# Methods

- Mussel Watch sites were selected to represent coastal areas that can be used to construct a nationwide assessment.
- Approximately half the sites are visited each year.
- Sediment samples are collected from Mussel Watch sites approximately once every 10 years, when new sites are established, or during special sampling events such as oil spills.
- Bivalve collection includes blue mussels in the Northeast Atlantic, and West Coast; oysters in the Middle Atlantic, Southeast Atlantic, Gulf of Mexico, Hawaii, and Puerto Rico; and zebra mussels in the Great Lakes.

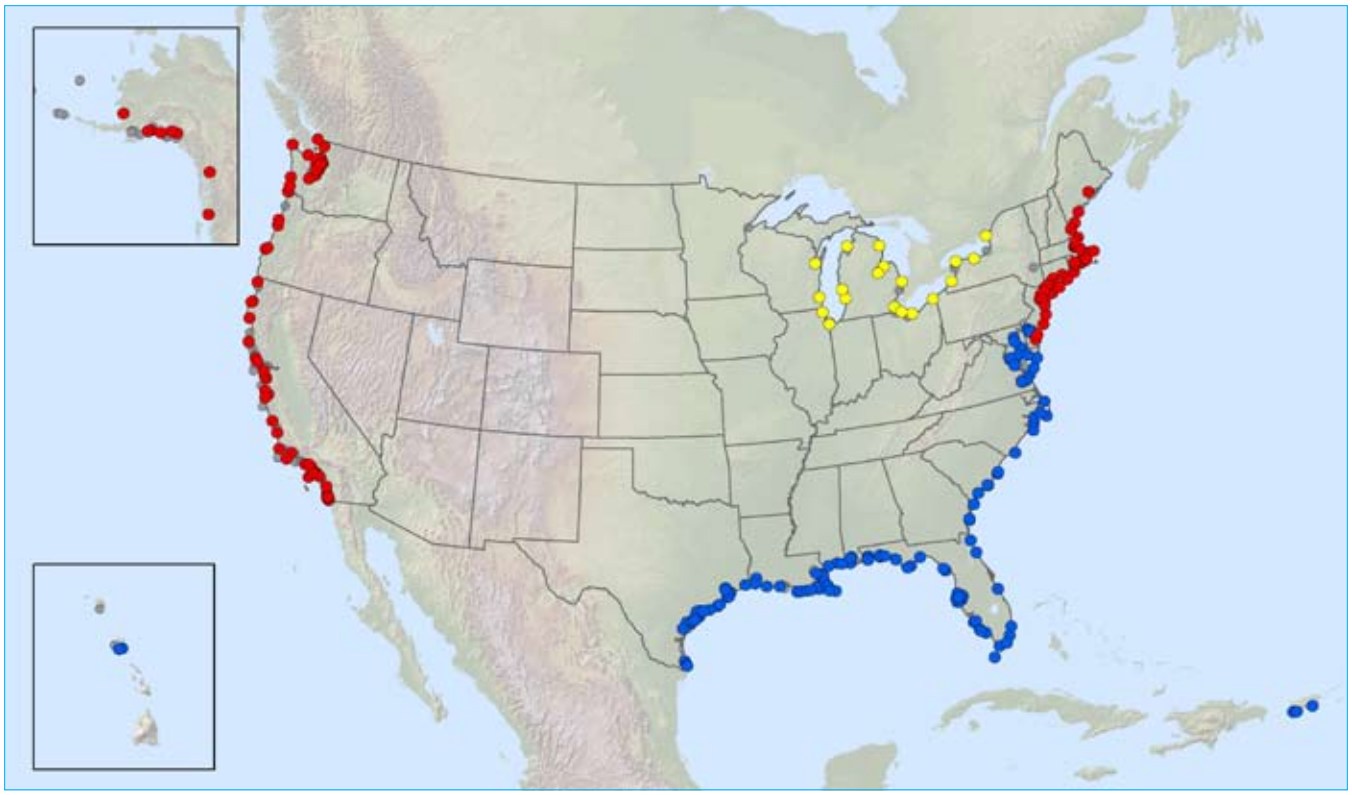

**Figure 2.** Distribution of ● zebra mussels, ● oysters, and ● mussels collected and measured as part of the Mussel Watch Program PBDE assessment. Mussel Watch Program sites not measured as part of this study (●).

## Species and Sites

Mussels and oysters are widely distributed along the coasts. They are integrators of contaminants where they live, and their use as indicators minimizes the problems inherent in comparing data from markedly different species (Berner et al., 1976; Farrington et al., 1980; Farrington, 1983; Tripp and Farrington, 1984). They are good indicators of environmental quality because contaminant levels in their tissue respond to changes in the ambient environment and accumulate with little metabolic transformation (Roesijadi et al., 1984; Sericano, 1993).

Mussel Watch sites were selected to represent coastal areas that can be used to construct a nationwide assessment. Sites selected for monitoring are generally 10 to 100 km apart along the entire U.S. coastline, including the Great Lakes, Puerto Rico, and Hawaii. Where possible, sites were selected to coincide with historic mussel and oyster monitoring locations from other programs, such as the U.S. EPA's Mussel Watch sites that were sampled from 1976 to 1978 (Goldberg et al., 1983), and to complement sites sampled through state programs, such as the California Mussel Watch Program (Martin, 1985).

Because one single species of mussel or oyster is not common to all coastal regions, a variety of species are collected to gain a national perspective. A target species is identified for each site based on abundance and ease of collection. Mussels (*Mytilus*

Table 3. Bivalves species used to assess national coastal PBDE concentrations.

| Target Species | Name used in this report |
|---|---|
| *Mytilus edulis, Mytilus californianus, Mytilus galloprovincialis,* and *Mytilus trossulus* | Mussels |
| *Crassostrea virginica, Ostrea sandvicensis, Crassostrea rhizophorae,* and *Chama sinuosa** | Oysters |
| *Dreissena polymorpha* and *Dreissena bugensis* | Zebra mussels |

* smooth-edge jewelbox collected from one site in the Florida Keys

*species*) are collected from the North Atlantic (Maine to Delaware) and Pacific coasts. Oysters are collected from Delaware Bay southward and along the Gulf Coast (*Crassostrea virginica)*, Hawaii (*Ostrea sandvicensis)*, and Puerto Rico *(Crassostrea rhizophorae)*. *Chama sinuosa* is collected from the Florida Keys and is classified along with oysters for this report. Zebra and quagga mussels (*Dreissena species*) are invasive species collected from the Great Lakes (Table 3; Figure 2). Oysters and *Mytilus* species range in size from 7 to 10 cm and 5 to 8 cm, respectively. Zebra mussels are smaller, typically 2 to 4 cm. Previous comparisons of contaminant accumulation between mussels and oysters showed large differences for trace metals, particularly zinc and copper, but the differences in organic contaminants were determined to be minor and not likely to affect comparison between species (O'Connor, 1992).

Although the U.S. coastline is extensive, relatively few species are currently required to determine a national contaminant perspective. It is possible to make spatial comparisons of organic contaminant concentrations across all sites, because Mussel Watch species bioaccumulate organic contaminants similarly (O'Connor, 1992).

Oysters and mussels are collected by hand or dredged from intertidal to shallow subtidal zones, brushed clean, packed in iced containers, and shipped to analytical laboratories within two days of collection. Approximately 20 oysters or 30 mussels are composited for each site from three stations. The bivalves are shucked, soft tissue is homogenized, and approximately 15 grams of wet tissue is extracted. Sample collection, preparation, and extraction protocols are described in detail in McDonald et al., (2006); Lauenstein et al., (1997); Lauenstein and Cantillo, (1993a-d and 1998).

Mussel Watch sites are sampled biennially with approximately half the sites visited in any one year. Annual collections are spatially distributed to provide a national snapshot (Figure 2). To provide a historical perspective and a current assessment, Mussel Watch samples from 1996 and 2004-2007 were measured respectively. Samples collected between 2004 and 2007 were aggregated and called 200X (Figure 3). Hence, 200X is a designation for samples collected from 2004 through 2007.

### Sediment Sites

Sediment samples are collected from Mussel Watch sites approximately once every 10 years, when new sites are established, or following extreme events such as oil spills. Bivalve and sediment sites are taken from areas in close proximity to one another. The top 3 cm of sediments, representing recent deposition, are used in this analysis. Three sediment grabs are collected from three

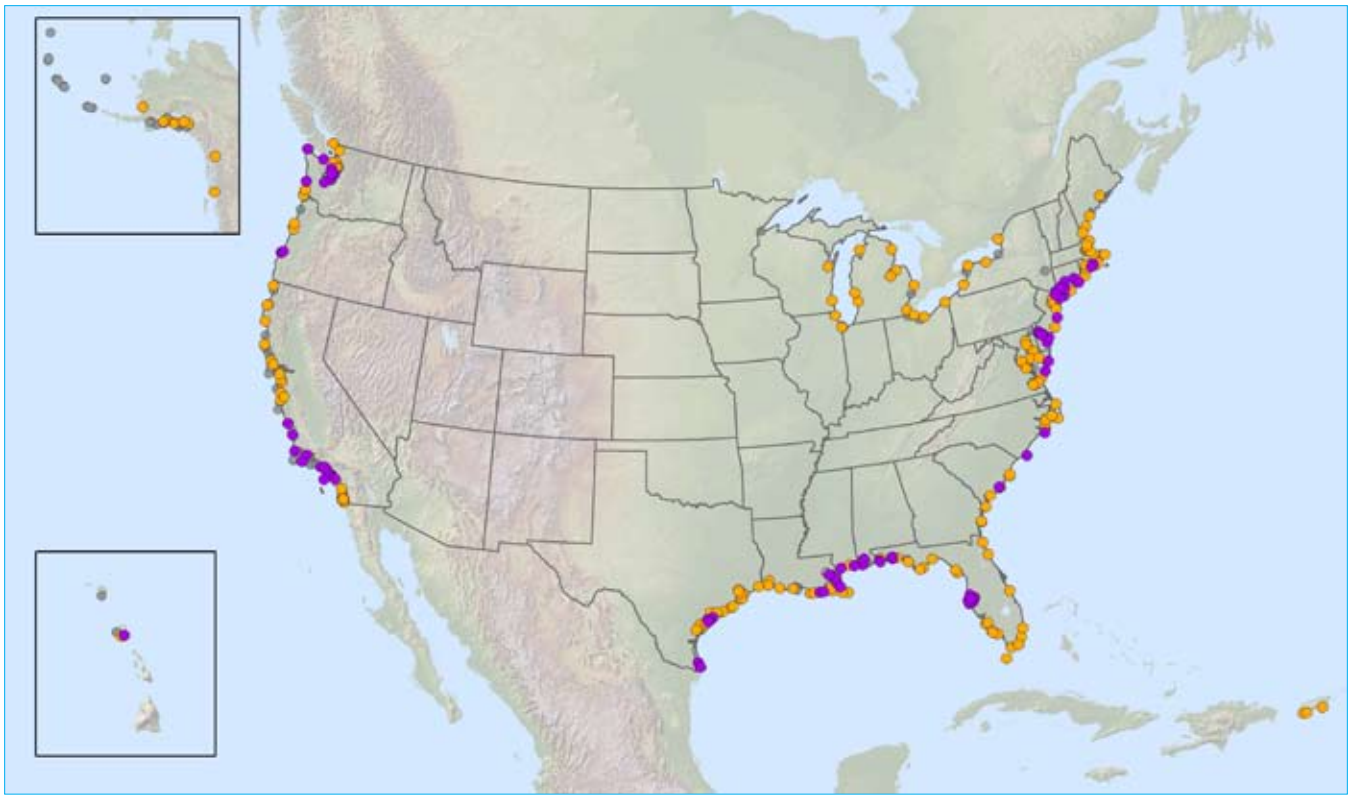

**Figure 3.** Number of biennial measurements taken at each of the 254 sites sampled in 1996 and 200X. Sites shown have one year (●) or two years (●) of data. Mussel Watch Program sites not measured as part of this study (●).

stations and composited. Sediment collection sites are located as near as possible to, but generally not more than, 2 km from the bivalve site, and located in low energy depositional areas.

### Analytical Methods

Analytical methods used by the Mussel Watch Program are provided in Appendix 2 and available online at http://NSandT.noaa.gov. Of the 209 possible PBDE congeners, 38 were analyzed for this report (Table 2) as a result of available standards, methodology and predominant congeners (Appendix 2). The co-eluting congeners 49 and 71 are labeled in this document as 49.

Higher substituted PBDEs, such as the octa, nona, and deca homologues that were not measured in this study, appear to accumulate preferentially in sediment. Other homologues, those measured in this study, more frequently accumulate in tissue (Zhu and Hites, 2005). Hence, this presentation provides a partial picture of the sediment-tissue relationship.

While PBDE's occur at ten different bromination levels, it was standard in early analyses to quantify the concentration of the first seven homologues. The more highly brominated forms or homologues (octa-, nona-, and deca-) were not generally measured because a standardized procedure for doing so did not readily exist. As the use of Deca, the most highly brominated form became the industry standard, the need to measure its presence in the environment

became increasingly important. A procedure for measuring high molecular weight PBDE's was developed for use in the NS&T and was implemented as a part of our protocols in 2007. Archived Mussel Watch samples have not yet been reanalyzed for these heavier compounds but could be should the need arise.

**Statistical Analysis**

Results from a Shapiro-Wilk W test for tissue (W = 0.233, prob < W =0.001) and sediment (W = 0.257, prob < W = 0.001) show that Mussel Watch chemistry data were not normally distributed; thus, nonparametric tests were used for statistical analyses.

All measurements below detection limits were considered to be zero. Maps for each year show the sum of all 38 PBDE (Table 2) congeners measured for this study. Mussel Watch sites were classified into three groups. Sites below detection limit were categorized as low. Sites above the detection limit were categorized as either medium or high through cluster analysis. Clustering was performed using Ward's Minimum Variance technique. The concentration in each cluster group was then tested using Wilcoxon analysis to ensure that medium and high categories were significantly different at the $\alpha = 0.05$ level. Statistical outliers were reanalyzed to ensure measurements were correct and then combined within the high category. Here, clustering was used to partition site-specific PBDE concentrations into a fixed number of "closely related" subsets. For purposes of highlighting regions with elevated PBDEs, clustering was limited to 2 subsets: high and medium.

Spearman's nonparametric statistical test was used to test for correlation between PBDE concentrations and human population. Population was derived from 2000 census data and represents population within a radius of 20 km of each site. Tests were considered significant at the $\alpha = 0.05$ level.

To evaluate overall PBDE contamination levels in watersheds throughout the Nation, a 2-way clustering (statistical classification) procedure was performed using paired sediment and tissue concentrations simultaneously. This procedure provided a descriptive yet objective technique to consider PBDE contamination levels as a function of the two measurements. Sediment and tissue concentrations from all sites within each unique watershed were averaged to develop the 2-way classification.

The Wilcoxon analysis was used to compare sediment and tissue concentrations from the watershed comparison analysis, significance was achieved at the $\alpha = 0.05$ level. To ensure comparable dimensions the dry weight measurements for sediment and tissue were used.


Mussels