Comparison of two analytical methods for measurement of chlorinated pesticides and PCB congeners in biological tissue- Trends in Boston Harbor lobster tissue

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REPORT

for

Comparison of Two Analytical Methods for Measurement of Chlorinated Pesticides and PCB Congeners in Biological Tissue — Trends in Boston Harbor lobster tissue

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EXECUTIVE SUMMARY

The Massachusetts Water Resources Authority (MWRA) has implemented a long-term biomonitoring program for fish and shellfish with the goal of providing data that may be used to assess the potential environmental impact of the diversion of effluent discharge into Massachusetts Bay. These data will be used to ensure that discharge from the new outfall does not result in adverse impacts to fish and shellfish by comparing values with established thresholds (MWRA 1997a). The objective of the biomonitoring program is to establish a baseline characterization of the health of three indicator species: winter flounder, lobster and mussels in Boston Harbor, Massachusetts Bay, and Cape Cod Bay through measurement of biological indicators and chemical body burdens.

While there have been stable or decreasing trends in the concentrations of most contaminants in the indicator species, one trend has led to some questions. Since 1995, the concentrations of polychlorinated biphenyls (PCBs) and DDTs in the hepatopancreas of lobster collected at Deer Island Flats (DIF) and the Outfall Site (OS) have steadily increased. A similar increase was not observed in muscle tissue nor was it found for other chemicals of concern in hepatopancreas such as chlorinated pesticides, (dieldrin or chlordanes) or for polynuclear aromatic hydrocarbons (PAHs).

This report documents the use of alternate analytical methods to determine if analytical artifacts could account for any or all of the observed trends in hepatopancreas burdens of PCBs. Other factors that may be related to the trend were also explored. These included seasonal migration patterns and behavior, the effect of collection date, and related sediment quality issues.

The objectives of this study were as follows:

- Assess and discuss changes in analytical methods and the potential implications to the MWRA biomonitoring program;
- Analyze a subset of archived lobster and flounder tissue extracts by GC/MS to evaluate the quality of data and compare results to those obtained using traditional GC/ECD methods.
- Use findings from analytical method comparisons to determine if the apparent increasing trend of PCBs and DDTs in lobster hepatopancreas in Boston Harbor and other sites is “real” or caused by artifacts of the analytical method used.

Battelle’s experience with the National Oceanic and Atmospheric Administration (NOAA) National Status and Trends (NS&T) Program methods for determination of chlorinated compounds by Gas Chromatography/Electron Capture Detection (GC/ECD) has revealed that the concentration of many congeners and pesticides are frequently overestimated due to coelution with other non-target PCB congeners, pesticides or other interfering compounds such as phthalate esters. This potential overestimation was investigated using a modern Gas Chromatography/Mass Spectrometry (GC/MS) method. Selected lobster and flounder extracts from the 1999 MWRA Harbor and Outfall Monitoring (HOM) program collections analyzed by this method determined that coelutions did bias the concentrations of some pesticides and PCB congeners were from conventional GC/ECD analyses.

Based on results from the GC/MS analyses, a subset of PCB congeners was chosen to represent congeners that are not affected by coelution using GC/ECD. Total PCB data in lobster tissue from 1992 through 1999, recalculated using these selected congeners, displayed the same increasing concentration pattern starting in 1995. This suggests that the observed trend is not an analytical artifact and that there is a measurable increase in levels of PCBs in the hepatopancreas of lobsters from the harbor since 1995.

This increasing trend in PCB concentrations in lobster hepatopancreas was further investigated by grouping congeners by similar metabolic and lipophilic characteristics (Weisbrod *et al*. 2000). The ratios of the means of these congener groupings through time indicate that the distribution of PCB congeners in lobster hepatopancreas collected at Deer Island Flats appear to have changed since 1992. Recent years show increasing levels of the more recalcitrant, group I PCBs. These are also the most bioaccumulatable PCBs as reflected in their high K_{ow} values.

Since analytical factors are not causing the observed trend, other potential reasons were evaluated. These included collection times, migratory behavior and the evaluation of potential food sources and levels of PCBs in sediments inhabited by the food resources of the lobsters. The potential impact of changing food resources and corresponding sediment concentrations was investigated by comparative analysis, using principal component analysis (PCA), of the patterns of PCBs present in the sediment and lobster in the Harbor over time. The overall pattern of PCBs in the sediments showed that the sediments collected in the vicinity of the OS are enriched in the more recalcitrant, high molecular weight PCB congeners relative to harbor sediments, which are comparatively more enriched in the lower chlorinated, "fresher" PCB congeners. PCA analysis of lobster data also revealed distinct patterns of PCBs in lobster collected at DI and at the OS indicating different PCB sources to the separate populations. Comparison of lobster and sediment PCB results appears to indicate that the OS lobster patterns are more similar to sediments from the vicinity of the OS while the DIF lobster patterns are more similar to those found in sediments from the nearshore harbor. The DIF PCB patterns observed in lobster collected in the later years tended to be more highly enriched in the more recalcitrant, high molecular weight PCB congener. This corresponding temporal pattern was not observed in sediments from the nearshore harbor. However, sediment PCB concentrations in this area of Boston Harbor are quite variable, and, depending on the specific collection location, may be representative of both point and non-point sources of PCBs which can be characterized by somewhat different PCB assemblages. Migratory behavior of lobster may also play a role in explaining the observed trends in hepatopancreas concentrations as lobsters in recent years have been collected somewhat later in the season and may have resulted in collection of lobsters that had recently been feeding in the more highly contaminated nearshore harbor. While it is impossible to know exactly where the lobsters collected might have recently been feeding, it is likely that lobsters feeding on organisms living in sediments significantly elevated in PCBs, such as those found in the nearshore harbor, would result in elevated body burdens of these compounds relative to lobsters feeding on organisms in the relatively clean, offshore sediments.

Various studies conducted in Boston Harbor and elsewhere suggest that lobsters tend to bioaccumulate compounds such as PCBs and DDTs primarily from their food. Following cessation of sludge discharge and the upgrade of wastewater treatment facilities, lobsters in the Harbor may have switched from a diet rich in animals impacted by the old discharges to other prey items in the nearshore parts of the Harbor where sediments are contaminated with higher levels of PCBs and DDTs. The PCBs and DDTs are sequestered primarily in the hepatopancreas and probably are released rapidly back to the environment when the lobsters move farther offshore in the autumn and consume less contaminated foods. The increasing trend over time in PCB and DDT concentrations in the hepatopancreas of lobsters, particularly at the Deer Island Flats site, probably reflects a combination of a change in the diet of the lobsters as the harbor cleanup proceeds and the fact that in recent years, lobsters have been collected later in the year.

1.0 INTRODUCTION

The measurement of anthropogenic contaminants in biological tissues is a challenging undertaking. The low concentrations at which contaminants are found in biological media, and the potential analytical interferences caused by the presence of co-extracted biomolecules complicate or preclude their measurement using standard EPA methods of chemical analysis. Because of these complications, a specialized suite of analytical methods has evolved over the last decade to facilitate accurate measurement of man-made chemicals in biological tissues. These methods, pioneered by the National Oceanic and Atmospheric Administration (NOAA) National Status and Trends (NS&T) Program and the US EPA Environmental Monitoring and Assessment Program (EMAP), utilize specialized techniques of sample preparation, cleanup, and instrumental analysis in order to measure industrial chemicals in biological media at levels of environmental concern. These methods are described in more detail later in this report.

Chlorinated pesticides and polychlorinated biphenyl (PCB) congeners (the individual chemicals that taken together make up commercial Aroclor formulations) are among the most difficult organic contaminants to measure in biological media, primarily because these chemicals are found in tissues at very low, though environmentally significant, concentrations. Thus, the problems that generally plague analysis of organic chemicals in tissues are exacerbated for these very low concentration chemicals of concern. The NOAA NS&T Program methods have historically relied upon high-resolution gas chromatography with electron capture detection (GC/ECD) to measure these particular chemicals. The GC/ECD technique has remained the method of choice for these measurements for the last decade because the ECD detector was one of the few capable of detecting chlorinated pesticides and PCB congeners in tissue media. Nonetheless, this GC/ECD technique suffers from well-documented limitations.

In the last 3 to 5 years, the sensitivity of mass spectrometers, used as the detection systems in gas chromatography/mass spectrometry (GC/MS), has risen dramatically. As such, the sensitivity of GC/MS systems is beginning to approach that of traditional GC/ECD. Thus, analytical chemists have the opportunity of exploiting the powerful selectivity of GC/MS for the analysis of chlorinated pesticides and PCB congeners in environmental media, including biological tissues.

As a result of the diversion of its wastewater discharge from Boston Harbor to Massachusetts Bay, the Massachusetts Water Resources Authority (MWRA) has implemented a long-term Harbor and Outfall Monitoring (HOM) Program for Massachusetts and Cape Cod Bays. The objective of the HOM Program is to (1) test for compliance with NPDES permit requirements; (2) test whether the impact of the discharge on the environment is within the bounds projected by the SEIS; and (3) test whether change within the system exceeds the Contingency Plan thresholds. One aspect of the MWRA HOM program is a long-term biomonitoring program for fish and shellfish (MWRA, 1991). The goal of the biomonitoring is to provide data that may be used to assess the potential environmental impact of effluent discharge into Massachusetts Bay. These data will be used to ensure that discharge from the new outfall does not result in adverse impacts to fish and shellfish by comparing values with established thresholds (MWRA 1997a). The objective of the fish and shellfish monitoring is to establish a baseline characterization of the health of three indicator species: winter flounder, lobster and mussels in Boston Harbor, Massachusetts Bay, and Cape Cod Bay through measurement of biological indicators and chemical body burdens. To adequately measure selected chemical compounds in these complex matrices (both muscle and organ tissues), NOAA NS&T methods were employed.

Over the last decade monitoring data has indicated generally stable or decreasing trends in the body burdens of toxic contaminants and the prevalence of adverse biological effects in flounder, lobster, and mussels (Lefkovitz *et al.* 2001) However, one trend has lead to some questions. Since 1995, the concentrations of PCBs and DDTs in the hepatopancreas of lobster collected from Boston Harbor and western Massachusetts Bay have steadily increased. This was curious because this increase was not observed in muscle tissue, nor

was it measured for other chlorinated pesticides, such as dieldrin or chlordanes, or for PAHs. Experience with the NS&T methods for determination of chlorinated compounds by GC/ECD has revealed that the concentrations of many PCB congeners and pesticides are frequently overestimated due to coelution with other non-target PCB congeners, pesticides or other interfering compounds such as phthalate esters. The newer more sensitive GC/MS analysis provides a means to evaluate whether these coelutions are contributing to the observed trend.

This report documents the use of a GC/MS system for the analysis of chlorinated pesticides and PCB congeners in lobster and flounder tissue. The use of GC/MS as a tool for improving accuracy and comparability in the measurement of these anthropogenic chlorinated organic compounds as part of the HOM monitoring program is described. Results for selected lobster samples are examined to determine if analytical artifacts during GC/ECD analyses could account for any bias in the measurement of PCB and chlorinated pesticides in lobster and whether the cause of the trend can be better understood.

1.1 Background

It has been clearly documented that standard methods of analysis such as those described in EPA SW-846 (USEPA1986a) or Contract Laboratory Program (CLP) statement of work (USEPA 1986b) cannot obtain the detection limits needed to achieve the goals of many of today's environmental monitoring and assessment programs, simply because those standard methods were designed for measurement of contaminants at high level, hazardous waste sites or for use in discharge regulatory compliance monitoring. In addition, there are no standard EPA methods for trace level analysis of tissues. Such methods are critical for assessing environmental quality, and ecological and human health risk.

Methods developed over the last 10 years by NOAA and EPA (NOAA 1993, 1998) and adopted by various agencies (USEPA 1991, 1993, 1998) have allowed scientists and regulators to meet the data quality objectives set for the various local and national monitoring and testing programs. While the established NOAA methods laid excellent groundwork for analysis of trace organic analytes in environmental matrices, there still exist some basic problems with measuring trace level chlorinated organic analytes by Gas Chromatography/Electron Capture Detection (GC/ECD). For example, the NOAA method for analysis of PCB congener and chlorinated pesticides relies on gas chromatography to separate individual compounds to an extent that allows accurate identification of each compound using the ECD detector. Because of the complex nature of the chemical mixtures under analysis and the non-selective nature of the detector, some non-target compounds can co-elute with chemicals of concern during GC analysis. In other words, compounds having the same or similar retention time can result in misidentification of target chemicals. In conventional GC/ECD analysis, the identification of target compounds is based strictly on chromatographic retention times and coelution can cause problems with quantitation of target chemicals. Even when a second column with different retention characteristics is used to confirm the identity and amount of an analyte, misidentification and errors in quantitation can and do occur, especially with complex samples.

One alternative to GC/ECD analysis of chlorinated organic compounds is medium resolution (MR) quadropole GC/MS. The nature of the MS detector, which detects and quantifies compounds and their respective ion fractions by their molecular mass, allows for quantitation of two coeluting compounds, as long as their masses are different. The GC/MS detector operating in selected ion mode (SIM) allows the detector to dwell in the mass range of selected compound-specific ions for longer periods of time, which affords greater sensitivity. Previous MRGC/MS detectors were limited in sensitivity for chlorinated compounds. However, with the introduction of more sensitive GC/MS instruments in the mid to late 90's, the detection limits by GC/MS for PCB congeners and pesticides are now nearly comparable to GC/ECD for PCBs, and only slightly higher for the chlorinated pesticides. The improvements in GC/MS

instrumentation mean that the selective power of the MS can now be used to overcome some of the problems encountered with GC/ECD while achieving environmentally relevant detection limits.

Notably, other GC/MS methods exist for analyzing chlorinated hydrocarbons including low resolution GC/MS coupled with negative chemical ionization (NCI) and high resolution (HR) GC/MS following EPA Method 1668. However, few GC/MS NCI systems exist in environmental laboratories, and the cost of HRGC/MS is by and large prohibitive. The MR quadropole GC/MS used for this study is available in most common analytical laboratories and offers a technically feasible, cost competitive alternative to traditional GC/ECD.

1.2 Objectives

This study was conducted to assist MWRA in explaining the observed trend of increases in PCBs and DDTs in lobster hepatopancreas. To accomplish the study two analytical objectives were established.

- Discuss changes in analytical methods and implications of selected methods to the MWRA program;
- Analyze a subset of archived lobster and flounder tissue extracts by GC/MS to evaluate the quality of the data and compare results to those obtained using traditional GC/ECD methods.

Using findings of the GC/MS analysis, a case study to understand the trends in MWRA HOM lobster concentrations since 1991 was conducted. The study assessed whether:

- the apparent increasing trend of PCBs and DDTs in lobster hepatopancreas in Boston Harbor and other sites are accurate or caused by biases in the analytical method used.
- sediment data, similarly treated, could be used to help explain the trends.
- other factors, such as migratory behavior, could help explain the observed trends.

2.0 EVALUATION OF GC/MS RESULTS FOR A SUBSET OF MWRA HOM3 TISSUE EXTRACTS

2.1 Methods

Lobster and flounder, two of three indicator species collected to define baseline conditions of aquatic organisms, were collected from each of the three core sites in Boston Harbor and the Bays: Deer Island Flats (DIF), the Outfall Site (OS) and East Cape Cod Bay (ECCB) (Figure 3-1). In general fifteen lobsters and fifteen flounder have been collected from each of the three core sampling sites and randomly divided into three groups of five animals for chemical analysis. Within each of the three groups, edible meat (tail and claw for lobster and fillet for flounder) and liver tissue (hepatopancreas for lobster) from the five animals were pooled by tissue type. However, in 1992 and 1993 analysis was done on individual lobsters with three per site collected in 1992 and between 2 and 10 per site in 1993. In all years, chemical analyses were performed on the composite samples of lobster (hepatopancreas and edible meat) and flounder (fillet and liver) for PCB congeners and chlorinated pesticides by GC/ECD (NOAA 1993, 1998).

During HOM I and III, Battelle has utilized the NOAA NS&T GC/ECD methods for measuring chlorinated pesticides and PCB congeners in lobster tissue. During HOM II, MWRA's contractor followed the same NOAA NS&T protocols. While the two contract laboratories have been responsible for the lobster and flounder tissue chemistry of the Fish and Shellfish program, both have utilized the same methodologies and successfully participated in interlaboratory comparisons. Thus, the lobster and flounder tissue residue data from among these three monitoring periods should be comparable and presumably can be used with confidence. Although the NOAA NS&T methods do not require second column confirmation of analytes, to improve data quality, a qualitative confirmation was performed in all years, with the exception of 1993 and 1994, when only selected pesticides (dieldrin specifically) were confirmed. Methods used in the F&S program and used in the 1999 measurement can be found in Lefkovitz *et al*. (2000).

In the present comparability exercise, GC/MS techniques were used to reanalyze selected archived samples of flounder liver, lobster edible meat and lobster hepatopancreas that had been collected during the 1999 monitoring season and previously analyzed following the traditional NOAA NS&T GC/ECD analyses. The specific chlorinated pesticide and PCB congeners of concern in this comparability exercise are listed in Table 2-1. These chemicals represent the standard list of chemicals of concern for the MWRA HOM program.

2.1.1 GC/MS Analysis for PCBs and Pesticides

The analysis of the target PCB congener and pesticide compounds was performed by capillary gas chromatography with a mass selective detector (GC/MS) using a Hewlett-Packard 6890 gas chromatograph fitted with HP5973 mass selective detector. The GC/MS analysis was performed using a 60-m, 0.25-mm internal diameter, 0.25-Φm film thickness, DB-5MS fused silica capillary column (J&W Scientific, Inc.). The GC was optimized for pesticide analysis through silanization of the inlet and modification of the inlet temperature and the oven temperature program. 1-µL of the sample extract was injected into the instrument. Prior to sample analysis, the GC/MS was tuned with perfluorotributylamine (PFTBA) and a minimum of a 5-point initial calibration consisting of the target compounds was established to demonstrate the linear range of the analysis. A mid-level calibration check standard was analyzed at least every 10 samples to monitor instrument response relative to the initial calibration. The MS detector was operated in the selected ion monitoring mode (SIM).

In the SIM mode of GC/MS operation, ions of interest are monitored in time windows during the GC/MS run. These time windows are preset based on standard runs in order to collect data on each analyte only

while that analyte is eluting from the GC. The masses (2 or 3 for each analyte) are selected to unambiguously identify the analyte of interest. In a normal full scan GC/MS run, the instrument spends about one millisecond on each mass peak (dwell time). In the SIM mode, the instrument spends about 40 milliseconds on each peak. As a result, the signal to noise of the system is increased with a resulting lower detection limit. Because of this improvement in signal to noise, the system gain can also be increased. The result is typically an increase of two orders of magnitude in overall sensitivity as compared to operation in the full scan mode. Table 2-2 shows the masses monitored and the retention times for the pesticides and individual PCB congeners monitored during the GC/MS SIM analyses.

The GC was equipped with a split/splitless inlet (with electronic pressure control) operated in the splitless mode. The GC condition for the analysis was:

Initial temperature:	60 ^{o}C
Initial time:	1 minute
Ramp Rate:	10 ^{o}C/minute to 140 ^{o}C; 1 ^{o}C/minute to 220 ^{o}C; 5 ^{o}C/minute to 290 ^{o}C
Final temperature:	290 ^{o}C; 10 minutes

Quantification of individual components was performed by the method of internal standard quantitation using the average relative response factors (RRFs) from the initial calibration. Final data are reported versus the appropriate surrogate compound to best represent the original field sample concentrations. The same surrogate internal standard (SIS) and recovery internal standard (RIS) compounds are used in the GC/MS analysis as in the traditional NOAA NS&T GC/ECD analysis method.

2.2 Results of GC/MS Analyses for PCBs and Selected Chlorinated Pesticides

The results of the 1999 flounder liver, lobster edible meat, and lobster hepatopancreas analyses produced for the HOM investigation using GC/ECD were compared with data produced using the GC/MS method described above. This comparison is used to evaluate the data quality that can be achieved with GC/MS and to identify cases where it is advantageous to use GC/MS versus GC/ECD for the analysis of chlorinated compounds, especially in highly contaminated tissue samples. As a secondary task in this exercise, the tissue samples under consideration were also analyzed for a list of over 100 PCB congeners, in an effort to better elucidate the true assemblage of PCB congeners found in the tissues of the animals collected during HOM III. The data also were used to better estimate "Total PCB" burdens in these tissues, and to derive a correlation factor between "Total PCB" and the sum of the traditional list of 18 congeners measured in the conventional HOM monitoring program.

2.2.1 Analytical Challenges

To accurately compare results from the two different analytical methods, it is important to understand and identify the potential problems inherent to both. The main limitation of the GC/ECD method is the fact that the ECD does not produce a signal specific to individual compounds, meaning that all analytes will respond similarly (with varying magnitudes) in the detector. Determination of compound-specific concentrations relies on good separation of the individual compounds of interest during chromatography. Often, the influences of matrix and other analytes can have a profound effect on the chromatography of target analytes. In complex matrices such as tissue samples, and when high levels of contamination are present, co-elution can compromise the quantification and identification of a target analyte.

There are many sources of possible co-elution during sample analysis. Three of the more common sources for co-elution/interference with a target analyte are:

- other target analytes (*e.g.*, 2,4-DDE and Endosulfan I)
- other known non-target analytes (*e.g.*, 4,4-DDE and PCBs 85/136)
- matrix/other environmental influences (*e.g.*, PCB180, PCB170 and various phthalates; and all other compounds that respond to the ECD)

Other environmental contaminants such as hydrocarbons can greatly influence the performance of the chromatography, resulting in negative peaks and poor chromatography. Depending on the chromatographic conditions and the matrix being analyzed, coelutions and interferences can vary from sample to sample or from instrument to instrument.

Confirmation (second column) analysis is often used to minimize the misidentification and matrix interferences encountered during GC/ECD analysis. The confirmation column has different polarity than the primary column, thus separating the target analytes and interferent peaks in a different retention order. The confirmation column approach, however, has it shortcomings in that it also suffers from co-elution problems. A list of coelutions identified by Battelle on the primary and confirmatory columns is provided in Table 2-3.

Conversely, GC/MS SIM provides specific analyte responses in the form of individual ion patterns. This specificity reduces the possible sources of interference. Thus influences of matrix and other analytes can have a minimal effect on the identification of target analytes. Figures 2-1 and 2-2 provide a pictorial representation of how GC/MS can separate overlapping peaks where GC/ECD cannot. The quantitation of PCB77 (a tetrachlorobiphenyl) monitoring M/e=292 and PCB110 (a pentachlorobiphenyl) monitoring M/e=326 would be a serious problem by ECD but with the MS in theory there is no problem since the compounds are of significantly different masses. However, in actual environmental samples, this coelution on the GC/MS also has its limits, because although the parent ions of these two compounds have different masses, one of the ion fragments of PCB110 (a loss of one chlorine) has the same mass as the parent ion of PCB 77. In environmental samples, the relative amount of PCB77 present is generally 10-50 times lower than that of PCB 110. In this case, the presence of PCB110 ion fragment is enough to mask entirely, the presence of the PCB 77 parent compound. This case is relatively rare but the possibility increases as the complexity of the sample increases. In most cases the selection of alternate mass peaks will solve the problem (2 or 3 mass peaks are typically monitored for each compound in the SIM mode of GC/MS operation).

The principal drawback to GC/MS SIM for analysis of chlorinated organics in tissue is sensitivity. While detection limits for PCB congeners, using the system described above, have been demonstrated to be comparable to those obtained by GC/ECD, the detection limits for some of the chlorinated pesticides is still somewhat higher. Because the GC/MS is less affected by coelution of analytes due to its inherent specificity, the sample can be further concentrated to achieve better detection. Concentrating GC/ECD samples also improves detection, but the interferences are increased in equal proportion. Without interference the GC/ECD would always be the method of choice.

Table 2-4 lists some representative detection limits obtained from this study. GC/MS detection limits for pesticides are on the order of 5 to 10 times higher than those obtained by GC/ECD. This difference is due to the fact that individual pesticide compounds tend to fragment resulting in reduced intensity for any given mass that can be monitored. PCBs, on the other hand, do not fragment significantly and most of the intensity resides with the molecular ion. These detection limits can be improved by optimizing the

sample preparation method to achieve greater concentration of the sample. This was not done for this study because samples were prepared prior to initiation of this investigation.

2.2.2 Quality of the Data

To compare results from both methods, a number of quality control samples were analyzed on both instruments. QC samples included procedural blanks, laboratory control samples (LCS) and standard reference materials (SRM – 1974a NIST Mussel Tissue). A complete set of QC samples was analyzed with each analytical batch (one batch per matrix). No analytes were detected in the procedural blanks for either GC/MS or GC/ECD. GC/MS results for both LCS and SRM samples (Appendix A) for PCB congeners compared very well with GC/ECD results. Example recoveries for both SRM and LCS samples analyzed with the lobster meat samples are provided in Table 2-5.

In general, the PCB congeners and most of the chlorinated pesticides (especially the DDTs) are quite stable in solvent for years. Recoveries of these compounds in both LCS (solvent blanks) and SRMs, which were extracted along with the original samples and stored along with the sample extracts were quite good and compared well with GC/ECD results. However, there were some exceptions. LCS recoveries for endrin aldehyde, endosulfan sulfate, and methoxychlor by GC/MS were all well below acceptable DQO criteria. Each of these analytes is unstable and because the extracts were analyzed outside of the 40-day EPA holding time, they may have degraded during storage. These three compounds are not part of the standard MWRA HOM list of analytes and were not included in this study.

Pesticide recoveries of most compounds in the SRM were also good and compared well with GC/ECD results; however, 4,4'-DDT was not recovered in any of the SRMs analyzed by GC/MS. This is most likely a detection limit limitation since 4,4'-DDT is certified below 5 ug/kg, which is below the reporting limit for these samples.

2.2.3 Comparison of GC/MS and GC/ECD Data for Lobster and Flounder Tissues

Extracts from lobster hepatopancreas, lobster edible meat and flounder livers, representing three composites of five animals each, collected from Deer Island in 1999, were analyzed by both GC/ECD and GC/MS. A comparison of analytical results using the two methods can be found in Tables 2-6, 2-7 and 2-8, respectively. Results of GC/MS analyses, including results of QC samples, are provided in Appendix A and results of corresponding GC/ECD analyses are provided in Appendix B.

Overall, results from GC/MS and GC/ECD were comparable. A number of notable exceptions were observed and are discussed below. These differences were generally overestimations of certain compounds in the GC/ECD analyses due to coelutions described previously. The largest discrepancies between the two data sets can be seen most clearly in the lobster hepatopancreas data from Deer Island (Figures 2-3 and 2-4). These tissues had the highest levels of both PCBs and pesticides relative to other tissue types and collection locations (Table 2-6). Certain low level PCB congeners and pesticides (*e.g.*, PCB8/5, PCB195 and trans-Nonachlor) analyzed by ECD were elevated relative to those analyzed by GC/MS, most likely due to matrix interference associated with a contaminated sample (Figures 2-3 and 2-4).

The high contamination level in Deer Island hepatopancreas samples appears to have caused elevations in the measured concentration of other analytes because of the presence of other non-target analytes. PCB87, a common congener in Aroclor mixtures, closely elutes with dieldrin in the GC/ECD analysis. Comparison of dieldrin values between the GC/ECD and GC/MS show elevated dieldrin on the GC/ECD, most likely a result of this coelution. Similarly the 4,4'-DDD value for the GC/ECD is elevated relative to the GC/MS value due to coelution with PCB114.

The last source of coelution related bias is evidenced by the elevated GC/ECD levels of PCB180 in the Deer Island hepatopancreas samples. A phthalate ester often coelutes with PCB180. The difference in the fragmentation pattern of the two compounds allows the GC/MS to differentiate between the phthalate from PCB180, whereas the GC/ECD data is compromised by this coelution.

The aggregate effects of these coelution biases on the GC/ECD data for hepatopancreas samples result in a high bias in measured concentrations of both total PCB and total DDTs. The mean total PCB value for Deer Island hepatopancreas, calculated as the sum of the NOAA NS&T congeners, was elevated by about 13% (using GC/ECD data as compared to the sum of the GC/MS results). Total DDT, calculated as the sum of the 2,4 and 4,4-related compounds, was also elevated by approximately 25% relative to the GC/MS results.

It is important to note that as the concentrations of contaminants in the tissues decrease, so do the interferences and the bias in the measurement of target compounds. For example, the hepatopancreas results for the Outfall and Cape Cod sites have similar results for trans-Nonachlor for both the GC/ECD and GC/MS (Table 2-6). The phthalate contamination so obvious in the Deer Island Hepatopancreas samples is not present in the samples from the other two sites; hence, the values derived by GC/MS and GC/ECD are similar. Results of the lobster edible tissue from Deer Island (Table 2-7) that have relatively low levels of PCBs and pesticides show good comparability between GC/ECD and GC/MS analyses.

Some of the same co-elution/interference problems seen in the lobster hepatopancreas data can be seen in the flounder liver data (Table 2-8). As illustrated in Figures 2-5 and 2-6, similar coelution issues are evident. These include elevated GC/ECD results for PCB 180, trans-Nonaclor and 4,4'-DDE. One difference in the Flounder GC/MS results compared to the lobster hepatopancreas results is the elevated GC/MS values for PCBs138 and PCB153 relative to the GC/ECD results. It appears that there was co-elution of PCB 132 and PCB153 on the GC/MS. Unfortunately, both of these congeners are hexachlorobiphenyls. The fragmentation patterns of these two congeners are identical and they could not be distinguished as separate compounds on the GC/MS. Since they appear to have been separated on the GC column during GC/ECD analysis and on the GC/MS for both lobster hepatopancreas and edible tissue analyses, it is likely that adjustments to the conditions of the GC/MS system would allow future separation of these compounds. A similar scenario applies to PCBs 138, 160 and 163, all hexachlorobiphenyls. This observation underscores the need for optimal gas chromatography in the analyses of chlorinated pesticides and PCB congeners—regardless if GC/ECD or GC/MS is used as the analytical method of choice. Few other coelutions of PCB congeners of similar chlorination level occur.

2.3 Using GC/MS to Measure an Extended List of PCB Congeners

The second task in this project was an investigation into the computation of "Total PCB". In the scientific literature, and indeed in the HOM Program, Total PCB is an operational term. In practical terms, various investigators or monitoring programs have used the sum of different numbers of congeners to derive Total PCB. This results both in incompatibility in Total PCB values among monitoring programs, but almost always an underestimation of the true amount of total PCB present in samples. As part of the NOAA National Status and Trends program, the relationship between the standard NOAA list of 18 congeners and a "True" PCB value was investigated (NOAA 1989) in mussels. Comparison of total PCB, calculated by GC/MS analysis of homologue groups, determined that the Total PCB could be estimated by multiplying the NS&T sum by approximately a factor of two.

In this investigation, we measured 107 individual PCB congeners to better quantify Total PCB in HOM samples. The 107 congeners measured represent more than 95% of total PCB in any Aroclor formulation; thus the sum of these 107 congeners is an excellent approximation of total PCB, and affords the

opportunity to derive a highly accurate HOM-specific correction factor between the sum of the 18 congeners measured in the HOM program and true total PCB.

The total PCB computed for three composites of the lobster hepatopancreas from Deer Island were, on average, 1.45 times greater using the sum of the 107 congeners compared to the sum of the NS&T 18 congener list. The Outfall composites of the lobster hepatopancreas were on average 1.37 times greater using the sum of the 107 congeners compared to the sum of the NS&T 18 congener list. A similar factor (1.38) is found with the Cape Cod composites of the lobster hepatopancreas. Even the edible tissue composites showed this trend, with a 1.47 factor between the 107-congener versus the 18-congener summation method. This same comparison using the NS&T 18 congeners quantified by GC/ECD, rather than by GC/MS, results in a slightly lower factor. Total PCBs were 1.18 times higher using the 107 GC/MS derived PCB total for both for lobster meat and lobster hepatopancreas. This difference is most likely due to the fact that the "Total PCB" determined from summing the 18 NS&T congeners quantified using GC/ECD results in an overestimation of some of the individual congeners, as discussed earlier in this report.

No congener summation method (short of one that measures all 209 theoretical PCB isomers) will be 100% accurate. The 107-congener method is an excellent estimation; however, using this method on a regular basis would be costly. Based on the data from this study, a more reasonable determination of "total PCB" could be estimated by multiplying the 18 NOAA congeners (determined by GC/MS) by a factor of 1.4.

Table 2-1. Target PCB congener and pesticide analytes.

MWRA HOM PCB Congener[a] and Pesticide Set			
PCB	**PCB**	**Pesticide**	**DDT**
8/5	138/160/163	Hexachlorobenzene	2,4'-DDD
18	153	gamma-BHC	4,4'-DDD
28	170/190	Heptachlor	2,4'-DDE
44	180	Endrin	4,4'-DDE
52	187/182	Aldrin	2,4'-DDT
66	195	Heptachlor Epoxide	4,4'-DDT
101/90	206	alpha-Chlordane	4,4'-DDMU
105	209	trans-Nonachlor	
118	126[b]	Dieldrin	
128	77[b]	Mirex	

[a] All congeners numbers are listed using the IUPAC nomenclature.
[b] These congeners were not included in this study due to their absence in the GC/MS calibration solutions.
[C] Coeluting congeners are listed in order of abundance in Aroclors 1242/1248/1254 (most abundant listed first). The most abundant single congener was used to calibrate the instrument for the co-eluting congener sets.

Table 2-2. GC/MS SIM analyte retention times and monitored masses.

Analyte	Retention Time	Masses to Monitor GC/MS
a-BHC	39.61	181,183,109
Cl2(08)	39.73	222,224
Hexachlorobenzene	40.86	284,142,249
b-BHC	43.89	181,183,109
g-BHC	44.78	183,181,109
Cl3(18)	46.64	258,260
d-BHC	48.78	183,181,109
Cl3(28)	54.69	258,260
Heptachlor	57.07	100,272,274
Cl4(52)	61.00	292,290
Cl4(49)	61.77	292,290
Aldrin	63.15	263,261,274
Cl4(44)	64.67	292,290
Heptachlor epoxide	70.65	353,355,351
Cl4(66)	72.67	292,290
g-Chlordane	75.10	373,375,272
4,4-DDMU	75.98	212,214,176
2,4 DDE	76.74	246,318,316
Endosulfan I	77.21	195,339,341
Cl5(101)	77.26	326,328
cis Chlordane	78.02	373,375,237
trans Nonachlor	79.06	409,407,237
Dieldrin	82.14	79,263,279
Cl5(87)	82.14	326,328
4,4 DDE	82.90	246,248,176
Cl4(77)	83.94	292,290
Cl5(110)	84.00	326,328
2,4 DDD	84.27	237,235,165
Endrin	86.01	263,82,81
Endosulfan II	87.84	337,339,341
Cl5(118)	89.00	326,328
4,4 DDD	90.71	235,237,165
2,4 DDT	91.16	235,237,165
Endrin aldehyde	91.51	67,345,250
Cl7(184)	93.28	394,396
Cl6(153)	93.39	360,362
Cl5(105)	93.82	326,328
Endosulfan sulfate	96.04	272,387,422
4,4 DDT	97.74	235,237,165
Cl6(138)	98.42	360,362
Cl6(129)	99.64	360,362

Table 2–2. GC/MS SIM analyte retention times and monitored masses. (continued)

Analyte	Retention Time	Masses to Monitor GC/MS
Cl5(126)	99.79	326,328
Cl7(187)	101.05	394,396
Cl7(183)	101.68	394,396
Cl6(128)	102.16	360,362
Endrin ketone	102.76	317,67,319
Methoxychlor	105.28	227,228,152
Cl7(180)	106.24	394,396
Mirex	107.82	272,237,274
Cl6(169)	107.83	360,362
Cl7(170)	108.39	394,396
Cl8(195)	111.14	428,430
Cl9(206)	114.36	462,464
Cl10(209)	116.21	498,500

Table 2-3. Potential co-eluting compounds on both primary and confirmatory GC/ECD columns.

Possible Primary Column (DB-5) Coelutions:		
a-BHC/PCB8	Heptachlor Epoxide/Oxychlordane	2,4-DDE/Endosulfan I
Dieldrin/PCB87	PCB77/PCB110	Oxydiazinon/Endrin
Cis-nonachlor/4,4-DDD	2,4-DDT/Endrin Aldehyde	Ethion/PCB184/PCB153/PCB 132
PCB126/PCB129	4,4-DDD/PCB114	4,4-DDE/PCB85/PCB136
Possible Confirmation Column (DB-1701) Coelutions:		
PCB34/PCB29	PCB39/PCB52	Oxychlordane/Dacthal/d-BHC
PCB8/PCB5	PCB12/PCB37	2,4-DDE/4,4-DDMU
PCB110/Dieldrin	PCB118/2,4/DDD/PCB184	2,4-DDT/Oxidiazinon
Endosulfan II/4,4-DDD/PC B138	PCB129/PCB187/PCB166	PCB128/Endrin Aldehyde

Table 2-4. Representative detection limits for PCBs and chlorinated pesticides by LRMS-SIM, GC/ECD and HRMS.

Analysis	Sample Weight (wet)	Low Cal. Standard	Final Extract Volume	PCB congener Reporting Limit (µg/kg dry wt based on low std.)	Pesticide Reporting Limit (µg/kg dry wt based on low std.)
MRMS-SIM					
Tissue	20 g	2 ng/mL	250 µL	0.5	5.0
ECD					
Tissue (NOAA 1998)	20 g	10 ng/mL	1,000 µL	0.5	1.0
HRMS					
Tissue (Method 1668)	20 g	1 ng/mL	100 µL	0.005	0.005

Table 2-5. Example SRM and blank dpike (LCS) recoveries for GC/MS and GC/ECD.

Standard Reference Material					
PCB/Pesticides	**Concentration (ng/g, dry weight)**		**Percent Difference from Certified Range (%)**		**NIST SRM 1974a Range of Certified Concentrations (a)**
	GCMS	**GC/ECD**	**GC/MS**	**GC/ECD**	**(Low - High)**
PCB44	79.2	74.3	0.0	0.0	65.3 - 80.1
PCB52	112.6	106.8	0.0	0.0	104 - 126
PCB66	104.6	101.7	0.0	0.0	97 - 105.8
PCB101/90	140.4	132.7	1.7	0.0	118.6 - 138
PCB105	53.5	56.0	0.0	0.0	49.6 - 56.4
PCB118	135.9	130.0	1.1	0.0	127.2 - 134.4
PCB128	20.9	22.7	0.0	0.0	18.6 - 25.4
PCB138/160/163	140.3	129.9	0.0	0.0	124 - 143
PCB153	133.4	161.8	3.1	5.9	137.6 - 152.8
PCB170/190	4.4	4.4	0.2	0.0	4.4 - 6.6
PCB180	12.6	22.0	5.4	5.2	13.3 - 20.9
PCB187/182	31.1	33.2	1.9	0.0	31.7 - 36.3
cis Chlordane	20.5	20.0	2.7	0.0	14.4 - 20.0
trans Nonachlor	19.5	20.7	0.0	0.0	14.4 - 21.6
4,4 DDD	29.3	49.8	20.3	1.1	45.7 - 56.7
4,4 DDE	50.3	52.9	0.0	0.0	36.7 - 49.3
4,4 DDT	ND	3.4	100.0	0.0	3.32 - 4.50

Laboratory Control Sample					
	Percent Recoveries (%)			**Percent Recoveries (%)**	
PCBs	**GC/MS**	**GC/ECD**	**Pesticides**	**GC/MS**	**GC/ECD**
PCB8/5	107	93	Hexachlorobenzene	115	110
PCB18	120	102	gamma-BHC	87	105
PCB28	125	107	Heptachlor	91	97
PCB44	120	108	Aldrin	103	104
PCB52	117	106	Heptachlor Epoxide	106	95
PCB66	117	108	4,4'-DDMU	115	103
PCB101/90	125	108	2,4'-DDE	119	111
PCB105	118	104	alpha-Chlordane	108	108
PCB118	113	101	trans-Nonachlor	101	109
PCB128	120	102	Dieldrin	106	109
PCB138/160/163	115	100	4,4'-DDE	93	105
PCB153	116	103	2,4'-DDD	95	100
PCB170/190	110	101	Endrin	84	93
PCB180	110	101	4,4'-DDD	78	101
PCB187/182	115	98	2,4'-DDT	73	117
PCB195	117	98	4,4'-DDT	67	95
PCB206	114	96	Mirex	90	92
PCB209	117	95			

[a]National Institute of Standards and Technology (NIST) 1974a - Mussel tissue; tissues certified using multiple analytical methods. Values listed represent a certified "range" rather than a "mean".

Table 2-6. GC/MS and GC/ECD mean lobster hepatopancreas concentrations from Deer Island, Outfall Site and East Cape Cod Bay, 1999.

Location	Deer Island Flats					Outfall Site					East Cape Cod Bay				
Sample Matrix:	Tissue		Tissue			Tissue		Tissue			Tissue		Tissue		
Detector:	GC/MS		GC/ECD			GC/MS		GC/ECD			GC/MS		GC/ECD		
Reporting Unit:	ng/g dry wt. mean	s.e.	ng/g dry wt. mean	s.e.	%RPD	ng/g dry wt. mean	s.e.	ng/g dry wt. mean	s.e.	%RPD	ng/g dry wt. mean	s.e.	ng/g dry wt. mean	s.e.	%RPD
PCB8/5	1.6	*0.2*	10.1	*0.8*	145	[a]		0.9		NA	[a]		[a]		NA
PCB18*	4.3	*0.3*	6.4	*0.3*	38	[a]		[a]		NA	[a]		[a]		NA
PCB28*	129.2	*20.1*	111.5	*15.3*	15	56.3	*10.3*	48.7	*7.9*	14	26.1	*2.7*	22.6	*2.2*	14
PCB44*	6.1	*1.1*	8.8	*1.0*	36	[a]		1.6	*0.4*	NA	[a]		0.4	*0.2*	NA
PCB52*	36.6	*0.9*	34.5	*0.8*	6	9.0	*0.5*	10.3	*0.7*	13	5.1	*1.3*	5.6	*1.1*	9
PCB66*	345.8	*40.4*	363.4	*38.8*	5	189.5	*26.2*	200.1	*32.2*	5	76.4	*10.5*	80.7	*10.0*	5
PCB101/90*	198.9	*10.7*	184.5	*1.5*	8	72.6	*4.4*	68.8	*4.9*	5	36.1	*3.5*	36.3	*3.4*	1
PCB105	573.9	*40.9*	624.4	*5.2*	8	354.3	*25.0*	355.0	*37.0*	0	139.8	*14.9*	135.8	*13.5*	3
PCB118*	1705.8	*118.9*	1898.0	*23.0*	11	1101.3	*68.7*	1031.8	*47.7*	7	499.0	*44.8*	474.1	*46.7*	5
PCB128*	307.5	*17.1*	316.1	*11.2*	3	232.1	*21.1*	227.3	*24.8*	2	121.4	*6.8*	109.9	*5.3*	10
PCB138/160/163	2141.8	*140.6*	2240.2	*52.6*	4	1499.1	*102.5*	1252.7	*108.0*	18	771.0	*45.7*	721.9	*50.2*	7
PCB153	2037.8	*105.4*	2299.5	*79.5*	12	1783.0	*178.5*	1564.7	*148.3*	13	919.2	*55.6*	872.0	*61.8*	5
PCB170/190	213.5	*6.7*	287.7	*2.8*	30	168.5	*21.0*	214.9	*31.5*	24	78.3	*6.5*	94.5	*7.4*	19
PCB180	632.8	*17.2*	1140.7	*47.1*	57	524.3	*62.5*	697.1	*98.1*	28	204.3	*26.4*	226.5	*27.1*	10
PCB187/182*	537.7	*25.8*	618.7	*13.3*	14	493.3	*25.9*	541.6	*22.2*	9	296.1	*9.9*	281.8	*16.4*	5
PCB195	20.8	*1.1*	33.6	*1.6*	47	26.0	*4.1*	44.2	*6.6*	52	13.5	*0.3*	24.1	*1.2*	57
PCB206	22.7	*1.2*	25.3	*2.1*	11	48.0	*5.2*	44.9	*8.6*	7	26.4	*1.3*	21.8	*0.7*	19
PCB209	6.0	*0.3*	6.8	*0.4*	13	18.6	*2.2*	16.5	*2.3*	12	13.9	*1.1*	11.0	*0.8*	24
Total PCB NS&T (b)	8922.8	*535.7*	10210.1	*127.4*	13	6576.0	*455.6*	6321.1	*450.1*	4	3226.5	*221.6*	3119.1	*238.8*	3
Total PCB-selected (c)	3271.9	*228.4*	3541.8	*69.3*	8	2154.1	*97.4*	2130.3	*105.5*	1	1060.2	*72.5*	1011.5	*79.2*	5
Total PCB – 107 (d)	12917.5	*807.0*	N/A	-	-	9001.4	*554.8*	N/A	-	-	4441.4		N/A	-	

Table 2–6. GC/MS and GC/ECD mean lobster hepatopancreas concentrations from Deer Island, Outfall Site and East Cape Cod Bay, 1999. (continued)

Location	Deer Island Flats					Outfall Site					East Cape Cod Bay				
Sample Matrix:	Tissue		Tissue			Tissue		Tissue			Tissue		Tissue		
Detector:	GC/MS		GC/ECD			GC/MS		GC/ECD			GC/MS		GC/ECD		
Reporting Unit:	ng/g dry wt. mean	s.e.	ng/g dry wt. mean	s.e.	%RPD	ng/g dry wt. mean	s.e.	ng/g dry wt. mean	s.e.	%RPD	ng/g dry wt. mean	s.e.	ng/g dry wt. mean	s.e.	%RPD
Hexachlorobenzene	5.5	*0.1*	7.0	*0.3*	23	8.3	*0.5*	8.8	*0.7*	6.0	6.9	*0.4*	7.0	*0.4*	1
gamma-BHC	[a]		[a]		NA	[a]		1.8	*0.9*	NA	[a]		2.5	*0.0*	NA
Heptachlor	[a]		[a]		NA	[a]		[a]		NA	[a]		[a]		NA
Endrin	[a]		[a]		NA	[a]		[a]		NA	[a]		[a]		NA
Aldrin	[a]		[a]		NA	[a]		[a]		NA	[a]		[a]		NA
Heptachlor Epoxide	[a]		4.5	*0.3*	NA	[a]		5.0	*0.2*	NA	[a]		4.0	*0.2*	NA
alpha-Chlordane	21.5	*2.4*	23.9	*2.1*	11	3.5		15.5	*0.4*	126	[a]		9.9	*0.8*	NA
trans-Nonachlor	79.2	*10.0*	109.6	*14.1*	32	31.9	*4.1*	37.5	*6.7*	16	19.7	*1.3*	17.9	*2.3*	9
Dieldrin	36.2	*4.5*	59.6	*3.7*	49	33.2	*3.3*	51.7	*6.3*	43	24.9	*1.3*	28.1	*1.9*	12
Mirex	15.9	*4.2*	11.4	*3.6*	33	13.5	*2.0*	9.8	*0.5*	32	13.5	*1.0*	6.9	*0.4*	64
2,4'-DDD	[a]		[a]		NA	[a]		[a]		NA	[a]		[a]		NA
4,4'-DDD	55.5	*7.7*	108.9	*7.5*	65	21.7	*1.6*	29.5	*15.2*	30	18.8	*2.1*	22.8	*2.5*	19
2,4'-DDE	[a]		[a]		NA	[a]		[a]		NA	[a]		[a]		NA
4,4'-DDE	898.4	*98.5*	1177.5	*24.0*	27	641.6	*34.8*	707.3	*60.1*	10	504.0	*19.4*	531.4	*30.4*	5
2,4'-DDT	[a]		[a]		NA	[a]		[a]		NA	[a]		[a]		NA
4,4'-DDT	17.9	*4.2*	11.4	*1.1*	44	12.6	*1.3*	9.1	*0.3*	33	3.4	*3.4*	4.8	*0.7*	36
4,4'-DDMU	46.3	*7.5*	43.4	*2.8*	7	16.0	*8.1*	12.4	*2.7*	25	26.4	*1.9*	17.5	*2.3*	41

[a] indicates not detected at or above detection limit
[b] all NS&T congeners
[c] selected congeners (indicated by "*")
[d] 107 congeners analyzed by GC/MS
s.e. = standard error
% RPD = relative percent difference
N/A not applicable

Table 2-7. GC/MS and GC/ECD mean lobster edible meat concentrations from Deer Island, 1999.

Detector:	GC/MS		GC/ECD		
Reporting Unit:	ng/g dry weight mean	*s.e.*	ng/g dry weight mean	*s.e.*	%RPD
PCB8/5	[a]		[a]		NA
PCB18*	[a]		[a]		NA
PCB28*	3.15	0.37	3.25	0.42	3
PCB44*	0.08	0.08	0.04	0.04	66
PCB52*	0.88	0.05	0.69	0.35	25
PCB66*	7.84	0.74	8.32	0.93	6
PCB101/90*	3.82	0.23	5.29	0.38	32
PCB105	11.54	0.77	11.23	1.04	3
PCB118*	35.80	2.62	32.00	2.53	11
PCB128*	6.17	0.38	5.95	0.59	4
PCB138/160/163	34.87	2.60	29.88	2.47	15
PCB153	30.99	1.95	28.10	2.00	10
PCB170/190	3.60	0.16	4.89	0.36	30
PCB180	9.48	0.59	14.04	1.69	39
PCB187/182*	9.58	0.43	9.01	0.50	6
PCB195	0.11	0.11	0.77	0.08	150
PCB206	[a]		0.55	0.08	NA
PCB209	0.20	0.01	0.22	0.13	8
Total PCB NS&T (b)	158.11	10.46	154.22	12.97	2
Total PCB-selected (c)	67.32	4.84	64.55	5.45	4
Total PCB – 107 (d)	231.54		N/A		
Hexachlorobenzene	0.28	0.05	0.47	0.02	51
gamma-BHC	[a]		[a]		NA
Heptachlor	[a]		[a]		NA
Endrin	[a]		[a]		NA
Aldrin	[a]		[a]		NA
Heptachlor Epoxide	[a]		0.83	0.08	NA
alpha-Chlordane	2.06	1.04	1.52	0.08	30
trans-Nonachlor	3.62	0.11	3.13	0.15	15
Dieldrin	5.16	0.24	6.79	0.06	27
Mirex	0.83	0.83	[a]		NA
2,4'-DDD	[a]		[a]		NA
4,4'-DDD	2.76	0.19	[a]		NA
2,4'-DDE	[a]		[a]		NA
4,4'-DDE	12.60	0.51	15.98	1.06	24
2,4'-DDT	[a]		[a]		NA
4,4'-DDT	[a]		[a]		NA
4,4'-DDMU	[a]		0.87	0.03	NA

[a] indicates not detected at or above detection limit
[b] all NS&T congeners
[c] selected congeners (indicated by "*")
[d] 107 congeners analyzed by GC/MS
s.e. = standard error

Table 2-8. GC/MS and GC/ECD mean flounder liver concentrations from Deer Island Flats, 1999.

Detector:	GC/MS		GC/ECD		
Reporting Unit:	ng/g dry weight mean	*s.e.*	ng/g dry weight mean	*s.e.*	%RPD
PCB8/5	[a]		[a]		NA
PCB18*	4.9	*2.5*	6.9	*0.7*	34
PCB28*	38.1	*1.9*	40.1	*1.5*	5
PCB44*	16.6	*1.5*	17.2	*2.2*	4
PCB52*	56.4	*3.6*	55.9	*3.8*	1
PCB66*	105.9	*1.9*	122.4	*1.0*	14
PCB101/90*	257.5	*10.6*	117.1	*22.5*	75
PCB105	181.7	*9.0*	227.5	*4.2*	22
PCB118*	546.9	*17.9*	467.5	*11.8*	16
PCB128*	91.8	*4.4*	86.5	*2.7*	6
PCB138/160/163	1001.9	*37.6*	778.8	*14.4*	25
PCB153	1049.1	*46.7*	392.1	*53.7*	91
PCB170/190	143.5	*13.9*	181.5	*9.1*	23
PCB180	404.9	*29.8*	1627.6	*126.8*	120
PCB187/182*	264.0	*8.6*	233.4	*6.8*	12
PCB195	16.2	*8.3*	34.3	*3.3*	72
PCB206	28.3	*4.1*	30.2	*3.8*	6
PCB209	7.8	*1.0*	8.7	*1.4*	11
Total PCB NS&T (b)	4215.6	*190.7*	4388.7	*152.1*	4
Total PCB-selected (c)	1382.1	*45.7*	1108.0	*64.3*	22
Total PCB – 107 (d)	7181.7	*308.3*	N/A		
Hexachlorobenzene	6.1	*0.2*	6.5	*0.3*	7
gamma-BHC	[a]		[a]		NA
Heptachlor	[a]		[a]		NA
Endrin	[a]		[a]		NA
Aldrin	[a]		[a]		NA
Heptachlor Epoxide	[a]		9.0	*0.2*	NA
alpha-Chlordane	66.4	*2.1*	77.8	*6.0*	16
trans-Nonachlor	92.5	*3.1*	139.1	*5.9*	40
Dieldrin	[a]	*7.2*	38.9	*14.8*	NA
Mirex	11.7	*0.3*	[a]		NA
2,4'-DDD	12.6	*0.7*	[a]		NA
4,4'-DDD	40.5	*2.0*	47.2	*7.4*	15
2,4'-DDE	[a]		[a]		NA
4,4'-DDE	272.4	*11.3*	393.4	*21.2*	36
2,4'-DDT	[a]		[a]		NA
4,4'-DDT	26.9	*1.2*	43.9	*2.6*	48
4,4'-DDMU	20.3	*0.6*	[a]		NA

[a] indicates not detected at or above detection limit
[b] all NS&T congeners
[c] selected congeners (indicated by "*")
[d] 107 congeners by GC/MS

Table 2-9. 107 PCB Congener List.

PCB Congener Set [a]				
1	42/37	89	136	183
3	43	91	137	185
4/10	**44**	92	**138/160/163**	**187/182**
6	45	95	141/179	189
7/9	46	97	146	191
8/5	47/75	99	149/123	193
12/13	48	100	151	194
16/32	49	**101/90**	**153**	**195/208**
17/15	51	**105**	156	197
18	**52**	107/147	158	198
19	53	110/77	167	199
21	56/60	114	169	200
22	59	**118**	**170/190**	201/157
24/27	63	119	171/202	203/196
25	**66**	124	172	205
26	70/76	**128**	173	**206**
28	74	129/126	174	207
29	82	130	175	**209**
31	83	131	176	
33/20	84	132	177	
40	85	134	178	
41/64/71	87/115/81	135/144	**180**	

[a] All congeners numbers are listed using the IUPAC nomenclature (**not** BZ numbers where different).

Bold PCB congeners indicate the 18 NOAA congeners

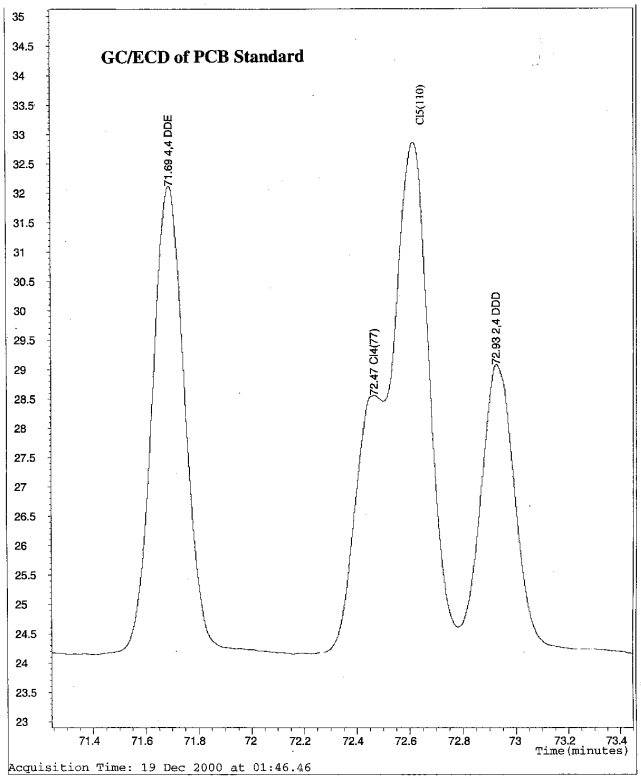

Figure 2-1. GC/ECD Chromatogram of PCB Standard.

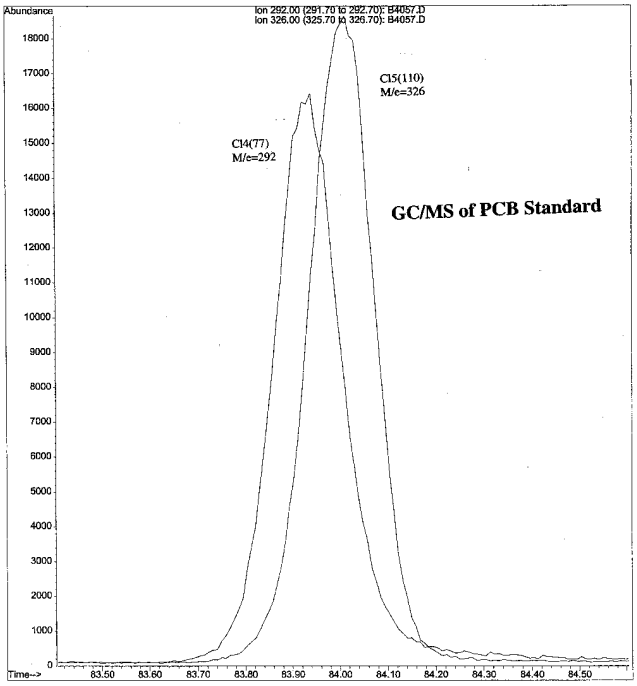

Figure 2-2. GC/MS Chromatogram of PCB Standard.

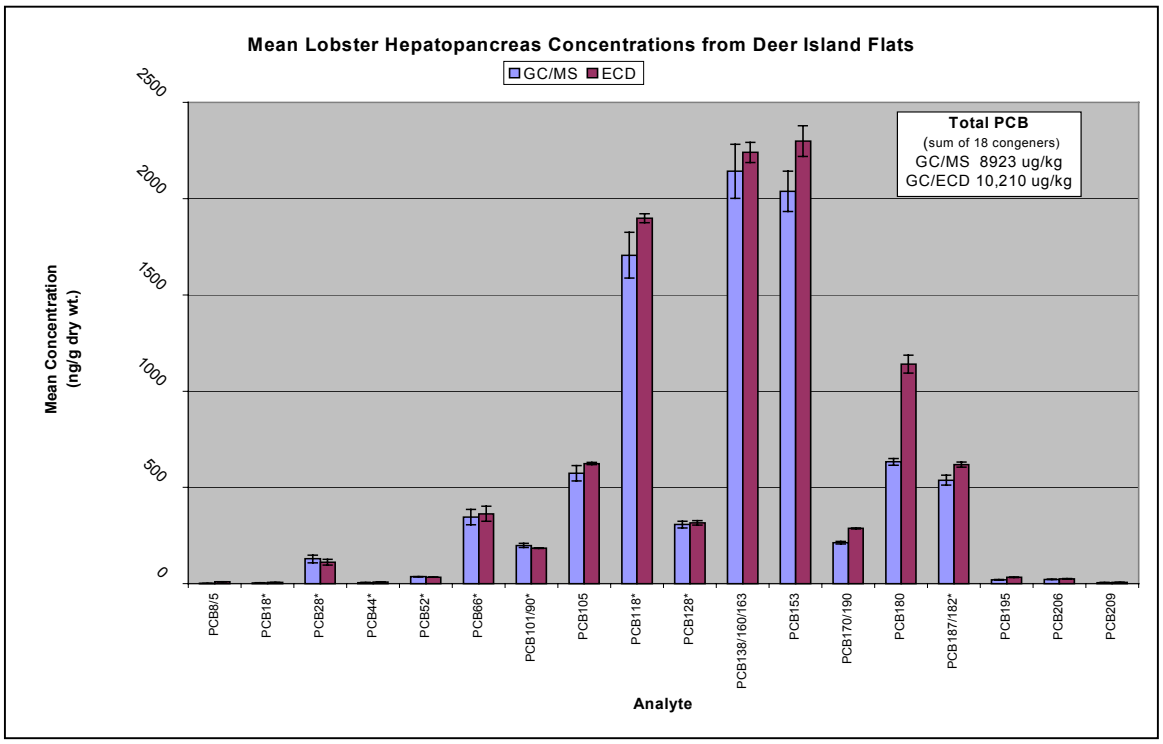

Figure 2-3. Comparison of mean lobster hepatopancreas concentrations of individual PCB congeners by GC/MS and GC/ECD from Deer Island Flats. (error bars represent one standard error).

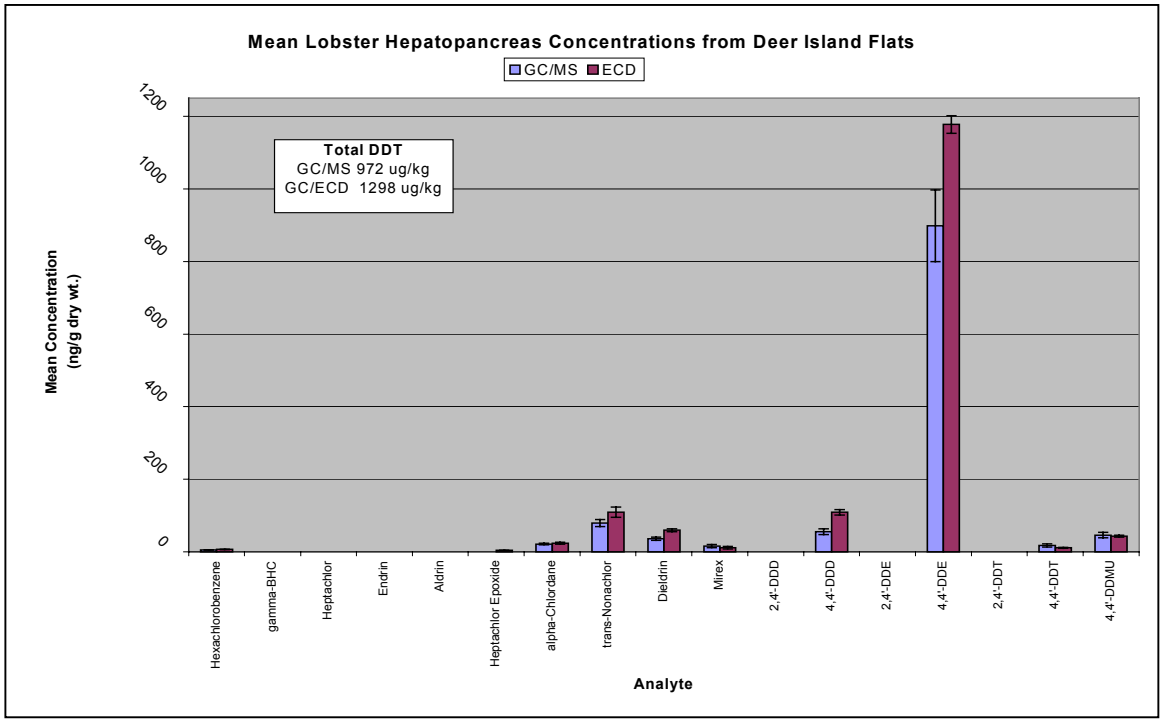

Figure 2-4. Comparison of mean lobster hepatopancreas concentrations of selected chlorinated pesticides by GC/MS and GC/ECD from Deer Island Flats. (error bars represent one standard error).

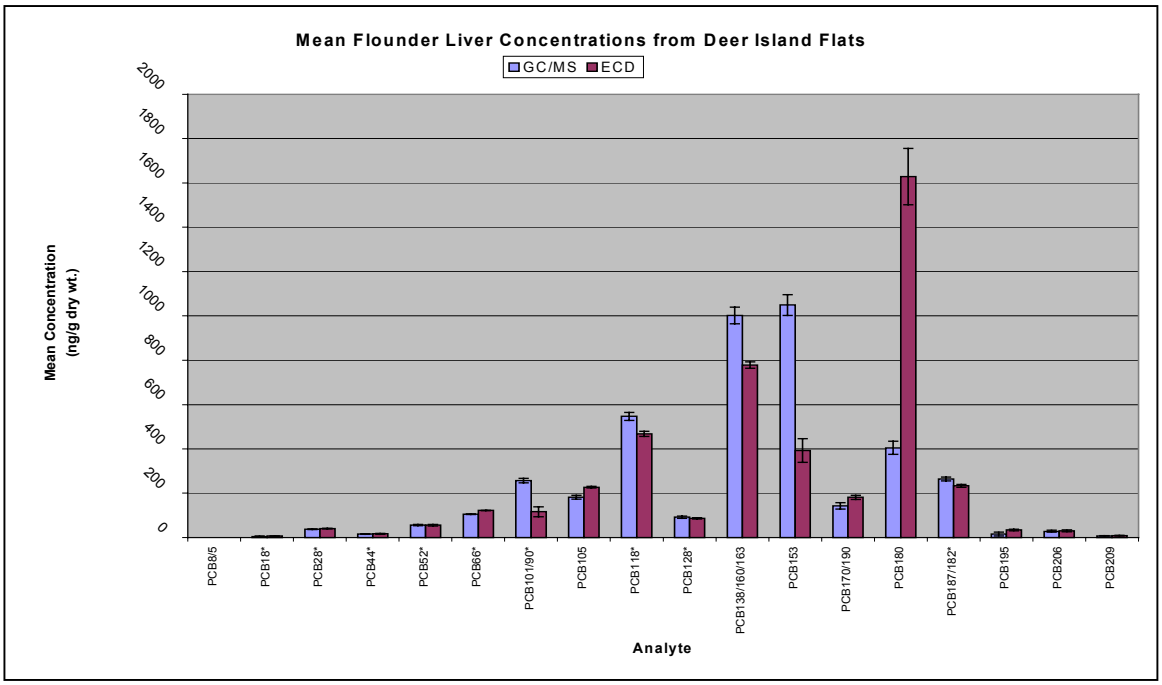

Figure 2-5. Comparison of mean flounder liver concentrations of individual PCB congeners by GC/MS and GC/ECD from Deer Island Flats. (error bars represent one standard error).

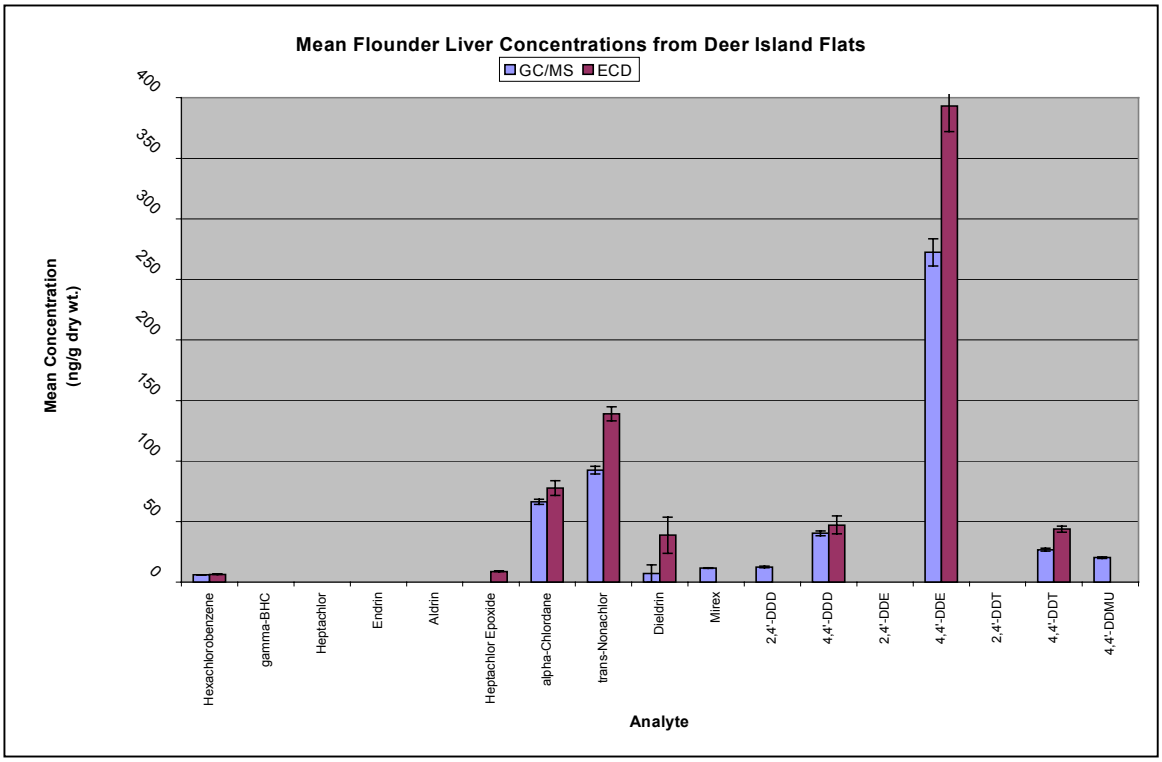

Figure 2-6. Comparison of mean flounder liver concentrations of selected chlorinated pesticides by GC/MS and GC/ECD from Deer Island Flats. (error bars represent one standard error).

3.0 CASE STUDY–CHLORINATED ORGANICS IN LOBSTER; MWRA HOM 1992–1999

3.1 Background

The Massachusetts Water Resources Authority (MWRA) performs a biomonitoring program for fish and shellfish that supports long-term evaluation of the MWRA effluent discharged into Massachusetts Bay. The goal of the biomonitoring program is to obtain baseline data that may be used to assess the potential environmental impact of the effluent discharge on Massachusetts Bay.

Despite decreases in effluent loadings of anthropogenic contaminants such as chlorinated organics as a result of cessation of sludge discharge, updated primary treatment, and secondary treatment (Butler *et al*. 1997, Sung *et al*. 1998, personal communication M. Hall; MWRA), PCB and DDT concentrations in lobster hepatopancreas tissue (Figure 3-1) have increased since 1992 (the beginning of the MWRA Harbor and Outfall Monitoring program) (Lefkovitz *et al*. 2001). This increase in PCB and DDT concentrations has only been observed in lobster hepatopancreas tissue, not in lobster meat, nor in either of the other two species monitored. Although the trend was observed at all three core monitoring locations (Deer Island, Outfall Site and Cape Cod Bay), it was most pronounced at the DI site. In addition, no corresponding increases were observed for any other chemicals including several other chlorinated pesticides and polynuclear aromatic hydrocarbons (PAHs), measured in the same tissue samples. The work described in Section 2 of this report focused on developing data for assessing if analytical bias could cause the observed trends in measured PCB and DDT concentrations in lobster hepatopancreas between 1992 and present. Those results will be discussed in this context in this section of the report. Further, other factors that could potentially affect the uptake of these chlorinated compounds were also explored, including the migratory habits of the lobsters, changes in collection times, and changes in feeding locations of lobsters and corresponding sediment quality.

3.2 Updating of Trends

Comparison of concentrations of certain organic constituents, specifically PCBs and DDTs, in hepatopancreas tissue at a monitoring station over time suggests a systematic increase in concentrations beginning in 1995 (Figures 3-2 and 3-3). In contrast, concentrations of total PCB and total DDT in lobster edible meat appear to have remained fairly constant at all locations since 1992 (Figures 3-4 and 3-5). Interestingly, total PAHs in hepatopancreas tissue appear to have decreased during the baseline period (Figure 3-6). Spatially, the mean concentrations of organic compounds (*i.e*., total PCB, total DDT) in lobster hepatopancreas across the study area since 1992 showed the same pattern for both edible and hepatopancreas tissue. The highest concentrations are generally found in samples from Deer Island and the lowest at East Cape Cod Bay.

As discussed in Section 2, one important question regarding the trends in PCB and DDT concentrations in hepatopancreas is whether these trends are biased in any way by analytical artifacts. This section further evaluates the PCB concentration trends using a combination of information gained from GC/MS analyses, evaluation of selected non-interfering congeners, and an exploration of the distribution of the congeners in samples over time and space.

3.2.1 Trend Analysis of PCBs Using Selected PCB Congeners

In an effort to determine if potential interferences from GC/ECD analyses might be responsible for the increasing trend in PCB and DDT concentrations in lobster hepatopancreas, a subset of congeners that were free from obvious coelutions or interferences were chosen for trend analysis. These congeners were selected based on GC/MS SIM analyses as described in Section 2. Nine congeners were chosen (PCB18, PCB28, PCB44, PCB52, PCB66, PCB118, PCB101, PCB128, PCB187) because they were confirmed to have no significant interferences in the GC/ECD (based on the corresponding measurements using

GC/MS). This set of congeners also covers the range of chlorination of PCBs and make up, on the average, 26% of the total PCB (based on the totals calculated using all 107 congeners quantified by GC/MS). Figure 3-7 shows the total PCB in lobster hepatopancreas based on the sum of this subset of nine congeners at each of the three locations from 1992 through 1999.

This subset of congeners shows the same pattern of increasing concentrations in total PCB starting in 1995 is observed. This suggests that analytical artifacts do not bias the observed trend noted earlier in the full PCB data set, and that indeed there may be a measurable increase in levels of PCBs and DDTs in the hepatopancreas of lobsters from the harbor.

3.2.2 Comparison of Congener Patterns Over Time

To further investigate the apparent increasing trend in total PCB concentrations in lobster hepatopancreas over time, the patterns of individual congeners in hepatopancreas samples were compared at each of the three locations over time.

Figure 3-8, shows the ratio of PCB 101 (a pentachloro-PCB) to three different congeners at Deer Island, the Outfall Site and East Cape Cod Bay in lobster hepatopancreas samples analyzed by GC/ECD. Based on the GC/MS data from this study, these congeners appear to be free from chromatographic interferences. At all stations, the ratio to all three congeners appears to be decreasing since1992. This would indicate that the relative proportion of the lighter weight (lower chlorinated) PCB congeners accumulated in the hepatopancreas tissue of the lobsters is decreasing over time.

Yet another perspective of the distribution of PCB over time can be gleaned by grouping the congeners by similar metabolic and lipophilic characteristics. This approach was used by Weisbrod *et al*. (2000) to explore the bioaccumulation patterns of PCBs in northwest Atlantic Pilot whales. Table 3-1 shows the four groupings of PCB congeners and their corresponding octanol-water partition coefficients (K_{ow}). As shown in Figure 3-9, the ratios of the various groups appear to have changed since 1992 (results of PCB grouping also provided in Appendix C). Recent trends appear to show increasing levels of the more recalcitrant, group I PCBs. Not surprisingly, these are also the most bioaccumulatable PCBs as reflected in their high K_{ow} values.

Both these observations suggest a similar change in the distribution of PCB congeners in lobster hepatopancreas over time. This shift toward a predominance of higher chlorinated, more recalcitrant PCB congeners may be a result of a number of factors that could have an affect on their bioaccumulation.

3.3 Other Factors Potentially Influencing the Observed Temporal PCB and DDT Trends in Lobster Hepatopancreas

3.3.1 Lobster Migration/Behavior

One factor that could affect the PCB signature is the source of the chemical. Exposure sources can be influenced by migratory patterns. Changes in sources, such as offshore sediments versus nearshore sediments, affect bioaccumulation of organic chemicals and could have an effect on the compositions of the PCB assemblage in lobster hepatopancreas over time. To that end, the relationship between collection date and migration trends of lobster in Boston Harbor was examined. Most of what is known about lobster migration in Massachusetts's coastal waters has been described by Estrella and Morrissey (1997). They report that offshore lobsters tend to move inshore in the spring. The migrations continue inshore in the warmer months until water temperatures begin to decline in the fall and the trend is reversed. The most extensive inshore migrations are by ripe ovigerous females, followed by females with immature external eggs, legal-sized males, and sublegal males. Sublegal and legal-sized nonovigerous females move the least amount. It has been suggested that these migrations may be related to optimizing the temperature regime to which eggs are exposed.

Inshore populations of lobsters, on the other hand, are relatively non-migratory (Krouse, 1980; Ennis, 1984, also see Estrella and Morrissey, 1997). Lobsters in nearshore waters of the Gulf of Maine were reported to make seasonal migrations averaging only 4.6 km (Cooper, 1970). During the early summer, most lobsters move into shallow coastal waters above the seasonal thermocline (about 9 m) with some animals moving into waters less than 5 m deep. In the autumn, they move offshore in response to decreasing water temperature and increasing water turbulence caused by storms.

As a result of their tagging studies Estrella and Morrissey (1997) found that many lobsters collected and tagged in offshore waters east of Cape Cod were recaptured in the nearshore waters of eastern Cape Cod Bay where they mixed with the endemic nearshore population. While there is substantial movement of lobsters in and out of Boston Harbor the population is thought to be mainly an inshore endemic population which seasonally moves out to the cooler, deeper waters of western Massachusetts Bay (pers. comm. B Estella). A few of Extrella and Morrissey's lobsters tagged east of Cape Cod were recaptured in the vicinity of the MWRA's Massachusetts Bay monitoring location. Lobster activity at this location has proven to be more specific to the spring and late summer/fall than at the other two monitoring sites. This and the previously mentioned information suggest that lobsters collected at this location may be a mixture of an offshore population collected during their seasonal migrations, as well as of an endemic nearshore population making their shorter movements into and out of deeper waters.

3.3.2 Changes in Lobster Collection Dates

Figure 3-10 shows the relative collection months at each station since 1992. While in the early years of the program lobsters were collected as early as April, more recently the majority of lobsters have been collected between mid-July and mid-September. No overall apparent trend in lobster length or weight measurements vs. collection date was evident nor was there any evidence of trends found at a given station. There is no indication from the sex ratios of the collected lobsters that there is a preponderance of females that might have come from offshore in any of the collections.

It is evident form Figure 3-11 that lobsters collected in the spring consistently had among the lowest concentrations of PCB's in their hepatopancreas. As will be discussed in more detail Section 3.3.4 the hepatopancreas tends to be an integrator of recent contaminant exposure. Low levels of PCBs found in the spring suggest that lobsters from all three sites had recently been exposed to the lower levels of PCBs expected in offshore waters and sediments. At OS and to a lesser extent at the Cape Cod Bay site, there appears to be a general tendency for PCBs to increase as the collection date moves later into the summer/fall (Figure 3-11). This is what might be expected as later collections would mean that the lobsters had spent more time in nearshore, generally more contaminated waters and sediments. The apparent steeper slope of the OS lobsters as compared to the Cape Cod lobsters suggests that OS lobsters had been exposed to higher levels of PCBs than Cape Cod lobsters. Lobsters from Boston Harbor show much more scatter than from the other two sites suggesting that in addition to collection date there may be other important factors impacting their uptake of PCBs.

3.3.3 Related Sediment Quality

Studies of sediment burdens of PCBs and DDTs in and around Boston Harbor since 1990 suggest that concentrations have not changed significantly over this time at a given location. Figures 3-12 and 3-13 show concentrations of PCBs and total DDTs, respectively, in sediments collected from Boston's Inner Harbor Dorchester Bay in 1994 and 1998 (Figure 3-15), adjacent to Deer Island in 1994 and 1998, and mean concentrations in sediments collected from near- and far-field locations relative to the new outfall location (Lefkovitz *et al.* 1999, Kropp *et al*. 2000). The relative concentrations of PCBs in sediments around the Harbor are quite different with substantially higher concentrations found in the nearshore sites.

The potential relationship between sediment quality and contaminant burdens in lobsters was investigated using principal component analyses (PCA). PCA is a chemometric technique for visualizing intersample

and intervariable relationships. It achieves this by reducing the "n" dimensionality of the data (where n = number of variables or samples, whichever is smaller) by finding linear combinations of the variables in the data set which account for the maximum amounts of variance. These linear combinations are the principle components. The 1st principle component (PC) accounts for the maximum amount of variance and each successive PC accounts for less of the remaining variance.

PCA yields a distribution of samples (*e.g.*, sediment samples) in n-dimensional space, where n is the number of variables (*e.g.*, PCB Group). The 1st PC is a line through this space upon which each sample point can be projected. The line's orientation is such that the variance of these projections is maximized. The 2nd PC is another line defining the next highest variance. These first two lines (*i.e.*, the 1st and 2nd PC) define a plane. These planes are called 'factor score plots' which are one 'end product' of PCA. The Euclidean distances between sample points on these factor score plots are representative of the variance captured in each PC. In simpler terms, samples which cluster together are chemically similar and outliers are chemically distinct. A factor loading is calculated for each variable (*e.g.*, PCB analyte group) contributing to each PC. A cross plot of the factor loadings for the first few PC's reveals the individual chemicals responsible for the variance in each PC. These factor loading plots are another 'end product' of PCA.

Figure 3-14 shows the factor score plot resulting from the analysis of a subset of the biologically significant PCB congeners listed in Table 3-1 found in lobster hepatopancreas samples from DI and OS from 1992 through 1999 and in sediments from both Boston Harbor and at selected "nearfield" (NF) locations adjacent to the OS (see figures 3-1 and 3-15 for sediment collection locations). The plot is separated into four quadrants (A, B, C, D) with the biologically significant PCB grouping (per Table 3-1) responsible for the variance noted for that area of the plot. Note that results from both lobster and sediment from the ECCB site were excluded from these analyses. The reason was that the relative concentrations of PCBs in both sediment and lobster from this site were low (compared to the Harbor area) and the differences evaluated using PCA were exaggerated and thereby tended to minimize the authentic differences among the Harbor area samples. In addition, PCB180 was also excluded from calculations of PCB grouping totals due to potential interferences often associated with this congener.

There is a distinct clustering of the lobster and sediment data in different quadrants. The distribution of PCBs in the sediments shows that the NF sediments (adjacent to OS) are clearly enriched in the more recalcitrant, Group I congeners and mostly cluster in Quadrant A, while the nearshore, inner harbor sediments are enriched in the group III and IV congeners and are focused in Quadrants B and D. The overall pattern of the higher molecular weight, more recalcitrant PCBs being predominant in the offshore sediments is not surprising since the sources of PCBs (which are presumably less weathered and relatively higher in the low molecular weight, lower chlorinated congeners) are found closer to shore and the PCBs found in offshore sediments are more likely to be enriched in the more highly weathered PCB assemblages.

PCA also revealed differences in the PCB distributions among OS and DIF lobster hepatopancreas samples (Figure 3-14). The lobster data, as opposed to the sediments, clearly clustered within quadrant C representing enrichment in the Group II, and to a lesser degree, Group III PCB congeners. These congeners represent some of the more highly bioaccumulatable congeners. Further inspection of the distribution of the lobster results shows that they cluster into two distinct patterns with the OS lobsters showing a slight enrichment in the Group I and II congeners compared with the DIF lobsters which tend more towards the Group II and III congeners. While there is some overlap the tendency for OS and DIF to cluster separately suggests exposure to different sources of PCBs. Interestingly in recent years, the PCB patterns observed in the lobsters from both sites have shifted to the left toward more recalcitrant Group I and II congeners.

Comparison of lobster and sediment PCB results appears to indicate that the OS lobsters are clustering closer to the sediments found near the OS site while the DIF lobsters are found clustering closer to the harbor sediments. Similar to DIF and OS lobster data there appears to be a tendency for nearfield sediment data to shift to the left toward Group I PCB over time (Figure 3-14).

The corresponding temporal pattern observed in DIF lobster of increasing relative concentrations of the Group I and II congeners was not observed in sediments from the Harbor. However, sediment PCB concentrations in this area of Boston Harbor are quite variable, and depending on the specific collection location, may be representative of either point or non-point sources of PCBs which can be characterized by somewhat different PCB assemblages. While it is impossible to know exactly where the lobsters collected might have recently been feeding, it is likely that lobsters feeding on organisms living in sediments significantly elevated in PCBs, such as those found in the nearshore harbor, would result in elevated body burdens of these compounds relative to lobsters feeding on organisms in the less contaminated sediments found offshore and around Deer Island. Supporting this conjecture are reliable reports of unusually high lobstering activity in and around the Inner Harbor in the weeks prior to the DIF collection in 1999 (personal communication M. Hall, MWRA). This was the year with the highest measured hepatopancreas concentrations of PCBs (Figure 3-2).

The observations made through PCA may be explained by changes in the PCB congener composition in water, sediments, and biota of Boston Harbor during the 1990s due to changes in the sources of PCBs to the Harbor and weathering processes affecting the residual PCBs remaining as the Harbor cleanup proceeded. The compositions of PCB congener mixtures that occur in the environment differ substantially from those of the original, technical Aroclor mixtures released to the environment. This is because the composition of PCB mixtures changes over time after release into the environment due to several processes collectively referred to as "environmental weathering." This weathering is a result of the combined effects of processes such as differential volatilization, solubility, sorption, anaerobic dechlorination, and metabolism, and results in changes in the composition of the PCB mixture over time and among trophic levels (Brown and Wagner, 1990; Bergen *et al*. 1998; Froese *et al*. 1998). Less chlorinated PCBs often are lost most rapidly due to volatilization and metabolism while more highly chlorinated PCBs often are more resistant to degradation and volatilization and adsorb more strongly to particulate matter. More highly chlorinated PCBs tend to bioaccumulate to higher concentrations than low molecular weight PCBs in tissues of animals, and may biomagnify in food webs. In addition, the temporal observation at DIF of relatively lower levels of the lower chlorinated congeners in later years could be a reflection of the reduced inputs of the lower chlorinated congeners to the harbor from the DI treatment plant as a result of sludge removal and completion of updated primary treatment and the initiation of secondary treatment in 1997-1998.

3.3.4 Bioaccumulation of PCBs by Lobsters

The trend of increasing concentrations of PCBs in lobster hepatopancreas with time since 1992 is greater for high molecular weight than for low molecular weight, more soluble, biodegradable PCBs. The most likely possibility is that the lobsters are bioaccumulating PCBs and DDTs from different sources, progressively farther inshore, each year. Another alternative is that the environmental sources of the PCBs in lobster tissues are undergoing microbial or abiotic degradation, or that lower molecular weight PCBs are being lost from the sediments. However, based on PCA (figure 3-14) there is no evidence to suggest a change in PCB composition in nearshore harbor sediments over time. It is possible that, as the cleanup of the Harbor has progressed, contributions of PCBs and DDTs as well as food for lobster prey have decreased and lobsters have moved farther into the harbor in search of food. The Inner Harbor sediments may contain more highly weathered PCB assemblages than the assemblages formerly discharged in treated wastewater.

Connolly (1991) modeled the food chain transfer and bioaccumulation of PCBs in lobsters and winter flounder from New Bedford Harbor, MA. The model successfully predicted the composition and concentrations of PCB congeners in lobster and flounder tissues. The model indicated that PCB concentrations in flounder and to a lesser extent lobster were derived from sediments where the animals lived. Dietary uptake (food chain transfer) exceeded uptake across the gills (bioconcentration) for the tri-, tetra-, penta-, and hexa-chlorobiphenyls, and was most pronounced for the higher molecular weight PCBs. Ingestion of contaminated food accounted for 55 to 80 percent of the PCBs in lobster tissues. The percentage from food increased with PCB congener molecular weight. However, the sediment accounted for only 35 percent of the trichlorobiphenyl and 65 percent of the hexachlorobiphenyl in the lobster tissues, indicating that the lobster diet includes both benthic and non-benthic prey. These results, if extrapolated to nearby Boston Harbor, suggest that Boston Harbor lobsters are accumulating high molecular weight PCBs from their food. Because PCB concentrations are higher in sediments in the nearshore relative to sediments in the vicinity of DIF or OS (Kropp 2001), it is likely that the food organisms in this area are more highly contaminated as well.

Between 1952 and 1991, increasing volumes of sludge were discharged into the channel immediately south of Deer Island (Figure 3-15). The discharge of sewage sludge caused severe organic enrichment of sediments near Deer Island. The sludge also introduced large amounts of chemical contaminants to the sediments. Organic enrichment of the sediments resulted in sediment hypoxia, a reduction of the diversity of benthic fauna, and massive stimulation of growth of a few opportunistic species, as has happened in many other sewage-affected coastal environments (Pearson and Rosenberg, 1978). Apparently, winter flounder found in the vicinity of the discharge fed actively on the opportunistic capitellid worms that fed directly on the sludge derived organic matter (Moore *et al*. 1996; Farrington, 1997). In this process, the flounder became heavily contaminated with metal and organic contaminants from the sewage sludge and suffered a wide variety of biochemical and histopathological lesions (Carr *et al*. 1991; Murchelano and Wolke, 1991; Moore *et al*. 1996).

Throughout the 1980's and early 1990's lobster landings from the Boston Harbor area ranked first among all Massachusetts regions (Estrella 1996). The majority of the catch from this region came from inshore of Deer Island with very heavy lobstering actively occurring in the immediate vicinity of the sludge discharge (pers.comm. B. Estrella). As was the case for winter flounder, it was likely that many lobsters collected from Boston Harbor in the early 1990's were utilizing prey items which had been directly feeding on or near the sludge and as a result, were exposed to the PCBs and other contaminants emanating from the discharges. The PCB assemblage in sludge and primary effluent would be expected to be dominated by the more soluble, low and middle weight PCB congeners. Thus, in the early 1990's many lobsters in Boston Harbor were most likely feeding on prey contaminated with lower molecular weight PCBs. With the cessation of the sludge discharge lobster activity appears to be more concentrated in the shallower, nearshore sediment with substantially higher levels of all PCB cogeners (Figure 3-12) including the more recalcitrant higher chlorinated PCBs

McLeese *et al*. (1980) measured the accumulation of two PCB congeners from food by lobsters. The lobsters were fed mussels, *Mytilus edulis*, containing nominal concentrations of 1.7 and 17 μg/g each of 2,2',4,5'-tetrachlorobiphenyl (TCBP) and 2,2',4,4',5,5'-hexachlorobiphenyl (HCBP). These PCB congeners have log octanol/water partition coefficients (log K_{ow}) of 5.85 and 6.92, respectively (Hawker and Connell, 1988), indicating a strong tendency to bioaccumulate and adsorb to the organic fraction of sediments. At low doses in the food (about 2.4 μg/feeding), dietary absorption efficiency (E_o) was 52% for TCBP and nearly 100% for DPCP. Values for E_o decreased with increasing dose in the food. Maximum concentration factors (concentration in tissues/concentration in food) also decreased with increasing dose in the food and were 5.1 and 7.3 for TCBP and HCBP, respectively, in the hepatopancreas of the lobsters after six weeks of feeding. Both compounds were released rapidly from the hepatopancreas when feeding on contaminated food was stopped. The TCBP was released more rapidly than the HCBP. The estimated half times for release of TCBP and HCBP from lobster

hepatopancreas after feeding with contaminated food was terminated were 4 and 17 weeks, respectively. Thus, concentrations of PCB, DDT and other nonpolar organic contaminants in the hepatopancreas integrate the recent exposure of lobsters to these contaminants in water, sediment, and especially food.

Several investigations have shown that PCBs, DDTs, and other highly nonpolar organic contaminants are bioaccumulated primarily in the hepatopancreas of lobsters during exposure to the contaminants in water, sediments, and food (Guarino *et al*. 1974; Clement *et al*. 1987; Rappe *et al*. 1991; King *et al*. 1993). More than 90% of the DDT administered in food to lobsters accumulated in the hepatopancreas. Only about 2.1% of the administered dose accumulated in muscle tissue (Guarino *et al*. 1974). These findings are similar to the relative amounts of chlorinated organics found in the meat versus the hepatopancreas of lobster from the present study of Boston Harbor. The preferential bioaccumulation of PCBs and DDTs in the hepatopancreas rather than other tissues of lobsters can be attributed to the much higher lipid concentration in the hepatopancreas than in other tissues, and to the role of the crustacean hepatopancreas in digestion of ingested food and storage of nutrients. The low concentration of PCBs and DDTs in muscle tissues of lobsters from the three study sites may indicate exposure to the contaminants in sediment, food, and water was of short duration.

Mean total lipid concentrations in muscle tissues of lobsters from the vicinity of Deer Island have varied annually from 1.9 percent in 1999 and 2000, to 19 percent in 1992 (Table 3-2). The high level in 1992, may be an analytical error, or it may indicate a lipid-rich diet (such as prey from areas of sewage sludge deposition). Concentrations of lipids in muscles of lobsters from the future outfall site and the Cape Cod Bay reference site also were high in 1992, suggesting that the data for that year may be anomalous. However, the overall temporal trend toward lower muscle lipid concentrations suggests the possibility of a shift in the lobster diet away from one derived in part from sewage organic matter toward one based more typical marine food items.

Lipid concentrations in hepatopancreas were always higher than those in lobster muscle tissue. Mean lipid concentrations in the hepatopancreas of lobsters from Deer Island ranged from 31 percent in 1999 to a high of 70.5 percent in 1994. There was no clear temporal trend in hepatopancreas lipid concentrations and lipid normalization did not smooth out the variability in PCB and DDT concentrations in lobster hepatopancreas and muscle. There was not a good correlation between lipid concentrations and concentrations of PCBs and DDTs in either hepatopancreas or muscle, though the hepatopancreas (high lipid) always contained higher contaminant concentrations than the muscle. These observations suggest that year-to-year variations in concentrations of PCBs and DDTs in muscle and hepatopancreas of lobsters from Boston Harbor reflect changes in exposure to bioavailable forms of these contaminants and are not a result of changes in lipid content and possibly corresponding health or condition of the lobsters.

The results of these studies suggest that Boston Harbor lobsters and lobsters from the Outfall site and eastern Cape Cod Bay are bioaccumulating PCBs and DDTs primarily from their food. The PCBs and DDTs are sequestered primarily in the hepatopancreas and probably are released rapidly back to the environment when the lobsters move farther offshore in the autumn and consume less contaminated foods. The increasing trend over time in PCB and DDT concentrations in the hepatopancreas of lobsters, particularly at the Deer Island Flats site, probably reflects a combination of a change in the source of food items of the lobster as the harbor cleanup proceeds and the fact that in recent years lobsters have been collected later in the year.

Table 3-1. PCB congeners grouped by lipophilic and metabolic characteristics.

		Group (a)	Log K_{ow} (b)
PCB Group I; no vicinal H, 2-4 ortho Cl; not metabolized			
2,2',3,3',4,4',5,5',6-nonachlorobiphenyl	CL9(206)	I	8.09
2,2',3,4',5,5',6-heptachlorobiphenyl	CL7(187)	I	7.17
2,2',3,4,4',5,5'-heptachlorobiphenyl	CL7(180)	I	7.36
2,2',4,4',5,5'-hexachlorobiphenyl	CL6(153)	I	6.92
decachlorobiphenyl	CL10(209)	I	8.18
PCB Group II: ortho-, meta-vicinal H, 2-3 ortho Cl, not metabolized			
2,2',3,3',4,4',5,6-octachlorobiphenyl	CL8(195)	II	7.56
2,2',3,3',4,4',5-heptachlorobiphenyl	CL7(170)	II	7.27
2,2',3,3',4,4'-hexachlorobiphenyl	CL6(128)	II	6.74
2,2',3,4,4',5-hexachlorobiphenyl	CL6(138)	II	6.83
PCB Group III; ortho-, meta-vicinal H, 0-1 Cl, metabolized			
2,3',4,4',5-pentachlorobiphenyl	CL5(118)	III	6.74
2,3',4,4'-tetrachlorobiphenyl	CL4(66)	III	6.2
2,3,3',4,4'-pentachlorobiphenyl	CL5(105)	III	6.65
2,4,4'-trichlorobiphenyl	CL3(28)	III	5.67
3,3',4,4',5-pentachlorobiphenyl	CL5(126)	III	6.89
3,3',4,4'-tetrachlorobiphenyl	CL4(77)	III	6.36
PCB Group IV; metal, -para-vicinal H, 2 ortho Cl, metabolized			
2,2',3,5'-tetrachlorobiphenyl	CL4(44)	IV	5.75
2,2',4,5,5'-pentachlorobiphenyl	CL5(101)	IV	6.38
2,2',5,5'-tetrachlorobiphenyl	CL4(52)	IV	5.84
2,2',5-trichlorobiphenyl	CL3(18)	IV	5.24

(a) Grouped based on position of vicinal hydrogens, number of ortho-chlorine substituents and known or proposed metabolism by cytochrome P450 (Weisenbrod *et al*. 2000).

(b) Known octanol-water partition coefficients (K_{ow}) from each analyte (Weisenbrod *et al*. 2000, Hawker and Connell, 1988).

Table 3-2. Lipids in Lobster Meat and Hepatopancreas Samples from DI OS and ECCB 1992-2000.

Lobster Meat	Deer Island Flats								
Lipids (% dry weight)	1992	1993	1994	1995	1996	1997	1998	1999	2000
Rep 1	16.2	3.2	10.9	4.4	3.8	4	4	2.2	1.9
Rep 2	19.6	1.6	9.7	5.5	3.4	3.1	3	1.6	1.6
Rep 3	21.8	2.7	6.2	4.9	4.2	3.1	6	1.9	2.1
AVG	19.2	2.5	8.9	4.9	3.8	3.4	4.3	1.9	1.9
	Outfall Site								
Rep 1	14.8	3.5	13.4	5.2	3.3	3.2	2	1.7	1.7
Rep 2	13.2	3.8	9.4	4.3	3.3	3.6	5	1.3	1.7
Rep 3	12.6			3.3	3.4	3.3	5	1.5	1.7
AVG	13.5	3.7	11.4	4.3	3.3	3.4	4.0	1.5	1.7
	East Cape Cod Bay								
Rep 1	13.6	6.8	5	5.1	3.3	3.4	4	2.6	2
Rep 2	26.9	4.8	4.8	4.4	3.2	3	4	1.7	2.3
Rep 3	8.3	4.5	4.9	4.5	3	3.5	3	1.8	1.9
Rep 4	16.3	2.8	4.9	4.7	3.2	3.3	3.7	2.0	2.1
Rep 5 AVG		7.6							
Rep 6		2.1							
Rep 7		0.4							
Rep 8		7.1							
Rep 9		4.1							
Rep 10		1.6							
AVG		4.2							

Hepatopancreas	Deer Island Flats								
Lipids (% dry weight)	1992	1993	1994	1995	1996	1997	1998	1999	2000
Rep 1	65.8	34.3	72.4	70.8	49.5	46.3	104 (1)	32.3	53.5
Rep 2	73.7	35.2	71.5	64.3	60.1	56.5	66	30	57.6
Rep 3	66.3	55.8	67.5	55.9	59.4	44.5	68	31.8	57.7
AVG	68.6	41.8	70.5	63.7	56.3	49.1	67.0	31.4	56.3
	Outfall Site								
Rep 1	57	56.2	59.2	70.9	47.4	64.2	68	30.2	42.7
Rep 2	47.1	45.3	56.5	60.4	54.1	62.8	70	58.7	52.9
Rep 3	79.2			61.8	52.4	44.7	60	40.8	56.8
AVG	61.1	50.8	57.9	64.4	51.3	57.2	66.0	43.2	50.8
	East Cape Cod Bay								
Rep 1	18.8	72.9	79	57.7	59.1	58.6	59	35.2	51.3
Rep 2	82.5	33.6	67.3	64.7	65.1	61	60	37.3	58.6
Rep 3	30.1	57.9	61.7	79.6	60.6	57.7	59	43.4	57.3
Rep 4		43.5							
Rep 5 AVG	43.8	65.5	69.3	67.3	61.6	59.1	59.3	38.6	55.7
Rep 6		33.7							
Rep 7		39.4							
Rep 8		40.3							
Rep 9		56.4							
Rep 10		67.2							
AVG		51.0							

[1] Value is suspect and is not used in calculation of mean.

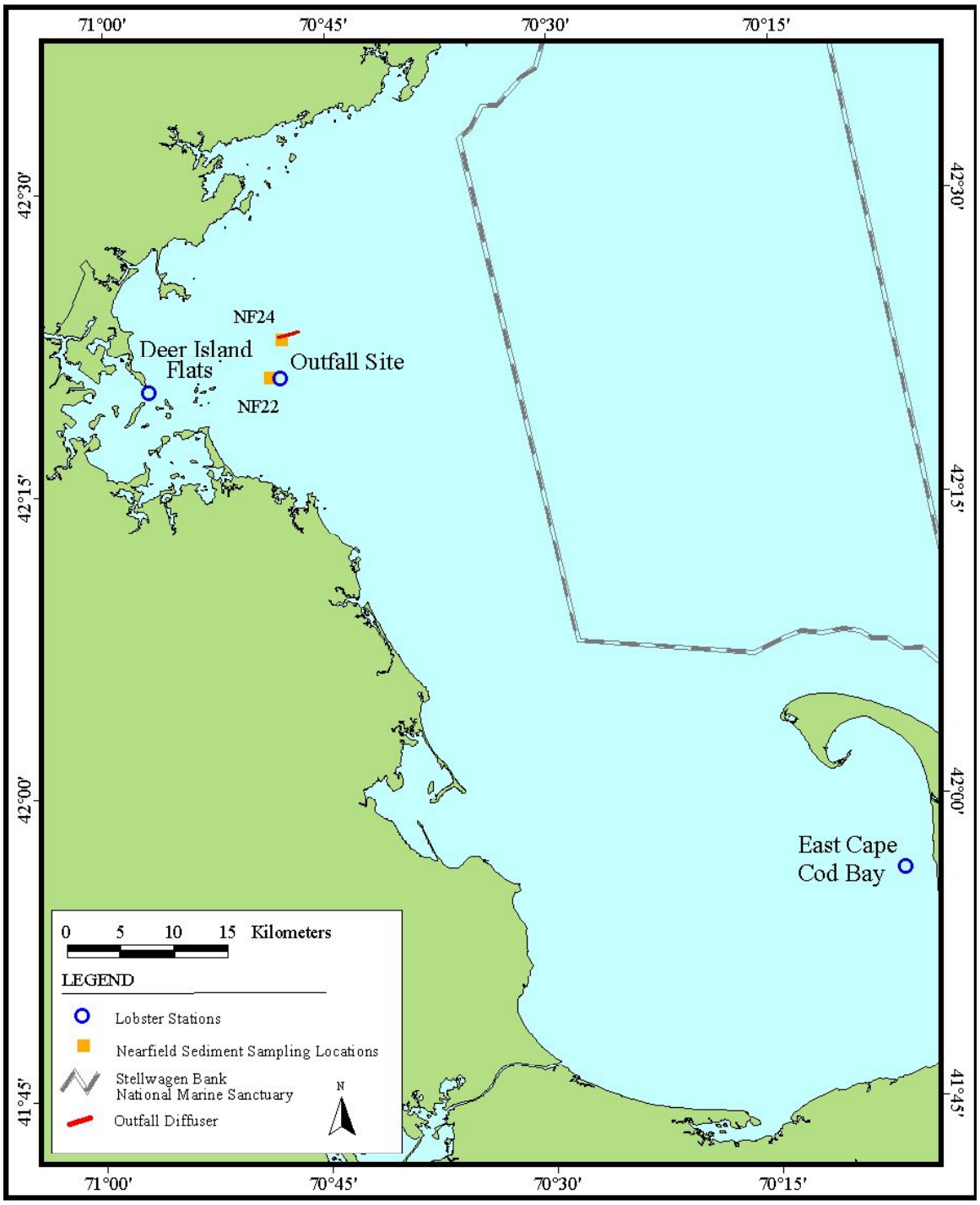

Figure 3-1. Collection locations of lobster as part of MWRA's harbor and outfall monitoring program

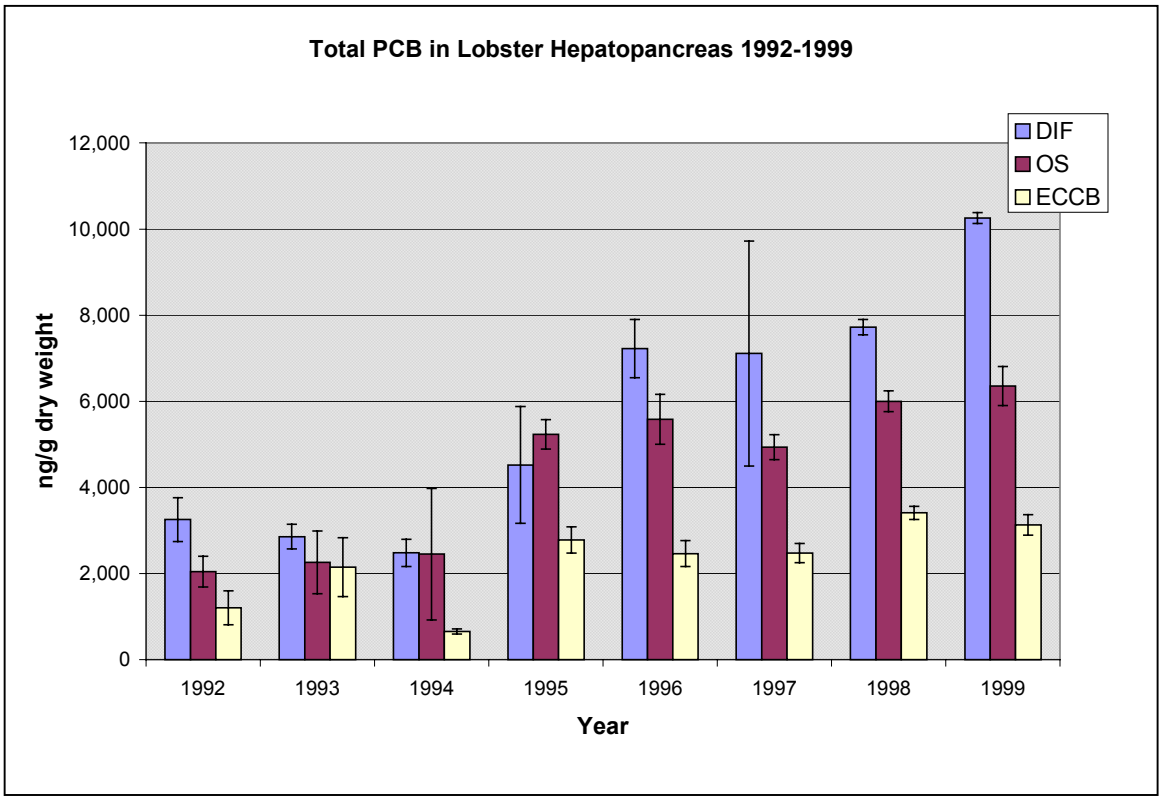

Figure 3-2. Total PCB in lobster hepatopancreas at DIF, OS and ECCB, 1992–1999.

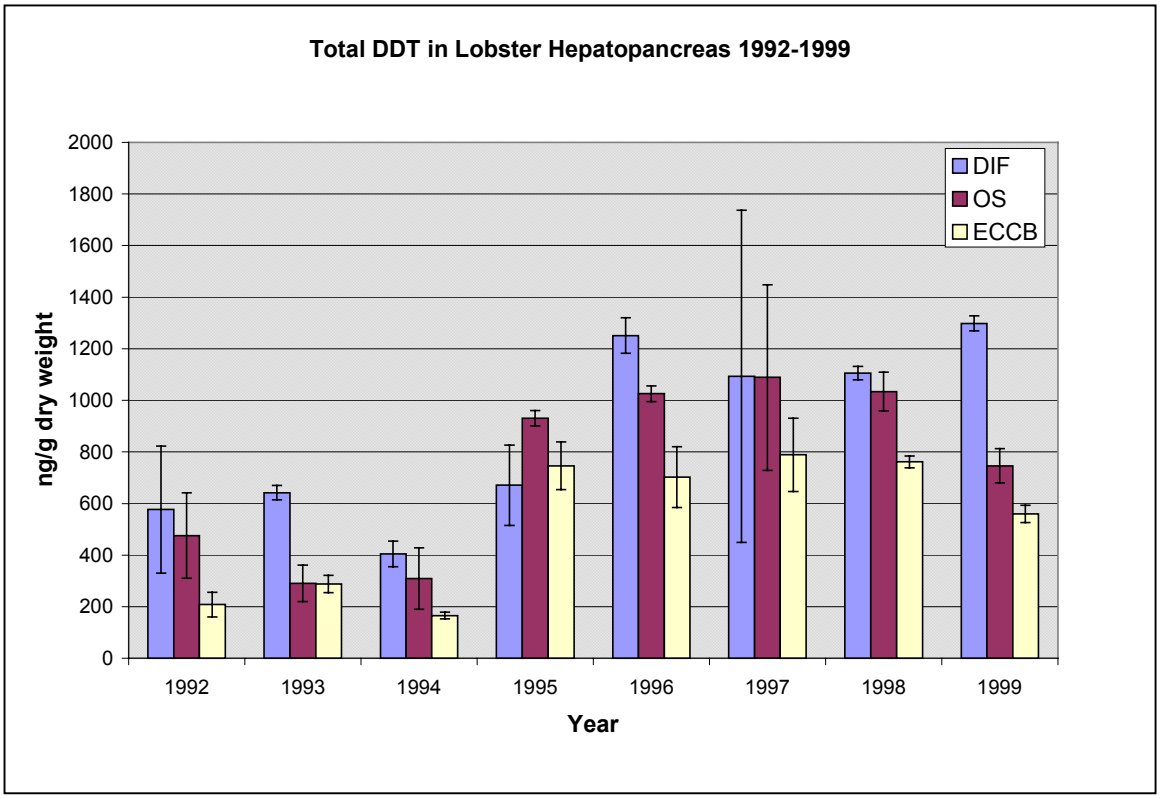

Figure 3-3. Total DDT in lobster hepatopancreas at DIF OS and ECCB, 1992–1999.

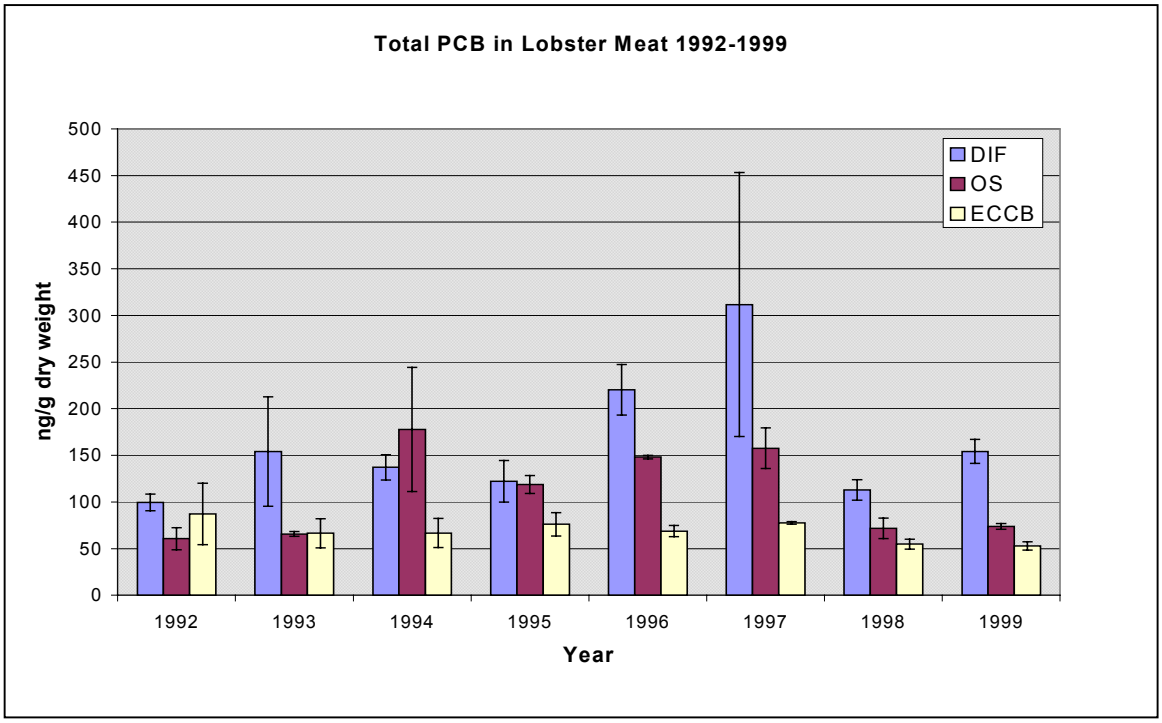

Figure 3-4. Total PCB in lobster edible meat at DIF, OS and ECCB, 1992–1999.

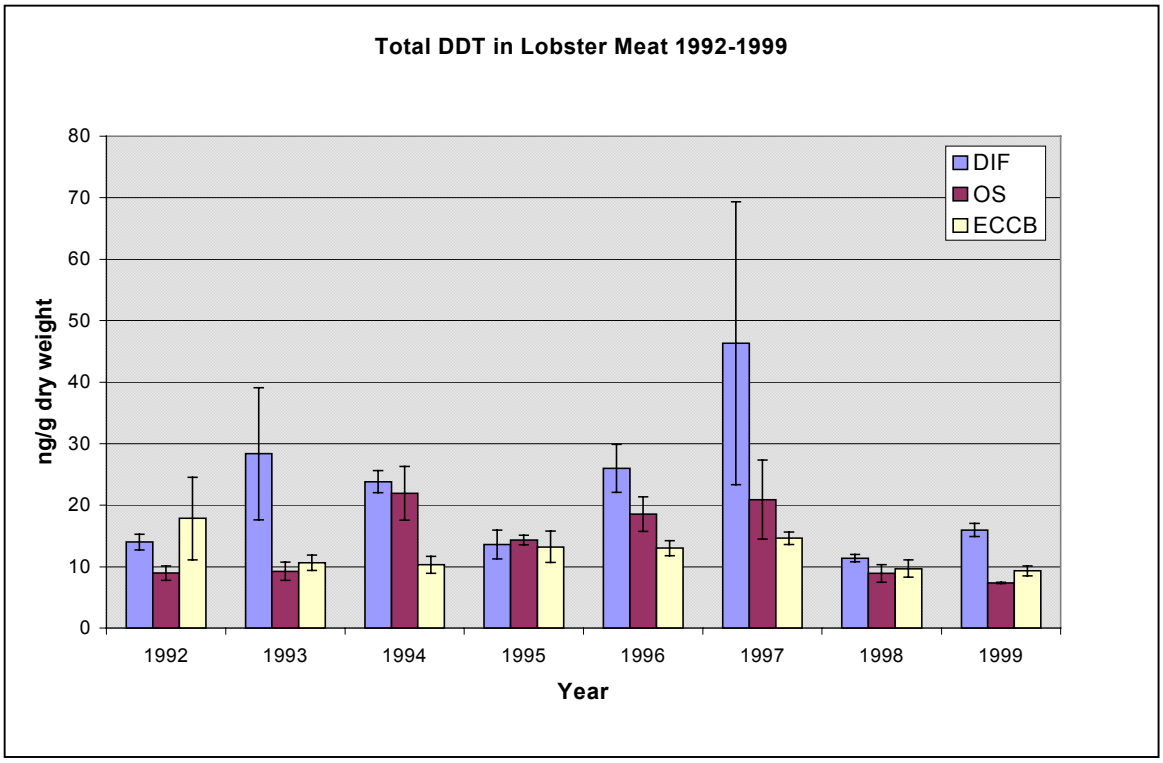

Figure 3-5. Total DDT in lobster edible meat at DIF, OS and ECCB, 1992–1999.

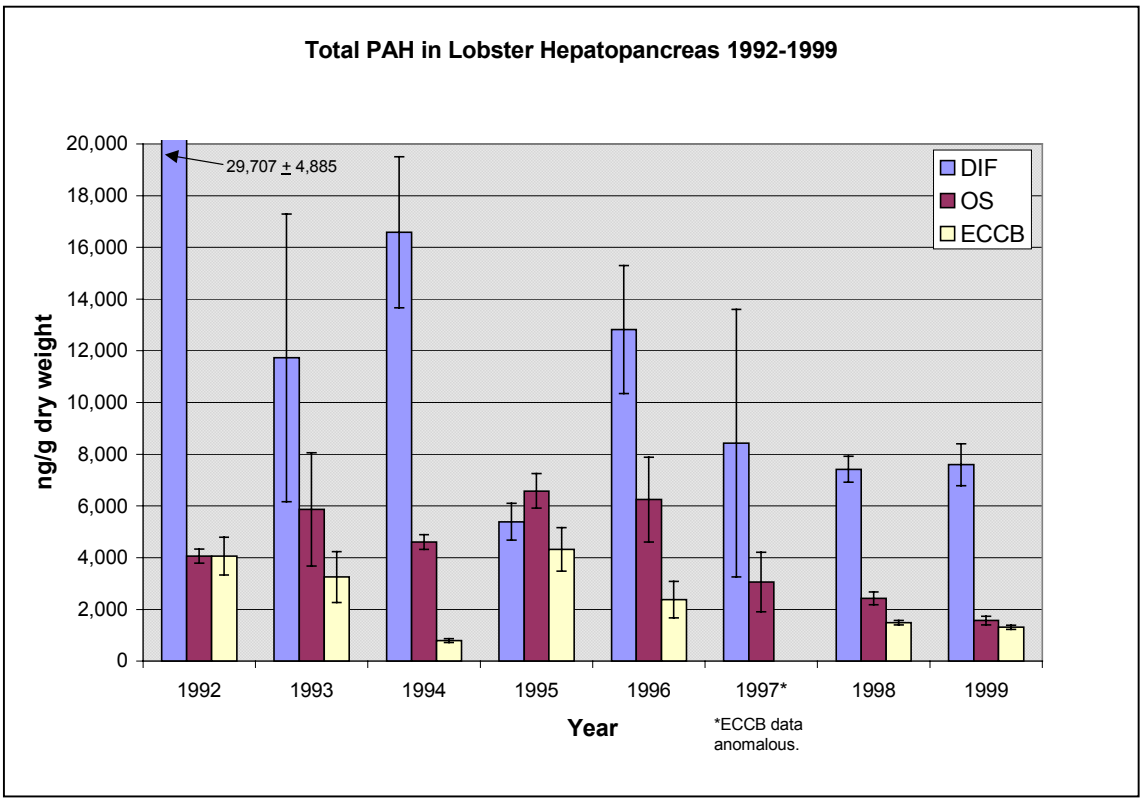

Figure 3-6. Total PAH in lobster hepatopancreas at DIF, OS and ECCB, 1992–1999.

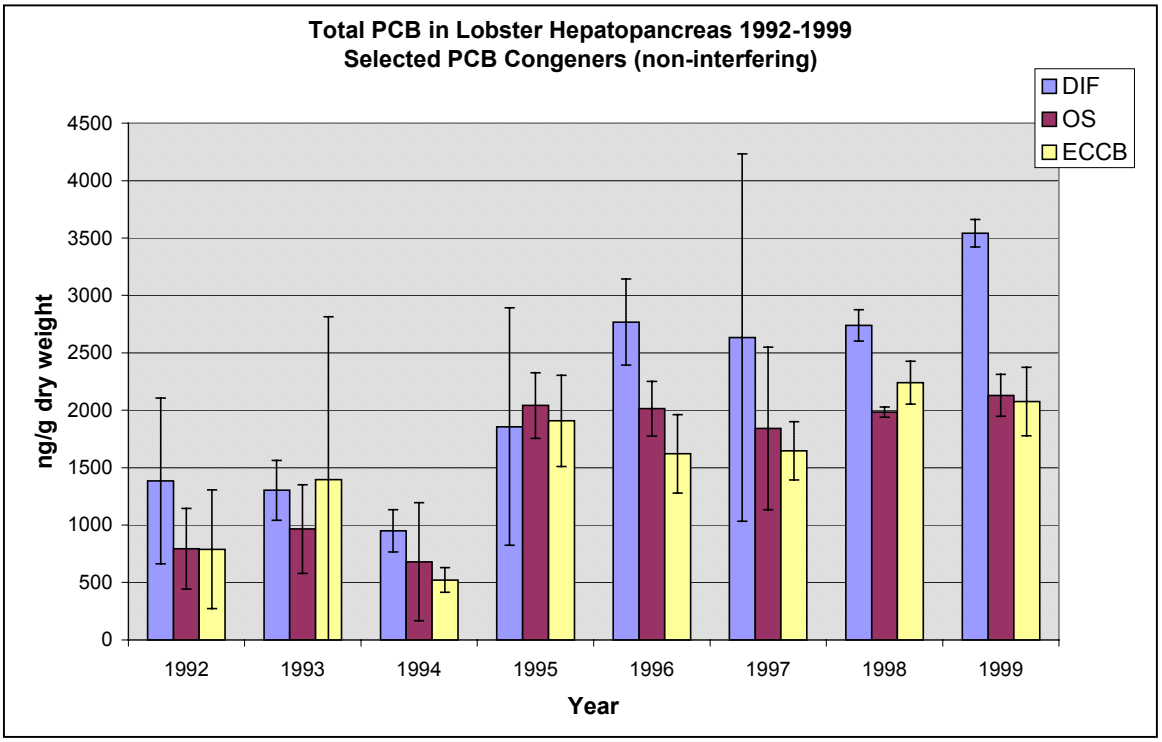

Figure 3-7. Total PCB in lobster hepatopancreas based on the sum of selected non-interfering PCB congeners at DIF, OS and ECCB 1992–1999.

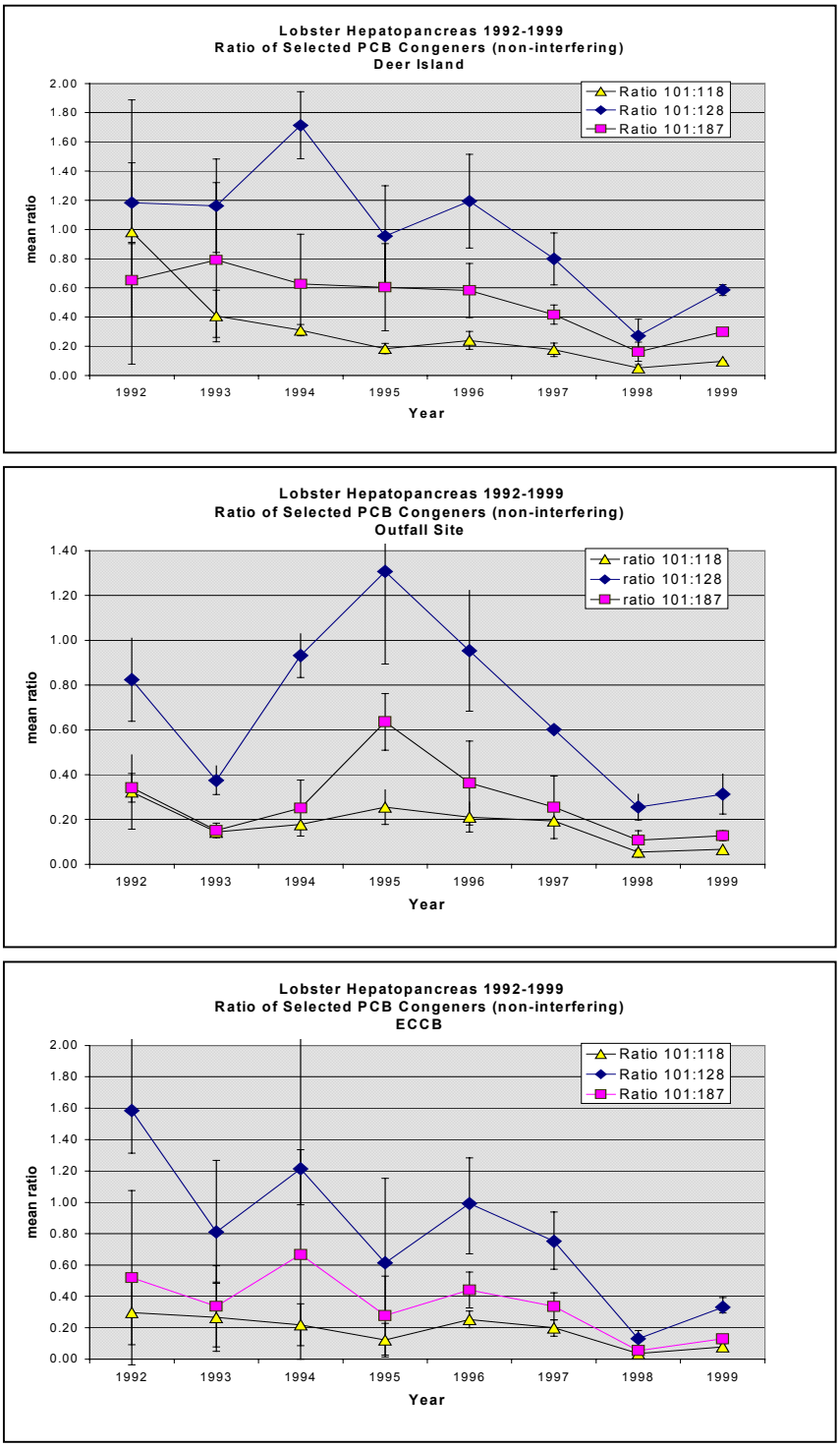

Figure 3-8. Ratio of PCB 101 to PCB 118 (C15), PCB 128 (C16) and PCB 187 (C17) in lobster hepatopancreas at DIF, OS and ECCB.

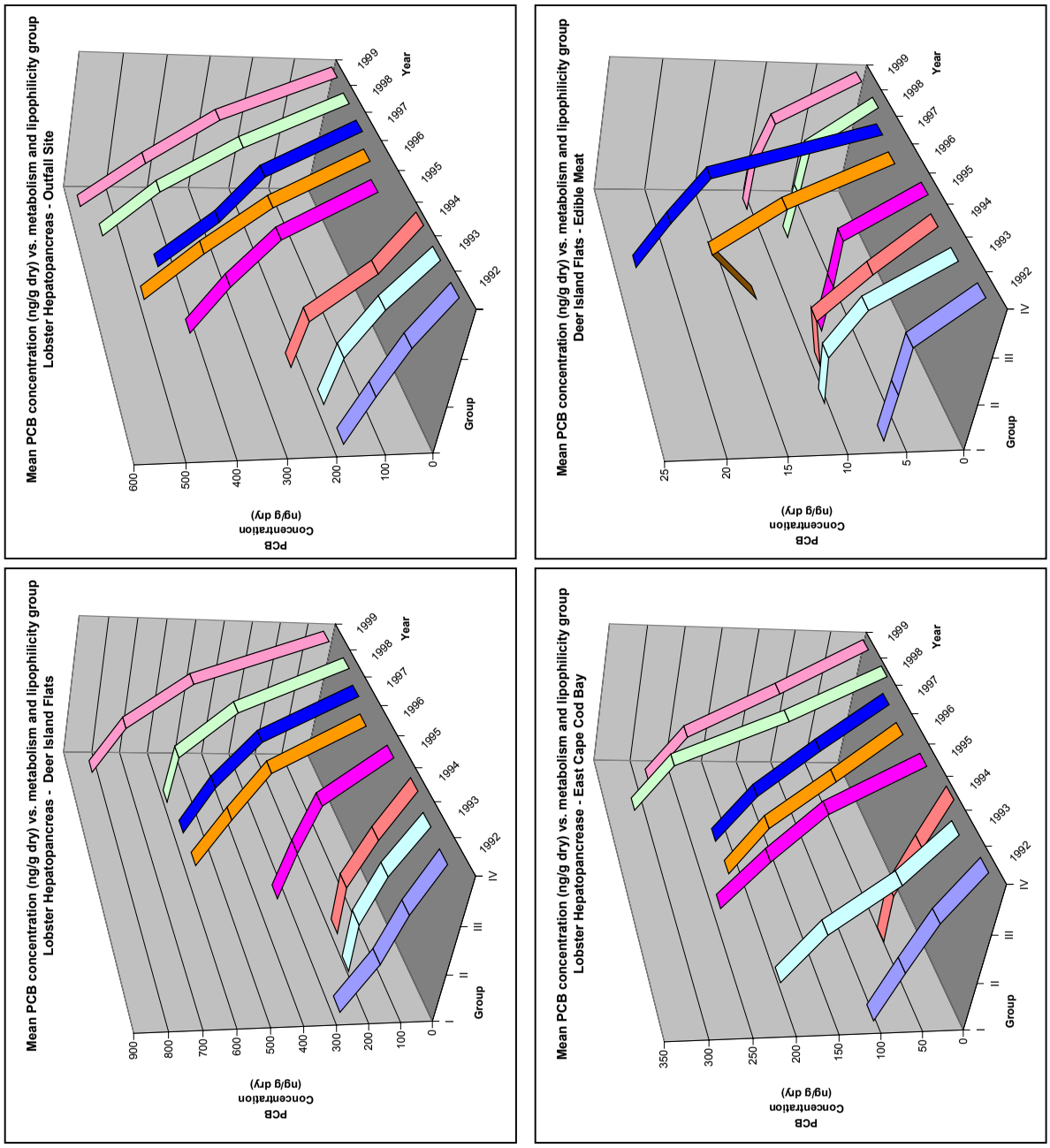

Figure 3-9. Mean PCB concentration in lobster hepatopancreas at DIF, OS and ECCB. PCBs summed by PCB Group (1 – most lipophilic, least metabolized; IV – least lipophilic, most easily metabolized).

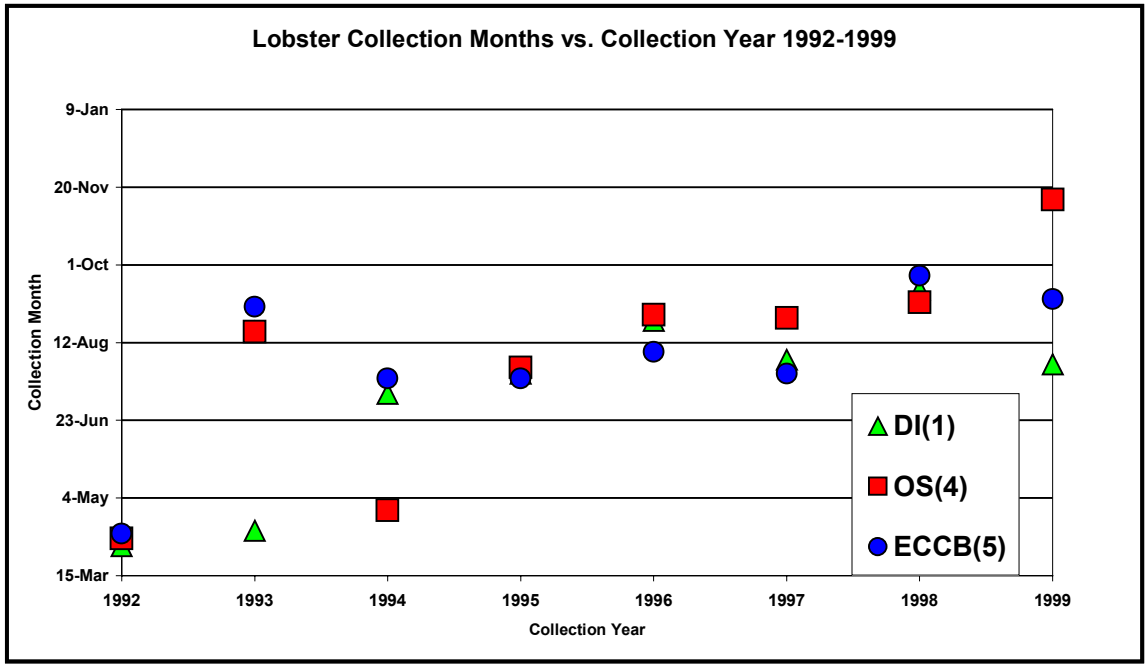

Figure 3-10. Lobster collection month plotted versus collection year 1992–2000.

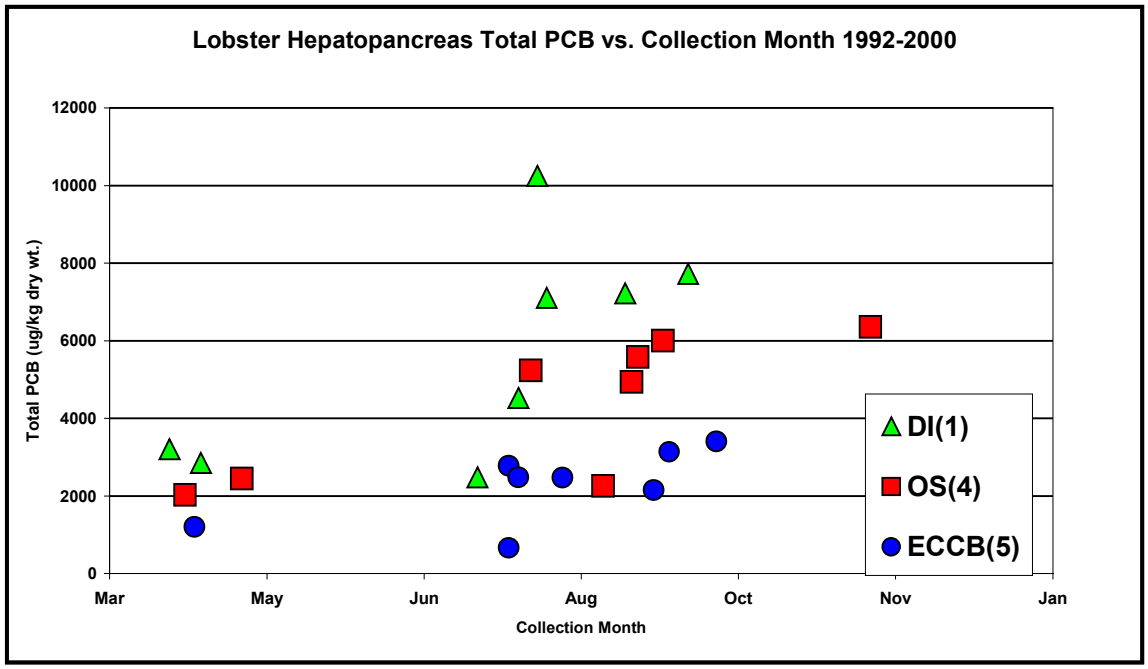

Figure 3-11. Lobster collection month plotted versus total hepatopancreas PCB concentration.

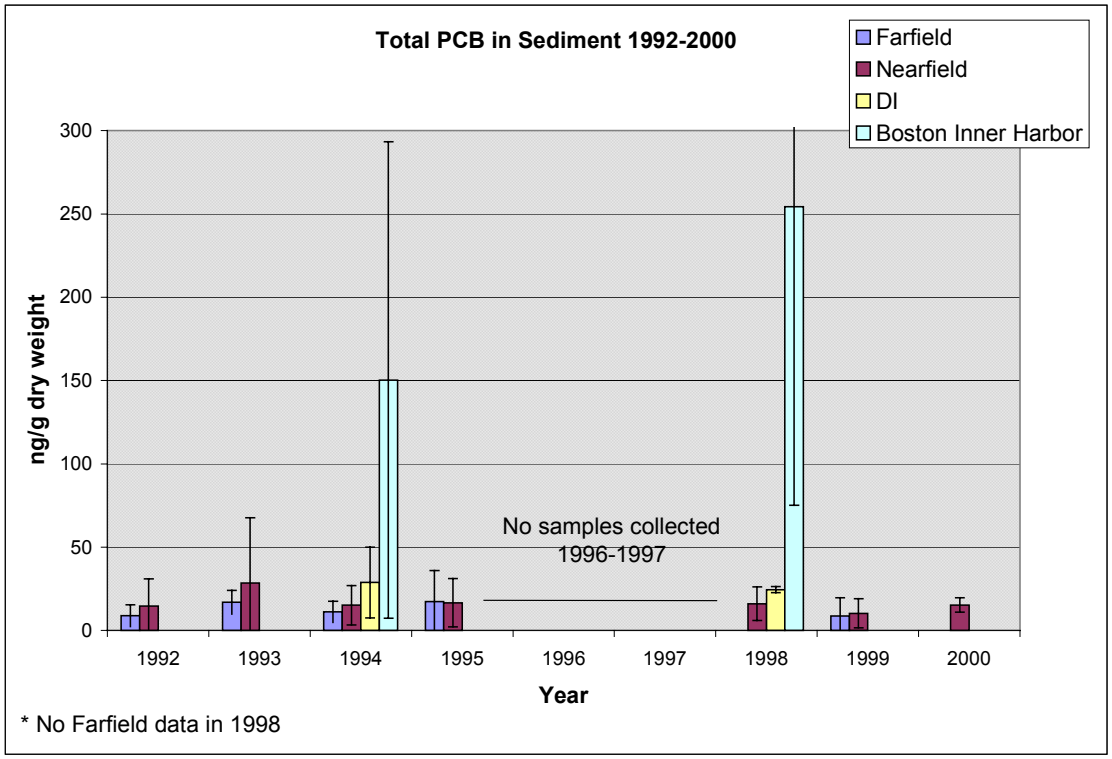

Figure 3-12. Total PCB in sediment from Boston Harbor 1992–1999.

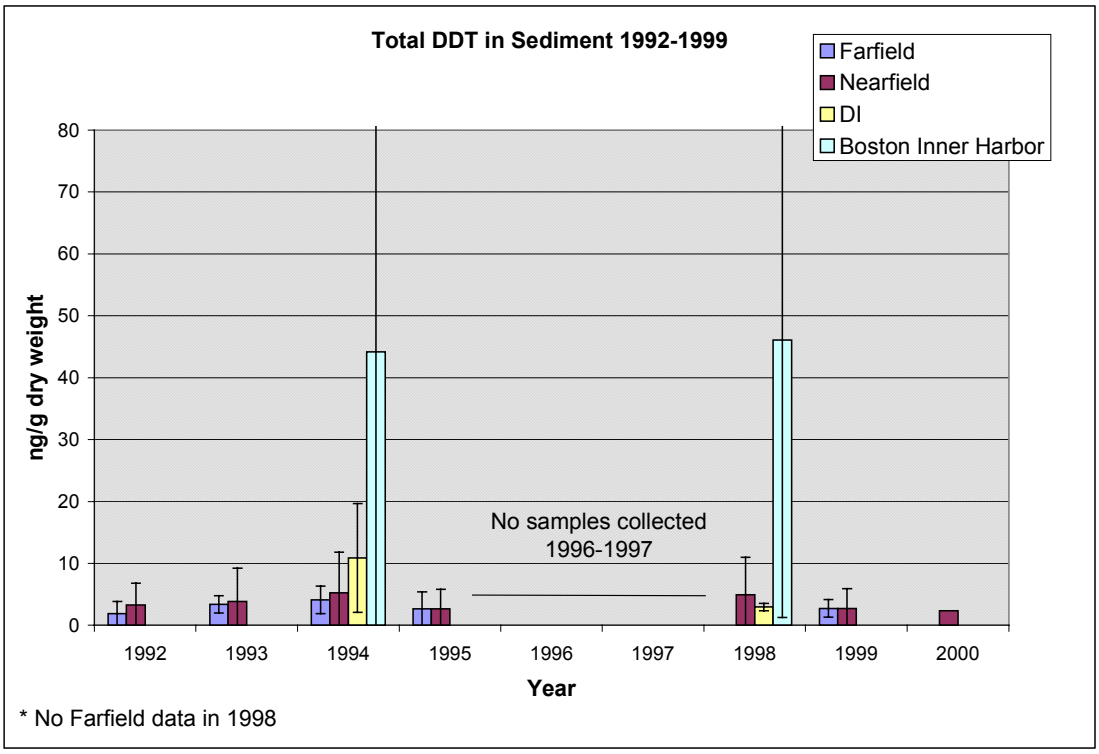

Figure 3-13. Total DDT in sediment from Boston Harbor 1992–1999.

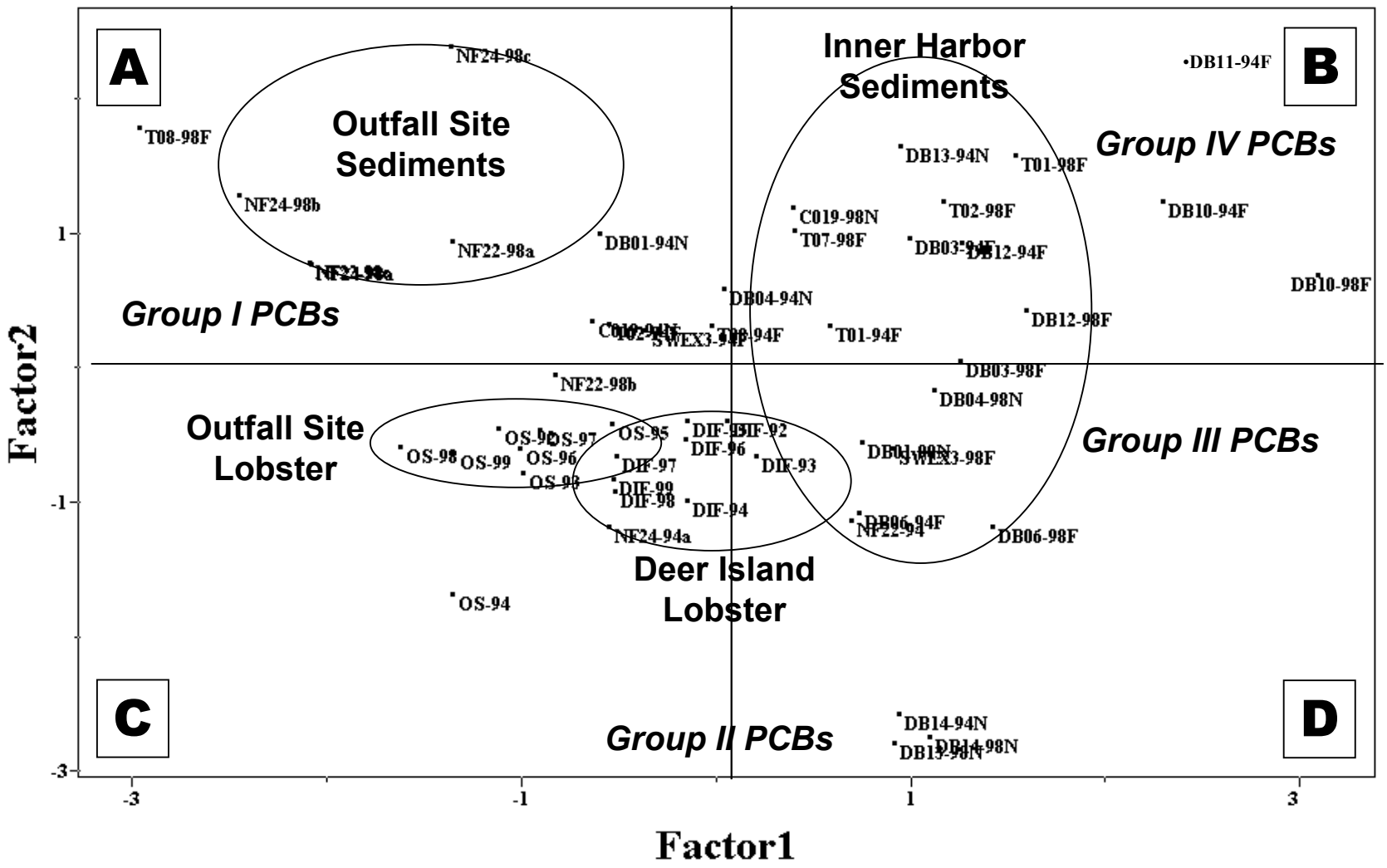

Figure 3-14. PCA analysis of sediment and lobster hepatopancreas PCB distribution (1st and 2nd PCs accounted for 41 and 35% of variance, respectively).

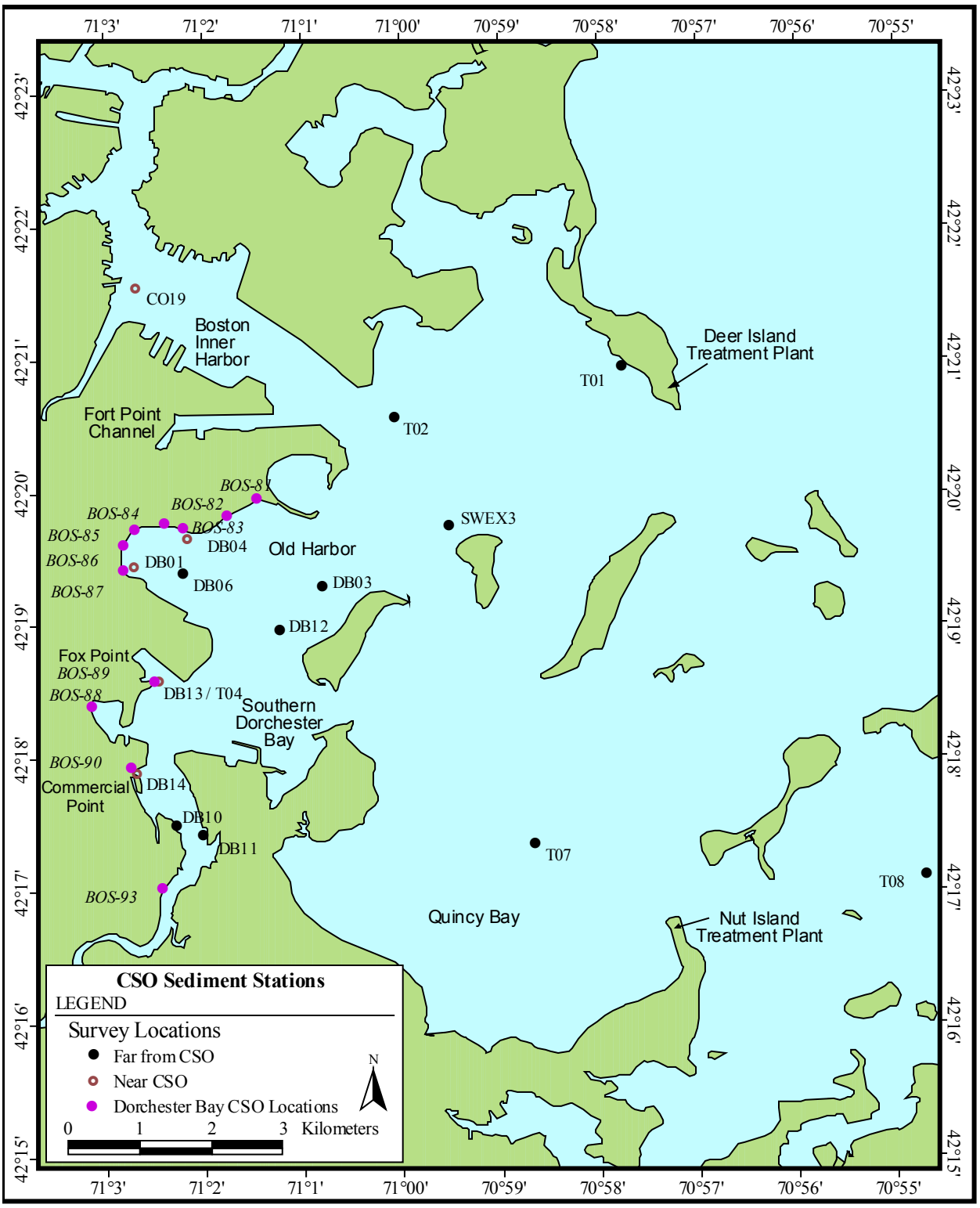

Figure 3-15. Boston Inner Harbor sediment sampling locations.

4.0 CONCLUSIONS

This study examined co-eluting compounds as a potential cause for an increasing trend in PCBs in lobster hepatopancreas in the face of decreasing loadings to Boston Harbor. The data indicate that while coelution is a clear problem, the trends are real and must be explained by other factors.

Concentrations of PCBs and DDTs in hepatopancreas of lobsters from the Deer Island Flats site, and to a lesser extent from the Outfall site, have risen gradually between 1992 and 1999. PCA analysis of well resolved PCB congeners indicate that assemblages observed in lobster hepatopancreas appear to reflect similar assemblages found in nearby sediments. Migratory habits in combination with changes in collection date and the PCB congener composition in water, sediments, and biota of Boston Harbor during the 1990s may play a role in explaining the observed trends. This trend probably is due to a change in the diet of the lobsters in the Harbor. Following cessation of the discharge of sludge and of effluent receiving only primary treatment, lobsters may have switched from a diet rich in organisms impacted by the sludge and wastewater to other prey in the more nearshore parts of the harbor where sediments are more highly contaminated with PCBs and DDTs.

4.1 Implications for MWRA HOM program

This investigation strongly suggests that GC/MS SIM offers advantages over traditional GC/ECD, in terms of accuracy and precision, in the measurement of PCB congeners and chlorinated pesticides in biological tissue. GC/MS SIM appears to be a viable, high quality alternative to GC/ECD analysis for determining chlorinated hydrocarbons in environmental samples. The advantages are especially evident when analyzing complex matrices that have high levels of PCBs as well as chlorinated pesticides. The results for both PCB congeners and chlorinated pesticides, based on analyses of quality control samples appear to be both highly accurate and precise. Analysis by GC/MS may avoid false positives often reported for chlorinated pesticides such as 4,4'-DDE and dieldrin, due to co-elution with certain PCB congeners as well as the false positives often reported for some PCB congeners due to co-elution with other PCBs or other environmental contaminants such as phthalate esters. If the MWRA chose to use the GC/MS method for future residue analysis in biological tissues, the Authority could expect to develop higher quality data for these contaminants. In addition, GC/MS SIM allows accurate and cost effective means for analyzing for a much larger list of PCB congeners, thus allowing a much more accurate estimate of Total PCBs.

The relationship between the highly accurate measurement of total PCB (determined by in-depth measurement of 107 PCB congeners that represent more than 95% of congeners in commercial Aroclors) and the sum of the concentrations of the 18 NOAA NS&T congeners measured as a routine matter in lobster tissues for the HOM monitoring program indicate that the sum of the 18 NOAA NS&T congeners represents about 70% of the total PCB (defined by the 107 congener measurement) present in samples from this study. The data suggest that the HOM program data could be re-calculated by applying a correction factor based on the above-mentioned relationship. This would give the Authority a better estimate of the total PCB concentrations in lobster tissues analyzed for the program.

4.2 Recommendations for Future Monitoring Programs

The results of this investigation demonstrate that the GC/MS method is comparable, and in some cases, superior to, traditional GC/ECD methods for the measurement of PCB and chlorinated pesticides in lobster tissue. The GC/MS method is particularly better than the GC/ECD method when analyzing complex, contaminated matrices — a condition common in tissues encountered in the HOM monitoring program. The GC/MS method also has the flexibility to measure, if deemed necessary, a much larger list

of PCB congeners than traditional GC/ECD should the Authority wish to better estimate total PCB in tissue samples. These observations suggest that the Authority can consider the GC/MS method a suitable and beneficial replacement method for traditional GC/ECD analysis of tissues for chlorinated organic compounds.

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