Penobscot River Estuary Lobster and Rock Crab Mercury Study

2014 Sampling Period Data Report

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Prepared by the Maine Center for Disease Control and Prevention
in collaboration with the Department of Marine Resources and the Department of Environmental Protection

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1. Executive Summary

In February 2014 the Maine Department of Marine Resources (DMR) closed an area in the upper Penobscot River estuary to lobster and crab harvesting due to elevated levels of methyl mercury in lobster tissue. Concern regarding mercury levels in lobster in the Penobscot River estuary was brought to the attention of the DMR through the Penobscot River Mercury Study (PRMS), a federal court-ordered study, released in 2013. The PRMS, as part of a federal lawsuit (*Maine People's Alliance and the Natural Resources Defense Council v. Mallinckrodt, Inc.*), evaluated mercury levels in water, soil, sediment, and biota in the Penobscot River from 2006 through 2012.

Following the release of the PRMS, study staff provided the DMR data for mercury levels in lobster (*Homarus americanus*) and crab (*Cancer irroratus*) collected in the Penobscot River estuary. The DMR, Maine Department of Environmental Protection (MEDEP) and Maine Center for Disease Control and Prevention (MECDC) reviewed these data and determined that lobster in the northern most areas of the estuary contained mercury at levels that would warrant a consumption advisory. PRMS data for mercury in crab was limited to areas further south in the estuary, below the current closure line. Mercury levels in these crabs suggested that crabs further north may have higher mercury levels, similar to lobster in the northern areas. As a precaution, the area north of a line from Wilson Point in Castine across to Fort Point in Stockton Springs was closed to both lobster and crab fishing.

As a result of the closure, the DMR along with the MEDEP and MECDC initiated a 2-year sampling plan to confirm the previous PRMS results for mercury levels in lobster, and to expand crab sampling to areas inside and directly adjacent to the closed area. Additionally, the DMR study aimed to identify potential seasonal changes in mercury levels in both lobster and crab, and assess any seasonal migrations in or out of the estuary for these two species. This data report provides a summary of the sampling plan, collection methods, laboratory mercury analysis, data analysis, and results of the DMR sampling conducted in 2014.

In general, results from the 2014 sampling period for mercury levels in lobster tissue confirm findings from the PRMS, and expand data for mercury levels in crab tissue. Specifically:

- Lobster collected from inside the closure contained higher mercury levels than lobster collected in areas south of the closure.
- Lobster contained the highest mercury levels in the spring/early summer with levels decreasing in the late summer and rebounding in the fall/early winter.
- Based on catch rates, lobster tended to migrate out of the northern areas in the winter and back in the summer/fall.
- Crab mercury levels were lower than expected based on previous PRMS results and were noticeably lower than those seen in lobster.
- Crab collected in the closed area had higher mercury levels than crab collected in areas south of the closure.
- Mercury levels in crabs steadily increased from spring to winter during the 2014 sampling.
- There appears to be no major seasonal crab migration in the estuary based on catch rates.

2. Introduction

In 2014 the Maine Department of Marine Resources (DMR) issued a limited closure on harvesting lobster and crab from the mouth of Penobscot River[1]. The limited closure encompasses an area in the Penobscot River estuary north of a line running between Fort Point in Stockton Springs and Wilson Point in Castine[2]. The closure was put into place after analysis and review of data released in 2013 from a Federal court ordered study that examined mercury levels in water, sediment, and biota in the Penobscot River estuary (Penobscot River Mercury Study (PRMS))[3]. Data from the PRMS indicated that lobster harvested from the now closed area had mercury levels above the Maine Center for Disease Control and Prevention (MECDC) action level for mercury in fish and shellfish. The PRMS provided minimal data on mercury levels in crab in the closure area, but as a precaution the area was also closed to crab fishing[1].

In response to the PRMS data and subsequent lobster and crab fisheries closure, the DMR, the Maine Department of Environmental Protection (MEDEP), and MECDC designed a sampling plan to confirm the PRMS lobster mercury data and to further examine mercury levels in crab (Rock crab) in the Penobscot River estuary. Sampling was to be conducted for two years, 2014 and 2015, and resulting data assessed thereafter. In addition to confirming the PRMS data, sample collections were designed to track seasonal changes and the spatial distribution of mercury in lobster and crab within the closure area and adjacent waters. This report details the sampling plan, collection methods, mercury analysis methods, and results from the 2014 sampling period.

[1] Chapter 25.65 Lobster and Crab Closure in Penobscot River.pdf

[2] http://www.maine.gov/dmr/news/2014/PenobscotClosure.htm

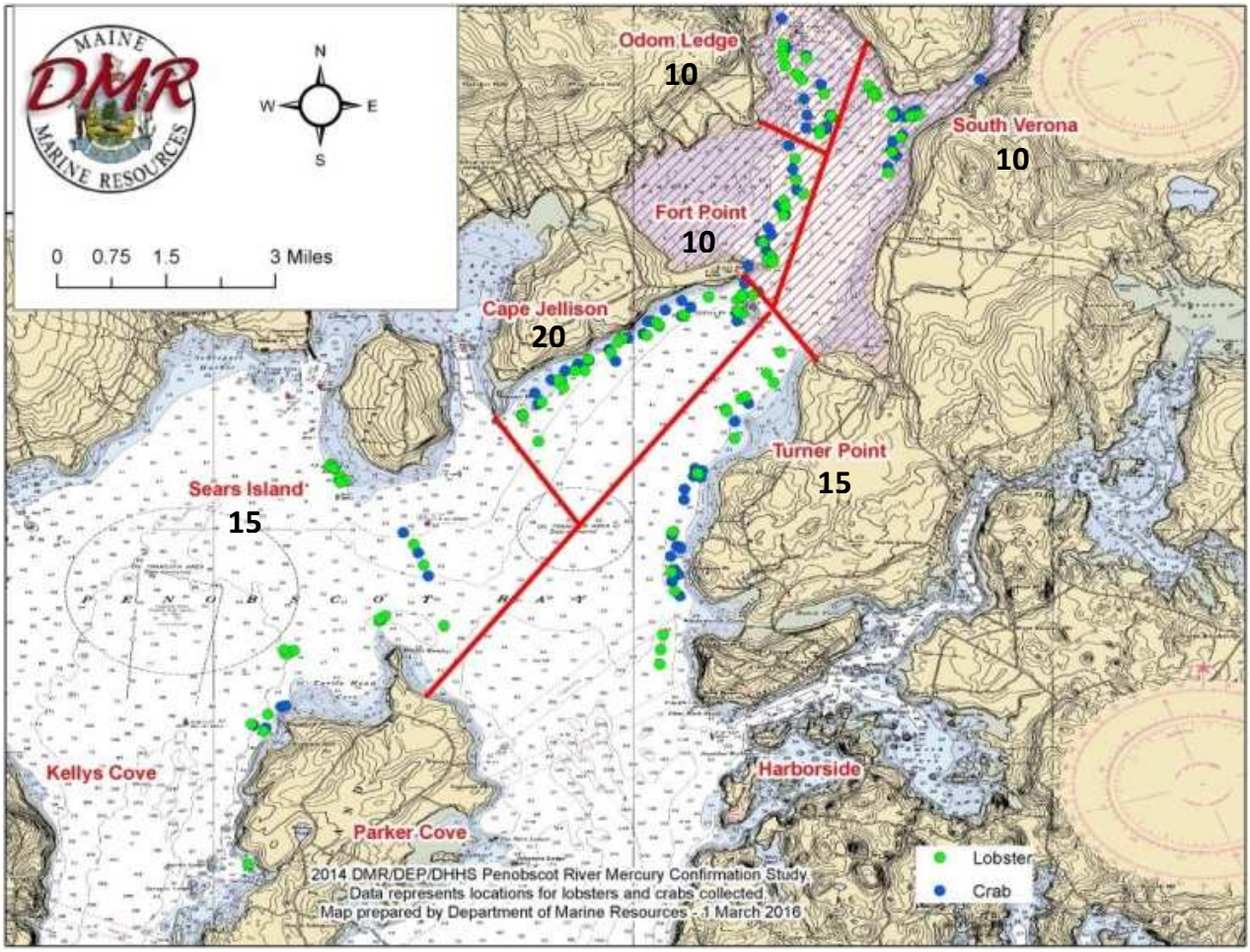
[3] http://www.maine.gov/dep/spills/holtrachem/river_study.html

3. Methods

3.1. Sampling design

Six regions were identified as priority areas for collection based on prior PRMS sampling locations; three areas inside the closure (Odom Ledge, South Verona, and Fort Point); and three outside the closure (Cape Jellison, Turner Point, and Sears Island) (Figure 1). These areas were consistent with previous PRMS collections, except in the case of Cape Jellison; an area previously un-sampled yet immediately adjacent to the closure. All lobster and crab samples were tracked with reference to these underlying areas. Based on previous PRMS data, site-specific target collection numbers of 10-20 individual lobster and crab for each sampling period were designated for the six sampling regions (Figure 1).

Figure 1. Collection areas and target collection numbers for 2014 DMR lobster and Rock crab sampling.



Segmented red lines designate general sampling areas for the 2014 DMR sampling, with individual sample collection sites for lobster (green dots) and crab (blue dots). Monthly target collection numbers for lobster and crab samples are indicated by numbers below sample area names. Thin red lines above the Cape Jellison and Turner Point areas specify the current closed area.

3.2. Sample collection

Part of the intent of this study was to sample quarterly to track seasonal changes in mercury concentrations. For a variety of reasons including weather, sampler availability, mechanical breakdowns, and lack of catch for target species (lobster) five rounds of sampling were conducted. Samples were collected on April 24 and 29; June 23 and 27; August 11 and 15; October 28 and 31; and, December 11 and 15, 2014.

During each sampling period, 74 single parlor lobster traps were set (as pairs) and distributed throughout the six collection areas. Trap locations changed in order to maximize catch relative to target sampling objectives. Traps were baited and hauled twice following a 3 to 5 night soak. Individual lobsters (*Homarus americanus)* and crabs (*Cancer irroratus)* were collected and uniquely tagged. Specimen data were recorded and included sample collection date, location (Lat/Lon), trap depth, size, sex, molt status, and cull status. Biological collections were made according to the target number assigned for each collection area/sampling site. There was no attempt to quantify catch per unit effort or other fishery dependent information.

An attempt was made to collect legal-sized lobsters (83-127 millimeter (mm) carapace length) and 'hard' large bodied male crabs (100-140 mm carapace width), as these are sizes consumed by the general public. For some sampling periods and sites legal-sized lobster sample targets were not achieved, largely due to lobster availability in the region. Accordingly, less than legal-sized lobster samples were collected to help meet target number goals.

Following collection, all live samples were placed in individual bags, on ice, transported to the DMR Boothbay Harbor Laboratory and immediately frozen in standard household freezers maintained at -20.5 C. The frozen samples were then transported to MEDEP facilities, as MEDEP freezer space became available, for tissue extraction and analysis.

3.3. Sample processing

The MEDEP received the live frozen lobster and crab samples in individual Ziploc poly bags, in large lots aggregated from each seasonal sampling period. The DMR attached individual sample ID tags, each with a unique 6 digit number to each specimen in the field upon collection. The unique sample ID was used to associate sample collection location, size, dissection, laboratory mercury concentrations, and other associated metadata to the individual samples. MEDEP stored the lobster and crab samples in freezer storage until dissections could be completed.

From one to several hours prior to dissection, the whole crustaceans were removed from the freezer in their individual bags, and allowed to partially thaw on the lab bench until tissue was workable for dissection. Individual crustaceans were removed from their Ziploc poly bags and placed atop the bag as further work proceeded. Animals were measured with calipers (carapace length to eye socket for lobsters and carapace width for crab) to the nearest 0.1 mm. Whole animals were weighed to the nearest 0.1 gram (g). Morphometric data was recorded along with the unique six digit DMR-assigned sample ID, which was affixed to each animal on a plastic tag placed around the claw. In the case when the claw had fallen off in the poly bag releasing the tag, the ID tag was retained by the bag and so could still be associated with the individual animal. Any abnormalities in specimens like one or no

claws, physical defects, or evidence of thawing or spoilage of the sample were noted on the dissection data sheets.

3.4. Dissection methods

Dissections were performed over an HDPE cutting board with the animal placed in the center and the cutting board within a drip pan. A washed stainless steel knife was used to split lobster claw and tail shell and remove muscle tissue, which was still partially frozen. Lobster claw muscle tissue was removed from the cheliped claws only. If only one cheliped claw was present on a lobster, tissue from that claw was used. When both cheliped claws were present, muscle tissue from both claws was used. Lobster tail muscle was removed via a mid-sagittal section through the shell and tail, allowing removal of the "vein" or digestive tract from the tail muscle sample, which was not included with muscle tissue for analysis.

Crab chelipeds were broken off the crab between the body and the medial terminus of the merus and a washed stainless steel knife was used to remove muscle tissue from the claws, the carpus, and the merus. When both cheliped claws were present, muscle tissue from both claws was used. All tissue from the described appendages was collected for analysis.

Dissection of each lobster produced separate muscle tissue samples for tail muscle and claw muscle, which were placed in individual, pre-cleaned glass jars provided by the laboratory. Each crab produced one muscle tissue sample taken from the chelipeds (both if available), which was placed in a pre-cleaned jar provided by the laboratory. The weight of each individual muscle tissue sample was measured on an analytical balance by taring pre-cleaned jars prior to sample addition. Jars containing muscle tissue were pre-labeled with sample ID and immediately returned to the freezer. Samples were retained frozen and shipped frozen in batches to Alpha Analytical, Mansfield, MA, the contracted laboratory, via their own courier service. Samples were received the same day following removal from the MEDEP freezer.

3.5. Mercury analysis

Alpha Analytical was responsible for tissue homogenization and measurement of total mercury in all lobster and crab tissue samples collected during the 2014 sampling year. The entire tissue sample provided in each jar was homogenized. A 1 g aliquot of the whole tissue homogenate was sub-sampled for digestion and analysis. Total mercury levels were measured using EPA method 1631E (Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry) (USEPA, 2002). Total solids and moisture content were measured using standard methodology[4]. Results for total mercury (mg/kg wet weight and mg/kg dry weight), percent moisture, and total solids were provided, as requested, to the MEDEP using the Department's Electronic Data Deliverable (EDD) format and one paper copy, including the lab narrative report, by mail.

[4] Alpha Analytical Labs Sample Reference Guide (Total solid and moisture content method: SM2540B/SM2540G)

3.6. Data validation and storage

Data for the 2014 sampling year, including laboratory quality control results, received from Alpha Analytical were reviewed and validated by the MEDEP and entered into the Environmental and Geographic Analysis Database (EGAD). The MEDEP maintains the EGAD to store and access various sampling and contamination data collected by the Department and other state agencies[5]. All data entered and stored in the EGAD goes through quality assurance/quality control procedures. Due to the review and validation processes, the EGAD is the principal database for storing and accessing data generated during the 2014 sampling year.

3.7. Laboratory quality assurance/quality control

The MEDEP requested that the contracted lab, Alpha Analytical, provide, at a minimum, the following quality assurance/quality control results:

a. Method blank results.
b. Laboratory control spike (LCS) samples, with percent recovery (% recovery) results between 80% and 120% using DORM-2, DORM-3, or DOLT-2 certified reference materials.
c. Matrix spike (MS) and matrix spike duplicate (MSD) samples, with % recovery results between 80% and 120% of added mercury.
d. Lab duplicates at a rate of 1 per 20 samples, with a relative percent difference (RPD) ≤25%.

3.7.1. Method blanks and laboratory control spikes

Alpha Analytical ran method blanks on a batch basis and provided the method blank results. All method blanks were non-detect and no laboratory qualifiers were noted for any method blank results. Laboratory control spike samples were run on a batch basis and results provided.

3.7.2. Matrix spikes and matrix spike duplicates

Matrix spike and matrix spike duplicate samples were run for both lobster claw and tail tissue, and crab claw tissue on a batch basis of 1 MS/MSD sample per 10 tissue samples. A total of 23 and 26 MS/MSD samples were run for lobster claw and tail tissue samples, respectively, and 44 MS/MSD samples for crab claw tissue samples.

Matrix spike samples measure potential bias introduced by the sample matrix, in this case lobster claw and tail tissue, and crab claw tissue, on the accuracy of the analytical method. Matrix bias is assessed by evaluating the % recovery between the measured native sample concentration and the measured spiked sample concentration, i.e., an aliquot of the native tissue sample spiked with a known amount of mercury[6]. Results for matrix spike % recovery should fall within an acceptable, predetermined range. For the 2014 mercury sampling analysis, the MEDEP requested that the % recovery from matrix spike

[5] MEDEP EGAD: http://www.maine.gov/dep/maps-data/egad/

[6] % Recovery for matrix spike samples:

$$\%\ \text{Recovery} = \left(\frac{\text{Matrix spike sample result} - \text{Native sample result}}{\text{Spike ammout added}}\right) \times 100$$

samples be between 80% and 120%. Alpha Analytical applied a laboratory QC % recovery range of 70% to 130% for matrix spike samples. Laboratory QC limits were used for data validation. Recovery results for matrix samples where the laboratory case narrative specified that the % recovery did not apply because the sample concentration is greater than four times the spike amount added were excluded from % recovery QC evaluations for matrix spike samples.

The average % recovery for matrix spike samples for lobster claw and tail tissue, and crab claw tissue were within the laboratory QC limits (Table 1). Percent recoveries for several individual MS and MSD samples were outside the laboratory QC limits (Table 2). For these individual matrix spike samples, the laboratory case narrative indicated that the associated LCS recoveries were within the acceptable range and no further action was taken. Native sample mercury concentrations with associated MS or MSD % recoveries that were outside the laboratory QC limits (70%-130%) were J-qualified during the data validation process[7].

For lobster claw tissue, while the average % recovery for matrix spike samples were within the laboratory QC limits, 7 out of 20 MS % recoveries and 10 out of 19 MSD % recoveries were <70% (Table 2). In comparison, only 2 MS and 2 MSD samples with lobster claw tissue had % recovery results >130% (Table 2). For these matrix spike samples with % recovery outside the laboratory QC limits, the laboratory case narrative indicated that the % recovery from corresponding LCS samples were normal. This suggests a potential low bias for lobster claw tissue. There was no substantial indication of a low or high bias for lobster tail tissue or crab tissue mercury levels from matrix spike recovery results.

Table 1. Percent recovery results for matrix spike and matrix spike duplicate samples.

Tissue	Analysis	N [a]	Mean	Standard deviation	Minimum	Maximum
Lobster Claw	Matrix Spike % Recovery	20	86.9	45.2	22.2	228.0
	Matrix Spike Duplicate % Recovery	19	78.1	34.7	29.0	167.0
Lobster Tail	Matrix Spike % Recovery	17	93.7	18.7	62.0	123.0
	Matrix Spike Duplicate % Recovery	18	97.2	23.4	39.0	126.0
Crab	Matrix Spike % Recovery	32	85.3	11.9	50.3	114.0
	Matrix Spike Duplicate % Recovery	32	85.0	26.8	28.6	189.0

[a] The sample number reflects MS and MSD samples with % recovery results excluded when the laboratory noted that the recovery for mercury does not apply because the sample concentration is greater than four times the spike amount added.

[7] MEDEP EGAD concentration valid qualifiers:
J = Associated value is estimated - may be due to factors such as holding time violations, blank contamination, etc.
R = Results are rejected during data validation due to serious analytical or sampling deficiencies
U = Not detected above the associated quantitation limit
* = QC results not within control limits
http://www.maine.gov/dep/maps-data/egad/documents/EGAD_Lookup_Tables.xlsx

Table 2. Number of matrix spike and matrix spike duplicate samples run and number and percent of samples with % recovery results within, less than, or greater than laboratory QC limits.

Tissue	Sample	Total number of MS/MSD samples	Number of MS/MSD samples with useable % Recovery results [a]	Number of samples with % Recovery w/in QC limits 70%-130% [b]	Number of samples with % Recovery <70% [b, c]	Number of samples with % Recovery >130% [b, c]
Lobster Claw	MS	23	20	11 (55%)	7 (35%)	2 (10%)
	MSD	23	19	7 (37%)	10 (53%)	2 (10%)
Lobster Tail	MS	26	17	16 (94%)	1 (6%)	0 (0%)
	MSD	26	18	16 (89%)	2 (11%)	0 (0%)
Crab	MS	44	33	31 (94%)	2 (6%)	0 (0%)
	MSD	44	32	26 (81%)	5 (16%)	1 (3%)

[a] Percent recovery results for individual MS or MSD samples were excluded if they were outside the laboratory QC limit and the lab indicated that the recovery for mercury does not apply because the sample concentration is greater than four times the spike amount added.

[b] The number and (%) are the number and percent of MS or MSD samples with useable % recovery results for each QC grouping, within, less than, or greater than laboratory QC limits.

[c] For % recovery results outside of the laboratory QC range, the laboratory case narrative indicated that the associated LCS recoveries were within the acceptable range and no further action was taken.

Matrix spike and matrix spike duplicate samples can be used to check analytical precision by evaluating the relative percent difference between the measured spiked sample and duplicate spiked sample concentrations and their average concentration with acceptable results falling below a predetermined QC limit[8]. The requested and laboratory QC limits for RPD from duplicate samples were ≤25% and ≤30%, respectively. Alpha Analytical provided RPD results for all MS/MSD samples. Laboratory QC limits for MS/MSD RPD were used for data validation.

The average RPD for MS/MSD samples for lobster claw and tail tissue, and crab tissue were below the requested RPD QC limit of ≤25% and met analytical QC goals (Table 3). For lobster tissue, only one MS/MSD RPD from a lobster claw tissue sample was above the RPD QC limit. The associated native sample was R-qualified for a MS/MSD RPD >25%, and both MS and MSD % recoveries outside the laboratory % recovery QC limits, 228% and 42%, respectively[7]. Results for this lobster claw tissue sample were rejected. For crab tissue, the RPD for 2 MS/MSD samples were above the RPD QC limit. While the MS/MSD RPD results for these two samples were qualified as *QC results not within control limits*, the native tissue sample results had no associated data validation qualifiers. Thus, the native sample results were accepted. Overall, for both lobster tail and claw tissue the RPD from MS/MSD samples tended to increase with decreasing mercury concentrations in the native sample (Figure 2).

[8] Relative Percent Difference (RPD) for MS/MSD samples:

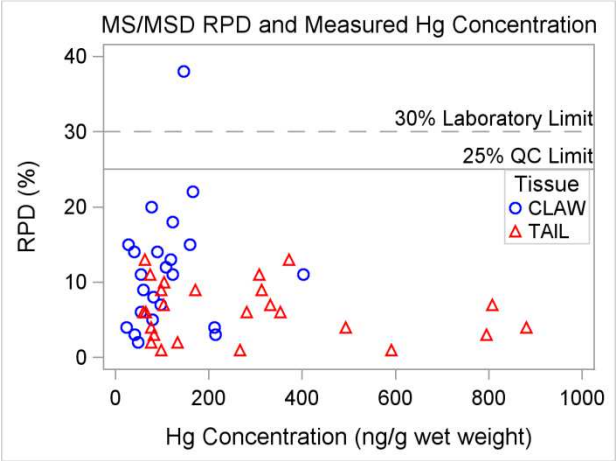
$$\text{RPD} = \left(\frac{\text{Matrix spike result} - \text{Matrix spike duplicate result}}{\text{Matrix spike result} + \text{Matrix spike duplicate result}/2}\right) \times 100$$

Table 3. Relative percent difference for matrix spike and matrix spike duplicate samples.

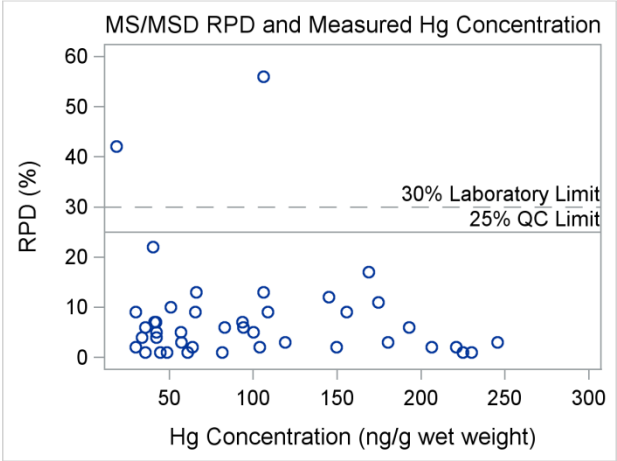
Tissue	N	Mean	Standard deviation	Minimum	Maximum
Lobster Claw	23	11.5	8.1	2.0	38.0
Lobster Tail	25	6.2	3.7	1.0	13.0
Crab	43	7.8	10.4	1.0	56.0

Figure 2. Relative percent difference for matrix spike/matrix spike duplicate samples compared with the measured native sample mercury concentration (ng/g wet weight).

A. Lobster claw and tail samples



B. Crab samples



Native sample mercury concentration plotted against the calculated RPD between MS and MSD samples. The 30% Laboratory Limit was the acceptable upper limit for RPD applied by Alpha Analytical. The 25% QC Limit was the MEDEP-requested upper limit for RPD.

3.7.3. Lab duplicates

Lab duplicates were run for both lobster and crab tissue samples at the requested rate of 1 duplicate per 20 samples. Twenty-four lab duplicates were run out of 461 lobster samples analyzed for total mercury, indicating 1 lab duplicate was run for approximately every 20 lobster tissue samples. For crab, 25 lab duplicates were run out of 393 crab samples, indicating 1 duplicate sample for approximately every 15 crab tissue samples.

Lab duplicates are a measure of analytical precision, which is typically assessed through evaluation of the RPD between the native sample concentration and lab duplicate sample concentration. The requested QC limit for lab duplicates was a RPD of ≤25%. The average RPD for lobster claw and tail samples was ≤25%, as was the average RPD for crab samples (Table 4). For several individual lab duplicates, the RPD was above the QC limit (Figure 3). Elevated RPDs were associated with native samples with relatively low mercury concentrations, indicating a decrease in precision with decreasing mercury levels in lobster and crab tissue (Figure 3). Alpha Analytical applied a lab duplicate RPD acceptance criteria of ≤30% and Q-qualified 2 lobster claw tissue and 2 crab tissue lab duplicate

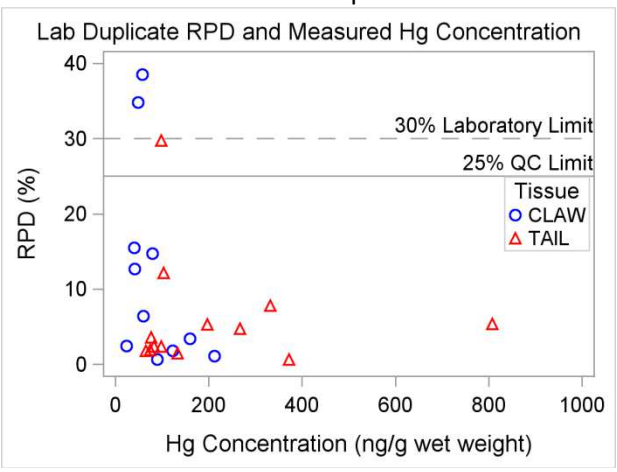
samples with RPDs >30%[9]. For these elevated RPDs, the laboratory case narrative noted that the elevated RPD has been attributed to the non-homogeneous nature of the sample utilized for the laboratory duplicate. Native sample mercury concentrations with associated lab duplicate RPDs >30% were J-qualified during data validation.

Table 4. Relative percent difference for lobster and crab lab duplicate samples.

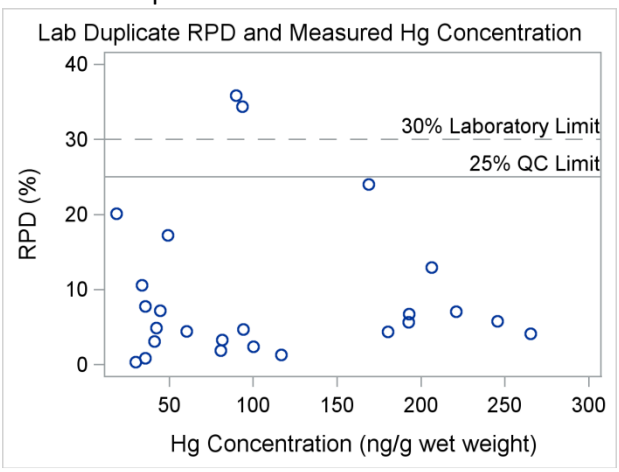
Tissue	N	Mean	Standard deviation	Minimum	Maximum
Lobster Claw	11	12.0	13.4	0.7	38.5
Lobster Tail	13	6.1	7.8	0.6	29.8
Crab	25	9.2	9.8	0.3	35.9

Figure 3. Relative percent difference for lab duplicate samples compared with the measured native sample mercury concentration (ng/g wet weight).

A. Lobster claw and tail samples



B. Crab samples



Native sample mercury concentrations plotted against the calculated lab duplicate RPD. The 30% Laboratory Limit was the acceptable upper limit for RPD for lab duplicates applied by Alpha Analytical. The 25% QC Limit was the MEDEP-requested upper limit for lab duplicate RPD.

3.8. Data analysis

Following the MEDEP data review, data validation, and entry process into the EGAD, data was accessed and extracted from the EGAD by the MECDC for analysis. Extracted datasets were transformed to combine results from the six individual sampling sites into a single dataset for analysis. Valid qualifiers for individual mercury measurements were reviewed for overall data usability. Only 3% of the native tissue mercury samples, 26 of 854 mercury measurements for lobster and crab tissue, were J-qualified. For all mercury tissue analyses, native sample mercury concentration results with associated J valid qualifiers were retained. One sample was R-qualified. Results from the single R-qualified sample were

[9] Alpha Analytical data qualifiers:
Q = The quality control sample exceeds the associated acceptance criteria.

rejected and excluded in all data analyses. Overall, 99% of the sample mercury measurements were considered useable. Native sample mercury concentration results were used for all analyses.

Total mercury levels for all tissue samples were measured on both a wet weight and dry weight basis. For consistency with data presented in the PRMS report and MECDC methodology for assessing fish and shellfish mercury concentrations, total mercury on a wet weight basis was used for all analyses. Mercury levels were reported as mg/kg and were converted to ng/g for consistency.

Lobster claw and tail mercury levels were analyzed separately, as previous data suggested that mercury concentrations differed between these two tissues. Due to the lack of legal size lobster catch for some sampling areas, lobster tail and claw data were stratified by legal size (legal size = ≥83 to ≤127mm carapace length and less than legal size = <83mm carapace length) to determine if there were any differences in mercury levels between legal size and less than legal size lobster.

To assess differences in mercury concentration between sampling areas, data were stratified into 6 groups based on sample area (Odom Ledge, South Verona, Fort Point, Cape Jellison, Turner Point, and Sears Island). For seasonal differences, due to the low number of lobster collected in April and December, April samples were combined with June samples, and December samples were combined with samples collected in October. Data were then stratified by sampling season defined as April and June = spring/early summer; August = late summer; and October and December = fall/early winter. For crabs, seasonality was assessed using these season groupings, as well as on a monthly basis because there was a sufficient number of crab collected at each sample area for each sampling month.

For descriptive statistics, the arithmetic mean, standard deviation, 95% upper and lower confidence limits on the mean, and minimum and maximum values were computed. For mercury level comparisons, distributions of total mercury in lobster and crab tissue were reviewed to assess normality. Distributions were positively skewed and were natural log (ln) transformed for statistical comparisons. Following ln-transformation data were normally distributed. For legal size and less than legal size lobster comparisons, independent t-tests were used to assess differences in the ln-transformed mean concentration between the two size groups. One-way ANOVA tests were used to compare ln-transformed mean mercury concentrations between sampling areas and between sampling seasons. Tukey's post-hoc comparisons were used to determine whether differences between individual groups were significantly different. For all statistical tests, the level of significance was set at $p < 0.05$. All analyses were performed with SAS (version 9.3).

4. Results and Discussion

4.1. Sample collection results

Sample collection during the 2014 period produced a total of 232 individual lobster samples from the six sampling areas. Lobster sampling was most productive during October, where almost all the site-specific target number goals were met (Table 5). Lobster sampling during April and December were the least productive months (Table 5). It appears that very few lobsters are present in the estuary in and above the Sears Island area in April. In June and August, lobster numbers increased in the more southern areas of Sears Island, Turner Point, and Cape Jellison. Lobster numbers increased in June and August in the more northern areas of Fort Point, South Verona, and Odom Ledge, but at a slower rate as compared to the southern areas. In October, lobster target numbers were successfully met in all areas. By December, lobster collection numbers decreased at each sampling area, suggesting that lobster are moving out of the estuary after October and don't start to move back until the late spring early summer months.

Legal-sized lobsters (≥83 - ≤127 mm carapace length) were preferentially selected over less than legal size, illegal (egg bearing and/or v-notch), and oversize (>127 mm carapace length) lobster. When prescribed area-specific target collections numbers were not met with legal-sized lobster collections, less than legal size lobsters were collected as close to the minimum size (83 mm carapace length) as possible. Overall, the number of legal-sized lobster collected at each sampling area was greater than the number of smaller, less than legal size lobster collected (Table 6). However, less than legal size lobsters were collected at a greater frequency in the three sampling sites outside the closed area (Table 6). The collection of mostly legal-sized lobster in the closed area is likely due to the decreased fishing pressure and a potential seasonal migration pattern where larger resident lobster remain in the northern reaches of the estuary longer than their smaller sized counterparts. In the areas outside the closure, 31% - 46% of the lobsters collected were less than legal size, suggesting that there is more fishing pressure for legal size lobster in these areas and/or a migration pattern where smaller sized lobster move into and remain in the southern section of the estuary.

A total of 393 individual male Rock crab samples from the six sampling areas were collected during the 2014 sampling year. Crab sampling showed no indication of seasonal movement in and out of the estuary. Target numbers for crab were met, or close to being met, during each sampling month for all areas (Table 5). This indicates that Rock crabs are fairly dispersed from Sears Island to Odom Ledge and tend not to move out of the Penobscot estuary, as was seen with lobster. With only two exceptions, all crabs collected were within the target size (≥100 - ≤140 mm carapace width) (Table 4).

Table 5. Monthly sample area collection numbers for lobster and crab.

		Sampling Month									
		April		June		August		October		December	
Sampling site	Target number	Lobster	Crab	Lobster	Crab	Lobster	Crab	Lobster	Crab	Lobster	Crab
Odom Ledge	10	0	10	11	10	6	10	10	10	4	10
South Verona	10	0	10	4	10	7	10	10	10	0	10
Fort Point	10	0	10	3	10	8	10	10	10	3	10
Cape Jellison	15	1	20	15	20	12	17	21	20	9	20
Turner Point	20	0	15	16	10	2	16	15	15	6	15
Sears Island	15	4	17	15	15	17	14	15	15	8	14
	Totals	5	82	64	75	52	77	81	80	30	76

Table 6. Number of legal size lobster and target size crab samples collected.

	Lobster samples [a]			Crab Samples [b]		
Sampling site	Legal size	< Legal size	> Legal size	Target size	< Target size	> Target size
Odom Ledge	27	4	0	49	0	1
South Verona [c]	14	6	0	49	1	0
Fort Point [c]	21	2	0	50	0	0
Cape Jellison	40	18	0	97	0	0
Turner Point	21	18	0	71	0	0
Sears Island	32	27	0	75	0	0

[a] Legal lobster size defined as ≥83 mm and ≤127 mm carapace length.

[b] Target crab size defined as ≥100 mm and ≤140 mm carapace width.

[c] No length measurement available for one lobster sample from Fort Point and one lobster sample from South Verona.

4.2. Mercury levels in lobster (*Homarus americanus*)

4.2.1. Mercury levels by legal size

Tissue, both tail and claw, from legal-sized lobster tended to have higher mercury levels than tissue from smaller, less than legal size lobster (Table 7). For lobster collected in the three areas inside the closure, the limited number of less than legal-sized lobster diminished the ability to detect any significant differences in mercury levels between the two size groups. For example, in the Fort Point area only two less than legal size lobsters were collected as compared to twenty-one legal-sized lobster. While in areas south of the closure, 31% - 46% of lobsters collected were less than legal size. For lobster from the Turner Point area, where the number of legal size and less than legal size lobster collected were nearly equivalent, the difference in mercury tissue levels between the two size groups was significant (Table 7). The difference in mercury levels was not as pronounced in lobster from the Cape Jellison and Sears

Island areas, but levels were slightly lower in less than legal-sized lobster. Overall, there was a clear indication that mercury levels were higher in claw and tail muscle tissue from legal-sized lobster as compared to less than legal size lobster.

Table 7. Mercury levels (ng/g wet weight) in legal size and less than legal size lobster by sampling area (listed north to south).

		Tail			Claw		
Sampling site	**Size grouping**	**N**	**Mean**	**Standard deviation**	**N**	**Mean**	**Standard deviation**
Odom Ledge	Legal size [a]	27	540.5	388.8	26	237.4	214.7
	Less than legal size	4	472.9	220.5	4	219.8	39.6
South Verona [b]	Legal size	14	533.7	584.8	14	203.5	245.9
	Less than legal size	6	357.0	268.6	6	154.9	87.4
Fort Point [b]	Legal size	21	422.5	411.6	20	189.8	219.8
	Less than legal size	2	108.0	11.4	1	41.2	-
Cape Jellison	Legal size	40	292.7	182.3	40	139.2	101.6
	Less than legal size	18	247.1	218.8	17	132.8	87.3
Turner Point	Legal size	21	302.6*	186.0	21	184.4*	157.1
	Less than legal size	18	124.8*	55.7	18	56.9*	23.9
Sears Island	Legal size	32	180.0	110.4	32	75.9	44.4
	Less than legal size	27	141.4	80.9	27	73.0	39.5

[a] Legal lobster size defined as ≥83 mm and ≤127 mm carapace length.

[b] No length measurement available for one lobster from Fort Point and one lobster from South Verona.

* Mean mercury concentration is significantly different between Legal and Less than Legal size lobster.

4.2.2. Mercury levels by sample area

Comparisons between sampling areas were restricted to legal-sized lobster to help reduce potential confounding from smaller, sublegal size lobster, which tended to have lower mercury tissue levels than the larger, legal-sized group. And the proportion of less than legal size lobster collected in each sampling area was greater in the sampling areas below the closure (less than legal size lobster collected: Cape Jellison - 31%, Turner Point - 46%, and Sears Island - 46%) as compared to the areas within the closure (less than legal size lobster collected: Odom Ledge - 13%, South Verona - 30%, and Fort Point - 9%). Additionally, from a human consumption perspective concern is focused on legal-sized lobster.

Mercury levels in lobster tissue from legal-sized lobster varied between sampling areas, with average concentrations in both tail and claw tissue decreasing from Odom Ledge down to Sears Island (Table 8). Similar to results from the PRMS, mercury levels in tail tissue were greater than levels in claw tissue (Table 8). Comparing mercury levels in tail tissue between sampling areas, average mercury levels in lobster from the Odom Ledge, South Verona, and Fort Point areas were not significantly different (Figure 4 and Table 9). Outside of the closure, mercury levels in lobster tail tissue from the previously un-sampled area of Cape Jellison were comparable to levels in lobster from the Turner Point area, an area

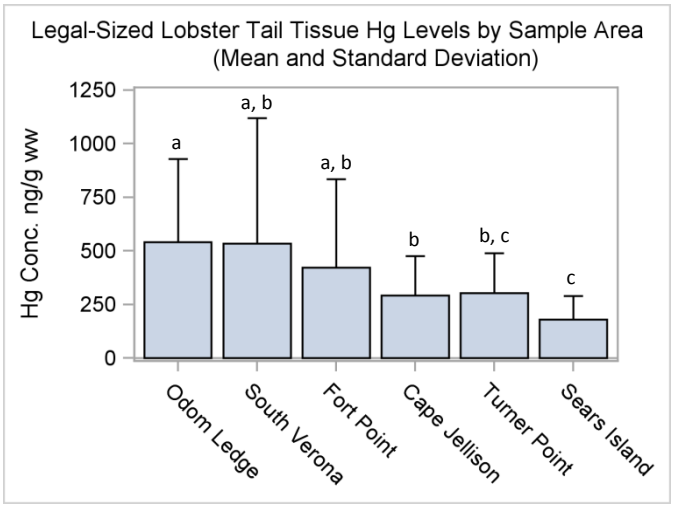
just east of Cape Jellison (Table 8). Average tail tissue mercury levels at Cape Jellison and Turner Point were significantly lower than levels at Odom Ledge, but not statistically different than levels at South Verona and Fort Point (Figure 4 and Table 9). Legal-sized lobster from the most southern area of Sears Island displayed the lowest average tail tissue level, which was significantly lower than all other areas expect the neighboring area of Turner Point (Figure 4 and Table 9). While mercury levels in lobster claw tissue decreased from north to south, only claw tissue samples from the Sears Island area had significantly lower mercury levels as compared to the all other areas (Figure 4 and Table 9).

Table 8. Mercury levels (ng/g wet weight) in legal-sized lobster tail and claw samples by sample area (listed north to south).

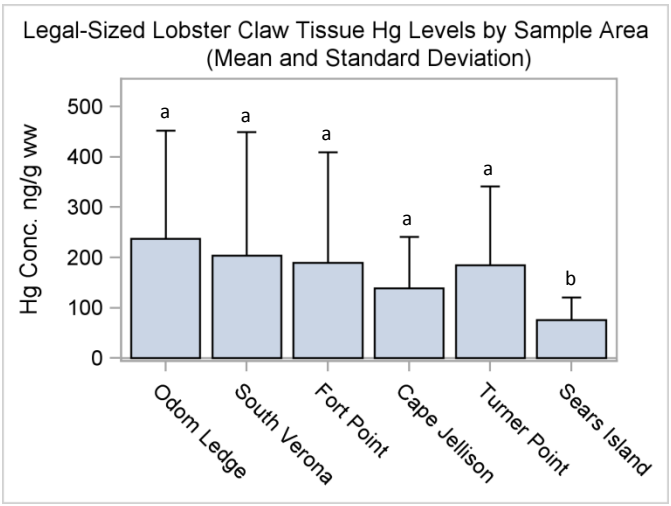
Sample site	Tissue	N	Mean	Standard deviation	95% lower CL	95% upper CL	Minimum	Maximum
Odom Ledge	Tail	27	540.5	388.8	386.7	694.3	85.5	1538.0
	Claw	26	237.4	214.7	150.7	324.2	26.0	742.9
South Verona	Tail	14	533.7	584.8	196.1	871.4	141.7	2432.0
	Claw	14	203.5	245.9	61.5	345.5	48.0	1008.0
Fort Point	Tail	21	422.5	411.6	235.1	609.8	112.0	1888.0
	Claw	20	189.8	219.8	86.9	292.7	12.5	1008.0
Cape Jellison	Tail	40	292.7	182.3	234.4	351.0	62.5	807.6
	Claw	40	139.2	101.6	106.7	171.7	43.0	496.8
Turner Point	Tail	21	302.6	186.0	218.0	387.3	52.8	794.4
	Claw	21	184.4	157.1	112.9	255.9	22.8	719.0
Sears Island	Tail	32	180.0	110.4	140.2	219.8	59.4	511.2
	Claw	32	75.9	44.4	59.9	91.9	20.3	215.2

Figure 4. Comparison of mean mercury levels (ng/g wet weight) in legal-sized lobster tail and claw tissue by sample area (listed north to south).

A. Tail



B. Claw



Letters above sample areas indicate results from multiple comparisons. Areas that share the same letter are not significantly different.

Table 9. Results from sample area multiple comparison tests for differences in mean mercury levels (ng/g wet weight) in tail and claw tissue samples from legal-sized lobster.

	Tail			Claw		
Sample site comparison	**Difference between means**	**95% Confidence interval**	**p-value**	**Difference between means**	**95% Confidence interval**	**p-value**
Odom Ledge - South Verona	0.11	-0.52-0.73	0.9963	0.18	-0.55-0.92	0.9785
Odom Ledge - Fort Point	0.30	-0.25-0.85	0.6188	0.33	-0.33-0.98	0.7035
Odom Ledge - Cape Jellison	0.56	0.09-1.03	0.0103	0.40	-0.16-0.95	0.3165
Odom Ledge - Turner Point	0.59	0.04-1.14	0.0297	0.21	-0.43-0.86	0.9306
Odom Ledge - Sears Island	1.05	0.56-1.55	<.0001	0.96	0.37-1.54	<.0001
South Verona - Fort Point	0.19	-0.46-0.85	0.9566	0.14	-0.63-0.91	0.9945
South Verona - Cape Jellison	0.45	-0.14-1.04	0.2345	0.21	-0.47-0.90	0.9477
South Verona - Turner Point	0.48	-0.17-1.14	0.2822	0.03	-0.73-0.79	1.0000
South Verona - Sears Island	0.95	0.34-1.55	0.0002	0.77	0.07-1.48	0.0233
Fort Point - Cape Jellison	0.26	-0.25-0.77	0.6866	0.07	-0.54-0.67	0.9995
Fort Point - Turner Point	0.29	-0.30-0.87	0.7170	-0.11	-0.80-0.58	0.9971
Fort Point - Sears Island	0.75	0.22-1.28	0.0011	0.63	0.001-1.26	0.0494
Cape Jellison - Turner Point	0.03	-0.48-0.54	1.0000	-0.18	-0.78-0.41	0.9508
Cape Jellison - Sears Island	0.49	0.04-0.94	0.0236	0.56	0.04-1.09	0.0278
Turner Point - Sears Island	0.46	-0.07-1.00	0.1272	0.74	0.12-1.36	0.0091

Multiple comparison tests were performed using ln-transformed mercury results.

4.2.3. Mercury levels by sample season

Seasonal changes in lobster tissue mercury levels were assessed for each sampling area individually, rather than combining results from all sites. This was done to control for potential confounding due to the significant differences in mercury levels in lobster tissue between specific areas (Figure 4 and Table 9). Similar to the sample area comparisons, seasonal changes were assessed in only legal-sized lobster. Overall, there was no clear seasonal gradient for mercury in lobster tissue at any one site (Table 10 and Figure 5). In general, mean mercury levels were higher in the spring/early summer (April and June) than in the late summer (August) (Table 10 and Figure 5). In the fall/early winter (October and December) mean mercury levels rebounded from the dip seen during late summer (Table 10 and Figure 7). For South Verona and Fort Point in the spring/early summer and Turner Point during the late summer, the sample size of only one or two legal-sized lobster limited the ability to assess seasonal fluctuations in mercury tissue levels for these individual sampling areas.

This unexpected pattern may reflect the presence of lobster that remained in an area over the winter and were collected in the spring/early summer season before migrating lobster moved into the area. Lobsters that remain in an area, particularly areas inside the closure, would presumably have higher

mercury levels. As lobsters from outside the affected area migrate into the upper estuary during the summer months they would have less time to accumulate mercury. Thus, the causality of the observed summer dip in mercury levels in lobster tissue may be partially due to a dilution effect of new lobster migrating north. Lobster molting, which generally occurs from June through September in the Gulf of Maine, could also influence mercury tissue levels and contribute to seasonal fluctuations.

Table 10. Mercury levels (ng/g wet weight) in legal-sized lobster tail and claw samples by sample season.

A. Tail

	Spring/Early Summer (April & June)			**Late Summer** (August)			**Fall/Early Winter** (October & December)		
Sampling site	**N**	**Mean**	**Standard deviation**	**N**	**Mean**	**Standard deviation**	**N**	**Mean**	**Standard deviation**
Odom Ledge	8	790.8*	424.6	6	294.1*	148.2	13	500.3	373.4
South Verona	1	2432.0	-	6	336.8	230.3	7	431.4	212.5
Fort Point	2	401.1	67.5	6	165.2*	68.5	13	544.5*	480.3
Cape Jellison	5	321.7	160.3	11	205.0	157.7	24	326.8	189.7
Turner Point	4	317.1	110.0	1	385.3	-	16	293.9	207.6
Sears Island	5	313.4*†	125.8	8	149.1*	95.2	19	157.9†	89.8

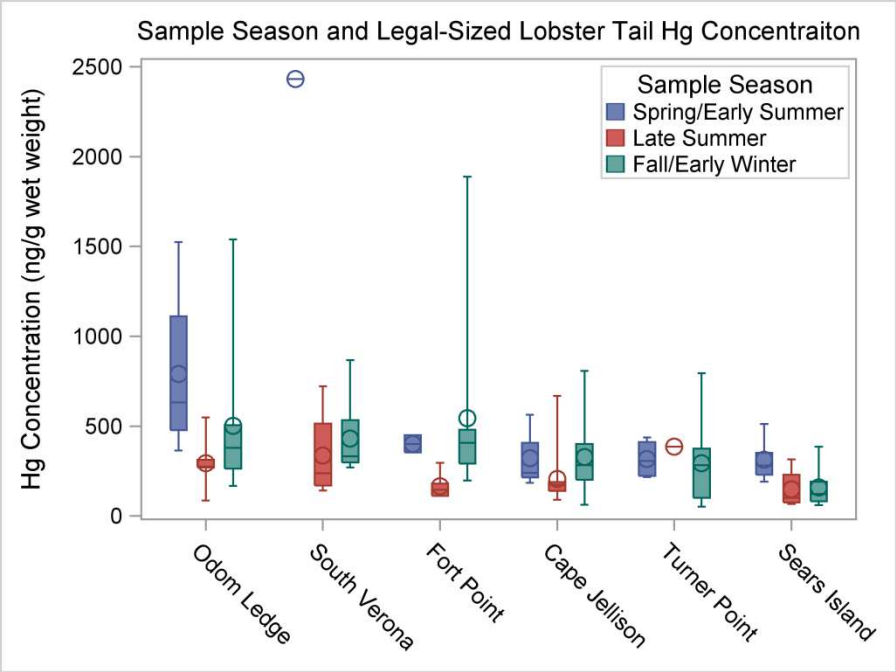
B. Claw

	Spring/Early Summer (April & June)			**Late Summer** (August)			**Fall/Early Winter** (October & December)		
Sampling site	**N**	**Mean**	**Standard deviation**	**N**	**Mean**	**Standard deviation**	**N**	**Mean**	**Standard deviation**
Odom Ledge	7	440.0*	276.9	6	96.9*	60.3	13	193.2	142.2
South Verona	1	1008.0	-	6	91.1*	46.7	7	184.8*	91.3
Fort Point	2	251.1	125.8	6	48.1*	22.0	12	250.5*	257.2
Cape Jellison	5	153.3*	98.9	11	89.1*†	86.6	24	159.3†	104.3
Turner Point	4	137.8	54.2	1	83.6	-	16	202.3	175.3
Sears Island	5	111.6*	60.3	8	66.0*	46.0	19	70.7	36.6

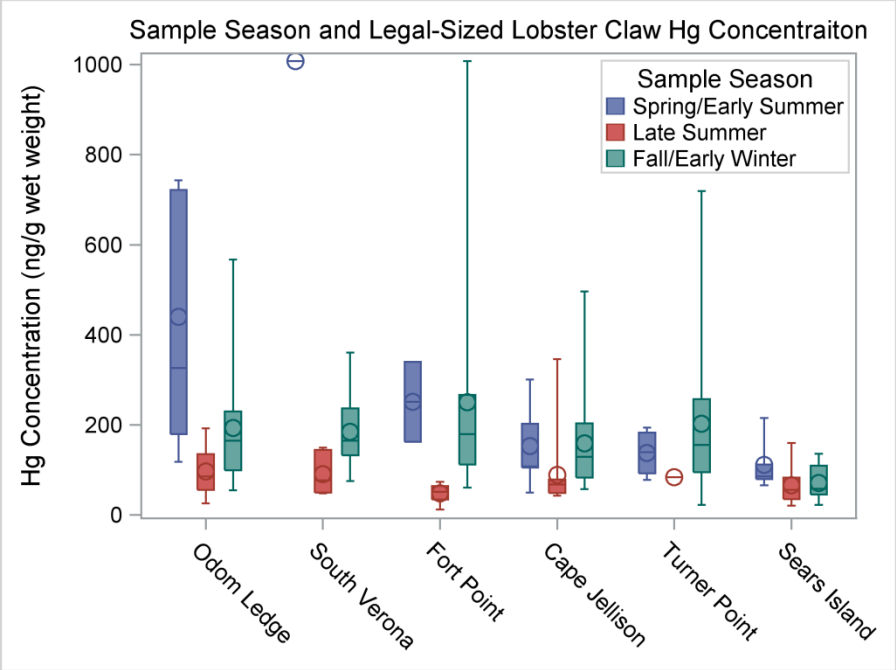
Average mercury levels were compared by season for individual sites separately. Mean mercury concentrations that share an * or † are significantly different ($p < 0.05$).

Figure 5. Seasonal changes in mercury levels (ng/g wet weight) in tail and claw tissue from legal-sized lobster by sample area.

A. Tail



B. Claw



Spring/Early summer = April and June sampling dates, Late Summer = August Sampling dates, Fall/Early Winter = October and December sampling dates. Bars extend to minimum and maximum values, circle indicates sample mean, line inside the box indicates the median and the bottom and top of the box indicate the 25th and 75th percentiles, respectively.

4.2.4. Comparison with previous PRMS lobster mercury data

Within the Penobscot River estuary the PRMS collected and measured total mercury in lobster tissue in 2006, 2008, 2009, 2010 and 2012[10] (PRMS, 2013b). During the first study year, 2006, lobsters were collected at Fort Point, Turner Point, Harborside, and Southwest Sears Island. In 2006, mercury was analyzed in all claw tissue samples, with only eight tail tissue samples analyzed for mercury. In 2008, 2009, 2010, and 2012 lobster collection was expanded north to include sampling areas near South Verona and Odom Ledge and further south/southwest to include areas in Kelly's Cove and Parker Cove. Mercury levels in both claw and tail tissue were analyzed for lobster collected in 2008 and 2009. In 2010, tail tissue was the primary muscle tissue analyzed, with mercury in claw tissue measured in approximately half of the lobster samples collected. For lobsters collected in 2012, mercury was analyzed solely in tail tissue. Lobster collections for the PRMS took place during the months of August, September, and October, with the majority of lobster collected in September for most sampling years.

Results from the 2006-2012 PRMS sampling and 2014 DMR sampling were similar, in that, mercury concentrations in lobster tail and claw tissue decreased geographically from north to south (Table 11 and Figure 6). Both the PRMS and DMR sampling indicated that lobster from the more northern areas of Odom Ledge, South Verona, and Fort Point had the highest average mercury levels (Table 11 and Figure 6). Mercury levels in lobster collected from the area of Cape Jellison, which was not sampled during the PRMS, were comparable to levels in lobster from the Turner Point area (Table 11 and Figure 6b).

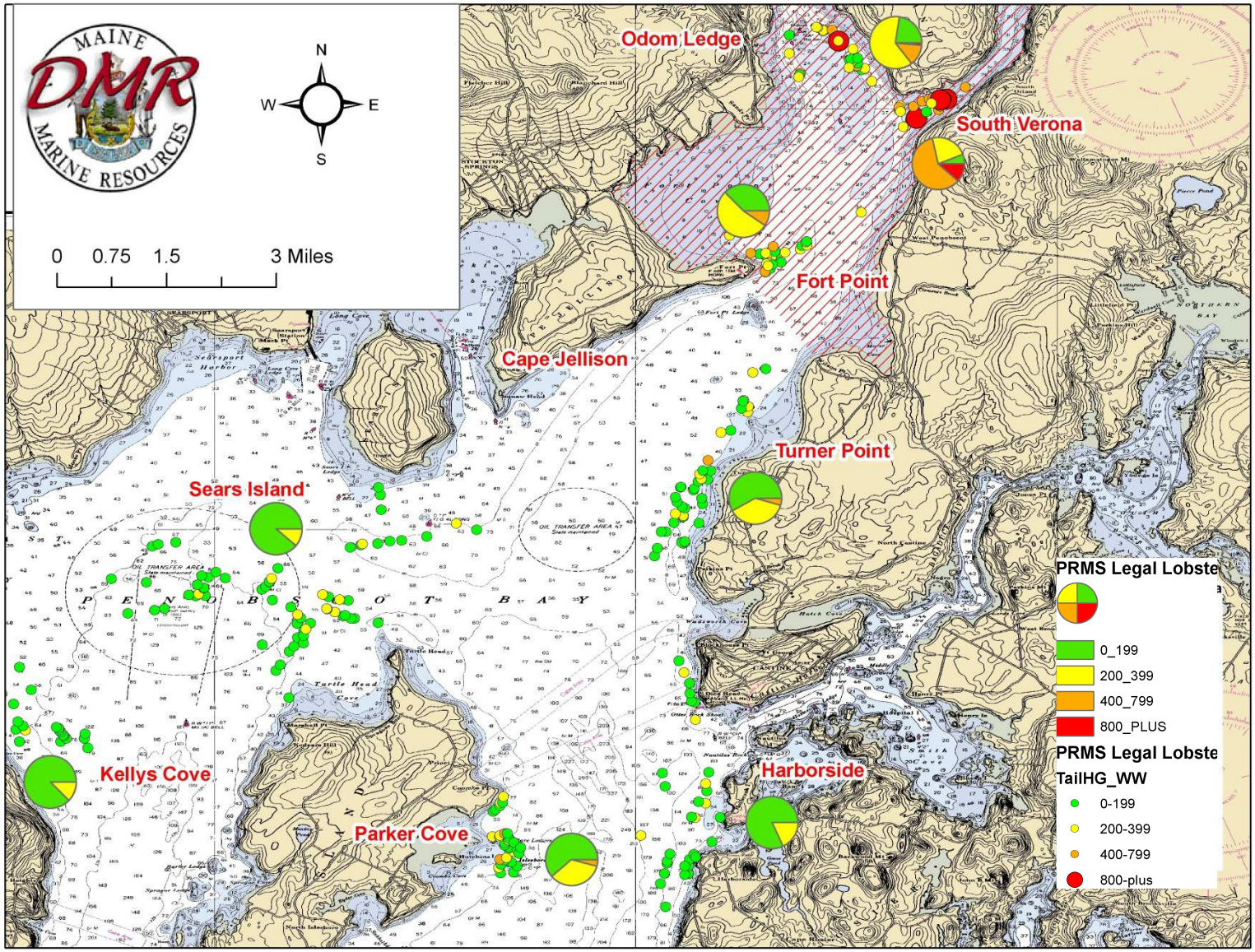
Table 11. Comparison of 2014 DMR and 2006-2012 PRMS mercury levels (ng/g wet weight) in tail and claw tissue from legal-sized lobster (≥83mm carapace length).

		Tail			Claw		
Sampling site	**Study**	**N**	**Mean**	**Standard deviation**	**N**	**Mean**	**Standard deviation**
Odom Ledge	2014 DMR	27	540.5	388.8	26	237.4	214.7
	PRMS	62	282.4	151.1	42	129.1	83.0
South Verona	2014 DMR	14	533.7	584.8	14	203.5	245.9
	PRMS	35	505.9	233.1	20	257.1	134.1
Fort Point	2014 DMR	21	422.5	411.6	20	189.8	219.8
	PRMS	43	247.2	130.2	23	122.0	63.8
Cape Jellison [a]	2014 DMR	40	292.7	182.3	40	139.2	101.6
	PRMS	-	-	-	-	-	-
Turner Point	2014 DMR	21	302.6	186.0	21	184.4	157.1
	PRMS	69	210.1	94.5	56	110.2	67.3
Sears Island	2014 DMR	32	180.0	110.4	32	75.9	44.4
	PRMS	89	119.8	57.8	79	57.4	75.3

[a] The PRMS did not sample for lobster in the Cape Jellison area.

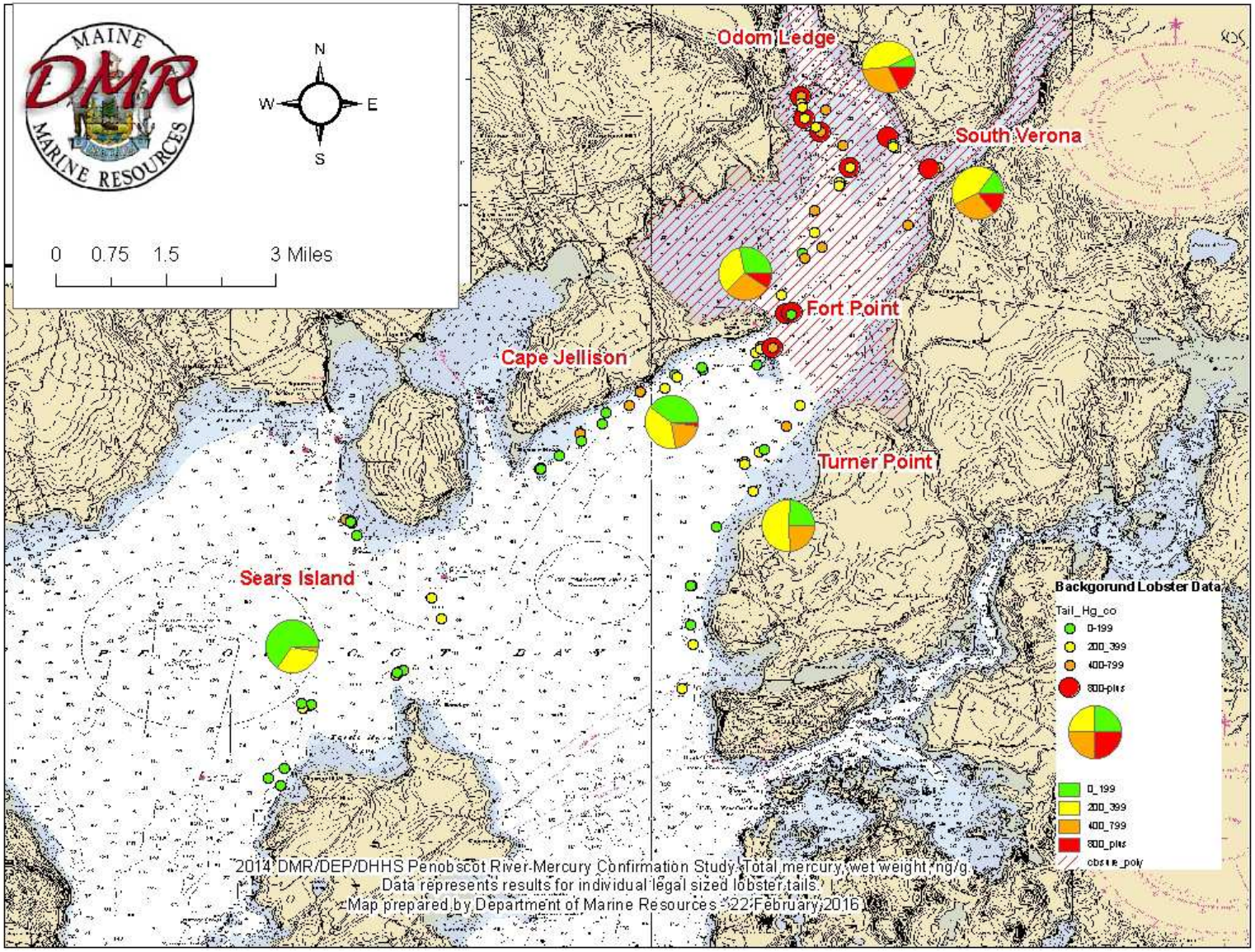
[10] Mercury data in lobsters collected in 2012 were not presented in the 2013 PRMS Final Report. Data for 2006-2012 sampling were provided by the PRMS study staff to the DMR, MEDEP, and MECDC upon request.

Figure 6a. Results from the Penobscot River Mercury Study (2006-2012) for total mercury in legal-sized lobster tail tissue.



Sample area mercury levels (ng/g wet weight) in legal-sized lobster tail tissue are summarized by pie charts. Colored dots indicate the maximum mercury level in legal-sized lobster tail tissue at each individual sample collection site.

Figure 6b. Results from the Maine Department of Maine Resource (2014) sampling for total mercury in legal-sized lobster tail tissue.



Sample area mercury levels (ng/g wet weight) in legal-sized lobster tail tissue are summarized by pie charts. Colored dots indicate the maximum mercury level in legal-sized lobster tail tissue at each individual sample collection site.

While similar trends were observed with both PRMS and DMR samplings, mercury levels in lobster tissue collected in 2014 were greater than levels from the PRMS, with the one exception of claw tissue from lobster in the South Verona area (Table 11). Higher mercury levels in DMR samples may be, in part, due to the sampling season. In 2014, the highest mean mercury levels were seen in lobster collected in April and June, with the lowest levels seen in August (Table 9). PRMS sampling took place during the late summer/early fall where mercury levels in lobster, as seen in 2014 DMR sampling, may be at their lowest levels, especially in the more northern areas (Figure 5). For several sampling areas, lobsters were collected in the same months during both the PRMS and 2014 DMR study (Table 12). Mercury levels were similar in lobster tail and claw tissue collected during the PRMS and DMR study in August in the Odom Ledge area (Table 12). In October, in the Odom Ledge area mercury levels were again higher in DMR lobster samples. For the South Verona area, mercury levels in lobster tail and claw tissue from the PRMS were slightly higher in October, while at Fort Point and Turner Point levels remained lower than those from the 2014 DMR sampling (Table 12). Differences in mercury levels in lobster tissue between DMR and PRMS sampling could also be partially attributable to differences in tissue collection and storage methods, hold times, and laboratory tissue preparation and analysis methodology.

Table 12. Mean mercury levels (ng/g wet weight) in tail and claw tissue samples from legal-sized lobster collected during equivalent sample months for 2014 DMR and 2006-2012 PRMS.

			Tail			Claw		
Sampling month	**Sampling site**	**Study**	**N**	**Mean**	**Standard deviation**	**N**	**Mean**	**Standard deviation**
August	Odom Ledge	2014 DMR	6	294.1	148.2	6	96.9	60.3
		PRMS	13	342.0	131.8	13	160.7	87.3
October	Odom Ledge	2014 DMR	9	546.0	422.4	9	191.1	149.7
		PRMS	16	289.3	206.3	16	103.3	73.9
October	South Verona	2014 DMR	7	431.4	212.5	7	184.8	91.3
		PRMS	13	526.7	209.2	13	252.8	143.0
October	Fort Point	2014 DMR	10	458.6	288.8	9	191.4	103.3
		PRMS	9	298.1	207.2	9	115.5	72.6
October	Turner Point	2014 DMR	10	274.9	258.4	10	168.1	134.1
		PRMS	30	205.2	89.7	30	99.1	62.9

4.3. Mercury in Rock Crab (*Cancer irroratus*)

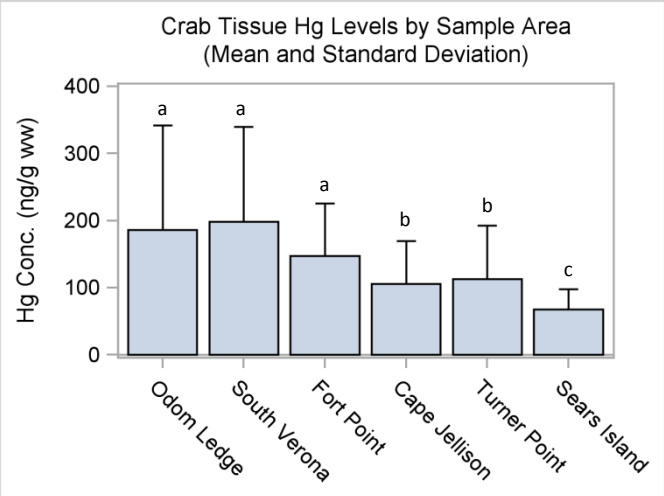
4.3.1. Mercury levels by sample area

Out of the six sampling areas, crabs collected from Odom Ledge and South Verona displayed the highest mercury levels (Table 13). Similar to lobster tissue, mercury levels tended to decreased by sample area from north to south, with the lowest mercury levels in crabs collected from Sears Island (Table 13). Within the closed area, mean mercury concentrations in crab tissue collected at Odom Ledge, South Verona, and Fort Point were not significantly different (Figure 7 and Table 14). The average mercury concentration from crabs collected at each area outside the closure was significantly lower than the average from each area within the closure (Figure 7 and Table 14). Comparing the three areas outside the closure, mercury levels in crabs from Cape Jellison and Turner point were similar, and average mercury concentrations from both areas were significantly higher than the average concentration in crabs from Sears Island (Figure 7 and Table 14).

Table 13. Mercury levels (ng/g wet weight) in crab tissue by sample area (listed north to south).

Sample site	N	Mean	Standard deviation	95% lower CL	95% upper CL	Minimum	Maximum
Odom Ledge	50	186.4	155.5	142.2	230.5	31.2	897.6
South Verona	50	198.2	141.3	158.0	238.3	35.6	699.6
Fort Point	50	147.6	78.0	125.4	169.7	35.0	392.6
Cape Jellison	97	105.9	63.8	93.0	118.8	23.6	289.2
Turner Point	71	112.6	79.9	93.7	131.5	23.6	381.6
Sears Island	75	67.5	30.2	60.6	74.5	18.6	184.6

Figure 7. Comparison of mercury levels (ng/g wet weight) in crab tissue by sampling area.



Letters above sample areas indicate results from multiple comparisons. Areas that share the same letter are not significantly different.

Table 14. Results from sample area multiple comparison tests for differences in mean mercury levels (ng/g wet weight) in crab claw tissue.

Sample site comparison	Difference between means	95% Confidence interval	p-value
Odom Ledge - South Verona	-0.10	-0.48-0.27	0.9686
Odom Ledge - Fort Point	0.10	-0.27-0.47	0.9734
Odom Ledge - Cape Jellison	0.47	0.14-0.80	0.0006
Odom Ledge - Turner Point	0.46	0.11-1.17	0.0023
Odom Ledge - Sears Island	0.82	0.48-0.58	<.0001
South Verona - Fort Point	0.20	-0.17-0.90	0.6277
South Verona - Cape Jellison	0.57	0.25-0.90	<.0001
South Verona - Turner Point	0.56	0.22-0.91	<.0001
South Verona - Sears Island	0.93	0.59-1.27	<.0001
Fort Point - Cape Jellison	0.37	0.04-0.70	0.0155
Fort Point - Turner Point	0.36	0.01-0.71	0.0365
Fort Point - Sears Island	0.72	0.38-1.07	<.0001
Cape Jellison - Turner Point	-0.01	-0.30-0.28	1.0000
Cape Jellison - Sears Island	0.35	0.06-0.64	0.0066
Turner Point - Sears Island	0.36	0.05-0.67	0.0109

Multiple comparison tests were performed using ln-transformed mercury results.

4.3.2. Mercury levels by sample season

There was a noticeable seasonal trend in mercury levels in crab tissue across all sampling areas. Crabs collected in the spring/early summer displayed the lowest mercury levels, with levels increasing in late summer and fall/early winter (Table 15 and Figure 8). Mean mercury concentrations in crabs collected during the late summer season were all significantly higher than crabs collected during the spring/early summer season (Table 15). While mean mercury levels were higher in crab collected in the fall/early winter at each site as compared to late summer, the increase was only significantly different for crab at Cape Jellison and Sears Island (Table 15).

Based on collection numbers for each sampling area and sampling month, crab did not display an obvious pattern of seasonal migration. Rather they remained dispersed throughout the estuary from April through December. Due to the consistent numbers of crab collected for each sampling month, seasonal changes in mercury levels in crabs were also assessed by sampling month (Figure 8). Although mercury levels were similar during April and June for most areas, there was a clear increase in mercury levels in crab tissue from April through December. This seasonal increase in mercury tissue levels, to some extent, may be due to temporal changes in mercury levels in water and sediment in the estuary. Rock crab molting, which typically occurs in the spring, may also be a contributing factor underlining the observed seasonal increase.

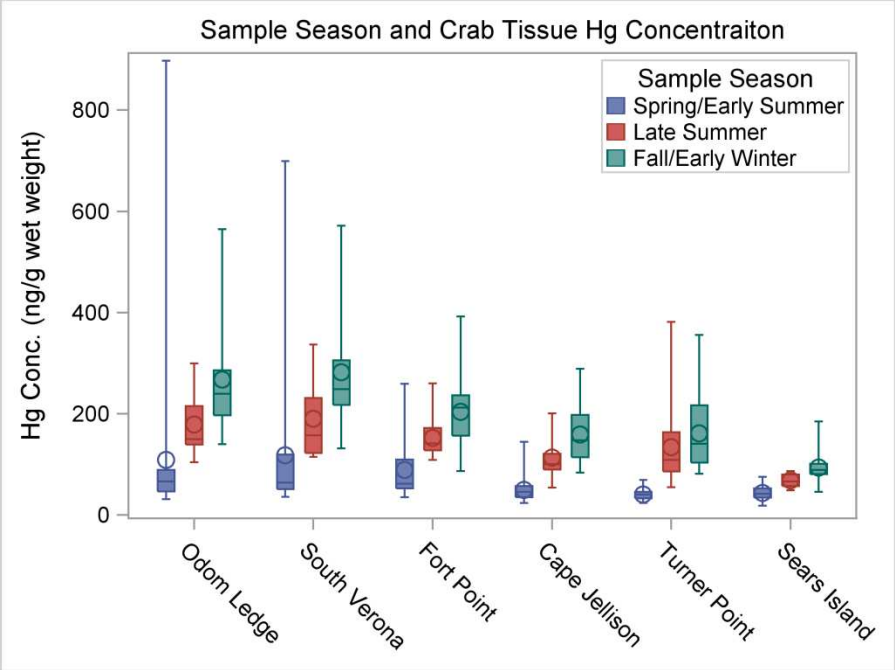
Table 15. Mercury levels (ng/g wet weight) in crab samples by season sampled.

	Spring/Early Summer (April & June)			**Late Summer** (August)			**Fall/Early Winter** (October & December)		
Sampling site	**N**	**Mean**	**Standard deviation**	**N**	**Mean**	**Standard deviation**	**N**	**Mean**	**Standard deviation**
Odom Ledge	20	109.0*†	188.0	10	178.4*	67.5	20	267.7†	107.5
South Verona	20	118.0*†	145.9	10	190.4*	81.2	20	282.2†	113.3
Fort Point	20	89.1*†	56.7	10	152.1*	43.8	20	203.8†	68.2
Cape Jellison	40	49.4*	21.4	17	113.7*	43.0	40	159.1*	51.0
Turner Point	25	40.4*†	10.1	16	133.8*	80.0	30	161.5†	69.3
Sears Island	32	43.6*	13.7	14	68.3*	11.9	29	93.5*	27.9

Average mercury levels were compared by season for individual sites separately. Mean mercury concentrations between sample seasons with the same indicator * or † are significantly different ($p < 0.05$).

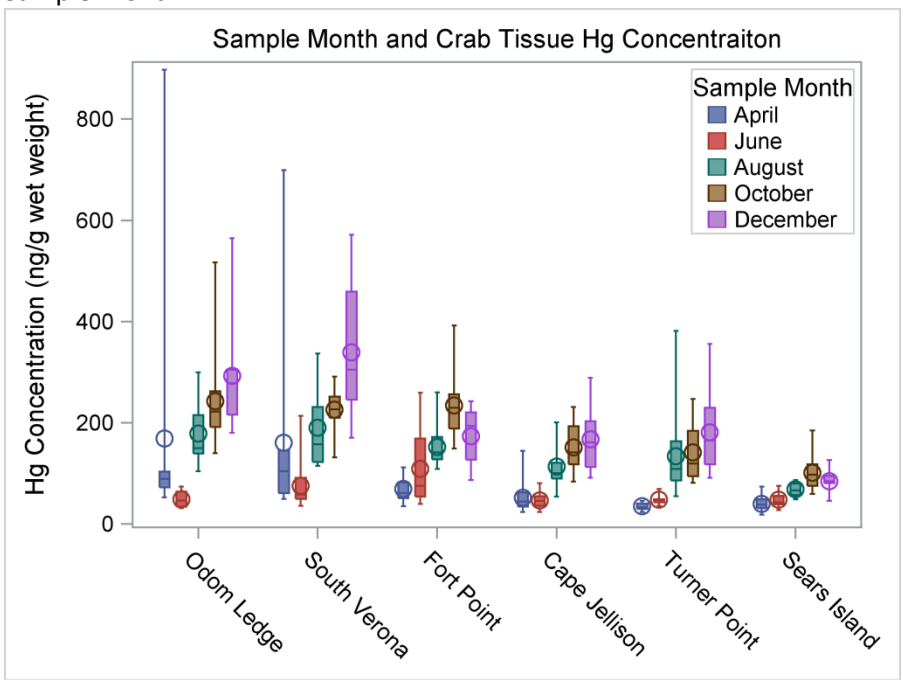
Figure 8. Seasonal changes in mercury levels (ng/g wet weight) in crab tissue by sampling area.

A. Sample Season



Spring/Early summer = April and June, Late Summer = August, Fall/Early Winter = October and December.

B. Sample Month



Bars extend to minimum and maximum values, circle symbol indicates sample mean, line inside the box indicates the median and the bottom and top of the box indicate the 25th and 75th percentiles.

4.3.3. Comparison with previous PRMS crab mercury data

The PRMS collected and measured mercury in several species of crab, including Rock crab (*Cancer irroratus*) (PRMS, 2009a). Crab collections took place during the September 2006 sampling period in the general areas of Turner Point, Southwest Sears Island, and North Isleboro (Figure 9a). Total mercury was analyzed in claw muscle tissue from male and female crabs (PRMS, 2009b).

Based on the 2006 PRMS sampling, where mean mercury levels in crab from Turner Point and Sears Island were > 200 ng/g wet weight, crab from more northern areas in the estuary were expected to have similar, if not higher, levels of mercury. However, at all six sampling areas mean mercury levels in crab collected in 2014 by the DMR were < 200 ng/g wet weight (Table 16). There was also a clear decreasing trend in mercury levels in crab from north to south with the highest levels in the Odom Ledge and South Verona areas (Table 16 and Figure 9b).

A potential explanation for the differences between the 2006 PRMS and 2014 DMR sampling for mercury levels in crab from the Turner Point and Sears Island areas may be seasonal timing. However, mean mercury levels in crab tissue from the 2006 PRMS sampling in September were greater than the mean levels for each sampling month in 2014, including October and December where the highest average levels were seen in the Turner Point and Sears Island areas (Figure 8). While the 2014 DMR sampling collected only male Rock crab, the PRMS collected both male and female Rock crabs and noted that female crabs contained higher claw tissue mercury levels than male crabs (PRMS, 2009b). Comparing mercury levels in male crabs only, results from the PRMS remained higher than the DMR sampling. However, average mercury levels from the PRMS were slightly reduced, particularly in the Sears Island area (Table 16). General differences in crab tissue collection and preparation methods, holding times and storage methods, and laboratory analysis procedures could also potentially underlie the differences in mercury levels in crab from 2006 PRMS and 2014 DMR sampling.

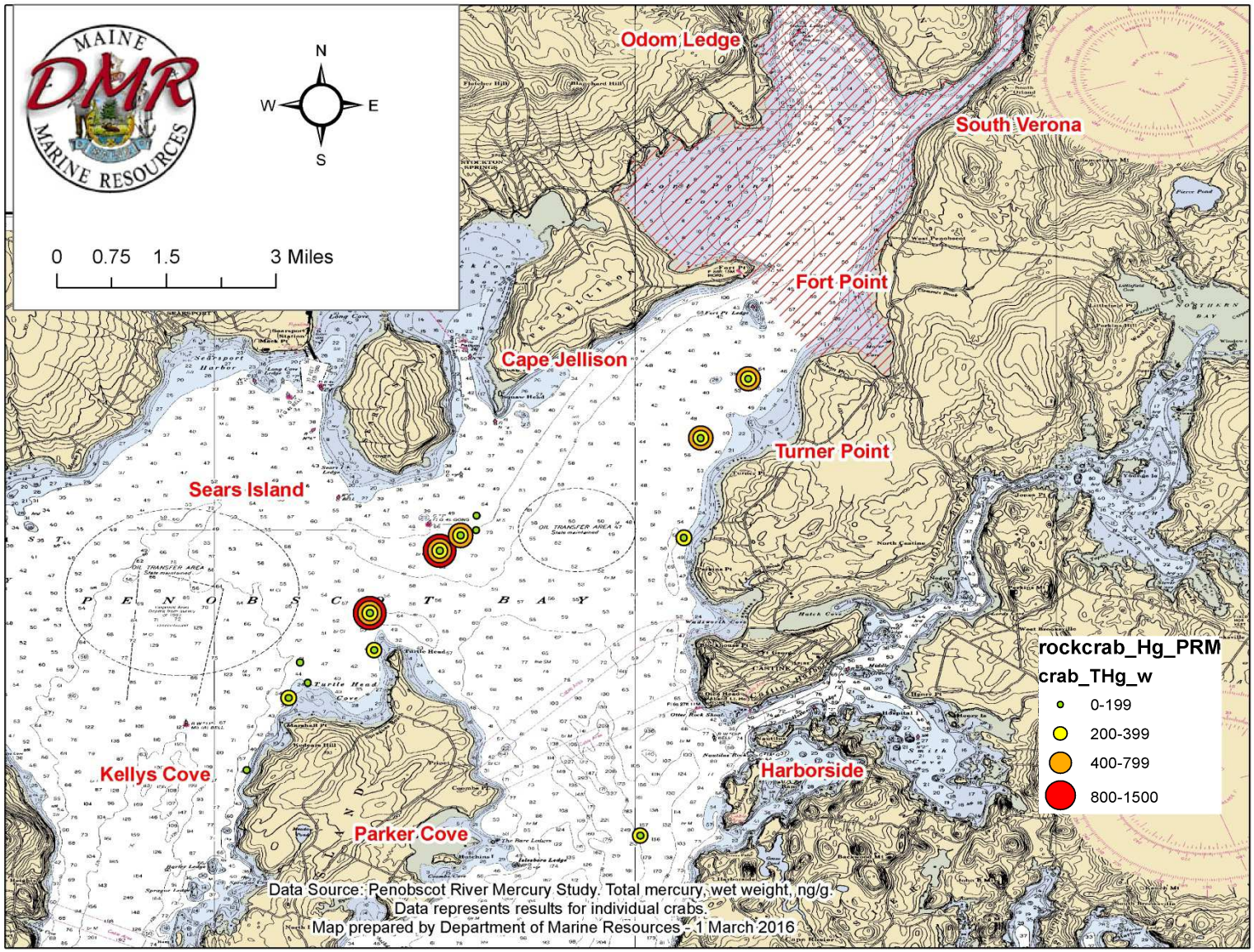
Table 16. Comparison of 2014 DMR and 2006 PRMS mercury levels (ng/g wet weight) in crab tissue.

Sample site	Study	N	Mean	Standard deviation	95% lower CL	95% upper CL	Minimum	Maximum
Odom Ledge	2014 DMR	50	186.4	155.5	142.2	230.5	31.2	897.6
	PRMS [a]	-	-	-	-	-	-	-
South Verona	2014 DMR	50	198.2	141.3	158.0	238.3	35.6	699.6
	PRMS [a]	-	-	-	-	-	-	-
Fort Point	2014 DMR	50	147.6	78.0	125.4	169.7	35.0	392.6
	PRMS [a]	-	-	-	-	-	-	-
Cape Jellison	2014 DMR	97	105.9	63.8	93.0	118.8	23.6	289.2
	PRMS [a]	-	-	-	-	-	-	-
Turner Point	2014 DMR	71	112.6	79.9	93.7	131.5	23.6	381.6
	PRMS - all [b]	26	223.8	145.9	164.8	282.7	46.0	572.0
	PRMS - male [b]	24	213.0	139.7	154.0	272.0	46.0	572.0
Sears Island	2014 DMR	75	67.5	30.2	60.6	74.5	18.6	184.6
	PRMS - all [b]	35	239.6	258.5	150.8	328.4	69.2	1340.0
	PRMS - male [b]	27	156.1	98.0	117.3	194.9	69.2	489.0

[a] The PRMS did not sample for Rock crab at Odom Ledge, South Verona, Fort Point or Cape Jellison.

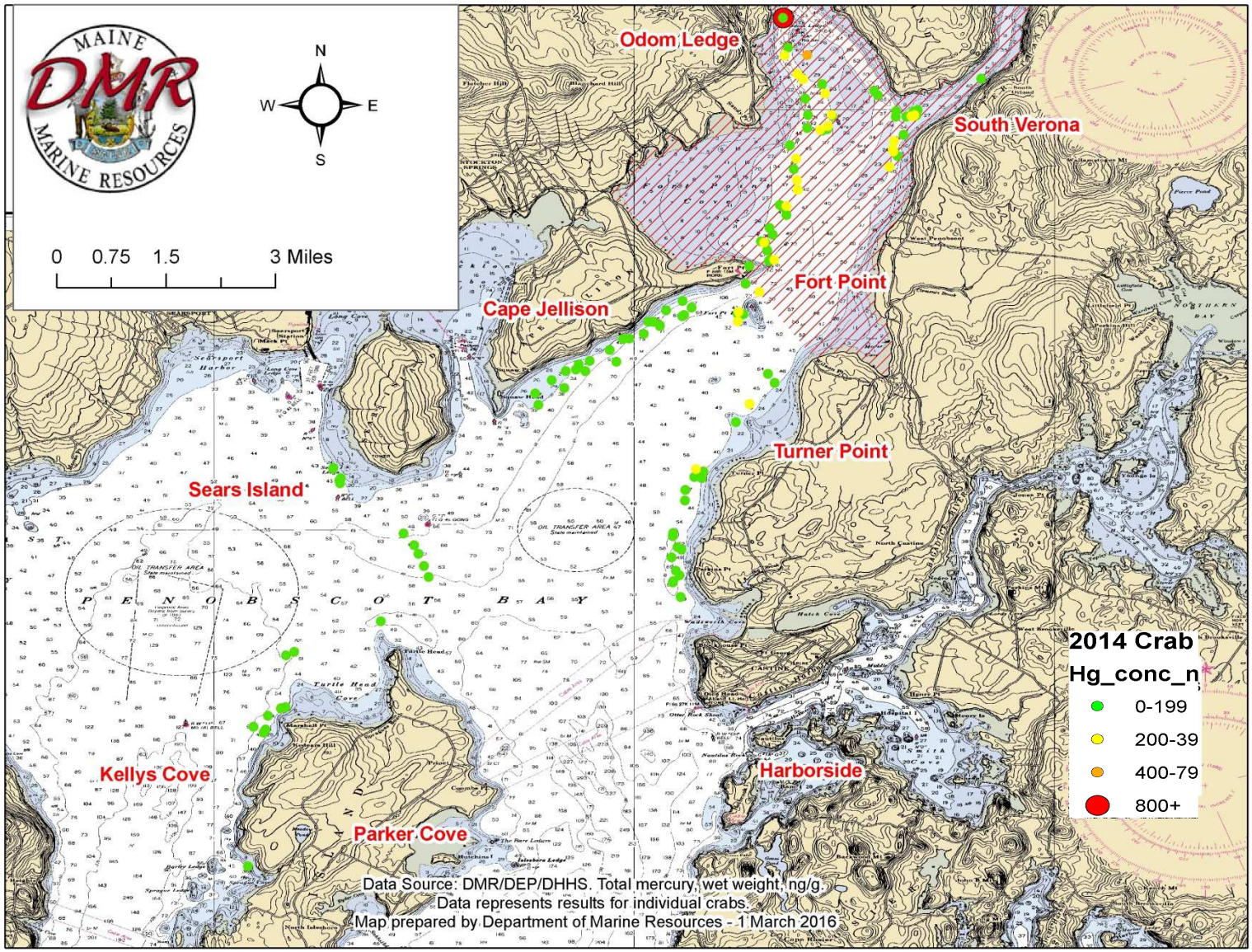
[b] The PRMS collected and measured mercury in both male and female Rock crab. The PRMS - all results are male and female crab tissue results combined, and the PRMS-male group comprises results male crabs only.

Figure 9a. Results from the Penobscot River Mercury Study (2006-2012) for total mercury in Rock crab claw tissue.



Concentric circles indicate mercury levels (ng/g wet weight) in Rock crab claw tissue at individual sample collection sites.

Figure 9b. Results from the Maine Department of Maine Resource (2014) sampling for total mercury in Rock crab claw tissue.



Colored dots indicate the maximum mercury level (ng/g wet weight) in Rock crab claw tissue at each individual sample collection site.

5. Conclusions

5.1. Lobster

The primary aim of the 2014 DMR lobster sampling was to confirm previous PRMS findings of elevated mercury levels in lobster tissue in the Penobscot River estuary with a geographic pattern of decreasing levels from north to south. Additional aims of the 2014 sampling were to characterize mercury levels in lobster collected in the Cape Jellison area, an area which had not been previously sampled, define potential seasonal fluctuations in mercury levels in lobster tissue, and generally assess lobster migratory patterns in the estuary.

Regarding these aims:

- The 2014 DMR sampling results largely confirmed the PRMS data. Mercury levels in lobster tissue collected from the three areas inside the closure (Odom Ledge, South Verona, and Fort Point) remain elevated and were higher than levels from lobster collected from areas south of the closure line (Cape Jellsion, Turner Point, and Sears Island).

- Similar to the PRMS data, mercury levels in lobster tissue tended to decrease geographically from north to south. Average mercury levels were the highest in lobster from Odom Ledge and South Verona areas and decreased by sampling area where the lowest average levels were found in lobster from the Sears Island area.

- Mercury levels in lobster collected from the previously un-sampled area around Cape Jellison were similar to levels in lobster from Turner Point, an area just to the east of Cape Jellison.

- Lobster tissue tended to contain the highest mercury levels in the spring/early summer with levels decreasing in the late summer and rebounding in the late fall/early winter. This seasonal pattern was observed at five of the six sampling areas.

- Lobsters appear to use the area seasonally with increased migration into the upper Penobscot River estuary in the summer and emigration in the winter.

5.2. Crab

The primary aim of the 2014 DMR Rock crab sampling was to confirm and expand the results of the limited 2006 PRMS Rock crab sampling where muscle tissue from crab collected around the Turner Point and Sears Island areas contained on average approximately 200 ng/g wet weight of mercury. These data suggested that crabs further north in the estuary could have elevated mercury levels, but no data was available for crabs in the areas of Odom Ledge, South Verona, Cape Jellison, or Fort Point. Similar to the lobster sampling, auxiliary aims for the 2014 crab sampling were to characterize potential seasonal changes in crab tissue mercury levels and identify any seasonal migrations in the estuary.

Regarding these aims:

- Rock crab from areas both inside and outside the closure contained lower levels of mercury than expected. Average levels in crab tissue from each sampling area were below 200 ng/g wet weight.

- Mercury levels in crab muscle tissue were the highest in the closure area and tended to decrease geographically from north to south.

- There was a clear seasonal increase from spring to winter in crab tissue mercury levels. At each sampling area, average mercury levels in crab tissue were the lowest in the spring/early summer, slightly higher in late summer, and the highest in the fall/early winter.

- Crab were present during each sampling month in all six sampling area suggesting there was no seasonal migration out of the estuary.

6. References

PRMS. 2009a. Penobscot River Mercury Study. Update to the Phase I Report – Part 1. Report to: Judge John Woodcock. U.S. District Court (District of Maine) Bangor, Maine. http://www.maine.gov/dep/spills/holtrachem/penobriver/penobsrivermercurystudy_phase_I_update_report_july_2009_part%201.pdf

PRMS. 2009b. Penobscot River Mercury Study. Phase I Update Report – Part 2. Report to: Judge John Woodcock. U.S. District Court (District of Maine) Bangor, Maine. http://www.maine.gov/dep/spills/holtrachem/penobriver/penobscot_river_mercurystudy_phase%20_I_updatereport%20_july_2009_part%202.pdf

PRMS. 2013a. Penobscot River Mercury Study. Mercury Contamination of the Penobscot River Estuary: Current Situation, Remediation Targets and Possible Remediation Procedures. Final Report. Executive Summary. http://www.maine.gov/dep/spills/holtrachem/penobriver/final/Executive%20Summary.pdf

PRMS. 2013b. Penobscot River Mercury Study. Chapter 14. Temporal and geographic trends in mercury in biota in the Penobscot estuary. http://www.maine.gov/dep/spills/holtrachem/penobriver/final/Chapter%2014.pdf

USEPA. 2002. Method 1631, Revision E: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry. Office of Water. EPA-821-R-02-019. http://water.epa.gov/scitech/methods/cwa/metals/mercury/upload/2007_07_10_methods_method_mercury_1631.pdf.