

Contaminant Concentrations in Osprey (*Pandion haliaetus*)
Eggs from Portland Harbor and Surrounding Areas:
Data Summary Report

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1.0 INTRODUCTION

This report summarizes data from an investigation of contaminants in osprey (*Pandion haliaetus*) eggs collected within the Portland Harbor Superfund Site Study Area (Portland Harbor Study Area), Multnomah Channel, and an upstream area along the mid-Willamette River in Oregon. This study was conducted to provide information for the remedial investigation and feasibility study (RI/FS) and Natural Resource Damage Assessment (NRDA) process for the Portland Harbor Superfund Site in Portland, Oregon. Under the RI/FS, the U.S. Environmental Protection Agency (EPA) required collection and analysis of osprey eggs from the Portland Harbor Superfund Site to facilitate the development of initial chemical concentrations as part of a long term post-remediation monitoring program, and to evaluate if incorporation of these tissue data would improve the accuracy of risk estimates for fish-eating birds presented in the final risk assessment. The study was conducted by the U.S. Geological Survey (USGS) and U.S. Fish and Wildlife Service (USFWS) in collaboration with the Portland Harbor Natural Resource Trustee Council (Trustees) and the EPA.

As top predators, ospreys are ideal indicators of ecosystem health, and their eggs have been used in the Pacific Northwest and other areas to characterize bioaccumulative contaminants and monitor trends in these chemicals over time (Elliott et al. 1998; 2000, Henny et al. 2003; 2004; 2008, Martin et. al 2003, Toschick et al. 2005). Ospreys along the Willamette River primarily feed on fish (nearly 100% of the diet are fish), and will bioaccumulate organochlorine contaminants into their tissues by consuming contaminated fish prey from waterways close to the nest site (Henny et al. 2003). Previous authors have suggested that ospreys could accumulate organochlorine pesticides to a greater extent on their wintering grounds in Mexico and Central America compared to the breeding grounds within the United States (Elliott et al. 2000). However, recent evaluations of prey items within the wintering territories of Columbia River ospreys equipped with satellite transmitters have shown that wintering site had no significant effect on egg contaminant concentrations (Elliott et al. 2007).

Contaminant bioaccumulation is considered to be most important during the month prior to egg laying, when female ospreys are feeding heavily following migration and building their lipid reserves from locally acquired lipid and protein (Drent and Daan 1980, Hobson et al. 1997, Evers et al. 2003). These reserves are tapped during egg formation, when mobilized contaminants are available to impact shell thickness and be deposited into the eggs. Elevated concentrations of DDE mobilized in the female during egg development can interfere with calcium supply to the eggshell gland, resulting in production of eggs with thin shells that are prone to cracking, breaking, and moisture loss which leads to embryo death (Wiemeyer et al. 1988, Lundholm 1997). Elevated concentrations of other organochlorine contaminants such as polychlorinated biphenyls (PCBs) and dioxins in the egg can also interfere with growth and survival of the developing embryo (Elliott et al. 1998; 2000).

Previously, osprey eggs have been collected within the upper Willamette Basin for contaminant analysis (Henny et al. 2003; 2009a), but relatively few eggs have been collected from nest sites within the Portland Harbor Superfund Site or Multnomah Channel specifically. The primary objectives of this study were to collect osprey eggs from the Portland Harbor Study Area and

vicinity and analyze egg contents for chemicals of concern to 1) establish initial chemical concentrations as part of a long term post-remediation monitoring program; 2) evaluate if incorporation of osprey egg tissue data would improve the accuracy of risk estimates for fish-eating birds presented in the final risk assessment; and 3) determine if egg contaminants exceed injury thresholds and evaluate extent of contamination in ospreys outside the Superfund site. This report provides a summary of the egg contaminant, eggshell, and productivity data collected from the Portland Harbor Study Area and vicinity and provides some data interpretation so the respective groups involved can more fully address specific objectives listed above. The report focuses on specific contaminants most associated with impacts to fish-eating birds based on previous investigations.

2.0 METHODS

2.1 Study Area

The study area included three river reaches along the Willamette River and vicinity in Oregon (Figure 1). The reaches included the Portland Harbor Study Area (Portland Harbor reach) along the lower Willamette River between river miles (RM) 2 and 12 (Figure 2), the Multnomah Channel between its confluence at the Columbia River at RM 1 and the Sauvie Island Bridge at RM 20.5 (Figure 3), and the mid-Willamette River from Grand Island at RM 68 to Darrow Rocks at RM 79 (Figure 4). The Portland Harbor reach was selected to specifically evaluate the influence of the Superfund site on contaminant concentrations in osprey eggs. The other sites were selected as comparison reaches to evaluate egg contaminants in a reach upstream of the Superfund site (mid-Willamette River) and in a reach potentially influenced from contaminants released from the Superfund site (Multnomah Channel). Target nest sites with safe access, landowner permission, and spatial distribution between nests were selected within each of these three river reaches. Although all eight nest sites that occurred within the Portland Harbor reach in 2008 were targeted for sampling, we were only able to access five nests successfully. Five eggs were also successfully collected from nest sites within each of the other two reaches, resulting in a total of 15 eggs collected from the three reaches (Table 1).

2.2 Productivity Surveys

Productivity surveys were conducted by USGS to determine nest success and nest outcome of ospreys where eggs were collected as well as other nests in the vicinity. Productivity of osprey is expressed as the number of young produced per occupied nest with known outcome. USGS conducts these surveys within the Willamette Basin to provide an indicator of the health of osprey populations and aquatic ecosystems (Grove et al. 2009). Two surveys are necessary to locate nests and document activity around nests, and to count the young at the nest. USGS conducted aerial, ground, and boat surveys in the Willamette River in 2008 to document productivity. The nesting status and productivity were determined following Postupalsky (1977) and details of the USGS productivity survey are reported in the *Draft Field sampling report for the collection of eggs and determination of productivity of osprey nesting within the Portland Harbor Superfund Site and vicinity* (Field Sampling Report) (Kaiser 2009).

Table 1. Characteristics of osprey eggs and chemical analyses conducted on eggs collected from Portland Harbor reach and vicinity in 2008 (adapted from Kaiser 2009).

Nest / Egg ID	RM	Coordinates (NAD83)		Clutch Size ¹	Egg Content Mass (g)	Egg Volume (mL)	Egg Adjustment Factor ²	Whole Egg Mass (g)	Eggshell Mass ³ (g)	Average Eggshell Thickness ⁴ (mm)	Embryo Development (Days) ⁵	Analyses ⁶
		Latitude	Longitude									
Portland Harbor Reach Egg Samples												
Collected: May 20, 2008 except W11 which was collected on May 28, 2008 Processed: June 13, 2008												
W3B	3.4	45.618142	-122.7964497	3-1	59.80	69.8	0.8567	68.18	7.36	0.517	21	OCP ^b , PCB, PBDEs, Dx,PCB209, THg
W6	5.8	45.588579	-122.7667377	3-1	58.66	66.1	0.8874	67.09	6.72	0.492	7	OCP ^b , PCB, PBDEs, Dx,PCB209, THg
W7A	7.2	45.573946	-122.7457653	3-1	56.73	65.7	0.8635	65.07	6.87	0.483	7	OCP ^b , PCB, PBDEs, Dx,PCB209, THg
W9B	8.5	45.564632	-122.7129607	3-1	60.45	70.2	0.8611	67.85	6.35	0.433	19	OCP ^b , PCB, PBDEs, Dx,PCB209, THg
W11	10.7	45.541439	-122.6909140	2-1	59.41	72.2	0.8229	69.14	7.93	0.567	7	OCP ^b , PCB, PBDEs, Dx,PCB209, THg
Multnomah Channel Egg Samples												
Collected: May 20, 2008 Processed: June 13, 2008												
MC-1B	1.6	45.845793	-122.7974236	3-1	58.27	64.8	0.8992	65.34	5.51 ^a	0.393	4	OCP, PCB, PBDEs, THg
MC-2B	2.6	45.831889	-122.8143055	2-1	66.31	74.4	0.8913	74.97	6.85	0.433	0-4	OCP, PCB, PBDEs, Dx,PCB209, THg
MC-9	9.0	45.757539	-122.8233607	3-1	60.41	70.2	0.8605	67.69	6.12	0.408	14	OCP, PCB, PBDEs, THg
MC-10B	10.2	45.746361	-122.8389166	3-1	61.05	75.0	0.8140	69.18	6.96	0.445	33	OCP, PCB, PBDEs, Dx,PCB209, THg
MC-20	19.2	45.644244	-122.8231093	3-1	71.71	79.4	0.9031	81.84	8.28 ^a	0.532	8	OCP, PCB, PBDEs, Dx,PCB209, THg
Mid-Willamette River (RM 68-79) Egg Samples												
Collected: May 16, 2008 Processed: June 13, 2008												
W23	69.2	45.113607	-123.0135010	2-1	59.63	74.0	0.8058	69.18	8.05	0.543	14	OCP, PCB, PBDEs, THg
W28	73.0	45.083093	-123.0722048	3-1	61.48	72.9	0.8433	70.36	7.33	0.499	14	OCP, PCB, PBDEs, THg
W30B	74.6	45.058436	-123.0581361	4-1	52.90	68.0	0.7779	60.28	6.23	0.460	38	OCP, PCB, PBDEs, Dx,PCB209, THg
W30C	75.3	45.048122	-123.0526841	2-1	51.64	60.8	0.8493	59.86	7.02	0.505	13	OCP, PCB, PBDEs, THg
W32	77.3	45.020934	-123.0698927	4-1	55.44	65.8	0.8425	63.34	6.98	0.483	28	OCP, PCB, PBDEs, Dx,PCB209, THg
¹ Clutch size minus egg collected. ² Egg adjustment factor (mass over volume) accounts for moisture loss during incubation; adjusted concentrations are reported as fresh weight (Stickel et al. 1973). ³ Measurement excludes minor loss of shell material along scalpel cut to harvest egg contents. ⁴ Measurement for MC-1B and MC-20 excludes apparent estimated loss of 2x10mm and 2x8mm portion of shell fragment, respectively. ⁵ Average of six measurements taken at three locations along the equator of each dried shell half using dial micrometer with rounded contacts. ⁶ Descriptive criteria for estimating age in days of embryo development for viable osprey egg samples established by Charles J. Henny, Ph.D., U.S. Geological Survey, Forest and Rangeland Experimental Science Center, Corvallis, Oregon. ⁷ Type of analysis conducted on egg sample. OCP = organochlorine pesticide scan analyzed by GLIER (and by Axys for the Portland Harbor eggs); PCB = 41 PCBs congeners analyzed by GLIER; PBDEs = selected polybrominated diphenyl ethers analyzed by Carleton; Dx = dioxins and furans analyzed by Axys; PCB209= all 209 PCB congeners analyzed by Axys; THg = total mercury analyzed by GLIER. ⁸ Analysis for organochlorine pesticides was completed by both Axys and GLIER for these eggs.												

2.3 Sample Collection and Processing

Egg collection and processing followed methods described in the *Field Sampling Plan for the Collection of Osprey Eggs from the Portland Harbor Superfund Site* (Field Sampling Plan) (Buck and Henny 2008) and the Field Sampling Report (Kaiser 2009). Osprey eggs were collected by staff from the USGS and USFWS in May 2008 coincident with a separate USGS study of osprey on the lower Columbia River. Eggs were transported in a padded container in coolers on blue or wet ice to the USGS, Forest and Rangeland Ecosystem Science Center's laboratory in Corvallis, Oregon on the same day of collection. The eggs were refrigerated until processing on June 13, 2008. Egg measurements (Table 1) were recorded and contents emptied into chemically-cleaned jars as described in the Field Sampling Plan. Egg contents were then stored at -18°C until shipment to analytical laboratories. Eggshells were set aside to dry for at least 30 days and eggshell thickness measured on all shells in accordance with the Field Sampling Plan.

2.4 Chemical Analysis

Osprey egg contents were analyzed for organochlorine (OC) pesticides, polychlorinated biphenyls (PCBs), brominated flame retardants (polybrominated diphenyl ethers or PBDEs), dioxins, furans, and mercury. Laboratory analyses were completed by the Great Lakes Institute for Environmental Research (GLIER) at University of Windsor, Ontario, Canada; Carleton University in Ontario, Canada; and Axys Laboratory at Sydney, British Columbia, Canada. GLIER and Carleton were selected to maintain consistency for comparing OC pesticide and PCB concentrations to egg values collected in previous studies conducted by USGS. Axys was selected to evaluate dioxins, furans, and the full suite of PCBs to maintain consistency with data previously collected by EPA within the Portland Harbor Study Area, and to represent PCBs that previously have not been characterized in osprey eggs from the Willamette River. GLIER received and homogenized all osprey egg samples and sent subsamples to the remaining laboratories. Each laboratory analyzed specific contaminants, although OC pesticides and some PCB congeners for some eggs were analyzed by both Axys and GLIER using slightly different methodologies (Table 1). IUPAC numbers for PCB congeners followed Ballschmiter and Zell (1980). The chemical analyses conducted by each laboratory are specifically described in the *Quality Assurance Project Plan (QAPP) for the Analysis of Osprey (Pandion haliaetus) Egg Tissue Collected from Portland Harbor and Surrounding Areas* (Buck 2008). The analytical methods and detection limits are briefly presented below.

GLIER analyzed OC pesticides and PCBs in all egg samples using column solid-liquid extraction with dichloromethane/hexane solid-liquid and clean-up and fractionation on Florisil column. Bulk lipids, when present, were removed by gel permeation chromatography. Analytes were quantified with electron-capture detection and confirmed using mass spectrometry.

Axys analyzed for dioxin, furans, and the full suite of 209 PCB congeners on seven of the egg samples (Table 1). In addition, Axys analyzed OC pesticides in the five samples from the Portland Harbor reach (Table 1). Extracts for these analytes were cleaned up and fractionated using a series of chromatographic columns including silica, Florisil, and alumina and gel permeation columns depending on the specific organic group. Column sequence was dependent

on the suite of target analytes isolated. Additional cleanup was conducted on the planar PCB analysis using carbon celite column to remove all non-coplanar PCBs. One resulting fraction was spiked with ^{13}C -labelled PCDD/F recovery (internal) standard and analyzed for PCDD/F according to EPA method 1613B by high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS). Another fraction was spiked with ^{13}C -labelled PCBs and pesticides and analyzed for PCBs according to EPA method 1668A and for pesticides by HRGC/HRMS. A third fraction was spiked with ^{13}C -labelled pesticides and analyzed for the most polar pesticides by HRGC/HRMS.

The PBDEs in egg tissue were analyzed at Carleton. Samples were extracted by accelerated solvent extraction using dichloromethane/hexane and removing bulk lipids by gel permeation chromatography. Targeted PBDEs in the final, isolated chemical fractions were determined by high resolution gas chromatography/low resolution mass spectrometry (electron capture negative ionization), using selected ion monitoring (SIM) for isotopic bromine anions $^{79}\text{Br}^-$ and $^{81}\text{Br}^-$, or, for BDE-209, using isotope ions of a pentabromophenoxy anion fragment (m/z 484 and 486) relative to that of the labeled surrogate ($^{13}\text{C}_{12}$ -BDE-209; m/z 494 and 496).

Total recoverable mercury was analyzed at GLIER by oxidizing egg tissue in digestion with sulphuric acid, nitric acid, potassium permanganate, and potassium persulphate. Mercury was quantified by cold vapor atomic absorption spectrophotometry.

Lipid analysis was conducted on all samples using methylene chloride, and percent moisture was determined by drying. Each laboratory analyzed samples for lipid, and percent moisture was conducted at GLIER and Carleton.

Laboratory quality control samples for contaminant analyses consisted of procedural blanks, replicate samples, standard reference materials, spiked samples, or other quality control measures in accordance with the QAPP (Buck 2008). Matrix spikes and replicate results were typically within the specified limits for this study. In general, analyte concentrations in egg samples with corresponding matrix spike recoveries outside specified boundaries were below or near detection limits or reported as estimated results. Osprey egg results were independently validated and quality control results reported in a separate document by EcoChem (2010). Results conducted on the PBDE analysis by Carleton were not independently verified; rather, control of data was evaluated based on the laboratory's standard reference materials and method blanks. Any anomalous results or values outside quality control boundaries are described by EcoChem (2010) and have been noted in the tables of the current report.

2.5 Data Analysis

Productivity data collected by USGS and documented in the Field Sampling Report (Kaiser 2009) were reported for the 2008 breeding season. Nest success was reported based on occupied nest sites (breeding pair documented as present near nest) and active nest sites (adult observed in incubating posture) (Postupalsky 1974, 1977). Productivity data were compared among the three reaches for 2008.

Concentrations of selected OC pesticides (primarily p,p'-DDE, hereafter reported as DDE), total PCBs (summation of 41 congeners), total PBDEs (summation of 15 congeners), dioxin-like compounds (7 dioxins, 10 furans, and 12 PCBs with dioxin-like activity), and mercury were the primary contaminants evaluated in all 15 osprey eggs in this report. These contaminants are associated with effects including eggshell thinning, embryo defects, or embryo mortality in osprey and other fish-eating birds. Concentrations of these primary contaminants were also compared to values found in eggs collected from previous studies conducted on osprey eggs in the Willamette and Columbia Rivers (Henny et al. 2003, 2004, 2008, 2009a, 2009b, Elliott et al. 1998, 2000). Concentrations of other contaminants in eggs were generally below values associated with effects, have unknown effects, or were below detection limits and are not presented in the text of this report. Results for all contaminants analyzed in osprey eggs are available electronically through the National Oceanic and Atmospheric Administration (NOAA) Query Manager/MARPLOT contaminant database for Portland Harbor. Download, install, and use instructions for Query Manager/MARPLOT and the Portland Harbor data set are available at <http://response.restoration.noaa.gov/watersheddownloads>.¹

Some of the contaminants were analyzed at two different laboratories as indicated in Table 1 and described in the QAPP (Buck 2008). GLIER reported results for OC pesticides on all 15 eggs collected, and Axys reported OC pesticide results for the five eggs from the Portland Harbor reach. In addition, Axys conducted a full PCB congener evaluation of all 209 PCB congeners (reported herein as total PCBs₂₀₉) on 10 eggs including five from the Portland Harbor reach, three from Multnomah Channel, and two from the mid-Willamette River. Results from GLIER are used in the comparisons made in the primary text in this report, along with the total PCBs₂₀₉ from Axys. Other results from Axys are available electronically in Query Manager/MARPLOT but are not used for statistical comparisons across river reaches, as insufficient samples were analyzed at two of the locations. As indicated above, some analytes were analyzed twice on a single egg by different laboratories. To separate these results in Query Manager/MARPLOT, the osprey information has been entered under two study names (“*Round 3/Injury Assessment Osprey Eggs*” and “*Injury Assessment Osprey Eggs*”) in the category “Available Studies.” Both these study names should be searched to include all the osprey egg results.

Eggshell thinning was determined as the percent change of eggshell thickness values in comparison to a reference eggshell thickness of 0.505 mm (determined before 1947 and the widespread use of DDT) for osprey eggshells from the eastern U.S. (Anderson and Hickey 1972). Eggshell thickness was also compared to DDE concentrations in eggs using simple linear regression.

¹ The Query Manager/MARPLOT installer (02/27/07), found at the bottom of the watershed downloads page via the provided link, should be downloaded to the user's desktop. Upon execution, the program will self-install. After installing Query Manager/MARPLOT, the three data files (database file, map file, dictionary) for Portland Harbor/Willamette River (see table on watershed downloads page) should be downloaded to the user's desktop and subsequently installed. As with the Query Manager/MARPLOT application, these files are self-installing. Upon completion of these steps, Query Manager/MARPLOT should be ready to use. More information on installing and using Query Manager /MARPLOT is available on the NOAA website. Geographic Information Systems (GIS) users should note that tools are also available for download from the NOAA website to enable easy import and projection of Query Manager exported .dbf files into ArcGIS 9.x.

Dioxin-like toxicity was assessed using 2,3,7,8-tetrachlorobenzo-*p*-dioxin (TCDD) toxic equivalent (TEQ) concentrations derived from avian-based toxic equivalency factors (TEF) suggested by Van den Berg et al. (1998) for PCDDs, PCDFs, and dioxin-like PCBs. TEQs were calculated based on contributions from seven dioxin and 10 furan congeners (reported as TEQ_{S_{Dx}}) and from contributions of those same dioxins and furans as well as 12 non-ortho and mono-ortho substituted PCB congeners (planar PCBs) exhibiting dioxin-like activity (Van den Berg et al. 1998). TEQs were compared to threshold values for osprey and other avian species, as well as to values from previous investigations along the lower Willamette River.

Eggshell thickness, concentrations of DDE and total PCBs, and TEQs in eggs from the Portland Harbor reach were compared to values from eggs collected at the other locations using analysis of variance (ANOVA) to evaluate influence of the location of breeding territory on egg parameters. Tukey's Studentized Range Test was used to separate means. It should be noted that statistical comparison of means between locations was based on small samples sizes which contained apparent outliers, and the results of mean comparisons should be treated with caution.

Graphing comparisons using scatterplots indicated natural log transformation improved linearity within each location for the primary contaminant data. Analytical data were transformed to natural log prior to statistical analysis, and geometric means are reported. Concentrations of dioxin-like compounds below detection or quantification limits were not used in the calculation of TEQs, and were not used in the summing of PCB congeners to calculate a total PCB value. Contaminant concentrations for each egg were adjusted for moisture and lipid loss using whole egg volume and mass measurements (Stickel et al. 1973) and reported as fresh weight. All egg concentrations (fresh weight) were reported as nanograms per gram (ng/g) for OC pesticides, total PCBs, and mercury, whereas dioxins, furans, and TEQs were reported as picograms per gram (pg/g). All statistical tests were performed at the 0.05 level of significance using the software program SYSTAT[®] 12.0 (SYSTAT[®] Software, Inc. 2007).

3.0 DATA SUMMARY

3.1 Productivity

The number of active nests (adult observed in incubation position) with known nesting outcome in the river reaches in 2008 included nine sites in the lower Willamette River from RM 1 to 11 (eight of these sites were included in the Portland Harbor reach from RM 2 to 11), 11 sites in the Multnomah Channel, and 22 sites in the mid-Willamette River (Table 2). Nest success was low in 2008 at all occupied sites when including sites where eggs were collected (ranging from 50 to 70%) but was much higher (1.56 young per occupied nest) for the mid-Willamette River when sites where eggs were collected were excluded (Table 2). Nest success was lowest in the Multnomah Channel area. Overall productivity ranged from about 0.9 to 1.3 young produced per occupied nest site (Table 2). Productivity for ospreys within all reaches in the study was much lower compared to previous years (Henny et al. 2009a).

Table 2. Reproductive success and productivity for ospreys at the Portland Harbor reach and vicinity in 2008 (adapted from Kaiser 2009).			
Reproductive Parameter	Portland Harbor Reach ¹	Multnomah Channel ²	Mid-Willamette River ³
Occupied nests ⁴	9	12	23
Active nests ⁴	9	11	22
% Active	100	92	96
Successful nests	6	6	16
% Successful (occupied)	66.7	50.0	69.6
% Successful (active)	66.7	54.5	72.7
No. advanced age nestlings	8	10	30
Productivity (occupied, w/ 1 egg collected)	0.80	1.20	0.40
Productivity (occupied, w/o egg collected)	1.00	0.57	1.56
Productivity (occupied, combined)	0.89	0.83	1.30
Productivity (active, w/ 1 egg collected)	0.80	1.20	0.40
Productivity (active, w/o egg collected)	1.00	1.00	1.65
Productivity (successful, w/ 1 egg collected)	1.00	2.00	1.00
Productivity (successful, w/o egg collected)	1.33	1.33	2.00
¹ Includes all Portland Harbor nests located along the lower Willamette River from River Mile (RM) 1.0 to 11.0.			
² Multnomah Channel nests located from RM 1 to 21.			
³ Mid-Willamette River nests located from RM 68 to 79.			
⁴ Occupied (breeding pair present) and Active (eggs laid with adult observed in incubating posture) terms follow definitions of Postupalsky (1974, 1977).			

3.2 Concentrations of selected organochlorine compounds, total PBDEs, and mercury

All osprey eggs collected contained detectable concentrations of OC pesticides, total PCBs, and total PBDEs (brominated flame retardants). Total PCBs and DDE were the most elevated contaminants, with values ranging up to two orders of magnitude above other OCs. DDT transformation products other than DDE ranged from 4.28 to 75.8 ng/g; all other OC pesticides were below 50 ng/g. Total PBDEs were present in all eggs ranging from 172 to 751 ng/g. Total mercury was below estimated threshold values of ≥ 500 ng/g wet weight (as reviewed in Henny et al. 2009a) and was only detected in four samples; two from Portland Harbor reach and two from Multnomah Channel.

Concentrations of DDE were not different ($P=0.42$) when compared across the three study locations (Table 3; Figure 5). Concentrations were relatively similar at these sites, although the highest DDE concentrations were found in an egg from the Multnomah Channel (2,410 ng/g) and an egg from the Portland Harbor reach (1,690 ng/g). In contrast, no eggs from the upstream site in the mid-Willamette River exceeded 800 ng/g. Concentrations in eggs were quite variable

Table 3. Eggshell thickness (mean \pm one standard deviation and extreme values) and concentrations (geometric mean and extreme values) of selected contaminants in osprey eggs collected from the Portland Harbor reach and vicinity in 2008. Values based on a sample size (n) of five eggs unless otherwise noted.

Parameter ¹	Portland Harbor Reach	Multnomah Channel	Mid-Willamette River
Eggshell			
Eggshell thickness (mm)	0.498 ^A \pm 0.049 (0.433 – 0.567)	0.442 ^A \pm 0.054 (0.393 – 0.532)	0.498 ^A \pm 0.031 (0.433 – 0.567)
Percent change ²	-1.3 (-14 – 12)	-12 (-22 – 5.4)	-1.4 (-8.9 – 7.5)
Contaminant (ng/g fresh weight)³			
p, p'-DDE	806 ^A (315 – 1,690)	749 ^A (323 – 2,410)	487 ^A (241 – 764)
Total PCBs	1,840 ^A (319 – 6,270)	531 ^B (334 – 688)	201 ^B 131 – 308
Total PCBs ₂₀₉	3,630 (477 – 16,900) n = 5	723 (461 – 1,020) n = 3	336 (276 – 410) n = 2
Total PBDEs	379 ^A (172 – 664)	263 ^A (186 – 352)	436 ^A (219 – 751)
Total mercury	NC (<20 – 80)	NC (<30 – 40)	NC (<20)
Contaminant (pg/g fresh weight)⁴			
2,3,7,8-TCDD	3.66 (2.86 – 4.90) n = 5	2.01 (1.16 – 3.63) n = 3	1.31 (0.88 – 1.94) n = 2
Avian-TEQs _{Dx}	21.0 (11.9 – 47.8) n = 5	7.77 (5.75 – 9.79) n = 3	20.5 (12.5 – 33.6) n = 2
Avian-TEQs	77.0 (55.2 – 105) n = 5	29.8 20.7 – 40.9 n = 3	30.7 (22.8 – 41.3) n = 2

¹ Means for eggshell thickness or contaminant concentrations with different letters among locations (across rows) are significantly different ($P < 0.05$). Means without letters were not compared due to insufficient sample size.

² Percent change from average eggshell thickness of 0.505 mm measured on osprey eggs collected prior to widespread use of DDT (Anderson and Hickey 1972).

³ Contaminants include total polychlorinated biphenyls (PCBs) as the sum of 41 congeners, total PCBs as the sum of 209 congeners (total PCBs₂₀₉), and total polybrominated diphenyl ethers (PBDEs) as the sum of 15 congeners.

⁴ Contaminants include 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) and toxic equivalents (TEQs) calculated based on avian toxicity factors, which combine dioxin-like activity from compounds exhibiting dioxin-like activity. Avian-TEQs_{Dx} sum dioxin-like toxicity based on only the dioxin and furan congeners, whereas the Avian-TEQs include contributions from dioxins, furans, and planar PCBs (which also exhibit dioxin-like activity).

in the Portland Harbor reach and Multnomah Channel, indicating large differences in DDE sources and concentrations in prey items within the individual foraging territories of an adult pair. Total PCBs were strongly influenced by location, with higher concentrations at the Portland Harbor reach compared to the mid-Willamette River ($P=0.001$; Figure 5) and the Multnomah Channel ($p=0.04$) (Table 3). The highest value (6,270 ng/g) of total PCBs was found in an egg sample (W9B) from Swan Island Lagoon (Figure 2). In contrast, an egg from the upstream end of the Portland Harbor reach at RM 11 was an outlier and had a total PCB concentration (319 ng/g) similar to eggs from the mid-Willamette River site. Total PCB concentrations in the Multnomah Channel were relatively low, and differences in means were not significant ($P=0.12$) compared to the mid-Willamette River.

Mean total PCB₂₀₉ concentrations determined by Axys were nearly double the total PCBs (based on summation of 41 congeners) determined by GLIER in the Portland Harbor reach, whereas the mean for these two PCB calculations at each of the other two reaches were more similar in concentration (Table 3; Figure 5). This difference reflects the much greater diversity and frequency of PCB congeners in the Portland Harbor reach compared to the other two reaches, and indicates that analysis of only 41 PCB congeners may not be appropriate for tissues collected from within the Portland Harbor reach.

Mean total PBDEs concentrations were not different ($P=0.20$) between river reaches, indicating location by river reach had no influence on PBDEs (Table 3, Figure 5). The maximum value of total PBDEs was found in the most upstream egg collected in the mid-Willamette River reach, indicating a local source for these compounds in the vicinity of the nest site. Mean total PBDEs from all three reaches appeared higher than the mean of 98 ng/g (range 55.2 to 275 ng/g) in osprey eggs collected in 2002 at reservoirs within the headwaters of the Willamette River (Henny et al. 2009b).

3.3 Dioxin and dioxin-like Contaminants

Dioxin-like contaminants including dioxins, furans, and planar PCBs were also present in all osprey eggs. Concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the most toxic dioxin congener, was relatively low compared to some other congeners and values were relatively similar between locations (Table 3). Octachlorinated dibenzo-*p*-dioxin (OCDD) was the most elevated congener in eggs, ranging from 18.2 to 3,530 pg/g. Within the Portland Harbor reach, OCDD ranged from 123 to 971 pg/g with increasing concentrations in upriver nests. However, OCDD contributed little to the overall dioxin-like activity due to its low TEF value. The highest OCDD concentrations occurred in the two nests from the mid-Willamette River, indicating a local source for this contaminant in upriver reaches. The 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (1,2,3,7,8-PCDD) contributed the most dioxin-like activity toward total TEQs, with contributions ranging from 5.22 to 33.1 pg/g. Concentrations of this congener were most elevated within the Portland Harbor reach, and increased from downstream to upstream within the reach.

The avian-based TEQ_{SDx} with contributions from only dioxin and furan congeners were similar in eggs from the Portland Harbor reach and in the two eggs from the mid-Willamette River, but

appeared lower in the three eggs from the Multnomah Channel (Table 3, Figure 6). The primary contributors to dioxin-like activity within these reaches were 1,2,3,7,8-PCDD and TCDD; other congeners of dioxins and furans contributed little to the TEQ values.

Overall dioxin-like activity (avian TEQs) including planar PCBs appeared much higher in eggs from the Portland Harbor reach compared to the other two reaches (Table 3, Figure 6). The minimum TEQ value from the Portland Harbor reach exceeded all TEQ values from the other reaches, and the geometric mean was more than double the other reaches. The higher TEQ value reflects the greater concentration and contribution of the planar PCBs in the Portland Harbor reach compared to the other two reaches. The total dioxin-like toxicity was influenced primarily by planar PCBs in the Portland Harbor reach and to some degree the Multnomah Channel. Planar PCBs had much less influence on the TEQ values at the mid-Willamette River sites, indicating PCBs may not be as prevalent at this site compared to downstream areas (Figure 6).

3.4 Eggshell Thickness

Eggshell thickness was not influenced by location and was not different ($P=0.12$) between river reaches (Table 3). Shell thickness was not related to DDE concentrations ($P=0.09$, $r=-0.46$, $n=15$) although there was a general trend of thinning as DDE concentrations increased (Figure 7). It is likely that additional samples would improve this relationship, as previous studies on osprey eggs in the lower Columbia River have shown a significant decline in thickness with increasing DDE concentrations (Henny et al. 2009a). Most eggs collected exhibited some degree of thinning compared to the reference value, but the greatest percent change (-12%) based on mean values was in the Multnomah Channel reach. One egg collected near the mouth of the Channel had a shell that was 22% thinner than reference values (Table 3).

3.5 Data Summary Assumptions and Disclaimer

It should be noted that chemical concentrations in osprey eggs reported in this study are considered representative of osprey exposure to contaminated prey items within the foraging area of the adults. The foraging range where the greatest degree of exposure occurs is considered to be within a 2-km radius from the nest site, and during 30 days prior to incubation. Therefore, eggs from different individual pairs within a reach may show remarkable differences in egg contaminants depending on the source of the contaminants and concentrations in prey items within their foraging range. The statistical results comparing river reaches are based on relatively small sample sizes which may contain outliers within a reach due to differences in foraging range and should be interpreted with caution. The statistical results are evidence of a general trend in the data; additional samples within the reaches could improve robustness of the dataset and reduce errors in interpretation. Increasing sample size of eggs may be possible in the future as more ospreys establish nest sites within the reach.

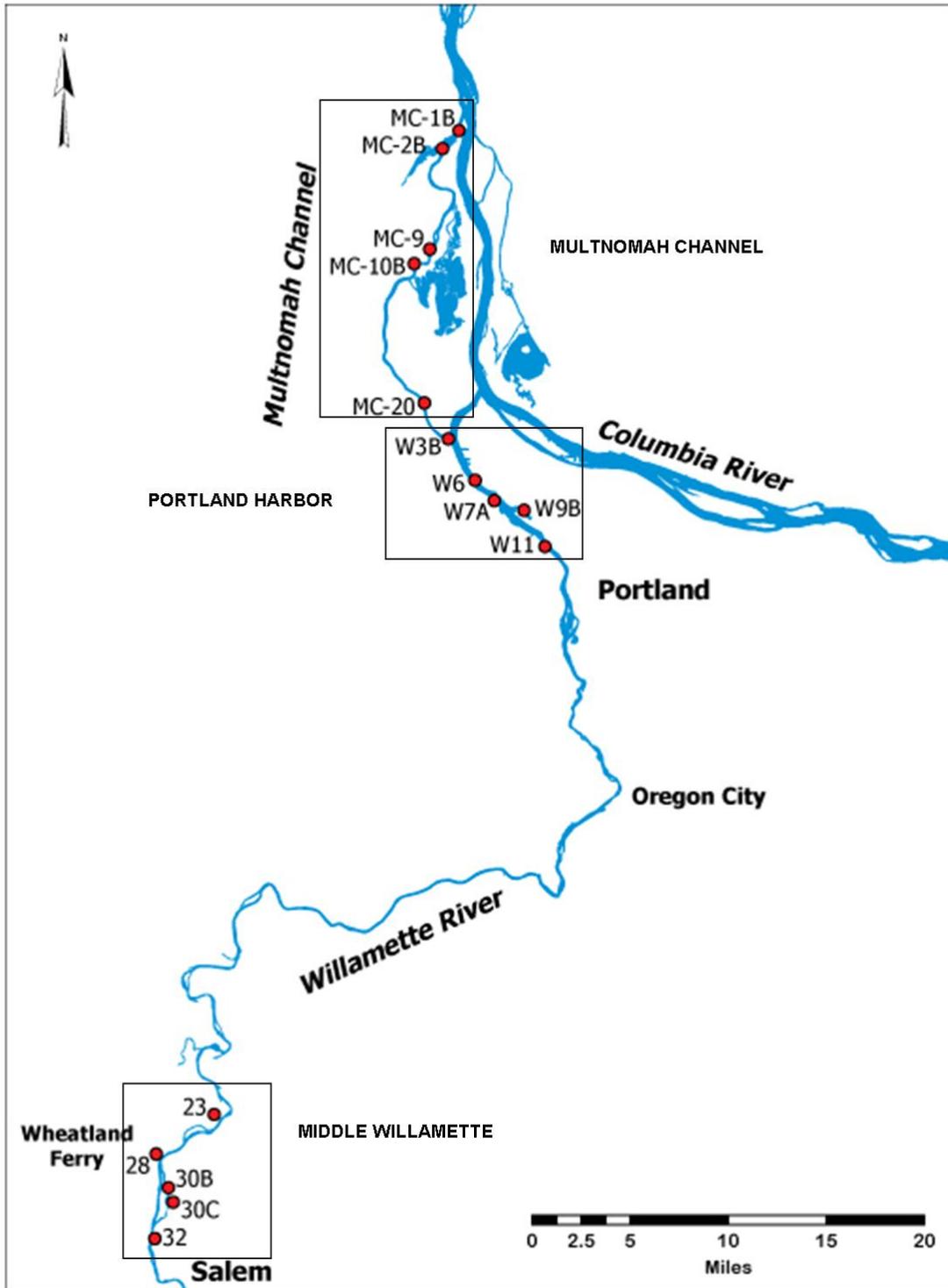


Figure 1. Location of osprey nest sites within river reaches of the Willamette River and Multnomah Channel in Oregon where osprey eggs were collected (adapted from Kaiser 2009).

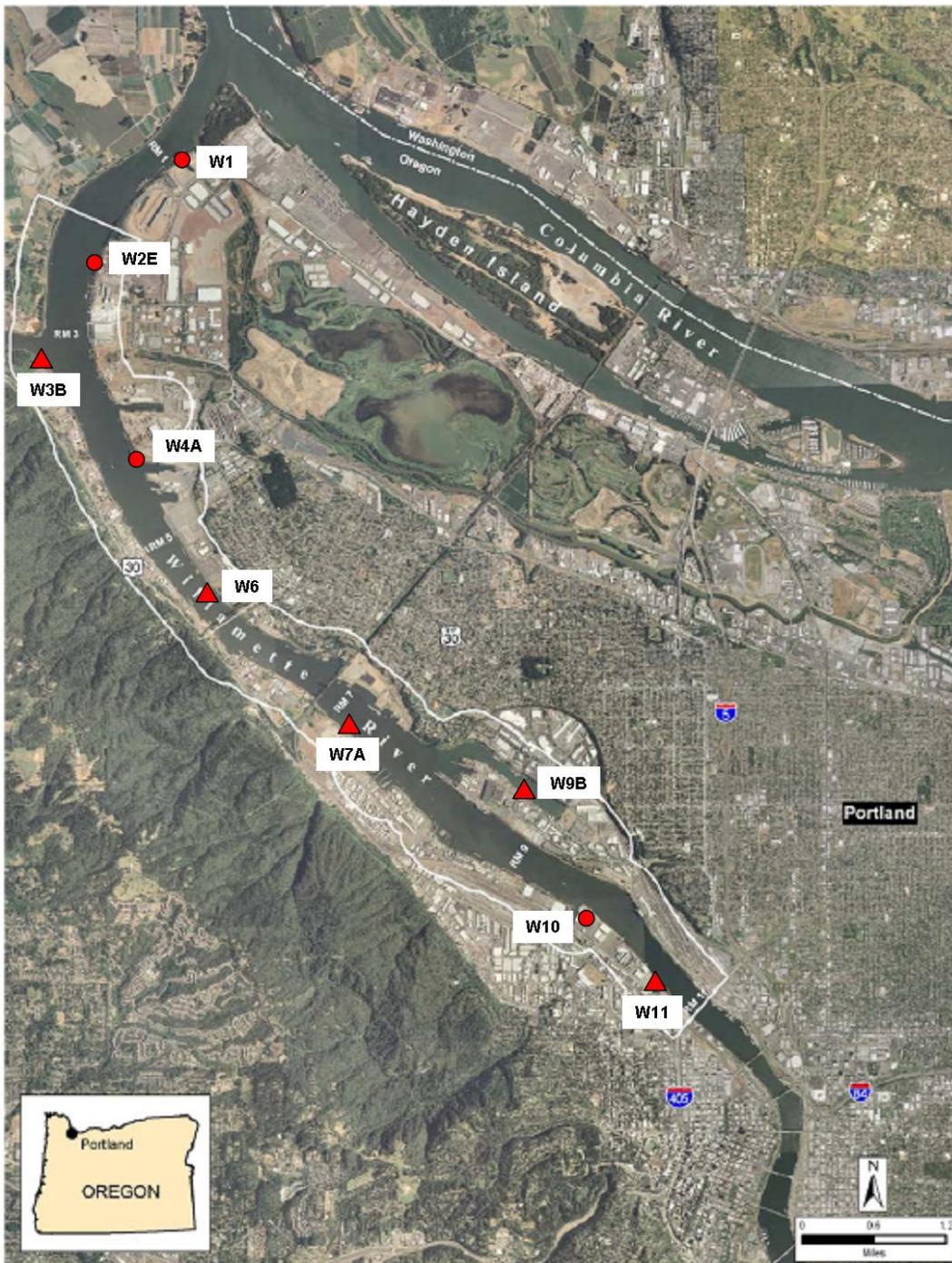


Figure 2. Osprey egg sampling sites (triangular shapes) and adjacent occupied nest sites (circular shapes) within Portland Harbor reach in 2008 (from Kaiser 2009).

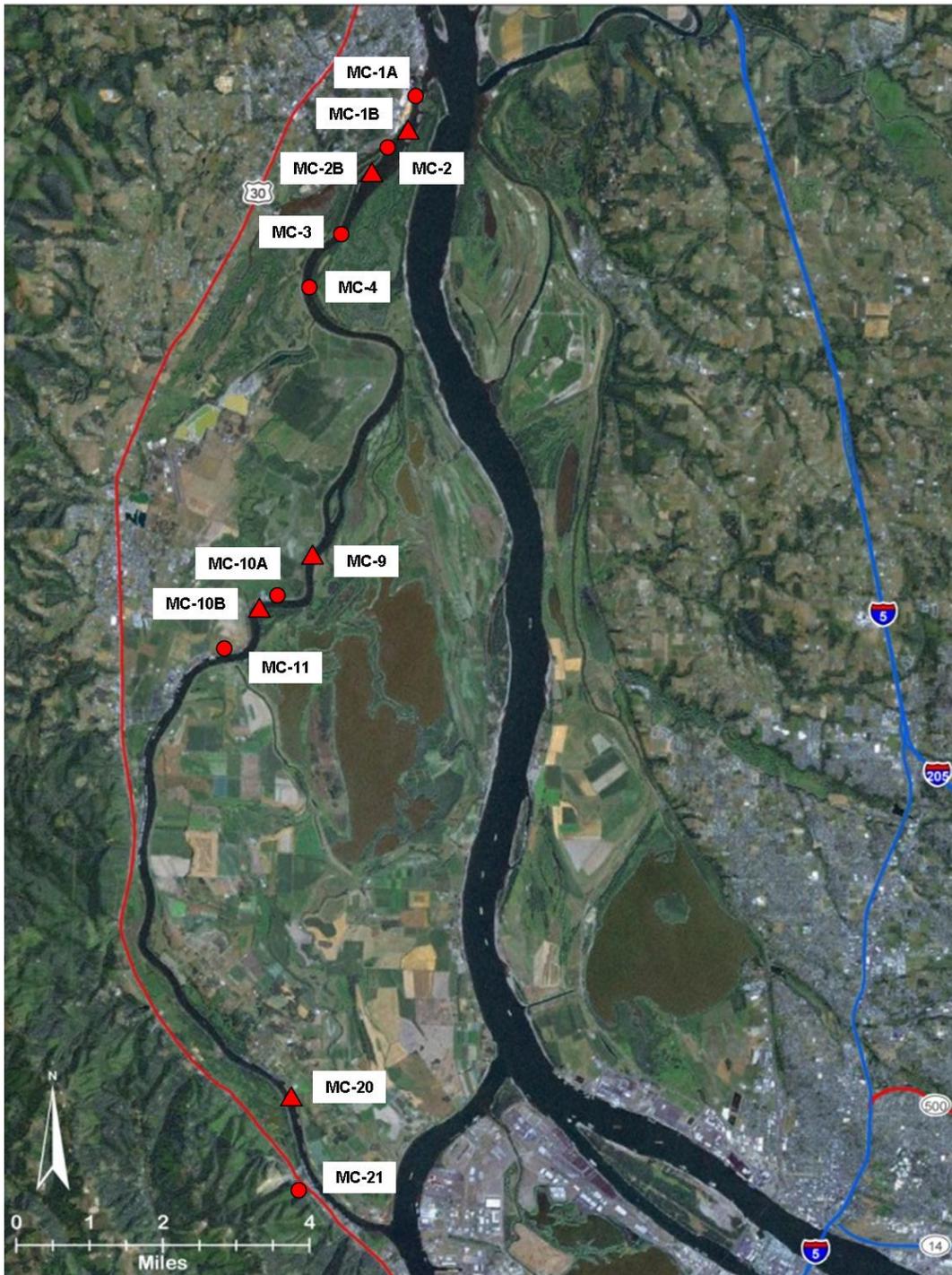


Figure 3. Osprey egg sampling sites (triangular shapes) and adjacent occupied nest sites (circular shapes) within Multnomah Channel, Oregon in 2008 (from Kaiser 2009).

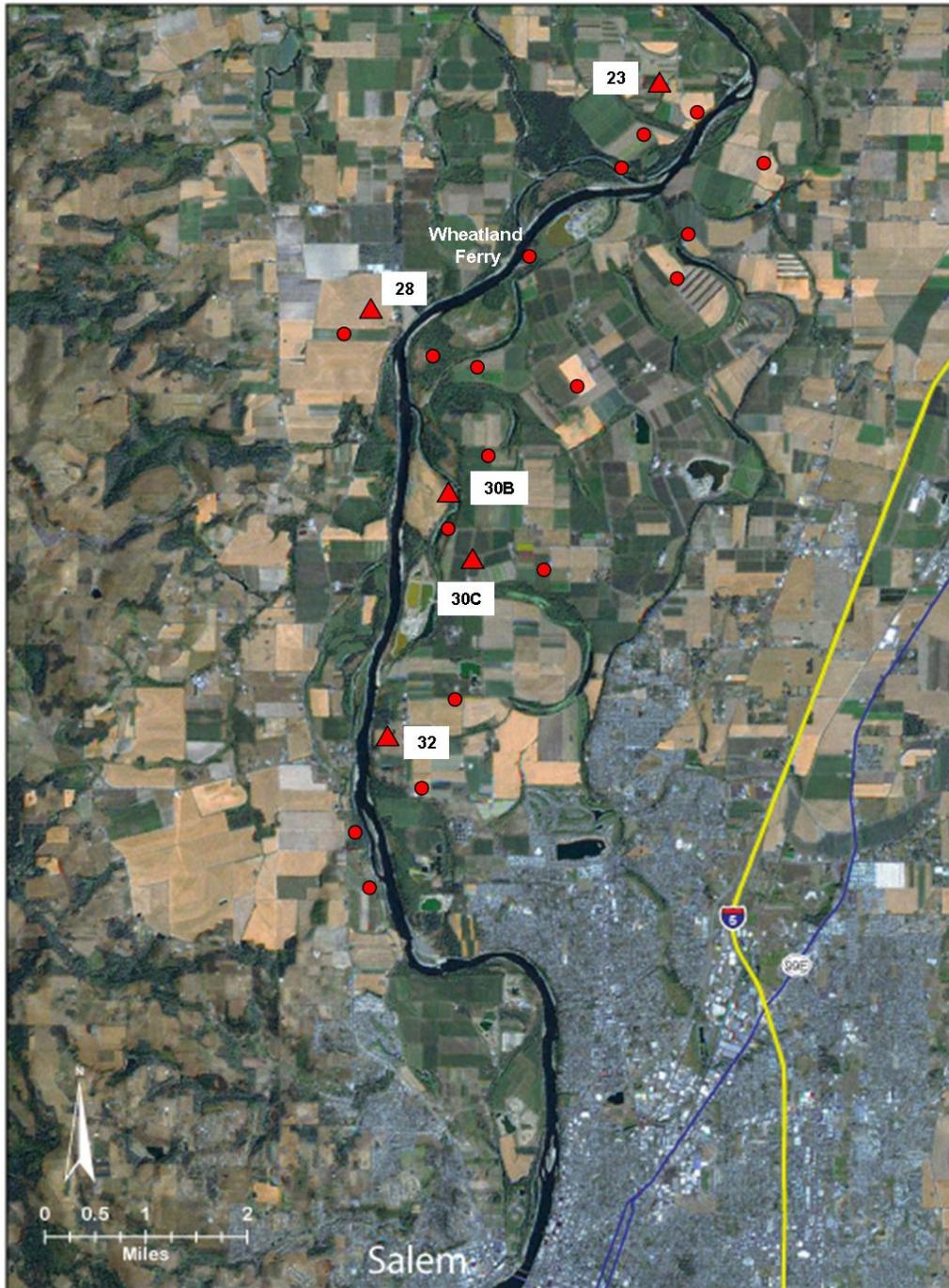


Figure 4. Osprey egg sampling sites (triangular shapes) and adjacent occupied nest sites (circular shapes) within the mid-Willamette River, Oregon in 2008 (from Kaiser 2009).

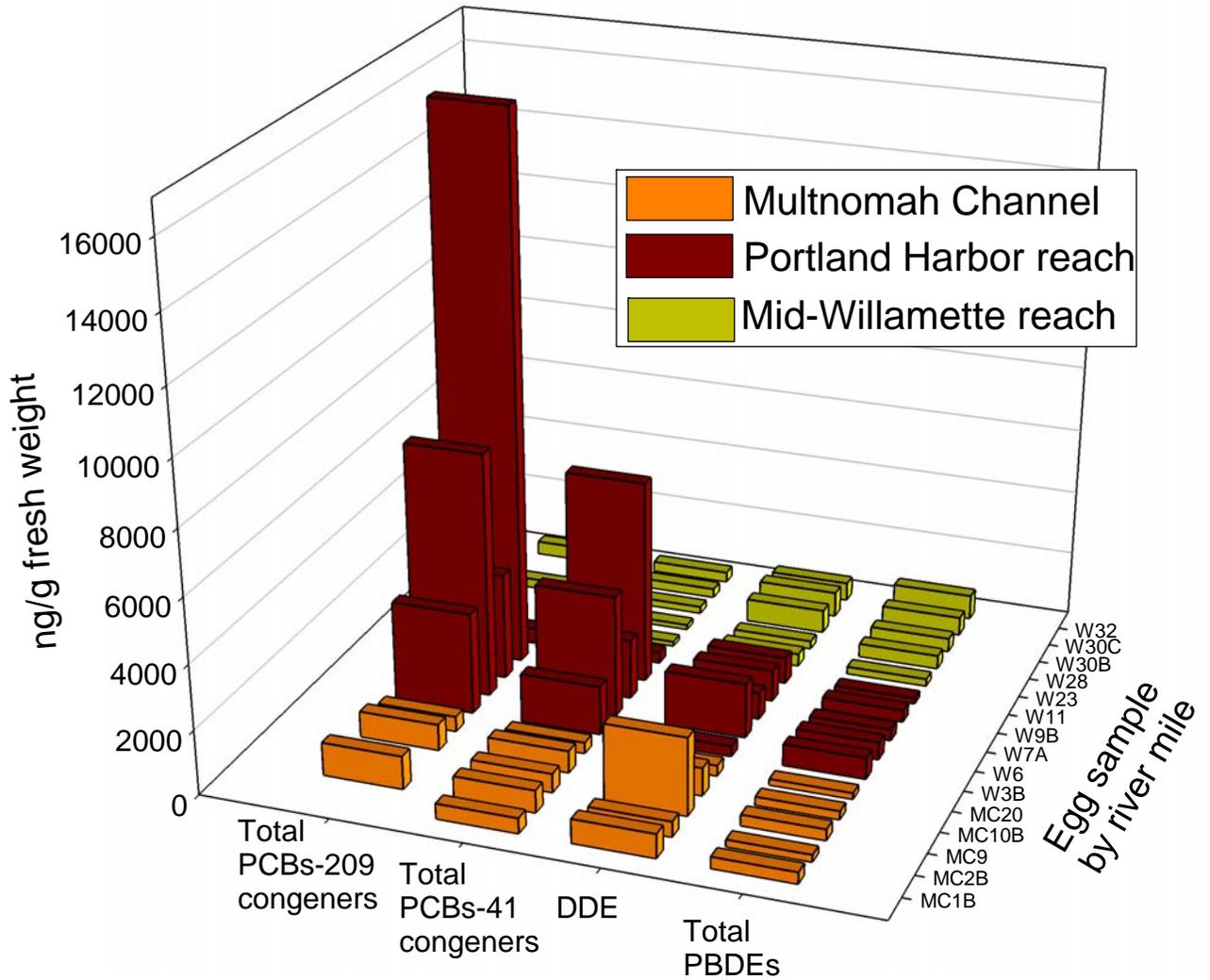


Figure 5. Concentrations of total polybrominated diphenyl ethers (PBDEs), DDE, and total polychlorinated biphenyls (PCBs) summed with either 41 congeners or all 209 congeners in osprey eggs collected in 2008 from the Portland Harbor reach and vicinity. Egg samples collected from the Multnomah Channel (MC1B, MC2B, MC9, MC10B, MC20), the Portland Harbor reach (W3B, W6, W7A, W9B, W11) and the mid-Willamette River (W23, W28, W30B, W30C, W32) are numbered consecutively by river mile on the graph.

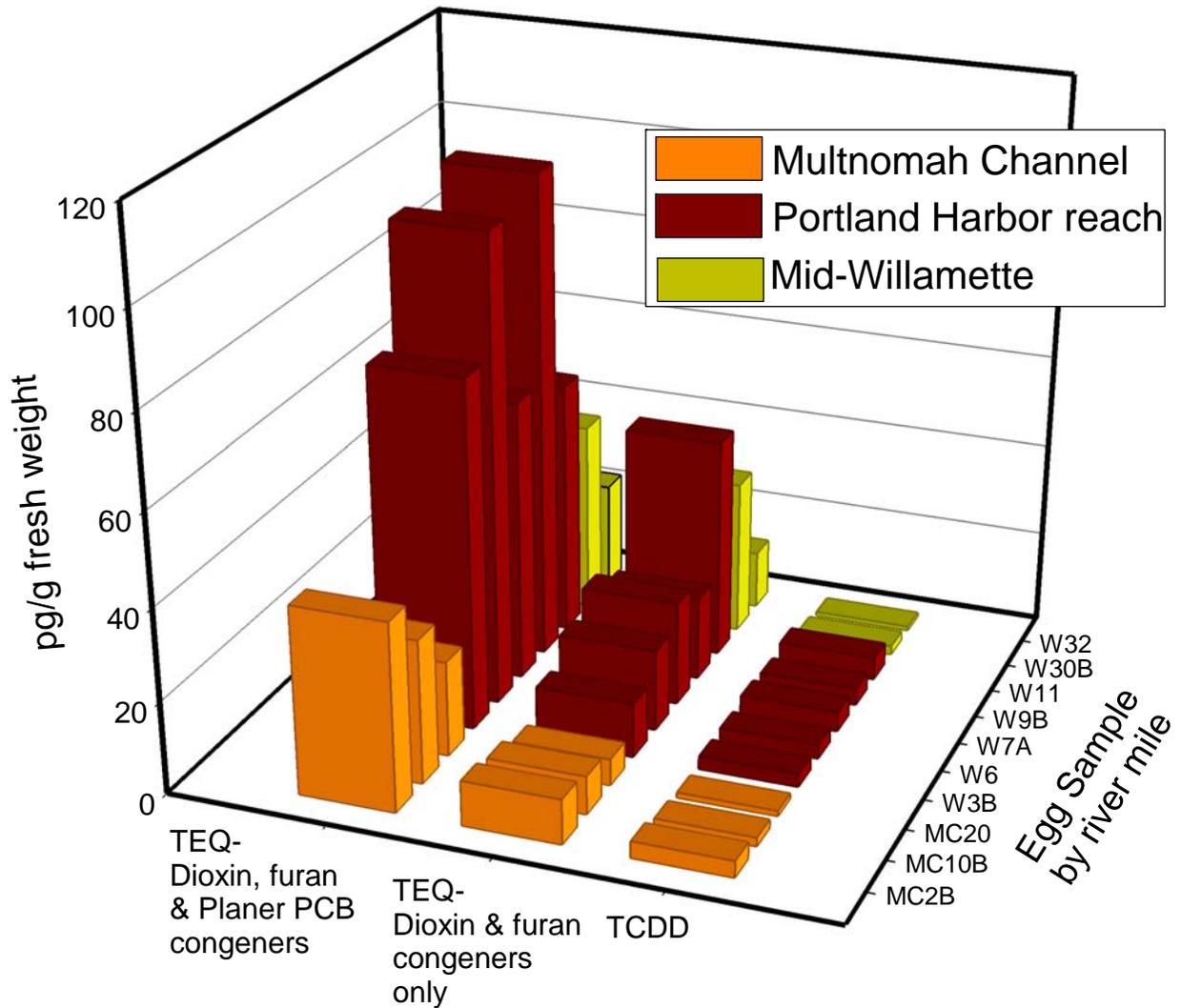


Figure 6. Concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and avian-based toxic equivalents (TEQs; calculated with dioxins and furans only or including planar PCBs), in osprey eggs from the Portland Harbor reach and vicinity in 2008. Egg samples collected from the Multnomah Channel (MC2B, MC10B, MC20), the Portland Harbor reach (W3B, W6, W7A, W9B, W11) and the mid-Willamette River (W30B and W32) are numbered consecutively by river mile on the graph.

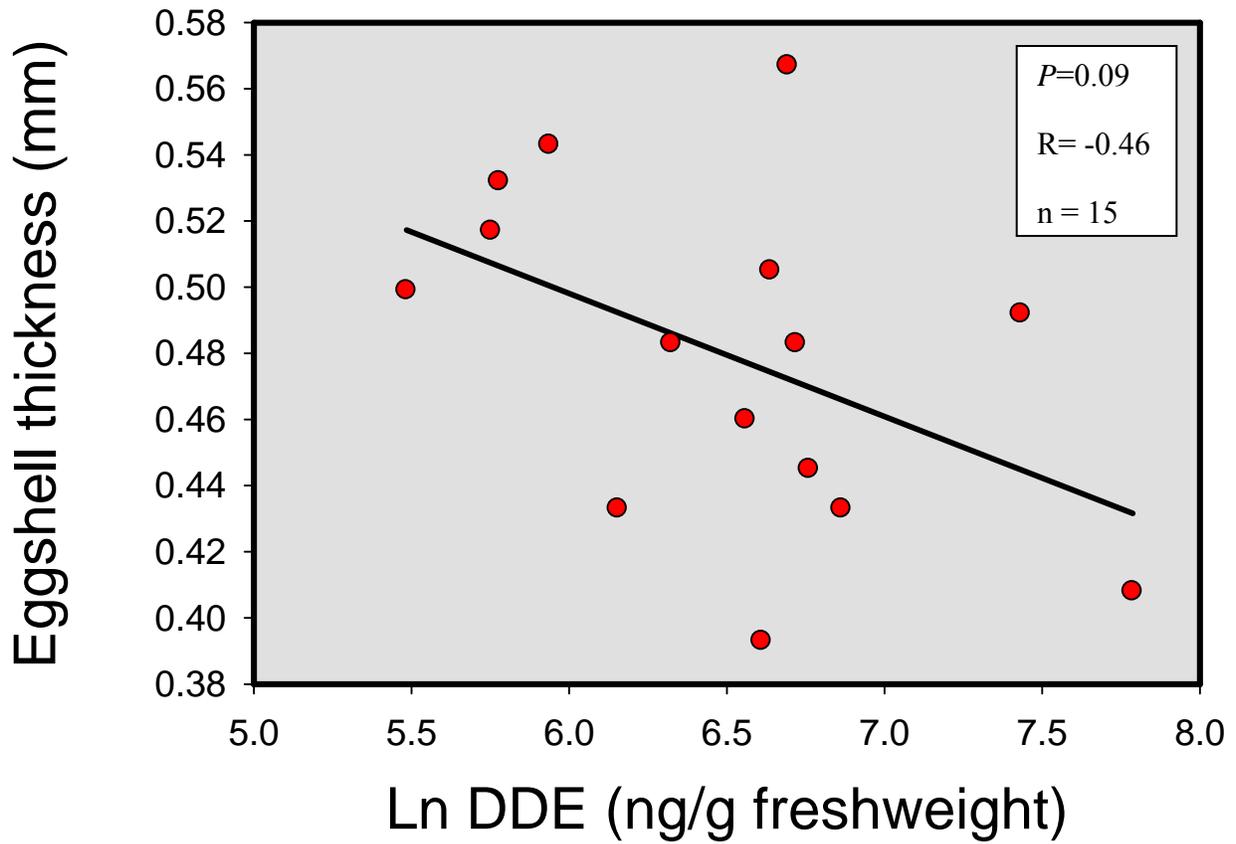


Figure 7. Regression of eggshell thickness and natural log of the DDE concentrations (Ln DDE) in 15 eggs of osprey from the Portland Harbor reach, the mid-Willamette River, and the Multnomah Channel.

4.0 REFERENCES

- Anderson, D.W. and J.J. Hickey. 1972. Eggshell changes in certain North American birds. *Proceedings of the International Ornithological Congress* 15: 514-540.
- Ballschmiter, K., and M. Zell. 1980. Analysis of polychlorinated biphenyls (PCB) by gas capillary chromatography. Composition of technical Aroclor- and Clophen-PCB mixtures. *Fresenius Zeitschrift Fur Analytische Chemie*. 302:20-31.
- Buck, J.A. 2008. Quality Assurance Project Plan (QAPP) for the analysis of osprey (*Pandion haliaetus*) egg tissue collected from Portland Harbor and surrounding areas. Prepared for the U.S. Environmental Protection Agency, Portland, Oregon by the U.S. Fish and Wildlife Service, Oregon Fish and Wildlife Office, Portland, Oregon.
- Buck, J.A. and C.J. Henny. 2008. Field sampling plan for the collection of osprey eggs from the Portland Harbor Superfund Site. Prepared for the U.S. Environmental Protection Agency, Portland, Oregon by the U.S. Fish and Wildlife Service, Oregon Fish and Wildlife Office, Portland, Oregon.
- Drent, R.H. and S. Daan. 1980. The prudent parent: Energetic adjustments in avian breeding. *Ardea* 68:225-252.
- EcoChem, Inc. 2010. Data validation report: Osprey (*Pandion haliaetus*) egg tissue analysis collected from Portland Harbor and surrounding areas. Prepared for the U.S. Fish and Wildlife Service, Oregon Fish and Wildlife Office, Portland, Oregon by EcoChem, Inc. Seattle, Washington.
- Elliott, J.E., M.M. Machmer, C.J. Henny, L.K. Wilson, and R.J. Norstrom. 1998. Contaminants in ospreys from the Pacific Northwest: I. Trends and patterns in polychlorinated dibenzo-*p*-dioxins and -dibenzofurans in eggs and plasma. *Archives of Environmental Contamination and Toxicology* 35:620-631.
- Elliott, J.E., M.M. Machmer, L.K. Wilson, and C.J. Henny. 2000. Contaminants in ospreys from the Pacific Northwest: II. Organochlorine pesticides, polychlorinated biphenyls, and mercury, 1991-1997. *Archives of Environmental Contamination and Toxicology* 38:93-106.
- Elliott, J.E., C.A. Morrissey, C.J. Henny, E.R. Inzunza, and P. Shaw. 2007. Satellite telemetry and prey sampling reveal contaminant sources to Pacific Northwest ospreys. *Ecological Applications* 17:1223-1233.
- Evers, D.C., K.M. Taylor, A. Major, R.J. Taylor, R.H. Poppenga, and A.M. Scheuhammer. 2003. Common loon eggs as indicators of methylmercury availability in North America. *Ecotoxicology* 12:69-81.

- Grove R.A., C.J. Henny, and J.L. Kaiser. 2009. Osprey: Worldwide sentinel species for assessing and monitoring environmental contaminants in rivers, lakes, reservoirs and estuaries. *Journal of Toxicology and Environmental Health Part B* 12:1-20.
- Henny, C.J., J.L. Kaiser, R.A. Grove, V.R. Bentley, and J.E. Elliott. 2003. Biomagnification factors (fish to osprey eggs from Willamette River, Oregon, U.S.A.) for PCDDs, PCDFs, PCBs and OC pesticides. *Environmental Monitoring and Assessment* 84:275-315.
- Henny, C.J., R.A. Grove, J.L. Kaiser, and V.R. Bentley. 2004. An evaluation of osprey eggs to determine spatial residue patterns and effects of contaminants along the lower Columbia River, U.S.A. Pages 369-388 in R.D. Chancellor, B.-U. Meyburg, editors. *Raptors Worldwide. Proceedings VI of the World Conference on Birds of Prey and Owls. World Working Group Birds of Prey (WWGBP) and BirdLife Hungary (MME), Budapest.*
- Henny, C.J., R.A. Grove, and J.L. Kaiser. 2008. Osprey distribution, abundance, reproductive success and contaminant burdens along lower Columbia River, 1997/1998 versus 2004. *Archives of Environmental Contamination and Toxicology* 54(3):525-534.
- Henny, C.J., J.L. Kaiser, and R.A. Grove. 2009a. PCDDs, PCDFs, PCBs, OC pesticides and mercury in fish and osprey eggs from Willamette River, Oregon (1993, 2001 and 2006) with calculated biomagnification factors. *Ecotoxicology* 18:151-173.
- Henny, C. J., J. L. Kaiser, R. A. Grove, B. L. Johnson, and R. J. Letcher. 2009b. Polybrominated diphenyl ether flame retardants in eggs may reduce reproductive success of ospreys in Oregon and Washington, USA. *Ecotoxicology* 18:802-813.
- Hobson, K.A., K.D. Hughes, and P.J. Ewins. 1997. Using stable-isotope analysis to identify endogenous and exogenous sources of nutrients in eggs of migratory birds: Applications to Great Slave Lake contaminant research. *The Auk* 114:467-478.
- Kaiser, J.L. 2009. Field sampling report for the collection of eggs and determination of productivity of osprey nesting within the Portland Harbor Superfund Site and vicinity. Draft report prepared for the U.S. Fish and Wildlife Service, Oregon Fish and Wildlife Office, Portland, Oregon by the U.S. Geological Survey, Forest and Rangeland Ecosystem Science Center, Corvallis, Oregon.
- Lundholm, C.E. 1997. DDE-induced eggshell thinning in birds: Effects of p,p'-DDE on the calcium and prostaglandin metabolism of the eggshell gland. *Comparative Biochemistry and Physiology C-Toxicology & Pharmacology* 118:113-128.
- Martin, P.A., S.R. De Solla, and P. Ewins. 2003. Chlorinated hydrocarbon contamination in osprey eggs and nestlings from the Canadian Great Lakes Basin, 1991–1995. *Ecotoxicology* 12: 209-224.

- Postupalsky, S. 1974. Raptor reproductive success: Some problems with methods, criteria, and terminology. Pages 21-31 *in* F.N. Hammerstrom, Jr., B.E. Harrell, R.R. Ohlendorff, editors. Management of Raptors: Proceedings of the Conference on Raptor Conservation Techniques, Raptor Research Report No. 2. Raptor Research Foundation, Vermillion, SD.
- Postupalsky, S. 1977. A critical review of problems in calculating osprey reproductive success. Pages 1-11 *in* J.C. Ogden, ed., Transactions North American Osprey Research Conference. Transactions and Proceedings Series, No. 2. National Park Service, Washington, D.C.
- Stickel, L.S., S.N. Wiemeyer, and L.J. Blus. 1973. Pesticide residues in eggs of wild birds: Adjustment for loss of moisture and lipid. *Bulletin of Environmental Contamination and Toxicology* 9:193-196.
- SYSTAT Software, Inc. 2007. Systat 12.0 for Windows. San Jose, California.
- Toschik, P.C., B.A. Rattner, P.C. McGowan, M.C. Christman, D.B. Carter, R.C. Hale, C.W. Matson, and M.A. Ottinger. 2005. Effects of contaminant exposure on reproductive success of ospreys (*Pandion haliaetus*) nesting in Delaware River and Bay, USA. *Environmental Toxicology and Chemistry* 24:617-628.
- van den Berg, M., L. Birnbaum, A.T.C. Bosveld, B. Brunstrom, P. Cook, M. Feeley, J.P. Giesy, A. Hanberg, R. Hasegawa, S.W. Kennedy, T. Kubiak, J.C. Larsen, F.X.R. van Leeuwen, A.K.D. Liem, C. Nolt, R.E. Peterson, L. Poellinger, S. Safe, D. Schrenk, D. Tillitt, M. Tysklind, M. Younes, F. Waern, and T. Zacharewski. 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environmental Health Perspectives* 106:775-792.
- Wiemeyer, S.N., C.M. Bunck, and A.J. Krynitsky. 1988. Organochlorine pesticides, polychlorinated-biphenyls, and mercury in osprey eggs 1970-79 and their relationships to shell thinning and productivity. *Archives of Environmental Contamination and Toxicology* 17(6): 767-787.