MODULE 3 RIVERS AND STREAMS

3.1.

AMBIENT BIOLOGICAL MONITORING

Ambient Biological Monitoring

The Ambient Biological Monitoring section is separate on our website at http://www.state.me.us/dep/blwq/docmonitoring/biomonitoring/index.htm.

3.2

FISH CONSUMPTION ADVISORIES

COPLANAR PCB

In 2004 the SWAT program was again integrated with the Dioxin Monitoring Program (DMP) that has been in effect since 1988. Fish samples collected at 17 DMP stations for dioxin analyses were also analyzed for coplanar PCBs in the SWAT program. All non-detects were calculated at half the detection limit. Dioxin toxic equivalents (DTEh) and coplanar PCB toxic equivalents (CTEh) were calculated using World Health Organization (1998) toxicity equivalency factors (TEFs). For comparison with the Bureau of Health (BOH) Fish Tissue Action Levels (FTAL) for protection of human consumers, the 95th upper confidence limits (95% UCL) were used. The 95%UCL DTEh are compared to the cancer action level, FTALc=1.5 ppt, and the 95%UCL TTEh (sum of both CTEh and DTEh) are compared to the reproductive and developmental action level, FTALr=1.8 ppt and both are compared against the potentially lower fish tissue action level (pFTAL=0.4 ppt) being considered by BOH.

SPECIES CODES

BNT	brown trout
EEL	eel
LMB	largemouth bass
RBT	rainbow trout
SMB	smallmouth bass
WHP	white perch
WHS	white sucker

STATION CODES

AGL	Androscoggin River at Gilead
ARP	Androscoggin River at Rumford Point
ARF	Androscoggin River at Rumford
ARY	Androscoggin River at Riley
AGI	Androscoggin River at GIP, Auburn
ALV	Androscoggin River at Livermore Falls
ALS	Androscoggin River at Lisbon Falls
ALW	Androscoggin Lake at Wayne
KNW	Kennebec River at Norridgewock
KFF	Kennebec River at Shawmut, Fairfield
KRS	Kennebec River at Sidney
PBW	Penobscot River at Woodville
PBL	Penobscot River at S Lincoln
PBV	Penobscot River at Veazie
SEN	E Br Sebasticook at Newport
SED	E Br Sebasticook at Detroit
SWP	W Br Sebasticook at Palmyra
SEB	Sebasticook River at Burnham

The results show that dioxin toxic equivalents (DTEh95ucl, upper 95% confidence limit with non-detects at ½ the detection level) and coplanar PCB toxic equivalents (CTEh95ucl, upper 95% confidence limit with non-detects at ½ the detection level) both separately and combined cause many samples to exceed the various FTALs (Figures 3.2.1 and 3.2.2). CTE appear more dominant than the DTE for bass from all rivers sampled and for suckers from the Kennebec and Penobscot rivers. But that is partly because the detection levels are higher for CTE, so that using non-detects at one-half of the detection level results in larger values. This is especially so for the Sebasticook River CTE which are much higher than those measured previously, when CTE detection levels were lower at a different lab. Attempts will be made to lower the detection limits in any analysis of future samples. Sources of PCBs are unknown but likely include atmospheric deposition.

Figure 3.2.1 Dioxin (DTE) and Coplanar PCB (CTE) toxic equivalents in smallmouth bass (and white perch WHP and rainbow trout RBT) from the Androscoggin (Axy), Kennebec (Kxy), Penobscot (Pxy), and Sebasticook (Sxy) rivers, 2004.

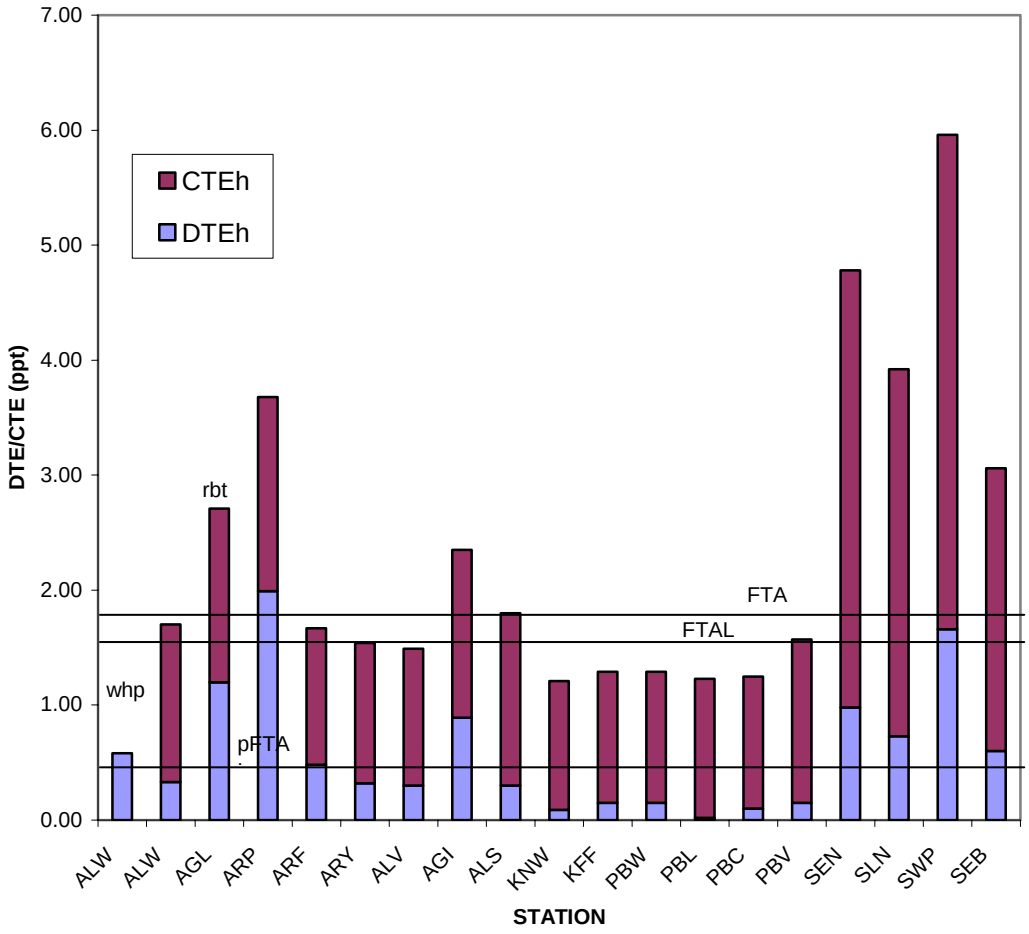
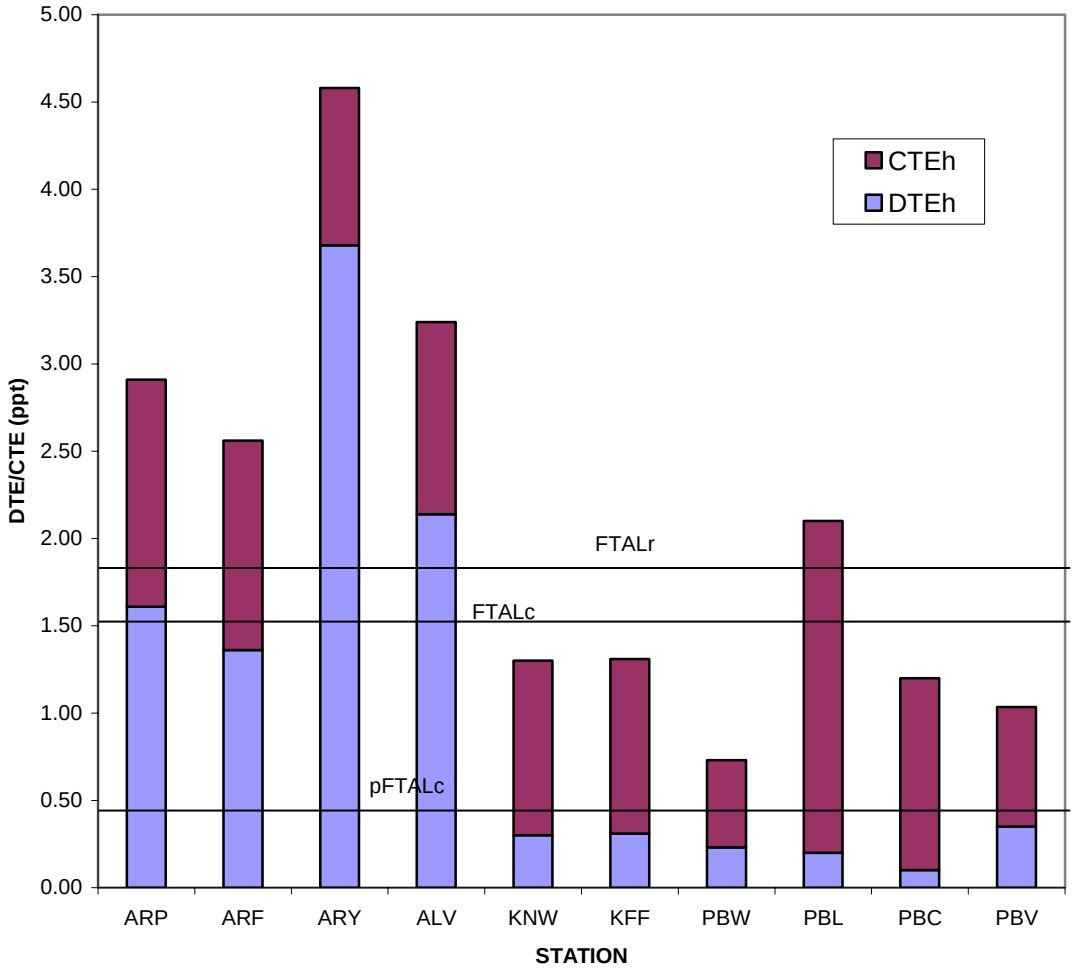


Figure 3.2.2. Dioxin (DTE) ans coplanar PCB (CTE) toxic equivalents in white suckers from the Androscoggin (Axy), Kennebec (Kxy), and Penobscot (PBy) rivers, 2004.



DIOXIN

Dioxins in rainbow trout at Gilead and in bass from three locations on the Sebasticook River were measured as part of the SWAT program but previously included in the 2004 final report of Maine's Dioxin Monitoring Program (DMP) available at http://www.maine.gov/dep/blwq/docmonitoring/dioxin/index.htm

RAW COPLANAR PCB DATA

DEP ID		AGL RBT 1 ng/Kg		AGL RBT 4 ng/Kg		AGL RBT 5 ng/Kg		AGL RBT 6 ng/Kg		AGL RBT 7 ng/Kg	AGL RBT ave
PCB IUPAC #											
77	<	19.6	<	19.8	<	19.7	<	19.8	<	19.9	
81	<	19.6	<	19.8	<	19.7	<	19.8	<	19.9	
105		357		288		326		490		278	
114		23.4		20.7		24.5		35.4	<	19.9	
118		1050		874		1070		1560		803	
123	<	19.6	<	19.8	<	19.7		30.6	<	19.9	
126	<	19.6	<	19.8	<	19.7	<	19.8	<	19.9	
156/157		284		224		328		526		256	
167		160		134		194		245		115	
169	<	19.6	<	19.8	<	19.7	<	19.8	<	19.9	
189		68.5		60.1		91.7		136		59.6	
CTEo		0.3035		0.246		0.3269		0.5048		0.2433	0.325
CTEd		2.469		2.429		2.505		2.684		2.443	2.506
CTEh		1.39		1.34		1.42		1.59		1.34	1.42
CTEh sd											0.11
CTEh confidence											0.09
CTEh 95 UCL											1.51
% FTAL											101
% Lipids		2.46		2.52		2.34		2.7		0.84	2.2
% Solids		25.2		23.9		24.3		25.3		23.6	24.5

DEP ID PCB IUPAC #
77
81
105
114
118
123
126
167
169
189
CTEo
CTEd
CTEh
CTEh sd
CTEh confidence
CTEh 95 UCL
% FTAL
% Lipids
% Solids

DEP ID		ARP-SMB-C1		ARP-SMB-C2		ARP-SMB-C3		ARP-SMB-C4		ARP-SMB-C5	ARP-SMB
PCB IUPAC #		ng/Kg		ng/Kg		ng/Kg		ng/Kg		ng/Kg	
77	<	19.7	<	19.7		24.3		22.2	<	19.9	
81	<	19.7	<	19.7	<	19.9	<	19.7	<	19.9	
105		426		402		529		523		472	
114		27.7		29.3		40.6		37		35.6	
118		1320		1270		1740		1650		1540	
123	<	19.7		19.7		21.3		22.3		25.2	
126	<	19.7	<	19.7	<	19.9	<	19.7	<	19.9	
156/157		449		419		606		547		567	
167		232		207		290		269		280	
169	<	19.7	<	19.7	<	19.9	<	19.7	<	19.9	
189		109		108		152		139		144	
CTEo		0.4259		0.4062		0.5731		0.53		0.5218	
CTEd		2.601		2.574		2.761		2.703		2.71	
CTEh		1.51		1.49		1.67		1.62		1.62	
CTEh sd											
CTEh confidence											
CTEh 95 UCL											
% FTAL											
% Lipids		1.21		1.07		2.12		2.31		1.53	
% Solids											

DEP ID		ARP-SMB-C6		ARP-SMB-C7		ARP-SMB-C8		ARP-SMB-C9		ARP-SMB-C10	
PCB IUPAC #		ng/Kg		ng/Kg		ng/Kg		ng/Kg		ng/Kg	
77		32.9		19.8	<	19.7		21.1	<	19.7	
81	<	19.7	<	19.6	<	19.7	<	19.7	<	19.7	
105		769		399		369		594		471	
114		55.5		31.1		28.7		44.8		36.4	
118		2440		1350		1270		1970		1560	
123		37.3	<	19.6		21.9		25.6		22.6	
126	<	19.7	<	19.6	<	19.7	<	19.7	<	19.7	
		831		487		424		650		579	
167		410		266		233		337		306	
169	<	19.7	<	19.6	<	19.7	<	19.7	<	19.7	
189		222		121		119		167		142	
CTEo		0.7975		0.4508		0.4068		0.6291		0.5305	0.527
CTEd		2.967		2.606		2.583		2.802		2.701	2.70
CTEh		1.88		1.53		1.49		1.72		1.62	1.61
CTEh sd											0.12
CTEh confidence											0.08
CTEh 95 UCL											1.69
% FTAL											113
% Lipids		2.22		2.11		2.17		1.86		1.33	1.8
% Solids		23		23		23.1		22.3		22.4	22.8

DEP ID PCB IUPAC #		ARF SMBC1 ng/Kg		ARF SMBC2 ng/Kg		ARF SMBC3 ng/Kg		ARF SMBC4 ng/Kg		ARF SMBC5 ng/Kg	ARF SMB ave
77	<	19.6	<	19.9	<	20	<	19.6	<	19.5	
81	<	19.6	<	19.9	<	20	<	19.6	<	19.5	
105		72		73.7		115		84.2		142	
114	<	19.6	<	19.9	<	20	<	19.6	<	19.5	
118		184		204		309		237		434	
123	<	19.6	<	19.9	<	20	<	19.6	<	19.5	
126	<	19.6	<	19.9	<	20	<	19.6	<	19.5	
156/157		45.1		55.3		75.6		65.2		108	
167		19.9		25.2		31.4		29.1		52.2	
169	<	19.6	<	19.9	<	20	<	19.6	<	19.5	
189	<	19.6	<	19.9	<	20	<	19.6		24.8	
CTEo		0.0483		0.05569		0.08054		0.06501		0.1144	
CTEd		2.22		2.267		2.295		2.243		2.275	
CTEh		1.13		1.16		1.19		1.15		1.19	
CTEh sd											
CTEh confidence											
CTEh 95 UCL											
% FTAL											
% Lipids		0.433		0.583		0.575		0.573		1	
% Solids		22		21.5		22.1		21.7		21.2	

DEP ID PCB IUPAC #		ARF SMBC6 ng/Kg		ARF SMBC7 ng/Kg		ARF SMBC8 ng/Kg		ARF SMBC9 ng/Kg		ARF SMBC1(ng/Kg	ARF SMB ave
77	<	19.6	<	19.9	<	19.6	<	19.7	<	19.7	
81	<	19.6	<	19.9	<	19.6	<	19.7	<	19.7	
105		88.3		128		147		99.1		124	
114	<	19.6	<	19.9	<	19.6	<	19.7	<	19.7	
118		242		395		424		294		393	
123	<	19.6	<	19.9	<	19.6	<	19.7	<	19.7	
126	<	19.6	<	19.9	<	19.6	<	19.7	<	19.7	
		62.4		102		121		90.6		113	
167		28.5		52.8		53.3		48		53	
169	<	19.6	<	19.9	<	19.6	<	19.7	<	19.7	
189	<	19.6		21.5		22.5		21		21.7	
CTEo		0.06448		0.1062		0.1205		0.08717		0.111	0.085
CTEd		2.237		2.31		2.29		2.266		2.295	2.270
CTEh		1.15		1.21		1.21		1.18		1.20	1.18
CTEh sd											0.03
CTEh confidence											0.02
CTEh 95 UCL											1.19
% FTAL											80
% Lipids		0.76		0.71		0.43		0.85		0.8	0.7
% Solids		21.3		21.7		20.7		21.6		22	21.6

DEP ID PCB IUPAC #		ARY SMBC1 ng/Kg		ARY SMBC2 ng/Kg		ARY SMBC3 ng/Kg		ARY SMBC4 ng/Kg		ARY SMBC5 ng/Kg	ARY SMB ave
77	<	19.7	<	20	<	20	<	19.7	<	19.6	
81	<	19.7	<	20	<	20	<	19.7	<	19.6	
105		101		130		82.2		129		131	
114	<	19.7	<	20	<	20	<	19.7	<	19.6	
118		357		466		300		437		442	
123	<	19.7	<	20	<	20	<	19.7	<	19.6	
126	<	19.7	<	20	<	20	<	19.7	<	19.6	
156/157		102		113		81.3		105		121	
167		49.8		54.3		40		47.8		54.7	
169	<	19.7	<	20	<	20	<	19.7	<	19.6	
189	<	19.7		20.7	<	20	<	19.7		20.9	
CTEo		0.0975		0.1187		0.07922		0.1097		0.1206	
CTEd		2.285		2.334		2.297		2.291		2.297	
CTEh		1.19		1.23		1.19		1.20		1.21	
CTEh sd											
CTEh confidence											
CTEh 95 UCL											
% FTAL											
% Lipids		0.19		0.33		0.21		0.75		1.12	
% Solids		20.6		21.4		19.7		20.1		20.1	

DEP ID PCB IUPAC #		ARY SMBC6 ng/Kg		ARY SMBC7 ng/Kg		ARY SMBC8 ng/Kg		ARY SMBC9 ng/Kg		ARY SMBC10 ng/Kg	
77	<	19.8	<	20	<	19.8	<	19.9	<	19.8	
81	<	19.8	<	20	<	19.8	<	19.9	<	19.8	
105		82		75.9		185		95.3		155	
114	<	19.8	<	20	<	19.8	<	19.9	<	19.8	
118		296		300		648		332		415	
123	<	19.8	<	20	<	19.8	<	19.9	<	19.8	
126	<	19.8	<	20	<	19.8	<	19.9	<	19.8	
		76.9		89.7		175		90.1		95.1	
167		33.7		35.1		70		39.3		27.6	
169	<	19.8	<	20	<	19.8	<	19.9	<	19.8	
189	<	19.8	<	20		30.5	<	19.9	<	19.8	
CTEo		0.07661		0.08277		0.1748		0.08817		0.1049	0.105
CTEd		2.272		2.299		2.364		2.292		2.303	2.303
CTEh		1.17		1.19		1.27		1.19		1.20	1.204
CTEh sd											0.03
CTEh confidence											0.02
CTEh 95 UCL											1.22
% FTAL											81
% Lipids		0.19		0.69		1.35		0.7		0.58	0.61
% Solids		20.8		19.5		20.7		19.8		20	20.3

DEP ID PCB IUPAC #		ALV SMBC1 ng/Kg		ALV SMBC2 ng/Kg		ALV SMBC3 ng/Kg		ALV SMBC4 ng/Kg		ALV SMBC5 ng/Kg	ALV SMB ave
77	<	19.6	<	19.8	<	19.9	<	20	<	19.9	
81	<	19.6	<	19.8	<	19.9	<	20	<	19.9	
105		50		42.1		53.1		62.2		33.6	
114	<	19.6	<	19.8	<	19.9	<	20	<	19.9	
118		158		135		180		206		93.1	
123	<	19.6	<	19.8	<	19.9	<	20	<	19.9	
126	<	19.6	<	19.8	<	19.9	<	20	<	19.9	
156/157		51.1		47.7		60.5		74.2		30.3	
167		27.4		25.5		35.4		42.8	<	19.9	
169	<	19.6	<	19.8	<	19.9	<	20	<	19.9	
189	<	19.6	<	19.8	<	19.9	<	20	<	19.9	
CTEo		0.0466		0.04177		0.05396		0.06432		0.02784	
CTEd		2.224		2.243		2.262		2.281		2.235	
CTEh		1.14		1.14		1.16		1.17		1.13	
CTEh sd											
CTEh confidence											
CTEh 95 UCL											
% FTAL											
% Lipids		0.61		0.613		0.569		0.329		0.498	
% Solids		20.3		20.4		20.7		20.4		20.4	

DEP ID PCB IUPAC #	SM	ALV SMBC6 ng/Kg		ALV SMBC7 ng/Kg		ALV SMBC8 ng/Kg		ALV SMBC9 ng/Kg		ALV SMBC10 ng/Kg	
77	<	19.9	<	19.8	<	20	<	19.9	<	19.4	
81	<	19.9	<	19.8	<	20	<	19.9	<	19.4	
105		46.8		63.3		160		112		31.2	
114	<	19.9	<	19.8	<	20	<	19.9	<	19.4	
118		135		204		461		368		99.4	
123	<	19.9	<	19.8	<	20	<	19.9	<	19.4	
126	<	19.9	<	19.8	<	20	<	19.9	<	19.4	
		46.5		69.9		142		113		33.8	
167		27.4		41.9		85		66.9	<	19.4	
169	<	19.9	<	19.8	<	20	<	19.9	<	19.4	
189	<	19.9	<	19.8		34.1		26	<	19.4	
CTEo		0.04175		0.06215		0.1372		0.1079		0.02996	0.061
CTEd		2.245		2.259		2.349		2.311		2.179	2.259
CTEh		1.14		1.16		1.24		1.21		1.10	1.160
CTEh sd											0.04
CTEh confidence											0.02
CTEh 95 UCL											1.19
% FTAL											79
% Lipids		0.53		0.393		0.384		0.512		0.248	0.5
% Solids		20.1		16.9		19		19.8		21.7	20.0

DEP ID PCB IUPAC #		AGI-SMB-01 ng/Kg		AGI-SMB-04 ng/Kg		AGI-SMB-05 ng/Kg	AGI-SMB ave
77	<	19.8	<	19.7	<	19.7	
81	<	19.8	<	19.7	<	19.7	
105		128		193		350	
114	<	19.8	<	19.7		26.3	
118		449		675		1220	
123	<	19.8	<	19.7	<	19.7	
126	<	19.8	<	19.7	<	19.7	
156/157		232		326		328	
167		144		183		174	
169	<	19.8	<	19.7	<	19.7	
189		46.1		88.4		70.1	
CTEo		0.1796		0.2602		0.3424	0.261
CTEd		2.376		2.439		2.515	2.443
CTEh		1.28		1.35		1.43	1.35
CTEh sd							0.08
CTEh confidence							0.10
CTEh 95 UCL							1.46
% FTAL							97
% Lipids		1.42		0.481		1.11	1.0
% Solids		23.4		21.2		22.7	22.4

DEP ID PCB IUPAC #		ALW SMBC1 ng/Kg		ALW SMBC6 ng/Kg	ALW SMB ave		ALW-WHP-01 ng/Kg
77	<	19.6	<	20		<	19.7
81	<	19.6	<	20		<	19.7
105		161		212			192
114	<	19.6	<	20		<	19.7
118		501		663			534
123	<	19.6	<	20		<	19.7
126	<	19.6	<	20		<	19.7
		181		262			201
167		107		159			92.6
169	<	19.6	<	20		<	19.7
189		43.3		61.4			54.8
CTEo		0.162		0.2261	0.194		0.1795
CTEd		2.335		2.439	2.387		2.366
CTEh		1.25		1.33	1.29		1.27
CTEh sd					0.06		
CTEh confidence					0.08		
CTEh 95 UCL					1.37		
% FTAL					92		
% Lipids		0.442		0.61	0.5		0.77
% Solids		20		19.4	19.7		21.6

DEP ID PCB IUPAC #		ALS-SMB-1 ng/Kg		ALS-SMB-2 ng/Kg		ALS-SMB-3 ng/Kg		ALS-SMB-4 ng/Kg		ALS-SMB-5 ng/Kg	ALS-SMB ave
77	<	20	<	19.7	<	19.9		25.8		24.3	
81	<	20	<	19.7	<	19.9	<	19.8	<	19.6	
105		606		398		413		413		458	
114		43.4		27.9		27.3		29.8		29.2	
118		1500		1270		1390		1200		1300	
123		33.4		23.2	<	19.9	<	19.8	<	19.6	
126	<	20	<	19.7	<	19.9	<	19.8	<	19.6	
156/157		427		271		314		272		295	
167		138		125		139		114		112	
169	<	20	<	19.7	<	19.9	<	19.8	<	19.6	
189		24.5		35.3		42.8		29.6		27.5	
CTEo		0.4527		0.3239		0.3567		0.3193		0.3445	0.359
CTEd		2.653		2.49		2.554		2.501		2.509	2.541
CTEh		1.55		1.41		1.46		1.41		1.43	1.450
CTEh sd											0.06
CTEh confidence											0.05
CTEh 95 UCL											1.50
% FTAL											100
% Lipids		0.897		0.48		0.93		0.555		0.855	0.7
% Solids		22.3		21.3		21.7		21.3		22.2	21.8

DEP ID	KNW SMBC1	KNW SMBC4	KNW SMBC7	KNW SMBC10	KNW SMBC1	KNW SMB
PCB IUPAC #	ng/Kg	ng/Kg	ng/Kg	ng/Kg	ng/Kg	
77	< 19.9	< 19.7	< 19.9	< 19.8	< 19.8	
81	< 19.9	< 19.7	< 19.9	< 19.8	< 19.8	
105	29.8	21.9	35.8	32.4	28.5	
114	< 19.9	< 19.7	< 19.9	< 19.8	< 19.8	
118	86.1	67.2	110	94.7	77.4	
123	< 19.9	< 19.7	< 19.9	< 19.8	< 19.8	
126	< 19.9	< 19.7	< 19.9	< 19.8	< 19.8	
156/157	< 19.9	< 19.7	20.2	< 19.8	< 19.8	
167	< 19.9	< 19.7	< 19.9	< 19.8	< 19.8	
169	< 19.9	< 19.7	< 19.9	< 19.8	< 19.8	
189	< 19.9	< 19.7	< 19.9	< 19.8	< 19.8	
CTEo	0.01159	0.008918	0.0247	0.0127	0.01058	
CTEd	2.226	2.199	2.227	2.223	2.211	
CTEh	1.12	1.10	1.13	1.12	1.11	
CTEh sd						
CTEh confidence						
CTEh 95 UCL						
% FTAL						
% Lipids	0.64	0.79	0.63	0.82	0.16	
% Solids	22.5	21.5	22.5	21.6	20.5	

DEP ID	KNW SMBC16	KNW SMBC19	KNW SMBC22	KNW SMBC25	KNW SMBC28	
PCB IUPAC #	ng/Kg	ng/Kg	ng/Kg	ng/Kg	ng/Kg	
77	< 19.5	< 19.6	< 19.9	< 19.9	< 19.9	
81	< 19.5	< 19.6	< 19.9	< 19.9	< 19.9	
105	37	< 19.6	38.3	< 19.9	62	
114	< 19.5	< 19.6	< 19.9	< 19.9	< 19.9	
118	102	49.4	101	36.2	162	
123	< 19.5	< 19.6	< 19.9	< 19.9	< 19.9	
126	< 19.5	< 19.6	< 19.9	< 19.9	< 19.9	
	21.1	< 19.6	< 19.9	< 19.9	33.3	
167	< 19.5	< 19.6	< 19.9	< 19.9	< 19.9	
169	< 19.5	< 19.6	< 19.9	< 19.9	< 19.9	
189	< 19.5	< 19.6	< 19.9	< 19.9	< 19.9	
CTEo	0.02441	0.004936	0.01394	0.003616	0.03904	**0.015**
CTEd	2.185	2.189	2.232	2.221	2.244	**2.216**
CTEh	1.10	1.10	1.12	1.11	1.14	**1.116**
CTEh sd						**0.01**
CTEh confidence						**0.01**
CTEh 95 UCL						**1.12**
% FTAL						**75**
% Lipids	0.23	0.65	0.67	0.66	0.85	**0.61**
% Solids	21.6	21.6	21.4	21.8	21.5	**21.7**

DEP ID PCB IUPAC #		KFF SMBC1 ng/Kg		KFF SMBC2 ng/Kg		KFF SMBC3 ng/Kg		KFF SMBC4 ng/Kg		KFF SMBC5 ng/Kg	KFF SMB ave
77	<	20	<	19.8	<	19.8	<	19.9	<	19.6	
81	<	20	<	19.8	<	19.8	<	19.9	<	19.6	
105		55.4		68.3		31.2		29.6		59.1	
114	<	20	<	19.8	<	19.8	<	19.9	<	19.6	
118		158		199		83.4		81.7		169	
123	<	20	<	19.8	<	19.8	<	19.9	<	19.6	
126	<	20	<	19.8	<	19.8	<	19.9	<	19.6	
156/157		28.9		35.1	<	19.8	<	19.9		32.1	
167	<	20	<	19.8	<	19.8	<	19.9	<	19.6	
169	<	20	<	19.8	<	19.8	<	19.9	<	19.6	
189	<	20	<	19.8	<	19.8	<	19.9	<	19.6	
CTEo		0.0358		0.04428		0.01145		0.01113		0.03889	
CTEd		2.249		2.241		2.22		2.231		2.216	
CTEh		1.14		1.14		1.12		1.12		1.13	
CTEh sd											
CTEh confidence											
CTEh 95 UCL											
% FTAL											
% Lipids		0.768		1		0.525		1.05		1	
% Solids		23.5		23.2		22.2		21.9		23.7	

DEP ID PCB IUPAC #		KFF SMBC6 ng/Kg		KFF SMBC7 ng/Kg		KFF SMBC8 ng/Kg		KFF SMBC9 ng/Kg		KFF SMBC10 ng/Kg	
77	<	20	<	20	<	19.9	<	19.9	<	19.9	
81	<	20	<	20	<	19.9	<	19.9	<	19.9	
105		36.8		32.8		60.5		39.7		52.7	
114	<	20	<	20	<	19.9	<	19.9	<	19.9	
118		95.5		91.4		160		101		143	
123	<	20	<	20	<	19.9	<	19.9	<	19.9	
126	<	20	<	20	<	19.9	<	19.9	<	19.9	
	<	20	<	20		29.7	<	19.9		32.1	
167	<	20	<	20	<	19.9	<	19.9	<	19.9	
169	<	20	<	20	<	19.9	<	19.9	<	19.9	
189	<	20	<	20	<	19.9	<	19.9	<	19.9	
CTEo		0.01323		0.01242		0.03695		0.01405		0.03563	**0.025**
CTEd		2.237		2.235		2.243		2.232		2.246	**2.235**
CTEh		1.13		1.12		1.14		1.12		1.14	**1.130**
CTEh sd											**0.01**
CTEh confidence											**0.01**
CTEh 95 UCL											**1.14**
% FTAL											**76**
% Lipids		1.04		0.865		1.37		1.31		0.98	**1.0**
% Solids		22.7		22.4		22.6		22.3		22	**22.7**

DEP ID		PBW SMBC1 ng/Kg		PBW SMBC6 ng/Kg	PBW SMB ave		PBL SMBC1 ng/Kg		PBL SMBC6 ng/Kg	PBL SMB ave
PCB IUPAC #										
77	<	19.6	<	19.9		<	20	<	19.8	
81	<	19.6	<	19.9		<	20	<	19.8	
105		31.5		30			112		136	
114	<	19.6	<	19.9		<	20	<	19.8	
118		110		103			327		314	
123	<	19.6	<	19.9		<	20	<	19.8	
126	<	19.6	<	19.9		<	20	<	19.8	
156/157		33.4		30.6			97.1		74.1	
167	<	19.6	<	19.9			48.7		27.3	
169	<	19.6	<	19.9		<	20	<	19.8	
189	<	19.6	<	19.9			23	<	19.8	
CTEo		0.03082		0.02865	0.030		0.09518		0.08228	0.089
CTEd		2.21		2.233	2.222		2.309		2.28	2.295
CTEh		1.12		1.13	1.13		1.20		1.18	1.19
CTEh sd					0.01					0.01
CTEh confidence					0.01					0.02
CTEh 95 UCL					1.14					1.21
% FTAL					76					81
% Lipids		0.37		0.52	0.4		1.51		0.82	1.2
% Solids		21		20.1	20.6		20.9		20.4	20.7

DEP ID PCB IUPAC #		PBC SMBC1 ng/Kg		PBC SMBC3 ng/Kg		PBC SMBC5 ng/Kg		PBC SMBC7 ng/Kg		PBC SMBC9 ng/Kg	PBC SMB ave
77	<	19.5	<	19.8	<	20	<	19.5	<	19.7	
81	<	19.5	<	19.8	<	20	<	19.5	<	19.7	
105		125		54.4		54.3		67		72.2	
114	<	19.5	<	19.8	<	20	<	19.5	<	19.7	
118		311		142		130		168		197	
123	<	19.5	<	19.8	<	20	<	19.5	<	19.7	
126	<	19.5	<	19.8	<	20	<	19.5	<	19.7	
156/157		60.1		28.7		28		32.6		38.8	
167		19.9	<	19.8	<	20	<	19.5	<	19.7	
169	<	19.5	<	19.8	<	20	<	19.5	<	19.7	
189	<	19.5	<	19.8	<	20	<	19.5	<	19.7	
CTEo		0.07388		0.03401		0.03244		0.03985		0.04636	
CTEd		2.241		2.235		2.247		2.208		2.236	
CTEh		1.16		1.13		1.14		1.12		1.14	
CTEh sd											
CTEh confidence											
CTEh 95 UCL											
% FTAL											
% Lipids		0.78		0.49		0.25		0.72		0.43	
% Solids		21.3		21.5		21.5		22.1		21	

DEP ID PCB IUPAC #		PBC SMBC11 ng/Kg		PBC SMBC13 ng/Kg		PBC SMBC15 ng/Kg		PBC SMBC17 ng/Kg		PBC SMBC19 ng/Kg	PBC SMB ave
77	<	19.7	<	19.8	<	19.8	<	19.9	<	19.5	
81	<	19.7	<	19.8	<	19.8	<	19.9	<	19.5	
105		132		46.3		79.2		64.3	<	19.5	
114	<	19.7	<	19.8	<	19.8	<	19.9	<	19.5	
118		345		123		209		158		23.1	
123	<	19.7	<	19.8	<	19.8	<	19.9	<	19.5	
126	<	19.7	<	19.8	<	19.8	<	19.9	<	19.5	
		62.6		23.6		38		30.8	<	19.5	
167		23.2	<	19.8	<	19.8	<	19.9	<	19.5	
169	<	19.7	<	19.8	<	19.8	<	19.9	<	19.5	
189	<	19.7	<	19.8	<	19.8	<	19.9	<	19.5	
CTEo		0.07918		0.0287		0.04777		0.03759		0.002313	0.042
CTEd		2.269		2.221		2.239		2.24		2.181	2.232
CTEh		1.17		1.12		1.14		1.14		1.09	1.14
CTEh sd											0.02
CTEh confidence											0.01
CTEh 95 UCL											1.15
% FTAL											77
% Lipids		0.23		0.37		0.48		0.42		0.44	0.5
% Solids		21.5		20.4		22.7		21.4		21.3	21.5

DEP ID PCB IUPAC #		PBV SMBC1 ng/Kg		PBV SMBC3 ng/Kg		PBV SMBC5 ng/Kg		PBV SMBC7 ng/Kg		PBV SMBC9 ng/Kg	PBV SMB ave
77	<	19.8	<	19.9	<	19.6	<	20	<	19.8	
81	<	19.8	<	19.9	<	19.6	<	20	<	19.8	
105		376		693		325		164		214	
114		30.1		55.4		27	<	20	<	19.8	
118		1180		1940		916		467		574	
123	<	19.8		34.8	<	19.6	<	20	<	19.8	
126	<	19.8	<	19.9	<	19.6	<	20	<	19.8	
156/157		310		427		184		93.1		139	
167		110		156		67.6		33.5		51	
169	<	19.8	<	19.9	<	19.6	<	20	<	19.8	
189		49		65		20.5	<	20	<	19.8	
CTEo		0.3314		0.5158		0.2322		0.11		0.1488	
CTEd		2.514		2.708		2.398		2.326		2.347	
CTEh		1.42		1.61		1.32		1.22		1.25	
CTEh sd											
CTEh confidence											
CTEh 95 UCL											
% FTAL											
% Lipids		0.42		0.66		0.26		0.59		0.65	
% Solids		21.3		22.6		22.3		22.3		22.4	

DEP ID PCB IUPAC #		PBV SMBC11 ng/Kg		PBV SMBC13 ng/Kg		PBV SMBC15 ng/Kg		PBV SMBC17 ng/Kg		PBV SMBC19 ng/Kg	PBV SMB ave
77	<	19.7	<	20	<	19.9	<	19.5	<	19.6	
81	<	19.7	<	20	<	19.9	<	19.5	<	19.6	
105		713		201		120		151		104	
114		43.9	<	20	<	19.9	<	19.5	<	19.6	
118		1910		568		376		495		282	
123		32.8	<	20	<	19.9	<	19.5	<	19.6	
126	<	19.7	<	20	<	19.9	<	19.5	<	19.6	
		392		114		107		109		54.9	
167		118		46.9		34.3		40.4		19.9	
169	<	19.7	<	20	<	19.9	<	19.5	<	19.6	
189		28	<	20	<	19.9	<	19.5	<	19.6	
CTEo		0.4873		0.1343		0.1036		0.1195		0.06623	0.225
CTEd		2.662		2.35		2.311		2.286		2.235	2.414
CTEh		1.57		1.24		1.21		1.20		1.15	1.32
CTEh sd											0.16
CTEh confidence											0.10
CTEh 95 UCL											1.42
% FTAL											95
% Lipids		0.63		1.86		0.57		0.72		0.81	0.7
% Solids		21.5		22.7		21.6		21.2		21.6	22.0

DEP ID		SEN-SMB-1 ng/Kg		SEN-SMB-2 ng/Kg		SEN-SMB-3 ng/Kg		SEN-SMB-4 ng/Kg		SEN-SMB-5 ng/Kg	SEN-SMB ave
PCB IUPAC #											
77		65.9		38.9		42.4		50.6		43.3	
81	<	19.7	<	19.9	<	19.5	<	19.9	<	19.9	
105		926		507		714		710		576	
114		59.6		33.1		52		53.1		42.1	
118		3590		2100		3310		2890		2280	
123		56.5	<	19.9		57.5		42		32.7	
126		27.1	<	19.9		26.1		28.4		26.3	
156/157		512		323		400		411		370	
167		360		222		293		214		181	
169	<	19.7	<	19.9	<	19.5	<	19.9	<	19.9	
189		47.1		34.9		44.2		21.9		20	
CTEo		3.471		0.4479		3.252		3.45		3.133	2.751
CTEd		3.669		2.637		3.449		3.651		3.334	3.348
CTEh		3.57		1.54		3.35		3.55		3.23	3.05
CTEh sd											0.85
CTEh confidence											0.75
CTEh 95 UCL											3.80
% FTAL											253
% Lipids		1.88		1.29		0.666		1.49		1.24	1.3
% Solids		22.7		21.8		20.7		22		23.4	22.1

DEP ID PCB IUPAC #		SLN SMB 1 ng/Kg		SLN SMB 2 ng/Kg		SLN SMB 3 ng/Kg		SLN SMB 4 ng/Kg		SLN SMB 5 ng/Kg	SLN SMB ave
77		41.7	<	19.6		68		39.4		83.5	
81	<	30.3	<	19.6	<	24.7	<	20	<	19.9	
105		354		110		544		571		989	
114		30.3	<	19.6		26.8		45.3		76	
118		2140		640		3000		3480		5780	
123	<	30.3	<	19.6		38.9		51.4		95.9	
126	<	30.3	<	19.6		32.9	<	20	<	19.9	
		297		90.9		456		463		738	
167		137		40.4		207		248		381	
169	<	30.3	<	19.6	<	24.7	<	20	<	19.9	
189	<	30.3	<	19.6		43.1		27.8		47.3	
CTEo		0.419		0.1208		3.903		0.674		1.11	1.245
CTEd		3.759		2.296		4.153		2.871		3.305	3.277
CTEh		2.09		1.21		4.03		1.77		2.21	2.26
CTEh sd											1.06
CTEh confidence											0.93
CTEh 95 UCL											3.19
% FTAL											213
% Lipids		1.07		0.97		2.25		1.31		4.12	1.9
% Solids		21.6		22		23.1		21.9		23.9	22.5

DEP ID PCB IUPAC #		SWP SMB 1 ng/Kg		SWP SMB 2 ng/Kg		SWP SMB 3 ng/Kg		SWP SMB 4 ng/Kg		SWP SMB 5 ng/Kg	SWP SMB ave
77	<	19.9		19.9	<	19.8		19.7		46.5	
81	<	19.9	<	19.4	<	19.8	<	19.6	<	19.7	
105		2660		851		265		363		5880	
114		173		62.4		22.3		26.7		351	
118		8260		3470		1780		1980		20300	
123		109		37.4	<	19.8	<	19.6		222	
126	<	19.9	<	19.4	<	19.8	<	19.6	<	19.7	
156/157		2220		522		355		248		3270	
167		858		220		173		131		1240	
169	<	19.9	<	19.4	<	19.8	<	19.6	<	19.7	
189		109		32.9		48.3		29.2		141	
CTEo		2.318		0.7355		0.4		0.3778		4.478	1.662
CTEd		4.513		2.876		2.583		2.543		6.651	3.833
CTEh		3.42		1.81		1.49		1.46		5.56	2.75
CTEh sd											1.77
CTEh confidence											1.55
CTEh 95 UCL											4.30
% FTAL											286
% Lipids		1.22		0.74		0.54		1.42		1.66	1.1
% Solids		22		21.7		18.9		22.6		23.6	21.8

DEP ID PCB IUPAC #		SEB SMB-1 ng/Kg		SEB SMB-2 ng/Kg		SEB SMB-3 ng/Kg		SEB SMB-4 ng/Kg		SEB SMB-5 ng/Kg	SEB SMB ave
77		70.7	<	19.7		47		43.7	<	19.3	
81	<	19.8	<	19.7	<	19.7	<	19.7	<	19.3	
105		517		217		403		649		197	
114		44.2	<	19.7		32.5		50	<	19.3	
118		1860		903		2150		3130		862	
123		32.5	<	19.7		29.4		43.5	<	19.3	
126	<	19.8	<	19.7	<	19.7		24.4	<	19.3	
		249		173		289		457		140	
167		109		80.6		150		231		69.7	
169	<	19.8	<	19.7	<	19.7	<	19.7	<	19.3	
189		21.6	<	19.7		28.6		40.4	<	19.3	
CTEo		0.398		0.1992		0.4284		3.089		0.1764	0.858
CTEd		2.581		2.379		2.593		3.288		2.318	2.632
CTEh		1.49		1.29		1.51		3.19		1.25	1.75
CTEh sd											0.82
CTEh confidence											0.71
CTEh 95 UCL											2.46
% FTAL											164
% Lipids		1.5		0.557		2.1		1.89		1.32	1.5
% Solids		23.4		22		25.6		25.1		23.9	24.0

STRIPED BASS AND BLUEFISH

The current fish consumption advisory issued by the Bureau of Health for striped bass and bluefish recommends consumption of no more than 2 meals per month driven by total PCB concentrations. DEP had total PCB data from 1995 to 2002 in striped bass along the Maine Coast (Table 3.2.2). Tissue from fish collected from 1995 were analyzed by the Midwestern Research Institute (MRI) by homologue analysis. The fish collected in 1996 to 2001 were analyzed by the Environmental Chemistry Lab (University of Maine at Orono) by homologue analysis. Fish collected in 2002 were analyzed by GERG at Texas A&M by analyzing all 209 congeners (some fish were analyzed by both methods). Data usually represent a mean of 5 individual fish.

In 2004 5 striped bass and 5 bluefish were collected from a number of rivers and analyzed by Pace Analytical Services (PAS) for all 209 congeners. Given the wide variation from year-to-year and lab-to-lab, to help compare past and present data 5 samples were split between GERG and PAS. Preliminary results show that the GERG results are all lower by an average of 28%, which is within the acceptable 30% relative percent difference data quality objective, although they were all biased low. But those were done by low resolution and would be expected to be lower than the high resolution analysis used by PAS since non-detects were taken at zero. The high resolution results from GERG are not yet available because the machine is down.

Comparison of the 2004 PCB levels for striped bass show concentrations are similar to those measured in 2002 but significantly higher than those measured earlier. For bluefish where there are fewer data, concentrations in 2004 were similar to those from 2001 and 2002 but significantly higher than those measured earlier. Given the measurement of all 209 congeners since 2002, it is likely those data are more accurate. All samples exceeded the Bureau of Health's FTAL (11 ppb) and most by a great amount.

Table 3.2.2 PCBs in marine fish from Maine estuaries, ppb average (95 ucl on the mean)

striped bass	Androscoggin	Kennebec	Penobscot	Royal	Sheepscot	Saco	Scarboro	York
Year								
1995		23 (30)						
1997		11 (14)						
1998	41 (43)	16 (17)				12.2	30.3	
1999		11 (12)						
2000	60 (72)				24 (28)	25 (32)		
2001			84					64
2002	288	93.2	279		149	135		103
2003								
2004	201	170	211	152				147

bluefish	Androscoggin	Kennebec	Penobscot	Royal	Sheepscot	Saco	Scarboro	York
Year								
1995		48.8						
1997								
1998							42.2	
1999								
2000								
2001		276						
2002		232			63.4 alewife	320		
2003								
2004						161		

2004 Raw Data

Field ID	Species	Length (mm)	Weight (g)	PCB ppb	% solids
ANDROSCOGGIN R mean	striped bass			201	22.5
ANDRO-R-STB-1		600	2150	162	22.7
ANDRO-R-STB-2		570	2000	307	23.8
ANDRO-R-STB-3		580	2050	226	22.6
ANDRO-R-STB-4		575	1875	108	21.8
ANDRO-R-STB-5		600	2050	200	21.6
KENNEBEC R mean	striped bass			170	22.6
KAG-STB-1		638	1700	244	25.4
KAG-STB-2		553	1825	208	21.7
KAG-STB-3		510	1200	144	22.8
KAG-STB-4		544	1525	126	21.9
KAG-STB-5		661	1990	126	21.2
PENOBSCOT R mean	striped bass			211	22.2
PENOBSCOT-R-STB-1		510	1300	150	22.2
PENOBSCOT-R-STB-2		595	1850	118	20.6
PENOBSCOT-R-STB-3		565	1700	143	20.2
PENOBSCOT-R-STB-4		595	2300	201	25.5
PENOBSCOT-R-STB-5		520	1275	444	22.3
ROYAL R mean	striped bass			152	22.8
ROYAL-R-STB-1		581	1875	141	21.3
ROYAL-R-STB-2		636	2625	181	23.8
ROYAL-R-STB-3		534	1500	154	23.1
ROYAL-R-STB-4		645	2750	183	23.2
ROYAL-R-STB-5		581	2050	100	22.8
YORK R mean	striped bass			147	22.5
YORKR-STB-1		645	2750	53.5	21.6
YORKR-STB-2		616	2175	260	23.9
YORKR-STB-3		678	2925	176	21.8
YORKR-STB-4		632	2450	143	22.4
YORKR-STB-5		643	2590	104	22.6
SACO R mean	bluefish			161	24.1
OOB-BLF-1		775		154	24.9
OOB-BLF-2		810		129	23.2
OOB-BLF-3		760		76.9	23.7
OOB-BLF-4		760		144	25.3
OOB-BLF-5		800		302	23.5

Mercury concentrations were much more constant from year-to-year and lab-to-lab than were the PCB data (Table 3.2.3). Concentrations in striped bass were relatively low compared to freshwater fish for top predators, but still exceeded the Bureau of Health's FTAL (0.2 ppm) for most samples. Concentrations in bluefish were slightly higher, but data are more limited.

Table 3.2.3 Mercury in marine fish from Maine estuaries, ppm average (95 ucl on the mean)

striped bass	Androscoggin	Kennebec	Penobscot	Royal	Sheepscot	Saco	Scarboro	York
Year								
1995		0.35						
1997		0.33						
1998	0.38	0.40					0.37	
1999		0.32						
2000	0.22				0.22	0.18		
2001			0.15					0.12
2002								
2003								
2004	0.24	0.23	0.32	0.17				0.21

bluefish	Androscoggin	Kennebec	Penobscot	Royal	Sheepscot	Saco	Scarboro	York
Year								
1995		0.53						
1997								
1998							0.33	
1999								
2000								
2001		0.39						
2002								
2003								
2004						0.48		

2004 Raw Data

Field ID	Species	Length (mm)	Weight (g)	HG (ppm)	% solids
ANDROSCOGGIN R mean	striped bass	600	2150	0.24	22.5
ANDRO-R-STB-1		570	2000	0.23	22.7
ANDRO-R-STB-2		580	2050	0.26	23.8
ANDRO-R-STB-3		575	1875	0.14	22.6
ANDRO-R-STB-4		600	2050	0.22	21.8
ANDRO-R-STB-5				0.33	21.6
KENNEBEC R mean	striped bass			0.23	22.6
KAG-STB-1		638	1700	0.31	25.4
KAG-STB-2		553	1825	0.14	21.7
KAG-STB-3		510	1200	0.16	22.8
KAG-STB-4		544	1525	0.28	21.9
KAG-STB-5		661	1990	0.26	21.2
PENOBSCOT R mean	striped bass			0.32	22.2
PENOBSCOT-R-STB-1		510	1300	0.16	22.2
PENOBSCOT-R-STB-2		595	1850	0.11	20.6
PENOBSCOT-R-STB-3		565	1700	0.14	20.2
PENOBSCOT-R-STB-4		595	2300	0.61	25.5
PENOBSCOT-R-STB-5		520	1275	0.58	22.3
ROYAL R mean	striped bass			0.17	22.8
ROYAL-R-STB-1		581	1875	0.12	21.3
ROYAL-R-STB-2		636	2625	0.25	23.8
ROYAL-R-STB-3		534	1500	0.16	23.1
ROYAL-R-STB-4		645	2750	0.22	23.2
ROYAL-R-STB-5		581	2050	0.12	22.8
YORK R mean	striped bass			0.21	22.5
YORKR-STB-1		645	2750	0.28	21.6
YORKR-STB-2		616	2175	0.24	23.9
YORKR-STB-3		678	2925	0.24	21.8
YORKR-STB-4		632	2450	0.13	22.4
YORKR-STB-5		643	2590	0.15	22.6
SACO R mean	bluefish			0.48	24.1
OOB-BLF-1		775		0.5	24.9
OOB-BLF-2		810		0.63	23.2
OOB-BLF-3		760		0.46	23.7
OOB-BLF-4		760		0.33	25.3
OOB-BLF-5		800		0.5	23.5

3.3

CUMMULATIVE EFFECTS DRIVEN ASSESSMENT OF FISH POPULATIONS

CUMMULATIVE EFFECTS ASSESSMENT OF FISH POPULATIONS

Introduction

The US Clean Water Act (CWA) and Maine statutes set an ultimate goal that point source discharges be eliminated where appropriate and an interim goal that all waters be 'fishable/swimmable'. Maine Water Quality Standards further require that all freshwaters be 'suitable for the designated uses of …fishing and ….as habitat for fish and other aquatic life' and be 'of sufficient quality to support …indigenous species of fish'. EPA and DEP interpret 'fishing' to mean that not only do fish have to be present, but also healthy and safe to eat in unlimited quantities. And in order to provide habitat… to support a species, water quality must ensure that the population is sustainable, by allowing adequate survival, growth, and reproduction.

In the past, most SWAT studies of fish have focused on measuring the effects of persistent, bioaccumulative and toxic, (PBT) contaminants on human consumers, i.e. assessment of attainment of the designated use 'fishing', with some consideration of impacts to wildlife consumers as well. Direct effects on fish populations have been measured or estimated by other DEP programs able to detect only relatively severe impacts on survival, growth, and reproduction. Several studies (Adams et al, 1992; Kavlock et al, 1996; Munkittrick et al, 1998; Rolland et al, 1997) have measured other more subtle effects on development, immune system function, and reproduction not normally seen in more typical stressor-based testing regimes historically used by DEP. These effects may be a result of long term exposure to relatively low levels of contaminants or cumulative effects of exposure to many low-level contaminants. These responses to pollutant challenge are often within the same magnitude as natural variation and therefore difficult to measure with the methods that are currently used. Many new techniques, such as a cumulative effects-driven assessment (CEA) of fish populations have been developed to measure some of these effects.

A CEA usually measures indicators of survival, growth, and reproduction. Age structure and mean age are measured as indicators of survival and measures of energy expenditure and storage are used as indicators of growth and reproduction. Energy expenditure measures include size and size at age as indicators of growth and gonadosomatic index (GSI), fecundity, and egg size as indicators of reproductive potential. Energy storage measures include condition factor (K) as an indicator of growth and liversomatic index (LSI) and lipid storage as indicators of both growth and reproductive potential (Munkittrick et al, 2000). Response patterns of all indicators provide an integrative assessment of overall performance that may reflect different types of stresses, such as exploitation, food limitation, recruitment failure, niche shift, metabolic disruption (Munkittrick et al, 2000). Levels of circulating sex steroids are also often used as biomarkers of reproductive potential, which is considered an index of potential population trends as is survival.

With the assistance of Environment Canada (EC), DEP has conducted CEAs of fish populations on the St John River in 1999-2001 that have indicated probable impacts to fish populations and identified a previously unknown source. In 2000 similar studies of the North Branch of Presque Isle Stream and Prestile Stream, where high concentrations of DDT, a known endocrine disruptor, have been previously found, indicated a potential population level effects as indicated by a significant reduction in gonad size in both streams compared to two reference streams with much lower DDT levels in fish.

When CEA studies began in Maine, the plan was to study what was considered the worst case first, and if no negative impacts were measured not to study the other rivers. The Androscoggin River was chosen to study first among the large industrial rivers because it has more (3) large pulp and paper mills for its size than the other major rivers and has historically had the poorest water quality. CEAs of white sucker populations in the Gulf Island Pond on the Androscoggin River from 2001-2003 did not show the evidence of endocrine disruption and metabolic redistribution found in a preliminary study in 1994. This result is possibly due to the change in bleaching technology from free chlorine to chlorine dioxide and improved waste treatment in the 3 upstream bleached kraft pulp and paper mills in the intervening years. Nor was there any evidence of endocrine disruption at any location below any of the mills in the rest of the river. There was evidence of increased eutrophication that correlated with increased nutrient levels downstream of the mills and associated municipalities (DEP, 2004).

Many studies have also documented effects of heavy metals, PAHs, sewage, and pulp and paper mill waste on fish immune systems (Voccia et al,1994; Holliday et al, 1998; Secombes et al, 1992; Ahmad et al, 1998). In 2002 and 2003 we looked at the spleen somatic index (SSI) and kidney somatic index (KSI) as rough indicators of immune system effects. There were significant decreases in SSI below the 2 most upstream mills for one or both sexes in 2002 and 2003, indicating potential immune system stress.

Studies of caged mussels in 2003 on the Androscoggin River showed no negative impacts on growth rate or induction of vitellin, a reproductive protein marker of endocrine disruption. This result is consistent with studies of fish in the river from 2001-2003 which also show no clear evidence of endocrine disruption. Studies of caged mussels in 2003 on the Kennebec River, however, did show induction of vitellin below a bleached kraft pulp and paper mill. Therefore, in 2004, a CEA was conducted on white suckers above and below the SAPPI Somerset bleached kraft pulp and paper mill on the Kennebec River.

Methods

The upstream station was approximately 5 miles above the mill but below the city of Skowhegan while the downstream stations was approximately 10 miles below the mill at the historic sampling site for the Dioxin Monitoring Program, with which sampling for the CEA was integrated. For each of the stations, 20 males and 20 females of each species were collected during fall recrudescence. Previous studies have determined that a sample size of 20 is sufficient to reduce the variance enough to detect a difference of 20-30% in the variables measured between stations. Fish were collected by gill net. Blood samples were collected, from live fish immobilized in a foam cradle, into heparinized Vacutainers and placed on ice for transport to the lab the same day. The fish were then killed with a blow to the head. The operculum was taken for aging. Livers were dissected out and weighed, for calculation of LSI, and then frozen in liquid nitrogen. Gonads were dissected out and weighed for calculation of GSI and a small sample ~1 cm square was taken and placed in 10% buffered formalin for storage. Head kidney and spleen were dissected out and weighed for calculation of KSI and SSI respectively.

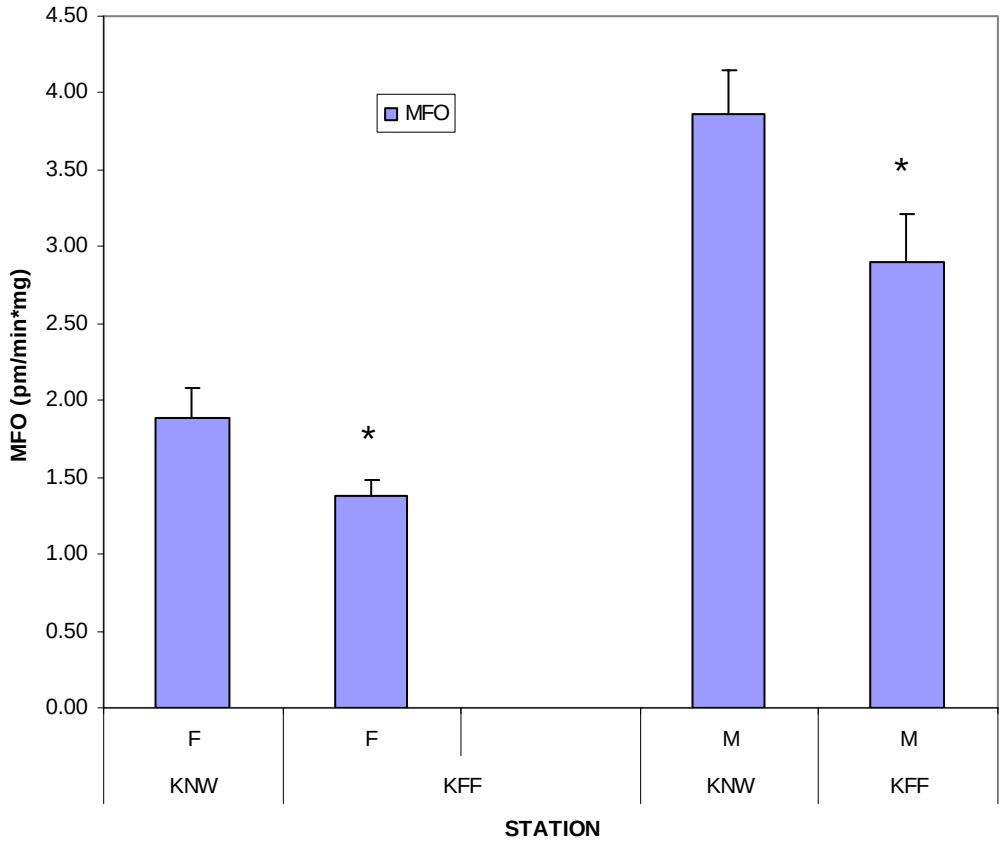
Later the same day in the lab, the samples were placed in proper storage to await analyses. Plasma was collected from the blood samples after centrifugation in the lab and then frozen at –20C for radioimmunoassay (RIA) analysis for T, 11-KT, E2, following the method of McMaster et al (1992) and

F following the method of Jardine (1996). Liver samples were stored at –80°C for MFO analysis as outlined by Munkittrick et al (1992). Gonad samples remained in formalin for further analyses. Histological samples of gonads will be prepared and examined for the presence of testis-ova as outlined in Gray and Metcalf (1997) or analysis of gonadal staging (McMaster, 2001). All laboratory analyses were performed by at Environment Canada's National Water Research Institute in Burlington, Ontario, Canada. Samples for aging were stored at –20°C until prepared and read in the DEP lab in Augusta, Maine.

Results

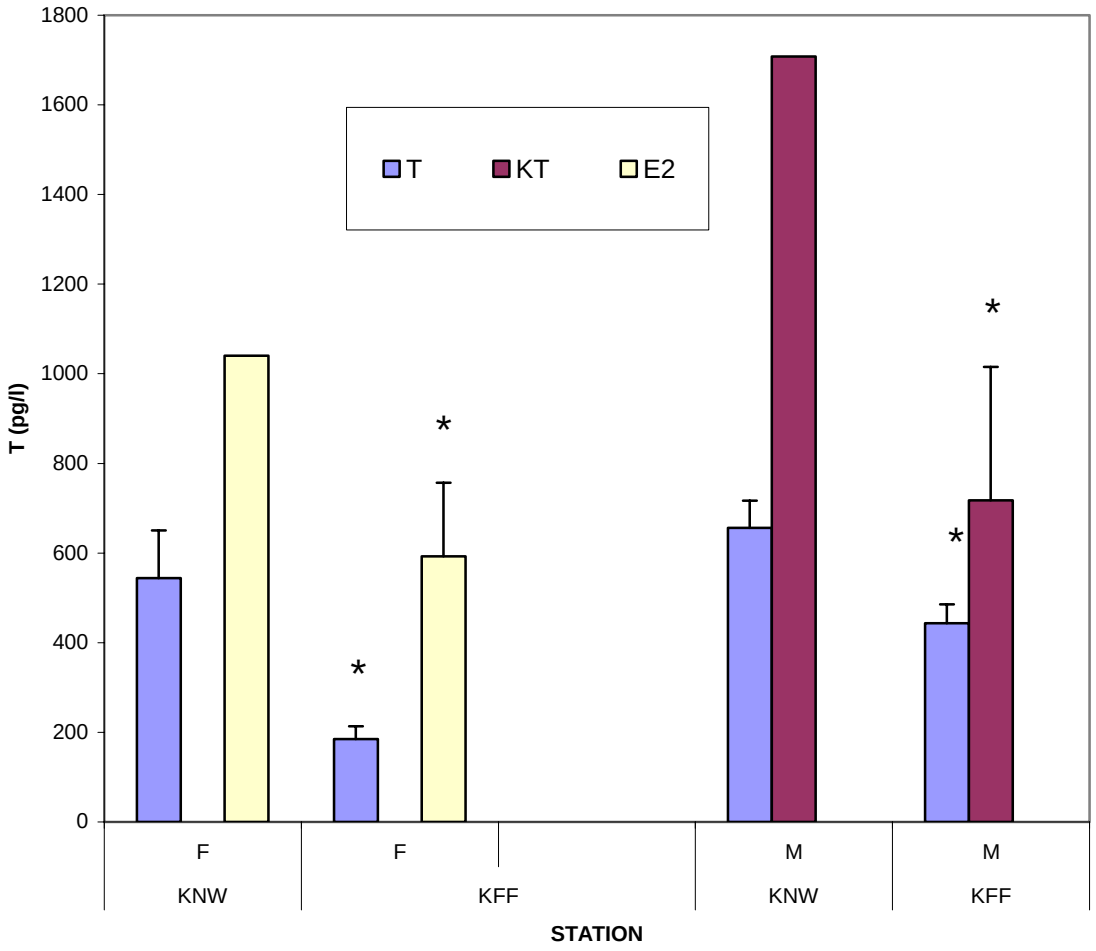
MFOs were significantly reduced at KFF, below the mill for both sexes (Figure 3.3.1). This is opposite of what was expected, that MFOs, as an indicator of exposure to pulp mill effluent, would be higher below the mill. It may be that with the changes in bleaching and improved process controls and wastewater treatment, that the potency of the effluent is no longer high enough to elicit a response.

Figure 3.3.1 MFO in male (M) and female (F) white suckers from the Kennebec River above (KNW) and below (KFF) the SAPPI Somerset pulp and paper mill, 2004



Concentrations of circulating levels of T were significantly reduced in both males and females, while 11-KT was significantly reduced in males and E2 significantly reduced in females at KFF both sexes (Figure 3.3.2). This finding is consistent with endocrine disruption, but the absence of MFO induction below the mill confounds interpretation of cause. Although KFF is 10 miles below the mill, there are no other known point or non-point sources in between that could reasonably be expected to cause this effect.

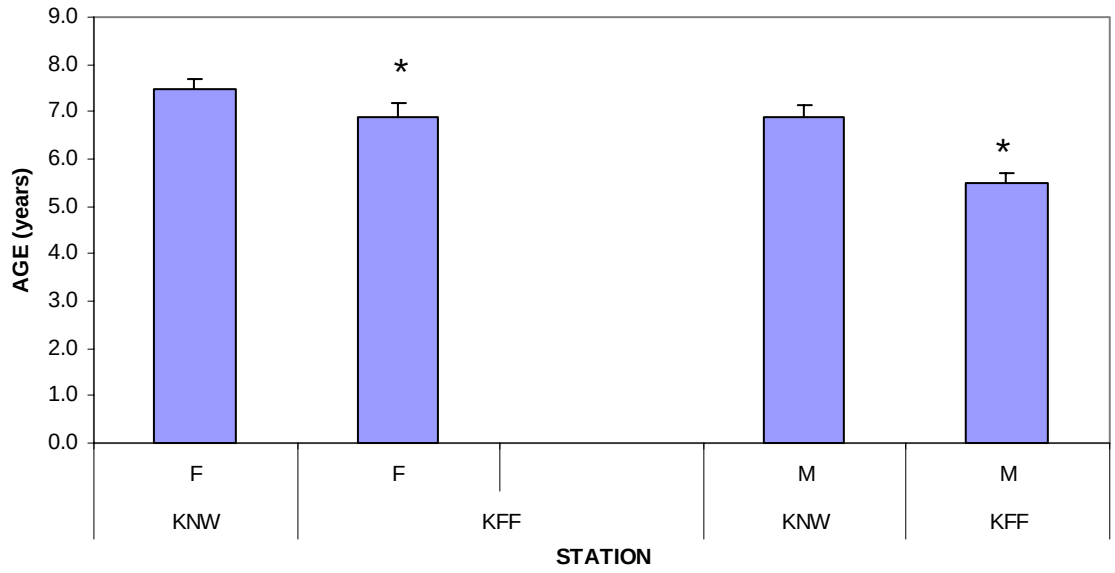
Figure 3.3.2 Testosterone (T), 11 ketotestosterone (KT) and estradiol (E2) in male (M) and female (F) white suckers from the Kennebec River above (KNW) and below (KFF) the SAPPI Somerset pulp and paper mill, 2004



Concentrations of cortisol (F) were significantly reduced at KFF in females but not males (not shown). The significance of this finding is not certain at this time. Cortisol is a steroid hormone that helps mobilize energy reserves diverting them from growth and reproduction to short term survival activities in times of stress. Cortisol might be elevated from capture and handling, but fish of both sexes at both stations were captured and handled similarly, so this should not be the cause of the differences.

Mean age as an indicator of survival was significantly reduced at KFF for both sexes (Figure 3.3.3). Munkittrick (2000) gives as two possible reasons 1) exploitation and 2) metabolic redistribution. Regarding exploitation, white suckers are not fished recreationally to any great extent. But there is a commercial fishery of suckers for lobster bait, although sucker traps have not been observed much on this reach of the river. That leaves metabolic redistribution of energy from survival towards growth or reproduction as mediated partly by cortisol levels as a possible cause. But cortisol levels were affected only for females, and are not consistent with age reduction for males.

Figure 3.3.3 Mean age of male (M) and femalie (F) white suckers sampled from the Kennebec River above (KNW) and below (KFF) the SAPPI Somerset pulp and paper mill 2004



Energy expenditure measures include size and size at age as indicators of growth and gonadosomatic index (GSI), fecundity, and egg size as indicators of reproductive potential. Mean length (size) did not change for females but was significantly reduced for males at KFF (Figure 3.3.4). GSI, however, was significantly increased at KFF for both sexes. It appears that energy expenditures were routed toward reproduction at the expense of growth in males.

Energy storage measures include condition factor (K) as an indicator of growth and liversomatic index (LSI) and lipid storage as indicators of both growth and reproductive potential. K was significantly increased for both sexes while LSI was significantly reduced for females (Figure 3.3.5). It appears that energy was routed from storage in the liver toward reproduction in females. In both sexes, then, increased K was a result of more energy being directed toward reproduction at KFF than at KNW, but it came from different compartments for males and females. Additional studies would be needed to verify these findings.

Figure 3.3.4 Length and gonadosomatic index (GSI) of male (M) and female (F) white suckers sampled from the Kennebec River above (KNW) and below (KFF) the SAPPI Somerset pulp and paper mill, 2004

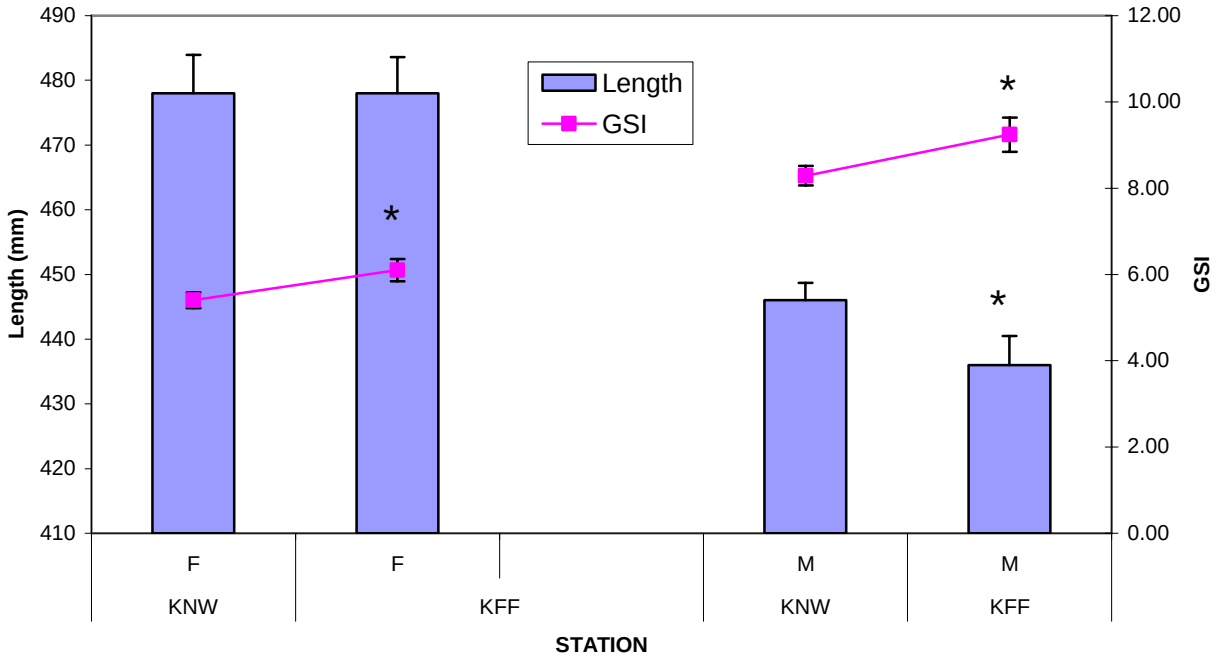
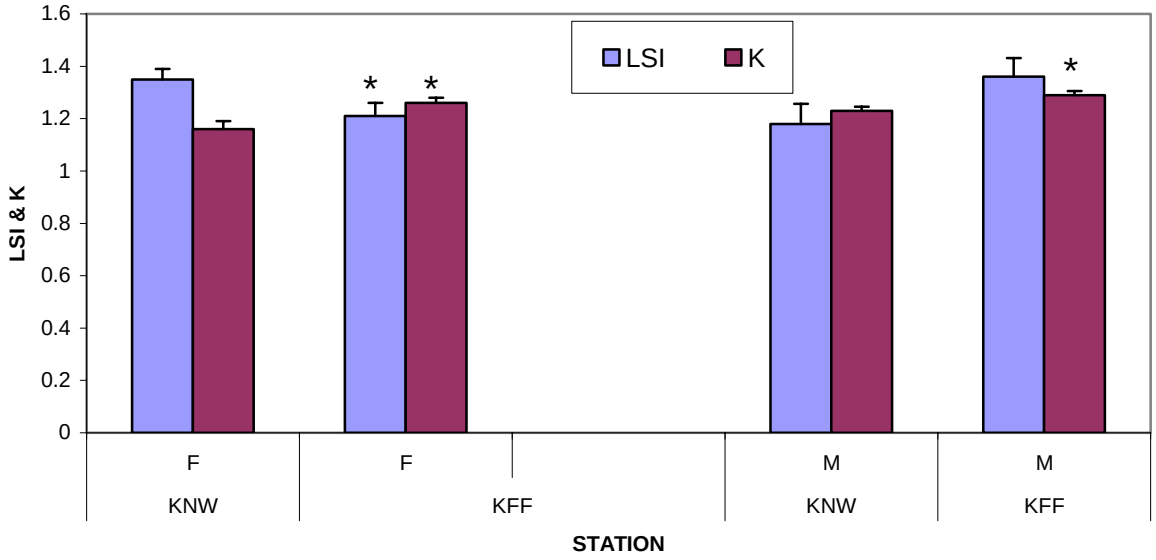
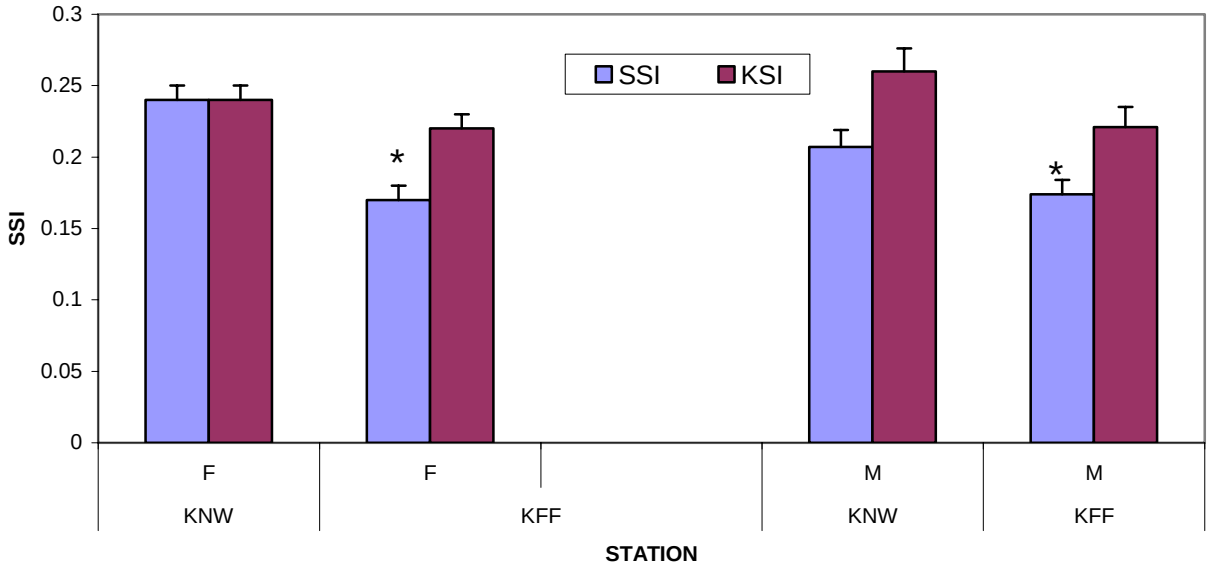


Figure 3.3.5 Liver somatic index (LSI) and condition factor (K) of male (M) and female (F) white suckers from the Kennebec River above (KNW) and below (KFF) the SAPPI Somerset pulp and paper mill, 2004



Interestingly, SSI was also significantly lower at KFF (Figure 3.3.6) and on some stations from the Androscoggin River reported previously (DEP, 2004). This finding is not inconsistent with the possible decreased immune system capacity found by Hannum in head kidneys (this report), although the mechanism is unclear since head kidney size (KSI) was no different between sites above and below the mills for either sex on either river.

Figure 3.3.6 Spleen somatic index (SSI) and head kidney somatic index (KSI) in male (M) and female (F) white suckers from the Kennebec River above (KNW) and below (KFF) the SAPPI Somerset pulp and paper mill, 2004



The survival indicator, energy expenditure indicators, and energy storage indicators responses measured in white suckers from KFF generally fit a pattern of metabolic disruption (Munkittrick, 2000) unlike the pattern of nutrient enrichment found on the Androscoggin in previous studies.
The induction of vitellin found in caged mussels in 2003 is not inconsistent with this pattern for white suckers in the Kennebec. Measurements of vitellogenin in the white suckers are pending at the lab. Indications of immune system suppression also indicate negative effects on white sucker populations below the mill on the Kennebec. Additional studies are warranted to verify these conclusions.

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3.4

FISH IMMUNOLOGY STUDY

Innate Immune Response Capacity of Fish from the Androscoggin and Kennebec Rivers

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March 22, 2005

Objectives

The primary goal of our research was to assess innate immune response capacity of fish from the Androscoggin and Kennebec rivers relative to areas of paper mill discharge. This collaborative project with Barry Mower of the Maine Department of Environmental Protection used fish from the same populations being sampled for dioxin levels as part of the Dioxin Monitoring Program. Because there is little data on innate immune responses in either of the fish species involved, we also sought to use this opportunity to generate an initial database on the variability of innate immune response capacity of smallmouth bass (*Micropterus dolomieu*) and white suckers (*Catostomus commersoni*) in the two rivers, and to examine white blood cell populations present in the anterior kidney of these species by flow cytometry.

Innate immunity and respiratory burst

In vertebrates, the innate immune system provides the first line of defense against infection by disease-causing microbes. Central to the innate immune response are several classes of white blood cells that detect and eliminate pathogenic microorganisms. Some of these cells, particularly macrophages and neutrophils, are referred to as phagocytes because of their ability to bind and engulf (phagocytose) foreign material. Phagocytes destroy internalized microbes using enzymes, antimicrobial peptides, and toxic oxygen-containing compounds such as hydrogen peroxide. Phagocytes generate these reactive oxygen compounds through a process known as respiratory burst.

Respiratory burst activity in resting, unstimulated phagocytes is relatively low. Contact with microbes (or artificial stimulation with reagents such as phorbol dibutyrate) triggers the respiratory burst response in these cells. Superoxide anion is a key intermediate in the respiratory burst reaction, and can be measured using nitro blue tetrazoleum (NBT). Colorless NBT solution turns blue in the presence of superoxide anion. Color change, indicative of the level of respiratory burst activity, can then be quantitated using a microplate reader.

Numerous environmental pollutants such as tributylin, metals, PCBs, and PAHs have been shown to suppress the innate immune response in fish, including phagocyte respiratory burst (Rice, 1996; Fournier, 1998; Regala, 2001; Dethloff, 2001; Zelikoff, 2002; Carlson 2002;). Thus, quantifying the innate immune response by measuring respiratory burst activity of white blood cells from the anterior kidney can be an effective method of assessing the effects of pollutants on fish health.

Methods

Fish Collection

Smallmouth bass (*Micropterus dolomieui*) and white suckers (*Catostomus commersoni*) were received from DEP researchers at three sites along the Androscoggin River in July of 2004. Fish were collected above the Mead paper mill in Rumford on July 7 and 9; and below the mill in Dixfield on July 13, 14 and 15, and Canton on July 19, 20 and 21. Smallmouth bass were collect at two sites along the Kennebec River in September of 2004. Fish were collected at the Norridgewock site, upstream of the Sappi paper mill in on September 14 and 16, then downstream in Fairfield on September 21 and 23. In both cases the upstream and downstream sites were separated by a dam, thus the upstream and downstream populations were not mixing. White suckers were caught in gill nets set the night before they were obtained. Smallmouth bass were caught on fishing lines same the day they were obtained. Fish were collected in the morning and transported to shore where our research team processed them immediately.

Isolation and Preparation of Head Kidneys Cells

Fish were placed in a 40 L cooler and anesthetized with Tricaine MS-222 (0.0784 mg/ml; Sigma-ALDRICH, St. Louis, MO), then killed by a blow to the head. Head kidneys were surgically removed and rinsed in Hank's buffered saline solution (HBSS) with 2 mM calcium (Sigma-ALDRICH, St. Louis, MO). Kidneys were then stored on ice in plastic tubes containing 10 ml HBSS for the return trip to Colby College.

At our laboratory, kidney tissues were disrupted on a scored petri dish with a syringe to liberate individual cells. Cell suspensions were transferred to 15 ml conical centrifuge tubes. After connective tissue settled out for approximately 1 min, the supernatant was transferred to another 15 ml centrifuge tube and spun at 300x g, 11˚C for 10 min on a Centra CL3R centrifuge. The resulting pellet was resuspended in 5 ml of ammonium chloride potassium solution (ACK) for 5 min to lyse red blood cells (RBCs). After 5 min, 5 ml of HBSS was added to stop the lysis and tubes were spun again as before. Two treatments with ACK were usually necessary to lyse the RBCs. After RBC lysis, cells were washed twice by resuspending the pellet in 10 ml HBSS and centrifuging as before. Remaining cells were resuspended in 10 ml HBSS. For counting, 10 ul of cell suspension was diluted 1:10 in HBSS and trypan blue (Sigma). Live leukocytes were counted on a hemacytometer. Cells were adjusted to final concentration of $1x10^7$/ml with HBSS.

Nitro Blue Tetrazoleum (NBT) Reduction Assay

100

at 1mg/ml in dimethyl sulfoxide (DMSO Sigma), was added as a stimulant to three of the wells. 60 ul of HBSS was added to the three unstimulated wells. All wells were mixed with a multichannel pipetter before being incubated for 20 min at room temperature under foil. After incubation, plates were spun at 300x g for 3 min at 11˚C. Supernatant was aspirated off and 120 ul of 2M KOH (Sigma) and 140 ul of DMSO (Sigma) were added to each well and mixed. Absorbance of each wells was read immediately on a Multiskan RC plate reader (Fisher Scientific) at dual wavelengths of 620 nm and 405 nm.

NBT Analysis and Statistics

The stimulation index (SI) was calculated for each fish by dividing the mean stimulated absorbance value from the NBT assay by the mean unstimulated absorbance value. P-values were determined using Mann-Whitney U test.

Flow Cytometry

Head kidney cell suspensions prepared for NBT assay (above) were diluted 1:10 into 1 ml phosphate buffered saline in 12 x 75 mm FACS tubes. Forward scatter and side scatter data were collected on 20,000 cells/sample using a B-D FACScalibur flow cytometer. Analysis was performed using CellQuest software.

Results: Respiratory burst

Androscoggin respiratory burst

In this study, stimulation index (SI) reflects the ability of phagocytic white blood cells to respond to artificial stimulation. The mean stimulation indexes were significantly higher in smallmouth bass from the Rumford site than bass from the downstream Dixfield and Canton sites (p=.04 and p=.03, respectively; Figure 1). There was no difference in SI between the Dixfield and Canton sites.

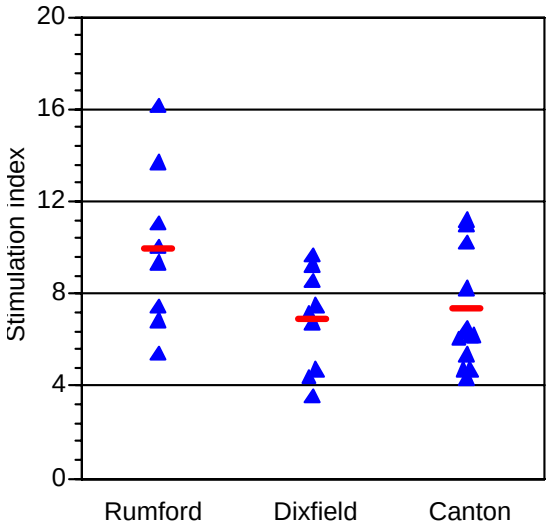

Figure 1. Stimulation indexes of head kidney cells from smallmouth bass collected at Rumford (n=8), Dixfield (n=9), and Canton (n=13) sites. Individual fish represented by triangles; bar represents mean value for each site.

Examination of respiratory burst levels in unstimulated head kidney cells revealed an interesting pattern. Respiratory burst activity in resting cells from bass collected at the Dixfield site was significantly higher than bass from the Rumford and Canton sites (p=.03 and p=.04, respectively; Figure 2). There was no significant difference between fish from Rumford and Canton sites. Fish were collected at Dixfield on three different days. Review of the raw data confirmed that fish with higher and lower unstimulated respiratory burst responses were collected on the same days; thus elevated resting respiratory burst activity in this set of the Dixfield bass was not due to a procedural variation on one day of testing.

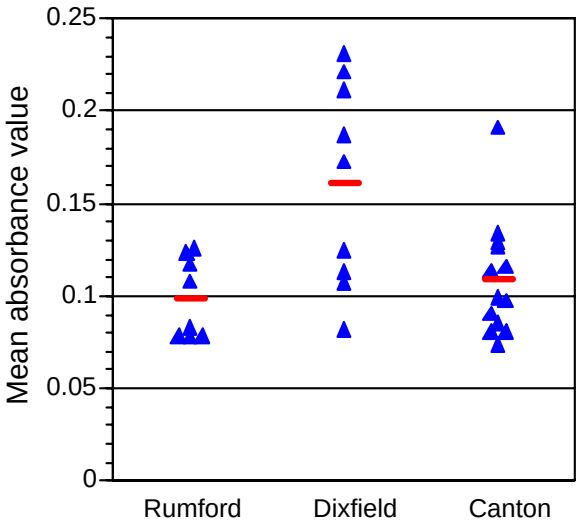

Figure 2. Mean absorbance values from NBT assay of unstimulated head kidney cells from smallmouth bass collected at Rumford (n=8), Dixfield (n=9), and Canton (n=13) sites. Individual fish are represented by triangles; bar represents mean value for each site.

We were concerned that trauma experienced by white suckers trapped overnight in gill nets would affect the functioning of their white blood cells in the NBT assay, as stress negatively impacts immune responses in most species, including fish. The initial results of NBT assays on white sucker head kidney cells supported this prediction, as the sucker SIs were very low compared to smallmouth bass (Figure 3). The highest sucker SIs were well below the lowest indexes of bass (as well as of perch and lake trout previously used in the NBT assay, data not shown). There was no significant difference between SIs of white sucker cells from any of the three sites.

When we later analyzed the respiratory burst activity of resting white sucker cells, they clearly exhibited the same pattern as smallmouth bass cells: the mean respiratory burst levels were substantially higher in unstimulated cells from the Dixfield site than from the Rumford and Canton sites (p=.06 and p=.02, respectively, Figure 4). Unlike SI data, the absorbance values reflecting unstimulated respiratory burst activity were comparable in bass and suckers. In both species, then, fish collected at the downstream site closest to the mill discharge displayed elevated respiratory burst activity, which can be indicative of oxidative stress (C.D.Rice, personal communication).

A.

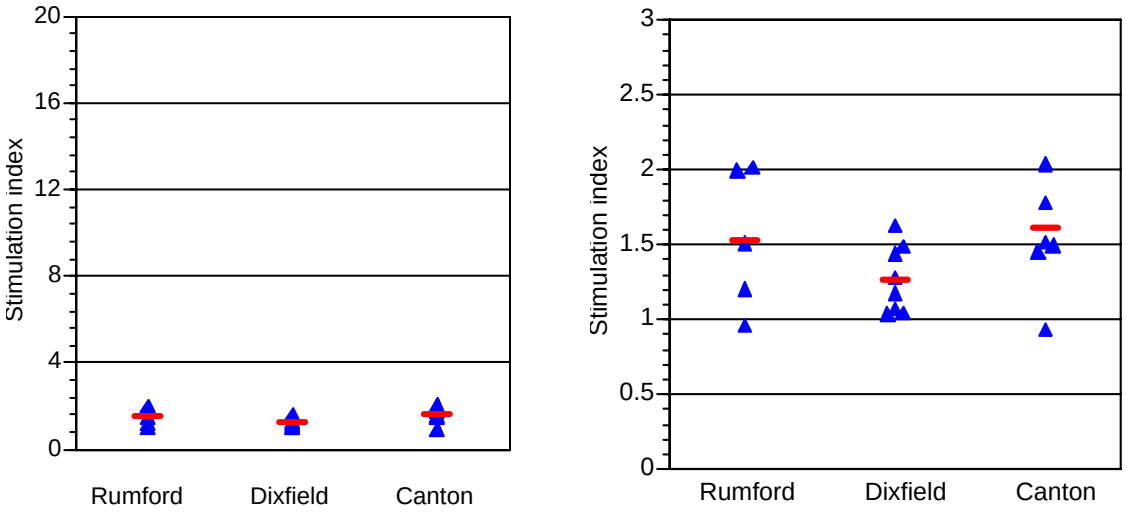

Figure 3. Stimulation indexes of head kidney cells from white suckers collected at Rumford (n=5), Dixfield (n=8), and Canton (n=7) sites. A, data presented on same SI scale as smallmouth bass (Figure 1); B, scaled to show SI patterns for each site. Individual fish represented by triangles; bar represents mean value for each site.

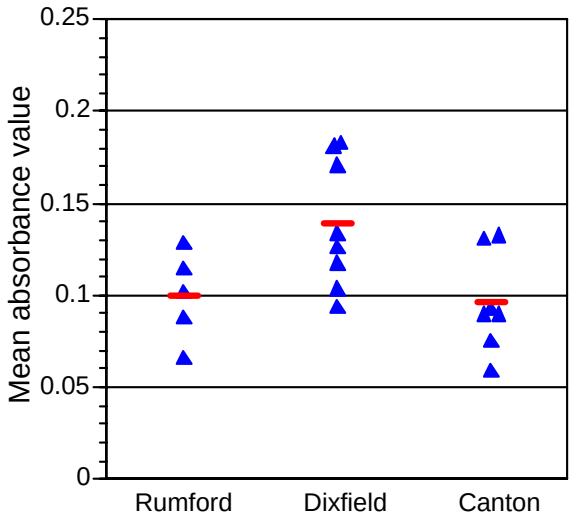

Figure 4. Mean absorbance values from NBT assay of unstimulated head kidney cells from white suckers collected at Rumford (n=5), Dixfield (n=8), and Canton (n=7) sites. Individual fish are represented by triangles; bar represents mean value for each site.

Kennebec respiratory burst

Smallmouth bass were collected at two sites, Norridgewock and Fairfield, in September 2004. The results of this study parallel those of the Androscoggin. The mean stimulation index was significantly higher in head kidney cells of bass from the upstream Norridgewock site than those from the downstream Fairfield site (p=.05; Figure 5). Additionally, the respiratory burst activity in unstimulated head kidney cells was significantly higher in bass from the Fairfield site than those from Norridgewock (p=.008, Figure 6).

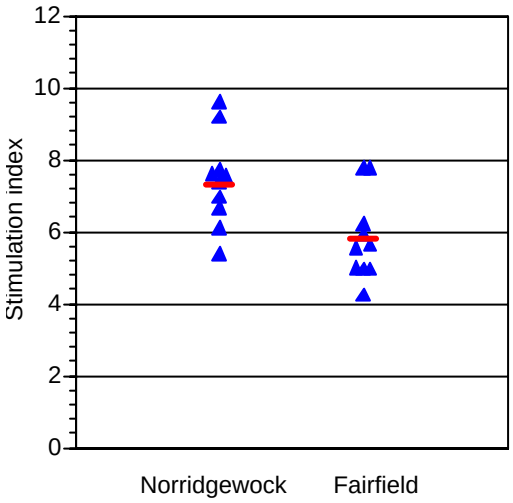

Figure 5. Stimulation indexes of head kidney cells from smallmouth bass collected at Norridgewock (n=10) and Fairfield (n=10) sites. Individual fish represented by triangles; bar represents mean SI value for each site.

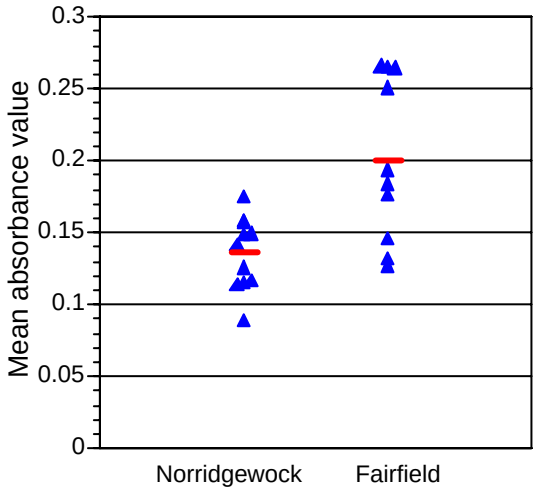

Figure 6. Mean absorbance values from NBT assay of unstimulated head kidney cells from smallmouth bass collected at Norridgewock (n=10) and Fairfield (n=10) sites. Individual fish are represented by triangles; bar represents mean value for each site.

The mean SI values were lower in the Kennebec bass than those from analogous sites on the Androscoggin; background respiratory burst levels in resting cells were slightly higher in Kennebec bass. However, several factors discourage direct comparisons of these numbers between the studies. Most notably, the studies were conducted two months apart. Lack of data on monthly/seasonal fluctuations in bass respiratory burst activity make it impossible to know whether the differences in Androscoggin and Kennebec numbers are due to timing, reagent differences, dissimilarity of the water environment, or intrinsic differences in the fish populations.

Results: Flow cytometric analysis of white blood cell populations

A flow cytometer allows characterization of each individual cell within a large, diverse cell sample. Typically, cells are marked with fluorescent tags specific for certain cell surface proteins. As the tagged cells are run single-file past a laser, the flow cytometer generates a profile of the tags on each cell. Since there are no fluorescent reagents available to differentiate populations of white blood cells from smallmouth bass or white suckers, our study relied on more basic forward and side scatter information generated by the flow cytometer. Forward scatter is a measure of the size of a particular cell; side scatter indicates the level of granularity of the cell cytoplasm. Different populations of white blood cells have characteristic forward vs. side scatter profiles: lymphocytes are smaller and less granular, while phagocytic cells (macrophages, neutrophils and other granulocytes) tend to be larger and more granular.

Our analysis of head kidney white blood cells from smallmouth bass showed the expected pattern of lymphocyte and macrophage/granulocyte populations, with some smaller cells and cellular debris visible at the lower left of the profiles (Figure 7, right panel). Profiles of white sucker head kidney cells (Figure 7, left panel) revealed a population of cells not seen in bass, nor in the landlocked salmon, perch or lake trout tested previously (data not shown). This group of cells appears to be highly granular and of varying size. We were unable to schedule use of a flow cytometer with cell sorting capacity at another institution prior to the completion of fish collection for this study, thus identification of the cell population(s) remains to be done.

Figure 8 illustrates the gates drawn around the lymphocyte (R2) and macrophage/granulocyte (phagocytic cells, in R3) populations in smallmouth bass. In Androscoggin bass, the mean percentage of cells in the macrophage/granulocyte population was significantly higher in fish from the Dixfield site than the Rumford ($p=.03$) and Dixfield ($p=.0005$) sites (Figure 9). There was no significant difference between the Rumord and Dixfield sites. We did not observe differences in this cell population in white suckers from the three sites. Despite the differences in SI and unstimulated respiratory burst levels in the Kennebec bass, there was no significant difference in the percentages of cells in the macrophage/granulocyte population from fish collected at the Norridgewock and Fairfield sites (data not shown).

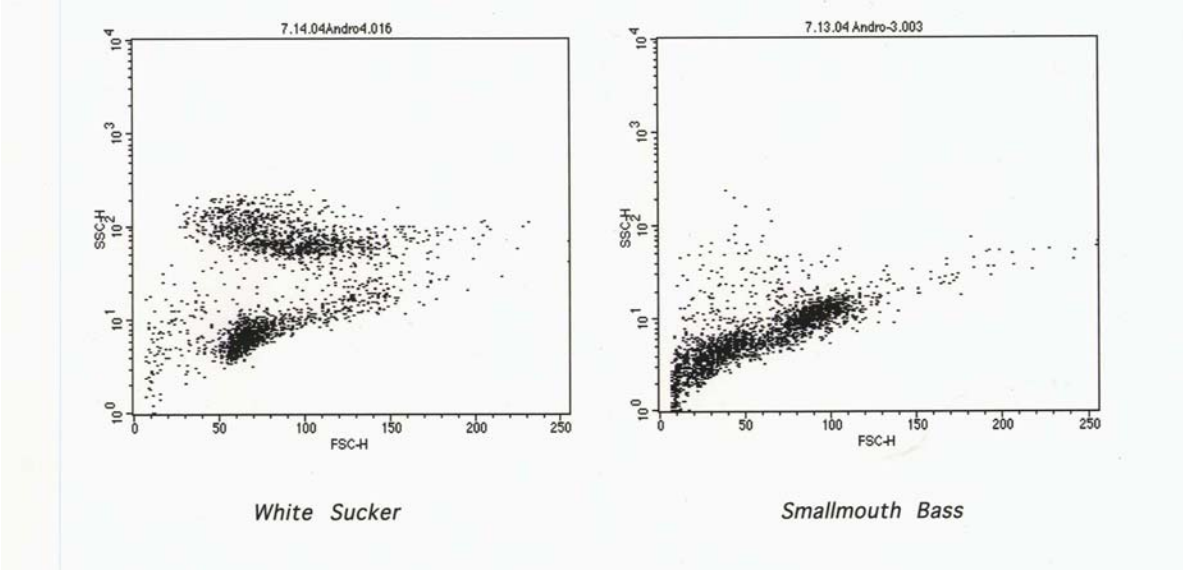

Figure 7. Flow cytometric scatter plot profiles of smallmouth bass and white sucker head kidney cells, showing forward scatter (size) on the x-axis and side scatter (granularity) on the y-axis. Each dot on the plots represents a single cell. Profiles shown are typical of individuals of each species.

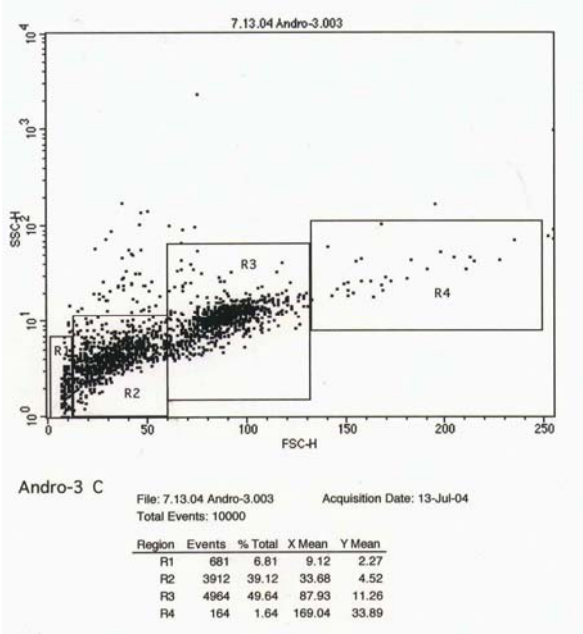

Figure 8. Flow cytometric scatter plot profile of a typical smallmouth bass showing forward scatter (size) on the x-axis and side scatter (granularity) on the y-axis. R3 gate encompasses the phagocytic cell populations, while gate R2 identifies the lymphocytes. Each dot on the plots represents a single cell.

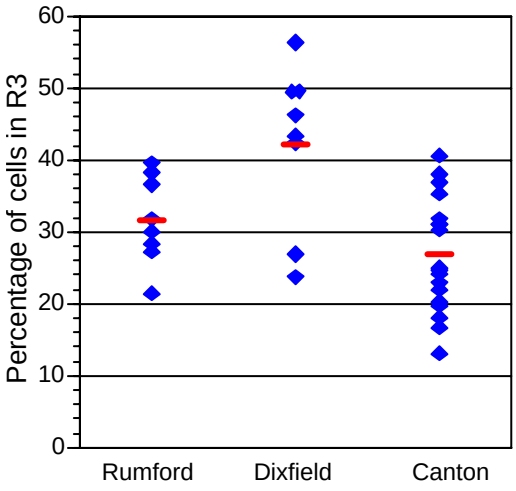

Figure 9. Percentage of head kidney cells fitting the macrophage/granulocyte (phagocytic cell) profile from smallmouth bass collected at Rumford (n=8), Dixfield (n=8), and Canton (n=18) sites. Individual fish are represented by triangles; bar represents mean value for each site.

Because macrophages and neutrophils are the primary head kidney phagocytic cells responsible for respiratory burst reactions, we speculated that the higher levels of background respiratory burst activity in bass cells from Dixfield was due to the higher percentage of phagocytic cells in samples from this site. The correlation between percentage of cells in R3 and resting respiratory burst levels is not significant, but shows a positive trend (R^2=.09, Figure 10).

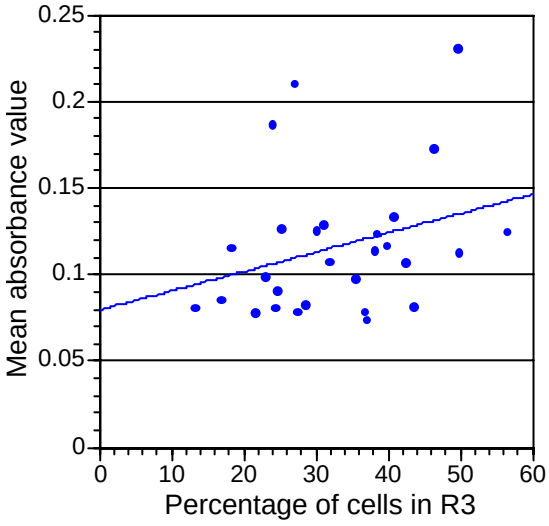

Figure 9. Comparison of head kidney cells fitting the macrophage/granulocyte (phagocytic cell) profile with the mean absorbance values from the NBT assay of unstimulated head kidney cells from smallmouth bass collected at the combined Androscoggin sites. Individual fish are represented by circles; line is linear regression.

Summary

We have found distinct differences in innate immune system activity, as measured by phagocyte superoxide anion production, in the anterior kidney cells of smallmouth bass and white suckers collected at sites above and below paper mill discharge on both the Kennebec and Androscoggin Rivers. Based on our work thus far, we cannot attribute these differences directly to components of the mill effluent. However, it is clear that factor(s) are causing suppression of respiratory burst capacity (SI) in bass from sites downstream of the mills, relative to upstream sites. White sucker cells responded very weakly to stimulation in culture, and did not show site to site variation in SI, possibly due to stress associated with harvest methods.

Most intriguing, a somewhat different pattern was observed in unstimulated cells of both bass and suckers from both rivers: background respiratory burst activity was significantly elevated in fish from Dixfield and Fairfield (closest to the sites of effluent discharge) suggestive of oxidative stress. Fish captured further downstream on the Androscoggin had lower resting superoxide anion levels, comparable to fish collected upstream of the mill. In Androscoggin bass, this pattern was matched by the percentage of anterior kidney cells within the macrophage/granulocyte profile by flow cytometry. It seemed possible that having greater proportions of phagocytes within the Dixfield cell samples could cause the observed elevated respiratory burst activity. The correlation between phagocytic populations and resting respiratory burst activity appears to be only weakly positive, likely not the primary reason for this pattern.

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3.5

POLAR ORGANIC CHEMICAL INTEGRATIVE SAMPLER

DETECTION OF PESTICIDES IN WASHINGTON COUNTY (MAINE) SURFACE WATERS USING POLAR ORGANIC CHEMICAL INTEGRATIVE SAMPLER (POCIS)

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University of Maine, Orono, ME 04469-5764

ABSTRACT

Since 1945 Maine environmental stakeholders have been trying to protect Atlantic Salmon (*Salmo solar*) againts pesticide-derived contamination in Downeast rivers. Accordingly, specialists of the University of Maine and the Board of Pesticide Control (BPC) have used grab sampling and Isco auto sampler to survey surface waters in Washington County. However, these traditional monitoring methods provide concentration estimates only for the time of sampling and do no not allow for an exposure assessment of aquatic lives to the contaminants. Therefore, in Summer 2004, we deployed a passive sampler, Polar Organic Chemical Integrative Sampler (POCIS) at eight sampling points in four water bodies at Washington County in order to come up with an alternative sampling methods for the pesticides used on wild and lowbush blueberries (*Vaccinium angustifolium*).

At each sampling point, two replicates comprising two POCIS each were deployed during 28 days in July 2004. After the retrieval, the admixture (or sorbent that sequesters the pesticides) have been extracted in organic solvents and quantified by GC/MS. Some pesticides like chlorothalonil and propiconazole were not detected at any site. Terbacil was detected at only one sampling point at Pork brook. Water concentration estimates for the replicates ranged from non-detect to 6.56 ng/L for phosmet and from non-detect to 739g/L for hexazinone. However, an ANOVA performed with the log-transformed data showed no significant difference between sites with regard to mean water concentrations of phosmet ($P = 0.260$). For hexazinone, the ANOVA did sugget a significant difference between sites ($P = 0.001$), but a 95% confidence interval constructed using the Bonferroni multiple comparison showed that only the Pleasant river lake site significantly differed from the others.

Although some uncertainties related to the calibration factors used in the calculations of pesticide concentrations in water and a slight instability in the variances of the data, the overall results show the capacity of the POCIS device to monitor the pesticide used on the blueberries at Washington County.

The full report is available as a separate file with the 2004 SWAT report at http://www.maine.gov/dep/blwq/docmonitoring/swat/index.htm

3.5

Caged Mussel Vitellin Study

CAGED MUSSEL VITELLIN STUDY -DEP

Increased vitellin production is an indication of potential endocrine disruption and reproductive effects in bivalves and is comparable to the induction of increased vitellogenin in fish. These chemical inducers mimic or interfere with endogenous hormones in vertebrates and invertebrates and may cause adverse biological effects. It is expected that the females may have some increase in vitellin when they are preparing for the next spawning cycle. However, excessive vitellin production in the females and the males, is an indication of adverse effects. (Salazar, 2004).

In the 2003 SWAT program, a caged mussel study was conducted on the Kennebec River to determine if the bleached kraft pulp and paper mill was discharging dioxin. The results showed that 2378-TCDD and 2378-TCDF, the dioxins historically discharge by this mill, were not significantly higher below the mill than above. However, there was an induction of vitellin, a reproductive protein marker potentially indicative of endocrine disruption below the mill. None of these effects were seen in a similar study on the Androscoggin River.

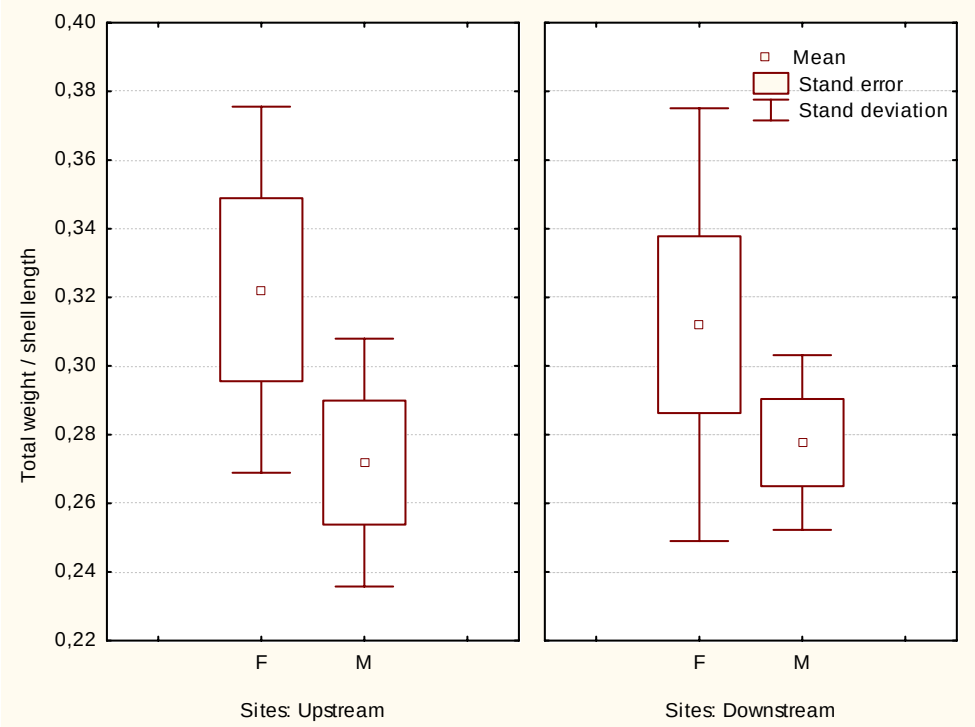
In 2004 again as part of the caged mussel dioxin study, we collected mussels from stations above and below the SAPPI- Somerset bleached kraft mill to be analyzed for vitellin. From the recommendations of a peer review pane, in 2004 the number of stations was reduced to two, one each above and below the mill. The downsteam station was the same that used in the dioxin above/below (A/B) fish test in order to make valid comparisons with the fish results as required by the A/B test. This meant that the station was not the same as those where the highest induction of vitellin was observed in 2003. Samples of fish collected from the same stations were to be analyzed for vitellogenin. This will allow comparison across species to confirm effects and establish options for future study. A total of 8 and 10 mussels were collected from the above and below stations respectively and wrapped in aluminum foil and frozen prior to shipment to the St. Lawrence Center for analysis.

The mussel vitellin assays were conducted by Francois Gagne, Christian Blaise, and Chantale Andre of Environment Canada's St. Lawrence Center, the developers of this biochemical assay, and who conducted the 2003 studies. Vitellogenin-like proteins were measured indirectly using the alkali-labile phosphate (ALP) assay. ALP was normalized for proteins, but these data were not as responsive. The ALP assay is an indirect method to determine the relative levels of vitellin in biological tissues. The ELISA is not performed because the available kits are for fish vitellogenin and the appropriate antibodies do not cross-react well with bivalves. The ALP assay, because it is indirect, is validated with gel electrophoresis where vitellogenin-like protein bands are quantified by densitometric analysis.

Results show no significant difference in condition factor, gonadosomatic index, or levels of vitellin above and below the mill (Figures 3.5.1-3.5.5). Fish data are not yet available. Once comparisons can be made between species, future monitoring needs will become clear.

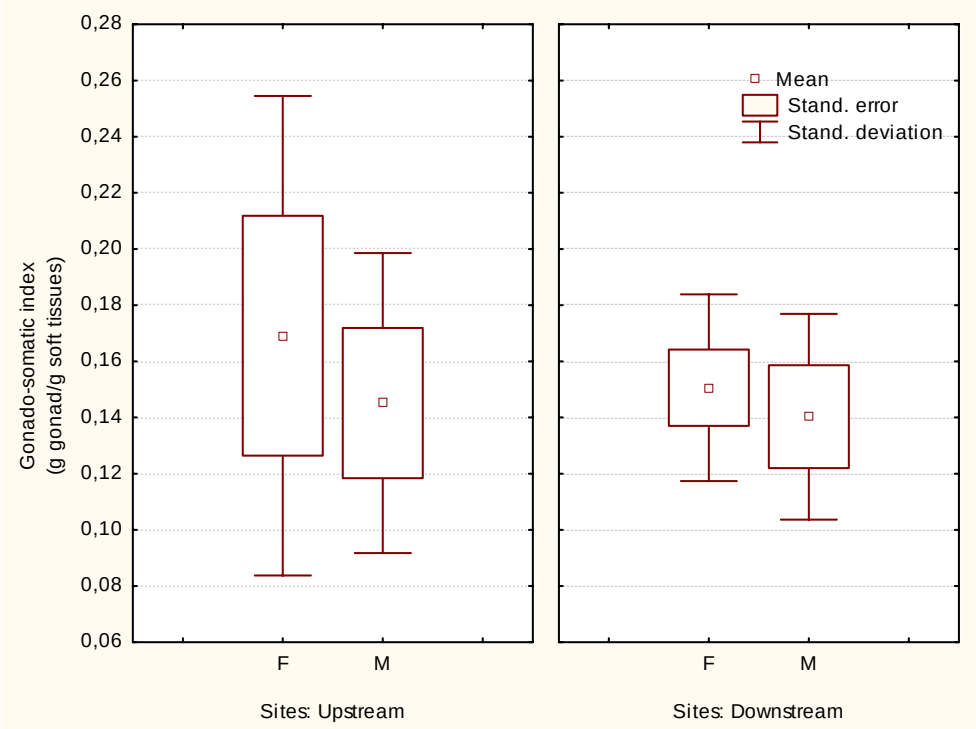
Upstream / downstream study design. Differences were examined using 2-way ANOVA.

Figure 3.5.1 Condition factor of caged mussels, Elliption complanata, above and below the SAPPI-Somerset bleached kraft pulp and paper mill, 2004



No significant difference was found between sites and gender.

Figure 3.5.2 Gonadosomatic index of caged mussels, Elliption complanata, above and below the SAPPI-Somerset bleached kraft pulp and paper mill, 2004



No significant difference was observed with sites and gender.

Figure 3.5.3 Vitellin-like proteins on a gonad weight basis of caged mussels, Elliption complanata, above and below the SAPPI-Somerset bleached kraft pulp and paper mill, 2004

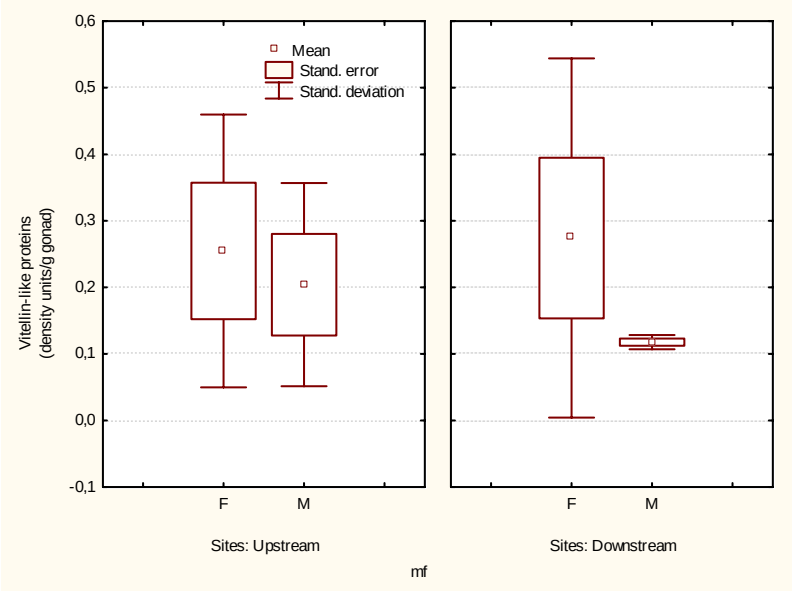
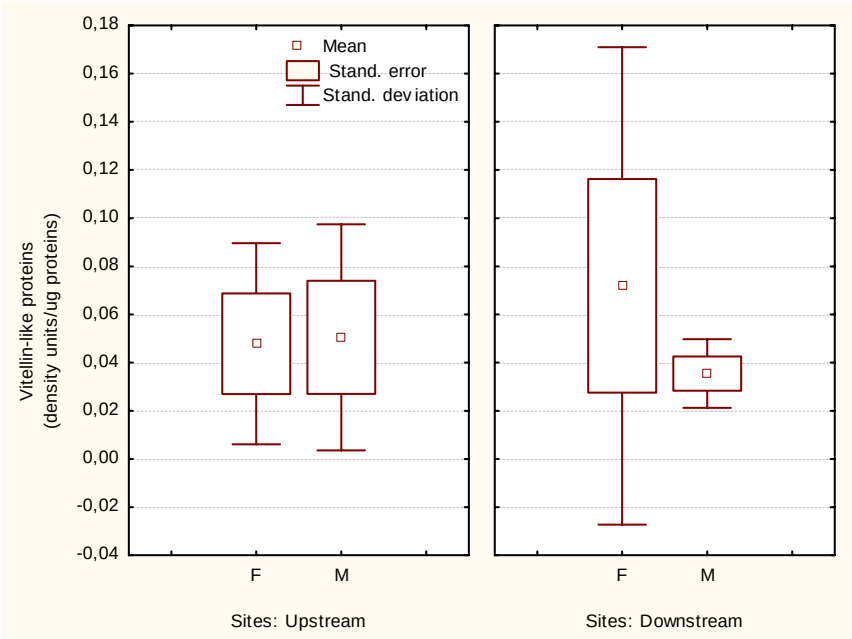
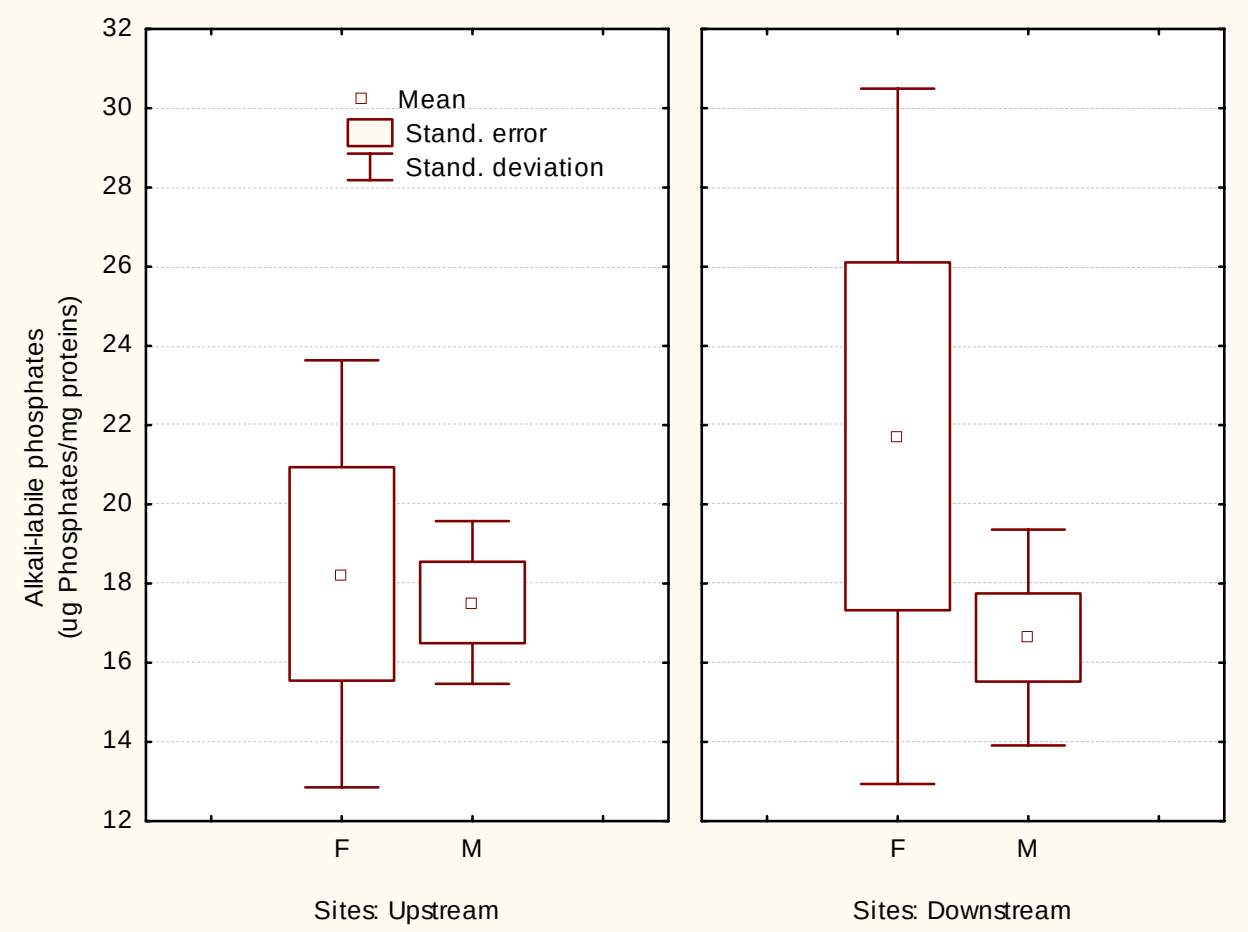


Figure 3.5.4 Vitellin-like proteins on total extracted proteins basis of caged mussels, Elliption complanata, above and below the SAPPI-Somerset bleached kraft pulp and paper mill, 2004



No significant differences were observed with sites and gender.

Figure 3.5.5 Alkali-labile phosphates (a generic and indirect assay for vitellin-like proteins)in caged mussels, Elliption complanata, above and below the SAPPI-Somerset bleached kraft pulp and paper mill, 2004



No significant differences were observed between sites and gender.

RAW DATA

SAMPLE	LENGTH	TOT WT	SHELL WT	TISSUE WT	GONAD WT	GSI	ALP	SEX
KHY-A	mm	g	g	g	g		ug/mg prot	
CM 4-3-1	60.53	20.94	11.25	9.69	1.69	0.17	7.43	F
CM 5-2-5	62.65	24.09	17.31	6.78	1.94	0.29	7.52	F
CM 6-2-5	63.40	18.59	8.47	10.12	0.91	0.09	6.83	F
CM 7-2-5	63.05	16.72	11.72	5.00	0.63	0.13	8.18	F
CM 4-4-1	61.00	18.07	10.26	7.81	1.55	0.20	5.71	I
CM 5-1-5	63.00	18.98	9.91	9.07	1.66	0.18	8.51	M
CM 6-1-15	62.00	16.58	8.31	8.27	0.89	0.11	6.24	M
CM 7-1-5	63.00	14.02	9.21	4.81	0.44	0.09	5.62	M
KFF-B								
CM 1-2-1	63.40	26.76	13.33	13.43	2.76	0.21	6.84	F
CM 2-2-1	63.55	18.30	8.64	9.66	1.32	0.14	6.87	F
CM 7-1-1	62.70	16.48	7.86	8.62	1.01	0.12	7.56	F
CM 9-1-1	63.00	22.08	11.66	10.42	1.45	0.14	8.99	F
CM 1-1-1	62.20	16.11	8.78	7.33	0.95	0.13	6.39	I
CM 2-1-1	62.70	18.18	8.50	9.68	1.70	0.18	5.01	I
CM 3-1-1	62.30	18.72	8.03	10.69	0.94	0.09	6.74	M
CM 9-2-1	62.70	18.51	8.38	10.13	1.48	0.15	6.39	M
CM 10-1-1	64.00	17.27	7.66	9.61	1.66	0.17	7.90	M
CM 10-2-1	62.30	15.28	7.70	7.58	1.17	0.15	5.20	M