

Report to the Joint Standing Committee on
Environment and Natural Resources
127th Legislature, First Session

Surface Water Ambient Toxics Monitoring Program 2014

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Introduction

This 2014 Surface Water Ambient Toxic (SWAT) monitoring program final report is organized into an Executive Summary, Introduction and 4 modules:

1. Marine and Estuarine
2. Lakes
3. Rivers and Streams
4. Special Studies (update from 2013)

The full report is available on the DEP website at

<http://www.maine.gov/dep/water/monitoring/toxics/swat/index.htm>

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EXECUTIVE SUMMARY

Maine's Surface Water Ambient Toxics (SWAT) monitoring program was established in 1993 (38 MRSA §420-B) and administered by the Department of Environmental Protection to determine the nature, scope and severity of toxic contamination in the surface waters and fisheries of the State. 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. The 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 five-year conceptual work plan in addition to annual work plans which are each reviewed by a Technical Advisory Group (TAG). The TAG is composed of 12 individuals, including two representatives with scientific backgrounds representing each of five various interests (business, municipal, conservation, public health and academic), and two legislators.

The SWAT program is divided into four modules: 1) Marine and Estuarine, 2) Lakes, 3) Rivers and Streams, and 4) Special Studies. This annual report follows the goals of the 2009 five-year conceptual plan, which are generally to continue to monitor previously identified and new toxic issues in the marine environment, lakes and ponds, and rivers and streams, including but not limited to providing baseline data for use by the Department of Marine Resources (DMR) in evaluating and assessing shellfish harvesting areas; providing fish and shellfish contaminants data to the Maine Center for Disease Control and Prevention (MCDC) for use in revising Maine's fish consumption advisories; and continuing biological assessment of rivers' and streams' attainment of Maine's Water Quality Standards.

This report more specifically presents the findings of the 2014 annual work plan recommended by the SWAT TAG in a meeting July 1, 2014. The 2014 work plan focused on monitoring of the first three modules only, including shellfish from known or suspected contaminated marine areas, dioxins and polychlorinated biphenyls (PCBs) in American shad from the Kennebec River, perflourinated compounds (PFCs) in reference waters as requested by MCDC, and biomonitoring of aquatic life in the St. John River basin and other waters in Maine needing monitoring for evaluation of discharge permits and other DEP programs. Following is a summary of key findings from the 2014 SWAT program for each of the three modules.

1. MARINE AND ESTUARINE

General Approach:

- In 2014, blue mussel tissue taken from Mill Creek, Falmouth; Navy Pier, Harpswell; and Mare Brook, Brunswick was analyzed for contaminants including metals, mercury, Polycyclic Aromatic Hydrocarbons (PAHs), and PCBs. Blue mussel tissue from Navy Pier and Mare Brook was also analyzed for PFCs.
- In 2014, softshell clam tissue taken from the Presumpscot River in Falmouth/Portland and from Strawberry Creek in Harpswell was tested and the results reported along with historical data from ten additional softshell clam sites sampled in 2004-05 and 2010-13. Clam tissue from Strawberry Creek was analyzed for contaminants including metals, mercury, PAHs, and PCBs. Clam tissue from the Presumpscot River was analyzed for metals.

Encouraging Results:

- PAH concentrations in mussel and clam tissues did not exceed the National Status and Trends (NS&T Musselwatch) nationwide 85th percentile at any of the four sites tested; therefore no sites were considered to be elevated. PAH levels in Maine shellfish tend to be low when compared to the national average.
- PCB concentrations in mussel and clam tissues did not exceed the NS&T Musselwatch 85th percentile at any site and were not considered to be elevated. PCB concentrations in mussel and clam tissue from all sites tested were below the MCDC fish tissue action level (FTAL) for cancer, indicating shellfish from all sites remained safe for human consumption with regard to PCBs.
- Testing for PFCs, emerging contaminants of concern, was new to the marine SWAT program in 2013 and continued at two blue mussel sites in 2014. Concentrations of 11 of 13 individual PFCs were below detection limits at both of the blue mussel sites tested in 2014.
- Mercury in blue mussel tissue at all three sites tested in 2014 was less than NS&T Musselwatch 85th percentile concentration, therefore no sites were considered elevated. Mercury levels in all 2014 mussel and clam tissue samples tested were below the MCDC methylmercury developmental FTAL for finfish, indicating shellfish at all sites remained safe for human consumption with regard to mercury.
- Lead in softshell clam tissue from Strawberry Cove in Harpswell was quite low. Examination of lead concentrations in SWAT-tested softshell clams has fostered a collaborative discussion between DEP, Maine Department of Marine Resources (DMR), MCDC, and industry about the appropriate tissue action level for lead in Maine softshell clams. Historic study work suggests that the edible portion of clam tissue is lower in lead than the soft tissue of the clam as a whole; however testing of the whole clam soft tissue

has been the norm in the SWAT program, which has focused mainly on environmental monitoring. Pending cooperative studies and ongoing exchange of expertise will help to advance this dialogue in the coming year.

Contaminants and Areas to Watch:

- Lead in softshell clam tissue from the Presumpscot River in Portland was determined to exceed the MCDC FTAL for lead in finfish. However, as noted above, these results were derived from whole-clam soft tissues and not just from the edible portion of the clam. Past work documenting that removal of the clam neck skin appears to significantly lower lead concentration is being investigated further by a collaborative team.
- The PFC perfluorooctane sulfonamide (PFOSA) was detected in mussel tissue from Navy Pier and from Mare Brook. The PFC perfluoroheptanoate (PFHpA) was detected in mussel tissue from one spatial sub-sample at Navy Pier. The 11 other PFCs for which testing was performed were all below the detection limit for those compounds.

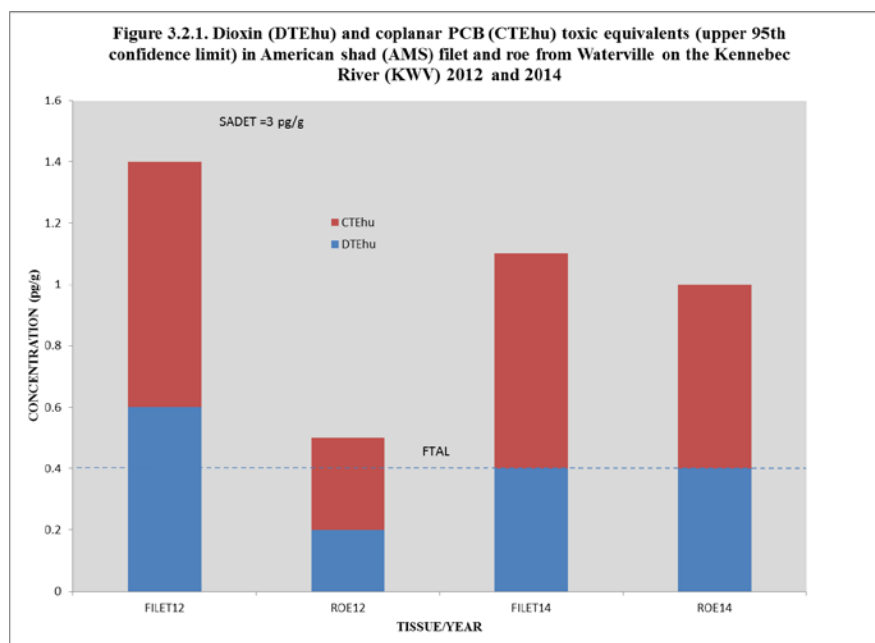
2. LAKES

- Some lake algal blooms produce cyanotoxins and are known as Harmful Algal Blooms (HABs). Cyanotoxins are secondary metabolites produced by cyanobacteria that may have hepatotoxic or neurotoxic effects, depending on the specific toxin present. In Maine, Microcystin LR (hepatotoxin) is thought to be most likely observed followed by Anatoxin (neurotoxin). In 2014, DEP undertook a study to better understand the distribution of cyanotoxins in Maine lakes. Single visit samples were obtained from thirty Central Maine Lakes, many with a history of blooms; seven of these lakes were revisited three times to obtain time-series data. A total of 140 samples were submitted for Microcystin analysis. Results obtained clearly indicate that some Maine lakes produce cyanotoxins when experiencing algal blooms. Highest Microcystin values were measured in the algal scums, where levels were detected up to 2.5 orders of magnitude greater than open water samples (maximum 17,696 ppb); open water Microcystin concentrations were often well above the WHO LOC of 1.0 ppb (maximum 38 ppb). The time-series data indicates substantial variation in the concentration of Microcystin during the life of the bloom. Highest concentrations were often observed in early September, although some blooms produced considerable Microcystin concentrations in late August.
- PFCs are a large (>200) class of highly persistent and mobile chemicals that have many specialized industrial and commercial uses in products that resist heat, stains, water, oil and grease; including hair conditioners, non-stick coatings, wetting agents, insulation, dust repellants, cleaners, anti-static agents, antifogging agents, and fire-fighting foams, among others. Exposure to PFCs has been correlated with increased cancers, thyroid disease, disrupted growth and development, and endocrine disruption in humans, and similar physiologically disruptive effects have been documented in amphibian, fish, and rodent models. PFCs are found all over the world, including the arctic and deep seas, which suggests they originate from both point and nonpoint sources such as sewage treatment plants and atmospheric deposition. Concentrations exceeding the MCDC's screening level

for protection of human consumers of fish have been found in fish at three military installations in Maine. To determine background levels elsewhere in Maine, in 2014 specimens of brook trout, brown bullhead (hornpout), and smallmouth bass were collected from three lakes or ponds each and analyzed for a suite of the most commonly detected PFCs. Results showed that concentrations were well below the screening level for all three species in all lakes and ponds sampled.

3. RIVERS AND STREAMS

- Forty stations were assessed for the condition of the benthic macroinvertebrate community. Thirty-two stations attained the aquatic life criteria of their assigned class.
- American shad fishery - There is a developing fishery for American shad in the Kennebec River and anglers have been inquiring about contamination in the fish. In 2014, concentrations of dioxins and dioxin-like coplanar PCBs in wet weight samples of both filets and roe of American shad collected in the Waterville area exceeded the MCDC FTAL for protection of human consumers (Figure 3.2.1). However, concentrations were below the Statewide Advisory Dioxin Equivalent Threshold (SADET), which is the concentration of dioxins and coplanar PCBs affording protection to human consumers equivalent to the level of protection experienced by consumers who follow the statewide fish consumption advisory for freshwater fish due to mercury contamination. As also shown, concentrations were somewhat different than had been found in 2012. When normalized to lipid, where these organic contaminants partition, concentrations were similar in both filets and roe for each year, although slightly higher in 2014 (not shown).



4. SPECIAL STUDIES

- Soft Plastic Lures – At the request of the Maine Department of Inland Fisheries and Wildlife (DIFW), in 2013 a study of the content and potential toxicity of plasticizers in soft plastic lures (SPLs), i.e. ‘rubber’ worms, was initiated with DIFW, DEP and the University of Maine. Results to date show evidence of phthalates in SPLs. Lake trout fed SPLs are currently being analyzed for uptake of phthalates and results are expected by late spring.

1.0 MARINE MODULE

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1.1 INTRODUCTION

Maine's coastline lies within and lends its name to the Gulf of Maine, a diverse and productive ecosystem. The Maine coast and the larger Gulf of Maine provide economic opportunities including commercial fisheries, aquaculture, recreational fisheries, commerce via shipping, and a wide variety of tourism activities. Maine includes the urbanized areas of Portland and Bangor, and has experienced growth and increased development in recent years, especially in the southwestern portion of the state's coastline. With increased development, increases in chemical contaminants discharged to the marine environment may occur. Some contaminants can also become concentrated as they move through the food chain, bioaccumulating at higher trophic levels and potentially impacting the viability of marine species and ecosystem health, and causing concern about potential consequences to human health. All these factors suggest that the monitoring of chemical contaminants is an important component of assessing the health of the marine environment in Maine.

1.1.1 Blue Mussels

Blue mussels (*Mytilus edulis*) have been relied upon extensively by the SWAT program (since 1986) and other monitoring programs as an indicator of exposure of marine environments to chemical pollutants. Mussels are ubiquitous and readily collected across the coast of Maine, as well as throughout the entire Gulf of Maine. Published information about contaminants in mussels provides some historical context and allows comparisons between geographic areas and over time. Since blue mussels are consumed as food by humans, they can be used to understand potential human exposure to contaminants. Mussels are sessile, allowing attribution of their contaminant burdens to the environment where they were collected. Mussels filter large volumes of water as they feed, allowing them to concentrate many chemicals from the water column or from sediments suspended in the water column. This allows detection in mussel tissue of contaminants that may be present below detection limits in particulate matter, sediment, or water. Use of mussels also provides insight into the biologically available portion of contaminants, which may not readily be discerned from background sediment or water concentrations.

This report presents and summarizes contaminant data from the collection and analysis of blue mussel tissue collected in 2014 from three sites along the Maine coast. All mussel tissue samples were analyzed for heavy metals (including mercury), PCBs, and PAHs; and two of the sites were analyzed for PFCs. In order to provide comparability of results from these 2014 samples, blue mussel contaminant levels from the SWAT program are compared to blue mussel contaminant levels in other programs including the Gulfwatch program ("Gulfwatch": Gulf of Maine Council on the Marine Environment) and the National Status & Trends Mussel Watch Program ("NS&T": National Oceanographic and Atmospheric Administration). This analysis provides a regional and national context to the Maine SWAT data.

1.1.2 Softshell Clams

Like blue mussels, softshell clams (*Mya arenaria*) are consumed as food by humans and can be used to understand potential human exposure to contaminants. Clams are sessile, allowing attribution of their contaminant burdens to the environment where they were collected. Like mussels, clams filter large volumes of water as they feed, allowing them to concentrate many chemicals from the water column or from sediments suspended in the water column. Softshell clam stations sampled by the SWAT program in recent years have been selected to characterize contaminant concentrations specifically in clam tissue, as opposed to blue mussel tissue which may or may not have been sampled previously in the same general area. Gulfwatch and SWAT softshell clam tissue contaminant data suggest that clams may have very different concentrations of some contaminants than blue mussel tissue taken from the same stations. This is an important point when considering the contaminant concentrations to which humans are exposed when consuming clams. Site selection for clam testing is typically driven by human consumption and exposure, and clams are used less than blue mussels in SWAT (or Gulfwatch) as a general environmental monitor or sentinel.

This report presents and summarizes contaminant data from the analysis of softshell clam tissue samples collected in 2014 from two sites on the Maine coast. Also presented are softshell clam contaminant data from ten additional sites sampled in 2004-05 and in 2010-13 by the SWAT program. Softshell clam tissue samples from one site were analyzed for metals, mercury, PAHs, and PCBs. Tissues from the second clam site were analyzed for metals. In order to provide comparability of results from the 2010-13 and 2004-05 samples, softshell clam contaminant concentrations from SWAT sampling are compared to contaminant concentrations from the Gulfwatch program to provide regional context.

The Maine Dept. of Marine Resources (DMR) has asked Maine DEP to sample clams in areas currently closed to shellfish harvest, which usually is due to the presence of bacterial contamination that prevents safe consumption of the clams by humans. Some significant clam resources demonstrate improving bacterial indicator counts or may be candidates for additional work to reduce bacterial contamination in the vicinity of the resource. Without corresponding SWAT contaminant data from clam tissue to document safe levels for human consumption, expenditure of resources to reduce bacterial contaminant sources might be premature if high SWAT contaminant concentrations are confirmed. Bacterial source cleanup efforts should be targeted to clam resources that already have been documented as safe for human consumption from a SWAT contaminant concentration perspective. As with mussels, testing clams at sites with low contaminant levels, which can only be determined post-sampling, still provides valuable data on background contaminant levels in clams and provides a baseline with which to compare more heavily contaminated sites.

1.2 METHODS

Sites sampled in recent years within the context of this program can be divided into three types based on the goals outlined above that drive the need for information. These types are Spatial, Temporal, and Follow-Up sites. Sites that have never been sampled (or that have not been sampled for eight or more years), have been sampled for only one analyte type, or have been sampled with no replication are classified as “Spatial” sites. The primary reason for sampling these sites is to provide data required to fill geographic gaps. Spatial sites enable a more complete picture of how contaminants vary along the Maine coastline, and provide screening data that can be used in assessing interest in testing these sites again in the future. Testing sites with low contaminant levels, which can only be determined post-sampling, still provides valuable data on background contaminant levels and provides a baseline with which to compare more heavily contaminated sites.

“Temporal” sites are locations where there is an interest in obtaining data to assess contaminant levels over time. These sites will be sampled on an accelerated schedule, with sampling occurring as often as biennially. More frequent data collection will provide more closely spaced data over time, which may permit trend analysis when sufficient data are acquired. Relatively few temporal sites will be sampled to minimize costs associated with higher frequency sampling.

“Follow-up” sites are those where previous SWAT contaminant levels (or results from another program like Gulfwatch) at the site or nearby indicate that additional sampling and analysis are warranted. Repeat sampling may occur at the same location in an attempt to confirm earlier results, or sampling of additional nearby sites might be used to determine local contaminant distribution. Follow-up sites may include sites in the Temporal or Spatial categories as well based on their historical sampling and data needs.

Resampling in subsequent years at Temporal or Follow-up sites does not occur at the exact sub-site replicate coordinates sampled previously, but varies somewhat due to distribution and quantity of mussels available in the target size range from year to year. Samples from a site include mussels taken from four distinct, sub-site replicates or locations within the site. The slight spatial variation in sub-site replicates sampled provides additional information regarding patchiness of contaminants, and arithmetic means across all four sub-site replicates are used to compare between years.

1.2.1 Blue Mussels

Blue mussel samples have been analyzed from more than 90 distinct locations sampled over the past 28 years. Blue mussels were collected from three sites during September, 2014. All three of the mussel sites had been sampled previously as part of the SWAT program and are shown in Table 1.2.1.1. A map of the blue mussel sampling locations is provided in Figure 1.2.1.1.

Methodology of field collection, morphometric measurement, and laboratory preparation of mussel samples has been provided in previous SWAT reports and in the Gulfwatch field manual (Sowles et al., 1997) and will be reviewed here to familiarize the reader with

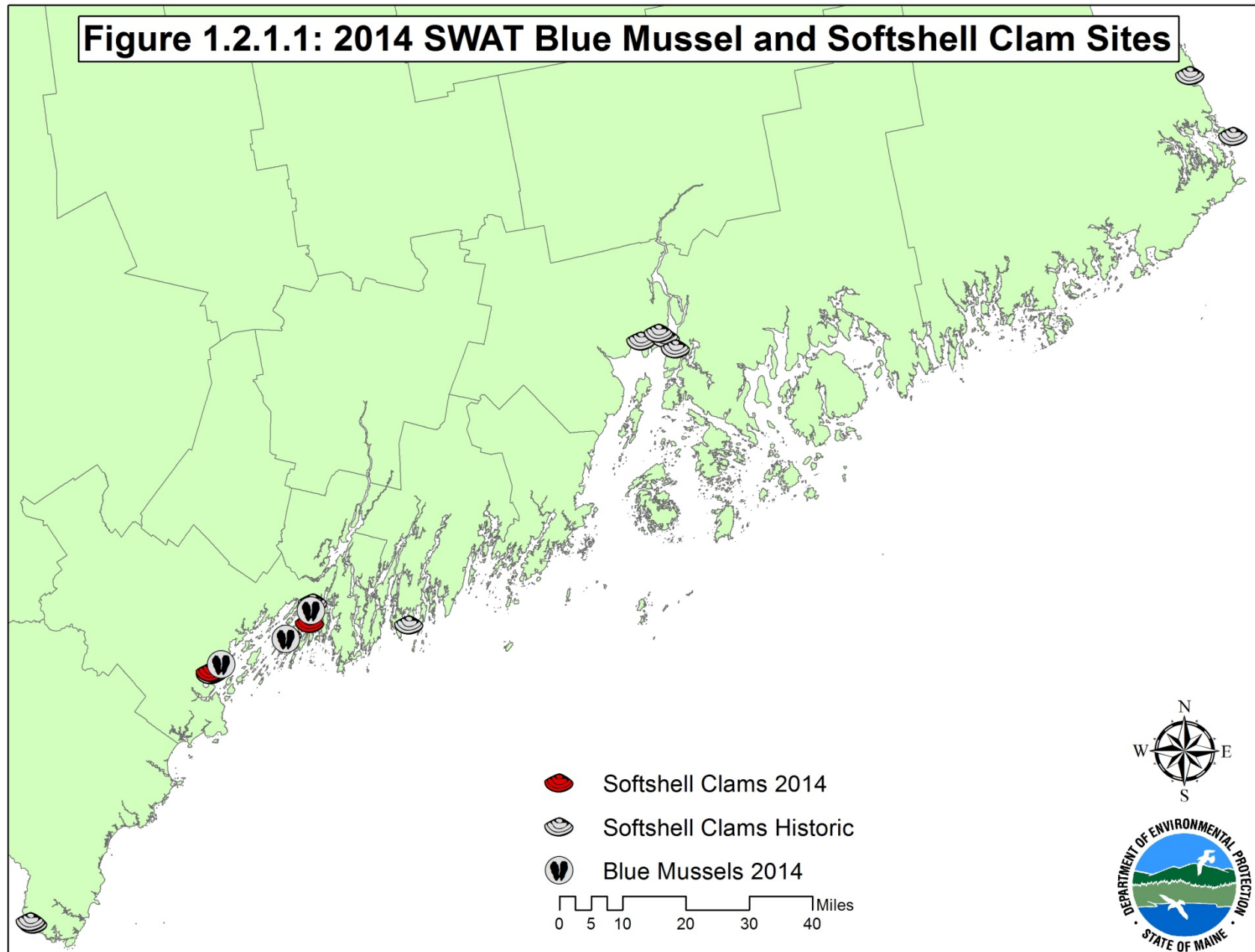
the general approaches used. SWAT mussel sampling is planned and conducted to control as much as possible any variability in factors that might cause a sample to be non-representative of the overall data being collected. Variations in mussel shell size, seasonal timing of collections relative to spawning, location within the intertidal zone, and sample location were all minimized to reduce conflicting signals in the contaminant data.

TABLE 1.2.1.1: 2014 SWAT Blue Mussel Sites						
<u>Site Name</u>	<u>Municipality</u>	<u>Station Code</u>	<u>West Longitude</u>	<u>North Latitude</u>	<u>Date Sampled</u>	<u>Site Type</u> ¹
Mill Creek	Falmouth	CBMCMC	-70.2214	43.7179	9/17/2014	T
Navy Pier	Harpswell	CBHWNP	-70.0177	43.7781	9/19/2014	F, S
Mare Brook	Brunswick	CBMBBH	-69.9391	43.8433	9/29/2014	F, S
1 S = Spatial, T = Temporal, F = Follow Up						

In order to characterize the contaminants present in a general area at the sampling site, mussels were collected along the shoreline from four distinct intra-site locations whenever possible. Sampling at Mill Creek, Falmouth, included only three areas due to a paucity of blue mussels. Gauges were used to sort mussels by shell length in the field, and mussels within a size range of 50-60 mm were selected for analysis. For metals analysis, a minimum of 20 mussels within the target size range were selected from each of the four intra-site locations (replicates) and placed in separate containers. For organics analysis including PAHs, PCBs, and PFCs, a minimum of 30 mussels were collected at each intra-site location. Mussels were washed in ambient sea water in a mesh or open bucket at the collection site to remove external debris and attached sediments. Mussel replicates were then transported to the laboratory in coolers (supplemented with ice packs in warmer weather). Mussels were not depurated prior to shucking to remove tissue for analysis.

Tissue sample processing was accomplished within 24 hours of field collections at all sites. At the laboratory, individual mussels were measured with calipers for length (anterior umbo to posterior growing edge) to the nearest 0.1 mm. Shell height and width (mm) and soft tissue wet weight (nearest 0.1 g) were also measured and recorded for ten mussels per replicate. All soft tissue was removed and combined with the soft tissue from mussels within the same replicate. Total soft tissue wet weights per replicate were recorded. Tissue composites were immediately placed in pre-cleaned glass jars and capped. Jars were pre-labeled and filled jars were stored at -5° C for up to two months until analysis.

Frozen mussel tissue was shipped overnight to the appropriate laboratory for analysis. Mussel tissues tested for PAHs, PCBs, and PFCs were analyzed by AXYS Analytical Services Ltd., Sidney, British Columbia. Mussel tissue tested for metals were analyzed by Alpha Analytical, Westborough, Massachusetts.



1.2.2 Softshell Clam

Softshell clams were collected at two sites in 2014. These locations were selected based on Maine DMR interest in potentially opening clam flats that had been closed due to high bacterial levels. The Presumpscot River was sampled previously by SWAT, although the area was resampled on a wider geographic scale to include flats where much of the historic clam resource was thought to exist. The second site, Strawberry Creek, was sampled to obtain first time data. In addition to the two sites sampled in 2014, this report includes data from ten softshell clam sites sampled in 2010-13 and 2004-05 (Table 1.2.2.1; Figure 1.2.1.1). These data are included to provide a broader context for softshell clam contaminant concentrations across the state.

TABLE 1.2.2.1: SWAT Softshell Clam Sites: 2004-05, 2010-14						
<u>Site Name</u>	<u>Municipality</u>	<u>Station Code</u>	<u>West Longitude</u>	<u>North Latitude</u>	<u>Date Sampled</u>	<u>Site Type</u> ¹
Mast Cove	Eliot	PQMCMC	-70.8048	43.1210	11/9/2004	S
Mast Cove	Eliot	PQMCMC	-70.7981	43.1155	10/16/2013	S
Presumpscot R.	Falmouth/Portland	CBPRMT	-70.2460	43.6981	10/9/2012	S
Presumpscot R.	Falmouth/Portland	CBPRMT	-70.2543	43.6943	10/25/2013	S
Presumpscot R.	Falmouth/Portland	CBPRMT	-70.2542	43.6999	10/30/2014	S
Navy Pier	Harpswell	CBHWNP	-70.0136	43.7870	11/12/2004	S
Mare Brook	Brunswick	CBMBBH	-69.9334	43.8617	10/11/2012	S
Strawberry Creek	Harpswell	CBHASC	-69.9434	43.8145	10/28/2014	S
Squirrel Island	Southport	MCBBSQ	-69.6290	43.8130	11/8/2004	S
Long Cove	Searsport	PBSTLC	-68.8938	44.4656	12/1/2005	S
Fort Point Cove	Stockton Springs	PBFPPF	-68.8150	44.4717	11/10/2005	S
Fort Point Cove	Stockton Springs	PBFPPF	-68.8372	44.4832	11/3/2011	F
Morse Cove	Penobscot/Castine	PBCAMC	-68.7835	44.4478	11/16/2010	S
Harris Cove	Eastport	PMHCHC	-66.9838	44.9171	11/9/2004	S
Mill Cove	Robbinston	PMSCMC	-67.1176	45.0580	11/29/2005	S
1 S = Spatial, T = Temporal, F = Follow Up						

Methodology of field collection, morphometric measurement, and laboratory preparation of mussel samples has been provided in previous SWAT reports and in the Gulfwatch field manual (Sowles et al., 1997), and any departures from that methodology in softshell clam sampling are noted in the following text. In order to characterize the contaminants present in a general area at the sampling station, softshell clams were collected from four distinct areas (replicates) along the shoreline at each site whenever possible. Clams at or above the commercial legal length of two inches (50.8 mm) were dug from each intra-site location. For metals analysis, a minimum of ten clams within the target size range were selected from each of the four intra-site locations and placed in separate containers. For organics analysis, a minimum of 20 clams was collected at each intra-site location. Replicates were washed in ambient sea water in a mesh or open bucket at the collection site to remove external debris and attached sediments. Clam replicates were then

transported to the laboratory in coolers (supplemented with ice packs in warmer weather). Clams were not depurated prior to shucking to remove tissue for analysis.

Tissue sample processing was accomplished within 24 hours of field collections. At the laboratory, individual clams were measured with calipers for length (longest shell measurement perpendicular to a line extending from the umbo to the growing edge) to the nearest 0.1 mm. Shell height and width (mm) and soft tissue wet weight (nearest 0.1 g) were also measured and recorded for ten clams in each replicate. All soft tissue was removed and combined with the soft tissue from the ten clams within the same replicate. Total soft tissue wet weights for each ten-clam replicate were recorded. For organics analysis (PAHs, PCBs, and PFCs), 10-20 clams were composited into a replicate to produce the 100 grams of tissue required for the analyses.

Tissue composite samples for metals analyses included ten clams per replicate, and tissue composite samples for organics analyses included 10-20 clams per replicate. For both metals and organics, four replicates were collected per sampling station. Tissue composites were immediately placed in pre-cleaned glass jars and capped. Jars were pre-labeled and filled jars were stored at -5° C for up to two months until analyses could be completed. Frozen tissue was shipped overnight to the laboratory for analysis. Softshell clam tissues tested for PAHs, PCBs, and PFCs in 2010-14 were analyzed by AXYS Analytical Services Ltd., Sidney, British Columbia. Clam tissues tested for metals in 2010-13 were analyzed by Pacific Northwest National Laboratory operated by Battelle, Sequim, Washington. Metals analyses in 2014 were completed by Alpha Analytical, Westborough, MA. Clam tissues tested in 2004-05 for both the metals and organic contaminants were analyzed by Pace Analytical, Minneapolis, MN.

1.3 RESULTS AND DISCUSSION

1.3.1 Metals

1.3.1.1 Blue Mussels

Mussel tissue samples collected in 2014 were analyzed for 11 metals: Silver (Ag), aluminum (Al), arsenic (Ar), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), mercury (Hg), nickel (Ni), and zinc (Zn). Results were compared to national NS&T (Kimbrough et al., 2008) and Gulf of Maine (Gulfwatch) (LeBlanc et al., 2009) blue mussel monitoring program data (collected through 2008, the most recent available) to place Maine SWAT data in a broader geographic context. From an environmental monitoring perspective, the concentration of an analyte in SWAT mussel tissue was considered elevated when that concentration exceeded the NS&T 85th percentile. This approach is consistent with the Gulfwatch program (LeBlanc et al., 2009).

1.3.1.1.1 Silver (Ag)

Silver was not detected at any of the three locations sampled in 2014. Silver laboratory reporting limits at the testing facility used in 2014 exceeded the Gulfwatch median and 85th percentile concentrations. Utilizing a different laboratory in previous years, typical silver concentrations detected often fell between the Gulfwatch median and 85th

percentile concentrations. In future, an alternate laboratory will be utilized which has a silver reporting limit low enough to provide detection of silver throughout the range expected in blue mussel tissue samples.

Higher silver concentrations in water and sediments have been shown to coincide with municipal sewage discharge (Sanudo-Wilhelmy and Flegal, 1992; Buchholtz ten Brink et al., 1997). The increasing use of silver, including nanosilver, in products like paints, caulking, and clothing makes monitoring silver of interest at present and in the future. Overall, silver concentrations in mussels from sampled locations appear to be relatively low. The highest Gulfwatch values, which came from sites in the Neponset River and Sandwich, Massachusetts exceeded the NS&T median but were below the NS&T 85th percentile.

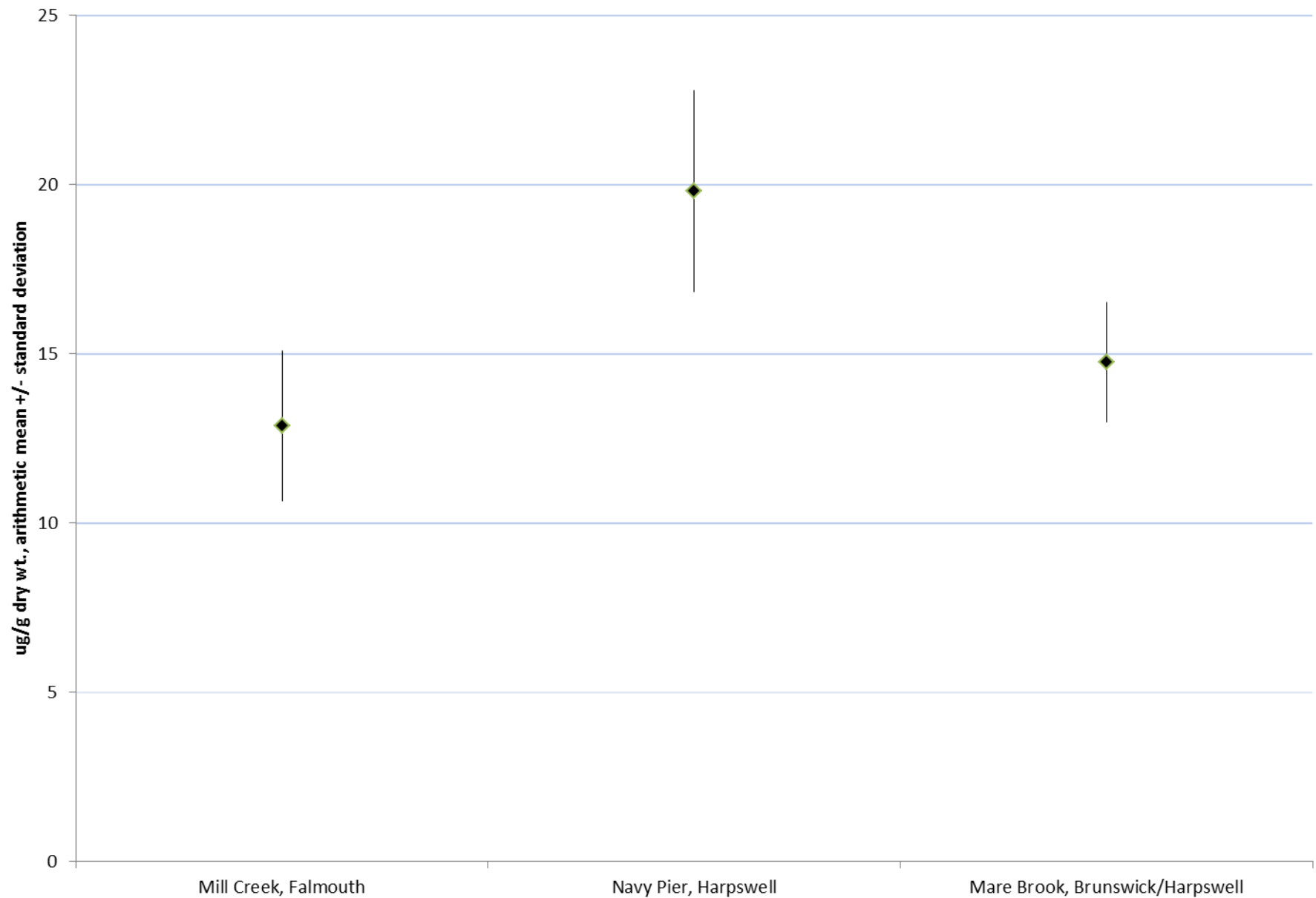
The MCDC silver non-cancer FTAL is 11 µg/g wet wt. for non-commercially caught fish. In prior sampling, the highest SWAT blue mussel tissue mean silver concentration, when expressed on a wet weight basis, was approximately three orders of magnitude below the 11 µg/g wet wt. FTAL.

1.3.1.1.2 Arsenic (As)

Arsenic was detected in mussel tissue at all three locations sampled in 2014 (Figure 1.3.1.1.2.1). Arsenic levels measured in mussels ranged from a low mean concentration of 12.87 µg/g dry wt. at Mill Cove, Falmouth, to a high mean concentration of 19.80 µg/g dry wt. at Navy Pier, Harpswell. While Gulfwatch does not monitor arsenic concentrations, they are tracked regionally and nationally by NS&T. In blue mussels, NS&T considers 12-22 parts per million dry wt. (directly comparable to SWAT µg/g data) to be in the mid-range of three ranges of arsenic concentration nationally (Kimbrough et al., 2008). All three blue mussel sites sampled in 2014 had arsenic concentrations which fell into the mid-range of the three NS&T ranges.

Nationally, the primary source for elevated levels of arsenic is crustal rock. In addition to natural sources, industrial pollution can contribute arsenic to the environment from preserved wood, semiconductors, pesticides, defoliants, pigments, antifouling paints, and veterinary medicines. Atmospheric sources include smelting, fossil fuel combustion, power generation, and pesticide application (Kimbrough et al., 2008).

For non-commercially caught finfish, MCDC reports a cancer FTAL of 0.014 µg/g and a non-cancer FTAL of 0.6 µg/g, both for inorganic arsenic (the most toxic form). Most fish tissue data and the SWAT blue mussel tissue data are analyzed for total arsenic, not inorganic arsenic. MCDC uses FDA's 1993 assumption that 10% of total arsenic in finfish is inorganic arsenic. Using this assumption, approximate inorganic arsenic concentrations for SWAT blue mussels were calculated by dividing wet weight concentrations by a factor of 10. Therefore, 2014 SWAT blue mussel inorganic arsenic concentrations are estimated to range from 0.19 µg/g wet wt. to 0.28 µg/g wet wt. All three sites exceeded the MCDC cancer FTAL of 0.014 µg/g wet wt.

Figure 1.3.1.1.2.1: Arsenic in 2014 SWAT Blue Mussels

Comparing recent data from all 60+ mussel sites sampled from 2007-14, the calculated inorganic arsenic concentrations in SWAT blue mussel tissue ranged from a low of 0.11 µg/g wet wt. (Bar Harbor, 2007) to a high of 0.33 µg/g wet wt. (Turnip Island, Georgetown, 2012). All SWAT sites sampled from 2007-14 had calculated blue mussel tissue inorganic arsenic concentrations exceeding the MCDC cancer action level of 0.014 µg/g wet wt. None of the three sites sampled in 2014 were calculated to have exceeded the MCDC non-cancer action level of 0.6 µg/g wet wt. for inorganic arsenic. Similarly, none of the 60 mussel stations sampled from 2007-13 were calculated to have exceeded the MCDC non-cancer FTAL. The MCDC non-commercially caught finfish FTALs applied here assume an 8 oz. meal eaten by the consumer on a weekly basis. Maine SWAT data indicate that this 8 oz. meal size would translate to approximately 45-50 mussels per meal.

1.3.1.1.3 Cadmium (Cd)

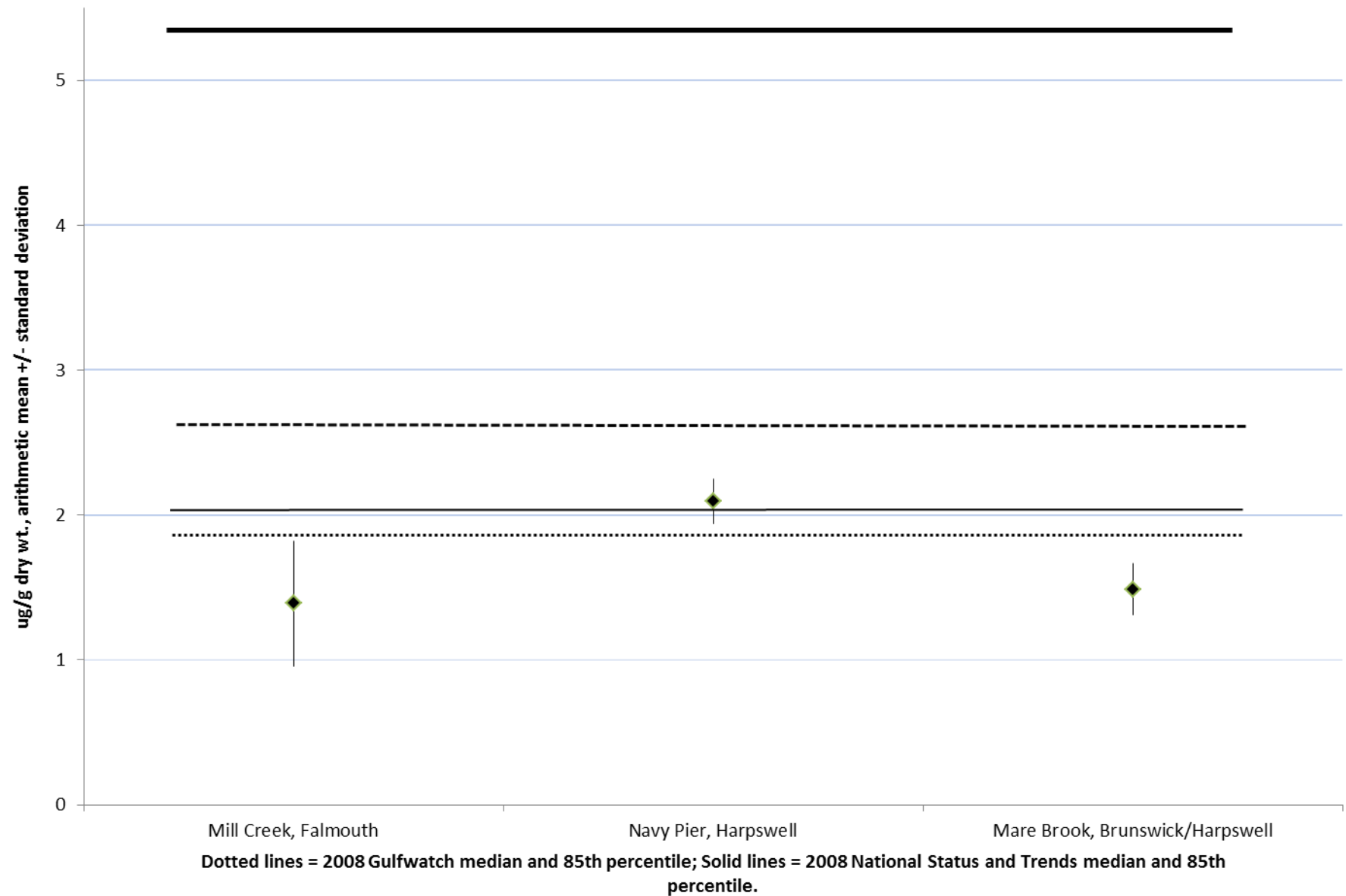
Cadmium was detected in samples taken at all three locations visited in 2014 (Figure 1.3.1.1.3.1). Cadmium levels measured in mussels ranged from a low mean concentration of 1.39 µg/g dry wt. at Mill Creek, Falmouth, to a high mean concentration of 2.10 µg/g dry wt. at Navy Pier, Harpswell. The cadmium concentrations at Mill Creek and at Mare Brook, Brunswick, fell below the 2008 Gulfwatch median, while the concentration at Navy Pier exceeded both the Gulfwatch and NS&T medians but fell below both 85th percentiles (Figure 1.3.1.1.3.1) (Kimbrough et al., 2008). Since tissue cadmium concentrations did not exceed the NS&T 85th percentile, no sites were considered elevated for cadmium.

Cadmium originates from crustal elements as rocks weather and is transported seaward by rivers, which account for approximately half of all cadmium sources worldwide. Cadmium is also released through forest fires and volcanic activity, with anthropogenic sources including manufacturing, fossil fuel combustion, and agriculture. Industrial sources include manufacture of batteries, plating, stabilizers, and nuclear power (Kimbrough et al., 2008).

From a human health perspective, the MCDC non-cancer FTAL for cadmium in non-commercially caught finfish is 2.2 µg/g wet wt. The FDA action level for clams, oysters, and mussels is 4 µg/g wet wt. (Kimbrough et al., 2008). The highest scoring 2014 SWAT site, Navy Pier, Harpswell, had a mean cadmium concentration of 0.30 µg/g wet wt., which is below the MCDC and FDA action levels.

1.3.1.1.4 Chromium (Cr)

Chromium was detected in samples taken at all three sites sampled in 2014. However, chromium concentrations at all three sites appeared to exceed previous statewide mussel tissue concentrations by at least a factor of three. Furthermore, all three mean concentrations exceeded the NS&T national 85th percentile by at least a factor of two. The data are considered unreliable at this time and reanalysis of samples will be undertaken by the laboratory to confirm concentrations.

Figure 1.3.1.1.3.1: Cadmium in 2014 SWAT Blue Mussels

Natural sources of chromium include leaching from soil and rock into surface waters. Chromium is released from textile, electroplating, and leather tanning industries. Chromium is used extensively in tanning leather and was frequently discharged with untreated tannery effluent during the last two centuries. Chromium persists in the marine environment in sediments near anthropogenic sources (Kimbrough et al., 2008).

From a human health perspective, the MCDC FTALs (7 µg/g cancer action level and 11 µg/g non-cancer action level) for chromium are based on chromium VI, and are not directly comparable to SWAT results, which measure total chromium (less toxic Cr III and more toxic Cr VI, combined).

1.3.1.1.5 Copper (Cu)

Copper was detected in tissue taken at all three SWAT mussel sites sampled in 2014 (Figure 1.3.1.1.5.1). Copper levels measured in mussels ranged from a low mean concentration of 6.08 µg/g dry wt. at Mill Cove, Falmouth, to a high mean concentration of 7.10 µg/g dry wt. at Mare Brook, Brunswick. Copper concentrations at Navy Pier, Harpswell, and Mare Brook, Brunswick, exceeded the Gulfwatch median but not the 85th percentile (LeBlanc et al., 2009). Mill Cove, Falmouth, had a copper concentration below the Gulfwatch median. SWAT copper concentrations at all three sites sampled in 2014 fell below the NS&T median and 85th percentile (Figure 1.3.1.1.5.2) (Kimbrough et al., 2008). None of the three sites sampled in 2014 was considered elevated for copper.

Copper occurs naturally and is ubiquitous throughout the marine environment. Copper in trace amounts is considered to be an important nutrient for plant and animal growth. Elevated copper concentrations can occur due to contributions from anthropogenic sources including mining, agriculture, sewage sludge, antifouling paint, fungicides, wood preservatives, and brake pads. With the reduction of the use of chromated copper arsenate (CCA) wood preservative subsequent to its being phased out by EPA, newer wood preservatives utilizing even higher levels of copper have come into use, including quaternary copper. Similarly, tributyltin marine bottom paint use was reduced in the 1980s, resulting in increased use of copper-based antifouling paints, and removal of asbestos from the manufacture of brake pads has been offset by increased usage of copper in their manufacture (Kimbrough et al., 2008).

Copper is not highly toxic to humans, though exposure can lead to some chronic effects. There is no recommended FDA safety level for human consumption for copper in fish or shellfish (Kimbrough et al., 2008), and MCDC does not report a FTAL for copper in non-commercially caught sportfish.

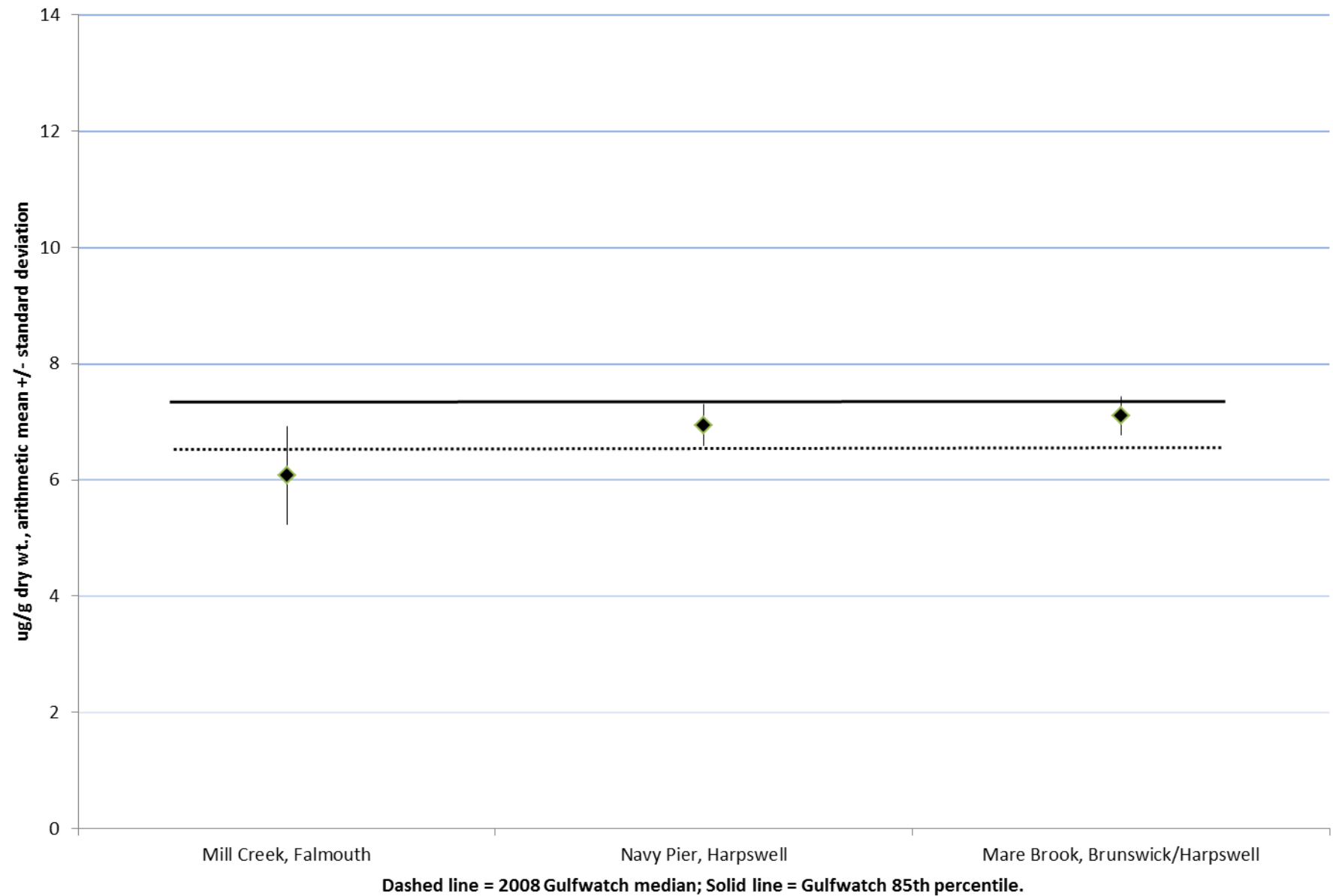
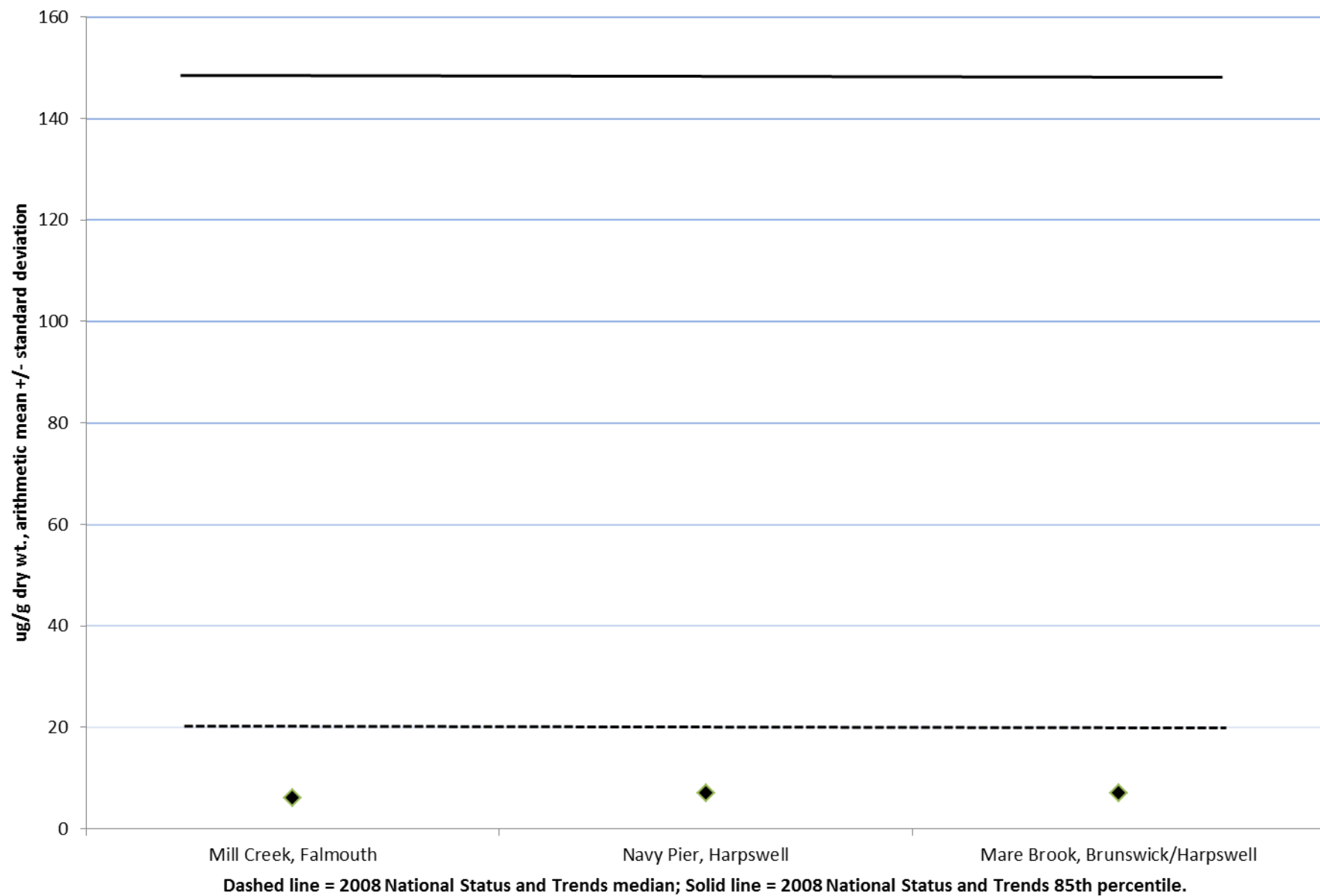
Figure 1.3.1.1.5.1: Copper in 2014 SWAT Blue Mussels

Figure 1.3.1.1.5.2: Copper in 2014 SWAT Blue Mussels

1.3.1.1.6 Iron (Fe) and Aluminum (Al)

Iron was detected in tissue from all three SWAT blue mussel sites sampled in 2014 (Figure 1.3.1.1.6.1). Iron concentrations measured in mussels ranged from a low mean concentration of 298 µg/g dry wt. at Mill Creek, Falmouth, to a high mean concentration of 659 µg/g dry wt. at Mare Brook, Brunswick. The iron concentration in samples from Mill Creek was below the Gulfwatch median, while the concentration in samples from Navy Pier, Harpswell, and Mare Brook exceeded the Gulfwatch median. Mare Brook samples also exceeded the Gulfwatch 85th percentile. Figure 1.3.1.1.6.1 also shows a comparison of SWAT mean iron concentrations to NS&T median and 85th percentile iron concentrations. The iron concentrations at both Navy Pier and Mare Brook were between the NS&T median and 85th percentile. No site had an iron concentration in mussel tissue that exceeded the NS&T national 85th percentile and consequently no site was considered elevated for iron.

Aluminum concentrations fell below the approximately 350 to 370 µg/g dry weight reporting limit (RL) provided by the laboratory. This was higher than previous RLs provided for blue mussel tissue analysis and so all three sites sampled in 2014 were reported as non-detects. The provided RL occurs between the Gulfwatch median and 85th percentile and between the NS&T median and 85th percentile. With this RL, none of the sites was considered to have a mussel tissue concentration elevated in aluminum, which would have resulted in detection of the aluminum concentration with this RL.

High iron and aluminum concentrations are usually associated with the intake of high levels of suspended sediments by mussels at sampled sites, with both metals being common components of crustal rocks and coastal sediments. This correlation has also been shown with gut depuration experiments conducted as part of Gulfwatch monitoring in previous years, indicating that some of the iron and aluminum is associated with gut contents and not bioaccumulated loads (Leblanc et al., 2009). Monitoring for iron and aluminum provides an important reference to gauge sediment intake by mussels, allowing iron and aluminum levels to be referenced if other more toxic metals or contaminants are detected in mussel tissue.

From a human health perspective, MCDC does not report FTALs for iron and aluminum.

1.3.1.1.7 Nickel (Ni)

Nickel was detected in tissue from all three SWAT blue mussel sites sampled in 2014 (Figure 1.3.1.1.7.1). Nickel levels measured in mussels ranged from a low mean concentration of 2.57 µg/g dry wt. at Mill Creek, Falmouth, to a high mean concentration of 3.02 µg/g dry wt. at Mare Brook, Brunswick. All three sites had nickel concentrations exceeding the Gulfwatch median and 85th percentile, and the NS&T median. None of the three sites had nickel concentrations exceeding the NS&T 85th percentile, so no SWAT sites were considered to be elevated for nickel. Higher nickel concentrations are probably associated with sediment ingestion, similar to iron and aluminum concentrations.

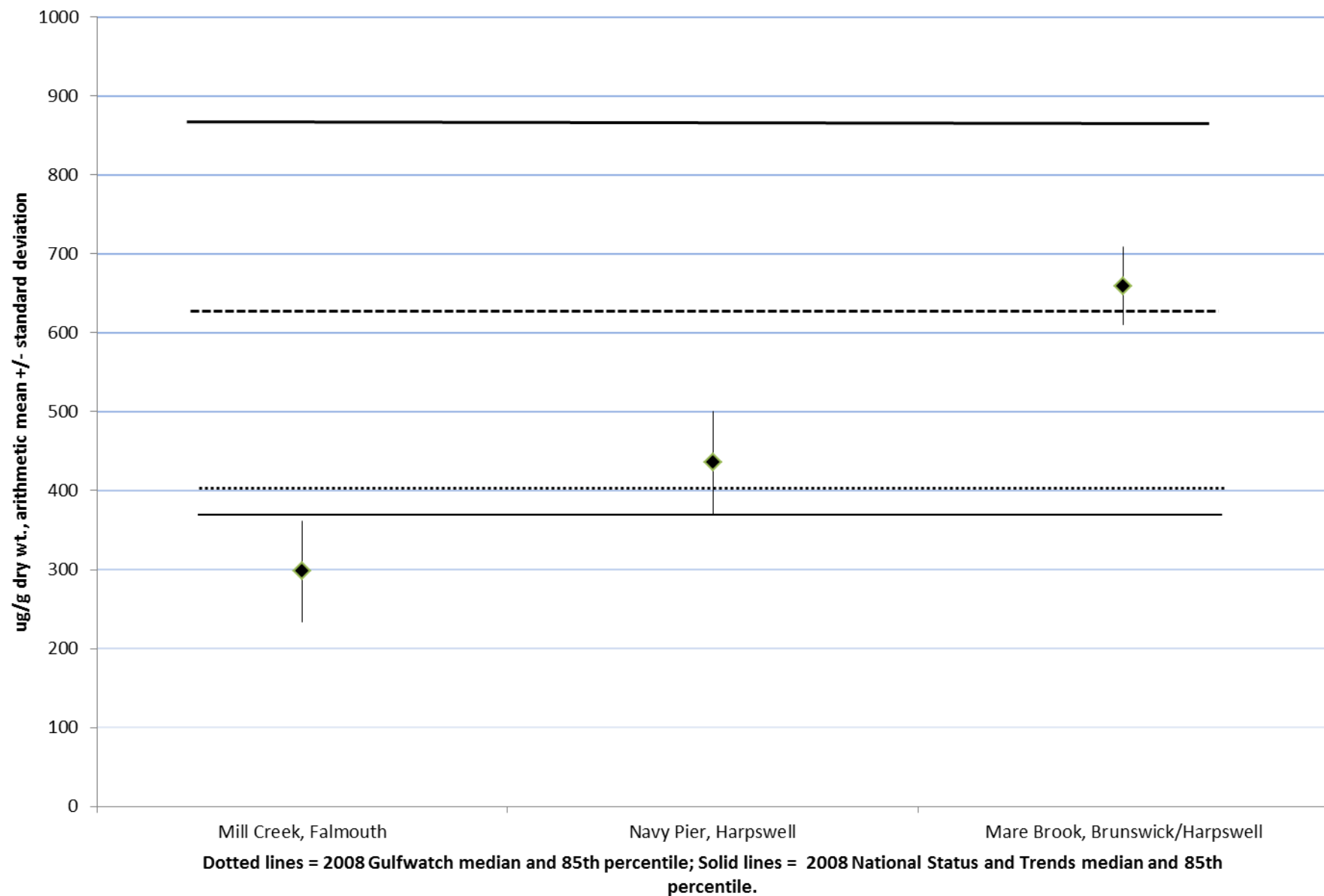
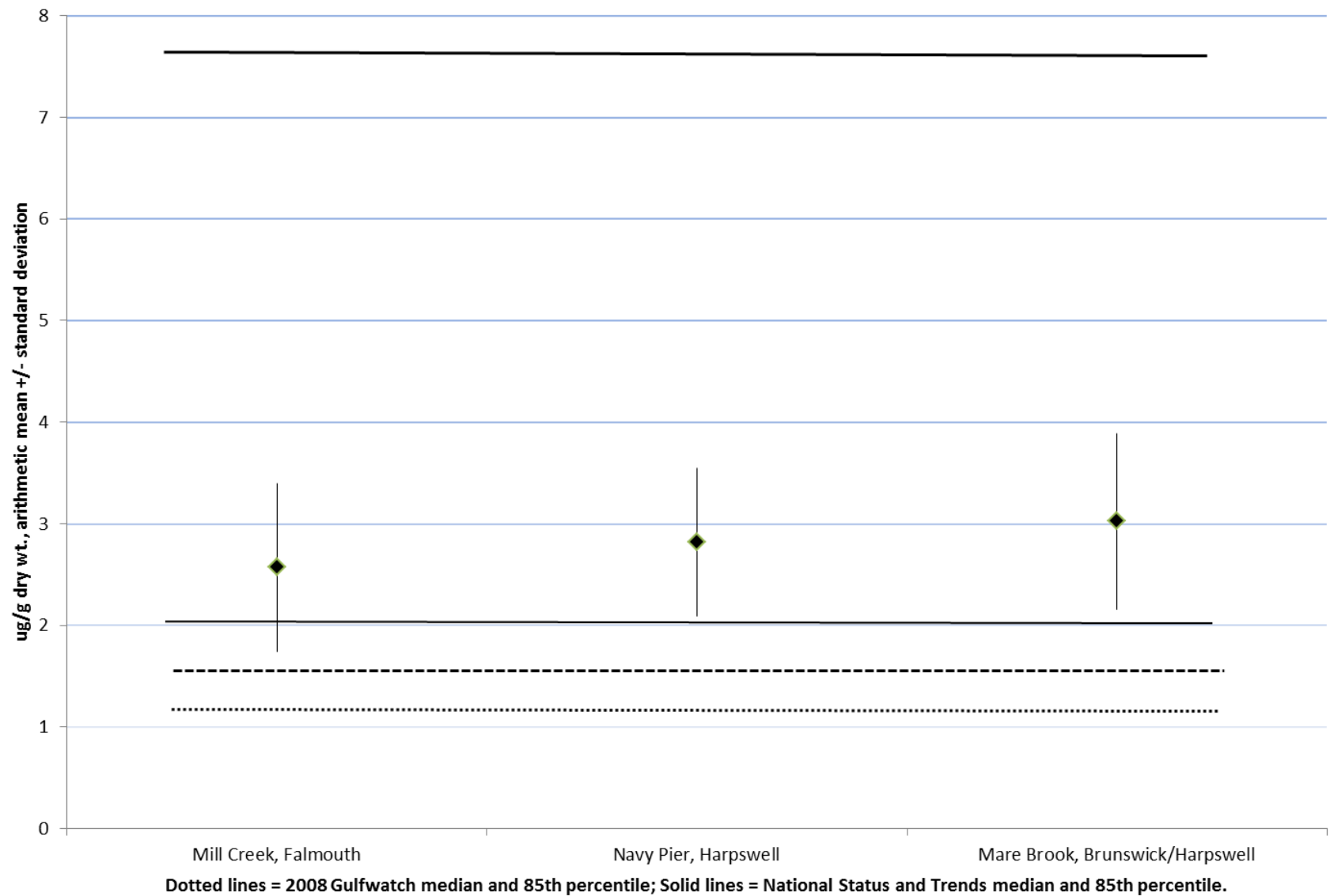
Figure 1.3.1.1.6.1: Iron in 2014 SWAT Blue Mussels

Figure 1.3.1.1.7.1: Nickel in 2014 SWAT Blue Mussels

Nickel occurs naturally in the environment and is essential to biological processes as a trace element. Nickel from soil and weathering of rocks enters rivers and provides the largest source of nickel to coastal waters. Nickel occurs in stainless steel, nickel-cadmium batteries, pigments, computers, wire, coins, and is used in electroplating. Elevated nickel concentrations occur in the Great Lakes and speculation about sources centers on air deposition from a large nickel smelting operation in Ontario, Canada (Kimbrough et al., 2008).

Nickel is not thought to bioaccumulate in the food chain; however, nickel can be harmful to humans in large doses, inducing effects including bronchitis and even cancer from long term exposure (Kimbrough et al., 2008). The MCDC reports a non-cancer FTAL for nickel in non-commercially caught finfish of 43 µg/g wet wt., which is more conservative than the FDA action level for shellfish of 80 µg/g wet weight. The maximum mean concentration detected by SWAT in 2014 of 0.13 µg/g wet wt. at Mare Brook is two orders of magnitude below the more conservative MCDC action level. MCDC does not report a cancer action level for nickel.

1.3.1.1.8 Lead (Pb)

Lead was detected in tissue from all three SWAT blue mussel sites sampled in 2014 (Figure 1.3.1.1.8.1). Lead levels measured in mussels ranged from a low mean concentration of 1.47 µg/g dry wt. at Navy Pier, Harpswell, to a high mean concentration of 1.92 µg/g dry wt. at Mare Brook, Brunswick. All three sites had lead concentrations less than the Gulfwatch median, but exceeding the NS&T median. None of the three sites had lead concentrations exceeding the NS&T 85th percentile and so none of the three blue mussel sites were considered elevated based on criteria in the SWAT and Gulfwatch programs (Figure 1.3.1.1.8.1).

Lead tissue concentrations from prior samples at a Temporal Site at Mill Creek, Falmouth, were compared to the 2014 concentration (Figure 1.3.1.1.8.2). Lead concentrations at Mill Creek appear to fluctuate somewhat from year to year, which is probably due to patchiness of contamination within the site. While more data will be required to demonstrate any trend, lead concentrations in mussel tissue at other Maine sites sampled in recent years suggest that concentrations are not increasing but have been relatively stable statewide (and Gulf-wide in the Gulfwatch program, as supported by longer-term data sets).

Upstream of the sampling site at Mill Creek, the upland area is developed with commercial land use including large office buildings and shopping malls, and Route 1 with substantial amounts of impervious surface that shed storm water. Repeated sampling at sites like Mill Creek should yield a more complete picture of trends in contaminants, including lead. Some inter-annual variability is to be expected especially with minor spatial differences between replicates. Contaminant patchiness may also be a factor in the variation in lead levels from year to year.

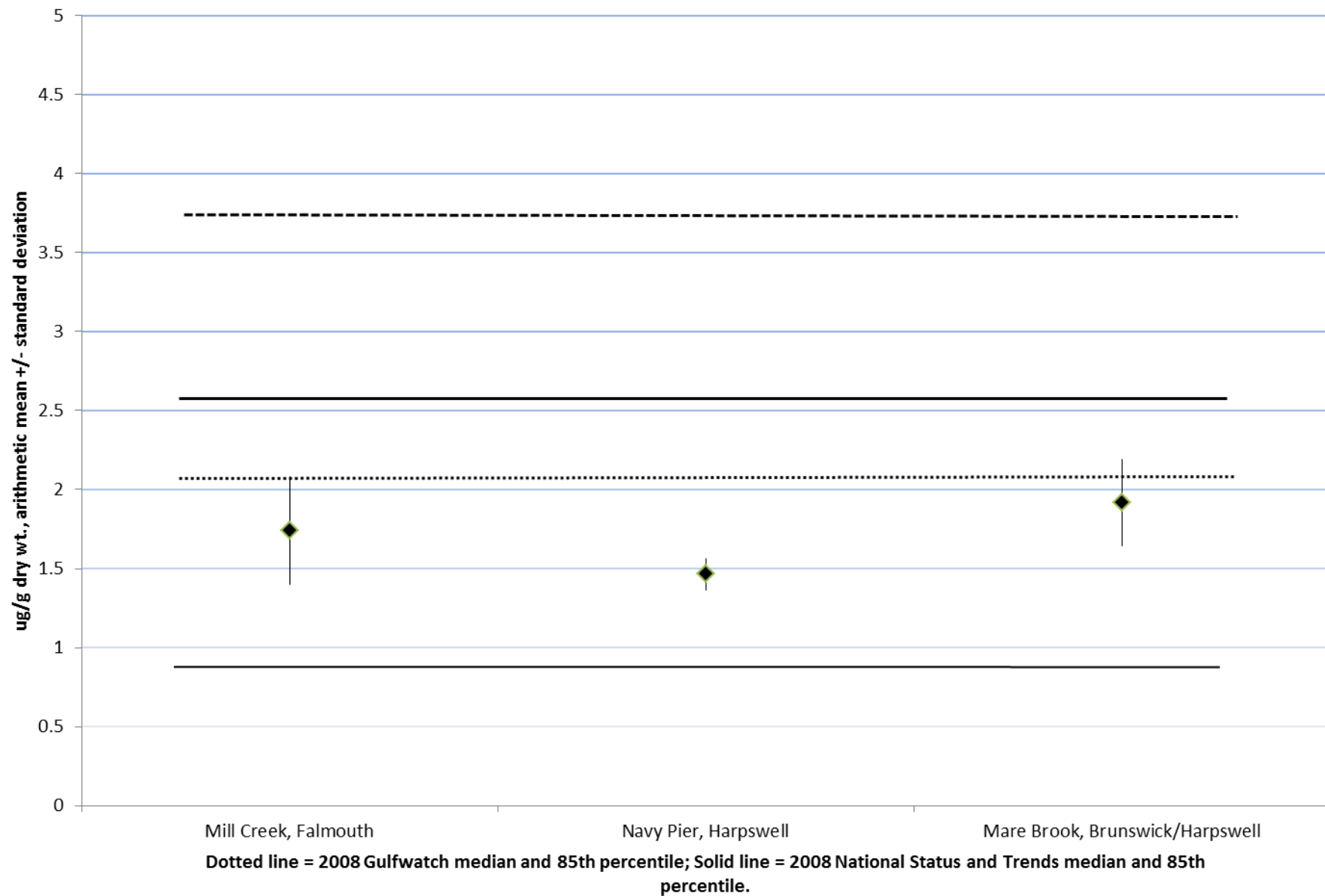
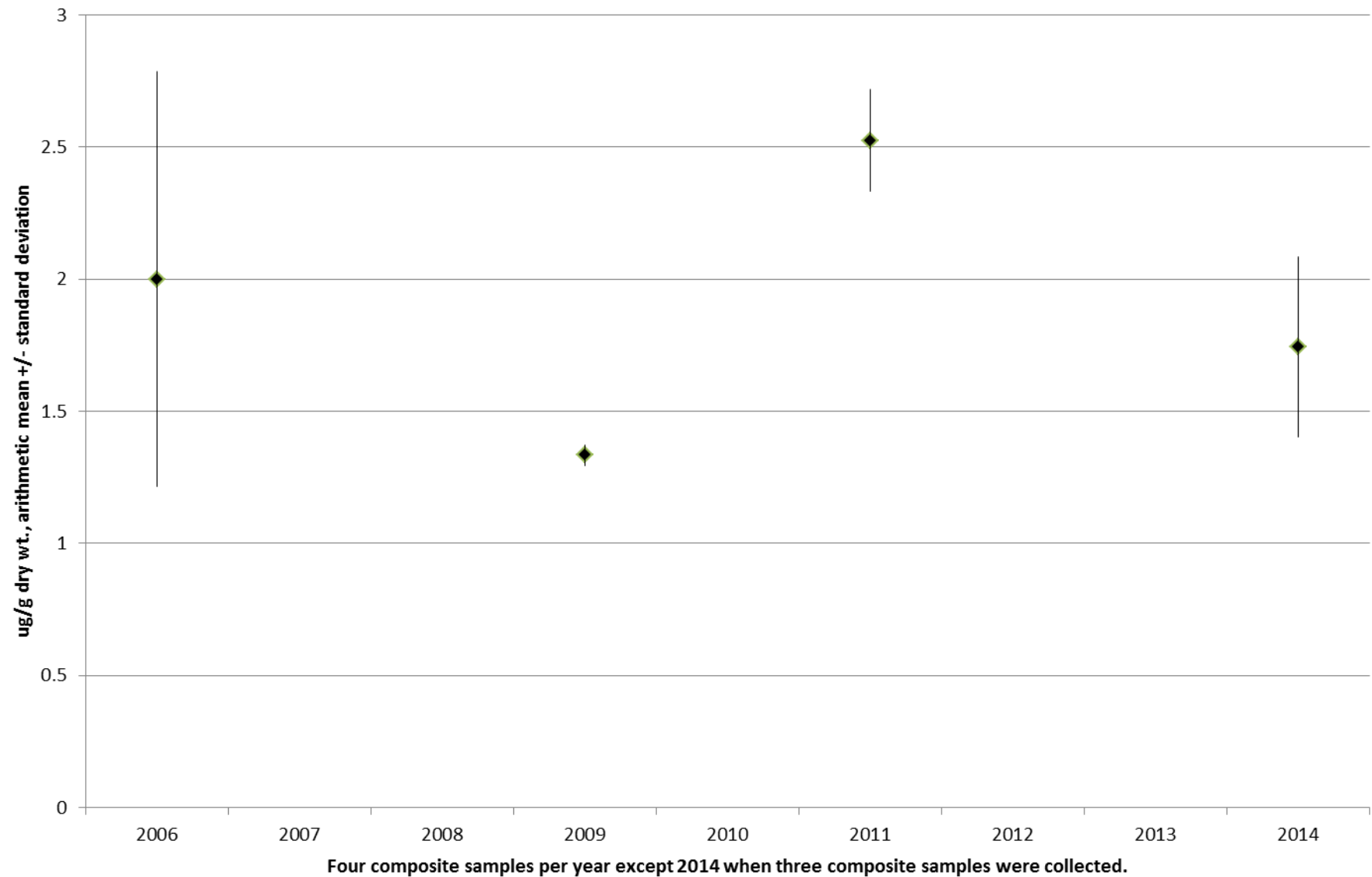
Figure 1.3.1.1.8.1: Lead in 2014 SWAT Blue Mussels

Figure 1.3.1.1.8.2: Trend in Blue Mussel Tissue Lead Concentrations from Mill Creek, Falmouth, 2006 - 2014



Lead occurs naturally in the earth's crust; however global lead concentrations in the environment have increased in the last century due to the use of leaded gasoline. Reduction in lead loading through regulation of leaded gasoline and lead paints has occurred in recent decades. Elevated lead levels in the environment also occur due to manufacturing, paints, lead solder, ammunition, plumbing, incineration and burning of fossil fuels. Lead loading in coastal waters is related to wastewater discharge, river runoff, atmospheric deposition, and natural weathering of crustal rock (Kimbrough et al., 2008).

From a human health perspective, the FDA action level for lead in clams, oysters, and mussels (molluscan shellfish) had been 1.7 µg/g wet wt. (Kimbrough et al., 2008). This limit apparently was dropped at the 2007 Interstate Shellfish Sanitation Conference. The more conservative MCDC lead FTAL in non-commercially caught sportfish is 0.6 µg/g wet wt., which is based on a blood lead concentration model. The highest mean concentration in the 2014 Maine SWAT mussel data, 0.27 µg/g wet wt. at Mare Brook, Brunswick, is less than half of the MCDC lead FTAL. The mean lead concentrations in tissues from the remaining two sites sampled in 2014 were slightly lower and also did not exceed the MCDC FTAL for lead.

Review of the 2007-14 SWAT blue mussel sampling data from 62 sites indicates that mean lead concentrations at eight sites equaled or exceeded the MCDC lead FTAL. Sites sampled in those years equaling or exceeding the MCDC FTAL for lead are:

Spring Point, S. Portland, 2007	0.6 ppm wet wt.
Spring Point, S. Portland, 2010	0.7 ppm wet wt.
Spring Point, S. Portland, 2012	0.6 ppm wet wt.
Middle Fore R., Portland, 2007	0.6 ppm wet wt.
East End Beach, Portland, 2007	0.8 ppm wet wt.
East End Beach, Portland, 2009	0.8 ppm wet wt.
East End Beach, Portland, 2011	0.9 ppm wet wt.
East End Beach, Portland, 2013	2.1 ppm wet wt.
Turnip Island, Georgetown, 2012	1.4 ppm wet wt.
Crockett Point, Rockland, 2007	1.1 ppm wet wt.
Crockett Point, Rockland, 2010	1.3 ppm wet wt.
Crockett Point, Rockland, 2011	1.1 ppm wet wt.
Ocean Pursuits Boat Yard, Rockland, 2013	0.6 ppm wet wt.
Town Landing, Rockland, 2013	0.9 ppm wet wt.

Camden Harbor, Camden, 2007	0.7 ppm wet wt.
Goose Falls, Brooksville, 2007	1.1 ppm wet wt.
Piscataqua River Back Channel, Kittery, 2008	0.6 ppm wet wt.

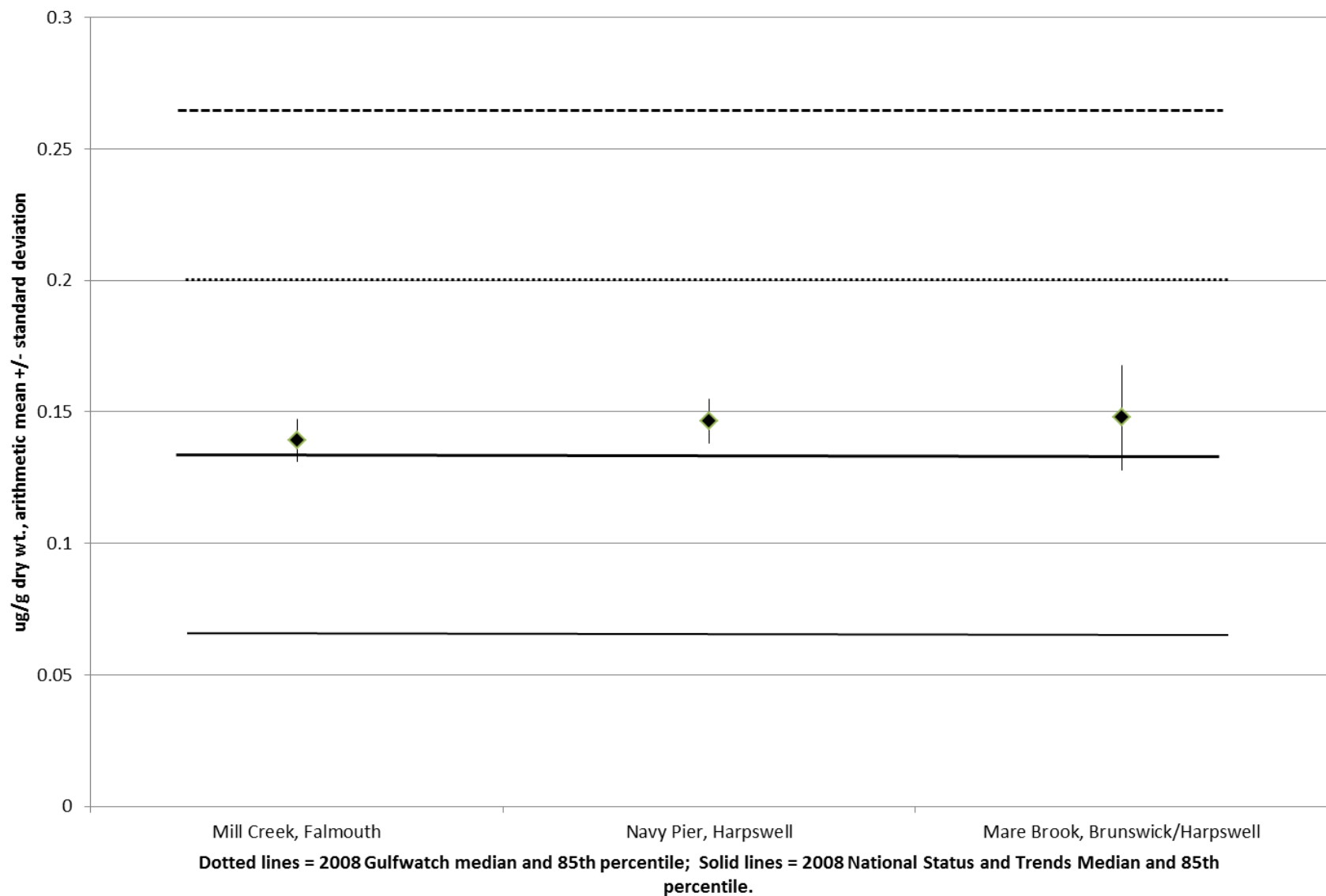
The MCDC lead FTAL is based on the consumer eating an 8 oz. meal. Maine SWAT data indicate that an 8 oz. meal would include approximately 45-50 mussels of the size tested by the SWAT program.

1.3.1.1.9 Mercury (Hg)

Mercury was detected in tissue from all three blue mussel sample locations tested in 2014 (Figure 1.3.1.1.9.1). Mercury levels measured in mussels ranged from a low mean concentration of 0.14 µg/g dry wt. at Mill Creek, Falmouth, to a high mean concentration of 0.15 µg/g dry wt. at Mare Brook, Brunswick. None of the three mercury concentrations exceeded the 2008 Gulfwatch median, and all three also fell below the Gulfwatch 85th percentile concentration. Figure 1.3.1.1.9.1 also compares 2014 SWAT blue mussel mercury concentrations to NS&T Mussel Watch median and 85th percentile values. The reader should note that Gulfwatch median and 85th percentile values actually exceed NS&T Mussel Watch median and 85th percentile values, respectively, since the northeastern US has relatively high mercury levels due to deposition of airborne mercury from a wide range of sources in the US Midwest. Based on the Gulfwatch and SWAT criteria of “elevated” contaminants being those above the NS&T 85th percentile, all three SWAT sites tested in 2014 would be considered elevated for mercury despite the more typical magnitude of their scores when compared to other northeast US samples from the Gulf of Maine.

Mercury occurs naturally in the environment; however, elevated levels are associated with anthropogenic sources. United States sources of mercury to the air include coal fired electrical power generation, incinerators, mining, landfills, and sewage sludge (Kimbrough et al., 2008).

From a human health perspective, the developmental methylmercury FTAL (more protective) used by the MCDC is 0.2 µg/g wet wt. for non-commercially caught finfish (fish filet). This FTAL assumes an 8 oz. meal size is consumed weekly. Maine SWAT data uses a total mercury value, which is a more complete measure of mercury than the methylmercury concentration, but includes this more toxic form. The highest mean blue mussel total tissue mercury concentration measured in Maine in 2014 was 0.021 µg/g wet wt. at Mare Brook, Brunswick. This compares favorably with the MCDC methylmercury developmental FTAL of 0.2 µg/g, assuming a similar meal size and frequency. To consume approximately 8 oz. of blue mussel tissue the consumer would need to eat approximately 45-50 mussels based on the mean mass per mussel collected by the SWAT program.

Figure 1.3.1.1.9.1: Mercury in 2014 SWAT Blue Mussels

1.3.1.1.10 Zinc (Zn)

Zinc was detected in tissues taken from all three locations sampled in 2014 (Figure 1.3.1.1.10.1). Zinc levels measured in mussels ranged from a low mean concentration of 66.8 µg/g dry wt. at Navy Pier, Harpswell, to a high mean concentration of 67.8 µg/g dry wt. at Mare Brook, Brunswick. None of the three SWAT blue mussel tissue zinc concentrations measured exceeded the 2008 Gulfwatch median or the 2008 Gulfwatch 85th percentile. Figure 1.3.1.1.10.2 shows 2014 Maine SWAT blue mussel zinc concentrations were all below the NS&T Mussel Watch median and 85th percentile.

Zinc is widespread in its distribution but elevated levels primarily originate from a variety of human activities including vehicle tire wear, electroplating and galvanized metals, industrial wastes, and drainage from mining (Kimbrough et al., 2008). Though an essential nutrient at low levels, higher levels in humans can cause anemia or pancreatic and kidney damage. Since humans do not bioaccumulate zinc, health impacts are normally associated with high doses. From a human health perspective, MCDC reports a non-cancer FTAL for zinc of 648 µg/g wet wt., which is higher than any wet wt. concentrations observed in SWAT blue mussel tissue. There is no recommended FDA safety level for zinc in fish (Kimbrough et al., 2008).

1.3.1.2 Softshell Clams

Two softshell clam sites were sampled in 2014: Presumpscot River, Portland, and Strawberry Creek, Harpswell. The samples were analyzed for 11 metals: Silver (Ag), aluminum (Al), arsenic (Ar), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), mercury (Hg), nickel (Ni), and zinc (Zn). Results for the two sites sampled in 2014 were compared to previous SWAT clam data from multiple sites sampled since 2004. In addition, results were compared to Gulf of Maine (Gulfwatch, see LeBlanc et al. 2009) softshell clam data to place Maine SWAT data in a regional context.

1.3.1.2.1 Silver (Ag)

Silver was detected at all sample locations visited historically (Figure 1.3.1.2.1.1). Silver measured in clams ranged from a low mean concentration of 0.13 µg/g dry wt. at Harris Cove, Eastport, to a high mean concentration of 2.39 µg/g dry wt. at Mast Cove, Eliot (2013). Silver mean concentrations at the two sites sampled in 2014, Presumpscot River, Portland, and Strawberry Creek, Harpswell, were quite different from each other. The Presumpscot River mean silver concentration was within the higher range of concentrations from previously sampled Maine clam sites, while the Strawberry Creek mean concentration was near the low end of the concentrations at the 11 historic sites. Silver mean concentrations in SWAT softshell clams were also compared to the Gulfwatch mean concentration for four sites sampled in 2008 (two in Maine and two in New Hampshire). The mean concentrations at Mast Cove, Eliot (2004 and 2013), and Presumpscot River, Falmouth/Portland (2012 and the new 2014 sample), exceeded the Gulfwatch mean (1.32 µg/g dry wt.). The silver concentration in clam tissue in Mast Cove appeared to be slightly higher than, though similar to the 2004 concentration. The silver concentration in Presumpscot clam tissue appeared to be slightly higher than, though similar to the 2013 concentration, which was also collected on the western shore of the estuary (as it was in 2013 and 2014). Presumpscot clam tissues predominantly came from the eastern portion of the estuary in 2012, while the 2013 tissues originated from clams collected on

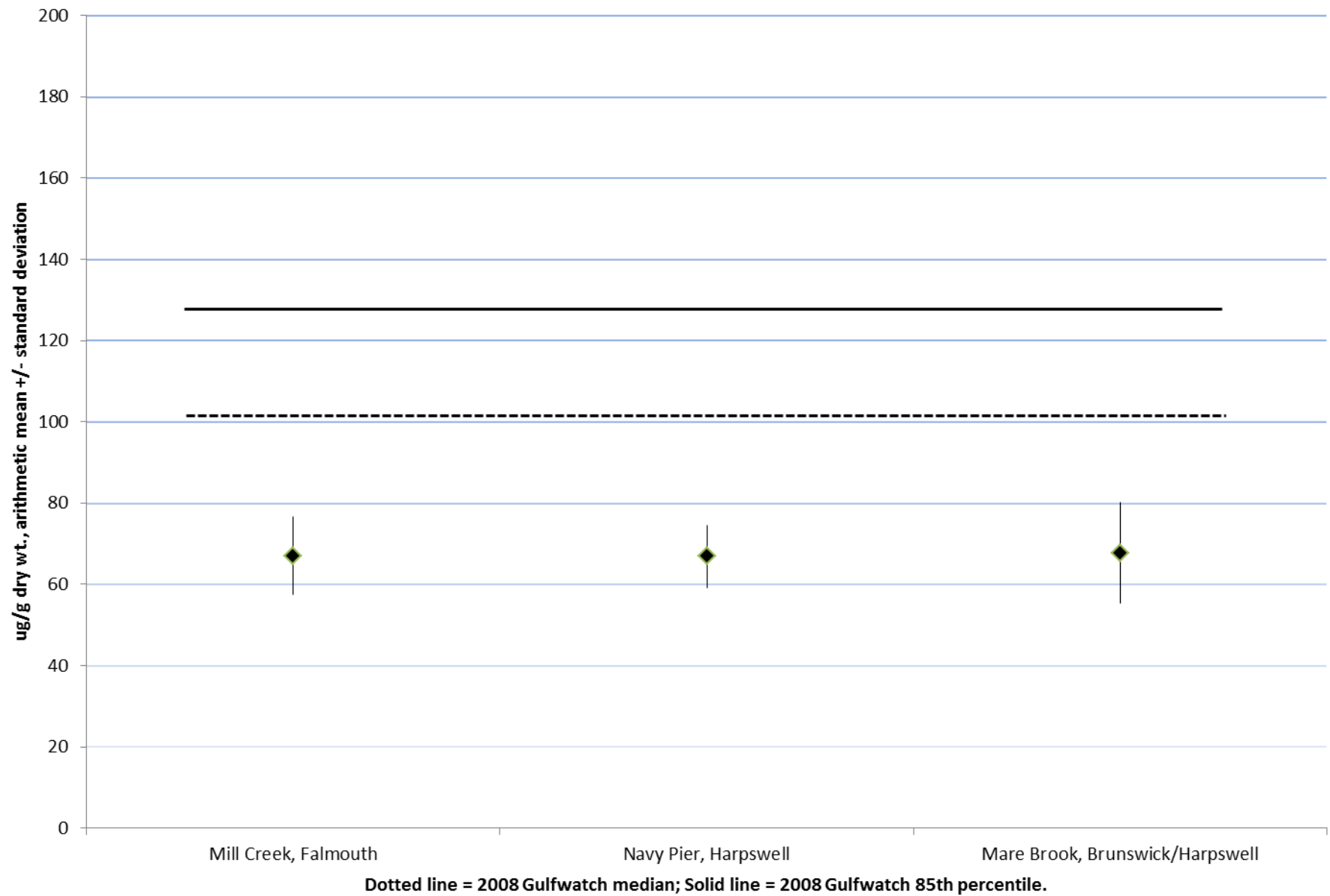
Figure 1.3.1.1.10.1: Zinc in 2014 SWAT Blue Mussels

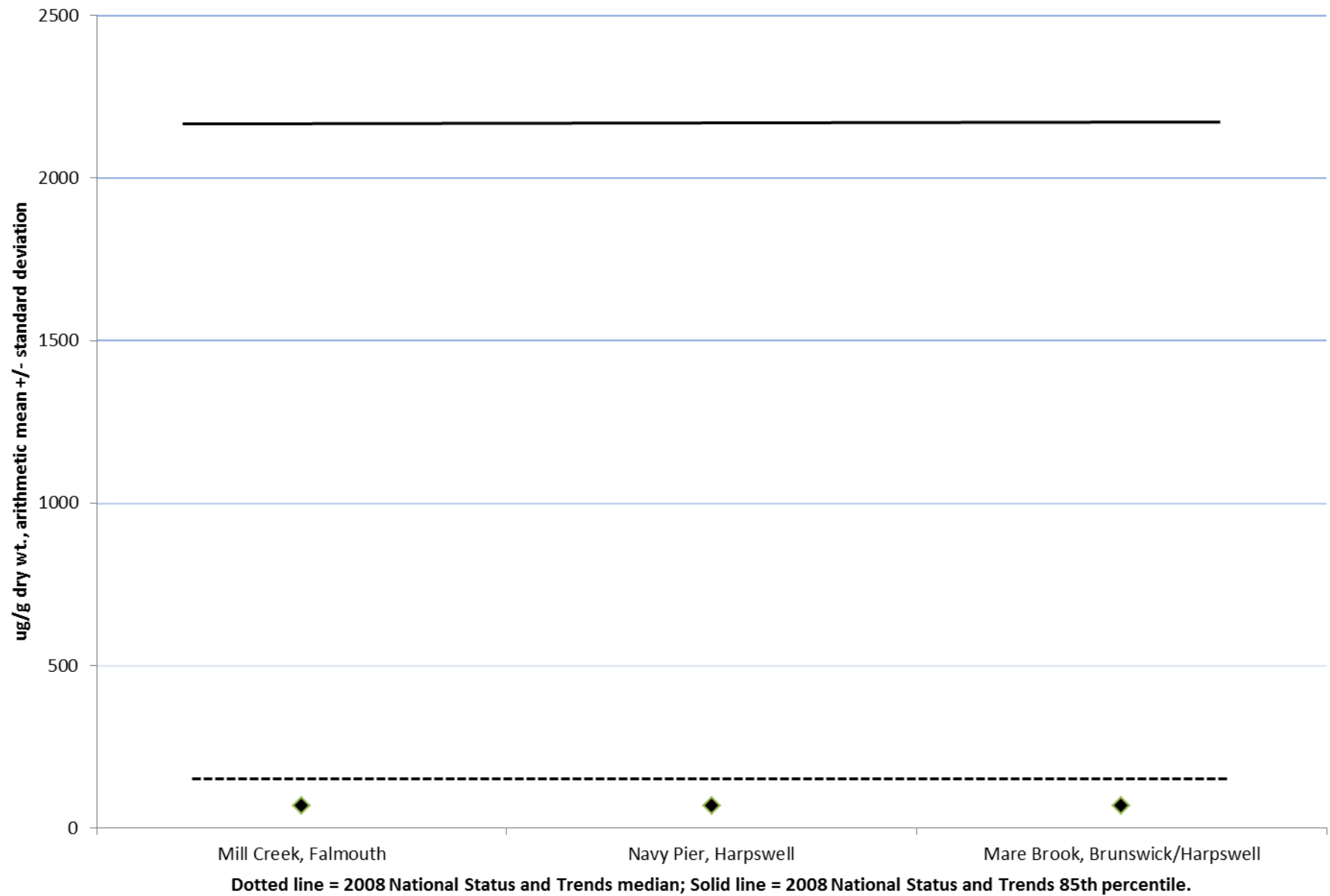
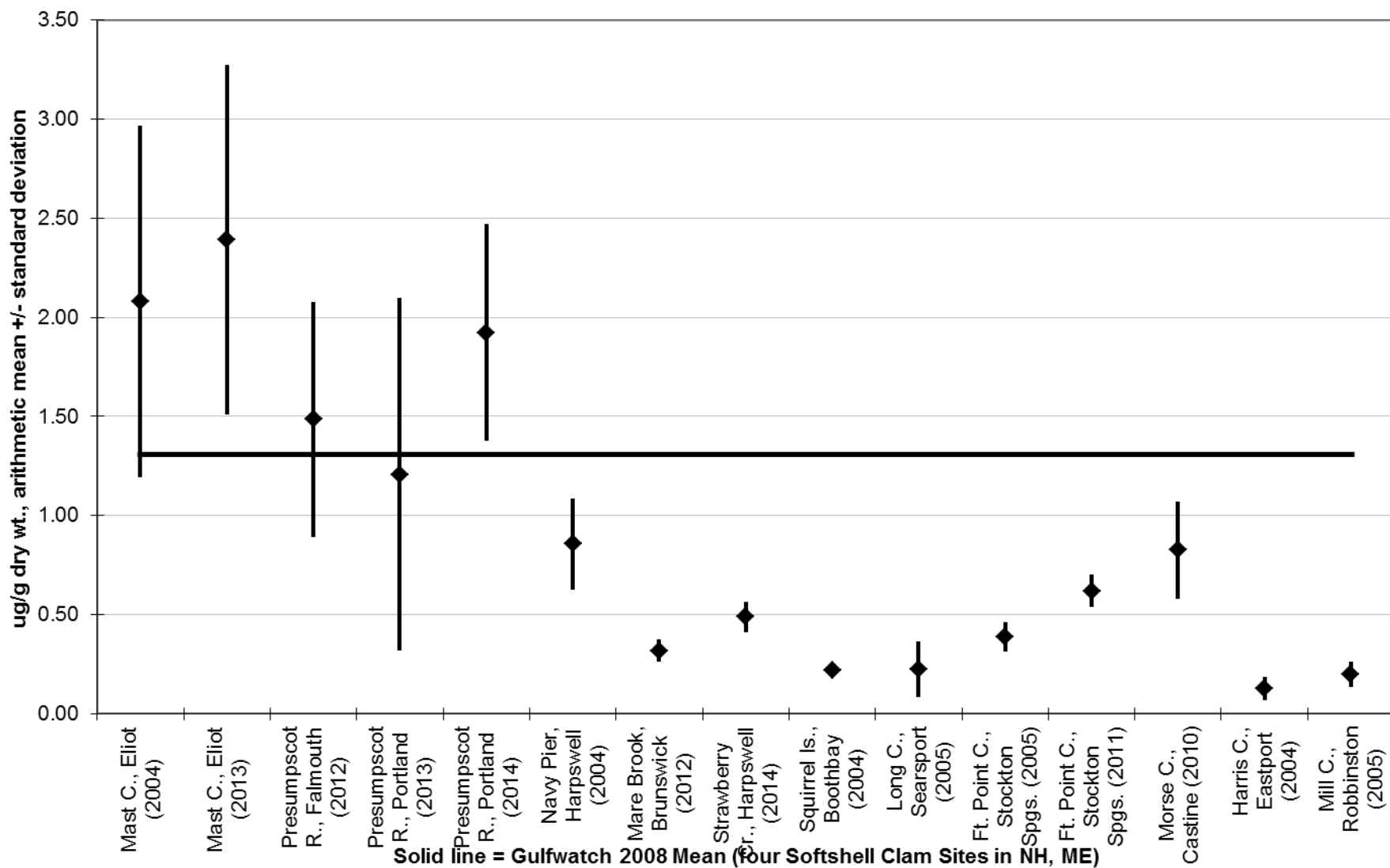
Figure 1.3.1.1.10.2: Zinc in 2014 SWAT Blue Mussels

Figure 1.3.1.2.1.1: Silver in SWAT Softshell Clams



the western portion of the estuary. Sampling in 2014 was concentrated in a specific area of the western shore of the estuary that was not as well represented in the 2013 sampling and includes an extensive flat that historically had a substantial clam resource and in which DMR has a strong interest. Variation from year to year may be an artifact of intra-site spatial variability (note length of whiskers representing standard deviation) or year to year variability.

Higher silver concentrations in water and sediments have been shown to coincide with municipal sewage discharge (Sanudo-Wilhelmy and Flegal, 1992; Buchholtz ten Brink et al., 1997). The increasing use of silver, including nanosilver, in products such as clothing, paints, and caulks, makes monitoring silver of interest at present and in the future. Silver concentrations in Maine softshell clams appear to be relatively low. The highest Gulfwatch values, which came from the two NH sites, were just over 2 µg/g dry wt., which is very similar to the Mast Cove, Eliot, SWAT site tissue concentration.

The Maine Center for Disease Control, Bureau of Health (MCDC) silver non-cancer FTAL is 11 µg/g wet wt. for non-commercially caught fish. The highest SWAT softshell clam tissue mean silver concentration, when expressed on a wet weight basis, is 0.43 µg/g wet wt. at Mast Cove, Eliot (2013). This concentration is over an order of magnitude below the 11 µg/g wet wt. FTAL, assuming the same meal size is applied.

1.3.1.2.2 Arsenic (As)

Arsenic was detected in clam tissue taken from the Presumpscot River, Portland, and from Strawberry Creek, Harpswell, in 2014. In previous years, analysis for arsenic was performed on clam tissues from only five sites: Presumpscot River, Portland (2012-13); Mast Cove, Eliot (2013); Mare Brook, Brunswick (2012); Fort Point Cove, Stockton Springs (2011); and Morse Cove, Castine (2010). Mean arsenic concentrations ranged from 9.97 µg/g dry wt. at Morse Cove (2010) to 92.95 µg/g dry wt. at Mare Brook (2012). Sites sampled in 2014 had mean arsenic concentrations in the middle of this range, 20.9 and 26.8 µg/g dry wt., at Strawberry Creek and the Presumpscot River respectively.

While Gulfwatch does not monitor arsenic in blue mussels or softshell clams in the Gulf of Maine, arsenic in mussels and oysters is tracked regionally and nationally by NS&T. In blue mussels, NS&T considers 5-11 parts per million dry wt. (directly comparable to SWAT µg/g data) to be in the lowest of three ranges of arsenic concentration within the region (Kimbrough et al., 2008). The mean arsenic concentration in softshell clams at Strawberry Creek (2014) and Morse Cove (2010) fell into this range, while the mean arsenic concentrations at Mast Cove (2013), the Presumpscot River (2012, 2013, and 2014), Fort Point Cove (2011), and Morse Cove (2010) fell into the lower end of the middle range of NS&T arsenic concentrations (23-41 ppm dry wt., Kimbrough et al., 2008). The mean arsenic concentration in softshell clams at Mare Brook, Brunswick (2012), 92.95 µg/g dry wt., fell above the high range used by NS&T regionally (23-41) and nationally (23-57 ppm). The NS&T ranges are based on mussels or oysters as regionally available. However, it is of interest to give a point of comparison for Maine clam data. Higher concentrations at Mare Brook may be related to very fine grained

sediments and sediment content in the clam gut, as clams are not depurated before tissue preparation for lab analysis.

Nationally, the primary source for elevated levels of arsenic is crustal rock. In addition to natural sources, industrial pollution can contribute arsenic to the environment from preserved wood, semiconductors, pesticides, defoliants, pigments, antifouling paints, and veterinary medicines. Atmospheric sources include smelting, fossil fuel combustion, power generation, and pesticide application (Kimbrough et al., 2008).

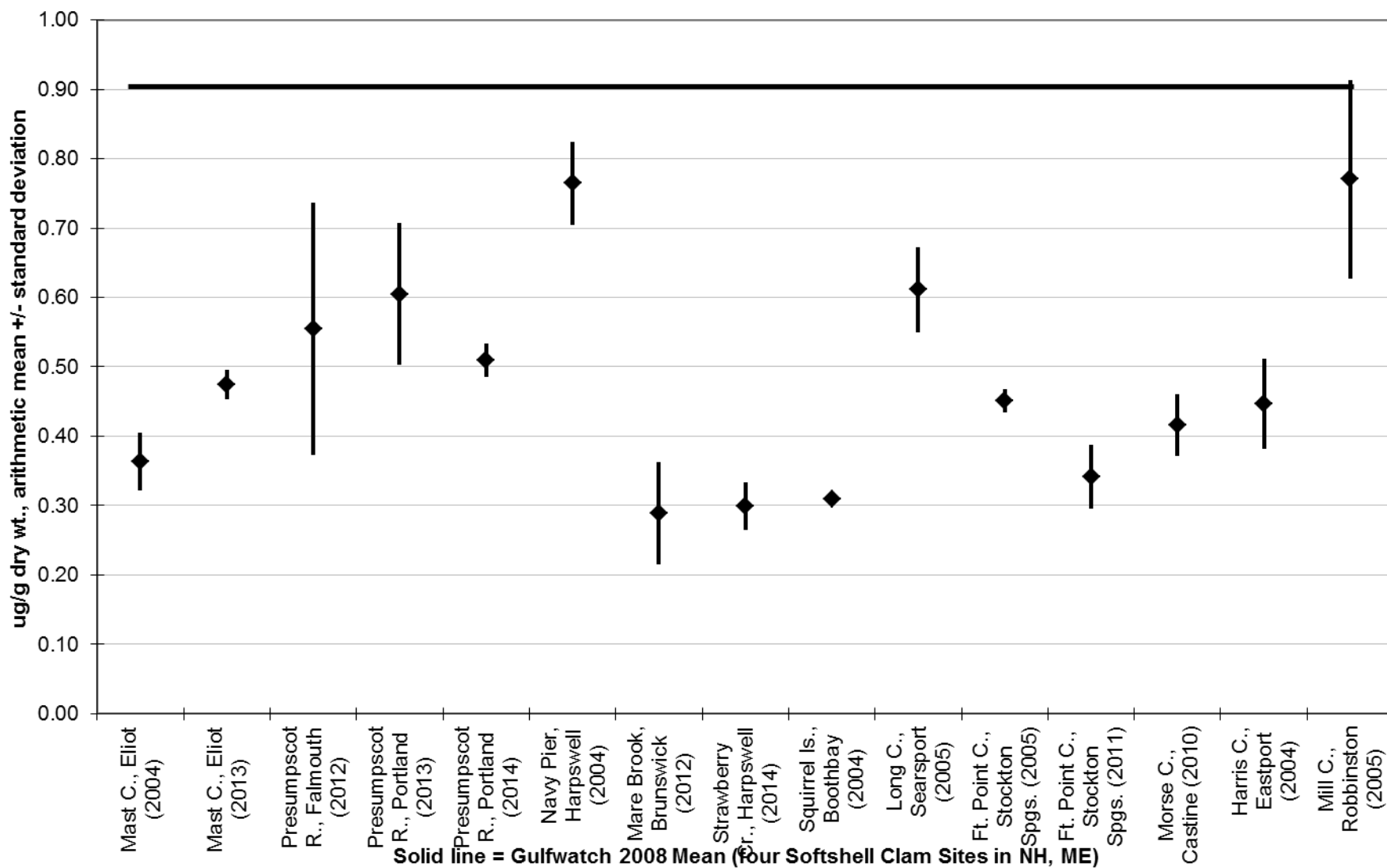
For non-commercially caught finfish, MCDC reports a cancer FTAL of 0.014 µg/g and a non-cancer FTAL of 0.6 µg/g, both for inorganic arsenic (the most toxic form). Most fish tissue data, including the SWAT blue mussel tissue data, are analyzed for total arsenic, not inorganic arsenic. MCDC uses FDA's 1993 assumption that 10% of total arsenic in finfish is inorganic arsenic. Using this assumption, approximate inorganic arsenic concentrations for SWAT softshell clams were calculated by dividing total arsenic wet weight concentrations by a factor of 10 to convert to inorganic arsenic wet weight concentrations. Using this methodology, the mean concentration of inorganic arsenic in Presumpscot River (2014) clams is estimated to be 0.38 µg/g wet wt., and the mean concentration of inorganic arsenic in Strawberry Creek (2014) clams is estimated to be 0.28 µg/g wet wt. Historically, all ten clam sites sampled for arsenic were calculated to exceed the MCDC cancer FTAL of 0.014 µg/g wet wt. Note that ever since arsenic data have been recorded as part of the SWAT program all blue mussel sites sampled have also exceeded the MCDC cancer FTAL. Only the Mare Brook, Brunswick (2012), estimated mean concentration of inorganic arsenic in clam tissue (1.56 µg/g wet wt.) exceeds the MCDC non-cancer action level of 0.6 µg/g wet wt. for inorganic arsenic. The MCDC non-commercially caught finfish FTALs applied here assume an 8 oz. meal eaten by the consumer on a weekly basis.

1.3.1.2.3 Cadmium (Cd)

Cadmium was detected in tissue from all 15 clam sampling events (11 locations) (Figure 1.3.1.2.3.1). Cadmium levels measured in softshell clams ranged from a low mean concentration of 0.29 µg/g dry wt. at Mare Brook, Brunswick (2012), to a high mean concentration of 0.77 µg/g dry wt. at Mill Cove, Robbinston. Mill Cove, Robbinston, and Navy Pier, Harpswell, were closest to the 2008 Gulfwatch median, with all 11 sites falling below that level. The mean cadmium concentration in clam tissue from samples taken at the Presumpscot River western shore in 2014 appeared to be similar to the mean from sampling in a broader western shore area (2013) and to the eastern portion of the river sampled (2012), although there is a great deal of variability in samples comprising the 2012-13 means.

Cadmium originates from crustal elements as rocks weather and is transported seaward by rivers, which account for approximately half of worldwide cadmium sources. Cadmium is also released through forest fires and volcanic activity, with anthropogenic sources including manufacturing, fossil fuel combustion, and agriculture. Industrial sources include manufacture of batteries, plating, stabilizers, and nuclear power (Kimbrough et al., 2008).

Figure 1.3.1.2.3.1: Cadmium in SWAT Softshell Clams



From a human health perspective, the MCDC non-cancer FTAL for cadmium in non-commercially caught finfish is 2.2 µg/g wet wt. The FDA action level for clams, oysters, and mussels is 4 µg/g wet wt. (Kimbrough et al., 2008). The highest scoring SWAT clam site, Mill Cove, Robbinston (2005), had a mean cadmium concentration of 0.088 µg/g wet wt., which was well below the MCDC and FDA action levels (4% of the more conservative MCDC non-cancer FTAL).

1.3.1.2.4 Chromium (Cr)

Chromium was detected in clam tissue from all 15 sampling events (11 locations) (Figure 1.3.1.2.4.1). Chromium levels measured in clam tissue ranged from a low mean concentration of 3.07 µg/g dry wt. at Fort Point Cove, Stockton Springs (2011), to a high mean concentration of 13.32 µg/g dry wt. at Mast Cove, Eliot (2004). Figure 1.3.1.2.4.1 depicts SWAT softshell clam chromium concentrations compared to the Gulfwatch 2008 mean concentration for four sites (two each in ME and NH). All but three clam sites, including the Presumpscot River in 2012, fell above the Gulfwatch 2008 mean. The mean concentration from the Presumpscot River in 2013 was slightly higher than the 2012 mean, while the 2014 mean was higher still. The 2013-14 samples came from the western half of the estuary and 2012 samples came from the eastern half, but this does not explain the large difference between 2013 and 2014, both from the western section of the estuary. This difference may be due to different specific areas sampled in the two subsequent years and to patchiness of contaminants, or it may be attributed to a change in the laboratory used in metals analysis in 2014. Strawberry Creek clams sampled in 2014 also had a relatively high chromium concentration. Blue mussel chromium concentrations were higher than anticipated in 2014 and mussel samples are being reanalyzed by the lab. Mean concentrations in samples taken from Mast Cove dropped noticeably from 2004 to 2013. The Fort Point Cove (2005) clam tissue chromium concentration was essentially the same as the Gulfwatch 2008 mean, while chromium concentrations appeared to be slightly lower in Fort Point Cove samples in 2011, falling below the Gulfwatch 2008 mean. The remaining sites were all above the Gulfwatch 2008 mean.

Natural sources of chromium include leaching from soil and rock into surface waters. Chromium is released from textile, electroplating, and leather tanning industries. Chromium is used extensively in tanning leather and was frequently discharged with untreated tannery effluent during the last two centuries. Chromium persists in the marine environment in sediments near anthropogenic sources (Kimbrough et al., 2008).

From a human health perspective, the MCDC FTALs (7 µg/g cancer action level and 11 µg/g non-cancer action level) for chromium are based on chromium VI, and are not directly comparable to SWAT results, which are for total chromium.

1.3.1.2.5 Copper (Cu)

Copper was detected in clam tissue samples taken at all 15 sampling events (11 locations) (Figure 1.3.1.2.5.1). Copper levels measured in clam tissue ranged from a low mean concentration of 7.31 µg/g dry wt. at Long Cove, Searsport (2005), to a high mean concentration of 33.6 µg/g dry wt. at the Presumpscot River, Portland (2014). Copper

Figure 1.3.1.2.4.1: Chromium in SWAT Softshell Clams

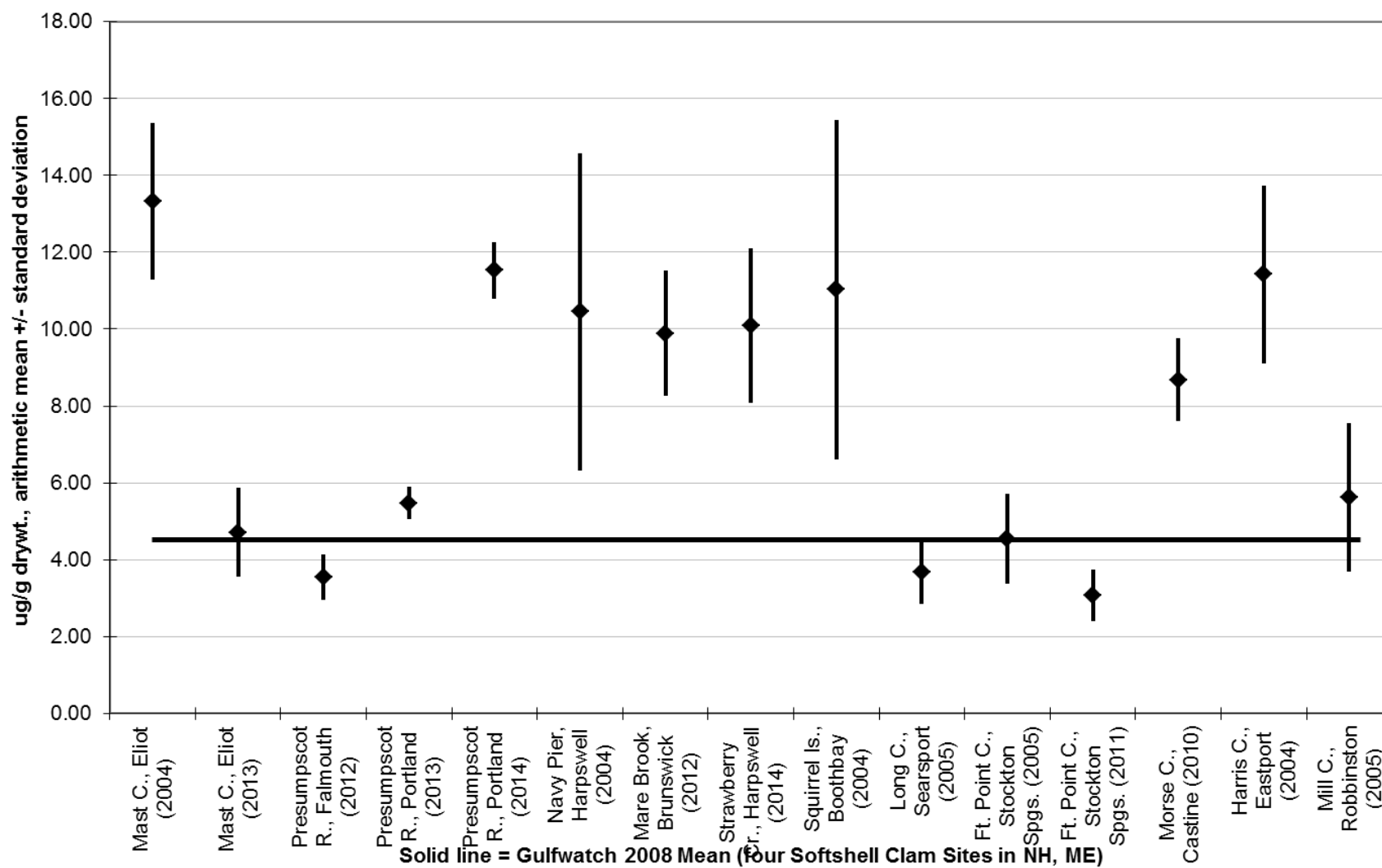
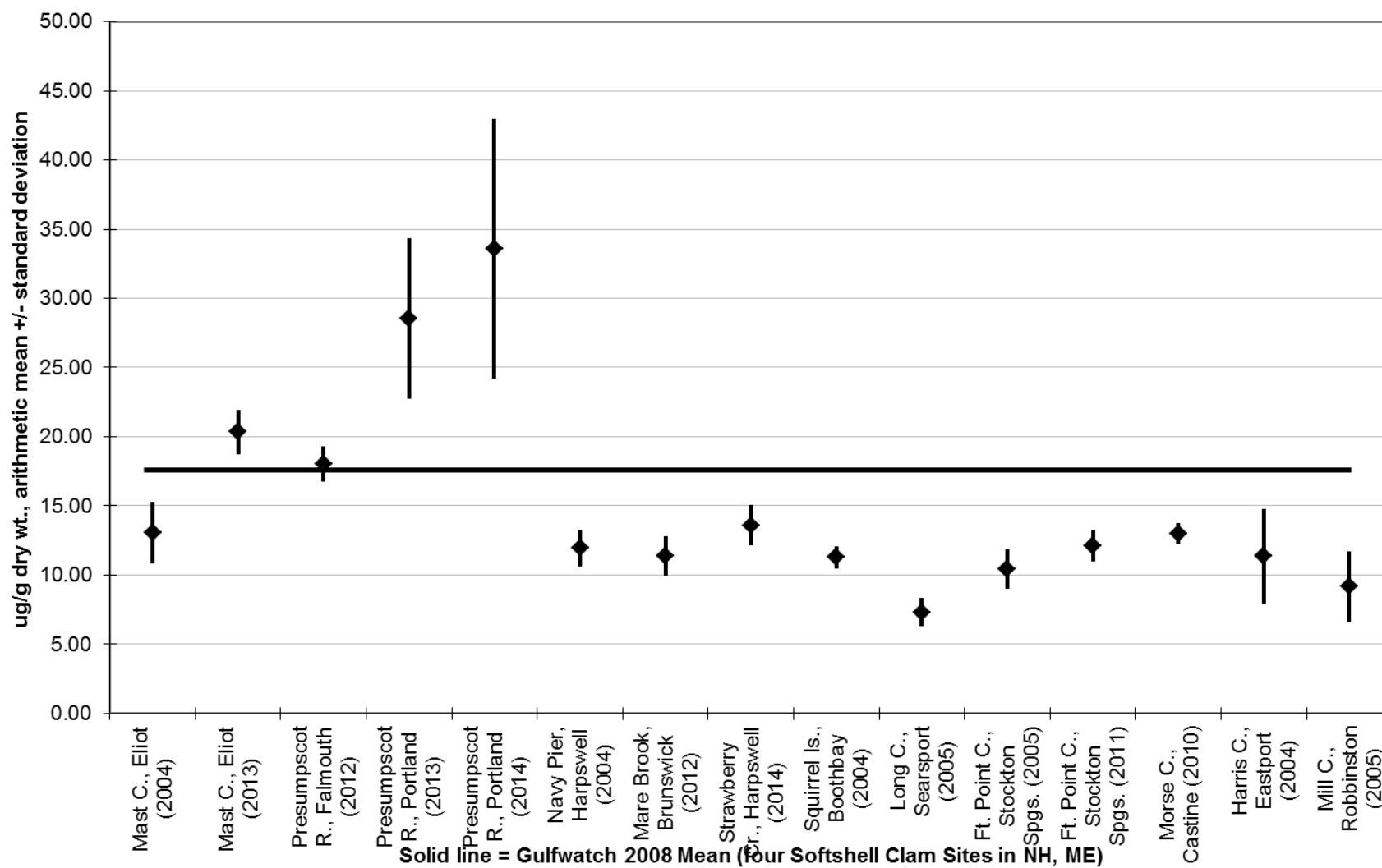


Figure 1.3.1.2.5.1: Copper in SWAT Softshell Clams



concentrations in clam tissue at 11 sites fell below the 2008 Gulfwatch mean, including Mast Cove when sampled in 2004 (LeBlanc et al., 2009). The Presumpscot River exceeded the Gulfwatch 2008 mean in all three years sampled, as did Mast Cove in 2013. The Mast Cove copper mean was higher in 2013 than in 2004. Differences in the Presumpscot River means may be attributed to the fact that in 2012, samples were from the east shore, while in 2013 the west shore was sampled; sub-site specific locations sampled in 2013 vs. 2014; differences in labs used in 2013 (and previously) vs. 2014; or patchiness in contaminants.

Copper occurs naturally and is ubiquitous throughout the marine environment. Copper, in trace amounts, is considered to be an important nutrient for plant and animal growth. Elevated copper concentrations can occur due to contributions from anthropogenic sources, including mining, agriculture, sewage sludge, antifouling paint, fungicides, wood preservatives, and brake pads. With the reduction of the use of chromated copper arsenate (CCA) wood preservative subsequent to its being phased out by EPA, newer wood preservatives utilizing even higher levels of copper have come into use, including quaternary copper. Similarly, tributyltin marine bottom paint use was reduced in the 1980s, resulting in increased use of copper-based antifouling paints, and removal of asbestos from the manufacture of brake pads has been offset by increased usage of copper in manufacturing brake pads (Kimbrough et al., 2008).

Copper is not highly toxic to humans, though exposure can lead to some chronic effects. There is no recommended FDA safety level for human consumption for copper in fish or shellfish (Kimbrough et al., 2008), and MCDC does not report a FTAL for copper in non-commercially caught sportfish.

1.3.1.2.6 Iron (Fe) and Aluminum (Al)

Iron was detected in clam tissue samples taken at all 15 sampling events (11 locations) (Figure 1.3.1.2.6.1). Iron concentrations measured in clam tissue ranged from a low mean concentration of 1,370 µg/g dry wt. at Squirrel Island, Boothbay (2004), to a high mean concentration of 26,145 µg/g dry wt. at the Presumpscot River, Portland (2013). Two SWAT sites had clam tissue iron concentrations that exceeded the 2008 Gulfwatch mean, including the Presumpscot River in 2013 and 2014 and Mare Brook, Brunswick, in 2012. Differences in iron concentrations in clam tissue from the western portion of the Presumpscot from 2013 to 2014 may be attributed to slightly different sub-sites sampled or to the use in 2014 of a different lab than had been used in analysis of previous years' samples. (Figure 1.3.1.2.6.1).

Aluminum concentrations detected in clams ranged from a low mean concentration of 563 µg/g dry wt. at Squirrel Island, Boothbay (2004), to a high mean concentration of 5,760 µg/g dry wt. at Mare Brook, Brunswick (2012) (Figure 1.3.1.2.6.2). Clam tissue from six sampling events, Mare Brook (2012), Presumpscot River (2012, 2013, and 2014), Mast Cove (2013), and Strawberry Creek (2014) had aluminum concentrations exceeding the 2008 Gulfwatch mean concentration. Mast Cove had a higher aluminum concentration in 2013 than 2004. Samples from the two opposite banks of the Presumpscot River estuary had similar aluminum concentrations in 2012 and 2013.

Figure 1.3.1.2.6.1: Iron in SWAT Softshell Clams

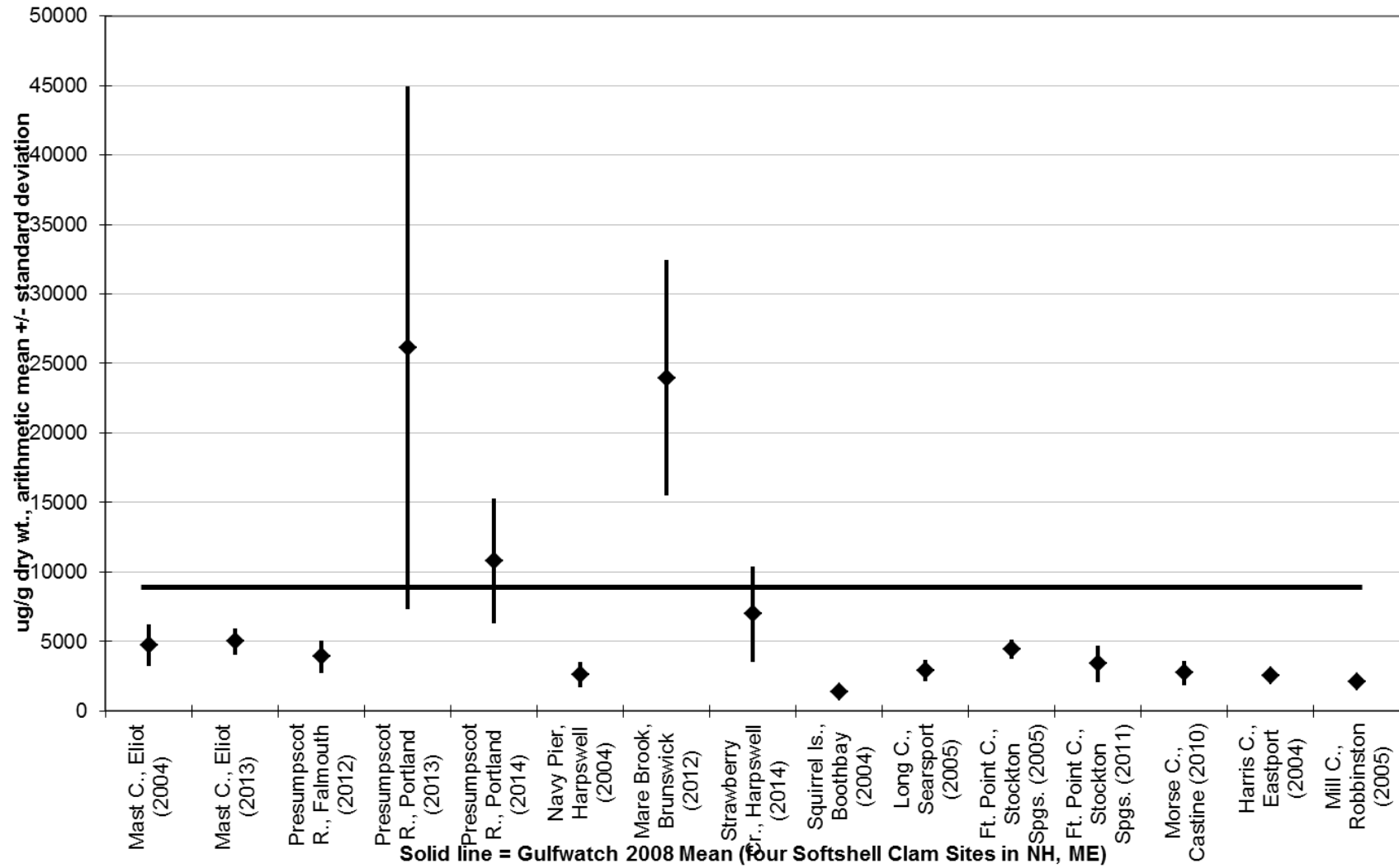
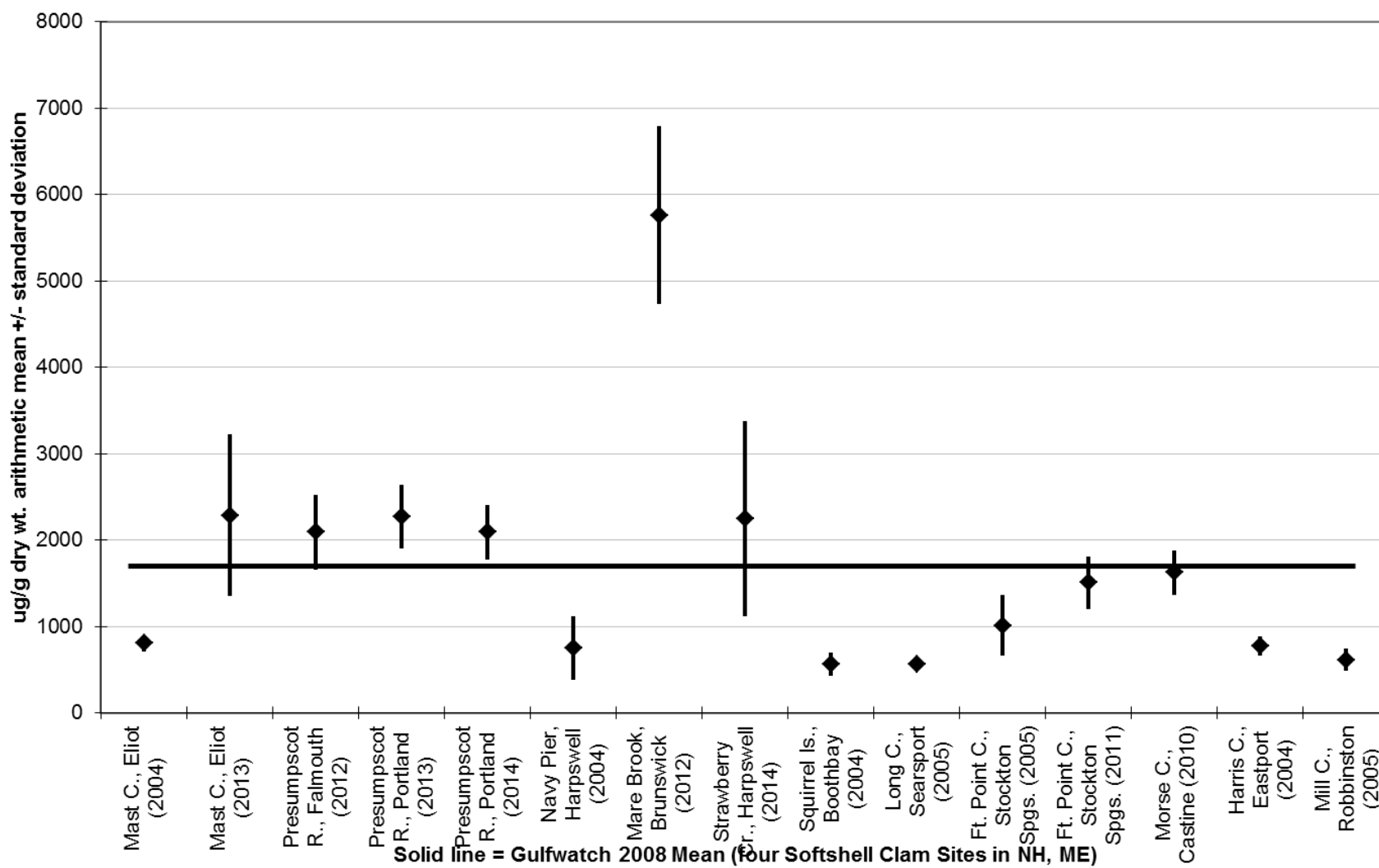


Figure 1.3.1.2.6.2: Aluminum in SWAT Softshell Clams



Samples from slightly different sub-sites on the west bank of the Presumpscot in 2013 and 2014 had similar aluminum concentrations despite a change in the lab used for sample analysis for metals between years.

High iron and aluminum concentrations are usually associated with the intake of high levels of suspended sediments by mussels and clams at sampled sites, since iron and aluminum are abundant crustal elements and therefore abundant in sediments. This correlation has also been shown with gut depuration experiments conducted as part of Gulfwatch monitoring in previous years, indicating that some of the iron and aluminum detected in SWAT samples is associated with gut contents and not bioaccumulated loads (LeBlanc et al., 2009). Sediment loading in clam gut contents may be quite a bit higher than in mussel gut contents, thus affecting aluminum and iron levels disproportionately in clam tissue concentrations since no depuration occurs prior to tissue removal.

Monitoring for iron and aluminum provides an important reference to gauge sediment intake by clams, allowing iron and aluminum levels to be referenced if other more toxic metals or contaminants are detected in tissue samples. If iron and aluminum concentrations are high, it is likely that a fraction of the contaminant load can be traced back to high sediment intake with some contamination coming from sediment in clam gut contents, rather than bioaccumulated contaminants from within clam tissue.

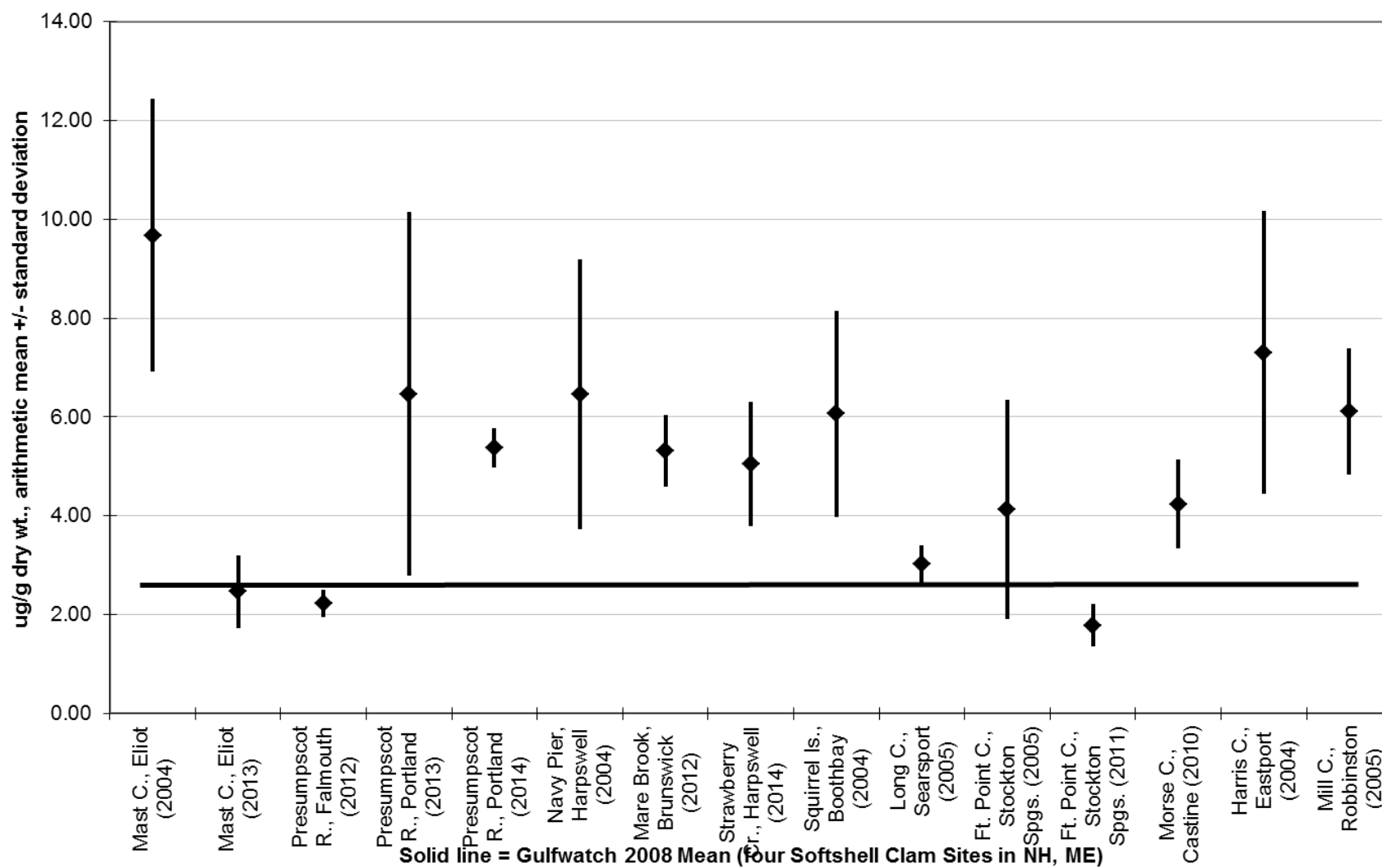
From a human health perspective, MCDC does not report FTALs for iron and aluminum.

1.3.1.2.7 Nickel (Ni)

Nickel was detected in clam tissue taken at all 15 sampling events (11 locations) (Figure 1.3.1.2.7.1). Nickel levels measured in clam tissues ranged from a low mean concentration of 1.79 µg/g dry wt. at Fort Point Cove, Stockton Springs (2011) to a high mean concentration of 9.68 µg/g dry wt. at Mast Cove, Eliot (2004). Maine SWAT clam tissue nickel concentrations were all higher than the 2008 Gulfwatch clam mean except for the Presumpscot River (2012), Fort Point Cove (2011), and Mast Cove (2013), which were below the Gulfwatch mean. The mean nickel concentration in samples from Mast Cove was lower in 2013 than in 2004. Presumpscot River nickel concentrations differed from 2012 to 2013, with the 2013 concentration from the western shore of the estuary being higher and showing more variability than that from 2012 from the eastern shore of the estuary. Nickel concentrations from the western shore of the estuary were similar in 2013 and 2014, despite slight differences in sub-site sampling location and a change of analysis labs. Higher nickel concentrations are probably associated with sediment ingestion, similar to iron and aluminum concentrations. The highest nickel concentration in the SWAT clam sites (Mast Cove, Eliot, 2004) was found at the same site as the highest iron concentration, indicating sediment in the clam gut may be a contributing factor to nickel concentration in the samples.

Nickel occurs naturally in the environment and is an essential trace element to biological processes. Nickel from soil and weathering of rocks enters rivers and provides the largest source of nickel to coastal waters. Nickel occurs in stainless steel, nickel-cadmium batteries, pigments, computers, wire, coins, and is used in electroplating. Elevated nickel

Figure 1.3.1.2.7.1: Nickel in SWAT Softshell Clams



concentrations occur in the Great Lakes and speculation about sources centers on air deposition from a large nickel smelting operation in Ontario, Canada (Kimbrough et al., 2008).

Nickel is not thought to bioaccumulate in the food chain, however, nickel can be harmful to humans in large doses, inducing effects including bronchitis and even cancer from long term exposure (Kimbrough et al., 2008). The MCDC reports a non-cancer FTAL for nickel in non-commercially caught finfish of 43 µg/g wet weight, which is more conservative than the FDA action level for shellfish of 80 µg/g wet weight. The maximum mean concentration detected by SWAT in clam tissue is 1.5 µg/g wet wt. at Mast Cove, Eliot, which is an order of magnitude lower than the more conservative MCDC action level. MCDC does not report a cancer action level for nickel.

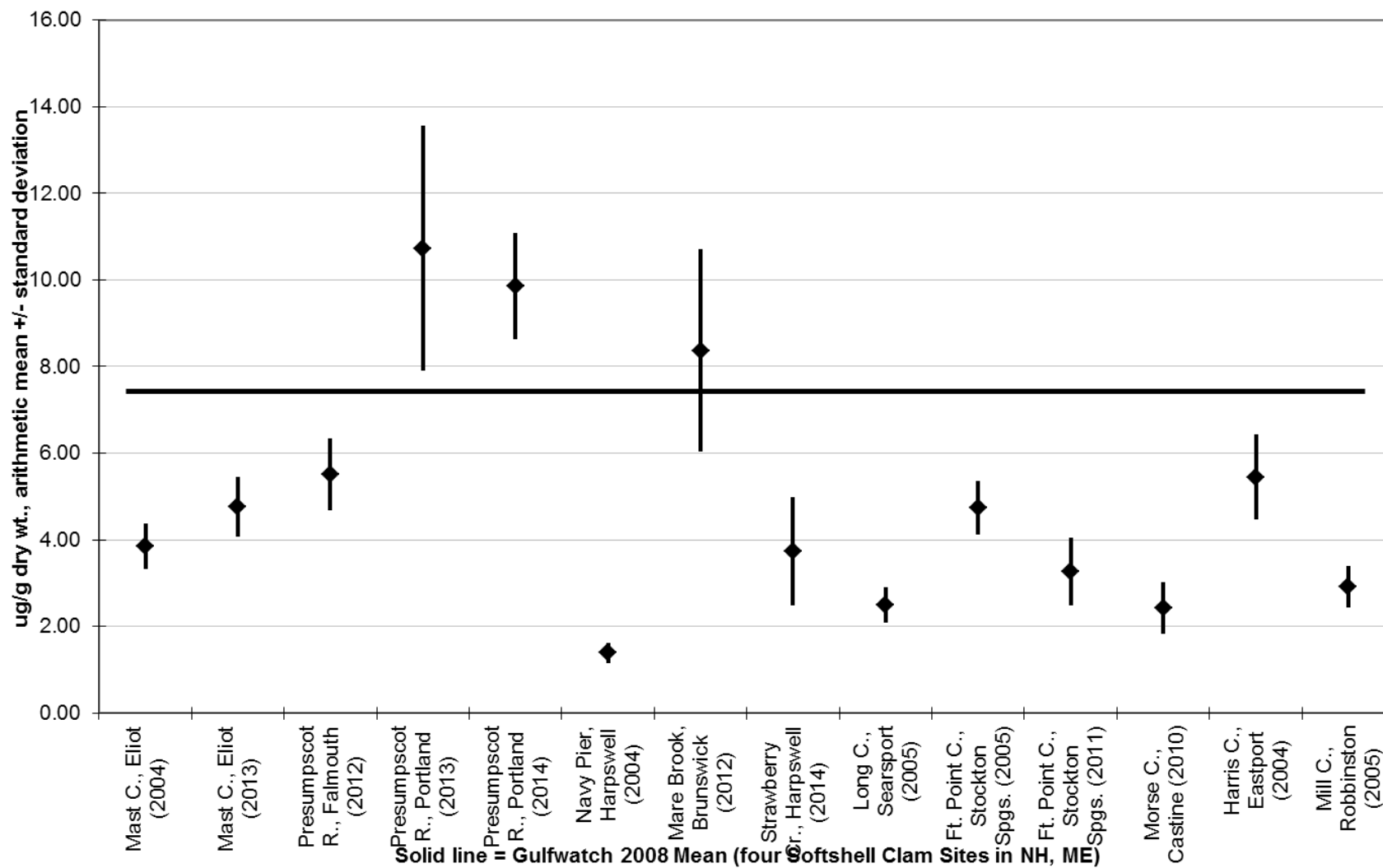
1.3.1.2.8 Lead (Pb)

Lead was detected in clam tissue samples taken at all 15 sampling events (11 locations) (Figure 1.3.1.2.8.1). Lead levels measured in clams ranged from a low mean concentration of 1.39 µg/g dry wt. at Navy Pier, Harpswell, (2004) to a high mean concentration of 10.73 µg/g dry wt. at the Presumpscot River, Portland, (2013). Mean lead clam tissue concentrations at all but two SWAT sites fell below the 2008 Gulfwatch mean. Only the Presumpscot River (in 2013 and 2014) and Mare Brook had mean lead tissue concentrations exceeding the 2008 Gulfwatch mean. Lead concentrations at Mast Cove were similar or slightly higher in 2013 compared to 2004. The Presumpscot River mean lead concentration was higher on the western shore of the estuary in 2013 and 2014 compared to the eastern shore in 2012.

Lead occurs naturally in the earth's crust; however, lead concentrations in the environment have increased globally in the last century due to the use of leaded gasoline. Reduction in lead loading through regulation of leaded gasoline and lead paints has occurred in recent decades. Elevated lead levels in the environment occur due to manufacturing, paints, lead solder, ammunition, plumbing, incineration and burning of fossil fuels. Lead loading in coastal waters is related to wastewater discharge, river runoff, atmospheric deposition, and natural weathering of crustal rock (Kimbrough et al., 2008).

From a human health perspective, the FDA action level for lead in clams, oysters, and mussels (molluscan shellfish) had been 1.7 µg/g wet wt. (Kimbrough et al., 2008). This limit apparently was eliminated at the 2007 Interstate Shellfish Sanitation Conference (ISSC). The more conservative MCDC lead FTAL in non-commercially caught sportfish is 0.6 µg/g wet wt., which is based on a blood lead concentration model. As presented in this and past SWAT reports, the SWAT program has tested whole softshell clam tissue, such that all tissue is included in the sample for contaminant analysis except the shell. On this whole clam tissue basis, the highest mean concentration in the Maine SWAT softshell clam data is 1.65 µg/g wet wt. in the Presumpscot River (2013), followed by 1.435 µg/g wet wt. in the Presumpscot River (2014), 1.413 µg/g wet wt. at Mare Brook, Brunswick (2012), and then followed by 0.873 µg/g wet wt. at the Presumpscot River, Portland (2012). These four whole clam tissue lead concentrations exceed the MCDC

Figure 1.3.1.2.8.1: Lead in SWAT Softshell Clams



lead FTAL for recreationally caught sportfish, as does Mast Cove (2013) (0.871 µg/g wet wt.), Harris Cove, Eastport (2004) (0.765 µg/g wet wt.), and Fort Point Cove, Searsport (2005) (0.647 µg/g wet wt.). Mast Cove, Eliot, (2004) (0.597 µg/g wet wt.) was at the MCDC lead FTAL. The other five historic SWAT softshell clam sites, when compared on a whole clam tissue basis, fell below the more conservative MCDC lead FTAL, as did the 2011 Fort Point Cove clam tissue sample (0.52 µg/g wet wt.). One replicate of four at Fort Point Cove in 2011 scored 0.65 µg/g wet wt. indicating considerable variability in the lead tissue concentrations, with some falling on either side of the MCDC lead FTAL.

SWAT has tested whole clams to adhere to the environmental monitoring standard and to facilitate comparison with Gulfwatch and NS&T clam contaminant data. In 1999 and 2000, studies conducted by DMR and supported in part by SWAT tested various softshell clam tissues to explore if the tested tissues contained different levels of several metals, including lead. Clams were tested with and without their skin tissue included in the analysis. Clam “edible portion”, which was all soft clam tissue with the exception of the clam skin, showed lower levels of some metals including lead when compared to whole clam tissue samples (like those whole clam tissue composites normally tested and presented in this and past SWAT reports). The study showed samples with neck skin included (clam with membrane) had lead concentrations of two to five times the concentration in samples without neck skin (edible portion). This becomes relevant when the human consumption of softshell clams is considered, as commercially shucked clams have their skins removed prior to use as fried clams and steamed clams normally have their skin removed by the consumer.

Utilizing this approach may be of interest particularly when considering clams containing concentrations that exceed the MCDC FTAL for lead since the skin or membrane, which is not consumed, appears to have a higher lead concentration than the edible portion. However, care must be taken in this approach, as the level of lead concentration varied from 2- to 5-fold between whole-clam and edible portion samples across the five areas sampled. Application of two and three fold reductions in whole clam SWAT lead concentrations are:

Site	Whole (µg/g wet wt.)	Edible	
		One Half	One Third
Presumpscot 2013	1.65	0.825	0.55
Presumpscot 2014	1.435	0.718	0.478
Mare Brook 2012	1.413	0.707	0.471
Presumpscot 2012	0.873	0.437	0.291

The choice of which factor is most appropriate to apply may be site specific, as demonstrated in the multi-site study. Future testing, if it is focused primarily on human health and consumption, might include the edible portion of the clam to document the specific local lead concentration. The most conservative two fold factor above reduces all but the highest three samples to a level below the MCDC FTAL of 0.6 µg/g wet wt. in recreationally caught sportfish. Application of the three fold factor reduces even the highest whole clam lead concentration to a converted edible portion concentration below the 0.6 MCDC FTAL. Another reasonable approach might be the development of a

softshell clam-specific FTAL, which would consider the frequency of consumption, meal size, and at-risk groups. The recreationally caught finfish FTAL applied above is that which is currently available from MCDC, but may include consumption, meal size, and risk groups that are not completely relevant to softshell clam consumption.

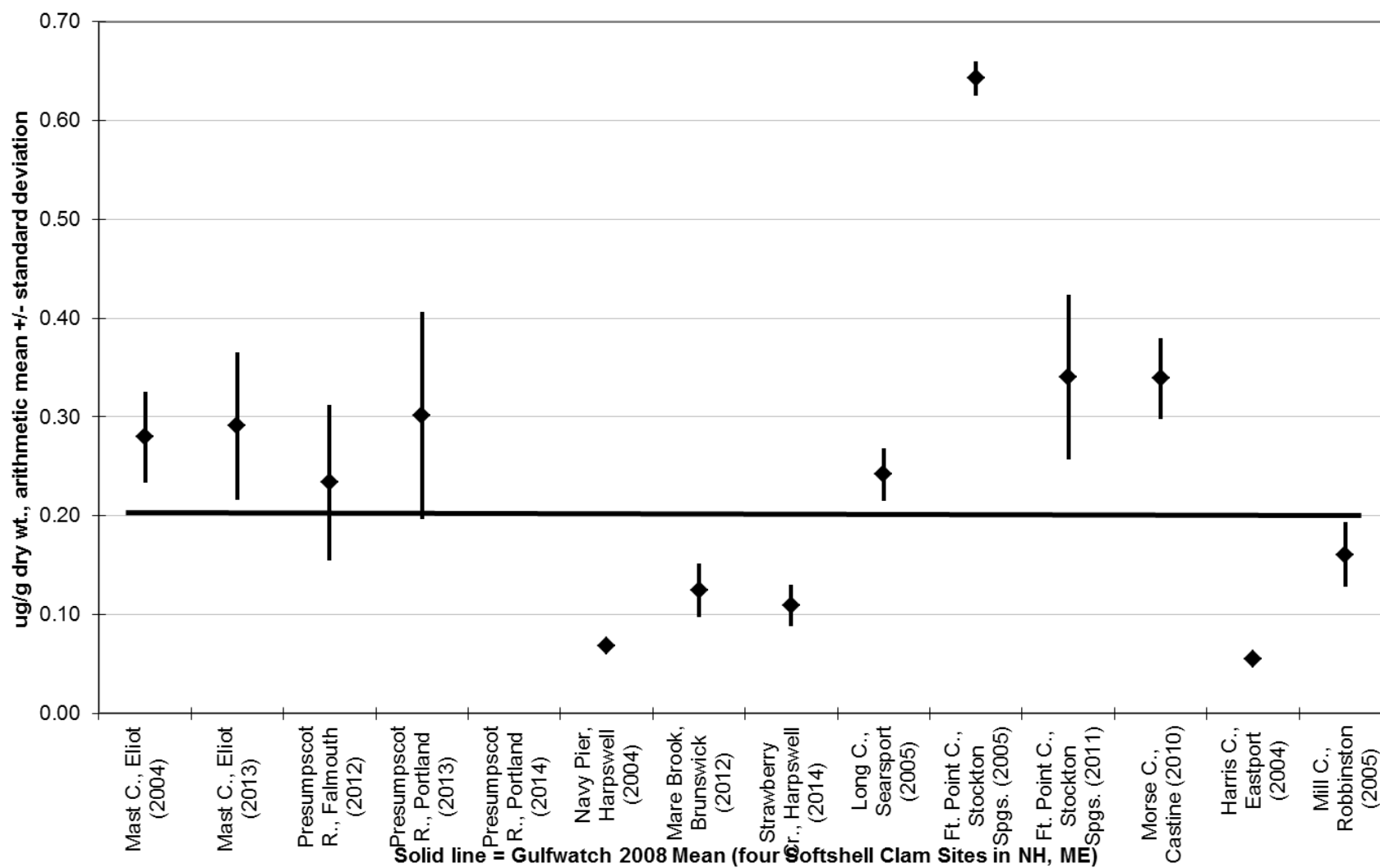
The MCDC FTAL is based on the consumer eating an 8 oz. meal weekly. Maine SWAT data indicate that an 8 oz. meal would include approximately 21 softshell clams of the size tested by the SWAT program.

1.3.1.2.9 Mercury (Hg)

Mercury was detected in clam tissue samples taken at all ten locations tested for its presence (Figure 1.3.1.2.9.1). Mercury was not included in the analysis of 2014 Presumpscot River clam tissue as other metals were of primary interest and because data had been collected in the previous two years. Mercury levels measured in clams ranged from a low mean concentration of 0.06 µg/g dry wt. at Harris Cove, Eastport (2004), to a high mean concentration of 0.64 µg/g dry wt. at Fort Point Cove, Stockton Springs (2005). High mercury concentrations in a variety of matrices have been documented in the Penobscot and are likely associated with the chloralkali process employed at the former Holtrachem site. Five sites had clam tissue concentrations that exceeded the 2008 Gulfwatch mean: the Presumpscot River, Portland (2012, 2013), Mast Cove, Eliot (2004, 2013), Long Cove, Searsport (2005), Fort Point Cove, Stockton Springs (2005, 2011), and Morse Cove, Castine (2010) (Figure 1.3.1.2.9.1). The mean mercury concentration at the Strawberry Creek, Harpswell, site tested in 2014 was one of the lower levels measured and did not exceed the 2008 Gulfwatch mean concentration.

Mercury occurs naturally in the environment; however elevated levels are associated with anthropogenic sources. United States sources of mercury to the air include coal fired electrical power generation, incinerators, mining, landfills, and sewage sludge (Kimbrough et al., 2008). From a human health perspective, the developmental methylmercury FTAL (more protective) used by the MCDC is 0.2 µg/g wet wt. for non-commercially caught finfish (fish filet). This FTAL assumes an 8 oz. meal is consumed weekly. Maine SWAT data uses a total mercury value, which is a more complete measure of mercury than the methylmercury concentration, but includes this more toxic form. Measuring total mercury is therefore more protective than measuring methylmercury alone. The highest mean softshell clam total tissue mercury concentration measured by SWAT in this Maine data set was 0.088 µg/g wet wt. at Fort Point Cove, Stockton Springs in 2005 (note that the 2011 concentration appears to be somewhat lower, which may be due to patchiness of contaminants, sampling variability, or inter-annual variability). The 2005 concentration compares favorably with the MCDC methylmercury developmental FTAL of 0.2 µg/g (less than half of the FTAL), assuming a similar meal size and frequency. To consume approximately 8oz. of tissue the consumer would need to eat approximately 21 softshell clams based on the mean mass per clam collected by the SWAT program.

Figure 1.3.1.2.9.1: Mercury in SWAT Softshell Clams



1.3.1.2.10 Zinc (Zn)

Zinc was detected in tissue taken at all 15 softshell clam sampling events (11 locations) (Figure 1.3.1.2.10.1). Zinc levels measured in clams ranged from a low mean concentration of 56.1 µg/g dry wt. at Squirrel Island, Boothbay in 2004, to a high mean concentration of 97.2 µg/g dry wt. at the Presumpscot River, Portland, in 2014. Zinc concentrations were similar at the Presumpscot River in 2012, 2013, and 2014, Mast Cove in 2004 and 2013, and Fort Point Cove in 2005 and 2011. Ten of 11 SWAT clam sites had zinc tissue concentrations that fell below the 2008 Gulfwatch mean, with only the Presumpscot River (2014) exceeding the Gulfwatch mean.

Zinc is widespread in its distribution but elevated levels primarily originate from a variety of human activities including vehicle tire wear, electroplating and galvanized metals, industrial wastes, and drainage from mining (Kimbrough et al., 2008). Though an essential nutrient at low levels, higher levels in humans can cause anemia or pancreatic and kidney damage. Since humans do not bioaccumulate zinc, health impacts are normally associated with high doses. From a human health perspective, MCDC reports a non-cancer FTAL for zinc of 648 µg/g wet wt., which is more than an order of magnitude higher than any wet wt. concentrations observed in SWAT clam tissue. There is no recommended FDA safety level for zinc in fish (Kimbrough et al., 2008).

1.3.2 Polycyclic Aromatic Compounds

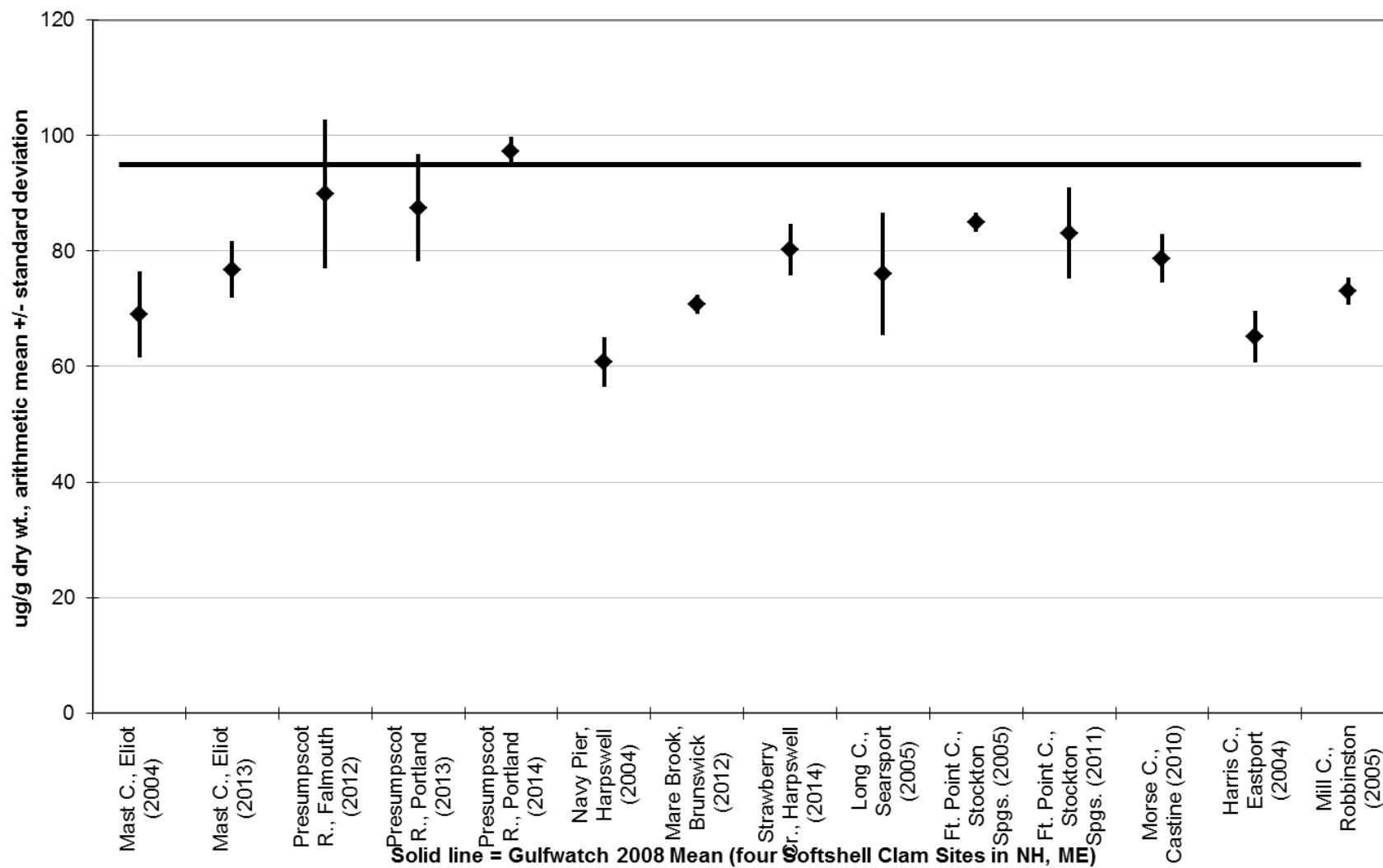
Polycyclic Aromatic Compounds (PAHs) occur in elevated concentrations near petroleum manufacturing, creosote use, and burning wood (Kimbrough et al., 2008). Though there are natural sources, including forest fires and volcanoes, anthropogenic sources, including automobile emissions, home heating, and coal-fired power plants, contribute to elevated levels of PAHs. As their name implies, polycyclic aromatic compounds are hydrocarbons composed of fused benzene rings, fusion of which may occur during combustion of other related compounds. However, they also occur in un-combusted coal and oil. PAHs in the environment are primarily from forest fires, coal-fired power plants, automobile exhaust, and spilled oil (Kimbrough et al., 2008).

1.3.2.1 Blue Mussels

Results were compared to national (NS&T) (Kimbrough et al., 2008) and Gulf of Maine (Gulfwatch) (LeBlanc et al., 2009) blue mussel monitoring program data (when available) in an effort to place Maine SWAT data in a national and regional context, respectively.

The NS&T and Gulfwatch programs utilize a subset of PAHs, summing results from 19, 24 and 40 individual PAHs to construct groups of PAHs to assess overall PAH concentrations and to compare regional and national concentrations. Smaller subsets of PAHs were utilized historically as a substitute for more complete sets as a cost saving measure. This report utilizes the Maine SWAT blue mussel tissue PAH data generated

Figure 1.3.1.2.10.1: Zinc in SWAT Softshell Clams



by AXYS Analytical, which includes 75 individual and summed alkylated PAHs. To compare Maine results to the NS&T and Gulfwatch lists of 19 unsubstituted (non-alkylated) PAHs, this report sums 19 non-alkylated PAHs from 2014 SWAT data. The summation of 19 PAHs is also useful for comparison to SWAT PAH data sets prior to 2009, as previous SWAT data included only 24 individual PAHs.

Both the Gulfwatch and NS&T programs utilize a summation of 24 PAHs, which in addition to the 19 non-alkylated PAHs previously mentioned also includes some alkylated PAHs (C1, C2, C3 Naphthalene, and C1-Phenanthrene). The 2014 SWAT PAH data can also be used to generate a summation for comparison with the Gulfwatch/NS&T summation of 40 PAHs, which includes even more alkylated PAHs. The corresponding SWAT data include 39 PAHs, the summation of which is the closest approximation possible. The difference between the Gulfwatch/NS&T summation and the SWAT summation is the absence of C4-Flourenes from the SWAT data set. This difference is considered to be relatively minor, and with some caution in interpretation, still allows comparison of SWAT data to regional and national data sets.

SWAT 2014 PAH data include additional alkylated PAHs as well, with a total of 75 PAHs included. The summation of 75 PAHs is presented and discussed in this report as “total PAHs.” Comparisons to other summations of lesser numbers of PAHs reviewed above are included to illustrate the wider data set provided by the greater level of PAH analysis obtained for SWAT sites in recent years, including 2010-14. Alkylated PAHs are typically associated with pyrogenic sources, rather than the more petrogenic sources associated with non-alkylated PAHs.

Table 1.3.2.1.1, “Analyzed PAHs and PAH Summation Calculations” shows comparisons between Gulfwatch/NS&T summation lists and SWAT summation lists, and details differences between the lists with footnotes and notes in the right column of the table.

Figure 1.3.2.1.1 shows the summation of the 19 non-alkylated PAHs, 24 PAHs, and 40 PAHs compared to the summation of all 75 (“total”) PAHs (including many alkylated PAHs) at the three 2014 SWAT blue mussel sites, Mill Creek, Falmouth, Navy Pier, Harpswell, and Mare Brook, Brunswick. The 19 summed non-alkylated PAHs and the total PAHs vary in a similar manner between sites, and the non-alkylated PAHs make up a small fraction of the total PAHs found at each site. The alkylated PAHs contribute the largest portion to the total PAHs, which is the difference between the sum of 19 PAHs and the total PAHs illustrated in Figure 1.3.2.1.1.

Total mean PAH concentrations were 296 ng/g dry wt. at Mill Creek, Falmouth, 112 ng/g dry wt. at Navy Pier, Harpswell, and 136 ng/g dry wt. at Mare Brook, Brunswick (Figure 1.3.2.1.1). The means of the sum of 19 non-alkylated PAHs were 110 ng/g dry wt. at Mill Creek, Falmouth, 37 ng/g dry wt. at Navy Pier, Harpswell, and 46 ng/g dry wt. at Mare Brook, Brunswick (Figure 1.3.2.1.1). The Gulfwatch program also utilized a summation of 24 PAHs in reports, the composition of which is outlined above. SWAT data were converted into this format and when 24 PAHs were summed, the mean

TABLE 1.3.2.1.1: Analyzed PAHs and PAH Summation Calculations

	SWAT				Gulfwatch, NS&T, SWAT Summations				
Parameter	2012-14	2010-11	2007-08	2004-05	ΣPAH19	ΣPAH24	ΣPAH40	Not Analyzed By Gulfwatch	Notes (See below list for more notes)
ACENAPHTHENE	x	x	x	x	x	x	x		
ACENAPHTHYLENE	x	x	x	x	x	x	x		
ANTHRACENE	x	x	x	x	x	x	x		
2-METHYLANTHRACENE	x	x						missing	
BENZ[A]ANTHRACENE	x	x	x	x	x	x	x		
DIBENZ(A,H)ANTHRACENE	x	x	x	x	x	x	x		
BIPHENYL	x	x	x	x	x	x	x		
BENZO[A]PYRENE	x	x	x	x	x	x	x		
BENZO(E)PYRENE	x	x	x	x	x	x	x		
7-METHYLBENZO[A]PYRENE	x	x						missing	
CHRYSENE	x	x	x	x	x	x	x		
1-METHYLCHRYSENE	x	x						missing	
5/6-METHYLCHRYSENE	x	x						missing	
5,9-DIMETHYLCHRYSENE	x	x						missing	
DIBENZOTHIOPHENE	x	x	1,2,3		x	x	x		
2,4-DIMETHYLDIBENZOTHIOPHENE	x	x						missing	
2/3-METHYLDIBENZOTHIOPHENES	x	x						missing	
FLUORANTHENE	x	x	x	x	x	x	x		
BENZO[B]FLUORANTHENES	x								SWAT split in 2012 from (B,J,K)
BENZO[J,K]FLUORANTHENES	x								SWAT split in 2012 from (B,J,K)
BENZO[B,J,K]FLUORANTHENES		x	x		x	x	x		in Gulfwatch list as BENZO[B]FLUORANTHENE and BENZO[K]FLUORANTHENE
3-METHYLFLUORANTHENE/BENZO[A]FLUORENE	x	x							
FLUORENE	x	x	x	x	x	x	x		
2-METHYLFLUORENE	x	x						missing	
1,7-DIMETHYLFLUORENE	x	x						missing	

TABLE 1.3.2.1.1: Analyzed PAHs and PAH Summation Calculations (continued)

	SWAT				Gulfwatch, NS&T, SWAT Summations				
Parameter	2012-14	2010-11	2007-08	2004-05	ΣPAH19	ΣPAH24	ΣPAH40	Not Analyzed By Gulfwatch	Notes (See below list for more notes)
NAPHTHALENE	x	x	x	x	x	x	x		
1-METHYLNAPHTHALENE	x	x	x					missing	
2-METHYLNAPHTHALENE	x	x	x					missing	
1,2-DIMETHYLNAPHTHALENE	x	x						missing	
2,6-DIMETHYLNAPHTHALENE	x	x	x					missing	
2,3,5-TRIMETHYLNAPHTHALENE	x	x	x					missing	
2,3,6-TRIMETHYLNAPHTHALENE	x	x						missing	
1,4,6,7-TETRAMETHYLNAPHTHALENE	x	x						missing	
PERYLENE	x	x	x	x		x	x		
BENZO[GH]PERYLENE	x	x	x	x	x	x	x		
PHENANTHRENE	x	x	x	x	x	x	x		
1-METHYLPHENANTHRENE	x	x	x					missing	
2-METHYLPHENANTHRENE	x	x						missing	
3-METHYLPHENANTHRENE	x	x						missing	
9/4-METHYLPHENANTHRENE	x	x						missing	
1,7-DIMETHYLPHENANTHRENE	x	x						missing	
1,8-DIMETHYLPHENANTHRENE	x	x						missing	
2,6-DIMETHYLPHENANTHRENE	x	x						missing	
3,6-DIMETHYLPHENANTHRENE	x	x						missing	
1,2,6-TRIMETHYLPHENANTHRENE	x	x						missing	
PYRENE	x	x	x	x	x	x	x		
INDENO[1,2,3-CD]PYRENE	x	x	x	x	x	x	x		

TABLE 1.3.2.1.1: Analyzed PAHs and PAH Summation Calculations (continued)

	SWAT				Gulfwatch, NS&T, SWAT Summations				
Parameter	2012-14	2010-11	2007-08	2004-05	ΣPAH19	ΣPAH24	ΣPAH40	Not Analyzed By Gulfwatch	Notes (See below list for more notes)
RETENE	x	x						missing	
C1-ACENAPHTHENES	x	x						missing	
C1-BENZO[A]ANTHRACENES/CHRYSENE	x	x	3				x		in Gulfwatch list as C1- CHRYSENE
C2-BENZO[A]ANTHRACENES/CHRYSENE	x	x	3				x		in Gulfwatch list as C2- CHRYSENE
C3-BENZO[A]ANTHRACENES/CHRYSENE	x	x	3				x		in Gulfwatch list as C3- CHRYSENE
C4-BENZO[A]ANTHRACENES/CHRYSENE	x	x	3				x		in Gulfwatch list as C4- CHRYSENE
C1-BENZOFLUORANTHENES/BENZOPYRENES	x	x						missing	
C2-BENZOFLUORANTHENES/BENZOPYRENES	x	x						missing	
C1-BIPHENYLS	x	x						missing	
C2-BIPHENYLS	x	x						missing	
C1-DIBENZOTHIOPHENES	x	x	3				x		
C2-DIBENZOTHIOPHENES	x	x	3				x		
C3-DIBENZOTHIOPHENES	x	x	3				x		
C4-DIBENZOTHIOPHENES	x	x						missing	
C1-FLUORANTHENES/PYRENES	x	x	3				x		
C2-FLUORANTHENES/PYRENES	x	x	3				x		
C3-FLUORANTHENES/PYRENES	x	x						missing	
C4-FLUORANTHENES/PYRENES	x	x						missing	
C1-FLUORENES	x	x	3				x		
C2-FLUORENES	x	x	3				x		
C3-FLUORENES	x	x	3				x		
C1-NAPHTHALENES	x	x	2,3			x	x		
C2-NAPHTHALENES	x	x	2,3			x	x		
C3-NAPHTHALENES	x	x	2,3			x	x		
C4-NAPHTHALENES	x	x						missing	

TABLE 1.3.2.1.1: Analyzed PAHs and PAH Summation Calculations (continued)

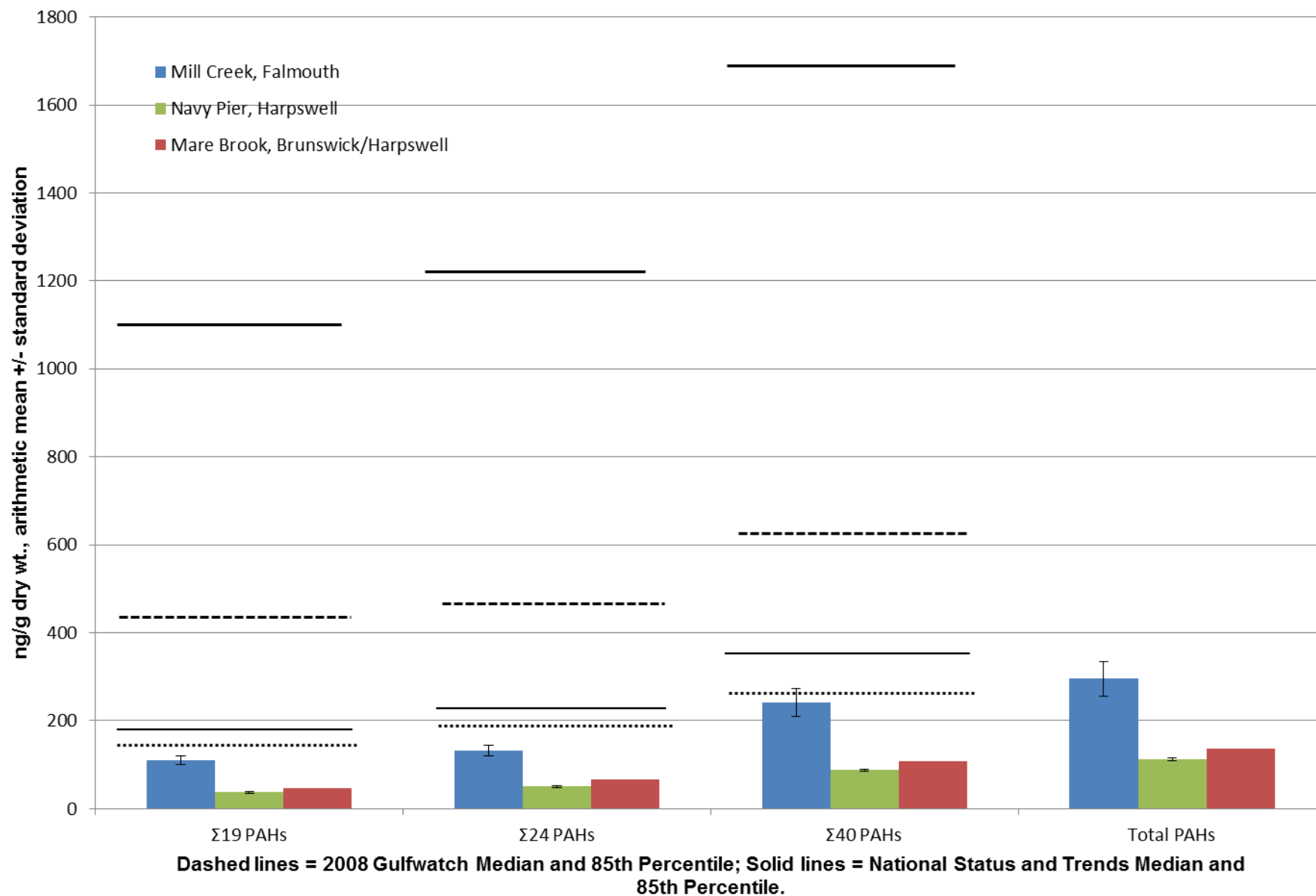
	SWAT				Gulfwatch, NS&T, SWAT Summations				
Parameter	2012-14	2010-11	2007-08	2004-05	ΣPAH19	ΣPAH24	ΣPAH40	Not Analyzed By Gulfwatch	Notes (See below list for more notes)
C1-PHENANTHRENES/ANTHRACENES	x	x	2,3			x	x		in Gulfwatch list as C1-PHENANTHRENE
C2-PHENANTHRENES/ANTHRACENES	x	x	3				x		in Gulfwatch list as C2-PHENANTHRENE
C3-PHENANTHRENES/ANTHRACENES	x	x	3				x		in Gulfwatch list as C3-PHENANTHRENE
C4-PHENANTHRENES/ANTHRACENES	x	x	3				x		in Gulfwatch list as C4-PHENANTHRENE
C4-FLUORENES			3				x		Not analyzed by SWAT

FOOTNOTES:

Prior to 2012: List of 'Sum PAH19' only has 18 compounds in it because we have BENZO[B]FLUORANTHENES and BENZO[K]FLUORANTHENES listed as one compound, BENZO[B,J,K]FLUORANTHENES; same applies to 'Sum PAH24' which has only 23 compounds. For 2012-14: List of 'Sum PAH19' has 19 compounds in it because we have BENZO[B]FLUORANTHENES and BENZO[J,K]FLUORANTHENES listed as two compounds. Same applies to 'Sum PAH24' which now has 24 compounds.

Prior to 2012: List of 'Sum PAH40' only has 38 compounds in it because we have BENZO[B]FLUORANTHENES and BENZO[K]FLUORANTHENES listed as one compound, BENZO[B,J,K]FLUORANTHENES and we do not have SWAT/AXYS data for C-4 FLUORENES (at bottom of above list). For 2012-14: List of 'Sum PAH40' has 39 compounds in it because we have BENZO[B]FLUORANTHENES and BENZO[J,K]FLUORANTHENES listed as two compounds, though we still do not have SWAT/AXYS data for C-4 FLUORENES (at bottom of above list)

In calculating the various summations, the approach used by SWAT is: Where SWAT has a slight variation from Gulfwatch in analytes, use the closest approximation to the Gulfwatch list as with the BENZO[B,J,K]FLUORANTHENES (prior to 2012), and the C1/2/3/4-BENZO[A]ANTHRACENES

Figure 1.3.2.1.1: PAHs in Blue Mussels

concentrations for the sum of 24 PAHs were 133 ng/g dry wt. at Mill Creek, Falmouth, 50 ng/g dry wt. at Navy Pier, Harpswell, and 66 ng/g dry wt. at Mare Brook, Brunswick (Figure 1.3.2.1.1).

Figure 1.3.2.1.1 also shows the summation of 40 PAHs compared to the summation of all 75 PAHs (Total PAHs) at the 2014 SWAT blue mussel sites. Both the 40 summed PAHs and the total PAHs vary in a similar manner between sites, but the sum of the 40 PAHs makes up the bulk of the total PAHs found at each site. The mean concentrations for the sum of 40 PAHs were 241 ng/g dry wt. at Mill Creek, Falmouth, 87 ng/g dry wt. at Navy Pier, Harpswell, and 107 ng/g dry wt. at Mare Brook, Brunswick (Figure 1.3.2.1.1).

Figure 1.3.2.1.1 compares the sum of 19 PAHs at the SWAT blue mussel sites sampled in 2014 to the Gulfwatch 2008 median and 85th percentile results. The sum of 19 PAHs from mussel tissue at all three sites sampled in 2014 was below the Gulfwatch median (154 ng/g dry wt.) and the Gulfwatch 85th percentile (429 ng/g dry wt.). The summation of non-alkylated PAHs is useful for putting Maine data into a regional, Gulf of Maine context. Figure 1.3.2.1.1 also compares the sum of 19 non-alkylated PAHs at the 2014 SWAT blue mussel sites to recent NS&T median and 85th percentile for 19 summed non-alkylated PAHs (2008 data, the most recent available). The sum of 19 PAHs in mussel tissue from all three sites sampled in 2014 was below the 2008 NS&T median of 180 ng/g dry wt. for 19 summed non-alkylated PAHs. None of the three SWAT mussel sites approached or exceeded the NS&T 85th percentile of 1,104 ng/g dry wt. for 19 summed PAHs.

Figure 1.3.2.1.1 compares the sum of 24 PAHs at the SWAT blue mussel sites sampled in 2014 to the Gulfwatch 2008 median and 85th percentile results. The sum of 24 PAHs from mussel tissue at all three sites sampled in 2014 was below the Gulfwatch 2008 median of 198 ng/g dry wt. for 24 summed PAHs. The summation of these PAHs is useful for putting Maine data into a regional, Gulf of Maine context. Figure 1.3.2.1.1 also compares the sum of 24 PAHs at the 2014 SWAT blue mussel sites to recent NS&T median and 85th percentile for 24 summed PAHs (2008 data, the most recent available). The sum of 24 PAHs from mussel tissue at all three sites sampled in 2014 was below the NS&T 2008 median of 247 ng/g dry wt. for 24 summed PAHs, and so none of the three sites approached or exceeded the NS&T 85th percentile of 1,216 ng/g dry wt. for 24 summed PAHs.

Figure 1.3.2.1.1 compares the sum of 40 PAHs at the SWAT blue mussel sites sampled in 2014 to the Gulfwatch 2008 median and 85th percentile results. The sum of 40 PAHs from mussel tissue at all three sites sampled in 2014 was below the Gulfwatch 2008 median of 260 ng/g dry wt. for 40 summed PAHs, although the sum of 40 PAHs from Mill Creek, Falmouth, was approaching the Gulfwatch mean. The sum of 40 PAHs at all three sites sampled in 2014 was below the Gulfwatch 85th percentile of 618 ng/g dry wt. for 40 summed PAHs.

The differences between the SWAT list of PAHs and the Gulfwatch list of PAHs available for the sum of 40 PAHs may be part of the reason why the SWAT sum of 40 PAHs is comparably high to the Gulfwatch sum of 40 PAHs. As noted in Table 1.3.2.1.1, SWAT utilizes C1 through C4-Benzo[A]Anthracenes/Chrysenes, where Gulfwatch utilizes C1 through C4-Chrysenes. Similarly, SWAT utilizes C1 through C4-Phenanthrenes/Anthracenes, where Gulfwatch utilizes C1 through C4-Phenanthrenes. It is likely that the additional summations of C1 through C4-Benzo[A]Anthracenes plus C1 through C4-Anthracenes included in the SWAT data are pushing the SWAT sum of 40 PAHs higher than the Gulfwatch equivalents. This result cannot be avoided due to the composition of the SWAT data, but should be noted when viewing the comparison in Figure 1.3.2.1.1.

Figure 1.3.2.1.1 also compares the sum of 40 PAHs at the 2014 SWAT mussel sites to recent NS&T median and 85th percentile for 40 summed PAHs (2008 data, the most recent available). The sum of 40 PAHs from mussel tissue at all three sites sampled in 2014 was below the NS&T 2008 median (353 ng/g dry wt.) for 40 summed PAHs, and so was also below the NS&T 85th percentile of 1,674 ng/g dry wt. for 40 summed PAHs.

For 2014 SWAT blue mussel sites, Figure 1.3.2.1.2 presents a graphic representation of selected PAHs expressed as a ratio. The equation used to derive the ratio is:

$$\text{Fluoranthene} + \text{Pyrene} / \Sigma(\text{Fluoranthene} + \text{Pyrene} + \text{C2-C4 Alkylphenanthrene})$$

This equation yields a numerical ratio, which is utilized to show relative concentrations of non-alkylated and alkylated PAHs. Values <0.1 are interpreted as a petrogenic (unburned fuel or petroleum) source, while values >0.2 are interpreted as a pyrogenic (combusted fuel) source of PAHs. All three SWAT blue mussel sites tested in 2014 have ratios above the 0.2 mark, which indicates a pyrogenic source of PAHs.

Toxicities of PAHs vary, with hundreds of compounds making up the pool of PAHs. Toxic responses in aquatic organisms may include reproductive inhibition, mutations, liver abnormalities, and even mortality. Exposure in the marine environment may be from spilled oil, boat exhaust, and runoff from urban areas. From a human health perspective, neither MCDC nor FDA have reported recommended safety levels for PAHs in fish or fish products (Kimbrough et al., 2008).

1.3.2.2 Softshell Clams

Results were compared to national (NS&T) (Kimbrough et al., 2008) shellfish data and Gulf of Maine (Gulfwatch) (LeBlanc et al., 2009) softshell clam data (when available) in an effort to place Maine SWAT data in a national and regional context, respectively. Differences in individual PAHs obtained from different laboratories and different years are described in depth in the previous section 1.3.2.1. The same approach was utilized to develop lists of PAHs in clam tissues presented in this section. Comparisons were made to NS&T and Gulfwatch programs when data sets were available.

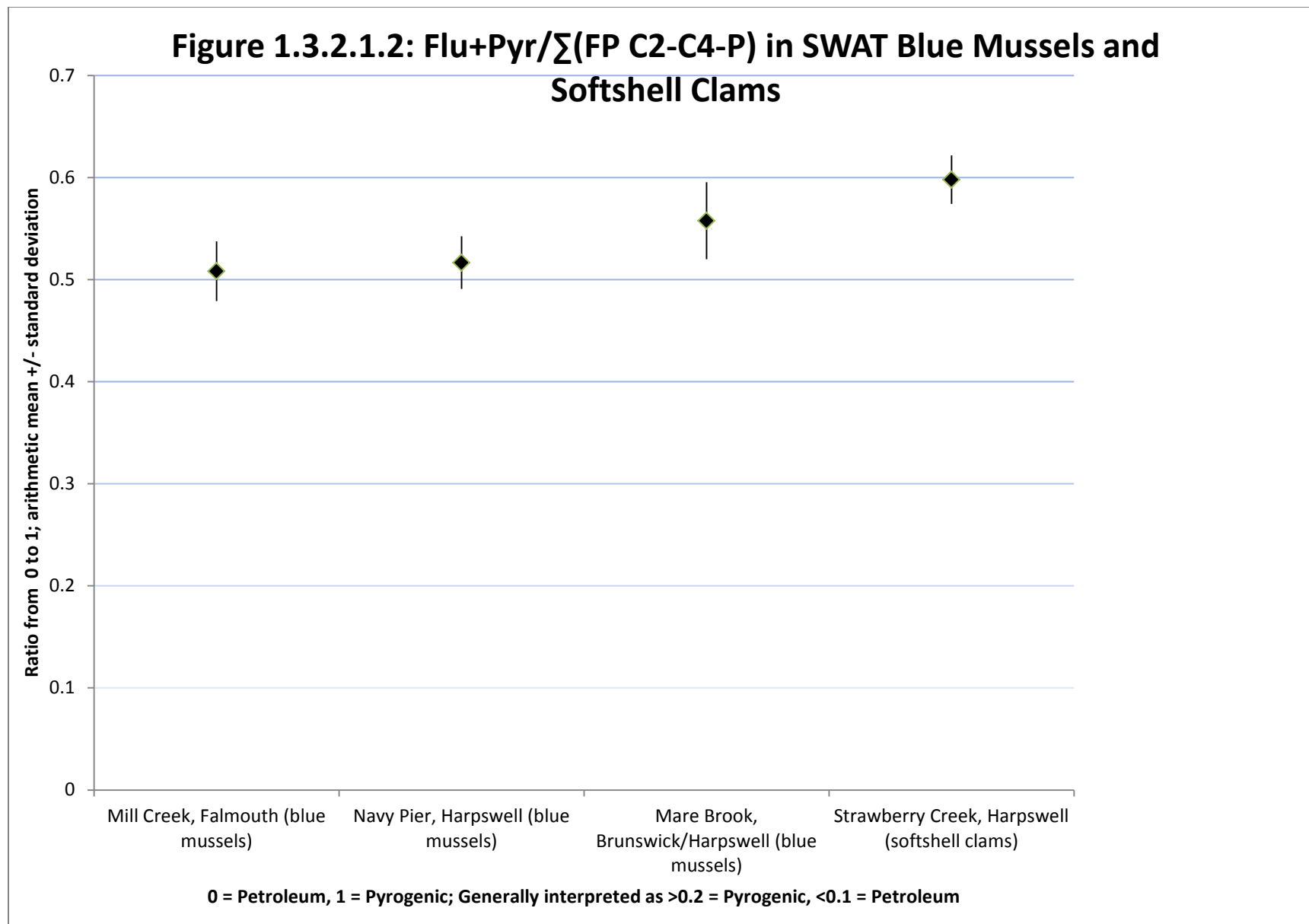


Table 1.3.2.1.1, “Analyzed PAHs and PAH Summation Calculations” shows comparisons between Gulfwatch/NS&T summation lists and SWAT summation lists, and details differences between the lists with footnotes and notes in the right column of the table.

Figure 1.3.2.2.1 shows the summation of the 19 non-alkylated PAHs at the one SWAT clam site sampled in 2014: Strawberry Creek, Harpswell. The Presumpscot River softshell clam site was tested for metals only and so no organic contaminant data is available for this site. The sum of 19 non-alkylated PAHs in clam tissue was 98 ng/g dry wt. at Strawberry Creek, Harpswell. Strawberry Creek was well below the 2008 Gulfwatch mean concentration for the sum of 19 non-alkylated PAHs (420 ng/g dry wt.), which was calculated for four sites (two in NH and two in ME).

In addition to the summation for 19 non-alkylated PAHs, Figure 1.3.2.2.1 includes summations of 24, 40, and total PAHs. The summation of 24 PAHs at Strawberry Creek was well below the 2008 Gulfwatch mean, and the sum of 40 PAHs was also well below the 2008 Gulfwatch mean (four sites, two in NH and two in ME). No summation of total PAHs is available for Gulfwatch data, so no mean can be calculated to present in Figure 1.3.2.2.1.

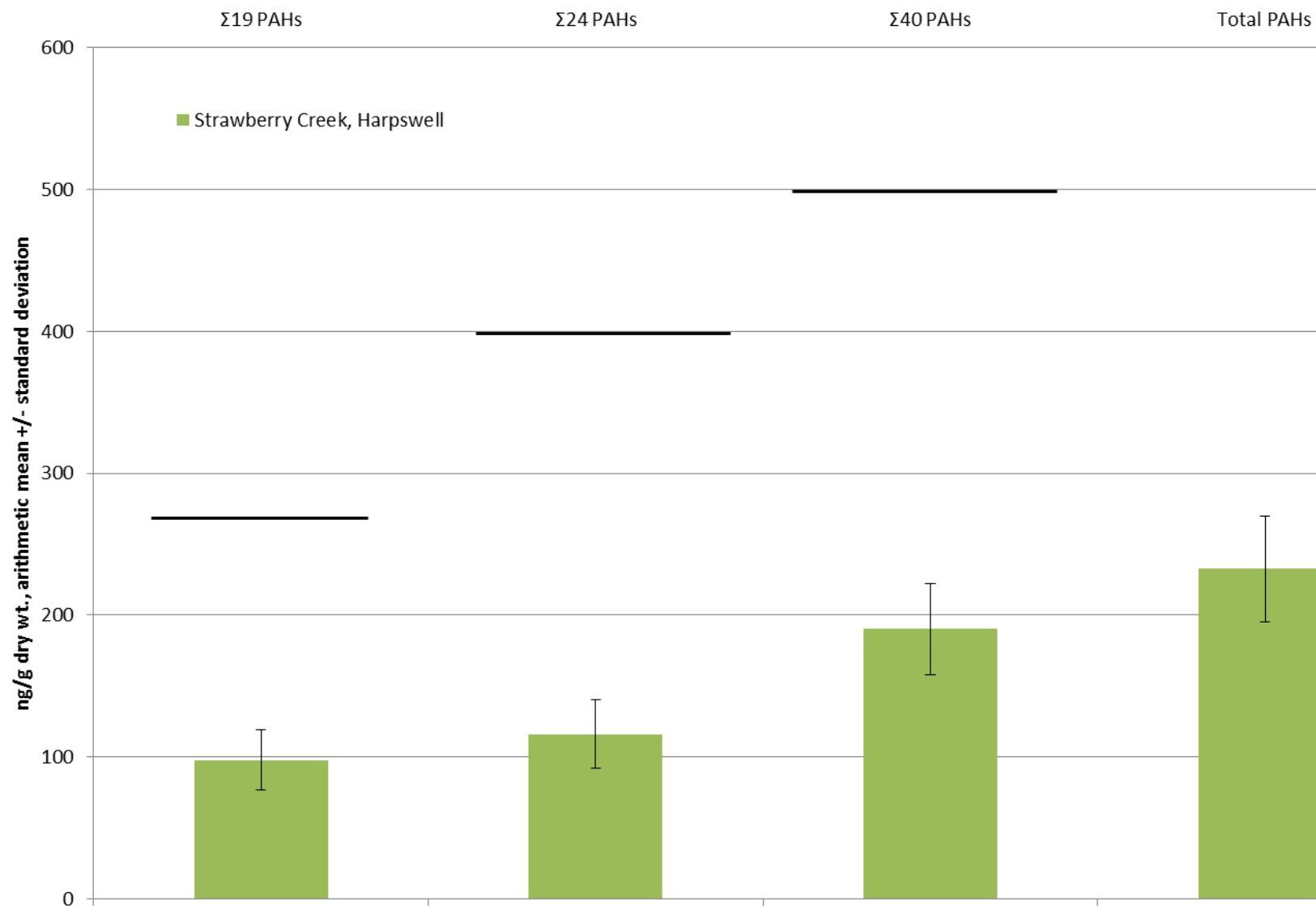
PAH results from Strawberry Creek, Harpswell, included the PAHs necessary to calculate the ratio discussed previously in the blue mussel PAH section to determine the likely source of PAHs. The equation used to derive the ratio is:

$$\text{Fluoranthene} + \text{Pyrene} / \Sigma(\text{Fluoranthene} + \text{Pyrene} + \text{C2-C4 Alkylphenanthrene})$$

This equation yields a numerical ratio, which is utilized to show relative concentrations of non-alkylated and alkylated PAHs. Values <0.1 are interpreted as a petrogenic (unburned fuel or petroleum) source, while values >0.2 are interpreted as a pyrogenic (combusted fuel) source of PAHs.

Since mussels (for PAH analysis) were sampled at two sites and clams were sampled at only one site in 2014, the calculated ratio for the Strawberry Creek clam site has been included in Figure 1.3.2.1.2. Strawberry Creek has a pyrogenic ratio approximately 0.6, indicating the major PAH components present in clam tissue at this site are from pyrogenic sources (Figure 1.3.2.1.2).

Toxicities of PAHs vary, with hundreds of compounds making up the pool of PAHs. Toxic responses in aquatic organisms may include reproductive inhibition, mutations, liver abnormalities, and even mortality. Exposure in the marine environment may be from spilled oil, boat exhaust, and runoff from urban areas. From a human health perspective, neither MCDC nor FDA have reported recommended safety levels for PAHs in fish or fish products (Kimbrough et al., 2008).

Figure 1.3.2.2.1: PAHs in Softshell Clams

Solid lines = Gulfwatch 2008 Means for $\Sigma 19$ PAHs, $\Sigma 24$ PAHs, and $\Sigma 40$ PAHs; (Four Softshell Clam Sites in NH and ME; No Σ Total PAHs Mean is Available for Gulfwatch).

1.3.3 Polychlorinated Biphenyls

Polychlorinated Biphenyls (PCBs) are synthetic organic compounds that consist of biphenyl with varying numbers of chlorine atoms. PCBs were manufactured from 1929 to 1977, though they were regulated in 1971 and new uses were banned in 1976. PCBs were used in electrical transformers and capacitors, and in lubricants and hydraulic fluids. They were also included in paints, adhesives, plasticizers, and flame retardants. Manufacturing of PCBs for flame retardants and lubricants was stopped in 1977. Current uses are in electrical equipment and transformers (Kimbrough et al., 2008).

1.3.3.1 Blue Mussels

This report utilizes the Maine SWAT blue mussel tissue PCB data generated by AXYS Analytical, which includes 209 PCB congeners, some of which co-elute and are represented as combinations of PCB congeners. Co-elution refers to congeners that are collected together and then not separated during the detection/quantitation process on the gas chromatograph (GC) trace. The NS&T and Gulfwatch programs utilize a subset of PCBs, summing scores from 24 peaks on the GC trace. The sum of these 24 GC peaks actually represents 31 PCB congeners since 7 of the 24 selected peaks contain two congeners each. These 31 summed PCB congeners will be called “Gulfwatch PCBs” or “NS&T PCBs” for the purposes of this report.

To compare Maine results to the NS&T and Gulfwatch PCBs, this report sums 35 congeners in the Maine SWAT PCB data, including 27 of 31 PCB congeners on the NS&T/Gulfwatch list, while including an additional 6 congeners that are not on the NS&T/Gulfwatch list. This difference is due to some congeners co-eluting differently or being summed differently at the various laboratories. These 35 summed congeners will be called “SWAT PCBs” for the purposes of this report.

Table 1.3.3.1.1 shows the list of PCB congeners used by NS&T and Gulfwatch compared to the list of PCB congeners reported by SWAT. Double numbers in the table represent co-elution or congeners that are quantified together within peaks on the GC output trace. Though the SWAT PCB and NS&T/Gulfwatch PCB congeners included in the summed lists are not identical, they are as close a comparison as possible. With some caution in data interpretation, this comparison may be used to place Maine SWAT blue mussel tissue PCB concentrations in a Gulf of Maine-wide and national perspective.

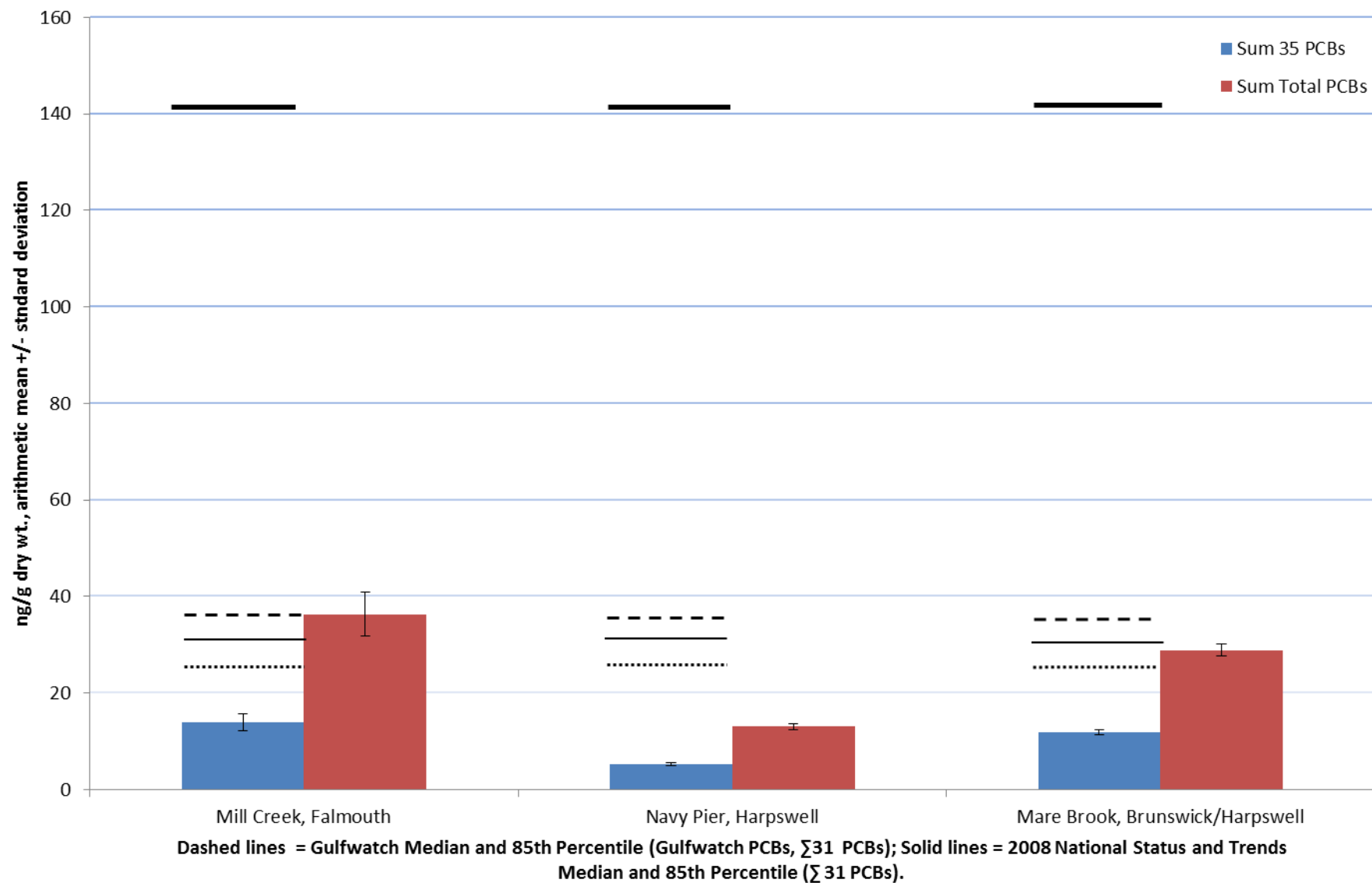
To illustrate what proportion of the total PCBs (209 congeners) the SWAT PCBs represent, Figure 1.3.3.1.1 shows the total PCBs next to the SWAT PCBs list used for comparison to Gulfwatch and NS&T data sets. Comparing the three mussel sites sampled for PCBs in 2014, the SWAT PCBs were 38.5%, 40.2%, and 41.4% of the total PCBs at Mill Creek, Falmouth, Navy Pier, Harpswell, and Mare Brook, Brunswick, respectively. Total PCB concentrations were 36.3 ng/g dry wt. at Mill Creek, 13.0 ng/g dry wt. at Navy Pier, and 28.9 ng/g dry wt. at Mare Brook (Figure 1.3.3.1.1).

Figure 1.3.3.1.1 compares the SWAT PCBs at the 2014 SWAT mussel sites to Gulfwatch median and 85th percentile for 2008 PCB data, the most recent available. None of the three 2014 SWAT mussel sites exceeded the Gulfwatch 2008 median of 24.1 ng/g dry

TABLE 1.3.3.1.1: Comparison of 35 PCBs Summed for SWAT to 31 PCBs Summed for National Status & Trends and Gulfwatch.

<u>SUM 35 PCBs</u> <u>“SWAT PCBs” List</u>	<u>SUM 31 PCBs</u> <u>“Gulfwatch, NS&T PCBs”</u> <u>List</u>
PCB-5	PCB-8/5
PCB-8	PCB-18/15
PCB-15	PCB-29
PCB 18/30	PCB-50
PCB 26/29	PCB-28
PCB 20/28	PCB-52
PCB 50/53	PCB-44
PCB-52	PCB-66/95
PCB-66	PCB-101/90
PCB-77	PCB-87
PCB-90/101/113	PCB-77
PCB-118	PCB-118
PCB-126	PCB-153/132
PCB-132	PCB-105
PCB-153/168	PCB-138
PCB-169	PCB-126
PCB-187	PCB-187
PCB-170	PCB-128
PCB-190	PCB-180
PCB-128/166	PCB-169
PCB-195	PCB-170/190
PCB-208	PCB-195/208
PCB-180/193	PCB-206
PCB-206	PCB-209
PCB-209	
PCB-105	
 <u>Unique to SWAT 35</u> <u>List</u>	 <u>Unique to GW and</u> <u>NS&T 31 List</u>
PCB-30	PCB-44
PCB-26	PCB-95
PCB-53	PCB-87
PCB-20	PCB-138
PCB-166	
PCB-193	

Figure 1.3.3.1.1.: SWAT PCBs ($\Sigma 35$ PCBs) and Total PCBs in 2014 SWAT Blue Mussels



wt., and consequently none of the sites tested in 2014 exceeded the Gulfwatch 85th percentile of 35.4 ng/g dry wt. for Gulfwatch PCBs.

Figure 1.3.3.1.1 also compares the SWAT PCBs at the 2014 SWAT sites to NS&T (NS&T) median and 85th percentile 2008 PCB data, the most recent available. None of the three SWAT sites exceeded the NS&T 2008 median, 29.2 ng/g dry wt., and none of the three exceeded the NS&T national 85th percentile, 14.1 ng/g dry wt.

Some areas in southern New England have higher levels of PCBs than Maine waters but are still relatively cleaner than the lower Hudson River/Raritan Bay system, which is heavily contaminated from PCBs moving downriver from the upper Hudson (Kimbrough et al., 2008).

From a human health perspective, the MCDC cancer FTAL for total PCBs for non-commercially caught finfish is 11 ng/g wet wt. (ppb), while the MCDC non-cancer FTAL for total PCBs is 43 ng/g wet wt. (ppb). The highest sum of total PCBs occurred at Mill Creek, Falmouth, which was 5.78 ng/g wet wt., and about half of the 11 ng/g wet wt. MCDC cancer FTAL for total PCBs, the lower, more conservative of the two FTALs.

1.3.3.2 Softshell Clams

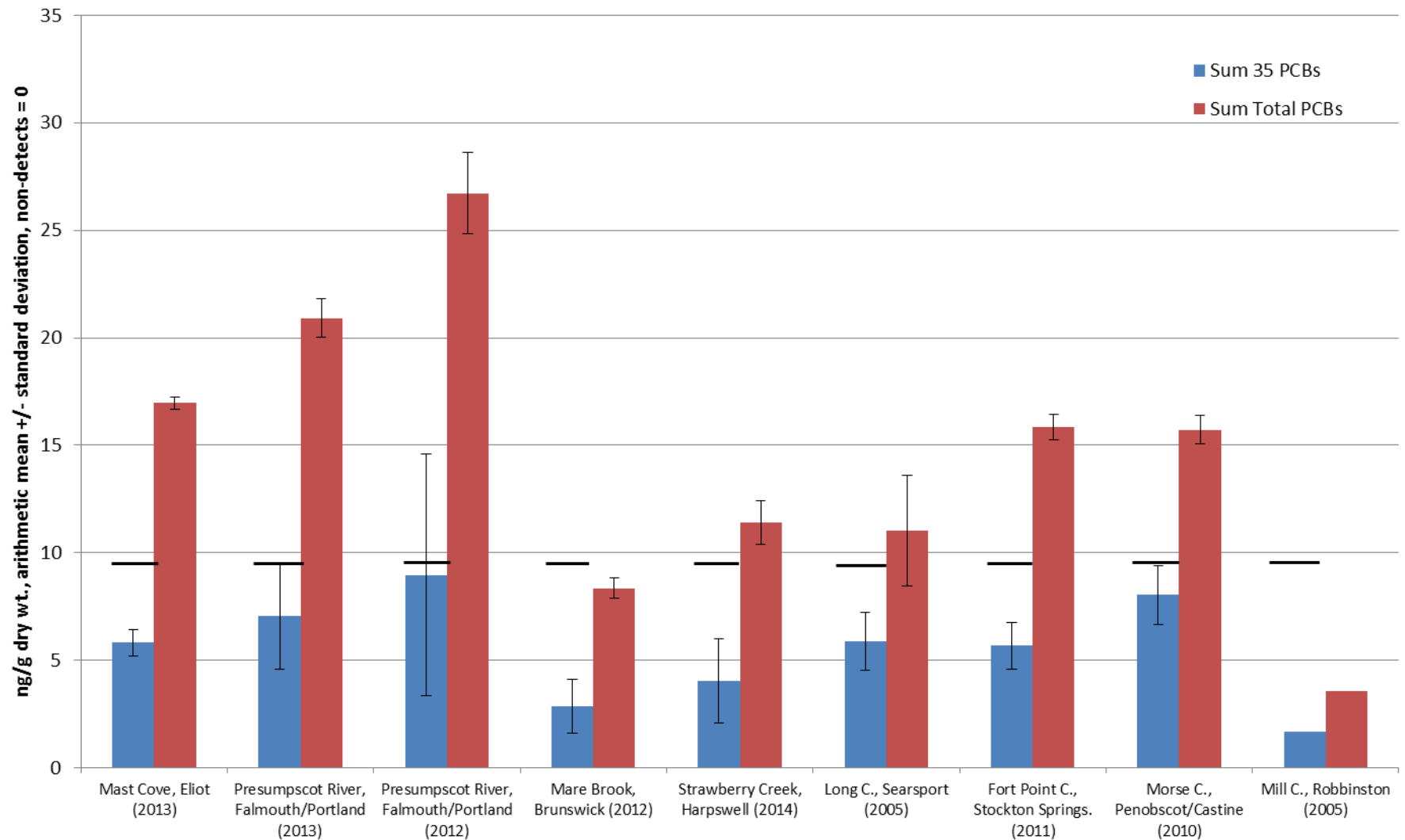
Softshell clams were tested for 209 PCBs from one site in 2014, Strawberry Creek, Harpswell, and results were compared to Gulf of Maine (Gulfwatch) (LeBlanc et al. 2009) softshell clam monitoring program data in an effort to place Maine SWAT data in a regional context. Summations of PCBs constructed for comparisons were previously discussed in Section 1.3.3.1. The same approach was utilized to construct clam PCB summations.

Table 1.3.3.1.1 shows the list of PCB congeners used by Gulfwatch compared to the list of PCB congeners reported by SWAT. Double numbers in the table represent co-elution or congeners that are quantified together within peaks on the GC output trace. Though the SWAT PCB and Gulfwatch PCB congeners included in the summed lists are not identical, they are as close a comparison as possible. With some caution in data interpretation, this comparison may be used to view Maine SWAT softshell clam tissue PCB concentrations in a Gulf of Maine-wide perspective.

To illustrate what proportion of the total PCBs (209 congeners) the SWAT PCBs represent, Figure 1.3.3.2.1 shows the total PCBs next to the SWAT PCBs list used for comparison to Gulfwatch. At Strawberry Creek, Harpswell, the SWAT PCBs were 35.3% of the total PCBs and the Total PCB concentration was 11.5 ng/g dry wt. (Figure 1.3.3.2.1).

Clams from Mill Cove and Long Cove were sampled in 2005, and analyzed at a different lab than was used for the 2010-12 clam tissues. The lab that analyzed the samples from the two early sites had much higher detection limits than the lab (AXYS Analytical) that analyzed the 2010-13 samples. In order to prevent the non-detects at Mill and Long Coves from inappropriately driving up the summations, as would have occurred if non-

Figure 1.3.3.2.1: Sum of 35 and Sum of Total PCBs in SWAT Softshell Clams



detects had been assigned a value of half the much higher detection limits used in that analysis, all non-detects were assigned a value of zero for these summations and subsequent PCB analysis of the clam samples.

Figure 1.3.3.2.1 compares the SWAT PCBs at the eight recently sampled SWAT clam sites to a Gulfwatch clam site sampled in 2008 in New Hampshire. All eight SWAT clam site summations of 35 PCBs fell below the summation for the one Gulfwatch site; though the 2012 Presumpscot summation was very close to the Gulfwatch summation (the 2013 Presumpscot summation was somewhat lower, but comparable). The 2014 clam site, Strawberry Creek, Harpswell, was well below the Gulfwatch clam site concentration. As noted above, comparison of 35 summed congeners from SWAT PCBs to 31 summed congeners from Gulfwatch PCBs is as close a comparison as possible due to differences in some PCBs co-eluting in different GC traces across laboratories. Gulfwatch non-detects were valued as half-detects, which will elevate the sum of 35 PCBs at North Mill Pond, NH, to some extent over the SWAT summations in which non-detects were valued at zero. Detection limits at the Gulfwatch site were lower than the older 2005 SWAT PCB analysis. Despite these differences, the summation of 35 SWAT congeners is useful for putting Maine data into a regional, Gulf of Maine context.

From a human health perspective, the MCDC cancer FTAL for total PCBs for non-commercially caught finfish is 11 ng/g wet wt., while the MCDC non-cancer FTAL for total PCBs is 43 ng/g wet wt. Of the seven SWAT clam sites sampled historically, the highest mean tissue concentration for total PCBs on a wet weight basis was 4.0 ng/g at the Presumpscot River, Portland (2012), which was less than half of the MCDC cancer FTAL of 11 ng/g wet wt. The 2014 site, Strawberry Creek, had a mean tissue concentration for total PCBs on a wet wt. basis of 1.7 ng/g, even further below the MCDC cancer FTAL.

1.3.4 Perfluorinated Compounds

Perfluorinated compounds or chemicals (PFCs) are organofluorine compounds that have fluorine substituted for all hydrogens where C-H bonds otherwise would occur in organic compounds. PFCs also have a functional group derived from the parent organic compound such that PFCs have properties of both fluorocarbons and the parent compound. The dual properties of PFCs make them useful in water, grease, and stain repellants (paper, fabric, and carpet treatments, notably Scotchgard by 3M), in the semiconductor industry, in firefighting foams, and as paint and other coating additives where flow is critical. Production of perfluorooctanesulfonyl fluoride related compounds, notably PFOSA (a sulfonamide), was terminated by 3M by 2003 but production overseas has continued or increased. While PFOSA was synthesized for use by industry, it is also created as a degradation byproduct of alkylated-perfluorooctanesulfonamides (which were used to treat paper, carpet, and fabric) through conversion into acetates and eventually to PFOSA.

Analysis for PFCs was suggested by the SWAT TAG for inclusion in 2013 marine SWAT investigations. With non-detects associated with 2013 softshell clam samples, and detection of PFOSA in blue mussels, further analysis of mussels was recommended by the TAG. This report includes data for two additional blue mussel sites tested in 2014. This report utilizes the Maine SWAT blue mussel tissue and softshell clam tissue PFC data generated by AXYS Analytical, which includes 13 compounds as presented in Table 1.3.4.1.1.

1.3.4.1 Blue Mussels

Blue mussels from two sites were tested for PFCs in 2014, Navy Pier, Harpswell, and Mare Brook, Brunswick. Both sites have a history of military activity: Navy Pier served as a fuel transfer site from ship to shore; and Mare Brook drains a portion of the former Brunswick Naval Air Station.

PFOSA was detected in three of four spatial replicates of mussel tissue collected at each of the two sites, Navy Pier and Mare Brook. Perfluoroheptanoate (PFHpA) was detected in only one of four spatial replicates at Navy Pier, Harpswell, and was not detected in any of the four spatial replicates at Mare Brook, Brunswick. The remaining PFCs were all below detection limits at

Table 1.3.4.1.1: LIST OF PERFLUORONATED COMPOUNDS AND THE RANGE OF SAMPLE SPECIFIC DETECTION LIMITS FOR 2014 SWAT BLUE MUSSELS

		<u>Non-Detects</u>	
		<u>Mussels</u>	
		<u>Low</u>	<u>High</u>
PERFLUOROBUTANE SULFONATE	NG/G	6.155	7.189
PERFLUOROBUTANOATE	NG/G	3.078	3.595
PERFLUORODECANOATE	NG/G	3.078	3.595
PERFLUORODODECANOATE	NG/G	3.078	3.595
PERFLUOROHEPTANOATE**	NG/G	3.078	3.595
PERFLUOROOCTANOATE	NG/G	3.078	3.595
PERFLUOROHEXANE SULFONATE	NG/G	6.155	7.189
PERFLUOROHEXANOATE	NG/G	3.078	3.595
PERFLUORONONANOATE	NG/G	3.078	3.595
PERFLUOROOCTANE SULFONATE	NG/G	6.155	7.189
PERFLUOROOCTANE SULFONAMIDE*	NG/G	3.992	4.314
PERFLUOROPENTANOATE	NG/G	3.078	3.595
PERFLUOROUNDECANOATE	NG/G	3.078	3.595

* Non-detect values for mussels are from one spatial replicate at Navy Pier, Harpswell, and one spatial replicate at Mare Brook, Brunswick, as each site had detects for three out of four spatial replicates.

** Non-detect values for mussels are from four spatial replicates at Mare Brook, Brunswick, and three spatial replicates at Navy Pier, Harpswell, as Navy Pier had one detect out of four spatial replicates.

both 2014 Blue Mussel sampling locations. Table 1.3.4.1.1 also shows the low and high values for the sample-specific detection limits for the PFCs for which analyses were performed. In general, sample-specific detection limits were approximately 3 to 7 parts per billion (ng/g) in

mussel tissue on a dry weight basis. PFOSA levels detected in tissue from Mare Brook ranged from 4.51 to 5.32 ng/g dry wt. with a mean of 4.87 ng/g dry wt. across the three spatial replicates where it was detected. PFOSA levels detected in tissue from Navy Pier ranged from 5.13 to 6.62 ng/g, with a mean of 5.80 ng/g dry wt. across the three spatial replicates that were detected. The only detect for PFHpA was 6.13 ng/g dry wt. from the Navy Pier, Harpswell.

PFHpA is a breakdown product of grease- and stain-proofing coatings on upholstered furniture, carpet, and food packaging material, is persistent in the environment, and is a seven-carbon version of PFOA. PFOSA has eight carbon atoms and breaks down into PFOS. It was an ingredient in the 3M Scotchgard formulation prior to its being discontinued and was used as a grease and water repellent in food packaging and other applications.

PFCs bioaccumulate and biomagnify through the food web. Testing of California *Mytilus spp.* tissue indicated >25% detection frequency for PFCs in samples and increasing concentrations with urbanization and proximity to stormwater discharge (Dodder et al., 2012). Total concentrations of PFCs ranged up to about 10 ppb, with some outliers above that range. Areas with mixed development topped out at total PFC concentrations of approximately 2 ng/g dry wt., while urban sites had higher total PFC concentrations approaching 9-10 ng/g dry wt. Two individual PFCs detected in the California study, PFDoDA and PFUnDA, had mean concentrations of 1.8 and 0.23 ng/g dry wt. respectively, which is roughly the same order of magnitude of the PFCs detected in recent SWAT mussel sampling in Maine (PFOSA – East End Beach (2013), Navy Pier and Mare Brook (2014), and PFHpA – Navy Pier (2014) (Dodder et al., 2012)). EPA has not released a fish tissue action level for PFCs.

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2.0 LAKES MODULE

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SPECIAL THANKS to the many department staff members and citizen volunteers who assisted us in this project.	
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2.1. HARMFUL ALGAE BLOOMS (HABs)

2.1.1 Background

Cyanotoxins are secondary metabolites produced by cyanobacteria (a/k/a bluegreen algae). In general, cyanotoxins have been measured during ‘bloom’ conditions when cell densities are highest. Such blooms are referred to as Harmful Algal Blooms or HABs in many areas of the country. Because triggers for toxin production are unclear, it has been difficult to associate production with routinely measured limnologic parameters such as chlorophyll-a, total phosphorus, cell counts, or Secchi disk transparency. Cyanotoxins may have hepatotoxic or neurotoxic effects, depending on the specific toxin present. In Maine, Microcystin LR (hepatotoxin) is thought to be most likely observed followed by Anatoxin (neurotoxin). There is some speculation that BMAA, another cyano-neurotoxin found in the Northeast, may cause long-term effects and has been associated with ALS, Parkinson’s disease, MS, and perhaps Alzheimer’s disease.

Blue-green algae are an opportunistic group of organisms. Populations attain high cell densities or bloom conditions when nutrient concentrations, particularly phosphorus, are elevated due to point-sources, non-point sources, internal sources (sediments) or a combination thereof. Most blue-greens have the ability to ‘fix’ nitrogen in specialized cells called heterocysts and have gas vesicles which they use to control buoyancy in the water column. They thrive under calm, sunny, warm conditions and create resting stages, some of which can accumulate phosphorus from the sediments for later use. Many species are not preferred food for zooplankton either due to size or palatability. With all of these features, blue-greens out-compete other algal species in more productive Maine lakes. Approximately 30 Maine lakes have annual algae blooms and another 20 have occasional blooms.

The DEP Lake Assessment Section undertook a multipurpose study in 2014 to better understand the distribution of cyanotoxins in Maine lakes and to test a possible surrogate for quickly identifying risk. There were four goals: 1) to implement a probability-based project so that results could be extrapolated to the larger population; 2) to test validity of using a portable fluorometer to determine photosynthetic pigment (chlorophyll and phycocyanin) concentrations in lake water obtained from 3-4 locations in each lake as a surrogate for cyanotoxins (cooperative regional study with EPA); 3) to determine concentrations of Microcystin LR in 140 samples (funded by SWAT), some of which came from the deep station where chlorophyll-a and total phosphorus concentrations would also be obtained; and 4) revisit a subset of lakes at least 4 times to evaluate time-series data. Goals 3 & 4 are the focus of this report.

2.1.2 Study Design

With assistance from EPA, 20 lakes greater than 150 acres in size were randomly selected from Kennebec County to target for this study. Another 4 lakes having a history of severe algal blooms were also targeted. Each lake was visited at least once in mid to late summer; samples were obtained from the deepest location in accordance with DEP Baseline monitoring protocols (including Secchi transparency, temperature/dissolved oxygen profiles, and water samples for chemistry including chlorophyll-a and total phosphorus); an additional sample was collected for pigment analysis using the EPA Fluorometer, and possible cyanotoxin analysis. Following EPA protocols for the fluorometric pigment analysis, a three-meter integrated sample was collected at

the deep spot as well as from two other deep locations; three one-meter integrated samples from two-meter-deep water along the down-wind shore; and, if surface scums were present, an additional three surface grabs. A total of 463 samples were collected for fluorometric analysis (3775 readings). DEP protocol deep-hole samples and the sample having the highest phycocyanin concentration (or highest fluorometric chlorophyll concentration if phycocyanin was non-existent) from each of the EPA protocol sample sets were frozen for possible microcystin analysis. Of the 192 samples frozen, funding was sufficient to analyze 140 samples.

Seven of these lakes were revisited at least four times to collect samples for time series analysis. Sample Stations were chosen to reflect areas in which various designated uses were most likely to occur. The deep hole and deep water stations were intended to characterize conditions that might be experienced by fishermen and water-skiers; the downwind shore stations characterize conditions one might experience swimming and wading; and downwind scums might represent conditions experienced wading and by pets/livestock drinking lake water. Accidental or intentional ingestion of lake water might occur at any of these locations.

2.1.3 Results

A considerable range of Microcystin LR concentrations were observed in the 140 samples submitted for analysis (Beagle Bioproducts, Columbus, OH). Descriptive statistics yielded a minimum of 0.08 ppb, maximum of 17,696 ppb, average of 1.04 ppb and a median of 241 ppb.

Figure 2.1.1 illustrates the distribution of microcystin in each of the lake stations monitored; note that the y-axis is log transformed to display the data in a manner that best depicts the distribution at each station. The World Health Organization (WHO) drinking water standard or level of concern (LOC) of 1 ppb is indicated by the red line which crosses the plot at $\text{Log}(1)=0$. Samples analyzed from the deep hole (DH), deep area (DA) and downwind shore (DS) had comparable distributions over a range of

Figure 2.1.1. Distribution of Microcystin LR Concentration by Sampling Station in 20 Maine Lakes, late summer 2014.

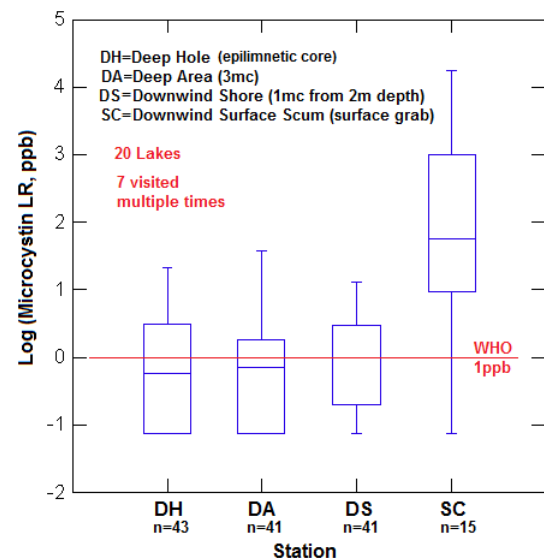
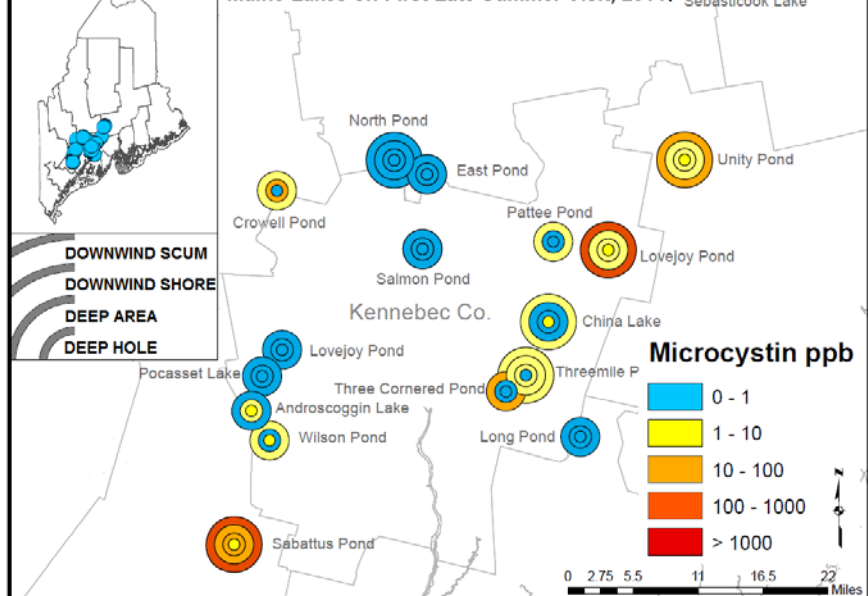


Figure 2.1.2. Microcystin LR Results by Sampling Station in 17 Central Maine Lakes on First Late Summer Visit, 2014.



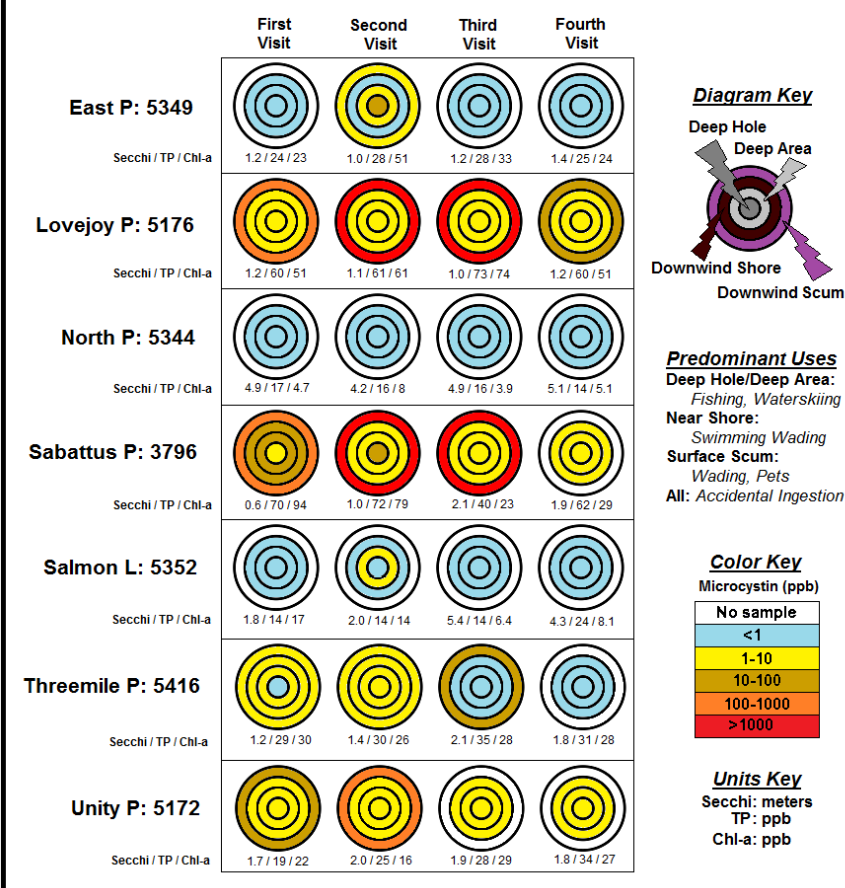
0.08 - 37.8 ppb. It is noteworthy that 54.4% of these samples exceeded the WHO LOC of 1 ppb; this is a much higher percentage of samples than measured during a pilot project in 2008 and 2009 [9%, or four of 45 (three of which were only slightly over 1.0 ppb)]. Nearly all of the surface scum samples (14 of 15, or 93%) exceeded the WHO LOC, not unexpected given the results from the aforementioned pilot project (six of six, or 100%).

Figure 2.1.2 illustrates first visit microcystin concentrations geographically in the Central Maine region. Concentric circles represent Sampling Stations (refer to key located just below the map of Maine). If no downwind scums were present, no outer circle appears. Sabattus Pond (Sabattus) clearly had the highest concentrations of microcystin followed by Lovejoy Pond (Albion) and then Unity Pond (Unity). Only 14 of the 20 Kennebec County probability-draw lakes were submitted for analysis because six had such low fluorometric pigment concentrations. Six of the fourteen lakes submitted for analysis had concentrations of microcystin below the WHO LOC at all stations.

Figure 2.1.3 illustrates time series data collected at seven lakes. Lakes were visited four times approximately every two weeks beginning in August of 2014. Again concentric circles are used to illustrate the sample station from which each concentration was measured. Sabattus Pond (Sabattus) and Lovejoy Pond (Albion) were found to have the highest concentrations of microcystin.

Concentrations were generally highest on the second visit for all lakes, except North Pond (Smithfield). Figure 2.1.4 presents actual microcystin concentrations for the same lakes shown in Figure 2.1.3. Note the extremely high concentrations measured at the second visits to Lovejoy (Albion) and Sabattus.

Figure 2.1.3. Time Series Concentrations of microcystin in seven Maine lakes. Lakes were visited 4 times with approximately 2 weeks between visits beginning in late August 2014. Samples were obtained from 3-4 locations on the lake represented by concentric circles. Secchi Transparency depth, Total Phosphorus and Chlorophyll_a concentrations from the Deep Hole are included below each sampling event.



2.1.4 Discussion

Results obtained during the 2014 study clearly indicate that some Maine lakes produce cyanotoxins when experiencing algal blooms. Highest Microcystin values were measured in the algal scums, the maximum (17,696 ppb) being nearly 2.5 orders of magnitude greater than the maximum observed from open water concentrations (38 ppb). In a few cases, the material collected that was thought to be an algal scum was found to be primarily insect casings and non-algal detritus, which would account for some of the lowest scum microcystin values (0.08). The open water microcystin concentrations were often well above the WHO LOC of 1.0 ppb.

The time-series data indicates substantial variation in the concentration of Microcystin during the life of the bloom. Highest concentrations were often observed in early September; nevertheless, some blooms produced considerable concentrations in late August. North Pond (Smithfield) experiences bloom conditions less frequently than the other time-series lakes and did not bloom in 2014. Salmon Lake (Belgrade) and East Pond (Oakland / Smithfield) produced bloom conditions in 2014, with East Pond sustaining low transparencies over the two-month period, but neither had Microcystin concentrations as high as Lovejoy Pond (Albion) and Sabattus Pond (Sabattus). It is possible that the blooms were caused by different blue-green species and/or that internal recycling of phosphorus (as evidenced by

the high Total Phosphorus concentrations shown in Figure 2.1.3) is a driver of blooms that become HABs. Microscopic algal species identification or genetic testing might lend some insight. [Note: samples were preserved but not sent for taxonomic analysis due to budget constraints; Dr. Susan Brawley's Algal Ecology class at the University of Maine (Orono) is currently performing genetic testing on 10 of the samples as a student community assistance project.]

Figure 2.1.4. Microcystin LR Concentrations (ppb) in Seven Maine Lakes from which Time Series Data was Collected during 2014.					
		Color Key			
MC(ppb)		<1	1-10	10-100	100-1000
Color		Blue	Yellow	Orange	Red
Lake & ID	Location	1st Visit	2nd Visit	3rd Visit	4th Visit
East: 5349	Deep Hole	0.08	12.37	0.08	0.08
	Deep Area	0.08	1.05	0.48	0.08
	Near Shore	0.08	0.48	0.08	0.93
	Surface Scum		9.67		
Lovejoy: 5176	Deep Hole	1.38	5.7	3	1.19
	Deep Area	1.83	2.94	1.81	1.68
	Near Shore	2.48	2.81	1.63	1.68
	Surface Scum	490.99	17696.08	1948.13	18.46
North: 5344	Deep Hole	0.08	0.08	0.08	0.08
	Deep Area	0.08	0.08	0.08	0.08
	Near Shore	0.08	0.08	0.08	0.08
Sabattus: 3796	Deep Hole	3.46	21.47	1.6	4.67
	Deep Area	37.84	3.34	1.82	7.35
	Near Shore	12.39	5.6	4.46	4.44
	Surface Scum	618.62	10605.58	1674.43	
Salmon: 5352	Deep Hole	0.63	0.08	0.35	0.35
	Deep Area	0.18	1.89	0.08	0.08
	Near Shore	0.99	0.08	0.27	0.2
Threemile: 5416	Deep Hole	0.64	1.68	0.33	0.3
	Deep Area	1.02	1.51	0.7	0.33
	Near Shore	1.48	2.31	0.61	0.27
	Surface Scum	9.26	2.7	56.9	
Unity: 5172	Deep Hole	3.16	7.88	3.14	6.42
	Deep Area	2.43	1.74	6.44	6.96
	Near Shore	3.2	2.13	7.44	4.97
	Surface Scum	22.53	272.9		

Preliminary statistical examination of the relationships between microcystin and each trophic indicator (Chlorophyll-a, Total Phosphorus and Secchi Transparency) obtained at the ‘deep hole’ station, indicates that they are all significant ($p =$ or < 0.05) with Chlorophyll-a and Total Phosphorus being positive and Secchi Transparency being negative. However, the Standard Error for each of these indicators is 3.6, 3.8, and 3.9 ppb of microcystin respectively, which is reasonable across the observations, and none of the indicators provide strong predictive value at the WHO LOC of 1.0 ppb.

In summary, these data indicate that some Maine lakes, which support algal blooms, have Microcystin LR concentrations in exceedance of the WHO LOC. Because of the health risk posed to humans, pets and livestock, cyanotoxin concentrations should be monitored in blooming lakes in future years. This year, EPA is in the process of determining LOCs for various Designated Uses this year; once LOCs are established, it is anticipated that DEP will work with Maine CDC to determine if/how risk should be communicated. These findings present an opportunity for Maine laboratories to provide a service to quickly and accurately measure cyanotoxin concentrations in water samples for DEP, concerned citizens, environmental organizations and municipalities.

2.2. PFCs IN FISH FROM MAINE LAKES AND PONDS

Introduction

Perfluorochemicals (PFCs) are a large (>200) class of highly persistent and mobile chemicals composed of fully fluorinated straight or branched carbon chains with different functional groups at one end. Consequently they may be hydrophilic, hydrophobic, and/or lipophilic. They have many specialized industrial and commercial uses for products that resist heat, stains, water, oil and grease, including hair conditioners, non-stick coatings, wetting agents, insulation, dust repellants, cleaners, anti-static agents, antifogging agents, and fire-fighting foams among others (Qi et al., 2011; Yingling, 2013).

PFCs are continuously emitted into the environment from point and nonpoint sources such as sewage treatment plants and atmospheric deposition, respectively (Ahrens and Bundschuh, 2014). In a study of sources of PFCs in major rivers of the world, Kimacjeva et al. (2012) found higher levels in industrial areas than in non-industrial areas. The most commonly detected PFCs are perfluorooctane sulfonate (PFOS) and to a lesser extent perfluorooctanoic acid (PFOA). Beginning in 2002, PFOS has been phased out in the US, Canada, and Europe, but its use has been increasing in China (Yingling, 2013).

PFCs have been found in humans and wildlife all over the world including the arctic and deep seas (Yingling, 2013), which suggests atmospheric sources (Houde et al., 2011). They have been correlated with increased cancers, thyroid disease, interference with normal growth and development, and endocrine disruption in humans (Yingling, 2013). There are also reports in the literature of high concentrations in invertebrates, fish, reptiles, and marine mammals worldwide (Houde et al. 2011).

PFCs with 8 or more carbons are considered bioaccumulative with sulfonates (e.g. PFOS) having a greater bioaccumulation rate than PFOA and other PFCs, indicating that the functional group is also important (Martin et al., 2013). Bioaccumulation of PFOS is considered similar to that of a moderately lipophilic substance (Houde et al., 2011). Bioaccumulation is higher in some tissues than others (liver>kidneys>whole blood>gill>carcass) but bioaccumulation factors in the carcass range up to ~2400 (Sharpe et al., 2010). PFC concentrations have been reported as high as 1900 ng/g wet wt. (Houde et al., 2011). Adverse effects in fish are not well known, but mortality, decreased fecundity, and histopathological alterations have been reported (Ahrens and Bundschuh, 2014; Sharpe et al. 2010).

MCDC has derived human health risk-based screening levels for PFOS and PFOA. Screening levels (SLs) were developed for exposures to soil, sediment, groundwater, surface water, and for the ingestion of fish. Health risk-based SLs for these PFCs are based on non-cancer effects because cancer toxicity values have not been established (Wadman, 2014). In a Maine study of streams near Loring Air Force Base (LAFB), where fire-fighting foams have been used, DEP found brook trout to have concentrations of PFOS ranging from 41-1080 ng/g wet wt. in exposed sites, all but one of which exceeded MCDC's SL for subsistence fishers (42 ng/g) and many of which exceeded MCDC's SL for a Maine recreational angler (175 ng/g), all based on upper level fish consumption rates for each group. Concentrations of PFOS in brook trout were at or below the SLs (0-43 ng/g) at a reference site (Akladiss, 2014).

Methods

To gather more data from reference sites and from other species, in 2014, six to ten brook trout, smallmouth bass, and brown bullhead were collected live from three lakes or ponds (one wetland) each via custom made minnow traps. Fish were immediately euthanized and placed in new clean plastic garbage bags on ice for transport back to the lab, where they were measured for length and weight, rinsed in tap water, wrapped in aluminum foil and frozen before shipment to AXYS Analytical Services laboratory in British Columbia, Canada. At the lab, fish were skinned, fileted and homogenized, combined into two composites of three to five fish each and analyzed for a suite of PFCs via AXYS Method MLA-043 Rev 08.

Results and Conclusions

Results show that concentrations of most PFCs were undetected (U) (Table 3.2.1). PFOS and perfluoroundecanoate were the most commonly detected, in four and five of nine waterbodies respectively. Both compounds were detected in one or two of the three samples for all three species. PFOS concentrations were well below MCDC's SLs and the concentrations found near LAFB. The magnitude of detected concentrations was no greater in the benthic omnivorous species brown bullhead (BBH) than in pelagic predators brook trout (BKT) and smallmouth bass (SMB).

Table 2.2.1 PERFLUORINATED COMPOUNDS (PFCs) IN FISH FROM MAINE LAKES (ng/g).

WATERBODY →	EGYPT P		PATTEN P		TMW		BALD MTN P		RANGELEY L		QUIMBY P		PITCHER P		SHEEPSCOT L		GREAT P	
SPECIES →	BBH		BBH		BBH		BKT		BKT		BKT		SMB		SMB		SMB	
PFC↓																		
PERFLUOROBUTANE SULFONATE	0.99	U	0.99	U	0.98	U	0.99	U	0.99	U	0.98	U	0.99	U	0.99	U	0.99	U
PERFLUOROBUTANOATE	0.49	U	0.50	U	0.49	U	0.49	U	0.49	U	0.49	U	0.49	U	0.49	U	0.50	U
PERFLUORODECANOATE	0.49	U	0.50	U	0.49	U	0.51	U	0.49	U	0.49	U	0.49	U	0.89		1.29	
PERFLUORODODECANOATE	0.49	U	0.50	U	0.49	U	1.26		0.49	U	0.49	U	0.49	U	1.87		1.47	
PERFLUOROHEPTANOATE	0.49	U	0.50	U	0.49	U	0.49	U	0.49	U	0.49	U	0.49	U	0.49	U	0.50	U
PERFLUOROHEXANE SULFONATE	0.99	U	0.99	U	0.98	U	0.99	U	0.99	U	0.98	U	0.99	U	0.99	U	0.99	U
PERFLUOROHEXANOATE	0.49	U	0.50	U	0.49	U	0.49	U	0.49	U	0.49	U	0.49	U	0.49	U	0.50	U
PERFLUORONONANOATE	0.49	U	0.50	U	0.49	U	0.49	U	0.49	U	0.49	U	0.49	U	0.49	U	0.50	U
PERFLUOROCTANE SULFONATE	0.99	U	2.57		0.98	U	3.30		0.99	U	0.98	U	1.27	U	3.15		4.71	
PERFLUOROCTANE SULFONAMIDE	0.59	U	0.59	U	0.59	U	0.59	U	0.59	U	0.59	U	0.59	U	0.59	U	0.60	U
PERFLUOROCTANOATE	0.49	U	0.50	U	0.49	U	0.49	U	0.49	U	0.49	U	0.49	U	0.49	U	0.50	U
PERFLUOROPENTANOATE	0.49	U	0.50	U	0.49	U	0.49	U	0.49	U	0.49	U	0.49	U	0.49	U	0.50	U
PERFLUOROUNDECANOATE	0.66		0.50	U	0.49	U	3.05		0.49	U	0.49	U	1.12		3.34		3.57	
LIPIDS	1.13		1.78		0.83		0.56		1.18		1.31		0.64		0.78		1.67	
MOISTURE	80.05		79.95		81.15		77.95		77.65		77.40		78.55		78.95		77.60	
TMW = THREEMILE WETLAND																		

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3.0 RIVERS AND STREAMS MODULE

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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 potentially impaired by toxic contamination. To establish a baseline for comparison, 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 that live on the stream bottom, such as mayflies, stoneflies, caddisflies, crayfish, snails, and leeches. In 2014, the Biological Monitoring Unit evaluated the condition of 40 sample locations, primarily in the St. John River basin.

The Biological Monitoring Unit uses a multivariate statistical model to analyze a benthic macroinvertebrate sample and predict whether a waterbody is attaining the biological criteria associated with its statutory class (DEP Rule Chapter 579). If a waterbody does not meet minimum state aquatic life criteria, Class C, then the model class is predicted as Non-Attainment (NA). Classes AA and A are treated the same in the model. Final determinations on aquatic life attainment of a waterbody are made accounting for factors that may allow adjustments to the model outcome.

Table 3.1.1 summarizes the results of ambient biological monitoring activities for the Rivers and Streams Module portion of the 2014 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.
- *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. By statute, Class AA and A waterbodies must support a “natural” biological community. Class B waterbodies must not display “detrimental changes in the resident biological community”. Class C waterbodies must “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. A final determination of non-attainment (NA) indicates that the sample did not meet the minimum Class C criteria. A final determination of Indeterminate (I) indicates that a final determination could not be made based on the aquatic community collected.
- *Attains Class* – “Yes” is given if the final determination is equal to or exceeds the Statutory Class. A Class B stream, for example, would receive a “Yes” if its final determination was either A or B. “No” is given if a stream does not attain its Statutory Class. A Class B stream, for example, would receive a “No” if its final determination was either C or NA.
- *Probable Cause* – The probable cause column lists potential stressors to benthic macroinvertebrate communities that have resulted in a final determination of non-attainment of a waterbody's assigned statutory class, based on the best professional judgment of the investigator. In some cases, a probable cause may not be related to toxic pollution but instead to other factors.

Field and water chemistry data for each sampling event (where available) are given in Tables 3.1.2 and 3.1.3, respectively. The data from Tables 3.1.1 to 3.1.3 is also summarized in reports for each sampling event, known as Aquatic Life Classification Attainment Reports, which are available in electronic format with the web-published version of this report:

(<http://www.maine.gov/dep/water/monitoring/toxics/swat/index.htm>). Continuous water temperature data are given in Figure 3.1.1. The attainment history of sampling stations prior to 2014, where available, is given in Table 3.1.4.

For more information about the Biological Monitoring Unit, please e-mail us at biome@maine.gov or visit our web site: <http://www.maine.gov/dep/water/monitoring/biomonitoring/>. The Data and Maps page of this website provides access to station information and available data via Google Earth.

3.1.2 Results Summary

The Biological Monitoring Unit concentrated its sampling in 2014 in the St. John River basin. Forty stations were sampled under the SWAT Program (Table 3.1.1).

Forty stations were analyzed in 2014 to determine aquatic life attainment, with thirty-two of these stations determined to be in attainment of their statutory class. Seventeen of these stations exceeded their assigned class. No licensing/relicensing issues have been found in waterbodies sampled below municipalities or industries. The streams that failed to attain their statutory class were all small rural or urban systems; summaries for these streams are found below.

Alder Brook, Presque Isle Station 1016

Alder Brook is a cold first order stream with a water quality classification goal of Class B. The sampling station is located below Route 1 across from the University of Maine, Presque Isle. The stream flows adjacent to agricultural fields and the University campus. Sampling in 2014 determined that Alder Brook meets the minimum Class C aquatic life criteria but does not meet the goal of Class B aquatic life criteria. The macroinvertebrate community is very diverse with the Generic Richness or number of different taxa totaling 64. However, the number of sensitive organisms is low. The Ephemeroptera, Plecoptera, and Trichoptera (EPT) Generic Richness, representing the number of mayflies, stoneflies, and caddisflies, is 13 with all but 3 taxa comprised of caddisflies. Forty percent of the community is comprised of the tolerant midges, *Parametriocnemus*, *Micropsectra*, *Tanytarsus* and the snail *Physa*. The level of Nitrate + Nitrite as N is very high at 1.5 mg/l and Total Phosphorus is 91 µg/l (Table 3.1.3). This is the first time Alder Brook has been sampled for aquatic life attainment status.

Amsden Brook, Fort Fairfield Station 1018

Amsden Brook is a second order stream with a water quality classification goal of Class B. It flows southwest into the Aroostook River in Fort Fairfield. Agricultural land use is very high in the watershed. Sampling in 2014 determined that Amsden Brook meets the minimum Class C aquatic life criteria but does not meet the goal of Class B aquatic life criteria. There are very few sensitive organisms present in the community, with tolerant midges comprising 71% of the community. Generic Richness in the community is low and EPT Generic Richness is represented by only one taxon, the mayfly *Baetis*. The dominant organism in the community is the tolerant midge *Cricotopus* which makes up 41% of the community. The heavy algae found on the rocks

probably favor this organism. The Nitrate + Nitrite as N level is very high at 5.4 mg/l and Total Phosphorus is high as well at 87 µg/l, indicating runoff from the agricultural fields in the watershed (Table 3.1.3). This is the first time Amsden Brook has been sampled for aquatic life attainment status.

Cowett Brook, Presque Isle Station 1021

Cowett Brook is a cold first order stream located in Presque Isle with a water quality classification goal of Class B. The stream flows west through a very concentrated agricultural area before entering the Aroostook River. Sampling in 2014 determined that Cowett Brook meets the minimum Class C aquatic life criteria but does not meet the goal of Class B aquatic life criteria. Generic Richness is low and EPT Generic Richness is represented by only three taxa. The tolerant mayfly *Baetis* is the most dominant organism in the community followed by the tolerant midge *Diplocladius*. The relative Richness of Diptera makes up 62% of the community. In general, Diptera are tolerant organisms that are more abundant in Class C systems. The Total Phosphorus in the system is high at 55 µg/l (Table 3.1.3). This is the first time Cowett Brook has been sampled for aquatic life attainment status.

Hacker Brook, Fort Fairfield Station 1024

Hacker Brook is a second order stream with a water quality classification goal of Class B. Hacker Brook flows southeast through an area of agricultural land use and then enters the Aroostook River in Fort Fairfield. Sampling in 2014 determined that Hacker Brook meets the minimum Class C criteria for aquatic life but does not meet the goal of Class B aquatic life criteria. Diversity, Generic Richness, and EPT Generic Richness are low. Eighty percent of the community is made up of two taxa, the collector-filterer caddisfly *Hydropsyche*, and the tolerant mayfly *Baetis*. The Nitrate + Nitrite as N level and Total Phosphorus are very high indicating agricultural runoff (Table 3.1.3). This is the first time Hacker Brook has been sampled for aquatic life attainment status.

Halfmoon Stream, Thorndike Station 697

Halfmoon Stream is a third order stream which flows west to the town of Unity, entering Sandy Stream and eventually Unity Pond. Above the Rt. 220 Bridge crossing in Thorndike, its water quality classification goal is Class A. The stream flows through a concentrated agricultural area consisting of large dairy farms. The station was sampled in 2003 and 2007 and met the Class A aquatic life criteria in both years. In 2012, the macroinvertebrate community showed signs of enrichment with the Total Mean Abundance of the sample totaling over 1300 individuals. Although Generic Richness was high, the total number of sensitive organisms was low compared to the Total Mean Abundance, and the most dominant taxa in the sample consisted of tolerant collector-filterers and scrapers (see SWAT 2012). Halfmoon Stream did not meet the Class A aquatic life criteria in 2012. The stream was sampled again during the 2013 field season. The Total Mean Abundance of the sample totaled over 1700 individuals. The Generic Richness dropped to 29 taxa as compared to 70 taxa in 2012. Possible explanations for this significant drop in Generic Richness were the combination of a 3-foot increase in water level due to a storm event in 2013, and the lack of algae attached to the rock bags during sample collection. In 2012, dense mats of algae were present on the samplers. The Chironomidae and some mayfly taxa use the algae as a food source and as attachment sites. In addition, the Nitrate + Nitrite as N level was high at 0.62 mg/l, indicating possible runoff from the agricultural fields. The 2014 sampling

determined that Halfmoon Stream did not meet the Class A aquatic life criteria. In 2014, Halfmoon Stream was indeterminate for Class A (.55) aquatic life criteria but was not raised to Class A based on abundance and dominant taxa found. The TMA of the 2014 sample was very high (3886 organisms/sampler) indicating very high enrichment. Although Generic Richness increased from 29 taxa in 2013 to 90 taxa in 2014, the makeup of the dominant taxa in the community consisted of organisms that are moderately to very tolerant to increases in organic loading. *Polypedilum*, a tolerant midge, made up 26% of the sample or over 1000 organisms per sampler. This high quality resource has shown a trend of increasing enrichment since 2003. Halfmoon Stream has been established as a long term monitoring station for the Biological Monitoring Unit. This should allow better understanding of community shifts due to agricultural inputs over time.

Hammond Brook, Hamlin Station 1025

Hammond Brook is a third order stream with a water quality classification goal of Class B. The stream flows north into the St. John River. Agriculture is the primary land use in the upper part of the watershed. Sampling in 2014 determined that Hammond Brook's macroinvertebrate community meets the Class C aquatic life criteria but does not attain the Class B criteria for aquatic life. The stream habitat is degraded as bank erosion is extreme in some areas of the watershed. In addition, the rock substrate is covered with algae. The macroinvertebrate community exhibits an enrichment effect. TMA is greater than 2400 organisms per rock bag sampler. Generic Richness is good but diversity is low. Sixty-four percent of the community consists of the filter feeding caddisfly *Hydropsyche* and the filter feeding midge *Cricotopus*. Sensitive taxa are not found in great enough numbers to meet the Class B aquatic life criteria. This is the first time Hammond Brook has been sampled for aquatic life attainment status.

Kennedy Brook, Presque Isle Station 646

Kennedy Brook is a second order stream with a water quality classification goal of Class B. It flows from east to west through Presque Isle and enters Presque Isle Stream. Land use in the watershed is primarily agricultural and urban. Sampling in 2014 determined that Kennedy Brook meets the minimum Class C aquatic life criteria but not the goal of Class B aquatic life criteria. Kennedy Brook shows signs of enrichment as the Total Mean Abundance is almost 1500 organisms/sampler. This is high for a second order system. Few of the taxa in the sample are comprised of sensitive organisms. The relative abundance of midges (Chironomidae) in the community is 60%. The majority of midges are tolerant of organic pollution. The midge *Rheotanytarsus* makes up 43% of the community. *Rheotanytarsus* is a collector filterer that does well when organic inputs are increasing in the system. The bottom substrate was covered with algae when the samplers were collected. The Nitrate + Nitrite as N level is high at 1.4 mg/l (Table 3.1.3). Kennedy Brook has been sampled in 2002, 2004, and 2009 and was determined to have attained Class B aquatic life criteria in each of those years (Table 3.1.4).

Perkins Stream, Waterville Station 977

Perkins Stream is a third order stream with a water quality classification goal of Class B. The stream flows through a highly impervious area, through the campus of Colby College, and finally to Messalonskee Stream. The Total Mean Abundance (48) and Generic Richness (14) are very low and do not meet the minimum criteria to run through the attainment model. Professional judgment is utilized when these minimum criteria are not met. Using this method, it was

determined that Perkins Stream does not meet the minimum Class C aquatic life criteria. There are very few sensitive taxa present in the community, as indicated by the stream's EPT Generic Richness of one. The majority of the community is made up of tolerant midges with almost half the sample comprised of the tolerant midge *Microtendipes*. Specific conductance is very high in the system (Table 3.1.2) as well as Total Dissolved Solids (Table 3.1.3). The habitat of the stream is compromised with the banks highly eroded and silts covering the substrate. Perkins Stream was previously sampled in 2012 and did not attain the Class C aquatic life criteria.

Table 3.1.1. 2014 SWAT Benthic Macroinvertebrate Biomonitoring Results

Waterbody	Town	Station	Log	Potential sources of pollution ¹	Statutory Class/ Final Determination	Attains Class?	Probable Cause
Alder Brook	Presque Isle	1016	2280	Agricultural NPS	B/C	N	Agricultural runoff
Allen Brook	Westfield	1017	2282	Agricultural NPS	B/A	Y	
Amsden Brook	Ft. Fairfield	1018	2272	Agricultural NPS	B/C	N	Agricultural runoff
Big Brook	Madawaska	728	2268	Agricultural NPS	B/B	Y	
Birch Brook	Presque Isle	1019	2264	Agricultural NPS	B/B	Y	
Bither Brook	New Limerick	1036	2294	Agricultural NPS	B/A	Y	
Cowett Brook	Presque Isle	1021	2276	Agricultural NPS	B/C	N	Agricultural runoff
Craig Brook	Littleton	1006	2286	Agricultural NPS	B/A	Y	
E. Br. Wesserunsett	Athens	486	2255	Long Term	B/A	Y	
Frost Brook	Westfield	1022	2283	Agricultural NPS	B/A	Y	
Getchell Brook	Easton	925	2285	Agricultural NPS	B/A	Y	
Gray Brook	Ft. Fairfield	1023	2273	Agricultural NPS	B/B	Y	
Hacker Brook	Ft. Fairfield	1024	2271	Agricultural NPS	B/C	N	Agricultural runoff
Halfmoon Stream	Thorndike	697	2256	Agricultural NPS/ Long Term	A/B	N	Agricultural runoff
Hammond Brook	Hamlin	1025	2266	Agricultural NPS	B/C	N	Agricultural runoff
Hill Brook	Houlton	556	2288	Agricultural NPS	B/A	Y	
Hockenhull Brook	Ft. Fairfield	1026	2274	Agricultural NPS	B/B	Y	
Kennebec River	Benton	196	2259	Municipal	B/A	Y	
Kennedy Brook	Presque Isle	646	2279	Urban NPS/Ag. NPS	B/C	N	Urban and Agricultural runoff
Limestone Stream	Limestone	47	2270	Agricultural NPS	C/C	Y	
Little Androscoggin River	Mechanic Falls	1033	2260	Municipal	B/B	Y	
Meduxnekeag River	Houlton	364	2290	Municipal	B/A	Y	
Meduxnekeag River	Houlton	1028	2293	Agricultural NPS	B/B	Y	
N. Br. Presque Isle Stream	Mapleton	687	2278	Agricultural NPS	B/A	Y	
North Fork McLean Brook	St. Agatha	922	2269	Agricultural NPS	B/B	Y	
Oliver Brook	Hodgdon	1005	2291	Agricultural NPS	B/A	Y	
Otter Brook	Caribou	1035	2265	Agricultural NPS	B/B	Y	

¹ NPS, non-point source pollution.

Table 3.1.1. 2014 SWAT Benthic Macroinvertebrate Biomonitoring Results

Waterbody	Town	Station	Log	Potential sources of pollution¹	Statutory Class/ Final Determination	Attains Class?	Probable Cause
Pearce Brook	Houlton	463	2292	Urban NPS	B/A	Y	
Perkins Stream	Waterville	977	2253	Urban NPS	B/NA	N	NPS toxics; Habitat
Piscataquis River	Dover Foxcroft	152	2258	Municipal	B/B	Y	
Piscataquis River	Sangerville	135	2257	Municipal	B/A	Y	
Presque Isle Stream	Presque Isle	197	2277	NPS	B/B	Y	
Prestile Stream	Blaine	3	2284	Municipal	B/B	Y	
Sheepscot River	N. Whitefield	74	2252	Long Term	AA/A	Y	
Smith Brook	Houlton	1007	2289	Agricultural NPS	B/A	Y	
Suiter Brook	Littleton	1029	2287	Agricultural NPS	B/A	Y	
Unnamed Brook	Presque Isle	1027	2275	Agricultural NPS	B/B	Y	
Unnamed Brook	Madawaska	1030	2267	NPS	B/A	Y	
W. Br. Sheepscot River	China	268	2254	Long Term	AA/A	Y	
Williams Brook	Presque Isle	1031	2281	Agricultural NPS	B/A	Y	

¹ NPS, non-point source pollution.

Table 3.1.2. 2014 SWAT Field Data

Measurements were obtained using handheld electronic meters. Highlighted values are of concern or do not attain criteria.

Site	Station	Log	Sample Deployment					Sample Retrieval				
			Date	Temperature Deg C	Dissolved Oxygen mg/l	Specific Conductance µS/cm	pH STU	Date	Temperature Deg C	Dissolved Oxygen mg/l	Specific Conductance µS/cm	pH STU
Alder Brook	1016	2280	7/22/2014	18.2	9.4	550	7.12	8/20/2014	14	9.17	600	7.78
Allen Brook	1017	2282	7/23/2014	18.7	8.8	359	8.4	8/20/2014	16.8	9.4	380	7.69
Amsden Brook	1018	2272	7/22/2014	13	10.6	456	8.62	8/19/2014	13.6	10.1	479	8.19
Big Brook	728	2268	7/21/2014	21.6	9	149	7.13	8/18/2014	17.6	9.36	153	7.56
Birch Brook	1019	2264	7/21/2014	13	9.5	450	7.04	8/18/2014	12	10	458	7.85
Bither Brook	1020	2294	7/24/2014	19.3	9.4	295	7.13	8/22/2014	18	8.9	303	6.81
Cowett Brook	1021	2276	7/22/2014	10	10.3	511	6.88	8/19/2014	10.9	10.3	510	6.96
Craig Brook	1006	2286	7/23/2014	21.3	8.4	351	8.31	8/21/2014	16.4	8.73	390	8.35
E. Branch Wesserunsett	486	2255	7/9/2014	22.2	8.4	47	7.15	8/6/2014	18.8	9	39	7.13
Frost Brook	1022	2283	7/23/2014	18.2	8.9	438	7.32	8/20/2014	16	9.3	459	7.43
Getchell Brook	925	2285	7/23/2014	13.8	9.1	528	8.15	8/20/2014	14.7	9.8	498	7.59
Gray Brook	1023	2273	7/22/2014	18.2	10	422	7.6	8/19/2014	14.3	11.54	429	8.3
Hacker Brook	1024	2271	7/22/2014	16.2	9.7	467	7.35	8/19/2014	15.3	9.32	472	7.6
Halfmoon Stream	697	2256	7/9/2014	25.1	9.4	102	7.27	8/6/2014	21.6	10.5	114	8.45
Hammond Brook	1025	2266	7/21/2014	20	9.5	216	7.48	8/18/2014	16.8	9.62	170	8.2
Hill Brook	556	2288	7/23/2014	20.6	8.2	367	8.28	8/21/2014	16.3	9.33	339	8.31
Hockenhull Brook	1026	2274	7/22/2014	18.5	10.5	405	7.13	8/19/2014	16.8	10.35	411	8.33
Kennebec River	196	2259	7/14/2014	21	8.7	55	7.11	8/11/2014	22.5	8.1	58	6.61
Kennedy Brook	646	2279	7/22/2014	20.3	9.3	549	8.18	8/20/2014	16.4	9.81	590	7.81

Table 3.1.2. 2014 SWAT Field Data (continued)

Site	Station	Log	Sample Deployment					Sample Retrieval				
			Date	Temperature Deg C	Dissolved Oxygen mg/l	Specific Conductance US/CM	pH STU	Date	Temperature Deg C	Dissolved Oxygen mg/l	Specific Conductance US/CM	pH STU
Limestone Stream	47	2270	7/21/2014	23	8.7	289	8.23	8/19/2014	16.3	9.63	260	7.93
Little Androscoggin River	1033	2260	7/14/2014	25	7.9	83	7.14	8/12/2014	22.3	8.4	84	6.9
Meduxnekeag River	364	2290	7/24/2014	23.4	8.2	177	7.97	8/22/2014	21.6	9.3	161	8.8
Meduxnekeag River	1028	2293	7/24/2014	21.7	9.6	210	7.9	8/21/2014	21.3	9.52	224	6.47
N. Br. Presque Isle Stream	687	2278	7/22/2014	25.3	10.5	267	8.52	8/20/2014	17	9.29	302	8.3
North Fork McLean Brook	922	2269	7/21/2014	15.2	8.4	250	7.2	8/18/2014	13.9	9.54	261	7.18
Oliver Brook	1005	2291	7/24/2014	20.4	8	330	7.1	8/21/2014	20.1	8.16	368	6.76
Otter Brook	1035	2265	7/21/2014	17.1	9.3	350	6.7	8/18/2014	14.6	9.77	337	7.98
Pearce Brook	463	2292	7/23/14	24.5	8.4	249	8.62	8/22/14	18.2	8.1	283	7.74
Perkins Stream	977	2253	7/9/2014	21.3	8	818	7.67	8/5/2014	23.5	7.6	862	7.17
Piscataquis River	152	2258	7/10/2014	24.3	9.4	31	7.13	8/7/2014	22.9	8.5	41	6.82
Piscataquis River	135	2257	7/10/2014	25	8.9	44	7.3	8/7/2014	23.9	10.5	61	8.5
Presque Isle Stream	197	2277	7/22/2014	25.3	9.7	167	8.17	8/19/2014	21.3	9.48	167	7.17
Prestile Stream	3	2284	7/23/2014	21.9	10.5	414	7.32	8/20/2014	21.9	12.41	363	8.52
Sheepscot River	74	2252	7/8/2014	22.4	7.9	41	6.89	8/5/2014	22.8	7.9	47	6.36
Smith Brook	1007	2289	7/23/2014	21.3	8.4	329	8.31	8/21/2014	17	9.14	369	8.16
Suitter Brook	1029	2287	7/23/2014	19.5	8.2	376	8.25	8/21/2014	15.9	9.18	415	7.55
Unnamed Brook	1027	2275	7/22/14	13.5	9.4	375	7.04	8/19/14	14.0	9.64	402	7.57
Unnamed Brook	1030	2267	7/21/2014	18.5	8.8	267	7.12	8/18/2014	16.3	9.67	268	7.95
W. Br. Sheepscot River	268	2254	7/8/2014	22.9	8.1	64	6.75	8/5/2014	22.5	8.2	69	7.03
Williams Brook	1031	2281	7/23/2014	18.9	8.4	404	8.02	8/20/2014	16.3	9.4	425	7.72

Table 3.1.3. 2014 SWAT Water Chemistry Data

Samples were analyzed by the Health & Environmental Testing Laboratory, Augusta, ME. Highlighted values indicate high results.

Waterbody	Station	Log	Sampling Date	DOC	NH ₃ -N	TKN	NO ₂ -NO ₃ -N	SRP	Total P	TSS	TDS
				mg/l	mg/l	mg/l	mg/l	µg/l	µg/l	mg/l	mg/l
Alder Brook	1016	2280	7/29/14			0.4	1.5	34	91		
Allen Brook	1017	2282	7/29/14			0.3	0.36	18	28		
Amsden Brook	1018	2272	7/8/14			0.6	5.4	35	87		
Big Brook	728	2268	7/9/14			0.5	0.45	4	25		
Birch Brook	1019	2264	7/31/14			0.2	4.5	18	26		
Cowett Brook	1021	2276	7/8/14			0.3	0.4	32	55		
Craig Brook	1006	2286	7/10/14			0.4	1.6	9	21		
E. Branch Wesserunsett Stream	486	2255	8/6/14	6.2	<0.01	0.4	0.02	1	21	2.8	56
Frost Brook	1022	2283	7/29/14			0.3	0.33	7	29		
Getchell Brook	925	2285	7/29/14			0.3	1.9	9	20		
Gray Brook	1023	2273	7/8/14			0.5	1.7	46	62		
Hacker Brook	1024	2271	7/8/14			0.5	4.3	64	100		
Halfmoon Stream	697	2256	8/6/14	2.1	0.01	0.3	0.55	3	12	2	84
Hammond Brook	1025	2266	7/30/14			0.5	0.24	3	24		
Hill Brook	556	2288	7/7/14			0.3	0.43	5	15		
Hockenhull Brook	1026	2274	7/8/14			0.5	1.9	24	42		
Kennebec River	196	2259	8/11/14	4.5	0.01	0.3	0.08	2	12	3.2	57
Kennedy Brook	646	2279	7/29/14			0.4	1.4	9	48		

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, TDS = total dissolved solids, "<" = constituent not detected at the reporting limit.

Table 3.1.3. 2014 SWAT Water Chemistry Data (continued)

Samples were analyzed by the Health & Environmental Testing Laboratory, Augusta, ME. Highlighted values indicate high results.

Waterbody	Station	Log	Sampling Date	DOC	NH ₃ -N	TKN	NO ₂ -NO ₃ -N	SRP	Total P	TSS	TDS
				mg/l	mg/l	mg/l	mg/l	µg/l	µg/l	mg/l	mg/l
Limestone Stream	47	2270	7/30/14			0.4	0.68	7	42		
Little Androscoggin River	1033	2260	8/12/14	3.5	0.01	0.3	0.14	3	17	<0.01	57
Meduxnekeag River	364	2290	7/28/14			0.2	0.32	1	7		
Meduxnekeag River	1028	2293	7/28/14			0.2	0.12	1	9		
N. Br. Presque Isle Stream	687	2278	7/31/14			2.2	0.35	5	26		
North Fork McLean Brook	922	2269	7/9/14			0.5	5.9	14	28		
Oliver Brook	1005	2291	7/7/14			0.4	0.44	9	18		
Pearce Brook	463	2292	7/28/14			0.2	0.08	2	11		
Perkins Stream	977	2253	8/5/14	3.9	0.01	0.3	0.09	4	30	5.1	510
Piscataquis River	135	2257	8/7/14	3.5	0.01	0.3	0.02	1	10	<0.01	51
Piscataquis River	152	2258	8/7/14	3.9	<0.01	0.3	0.02	17	32	<0.01	56
Presque Isle Stream	197	2277	7/30/14			0.4	0.15	3	35		
Prestile Stream	3	2284	8/20/14			0.2	0.53	2	23		
Sheepscot River	74	2252	8/5/14	5	0.01	0.3	0.02	2	12	<0.01	52
Smith Brook	1007	2289	7/10/14			0.6	0.42	4	14		
Unnamed Brook (Presque Isle)	1027	2275	7/8/14			0.3	3.3	20	36		
Unnamed Brook (Madawaska)	1030	2267	7/9/14			0.6	1.5	14	29		
W. Br. Sheepscot River	268	2254	6/24/14	4.4	0.01	0.3	0.02	2	11	<0.01	60
Williams Brook	1031	2281	7/29/14			0.5	0.61	8	43		

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, TDS = total dissolved solids, "<" = constituent not detected at the reporting limit.

Figure 3.1.1. 2014 In-Stream Continuous Temperature Data

Please note: all data are in degrees Celsius and maximum value of Y-axis varies amongst graphs.

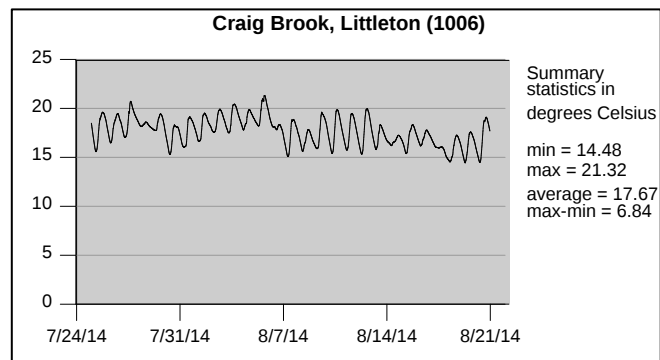
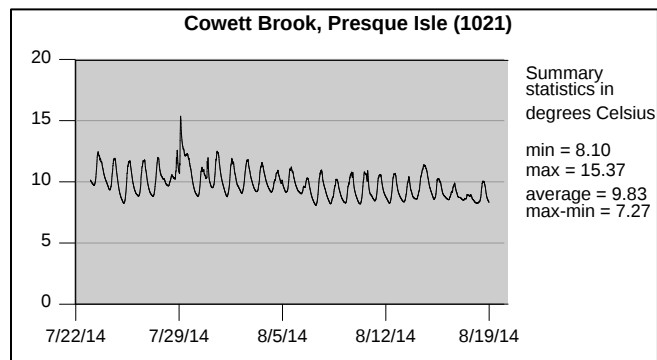
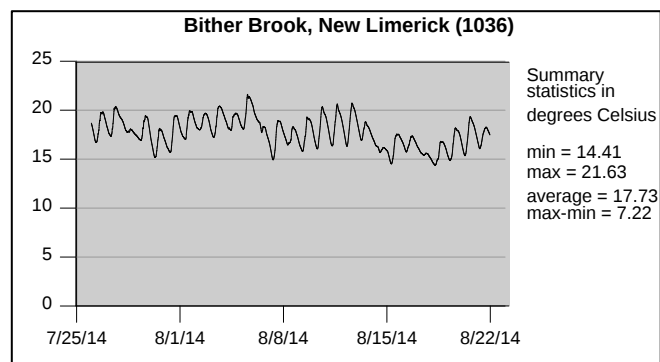
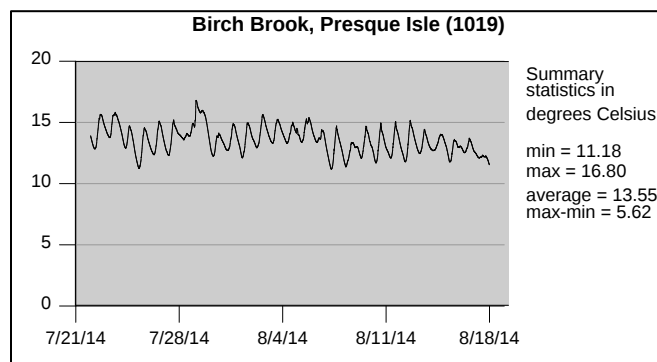
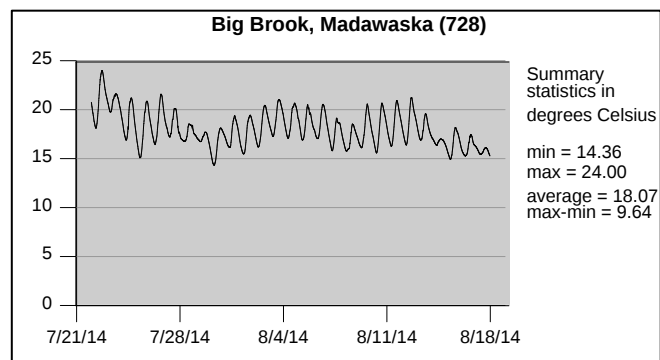
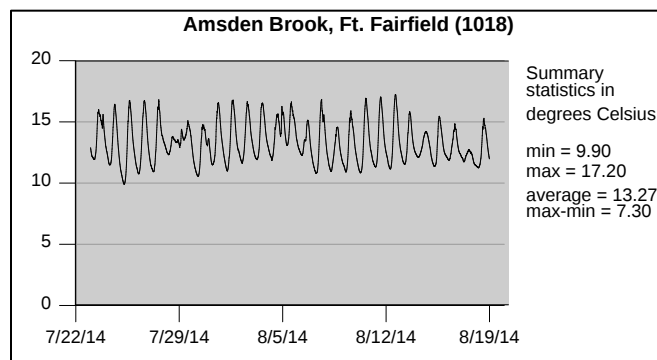
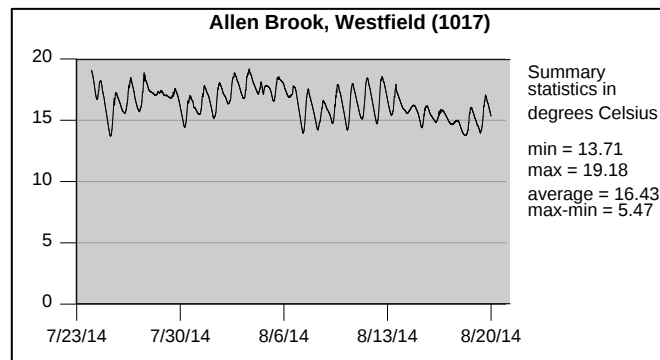
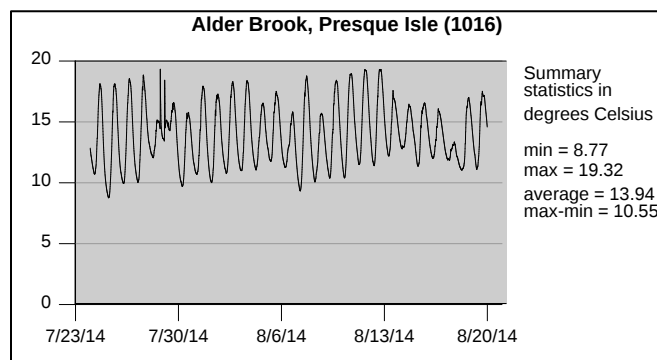


Figure 3.1.1. 2014 In-Stream Continuous Temperature Data (continued)

Please note: all data are in degrees Celsius and maximum value of Y-axis varies amongst graphs.

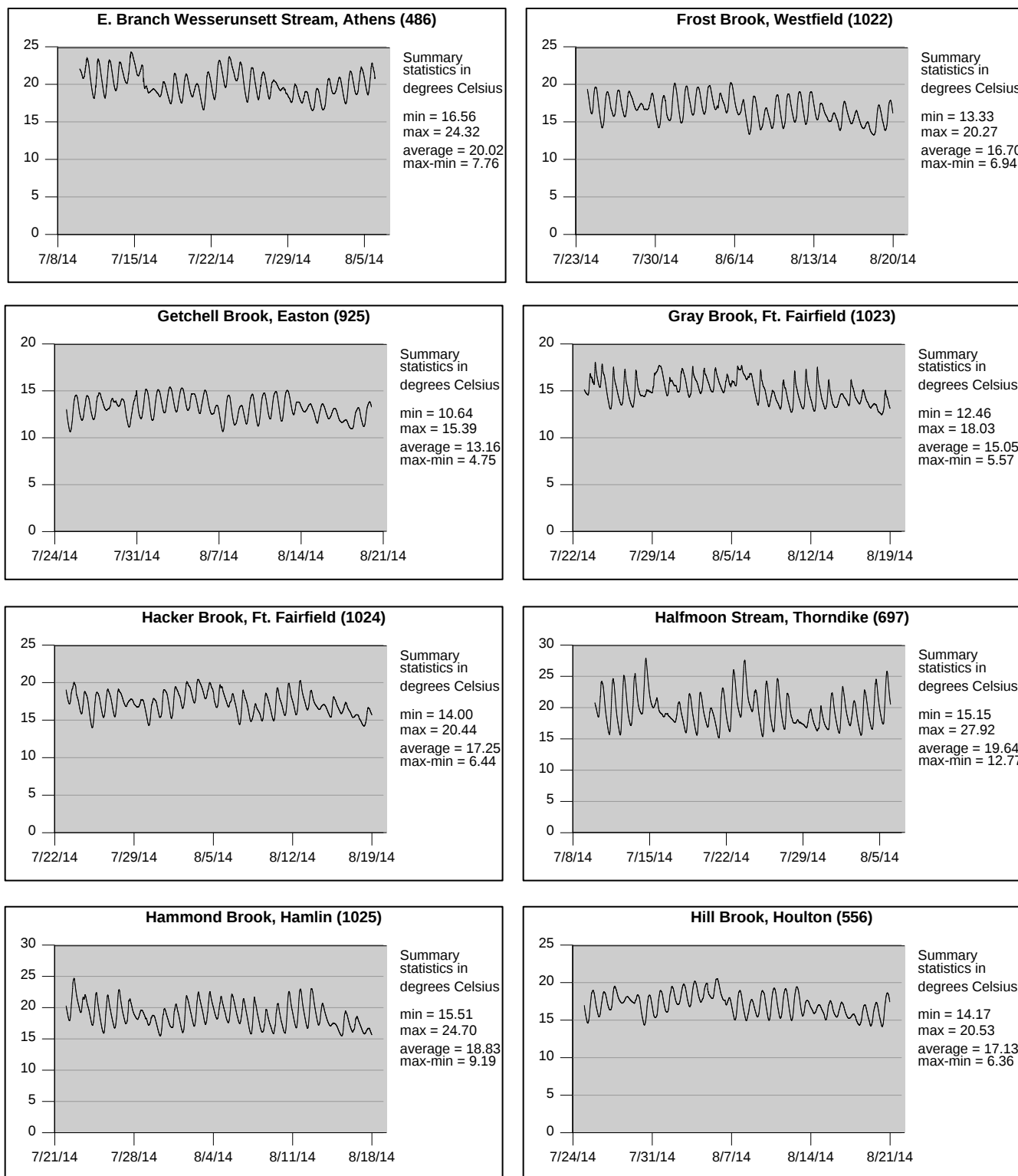


Figure 3.1.1. 2014 In-Stream Continuous Temperature Data (continued)

Please note: all data are in degrees Celsius and maximum value of Y-axis varies amongst graphs.

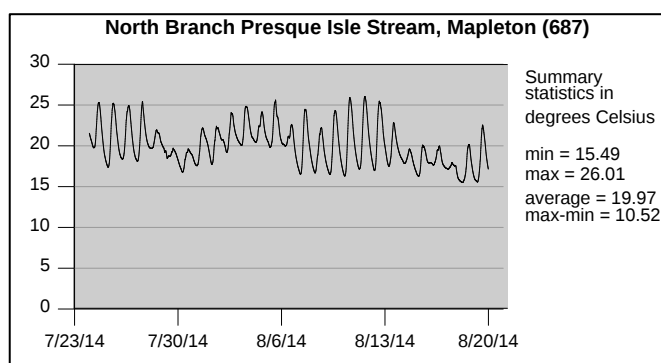
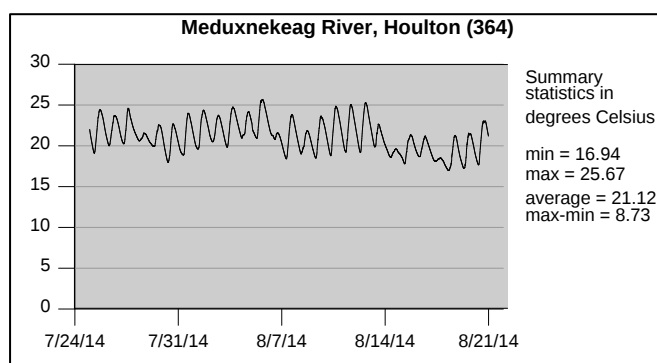
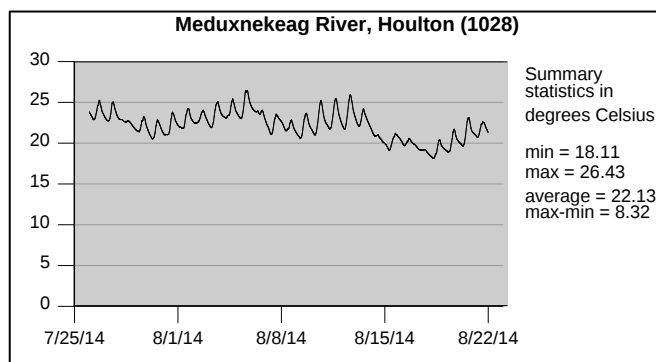
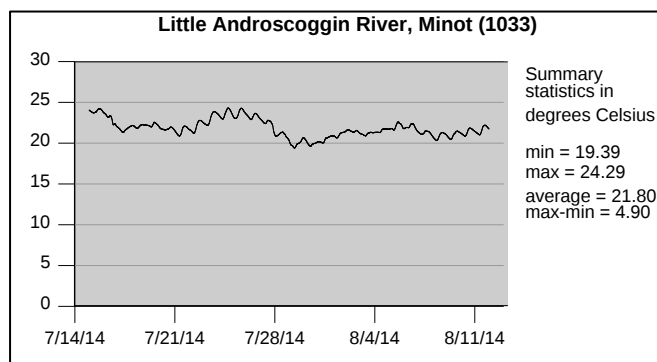
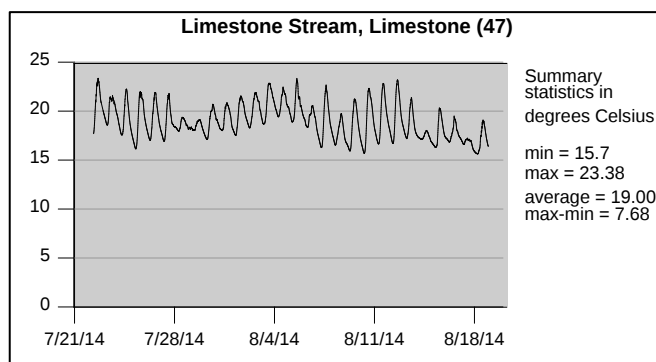
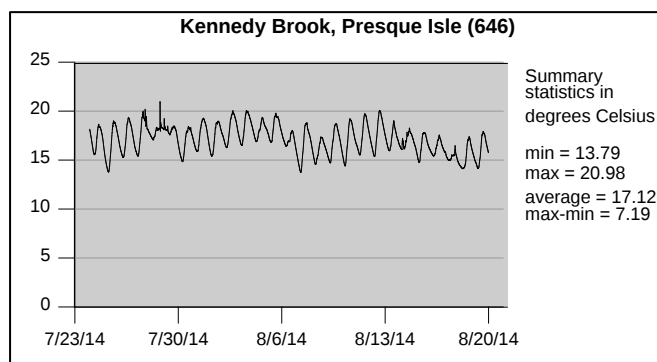
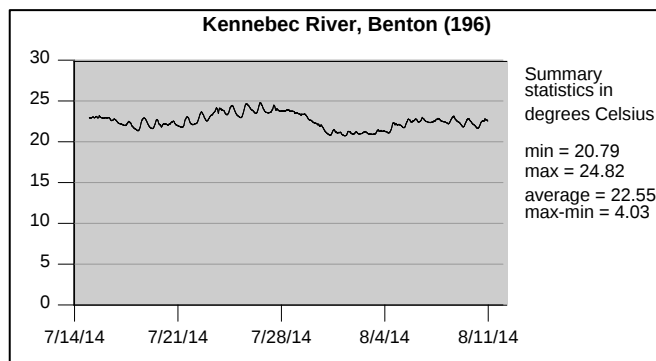
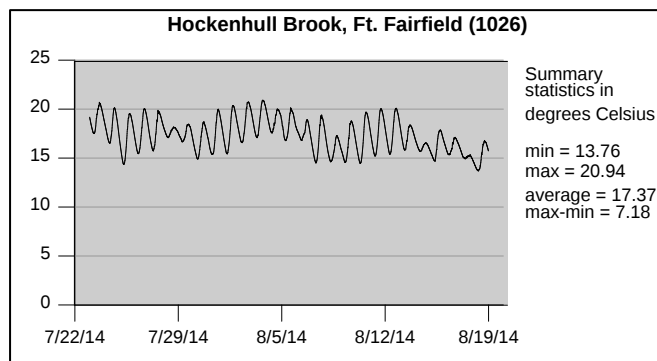


Figure 3.1.1. 2014 In-Stream Continuous Temperature Data (continued)

Please note: all data are in degrees Celsius and maximum value of Y-axis varies amongst graphs.

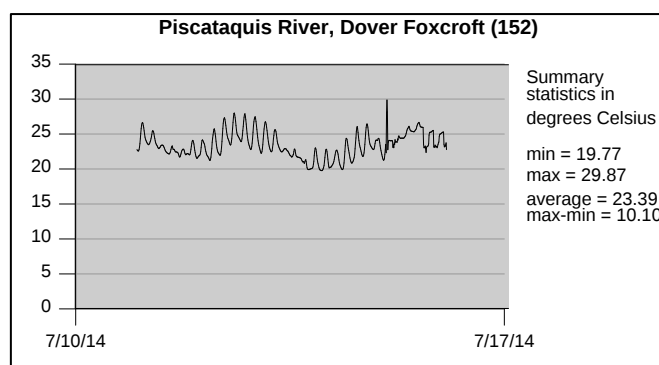
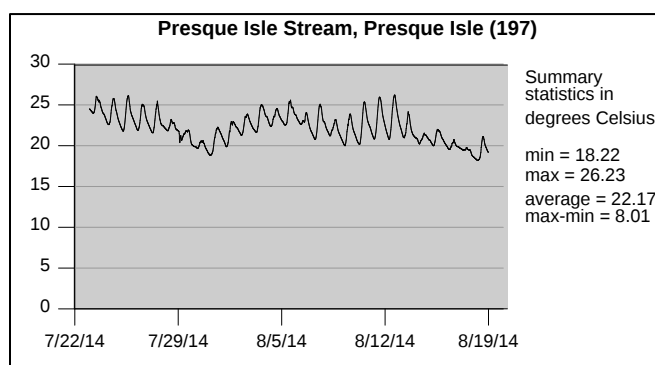
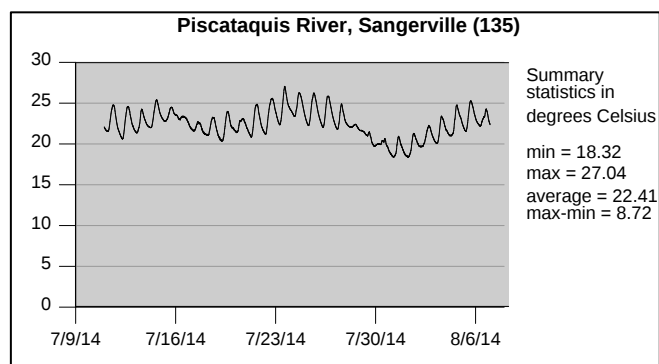
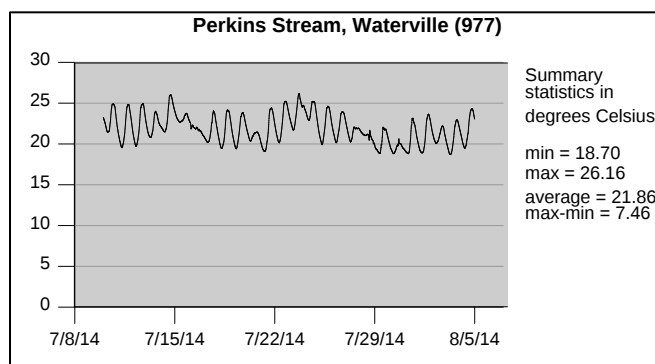
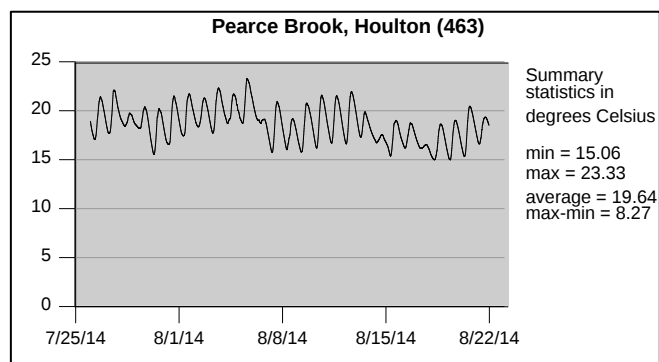
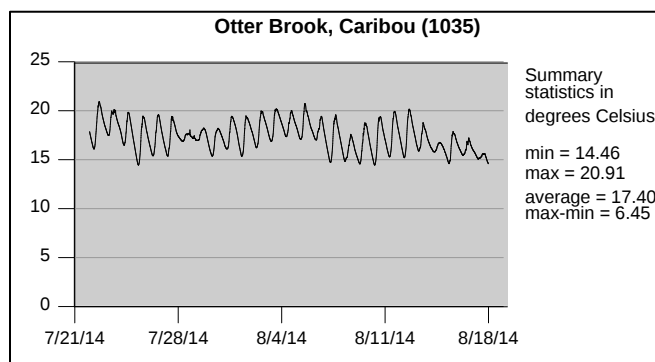
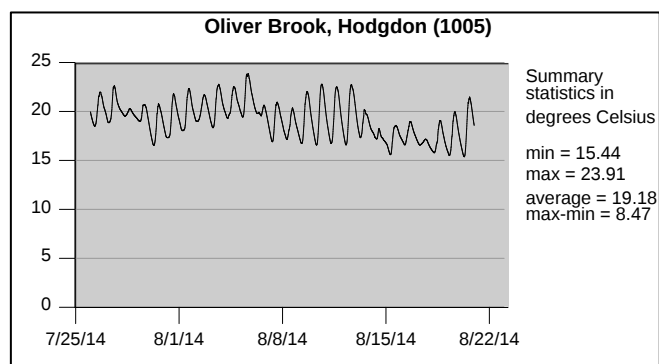
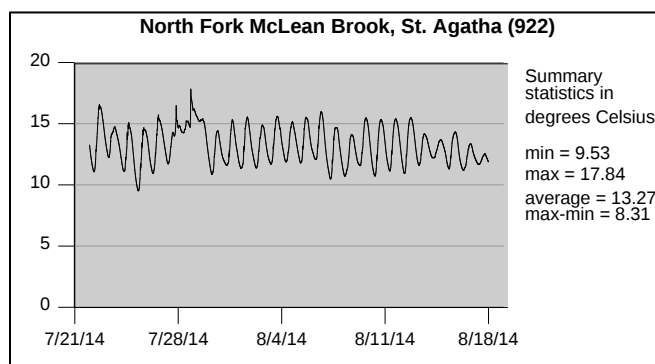
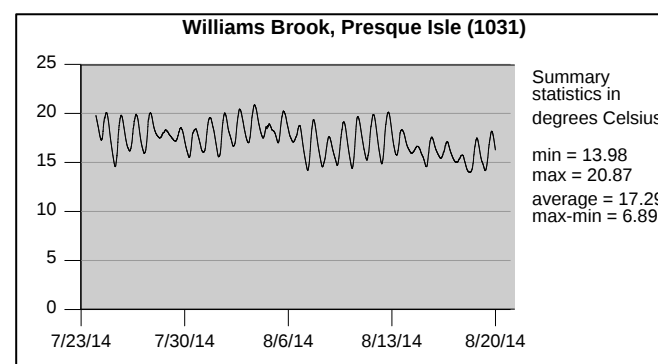
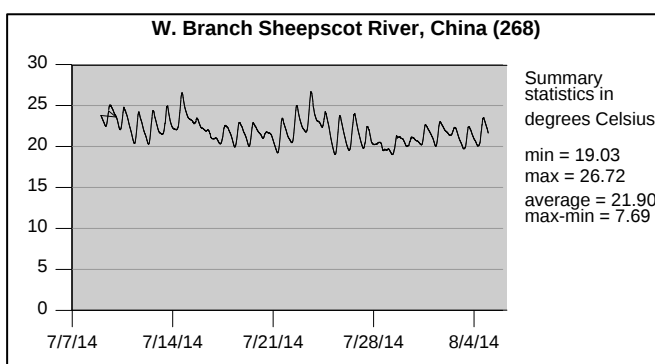
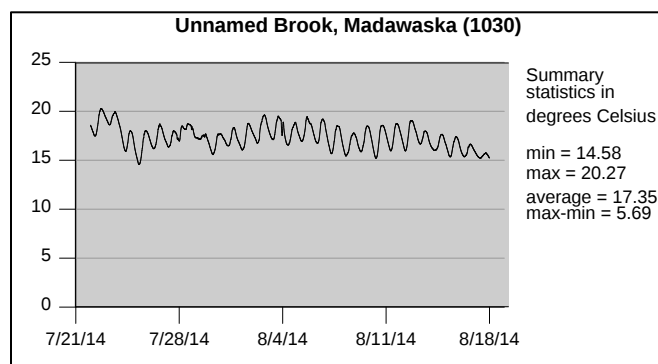
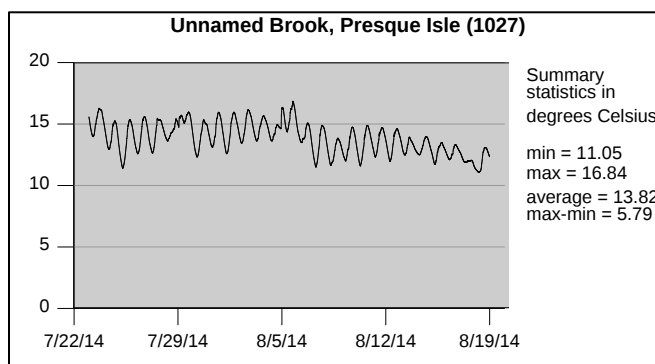
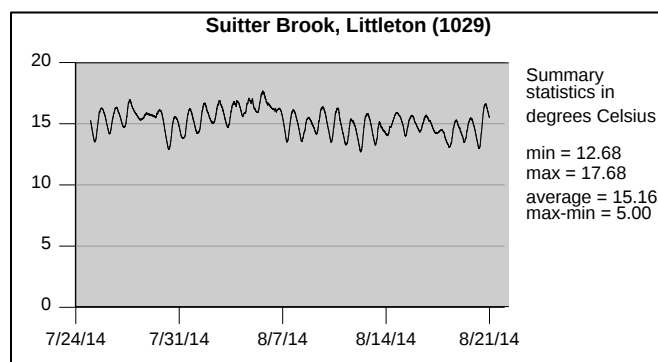
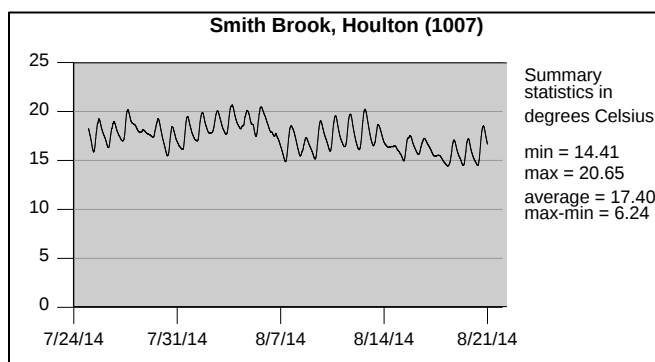
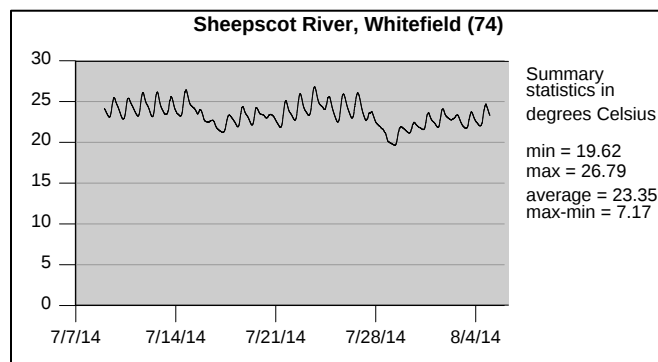
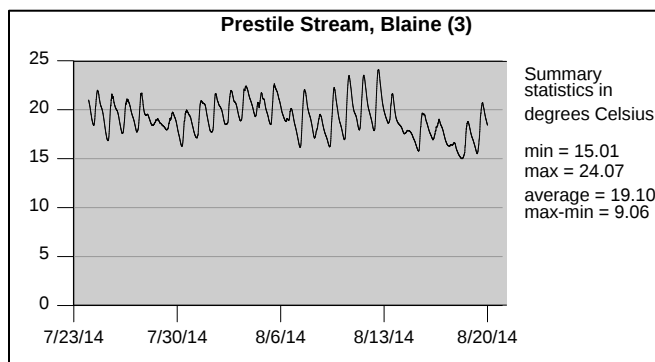


Figure 3.1.1. 2014 In-Stream Continuous Temperature Data (continued)

Please note: all data are in degrees Celsius and maximum value of Y-axis varies amongst graphs.



3.1.3 Attainment History of Sampling Stations prior to 2014

The table below provides the attainment history for sampling stations that have been sampled in the past.

Table 3.1.4. Past Attainment History

Waterbody	Station	Attained Class	Did not Attain Class	Indeterminate Result
Big Brook	728	2004		
E. Branch Wesserunsett Stream	486	2001, 2007, 2012, 2013		
Getchell Brook	925	2009		
Halfmoon Stream	697	2003, 2007	2012, 2013	
Kennebec River	196	1992, 1997, 2007, 2012		
Kennedy Brook	646	2002, 2004, 2009		
Limestone Stream	47	1983, 2004		
Meduxnekeag River	364	1998, 1999, 2000, 2004		
North Fork McLean Brook	922		2009	
Pearce Brook	463	1999, 2000, 2004		
Perkins Stream	977		2012	
Piscataquis River	152	1991, 1993, 1995, 2006, 2011		
Piscataquis River	135	1989, 1990, 1996, 2006		
Presque Isle Stream	197	1993, 1994		
Prestile Stream	3	1994, 1999	1983, 2009	
Sheepscot River	74	1987, 1988, 1989, 1990, 1992, 1995, 1996, 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013	1984, 1985, 1986, 1991, 1993, 1994, 1997	
W. Br. Sheepscot River	268	1996, 1997, 1998, 1999, 2001, 2002, 2005, 2007, 2009, 2010, 2011, 2012, 2013	2000, 2003, 2004, 2006, 2008	1995

3.2 FISH CONSUMPTION ADVISORIES

(Requested by Maine Center for Disease Control and Prevention)

3.2.1. American Shad in Kennebec River

Dioxins and Coplanar PCBs

The Department of Marine Resources had a project for several years to restore American shad (AMS) to the Kennebec River. In the last few years anglers have been catching many AMS in the Kennebec River at Waterville (KWV) and have asked about the risk of consuming them. In 2012 a total of ten AMS were collected from the KWV. Roe (gonad) of females and filet (muscle tissue) of both males and females were analyzed for dioxins (DTEhu = dioxin toxic equivalents with non-detects at $\frac{1}{2}$ of the detection limit, upper 95th confidence limit) and coplanar PCBs (CTEhu = coplanar PCB toxic equivalents with non-detects at $\frac{1}{2}$ of the detection limit, upper 95th confidence limit). The dioxin concentration in filets exceeded the MCDC's FTAL for dioxin-like compounds whereas the concentration in roe did not. The coplanar PCB concentration in filets alone did exceed the FTAL; the concentration in roe alone did not exceed the FTAL, but summing of the two contaminants DTEhu and CTEhu resulted in a slight exceedance of the FTAL. The sum of both contaminants was well below the statewide (mercury) advisory dioxin equivalent threshold (SADET= 3 pg/g), which is the concentration of dioxins and coplanar PCBs affording protection to human consumers equivalent to the protection to consumers achieved by following the statewide fish consumption advisory for freshwater fish due to mercury contamination. Similarly, concentrations of total PCBs were higher in filets than in roe and exceeded the FTAL, whereas the concentrations in roe were at or only slightly above the FTAL.

These results were the reverse of what was expected. Concentrations of both dioxins and PCBs, lipophilic organic compounds, were expected to be higher in the roe, since roe typically has a higher lipid content than muscle tissue. In fact, lipid content was found to be higher in the filets, and consequently the lipid-normalized concentrations were similar in both tissues. The study was to be repeated in 2013, but the fish were spawned out by the time sampling was scheduled.

In 2014, 10 male and 10 female AMS were collected and combined into two composites of five fish for each sex. Muscle tissue filets of both males and females and roe of females were analyzed for dioxins and for total and coplanar PCBs. The wet weight concentration of dioxins was at the FTAL for both filets and roe (Figure 3.2.1). Coplanar PCBs exceeded the FTAL alone for both filets and roe. The sum of both DTEhu and CTEhu was below the SADET. Concentrations were slightly lower in filets and somewhat higher in roe than had been measured in 2012. Surprisingly, lipid content was higher in filets than in the roe. Consequently, when normalized to lipid content, concentrations were similar in both filets and roe each year, but slightly higher in 2014 (Figure 3.2.2).

Total PCBs

Total PCBs (wet weight) exceeded MCDC's FTAL (11 $\mu\text{g/g}$) in both filets and roe of AMS (Figure 3.2.3). Concentrations were lower in filets than had been measured in 2012 but higher in roe. On a lipid weight basis, concentrations were higher in both filets and roe in 2014 than in 2012 (Figure 3.2.4).

Figure 3.2.1. Dioxin (DTEhu) and coplanar PCB (CTEhu) toxic equivalents (upper 95th confidence limit) in American shad (AMS) filet and roe from Waterville on the Kennebec River (KWV) 2012 and 2014

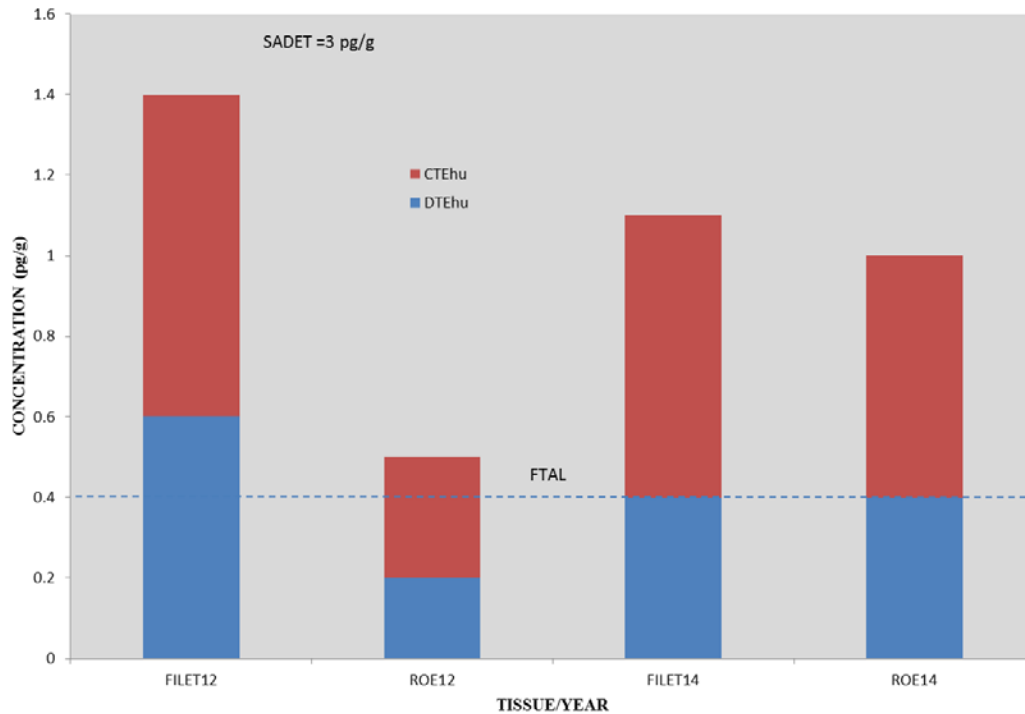
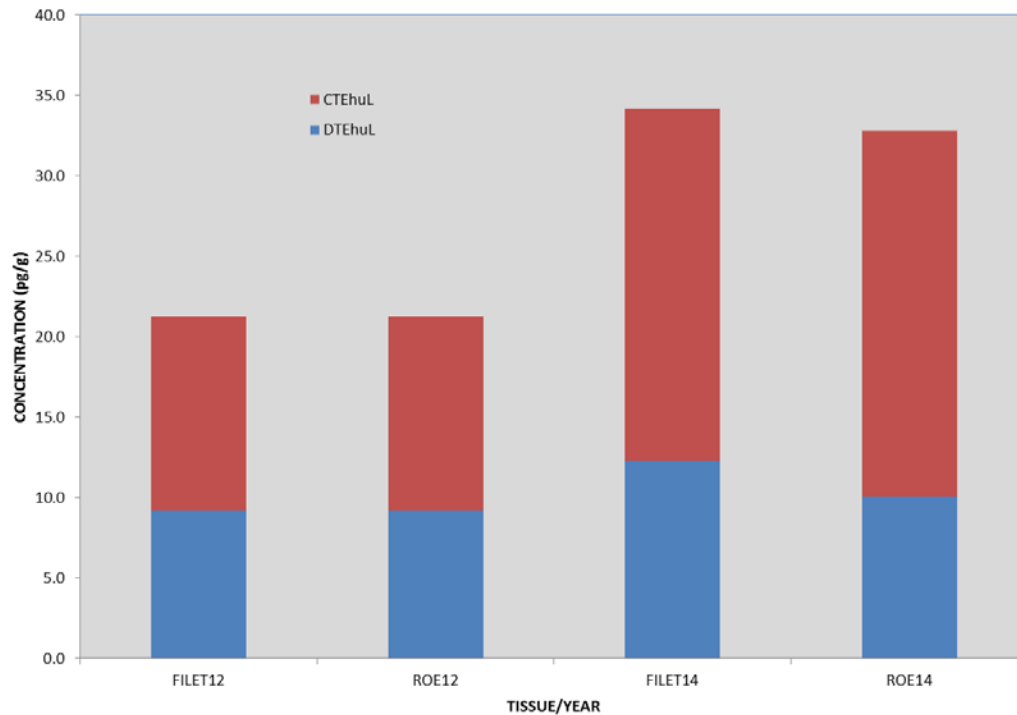
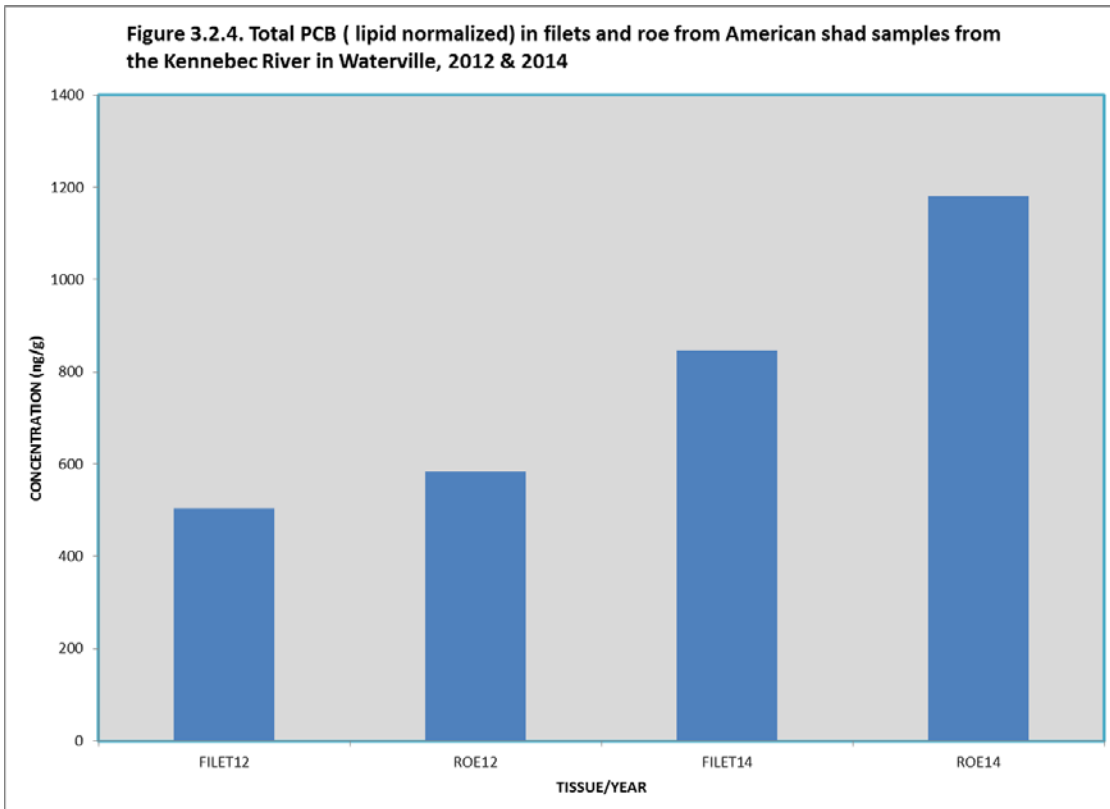
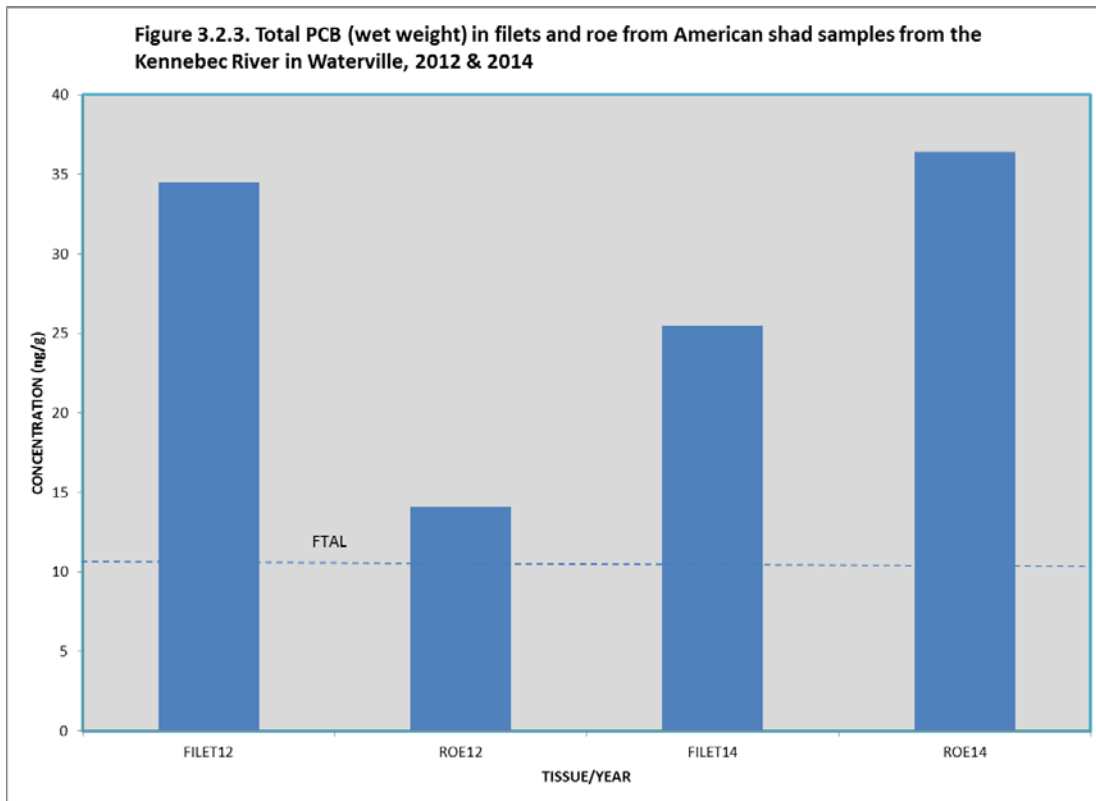


Figure 3.2.2. Lipid normalized Dioxin (DTEhuL) and coplanar PCB (CTEhuL) toxic equivalents (upper 95th confidence limit) in American shad (AMS) filet and roe from Waterville on the Kennebec River (KWV) 2012 and 2014





4.0 SPECIAL STUDIES

	<u>PAGE</u>
4.1 SOFT PLASTIC LURES (SPL) STUDY	105
PRINCIPAL INVESTIGATORS	Dana DeGraff, DIFW Dr. Lawrence LeBlanc, UMO
TECHNICAL ASSISTANTS	Colby Wells, DIFW

4.1 SOFT PLASTIC LURES (SPL) STUDY (continued from 2013)

Ingestion of Biodegradable and Non-Biodegradable Soft Plastic Lures by Salmonids Analysis of Plasticizer Concentrations in Edible Fish Tissue Dr. Lawrence LeBlanc, University of Maine

Introduction

Soft plastic lures (SPLs) are popular tackle in many sport fisheries in North America. In Maine, SPLs are used frequently in the bass fishery and are often lost to the aquatic environment when lines accidentally break, when SPLs become hooked on underwater structures, or when old or heavily used SPLs disengage from the line during casting. Lost and discarded SPLs have been documented extensively in many Maine lakes (F. Brautigam and J. Seiders, pers. comm., Maine Department of Inland Fisheries and Wildlife) and the ingestion of these SPLs by salmonids is a growing concern for anglers and fisheries managers (Danner et al., 2009). SPLs are produced by many manufacturers and are widely distributed among retailers. SPLs are highly variable in size, color, shape, scent, elasticity, and chemical constituency. Whereas the effects of ingested SPLs on brook trout (*Salvelinus fontinalis*) growth and condition factor has been documented (Danner et al., 2009), the bioaccumulation of chemicals leached from ingested SPLs has not. No data on the effects of chemicals leached from ingested SPLs were found from literature searches using search terms “fish health”, “plastic lure leachates”, “plastic lure toxicity” and “plastic lure leachates”.

Plasticizers, such as phthalates, or phthalate esters, are low-molecular weight polymers; Di-*n*-butyl phthalate (DnBP) and di-(2-ethylhexyl)-phthalate (DEHP) are the major chemical constituents of plastics (Metcalf et al., 1973; Stalling et al., 1973; Chandra et al., 2012). Phthalates are frequently used in soft plastics and are used to render SPLs flexible (Danner et al., 2009; Chandra et al., 2012). Phthalates represent 69% of plasticizer use in the United States, 92% in Western Europe, and 81% in Japan (Johnson et al., 2010). The widespread use of phthalate products globally has, within a few decades, resulted in global contamination by this class of compounds (Bell, 1982). Phthalates may comprise 10-40% of the total weight of consumer products (Metcalf et al., 1973; Johnson et al., 2010), and likely comprise a substantial proportion of SPL weight based on the requirement of an SPL to be extremely flexible and “life like”.

Recently, fiber-reinforced and biodegradable SPLs have been developed to reduce the potential of SPL loss and spread of harmful chemicals into aquatic environments. The constituents of biodegradable SPLs are proprietary and not fully advertised and vary between manufacturers. Additionally, there are no established standards for what constitutes a biodegradable SPL and likely there is no, or very limited, information on the time period required for SPLs to biodegrade. Some producers, such as Big Bite Baits, Inc., claim their product, Biobait[®], is 100% biodegradable and made from all natural ingredients. However, a review of Biobait[®] quotes the manufacturer as saying that the SPL is a blend of plastic (i.e. 15% polyvinyl-chloride [PVC]) and natural plasticizers (i.e. 85% fish and vegetable oils; DeWitt, 2008). Even small percentages of PVC in SPLs are of concern; DEHP is the most common plasticizer in PVC formulation for

many consumer products (Metcalf et al., 1973; Carnevali et al., 2010). The harmful effects of phthalate esters on the environment and human health are well documented and summarized (Metcalf et al., 1973; Blount et al., 2000; Ghorpade et al., 2002; Duty et al., 2003; Lee et al., 2005; Norman et al., 2007; Lithner et al., 2009; Oehlmann et al., 2009).

Manufacturers are not currently required to list the ingredients of SPLs, which makes evaluating the effects of discarded SPLs on aquatic biota difficult to determine. However, the negative effects of phthalates on both terrestrial and aquatic organisms have been documented in many studies. For example, DEHP has been shown to have diverse biochemical effects in rats, rabbits and pigs, including inhibition of cholesterologenesis in liver, testes, and adrenal gland, decreased plasma cholesterol levels, and increased fatty acid oxidation in liver mitochondria (Bell, 1982). In the aquatic environment, DEHP can bioaccumulate in a variety of plants and animals (Oehlmann et al., 2009). DEHP degrades very slowly in algae, *Daphnia* spp., mosquito larvae, snails, and clams; it closely resembles Dichloro-Diphenyl-Trichloroethane (DDT) in rate of uptake and storage in the lipids of plants and animals; and it is concentrated through food chains (Metcalf et al., 1973). Exposure of channel catfish (*Ictalurus punctatus*) to 1 µg/l of DEHP for 24 hours resulted in tissue residues of 2.6 µg/g (Stalling et al., 1973). Exposure of early life-stages of Atlantic salmon (*Salmo salar*) to DEHP has been shown to interfere with gonad differentiation and cause intersex (ovo-testis) individuals (Norman et al., 2007). In recent studies, plastic leachates have caused acute toxic effects for *Daphnia magna*; of 15 different plastic types tested, PVC was one of two plastics that displayed toxicity in *D. magna* (Lithner et al., 2009). Environmentally relevant doses have also been shown to affect vitellogenesis in zebrafish (*Danio rerio*) in the laboratory (Carnevali et al., 2010).

Phthalates are widespread in aquatic environments worldwide, and fish are exposed to phthalates via water, food, and/or sediments, depending on their ecological niche (Oehlmann et al., 2009). In wild fish in the Netherlands, median DEHP ranged from 1.7 µg/kg to 141 µg/kg wet wt.; however biotransformation of DEHP in fish appeared to be relatively fast (Peijnenburg and Struijs, 2006). Some biodegradable SPL manufacturers may have eliminated or greatly reduced phthalate use in their products; however some proportion of plastics like PVC, or more importantly phthalate esters found in plastics, may still be used in the production of SPLs which could continue to leach phthalates into the aquatic environment. Throughout the United States, fish consumption advisories are listed for various freshwater and marine fish species that contain chemicals such as mercury, polychlorinated biphenyls (PCBs), and dioxins, that could cause human health risks (EPA, 2012). Based on the chemical constituency of SPLs, the ubiquity of SPLs as lost or discarded fishing tackle in Maine lakes and ponds, and the well documented environmental and human health impacts from phthalate esters, the purpose of this study is to document the bioconcentration of phthalates into edible fish tissue resulting from ingestion of SPLs by hatchery and wild salmonids.

Methodology

The study was divided into three tasks which required development of suitable methods.

Task 1: Plasticizer Identification Study

Task one was to identify the most common plasticizers in biodegradable (e.g., Gulp[®] brand) and non-biodegradable (e.g., Z-Man[®] brand) SPLs. This work was mostly completed in summer 2014.

Task 2: Bioavailability Study

Task two was to determine the potential bioavailability and uptake of toxic plasticizers in each type of SPL into fish muscle tissue. SPLs were provided by DIFW. This work was mostly completed by fall 2014.

Task 3: Fish Tissue Analysis

Task three was to determine fish muscle tissue concentrations of the most significant plasticizers identified in Tasks 1 and/or 2 after collaboration with DEP and DIFW. DIFW was to conduct an SPL fish exposure study beginning in summer 2014 to be completed by fall 2014 with subsequent chemical analysis to be completed by spring 2015. DIFW intended to feed biodegradable and non-biodegradable SPLs to hatchery fish and have the muscle tissue sampled for toxic plasticizers at various sampling time intervals as shown below (Table 1). There were to be 15 fish from each treatment and sampling interval to be sent to the analytical chemistry lab at the end of each sampling interval, except for the T=0 fish when only fish from Treatment 3 would be available to be collected at T=2 days. The lab would filet, homogenize, subsample, and combine the 15 fish from each time/treatment into 3 composites of 5 fish each, for a total of 39 tissue samples, prior to chemical analysis.

Table 1. Number of fish sampled for each treatment group at each sampling interval.

Sampling Interval (days)	Treatment 1 Biodegradable SPL (# Fish Sampled)	Treatment 2 Non-Biodegrade. SPL (# Fish Sampled)	Treatment 3 Control Group (# Fish Sampled)
t = 0	0	0	15
7	15	15	15
30	15	15	15
60	15	15	15
120	15	15	15

The study was to be similar to that described by Danner et al. (2009), with the addition of chemical analysis of fish. Study fish would be taken from lots which were tested for pathogens of regulatory concern in Maine prior to commencing the study, according to fish health inspection procedures. All study fish were to be of the same strain, spawning group, and age. Study and control groups of spring yearling brook trout were to be kept in identical four-foot diameter flow-through tanks at the DIFW Governor Hill Fish Hatchery. Hatchery staff would

provide fish feeding regimens and monitor photoperiod and water quality parameters including dissolved oxygen and water temperature.

Two study groups of 90 spring yearling brook trout each would be obtained from the DIFW Governor Hill Fish Hatchery in June 2013. Fish were to be anesthetized (using either MS-222 or Aqui-S 20e) and force-fed popular biodegradable (e.g., Gulp[®] brand; Group 1) and non-biodegradable (e.g., Z-Man[®] brand; Group 2) SPLs. A control group of 95 spring yearling brook trout would be kept in identical conditions as the study groups (Table 1). All study and control group fish were to be fed a regular feed diet throughout the study period according to established hatchery regimens.

Fish samples were to be obtained by euthanizing fish in an overdose of MS-222 or Aqui-S 20e. Fish will be processed and prepared at the DIFW Fish Health Diagnostic Laboratory according to DEP, Surface Water Ambient Toxics (SWAT) protocols. Each fish would be measured for total length (mm) and wet weight (g) and placed on wet ice for immediate necropsy following methods outlined by Danner et al. (2009). One composite sample of 5 control fish was to be collected at $t = 0$ in order to screen for background phthalate concentration levels ([phthalate]) in fish tissue. Following, triplicate composites of 5 fish each (15 fish total) would be collected from each of the study and control groups at specific time intervals (i.e. 7, 30, 60 and 120 days). All composite fish tissue samples were to be analyzed for phthalates.

Data Analysis

Data were to be presented in graphical or tabular formats. Fish tissue [phthalate] would be directly compared between all laboratory study and control fish. The effects SPL ingestion on tissue [phthalate] between study groups for each sampling interval would be analyzed by one-way Analysis of Variance (ANOVA). Additionally, effects of SPL ingestion on fish weight and length would be analyzed by one-way ANOVA. When effects are significant, ($p < 0.05$) a Tukey's *a posteriori* multiple range test would be used for comparisons. Correlations between SPL exposure time and [phthalate] in fish tissue would be made using the Pearson-type simple correlation model. [Phthalate] in fish tissue would be compared to established detection limits and exposure limits for human health risk. The results of study fish necropsies would be summarized and compared between study and control groups. General comparisons between field and laboratory [phthalate] data would be made when possible and/or appropriate.

Results and Discussion

Due to equipment malfunctions and difficulty in the trout feeding trials, the project is not yet complete; however results are expected to be available by late spring 2015. The following discussion includes progress to date.

Task 1: Plasticizer Identification Study

Phthalate mixes 8060 and 8061 were purchased and individual phthalates were identified in the mixes. Quantitative solutions were made up that included the following analytes:

Dimethyl Phthalate (DMP)
Diethyl Phthalate (DEP)
Diisobutyl Phthalate
Dibutyl Phthalate (DBP)
Bis(2methoxyethyl)Phthalate
Bis(4-methyl-2-pentyl)Phthalate
Bis(2-ethoxyethyl)Phthalate
Diamyl Phthalate
Di-n-hexyl Phthalate
Benzo Butyl Phthalate (BBP)
Hexyl-2ethylhexyl Phthalate
bis(2-n-butoxyethyl) Phthalate
Dicyclohexyl Phthalate
Diethylhexyl Phthalate (DEHP)
Dioctyl Phthalate (DOP)
Dinonyl Phthalate

Using these standards, a cleanup column was devised using 2 grams of 100% activated silica gel (200-300 mesh) for the purpose of removing interfering matrix from extracts of the soft plastic lures. It was determined that matrix removal was effectively accomplished by placing an extract on the column and washing it with 10 ml of 50:50 hexane:methylene chloride prior to eluting the phthalate fraction. The phthalate fraction was then extracted from the silica gel using 10 ml of 80:20 methylene chloride:ethyl acetate mixture.

Next, three types of SPLs purchased from sporting goods stores were extracted in order to identify major marker compounds for the fish feeding study. The brand names of the SPLs extracted to date are Zoom, Z-man and Gary Yamamoto, none of which are advertised as being biodegradable. A fourth brand, Gulp, is advertised as biodegradable and has yet to be analyzed. Samples were extracted using accelerated solvent extraction (Dionex ASE 200 system). Acetonitrile was used as the extraction solvent, as it has been shown to be effective for extracting hydrophobic analytes while leaving behind substantial amounts of background matrix. The samples were then re-extracted with solvents typically used for base-neutral extractions (a 50:50 methylene chloride:acetone mixture) to verify no analytes of interest were left behind. In addition, two laboratory sample blanks were also processed.

Acetonitrile extracts were placed in a separatory funnel containing 150 ml of MilliQ water, and then back-extracted with 3 x 75 ml of hexane. Hexane extracts were combined, reduced in volume, and placed on the silica gel cleanup column described above. The water was then acidified and re-extracted with hexane.

Instrumental analysis for these preliminary trials was performed using a gas chromatography/mass spectroscopy (GC/MS) system (Agilent 6890/5973) using electron ionization. Using the

standard mixes, a selected ion monitoring (SIM) method was developed to eliminate as much of the effects of the background matrix as possible, while retaining critical spectral information.

Initial results showed no identifiable target phthalate compounds present in any of the sample lures. Instrumental traces from the mass spectrometry analyses revealed large unresolved “humps” with several partly resolved peaks. Pattern identification software identified many of these partly resolved peaks as dicarboxylic acid esters, which are phthalate esters. Similar results have been found in other studies examining residual phthalates in environmental samples (e.g., Lin et al., 2003). These authors concluded that these patterns reflect higher molecular weight phthalate isomeric mixtures, and reported a method of quantification, based upon the carbon number of the straight or branched chain portion of the phthalate molecule, using LC/MS/MS (liquid chromatography/tandem mass spectroscopy). Similar results were found in the methylene chloride:acetone extracts. Based upon the intensity of the signal, the ZMan and the Gary Yamamoto brands were suggested as being the best for the feeding study, along with the Gulp (biodegradable) brand. Analysis of the Gulp lures is pending.

Analytical standards of higher molecular weight isomeric phthalate mixtures commercially available were ordered and work commenced on developing an LC/MS/MS instrumental procedure. With patterns matching between isomeric standards and samples of fish lures, phthalates were quantified as described above, by separating isomers based upon the carbon number of the hydrocarbon chain moiety – i.e., the sum of C9 phthalates, C10 phthalates, etc. The LC/MS/MS provided a quantitative confirmation of the qualitative identification of the mixed isomers made by GC/MS. The tandem (MS/MS) portion of the mass spectrometry in the LC/MS allows for a two-stage decomposition of the target analytes. In the first MS analysis a characteristic fragment is isolated and ionized a second time to produce a highly specific mass fingerprint with much lower background interference. Results are presented below.

Concentration (ppm=µg/g)				
	C7	C8	C9	C10
Zman	13.1	1.61	28.96	0.93
Zoom	527	4.39	338	39.4
Gary Yamamoto	775	0.80	343	0.44
Gulp	NA	NA	NA	NA

These numbers should be considered as preliminary, as quantitation was performed using external standards, with 5-point standard curves for each mixed isomer standard. Parts-per-million concentrations of C7-C10 isomers were observed in the 3 brands analyzed via LC/MS. The distribution is dominated by those isomer groupings with odd-numbered alkyl side chains (C7 and C9), with lesser amounts of the C8 and C10 isomers. Laboratory blanks thus far showed small (1-10 ppb) concentrations of diethyl phthalate and slightly higher (10-50 ppb) concentrations of diethylhexyl phthalate and dioctyl phthalate.

Scanning for the presence of bisphenol A by both GC/MS and LC/MS/MS did not detect its presence in any of the lure samples (3 different brands of SPLs – Z-man, Gary Yamamoto, and Zoom – were extracted).

Task 2: Bioavailability Study

The Bioavailability Study initially focused on studies of desorption into distilled (DI) laboratory water conducted with Zoom, Gary Yamamoto and Gulp brand lures. Samples were placed in a covered one-liter beaker, along with 800 ml of DI water (both neutral and acidified) and agitated gently for three days (72 hours). At that point the water was removed and the sample extracted with 3 x 75 ml of hexane. Fresh DI water was added to the beaker and SPLs were allowed to desorb for another 72 hours.

There was evidence of isomeric mixtures in the initial desorption studies. For the Zoom and Gary Yamamoto brands there was evidence of desorption of the C7 isomer, and lesser amounts of the C8 and C9 isomers as shown below.

Desorption (%)				
	C7	C8	C9	C10
Zoom	14.1	2.5	1.0	
Zoom acid water	5.9	0.6	0.2	
Gary Yamamoto	24.7	0.2		
Gulp	ND	ND	ND	ND

Values are expressed as the percentage of the total mass found in the lures desorbed into the water. The Zman desorption extracts were not analyzed by LC/MS due to loss of the extracts to evaporation. This experiment has been repeated (under neutral and acidic conditions), using the Zman SPLs (which were used in the fish feeding experiment), allowing desorption of phthalates to occur in the presence of distilled water and tenax resin, which provides an infinite sink for the phthalate compounds to desorb onto (thereby generating a maximum estimate of desorption). The tenax resin was isolated and extracted for phthalate analysis. These extracts are awaiting analysis via LC/MS. Samples of the Gulp SPLs were desorbed into water, and there was no evidence of any of the phthalate isomers in the desorption extracts when analyzed by LC/MS.

Task 3: Fish Tissue Study

Fish Exposures

In June 2014, spring yearling Maine Hatchery Strain brook trout (25.4 ± 2.60 cm total length) were obtained from the DIFW Governor Hill Fish Hatchery, Augusta, Maine, one of the department's broodfish hatcheries. These brook trout were evaluated for their potential to be used in this study following methods for SPL ingestion described by Danner et al. (2009) and also by directly force-feeding SPLs using sterilized stainless steel 22.86 cm Doyen intestinal forceps. The brook trout however did not actively feed on provided SPLs and were too small for force-feeding SPLs; consequently brook trout were deemed unsuitable for this study. In July and August 2014, adult broodstock lake trout (*Salvelinus namaycush*; ages IV⁺ - IX⁺) from the same hatchery were utilized.

All study lake trout were housed in an outdoor raceway exposed to ambient light for the duration of this 4 month study. Water at the hatchery was supplied from a spring source and water

temperature was monitored daily. Mean water temperatures ranged from 11.3 °C in July 2014 to 6.7 °C in January 2015. All study fish were fed a diet of Bio-Oregon® BioBrood (6.0 mm pellet; 48% protein, 20% oil) feed over the duration of the experiment. In July 2014, fish were fed on average 0.22% of their body weight per day. As fish require less feed later in the summer season, after July fish were fed on average 0.9% of their body weight per day.

Study Design

To facilitate fish marking, length and weight measurements and SPL force-feeding, all fish were anesthetized with tricaine methanesulfonate (MS-222; Tricaine-S, Western Chemical, Inc., Ferndale, Washington) following manufacturer-suggested dosing for salmonid fishes and under direction of the DIFW veterinarian. Fish in each treatment and control group received unique fin clips (caudal tail, adipose, or no clip) to differentiate each group. Eighteen (65.9 ± 7.96 cm, 15.9 ± 6.20 kg) and 25 (57.4 ± 9.30 cm, 7.2 ± 1.4 kg) fish were used in treatment groups 1 and 2, respectively. Ten additional lake trout (40.8 ± 5.81 cm) were utilized as the control group. The male:female ratio was 3:4. Following fish measurements, the fish in Treatment Group 1 were force-fed popular worm-style SPLs (12.7 cm length; 10.5 g; 20 cc) that are not currently advertised as either containing or not containing phthalates. The fish in Treatment Group 2 were force-fed a popular worm-style brand of SPL (15.24 cm length; 10 cc) that are specifically advertised as not containing phthalates. Treatment Group 1 and Treatment Group 2 fish received five (100 cc) and two (40 cc) SPLs per fish, respectively. The number, and more specifically, the volume of SPLs fed to each fish of each treatment group was based on mean wild lake trout volumetric stomach content data from comparably sized fish, obtained from the DIFW stomach content database. Initially, Treatment Group 2 fish were force-fed worm-style SPLs; however, soon after feeding most fish had either excreted or regurgitated these SPLs. Following, additional Treatment Group 2 fish were force-fed lizard-style SPLs of the same brand advertised not to contain phthalates. All treatment group fish were force-fed SPLs using stainless steel 22.86 cm Doyen intestinal forceps.

Regurgitated and excreted SPLs were collected and counted daily. Fish health and condition were monitored visually throughout the duration of the study and any adverse health effects were documented. There were no mortalities in any of the study groups.

Fish Collection and Processing

At each sampling time, fish were euthanized at the point of collection at the hatchery with an overdose of MS-222 and were immediately processed and prepared at the DIFW Fish Health Diagnostic Laboratory according to DEP, Surface Water Ambient Toxics (SWAT) protocols. Each study fish was measured and weighed in the laboratory prior to necropsy. One composite sample of five control fish was collected at each sampling event in order to screen for background [phthalate] in fish tissue, liver, and blood samples. Only Treatment Group fish that had retained SPLs after 120 days were processed and screened for in-tissue, liver, and blood samples. All fish were dissected immediately after euthanasia, and the ingested masses of SPLs in the gastrointestinal (GI) tract were noted but not counted to avoid unnecessary sample contamination. A brief external and internal examination of each fish in Groups 1 and 2 was performed at this time. Only individual fish that had SPLs in their GI tract had whole-blood

samples taken prior to being delivered to the analytical laboratory for processing. Whole-blood samples were collected directly from the caudal vein of each individual fish into 20 cc glass tubes, filling each tube approximately two-thirds full. Individual fish were wrapped whole in tin foil following MDEP SWAT protocol and submitted to the University of Maine for tissue processing and analysis.

Animal Care and Use

A peer review group composed of DIFW fisheries biologists, hatchery personnel, and a veterinarian, reviewed this study prior to initiation and served as the Animal Care and Use Committee (ACUC) during the study. All fish housed at the Governor Hill Hatchery were tested and found to be negative for pathogens of regulatory concern in Maine prior to commencing the study according to fish health inspection procedures (USFWS and AFS–FHS Blue Book 2012).

Results

Eleven of eighteen fish from Treatment Group 1 (61.1%) retained SPLs in their GI tract after 4 months. Of these, one fish had a total of six SPLs in its GI tract. This fish had freely consumed an additional SPL regurgitated by a different fish. Seven fish had retained between three and five SPLs in their GI tracts, and three fish had between 1 and 2 SPLs retained in their GI tract. The location of the majority of SPLs retained by Treatment Group 1 fish was in the stomach, however one SPL was found in the lower GI tract of one fish.

Thirteen of twenty-five fish from Treatment Group 2 (52.0%) retained SPLs in their GI tract after 4 months. Of these, only three fish retained each of the two lizard SPLs. All the remaining fish had only one worm SPL or one lizard SPL retained in their GI tract, except that one fish had both a worm SPL and a lizard SPL in its GI tract. This fish had been force-fed a worm SPL and later had freely consumed a lizard SPL that had been regurgitated by a different fish. The location of all SPLs retained by fish in Treatment Group 2 was in the stomach. No SPLs were found in the lower GI tracts of fish from Treatment Group 2.

No significant differences in length or weight of study fish were documented at the conclusion of this study. No considerable abnormalities were noted externally or in the internal organs of the coelomic cavity, other than mild congestion of the blood vessels supplying the stomach and lower GI tract in a small number of the treatment group fish.

Fish Tissue Chemical Analyses

Chemical analyses of fish tissue for phthalates is currently in process and will be reported by mid-May.

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