27.78	85.04
max	87.51	86.14	87.94	86.82	87.28	87.27	87.78	89.31	88.47	87.95	87.09	87.37	45.45	89.31
stdev	0.98	0.14	0.98	0.47	0.25	0.52	0.35	2.43	1.24	0.72	0.68	0.42	8.85	0.908
count	3	3	3	3	3	3	3	3	2	3	3	3	3	35
95% CI	1.11	0.16	1.11	0.53	0.28	0.59	0.39	2.75	1.72	0.82	0.77	0.47	10.02	0.301

EOT length growth rates by station ranged from 0.001 mm/wk at Station 9 to 0.065 mm/wk at Station 2. As with shell length, length growth rates were higher at Station 2 when compared to all other stations (Table 5).

4.3.2 Whole-Animal Wet-Weight (WAWW)

There was no statistically significant difference in mean WAWWs among individual cages or among stations at the beginning of the test. Mean WAWW increased at all stations during the 67-day exposure period. When compared to the beginning-of-test measurements, there was a significant increase in WAWW at all stations ($p < 0.0001$), with an average increase in shell length across all stations of approximately 0.82 g-wet. No statistically significant differences in WAWW were found among stations at the end of the.

EOT WAWW growth rates by station ranged from 40 mg/wk at Station 7 to 106 mg/wk at Station 4 (Table 5). WAWW growth rates were significantly lower at Stations 6, 7, and 9 when compared to Stations 2, 3, 4, and 12, where growth rates exceeded 84 mg/wk.

4.3.3 Wet Tissue Weights

Mean whole soft tissue weight at the start of the test was estimated at 4.69 g-wet (Table 5) based on the tissue weights from the 60 baseline BOT measurements. Based on this estimated BOT value, there was a significant increase in EOT tissue weight at all stations when compared to the BOT tissue weights. EOT tissue weights were significantly lower at Station 2 than at Stations 8 and 12, while EOT tissue weights were significantly higher at Station 8 than at Stations 3 and 9. Tissue weights were similar among all other stations.

4.3.4 Shell Weight

Mean shell weight at the start of the test was estimated at 8.74 g-wet (Table 5) based on the shell weights from the 60 baseline BOT measurements. Based on this estimated BOT value, mean shell weights increased at all stations except Stations 9, 11, and 12 during the 67-day exposure period. Only mussels at Station 5 had a significant increase in shell weight when compared to the BOT estimate. Although results of the ANOVA on EOT shell weights indicated a statistically significant difference among stations, no significant differences were found with the Tukey-Kramer multiple comparison test.

4.3.5 Percent Lipids & Moisture in Soft Tissues

The percent lipids measured in mussels were extremely low and are highly questionable. Mean percent lipids at the start of the test was estimated at 0.36% (Table 5) based on the analysis of the three composite BOT tissue samples. At the end of the test, percent lipids ranged from 0.004% at Station 1 to 0.51% at Station 5, with mussels at nine stations reportedly having lipid concentrations less than 0.07%. Using these results and the estimated BOT value, mussels at all stations except three had a decline in percent lipids during the 67-day exposure period.

Mean percent moisture at the start of the test was estimated at 36.3% (Table 5) based on the analysis of the three composite BOT tissue samples. This value also appears extremely low and highly questionable. At the end of the test, percent moisture ranged from 86.3% at Station

4 to 87.6% at Station 9. Percent moisture increased in mussels at all stations compared to the BOT value, however it is very likely that the BOT value is in error.

4.4 Water Temperature

All water temperature monitors attached to the mussel cages were retrieved, as well as those deployed at the effluent outfall and 100 feet downstream of the outfall. The mean, minimum, maximum, and range in water temperature at each station are provided in Table 6. Water temperatures ranged between 12.4 and 26.0°C with the highest temperature measured at Station 5. The highest temperatures were measured from the time of deployment in July through August, although water temperature was not consistent. Water temperature fluctuated considerably above and below 23°C (Figure 6). These conditions my reflect rain events which had an immediate cooling effect on river water temperature (i.e., water temperatures decreased to less than 22°C). Water temperatures increased quickly to the normal range of about 24°C. Towards the end of August, water temperature decreased to about 20°C at all stations, and continued to decrease until mid-September when a slight elevation was measured (i.e., approximately 22°C). Water temperatures continued to decrease after this brief spike. Water temperatures were similar at all stations during the entire deployment period.

Table 6. Mean, minimum, maximum, and range in water temperature (°C).

	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6	Station 7	Station 8	Station 9	Station 10	Station 11	Station 12
Mean	20.6	20.7	20.7	20.7	21.2	21.0	21.0	21.1	21.1	21.2	21.0	21.1
Min	12.4	12.9	12.7	12.6	13.2	12.9	12.7	12.8	12.8	13.4	13.3	13.4
Max	25.3	25.6	25.4	25.4	26.0	25.9	25.6	25.8	25.7	25.7	25.5	25.7
Range	12.9	12.7	12.7	12.9	12.8	13.0	12.9	13.0	12.9	12.4	12.3	12.3

Based on results of the ANOVA, there were no statistically significant differences in daily average water temperatures across stations ($p = 0.9674$). The non-parametric ANOVA showed that the range in daily water temperature at Station 11 was significantly different than that at Stations 1, 2, and 8.

5.0 DISCUSSION

With respect to the purpose and objectives of this study, the results demonstrate that transplanted mussels are a viable option to monitor the effluent discharged by kraft mills and provide detailed information over fine spatial scales that cannot be provided by collecting fish above and below dams creating these impoundments. The absence of 2,3,7,8-TCDD and the very low concentrations of 2,3,7,8-TCDF in mussels deployed within the impoundment and below the mill suggest that these congeners are not currently being discharged by this mill. OCDD was the predominant congener in all tissue samples. However, OCDD is primarily associated with combustion processes and not necessarily an indication of dioxins-furans present in mill effluent. The limited accumulation of this congener by mussels at most stations suggests that these activities are fairly restricted along the stretch of the Androscoggin tested, except in the vicinity of Station 8 where OCDD concentrations were highest. This study was unique in that the stations spanned four dams over a distance of approximately 5.5 miles. An interesting observation was that for nearly all congeners, total PCDD-Fs, and total TEQs, the concentrations were higher directly downstream of each dam.

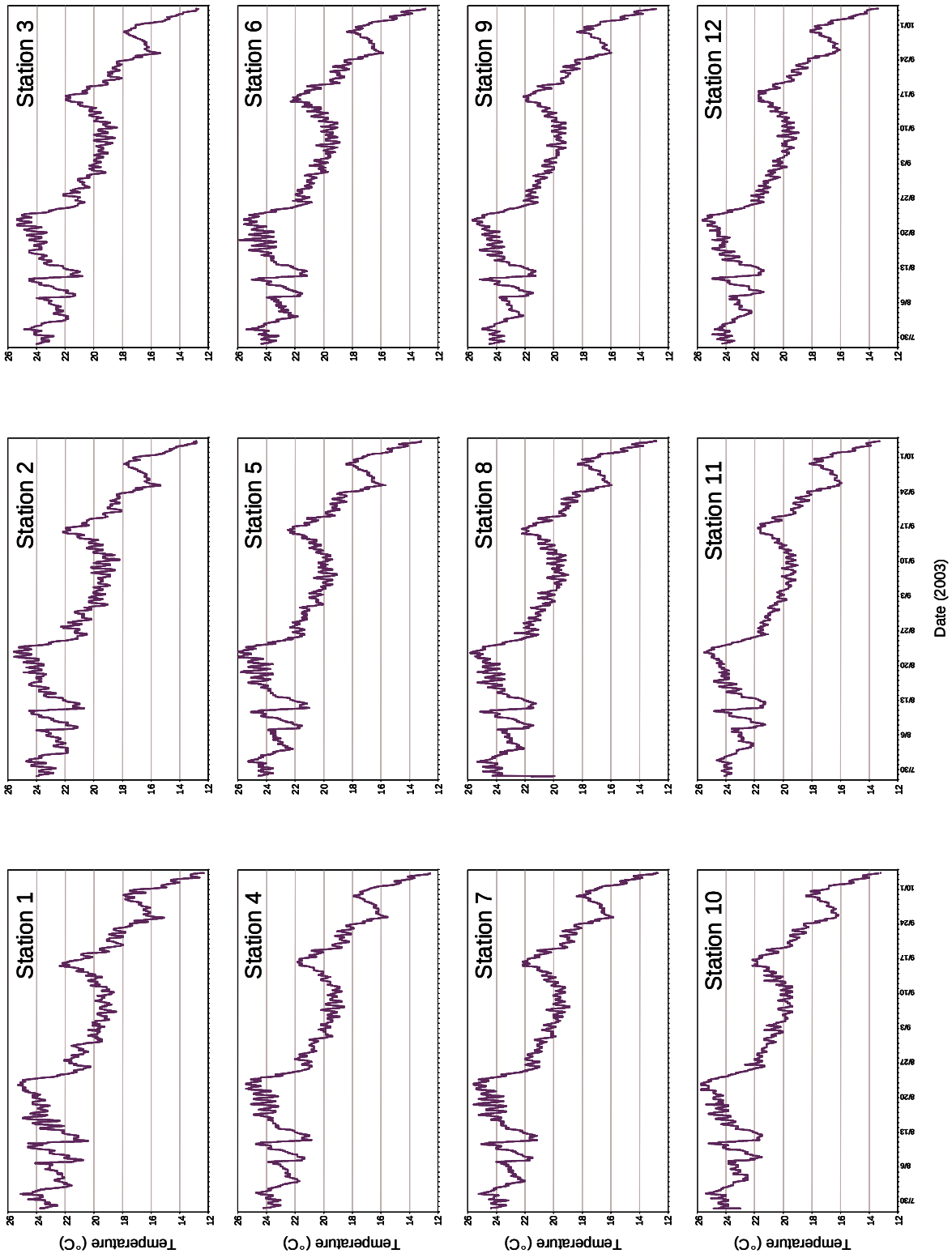

Figure 6. Mean daily temperatures by station.

The discussion will emphasize tissue chemistry, specifically the most toxic dioxin-furan congeners generally believed to be related to kraft mill processes, i.e., 2,3,7,8-TCDD and 2,3,7,8-TCDF. The discussion will also focus on results from the impoundment, which are believed to be the most relevant.

5.1 Above-Below Comparisons

Because 2,3,7,8-TCDD was not found at any station, the mussel tissue chemistry data strongly suggest that the Jay Mill is not a source of the most toxic dioxin congener commonly associated with kraft mill effluents. Although 2,3,7,8-TCDF, the most toxic furan congener commonly associated with kraft mills, was accumulated by mussels at all stations, the mussel tissue chemistry data strongly suggest that the mill is not a source of this compound either. Regardless of which stations within the impoundment were used for the above-below comparisons, concentrations of 2,3,7,8-TCDF were never significantly higher in mussel tissues below the mill.

5.2 Measured Gradients

There could not possibly be any gradient for 2,3,7,8-TCDD because it was not found at any station. While there was an apparent gradient below the mill in 2,3,7,8-TCDF on a lipid-normalized basis, the extreme variability in the lipid data preclude any conclusion that this gradient was real. Furthermore, the lipid-normalized data were not corrected for dry weight because those data were questionable as well. The most reliable 2,3,7,8-TCDF data were the non-normalized values. There was no indication of a gradient with distance from the mill for these data that might suggest it as a source.

5.3 Effects of Dams

An interesting observation was that for nearly all congeners, total PCDD-Fs, and total TEQs, the concentrations were higher directly downstream of each dam. While there was no increase or decrease in any of these parameters in close proximity to the mill, there were substantial increases below each dam. The dams may make a difference in the uptake of dioxins or furans by mussels, but it can not be determined whether the increased uptake was due to elevated concentrations in these areas, to the barrier changing the physical-chemical characteristics of the area, or a combination of both.

5.4 Tissue Chemistry Issues

Of the three caged mussel studies conducted between 2000 and 2003 on the Kennebec and Androscoggin Rivers, the tissue chemistry results from the Androscoggin study are the most believable. Nevertheless, there are some remaining tissue chemistry issues that should be addressed in the future: 1) measured concentrations of individual congeners, 2) number of congeners detected, 3) tissue mass required for maximizing detection, and 4) protocols for determining percent lipids and percent moisture.

In the 2000 Kennebec study (Applied Biomonitoring 2002), the beginning-of-test mussel tissue samples analyzed by the University of Maine, Orono (UMO), had no detectable dioxins-furans.

DEP was skeptical that caged mussels were reasonable surrogates for fish because all previous fish samples had some detectable dioxins-furans, although fish from lake Nequasset were never analyzed. In the 2003 Kennebec study, Pace Analytical reported concentrations for beginning-of-test mussel tissue samples that in some cases were higher than those measured a the end of the test. The data from Columbia Analytical for the Androscoggin beginning-of-test samples showed detectable dioxins-furans, and based on these data, the concentrations of measured congeners increased at all stations during the deployment period. Columbia Analytical also provided individual data sheets for each sample which demonstrated that the sensitivity of the analytical instrument varied with each batch of samples analyzed. These details were not provided by either UMO or PACE.

Regarding tissue mass concerns, Columbia Analytical re-analyzed all Androscoggin samples using more tissue mass (25 g instead of 10 g), and the number of congeners and total concentrations increased significantly. It is not clear whether sufficient tissue mass was used in the second analysis because the number of congeners detected was still approximately half that detected by UMO for the 2000 Kennebec study (Applied Biomonitoring 2002).

The percent lipid values reported by Columbia Analytical do not appear realistic and are inconsistent with previous studies. The values are extremely low with a high degree of variability among replicates. The only possible decreasing gradient detected was for lipid-normalized 2,3,7,8-TCDF, but the data are too variable to establish a clear gradient. The standard caged mussel protocol suggests dry-weight normalization of tissue chemistry data, but the percent moisture data were also not consistent with previous studies.

In the final analysis, the dioxin-furan tissue chemistry results provided by Columbia Analytical for the caged mussels were the most believable, the most consistent and the most useful. However, some questions still remain regarding the details of normalization and the amount of tissue required to maximize detection of dioxin-furan congeners.

6.0 SUMMARY AND CONCLUSIONS

Caged mussels deployed in the Androscoggin River survived, grew, and accumulated some dioxin-furan congeners in their tissues. Results from this study strongly suggest that the Jay mill is not a source for any dioxin-furan congener. Even though some questions remain with respect to the details of the analytical results, the weight of evidence shows that the caged mussel approach is a viable surrogate for fish testing in the dioxin monitoring program.

7.0 REFERENCES

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