DEPLW0756

SURFACE WATER AMBIENT TOXIC MONITORING PROGRAM

FINAL REPORT
EXECUTIVE SUMMARY
2005

DIVISION OF ENVIRONMENTAL ASSESSMENT
MAINE DEPARTMENT OF ENVIRONMENTAL PROTECTION
AUGUSTA, MAINE 04333

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INTRODUCTION

This 2005 Surface Water Ambient Toxic (SWAT) monitoring program final report is organized into this Executive Summary (with introduction and table of contents) and 4 modules, 1) Marine & Estuarine 2) Lakes, 3) Rivers & Streams, and 4) Special Studies. The full report is available on DEP's website at http://www.maine.gov/dep/blwq/docmonitoring/swat/index.htm

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Acknowledgements
Collection of samples was conducted by the principal investigators and technical assistants listed (DEP staff unless otherwise specified) assisted by the Department of Inland Fisheries and Wildlife, and the Penobscot Indian Nation.

Chemical analyses were performed by Pace Analytical Services, Minneapolis, Minnesota or other laboratories as listed in reports in individual sections.

EXECUTIVE SUMMARY

Maine's Surface Water Ambient Toxics (SWAT) monitoring program was established in 1993 (38 MRSA §420-B) to determine the nature, scope and severity of toxic contamination in the surface waters and fisheries of the State. The program must be designed to comprehensively monitor the lakes, rivers and streams and marine and estuarine waters of the State on an ongoing basis. The program must incorporate testing for suspected toxic contamination in biological tissue and sediment, may include testing of the water column and must include biomonitoring and the monitoring of the health of individual organisms that may serve as indicators of toxic contamination. This program must collect data sufficient to support assessment of the risks to human and ecological health posed by the direct and indirect discharge of toxic contaminants.

The Commissioner of the Department of Environmental Protection (DEP) must prepare a 5-year conceptual workplan that outlines monitoring approach for the following 5 years. The Commissioner must also develop annual workplans that define the work to be accomplished each year. A Technical Advisory Group (TAG), composed of 10 individuals with scientific backgrounds representing various interests and 1 legislator, is established to advise the Commissioner on the development of the 5-year framework and annual workplans.

The first 5-year framework, for the period 1994-1998, was an initial sampling of all watersheds in the state. The 5-year plans for the periods 1999-2003 and 2004-2008 were focused on problems discovered in the initial periods and were designed to confirm the initial findings and establish background conditions. Once those are established and a sufficient amount of time has elapsed, 5-10 years depending on what if any action has occurred to solve the problem, repeat sampling may be conducted to determine if the problem has been solved. The program also explores new issues as they are identified.

The SWAT program is divided into 4 modules, 1) Marine and Estuarine, 2) Lakes, 3) Rivers and Streams, and 4) Special Studies. This annual report follows the outline of the 2005 workplan recommended by the SWAT TAG in a meeting June 9, 2005. Following is a summary of key findings from the 2005 SWAT program for each module.

1. MARINE AND ESTUARINE

- Sediment and softshell clam tissue monitoring occurred at 3 stations along the coast in 2005. Locations were selected in consultation with DMR and consisted of areas where the acquisition of toxics data would allow the update of information concerning closed areas and might potentially allow opening areas to commercial clam harvest. Results will be provided to DMR and the state toxicologist for analysis, update of flat closures, and other appropriate action.

- Sediment monitoring occurred at three areas of the coast in 2005. Spruce Creek, Kittery (four locations), and Barberry Creek and Clark's Pond, South Portland (one location each), were sampled for sediments in 2005.

- Lobster collections and analysis occurred at 13 stations over the eastern half of the Maine coast in conjunction with the EPA National Coastal Assessment. DEP recently received raw lobster data from the contracted laboratory. Pending review of the results, the data will be provided to the state toxicologist for use in updating public health advisories. It will also be posted on the DEP SWAT web site.

2. LAKES

- A cooperative study of bald eagles with the Maine Department of Inland Fisheries and Wildlife, US Fish and Wildlife Service, Passamaquoddy Tribe, Penobscot Nation, FPL Energy, and BioDiversity Research Institute found that concentrations of mercury in 19-30% of Maine eagles exceeds safe levels. Despite increasing populations in recent years, recovery has been limited by mercury levels, which are no lower than when last monitored in the early 1990s.

3. RIVERS AND STREAMS

- Thirty-nine stations were assessed for the condition of the benthic macroinvertebrate community. Results have been received to date (March 14, 2006) for seven stations. Four of the seven stations (57%) reported failed to attain the aquatic life standards of their assigned class.

- Striped bass and bluefish exceed the Maine Center for Disease Control and Prevention's (MCDC) fish tissue action levels for mercury and PCBs in estuaries all along the coast. MCDC is leading a process with all other Atlantic coast states with significant fisheries for these fish to explore the desirability of a coast wide fish consumption advisory, since these species are coast wide migrants.

- A Cummulative Effects-driven Assessment of fish populations above and below Lincoln found possible evidence of endocrine disruption and needs to be repeated.

- A repeat study of immune function in fish did not find any differences in innate immune system activity of smallmouth bass collected at sites above and below paper mill and municipal discharges on both the Kennebec and Androscoggin Rivers. This is unlike the results of previous work in 2004, when there was evidence of immune system disruption. The study needs to be repeated.

- A caged mussel study indicated endocrine disruption below a pulp and paper mill on the Kennebec River. The study needs to be repeated to confirm the findings.

4. SPECIAL STUDIES (from 2004)

- A study of 135 dead waterbirds of various species found no evidence of organophosphate or carbamate pesticide exposure. DDE was the highest organochlorine pesticide found in the present study. Overall, while organochlorine levels in the birds of this study are below sublethal ranges, values are elevated in some individuals. Two individuals (double-crested cormorant and hooded merganser) had DDE levels within the range of those associated with egg-shell thinning in double-crested cormorants. In addition, levels are similar to those found to impair immune response in herring gulls and glaucous gulls.

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MODULE 1 MARINE

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1.1 2005 Marine Sediment, Shellfish, and Lobster Tissue Analysis

This draft report contains data on freshwater sediments, marine sediments, softshell clam (*Mya arenaria*) tissue, and lobster (*Homerus americanus*) tissues collected in 2005. DEP is still waiting for some clam and lobster (*Homerus americanus*) tissue data from the contracted laboratory. Tissue data will be reported as they are received and reviewed.

The following sites were sampled for sediment in 2005: Spruce Creek, Kittery (three replicates at four locations); Barberry Creek, South Portland (three replicates); and Clarks Pond, South Portland (three replicates). Spruce Creek is a tidal estuary. The South Portland sites were fresh water sites, sampled above the salt water estuarine area to determine contaminant loading coming from extensive upland development in these areas and potentially entering the Fore River estuary. Sites were samples on the following dates:

Location	Date Sampled
Spruce Creek, Kittery	11/17/05
Barberry Creek, South Portland	12/07/05
Clarks Pond, South Portland	12/07/05

Sediment taken from Spruce Creek, Kittery; Barberry Creek, South Portland; and Clarks Pond, South Portland; were analyzed for: Mercury, heavy metals, PAHs, pesticides, and PCBs.

The following sediment and clam sites were sampled in 2005: Long Cove, Searsport; Fort Point Cove, Stockton Springs; and Mill Cove, Robbinston. All samples consisted of three replicate samples. Sites were sampled on the following dates:

Location	Date Sampled
Long Cove, Searsport	12/01/05
Fort Point Cove, Stockton Springs	11/10/05
Mill Cove, Robbinston	11/29/05

Sediment and clam tissue from Long Cove, Searsport; Fort Point Cove, Stockton Springs; and Mill Cove, Robbinston; were analyzed for: Mercury, heavy metals, PAHs, pesticides, and PCBs. Sediment and clam tissue from Mill Cove, Robbinston, were analyzed for dioxins and furans.

Lobsters were collected as part of the National Coastal Assessment (NCA) on the eastern half of the Maine coast in 2005. Thirteen stations were sampled over the eastern half of the coast, and DEP dissected lobsters into hepatopancreas, muscle, and offal tissues. Whenever possible, lobster samples were composites of seven individual animals, though a some samples contained fewer lobsters. EPA, as part of the NCA program, will analyze lobster muscle tissue for: Mercury, heavy metals, PAHs, pesticides, and PCBs. As part of the SWAT program, DEP analyzed the lobster muscle tissue for: Dioxins, furans, coplanar PCBs, and PBDEs. In addition, as part of the SWAT program, DEP analyzed

lobster hepatopancreas for: Mercury, heavy metals, PAHs, pesticides, PCBs, dioxins/furans, coplanar PCBs, and PBDEs. No lobster data have been received from the contracted lab. Lobster data will be presented upon their receipt, analysis, and review and are not contained in this draft report. In 2004, there was insufficient hepatopancreas tissue to allow the analysis of hepatopancreas for PBDEs. Sampling in 2005 included acquisition of up to seven lobsters at each site, rather than the five collected in 2004. This change appears to have provided adequate tissue to allow PBDE analysis (along with other planned analyses) in nearly all samples. Some samples did not contain sufficient mass of hepatopancreas to allow all analyses, but these were locations were the target of seven lobsters was not achieved.

MODULE 2 LAKES

2.2

MERCURY IN BALD EAGLES

Evaluating exposure of Maine's Bald Eagle population to Mercury: assessing impacts on productivity and spatial exposure patterns.

(REPORT BRI – 2006-02)

Submitted to:

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1.0 Executive Summary

This report summarizes findings from an ongoing eagle mercury monitoring and impacts study supported by non-profit (BioDiversity Research Institute), state (Maine Dept. of Inland Fisheries and Wildlife, Maine Dept. of Environmental Protection), federal (US Fish and Wildlife Service) and industry (FPL Energy Maine Hydro) agencies. Substantial support for this project was provided by the Maine Department of Environmental Protection.

We collected and analyzed mercury concentrations in Bald Eagle nestling blood, shed adult feathers, and abandoned eggs from freshwater-based Bald Eagle (*Haliaeetus leucocephalus*) nests in Maine (2001-2005) to (1) evaluate dietary exposure to mercury (Hg) (2) assess if Hg exposure might be negatively impacting eagle productivity in Maine, and (3) evaluate spatial and temporal Hg trends in Maine. The following is a summary of current findings:

- *Nestling eagle Hg exposure:* **Maine Bald Eagle nestlings and adults are exposed to elevated levels of methylmercury via the freshwater foodweb. Eagles in lacustrine habitats are particularly at risk. Blood mercury exposure levels of Maine eaglets is higher than many regional comparisons, and most similar to populations associated with significant point source pollution problems (e.g., Hg mines, dredging).**

- *Adult eagle Hg exposure - feathers:* **Exposure levels in Maine's adult Bald Eagles (as indicated by shed adult feathers) is elevated in comparison to virtually all comparison populations. As found in eaglet blood, mean Hg concentrations in Maine adult eagle feathers are most comparable to levels found at a site associated with a Hg mine (Pinchi Lake, BC).**

- *Hg in Eggs:* **Hg in abandoned Bald Eagle eggs from Maine study sites is elevated compared to most populations in the U.S.**

- *Hg-Productivity Relationships: potential impacts:* **We document significant negative relationships between eagle blood Hg and 3,5, and 10- year eagle productivity (chicks fledged/occupied nest). This has not been documented in other eagle populations and suggests that Maine's eagle population may be experiencing reproductive impacts due to Hg exposure despite population growth.**

- *Spatial Patterns:* **Eaglet blood mercury levels were significantly different among 10 Maine watersheds, but sample sizes preclude powerful analyses. Eaglet mercury exposure in Maine highlights geographic mercury "hot spots" that demonstrate a general agreement with Hg findings in common loons and fish.**

- *Long-term trends:* **Mercury bioavailability as indicated by nestling blood does not appear to be markedly different in lacustrine habitats during 2001-2005 in comparison to**

1991-1992. Riverine comparisons suggest that levels are likely the same or higher than 1991-1992 levels.

- *Proportions of sampled eaglets at levels of concern:* **Our findings suggest that Maine's Bald Eagle population is within the range of negative impacts. Our findings suggest that between 19-30% of eaglets sampled in lacustrine habitats contain blood mercury levels designated as elevated or higher (>0.70 ppm), and 4-9% of those sampled are highly elevated.**
- *Proportions of sampled eaglets at levels of concern:* **Feather mercury concentrations up to 87 ppm indicate a substantial proportion of Maine's adult eagle population are bioaccumulating mercury; 78% of feathers are >20 ppm, 38% are >40 ppm, and 21% were at or above 60 ppm; these levels are highly elevated and suggestive of impacts.**
- *Eagles as Hg indicators:* **Bald eagles are good biomonitors of spatial and temporal patterns of Hg bioavailability in Maine's aquatic ecosystems.**

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

MODULE 3 RIVERS AND STREAMS

3.1.

AMBIENT BIOLOGICAL MONITORING

AMBIENT BIOLOGICAL MONITORING

As part of the SWAT program, the Biological Monitoring Unit evaluates benthic macroinvertebrate communities of Maine streams and rivers to determine if they are impaired by toxic contamination. Benthic macroinvertebrates are animals without backbones that can be seen with the naked eye and live on the stream bottom, such as mayflies, stoneflies, caddisflies, crayfish, snails, and leeches. In 2005, we evaluated the condition of 39 sample locations, primarily in the Saco, Piscataqua, and Presumpscot River basins.

Table 3.1.1 summarizes the results of biological monitoring activities for the 2005 SWAT Program, sorted by waterbody name. Column headings of Table 3.1.1 are described below:

- *Station* – Since waterbodies are sometimes sampled in more than one location, each sampling location is assigned a unique "Station" number.
- *Log* – Each sample event is assigned a unique "Log" number.
- *Map* – The "Map" number refers to Maps 1 through 24, which are located after the tables.
- *Location* – Some Stations are located upstream or downstream of potential sources of pollution, which are called "Issues".
- *Issue* – Issues are potential sources of pollution.
- *Statutory Class* – The state legislature has assigned a statutory class, either AA, A, B, or C, to every Maine stream and river. Class AA and A waterbodies shall support a "natural" biological community. Class B waterbodies shall not display "detrimental changes in the resident biological community". Class C waterbodies shall "maintain the structure and function of the resident biological community".
- *Model Class* – The Biological Monitoring Unit uses a multivariate statistical model, called BioME, to analyze a benthic macroinvertebrate sample and predict if a waterbody is attaining the biological criteria associated with its statutory class. The Model Class is the final determination of the BioME model. If a stream does not meet minimum state criteria, Class C, then the Model Class is non-attainment (NA). AA and A are treated the same in the model.
- *Attains Class* – "Y" is given if the Model Class is equal to or exceeds the Statutory Class. A Class B stream, for example, would receive a "Y" if its Model Class was either A or B. "N" is given if a stream does not attain its Statutory Class. A Class B stream, for example, would receive an "N" if its Model Class was either C or NA. A dash ("-") is given if the sample was disturbed or provided insufficient information.
- *Probable Cause* – The probable cause column lists potential stressors to benthic macroinvertebrate communities, based on best professional judgement. In some cases, a probable cause may not be related to toxic pollution but instead to poor habitat conditions.

Data reports for each sampling event (Aquatic Life Classification Attainment Reports) are available in electronic format with the web version of this report. Supporting water chemistry data are given in Table 3.1.2. Water temperature data are given in Figure 3.1.1. For more information about the Biological Monitoring Unit, please e-mail us at biome@maine.gov or visit our web site: http://www.state.me.us/dep/blwq/docmonitoring/biomonitoring/index.htm.

Results Summary

- Thirty-nine stations were assessed for the condition of the benthic macroinvertebrate community.
- Results have been received to date for seven stations.
- Four of the seven stations (57%) reported failed to attain the aquatic life standards of their assigned class.

Historical Notes (not all of the samples listed below collected under the SWAT Program)

- Back Brook (Station 107) attained (exceeded) class in 1987.
- Birch Stream (Station 312) failed to attain class in 1997, 1999, 2001, 2003, and 2004.
- Branch Brook (Station 106) attained class in 1987 and failed to attain class in 2000.
- Brown Brook (Station 445) failed to attain class in 2000.
- Cascade Brook (Station 434) attained class in 2000.
- Cascade Brook (Station 435) attained (exceeded) class in 2000.
- Frost Gulley (Station 303) failed to attain class in 1998 and attained class in 2000.
- Frost Gulley(Station 304) failed to attain class in 1998 and attained class in 2000.
- Goosefare Brook(Station 271) failed to attain class in 1995, 1998, and 2000.
- Goosefare Brook (Station 337) attained class in 1998 and failed to attain class in 2000.
- Goosefare Brook (Station 48) attained class in 1984, 1986, 1994, 1995, 1998, and 2000.
- Great Works River (Station 439) attained class in 2000.
- Kennebunk River (Station 469) attained class in 2000.
- Kennebunk River (Station 270) attained class in 1995 and 2000.
- Little Ossippee River (Station 447) attained class in 2000.
- Little Ossippee River (Station 446) failed to attain class in 2000.
- Little River (Station 440) attained class in 2000.
- Martin Stream (Station 755) failed to attain class in 2004.
- Martin Stream (Station 756) failed to meet minimum abundance criteria in 2004. Resampled in 2005.
- Merriland River (Station 436) attained class in 2000.
- Merriland River (Station 437) attained class in 2000.
- Mousam River (Station 388) attained class in 1999.
- Mousam River (Station 259) failed to attain class in 1995 and attained class in 1999.
- Presumpscot River (Station 72) failed to attain class in 1984, 1994, 1995, and 1996.
- Red Brook (Station 219) attained class in 1994 and failed to attain class in 1999.
- Salmon Falls River (Station 52) failed to attain class in 1984, 1992, and 1995.
- Sheepscot River (Station 74) attained class in 1987, 1989, 1990, 1992, 1995, 1996, 1998, 1999, 2000, 2001, 2002, 2003, and 2004. It failed to attain class in 1984, 1985, 1986, 1988, 1991, 1993, 1994, and 1997.
- Stroudwater River (Station 240) attained class in 1992.
- Thatcher Brook (Station 451) attained class in 2000.
- Trout Brook (Station 675) failed to attain class in 2003 and 2004.
- Webhannet River (Station 438) attained class in 2000.
- West Branch Sheepscot River (Station 268) attained class in 1995, 1996, 1997, 1998, 1999, 2001, and 2002. It failed to attain class in 2000, 2003, and 2004.

TABLE 3.1.1 - 2005 SWAT Benthic Macroinvertebrate Biomonitoring Results

Name	**Map**	Station	**Log**	Town	**Location**	**Issue**[1]	**Statutory Class/ Model Class**	**Attains Class?**	Probable Cause[1]
Back Brook	1	107	1496	Limington		Reference	B /		
Birch Stream	2	312	1319	Bangor	downstream	Urban NPS; Airport	B / NA	N	NPS Toxics; Habitat
Branch Brook	3	106	1341	Sanford		NPS	A /		
Brown Brook	4	445	1357	Limerick		Munic/Ind/ Urb NPS/Imp	B /		
Cascade Brook	5	434	1337	Saco	Above	Control	B /		
Cascade Brook	5	435	1338	Saco	Below	Urban NPS/ Turnpike	B /		
East Branch West Brook	6	798	1331	Biddeford		NPS	B /		
Emery's Brook	7	794	1354	So. Berwick		Reference	B/		
Frost Gulley	8	303	1499	Freeport	Above	NPS	A /		
Frost Gulley	8	304	1500	Freeport	Below	NPS	A /		
Goosefare Brook	5	271	1333	Saco	Below	Urban NPS/In-Place Contamination	B /		
Goosefare Brook	5	337	1334	Saco		NPS/ Turnpike	B /		
Goosefare Brook	5	48	1335	Saco	Above	Control	B /		
Great Works River	9	439	1355	No. Berwick		NPS	B /		
Kennebunk River	10	469	1328	Arundel		NPS	B /		
Kennebunk River	10	270	1329	Kennebunk		Urban NPS/ Turnpike	B /		
Little Ossippee River	4	447	1497	Limerick		NPS	B /		
Little Ossippee River	4	446	1498	Limington		Munic/Ind/ NPS/Imp	B /		

[1] NPS = non-point source pollution

Name	**Map**	Station	**Log**	**Town**	**Location**	**Issue**[1]	**Statutory Class/ Model Class**	**Attains Class?**	Probable Cause[1]
Little River	11	440	1342	Lebanon		NPS	B /		
Martin Stream	12	755	1317	Dixmont	upstream	Agric NPS	A / B	N	
Martin Stream	12	756	1318	Dixmont	downstream	Agric NPS	A / B	N	
Merriland River	13	436	1324	Wells		NPS	A /		
Merriland River	13	437	1325	Wells		NPS	A /		
Mousam River	14	388	1339	Springvale		NPS	B /		
Mousam River	14	259	1340	Sanford		Urban NPS	C /		
Nonesuch River	15	788	1323	Scar-borough		NPS	B		
Presumpscot River	16	72	1501	Westbrook		Munic/Ind/ Urban NPS	C / C	Y	
Presumpscot River	16	802	1502	Falmouth		Munic/Ind/ Urban NPS	C / B	Y	
Red Brook	15	219	1322	Scar-borough		Landfill/NPS	C /		
Salmon Falls River	17	52	1356	Berwick		Municipal	C		
Sheepscot River	18	74	1314	N. Whitefield		Reference	AA /		
Stroudwater River	15	796	1348	Gorham	above	NPS	B /		
Stroudwater River	15	240	1349	Gorham		NPS/In Place Contamin.	B /		
Swan Pond Brook	6	786	1327	Biddeford		NPS	B / B	Y	
Thatcher Brook	6	451	1332	Biddeford		Urban NPS	B /		
Trout Brook	19	675	1320	So. Portland	upstream	Urban NPS	C / NA	N	NPS Toxics
Webhannet River	13	438	1326	Wells		NPS/Turnpike	B /		
W. Br. Sheepscot River	20	268	1315	China		Reference	AA /		
West Brook	6	797	1330	Biddeford		Urban NPS	B /		

[1] NPS = non-point source pollution

TABLE 3.1.2 - 2005 SWAT Nutrients and Solids Data

Log	Waterbody	Sampling Date	DOC	NH_3-N	TKN	NO_2-NO_3-N	OPO_{-4}	Total P	TSS	TDS
			mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
1496	Back Brook	8/24/2005	2.0	0.01	0.1	0.15	0.004	0.011	~0.2	35
1319	Birch Stream, up	8/8/2005	5.2	0.01	0.3	0.23	0.003	0.019	~1.1	400
1341	Branch Brook	8/17/2005	1.9	0.01	0.2	0.31	0.003	0.007	~1.5	50
1337	Cascade Brook (above)	8/16/2005	8.8	0.07	0.7	0.42	0.023	0.065	~2.0	180
1338	Cascade Brook (below)	8/16/2005	8.2	0.09	0.7	0.32	0.010	0.062	5.0	270
1331	East Branch West Brook	8/15/2005	5.1	0.02	0.5	0.31	0.003	0.047	10	180
1499	Frost Gulley (above)	8/25/2005	1.9	<0.01	0.2	0.46	0.004	0.007	~0.1	220
1500	Frost Gulley (below)	8/25/2005	1.7	0.01	0.2	0.47	0.007	0.012	~0.5	250
1335	Goosefare Brook (above)	8/16/2005	3.1	0.03	1.1	0.47	0.006	0.099	~62	100
1333	Goosefare Brook (below)	8/16/2005	3.8	0.09	0.4	0.27	0.003	0.018	4.0	370
1355	Great Works River	8/23/2005	3.7	0.01	0.3	0.09	0.003	0.016	~1.0	82
1355	Great Works River DUP	8/23/2005	4.4	0.01	0.3	0.09	0.002	0.016	~0.2	88
1498	Little Ossippee River	8/24/2005	3.6	<0.01	0.3	<0.01	0.001	0.009	~0.2	31
1317	Martin Stream (above)	8/8/2005	4.6	<0.01	0.2	0.05	0.002	0.012	~1.9	80
1318	Martin Stream (below)	8/8/2005	4.3	<0.01	0.3	0.05	0.002	0.012	2.0	80
1325	Merriland River (below)	8/11/2005	7.1	0.01	0.4	0.11	0.006	0.026	3.0	86
1324	Merriland River	8/11/2005	6.7	0.01	0.3	0.12	0.008	0.024	~0.0	62
1340	Mousam River	8/17/2005	4.3	0.04	0.3	0.06	0.009	0.011	~1.1	68
1356	Salmon Falls River	8/23/2005	8.1	0.09	1.1	0.32	0.003	0.021	~0.3	~4
1349	Stroudwater River (below)	8/22/2005	2.5	0.02	0.2	0.72	0.004	0.021	2.0	88

DOC = dissolved organic carbon, NH_3-N = ammonia-nitrogen, TKN = total Kjeldahl nitrogen, NO_2-NO_3-N = nitrite-nitrate-nitrogen, OPO_{-4} = Ortho-phosphate, Total P = total phosphorus, TSS = total suspended solids, and TDS = total dissolved solids.

More detail including maps of sampling stations, temperature data, and raw macroinvertebrate data are available in the Ambient Biological Monitoring section on our website at http://www.state.me.us/dep/blwq/docmonitoring/swat/index.htm

3.2

FISH CONSUMPTION ADVISORIES

COPLANAR PCB

In 2005 the SWAT program was again integrated with the Dioxin Monitoring Program (DMP) that has been in effect since 1988. Fish samples collected at 12 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 Maine Center for Disease Control and Prevention's (MCDC) (formerly Maine Bureau of Health) 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 MCDC.

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 R at Gilead
ARF Androscoggin R at Rumford
ARY Androscoggin R at Riley
AGI Androscoggin R at GIP, Auburn
ALV Androscoggin R at Livermore Falls
ALW Androscoggin Lake at Wayne
KFF Kennebec R at Shawmut, Fairfield
PBW Penobscot R at Woodville
PBL Penobscot R at S Lincoln
PBV Penobscot R at Veazie
SWP W Br Sebasticook R at Palmyra
SBN Sebasticook R at Burnham

The results show that dioxin (toxic equivalents, upper 95% confidence limit with non-detects at ½ the detection level) and coplanar PCB (toxic equivalents, upper 95% confidence limit with non-detects at ½ the detection level) both separately and combined cause many samples to exceed the pFTALs and some to exceed the FTAL and FTALr (Figures 3.2.1 and 3.2.2, Table 3.2.1). The contribution of each varies with station, species and (not shown) year. Concentrations of coplanar PCB are within the wide range of those seen in previous years. Coplanar PCB concentrations at SWP are much lower than those in 2004, which were unusually high. Sources of PCBs are unknown but likely include atmospheric deposition.

Figure 3.2.1. Dioxin and coplanar PCB toxic equivalents in smallmouth bass (and white perch WHP, brown trout BNT and rainbow trout RBT) from the Androscoggin (Axy), Kennebec (Kxy), Penobscot (Pxy) and Sebasticook (Sxy) rivers, 2005

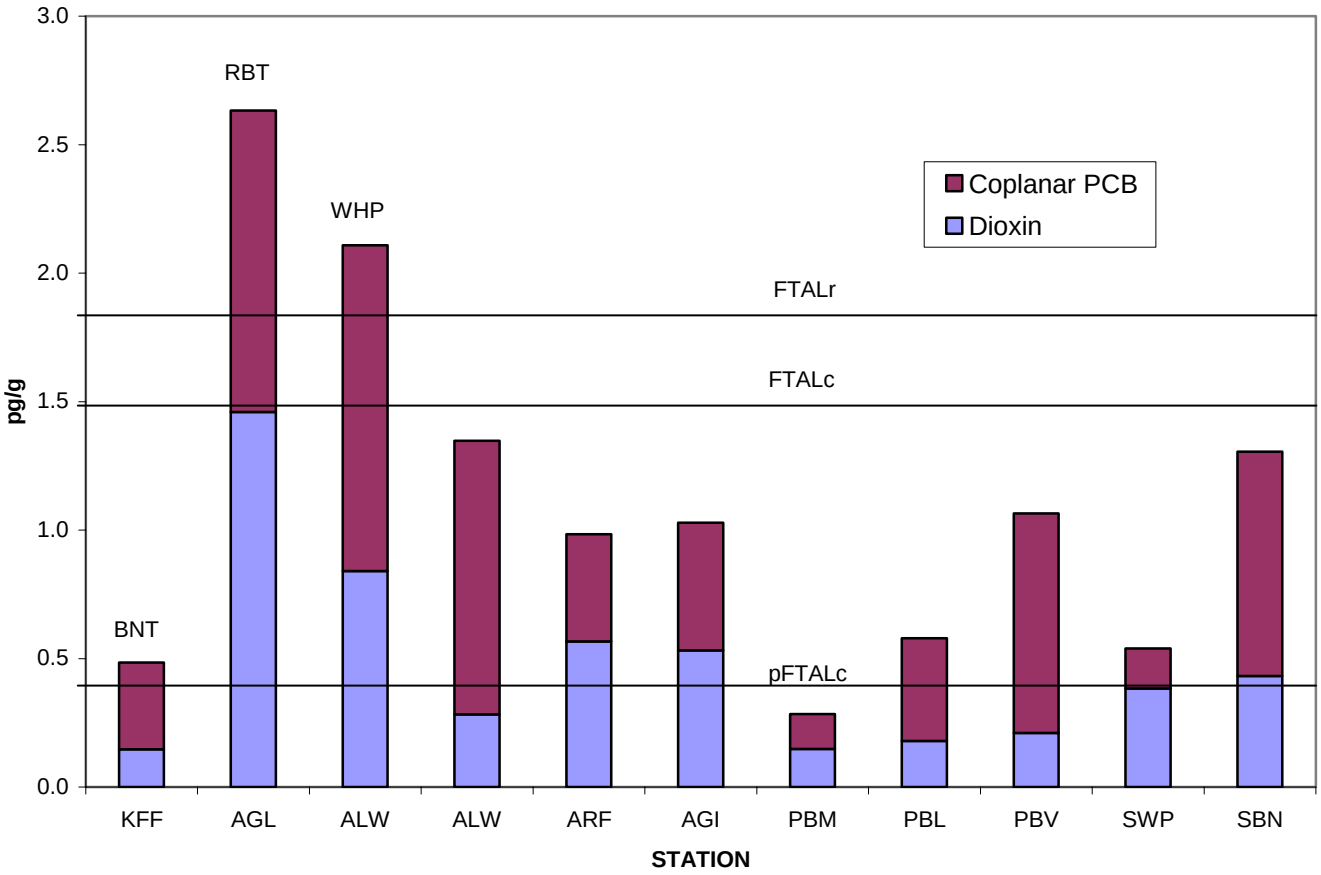
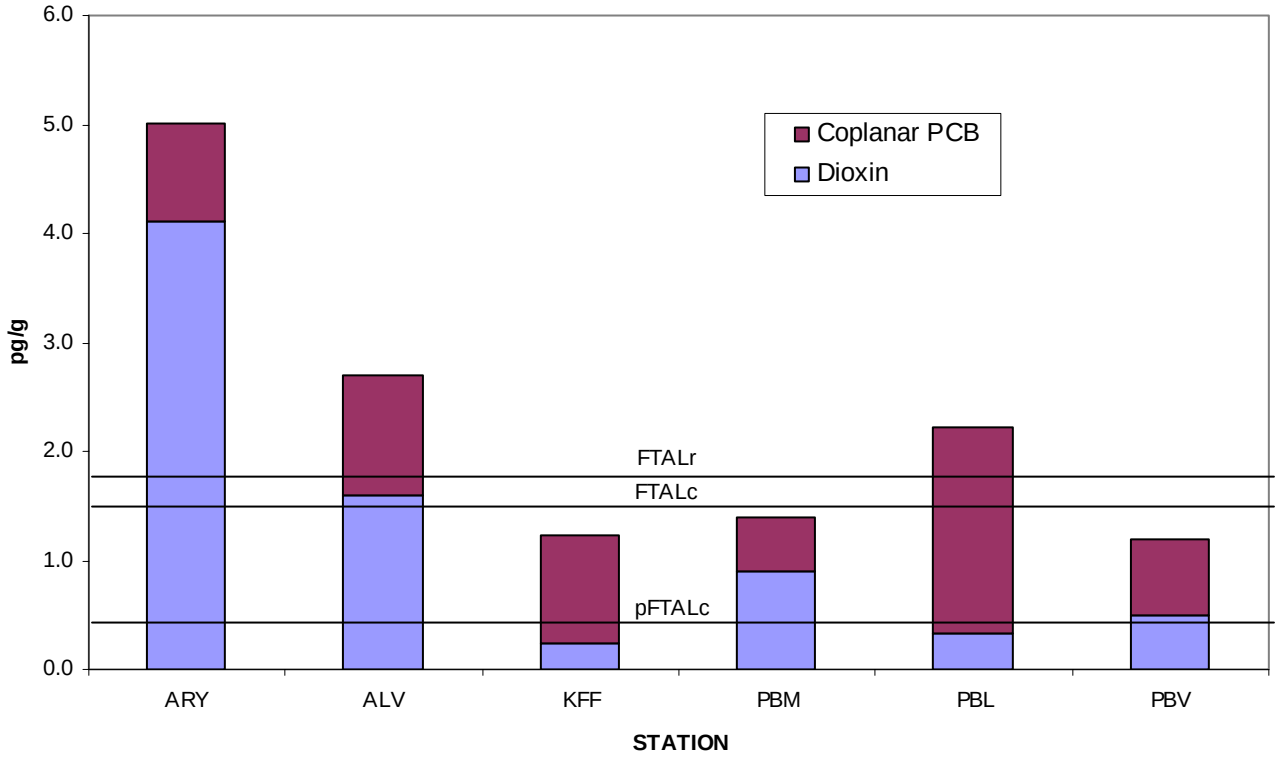


Figure 2. Dioxin and coplanar PCB toxic equivalents in white suckers from the Androscoggin (Axy), Kennebec (Kxy), and Penobscot (Pxy) rivers, 2005



DIOXIN

Dioxin concentrations in rainbow trout at Gilead and in bass from two locations on the Sebasticook River were measured as part of the SWAT program but are discussed in detail in the 2005 final report of Maine's Dioxin Monitoring Program (DMP) available at http://www.maine.gov/dep/blwq/docmonitoring/dioxin/index.htm

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). Beginning in 2003, samples have been analyzed by Pace Analytical Services for all 209 congeners. Data usually represent a mean of 5 individual fish.

In 2005 a total of 5 striped bass and 5 bluefish were collected from four and one rivers respectively, and analyzed by Pace Analytical Services (PAS) for all 209 PCB congeners (and mercury). Given the wide variation in PCBs in fish from year-to-year and lab-to-lab, to help compare past and present data, in 2004 5 samples were split between GERG and PAS. Preliminary results showed that the GERG results were all lower by an average of 28%, which is within the acceptable 30% relative percent difference data quality objective, but biased low. The samples were analyzed by low resolution, however, 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 showed much better correspondence between labs (see Special Studies section 4.2 of this report) and corroborate the PAS data (since 2002) from the past and present.

Comparison of the 2005 PCB levels for striped bass show concentrations are generally similar to those measured since 2002 but significantly higher than those measured earlier (Tables 3.2.2, 3.3.3). The Scarboro River is close to the Saco River; therefore, results from the Scarboro River can be compared to prior results from either river. For bluefish where there are fewer data, concentrations in 2005 were similar to those since 2001 but significantly higher than those measured earlier. Given the measurement of all 209 congeners since 2002, it is likely data since then are more accurate. All samples exceeded the Bureau of Health's FTAL (11 ppb) and most samples 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
2005		269	110				262	108

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		
2005						313		

Table 3.2.3 PCBs in striped bass (STB) and bluefish (BLF) , 2005

Field ID	LENGTH mm	WEIGHT g	PCB ng/g	% LIPID
KENNEBEC R AT AUGUSTA				
KAG STB 1	567		336	0.15
KAG STB 2	579		109	0.19
KAG STB 3	629		172	1.27
KAG STB 4	635		149	0.12
KAG STB 5	625		200	0.29
MEAN			**193**	
STD			**86.6**	
CONFIDENCE INTERVAL			**75.9**	
95 UCL			**269**	
PENOBSCOT R AT ORRINGTON				
PBO STB 1	625			
PBO STB 2	543			
PBO STB 3	598		63.2	0.44
PBO STB 4	647		119	0.34
PBO STB 5	527		60.4	0.59
MEAN			**80.7**	
STD			**32.8**	
CONFIDENCE INTERVAL			**28.8**	
95 UCL			**110**	
SCARBORO R				
SCARBROUGH STB 1	514		254	0.28
SCARBROUGH STB 2	542		125	0.45
SCARBROUGH STB 3	508		293	0.31
SCARBROUGH STB 4	511		175	0.31
SCARBROUGH STB 5	513		104	0.24
MEAN			**190**	
STD			**81.7**	
CONFIDENCE INTERVAL			**71.6**	
95 UCL			**262**	
YORK R				
YORK STB 1	621		99.60	1.10
YORK STB 2	539		108.30	0.24
YORK STB 3	586		109.5	0.31
YORK STB 4	582		97.9	0.33
YORK STB 5	583		88.2	0.64
MEAN			**101**	
STD			**8.67**	
CONFIDENCE INTERVAL			**7.60**	
95 UCL			**108**	
SACO R				
OOB BLF 1	860	4600		5.3
OOB BLF 2	800	3640	342	2.3
OOB BLF 4	715	3000	110	1.0
OOB BLF 5	700	2900		8.2
OOB BLF 7	710	3100	486	2.2
MEAN			**313**	
STD			**190**	
CONFIDENCE INTERVAL			**166**	
95 UCL			**479**	

The upper 95th confidence level (95 UCL) mercury concentrations in striped bass and bluefish in 2005 were similar to those from previous years (Tables 3.2.2). Concentrations for striped bass from the Penobscot River was slightly higher than those for the other rivers primarily due to the greater variation among the results of the 5 samples from the Penobscot River than the other rivers (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.

Table 3.2.4 Mercury in marine fish from Maine estuaries, mg/kg 95th 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
2005		0.28	0.44				0.28	0.15

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		
2005						0.43		

Table 3.2.5 Mercury in striped bass (STB) and bluefish (BLF) , 2005

Field ID	LENGTH	WEIGHT	HG	% SOLIDS
	mm	g	mg/kg	
KENNEBEC R AT AUGUSTA				
KAG STB 1	567		0.26	21.6
KAG STB 2	579		0.16	20.9
KAG STB 3	629		0.23	22.8
KAG STB 4	635		0.10	20.4
KAG STB 5	625		0.29	22.5
MEAN			**0.21**	
STD			**0.08**	
CONFIDENCE INTERVAL			**0.07**	
95 UCL			**0.28**	
PENOBSCOT R AT ORRINGTON				
PBO STB 1	625		0.12	22.8
PBO STB 2	543		0.11	21.7
PBO STB 3	598		0.54	23.1
PBO STB 4	647		0.13	22.7
PBO STB 5	527		0.43	23.2
MEAN			**0.27**	
STD			**0.20**	
CONFIDENCE INTERVAL			**0.18**	
95 UCL			**0.44**	
SCARBORO R				
SCARBROUGH STB 1	514		0.26	22.0
SCARBROUGH STB 2	542		0.18	21.1
SCARBROUGH STB 3	508		0.24	22.2
SCARBROUGH STB 4	511		0.10	20.9
SCARBROUGH STB 5	513		0.30	22.9
MEAN			**0.22**	
STD			**0.08**	
CONFIDENCE INTERVAL			**0.07**	
95 UCL			**0.28**	
SACO R				
OOB BLF 1	860	4600	0.39	27.0
OOB BLF 2	800	3640	0.37	25.9
OOB BLF 4	715	3000	0.47	24.1
OOB BLF 5	700	2900	0.32	29.5
OOB BLF 7	710	3100	0.24	25.7
MEAN			**0.36**	
STD			**0.09**	
CONFIDENCE INTERVAL			**0.07**	
95 UCL			**0.43**	
YORK R				
YORK STB 1	621		0.06	22.0
YORK STB 2	539		0.08	21.4
YORK STB 3	586		0.16	20.6
YORK STB 4	582		0.16	20.3
YORK STB 5	583		0.11	21.0
MEAN			**0.11**	
STD			**0.05**	
CONFIDENCE INTERVAL			**0.04**	
95 UCL			**0.15**	

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, toxic, and bioaccumulative (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 more subtle effects may be a result of long term or cumulative exposure to relatively low levels of 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 measures indicators of survival, growth, and reproduction. Age structure and mean age are measured as indicators of survival. 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 while 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, along with survival, is considered an index of potential population trends.

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.

For Maine's major industrial rivers, the initial 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 had 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 host municipalities (DEP, 2004).

Studies of caged mussels in 2003 on the Androscoggin River showed no negative impacts on growth rate or induction of vitellin, a reproductive protein biomarker of endocrine disruption. This result is consistent with the CEA of fish populations in the river from 2001-2003. Studies of caged mussels in 2003 on the Kennebec River, however, did show induction of vitellin below a bleached kraft pulp and paper mill, evidence of endocrine disruption. 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. The results indicated possible endocrine disruption of survival, growth, and reproduction, as mean age, length in males, and levels of circulating sex steroids were reduced below the mill. Yet the results were not conclusive since MFOs, an indicator of exposure to bleached kraft mill effluent and other xenobiotics, and LSI in females were reduced below the mill while GSI and K actually increased, indicating a shift in energy storage and utilization and/or nutrient enrichment. This study needs to be repeated. A caged mussel study did not show induction of vitellin seen in 2003, but the stations were different between the two years due to other priorities. The study was repeated in 2005 and showed that **?? (Data not yet received).**

Since there is some evidence of endocrine disruption below the bleached kraft mill on the Kennebec River, in 2005 a CEA was conducted above and below the Lincoln Paper and Tissue bleached kraft mill on the Penobscot River. Fish samples were collected in conjunction with the dioxin above/below (A/B) test, which allowed sampling effort and use of fish for both studies. The Environmental Effects Monitoring (EEM) program in Canada require all pulp and paper mills to conduct CEA of two species for each mill. Our 2005 Penobscot River CEA included two species as well, smallmouth bass and white sucker. Also a caged mussel study was initiated for the Penobscot for the A/B dioxin test and measurement of vitellin, but heavy fall rains and subsequent flooding prevented retrieval of the mussels.

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). We have measured the spleen somatic index (SSI) and kidney somatic index (KSI) from white suckers from the Androscoggin River from 2002-2003, the Kennebec River in 2004, and Penobscot River in 2005 as rough indicators of immune system effects. There were significant decreases in SSI below the 2 most upstream mills on the Androscoggin for one or both sexes in 2002 and 2003, indicating potential immune system stress. Similarly, SSI was decreased below the SAPPI Somerset bleached kraft mill on the Kennebec River in 2004 not inconsistent with the possible decreased immune system capacity found by Hannum in head kidneys (SWAT, 2004), although the mechanism is unclear since head kidney size (KSI) in our study was no different between sites above

and below the mills for either sex on either river. Both SSI and KSI were measured on both species from the Penobscot River in 2005.

Methods

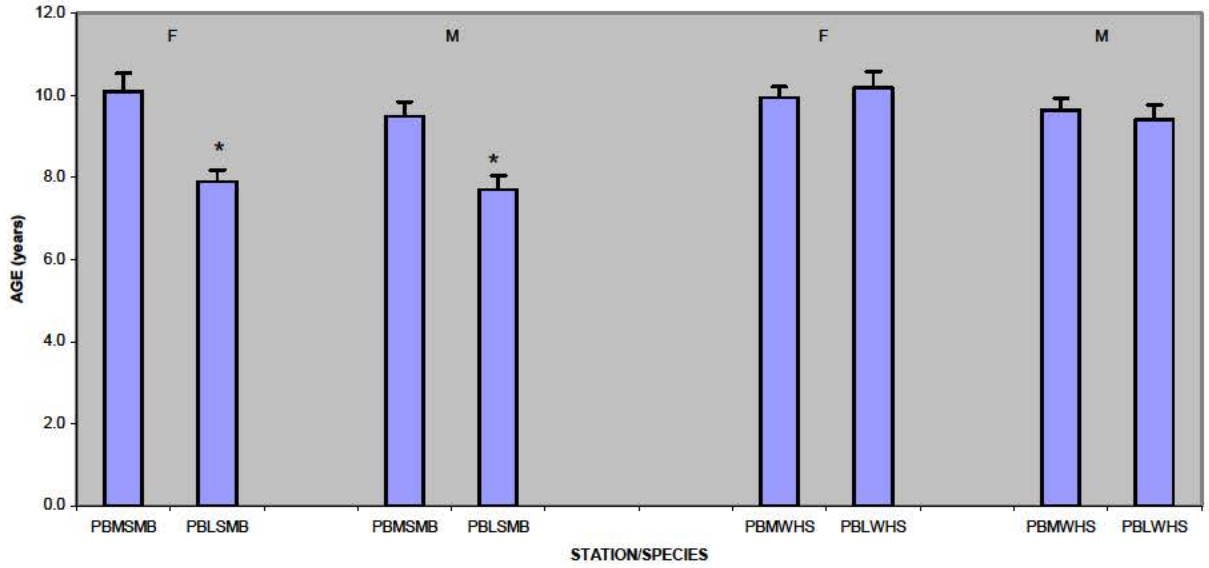
The station above the mill was above the Mattaceunk dam but below Millinockett and the confluence with the East Branch of the Penobscot River while the downstream station was approximately 3 miles below the mill at the historic sampling site for the Dioxin Monitoring Program, with which sampling for 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 (and scales from bass) were collected 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 in suckers and spleen in both species 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 circulating sex steroids (testosterone T, 11 ketotestosterone 11-KT, and estradiol 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 (CYP1A) analysis as outlined by Munkittrick et al (1992). Gonad samples remained in formalin for further analyses. Histological samples of gonads were 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 –20C until prepared and read in the DEP lab in Augusta, Maine.

Results

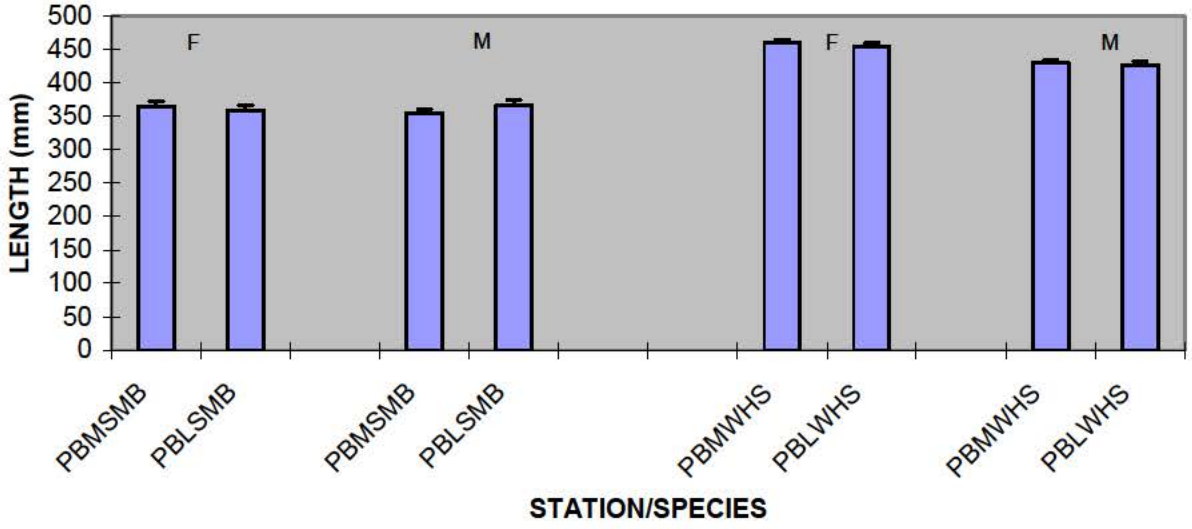
Mean age as an indicator of survival was significantly reduced in smallmouth bass below the mill at PBL compared to above the mill at PBM for both sexes, but there was no such reduction for suckers (Figure 3.3.1). Munkittrick (2000) gives as two possible reasons for reduced survival, 1) exploitation and 2) metabolic redistribution. Since PBL is at a public boat ramp popularly used by anglers, exploitation may be the reason for lower age in bass and not in suckers, which are less desired by anglers.

Figure 3.3.1. Mean age of Male and Female smallmouth bass (SMB) and white suckers (WHS) from the Penobscot River above (PBM) and below (PBL) the Lincoln Paper & Tissue mill, 2005



Indicators of growth include measures of energy expenditure (size and size at age) and measures of energy storage (condition factor K and liversomatic index LSI). Mean size as measured by length did not change for either sex for either species from PBM to PBL (Figure 3.3.2), which, in light of reduced age of bass at PBL, seems to indicate increased growth rate of bass at PBL.

Figure 3.3.2. Mean length in Male and Female smallmouth bass (SMB) and white suckers (WHS) from the Penobscot River above (PBM) and below (PBL) the Lincoln Paper and Tissue mill, 2005



To the contrary, mean K was significantly greater in male bass and both male and female suckers at PBL (Figure 3.3.3). Likewise, mean LSI was greater for female bass (and marginally for males, p=0.09) and both female and male suckers at PBL. Increased levels of energy storage at PBL are correlated with increased nutrient enrichment as indicated by specific conductance, total phosphorus (TP) and total nitrogen (TN) in river water (Table 3.3.1). Although water samples were collected on one date only, the data are consistent with the relatively large mass of nutrients discharged from the pulp and paper mill combined with lesser amounts from the Lincoln municipal discharge.

Figure 3.3.3 Mean condition factor (K) and liver somatic index (LSI) in Male and Female smallmouth bass (SMB) and white suckers (WHS) from the Penobscot River above (PBM) and below (PBL) the Lincoln Paper & Tissue mill, 2005

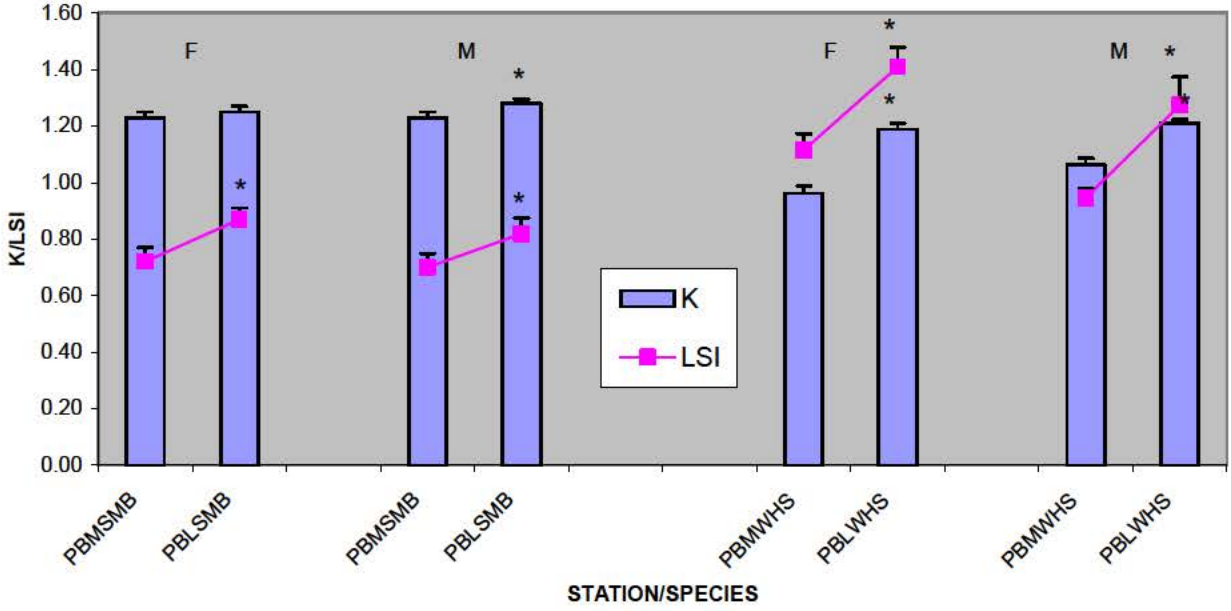
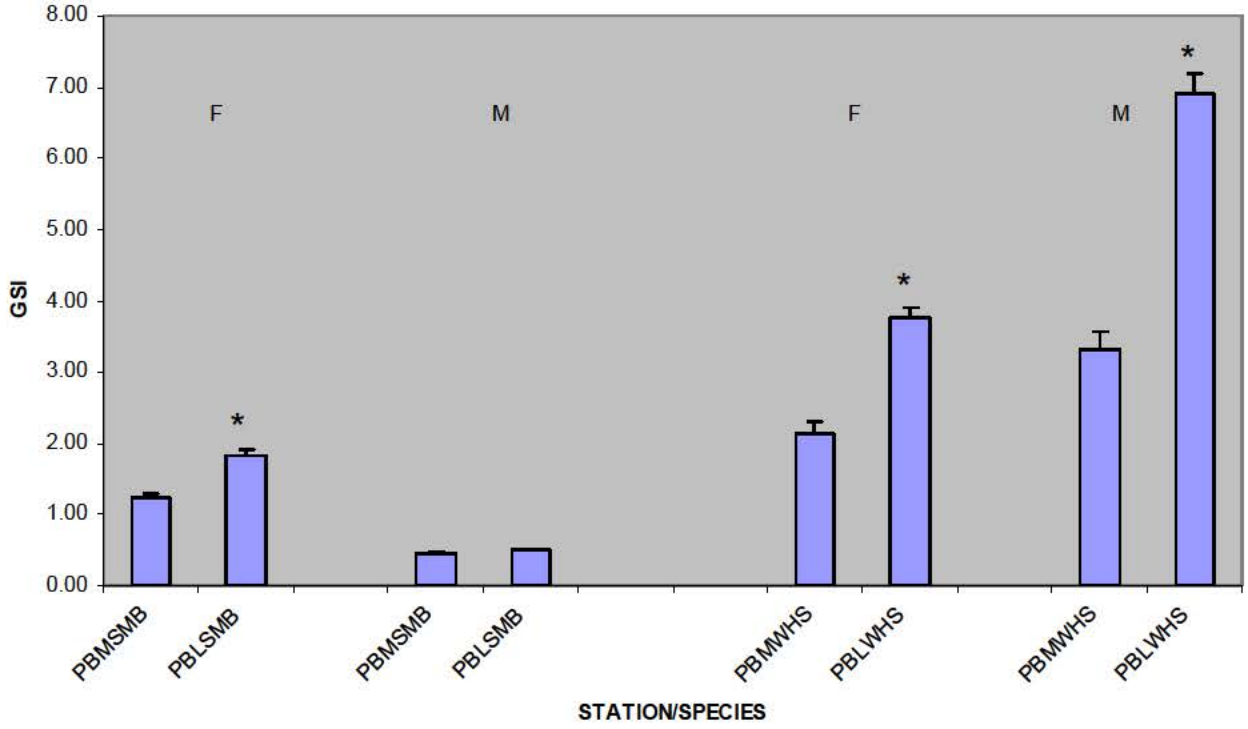


Table 3.3.1

station	DATE	TEMP C	COND us	SP COND us	TP ug/l	NO2/NO3 ug/l	TKN ug/l	TN ug/l
PBM (PBW)	9/12/2005	23	28	29	0.011	0.03	0.3	0.33
PBL	9/12/2005	21	75	81	0.025	0.03	0.4	0.43

Indicators of reproduction also include measures of energy expenditure (gonadosomatic index (GSI), fecundity, and egg size) and measures of energy storage (LSI and lipid storage). GSI was significantly increased at PBL in female bass and in both female and male suckers (Figure 3.3.4).

Figure 3.3.4. Mean gonadosomatic index (GSI) in Male and Female smallmouth bass (SMB) and white suckers (WHS) from the Penobscot River above (PBM) and below (PBL) the Lincoln Paper & Tissue mill, 2005



The CYP1A, cortisol, and gonad data have not yet been received from the lab.

There was no significant difference in mean concentrations of T in male or female smallmouth bass or in E2 concentrations in female smallmouth bass between the stations above (PBM) and below (PBL) the mill (Figure 3.3.1). Concentrations in female smallmouth bass appeared to be lower at PBL using a t-test ($p=0.040$) but lack of homogeneity of variances required use of non-parametric test (Mann-Whitney) which resulted in no significant difference ($p=0.176$). But 11KT was significantly higher at PBL. Concentrations of all three steroids were significantly higher in white suckers below the mill at PBL, however (Figure 3.3.2).

Figure 3.3.5 Mean testesterone (T), 11 ketotestosterone (KT), and estradiol (E2) in Male and Female smallmouth bass from the Penobscot River above (PBM) and below (PBL) Lincoln Paper and Tissue mill

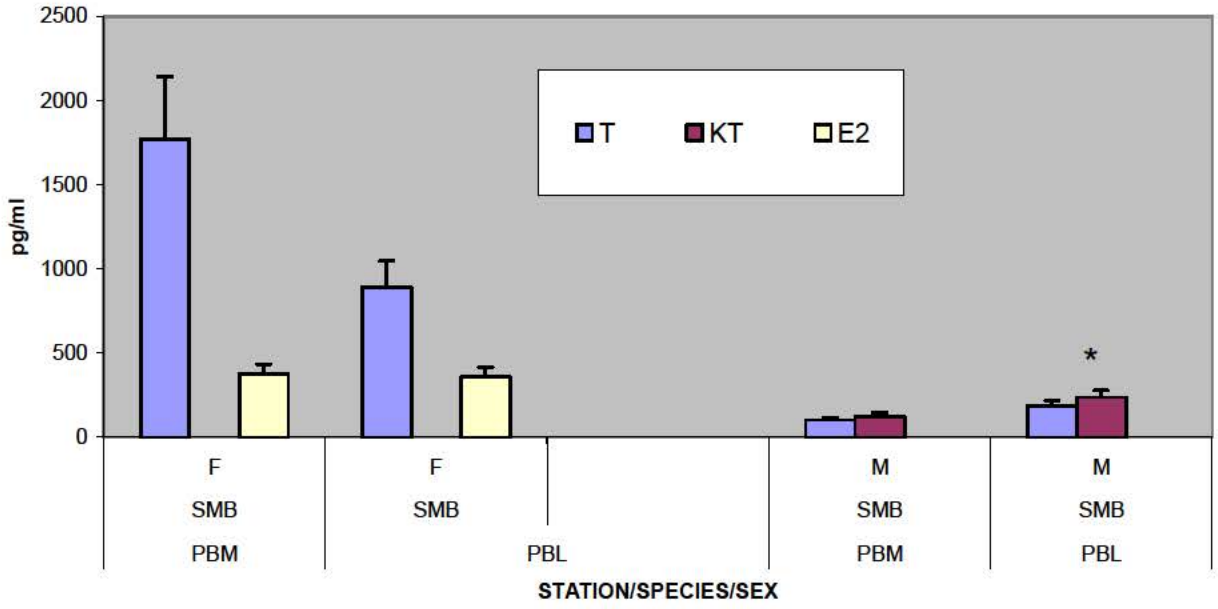
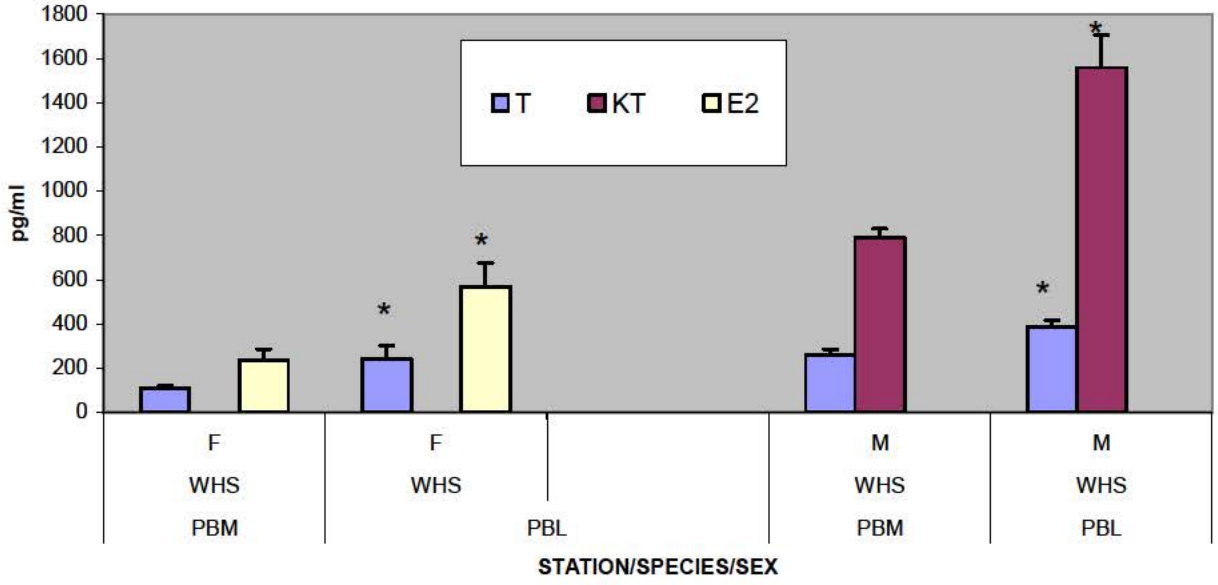


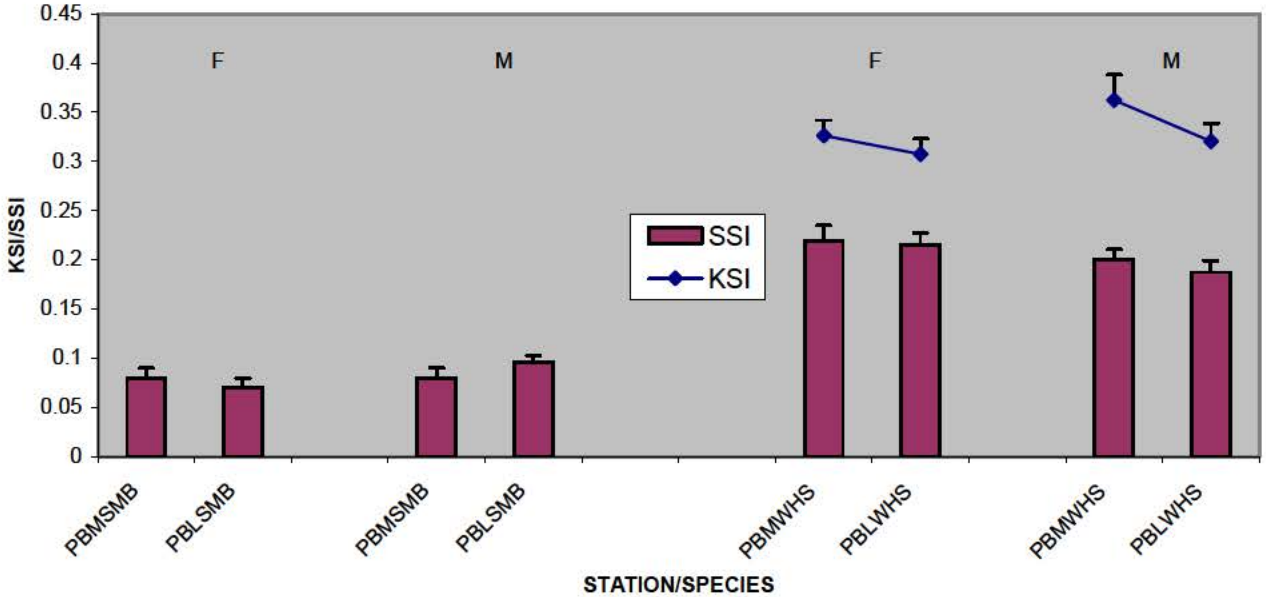
Figure 3.3.6 Mean testesterone (T), 11 ketotestosterone (KT), and estradiol (E2) in Male and Female white suckers from the Penobscot River above (PBM) and below (PBL) the Lincoln Paper and Tissue mill



Increased productivity of the system at PBL due to nutrient discharges from the mill and treated municipal wastewater from the Town of Lincoln resulted in increased energy storage in both smallmouth bass and white suckers. Energy utilization was also elevated at PBL but differently for each species. In both male and female smallmouth bass increased energy was channeled towards growth, as indicated by increased length at age. In both male and female white suckers and female bass increased energy was channeled toward increased reproduction, as indicated by GSI. The steroid data are consistent with GSI for white suckers but not for bass. This may be due to species differences or reflect the paucity of data. These responses for bass fit a pattern of exploitation, caused by mortality or eutrophication, both of which occur at PBL. The responses also fit a pattern of metabolic disruption, but it seems unlikely given lack of correspondence with the steroid data, although the data are limited. For suckers the pattern of responses most closely resembles one of metabolic disruption with energy directed mostly toward reproduction and little toward growth. These results may be skewed by sampling bias however. Due to use of the same suckers for dioxin contaminant analysis, sampling was not random, but directed towards certain narrow size ranges. Therefore, lack of length differences may not represent the characteristic of the true populations at either site. The study needs to be repeated to confirm the results.

There was no difference in head kidney somatic index (KSI) or spleen somatic index (SSI) between PBM and PBL for either sex of either species (Figure 3.3.7). This finding is unlike that from the Kennebec and Androscoggin rivers in previous years where SSI was significantly lower below the mills and host municipalities, both of which were larger than Lincoln on the Penobscot River.

Figure 3.3.7. Mean head kidney somatic index (KSI) and spleen somatic index (SSI) in Male and Female smallmouth bass (SMB) and white suckers (WHS) from the Penobscot River above (PBM) and below (PBL) the Lincoln Paper & Tissue mill, 2005



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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 16, 2006

Objectives

The goal of our research was to continue assessment of innate immune response capacity of fish from the Androscoggin and Kennebec rivers relative to areas of paper mill discharge. In 2004, we collaborated with Barry Mower of the Maine Department of Environmental Protection to perform an initial investigation of the innate immune capacity of white suckers (*Catostomus commersoni*) and smallmouth bass (*Micropterus dolomieui*) from the Androscoggin and Kennebec Rivers. On both rivers, we found the capacity of stimulated anterior kidney cells to produce reactive oxygen species (ROS) was significantly lower in bass collected downstream of paper mill sites than in bass from upstream sites. This suggested a reduced relative innate immune function in fish from downstream sites compared to upstream sites. When we examined background levels of ROS in unstimulated, resting cells we found that in both species ROS levels were significantly elevated at sites immediately downstream from mill effluent discharge, indicative of cellular oxidative stress. Flow cytometric analysis showed that percentages of phagocytic cells in the anterior kidney were higher in downstream Androscoggin bass than upstream bass, suggesting a skewing of white blood cell populations at this site.

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 superoxide anion and hydrogen peroxide. Phagocytes generate these reactive oxygen species (ROS) through a process known as respiratory burst.

ROS production 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*) were received from DEP researchers at four sites along the Androscoggin River in July and August of 2005. Bass were collected above the Mead paper mill at Rumford Point on July 12 and August 2; below the mill in Dixfield on July 12; in Canton on July 17 and August 4, and at Pine Island below the International Paper Company mill in Jay on August 8. Smallmouth bass and white suckers (*Catostomus commersoni*) were collect at two sites along the Kennebec River in September and November, respectively. Bass were collected at the Norridgewock site, upstream of the SAPPI paper mill in on September 22, and downstream in Fairfield on September 15. Suckers were collected from Norrigewock on November 17 and from Fairfield on November 11. In both cases the upstream and downstream sites were separated by a dam, thus the upstream and downstream populations were not mixing.

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 ul of cell suspension ($1x10^7$cells/ml) from each fish was plated to six wells of a 96-well plate. 140 ul of 176 ug/ml NBT solution (Sigma) in HBSS was also added to each well. 60 ul of phorbol 12,13-dibutytate (Sigma) 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. There was no significant difference in the mean stimulation indexes of smallmouth bass from any of the four Androscoggin sites (Table 1).

Table 1: Stimulation indexes of head kidney cells from smallmouth bass collected at four sites on the Androscoggin River.

	n	Mean SI	range
Rumford point	15	5.29	1.94 – 9.48
Dixfield	6	4.88	2.59 – 7.69
Canton	20	4.90	2.81 – 10.38
Pine Island	10	4.96	3.47 – 7.03

Examination of respiratory burst levels in unstimulated head kidney cells revealed an unexpected pattern. There was no significant difference in respiratory burst activity in resting cells from bass collected at the Rumford Point site and the Dixfield site, immediately downstream of the Mead paper mill; this was not the case in 2004, when the downstream site showed elevated ROS in unstimulated cells.

Kennebec respiratory burst

Smallmouth bass were collected at two sites, Norridgewock and Fairfield, on September 22 and September 15, respectively. The results of this study parallel those of the Androscoggin. There was no significant difference in mean stimulation index in head kidney cells of bass from the upstream Norridgewock site than those from the downstream Fairfield site (Table 2). Because white sucker head kidney cells do not repond vigorously to the NBT assay, we can only observe the levels of ROS in

unstimulated resting cells. Unlike our 2004 results, there were no significant differences between ROS levels in the cells from upstream and downstream fish.

Table 2: Stimulation indexes of head kidney cells from smallmouth bass collected at two sites on the Kennebec River.

	n	Mean SI	range
Norrigewock	8	5.86	4.80 – 7.33
Fairfield	9	5.98	3.29 – 9.70

The mean SI values were slightly higher in the Kennebec bass than those from analogous sites on the Androscoggin; background respiratory burst levels in resting cells were similar 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.

Summary

We did not find differences in innate immune system activity, as measured by phagocyte superoxide anion production, in the anterior kidney cells of smallmouth bass collected at sites above and below paper mill discharge on both the Kennebec and Androscoggin Rivers. This is unlike the results of our previous work in 2004, when there were lower stimulation indexes at sites immediately downstream of the two mills.

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Support

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3.5

Caged Mussel Vitellin Study

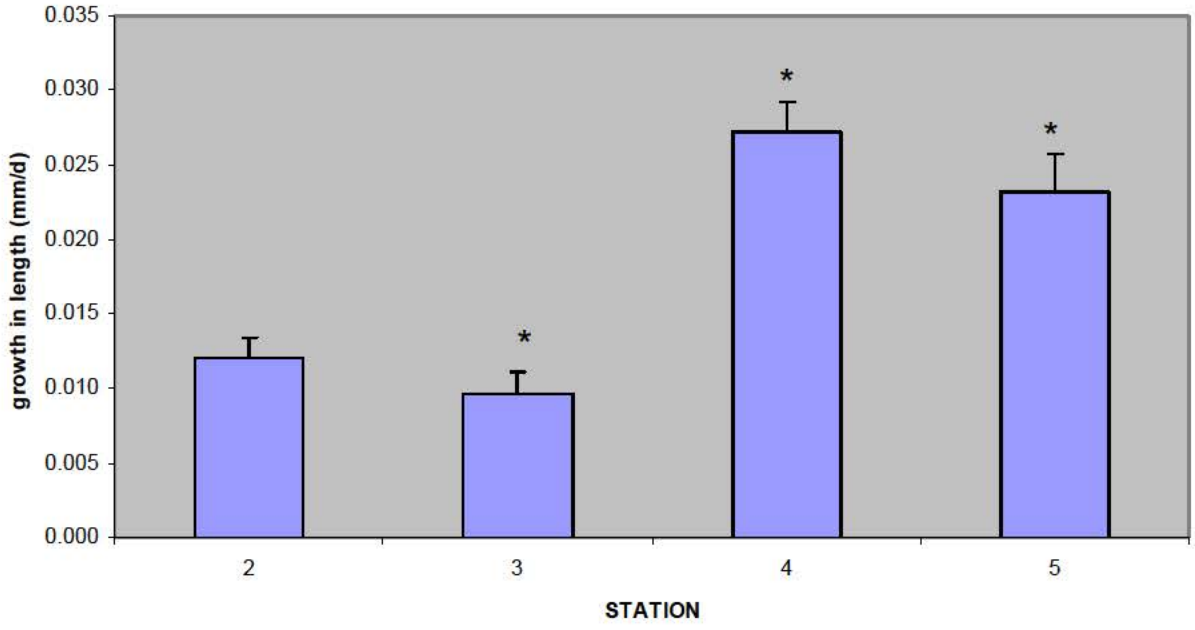
CAGED MUSSEL VITELLIN STUDY -DEP

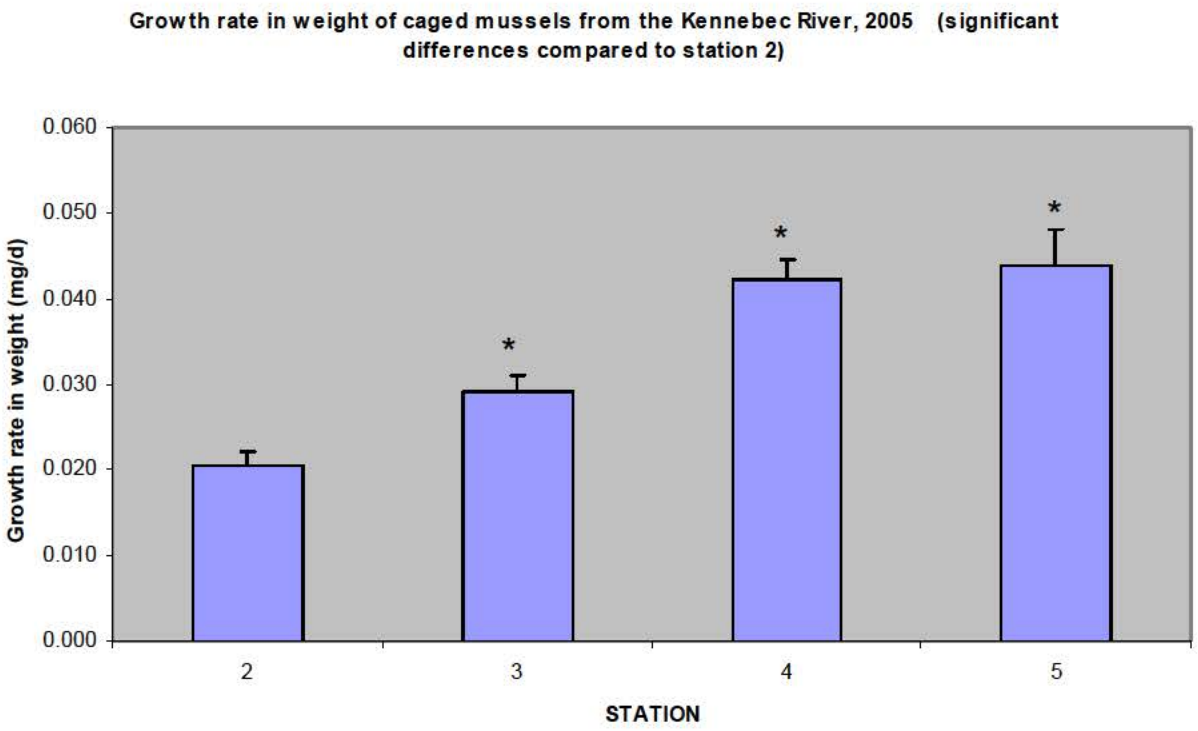
In 2003 a study with caged mussels detected a significant induction of vitellin, a reproductive protein normally found in females, in a subsample of both males and females at stations 3 and 4, ~ 0.08 miles and 2.5 miles below the SAPPI bleached kraft pulp and paper mill on the Kennebec River respectively compared to stations 1 and 2, ~ 13 and 5 miles above the mill respectively. Growth of whole animal length and weight, shell weight, and wet tissue weight were elevated at station 5, ~ 5 miles below the mill. A repeat study in 2004 found no such induction at station 6, ~ 11 miles below the mill, compared to station 2 and there was no difference in condition factor or relative gonad size between the stations. The 2003 study was repeated in 2005 at stations 2-5 with results as indicated below.

Results

Growth rates for both length and/or whole animal wet weight were significantly greater below the mill than above (Figures 3.5.1, 3.5.2) similar to that for station 5 in 2003 and results for condition factor in fish at station 6 in 2004.

Figure 3.5.1. Growth rates for length of caged mussels in the Kennebec River, 2005 (significant differences compared to station 2)

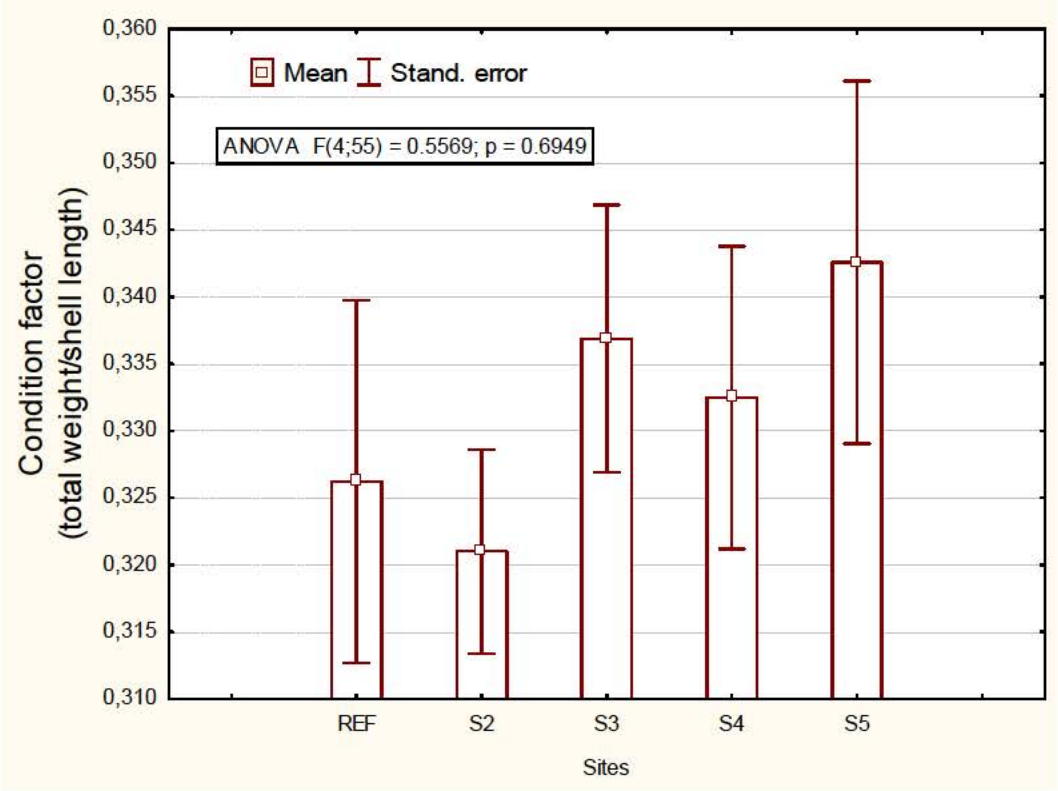




Notes for the following analyses made by the St. Lawrence Center:

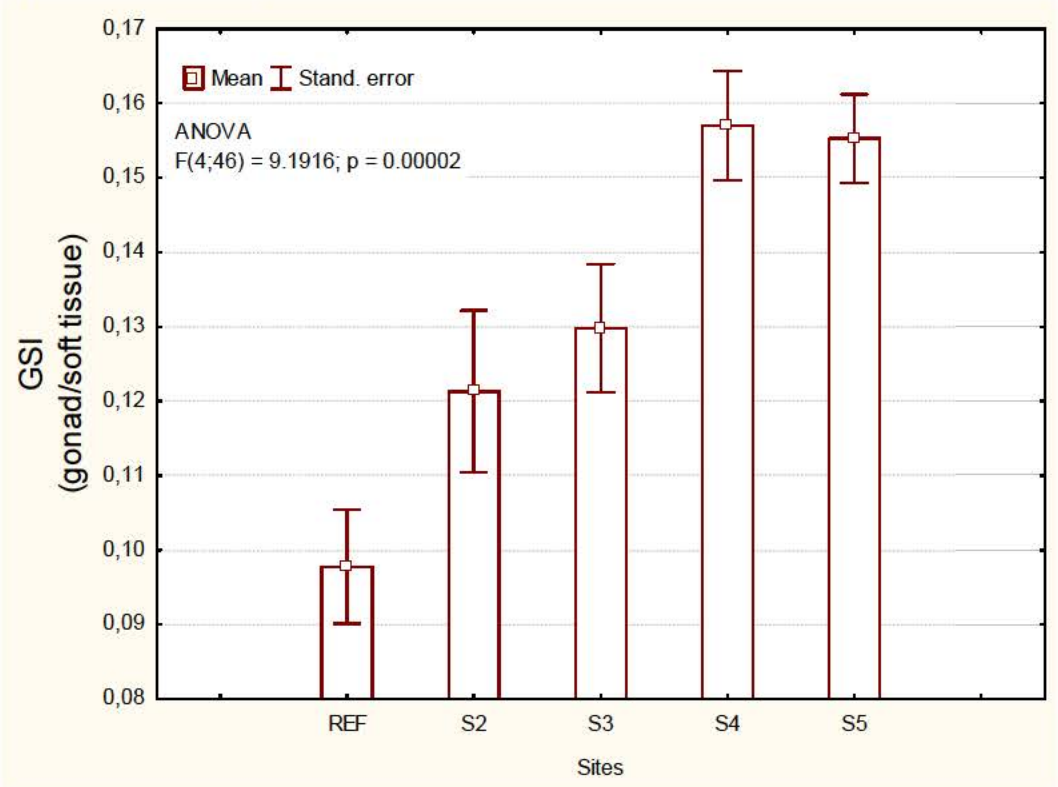
1) Data were screened for aberrant/extreme values and compared with a reference material to eliminate any methodological variations;
2) Data were analyzed using a 2-way ANOVA where the main effects were sites and gender. Sexing gave three groupings : Indeterminate, Males and Females.

1) Condition factor



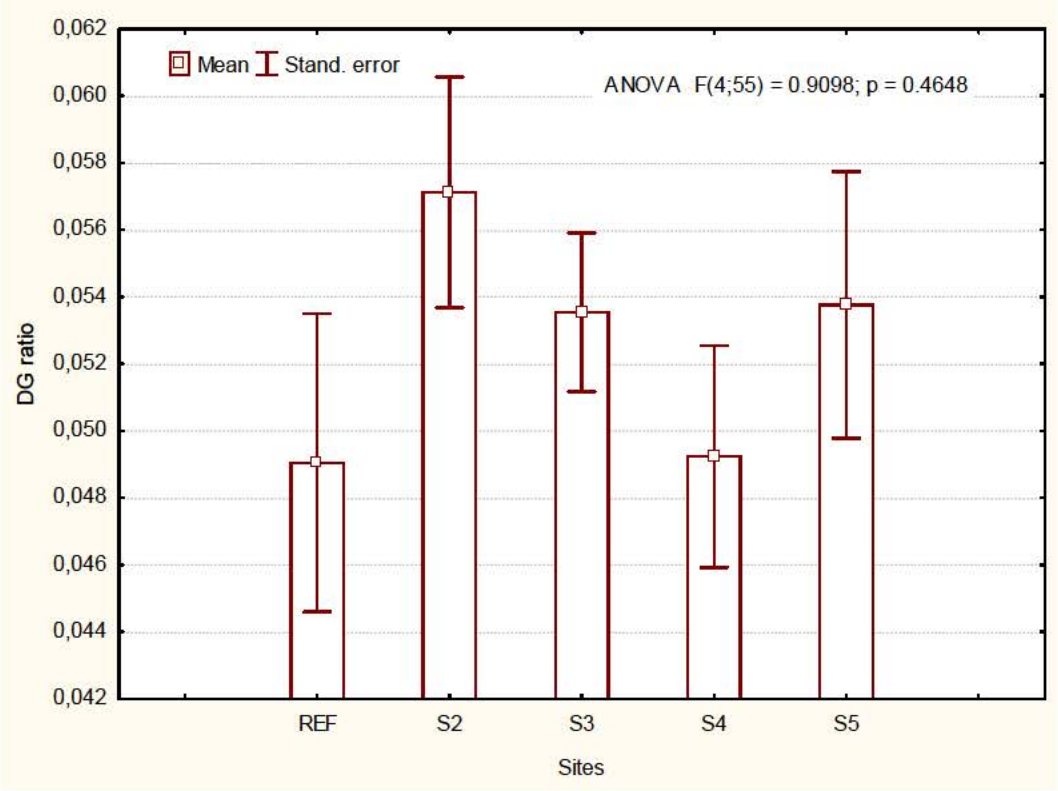
No significant change was observed with the condition factor for either sites or gender. However, sites S3, S4 and S5 appeared somewhat higher than REF and S2.

2) Gonado-somatic index



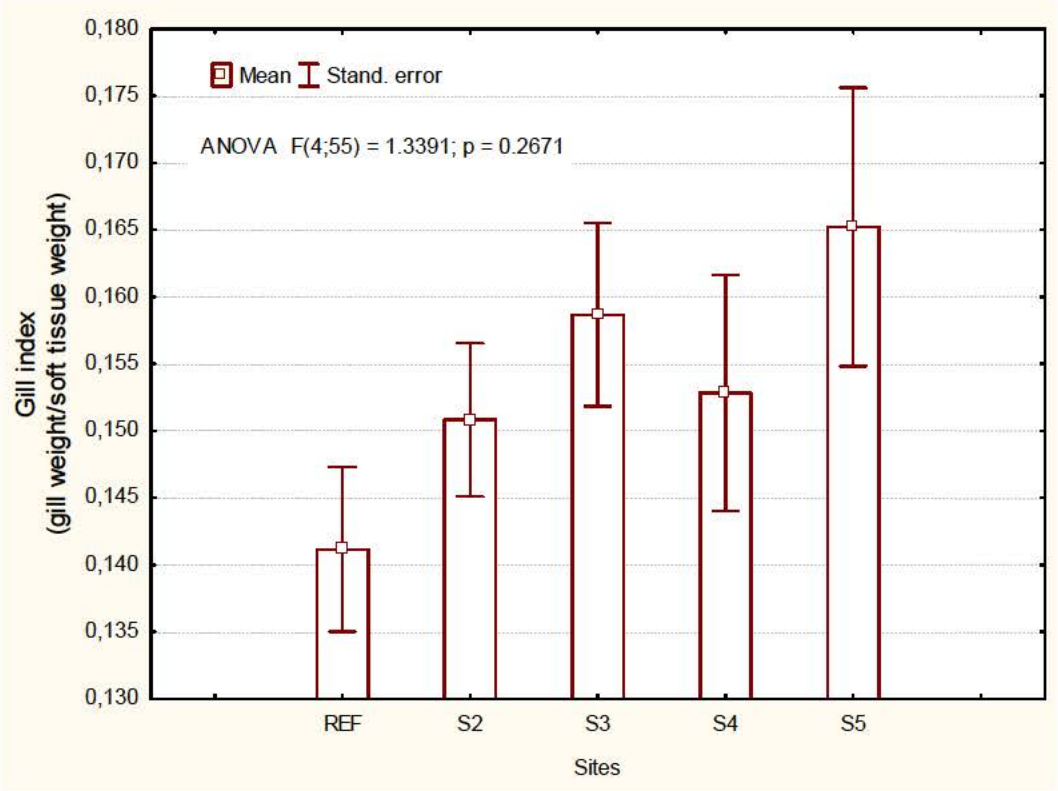
The gonado-somatic index was significantly increased at all sites in respect to the reference site. Sites S4 and S5 have the strongest responses. Along with vitellogenin-like proteins (see below), this could indicate the presence of estrogenic compounds. 2-way ANOVA revealed that gender had no effects but the sites were significant.

3) Digestive gland index

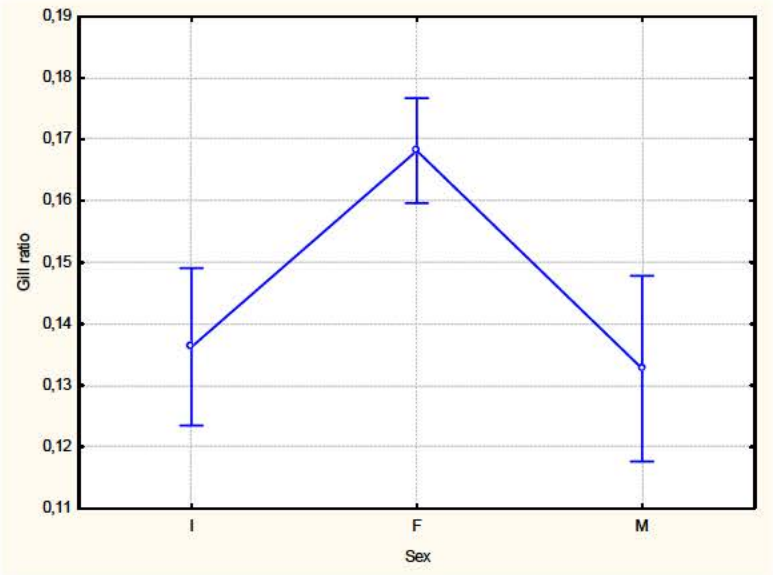


2-Way ANOVA did not reveal any significant effects for either sites or gender.

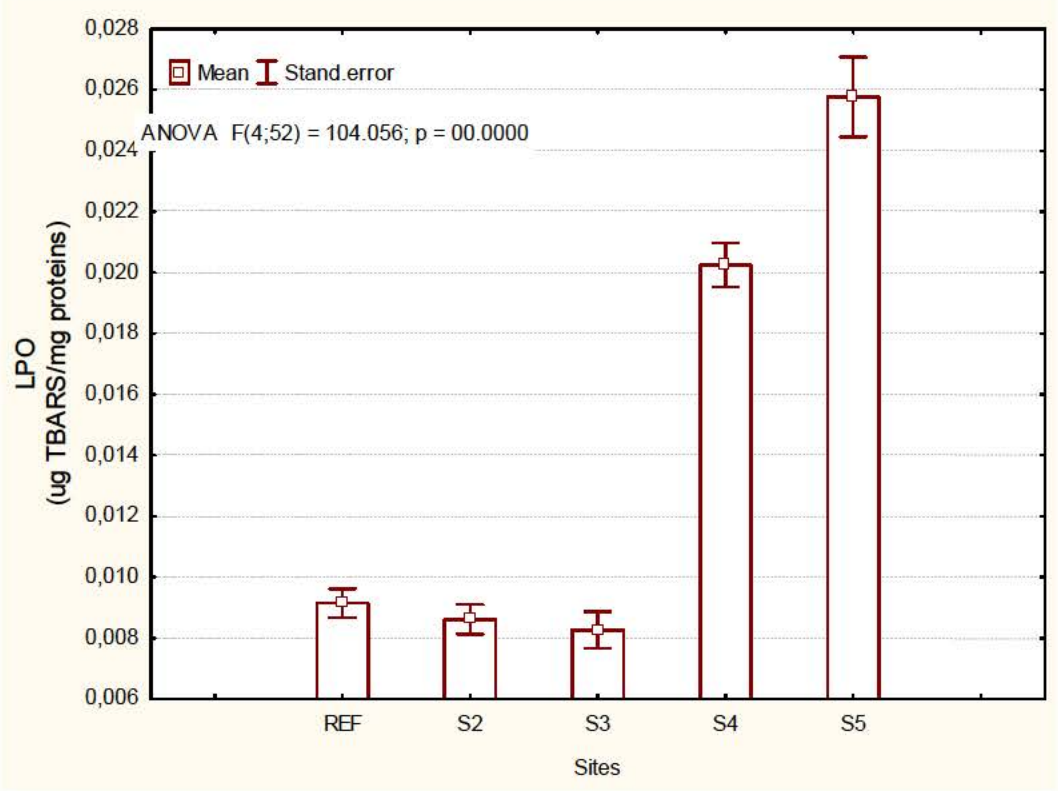
4) Gill index



2-Way ANOVA revealed a positive effects for gender only. The gill index was higher in females:

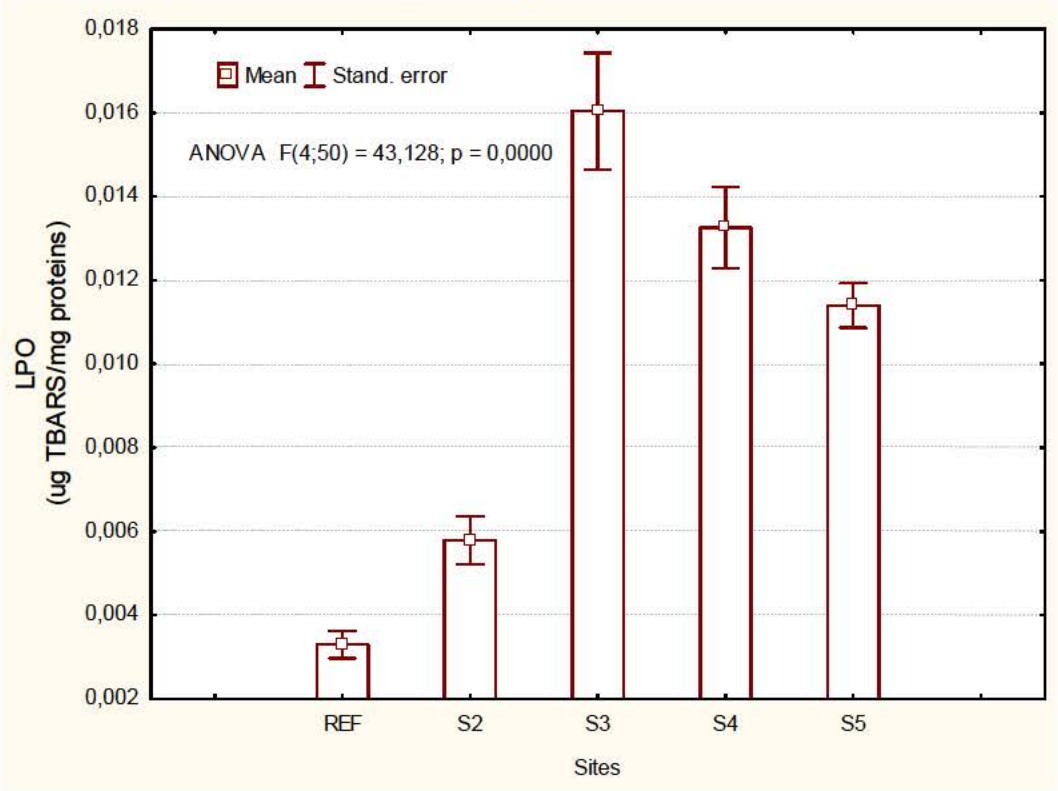


5) Lipid peroxidation in digestive gland



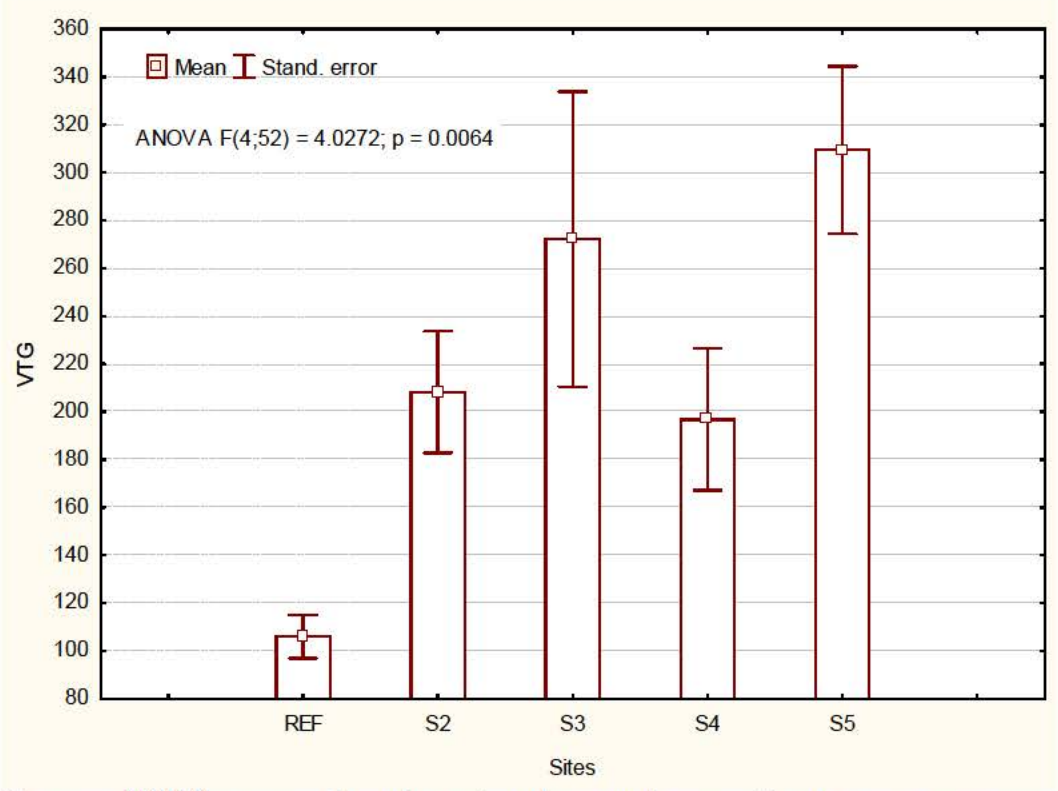
2-way ANOVA reveals only a significant effect for sites (no effects for gender). Sites S4 and S5 were highly affected in respect to others. Toxicity is occurring at these sites perhaps through suspended particles.

5) Lipid peroxidation in gills



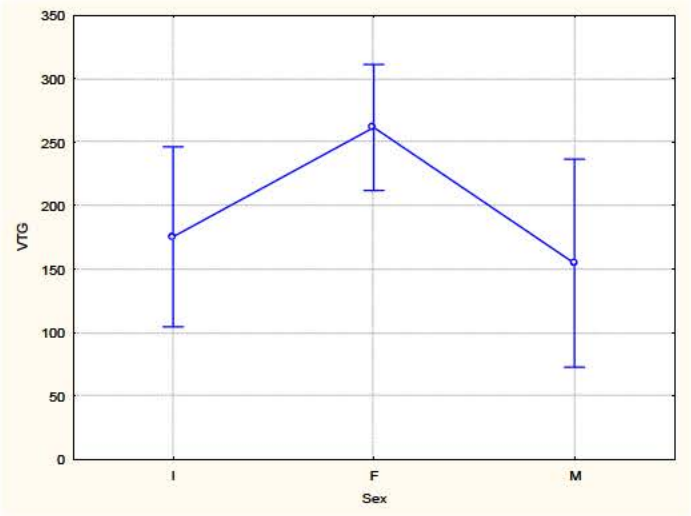
2-way ANOVA reveals only a significant effect for sites (no effects for gender). Sites S3, S4 and S5 were highly affected in respect to others. Toxicity is occurring at these sites perhaps through low molecular weight suspended solids (colloids) or dissolved chemicals.

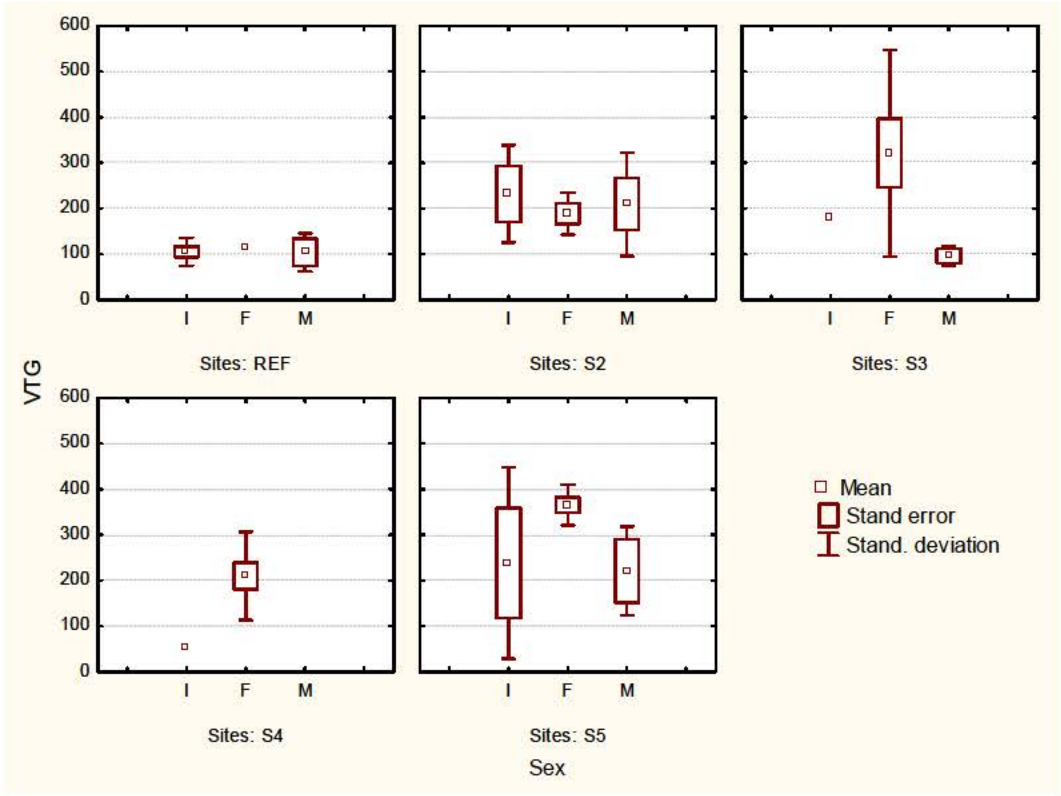
6) Vitellogenin-like proteins



2-way ANOVA reveals that both gender and sites were significant. All sites had increased VTG-like proteins in respect to the reference site. The GSI was not significantly correlated with Vtg-like proteins however.

As expected, females had more VTG-like proteins that males and the indeterminate sex.





We observe that females respond more than males at the sites examined. The indifferentiated sex is stable thoruoughout the study sites with perhaps a slight increase at site S5. Based on the responses obtained for the indeterminate sex group, this group appears closer to males than females.

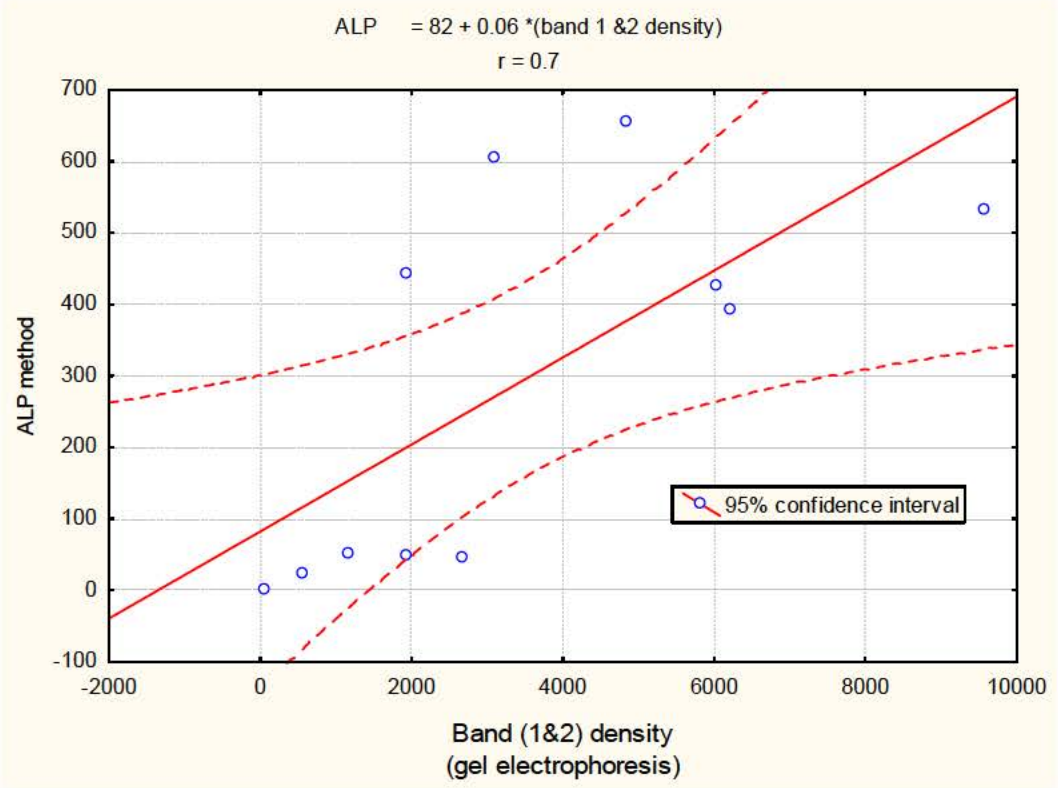
7) Correlation analysis

Correlations (Data Ed Friedman.sta) Significant at p < 0.05

	CF	GSI	DG ratio	Gill ratio	LPO DG	LPO gill	VTG
CF	1,0000	-,0642	**-,2646**	-,0521	,0596	,1689	,1468
	N=60	N=51	**N=60**	N=60	N=57	N=55	N=57
	p= ---	p=,654	**p=,041**	p=,693	p=,660	p=,218	p=,276
GSI	-,0642	1,0000	-,0701	-,0802	**,5685**	**,4722**	,1407
	N=51	N=51	N=51	N=51	**N=48**	**N=46**	N=48
	p=,654	p= ---	p=,625	p=,576	**p=,000**	**p=,001**	p=,340
DG ratio	**-,2646**	-,0701	1,0000	**,3957**	-,0528	,0356	,1783
	N=60	N=51	N=60	**N=60**	N=57	N=55	N=57
	p=,041	p=,625	p= ---	**p=,002**	p=,696	p=,797	p=,185
Gill ratio	-,0521	-,0802	**,3957**	1,0000	,0444	**,2944**	**,4673**
	N=60	N=51	**N=60**	N=60	N=57	**N=55**	**N=57**
	p=,693	p=,576	**p=,002**	p= ---	p=,743	**p=,029**	**p=,000**
LPO DG	,0596	**,5685**	-,0528	,0444	1,0000	**,2723**	***,2382***
	N=57	**N=48**	N=57	N=57	N=57	**N=52**	***N=54***
	p=,660	**p=,000**	p=,696	p=,743	p= ---	**p=,051**	***p=,083***
LPO gill	,1689	**,4722**	,0356	**,2944**	**,2723**	1,0000	**,3148**
	N=55	**N=46**	N=55	**N=55**	**N=52**	N=55	**N=52**
	p=,218	**p=,001**	p=,797	**p=,029**	**p=,051**	p= ---	**p=,023**
VTG	,1468	,1407	,1783	**,4673**	***,2382***	**,3148**	1,0000
	N=57	N=48	N=57	**N=57**	***N=54***	**N=52**	N=57
	p=,276	p=,340	p=,185	**p=,000**	***p=,083***	**p=,023**	p= ---

The biomarkers of damage (lipid peroxidation) are significantly related with
GSI and Vtg-like proteins suggesting that increased Vtg by oestrogens leads to oxidative stress (it is damaging to the mussels). Interestingly, Vtg-like proteins were also positively linked to the gill weight index (increased respiration to support vitellogenin production?)

The following graph shows the linear and significant ($p<0.05$) relation between the indirect alkali-labile phosphate assay and the appearance of high molecular weight bands (ressembling those of rainbow trout lipovitellin and phosvitin). This validates the assay that the indirect method is linked to vitellogenin-like protein bands.



MODULE 4 SPECIAL STUDIES

Monitoring Pesticide and PCB Exposure in Birds Found Dead or Presenting to Rehabilitators

Report to Maine DEP SWAT Program

Prepared by

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December 2005

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Executive Summary

In Maine, little study of pesticides in birds has been conducted, despite past evidence of organochlorine burdens in marine life and current use of highly toxic pesticides in agricultural settings. Wildlife organizations, rehabilitators and state agencies frequently receive calls reporting dead or injured birds. This provides an opportunity to conveniently and economically sample a diversity of species from a broad geographic range. Further, birds that are injured or otherwise debilitated may be more likely than healthy birds to have underlying contaminant burdens that predisposed them to injury or disease.

In this study, 135 dead birds were collected between July 2004 and July 2005. Fifty-four of these were tested for exposure to organophosphates and carbamates, 28 were tested for organochlorine pesticides, and 8 of these were additionally tested for PCBs. There was no evidence of organophosphate or carbamate exposure in the birds of this study. More focused testing of birds in habitats downstream of agricultural lands will be required to assess the importance of these pesticides to birds.

DDE was the highest OC found in the present study. Overall, while OC levels in the birds of this study are below sublethal ranges, values are elevated in some individuals. Two individuals (double-crested cormorant and hooded merganser) had DDE levels within the range of those associated with egg-shell thinning in double-crested cormorants. In addition, levels are similar to those found to impair immune response in herring gulls and glaucous gulls. Immune effects, even if small, may contribute to the entire suite of stressors that negatively affect populations.

Future study focused on these species could look at biomarkers of immune response relative to OC loads. Such a study could not only provide information on OC effects, but also be used as a screen to identify populations that might be exposed to other immune-modulating contaminants, such as polybrominated diphenyl ether (PBDE), methylmercury, polycyclic aromatic hydrocarbons (PAH), and some pharmaceuticals.

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1. Introduction

Birds play an important role as sentinel species for contaminants in the environment, and are extremely sensitive indicators for certain classes of pesticides, namely the organochlorines (OC) and cholinesterase-inhibiting organophosphates (OPs) and carbamates (CBs). The most notorious of the organochlorine pestic ides is DDT which with its metabolites, is well known for its effects on reproduction and egg-shell thinning. Thresholds for OCs and toxic effects have been established in many species for determination of lethality and reproductive effects, and serial monitoring of OC levels in individual ecosystems can be used to monitor ongoing OC burden and assess population risks. Lethal and reproductive effects are associated with high body burdens of OCs, however recent study has drawn attention to immune effects of lower levels of OCs in birds, with potentially significant implications on health (Fowles 1997).

A second class of pesticides that is highly toxic to birds is the cholinesterase inhibiting organophosphates (OP) and carbamates (CB). Widespread use of OP and CB insecticides has been implicated in the morbidity and mortality of numerous species of birds, which are highly sensitive to toxicity from these pesticides. In Maine, OPs and CBs are used heavily in apple and blueberry agriculture, and sufficient evidence exists to suspect agricultural OP/CB use has measurable impacts on birds in Maine. Recent study by Mineau (*in review*) ranks apple and blueberry agriculture 5th and 24th, respectively, in terms of the lethal risk to birds. This ranking system accounts for relative acreage devoted to each crop on a national scale, thus Maine's risk from blueberries is increased relative to other states. According to Mineau, risk posed by blueberry farming is growing due to increasing intensity of treatment and use of more highly toxic OPs such as phosmet, diazinon and azinphos-methyl. Avian impact from OPs has been detected in birds in habitats downstream from agricultural lands, and sublethal exposure can cause debilitation and disease. In black-crowned night herons, normally beneficial nest beetles feed opportunistically on live tissue of chicks made lethargic by pesticide exposure (Parsons et al. 2000).

2. Survey Purpose

In Maine, little study of pesticides in birds has been conducted, despite past evidence of organochlorine burdens in marine life and current use of highly toxic pesticides in agricultural settings. The project reported upon herein represents a survey of pesticide exposure among injured or found-dead birds brought to rehabilitators. Use of injured and diseased birds allowed for economic sampling of a diversity of species and geographic locales for the purpose of identifying populations potentially at risk. Further, we suspected that birds that are injured or otherwise debilitated might be more likely to have elevated contaminant burdens that could have increased their susceptibility to trauma or disease.

Objectives of this survey were to:

1. Identify avian populations at risk for pesticide impacts by establishing baseline indices for multiple species across a diverse geographic range, and

2. Assess the utility of avian incident monitoring for detecting poisoning events associated with OP/CB pesticides.

3. Methods

Sample collection A total of 135 dead birds were collected between July 2004 and July 2005 from various locations via collaborating entities serving as freezer depot sites. Birds were either found dead by the public and brought to one of these freezer depots pending collection, or else died or were euthanized at a wildlife rehabilitation center and were submitted for the study. Birds were either necropsied before freezing at -30°C or frozen immediately and decapitated and thawed (with heads left in freezer storage) for necropsy. All birds were necropsied and gross changes recorded. Cause of death was established based on a combination of clinical history and post-mortem findings. Location by town, and in sometimes street address, where the bird was found is known for 94 of these.

Cholinesterase assays Heads were moved to -70°C within a month of initial freezing, for storage pending cholinesterase analysis. Heads were shipped on dry ice to the testing lab. Brain cholinesterase was assayed using a modified Ellman technique, with 2-PAM reactivation in approximately 30% of samples (Ellman et al. 1961, Gard and Hooper 1993).

Organochlorine Scan During necropsy, livers were collected in either Teflon sheets or chemical free jars (I-Chem, division of Nalge, New Castle, Delaware 19720) and stored at -20°C pending analysis. Samples were tested in two batches. The first batch was sent to Mississippi State Chemical Laboratory where samples were assayed for organochlorines and total PCB. This scan included HCB, alpha BHC, gamma BHC, beta BHC, delta BHC, Oxychlordane, Heptachlor Epoxide, gamma Chlordane, trans-Nonachlor, Toxaphene, Aroclor – 1242, Aroclor – 1248, Aroclor – 1254, Aroclor – 1260, Total PCBs as Aroclors, o,p'-DDE, alpha Chlordane, p,p'-DDE, Dieldrin, o,p'-DDD, Endrin, cis-Nonachlor, o,p'-DDT, p,p'-DDD, p,p'-DDT, and Mirex. The second batch of samples was sent to the University of Pennsylvania New Bolton Center. There samples were assayed for organochlorine s only. The scan included alpha BHC, Lindane, beta BHC, Heptachlor, Aldrin, Oxychlordane, Heptachlor epoxide, alpha Chlordane, p,p'-DDE, Dieldrin, Endrin, p,p'-DDD, p,p'-DDT, and Methoxychlor. Summary statistics (means and ranges) were determined with non-detect values substituted with one half of the detection limit (Stout and Trust 2002). All results except those for some great blue herons, double-crested cormorants and an osprey had minimum detection limit of 0.020ug/gm (ppm). Samples from six great blue herons, two double-crested cormorants and one osprey were sent to a different lab, where minimum detection limit was 0.010ug/gm.

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4. Results

Primary cause of death or injury (leading to euthanasia) was assigned as unknown trauma (n=47), fishing gear (n=4), trash obstruction (n=2), power line (n=3), collision with vehicle (n=8), shot (n=5), other foreign body (n=2), attack by children (n=1), and other (including undetermined; n=63).

4.1.Cholinesterase

Fifty-four water birds were tested for inhabitation of brain cholinesterase. Significant species differences exist for normal cholinesterase levels and normal reference values have been published for only five species we tested. These are double-crested cormorant, black duck, mallard, herring gull, and ring-billed gull. For other species we established normals by calculating means and looking for values falling greater than 40% below the mean.

4.2. Organic Compounds

Twenty-eight birds were screened for organochlorine pesticide residues (Table 1). These birds were sampled from diverse locations across Maine (Figure 1). DDE was the most commonly occurring OC and was detected in 24 birds. DDE values ranged from undetectable to 3.45ug/gm wet wt., and PCB values ranged from 0.077-2.6ug/gm wet wt. DDE was analyzed by ANOVA for relationship to body condition, sex and age. Birds were scored 1-3 based on pectoral mass, with 1 being emaciated, 2 being moderately thin and 3 being normal. PCB but not DDE was negatively associated with body score, with significantly higher levels in birds with a body score of 1 than 3 ($p=0.04$). There was no difference in PCB or DDE levels between sexes or between first year and older birds. However, first year birds had among the highest OC levels, with the two highest PCB levels found in juvenile great blue herons.

The three highest DDE leve ls were found in a juvenile double-crested cormorant in South Portland (5/27/05), an adult hooded merganser in Nobleboro (6/16/05), and a hatchling osprey in Nobleboro (6/21/05). The three highest PCB levels were two juvenile great blue herons found in Boothbay Harbor and Greene in 9/29/04 and 10/11/04, respectively, and a juvenile double-crested cormorant found in Portland in November 2004.

There was no relationship between DDE or PCB levels in relation to body score. There was no evidence that species feeding lower on the trophic scale (e.g. mallard and wood duck) had lower contaminant burdens, though the numbers were very small.

5. Discussion

DDE was the highest OC found in the present study. Of the DDT metabolites, this is one of the most toxic and extensively studied (Blus, 1996). Overall, while OC levels in the birds of this study are below sublethal ranges, values are elevated in some individuals. Several individuals had DDE levels within the range of those associated with egg-shell thinning in double-crested cormorants (Custer et al. 2000). In addition, levels are within the range of those found to impair immune response. DDE levels in herring gull eggs between 0.6 to 7.4 ug/gm are positively associated with heterophil and lymphocyte counts (Grasman et al. 1996, 2000), as are blood DDE levels in glaucous gulls averaging 0.062 ug/gm (Bustnes et al. 2004). PCBs are also known to influence a variety of immune responses (Fowles et al. 1997). In the present study, two individuals had liver PCB levels (2.1ug/gm, 2.6ug/gm) that approach the level (3.5 ug/gm) shown to be associated with elevated heterophils and lymphocytes in glaucous gulls (Bustnes et al. 2004).

Species-specific comparisons with previously published studies of herring gulls and double-crested cormorants show overlapping levels of PCBs and DDE levels. Herring gulls collected in 1966 from Lincoln, Sagadahoc, and Washington counties by Wiemeyer et al. (1978) had DDE and PCBs concentrations ranging from 1.7-5.9ug/gm and 2.8-17ug/gm, respectively. Herring gulls collected from the Bay of Fundy in the Gulf of Maine area had mean liver concentrations of DDE of 2.08ug/gm and liver PCB concentrations of 6.50ug/gm (Zitko and Choi 1972; Zitko and Hutzinger 1972).

Double-crested cormorants collected from the Bay of Fundy in the Gulf of Maine area had mean liver DDE of 4.16ug/gm and liver PCB of 2.13ug/gm (Zitko and Choi 1972; Zitko and Hutzinger 1972). Double-crested cormorants collected from Muscongus Bay in 1966, contained tissue concentrations of DDE ranging from 1.5ug/gm in the brain to 6.5ug/gm in gonads (Kury 1969). In 1967 Kury (1969) found mean DDE concentrations of 0.34ug/gm in brain. In that study, researchers concluded exposure to the pesticides occurred in some other area, possibly its wintering grounds in Florida. In the present study, juvenile birds are among those with the highest contaminant levels, suggesting exposure is significant in Maine.

There was no evidence of OP/CB exposure in the birds of this study. Behavioral effects from these compounds can be seen at sublethal doses, and typically these are associated with 40% or greater reduction of enzymatic activity and 2-PAM reactivation (Walker 2003). These levels were not seen in the present study, however our findings should not be taken to assume no exposure in Maine. At the same time, it can be concluded that avian incident monitoring is not a sensitive tool for detecting effects from this class of pesticides. More focused testing of birds in habitats downstream of agricultural lands will be required to assess the importance of these pesticides.

OC levels in this study were potentially high enough in several individuals to cause immune effects, which, even if small, may contribute to the entire suite of stressors that negatively affect populations. Future study focused on these species in which values were elevated could look at biomarkers of immune response relative to OC loads. Such a study could not only provide information on OC effects, but also be used to identify populations that might be exposed to other immune-modulating contaminants, such as polybrominated diphenyl ether (PBDE), methylmercury, polycyclic aromatic hydrocarbons (PAH), and some pharmaceuticals.

Great blue herons and double-crested cormorants were among those with elevated contaminant levels, which is not unsurprising given their location high on the food web. Previous study has shown higher OC exposure among great blue herons and double-crested cormorants in estuarine vs. non-estuarine habitats, presumably due to greater bioaccumulation rates due to particulate deposition from watershed and higher biomagnification rates due to species differences at lower trophic levels, as well as past agricultural use (Harris et al. 2003). For this reason, estuarine populations should be targeted to maximize detection of bioaccumulative compounds.

There are several limitations to the present study. First, comparisons based on location, species and sex are difficult due to small sample size, and pooling data among species prevents statistical determination of inter- or intraspecific variability of contamination levels. Additionally, some of the study birds were thin, causing artifact of at least elevated PCB levels. PCB and DDE concentrations are influenced by body condition, with higher concentrations found in birds with low fat scores (Wienburg and Shore 2004). It is likely DDE levels in thin birds were also elevated as artifact this study, though this was not

statistically significant. Bustnes (2003) found in glaucous gulls changes in body condition and amount of blood lipids were of lesser importance than trophic level. Finally, birds were collected in different seasons, so that while season is unlikely to significantly affect contaminant levels, increased movement of birds in late summer and early fall prevents locating source of exposure.

6. Summary

Overall, while OC levels in the birds of this study are below lethal ranges, values are elevated in individuals of some species (double crested cormorant, great blue heron, hooded merganser, and osprey). Further study of those species is warranted to detect immune effects of documented organochlorines, as well as to screen for other immunomodulating contaminants of concern in Maine. To measure impact of agricultural OP/CB use, more focused study of birds inhabiting waters adjacent to agricultural lands is recommended.

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Figure 1. Origin of birds screened for organochlorines.

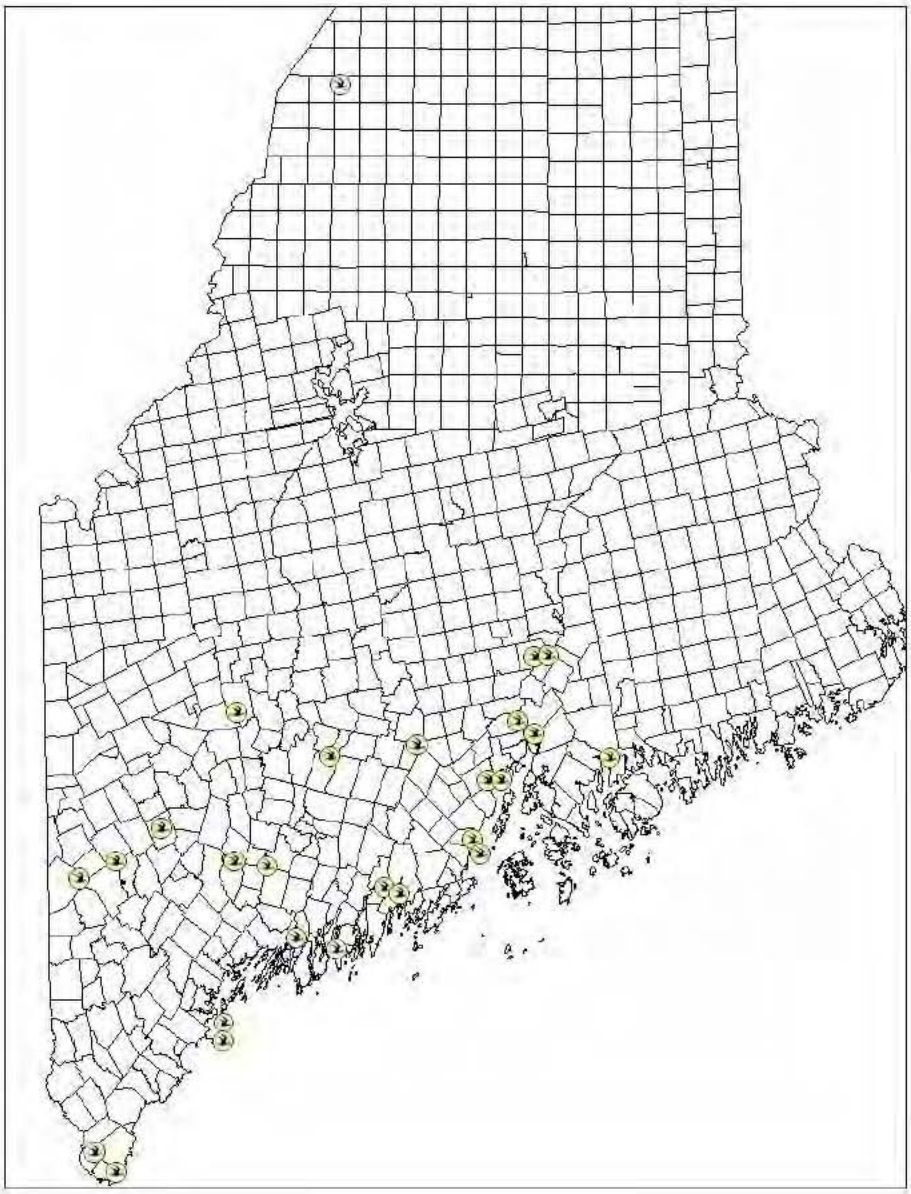

Table 1. Hepatic OC concentrations (mean (range), ug/gm wet wt). N/A=not assayed . Values preceded by "<" are below the method detection limit.

	uck		led	n	n	ser
Bs						
ne						
ordane		0.039)				
		.435)			.633)	
hlor epoxide	=1	=2	=1	=1	=3	=1

		ue heron	crested cormoran	illed murre	gull
Bs	1	077-2.6) n=6	63-1.4) n=2		
	<0.010-0.024) n=3	0.010-0.010) n=6	=4	=1	=4
lordane	N=3	=6	=4	=1	=4
ordane	N=3	0.010-0.027) n=6	0.010-0.014) n=4	=1	=4
	0.023-1.450) n=3	012-0.690) n=6	110-2.161) n=4	1	052-0.776) n=4
	N=3	=6	=4	=1	=4
hlor epoxide	N=3	0.010-0.012) n=6	=4	=1	=4

*includes nestling

4.2 PCB LABORATORY COMPARISONS

The Maine Center for Disease Control (MCDC, formerly Maine Bureau of Health) issued several fish consumption advisories for various rivers in Maine and for striped bass and bluefish in general based wholly or partially on high levels of PCBs found from the SWAT program. Over the years samples have been analyzed by several different labs, often with different results, although each had a quality assurance (QA) plan and met the requirements. To determine if the current lab used by DEP, Pace Analytical Services (PAS) was providing credible PCB data, 5 samples were split and sent to PAS and the Geochemical and Environmental Research Group lab at Texas A&M University (TAMU), a lab approved for use for these analyses by the US Fish and Wildlife Service. The samples were to be analyzed by high resolution gas chromatography mass spectrometry (HR GC/MS).

Equipment issues at TAMU delayed HR analyses, and in the interim, preliminary low resolution (LR GC/MS) results were delivered to DEP. The results were biased low compared to the PAS results, but within the 30% relative percent difference (RPD) allowed by the QA plan (Table 4.2.1). HR results were biased high but much closer to the PAS results than were the LR results and well within the RPD required. These results indicate that the results from PAS are likely reasonably accurate.

Table 4.2.1. 2004 PCBs in striped bass and bluefish from Maine rivers

STATION	SPECIES	PAS PCB ppt	TAMU PCB LR ppt	RPD %	PAS PCB ppt	TAMU PCB HR	RPD %
Androscoggin R at Brunswick	STB	201			201		
	ARB-STB-03	226	183	21.0	226	243	-7.2
Kennebec R at Augusta	STB	170			170		
	KAG-STB-02	208	151	31.8	208	226	-8.3
Penobscot R at Orrington	STB	211			211		
	PBO-STB-04	201	140	35.8	201	215	-6.7
Royal R at Yarmouth	STB	152			152		
York R at York	STB	147			147		
	YRY-STB-05	104	84.5	20.7	104	123	-16.7
Old Orchard Beach	BLF	161			161		
	OOB-BLF-01	154	109	34.2	154	174	-12.2
AVE				28.7			-10.2

PCB LR= low resolution GC/MS
PCB HR= high resolution GC/MS