



Surface Water Ambient Toxic Monitoring Program

2007 Report

Maine Department of Environmental Protection
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INTRODUCTION

This 2007 Surface Water Ambient Toxic (SWAT) monitoring program final report is organized into an Executive Summary (with introduction and table of contents) and three modules:

1. Marine & Estuarine
2. Lakes, and
3. Rivers & Streams.

The full report is available on the Maine Department of Environmental Protection's (DEP's) website at www.maine.gov/dep/blwq/docmonitoring/swat/index.htm

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Chemical analyses were performed by AXYS Analytical Services, Sidney, British Columbia or other laboratories as listed in reports in individual sections.

EXECUTIVE SUMMARY

Maine's Surface Water Ambient Toxics (SWAT) monitoring program was established by the Maine Legislature in 1993 (see 38 MRS §420-B) to determine the nature, scope and severity of toxic contamination in the surface waters and fisheries of the state of Maine. The authorizing statute states that 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 Maine Department of Environmental Protection (DEP) must prepare a 5-year conceptual workplan in addition to annual workplans which are each reviewed by the SWAT Technical Advisory Group (TAG) comprised of ten individuals with scientific backgrounds representing various interests plus two legislators.

The 2007 SWAT program is divided into 3 modules, 1) Marine and Estuarine, 2) Lakes, and 3) Rivers and Streams. This annual report follows the outline of the 2007 workplan recommended by the SWAT TAG in a meeting held June 27, 2007.

Following is a summary of key findings from the 2007 SWAT program for each module.

1. MARINE AND ESTUARINE

- From a two-year cooperative study funded by the federal Environmental Protection Agency (EPA) and SWAT, contaminants in lobster along the Maine coast including mercury, cadmium, and lead levels in lobster tomalley were examined. Elevated mercury levels in tomalley appear to persist in Penobscot Bay. Lead levels in tomalley appear to be decreasing.
- In a study of contaminants in birds along the Maine coast, mercury and chemicals used as flame-retardants, industrial repellents, transformer coolants (now banned) and pesticides were found in all 23 species studied. Many of the concentrations found were above those documented to have adverse effects. Common loon, Atlantic puffin, piping plover, belted kingfisher, great black-backed gull, peregrine falcon, and bald eagle had the highest contaminant levels. Overall, eagles carried the greatest load and, for many compounds, had levels multiple times higher than other species. However, contaminants banned 30 years ago, DDT and PCBs, are decreasing in the environment.

2. LAKES

- Samples of trout collected for mercury analysis for the Maine Center for Disease Control and Prevention (MCDC) to use in review of Maine's statewide fish consumption advisory were not analyzed due to a subsequent budget reduction per the Governor's rescission order to curtail state spending to meet the budget shortfall.

3. RIVERS AND STREAMS

- Forty stations, primarily in the Kennebec River basin, were assessed for the condition of the benthic macroinvertebrate community. Results have been received for 36 station and of those, 8 (22%) have failed to attain the aquatic life standards of their assigned class.
- Dioxins and coplanar PCBs analyzed under Maine's Dioxin Monitoring Program and SWAT program continue to show reductions in some rivers, but exceed the MCDC's new fish tissue action level (FTAL) of 0.4 ppb in others. MCDC expects to revise the fish consumption advisories this year.
- Samples of rainbow smelt and smallmouth bass from the Kennebec River at Hallowell, Gardiner, and Richmond all exceeded the MCDC's FTAL for PCBs for setting fish consumption advisories. Concentrations in brown trout at Norridgewock marginally exceeded the FTAL. Samples of rainbow smelt from the Penobscot at Bucksport were lower than those from the Kennebec but still slightly above the FTAL.
- There is a fish consumption advisory for several Aroostook County rivers and streams issued by the MCDC because of residuals of DDT used decades ago. Samples of trout from several Aroostook County rivers and streams sampled in 2007 still exceeded MCDC's FTAL for DDT used for setting the advisories.
- A cumulative effects assessment (collectively looking at survival, growth, and reproduction) of a fish population in the Presumpscot River found some differences above and below the runoff and discharges from the city of Westbrook and SD Warren paper mill. Catch rates were much lower and there were few males captured below Westbrook. Additional study is needed to determine the significance and cause of these results.

1.0 MARINE MODULE

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1.1

SHELLFISH TISSUE AND SEDIMENT ANALYSIS

1.1 ASSESSMENT OF CONTAMINANT LEVELS IN LOBSTER TISSUE

(This study was funded by the DEP's Maine Inland and Coastal Surface Water Oil Clean-up Fund)

1.1.1 Introduction

This draft report contains data on American lobster (*Homarus americanus*) tissues collected in 2005 and 2006. Lobsters were collected as part of EPA's National Coastal Assessment (NCA), which also provides data on water column parameters, sediment chemistry, and benthic community structure. Through NCA, lobsters were collected in Maine rather than finfish, which are the usual target for analysis within most other states' waters.

Lobster was selected to provide information concerning the quality of the benthic environment and because Maine has a fish consumption advisory on lobster hepatopancreas (tomalley) tissue. As predators and scavengers of benthic infauna and detritus on the sea bottom, lobsters ingest toxic contaminants and bioaccumulate those contaminants in their body tissues. Lobsters are ubiquitous along the Maine coast, allowing collections to take place along the entire coast and facilitating geographic comparisons. The lobster fishery is Maine's premier fishery, with the highest landed value of any commercial fishery in the state. In addition, Maine lobstermen strive to provide a high quality product and determining and assuring the quality of this product is of importance to the future sustainability of the fishery. This project builds upon earlier work done by DEP in 1994-1996 on contaminants in lobster tissues at several locations along the Maine coast.

1.1.2 Methods

Because of the extensive, indented coastline and logistical concerns associated with sampling such a large area, the NCA sampling design divided the Maine coast into two segments. This design allowed collection of all parameters at up to 25 stations representing half the coast in each of two years, 2005 and 2006. Lobsters were collected on the northeastern half of the Maine coast in 2005 (14 stations) and the southwestern half of the Maine coast in 2006 (19 stations). All sampling stations were selected through a computer generated randomized sampling scheme, which NCA provided to allow each selected station to be used to characterize the segment of the coast from which it was chosen. Sampling occurred in late summer before lobsters migrated to deeper offshore waters. Up to seven lobsters per station were collected to provide adequate tissue (specifically the hepatopancreas) for achieving laboratory detection limits. Smaller, legal lobsters that just recruited to the fishery (the smallest lobsters that can be legally retained in the fishery, approximately 1 to 1.25 lbs.) were selected for inclusion in the composite samples at each station. Smaller lobsters are thought to have a smaller home range of perhaps less than 1-2 km. Using smaller animals in late summer would limit the integration of contaminants to a smaller area within that home range. Up to seven lobsters were purchased directly from the lobstermen, who agreed to provide lobsters from within the vicinity of the sampling station after the location was provided via use of a map or from station coordinates. Lobsters were individually triple wrapped in aluminum foil, labeled with station ID and individual lobster number, placed in a poly bag, and frozen live at -20°C.

DEP dissected lobsters into hepatopancreas, muscle, and offal tissues. Whenever possible, lobster samples were composites of seven individual animals, though some samples contained fewer lobsters. EPA, as part of the NCA program, analyzed lobster muscle tissue for: mercury, heavy metals, PAHs, pesticides, and PCBs. As part of the SWAT program, DEP provided additional analysis of lobster muscle tissue for: dioxins, furans, coplanar PCBs, and PBDEs. In addition, as part of the SWAT program, DEP analyzed lobster hepatopancreas for: mercury, other heavy metals, PAHs, pesticides, PCBs, dioxins/furans, coplanar PCBs, and PBDEs. This report includes data analysis for selected heavy metals and mercury in lobster hepatopancreas tissue from the 2005 and 2006 sampling along the Maine coast. Additional lobster data will be examined in future reports.

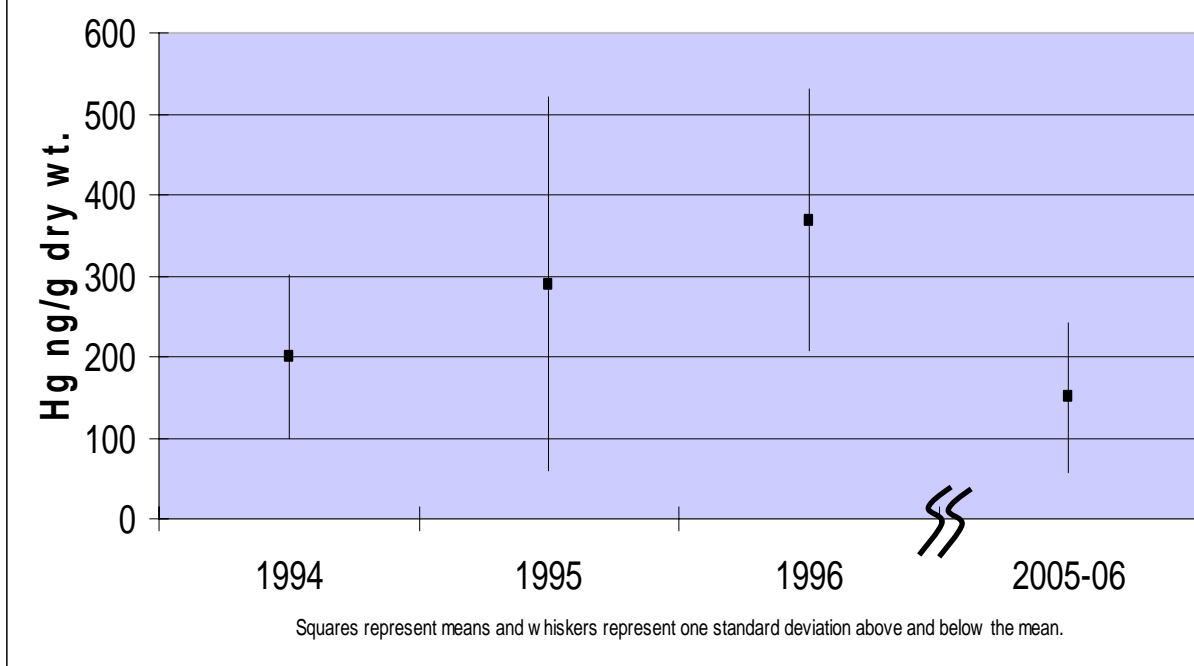
1.1.3 Results and Discussion

Consistent with the approach used in the examination of lobster tissue in the 1994-95 SWAT reports, variation within 3 standard deviations above the arithmetic mean concentration was considered part of natural variability. Assuming a normal distribution, 3 standard deviations would include 99.7% of concentration values, with those lying outside that range expected to occur less than 0.3% of the time. Means for 2005-06 metals concentrations were calculated from all samples collected across the Maine coast (n=33) in 2005-06, since no reference samples were collected and sampling design was randomized as part of the NCA sampling scheme. The 1994-95 mean was calculated from an assigned reference population collected in each of those years. Though the 2005-06 data set includes more sites, the 1994-96 work included 4 replicate samples at each site, allowing a measure of variance at each location sampled. Sites included in the current analysis were also composited samples but due to cost constraints, only one composited sample per station was collected and analyzed.

1.1.3.1 Mercury

As shown in Figure 1, the mean concentrations of mercury (Hg) in lobster tomalley (dry weight) sampled in 1994 through 1996 were 200 ng/g (1994), 290 ng/g (1995), and 369 ng/g (1996), although the 1996 values were calculated from the complete list of all sites sampled and are not a subset categorized as reference sites as had been done in 1994-95. The mean of mercury in lobster tomalley for all sites (n=33) sampled in the current data set (2005-06) is 150 ng/g dry wt. Several factors may influence the lower mean mercury concentrations observed in the recent data set. Concentrations from east of the Penobscot River are generally much lower than those to the southwest

Figure 1. Mean Lobster Tomalley Mercury Concentration

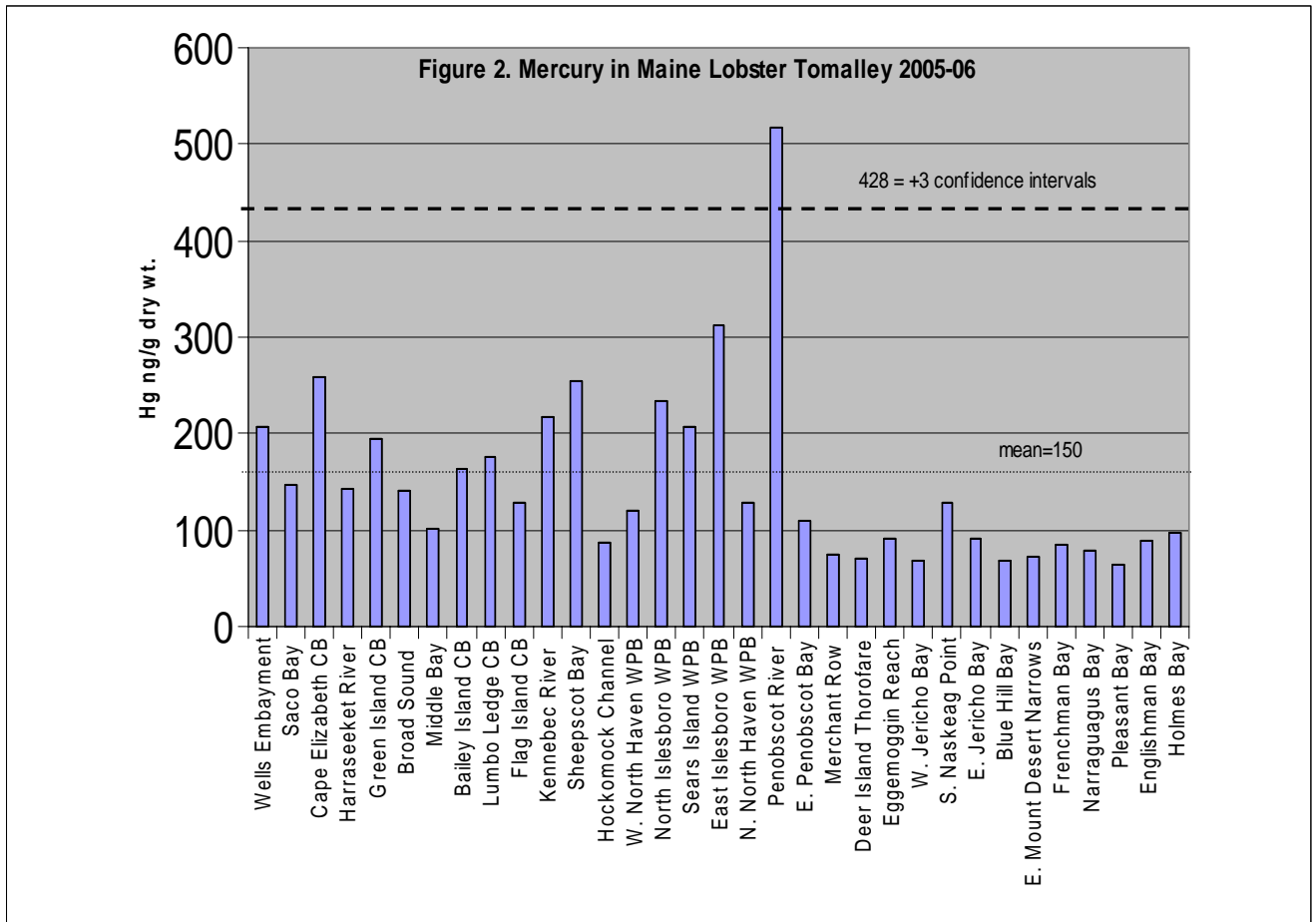


of the Penobscot. The addition of many sites from eastern Maine in the new randomized sampling approach depresses the mean. Two separate laboratories were utilized in 2005 and 2006 to analyze the samples, with the sites including the Penobscot and west of the Penobscot analyzed in 2006 and the sites east of the Penobscot analyzed in 2005. However, despite generally lower concentrations east of the Penobscot in 2005 compared to the remainder of the Maine coast, the limited amount of sampling previously conducted in 1994-96 east of the Penobscot also showed generally lower concentrations than more heavily sampled western areas of the coast. Brooklin, Corea, Machias, and St. Croix all had mercury concentrations in tomalley lower than the means for the years they were sampled (1994-1996 data). General range of mercury concentrations in tomalley east of the Penobscot falls at or just below the lowest scores west of the Penobscot.

Of the 33 sites sampled in 2005-06, only one site exceeds the hypothetical “natural” threshold of three standard deviations above the arithmetic mean concentration (see Figure 2). Mercury in lobster tomalley at the Penobscot River site near Verona exceeded this threshold (428 ng/g dry weight) with a result of 517 ng/g dry weight. This result represents one composited sample, while previous testing had averaged scores from four composites of five lobsters each at each station (1994-96 data). Several sites within upper Penobscot Bay area have had relatively high results for tomalley mercury in several rounds of testing since 1994. Castine (1994), Verona Island (1995) and Fort Point (1996) have all had scores between 400 and 600

ng/g dry weight mercury in tomalley. In combination, this suggests there may be an elevated level of mercury present in upper Penobscot Bay.

When the 2005-06 tomalley mercury levels (total mercury) are compared on a wet weight basis to Maine’s non-cancer action levels for methylmercury, the levels found in the tomalley samples are still below the more conservative action level for children and pregnant females (0.2 ppm wet weight). For comparison, the highest score coast-wide at Verona, Penobscot River, was 0.131 ppm (wet weight). Since fish are not capable of methylation of mercury, direct comparison between total mercury results and the methylmercury action level is possible. Results from lobster muscle tissue analysis should be reviewed when this data becomes available from EPA. Muscle tissue was analyzed by the contracted EPA laboratory while Maine DEP/SWAT provided analyses of the tomalley for the 2005-06 samples. Earlier SWAT reports suggest tomalley may contain half the amount of mercury on average as muscle tissue, so an examination of muscle tissue scores would be of interest from a human health perspective. Since the above tomalley score for Verona Island is approximately half of the non-cancer action level for methylmercury, it is possible that the forthcoming lobster muscle tissue results may fall near the Maine action level for mercury.



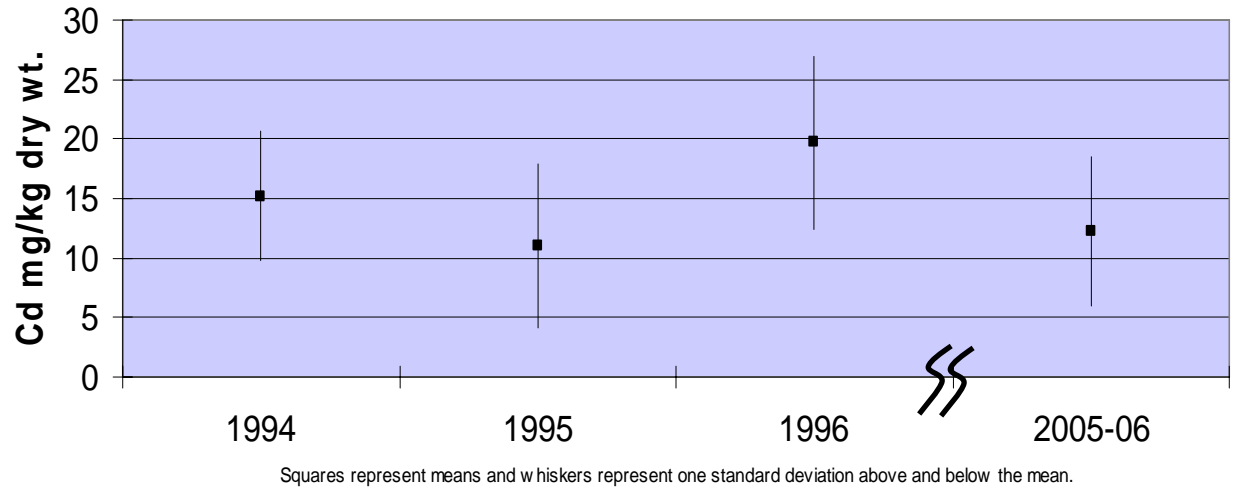
1.1.3.2 Cadmium

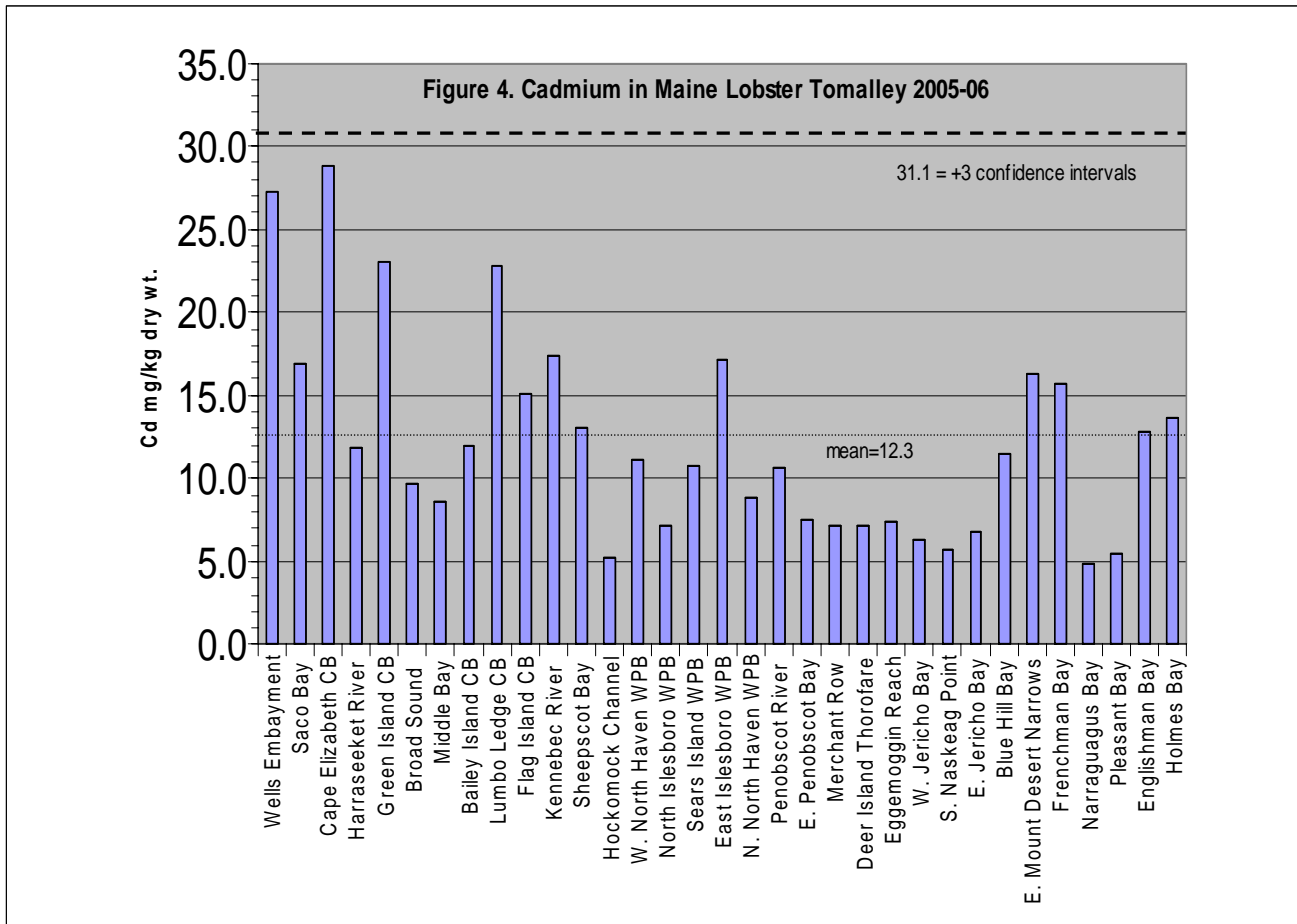
Mean concentrations of cadmium in lobster tomalley (dry weight) sampled in 1994 through 1996 were 15.2 mg/kg (1994), 11.1 mg/kg (1995), and 19.7 mg/kg (1996), although the 1996 values were calculated from the complete list of all sites sampled and not a subset categorized as reference sites as had been done in 1994-95 (Figure 3). The mean concentration of cadmium in lobster tomalley for all sites (n=33) sampled in the current data set (2005-06) is 12.3 mg/kg dry wt.

Of the 33 sites sampled in 2005-06, no site exceeded the hypothetical “natural” threshold for cadmium of three standard deviations above the arithmetic mean concentration (see Figure 4). Two sites within the 2005-06 data, Wells Embayment and Cape Elizabeth, had concentrations above 25 mg/kg dry wt. However, concentrations in tomalley over 25 mg/kg dry wt. were also observed in 1996 at Brave Boat Harbor and Corea.

When the 2005-06 cadmium levels are compared on a wet weight basis to Maine’s non-cancer action levels for cadmium, the levels found in the tomalley samples frequently exceed the 2.2 ppm action level. However, the action level is based on a reference dose of 1×10^{-3} mg/kg/day, which may be an unrealistically high dose when applied to lobster tomalley consumption. Results from lobster muscle tissue analysis should be reviewed when this data becomes available from EPA. Muscle tissue was analyzed by the contracted EPA laboratory while Maine DEP/SWAT provided analyses of the tomalley for the 2005-06 samples. Earlier SWAT reports suggest tomalley may contain 100 times the concentration of cadmium as lobster muscle tissue, so muscle tissue levels of cadmium may be quite low and would be expected to fall below the action level.

Figure 3. Mean Lobster Tomalley Cadmium Concentration





1.1.3.3 Lead

Mean concentrations of lead in lobster tomalley (dry weight) sampled in 1994 through 1996 were 0.66 mg/kg (1994), 0.75 mg/kg (1995), 1.02 mg/kg (1996), although the 1996 values were calculated from the complete list of all sites sampled and not a subset categorized as references sites as had been done in 1994-95 (Figure 5). The mean for lead in lobster tomalley for all sites (n=33) sampled in the current data set (2005-06) is 0.099 mg/kg dry wt. The mean from the newer data is nearly an order of magnitude lower than the previous means from the 1994-96 data sets. Four stations tested in 2005 resulted in non-detectable concentrations for lead, so half the minimum detection limit value was substituted to calculate the mean for the 05-06 data set. This depresses the mean somewhat. It should also be noted that most samples in the data set also had lead present in the laboratory blanks, but this should not have had a substantial impact on the data.

Of the 33 sites sampled in 2005-06, no site exceeded the hypothetical “natural” threshold for lead of three standard deviations above the arithmetic mean concentration (Figure 6). Two sites within the 2005-06 data., Wells Embayment and Cape Elizabeth, had concentrations above 0.18 mg/kg wet wt. However, concentrations in tomalley over 0.5 mg/kg wet wt. were the norm in the 1994-96 data, as reflected by the mean values discussed above. Further

investigation of existing lead data, in lobster muscle (when data is released by EPA) and in other matrices such as blue mussel and sediments, should be undertaken to determine if lead levels have in fact declined.

When the 2005-06 tomalley lead levels are compared on a wet weight basis to Maine's action level for lead, the levels found in the tomalley samples are an order of magnitude below the 0.6 ppm action level. For comparison, the highest score coast-wide at Wells was 0.050 ppm (wet weight). Results from lobster muscle tissue analysis should be reviewed when this data becomes available from EPA. Muscle tissue was analyzed by the contracted EPA laboratory while Maine DEP/SWAT provided analyses of the tomalley for the 2005-06 samples. Earlier SWAT reports suggest tomalley may contain a similar concentration of lead as lobster muscle tissue, so muscle tissue levels of lead may also be quite low if lead levels in muscle are still similar to tomalley levels.

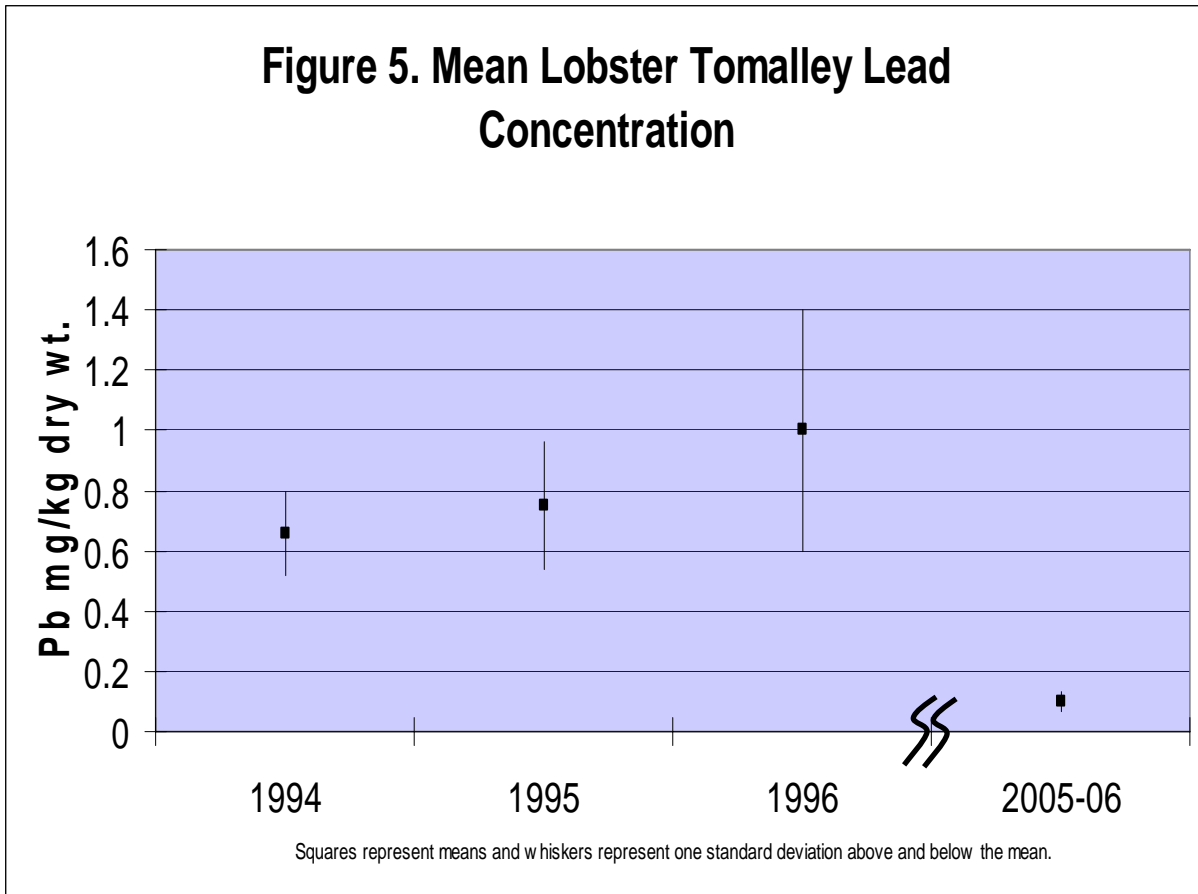
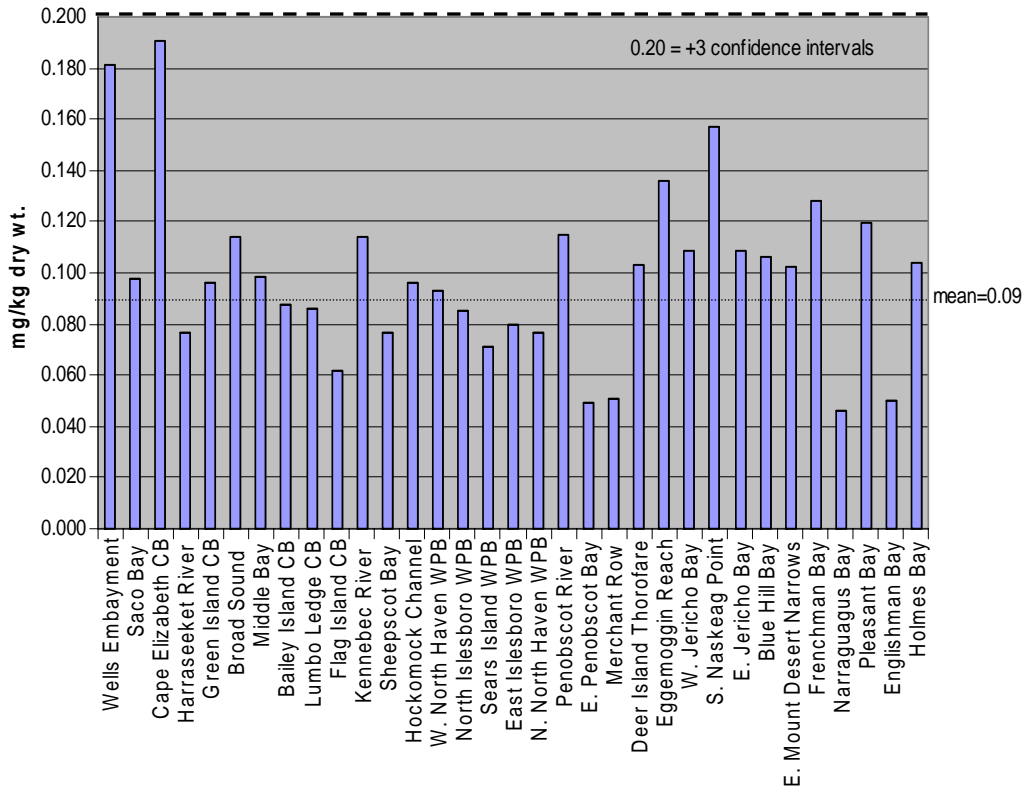


Figure 6. Lead in Maine Lobster Tomalley 2005-06



1.2

CONTAMINANTS IN MAINE'S ECOSYSTEMS

1.2 CONTAMINANTS IN MAINE'S ECOSYSTEMS

Preliminary findings of contaminant screening in Maine Birds, 2007 Field Season. A report presented by W. Goodale of the BioDiversity Research Institute, Gorham, Maine in 2008.

1. Executive Summary and Primary Findings (excerpted from the report)

Starting in May 2007, BioDiversity Research Institute (BRI) and collaborators initiated a broadbased contaminant study on Maine birds, measuring both historical and emerging chemicals. This comprehensive project measured 192 synthetic contaminants in 23 species across Maine to determine in which species, habitats, and locations these anthropogenic compounds are concentrating. The compounds we analyzed in 60 egg composites were mercury (Hg), polychlorinated biphenyls (PCB), polybrominated diphenyl ethers (PBDE), perfluorinated compounds (PFCs), and organochlorine pesticides (OCs). Our preliminary findings are:

- Hg, PCBs, PBDEs, PFCs, and OCs are found in all species sampled across marine, estuarine, riverine, lacustrine (lake), and terrestrial ecosystems; these are the first records of PFCs in Maine birds.
- Hg, PCBs, PFCs are all found at levels that may cause adverse effects—there are currently no established adverse effects thresholds established for PBDEs in bird eggs. OCs are all significantly below adverse effects thresholds.
- Our Hg, PCB, and OC levels were generally consistent with levels recorded around the country. Certain species had PBDEs higher than other locations, while other species had lower levels. PFOS have not been widely studied in eggs; therefore, we could not directly compare our results to other areas.
- The total PCBs levels we recorded are lower than those in the past, indicating a continued decline in PCBs.
- Bald eagles have the highest overall contaminant load of the 23 species measured.
- We found all of the compounds across the entire state, but overall contaminant loading tends to be highest in southern coastal Maine. This geographic pattern suggests that these compounds are entering the environment both through atmospheric deposition, because they are found across the entire state, and through local point sources, because we detected higher levels in urban and industrial areas.
- PCBs, PBDEs, PFCs, and OCs levels are positively correlated, indicating that birds with high levels of one compound tend to have higher levels of the others. PBDEs and PCB have the strongest relationship.
- Birds that feed on terrestrial prey accumulated higher PBDEs; DecaBDE is found in eight species with gulls and peregrine falcon having the highest levels.
- Of the samples we analyzed, birds feeding in estuaries have the lowest contaminant levels.
- The mouth of the Kennebec and Isles of Shoals tended to have high concentrations of contaminants.

The full report may be seen under the title in the Marine Module of this 2007 SWAT report at www.maine.gov/dep/blwq/docmonitoring/swat/index.htm .

2.0 LAKES MODULE

2.1 Fish Consumption Advisories

At the request of the Maine Center for Disease Control and Prevention (MCDC) to provide data for refinement of Maine's Statewide Fish Consumption Advisory, five trout from each of seven lakes were collected for analysis for mercury. Subsequently, a Governor's budget rescission order resulted in a reduction of the available funds for the 2007 SWAT program and the samples were not analyzed.

3.0 RIVERS AND STREAMS MODULE

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| | TECHNICAL ASSISTANTS | John Reynolds Joseph Glowa Ryan Burton |

3.1

AMBIENT BIOLOGICAL MONITORING

3.1 AMBIENT BIOLOGICAL MONITORING

3.1.1 Background

As part of the SWAT program, DEP's Biological Monitoring Unit evaluates benthic macroinvertebrate communities of Maine streams and rivers to determine if they are impaired by toxic contamination. For reasons of comparability, a small number of unimpaired reference sites are also evaluated. 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 2007, we evaluated the condition of 40 sample locations, primarily in the Kennebec River basin.

The Biological Monitoring Unit uses a multivariate statistical model to analyze a benthic macroinvertebrate sample and predict if a waterbody is attaining the biological criteria associated with its statutory class. If a waterbody does not meet any of the minimum state aquatic life criteria, then the model class is predicted as Non-Attainment (NA). Final decisions on aquatic life attainment of a waterbody are made accounting for factors that may allow adjustments to the model outcome. This is called the "final determination." Aquatic life classes AA and A are treated the same in the model.

Table 3.1.1 summarizes the results of biological monitoring activities for the 2007 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.
- *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."
- *Final determination* – The final decision on aquatic life attainment of a waterbody. This decision accounts for factors that may allow adjustments to the model outcome.
- *Attains Class* – "Y" is given if the final determination is equal to or exceeds the Statutory Class. A Class B stream, for example, would receive a "Y" if its Final determination 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 final determination was either C or NA.
- *Probable Cause* – The probable cause column lists potential stressors to benthic macroinvertebrate communities, based on best professional judgment. 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, known as 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 (available in electronic format with the web version of this report). For more information about the DEP's Biological Monitoring Unit, please e-mail biome@maine.gov or

visit our web site: www.state.me.us/dep/blwq/docmonitoring/biomonitoring/index.htm. The Data and Maps page of this website provides access to station information and available data via Google Earth.

3.1.2 Results Summary

- Forty stations were assessed for the condition of the benthic macroinvertebrate community.
- Eight of the 40 stations (20 %) failed to attain the aquatic life standards of their assigned class.

TABLE 3.1.1 - 2007 SWAT Benthic Macroinvertebrate Biomonitoring Results

| Waterbody | Town | Station | Log | Issue ¹ | Statutory Class / Final Determination | Attains Class? | Probable Cause ¹ |
|---------------------------------|--------------------|---------|------|-----------------------|---------------------------------------|----------------|-----------------------------|
| Birch Stream | Bangor | 312 | 1646 | Urban NPS; Airport | B / C | N | NPS toxics; habitat |
| Birch Stream | Bangor | 682 | 1647 | Urban NPS; Airport | B / NA | N | NPS toxics; habitat |
| Bond Brook | Augusta | 30 | 1636 | Urban NPS | B / B | Y | |
| Bond Brook | Augusta | 597 | 1637 | Urban NPS | B / B | Y | |
| Bond Brook - Unnamed Tributary | Augusta | 489 | 1638 | Urban NPS | B / NA | N | NPS toxics |
| Burgess Brook | Lily Bay TWP | 834 | 1670 | Reference | A / A | Y | |
| Carrabassett River | Kingfield | 16 | 1669 | Municipal/ NPS | A / A | Y | |
| Carrying Place Stream | Carrying Place TWP | 768 | 1668 | NPS (logging) | A / A | Y | |
| China Lake Outlet Stream | Winslow | 604 | 1655 | Municipal | B / B | Y | |
| Cobbosseecontee Stream | Gardiner | 253 | 1640 | Urban NPS | B / NA | N | NPS toxics/ hydro |
| Ducktrap River | Lincolnton | 626 | 1676 | Reference | AA / A | Y | |
| East Branch Sebasticook River | Corinna | 20 | 1644 | Superfund Site | C / C | Y | |
| East Branch Sebasticook River | Corinna | 194 | 1643 | Superfund Site | C / C | Y | |
| East Branch Wesserunsett Stream | Athens | 486 | 1662 | Reference | B / A | Y | |
| Halfmoon Stream | Thorndike | 697 | 1648 | Agricultural NPS | A / A | Y | |
| Kennebec River | Bingham | 636 | 1667 | Municipal | A / A | Y | |
| Kennebec River | Norridgewock | 22 | 1656 | Municipal/ Industrial | B / A | Y | |

¹ NPS is non-point source pollution.

| Waterbody | Town | Station | Log | Issue | Statutory Class/Final Determination | Attains Class? | Probable Cause |
|---------------------------------|---------------------|----------------|------------|----------------------------|--|-----------------------|-----------------------|
| Kennebec River | Benton | 196 | 1651 | Municipal/Industrial | C / B | Y | |
| Kennebec River | Sidney | 848 | 1679 | Municipal/Industrial | B / B | Y | |
| Kennebec River | Augusta | 785 | 1680 | Municipal/Industrial | B / B | Y | |
| Kennedy Brook | Augusta | 620 | 1642 | Urban NPS | B / C | N | NPS |
| Lily Bay Brook | Lily Bay TWP | 844 | 1673 | Reference | A / A | Y | |
| Little River | Belfast | 850 | 1677 | Agricultural NPS | B / B | Y | |
| North Brook | Lily Bay TWP | 843 | 1672 | Reference | A / A | Y | |
| Orbeton Stream | Madrid TWP | 840 | 1666 | Reference | A / A | Y | |
| Passagassawakeag River | Belfast | 430 | 1678 | Agricultural NPS | B / B | Y | |
| Penobscot River | Woodville | 399 | 1628 | Industrial/Municipal | C / C | Y | |
| Riggs Brook | Augusta | 599 | 1634 | Urban NPS | B / C | N | NPS toxics |
| Sandy River | Phillips | 17 | 1658 | Reference | AA / A | Y | |
| Sandy River | Farmington | 572 | 1659 | Municipal/Agricultural NPS | B / A | Y | |
| Sandy River | New Sharon | 617 | 1660 | Municipal/Agricultural NPS | B / B | Y | |
| Sebasticook River | Benton | 299 | 1654 | Municipal/NPS | C / B | Y | |
| Sheepscot River | Whitefield | 74 | 1629 | Reference | AA / A | Y | |
| South Branch Carrabassett River | Carrabassett Valley | 836 | 1664 | Reference | AA / A | Y | |
| Wesserunsett Stream | Cornville | 488 | 1663 | Agricultural NPS | B / A | Y | |
| West Branch Sebasticook River | Pittsfield | 27 | 1652 | Municipal/NPS | C / B | Y | |
| West Branch Sheepscot River | China | 268 | 1630 | Reference | AA / A | Y | |
| Whitney Brook | Augusta | 601 | 1633 | Urban NPS | B / NA | N | NPS toxics; habitat |
| Whitten Brook | Skowhegan | 628 | 1661 | Urban NPS | B / NA | N | NPS |
| Wilson Stream | Wilton | 34 | 1657 | Agricultural NPS | C / B | Y | |

3.1.3 Attainment History of Sampling Stations prior to 2007

- Birch Stream (Station 312) failed to attain class in 1997, 1999, 2001, 2003, 2004, 2005 and 2006.
- Birch Stream (Station 682) failed to attain class in 2003 and 2004.
- Bond Brook (Station 30) attained class in 1983, 1991, 1992, 1997, and 2002.
- Bond Brook (Station 597) attained class in 2002.
- Bond Brook – Unnamed Tributary (Station 489) failed to attain class in 2001 and 2002.
- Carrabassett River (Station 16) attained class in 1983 and 1997.
- Carrying Place Stream (Station 768) attained class in 2004.
- China Lake Outlet Stream (Station 604) attained class in 2002.
- Cobbosseecontee Stream (Station 253) failed to attain class in 1997.
- Ducktrap River (Station 626) failed to attain class in 2002.
- East Branch Sebasticook River (Station 20) failed to attain class in 1983, 1984, 1987, 1988, 1989, 1990, 1992, and 1994. It attained class in 1993, 1997, and 2003.
- East Branch Sebasticook River (Station 194) attained class in 1993, 1997, and 2003. It failed to attain class in 1994.
- East Branch Wesserunsett Stream (Station 486) attained class in 2001.
- Halfmoon Stream (Station 697) attained class in 2003.
- Kennebec River (Station 22) attained class in 1983 and 1997.
- Kennebec River (Station 196) attained class in 1992 and 1997.
- Kennebec River (Station 636) attained class in 2002.
- Kennebec River (Station 785) attained class in 1999, 2000, 2001, and 2002.
- Kennedy Brook (Station 620) attained class in 2002. It failed to attain class in 2004.
- Passagassawakeag River (Station 430) attained class in 2000.
- Penobscot River (Station 399) failed to attain class in 2000
- Sandy River (Station 17) attained class in 1983 and 2000.
- Sandy River (Station 572) failed to attain class in 2000.
- Sebasticook River (Station 299) attained class in 1997 and 2002.
- Sheepscot River (Station 74) attained class in 1985, 1987, 1988, 1989, 1990, 1992, 1995, 1996, 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005 and 2006. It failed to attain class in 1984, 1986, 1991, 1993, 1994, and 1997.
- Wesserunsett Stream (Station 488) attained class in 2001.
- West Branch Sebasticook River (Station 27) attained class in 1983 and 2002.
- West Branch Sheepscot River (Station 268) attained class in 1996, 1997, 1998, 1999, 2001, 2002, and 2005. It failed to attain class in 2000, 2003, 2004 and 2006.
- Whitten Brook (Station 628) failed to attain class in 2002.
- Wilson Stream (Station 34) attained class in 1983, 1991, and 1997.

TABLE 3.1.2 - 2007 Water chemistry data associated with biomonitoring sites

Please note that data for dissolved oxygen, specific conductance and pH (where available) are shown in the Aquatic Life Classification Attainment Report (pdf format) for each log number.

| Waterbody | Log | Sampling Date | DOC | NH ₃ -N | TKN | NO ₂ -NO ₃ -N | SRP | Total P | TSS | TDS |
|---------------------------------|------|---------------|------|--------------------|------|-------------------------------------|------|---------|------|------|
| Unit of measurement | | | mg/L | mg/L | mg/L | mg/L | ug/L | mg/L | mg/L | mg/L |
| Birch Stream | 1646 | 8/13/2007 | 6.9 | 0.04 | 0.7 | 0.26 | 2 | 0.042 | 6.8 | 330 |
| Bond Brook | 1637 | 8/8/2007 | 3.7 | 0.01 | 0.4 | 0.15 | 4 | 0.022 | 6.2 | 110 |
| Bond Brook - Unnamed Tributary | 1638 | 8/8/2007 | 6.1 | 0.05 | 0.9 | 2.00 | 6 | 0.048 | 17 | 230 |
| Burgess Brook | 1671 | 8/22/2007 | 12.0 | 0.03 | 0.9 | 0.01 | 2 | 0.028 | 13 | 59 |
| China Lake Outlet Stream | 1655 | 8/14/2007 | 3.8 | <0.01 | 0.5 | 0.05 | 9 | 0.024 | <2 | 50 |
| East Branch Wesserunsett Stream | 1662 | 8/16/2007 | 4.8 | <0.01 | 0.4 | 0.03 | 1 | 0.005 | <2 | 94 |
| Halfmoon Stream | 1648 | 8/13/2007 | 2.8 | 0.02 | 0.4 | 0.58 | 1 | 0.01 | 5.4 | 47 |
| Kennebec River | 1651 | 8/14/2007 | 6.0 | 0.01 | 0.6 | 0.07 | 13 | 0.033 | 2.2 | 41 |
| Kennebec River | 1656 | 8/15/2007 | 4.8 | 0.02 | 0.4 | 0.08 | 15 | 0.026 | <2 | 21 |
| Kennedy Brook | 1642 | 8/9/2007 | 2.7 | 0.01 | 0.3 | 0.82 | 9 | 0.012 | <2 | 330 |
| Lily Bay Brook | 1673 | 8/22/2007 | 2.0 | 0.01 | 0.1 | 0.07 | 2 | 0.006 | <2 | 40 |
| Orbeton Stream | 1666 | 8/20/2007 | 2.2 | 0.01 | 0.2 | 0.04 | 2 | 0.004 | <2 | 55 |
| Riggs Brook | 1634 | 8/8/2007 | 8.1 | 0.02 | 0.7 | 0.15 | 7 | 0.051 | 4.6 | 100 |
| Sandy River | 1658 | 8/15/2007 | 2.2 | <0.01 | 0.3 | 0.05 | 1 | 0.003 | <2 | 20 |
| Sandy River | 1659 | 8/15/2007 | 2.7 | 0.01 | 0.3 | 0.12 | 1 | 0.019 | 3.4 | 28 |
| Sebasticook River | 1654 | 8/14/2007 | 5.6 | <0.01 | 0.9 | 0.02 | 1 | 0.027 | 4.2 | 54 |
| Sheepscot River | 1629 | 8/7/2007 | 4.5 | 0.01 | 0.4 | 0.02 | 1 | 0.011 | <2 | 53 |
| South Branch Carrabassett River | 1664 | 8/20/2007 | 2.3 | 0.01 | 0.1 | 0.05 | 1 | 0.004 | <2 | 51 |
| West Branch Sebasticook River | 1652 | 8/14/2007 | 4.4 | 0.01 | 0.5 | 0.05 | <1 | 0.01 | <2 | 49 |
| West Branch Sheepscot River | 1630 | 8/7/2007 | 4.7 | 0.01 | 0.5 | 0.04 | 1 | 0.01 | <2 | 34 |

DOC = dissolved organic carbon, NH₃-N = ammonia-nitrogen, TKN = total Kjeldahl-nitrogen, NO₂-NO₃-N = nitrite-nitrate-nitrogen, SRP = soluble reactive phosphorus (ortho-phosphate), Total P = total phosphorus, TSS = total suspended solids, and TDS = total dissolved solids.

3.2

FISH CONSUMPTION ADVISORIES

3.2 FISH CONSUMPTION ADVISORIES

3.2.1 Dioxins and Coplanar PCBs

In 2007 the SWAT program was integrated with Maine's Dioxin Monitoring Program (DMP) that has been in effect since 1988. Fish samples collected at ten DMP stations were analyzed for dioxins and coplanar polychlorinated biphenyls (PCBs) in the SWAT program. Additional samples were collected from 4 DMP stations and 7 new SWAT stations for analysis for dioxins and PCB as well. We were unable to collect enough brown trout from the Kennebec River below Winslow for the SWAT program and Augusta for the DMP for dioxin and PCB analysis.

The results were published in a report to the Maine Legislature's Natural Resources Committee by the Maine Center for Disease Control and Prevention (MCDC) in January 2008. The report, *Evaluation of the Health Implications of Levels of Polychlorinated Dibenzo-p-Dioxins (dioxins) and Polychlorinated Dibenzofurans (furans) in Fish from Maine Rivers*, may be seen at www.maine.gov/dep/blwq/docmonitoring/dioxin/index.htm . The report summarizes the development of MCDC's revised fish tissue action level (FTAL) of 0.4 pptr for dioxin and compares it to recent data, with implications for the fish consumption advisories currently being reviewed for revision for certain rivers.

3.2.2 Berwick and Somersworth, N.H. Sludge

The goal of the DMP is "to determine the nature of dioxin contamination in the waters and fisheries of the State." Charged with administration of the program, DEP is required to sample fish once a year below no more than 12 bleached pulp mills, municipal wastewater treatment plants, or other known or likely sources of dioxin. DEP policy is that once identified as a discharger of dioxin, a facility is considered to be a continuing discharger until it provides data showing otherwise. There are two ways a facility may demonstrate that it is no longer a discharger: (1) For facilities that are the only source to a river reach, concentrations in fish must be not measurably higher below the discharge than above, or (2) For facilities on river reaches with other dischargers, concentrations in sludge must be below a value of 1 pptr for 4 quarters over a 2 year period.

In 1989, high levels of dioxins and furans were discovered in the Berwick Sewer District's sludge and subsequently levels in fish below the discharge were found to be significantly higher than in fish above the discharge. Consequently, the former Maine Bureau of Health (now MCDC) issued a fish consumption advisory for the Salmon Falls River below the District's wastewater treatment plant discharge due to elevated levels of dioxins and dioxin-like coplanar PCBs. The District has therefore been identified as a source, included in the annual DMP and assessed fees associated with the laboratory costs of analysis of fish for dioxins and furans. The District does not pay for the coplanar PCBs, which are funded in the SWAT program by the state's General Fund.

In 2005 Prime Tanning Company, a tannery that discharges pretreated wastewater to the District's wastewater treatment plant and the likely source of the dioxins, suggested that there were other historical, if not current, sources on the river, which might be responsible for the elevated levels in fish. Subsequently, data from 1999 were discovered that documented historically elevated levels in sludge from the Somersworth, New Hampshire wastewater treatment plant, just downstream of the Berwick discharge and above the historical fish sampling station. In order to determine the relative

contributions of both discharges,, sludge samples were collected quarterly from each facility for a period of 2 years, beginning in late 2005 and continuing through 2007, rather than continuing to sample fish. The costs of analyzing the samples from the Berwick wastewater treatment plant were borne by Prime Tanning as part of the DMP, but the costs of the Somersworth samples were borne by SWAT.

Results show that concentrations of dioxins and furans fluctuate somewhat between dates (Table 3.2.1). With the exception of 2 dates, there was little difference in concentrations between the two facilities. However, on 12/30/06 and 4/4/07 dioxin toxic equivalents calculated with non-detects at zero (DTEo) and at the detection limit (DTEd) were much higher in sludge from the Berwick treatment plant. This was due to extremely high values of 123478 Hx-CDF, a congener not normally found in elevated amounts. That this was probably real and not an analytical error is supported by the facts that (1) these levels were seen in two samples just months apart, (2) were seen in a duplicate sample on 4/4/07, and 3) were not seen in Somersworth sludge samples taken and analyzed at the same time. Interestingly, these levels were not repeated in samples collected from Berwick on 7/11/07 or 10/18/07. While, except for these two dates and 7/11/08 where DTE's were high for Somersworth due to an seldom high level of a HpCDD, levels are not significantly different between the two facilities, levels at both exceed the monitoring threshold of 1 ppt.

Table 3.2.1. Dioxins and furans in Berwick, Me and Somersworth, NH wastewater treatment plant sludge (ng/kg dw)

| date | Berwick sludge | Berwick sludge | Berwick sludge | Berwick sludge | Somersworth sludge | Somersworth sludge | Somersworth sludge | Somersworth sludge |
|----------|-------------------|-------------------|-------------------|-------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| | TCDD | TCDF | DTEo | DTEd | TCDD | TCDF | DTEo | DTEd |
| 8/19/05 | <0.40 | 1.8 | 4.0 | 5.1 | <0.52 | 2.1 | 5.8 | 6.4 |
| 12/21/05 | <0.68 | 1.2 | 4.1 | 6.2 | <0.15 | 0.9 | 1.5 | 2.5 |
| 3/29/06 | <0.43 | 1.4 | 4.4 | 4.8 | <0.39 | 1.1 | 4.7 | 5.1 |
| 6/29/06 | <0.60 | <0.9 | 4.2 | 6.9 | <0.34 | 1.4 | 5.4 | 6.9 |
| 12/30/06 | 0.20 | 1.1 | 33.8 | 33.8 | 0.305 | 1.4 | 7.9 | 7.9 |
| 4/4/07 | <0.03 | 0.3 | 16.5 | 16.5 | 0.05 | 0.2 | 0.9 | 0.9 |
| 7/11/07 | 0.25 | 0.7 | 7.5 | 7.5 | 0.65 | 2.0 | 13.9 | 13.9 |
| 10/18/07 | 0.22 | 0.7 | 3.4 | 3.4 | 0.68 | 1.8 | 9.0 | 9.0 |

TCDD = 2378-tetrachlorodibenzo-p-dioxin

TCDF = 2378-tetrachlorodibenzofuran

DTEo = dioxin toxic equivalents with non-detects at 0

DTEd = dioxin toxic equivalents with non-detects at the DL

Samples of largemouth bass were collected from the Salmon Falls River below Berwick as part of the SWAT program in 2007. As previously reported by the MCDC in their 2007 report referenced above, concentrations of dioxins were just below the revised FTAL of 0.4 ppt, but when combined with coplanar PCBs, the total exceeds the FTAL. MCDC is currently reviewing the data and will revise the fish consumption advisories within the next few months if necessary. Sampling of the river for fish may need to be continued.

3.2.3 Total PCBs

The current fish consumption advisory issued by the MCDC for the Kennebec River is for the limited consumption of trout (5-6 meals per year) and bass (1-2 meals a month) from Shawmut Dam in Fairfield to Augusta and no consumption of any fish from Augusta to the Chops. This advisory is based on a mix of contaminants. As some of the concentrations appear to have decreased over time, the Kennebec River is a likely candidate for revision. MCDC requested sampling of brown trout from Norridgewock, Fairfield, and Augusta, but samples were successfully collected only from Norridgewock. In addition, MCDC requested sampling of smallmouth bass from Norridgewock to Richmond to determine the upstream-downstream limits of any revised advisory, but because of sampling limitations, bass were captured only at Gardiner and Richmond.

There is a popular commercial winter ice shack fishery and spring dipnet fishery for rainbow smelt on many rivers and coastal streams where many fish are caught for consumption. Sampling in 2005 found total PCB levels in Kennebec River rainbow smelt exceeding MCDC's FTAL of 11ng/g. MCDC requested samples of rainbow smelt from the Kennebec and from another location (e.g., the Penobscot River) in 2007 to determine if this is a localized or general phenomenon.

The fish consumption advisory on the Salmon Falls (based on data from South Berwick) has been based on only several data points. Concentrations upstream appear to be relatively low. Concentrations at South Berwick seem to vary considerably, with the most recent data showing relatively low levels of total PCBs. MCDC requested additional sampling of fish from the river at South Berwick.

Results show that concentrations of total PCBs in brown trout at Norridgewock and smallmouth bass at Gardiner on the Kennebec River are similar to those found in previous years (Table 3.2.2). Concentrations in bass at Richmond are slightly lower than those found at Gardiner. Concentrations in rainbow smelt at Hallowell are intermediate to those in bass upstream at Augusta and downstream at Richmond and slightly higher than found in 2005. This was surprising given that these rainbow smelt are thought to inhabit the estuary for most of the year, which, because of dilution, should reduce their exposure to contaminants from upriver. Concentrations in rainbow smelt at Bucksport on the Penobscot River were significantly lower than in smelt at Hallowell on the Kennebec, perhaps because the Penobscot fish were in captured in the estuary lower in the river. Samples of largemouth bass at S. Berwick were within the bounds of those of past years. All sample results exceed the FTAL.

Table 3.2.2 Total PCBs in fish from some Maine rivers, mean ng/g
(95th upper confidence level on the mean or max if n=2)

KENNEBEC

| Year | Species | Norridgewock | Skowhegan | Fairfield | Sidney | Augusta | Hallowell | Gardiner | Richmond |
|------|---------|--------------|-----------|-----------|-----------|-------------|-----------|-----------|----------|
| 1994 | BNT | | | 300 | | | | | |
| 1997 | BNT | | | 93 (107) | | 54.6 (70.9) | | | |
| 1999 | BNT | | | | | 55 (71) | | | |
| 2000 | BNT | | | | 34 (45) | | | | |
| 2002 | BNT | 7.9 | | 10.2 | | | | | |
| 2007 | BNT | 9.5 (14) | | | | | | | |
| 1994 | SMB | | | 4.5 | 8.6 | 604 | | | |
| 1997 | SMB | | 3.7 (4.5) | 4.0 (4.9) | 6.1 (7.2) | 342 (357) | | | |
| 1999 | SMB | | | | | 263 (323) | | 179 (227) | |
| 2000 | SMB | | | | 32 (42) | | | | |
| 2002 | SMB | 1.6 | | 1.7 | 19.5 | 111 | | 47.5 | |
| 2006 | SMB | | | | 7.5 (10) | 83 (142) | | 51 (75) | |
| 2007 | SMB | | | | | | | 52 (70) | 44 (64) |
| 2005 | SLT | | | | | | 46 (64) | | |
| 2007 | SLT | | | | | | 60 (83) | | |

PENOBSCOT

| Year | Species | Bucksport | | | | | | | |
|------|---------|-----------|--|--|--|--|--|--|--|
| 2007 | SLT | 27 (27) | | | | | | | |

SALMON FALLS

| Year | Species | Acton | Northeast Spaulding P | | | Berwick | S. Berwick |
|------|---------|-------|-----------------------|--|-----------|---------|------------|
| 1997 | SMB | 5 (6) | | | | | 75 |
| 1997 | CHP | | | | | | 47 (53) |
| 2000 | SMB | | | | | | 83 (100) |
| 2002 | SMB | | | | | 110 | |
| 2002 | WHP | | 23.4 | | | | |
| 2006 | LMB | | | | 25.5 (49) | | 33.2 (44) |
| 2007 | LMB | | | | | | 47 (61) |

Species Legend

- BNT brown trout
- LMB largemouth bass
- SLT rainbow smelt
- SMB smallmouth bass
- WHP white perch

3.2.4 DDT in Aroostook County Rivers

Detectable concentrations of DDE, a breakdown product of DDT, are still found throughout the United States, 40 years after it was banned. It is still manufactured and sold to third world countries for control of human disease vectors, and some may travel back to the US via evasion, long-range transport, and atmospheric deposition. Based on previous data collected in the SWAT program, levels exceeding the MCDC's cancer based FTAL for total DDTs (64 ppb) were found in several rivers and streams in Aroostook County, a legacy from past use. Consequently, MCDC has issued fish consumption advisories (FCAs) for the Meduxnekeag River, North Branch Presque Isle Stream, and Prestile Stream. Since then additional data for Everett Brook in Ft. Fairfield and Prestile Brook in Caribou have shown exceedances of the FTAL. To provide a current assessment of DDT levels in County rivers and streams for potential revisions to the the FCAs, MCDC requested resampling of brook trout from these rivers and streams in 2007. Results show that concentrations were generally similar to previous data with one exception. At the Prestile Stream at Mars Hill concentrations are considerably lower than sampled in 1996, but not much different than that at Westfield in 2000 (Table 3.2.3).

Table 3.2.3 Total DDT (DDD+DDE+DDT) in Fish from Aroostook Co. Rivers and Streams, ug/kg

| Year | Species | N. Br. Presque Isle Str Mapleton | Meduxnekeag R. S Br Hodgdon | Meduxnekeag R Houlton | Prestile Str Mars Hill | Prestile Str Westfield | Everett Bk Ft. Fairfield | Prestile Bk Caribou |
|------|---------|--|-----------------------------------|-----------------------------|------------------------------|------------------------------|--------------------------------|---------------------------|
| 1995 | BKT | 142 | | 82 | | | | |
| 1995 | BNT | | | 98 | | | | |
| 1996 | BKT | | | | 260 | | | |
| 1997 | BKT | | | | | | | |
| 2000 | BKT | 44 | | | | 96 | 242 | |
| 2003 | BKT | | | | | | | 170 |
| 2007 | BKT | 65 | 25 | | 88 | | 196 | 140 |

Species Legend

BKT brook trout

Given the historical extent of agriculture in the County, there is likely little difference between results at Westfield and Mars Hill, and the 2000 and 2007 data are likely more representative of the Prestile Stream than are the 1996 data. All 2007 results exceed MCDC's FTAL with one exception. The South Branch Meduxnekeag River at Hodgdon had relatively low concentrations, probably due to the predominant forested land use in the watershed compared to much greater agricultural land use in the main stem Meduxnekeag, which was sampled in 1995.

3.3

CUMULATIVE EFFECTS-DRIVEN ASSESSMENT OF FISH POPULATIONS

3.3 CUMULATIVE EFFECTS ASSESSMENT OF FISH POPULATIONS

3.3.1 Introduction

The federal 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 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. the 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 in wild populations. Many new techniques, such as a cumulative effects 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. Indicators of growth and reproduction include measures of energy expenditure and storage. 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, and metabolic disruption (Munkittrick et al, 2000). Levels of circulating sex steroids are also often used as biomarkers of reproductive potential which, along with survival, are considered an index of potential population trends.

With the assistance of Environment Canada (EC), DEP conducted CEAs of fish populations on the St John River in 1999-2001 that indicated probable impacts to fish populations and identified a previously unknown source of pollution in Canada (a poultry processing plant), that negatively affected fish populations in the river. 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 potential population level effects by a significant reduction in gonad size in both streams compared to two reference streams with much lower DDT levels in fish.

To undertake a CEA for Maine's major industrial rivers, it was decided to evaluate the most impacted river, and if no negative impacts were measured, to not study the other rivers. The Androscoggin River was chosen to study because it had more large pulp and paper mills (three) for its size than the other major rivers and has historically had poor 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 may be due to the change in technology to elemental chlorine free (ECF) bleaching and improved waste treatment in the three upstream bleached kraft pulp and paper mills in the intervening years. There was also no evidence of endocrine disruption at any location below any of the mills along the rest of the river. There was, however, 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, thereby providing 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 other responses showed no such evidence. Consequently, this study was repeated in 2006 but the effects seen in 2004 were not observed.

A caged mussel study in the Kennebec River in 2004 did not show induction of vitellin seen in 2003, but the stations were different between the two years due to other study priorities. The study was repeated in 2005 and showed induction of vitellin below the mill as in 2003, although at station 5 rather than stations 3 and 4, for both males and females. In addition, lipid peroxidation, an indicator of toxicity, was elevated at all three stations below the mill. Growth in length and/or weight was increased at all stations below the mill. This study was repeated in 2006 but did not show induction at any station below the mill.

Because there was some evidence of endocrine disruption from the Kennebec in the caged mussel study during 2003 and in some responses in the fish study in 2004, it seemed prudent to sample the Penobscot as well. Consequently, in 2005 and 2006 CEAs were 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 in 2005, which allowed for a coordinated sampling effort and use of fish for both studies. The Environmental Effects Monitoring (EEM) program in Canada requires all pulp and paper mills to conduct a CEA of two species for each mill. Our 2005 Penobscot River CEA included two species as well, smallmouth bass and white sucker. Results showed increased eutrophication below the mill for both years. 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 statistically significant decreases in SSI below the two most upstream mills on the Androscoggin for one or both sexes reported 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. There was no such difference in 2006. Both SSI and KSI, measured on both species from the Penobscot River in 2005 and 2006, showed a marginal reduction in SSI below the mill in 2005 and a significant increase in 2006. The results of the SSI are therefore not consistent from year to year at either river. Additional study with more sophisticated assays, such as those conducted by Hannum, are needed.

Another method of determining the impact of stressors on fish populations is through studies of fish communities. For the last several years, Chris Yoder, Midwest Biodiversity Institute, and Brandon Kulik, Kleinschmidt Associates, have been conducting fish assemblage studies on large rivers in Maine and the rest of New England, for the purpose of developing an Index of Biological Integrity of fish communities, under an EPA grant in consultation with Maine DEP and the Department of Inland Fisheries and Wildlife. In 2006, their study of the Presumpscot River documented reduced catch rates of fish in the river below Westbrook compared to the river above the City. Consequently, it was decided that further investigation was necessary, and in 2007 a CEA was conducted on the Presumpscot River. The SAPPI Westbrook pulp mill closed in 1999, leaving the paper mill and city of Westbrook as the major dischargers into the river. The Presumpscot River is much smaller than the Androscoggin, Kennebec, and Penobscot and consequently wastewater is a larger proportion of the Presumpscot River than the others, and therefore more likely to have an effect on fish populations.

3.3.2 Methods

In September 2007, white suckers were collected from the Presumpscot River at Windham (station PWD) and Westbrook (station PWB), above and below the discharges from the municipal wastewater treatment plant in the city of Westbrook and the SAPPI Westbrook SD Warren paper mill,. At PWD, 20 males and 20 females were collected during fall recrudescence. At PWB, 20 females were collected, but only 3 males were collected despite a 2 week sampling period. Previous studies have determined that a sample size of 20 is sufficient to reduce the variance enough to detect a difference of approximately 25% in the variables measured between stations.

Fish were collected by gill net. Blood samples were collected via caudal puncture with a syringe from live fish immobilized in a foam cradle, transferred to heparinized Vacutainers and placed on ice for transport to the lab the same day. The fish were then killed by concussion. The operculum was collected for aging. Livers were dissected out, weighed for calculation of LSI, and then frozen in liquid nitrogen. Gonads were dissected out, weighed for calculation of GSI, and a small sample approximately 1 cm square was taken and placed in 10% buffered formalin for storage. Head kidney

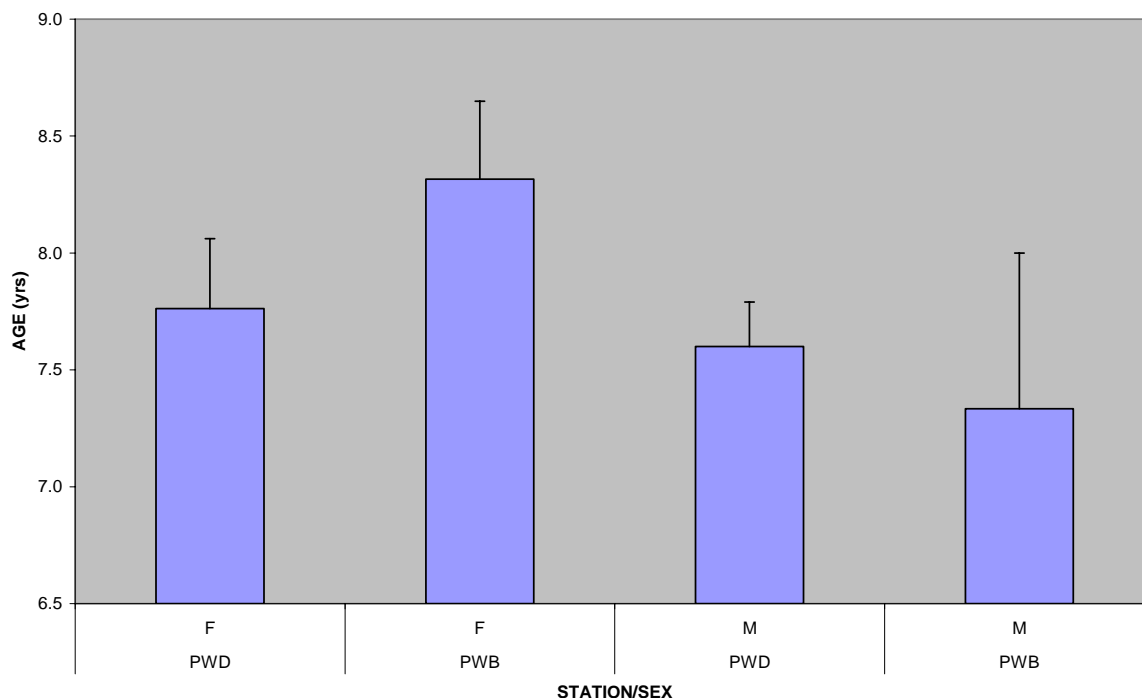
in suckers and spleen in both species were dissected out and weighed for calculation of KSI and SSI respectively.

Later the same day in the lab, the samples were placed in proper storage to await analysis. Plasma was collected from the blood samples after centrifugation in the lab and then frozen at -20°C for radioimmunoassay (RIA) analysis for circulating sex steroids (testosterone T, 11 ketotestosterone 11-KT, and estradiol E2) following the methods of McMaster, et al. (1992) and Jardine (1996). Liver samples were stored at -80°C for mixed function oxidase MFO (CYP1A) enzyme 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 at Environment Canada's National Water Research Institute in Burlington, Ontario, Canada. Samples for aging were stored at -20°C until prepared and read in the DEP lab in Augusta, Maine.

3.3.3 Results

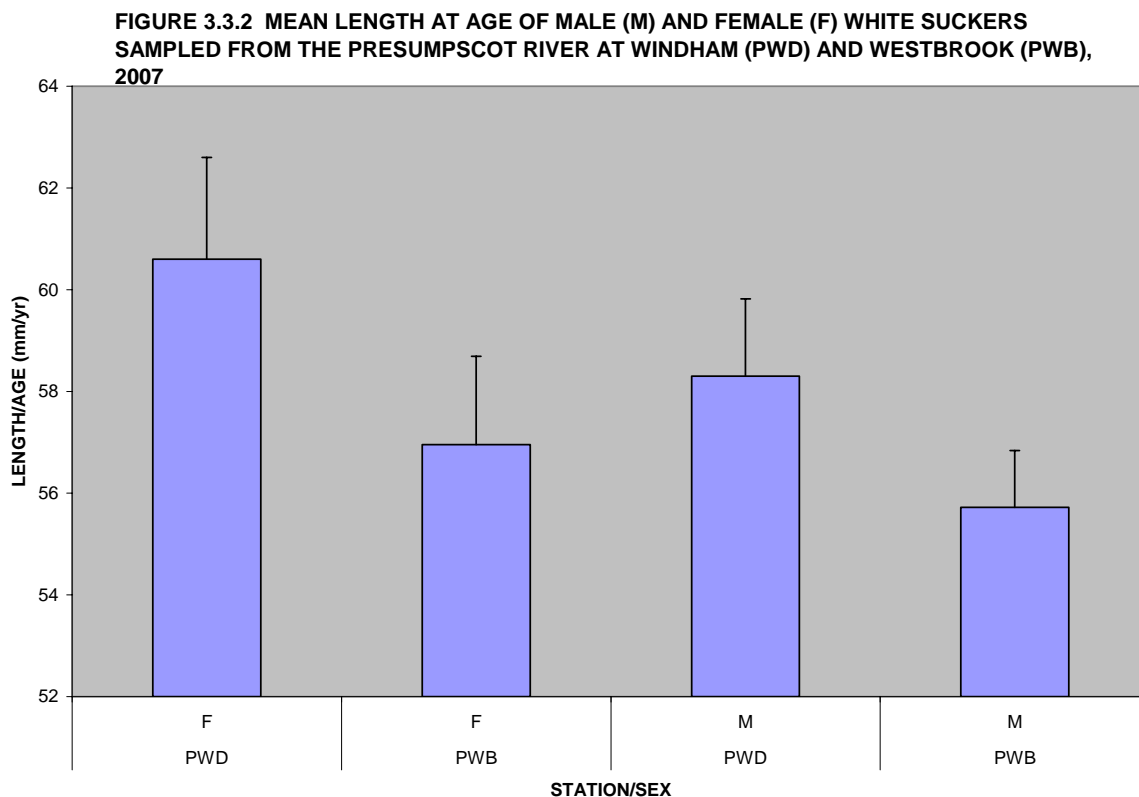
The small sample size for males at PWB ($n=3$), negates any final conclusions for males, but data are presented to show potential difference between stations and for comparison of data between sexes. There was no difference in mean age, an indicator of survival, between the stations for females (Figure 3.3.1).

Figure 3.3.1 MEAN AGE OF MALE (M) AND FEMALE (F) WHITE SUCKERS SAMPLED FROM THE PRESUMPCOT RIVER AT WINDHAM (PWD) AND WESTBROOK (PWB), 2007



This is an interesting finding given that the catch rate for females was much lower at PWB than at PWD, which would suggest a smaller population and lower survival or recruitment. At PWD we collected 20 males and 20 females in two overnight gill net sets with 1000 feet of 2 in bar mesh monofilament gill nets. At PWB we collected 20 females and only three males in eight overnight sets with the same sampling gear. This finding is similar to lower catch rates at PWB in 2006 in Chris Yoder’s fish assemblage study using an electrofishing boat (Personal communication with field crew).

There was no difference between stations of length at age, an indicator of energy utilization for growth (Figure 3.3.2). Yet there was a significant increase in the gonadosomatic index (GSI), the relative size of the gonad to body size, an indicator of energy utilization for reproduction, at PWB, implying increased reproductive potential (Figure 3.3.3).



There was a significant increase in condition factor (K), an indicator of energy storage at PWB (Figure 3.3.4). But there was no similar increase in the liversomatic index (LSI), another indicator of energy storage, at PWB (Figure 3.3.5). These results are somewhat aberrant of common responses to eutrophication, which include increases in GSI, K, and LSI. Water quality data (Table 3.3.1) and visual observations clearly document that the river is much more enriched at PWB than at PWD. One possible explanation for such unusual responses is a suggestion by mill personnel that the habitat at PWB and PWD are different, with the latter being more influenced by marine clay deposits than the former.

FIGURE 3.3.3 MEAN GSI IN MALE (M) AND FEMALE (F) WHITE SUCKERS SAMPLED FROM THE PRESUMPCOT RIVER AT WINDHAM (PWD) AND WESTBROOK (PWB), 2007

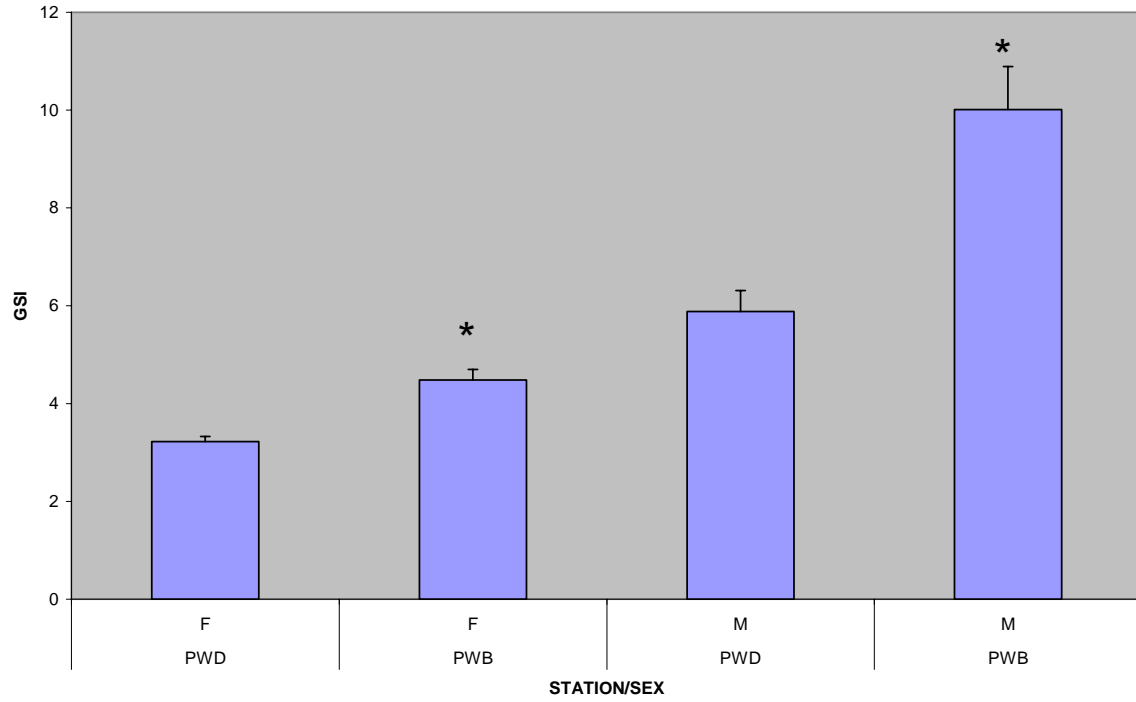


FIGURE 3.3.4. MEAN CONDITION FACTOR (K) IN MALE (M) AND FEMALE (F) WHITE SUCKERS SAMPLED FROM THE PRESUMPSCOT RIVER AT WINDHAM (PWD) AND WESTBROOK (PWB), 2007

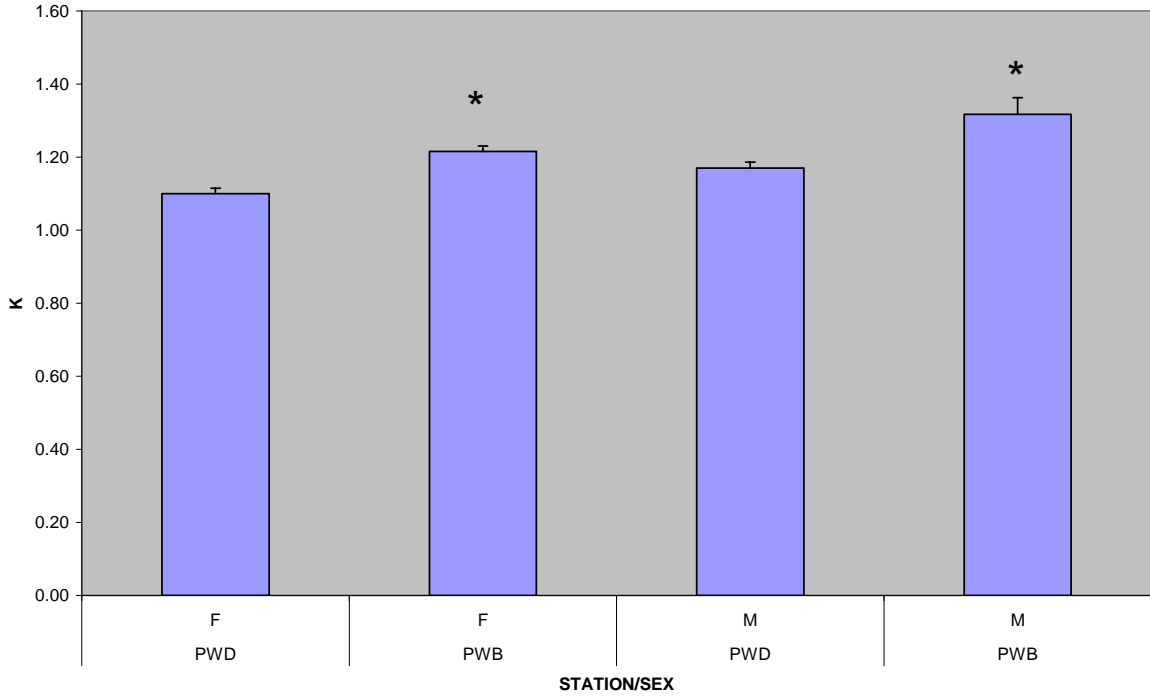
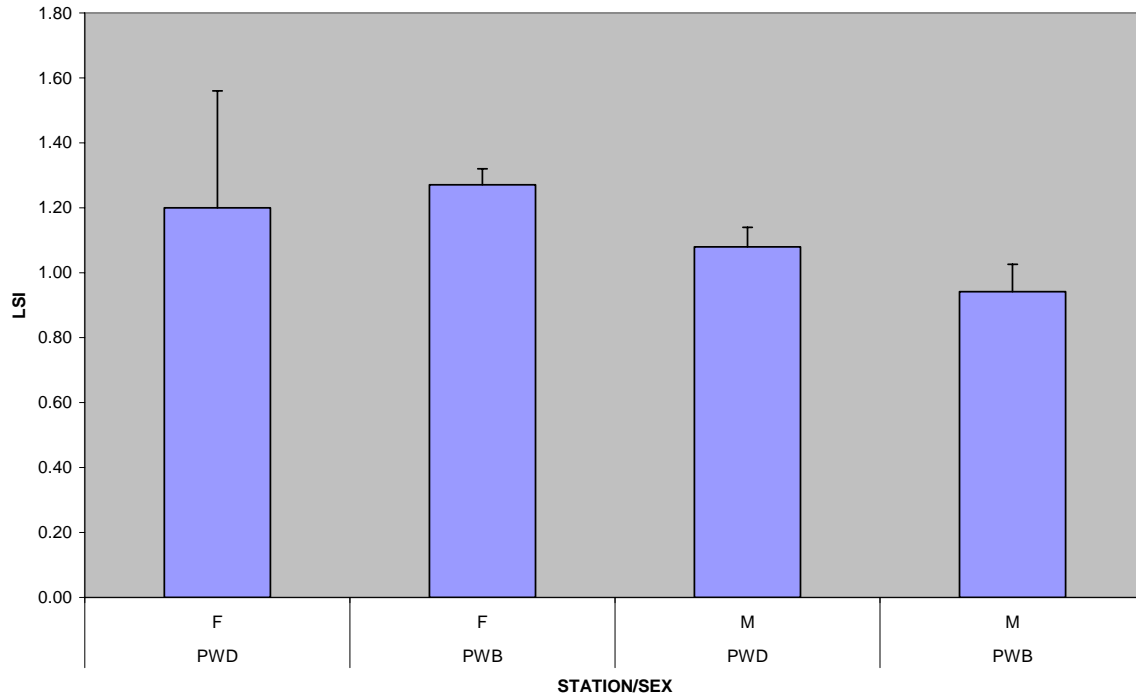


FIGURE 3.3.5. MEAN LSI IN MALE (M) AND FEMALE (F) WHITE SUCKERS SAMPLED FROM THE PRESUMPCOT RIVER AT WINDHAM (PWD) AND WESTBROOK (PWB), 2007



Given the same finding of lower catch rates with our study as those found by Yoder and Kulic in 2006 and of capture of fewer males below Westbrook than above in our study, there is an indication that there is something affecting fish populations at PWB. The effect may be due to habitat, although according to mill personnel, there is some habitat above the mill that is similar to that below and which the 2006 survey sampled. The Presumpscot River is much smaller than the Androscoggin, Kennebec, and Penobscot rivers studied in recent years where eutrophication was the major impact of discharges from primarily the bleached kraft pulp and paper mills. While the SAPPI Westbrook pulp mill closed in 1999, there may be a residual effect from past discharges. There may be an effect from Westbrook's municipal wastewater treatment plant discharges. Whole effluent toxicity (WET) tests from both facilities do not show potential toxicity with the effluent, but WET tests typically detect only relatively gross effects. And there is a possibility of effects from urban runoff from the highly developed city of Westbrook. Most likely the cause of any impaired fish population is a combination of factors. Further study is warranted to determine if the effect continues and the likely cause.

Table 3.3.1 Water Quality in samples from the Presumpscot River 2007
at Windham (PWD) and Westbrook (PWB), 2007

| STATION | DATE | SP COND | TA | TP | NO2/NO3 | TKN | TN | COMMENTS |
|---------|-----------|---------|-----|-------|---------|-----|------|-------------|
| | | us | ppm | ppb | ppb | ppb | ppb | |
| PWD | 9/20/2007 | 47 | | | | | | |
| | 9/25/2007 | 48 | 7 | 0.004 | 0.02 | 0.3 | 0.32 | Qr 330=>660 |
| PWB | 9/20/2007 | 74 | | | | | | |
| | 9/25/2007 | 69 | 15 | 0.048 | 0.25 | 0.4 | 0.65 | Qr 330=>660 |

Qr = flow in river

References

- Adams, S.M., W.D. Crumby, M.S.Greeley Jr., L.R. Shugart, and C.F. Saylor, 1992. Responses of Fish Populations and Communities to Pulp Mill Effluents: A Holistic Assessment. *Ecotoxicology and Environmental Safety* 24:347-360.
- Ahmad, T.M., M. Athar, N.Z. Khan, and S. Raisuddin, 1998. Responses of circulating fish phagocytes to paper mill effluent exposure. *Bull. Environ. Contam. Toxicol.* 61: 746-753.
- DEP, 2004. Surface Waters Ambient Toxics Monitoring Program Final Report, 2002-2003, Maine Department of Environmental Protection, Augusta, Maine, December 2004.
- Gray, MA and CD Metcalf, 1997. Induction of testis-ova in Japanese medaka (*Oryzias latipes*) exposed to p-nonylphenol. *Env. Toxicol. Chem.* 16(4):1082-1086.
- Jardine, JJ, GJ Van Der Kraak, and KR Munkittrick, 1996. Impact of capture, handling, confinement, and a three day recovery period on general indicators of stress and reproductive steroids in white sucker exposed to bleached kraft mill effluent. *Ecotoxicol. Environ. Safe* 33:287-298.
- Holliday, S.D., S.A. Smith, E.G. Besteman, A.S.M.I. Deyab, R.M. Gogal, T. Hrubec, J.L. Robertson, and S.A. Ahmed, 1998. Benzoapyrene-induced hypocellularity of the pronephros in tilapia (*Oreochromis niloticus*) is accompanied by alterations in stromal and parenchymal cells and by enhanced cell apoptosis. *Vet. Immunology and Immunopathology* 64(1):69-82.
- Kavlock, R.J., G.P. Daston, C. DeRosa, P. Fennes-Crisp, L. E. Gray, S. Kaattari, G. Lucier, M. Luster, M.J. Mac, C. Maczka, R. Miller, J. Moore, R. Rolland, G. Scott, D.M. Sheehan, T. Sinks, and H.A. Tilson, 1996. Research needs for the risk assessment of health and environmental effects of endocrine disruptors: A report of the US EPA sponsored workshop. *Env. Health Perspectives* 104 supp 715-
- McMaster, M, GJ Van Der Kraak, and KR Munkittrick, 1996. An epidemiological evaluation of the biochemical basis for steroid hormonal depressions in fish exposed to industrial wastes. *J. Great Lakes Res.* 22(2):153-171.
- McMaster, ME, KR Munkittrick, and GJ Van Der Kraak, 1992. Protocol for measuring circulating levels of gonadal sex steroids in fish. *Can. Tech. Rept. Fish. Aquat. Sci.* 1836.
- McMaster, M, 2001. National Water Research Institute, Canada Center for Inland Waters, Environment Canada, Burlington, Ontario. Personal communication.
- Munkittrick, KR, GJ Van Der Kraak, ME McMaster, and CB Portt, 1992. Response of hepatic MFO activity and plasma sex steroids to secondary treatment of bleached kraft pulp mill effluent and mill shutdowns. *Env. Toxicol. Chem.* 11:1427-1439.

Munkittrick, K.A., M.E. McMaster, L.H. McCarthy, M.R. Servos, and G.J. Van Der Kraak, 1998. An overview of recent studies on the potential of pulp-mill effluents to alter reproductive parameters in fish. *J. of Toxicology and Environmental Health, Part B*, 1:347-371.

Munkittrick, K.A., M.E. McMaster, G. Van Der Kraak, C. Portt, W. N. Gibbons, A. Farwell, and M. Gray, 2000. Development of methods for effects driven cumulative effects assessment using fish populations: Moose River project. Technical Publication, SETAC Press, Pensacola, Fla. 236 pp.

Rolland, R.M., M. Gilbertson, and R.E. Peterson editors, 1997. Chemically Induced Alterations in Functional Development and Reproduction of Fishes. Proceedings from a session at the 1995 Wingspread Conference Center, 21-23 July 1995, Racine Wi. Published by the Society of Environmental Toxicology and Chemistry (SETAC), Pensacola, Florida.

Secombes, C.J., T.C. Fletcher, A. White, M.J. Costello, R. Stagg, and D.F. Houlihan, 1992. Effects of sewage sludge on immune responses in the dab, *Limanda limanda* L. *Aquatic Toxicology* 23:217-230.

Voccia, I., K. Krzystyniak, M. Dunier, and M. Fournier, 1994. In vitro mercury-related cytotoxicity and functional impairment of the immune cells of rainbow trout (*Oncorhynchus mykiss*). *Aqu. Tox.* 29(1-2):37-48.

3.4

CAGED MUSSEL VITELLIN STUDY

(FROM 2006)

3.4 CAGED MUSSEL VITELLIN STUDY (Performed by the DEP in 2006)

3.4.1 Background

This caged mussel vitellin study was actually performed in 2006 but the lab results were not available until after the 2006 report was made public. The entire report is provided here.

In 2003, a study with caged mussels detected a significant induction of vitellin, a vitellogenin-like reproductive protein normally found in females, in a subsample of both males and females at stations 3 and 4, approximately 0.08 miles and 2.5 miles below the SAPPI Somerset bleached kraft pulp and paper mill on the Kennebec River in Skowhegan respectively, compared to stations KR1 and KR2, approximately 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 KR5, approximately 5 miles below the mill. A repeat study in 2004 found no such induction at station KR6, approximately 11 miles below the mill, compared to KR2, and there was no difference in condition factor or relative gonad size (GSI) between the stations. A repeat study in 2005 found increased growth in shell length and whole animal wet weight at all stations sampled below the mill (KR3 through KR5). ALP (vitellin) total mass was greater at KR5 than the upstream station KR2, but ALP normalized to protein was no different between the two. In 2005, there was a significant increase in lipid peroxidation at all stations below the mill. The study was repeated in 2006 including all stations KR2 through KR6. A similar study was attempted in the Penobscot River above (PBW) or below (PBL and PBC) the Lincoln Paper and Tissue bleached kraft pulp and paper mill at Lincoln in 2005, but samples were lost in a flood. The study was repeated in 2006.

3.4.2 Results

In 2006 in the Kennebec River, unlike 2005, growth rates for length were no different among the stations (Figure 3.4.1). Growth rates for whole animal wet weight were significantly greater below the mill (as far downstream as KR5) than above, (Figure 3.4.2) similar to that for KR5 in 2003 and all stations below the mill in 2005. In the Penobscot River there was no difference in growth for either shell length or whole animal wet weight among the stations, due to high variability among the stations (Figures 3.4.3 and 3.4.4). The indication of enrichment in the Kennebec below the mill and not in the Penobscot below the mill was opposite that seen with the fish for both rivers.

Figure 3.4.1. Growth rates in length of caged mussels in the Kennebec River, 2006

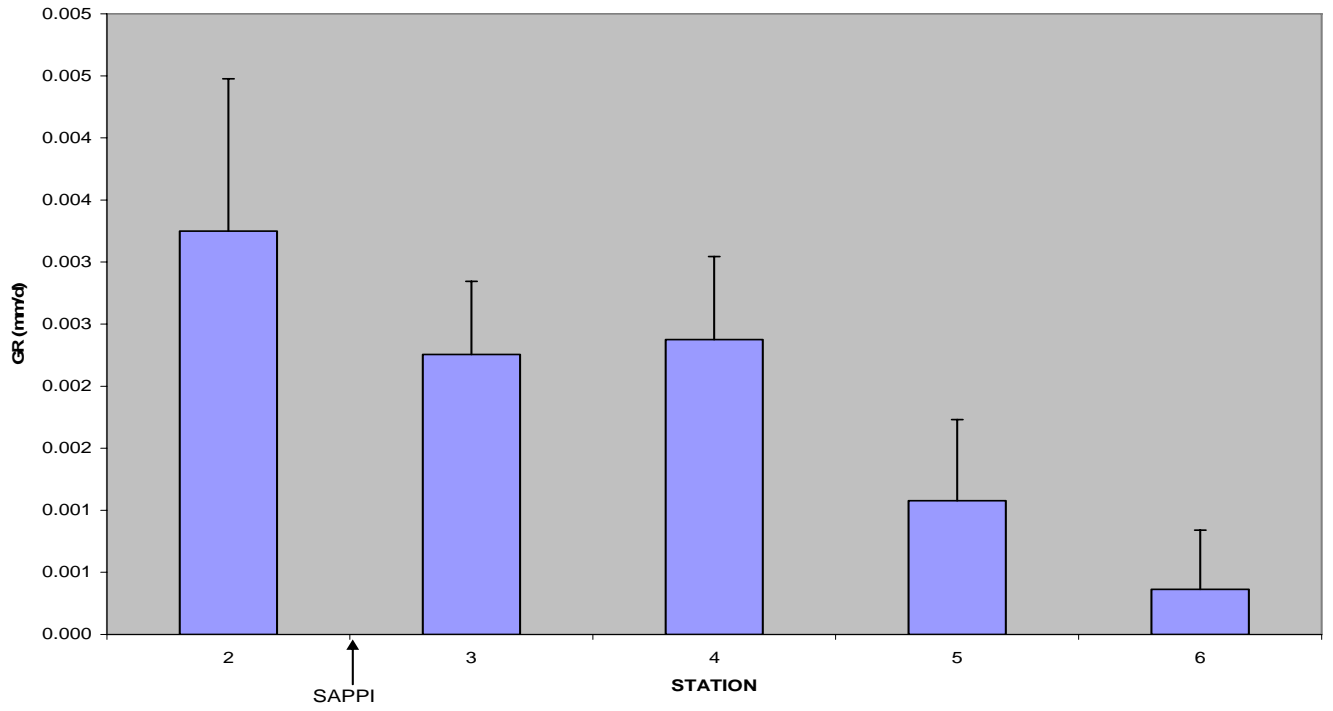


Figure 3.4.2. Growth rate in weight of caged mussels in the Kennebec River, 2006

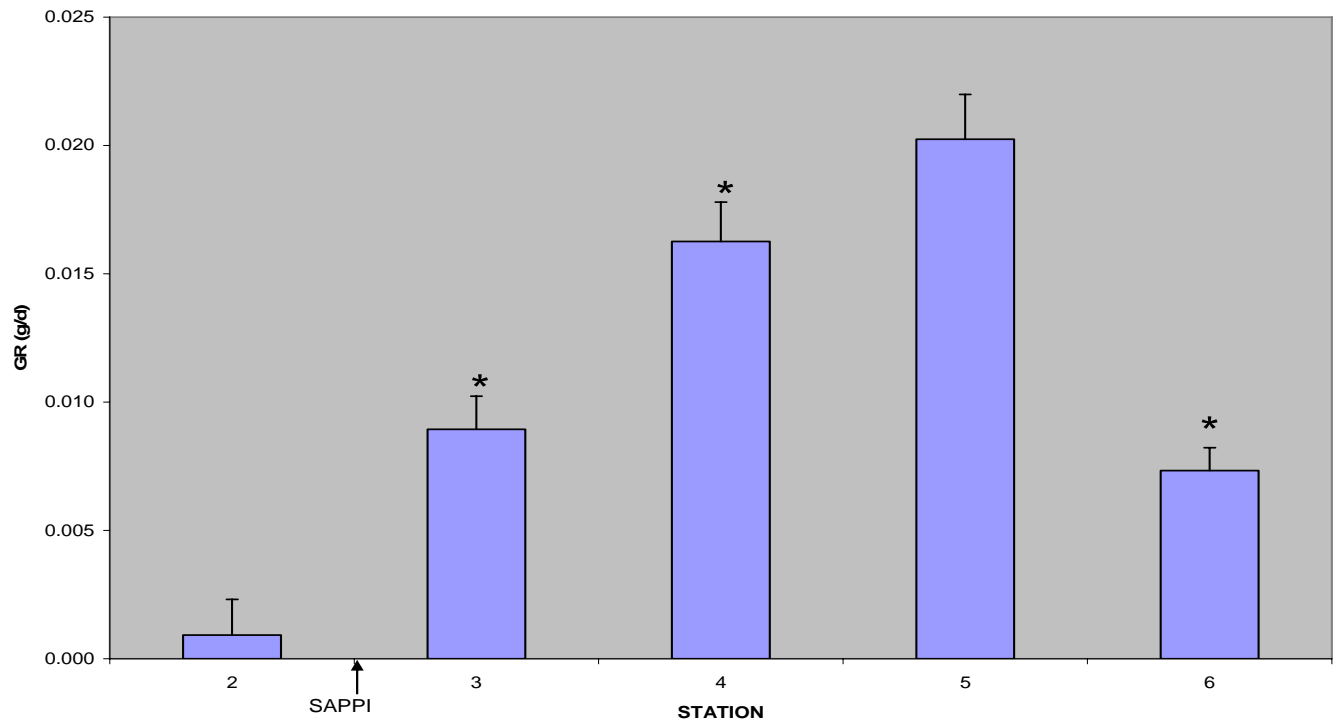


Figure 3.4.3. Growth rate in length of caged mussels in the Penobscot River, 2006 (mean+se)

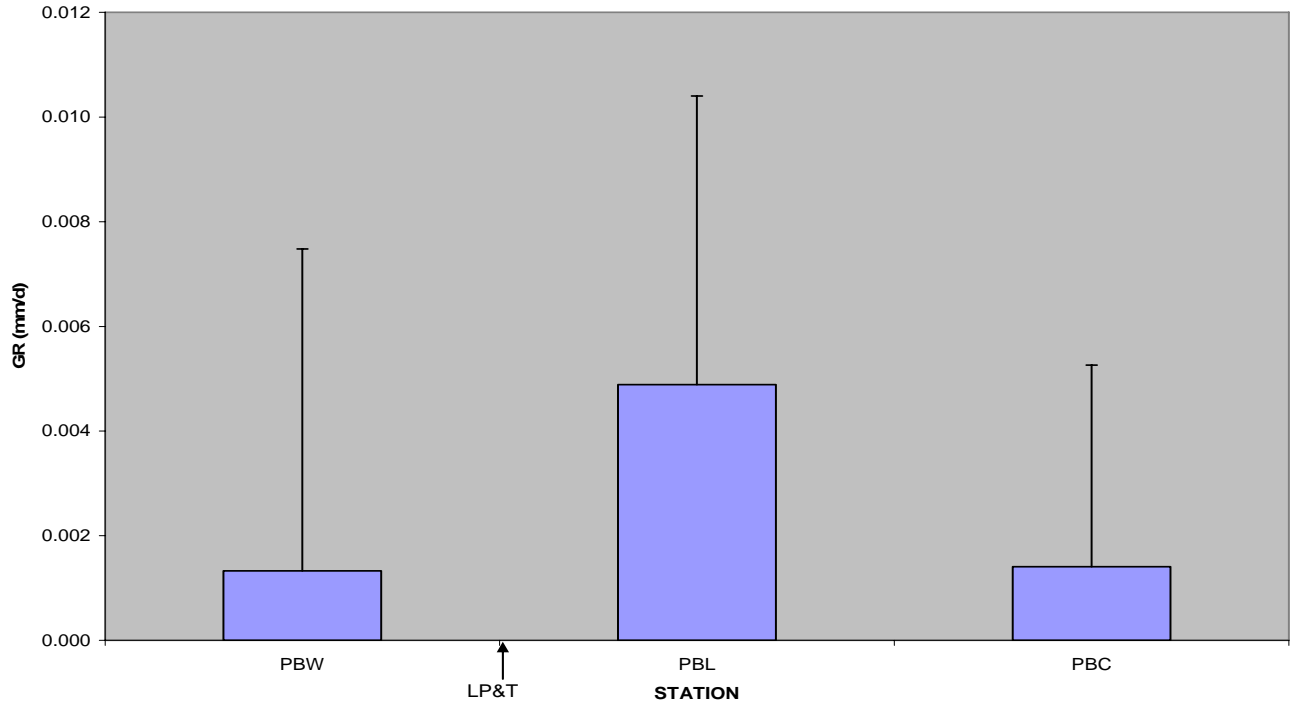
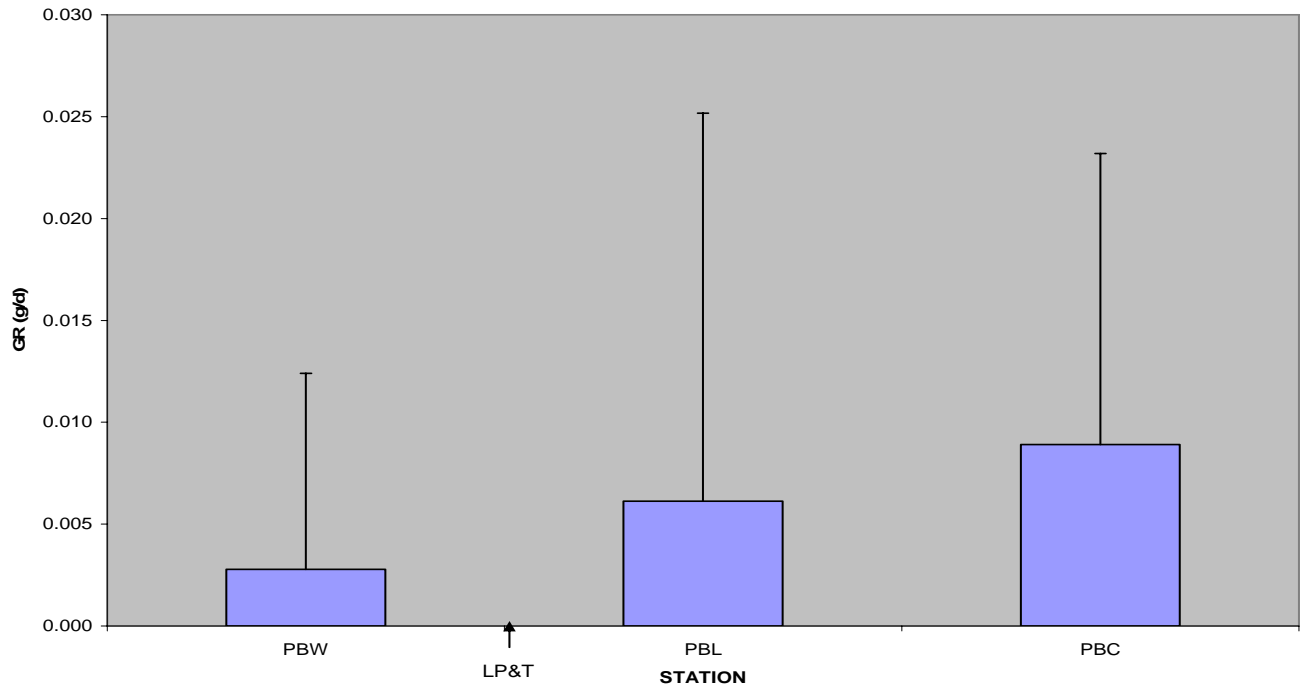


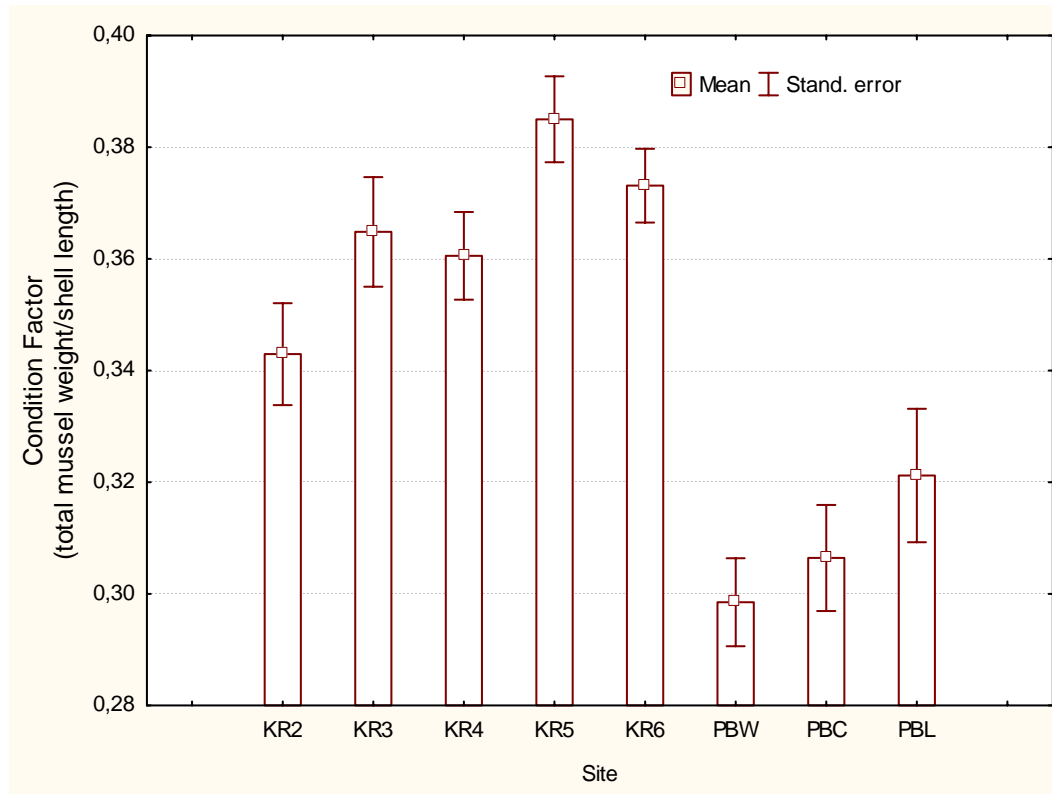
Figure 3.4.4. Growth rate in weight of caged mussels in the Penobscot River, 2006 (mean+se)



The following analyses were performed by Dr. Francoise Gagne of Environment Canada's St. Lawrence Center in Montreal, Quebec:

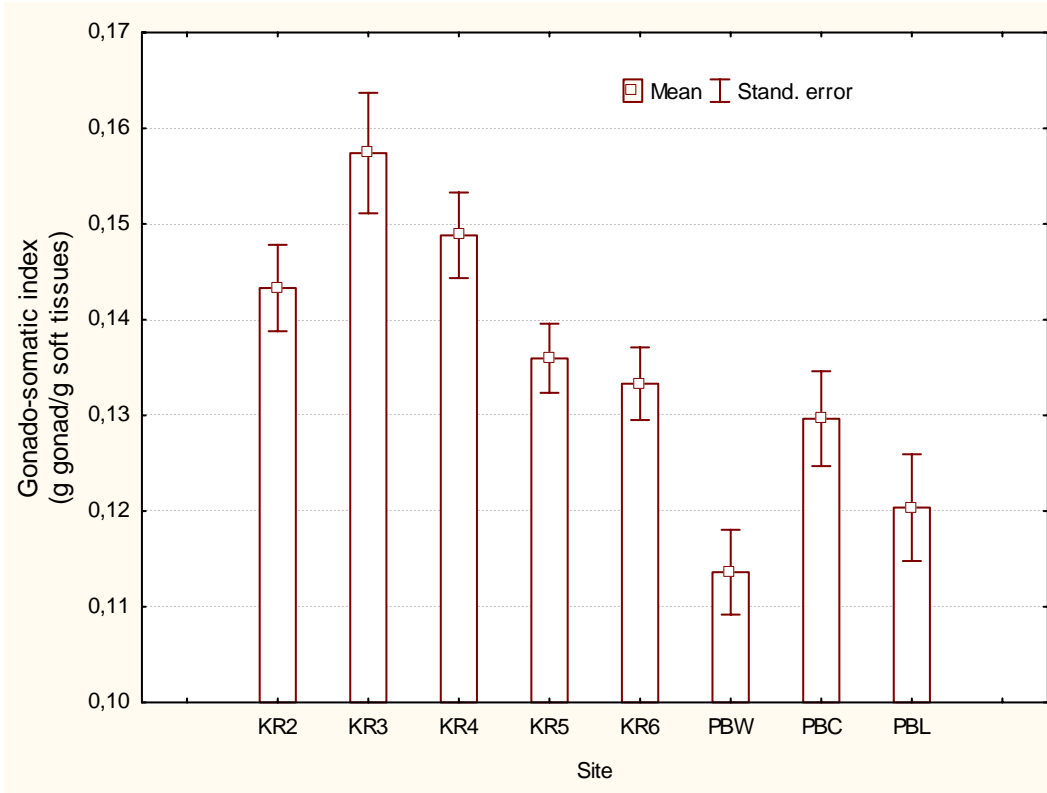
Mussels were analyzed for sex, condition factor, gonado-somatic index, vitellogenin-like proteins in the gonad, lipid peroxidation in gills and digestive gland. Data were screened with the Statistica software (version 7) to remove extreme outliers. Data were then analyzed using 2-way ANOVA statistic for sex- and site-related effects. This study was blinded so information on each site was unknown to the analysis laboratory.

1) Condition factor



2-way ANOVA revealed that site was significant ($p < 0.001$) while gender was not significant ($p > 0.1$). There was an increase in condition factor from the reference stations in both the Kennebec (KR2) and Penobscot (PBW) rivers to the other stations below the discharges, similar to the increase in weight.

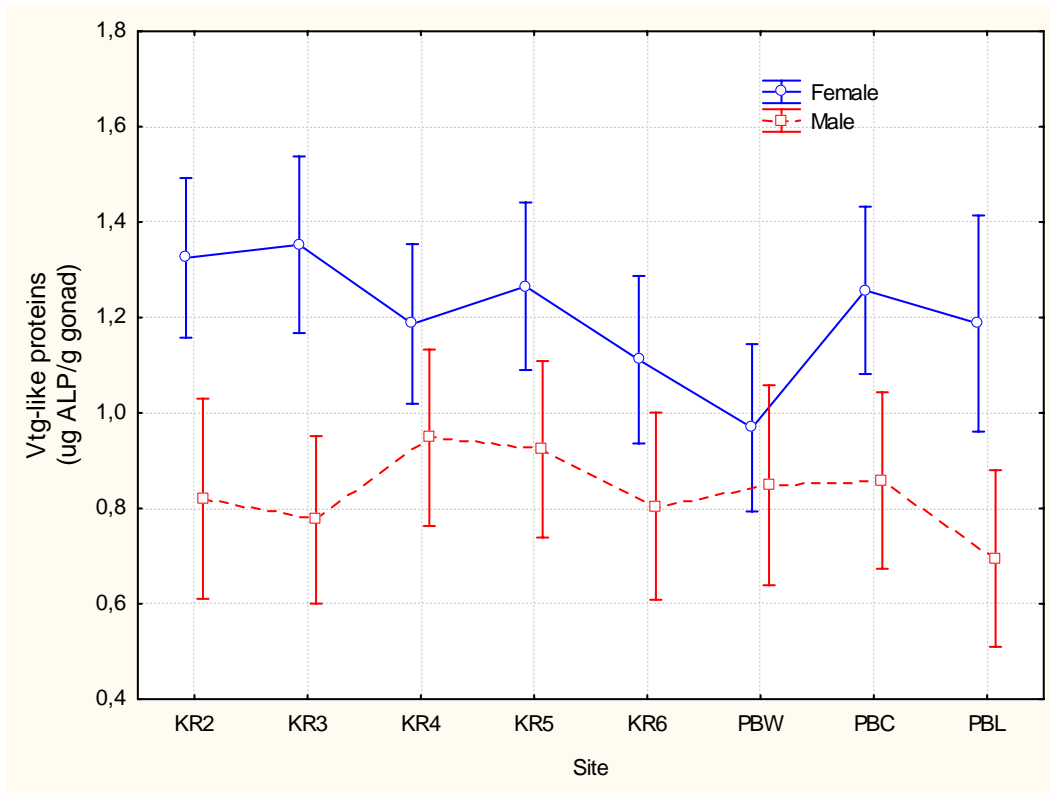
2) Gonado-somatic index (GSI)



2-way ANOVA revealed that site and gender was significant ($p < 0.001$) with a significant interaction of site and sex. GSI showed the same relationship among stations as did the growth in weight and condition factor, all showing increased enrichment below the discharges that was seen with the fish studies and water quality data.

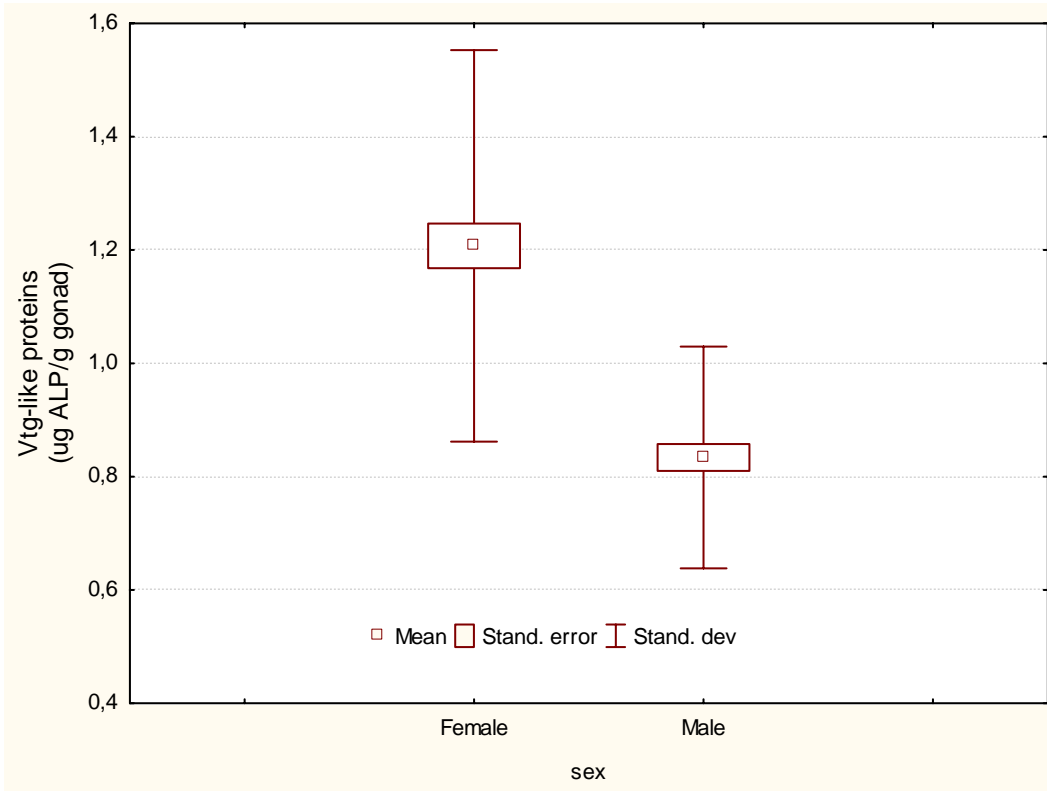
3) Vitellogenin-like proteins

3.1) Alkali-labile phosphates (indirect assay for vitellogenin)



2-way ANOVA revealed that only gender was significant while site was not significant. Females contain more ALP than males and do not vary significantly with site. The lack of induction of vitellogenin-like proteins (vitellin) was different than seen in previous studies, but there does not seem to be a consistent pattern among the years.

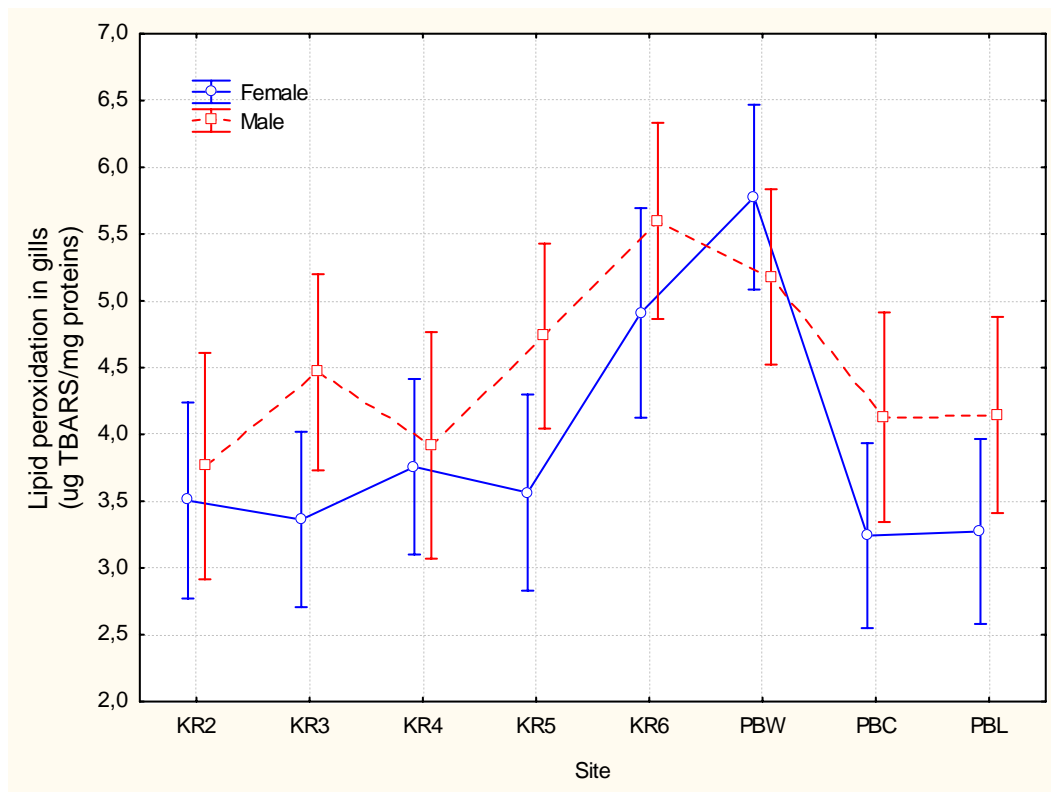
3.2) Sex difference



Vitellogenin-like proteins in females are significantly higher than males as determined with the alkali-labile phosphate assay (a validation step).

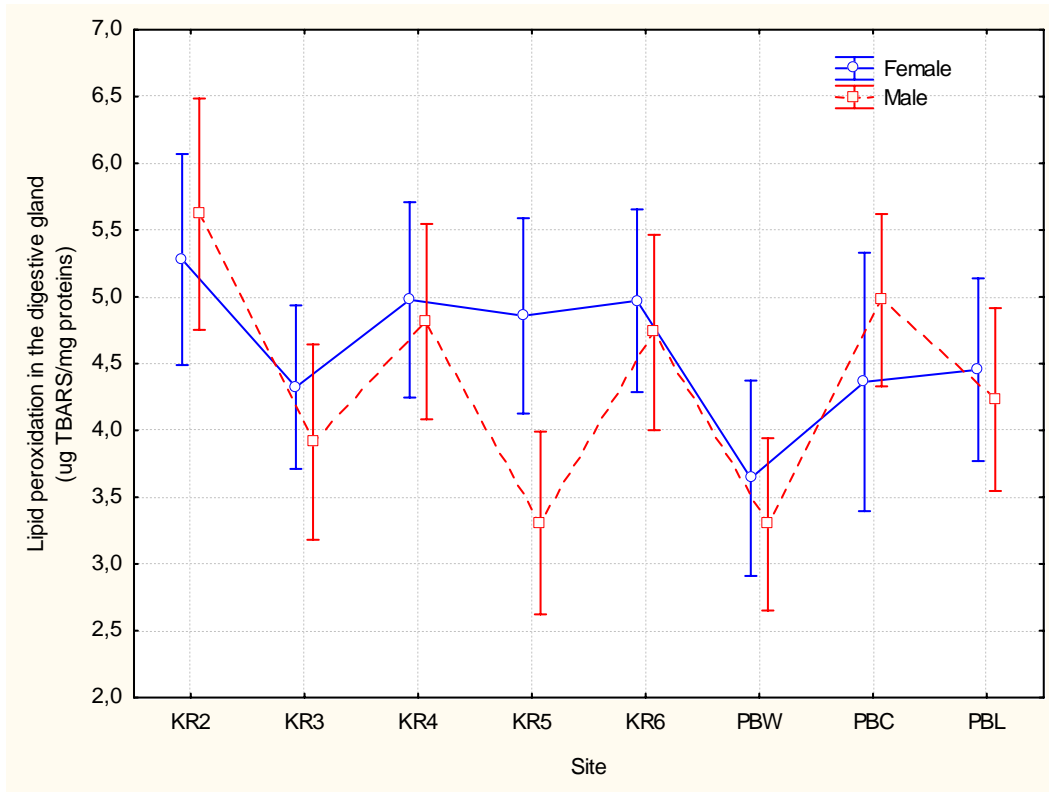
4) Oxidative stress

4.1) In gills



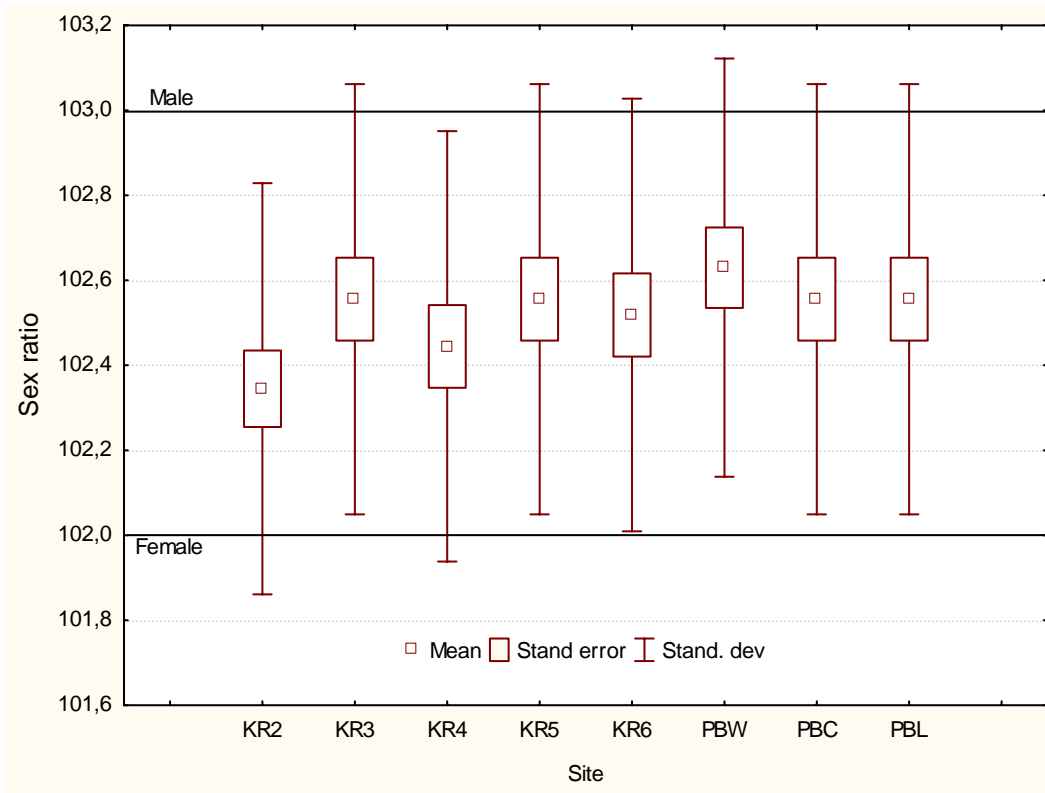
2-way ANOVA revealed that both sex and site were significant at $p < 0.01$ level. More lipid peroxidation (LPO) was observed in males than in females but LPO was induced in both sexes at sites KR6 and PBW. KR6 is the lowermost of 4 stations below the SAPPi Somerset mill's discharge, and these results differ from those of 2005, where LPO was found immediately below the outfall. PBW is above the Lincoln Paper and Tissue discharge, but below Millinockett and East Millinockett where there are discharges of municipal and pulp and paper mill wastewaters.

4.2) In the digestive gland



2-way ANOVA revealed that only sites differed significantly while no significant gender related effects were obtained.

5) Sex ratio



The sex ratio was constant throughout the study sites (ANOVA: $p > 0.1$). This confirms that the number of males or females did not vary significantly within each site.

3.4.3 Conclusions

The caged mussel studies on the Kennebec River in 2006 corroborated the CEA fish studies and water quality data documenting nutrient enrichment. In the Kennebec, induction of vitellogenin from 2003 and 2005 and increased lipid peroxidation in 2005 below the mill was not repeated in 2006. Therefore, there is no evidence of consistent endocrine disruption from the SAPPI Somerset discharge in Skowhegan. Nor was there induction of vitellogenin or lipid peroxidation below the Lincoln Paper and Tissue mill in Lincoln. Lipid peroxidation below Millinockett may warrant further investigation.