Contaminant screening of osprey eggs in Casco Bay, Maine

2009 Field Season

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Contaminant screening of osprey eggs in Casco Bay, Maine
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1. Executive Summary and Primary Findings

Starting in May 2009, BioDiversity Research Institute (BRI) expanded upon the 2007 and 2008 broad-based contaminant study on Maine birds, measuring both historical and emerging chemicals. Out of the 23 species studied in the first two years, we determined that osprey (*Pandion haliaetus*) foraging in Casco Bay required additional study in 2009. We selected osprey because they act as bioidicators of the marine habitat. The compounds we analyzed in ten eggs from Casco Bay were mercury (results pending), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), perfluorinated compounds (PFCs), and organochlorine pesticides (OCs). The results in this report also include the six osprey eggs we analyzed in 2007.

Our preliminary findings are:

- PCBs, PBDEs, PFCs, and OCs were detected in all samples.
- Deca-BDE was found in ten out of 12 eggs in Casco Bay, indicating that deca is bioaccumulating in wildlife.
- PFOS level on Flag Island had the highest level detected in Maine wildlife—75% of the eggs had PFOS levels above effects thresholds established for chickens.
- Levels of PBDEs and PFCs tended to be higher in Casco Bay than mid-coast Maine.
- The osprey samples did not show a specific spatial pattern, suggesting that within the marine ecosystem, contaminant levels may be dictated by point sources, watershed characteristics, and/or food web dynamics.
- Like the 2007 and 2008 results, PCB, PBDE, PFC, and OC levels are positively correlated, indicating that birds with high levels of one compound tend to have higher levels of the others. PBDEs and PCBs have one of the strongest relationships.

2. INTRODUCTION

2.1 Project overview

With financial support from Casco Bay Estuary Partnership, BioDiversity Research Institute (BRI) assessed current levels of nearly 200 contemporary and traditional contaminants in ten viable osprey (*Pandion haliaetus*) eggs across Casco Bay.

Today, scientists are increasingly aware that a new suite of anthropogenic chemicals – flame retardants (PBDEs), and surface treatments (PFCs) – persist in our environment alongside transformer coolants (PCBs), organic pesticides (OCs) and heavy metals (e.g. mercury). Both field and laboratory studies have documented that these contaminants bioaccumulate and biomagnify in wildlife and can have significant negative effects on immune, endocrine, and neurological systems. Despite several recent studies, researchers still have a poor understanding how these new contaminants are distributed in the environment and how they vary by region.

In 2008, with Casco Bay Estuary Partnership support, BRI released a first-of-its-kind study that measured PBDEs, PFCs, PCBs, OCs, and mercury in 23 species of Maine birds. This study detected all of these contaminants in all species and recorded PFCs for the first time in Maine.

Primary findings of the study:

- Birds in coastal southern Maine tended to have higher contaminant levels than the rest of the state.
- Within the greater Portland area, osprey had some of the highest in PCB, PBDE, and PFOS levels out of 14 species.
- Out of the six osprey samples collected in coastal Maine, the egg collected at Bug Light had the highest total contaminant load and had PFOS levels three times higher than an adverse effects threshold (Goodale 2008).

These findings indicated that further sampling within Casco Bay was critical to determine if the high contaminant levels from Bug Light are also found in other areas of Casco Bay.

2.1.1 Species selection

Ospreys are an optimal species to monitor contaminants because they are long-lived fish-eaters that feed at the top of the food chain. As a result, their eggs are reflective of contaminants in the aquatic food web in which they feed. Additionally, since they are conspicuous and often adapt to development and human disturbance, we are able to survey them and collect samples with relative ease.

2.1.2 Tissue selection

As with our 2008 study, we collected and analyze eggs. Eggs are an optimal choice for organic contaminant analyses because many of the pollutants are lipophilic (binding to fat), and eggs can be collected easily and non-lethally. Eggs are used extensively for contaminant studies (Wiemeyer 1996, Kannan et al. 2001, Braune et al. 2002, Evers et al. 2003, and Braune 2007) because female birds depurate contaminant burdens into their eggs and eggs represent the contaminants present in the bird's breeding territory diet (Hobson et. al 1997, Evers et al. 2003). Research has confirmed that tissues of breeding ospreys reflect contamination gathered locally (Elliott et al. 2007).

2.1.3 Conservation value

Since contaminant levels in osprey can be readily compared to other organisms with complex neurological systems, ospreys provide an index of the extent to which wildlife are exposed to contaminants entering ecosystems. Results from this study will aid in determining geographic patterns of contaminant exposure and evaluate areas that require in-depth study. Within Casco Bay, these results will evaluate if areas closer to development (i.e. Portland) have higher contaminant exposure and if wildlife within Casco Bay are accumulating contaminants above adverse effects thresholds.

2.1.4 Project Goals

- Determine spatial variation of contaminants in Casco Bay using osprey as bioindicators
- Determine contaminant exposure and risk to ospreys nesting in Casco Bay and four sites of varying industrialization along the Atlantic seaboard.

2.2 Chemical Interaction

Researchers have studied the effects of many of the contaminants analyzed in this study on behavior, reproductive success, organ function, and acute toxicity. However, a number of studies have also attempted to determine if multiple compounds interact to create physiological effects greater than their sum. Researchers found that organochlorine pesticides can interact with each other to create either an additive or synergistic effect (Blus 2003). Epidemiological studies on human children (Grandjean et al. 2001, Stewart et al. 2003, Roegge et al. 2004), and laboratory studies on animals (Bemis and Seegal 1999, Costa et al. 2007) indicate that PCBs and methylmercury may act synergistically or additively. Additionally, researchers have found that PCB 52 can interact with PBDE 99 to enhance neurobehavioral defects in mice (Eriksson et al. 2006) . These studies suggests that many of the compounds analyzed in this study can interact to create an effect greater than one contaminant alone.

2.3 Review of compounds measured

2.3.1 Hg

Mercury is a naturally occurring heavy metal that has been mobilized into the environment by anthropogenic activities. Due to its unique properties, mercury is used in many products such as thermostats and dental fillings. It is also used in mining, and is released to the environment through the combustion of fossil fuels.

Generally attributed to anthropogenic input (Lockhart et al. 1998), mercury (Hg) levels in the North Atlantic have doubled over the last 100 years (Asmund and Nielsen 2000) and are increasing by nearly 1.5% a year (Slemr and Langer 1992) with peak levels in Maine recorded after 1970 (Perry et al. 2005). This historical increase has been documented in North Atlantic seabirds (Thompson and Furness 1992, Monteiro and Furness 1997), Canadian Arctic seabirds (Braune 2007) with local Hg deposition causing high rates of increase in biota (Frederick et al. 2004, Evers et al. 2007). This increase of global Hg levels since the 1900s is of concern because mercury is a persistent toxic heavy metal that both bioaccumulates and biomagnifies[1] in wildlife, and has neurological and reproductive impacts (Wolfe et al. 2007).

Researchers have documented Hg in the Maine sediment (Perry et al. 2005), water (Dennis et al. 2005), crayfish (Pennuto et al. 2005), fish (Kamman et al. 2005), salamanders (Bank et al. 2005), birds (Evers et al. 2005), and mammals (Yates et al. 2005). In addition Hg hot spots have been documented in Maine (Evers et al. 2007).

2.3.2 PCBs

Polychlorinated biphenyls (PCBs) are synthetic chlorinated aromatic hydrocarbons that were first created in 1881; between 1930 and 1975 680 million kilograms were manufactured in the United States (Hoffman et al. 1996). Because of PCBs unique chemical properties they were used in a many industrial processes such as heat transfer agents, lubricants, dielectric agents, flame retardants, plasticizers, water proofing material, and most notably for cooling in electrical transformers (Hoffman et al. 1996). They are resistant to chemical breakdown, and have high thermal stability, low vapor pressure, flammability, and solubility (Niimi 1996). PCBs consist of two benzene (phenyl) rings connected by a carbon bond to which chlorine atoms are connected. The number of chlorine atoms provide the base for the 209 PCB congeners (Rice et al. 2003).

Originating from industrial leaks, sewage runoff, landfills, and incinerators, researchers have detected PCBs worldwide in the atmosphere, water, fish, birds, mammals, and humans (Hoffman et al 1996). Because of PCBs chemical structure, they are extremely persistent in the environment and resist being broken down by bacteria or chemicals.

[1] Builds up exponentially when one organism eats another.

However, PCBs are easily absorbed into the fat of plankton and enter the wood web (Hoffman et al 1996) and are eventually consumed by wildlife and humans.

In wildlife, PCBs both bioaccumulate and biomagnify. Piscivorous (fish eating) birds are most exposed to PCBs, and eagles and other top trophic level predators are particularly vulnerable to accumulating elevated levels. PCBs are extremely toxic to biota, causing wasting, immune effects, reduced reproduction, and liver damage (Hoffman et al 1996). In birds PCBs reduce egg hatchability, increase liver size, and affect thyroid and spleen function (Hoffman et al 1996). Researchers have observed similar effects in mammals with PCBs reducing reproductive success, and at high levels can lead to death (Kamrin and Ringer 1996). Because of these known effects, PCBs were banned in the United States in 1979 (Rice et al. 2003). Today in Maine PCBs are still widely detected in wildlife. They have been detected in mussels (Chase et al. 2001), seabirds, shorebirds (Mierzykowski and Carr 2004), eagle (Matz 1998), porpoise (Westgate et al. 1997), dolphin, and pilot whale (Weisbrod et al. 2001).

2.3.3 PBDEs

Polybrominated diphenyl ethers (PBDEs) are brominated flame retardants that are used in both commercial and residential textiles and electronics. They work by slowing combustion by releasing hydrogen bromide gas, which interferes with the chemical reaction that spreads fire (Janssen 2005). PBDEs consist of two benzene rings linked by an oxygen atom and can have up to ten attached bromine atoms (Hellstrom 2000). This stable structure causes the molecules to be lipophilic (fat loving) and consequently subject to bioaccumulation (Karlsson et al. 2006). The three primary types of PBDEs are penta-BDE, octa-BDE, and deca-BDE. Penta has been primarily used in polyurethane foam (up to 30% in weight) that is used in couches, carpets, and mattresses; octa is used in computer monitor plastics; and deca, which makes up 83% of global PBDE production, is used in electronic equipment (Johnson-Restrepo et al. 2005). Deca-BDE is an off-white crystalline powder that is usually 10-15% of the weight of the host material and is an additive flame retardant that does not chemically bond to its host material. Consequently, deca-BDE migrates into the environment (DEP 2007). PBDEs enter the environment through atmosphere deposition, wastewater treatment facilities, and runoff (Anderson and MacRae 2006).

PBDEs are found globally in humans, wildlife, and the environment. They have been found in whales, Tasmanian devils, fish, and falcons in Australia (Symons et al. 2004); terns in San Francisco Bay (She et al. 2004); guillemots in the Baltic Sea (Sellstrom et al. 2003); peregrine falcons in Sweden (Sellstrom et al. 2001); marine fish in Florida (Johnson-Restrepo et al. 2005); seabirds in Norway (Murvoll 2006); birds of prey in Belgium (Voorspoels et al. 2004); birds of prey in China (Chen et al. 2007); fish in Maine's Penobscot River (Anderson and MacRae 2006); and Arctic fox in Greenland and Russia (Lifgren 2005).

Laboratory studies have documented health effects of PBDEs, generally at levels higher than currently observed in the environment. Rats fed penta-BDE had reduced growth, diarrhea, reduced activity, tremors, red stained eye edges, and chewed continuously. Those animals that received repeated doses had changes in hepatic and thyroid size and histology as well as immunological effects. Rats fed octa-BDE had enlarged livers, and fetuses with bent ribs, limp bones, and rear limb malformations. Although health effects were observed at higher doses, animals dosed with deca-BDE had enlarged livers, and hyaline degeneration in kidneys. Those fed deca-BDE for 103 weeks at high doses developed tumors as well as an increase in thyroid, hepatic and pancreatic adenomas (Darnerud 2003). A dosing study on kestrels found changes in thyroid levels and concludes: "Concentrations of PBDE congeners in wild birds may alter thyroid hormone and vitamin A concentrations, glutathione metabolism and oxidative stress (Fernie et al. 2005)." Because of these effects, penta and octa were voluntarily phased out in 2004 (EPA website), and deca was partially banned in Maine and Washington State in 2007.

2.3.4 PFCs

Perflorinated chemicals (PFCs) have been produced for over 50 years for their repellant properties and are used as stain repelents, cleaning agents, floor polish, fire-fighting foam, and in photography (Tao et al. 2006). Most commonly used PFCs are derived from perfluorooctanesulfonyl fluoride (POSF), which have extremely strong carbon-fluorine bonds. These strong bonds make the PFCs highly resistant to environmental and metabolic degradation (Butenhoff et al. 2006) and are consequently environmentally persistent (Kannan et al. 2002). Of the PFC congeners, perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) are of greatest concern because of their global abundance and bioaccumulation (Giesy and Kannan 2001, Kannan et al. 2002, and Tao et al. 2006).

Annual estimated production of POSF in 2000 was greater than 5000 tons (Tao et al. 2006), but by 2002 the 3M Company—the primary manufacture of POSF—discontinued production (Butenhoff et al. 2006). However, some PFOS is still produced outside of the United States for applications where there are no alternatives (Butenhoff et al. 2006) and other PFC are still produced and used in the United States (Kannan pers. com.). PFCs are transported in the environment through ocean currents and the atmospheric circulation (Toa et al. 2006) and may enter the environment through similar pathways as PBDEs.

Although there has been no analysis of PFCs in Maine, they have been documented in wildlife in the Southern Ocean and Antarctica (Toa et, 2006), Artic, North America, Pacific Ocean, Japan, Europe (Giesy and Kannan 2002), seaotters in California (Kannan et al. 2006), birds in Japan and Korea (Kannan et al. 2002), and in fish and pelicans in Columbia (Olivero-Verbel et al. 2006).

PFOS are documented to have health effects in wildlife. Hen eggs injected with PFOS had significantly lower hatching success (Molina et al. 2006). Quail exposed to PFOS through diet had increased liver weight and, at high levels, died (Newsted et al. 2007). In

California, diseased sea otters were positively associated with elevated PFOS levels (Kannan et al. 2006).

2.3.5 OCs

Organochlorine pesticides (OCs) are used primarily for insect control, are extremely persistent in the environment, and bioaccumulate in wildlife (Blus 2003). The five major groups are dichlorodiphenyltrichloroethane (DDT), hexachlorocyclohexane (HCH), cyclodienes, toxephene, and chlordecone.

2.3.6 HCH

Hexachlorocyclohexane (HCH) is an insecticide that is currently used in agriculture—the most wildly used form is lindane. Unlike other OCs pesticides, lindane has a short half-life and rapidly degrades after use. Consequently, lindane is rarely found in wildlife. However, in some laboratory studies lindane has reduced hatching success, increased embryo mortality, and caused egg shell thinning in chickens. In other studies researchers documented little effects (Blus 2003).

2.3.7 HCB

Hexachlorobenzene (HCB) is a fungicide used most commonly on seed grains, is an industrial waste product, and is used in the manufacture of tire rubber (Wiemeyer 1996). HCB is persistent in the environment and experimental studies have documented death and significant effects in birds. Quail fed high doses of HCB had weight loss, ruffling of feathers, and tremors. Birds fed a lower does had reduced hatchability of eggs and sterile eggs (Wiemeyer 1996).

2.3.8 Chlordane

Chlordane is composed of number of OCs and has been used since the 1940s (Blus 2003). In 1978 most chlordane was restricted in the United States; all chlordanes are now banned (Wiemeyer 1996). The most toxic metabolite is oxychlordane (Wiemeyer 1996). In the past chlordane was used extensively on lawns, golf courses, and crops, and is persistent in the environment. The most measured effect in experimental settings is death. As recently as 1997 over 400 birds died from eating beetles with high chlordane residues in an area that had been treated in the past (Blus 2003).

2.3.9 DDT

Dichlorodiphenyltrichloroethane (DDT) was first synthesized in 1874, used as an insecticide in 1939, used extensively in agriculture after World War II (Blus 1996), and banned in the United States in 1972 (Blus 2003). Despite the well-documented effects on wildlife, DDT is still used in a number of countries. After application DDT breaks down to DDE. DDE has been well documented to cause egg shell thinning, which causes eggs to break during incubation. Because of the persistent nature of DDE, it is still widely detected in birds although at levels generally below effects thresholds (Blus 2003).

2.4 Birds as bioindicators of the environmental contaminants

Birds are commonly used as indicators of Hg and other contaminants in the environment (Scheuhammer 1987, Furness and Camphuysen 1997, Wolfe et al. 1998, Cifuentes et al. 2003, Braune 2007, Evers et al. 2005, and Sheuhammer et al. 2007, and Wolfe et al. 2007). The species we selected for this contaminant screening represent distinct foraging guilds and ecosystems across Maine. Additionally, some of the species we selected are high trophic level predators that may accumulate contaminants at higher levels. In total the 23 species of birds in our study indicate the contaminants other biota, and people—through consuming fish and game—may be exposed to.

2.5 Eggs as indicators of local contaminants

Eggs are used extensively for contaminant studies (Wiemeyer 1996, Kannan et al. 2001, Braune et al. 2002, Evers et al. 2003, and Braune 2007) because female birds depurate lipophilic contaminants into their eggs. For most species, all of the egg nutrients are allocated from exogenous (i.e. recent dietary uptake) rather than endogenous (reserves acquired during migration and on winter grounds) sources (Bond et al. 2007, Hobson 2006, Hobson et al. 2000, and Hobson et al. 1997). Consequently, egg contaminant residues represent the contaminants present in the bird's breeding territory diet (Hobson et. al 1997). These findings are supported by Evers et al. (2003), which found a strong relationship between common loon egg Hg levels and female Hg blood levels (blood represents recent dietary uptake). The exception is species that arrive on the breeding ground and immediately lay eggs (Hobson 2006). The species in our study are all present at their breeding site for at least two weeks prior to laying eggs (Table 1). Therefore, the results presented in this report represent contaminant levels of the birds within their foraging range during the breeding season in Maine.

4. METHODS

4.1 Field

We collected viable and nonviable eggs from osprey in 2007 and 2009 (Table 1, Figure 1). The eggs were collected by BRI staff and placed in polyethylene bags and sent with dry ice to the Wadsworth Center (New York State Department of Health) for analysis (see below for methods). BRI currently has state and federal collection permits.

Table 1. Samples collected.

Location	Year	Location
Penobscot Bay	2007	Fort Point
Penobscot Bay	2007	Hog Island
Penobscot Bay	2007	Verso Mill
Kennebec River Mouth	2007	S. Sugarloaf Island
Casco Bay	2007	Bug Light, S. Portland
Casco Bay	2007	Fore River, Airport
Casco Bay	2009	Cliff Island
Casco Bay	2009	Crow Island
Casco Bay	2009	Falmouth Harbor
Casco Bay	2009	Flag Island
Casco Bay	2009	Fore River-East
Casco Bay	2009	Fore River-West
Casco Bay	2009	French Island
Casco Bay	2009	House Island
Casco Bay	2009	Ragged Island
Casco Bay	2009	Wolfe's Neck State Park

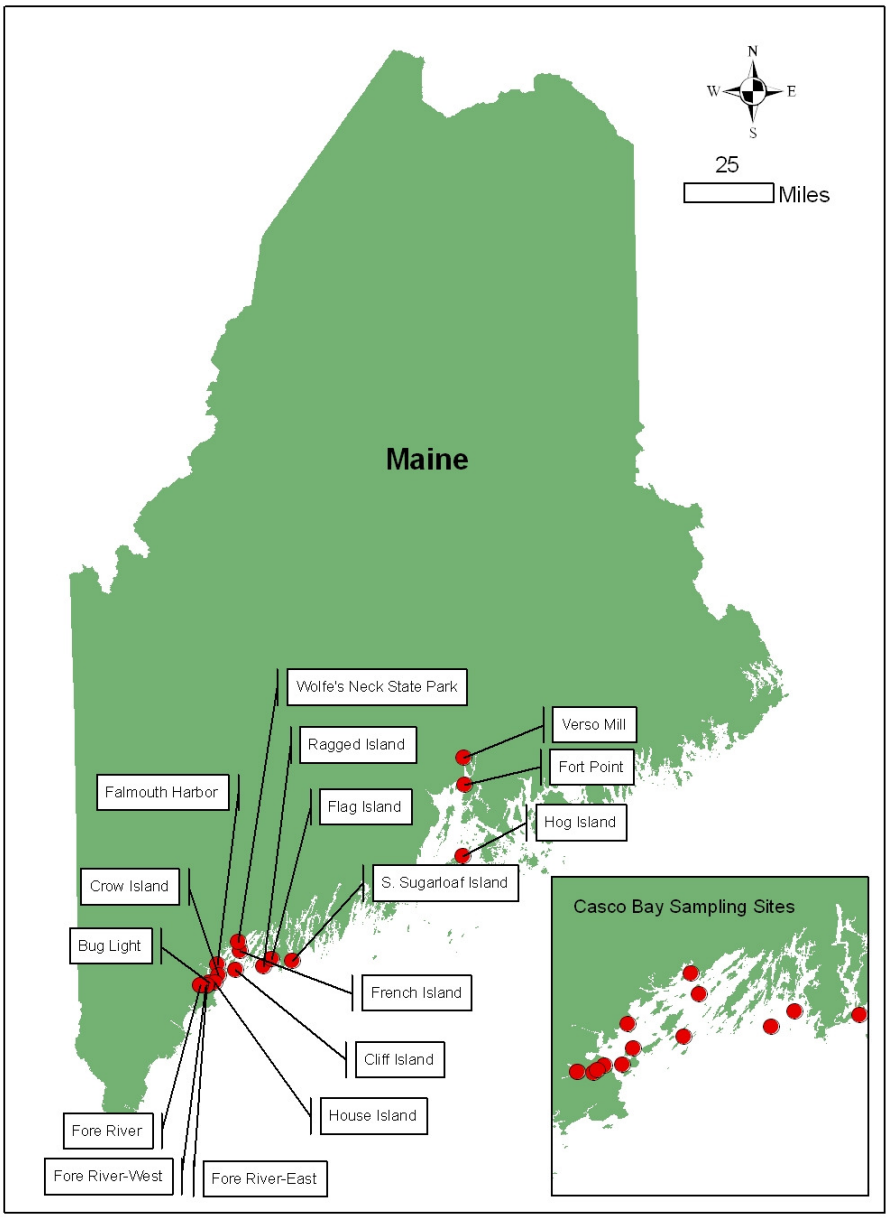


Figure 1. Sampling sites.

4.2 Statistics

We performed statistics with JMP (SAS Institute Inc., 2001). The range of each contaminant was displayed in three categories determined by natural breaks within the contaminant range for each species. Trends were evaluated qualitatively.

4.3 Egg morphometric measurements

An hand-held caliper, capable of recording the 0.1 of a mm was used to determine the length and width. The egg length was measured from tip to tip of the egg. The width was measured from the widest point of the egg. A digital balance capable of weighing to the 0.1 of a gram was used to measure weight of the eggs with shell (whole egg) and without shell (content weight). Graduated measuring cylinders with Milli-Q water was used to determine the volume of eggs, determined as the volume of water displaced (recorded in ml). Developmental stage of the eggs were recorded as a ranking of the developmental stage of the embryo. An embryological development scale used for common loon and waterfowl eggs was used to assess the developmental stage and ranked as NA, 0,1,2,3,4,and 5 as below:

NA (not assessable): Developmental stage could not be determined. Contents were gray or yellowish-tan in color and typically had a foul smell. A darker color suggested some degree of development had occurred, whereas a yellow homogeneous liquid may be sifted through and if no dark spots or hardened areas were found we classified the egg as infertile (0).

0: No development was evident. Egg had a yellow/orange or yellow/tan yolk (intact or broken down into a liquid). A translucent jelly-like mass surrounded the yolk sac and showed no sign of embryonic development (e.g. mass not dark or hardened).

1: Embryo was viable (length was up to 1.5 cm). The jelly like mass (embryo) was dense and hardened. Small dark (red) eyespots may be visible at this stage.

2: Developing embryo (length was 1.5 – 2.0) has an apparent central nervous system. Cranial development and visible eyes are apparent. Feathers are absent.

3: The embryo shows advanced development (length was 2-3 cm). Bill was developed (e.g. egg tooth present but soft). Legs and wings were visible but not fully developed. Some feathers were present (first seen in tail).

4: The fully developed embryo was completely covered by feathers. Appendages were completely developed. Vent, preen gland was visible. A small portion of yolk sac remained attached to belly.

4.4 Analysis of egg moisture and lipid contents

After the determination of morphometric parameters on each of the eggs, some samples collected from the same location and same species were pooled and homogenized using a homogenizer and composites were prepared. The composites were used the analysis of trace organic contaminants and mercury. Homogenized egg samples (in most cases 10-11 g; for some samples only 5 g was used due to the availability) were extracted with dichloromethane and hexane (1:3; 400 mL) in a Soxhlet apparatus for 16 h after spiking the samples with surrogate standards (PCB-30 and PCB204). The extracts were concentrated to 10 mL and 1 ml of the aliquot was taken for the analysis of lipid content by gravimetry. An aliquot of the egg homogenate (approximately 2 g) was also taken and freeze-dried to measure the moisture content.

4.5 Analysis of PCBs, PBDEs and organochlorine pesticides

Details of the analytical methods have been described elsewhere (Kannan et al., 2005; 2007). An aliquot of the sample extract was spiked with ^{13}C-labeled PCB congeners 3, 15, 31, 52, 118, 153, 180, 194, 206, 209, and ^{13}C-labeled PBDE congeners 3, 15, 28, 47, 99, 100, 118, and 153 as internal standards. PCB congeners 30 (2,4,6-triCB) and 204 (2,2',3,4,4',5,6,6'-octaCB) were spiked as surrogate standards. The sample extracts was then purified by passage through a series of layers of silica gel (Davisil, 100-200 mesh, Aldrich, WI; 1 g of silica gel, 2 g of 40% acidic-silica gel, 2 g of 20% acidic-silica gel, and 1 g of silica gel at the top). The analytes were then eluted using 150 mL of 20% dichloromethane in hexane. The extracts were then concentrated using a rotary evaporator and treated with sulfuric acid (5 mL) and further concentrated to 1 mL for the analysis of PCBs and PBDEs. Another portion of the extract was passed through silica gel (2 g) by elution with 20% dichloromethane in hexane; it was then treated with sulfuric acid, for the analysis of organochlorine pesticides.

Extracts were injected into a gas chromatograph (Hewlett-Packed 6890) coupled with a mass-selective detector (Hewlett-Packed, series 5973) for the determination of PCBs and PBDEs. A capillary column coated with RTX-5MS (30 m x 0.25 mm i.d. x 0.25 µm film thickness; Restek Corp, Bellefonte, PA) was used for the separation of individual isomers. The column oven temperature was programmed from 100°C (1 min) to 160°C (3 min) at a rate of 4°C/min, and then to 250°C at 3°C/min, with a final hold time of 5 min for PCBs. For PBDEs, the column temperature was programmed from 100 °C (1 min) to 160°C (3 min) at a rate of 10°C/min, and then to 260°C at 2°C/min, with a final hold time of 5 min. The MS was operated in an electron impact (70 eV), selected ion monitoring mode. An equivalent mixture of Kanechlor (KC300, 400, 500, and 600) with known PCB composition was used in the identification of PCB congeners. One hundred and fifty four isomers of PCBs with 35 coleuting pairs (IUPAC number in the order of GC-MS elution: 4+10, 9+7, 6, 5+8, 19, 18, 17, 15, 24+27,16+32 ,26,25,28+31, 20+33+53, 22, 36, 37, 54, 50,53,51,45, 52+73, 46+69, 49+43, 47+48+75, 44, 59+42, 41+64, 40+57, 67, 63, 74+61, 70+76, 66+80, 60+56, 77, 104, 98+102, 93+95, 91, 92, 84, 90+101+89, 99, 86+97, 97+113, 87+117+125+116+111+115, 85+120, 110, 82, 124, 107,

118+106, 114+122, 105+127, 126, 155, 136, 151, 135+144, 149+139, 134, 133, 146+161, 153, 132+168, 141, 137, 130, 138+164+163, 158, 129, 128, 167, 156, 157, 169, 188, 179, 176, 178, 187+182, 183, 185, 174, 177, 171, 173, 172+192, 180, 193, 191, 170, 190, 189, 202, 201, 197, 200, 198, 199, 196+203, 195, 194, 205, 208, 207, 206, and 209), including mono-*ortho* PCB congeners (105, 118, 189) were analyzed. Quantification of PCB congeners was based on external calibration standards containing known concentrations of di- through deca-CB congeners. Concentrations of individually resolved peaks of PCB isomers were summed to obtain total PCB concentrations. PBDE congeners were monitored at molecular ion clusters, $[M]^+$ and $[M+2]^+$ or $[M+4]^+$. Tri- through hexa-PBDE congeners analyzed in this study were 28, 30, 47, 66, 85, 99, 100, 138, 153, and 154 were targeted for analysis. Hepta- through deca-BDE congeners (183, 203, and 209) were analyzed using a Agilent Technologies 6890N gas chromatograph-electron capture detector (GC-ECD). PBDE congeners were quantified using an external calibration standard. Organochlorine pesticides were analyzed using a Agilent Technologies 6890N gas chromatograph-electron capture detector (GC-ECD; for HCH isomers) and a gas chromatograph (Hewlett-Packed 6890) coupled with a mass-selective detector (Hewlett-Packed, series 5973) for DDTs, chlordanes and HCB. A capillary column coated with DB-5 (30 m x 0.25 mm i.d. x 0.25 µm film thickness) was used for the separation of pesticides. Concentrations were calculated from the peak area of the sample to that of the corresponding external standard. DDTs refers to the sum of *p,p'*-DDE, *p,p'*-DDT and *p,p'*-DDD; chlordanes to the sum of *cis*-chlordane, *cis*-nonachlor, *trans*-nonachlor, and oxychlordane; HCHs to the sum of α-,β-, and γ-isomers. PCB and PBDE congeners are represented by their IUPAC numbers.

4.6 PCB and PBDE quality assurance and quality control

The extraction, clean-up, and fractionation steps were evaluated by measurement of the absolute recoveries of the compounds spiked and passed through the entire analytical procedure. Mean (± standard deviation) recoveries of ^{13}C-labeled PCB congeners #30, 118, 153, and 194 spiked into the samples were 80 ± 14%, 82 ± 17%, 89 ± 12%, and 91 ± 14%, respectively. Recoveries of surrogate PCB congeners CB-30 and CB-204 spiked into the egg samples prior to extraction were 72±10%. Mean (± standard deviation) recoveries of ^{13}C-labeled PBDE congeners 28 and 47 were 92 ± 14% and 91 ± 14%, respectively. Overall recoveries of PBDEs ranged from 82 to 103%. The reported concentrations of PCBs, PBDEs and pesticides were corrected for the recoveries of surrogate standards (CB-30 and CB-204). Recoveries of organochlorine pesticides through the analytical procedure ranged from 85 to 110%. Procedural blanks were analyzed for every set of 10 samples, as a check for interferences. Calculated concentrations were reported as below the limit of detection, if either the observed isotope ratio was not within ±20% of the theoretical-ratio, or the peak area was not greater than the specified threshold (3 times the noise). Known concentrations of PCBs, PBDEs, and organochlorine pesticides were spiked into selected samples (matrix spikes) and passed through the entire analytical procedures to calculate the recoveries. Recoveries of all of the target compounds spiked into egg matrixes were between 84 and 106% with a standard deviation of <15%. The quantitation limits of individual PBDE

congeners varied from 10 to 500 pg/g, wet wt. The quantitation limit for organochlorine pesticides varied from 50 to 1000 pg/g, wet wt.

4.7 Analysis of perfluorinated compounds:

Potassium salts of PFOS (86.4%), PFOA (98%), PFOSA (95%), PFHxS (99.9%), and PFBS (99%) were provided by the 3M Company (St. Paul, MN). PFHpA, PFNA, PFDA, and PFUnDA were from Fluorochem Ltd (≥95% purity, Derbyshire, UK). $^{13}C_4$-PFOS, $^{13}C_4$-PFOA (99% purity, Wellington Laboratories, Guelph, ON, Canada), $^{13}C_4$-PFNA and $^{13}C_4$-PFDA were used as internal standards and were spiked into egg samples prior to the addition of reagents for extraction.

PFCs in eggs were analyzed following the method described elsewhere (Tao et al., 2007). Egg homogenates (0.3-0.5 g) were taken in 15-mL polypropylene (PP) tubes and 5 ng of internal standards ($^{13}C_4$-PFOS, $^{13}C_4$-PFOA, $^{13}C_2$-PFDA, and $^{13}C_2$-PFNA), 2 mL of 0.25 M sodium carbonate buffer, and 1 mL of 0.5 M tetrabutylammonium hydrogensulfate solution (adjusted to pH 10) were mixed. Sample was then extracted with 5 mL of methyl-tert-butyl ether (MTBE) by shaking vigorously for 45 min. The MTBE layer was separated by centrifugation at 3500 rpm for 5 min and then transferred into another PP tube. The extraction was repeated twice with another 3 mL of MTBE. The MTBE extract was combined and evaporated to near-dryness under a gentle stream of nitrogen and then reconstituted with 1 mL of methanol. The sample was vortexed for 30 sec and filtered through a 0.2-µm nylon filter into an autosampler vial. Matrix-matched calibration standards (seven points ranging from 0.5 ng/mL to 75 ng/mL) were prepared by spiking different amounts of calibration standards into a sample that contained no quantifiable amount of the target analytes; these standards were passed through the entire analytical procedure along with the samples.

Analytes were detected and quantified using an Agilent 1100 series high-performance liquid chromatography (HPLC) coupled with an Applied Biosystems API 2000 electrospray triple-quadrupole mass spectrometer (ESI-MS/MS). Ten microliters of the extract were injected onto a 50 x 2 mm (5 µm) Keystone Betasil® C18 column. The mobile phase was 2 mM ammonium acetate/methanol starting at 10% methanol, at a flow rate of 300 µL/min. The gradient increased to 100% methanol at 10 min and was held for 2 min, and then reversed back to 10% methanol. The MS/MS was operated in electrospray negative ion mode. Target compounds were determined by multiple reaction monitoring (MRM). The MRM transitions were 299>80 for PFBS, 399>80 for PFHS, 499>99 for PFOS, 503>99 for $^{13}C_4$-PFOS, 599>99 for PFDS, 498>78 for PFOSA, 363>169 for PFHpA, 369>169 for PFOA, 372>172 for $^{13}C_4$-PFOA, 463>219 for PFNA, 513>219 for PFDA, 563>169 for PFUnDA, and 613>169 for PFDoDA. Samples were injected twice, to monitor sulfonates and carboxylates separately, and PFBS was monitored in both of the injections. A mid-point calibration standard was injected after every 10 samples to check for the instrumental response and drift. Calibration standards were injected daily before and after the analysis.

The egg samples were quantified with the quadratic regression fit analysis weighted by 1/x of a matrix-extracted calibration curve. The limit of quantitation (LOQ) was determined as the lowest acceptable standard in the calibration curve that is defined as a standard within ±30% of the theoretical value, and that has a peak area twice as great as the analyte peak area in blanks. LOQs for PFCs were 0.28 to 0.6 ng/g, wet wt, except for PFDS and PFBS, for which the LOQs were 0.94 and 1.12 ng/g, wet wt, respectively.

4.8 PFC quality assurance and quality control

Matrix spikes (6 egg composites) were performed for egg samples. Known amounts of mixed PFC standards (20 ng each) were spiked into sample matrices before extraction and were passed through the entire analytical procedure. Recoveries of PFCs spiked into egg homogenates and passed through the entire analytical procedure are shown in Table 3. The recoveries of all the PFCs were acceptable except for PFBS, which had a low recovery; however, PFBS does not bioaccumulate in tissues and also had not been detected in biological samples. Four 13C-labeled internal standards were spiked into all samples before the extraction, and the recoveries of internal standards are also shown in Table 3. Reported concentrations of PFCs in egg samples were not corrected for the recoveries of internal standards. Blanks were analyzed by passing Milli-Q water and reagents through the whole analytical procedure. Blanks contained trace levels of PFOA (<100 pg). Reported concentrations for PFOA in egg samples were subtracted from the mean blank values. A midpoint calibration standard was injected after every 10 samples to check for instrumental stability, response and drift. Calibration standards were injected daily before and after the analysis.

4.9 Mercury analysis

Egg composites were freeze-dried and homogenized; an aliquot (~0.1 g) of the sample was weighed in a vial lined with Teflon®. Samples were digested overnight in concentrated nitric acid (2 mL). Samples were then further digested in a microwave oven for 7 min at 200 W; this step was repeated three times. Concentrations of Hg were determined by a cold vapor atomic absorption spectrometer (Model HG-3000; Sanso, Tsukuba, Japan). The limit of quantification was 50 ng/g, dry wt. Accuracy of the analysis was examined by analyzing Certified Reference Materials: dogfish muscle (DORM2; National Research Council, Ottawa, ON, Canada) and bovine liver (SRM1577b; National Institute of Standards and Technology, Gaithersburg, MD, USA) along with the samples. The overall analytical scheme used for the analysis of egg samples is shown in Figure 3.

5. RESULTS AND DISCUSSION

Overall the osprey contaminant load recorded in this study is consistent with Goodale 2008 and 2009. Compared to other species, osprey were in the upper third of overall contaminant load, but were lower than bald eagles (*Haliaeetus leucocephalus*), peregrine falcons (*Falco peregrinus*), and great black-backed gulls (*Larus marinus*) (Figure 3).

5.1 Relationship between compounds (Figure 2)

Similar to the results in Goodale 2008 & 2009, we found a significant positive relationship between several of the organic compounds. These osprey results show a significant relationship between total PCBs and PBDEs as well as PFOS, total chloradane, and DDT. This is consistent in studies conducted with OCs, which show that the pesticides are positively correlated in animal tissue (Blus 2003). This is of particular interest because in mice PCBs and PBDEs are demonstrated to interact, and together, at low doses can enhance developmental neurobehavioral defects (Eriksson et al. 2006). Additionally, researchers have also found that organochlorine pesticides (both DDE and chlordane are OCs) interact (Blus 2003).

The simultaneous increase in these compounds may be caused by a number of factors, including the similar chemical structure of PCBs and PBDEs, and their similar pattern of bioaccumulation. PCBs, PBDEs, and DDE are all composed of two benzene rings, but in PCBs the benzene rings are connected with a carbon bond, while in PBDEs there is an oxygen atom. PBDEs have attached bromine atoms, while PCBs have attached chlorines. This similar structure may mean that they move through the environment in a similar pattern.

PCBs and OCs have been extensively studied (Hoffman et al. 1996, Wiemeyer 1996, Blus 2003), but only recently have PBDEs been studied in wildlife. The positive relationship between these compounds suggests that species and geographic areas that have been documented to have high PCB levels may also have elevated PBDEs.

5.2 Hg

Hg is still being analyzed by the lab.

5.3 PCB (Figure 4, 5)

5.3.1 Comparison to known effects thresholds

The effects of PCBs on wildlife have been well studied (Blus 2003). Studies on bird eggs have shown chickens are particularly sensitive to total PCB levels and can show effects at 1,000-5,000 ng/g (ww) (Hoffman et al. 1996). In the field, total PCB levels have shown

effects ranging from 8,000 – 20,000 ng/g in terns and other species (Hoffman et al 1996). Our results indicate that osprey eggs (total PCB 471 – 2666 ng/g, ww) are likely below adverse affects threshold.

5.3.2 Comparison with other studies

Our mean osprey total PCB level (1,142 ng/g, ww) is lower than 1998 results in New Jersey (1,800-3,200 ng/g, ww)(Clark et al. 2001) and in the Great Lakes (5,000 ng/g, ww)(Martin et al. 2004), but comparable to than those previously detected in inland Maine (1,150 ng/g , ww) (Mierzykowski 2006).

Our total PCB results are lower than those detected in eagles in Maine between 1994-1996 [330-45,398 ng/g (fww)(Matz 1998)], and lower than those detected in eagles foraging on the Penobcot River, Maine [6,230 – 11,410 ng/g (fww)(Mierzykowski and Carr 2002)].

5.3.3 Spatial variation

There is not a general spatial trend for the osprey results in Casco Bay, but eggs collected from the Fore River near Portland did tend to have higher PCBs concentrations. These results suggest that individuals' exposure can be dramatically different because of potential point sources, specific watershed characteristics, or food web dynamics.

5.4 PBDEs (Figure 6, 7, 8, 9)

5.4.1 Comparison to known effects thresholds

Laboratory study in kestrels found negative physiological effects in chicks that had 1,500 ng/g total PBDE injected into their egg and were fed 100 ng/g per day (Fernie et al. 2005). Studies on chickens, mallards, and kestrels found that the lowest observed effect level for hatching success is 1,800 ng/g penta-BDE (McKernan et al. 2009). Our egg total PBDE residues, ranging from 75-393 ng/g (ww), are not as high as dosing studies. Compared to the results in Goodale 2008 and 2009, osprey had one of the highest total PBDE levels in Maine.

5.4.2 Comparison with other studies

Our mean osprey level, 217 ng/g (ww) is comparably to results from the Pacific Northwest (203-299 ng.g, ww)(Henny et al. 2009) and higher than results from Norway (103 ng/g, ww) (Herzke et al. 2005).

5.4.3 Spatial variation

PBDEs are distributed across Casco Bay in a similar pattern as PCBs and PFOS. There is not a general spatial trend for osprey across the Bay, with some of the most elevated levels in relative close proximity to lower levels. These results suggest that individuals' exposure can be dramatically different because of potential point sources, specific watershed characteristics, or food web dynamics.

5.4.4 Congener patterns

We detected deca-BDE (209) in all ten 2009 Casco Bay osprey eggs. BDE 47, 99, 100 made up the majority of the samples and there was a general consistent pattern between sites. This pattern was also consistent with near shore marine piscovores such as double-crested cormorant, black guillemot, herring gull, and great black-backed gull. Research has demonstrated that bacteria can cause deca to breakdown into the more toxic lower brominated congeners (He et al. 2006); consequently the levels of tetra- and octa-BDE that we recorded may have originated from deca.

5.5 PFC (Figure 10, 11)

5.5.1 Comparison to known effects thresholds

PFCs have only recently been identified as a persistent bioaccumulative contaminant of concern. Consequently, few studies have been conducted on effects in bird eggs. However, a study that injected perfluorooctane sulfonate (PFOS) in white leghorn chicken eggs—known to be particularly sensitive to contaminants—determined, based on reduced hatchability, that the lowest-observed adverse-effects level (LOAEL) was 0.1 ug/g or 100 ng/g (ww). The species we studied may be either more or less sensitive than the chickens.

Twelve out of our 16 (75%) osprey eggs have PFOS values above the LOAEL of 100 ng/g (ww)—75% of the Casco Bay eggs were also above 100 ng/g, ww. One sample of note is the osprey egg from Flag Island which had a PFOS level of 2545 ng/g, ww which is seven times greater than the mean.

5.5.2 Comparison with other studies

Only one study looked specifically at PFOS in bird eggs and our results are comparable to double-crested cormorants in the Great Lake region [157 ng/g (ww), yolk] and ring-

billed gull [67 ng/g (ww, yolk) (Kannan et al. 2001)]. Whole egg contaminant levels are lower than yolk levels.

These 2009 osprey results are consistent with Goodale 2008 and 2009 and demonstrate that osprey have one of the highest levels of PFOS of birds tested in Maine. Only bald eagles and belted kingfishers (*Megaceryle alcyon*) were higher. The high level detected on Flag Island is the highest record in Maine wildlife, and appears to be one of the highest bird egg levels detected in the world. The reason this particularly high level is not clear. Since birds can specialize in feeding on particular prey and areas, the Flag Island osprey may have foraged in a highly contaminated site. Although eggs generally reflect recent dietary uptake (see introduction), a bird that fed in a highly contaminated site during migration or in the wintering grounds does have a higher overall contaminant body burden which may be reflected in egg values. Note: this Flag Island egg sample also had high levels of organochlorine pesticides.

5.5.3 Spatial variation

PFOS are distributed across Casco Bay in a similar pattern as PCBs and PBDEs. There is not a general spatial trend, with some of the elevated levels in relative close proximity to lower levels. These results suggest that individuals' exposure can be dramatically different because of potential point sources, specific watershed characteristics, or food web dynamics.

5.6 Organochlorine pesticides (Figure 12, 13)

5.6.1 Comparison to known effects thresholds

Although the OCs tested are present in all species (except HCH), the samples are well below known effects thresholds. HCH are not detected in any samples. This is consistent with other studies that have not detected HCH, because it has a short half-life (Blus 2003).

Goodale 2008 results showed that the HCB range for 23 Maine bird species is 0.75 – 20.33 ng/g (ww), which is significantly below the effects threshold of 35,000 ng/g (ww) (Wiemeyer 1996). All the 2009 osprey samples fell within this range with the exception of Flag Island which had a value of 43 ng/g, ww.

Goodale 2008 results showed that the chlordane residues range for all species is 1.81 – 259.51 ng/g (ww), which is significantly below the effects threshold of 2,000 ng/g (ww) (Blus 2003). All the 2009 Casco Bay osprey samples fell within this range.

Goodale 2008 results showed that the DDE residues range for all species is 9.91 – 2,072.44 ng/g (ww), which is significantly below the effects threshold of 3,000 -30,000 ng/g (ww) (Blus 2003). All the 2009 Casco Bay osprey samples fell within this range (77-977 ng/g, ww). Studies indicate that slight egg shell thinning is possible at lower levels. Depending on the species, no eggshell thinning is seen below 100 to 2000 ng/g (Blus 1996). Flag Island had the highest level.

5.6.2 Comparison with other studies

The levels of OC measured in our study are generally in the range detected in other studies (see Goodale 2008 and 2009).

5.6.3 Spatial variation

DDEs are distributed across Casco Bay in a similar pattern as PCBs, PBDEs, and PFOS. There is not a general spatial tend, with some of the most elevated levels in relative close proximity to lower levels. These results suggest that individuals' exposure can be dramatically different because of potential point sources, specific watershed characteristics, or food web dynamics.

5.7 Overall conclusions

Our 2009 osprey results confirm findings in Goodale 2008 and 2009 that both established (Hg, PCBs, chlordane, HCB, DEE) and emerging (PBDEs, PFCs) toxic pollutants of concern, including deca-BDE, are bioaccumulating in Maine birds and that that PFCs are pervasive. Prior to our study, PFCs had not been tested in Maine birds.

These results also confirm that Maine osprey continue to be at risk from elevated levels of PFOS—75% of the eggs were above adverse effects thresholds. Of particular note is the one sample from Flag Island that had a PFOS level more than twenty-five times above the adverse effects threshold established for chickens (osprey may be more or less sensitive than chickens).

Overall these data do not show a consistent spatial pattern for osprey within Casco Bay. The samples from the Fore River near Portland did tend to have higher levels of PCBs, PBDEs, PFOS, and DDE, but there was not a distinct gradient from Portland across the bay. These results suggest that individuals' exposure can be dramatically different because of potential point sources, specific watershed characteristics, and/or food web dynamics.

Although the osprey data set is not large enough to conduct a rigorous statistical analysis, two of the emerging contaminants PBDEs and PFOS did have higher levels in Casco Bay than mid-coast Maine. This is consistent with the Goodale 2008, which found that birds

in southern Maine tended to have higher contaminant loads than birds in the rest of the state.

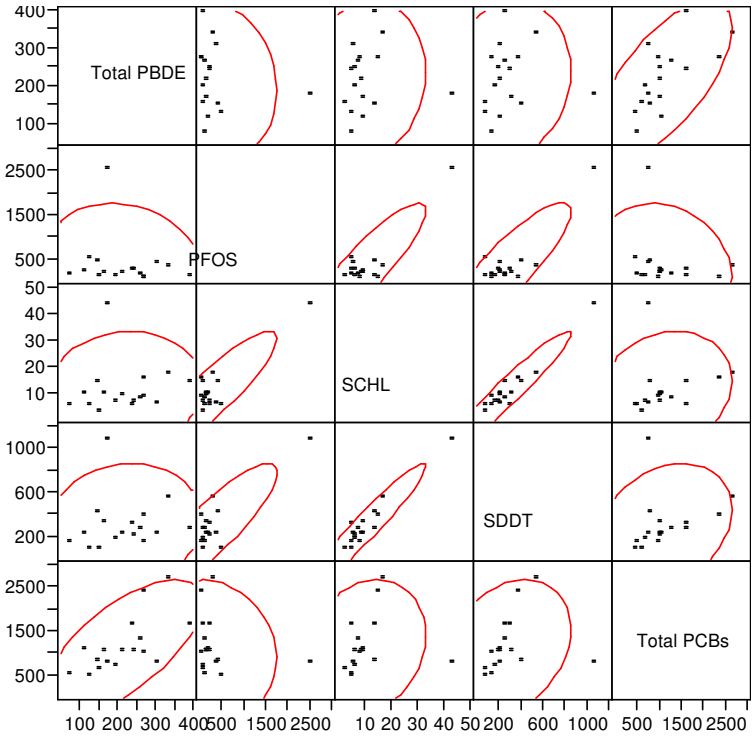
These 2009 osprey results continue to show that many of the compounds we measured increase in concert with each other. One of strongest relationship we found is between PCBs and PBDEs, indicating that species and areas with high PCB levels may also have high PBDE levels. These relationships suggest that some species may have higher levels simultaneously of multiple compounds, which together may have greater negative impact on reproductive success, the neurological system, endocrine function, and overall physiology. Consequently, high trophic level predators may have a combined negative effect of these compounds despite having individual contaminants below known effects thresholds.

In summary, our results indicate that both historical and emerging chemicals of concern are accumulating in birds that forage in diverse ecosystems across the entire state of Maine.

6. ACKNOWLEDGEMENTS

We would like to thank the field staff of BioDiversity Research Institute and the generous funding support from the Casco Bay Estuary Partnership.

7. FIGURES AND TABLES



Variable	by Variable	Correlation	Count	Signif Prob
SCHL	Total PBDE	0.0941	16	0.7196
SDDT	Total PBDE	0.1134	16	0.6649
PFOS	Total PBDE	-0.1442	16	0.5808
Total PCBs	SCHL	0.1777	16	0.4951
Total PCBs	PFOS	-0.1875	16	0.4712
Total PCBs	SDDT	0.265	16	0.3039
Total PCBs	Total PBDE	0.6613	16	0.0038
SCHL	PFOS	0.8818	16	0
SDDT	PFOS	0.8593	16	0
SDDT	SCHL	0.9582	16	0

Figure 2. Correlation between compounds. In the graph, the stronger relationships have tight ovals while poor relationships have circles. The closer the correlation value is to 1 the stronger the relationship. Rows highlighted in gray are significantly related.

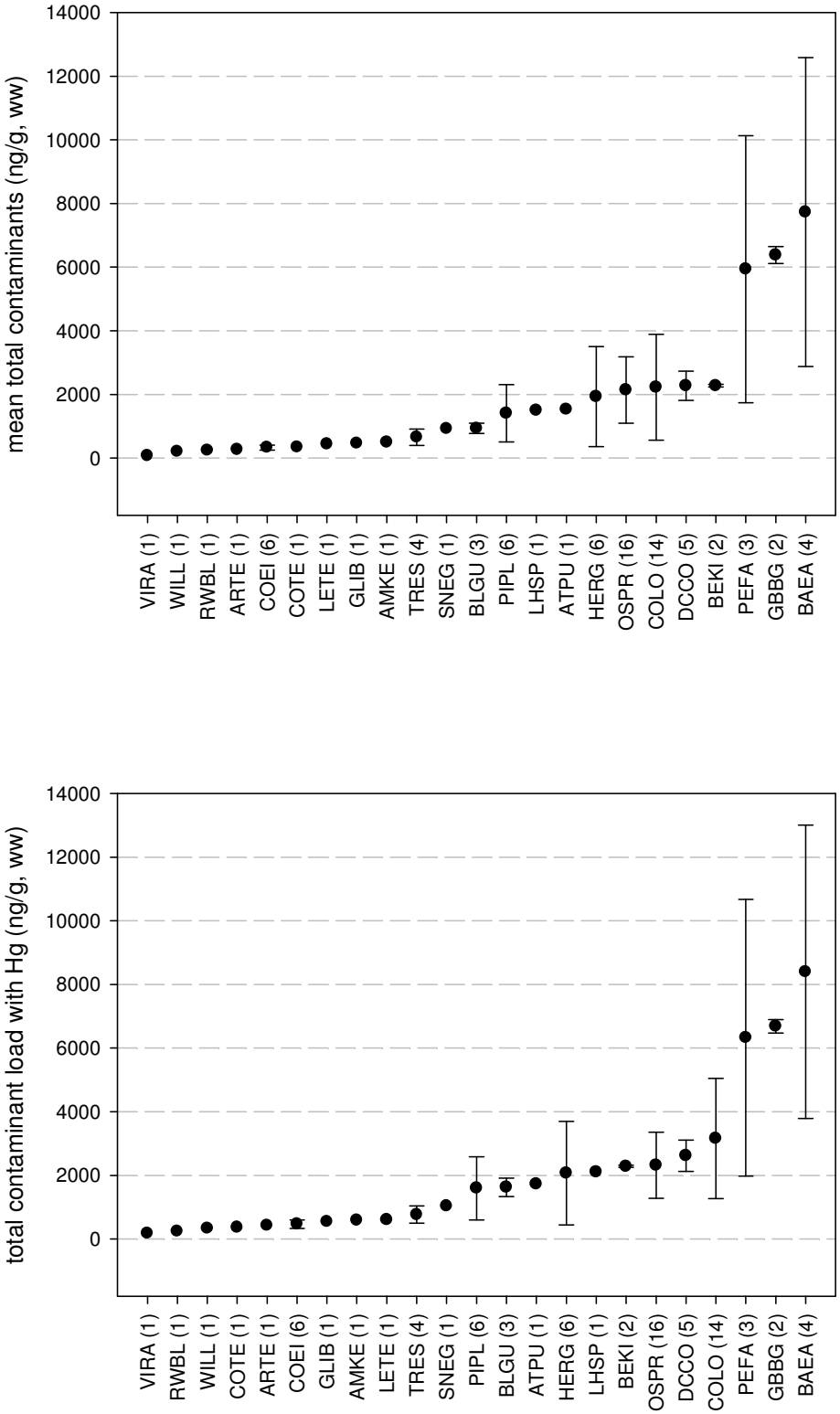


Figure 3. Osprey results compared to the data set as a whole. These data are the sum of all contaminants and are not weighed by the toxicity of different contaminants.

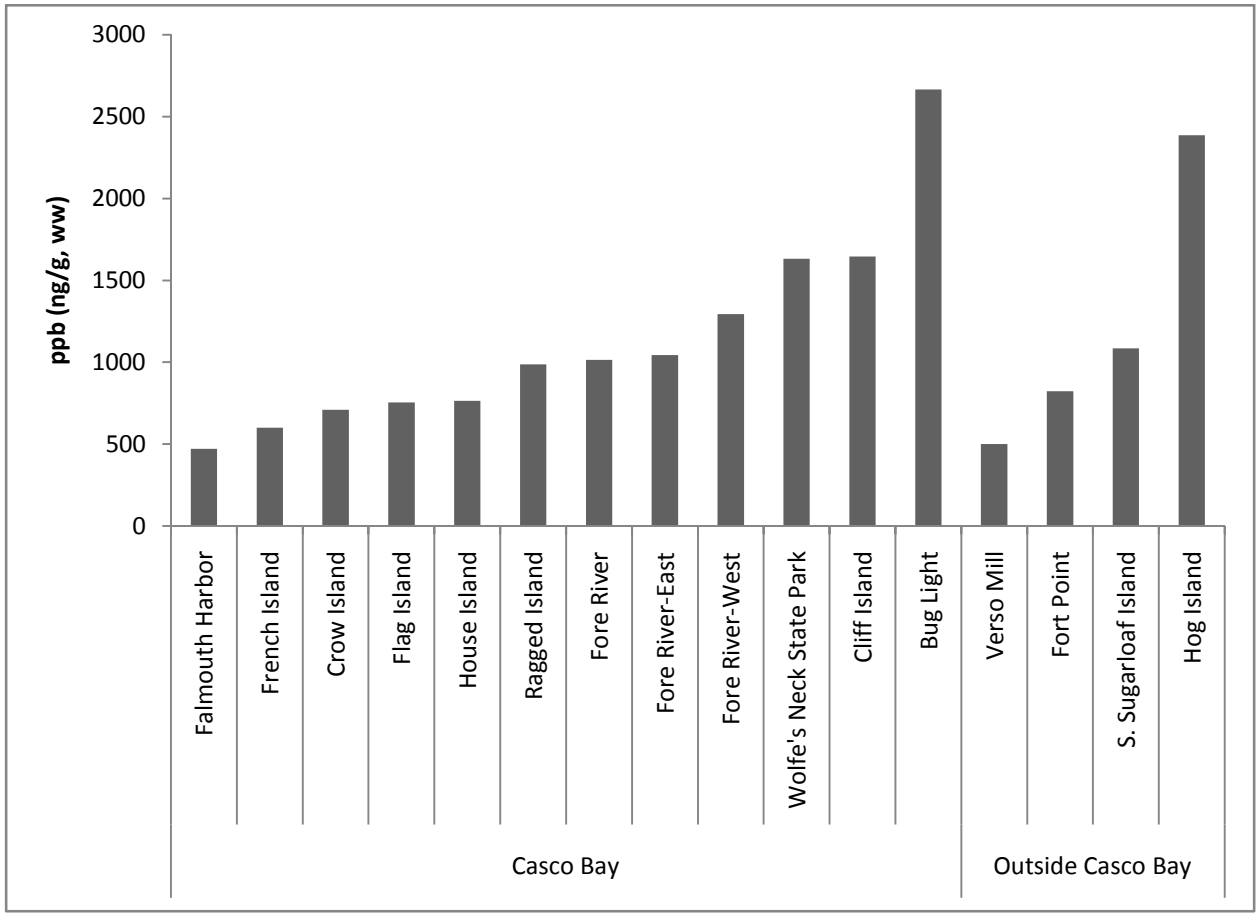


Figure 4. Total PCBs by site.

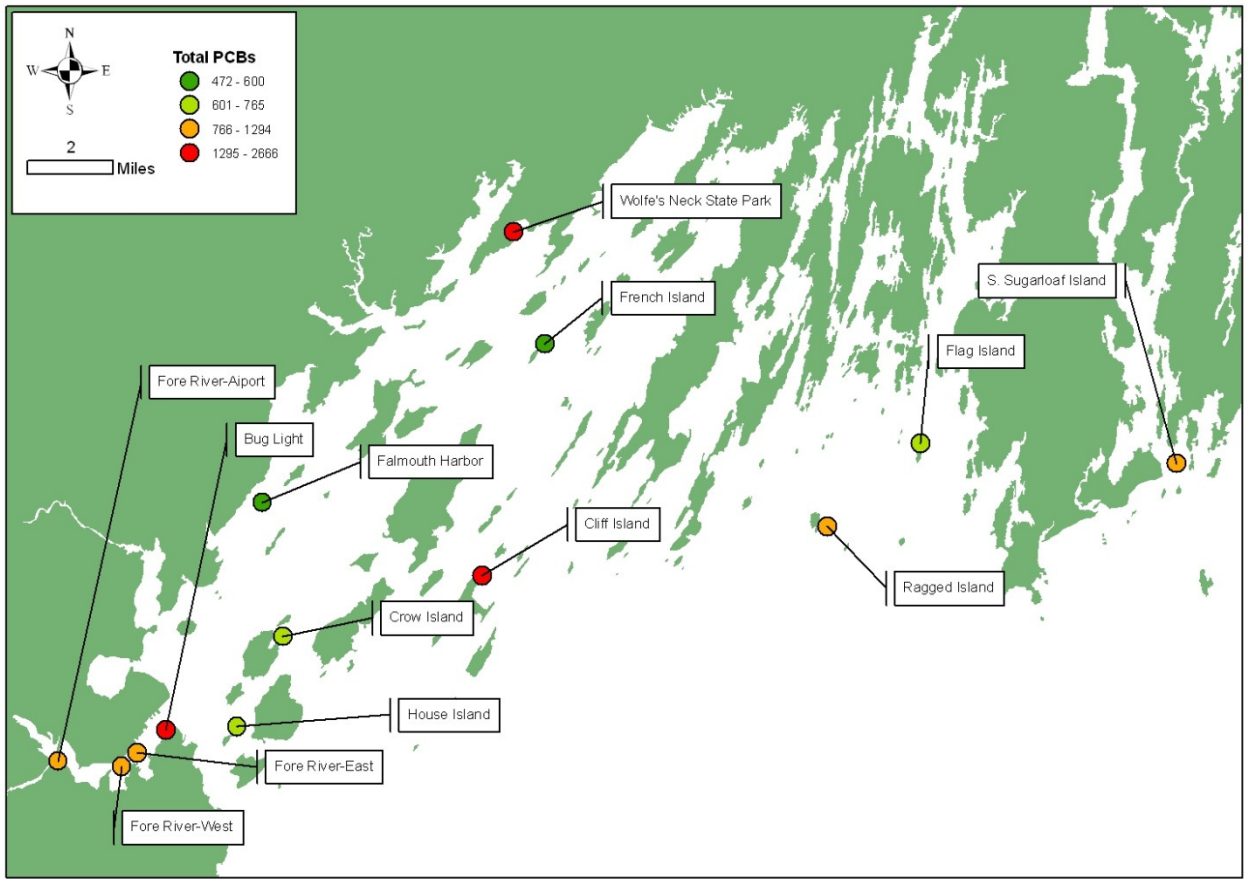


Figure 5. Map of Casco Bay total PCB levels.

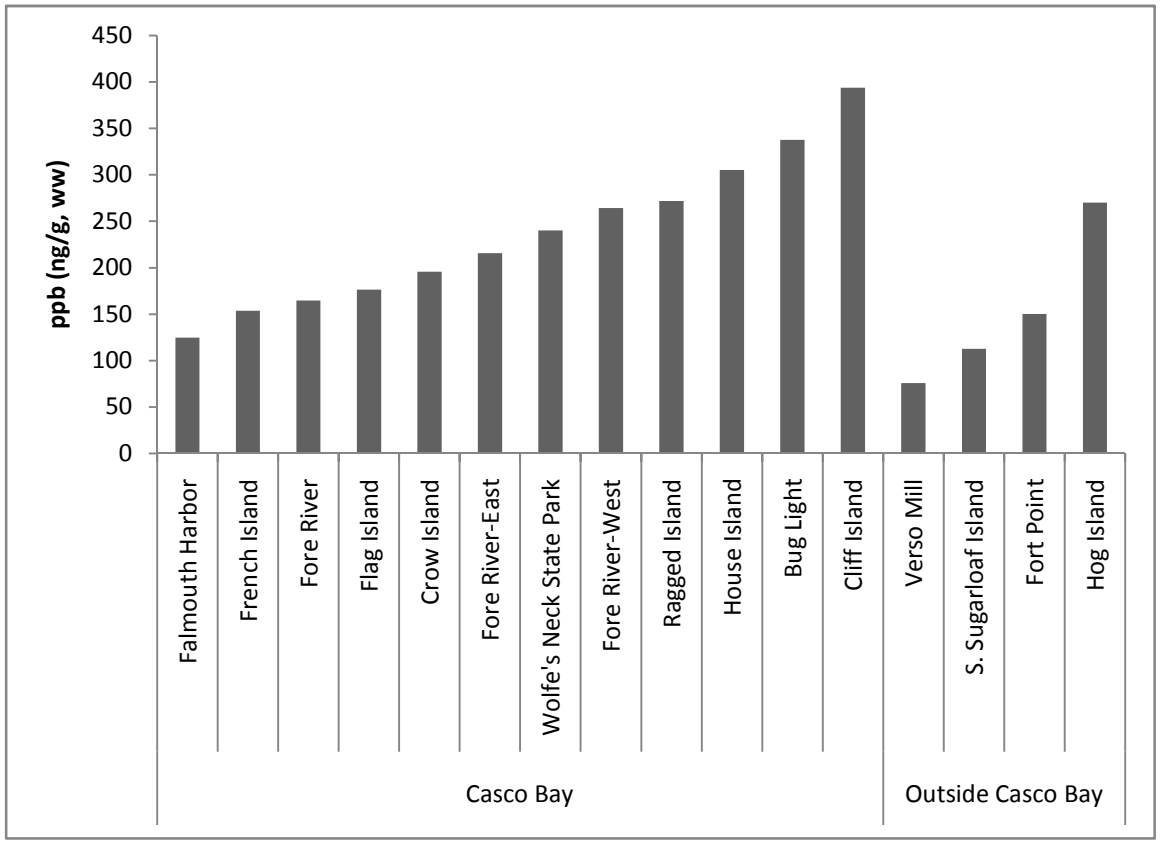


Figure 6.Total PBDE's by site.

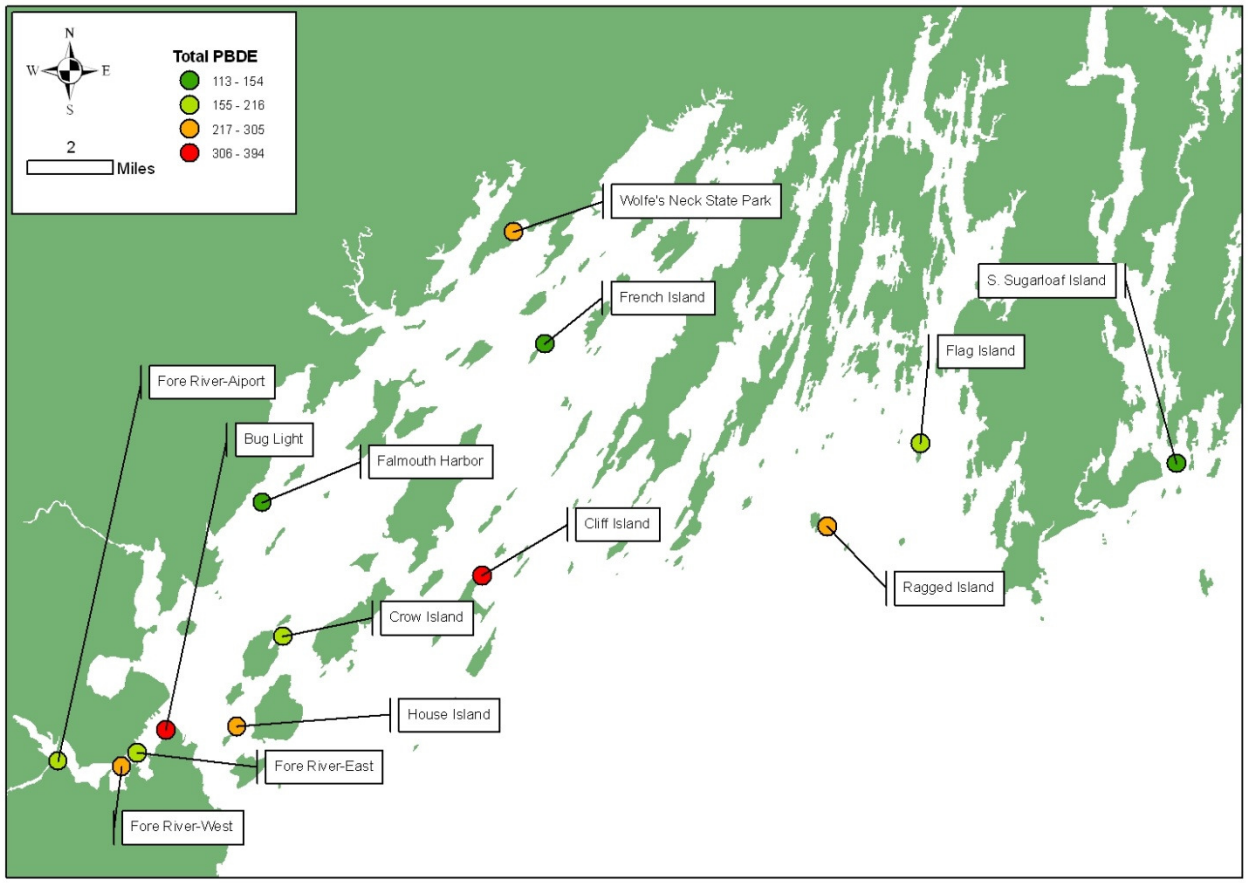


Figure 7. Map of Casco Bay total PBDEs.

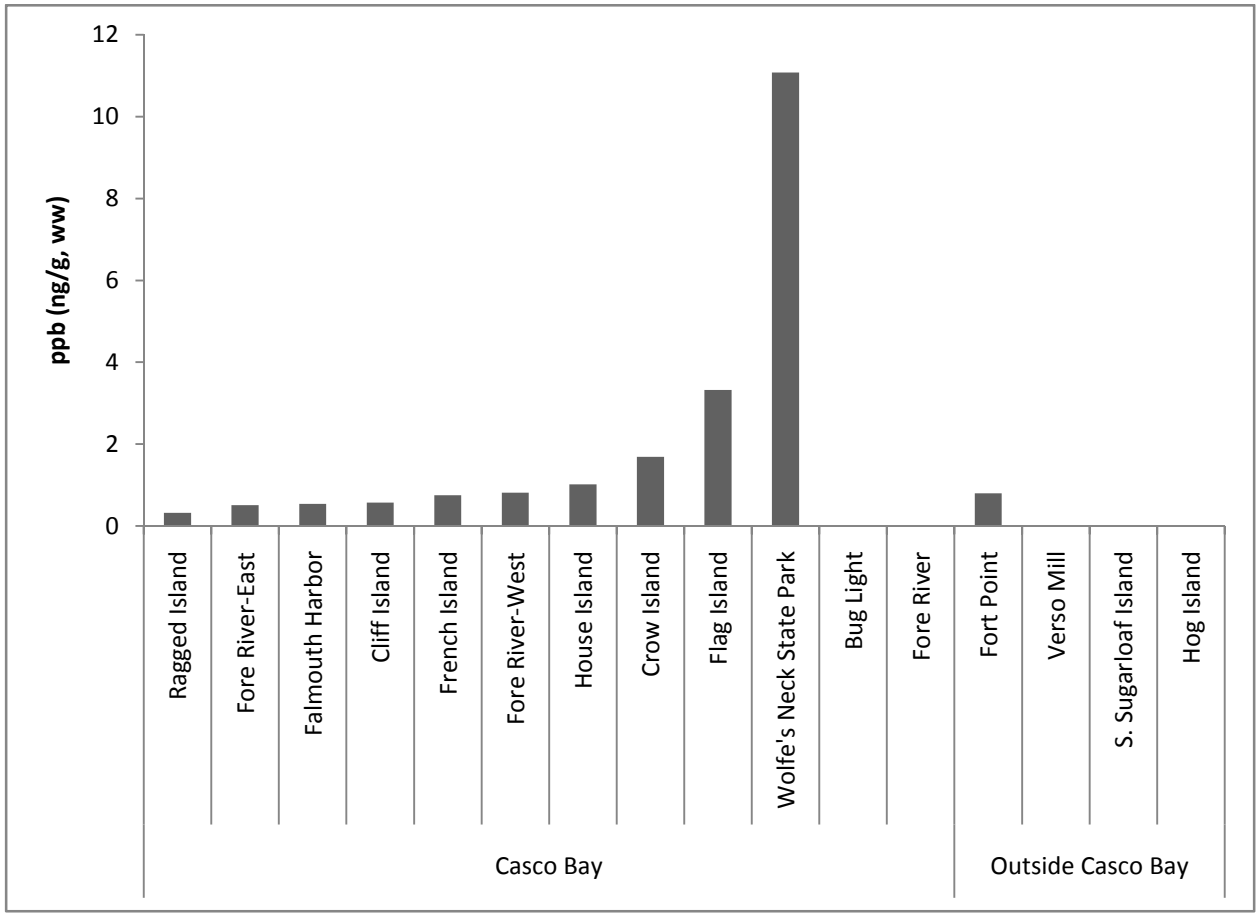


Figure 8. decaBDE levels by site.

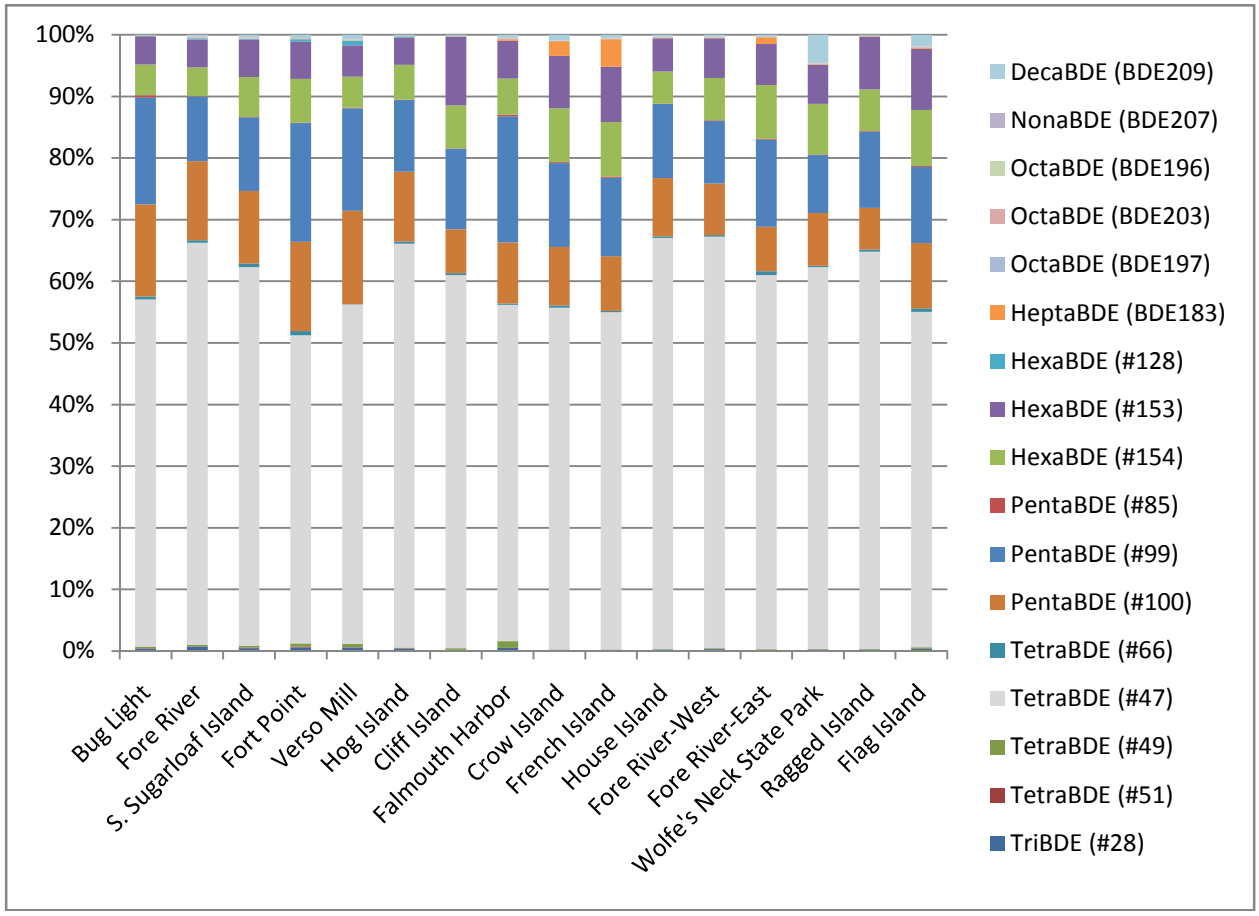


Figure 9. % of PBDE congeners.

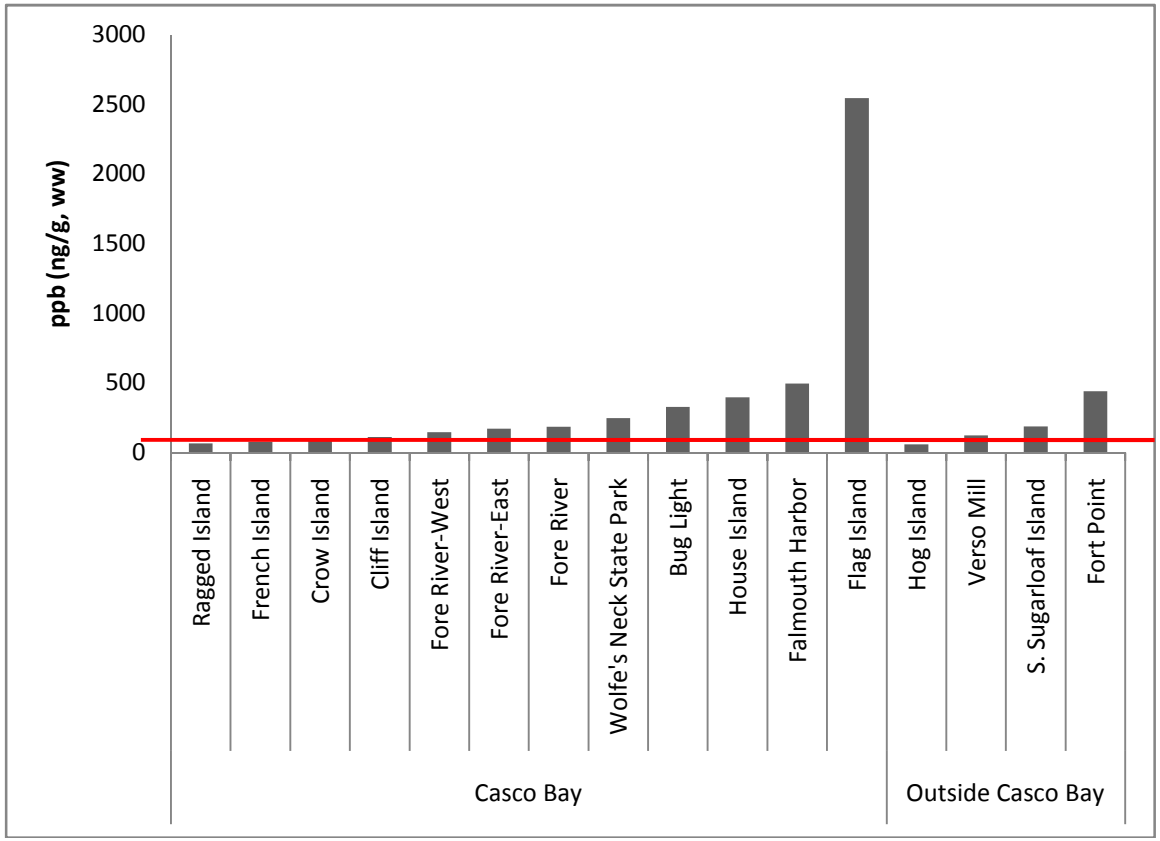


Figure 10. PFOS levels by site. Adverse effects threshold for chickens (Molina et al. 2006).

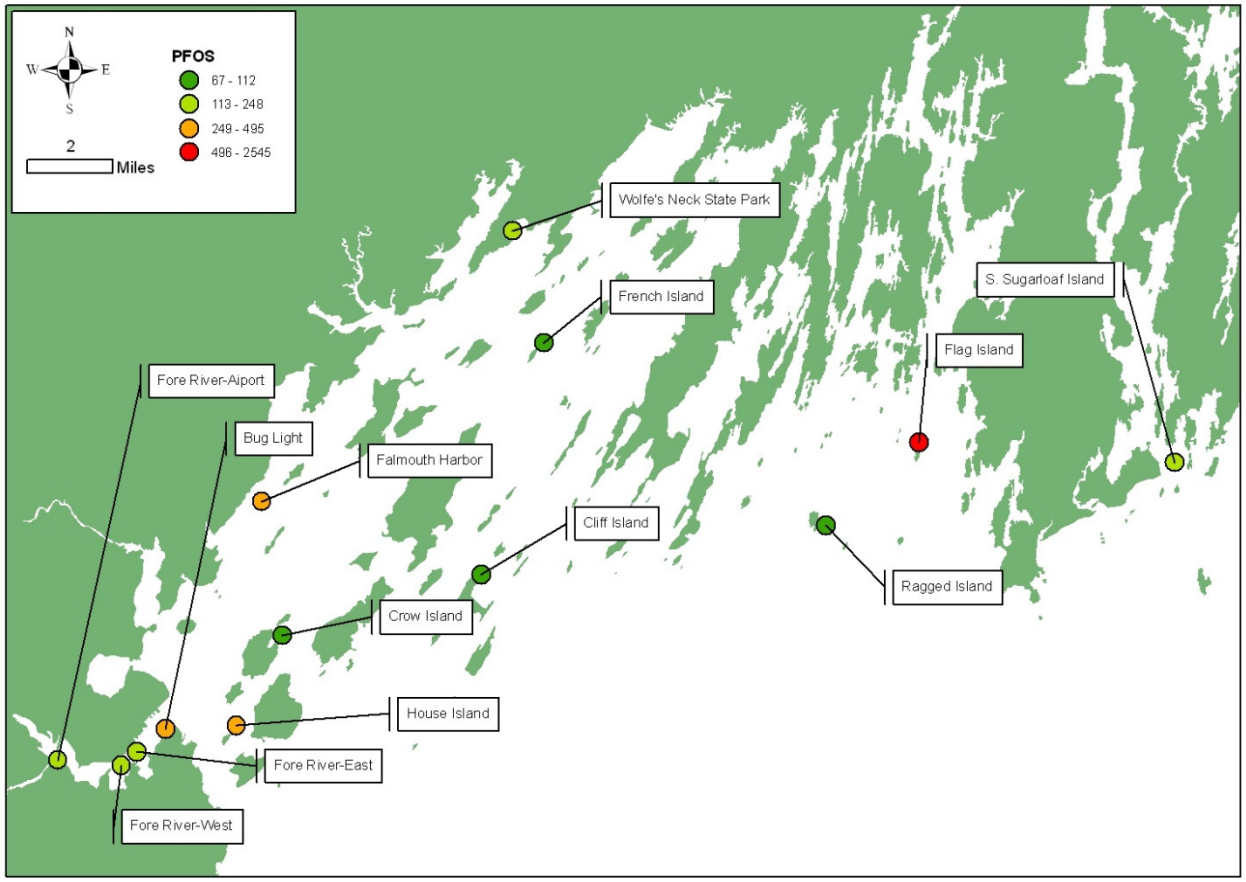


Figure 11. Map of Casco Bay PFOS levels.

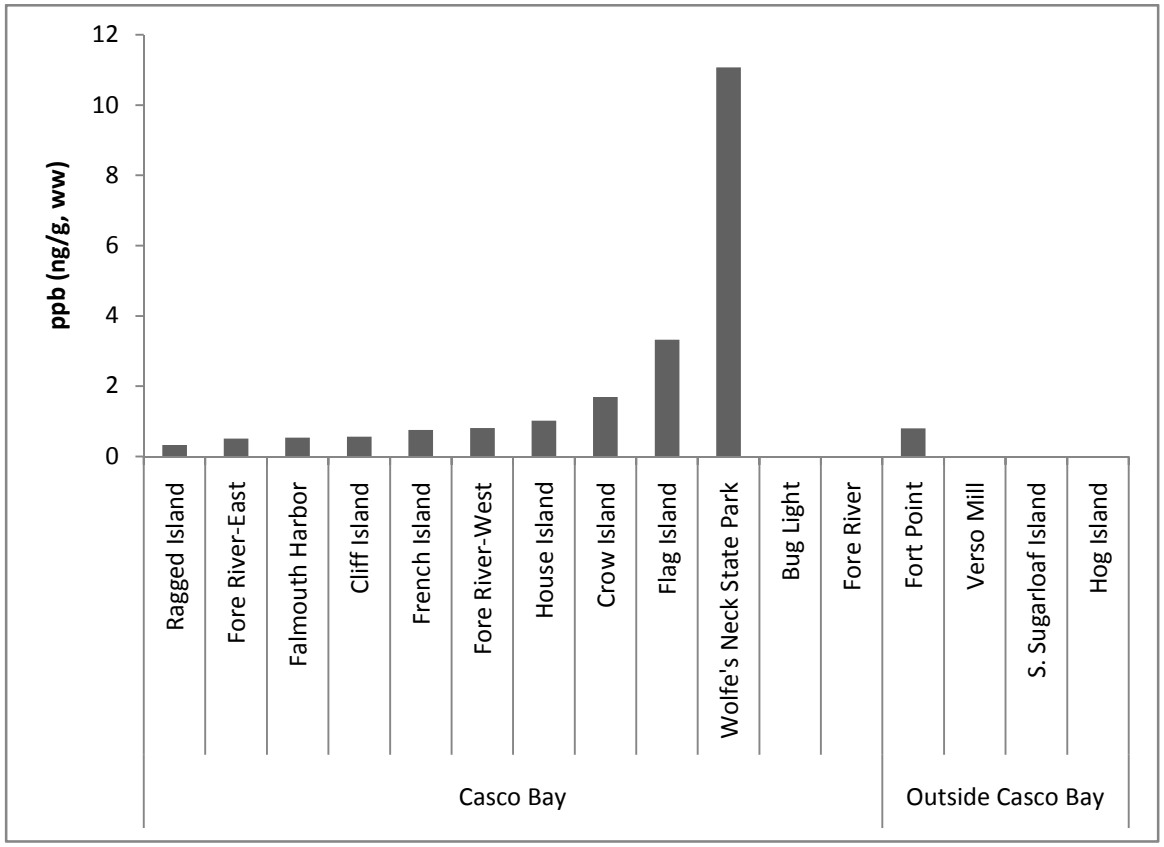


Figure 12. Total DDT levels by site.

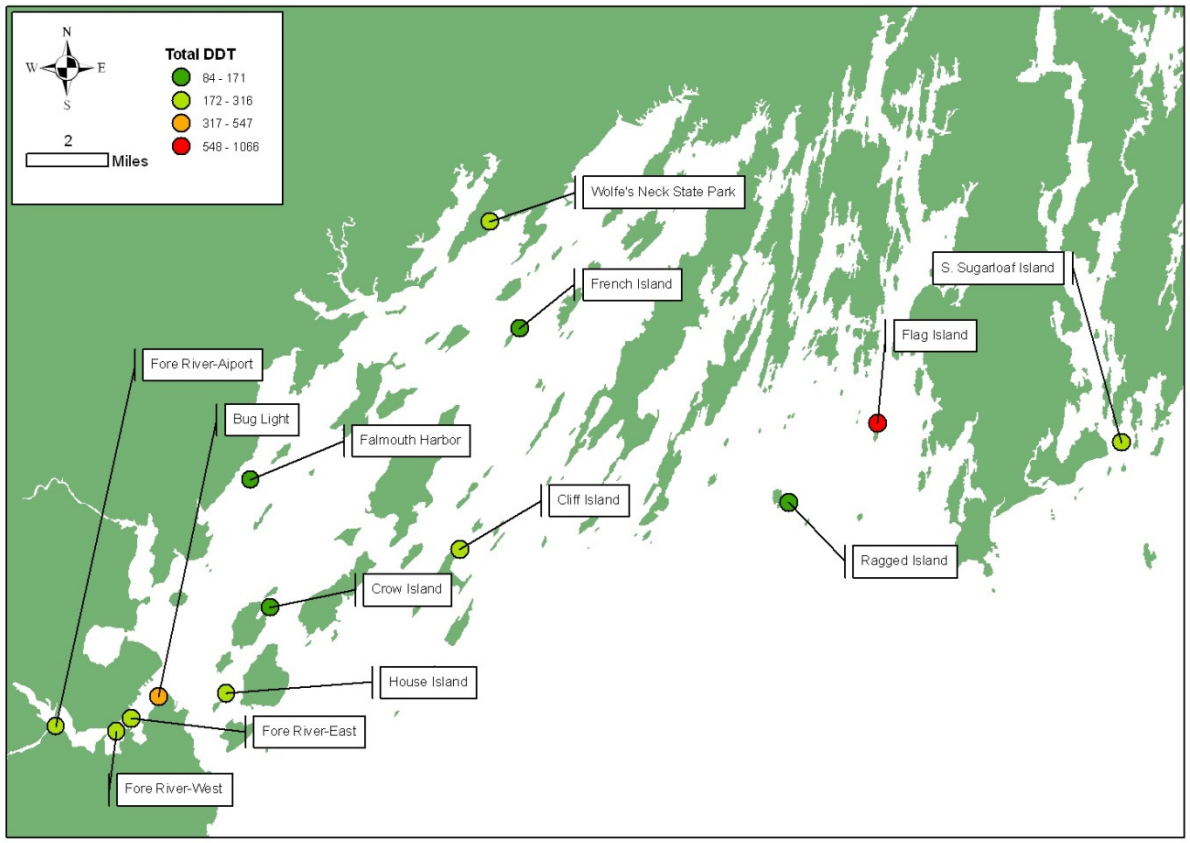


Figure 13. Map of Casco Bay total DDT levels.

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