2012
Fish and Shellfish Report

Massachusetts Water Resources Authority
Environmental Quality Department
Report 2013-08

Citation:

Hall M.P., Lao Y., Moore M.J., 2013. **2012 fish and shellfish report**. Boston: Massachusetts Water Resources Authority. Report 2013-08. 47p.

2012

FISH AND SHELLFISH REPORT

submitted to

MWRA Water Resources Authority
Environmental Quality Department
100 First Avenue
Charlestown Navy Yard
Boston, MA 02129
(617) 242-6000

prepared by

Maurice P. Hall[1]
Yong Lao[1]
Dr. Michael J. Moore[2]

[1]MWRA
100 First Ave.
Boston, MA 02129
(617) 788-4944

[2]Woods Hole Oceanographic Institution
Woods Hole, MA 02543

August 2013

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EXECUTIVE SUMMARY

The objective of the MWRA Fish and Shellfish monitoring program is to define the post-discharge condition of three indicator species: winter flounder (*Pseudopleuronectes americanus*), lobster (*Homarus americanus*), and blue mussel (*Mytilus edulis*). Flounder and lobster specimens were collected from three core sites in Boston Harbor and the Bays: Deer Island Flats (DIF), the Outfall Site (OS), and East Cape Cod Bay (ECCB). Flounder were also collected at one ancillary site off Nantasket Beach (NB), to provide information on flounder in the general area of the former Deer Island outfall. Caged mussels, collected from Pemaquid Point, ME, were deployed at sites in Boston Harbor and the bays to evaluate bioaccumulation potential.

This report details chemical contaminant results from flounder edible meat and liver, lobster edible meat and hepatopancreas, and whole mussels. Flounder liver histological results are also discussed. The monitored parameters were examined for spatial distribution among stations in 2012 and inter-annual variations from previous monitoring data. In addition, body burdens of certain pesticides, PCBs, PAHs, lead, and mercury were compared to FDA Action Limits and Contingency Plan (MWRA 2001a) threshold values to evaluate potential risk.

Flounder
Winter flounder were collected at the four monitoring locations (DIF, NB, OS, and ECCB). With the exception of the younger fish collected from Nantasket Beach, fish at the other stations were of approximately the same average age. As in past years fish collected off Deer Island were longer and heavier than those from other stations. In 2012 only two fish from Nantasket and three from the Outfall Site had ventral lesions. These ulcers were first observed in marked numbers in 2003 but have become less frequent since 2005. The fish at all stations were predominantly females.

In 2012 no liver neoplasms were reported and the relatively low prevalence of hydropic vacuolation suggests a steady system-wide reduction in the contaminant–associated pathology over the past two decades. While lower than in the 1990's centrotubular hydropic vacuolation (CHV) remains highest in fish collected off Deer Island but remains low in the vicinity of the Massachusetts Bay Outfall Site (OS).

Fifteen winter flounder were collected at each location and edible meat and liver were analyzed for chemical contaminants. In general mean concentrations of organic compounds in fillets were highest off Deer Island and lowest at Eastern Cape Cod Bay (ECCB), a pattern observed throughout this program. Despite somewhat elevated tissue concentrations at most stations in 2012 chlordane and DDT continue to show long term decreases throughout the region. Metal tissue burdens were less predictable. Concentrations of mercury in flounder meat were similar at all stations. Of the metals measured in livers the only apparent trend is a possible region-wide increase in nickel. All flounder edible tissue contaminant levels remain well below the federal action limits and the MWRA Threshold Levels and indicate no risk for human consumption.

Lobster
Twenty one lobsters were collected at each of the three core monitoring stations (DIF, OS, and ECCB). The average size as determined by carapace length was identical at all stations. The percentage of females varied from 52% off Deer Island (DIF) to 71% at the other two stations. One lobster collected from OS had indications of shell erosion. No other deleterious external conditions were noted.

Similar to the 2012 flounder results and historical lobster results organic contaminants tended to be highest in lobster collected in Boston Harbor and lowest in those from Cape Cod Bay. Chlordane and DDTs continue to show downward trends throughout the region. Total PCBs and mercury were low in

lobster meat at all sites. The highest concentrations of several metals in lobster hepatopancreas were from lobsters collected at OS. The very high concentration of nickel measured from ECCB lobsters accentuated a possible long-term region-wide increase for that metal. PAHs were very high in the lobsters collected at DIF but low at the other two stations. Lobster edible tissue concentrations were well below MWRA Caution and Warning Thresholds and FDA Action Limits for human consumption.

Mussels

Mussels were collected at Pemaquid Point, Maine and deployed in arrays at Deer Island (DIL), Boston Inner Harbor (IH), Outfall Site (OSM), and "B" Buoy (LNB). A full set of arrays was successfully retrieved at sixty days from all locations. Mussel survival at all stations was between 90 and 100%.

The 2012 data were similar to previous years with the highest contaminant levels generally observed in mussels deployed in Boston's Inner Harbor with lower concentrations in Massachusetts Bay. With the exception of lead, mussels from Maine had the lowest concentrations. Trend results suggest decreasing trends of total chlordane, DDT, and PCBs at all sites. PAHs have decreased in mussels deployed at DIL. At OS mussels bio-accumulated only slightly higher concentrations of HMW PAHs than they did prior to wastewater diversion. However, concentrations remain well below the MWRA Caution Threshold. There were no threshold exceedances in 2012.

1.0 INTRODUCTION

The Massachusetts Water Resources Authority (MWRA) has implemented a long-term Harbor and Outfall Monitoring (HOM) Program for Massachusetts and Cape Cod Bays. The objectives of the HOM Program are to assess whether the environmental impacts of the MWRA discharge are consistent with SEIS projections and whether the impacts exceed any Contingency Plan thresholds (MWRA 2001a). A detailed description of the monitoring and its rationale is provided in the Effluent Outfall Monitoring Plan developed for the baseline period and the post-discharge monitoring plan (MWRA 1997, 2004).

One aspect of the MWRA HOM program is a long-term monitoring program for fish and shellfish (MWRA 1991). The goal of the fish and shellfish monitoring is to provide data to assess environmental impact of effluent discharge into Massachusetts Bay. These data are 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 2001a and b).

The objective of the fish and shellfish monitoring is to define the condition of three indicator species: winter flounder (*Pseudopleuronectes americanus),* lobster (*Homarus americanus),* and blue mussel *(Mytilus edulis*). Measured parameters include length, weight, the presence of external or internal disease, and inorganic and organic contaminant tissue concentrations. This characterization of the health of winter flounder, lobster, and mussel in Boston Harbor, Massachusetts Bay, and Cape Cod Bay (hereafter: Boston Harbor and the Bays) forms the basis for assessing changes resulting from the relocation of the outfall discharge.

The scope of the 2012 Fish and Shellfish Report is focused primarily on assessing changes, if any, as a result of relocation of the MWRA wastewater discharge. The report first provides a summary of the survey and laboratory methods (Section 2). Section 3 presents the results of monitoring data from surveys conducted during 2012, as well as selected data from previous studies. References can be found in Section 4. All historical data are reported in Appendices A - E.

2.0 METHODS

The methods and protocols used in the 2012 surveys conducted to collect biological specimens are similar to and consistent with previously used methods. More detailed descriptions of the methods are contained in *Quality Assurance Project Plan (QAPP) for Fish and Shellfish Monitoring: 2011-2013. (Nestler et al.* 2011*)* and *Quality assurance project plan (QAPP) for Chemistry Analyses for fish and shellfish Monitoring* (Lao *et al*., 2012).

2.1 Winter Flounder Monitoring

Winter flounder *(Pseudopleuronectes americanus)* were collected from four locations in Boston Harbor and the Bays to obtain specimens for age, weight, and length determination, gross examination of health, histology of livers, and chemical analyses of tissues to determine contaminant exposure. Chemical data were used to determine whether contaminant tissue burdens have changed since the startup of the Massachusetts Bay outfall and whether these concentrations approach human health consumption limits.

2.1.1 Stations and Sampling

The 2012 flounder survey was conducted between April 24 and April 26, 2012. Four sites were sampled to collect winter flounder for histological and chemical analyses:

- Deer Island Flats (DIF)
- Off Nantasket Beach (NB)
- Massachusetts Bay Outfall Site (OS)
- East Cape Cod Bay (ECCB)

Table 2-1 provides the planned and actual sampling sites and locations for the 2012 flounder sampling. Adjustments in location and time were made to maximize collection efforts in an attempt to collect the required 50 flounder per site. Figure 1 displays the actual monitoring locations.

At each of the four designated sampling sites, otter-trawl tows were conducted from the F/V *Harvest Moon* (captained by Mr. Mark Carroll) to collect 50 sexually mature (4-5 years old, total length ≥30 cm) winter flounder. Thirty-five fish at each station were assigned unique identification numbers to indicate date, time, and site of collection. These fish were killed at sea by cervical section and used for histological processing. They were examined externally and their external condition was noted prior to histological processing. The gonads of each flounder were examined to determine sexual maturity. All specimens were weighed, and total and standard lengths were determined. Scales and otoliths were then taken from each specimen for age determination.

Of the 50 flounder collected from each station, 15 were designated for tissue chemical analysis. Because contaminant-free conditions were not available on board the vessel, the fish used for chemical analysis were maintained alive on-board (on ice) and transported to Envirosystems Inc. (Hampton, New Hampshire) for histological and organ dissections. These fish were also examined for external condition in the laboratory.

2.1.2 Age Determination

Scales from each specimen were collected for age determination. Scales were removed after first removing any mucus, debris, and epidermis from the dorsum of the caudal peduncle by wiping in the direction of the tail with a blunt-edged table knife. Scales were then collected from the cleaned area by applying quick, firm scraping motions in the direction of the head. The loosened scales were placed in

the labeled age-sample envelope by inserting the knife between the liner of the sample envelope and scraping off the scales. At the time of processing, otoliths were also removed from the inner ear. The age of each flounder was determined by scientists at the National Marine Fisheries Services (NMFS) in Woods Hole, Massachusetts.

2.1.3 Dissection of Fish

The flounder tissues were removed in the laboratory under contaminant-free conditions. Tissue processing was conducted in a Class-100 clean room. The fillets (muscle) were removed from the flounder and the skin was removed from the fillet, using a pre-cleaned (*i.e*., rinsed with 10% HCL, Milli-Q (18 meg-ohm) water, acetone, DCM, and hexane) stainless steel knife.

From each site, three composites were prepared; each composed of approximately equal masses of top and bottom tissue from five randomly chosen fish. Homogenization was performed using a stainless steel TEKMAR® tissuemizer. Each composite was placed in a sample container clearly identified with the unique sample identifier.

Livers from the 15 fish selected for chemical analyses were removed using a titanium knife and processed for chemical analysis, after sectioning for histopathology analysis. (Livers from the fish not used for chemical analyses were removed shipboard and processed for histology as described below). Following the removal of three individual slices of liver for histology analysis, the remaining liver tissue from each fish was homogenized by finely chopping with the titanium knife and three separate composites per station were formed to correspond to the composites made for the fillets (*i.e.,* the livers of the same five specimens used for each edible tissue composite were combined). This was done to ensure comparability between fillet and liver chemical analyses. Each composite was placed in a sample container clearly identified with the unique sample identifier. This resulted in 24 pooled samples for analysis in 2012 (12 pooled fillets and 12 pooled livers). The homogenized tissue and liver samples were frozen and stored. Any remaining tissue from each specimen was archived frozen in case additional analysis was required.

2.1.4 Histological Processing

After the fish were completely examined and scales and otoliths removed, the livers were removed (either on-board the ship or in the lab, as described above) and examined for visible gross abnormalities ("Gross Liver Lesion"). The livers were then preserved in 10% neutral buffered formalin for histological analysis. Liver samples from each fish were placed in a separate clearly labeled sample container.

2.1.5 Histological Analysis

Livers of 50 flounder from each site were prepared for histological analysis by Experimental Pathology Laboratories in Herndon, VA. Transverse sections of flounder livers fixed as part of tissue sample processing were removed from the buffered formalin after at least 24 hours, rinsed in running tap water, dehydrated through a series of ethanols, cleared in xylene, and embedded in paraffin. Paraffin-embedded material was sectioned on a rotary microtome at a thickness of 5 μm. Each block contained three liver slices, resulting in one slide with three slices per slide per fish, for a total of 200 slides (50 fish X 4 sites). The sections were stained in hematoxylin and eosin.

Each slide was examined under bright-field illumination at 25x, 100x, and 200x magnification to quantify the presence and extent of:

- Three types of vacuolation (centrotubular, tubular, and focal)
- Macrophage aggregation
- Biliary duct proliferation
- Neoplasia

The severity of each lesion was rated on a scale of 0 to 4, where: 0 = absent; 1 = minor; 2 = moderate; 3 = severe; and 4 = extreme. For each lesion and each fish, a histopathological index was then calculated as a mean of scores from three slices on one slide.

2.1.6 Tissue Processing and Chemical Analyses

Chemical analyses were performed on composite samples of flounder from all stations. Two tissue types (fillet and liver) were analyzed. Flounder fillet and livers were analyzed for PCBs/Pesticides, lipids, and mercury. In addition, flounder livers were analyzed for PAHs, lead, silver, cadmium, chromium, copper, nickel, and zinc. The individual steps involved in the tissue processing and chemical analyses of these samples are detailed in Section 2.4.

2.1.7 Data Reduction and Statistical Analyses

Data reduction was conducted as described in the Fish and Shellfish Monitoring CW/QAPP (Nestler *et al.* 2011) and in Section 2.5 of this report. Histopathological indices and prevalence of lesions were compared among groups of flounder by station. Chemical constituents were presented graphically and compared among stations and over time.

In addition to reporting the prevalence and lesion index of hydropic vacuolation, historical data has included several other lesions, including macrophage aggregates, biliary proliferation, neoplasia, and balloon hepatocytes.

2.2 Lobster Monitoring

Lobsters *(Homarus americanus)* were collected from three sampling sites for gross examination (to determine specimen health) and chemical analyses (to determine tissue burden of contaminants).

2.2.1 Stations and Sampling

Lobsters were collected on August 1, 2012 (ECCB), August 7, 2012 (OS), and October 9, 2012 (DIF). The animals were captured in traps deployed at each location by local lobstermen (Mr. William Lister, Mr. Larry Bradley, and Mr. Harry Crispo, respectively.

Table 2-2 provides the planned and actual sampling locations for the lobster surveys. Figure 2-2 illustrates the actual sampling locations in Boston Harbor and the Bays. Adjustments in location and time were made to maximize collection efforts. In the case of DIF the warm spring and early summer water temperatures reportedly drove the lobsters off Deer Island Flats (DIF) into deeper waters earlier than normal. After consultation with the Massachusetts Division of Marine Fisheries lobster collections took place in the deeper channel of President Roads, up to one mile from the planned collection location.

Individual lobsters retained for analyses were assigned a unique identification number to indicate date, time, and site of collection. Lobsters were measured for carapace length and weight (DIF only), and gender was determined. Lobster specimens were visually examined and the condition noted. Processing of the hepatopancreas and the edible tail and claw meat tissue samples was conducted in the laboratory (Envirosystems, Inc.).

2.2.2 Size and Sex Determination

Carapace length was determined with calipers by measuring the distance from the posterior of the eye socket to the midpoint of the posterior of the carapace. Measurements were recorded to the nearest millimeter. Specimen weight was recorded to the nearest gram. Specimens were visually examined for the presence and severity of gross external abnormalities, such as black gill disease, shell erosion, and parasites. Data for each specimen were recorded on a lobster sample collection log.

2.2.3 Dissection of Lobster

The hepatopancreas was removed and frozen for chemical analysis. The tail and claw meat (edible tissue) was stored frozen in the shells until processed in the laboratory. Samples were placed in sample containers that were clearly identified with a conventional label containing the pertinent sample information.

The lobsters collected at each site were randomly divided into three groups of seven lobsters each. Within each of the three groups, edible meat (tail and claw) and hepatopancreas from the same seven lobsters were pooled by tissue type. Homogenization of lobster meat was performed using a stainless steel TEKMAR® tissuemizer. Hepatopancreas samples were homogenized using a titanium knife to avoid metals contamination. Each composite was placed in a sample container clearly identified with the unique sample identifier. This resulted in 18 pooled samples for analysis in 2012 (nine edible meat samples and nine hepatopancreas samples).

2.2.4 Tissue Processing and Chemical Analyses

Chemical analyses were performed on the composite samples of lobster (edible meat and hepatopancreas). Edible lobster meat and hepatopancreas were analyzed for PCBs/Pesticides, lipids, and mercury. In addition, hepatopancreas samples were analyzed for PAHs, lead, silver, cadmium, chromium, copper, nickel, and zinc. The individual steps involved in the tissue processing and chemical analyses of these samples are detailed in Section 2.4.

2.2.5 Data Reduction and Statistical Analyses

Data reduction was conducted as described in the Fish and Shellfish Monitoring CW/QAPP (Nestler *et al.* 2011) and Section 2.5 of this report. Chemical constituents were presented graphically and compared among stations and over time.

2.3 Mussel Bioaccumulation Monitoring

Blue mussels *(Mytilus edulis)* were collected from Pemaquid Point, ME and deployed in suspended cages at four sites in Boston Harbor and the bays. Mussels were recovered for determination of short-term accumulation of anthropogenic contaminants in soft tissues.

2.3.1 Stations and Reference Area

2012 pre-deployment mussels were collected from a reference site in Pemaquid Point, ME and were deployed at four sites:

- Deer Island Light (DIL)
- Outfall Site (OSM)
- Outfall Site “B” Buoy – 1 km south of the outfall risers (LNB)
- Boston Inner Harbor (IH)

Table 2-3 provides the planned and actual sampling sites and locations. Figure 2-3 illustrates the sampling locations in Boston Harbor and Massachusetts Bay.

2.3.2 Mussel Collection

On June 29, 2012, approximately 1600 mussels were collected from Pemaquid Point, ME for deployment and organic and inorganic chemical analyses. Mussels between 55 and 65 mm in length were harvested during low tide with 100 mussels individually checked for length. A sub-sample of 100 mussels was randomly selected and set aside for pre-deployment chemical analyses.

2.3.3 Mussel Deployment

After collection, the mussels were randomly distributed to plastic cages for deployment as an array (i.e., set of cages) in sufficient number to provide the necessary biological material. At least 10% additional mussels were included to account for potential mortality. Mussels were deployed on July 1 and 2 in replicate arrays at the four sites (Figures 2-3 and 2-4). Table 2-4 lists the minimum numbers of mussels and the number of cages and corresponding arrays that were deployed at each location. Each array was deployed on a separate mooring and each with enough mussels to provide sufficient tissue to complete the study. The locations of the arrays were recorded using Differential Global Positioning System (DGPS).

At OSM, four arrays (OS-M1, OS-M2, OS-M3, and OS-M6) were deployed at various locations just south of the diffuser heads and one approximately 1 km away at the "B" buoy (LNB) (Figure 2-4). This deployment scheme was used to better understand the spatial variability of contaminant concentrations along the length of the outfall as well as to reduce the possibility of accidental loss of all arrays.

2.3.4 Mussel Retrieval

Mussel retrieval was planned for two occasions with a collection of up to one half of the mussels per station at 42-days to provide tissue only in the event of failure to recover cages at the planned 60-day retrievals. The 42-day retrieval occurred on August 12 and the 60-day retrieval occurred on August 30.

2.3.5 Tissue Processing and Chemical Analyses

Individual mussels were pooled into a single composite for organic and inorganic analyses. A total of four replicate samples of 25 mussels each, were created with mussels deployed and collected at DIL, IH, and LNB. At OS, eight pooled samples of 25 mussels each were created; four composites were created from the OS-M3 deployment, and two composites each from the OS-M1 and OS-M6 deployments. Four pooled replicates of Pemaquid Point pre-deployment mussels were also analyzed for organic and inorganic parameters.

Mussel composites were prepared from individual mussels by removing attached material and byssal threads. All soft tissue, including fluids, was placed directly into a clean glass jar. Mussel composite samples were prepared for both organic and inorganic chemical analyses by homogenization of the 25 mussels using a Titanium Tekmar "tissumizer" rinsed with methanol and de-ionized water prior to use. The homogenate was separated into aliquots using a titanium or Teflon utensil and placed in a pre-cleaned 4 ounce plastic jar. All composite splits were stored frozen prior to analysis.

The tissue composites were analyzed for PCBs/Pesticides, PAHs, lipids, mercury, and lead. The individual steps involved in the tissue processing and chemical analyses of these samples are detailed in Section 2.4.

2.3.6 Data Reduction and Statistical Analyses

The extent of bioaccumulation of contaminants in the mussels was evaluated using the data reduction methods described in the Fish and Shellfish Monitoring CW/QAPP (Nestler *et al.* 2011) and in Section 2.5 of this report. Chemical concentrations by constituent were presented graphically and compared among stations and over time.

2.4 Chemical Analyses of Tissue Samples

Table 2-5 summarizes the analyses performed on each type of tissue sample. The methods, references and specific analytes are listed in Table 2-7 and 2-8. The same analytical methods were used for all tissues.

2.4.1 Organic Tissue Extraction

The MWRA Central Laboratory performed all organic fish and shellfish tissue chemistry analyses. Tissue samples are extracted for PAH, chlorinated pesticides, and PCB congeners following MWRA Central Lab SOP #1189.0 (MWRA, 2004a). This extraction method utilizes sonication, and is based on EPA Method 3550B. Between 2 and 16 g of homogenized tissue is mixed with sodium sulfate and is serially extracted with methylene chloride (DCM) using sonication techniques. The sample is weighed in an extraction vessel, mixed with the appropriate amount of sodium sulfate to achieve a free-flowing consistency, and spiked with the surrogate compounds. Methylene chloride is added and the sample is sonicated using the ultrasonic disruptor. The extract is decanted in an Erlenmeyer flask through a powder funnel containing glass wool and sodium sulfate to remove any water and solid particles. After each extraction (total of three solvent additions) the filtered solvent is combined in the flask. If a percent lipids determination is to be performed, 10 mL of the total extract is removed and transferred to an aluminum weighing dish. The solvent is allowed to evaporate overnight and the pan is weighed for the percent lipids determination. The remaining extract is measured in a graduated cylinder and then concentrated to 1 mL using the TurboVap automatic concentrator technique. This concentrated extract is then processed through a silica gel cartridge and concentrated to 1.0 mL using the N-Vap automatic concentrator technique. The post-cleanup extracts are then split 50:50 for analysis by the PAH and pesticide/congener methods.

2.4.2 Metals Tissue Digestion and Analyses

The MWRA Central Laboratory performed metals digestions and analyses for Ag, Cd, Cr, Cu, Hg, Ni, Pb, and Zn. Tissue samples are prepared by weighing, freeze drying, and then weighing again to determine the dry weight. Tissue samples for Ag, Cd, Cr, Cu, Ni, Pb, and Zn are digested using a nitric acid digestion according to MWRA Central Lab SOP #1195.0 (MWRA, 2004b). A 500 to 1000 mg aliquot of each homogeneous lyophilized sample is combined with 5 mL HNO_3 and 5 mL water in a Teflon microwave vessel. Samples are cold-digested in this acid mixture overnight. Samples are then microwave digested for approximately 30 minutes. After heating and cooling, samples are filtered through Whatman #541 filters and rinsed with Milli-Q water (final volume is 50 mL).

Samples for mercury analysis are digested according to MWRA Central Lab SOP #1236.0 (MWRA, 2006). A 200 mg lyophilized aliquot is cold-digested with 15 mL dilute HNO_3 and H_2SO_4 overnight. Samples are heated in a 58^{o}C waterbath for 1 hour, and then heated again at 80^{o}C for an additional 30 minutes. Cooled samples are further oxidized with $KMnO_4$ and $K_2S_2O_8$ overnight. Deionized water is added to bring the final sample volume to 50 mL. For analysis by ICP digestates are analyzed according to MWRA Central Lab SOP #1008.3 (MWRA, 2008a). Elements that are undetected by ICP may be analyzed by GFA for lower reporting limits according to MWRA Central Lab SOP #1150.3 (MWRA, 2008b). Results are reported as μg/g dry-weight.

Analysis of Mercury - The digested sample is mixed with a reducing agent in-line to release elemental Hg vapor. Mercury is quantified by atomic absorption at 254 nm. Results are reported as μg/g dry-weight. Samples are analyzed according to MWRA Central Lab SOP #1049.3 (MWRA, 2008c).

2.4.3 Organic Analyses

PAH Analysis - Sample extracts are analyzed for PAH compounds by gas chromatography/mass spectrometry (GC/MS) operating in the selected-ion-monitoring (SIM) mode, using a 30m Rtx-5 column (or equivalent) and an Agilent 5973 detector (or equivalent) according to MWRA Central Lab SOP #1030.3 (MWRA, 2004c). The PAH compounds are quantified using the internal standard method. Concentrations of the substituted PAH homologues are determined by summing the total area of each homologue and using the response factor of the parent PAH compound.

PCB/Pesticide Analysis – Pesticides and PCB congeners are analyzed by gas chromatography/mass spectrometry (GC/MS) operating in the selected-ion-monitoring (SIM) mode, using a 60m Rtx-5 column (or equivalent) and an Agilent 5973 detector (or equivalent) according to MWRA Central Lab SOP #1173.3 (MWRA, 2004d). Two separate analyses are performed, one to determine the pesticide compounds and one for the PCB congeners. Concentrations for all target analytes are determined using the internal standard method.

All PAH, PCB congener, and pesticide results are reported in micrograms per kilogram (μg/kg) on a dry weight basis, which is determined during metals analysis.

2.5 General Data Treatment and Reduction

This section describes the data reduction performed on 2012 Fish and Shellfish data, as well as historical data, as part of the MWRA Harbor and Outfall Monitoring Project.

Specifics of data handling are as follows:

- All 2012 chemical data were generated by MWRA's Department of Laboratory Services and loaded directly into the HOM database.
- Mussel data for all OS 60-day deployment locations (e.g. OS-M1, OS-M3, OS-M6) were averaged for both 2012 and time-series plots.
- All fish and shellfish data (2012 and historical) were extracted directly from the HOM database and exported into Excel files, where graphical presentations and statistical analyses were preformed.
- All laboratory duplicates for pre-1998 data were averaged for reporting and calculating. No laboratory duplicate data were entered for post-1998 data.
- Error bars in all graphical presentations represent standard errors. Means and sample size by station and year are reported in the Appendices.
- 1992 flounder collection consisted of three individual fish and a composite of seven fish. Results were calculated by treating the composite as seven individual fish and averaging those values with the values of the other three individual fish (i.e., [(7*val1 +val2 + val3 + val4)/10]). MWRA decided that the appropriate standard error and *n* values for this composite are null. This manipulation was done in the script used to query the data from the database and is not reflected in the EM&MS database.

- 1993 lobster selection consisted of two animals collected in June and one in August. Results were calculated by taking the average of these three animals (n = 3).
- Total PCB was calculated as the sum of twenty PCB congeners (Table 2-7).
- Total DDT was calculated as the sum of six DDT-related compounds: 2,4'-DDD, 4,4'-DDD, 2,4'-DDE, 4,4'-DDE, 2,4'-DDT, and 4,4'-DDT (Table 2-7).
- Total chlordane was calculated as the sum of four compounds: heptachlor, heptachlor epoxide, cis-chlordane, and trans-nonachlor (Table 2-7).
- Sums of PAHs were calculated using several groupings. The "Total PAH List" and the "Historical NOAA List" are presented in Table 2-8.
- In 1995, the individual five alkylated PAHs on the "Historical NOAA List" were not measured in mussels. Instead, the C1-, C2-, and C3-alkylated naphthalene homologue groups were quantified. To make 1995 results more comparable to the "Historical NOAA List", values for the individual alkylated naphthalene compounds were estimated using ratios of the individuals to their respective homologue groups from 1996 and 1997 data sets.
- All organic data (i.e., PAHs, PCBs, and pesticides) are surrogate recovery corrected.
- The "s" qualifier was used to indicate suspect data. Unless otherwise noted, only "s"-flagged data were excluded in calculations, tables, or graphs presented in this report.
- All non-detects used in calculations and trend analyses in this report were treated as zero.
- All data entered into the database are in dry weight units.
- Wet weight tissue concentrations were calculated from the wet/dry ratio and used in comparison to MWRA Contingency Plan Thresholds.
- Years in which composite samples were made up of only one animal were excluded from temporal plots.

Table 2-1. Planned and actual sampling locations for flounder survey

Station ID	Sampling Site	Number of Tows	Planned Locations		Actual Locations[1]	
			N Latitude	W Longitude	N Latitude	W Longitude
DIF	Deer Island Flats	3	42º20.4’	70º58.4’	42º20.9’	70º58.1’
NB	Off Nantasket Beach	4	42º17.6’	70º52.2’	42º17.4’	70º52.0’
OS	Outfall Site	2	42º23.1’	70º49.3’	42º23.1’	70º49.6’
ECCB	East Cape Cod Bay	1	41º56.2’	70º06.6’	41º56.8’	70º07.3’

[1]Based on an average of the Latitude and Longitude of several tows

Table 2-2. Planned and actual sampling locations for lobster survey

Station ID	Sampling Site	Planned Location		Actual Location	
		N Latitude	W Longitude	N Latitude	W Longitude
DIF	Deer Island Flats	42º20.4’	70º58.4’	42º20.4’	70º57.4’
OS	Outfall Site	42º23.1’	70º49.3’	42º22.6’	70º47.8’
ECCB	East Cape Cod Bay	41º56.2’	70º06.6’	41º55.9’	70º15.5’

Table 2-3. Planned and actual sampling locations for mussel survey

Station ID	Sampling Site	Planned Location		Actual Location	
		N Latitude	W Longitude	N Latitude	W Longitude
DIL	Deer Island Light	42º20.4’	70º57.2’	42º20.4’	71º57.2’
OS-M3	Outfall Site - Mussel Array 3	42º23.17’	70º47.47’	42°23.16’	70°47.46’
OS-M6	Outfall Site - Mussel Array 6	42º23.26’	70º46.99’	42°23.26’	70°46.99’
LNB	LNB“B” Buoy	42º22.67’	70º47.13’	42º22.7’	70º47.1’
IH	Boston Inner Harbor	42º21.5’	71º02.9’	42°21.5’	71°02.9’
SP	Pemaquid Point, ME	43º52.8’	69º31.1’	43º52.8’	69º31.1’

Table 2-4. Summary of mussel deployment scheme

Site	Description/ Location	Water Depth[a]	Cage Height Above Bottom	# Arrays	# Cages/Array	# Mussels/ Cage
DIL	Deer Island Light	2-5 m	<1-1.5m	3	2	60
OS	Outfall Site	33m	12m	4	3	60
LNB	"B" Buoy	33m	12 m	2	2	60
IH	Boston Inner Harbor	8-11m	1.5-4.5m[b]	2	2	60

[a] Arrays rise and fall with tide, so that they are at a constant depth below the water surface.
[b] Based on historical data.

Table 2-5. Summary of chemical analyses performed

Sample Type	Number of Samples	Metals (1) (other than Hg and Pb)	Hg	Pb	PCBs	PAHs	Pesticides	Lipids
Flounder Meat	12	NR	*	NR	*	NR	*	*
Flounder Liver	12	*	*	*	*	*	*	*
Lobster Meat	9	NR	*	NR	*	NR	*	*
Lobster Hepatopancreas	9	*	*	*	*	*	*	*
Mussel Tissue	24	NR	*	*	*	*	*	*

*Targeted for Analysis; NR = Not Required
(1) Additional metals: Ag, Cd, Cr, Cu, Ni, and Zn

Table 2-6. Summary of analytical methods.

Parameter	Unit of Measurement	Method	Reference
Organic Analyses			
Organic Extraction	NA	Tissuemize/Methylene Chloride	MWRA (2004a), SOP 1189.0
Polycyclic Aromatic Hydrocarbons (PAH)	ng/g dry wt.	GC/MS-SIM	MWRA (2004c), SOP 1030.3
Polychlorinated Biphenyls (PCB)/Pesticides	ng/g dry wt.	GC/MS-SIM	MWRA (2004d), SOP 1173.3
Metals Analyses			
Digestion: Ag, Cd, Cr, Cu, Ni, Pb, Zn	NA	Microwave digestion Nitric acid	MWRA (2004b), SOP 1195.0
Digestion: Hg	NA	Nitric acid, sulfuric acid	MWRA (2006), SOP 1236.0
Analysis: Ag, Cd, Cr, Cu, Ni, Pb, Zn	µg/g dry wt	ICP AES, GFA as needed	MWRA (2008a, b), SOP 1008.3, 1150.3
Analysis: Hg	µg/g dry wt	CVA	MWRA (2008c), SOP 1049.3
Ancillary Parameters			
Lipids	% by dry weight	Gravimetric	MWRA (2004a), SOP 1189.0
Dry Weight	% by dry weight	Freeze drying	MWRA (2004b), SOP 1195.0

Table 2-7. Specific chemical analytes

Chemical Analytes	
Trace Metals[a]	Polynuclear Aromatic Hydrocarbons (PAHs) (continued)
Ag Silver	C_1-Phenanthrenes/anthracenes
Cd Cadmium	C_2-Phenanthrenes/anthracenes
Cr Chromium	C_3-Phenanthrenes/anthracenes
Cu Copper	C_4-Phenanthrenes/anthracenes
Hg Mercury[b,d]	Dibenzothiophene
Ni Nickel	C_1-dibenzothiophenes
Pb Lead[d]	C_2-dibenzothiophenes
Zn Zinc	C_3-dibenzothiophenes
Polychlorinated biphenyls (PCBs)[c,d]	Fluoranthene
2,4'-Cl_2(8)	Pyrene
2,2',5-Cl_3(18)	C_1-fluoranthenes/pyrenes
2,4,4'-Cl_3(28)	C_2-fluoranthenes/pyrenes
2,2',3,5'-Cl_4(44)	C_3-fluoranthenes/pyrenes
2,2',5,5'-Cl_4(52)	Benzo[*a*]anthracene
2,3',4,4'-Cl_4(66)	Chrysene
3,3',4,4'-Cl_4(77)	C_1-chrysenes
2,2'4,5,5'-Cl_5(101)	C_2-chrysenes
2,3,3',4,4'-Cl_5(105)	C_3-chrysenes
2,3',4,4'5-Cl_5(118)	C_4-chrysenes
3,3',4,4',5-Cl_5(126)	Benzo[*b*]fluoranthene
2,2',3,3',4,4'-Cl_6(128)	Benzo[*k*]fluoranthene
2,2',3,4,4',5'-Cl_6(138)	Benzo[*a*]pyrene
2,2'4,4',5,5'-Cl_6(153)	Dibenzo[*a,h*]anthracene
2,2'3,3',4,4',5-Cl_7(170)	Benzo[g,h,i]perylene
2,2',3,4,4',5,5'-Cl_7(180)	Indeno[1,2,3-*c,d*]pyrene
2,2',3,4',5,5',6-Cl_7(187)	Perylene
2,2',3,3',4,4',5,6-Cl_8(195)	Biphenyl
2,2',3,3'4,4',5,5',6-Cl_9(206)	Benzo[*e*]pyrene
Decachlorobiphenyl-Cl_{10}(209)	Dibenzofuran
Polynuclear Aromatic Hydrocarbons (PAHs)[a,d]	Benzothiazole
Naphthalene	Pesticides[c,d]
C_1-naphthalenes	Hexachlorobenzene
C_2-naphthalenes	Lindane
C_3-naphthalenes	Endrin
C_4-naphthalenes	Aldrin
1-methylnaphthalenes[e]	Dieldrin
2-methylnaphthalenes[e]	Mirex
2,6-methylnaphthalenes[e]	Heptachlor
2,3,5-methylnaphthalenes[e]	Heptachlor epoxide
Acenaphthylene	cis-chlordane
Acenaphthene	trans-nonachlor
Fluorene	2,4'-DDD
C_1-fluorenes	4,4'-DDD
C_2-fluorenes	2,4'-DDE
C_3-fluorenes	4,4'-DDE
Phenanthrene	2,4'-DDT
1-methylphenanthrene[e]	4,4'-DDT
Anthracene	DDMU
	Lipids[c,d]

[a] Flounder liver; lobster hepatopancreas
[b] Flounder and lobster edible tissue
[c] Flounder edible tissue and liver; lobster edible tissue and hepatopancreas
[d] Mussel soft tissue
[e] Measured in mussel tissue in 1992–1994 and 1996–2009

Table 2-8. Summary of PAH lists of analytes

Total PAH List	"Historical" NOAA PAH List
Low Molecular Weight PAHs	**Low Molecular Weight PAHs**
1-methylnaphthalene*	1-methylnaphthalene
1-methylphenanthrene*	1-methylphenanthrene
2,3,5-trimethylnaphthalene*	2,3,5-trimethylnaphthalene
2,6-dimethylnaphthalene*	2,6-dimethylnaphthalene
2-methylnaphthalene*	2-methylnaphthalene
Acenaphthene	Acenaphthene
Acenaphthylene	Acenapthylene
Anthracene	Anthracene
Benzothiozole*	
Biphenyl	Biphenyl
C1-dibenzothiophenes	
C1-fluorenes	
C1-naphthalenes	
C1-phenanthrenes/anthracenes	
C2-dibenzothiophenes	
C2-fluorenes	
C2-naphthalenes	
C2-phenanthrenes/anthracenes	
C3-dibenzothiophenes	
C3-fluorenes	
C3-naphthalenes	
C3-phenanthrenes/anthracenes	
C4-naphthalenes	
C4-phenanthrenes/anthracenes	
Dibenzofuran	
Dbenzothiophene	
Fluorene	Fluorene
Naphthalene	Naphthalene
Phenanthrene	Phenanthrene
High Molecular Weight PAHs	**High Molecular Weight PAHs**
Benzo(a)anthracene	Benzo(a)anthracene
Benzo(a)pyrene	Benzo(a)pyrene
Benzo(b)fluoranthene	Benzo(b)fluoranthene
Benzo(e)pyrene	Benzo(e)pyrene
Benzo(g,h,i)perylene	Benzo(g,h,i)perylene
Benzo(k)fluoranthene	Benzo(k)fluoranthene
C1-chrysenes	
C1-fluoranthenes/pyrenes	
C2-chrysenes	
C2-fluoranthenes/pyrenes	
C3-chrysenes	
C3-fluoranthenes/pyrenes	
C4-chrysenes	
Chrysene	Chrysene
Dibenzo(a,h)anthracene	Dibenzo(a,h)anthracene
Fluoranthene	Fluoranthene
Indeno(1,2,3-c,d)pyrene	Indeno(1,2,3-c,d)pyrene
Perylene	Perylene
Pyrene	Pyrene

* **Not Included in Total PAH**

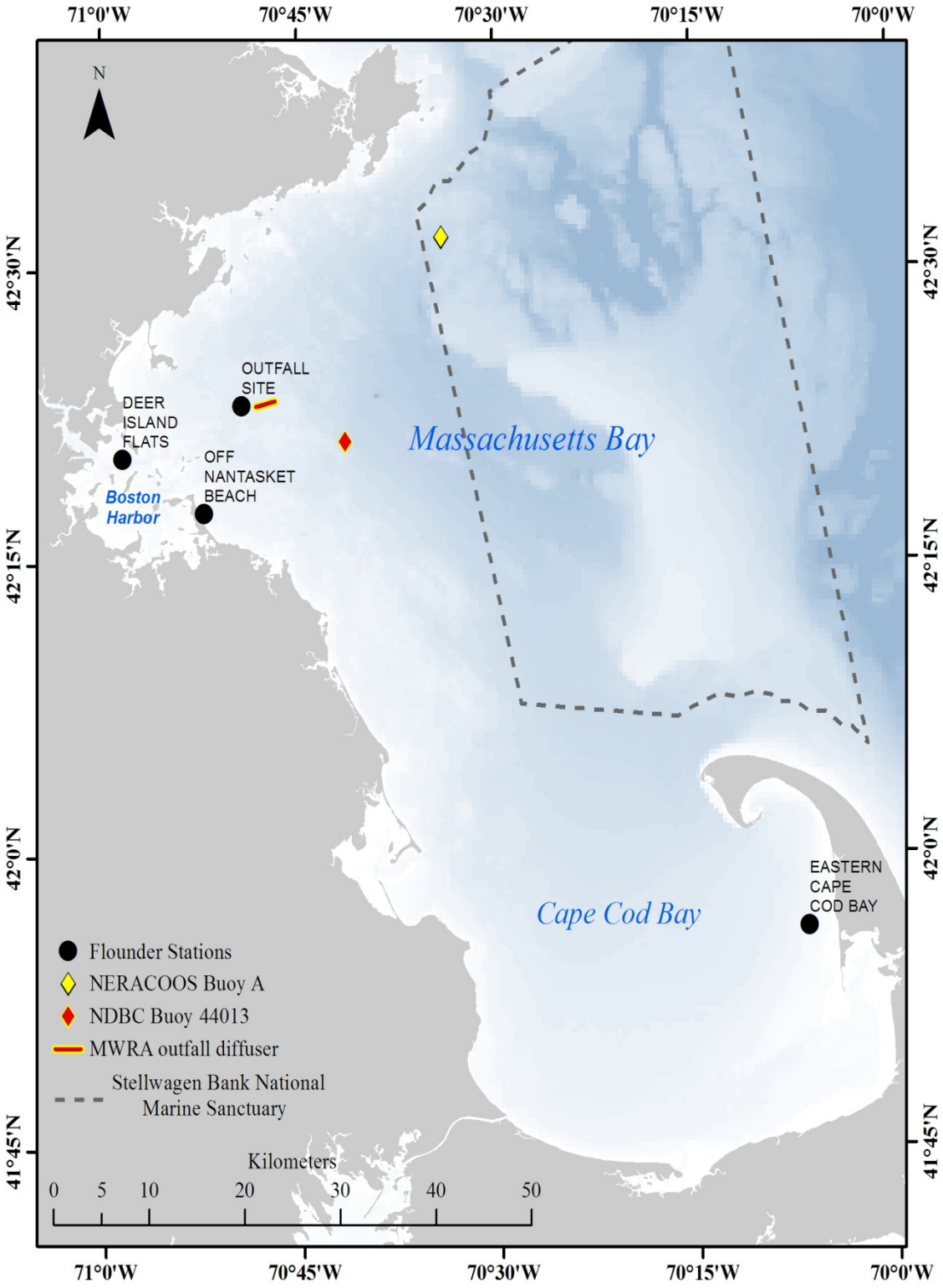

Figure 2-1. 2012 Flounder Monitoring Locations.

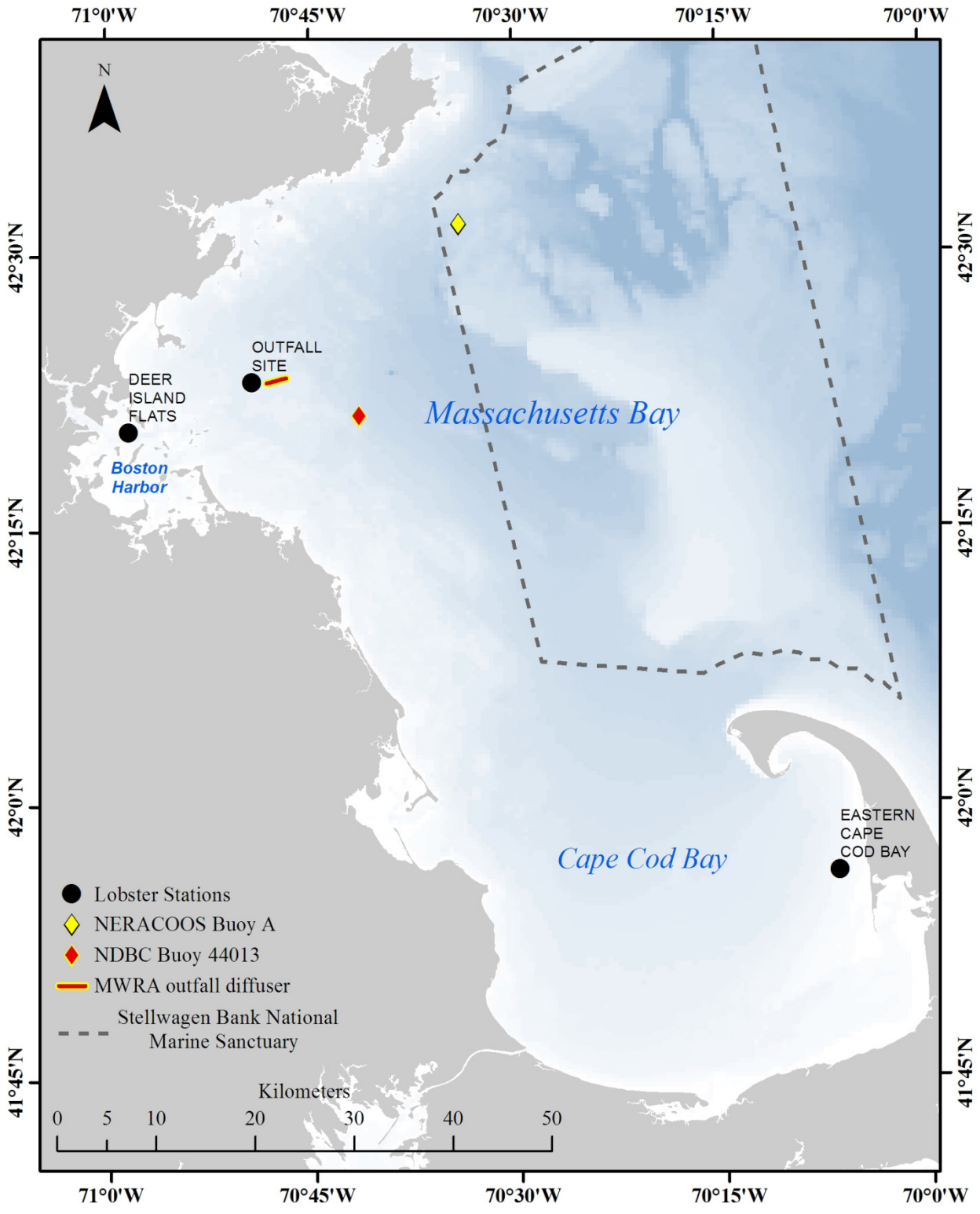

Figure 2-2. 2012 Lobster Monitoring Locations.

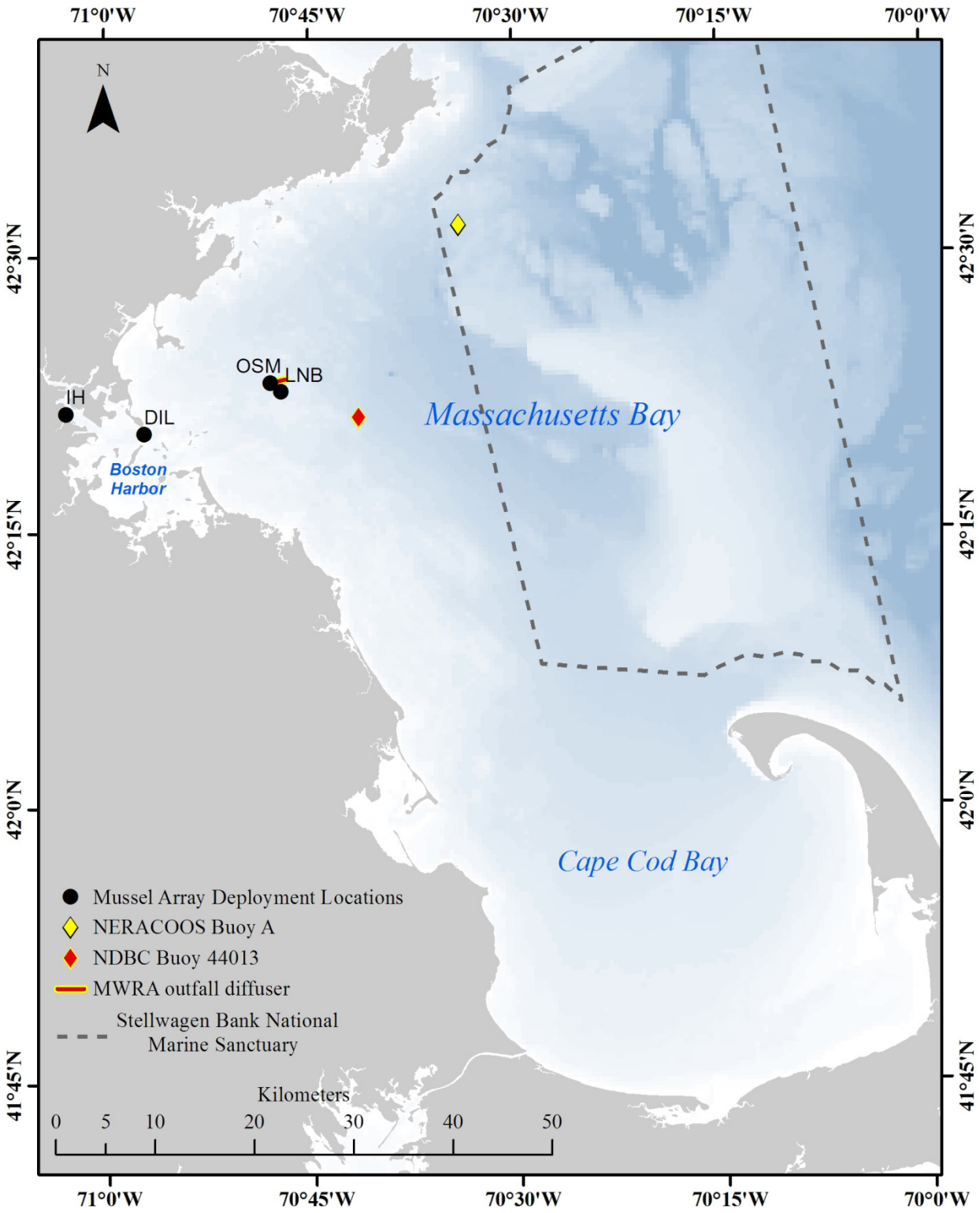

Figure 2-3. 2012 Mussel Deployment Locations.

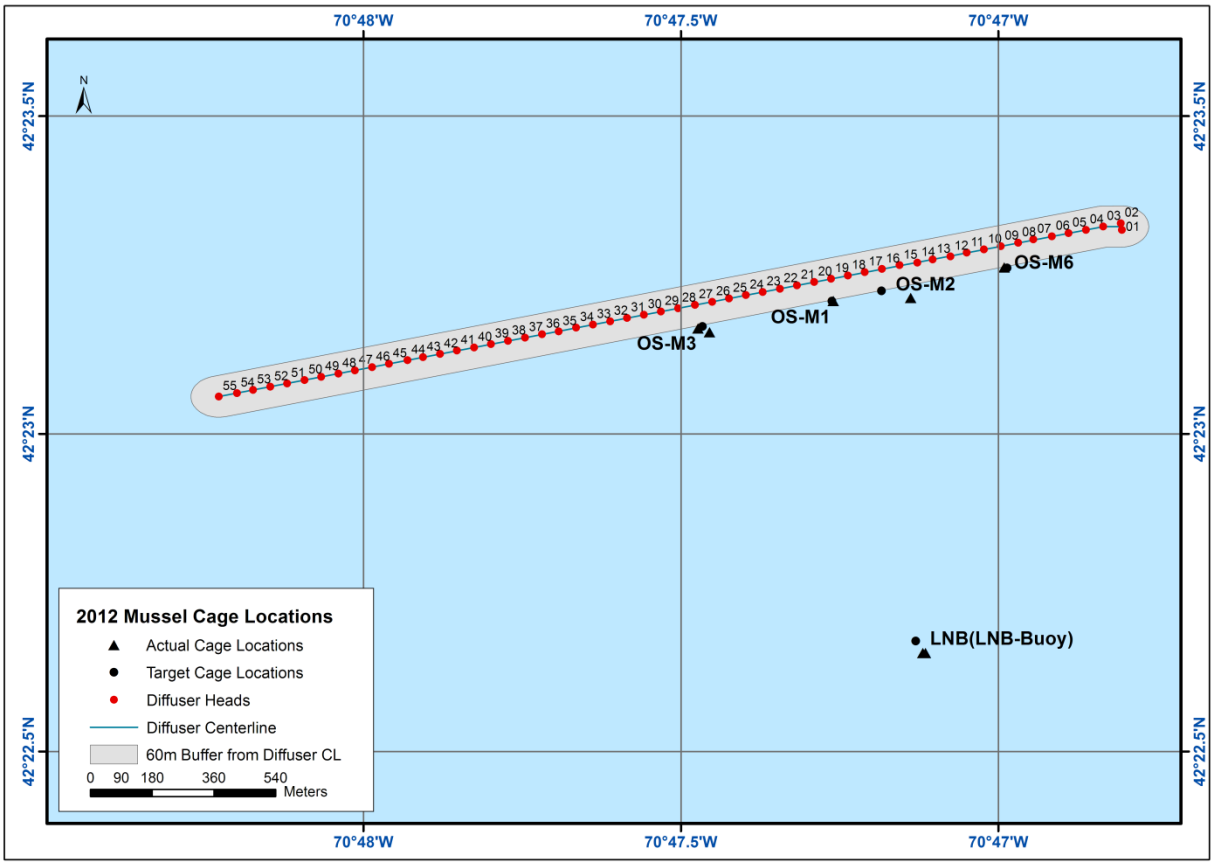

Figure 2-4. Mussel Deployment Locations at OS in 2012.

3.0 RESULTS

3.1 Winter Flounder

3.1.1 Fish Collected

Winter flounder, each a minimum 30 cm in length, were collected between April 24 and 26, 2012 at four stations in the study area (Figure 2-1**).** The catch per unit effort (CPUE), defined as the number of fish obtained per minute of bottom trawling time, is displayed in Figure 3-1. For the third consecutive year the 2012 CPUE at OS and NB remained near the low end of their historical ranges. In contrast for the fourth consecutive year CPUE at Cape Cod Bay was high compared to its historical range. CPUE at DIF was the highest since its peak of over 3 fish per minute in 2005. However, that value is still well below the 10 fish per minute reported in the late 1980's (pers. Obs. – M. Moore).

3.1.2 Physical Characteristics

Mean age, total length, and weight of the winter flounder collected at the four stations are provided in Table 3-1. In 2012 the numerically oldest, longest, and heaviest fish were collected from DIF and ECCB. Fish from ECCB were noticeably older than the long-term average at this station, but younger than the historical high of the 2011 catch. At all other stations the average age was very close to the historical mean (Figure 3-2).

In 2012 average fish weights were less than the historical average at all stations, but only at OS were they substantially lower (Figure 3-3). As in many past years fish collected at DIF were heavier than at other locations even though they were on average no older. Another factor besides age that contributes to determination of average weight is the proportion of female fish collected, since females reach a larger adult size than males (Pereira *et al*. 1999). For the fourth consecutive year the percent of female fish was relatively low (<80%) at OS (Figure 3-4) which likely contributed to the very low average weight for fish collected at this station.

3.1.3 External Condition

The prevalence of external abnormalities (*i.e*., bent fin ray, fin erosion, blind side ulcers, lymphocystis) at each station is presented in Table 3-2. Fish collected off Deer Island were more likely to be affected by bent fin ray and fin erosion than those collected from other stations. Fin erosion is a useful parameter in detecting sub-optimal water quality conditions (Bosakowski and Wagner 1994) and its prevalence has often been higher at DIF than at other stations (Figure 3-5). The Outfall Site again had the highest prevalence of lymphocysits, a virus caused swelling of epithelial cells.

The prevalence of blind side ulcers, first observed in 2003 (see Moore *et al.* 2004) was low again in 2012. As in several past years the highest prevalence was at OS. While we do not understand the cause of these ulcers there may be some indirect link between the ulcer prevalence and winter bottom water temperature. Figure 3-6 suggests that the highest prevalence of ulcerated fish at OS is found when the early February bottom water temperatures at a water column monitoring site close to the flounder collection site are coldest. Future monitoring will determine if this relationship continues.

3.1.4 Liver Histopathology

Winter flounder have historically been used as a sentinel of chemical contamination impact on the marine environment. Chang *et al*. (1998) and Myers *et al.* (1998) discuss the positive relationships observed in the NOAA National Benthic Surveillance Program between the prevalence of liver neoplasm and pre-neoplasm and concentrations of toxic contaminants. The high prevalence of liver neoplasm in Boston

Harbor winter flounder reported by Murchelano and Wolke (1985) was one of several important findings which resulted in the Boston Harbor "clean-up" project.

In 2012 neoplasm were again not observed at any of the stations sampled (Table 3-3). Neoplasia has always been rare or absent from all sites other than DIF with none ever detected at OS. Since 1995 only one fish at DIF has been observed to have a liver neoplasm.

Also displayed in Table 3-3 is the prevalence of other liver abnormalities enumerated in 2012. Hydropic vacuolation (HV), lesions also positively associated with chemical contaminants, have been one of the principal abnormalities monitored throughout this program. HVs range from the least severe centrotubular hydropic vacuolation (CHV) to the most severe focal HV. As in past years the prevalence of CHV and the moderately severe tubular HV was highest in fish collected from the harbor. However, the long term trend at DIF, as well as at NB and OS is a decrease in the prevalence of CHV (Figure 3-7). Fish collected from Cape Cod have shown a consistently low prevalence since the inception of this program.

Focal HV were absent at all stations in 2012 (Table 3-3). Both biliary proliferation and macrophage aggregation were highest in fish from OS and lowest in fish from ECCB. Neither abnormality has shown meaningful long-term trends at any station.

3.1.5 Tissue Contaminant Levels

In this section the body burdens of selected contaminants measured in 2012 are presented and discussed within the context of historical trends. The trend plots begin in the year 1995 when the number of organisms per replicate increased from one to five. Beginning in 2004 contaminants have been measured every third year at all stations except Nantasket Beach (NB) where they have been measured every three years since the inception of this program. All winter flounder body burden data collected during this program are presented in Appendices A and B.

Edible fillet - As in past years fillets from fish collected off of Deer Island had the highest concentrations of organic contaminants and those from Cape Cod Bay the lowest. The 2012 results for total chlordane (the sum of alpha chlordane, trans-nonachlor, heptachlor, and heptachlor epoxide) and 4-4 DDE (the predominant DDT breakdown isomer) continued a downward trend (Figures 3-8 and 3-9). The observed decreases appear not to be related to the diversion of the MWRA's effluent into Massachusetts Bay in September 2000. A Before/After Control Impact (BACI) study concluded that based upon data through 2006 changes observed in the harbor and Massachusetts Bay were not statistically different from those at the control station in Eastern Cape Cod Bay (Kane-Driscoll *et al*. 2008). These region-wide decreases were anticipated given that these compounds have been banned in the United States - DDTs since 1972 and chlordane since 1988. The similarity of the station trends may also reflect subtle differences in analytical techniques and reporting limits employed by the several laboratories used during this program. For example the PCB and pesticide analyses conducted since 2009 used a technique, MS-SIM, which eliminates interferences that may have been quantified in earlier years. Also, there is evidence from the National Marine Fisheries Service that flounder in the Gulf of Maine have been wintering in deeper, offshore waters (Nye *et al*. 2009). This suggests the possibility that the fish collected in this program may be spending more time in less contaminated environments which would result in a decrease in exposure to bio-accumulating contaminants.

Surprisingly, in 2012 tissue concentrations of PCBs in fish collected at DIF, NB, and OS were among the highest observed during this program (Figure 3-10). Only at ECCB did tissue concentrations remain low.

In 2012 mercury concentrations in fillet were nearly identical at all four stations (Figure 3-11). No temporal trends are evident at any of the stations.

Liver - Spatial and temporal patterns of organic contaminants in flounder liver tend to be similar to those in the fillets. In 2012, despite an unexpected increase in total chlordane at DIF and to a lesser extent at NB, the decades long decrease in total chlordane concentration at all stations continues (Figure 3-12). Total PCBs in livers collected from DIF also increased in 2012, as they did for PCBs in DIF meat tissue (Figure 3-13). Total DDTs and LMW PAHs also increased from 2009 to 2012 at several stations (Appendix B).

Unlike for organics, the body burdens of metals have had ambiguous temporal trends and the spatial pattern has been less predictable (see Appendix B). For example, body burdens of silver have historically been highest in fish from OS and often lowest in fish from DIF (Figure 3-14). The most recent data now suggests that there may be a downwards trend at OS and possibly at NB with no apparent trends at the other two stations. In 2012 the highest concentrations were observed at ECCB. Another example is nickel (Figure 3-15) for which there is a hint of an increase at several stations, especially at OS. As has often been the case, DIF had one of the lower concentrations. These counter intuitive results probably reflect less on the total concentrations of metals in the respective environments and more on their bio-availability. Solids and organic matter will bind up metals in non-bioavailable forms decreasing their actual bioaccumulation potential. Inshore environments, like Boston Harbor have higher levels of these binding compounds than the environment near the Outfall or Cape Cod Bay (see Hunt *et al*. 2006).

Myers *et al.* (1998) and Chang *et al*. 1998) suggest that organics, especially pesticides and PAHs, show the strongest positive association with neoplasms and cellular vacuolations. The similarity in trend line of CHVs and some organic contaminants in our study are also suggestive that organics rather than metals are more closely associated with the liver vacuolations.

3.2 Lobster

3.2.1 Size, Sex, and External Conditions

Weight, carapace length, and sex were determined for 21 lobsters from each of the three sites (Table 3-4). The average carapace length was identical at all locations which was typical of historical conditions. Weights were collected only from Deer Island lobsters with the average weight in 2012 only slightly above the historical average. Lobsters at all three locations were predominantly female. This was typical of historical DIF and OS results but differed markedly from past ECCB data when males predominated. Visual inspection of the lobsters indicated one animal from OS had signs of shell erosion. No other deleterious external conditions were reported at any location.

3.2.2 Tissue Contaminant Levels

In this section the body burdens of selected contaminants measured in 2012 are presented and discussed within the context of historical trends. The trend plots present data beginning in 1994 when the number

of organisms per replicate increased from one to five. All lobster body burden data collected during this program can be found in Appendices C and D.

Edible Meat - Since the inception of this program organic contaminants in lobster meat have tended to be highest in animals collected off Deer Island and lowest in those from Cape Cod Bay. In 2012 while lobsters from off Deer Island still had the highest concentrations of total chlordane (Figure 3-16), total DDT (Figure 3-17), and total PCB (3-18), concentrations of these compounds at all three sites were at or near historic lows. For both total chlordane and total DDT we continue to observe an apparent long-term decrease at all stations. The stark decrease in 1995 total chlordane at all three stations seems likely an unresolved analytical issue and likely misrepresents actual tissue concentrations. A BACI analysis reported in Kane-Driscoll *et al.* (2008) concluded that these changes were not due to relocation of the outfall since decreases in the harbor and Massachusetts Bay were statistically no different than changes at the control station in Cape Cod Bay.

In 2012 PCB concentrations at all three stations were among the lowest observed during this program. However, PCBs in lobster meat have shown no clear trend at any of the three stations (Figure 3-18). In 2009 PCBs in Deer Island lobster meat had the highest average observed during this program. Only one of the three replicates was high, a phenomenon similar to that observed at OS in 2003. At that time analysis of tissue from each individual lobster demonstrated that the high level of PCBs in the replicate came from only one of the five lobsters in the composited replicate (Wisneski *et al.* 2004). Given that adult lobster can be highly migratory, moving inshore in the early summer and offshore in the fall, it is difficult to assess with certainty where a given lobster has been exposed to anthropogenic contaminants (see Mitchell *et al.* 1998 and Lavalli and Kropp 1999 for further discussion of lobster biology and migration).

There is some indication that mercury in lobster meat at all three stations may be trending lower since the late 1990's (Figure 3-19). In 2012 concentrations at the three sites were very similar.

Hepatopancreas - Unlike 2003 and 2009 when mean PCB concentrations at DIF and OS were high, 2012 levels were relativelyl low at all sites (Figure 3-20). Likewise, total chlordane (Figure 3-21) and DDT (Appendix D) concentrations were among the lowest observed during this program. In 2012 Dieldrin was not detected in tissue from any station (Appendix D). While High Molecular Weight (combusted) PAHs were at or near historic lows at OS and ECCB, concentrations in DIF lobsters were extremely high, 30% higher than in any past year (Appendix D). Low Molecular Weight (non-combusted) PAHs remained low at OS but were elevated at the other two stations. The elevations were in part due to apparent contamination of several compounds which was reflected in QA/QC analytic blanks.

Like for metals in flounder liver, the spatial and temporal pattern of metals in lobster hepatopancreas has been less predictable than for organic contaminants. Cadmium has tended to be highest around the Outfall and lowest in Boston Harbor (Figure 3-22), copper highest at either OS or DIF and lowest at Cape Cod Bay (Figure 3-23), and nickel highest at OS or ECCB and lowest in the harbor (Figure 3-24). In 2012 concentrations of cadmium, copper, lead and zinc (Appendix D) were the highest at OS and among the highest at any station since the program's inception. There is a possible suggestion of an upward trend at OS for all four metals. Concentrations of chromium and mercury were also highest at OS in 2012 but no trend is apparent (Appendix D). Nickel (Figure 3-24) also appears to be trending up in lobsters collected from OS but in 2012 concentrations at ECCB spiked to the highest level reported from any station during this program. In contrast silver concentrations at all stations were the lowest during the monitoring period.

3.3 Blue Mussel

3.3.1 Mussel Survival

Samples were successfully collected at all stations on August 12 (42-day retrieval) and August 30 (60-day retrieval). The only mortality recorded during either retrieval was the loss of 9% of the mussels collected at the Inner Harbor (IH) site on August 12 (Table 3-5).

3.3.2 Tissue Contaminant Levels

2012 Spatial Comparison – Blue mussels passively filter ambient waters and readily bio-accumulate contaminants in those waters, making them an excellent and commonly employed tool for assessing spatial patterns in water quality (O'Connor and Lauenstein 2006). For this reason the MWRA has used caged mussels as a "controlled experiment", deploying mussels from a clean environment at various locations, collecting them after a set period of time, and determining the extent of contaminant bioaccumulation.

The 2012 results were largely consistent with past years. Both mercury and lead were highest in mussels deployed at IH and lowest in mussels deployed south of the outfall at LNB (Figures 3-25 and 3-26). Mercury in OSM mussels was roughly equal to the background mussels, but lead was much lower.

Mussels at all stations bio-accumulated total PCBs with the largest increases in the harbor, especially at IH (Figure 3-27). Total chlordane was not detected in the source mussels (PE) but did bio-accumulate to similar levels at all sites except IH, where concentrations were much higher (Figure 3- 28). There was no apparent increase of DDTs at either of the sites near the effluent discharge. In contrast mussels from the harbor, especially IH had much higher DDT levels (Figure 3-29). Both Low Molecular Weight (LMW) and High Molecular Weight (HMW) PAHs were similar at all sites except IH where they were much higher (Figures 3-30 and 3-31).

In general the mussel results suggest increasing better water quality as we move from Boston's Inner Harbor to Outer Boston Harbor to Massachusetts Bay to Coastal Maine.

Inter-annual comparison – This study is intended to assess whether there have been changes in water quality either at the new (OS) or old (DIL) discharge locations as a result of the diversion of wastewater discharge in September of 2000. A BACI analysis based on data through 2006 suggested that after controlling for data from a control station in Cape Cod Bay (CCB) there had been an increase of lead, PCBs, chlordane, DDE, and HMW PAHs in mussels deployed at the Outfall Site. At Deer Island only chlordane had shown a significant decrease (Kane-Driscoll *et al.* 2008).

Total chlordane continued its decrease at DIL (Figure 3-32). While there were clear increases in chlordane in mussels deployed near the outfall in 2001-2003, concentrations are now lower than those observed prior to effluent diversion. Likewise PCB and DDT (Figures3-33 and 3-34) concentrations continued decreasing at all three sites and in 2012 were at the lowest levels observed since the inception of the program. Both Low (LMW) and High (HMW) Molecular Weight PAHs continued a decreasing trend in mussels deployed at DIL (Figure 3-35 and 3-36). At OS and LNB, LMW PAHs remained low in 2012, and HMW PAHs continued decreasing since the post-diversion peaks in 2001 to 2003.

3.4 Comparison to Thresholds

The U.S. Food and Drug Administration (FDA) has set action limits for the maximum tissue concentrations of specific contaminants in the edible portions of fish and fishery products. For the MWRA monitoring program, Caution and Warning thresholds have been set for tissue contaminant concentrations (organic and inorganic) and liver disease incidence (MWRA 2001a, MWRA 2001b). These thresholds are derived from either the FDA Action Limits, when available, or from the baseline mean of contaminant concentrations at OS. These two levels provide reference benchmarks for detecting adverse changes (and their potential human health risks) of the outfall discharge.

All thresholds for flounder fillet (Table 3-6) and lobster meat (Table 3-7) have been easily met since outfall start-up. While there were mussel threshold exceedances for total chlordane (2001) and PAH (2001, 2002, and 2003), there have been no exceedances since that time. In 2012 all thresholds were met (Table 3-8).

Table 3-1. Summary of physical characteristics of flounder collected in 2012

Station Name		DIF	NB	OS	ECCB
Sample size		50	50	50	50
Age (years)	Mean	4.9	4.3	4.7	5.0
	Std. Dev.	0.9	0.9	0.8	1.2
Total Length (mm)	Mean	377.1[a]	352.9	336.5	360.0
	Std. Dev.	30.8	31.4	27.6	36.8
Weight (g)	Mean	607.1	501.6	447.2	529.0
	Std. Dev.	140.8	130.0	126.6	159.7

Std. Dev. = Standard Deviation
[a] N=49

Table 3-2. Prevalence (%) of external flounder conditions

Station	Bent Fin Ray	Fin Erosion	Blind Side Ulcers	Lymphocystis
DIF	22	44	0	34
NB	8	22	4	20
OS	2	8	6	54
ECCB	20	26	0	26

Sample size – 50 fish at each station

Table 3-3. Prevalence (%) of liver abnormalities

Station		DIF	NB	OS	ECCB
N		50	50	50	50
Abnormality	Neoplasm	0	0	0	0
	Focal HV	0	0	0	0
	Tubular HV	14	8	6	0
	Centrotubular HV	24	20	12	6
	Macrophage Aggregation	62	60	70	50
	Biliary Proliferation	14	8	26	4

Table 3-4. Mean length and weight, and % females of lobsters collected in 2012

Parameter	DIF			OS			ECCB		
	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N
Carapace Length (mm)	88	2	21	88	3	21	88	3	21
Weight (g)	526	53	21	ND	ND	21	ND	ND	21
Percent female (%)	52		21	71		21	71		21

ND = No Data

Table 3-5. 2012 caged mussels survival data

Collection	Site	Total Mussels	Dead Mussels	Survival Rate
42-day	IH	100	9	91%
	DIL	100	0	100%
	OS	100	0	100%
	LNB	100	0	100%
60-day	IH	100	0	100%
	DIL	100	0	100%
	OS	300	0	100%
	LNB	100	0	100%

Table 3-6. Comparison of 2012 flounder fillet results to MWRA Caution Levels

	Caution Threshold		2012 Results
Chlordane	**484**	**ng/g lipid**	**73.6**
DDT	**1552**	**ng/g lipid**	**369**
Dieldrin	**127**	**ng/g lipid**	**0**
PCB	**1000**	**ng/g wet**	**41.3**
Mercury	**0.5**	**ng/g wet**	**0.065**
Liver Disease	**44.9**	**%**	**12**

Table 3-7. Comparison of 2012 lobster meat results to MWRA Caution Levels

	Caution Threshold		2012 Results
Chlordane	**150**	**ng/g lipid**	**0**
DDT	**683**	**ng/g lipid**	**45.1**
Dieldrin	**322**	**ng/g lipid**	**0**
PCB	**1000**	**ng/g wet**	**8.57**
Mercury	**0.5**	**ng/g wet**	**0.115**

Table 3-8. Comparison of 2012 mussel results to MWRA Caution Levels

	Caution Threshold		2012 Results
Chlordane	**205**	**ng/g lipid**	**40.5**
DDT	**483**	**ng/g lipid**	**33.0**
Dieldrin	**50**	**ng/g lipid**	**0**
PAH	**2160**	**ng/g lipid**	**1020**
PCB	**1000**	**ng/g wet**	**2.4**
Mercury	**0.5**	**ng/g wet**	**0.016**
Lead	**2.0**	**ng/g wet**	**0.216**

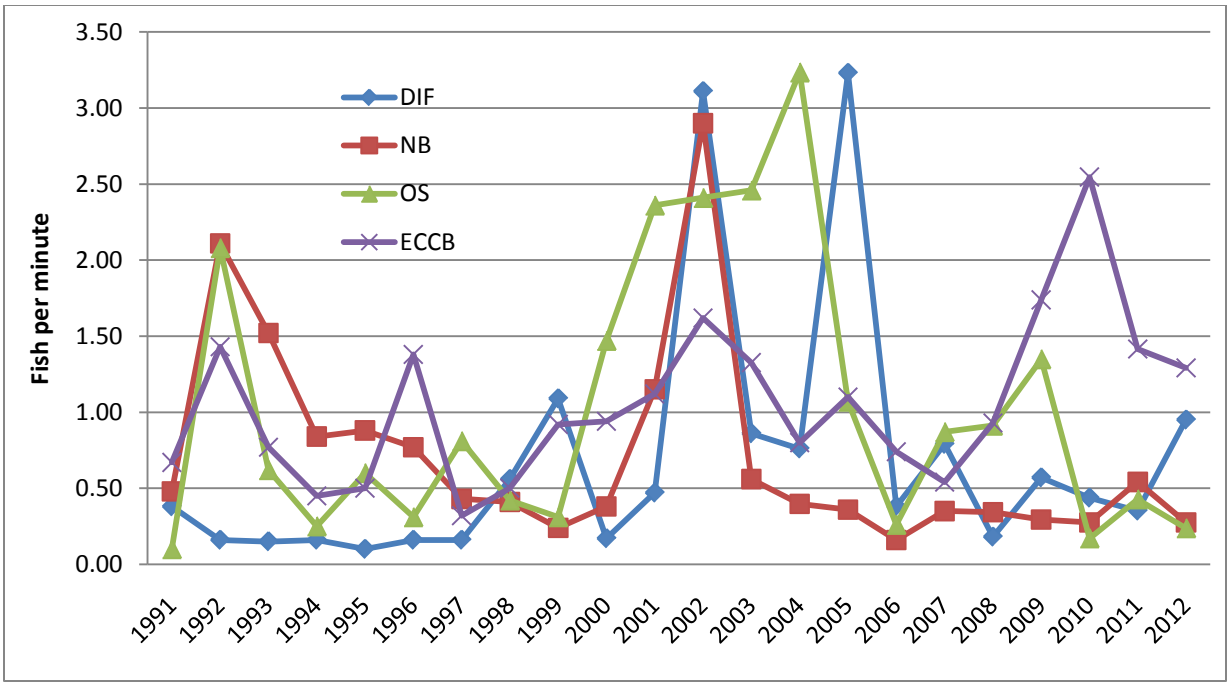

Figure 3-1. Flounder Catch Per Unit Effort (1991–2012)

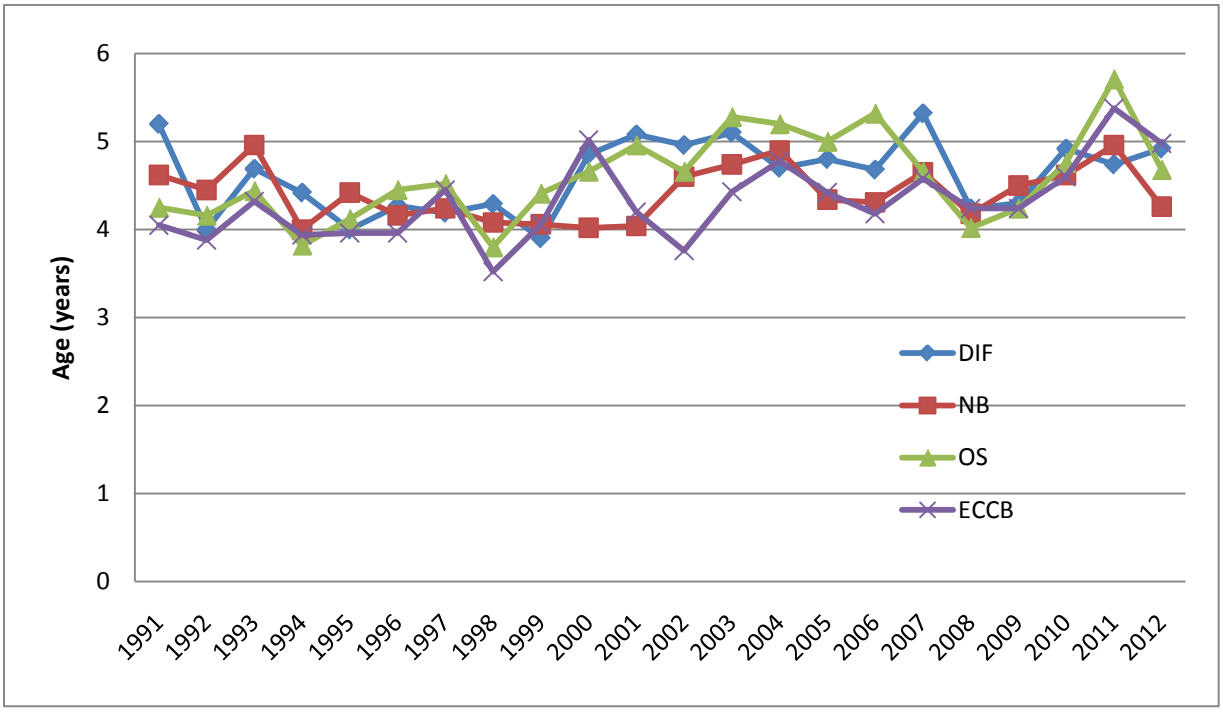

Figure 3-2. Average flounder age (1991-2012)

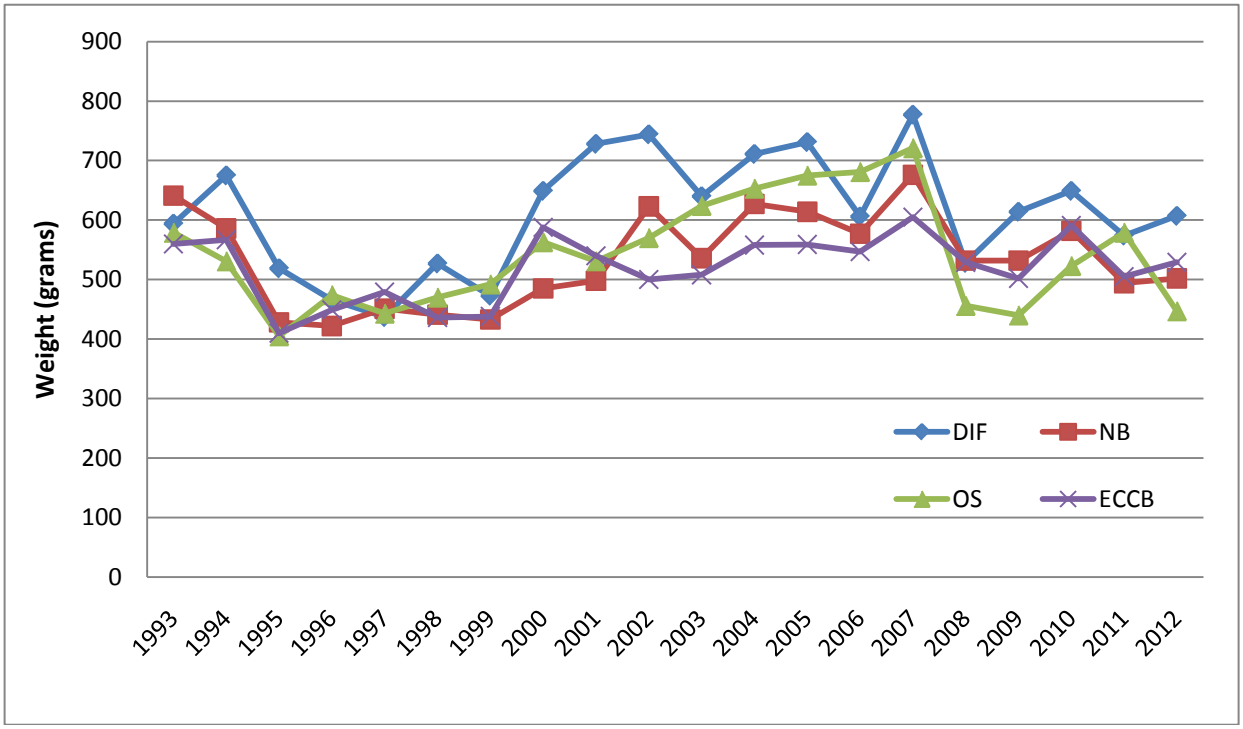

Figure 3-3. Average flounder weight (1993-2012)

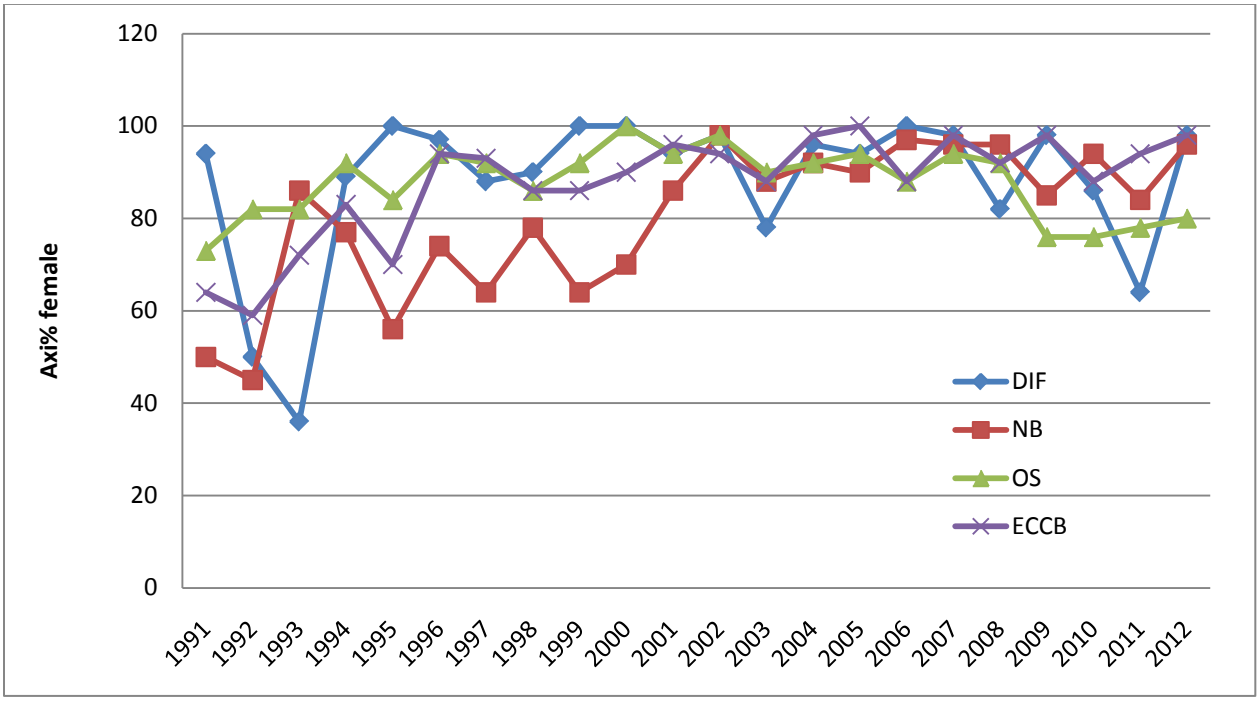

Figure 3-4. Percentage of female flounder (1991-2012)

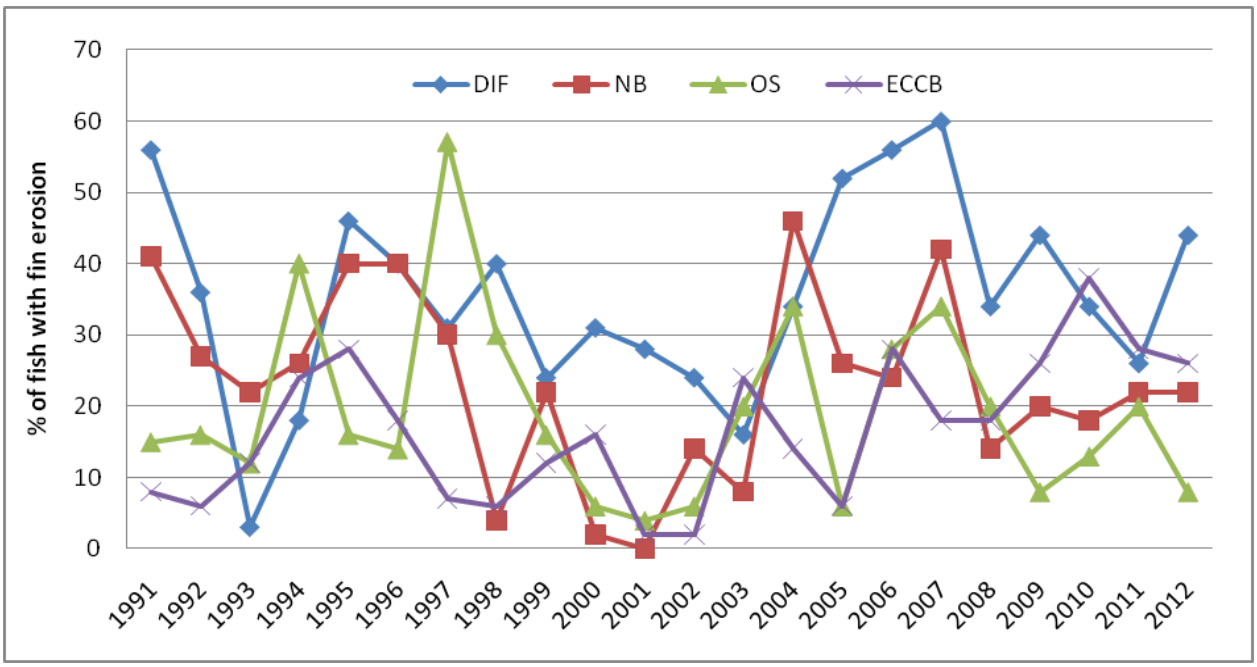

Figure 3-5. Prevalence (%) of flounder fin erosion (1991-2012)

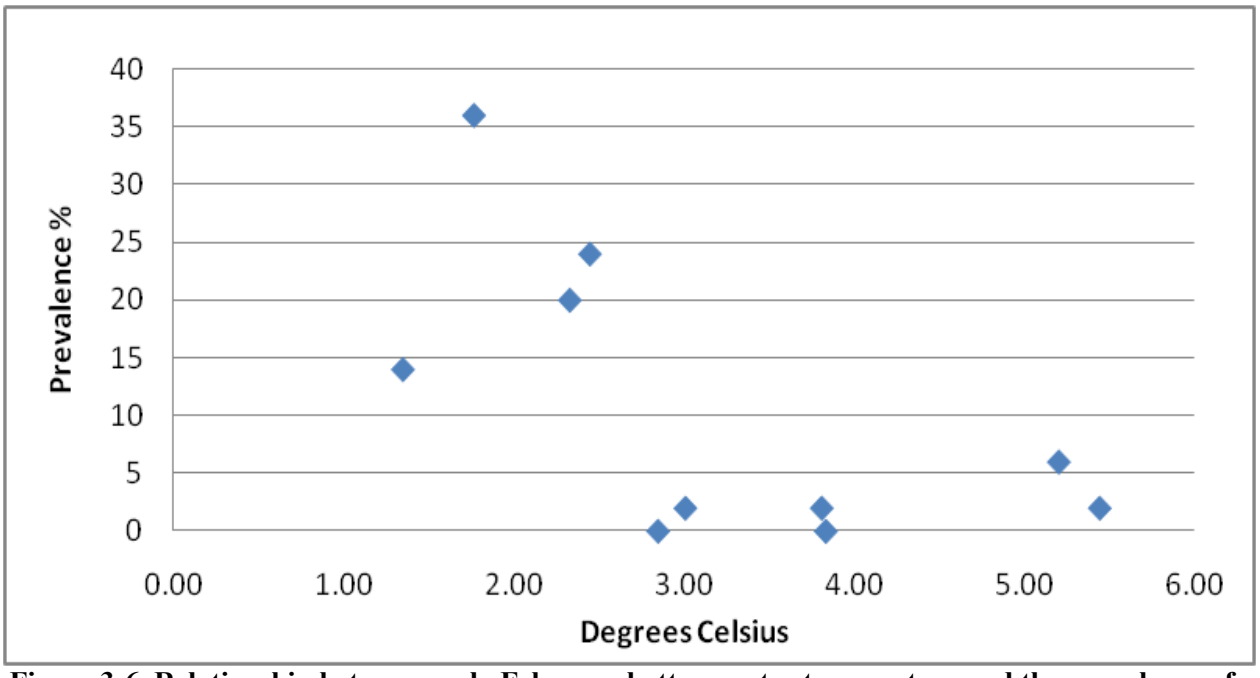

Figure 3-6. Relationship between early February bottom water temperature and the prevalence of fish with blind-side ulcers at OS (2003-2012).

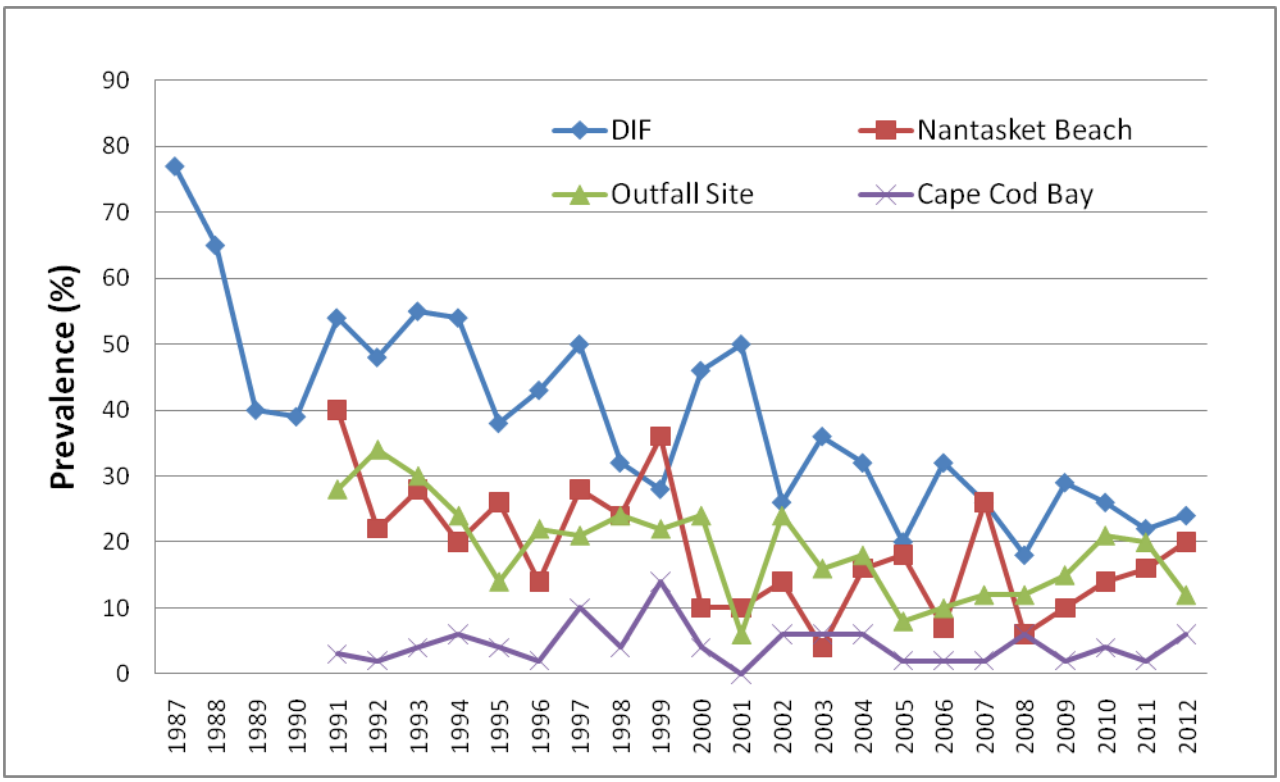

Figure 3-7. Prevalence of flounder CHV (1987-2012)

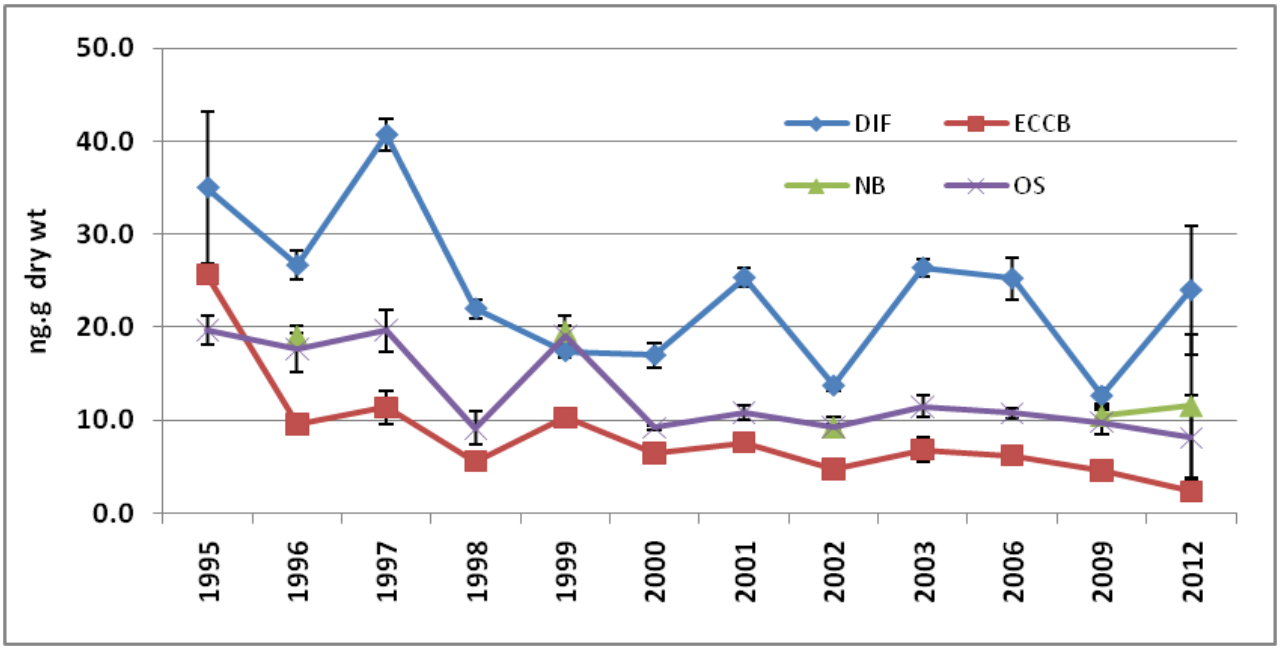

Figure 3-8. Total chlordane in flounder fillet (1995-2012)

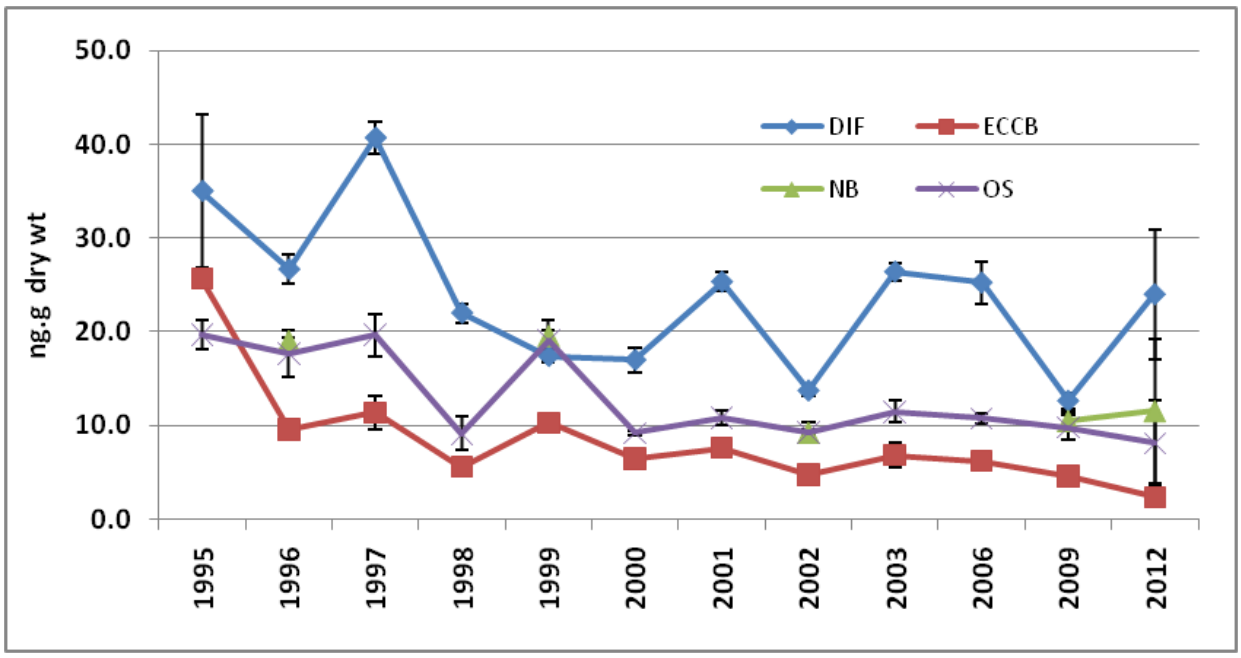

Figure 3-9. 4-4 DDE in flounder fillet (1995-2012)

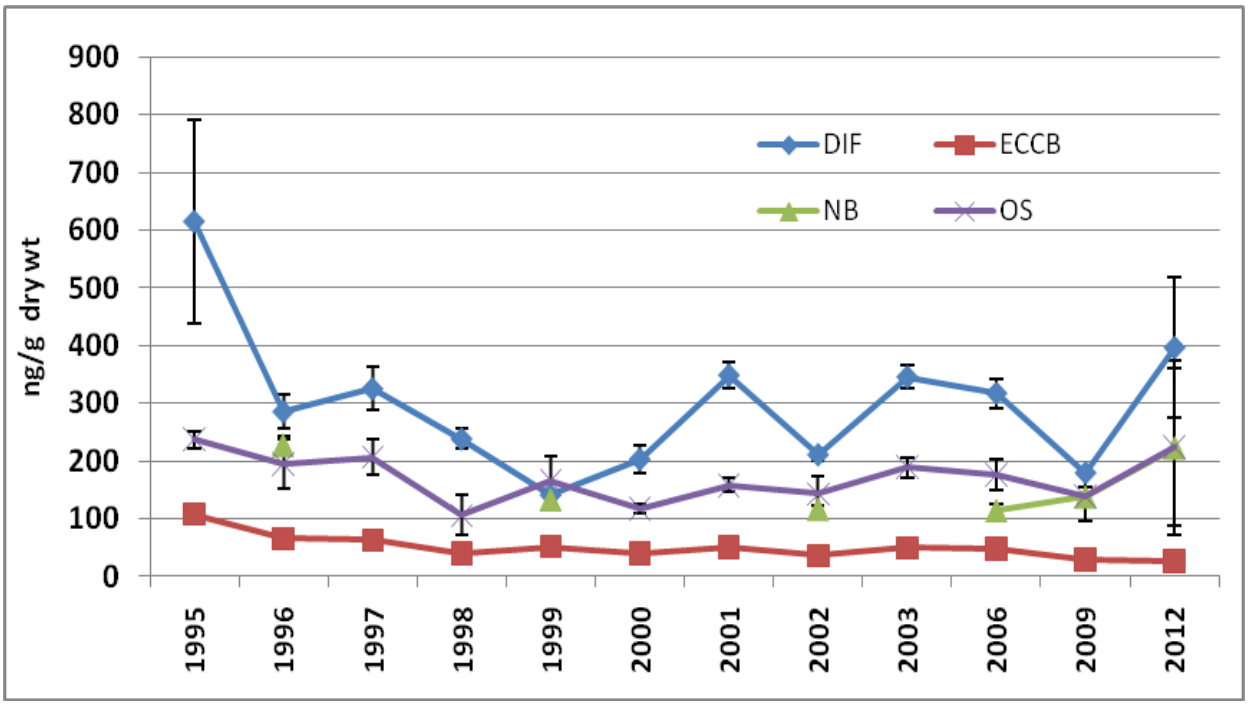

Figure 3-10. Total PCB in flounder fillet (1995-2012)

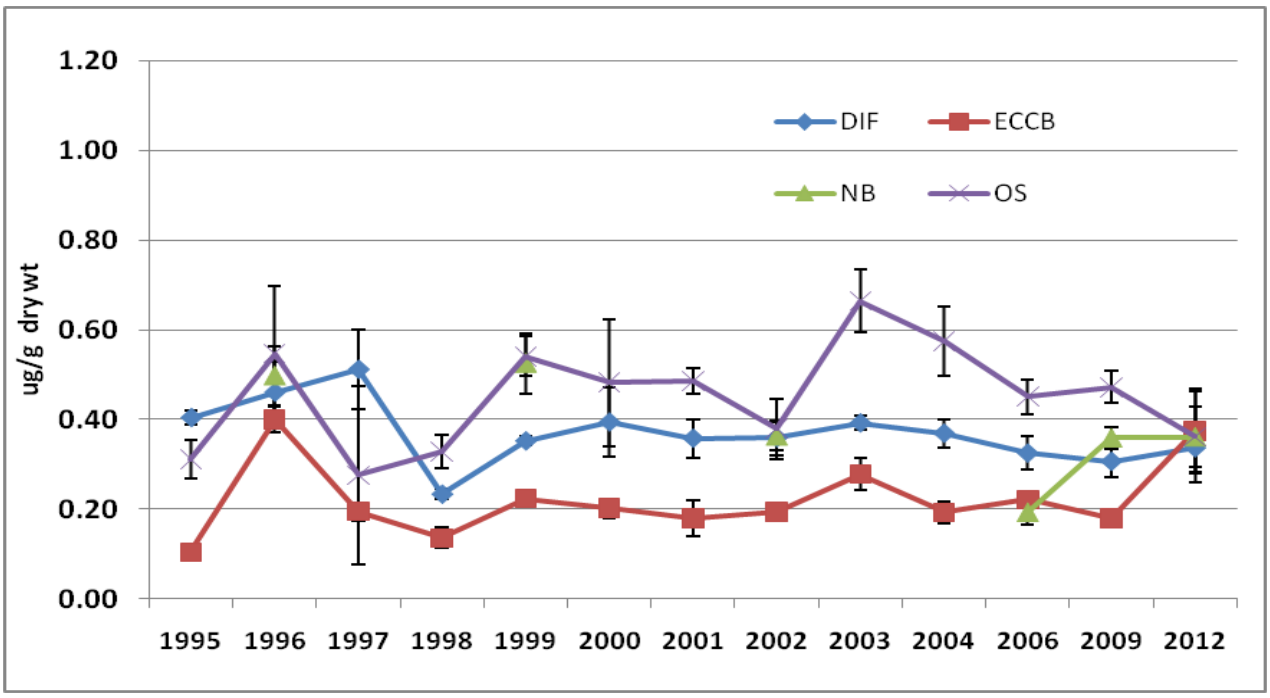

Figure 3-11. Mercury in flounder fillet (1995-2012)

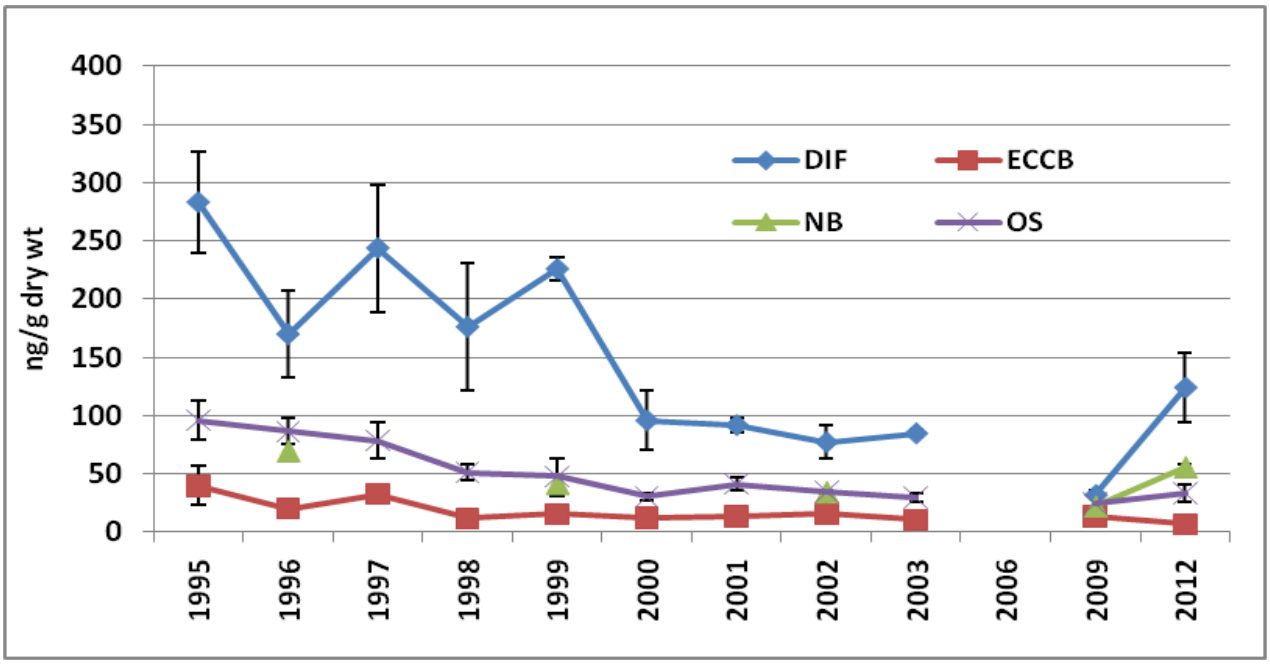

Figure 3-12. Total Chlordane in flounder liver (1995-2012)

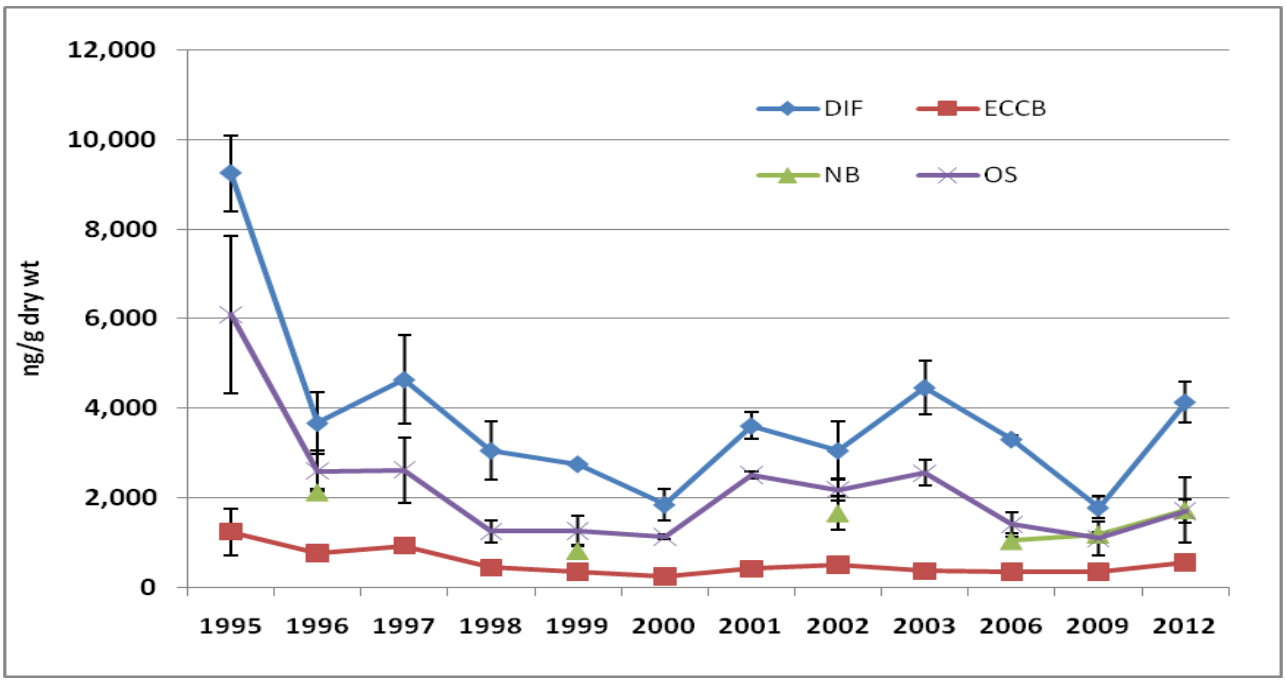

Figure 3-13. Total PCBs in flounder liver (1995-2012)

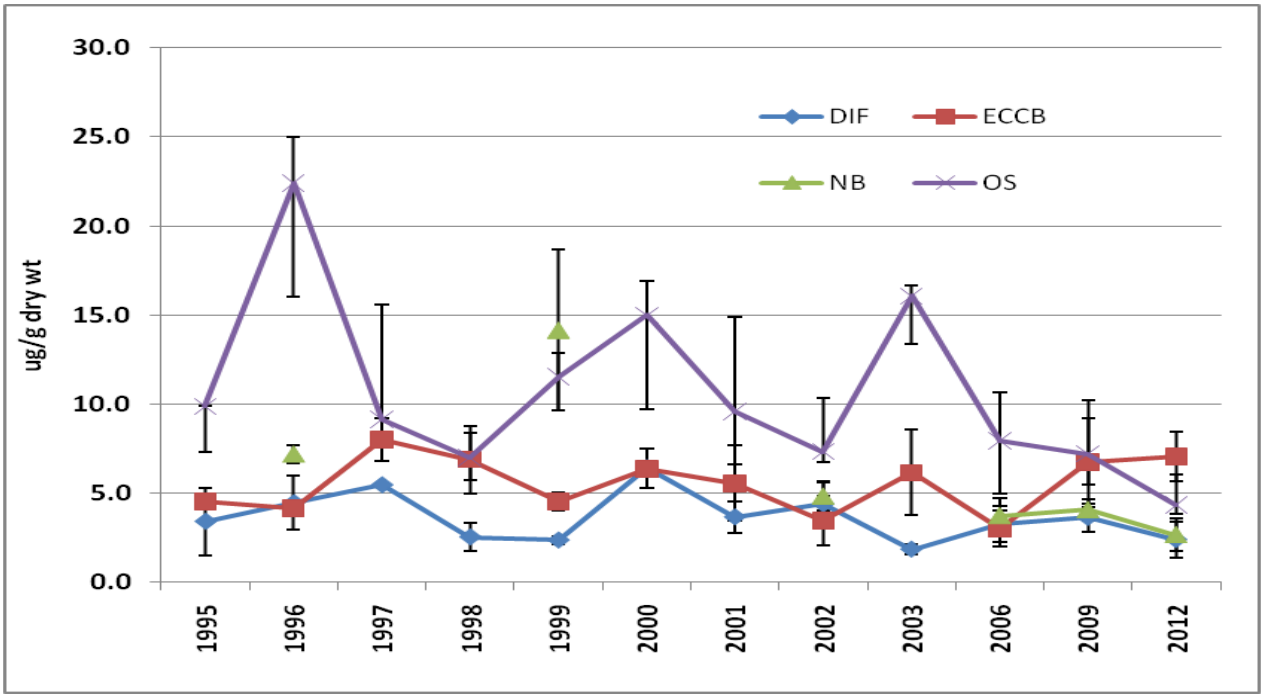

Figure 3-14. Silver in flounder liver (1995-2012)

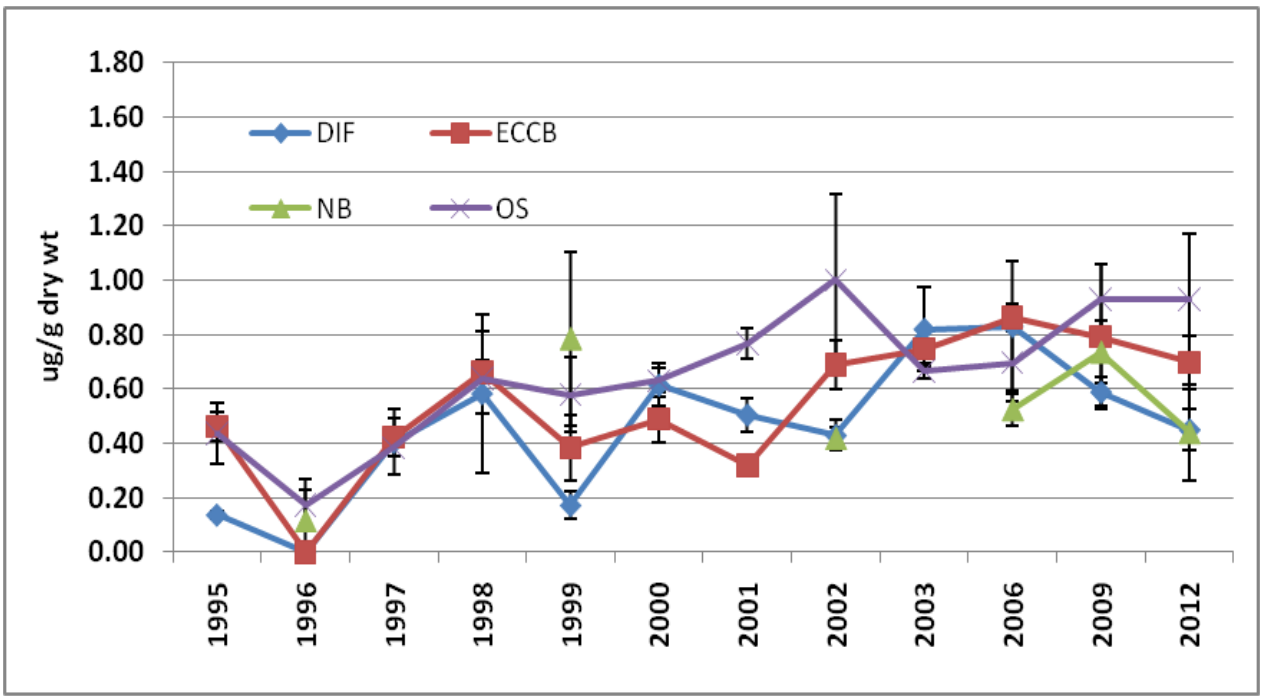

Figure 3-15. Nickel in flounder liver (1995-2012)

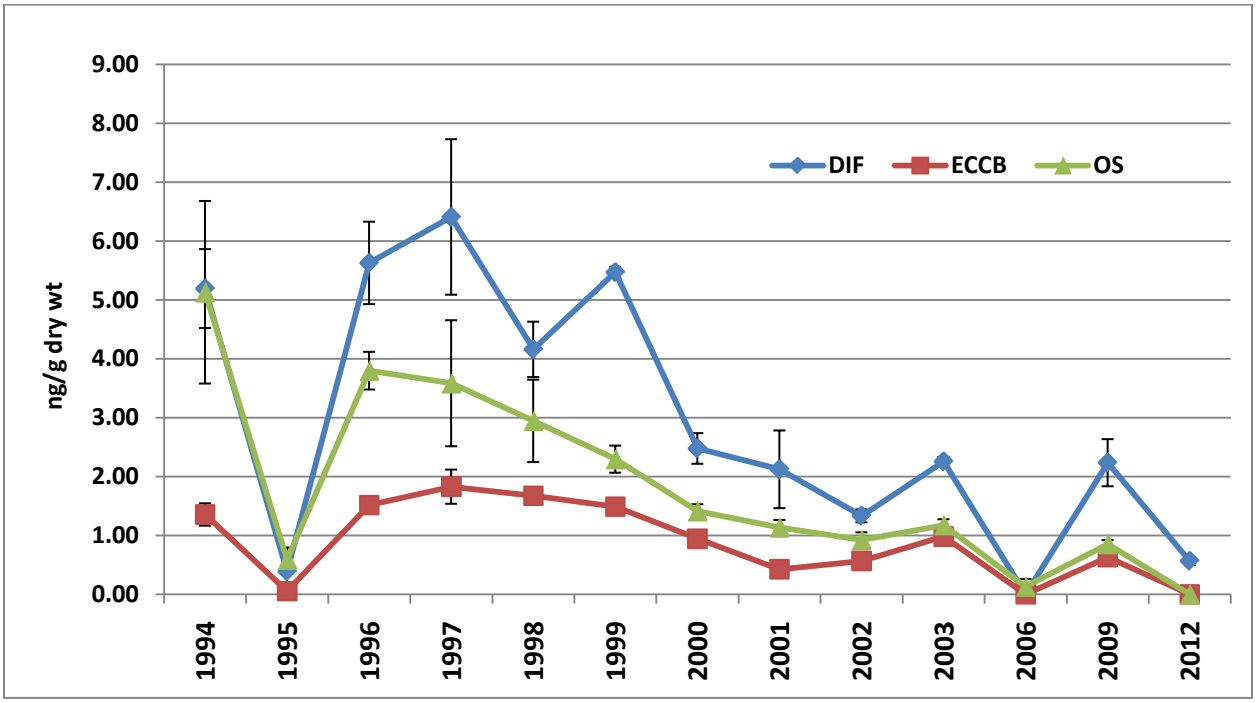

Figure 3-16. Total Chlordane in lobster meat (1994-2012)

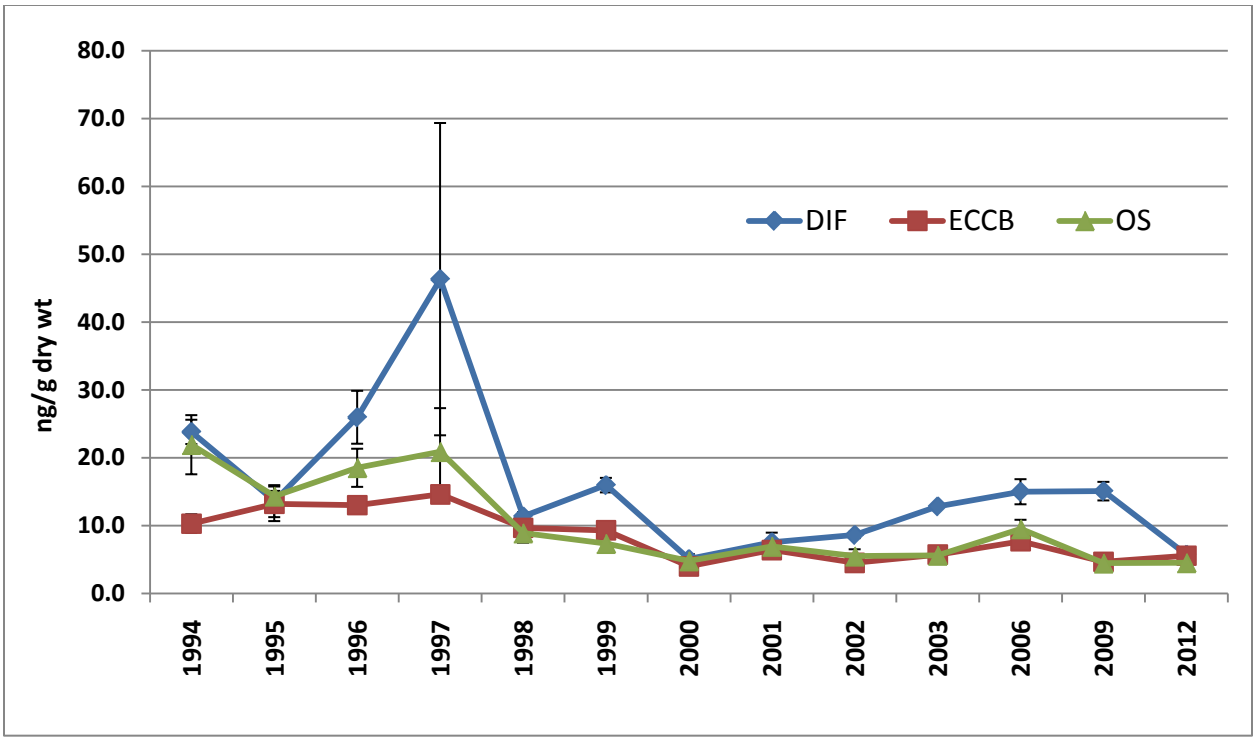

Figure 3-17. Total DDT in lobster meat (1994-2012)

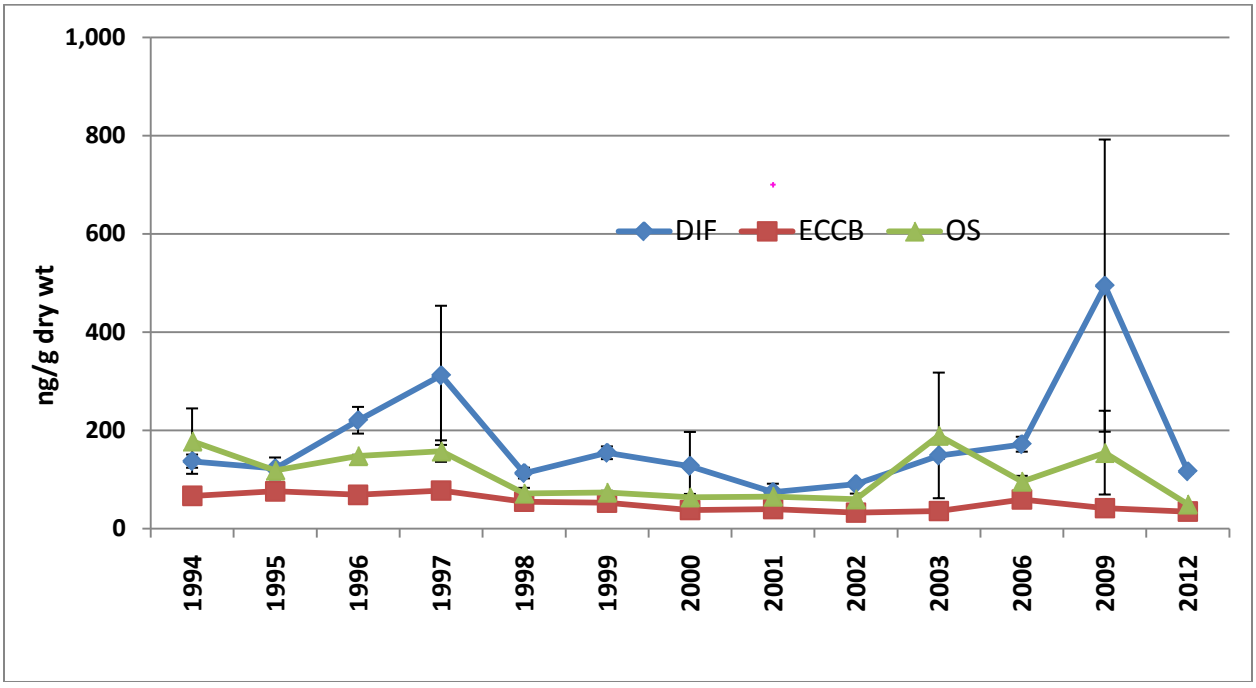

Figure 3-18. Total PCBs in lobster meat (1994-2012)

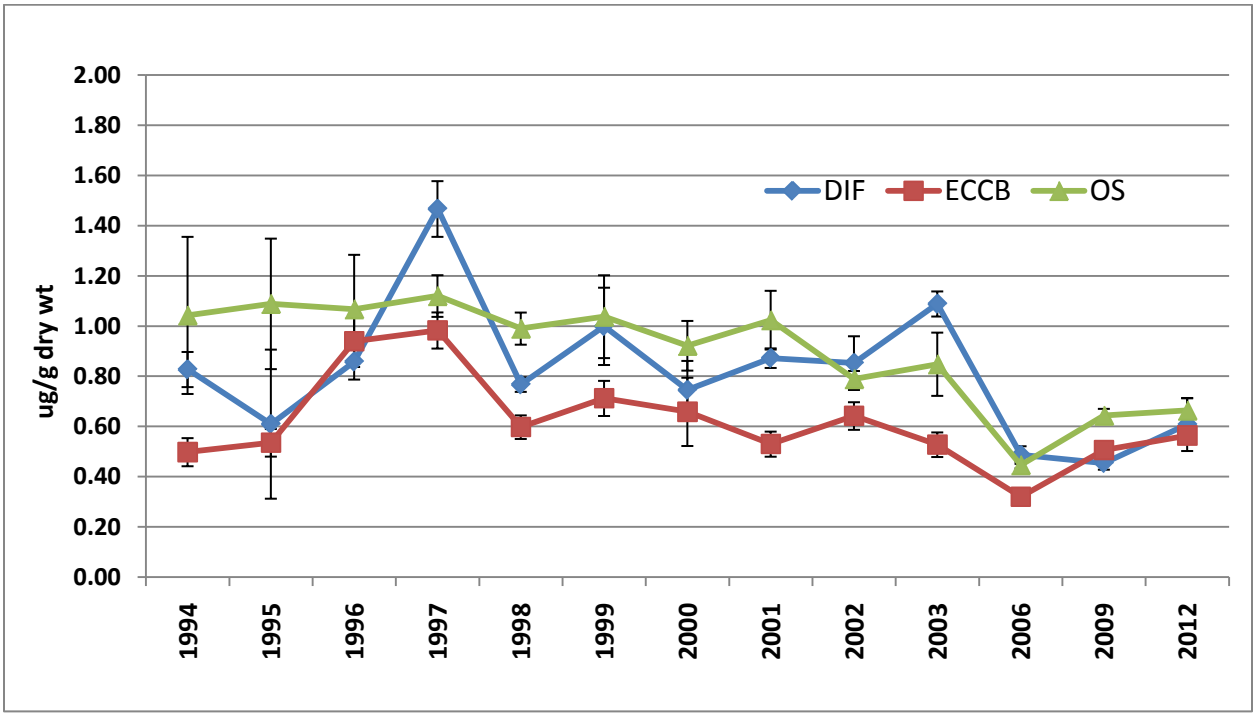

Figure 3-19. Mercury in lobster meat (1994-2012)

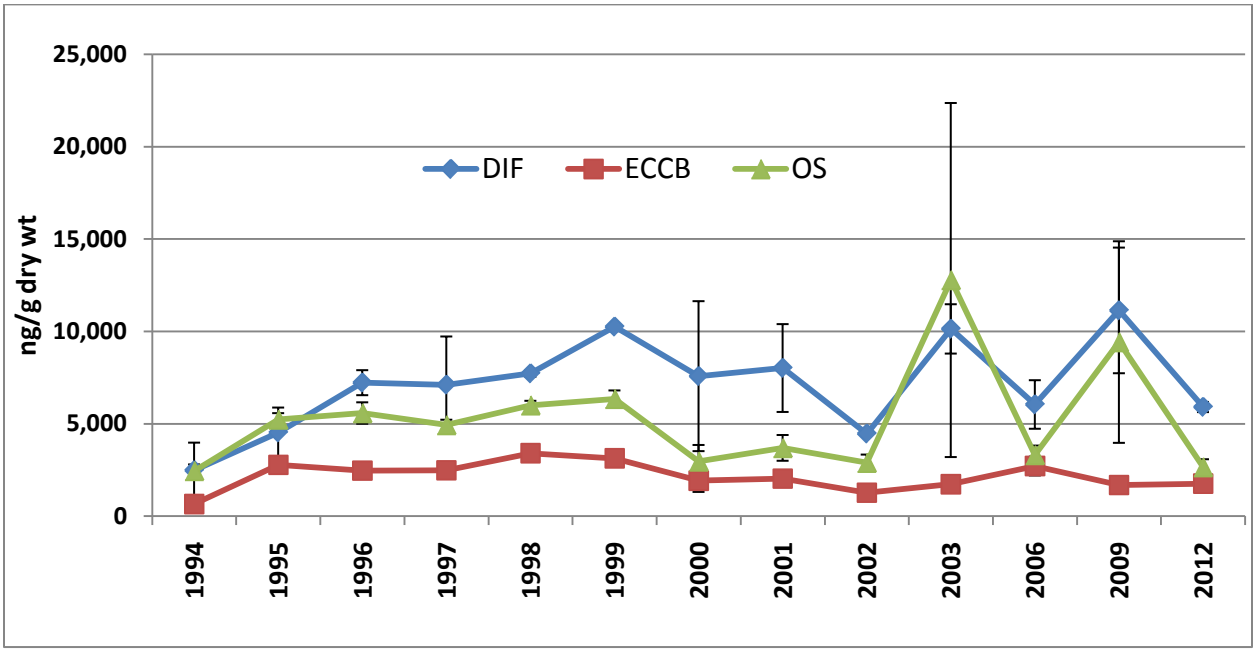

Figure 3-20. Total PCBs in lobster hepatopancreas (1994-2012)

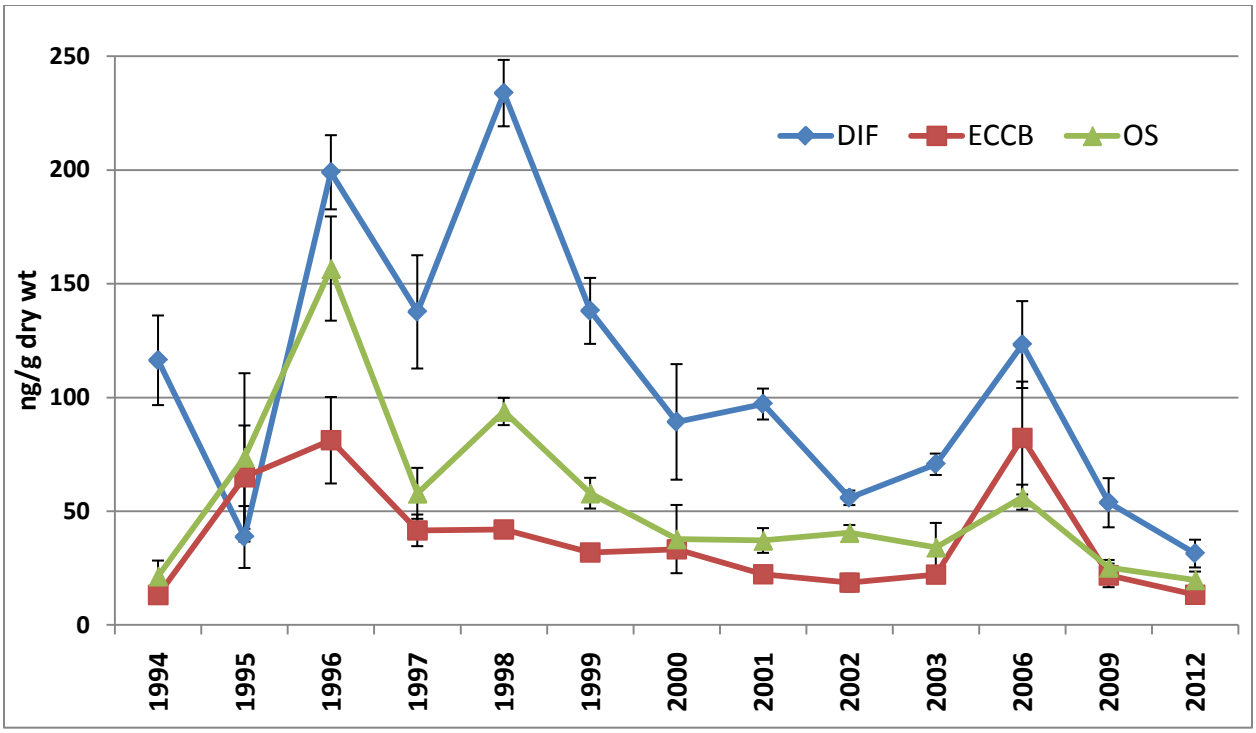

Figure 3-21. Total chlordane in lobster hepatopancreas (1994-2012)

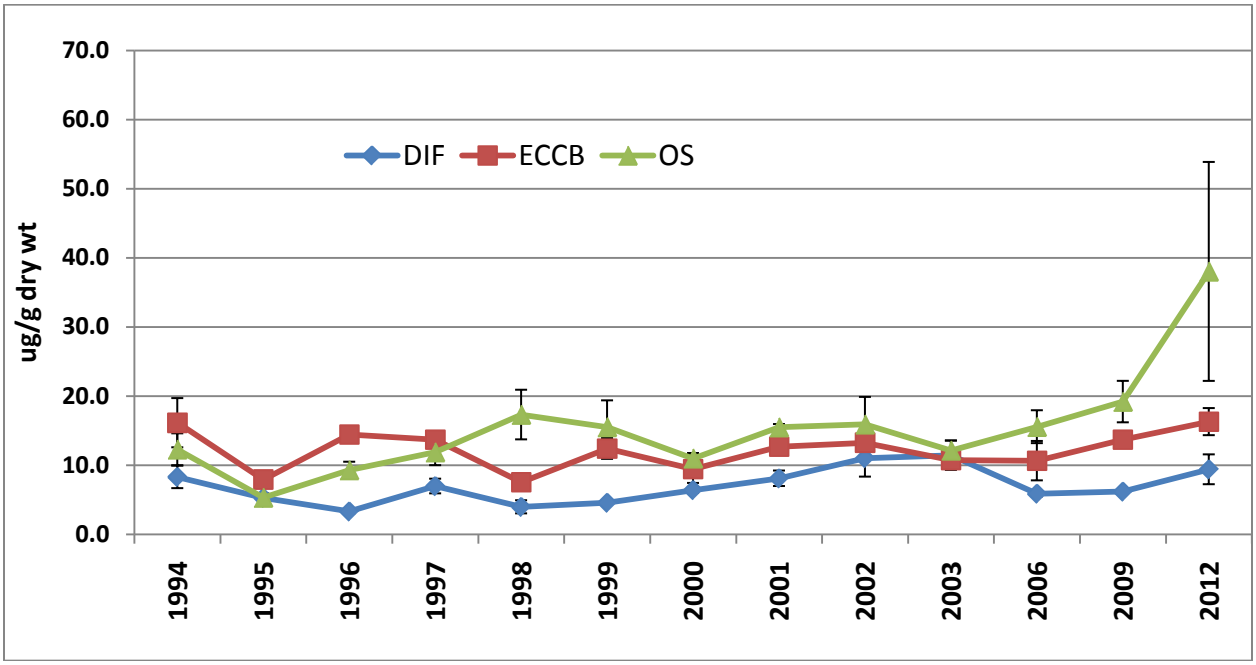

Figure 3-22. Cadmium in lobster hepatopancreas (1994-2012)

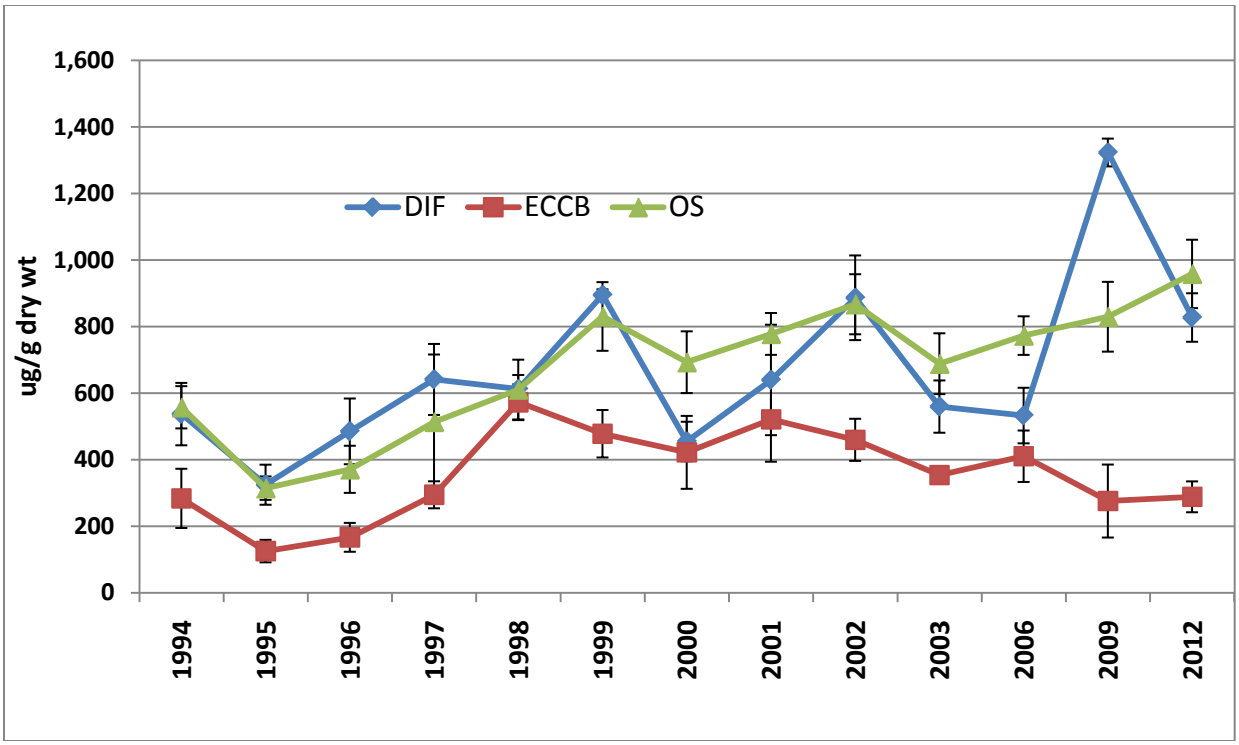

Figure 3-23. Copper in lobster hepatopancreas (1994-2012)

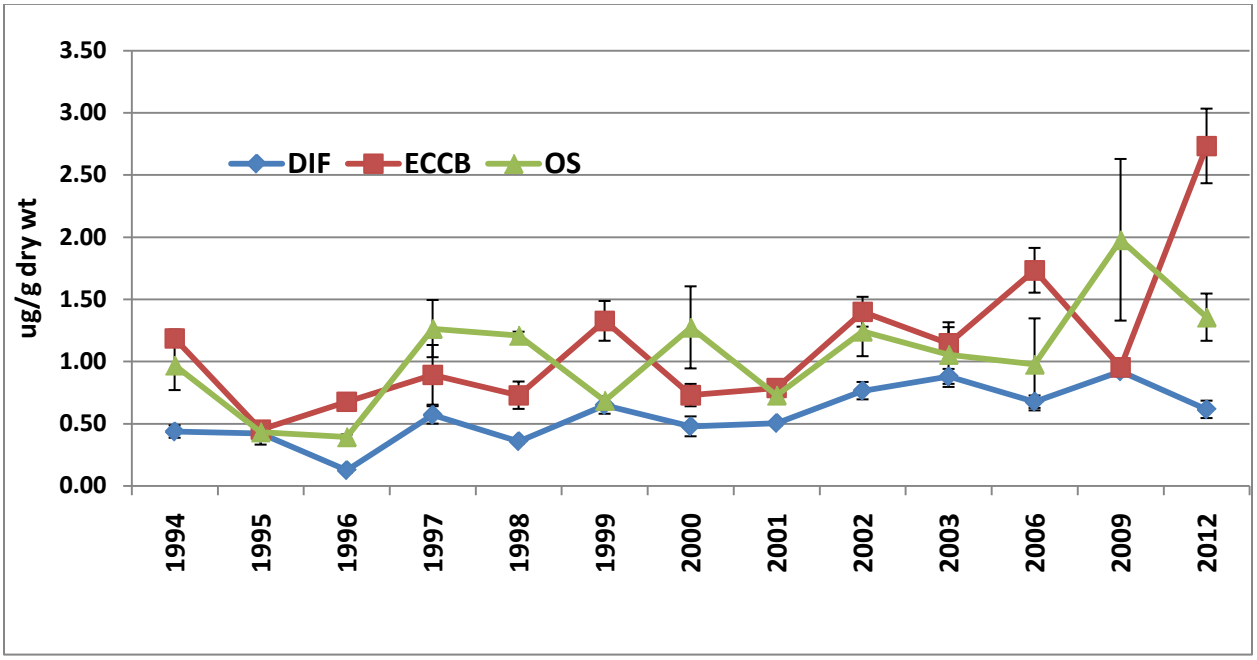

Figure 3-24. Nickel in lobster hepatopancreas (1994-2012)

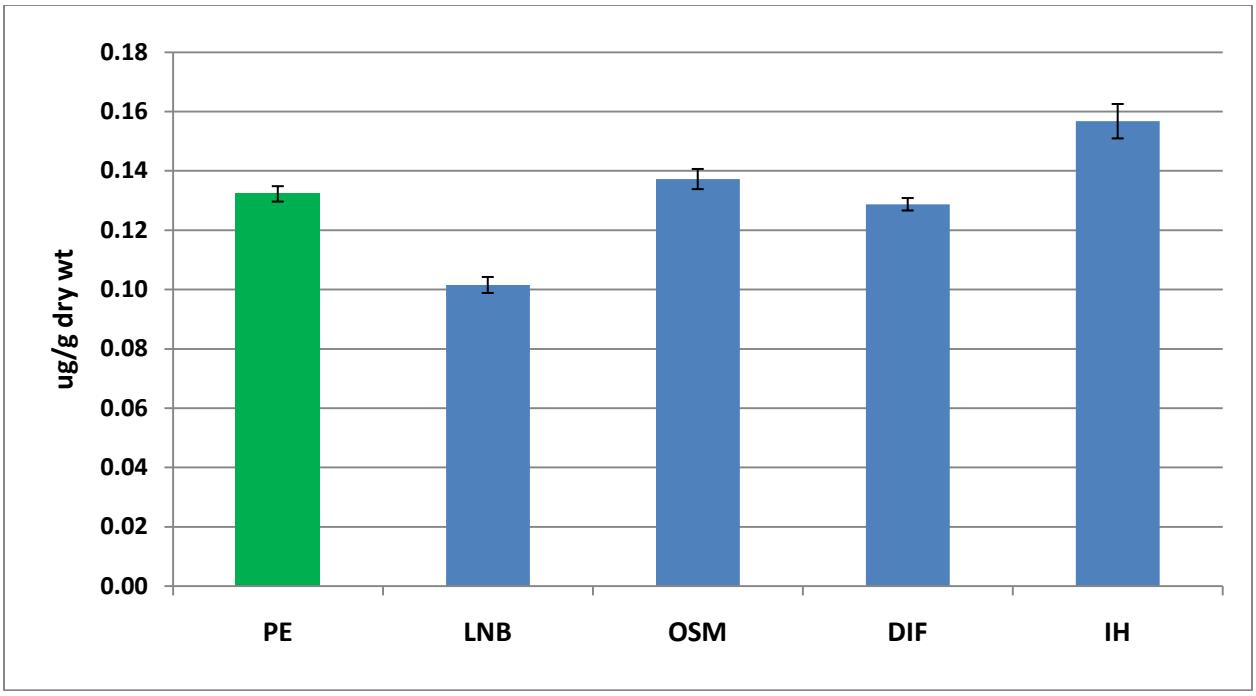

Figure 3-25. Mercury bioaccumulation in mussels (2012)

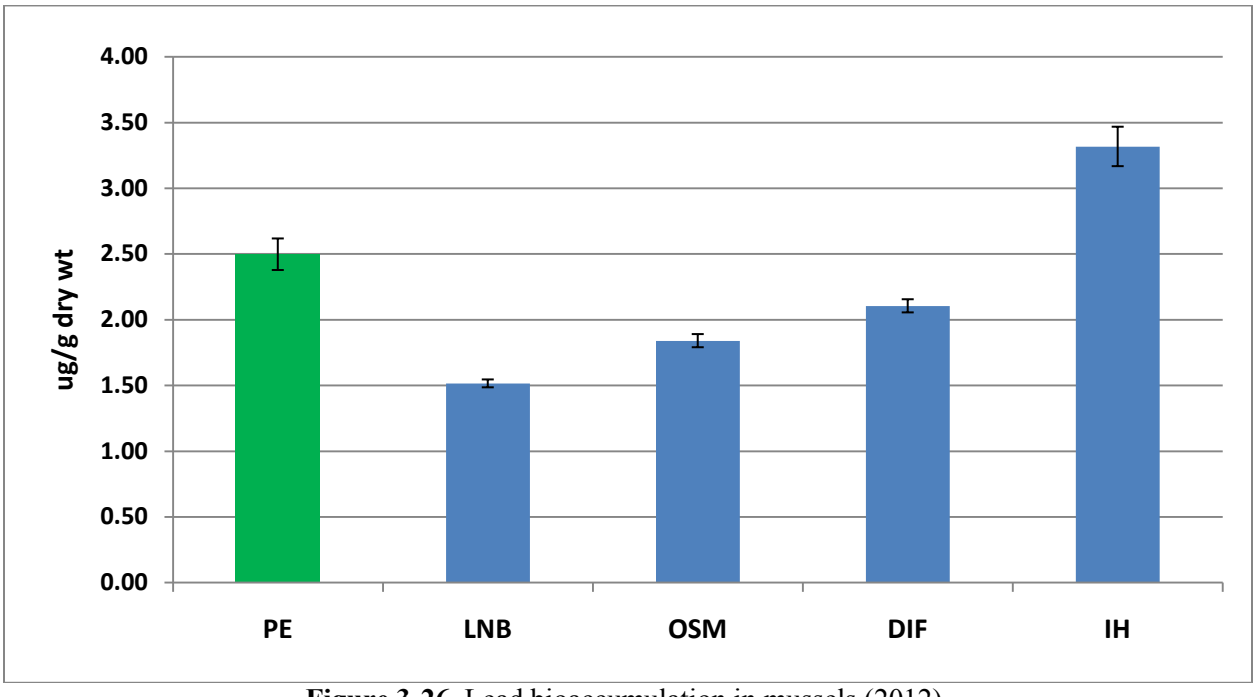

Figure 3-26. Lead bioaccumulation in mussels (2012)

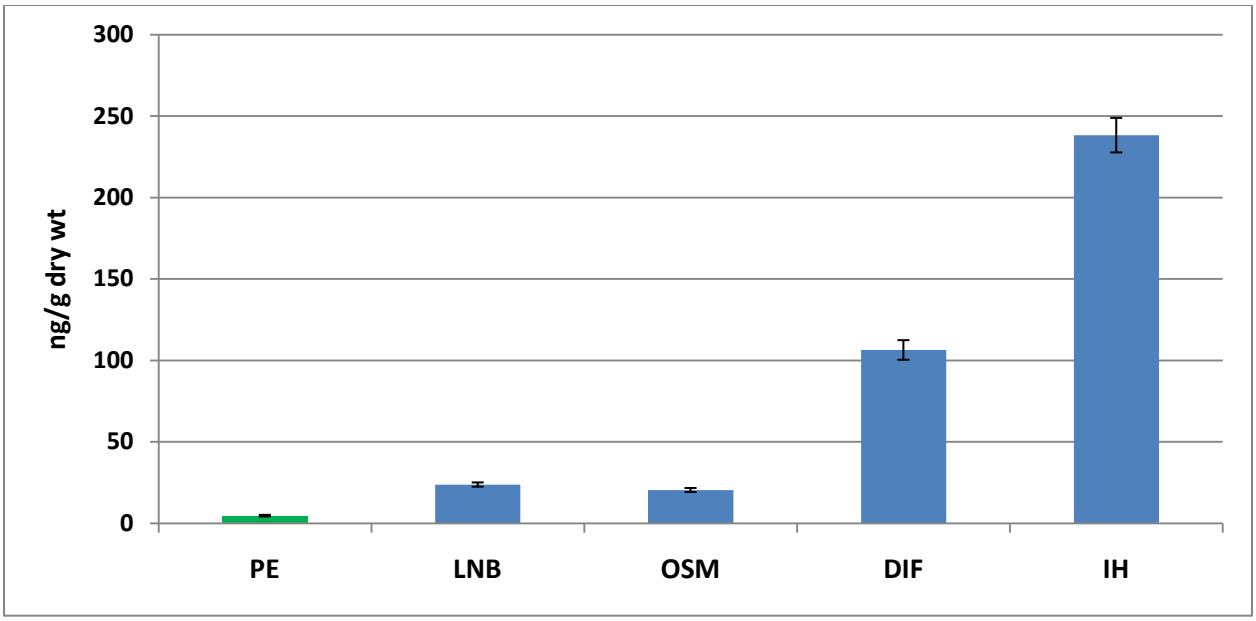

Figure 3-27. Total PCB bioaccumulation in mussels (2012)

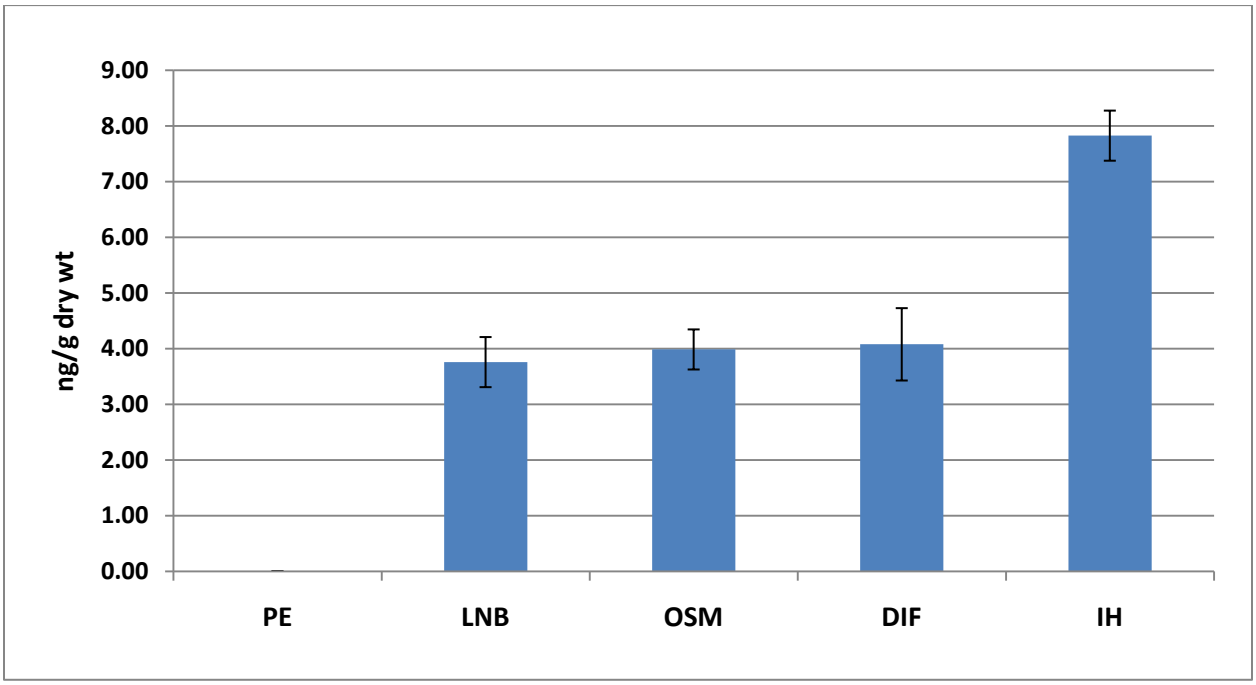

Figure 3-28. Total chlordane bioaccumulation in mussels (2012)

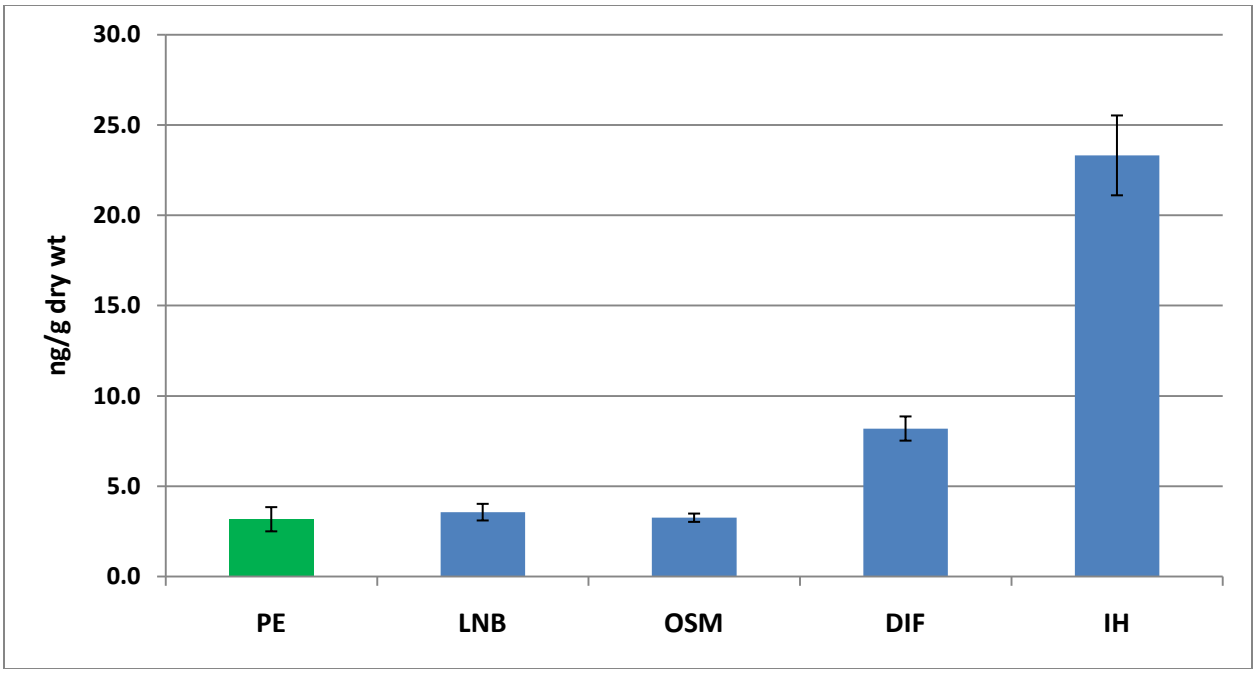

Figure 3-29. Total DDT bioaccumulation in mussels (2012)

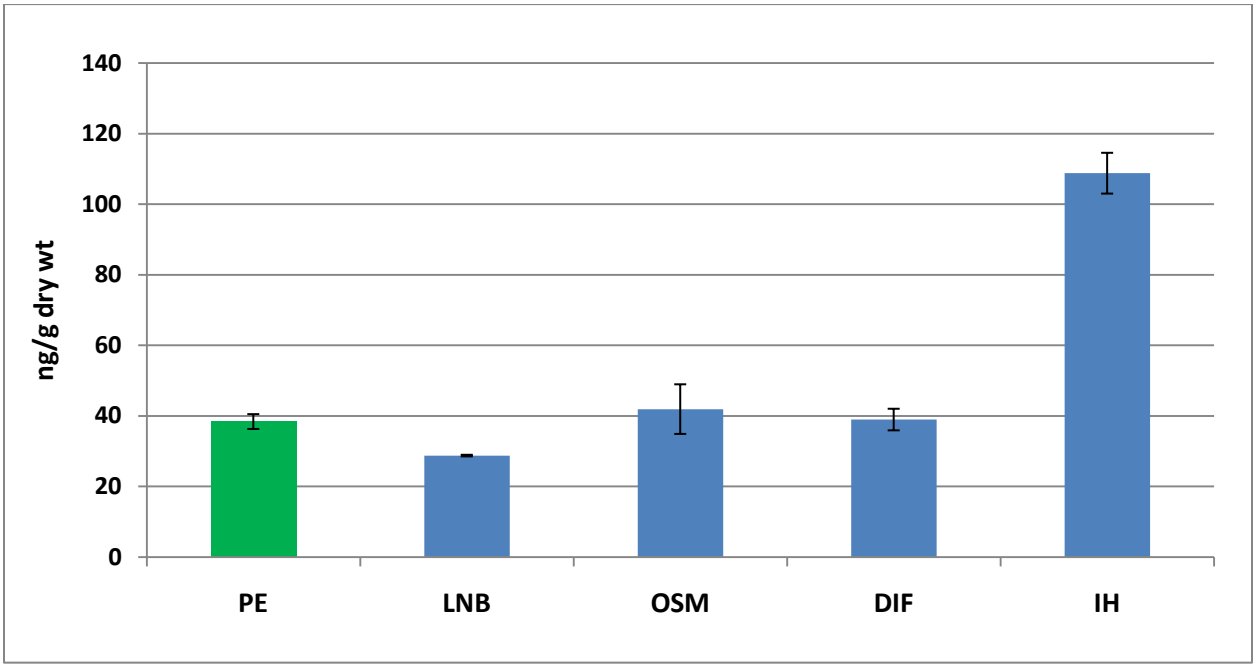

Figure 3-30. Total NOAA LMW PAH bioaccumulation in mussels (2012)

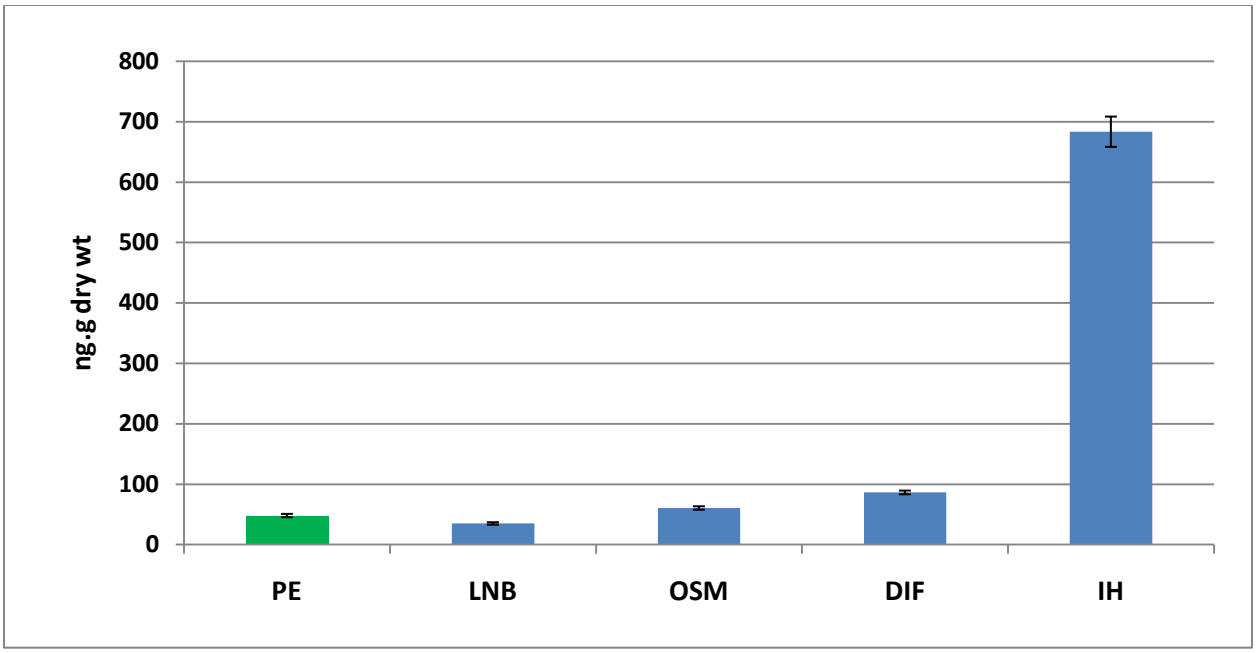

Figure 3-31. Total NOAA HMW PAH bioaccumulation in mussels (2012)

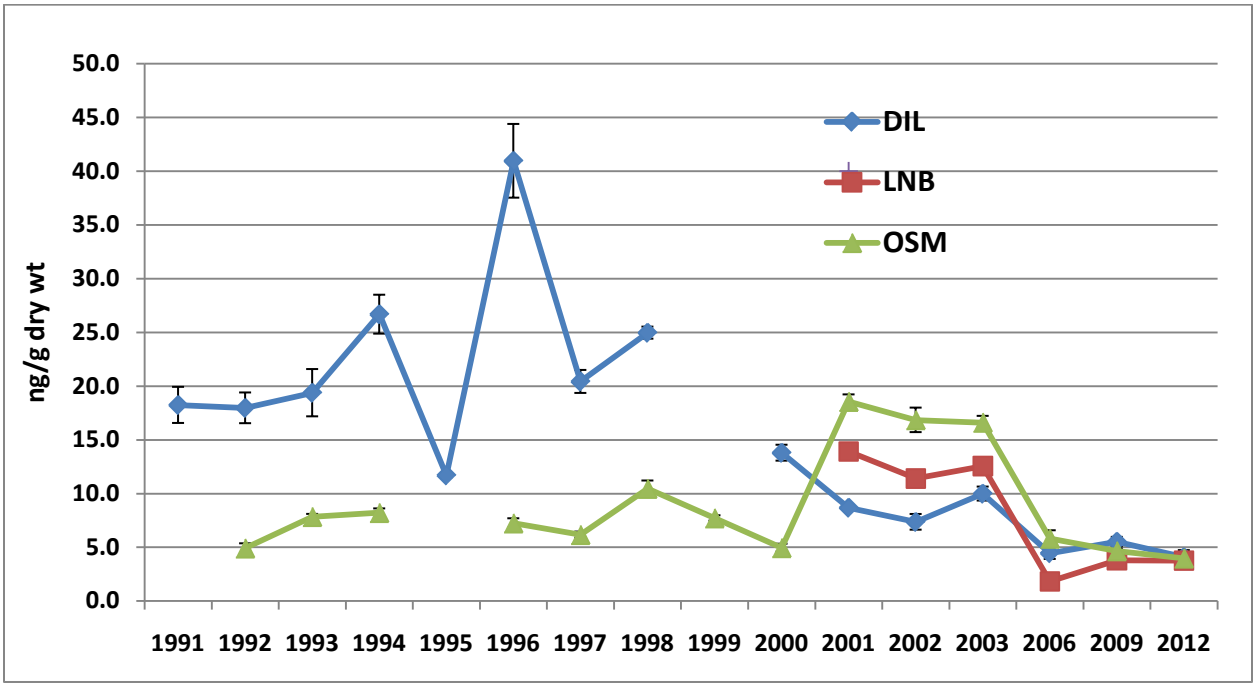

Figure 3-32. Total chlordane trends in mussels (1991-2012)

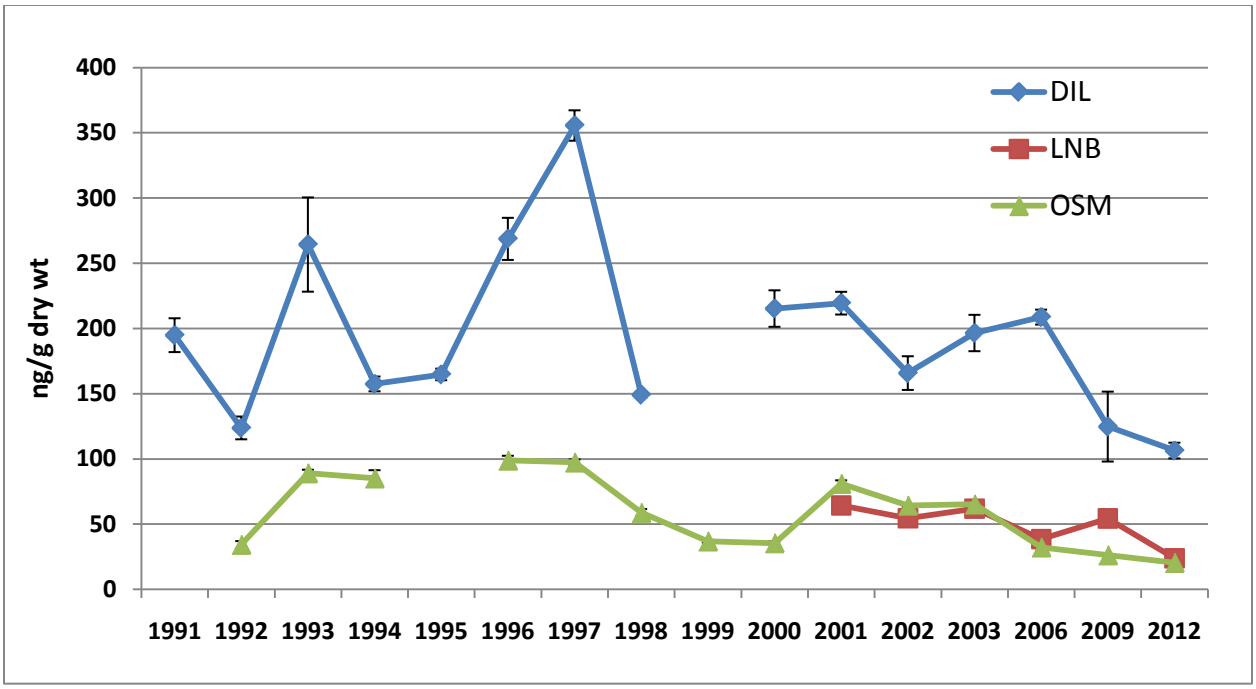

Figure 3-33. Total PCB trends in mussels (1991-2012)

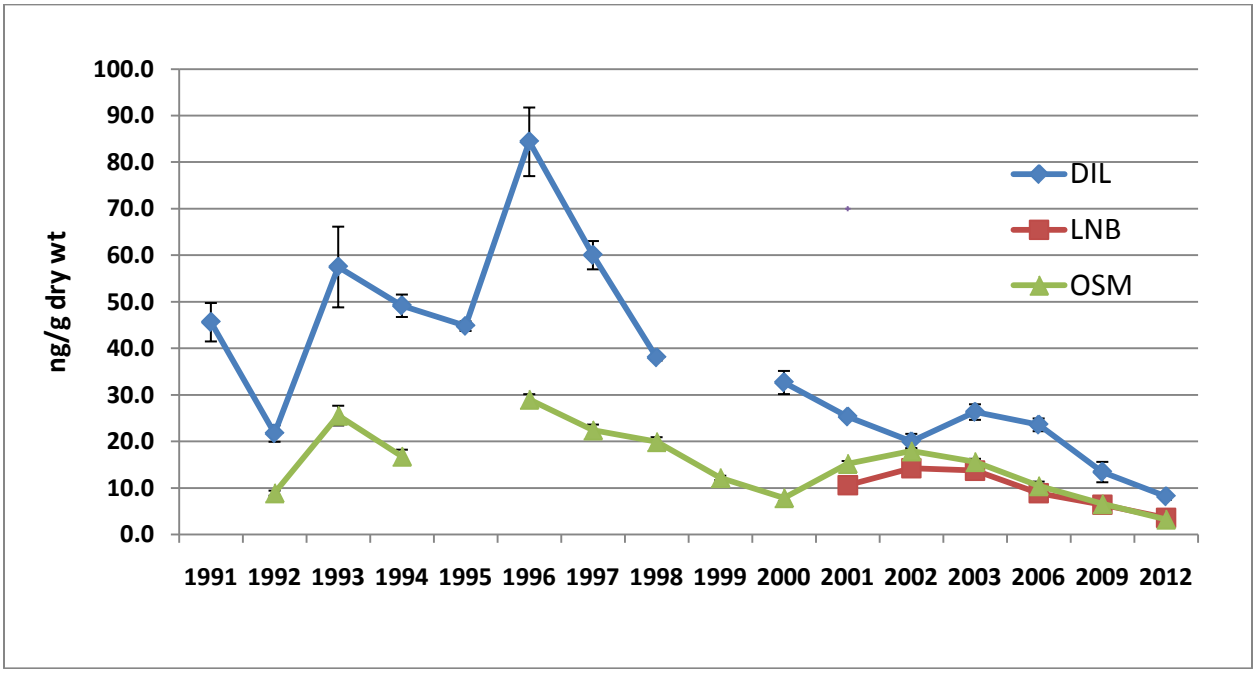

Figure 3-34. Total DDT trends in mussels (1991-2012)

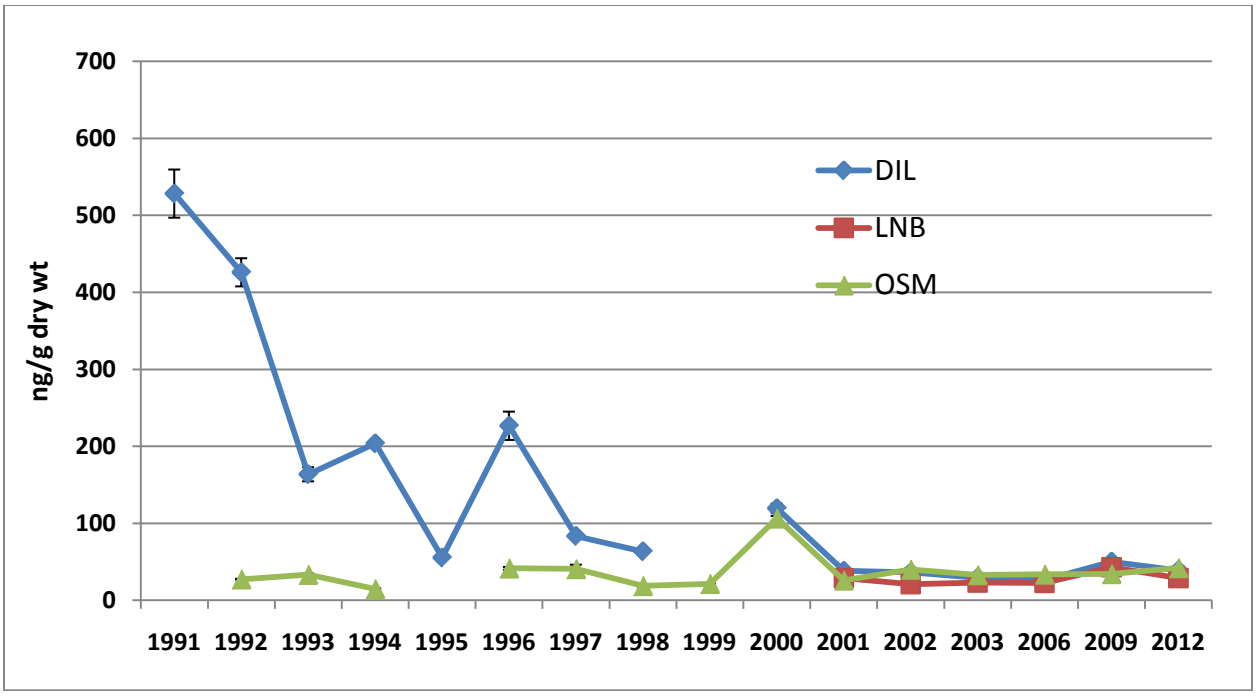

Figure 3-35. Total LMW PAH trends in mussels (1991-2012)

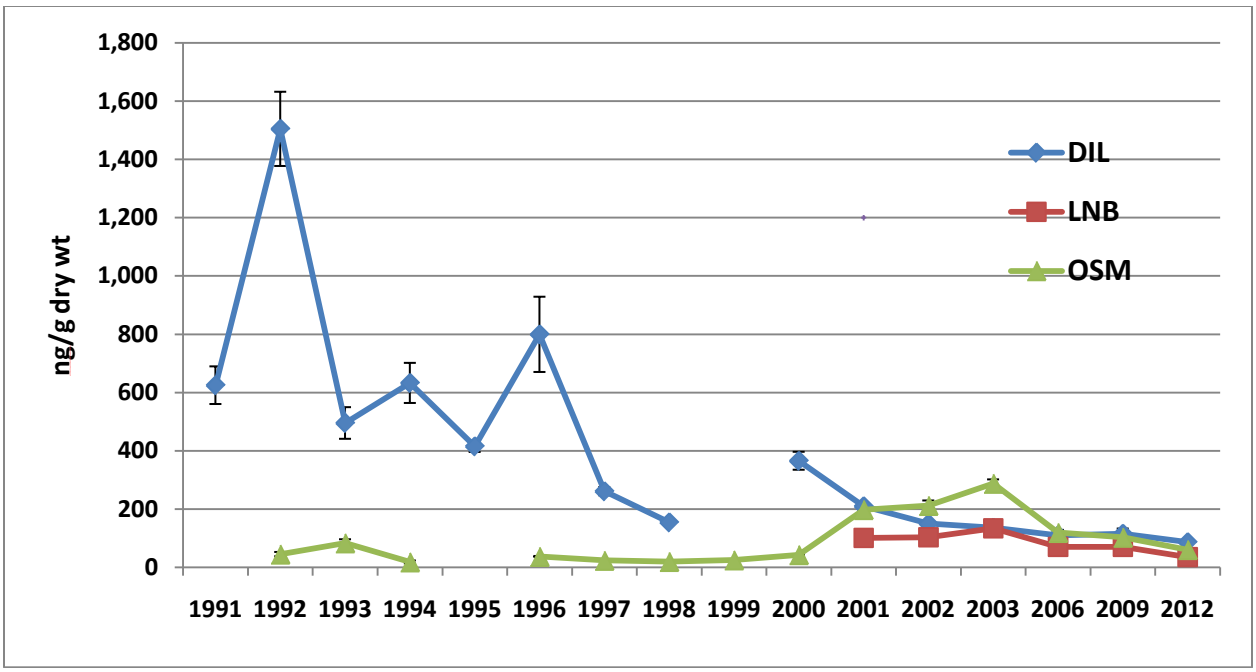

Figure 3-36. Total HMW PAH trends in mussels (1991-2012)

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Massachusetts Water Resources Authority
Charlestown Navy Yard
100 First Avenue
Boston, MA 02129
(617) 242-6000
http://www.mwra.state.ma.us