

DATA REPORT

FOR

LOWER COLUMBIA JUVENILE SALMON

PERSISTENT ORGANIC POLLUTANT EXPOSURE

ASSESSMENT

Prepared By

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For

NOAA Damage Assessment Center
Portland Harbor Natural Resource Trustees

Project Background and Objectives

Portland Harbor is located along the lower Willamette River in Portland, Oregon. Sediments in the Portland Harbor area have been found to have elevated levels of hazardous substances such as PCBs, pesticides, herbicides, dioxins and furans, metals, tributyltin, and PAHs (LWG 2007). In December 2000, the Environmental Protection Agency listed the site on the National Priorities List as a Superfund site. The remedial investigation has encompassed a reach of the lower Willamette River that extends from river mile 1.0 up to approximately river mile 11.8.

The lower Willamette River is a migration corridor and juvenile rearing area for anadromous salmonids, including several stocks that are listed as threatened species under the Endangered Species Act. Wild (unmarked) juvenile Chinook salmon from the lower Willamette River and lower Columbia River ESUs (listed as threatened species under the ESA in March 1998), are among the most frequently observed salmon stocks in the area, and sampling information from the lower Willamette River suggests that these subyearling Chinook salmon reside there for an extended period of time (Freisen *et al.* 2005). Because of their life history characteristics, subyearling Chinook salmon have a high potential for contaminant exposure and subsequent injury in the Portland Harbor area via contact with contaminated sediments and ingestion of contaminated benthic invertebrates. Injury assessment studies in Portland Harbor have confirmed elevated body burdens of bioaccumulative contaminants in juvenile Chinook from some sampling sites (LWG 2007).

There is also concern about how contaminants may be affecting salmon stocks that migrate down the Columbia River and spend time in the area near and below the Willamette/Columbia confluence area, a region that could be impacted by pollutants from Portland Harbor as well as urban and industrial areas near Vancouver and Portland on the Columbia River. A monitoring study by NOAA and the Lower Columbia River Estuary Partnership showed that juvenile Chinook salmon from several Columbia River stocks, collected from sites below the Willamette/Columbia confluence area have increased body burdens of PBDEs and PCBs as compared to concentrations in fish from the same stocks collected above the confluence, in the Columbia Gorge (LCREP 2007). The same study found elevated concentrations of PAH metabolites in bile of juvenile Chinook collected from sites in the area between the Portland/Vancouver area and Longview.

The objective of the current study was to characterize exposure to persistent organic pollutants (POPs) in juvenile salmon from above and below the Willamette/ Columbia confluence area. This report covers the data on concentrations of bioaccumulative contaminants in salmon (e.g., PCBs, DDTs, and PBDEs); PAH exposure data were presented in an earlier report. This research will help to determine whether the urban areas near Portland and Vancouver are major sources of exposure to PCBs, DDTs, PBDEs, and related contaminants for outmigrant salmon, and may provide some information on differences in exposure in salmon rearing in the lower Willamette River as compared to those that are from other locations on the Columbia River. These data will also form a basis for estimating the likely level of injury in these fish, based on other

studies of health effects of PCBs, DDTs, and PBDEs on salmonids (Meador *et al.* 2002, Beckvar *et al.* 2005, Johnson *et al.* 2007).

Methods

Salmon Sampling

The juvenile Chinook salmon analyzed in the study were collected in the spring of 2008, as part of a larger salmon monitoring program conducted by NOAA Fisheries and the Lower Columbia Estuary Partnership (LCREP) with support from the Bonneville Power Administration. Juvenile salmon were sampled from three sites in Columbia Gorge (Franz Lake, Beacon Rock Slough, and Mirror Lake), from the Willamette/ Columbia confluence area at one site on the Washington side of the river and one on the Oregon side of the river; from Hayden Island and Sauvie Island; and from Campbell Sough near Ridgefield Wildlife refuge and Sandy Island near Goble, Oregon (2007 only). All sites surveyed for juvenile salmonids are shown in Figure 1. Sites that have been analyzed to date for persistent organic pollutants are shown in Figure 2; those where bile samples were collected are listed in Table 1.

Salmon were collected by beach seine generally following the procedures described in the Puget Sound Protocols (PTI 1990), and by Varanasi *et al.* (1993). Fish captured from the sites were held alive in aerated river water until necropsies could be conducted. Fish processing was done on-site in mobile lab facilities.

At each site, or for every collection period, approximately 10-30 juvenile Chinook salmon bodies were collected for chemical analyses. Initially, fish were weighed (g) and measured (fork length in mm), then euthanized with MS-222. The stomach and gastrointestinal tract were removed and stomach contents were extracted and saved for chemical and taxonomy analyses. These internal organs were then placed back into the visceral cavity of the carcass, so the tissue analyzed would consist of the whole body minus stomach contents. The carcasses containing the internal organs were individually wrapped in foil, and labeled. Caudal fin samples were collected in ethanol for genetic analyses. Body samples were placed in a cooler with dry ice for transport back to the NWFSC laboratory in Seattle. At the laboratory, body samples were stored at -80 °C until chemical analyses were performed.

Sample Analyses

Sample preparation.— For lipid and chemical analyses, individual salmon bodies (carcass plus internal organs) were combined to produce composite samples consisting of 3-5 fish each, in which all individuals were from the same stock, the same site, and the same sampling time. The proportions of samples from each genetic stock at each sampling site are shown in Table 2; genetic origin was estimated as described in Teel *et al.* (2009).

Lipid determination.— The amount of total, non-volatile extractable lipid (reported as

percent lipid) in the body composites was determined by gravimetric analysis as described in Sloan *et al.* (2004). Lipid classes were determined using thin layer chromatography/flame ionization detection (TLC/FID) with Iatroskan analysis, as described in Ylitalo *et al.* (2005). The TLC/FID analysis also provided an estimate of percent lipid content.

Chemical contaminants in feed and body samples. —Body composite samples were analyzed by gas chromatography/mass spectrometry (GC/MS) for PCB congeners, DDTs and DDT isomers, and other organochlorine (OC) pesticides (hexachlorocyclohexanes, hexachlorobenzene (HCB), chlordanes, aldrin, dieldrin, mirex, and endosulfans) as described by Sloan *et al.* (2005). PBDEs were measured similarly and concurrently in the GC/MS analyses. A total of 47 individual PCB congeners were measured [International Union of Pure and Applied Chemistry (IUPAC) numbers 17, 18, 28, 31, 33, 44, 49, 52, 66, 70, 74, 82, 87, 95, 99, 101/90, 105, 110, 118, 128, 138/163/164, 149, 151, 153/132, 156, 158, 170/190, 171, 177, 180, 183, 187/159/182, 191, 194, 195, 199, 205, 206, 208, 209]. Hexachlorocyclohexanes (HCHs) measured included α -HCH, β -HCH, and γ -HCH (lindane). Dichloro-diphenyl-trichloroethanes measured included *p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, *o,p'*-DDD, *o,p'*-DDE and *o,p'*-DDT. Chlordanes and related compounds measured included heptachlor, heptachlor epoxide, g-chlordane, a-chlordane, oxychlordane, *cis*-nonachlor, *trans*-nonachlor and nonachlor III. A total of 10 individual PBDE congeners were measured (IUPAC numbers 28, 47, 49, 66, 85, 99, 100, 153, 154, 183). In body samples, the limits of quantitation (LOQs) ranged from less than 0.059 to less than 0.34 ng/g wet wt for individual PCB congeners, from less than 0.23 to less than 0.35 ng/g wet wt for DDTs, aldrin, dieldrin, chlordanes, mirex, and HCHs, from less than 0.64 to less than 0.93 ng/g, wet wt for endosulfan I, and from less than 0.28 to less than 0.34 ng/g wet wt for HCB.

Summed PCBs (Σ PCBs) were calculated by adding the concentrations of 17 commonly detected chlorobiphenyl congeners (IUPAC numbers 18, 28, 44, 52, 95, 101, 105, 118, 128, 138, 153, 170, 180, 187, 195, 206, 209), and multiplying the result by two. This formula provides a good estimate of the total PCBs in a typical environmental sample of sediments or animals feeding on lower trophic levels (Lauenstein *et al.* 1993). Summed PCBs (Σ PCBs) were calculated by adding the concentrations of 47 measured congeners. Summed DDTs (Σ DDTs) were calculated by summing the concentrations of *p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, *o,p'*-DDD, *o,p'*-DDE and *o,p'*-DDT. Summed chlordanes (Σ CHLDs) were determined by adding the concentrations of heptachlor, heptachlor epoxide, g-chlordane, a-chlordane, oxychlordane, *cis*-nonachlor, *trans*-nonachlor and nonachlor III. Summed hexachlorocyclohexanes (Σ HCHs) were calculated by adding the concentrations of a-HCH, b-HCH and lindane (g-HCH). Summed PBDEs (Σ PBDEs) were calculated by adding the concentrations of the 10 PBDE congeners measured.

To monitor the accuracy of the GC/MS method, a National Institute of Standards and Technology (NIST) Standard Reference Material blue mussel (*Mytilus edulis*) homogenate (SRM 1974b) and fish tissue homogenate (NIST SRM 1947) were analyzed with each sample set and the results met laboratory criteria (Sloan *et al.* 2006). One of the eight of feed samples (12.5%) was analyzed in duplicate to measure the precision of the method, and the laboratory quality assurance criteria were met for all analytes

measured in the feed samples. Method blanks also met laboratory criteria. Quality assurance procedures and criteria are described in detail in Sloan *et al.* (2006). The percent recoveries of the surrogate standards ranged from 75 – 106%.

To adjust for the influence of lipid on toxicity, we normalized body contaminant concentrations for lipid, and relied primarily on lipid-normalized data to evaluate potential health effects of toxicants on juvenile salmon. Wet-weight data are also presented to facilitate comparison with other studies, and to evaluate risks to predators who consume salmon that have accumulated toxicants.

Results

Average concentrations of PCBs, DDTs, and PBDEs in (ng/g wet wt and ng/g lipid) in whole bodies of salmon from five of the 2008 sampling sites are shown in Figures 4-6. In general, concentrations of all three classes of contaminants tended to be higher in salmon from the two confluence sites than in salmon from either the Gorge sites (Franz Lake and Mirror Lake) or the Ridgefield site downstream. However, statistically significant intersite differences in body concentrations were observed only for DDTs and PBDEs, because of the highly variable PCB concentrations in samples from the confluence sites. Additional organochlorine pesticides measured in the juvenile salmon composites samples included Σ CHLDs, Σ HCHs, HCB, mirex, aldrin, dieldrin, and endosulfan (Table 3). Mirex, endosulfan, aldrin, and Σ HCHs were <LOQ at all sites. Σ CHLDs, HCB, and dieldrin were present at low concentrations (0.2-2.7 ng/g wet wt) at some sites, with highest levels generally found in fish from the confluence sites.

The concentrations of PCBs and DDTs we observed in juvenile salmon from some sites suggest these fish may be at risk for toxic effects of these contaminants. Meador *et al.* (2002) suggest a body concentration of 2400 ng/g lipid as a threshold effect level for PCB exposure. If we apply this criterion to PCB data shown in Figure 4, salmon from both confluence sites may be at risk for PCB-associated injury; in fish from the other sites, PCB levels are below threshold. In the case of DDTs, based on evaluations by Beckvar *et al.* (2005) and the Science Center (see Johnson *et al.* 2007) we estimated that the threshold concentration for injury from DDTs would be 500-600 ng/g wet wt, or approximately 5,000-6,000 ng/g lipid, assuming that the fish on which these thresholds were had a lipid content of about 10%, which is in the typical range for laboratory-reared salmonids (Meador *et al.* 2002). Body concentrations of DDTs were below this level in most of the samples we analyzed, but were within this range in samples from the Confluence Washington site.

Figure 7 shows the lipid content of the 2008 salmon body samples analyzed to date. Lipid content tended to be higher in samples from Franz Lake and Ridgefield than from in those from Mirror Lake and the confluence sites, although the differences were not statistically significant. Also, in salmon from these two sites, triglycerides made up a higher percentage of total lipids than in salmon from the other sites, an indicator of healthy energy stores.

Figures 8-10 show levels of PCBs, DDTs, and PBDEs in Chinook salmon from the lower Columbia River and lower Willamette River sites sampled both in 2008 and in 2005, as part of the LCREP Salmon and Water Quality study (LCREP 2007). Salmon samples analyzed from four lower Willamette River sites analyzed by the Lower Willamette Group (LWG 2007) are also included for comparison. In all studies, POPs levels tended to highest in salmon from the Columbia River near the confluence, although there was considerable variation from site to site in the different regions that were sampled. Figure 11 shows these data with sites grouped by region (Gorge, Lower Willamette, Confluence WA, Confluence OR, and Below Confluence), so patterns can be observed more easily. Contaminant concentrations in fish from the Gorge are consistently lower in than fish from other regions, and highest contaminant concentrations are generally in the lower Willamette River or confluence sites.

Figure 12 shows levels of PCBs, DDTs, and PBDEs in Chinook salmon from different stocks at sites in and below the Columbia Gorge. These data include samples from both 2007-2008 and 2005 to provide the largest data set possible. Salmon stocks included in this comparison are fall Chinook Spring Creek, West Cascades, Snake River, and Upper Columbia groups. The Spring Creek and West Cascades groups are both lower Columbia River stocks. Two-way ANOVA indicated that while concentrations of PCBs, DDTs, and PBDEs did not differ significantly among stocks, concentrations of all these contaminants were significantly higher in fish collected from sites below the Columbia Gorge than in sites within the Columbia Gorge. For region (i.e. the Gorge vs. the lower river, below the Gorge) $0.0001 \leq p \leq 0.0021$, while for stock $0.1571 \leq p \leq 0.6297$.

In summary, these data show that exposure to POPs is generally greater in juvenile salmon from sites near or below the Willamette/Columbia confluence area than in fish above this area (i.e., from the Columbia Gorge). This is true for salmon from Snake River and Upper Columbia River stocks as well as Lower Columbia River stocks. Moreover, some fish from the area around the confluence are at risk for injury.

References

Beckvar N., T.M. Dillon, and L. B. Read. 2005. Approaches for linking whole-body fish residues of mercury or DDT to biological effects thresholds. *Environmental Toxicology and Chemistry* 24:2094-2105.

Friesen, T.A., J.S. Vile and A.L. Pribyl. 2005. Migratory Behavior, Timing, Rearing, and Habitat Use of Juvenile Salmonids in the Lower Willamette River. In: Friesen, T.A. (Ed). 2005. *Biology, Behavior, and Resources of Resident and Anadromous Fish in the Lower Willamette River Final Report of Research, 2000-2004*. Prepared by Oregon Department of Fish and Wildlife for the City of Portland.

Johnson, L. L., G. M. Ylitalo, C. A. Sloan, B. F. Anulacion, A. N. Kagley, M. R. Arkoosh, T. A. Lundrigan, K. Larson, M. D. Siipola, T. K. Collier. 2007. Persistent organic pollutants in outmigrant juvenile chinook salmon from the Lower Columbia Estuary, USA. *Science of the Total Environment* 374:342-366.

LCREP (Lower Columbia River Estuary Partnership). 2007. *Lower Columbia River Estuary Ecosystem Monitoring: Water Quality and Salmon Sampling Report*. Prepared by the Lower Columbia River Estuary Partnership, Portland, OR, 76 p.

LWG (Lower Willamette Group) 2007. *Portland Harbor RI/FS Comprehensive Round 2 Site Characterization Summary And Data Gaps Analysis Report*. Prepared by Integral Consulting Inc. and Windward, Environmental LLC. for Lower Willamette Group. February 30, 2007.

Meador, J. P., T. K. Collier, J. E. Stein. 2002. Determination of a tissue and sediment threshold for tributyltin to protect prey species for juvenile salmonids listed by the U. S. Endangered Species Act. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 12:539-551 Meador *et al.* 2002

PTI, 1990. *Recommended Guidelines for Sampling Soft-Bottom Demersal Fishes by Beach Seine and Trawl in Puget Sound*. Prepared for E.P.A.; Puget Sound Estuary Program. In: *Recommended Protocols for Measuring Selected Environmental Variables in Puget Sound*. Vol 2.

Sloan, C. A., D. W. Brown, R. W. Pearce, R. H. Boyer, J. L. Bolton, D. G. Burrows, D. P. Herman, M. M. Krahn. 2004. Extraction, cleanup, and gas chromatography/mass spectrometry analysis of sediments and tissues for organic contaminants. U.S. Dept. of Commerce, NOAA Tech. Memo., NMFS-NWFSC-59, 47 p

Sloan, C. A., D. W. Brown, R. W. Pearce, R. H. Boyer, J. L. Bolton, D. G. Burrows, D. P. Herman, M. M. Krahn. 2005. Determining aromatic hydrocarbons and chlorinated hydrocarbons in sediments and tissues using accelerated solvent extraction and gas chromatography/mass spectrometry. In *Techniques in Aquatic Toxicology*. CRC Press, Boca Raton, FL

Sloan, C.A., D.W. Brown, G.M. Ylitalo, J. Buzitis, D.P. Herman, D.G. Burrows, G.K. Yanagida, R.W. Pearce, J.L. Bolton, R.H. Boyer, and M. M. Krahn. 2006. Quality assurance plan for analyses of environmental samples for polycyclic aromatic compounds, persistent organic pollutants, fatty acids, stable isotope ratios, lipid classes, and metabolites of polycyclic aromatic compounds. U.S. Dept. of Commerce, NOAA Tech. Memo., NMFS-NWFSC-77, 30 p.

Teel, D.J., C. Baker, D.R. Kuligowski, T.A. Friesen, and B. Shields. 2009. Genetic Stock Composition of Subyearling Chinook Salmon in Seasonal Floodplain Wetlands of the Lower Willamette River. *Transactions of the American Fisheries Society* 138:211-217.

Varanasi, U., E. Casillas, M.R. Arkoosh, T. Hom, D.A. Misitano, D.W. Brown, S-L. Chan, T.K. Collier, B.B. McCain, and J.E. Stein. 1993. Contaminant exposure and associated biological effects in juvenile chinook salmon (*Oncorhynchus tshawytscha*) from urban and nonurban estuaries of Puget Sound. U. S. Dept. of Commerce, NOAA Tech. Memo. NMFS-NWFSC-8, 112 p.

Ylitalo, G. M., G. K. Yanagida, L. C. Hufnagle Jr., M. M. Krahn. 2005. Determination of lipid classes and lipid content in tissues of aquatic organisms using a thin layer chromatography/flame ionization detection (TLC/FID) microlipid method. Pages 227-237 in Ostrander, G. K. (Ed.) *Techniques in Aquatic Toxicology*. CRC Press, Boca Raton, FL.

Table 1. Site locations where Chinook body samples were collected.

Site Name	Latitude	Longitude	Composites Analyzed to Date
Franz Lake, WA	45.600583°	-122.103067°	1
Beacon Slough, WA	45.628217°	-122.012150°	0
Pierce Island, OR	45.620967°	-122.01080°	0
Sand Island, OR	45.553350°	-122.21112°	0
Mirror Lake, OR	45.542700°	-122.245050°	2
Confluence WA	45.667533°	-122.762017°	4
Confluence OR	45.673267°	-122.775617°	5
Hayden Island, OR	45.632367°	-122.732703°	0
Sauvie Island, OR	45.625422°	-122.794713°	0
Ridgefield, WA	45.783867°	-122.754850°	1
Sandy Island, OR	46.015111°	-122.868472°	0

Table 2. Concentrations of organochlorine pesticides and PBDEs (ng/g, wet wt) determined by GC/MS in bodies of juvenile fall Chinook salmon from Columbia River sites sampled in 2008. <LOQ = less than lower quantitation limit. Σ CHLDS = summed chlordanes; HCB = hexachlorobenzene. Mirex, endosulfan, and hexachlorocyclohexanes (HCHs), including α -HCH, β -HCH, and γ -HCH, were also measured, but were <LOQ at all sites. Body samples are composites of 2-5 fish each.

Site	Dieldrin (ng/g wet wt)	Σ CHLDS ^a (ng/g wet wt)	HCB (ng/g wet wt)
Franz Lake (n=1)	<LOQ	<LOQ	<LOQ
Mirror Lake (n=2)	<LOQ	0.24 ± 0.04	<LOQ
Confluence-WA (n=4)	<LOQ	0.72 ± 0.68	0.24 ± 0.18
Confluence-OR (n=5)	0.89 ± 0.45	2.7 ± 1.5	0.43 ± 0.18
Ridgefield (n=1)	0.31	0.69	0.39

^aincludes heptachlor, heptachlor epoxide, γ -chlordane, α -chlordane, oxychlordane, *cis*-nonachlor, *trans*-nonachlor and nonachlor III.

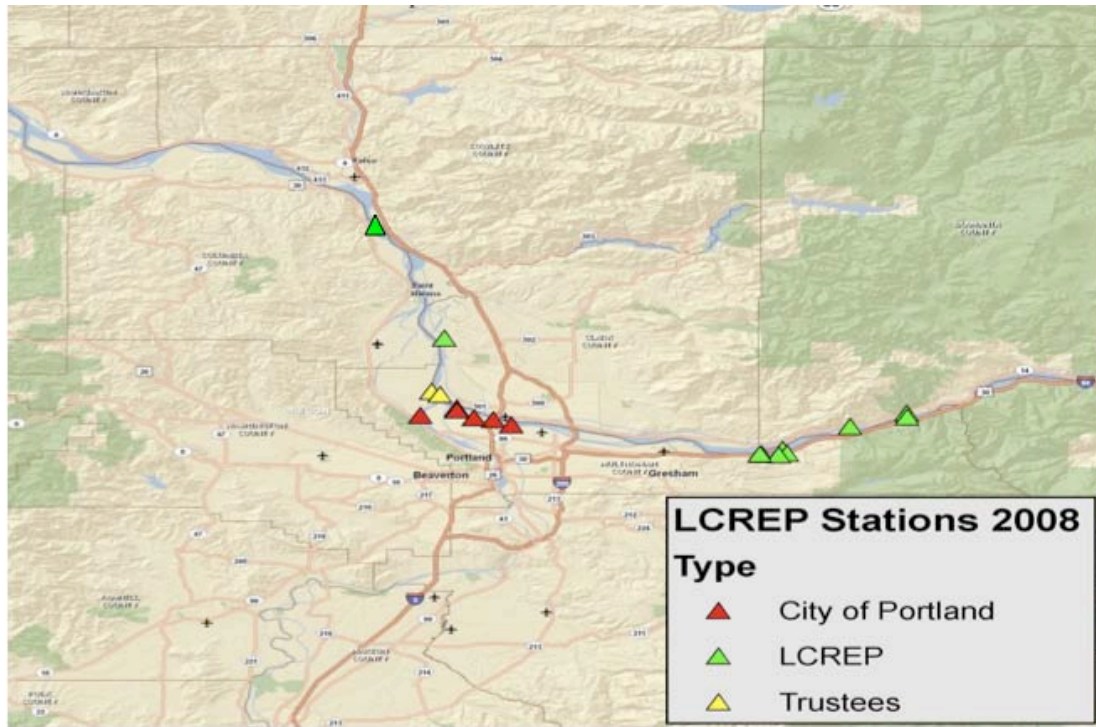


Figure 1. Juvenile salmon sampling sites

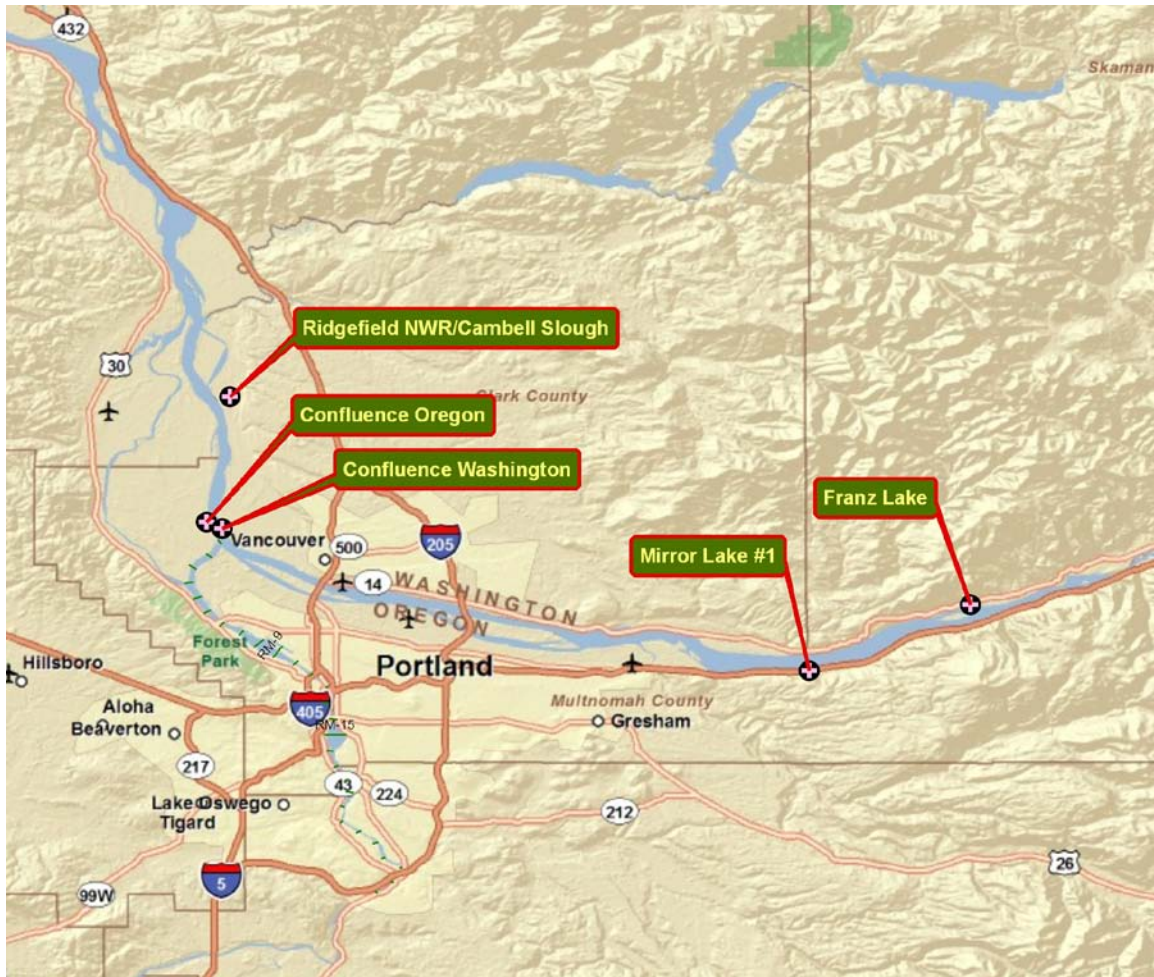


Figure 2. Juvenile salmon sampling sites from 2008 that have been analyzed for persistent organic pollutants. Additional composites are available for analysis.

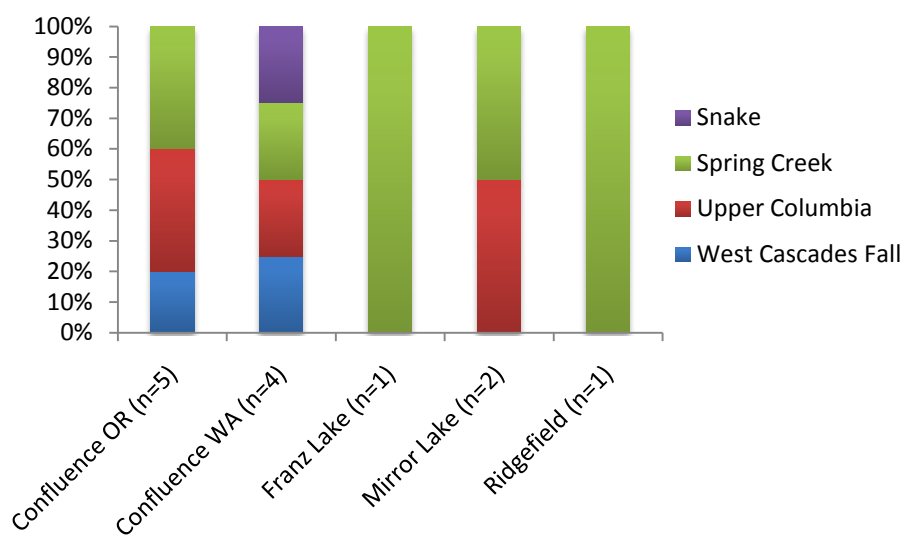


Figure 3. Genetic stocks of juvenile salmon in body composites from 2008 sampling sites.

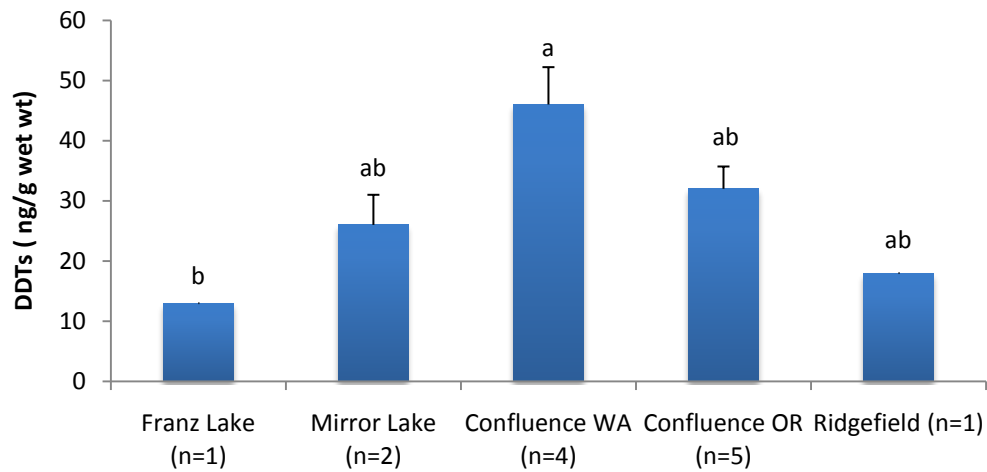
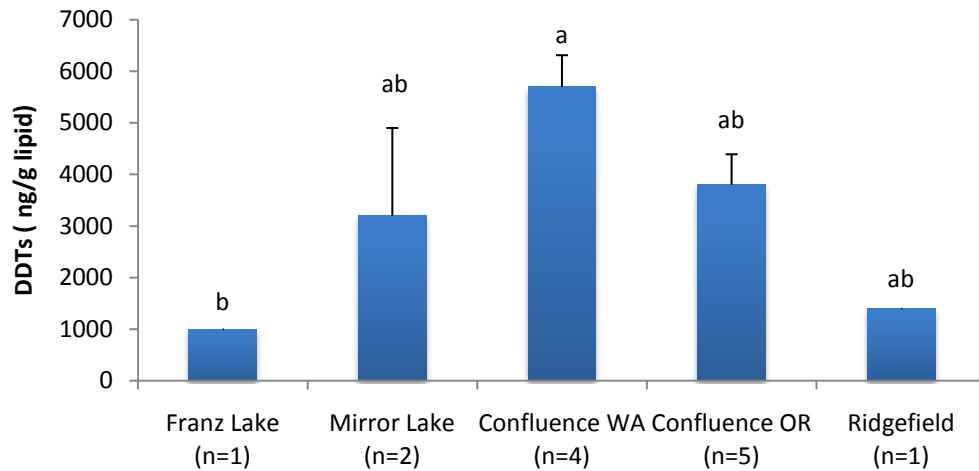


Figure 4. Mean concentrations (\pm SE) of DDTs in ng/g wet wt and ng/g lipid, in whole bodies of juvenile Chinook salmon from Lower Columbia River sites. All fish included in these composites are unmarked, presumably wild fish. Values with different letter superscripts are significantly different ($p < 0.05$, ANOVA and Tukey-Kramer test).

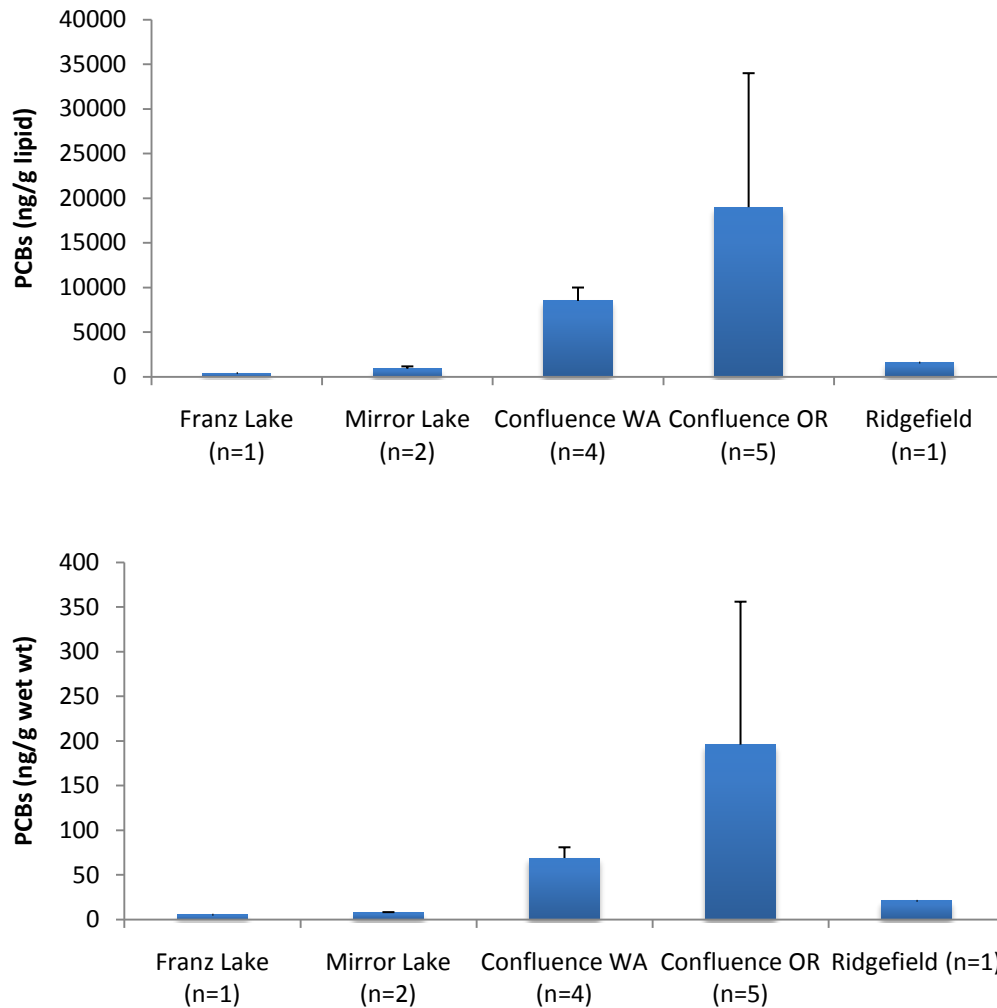


Figure 5. Mean concentrations (\pm SE) of PCBs in ng/g wet wt and ng/g lipid, in composite body samples of subyearling Chinook salmon from Lower Columbia River sites. All fish included in these composites are unmarked, presumably wild fish. Values with different letter superscripts are significantly different ($p < 0.05$, ANOVA and Tukey-Kramer test).

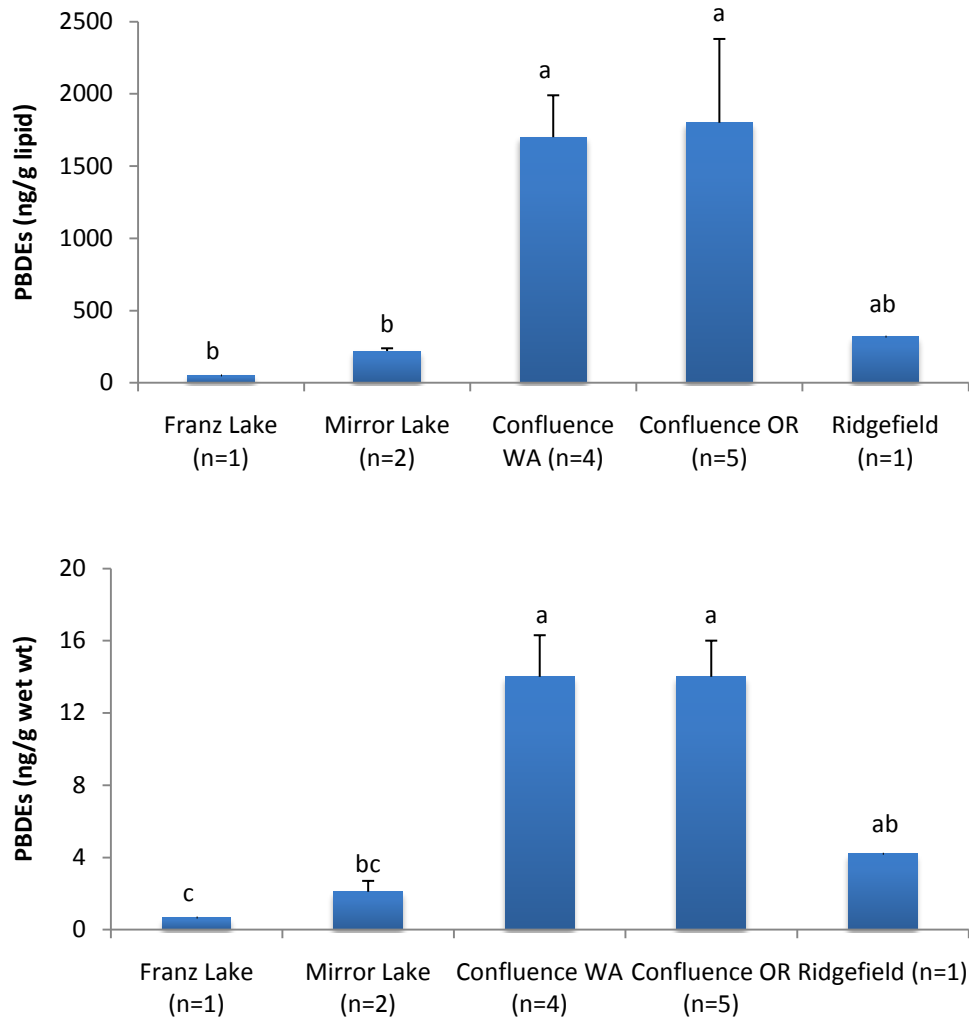


Figure 6. Mean concentrations (\pm SE) of PBDEs in ng/g wet wt and ng/g lipid, in composite body samples of subyearling Chinook salmon from Lower Columbia River sites. All fish included in these composites are unmarked, presumably wild fish. Values with different letter superscripts are significantly different ($p < 0.05$, ANOVA and Tukey-Kramer test).

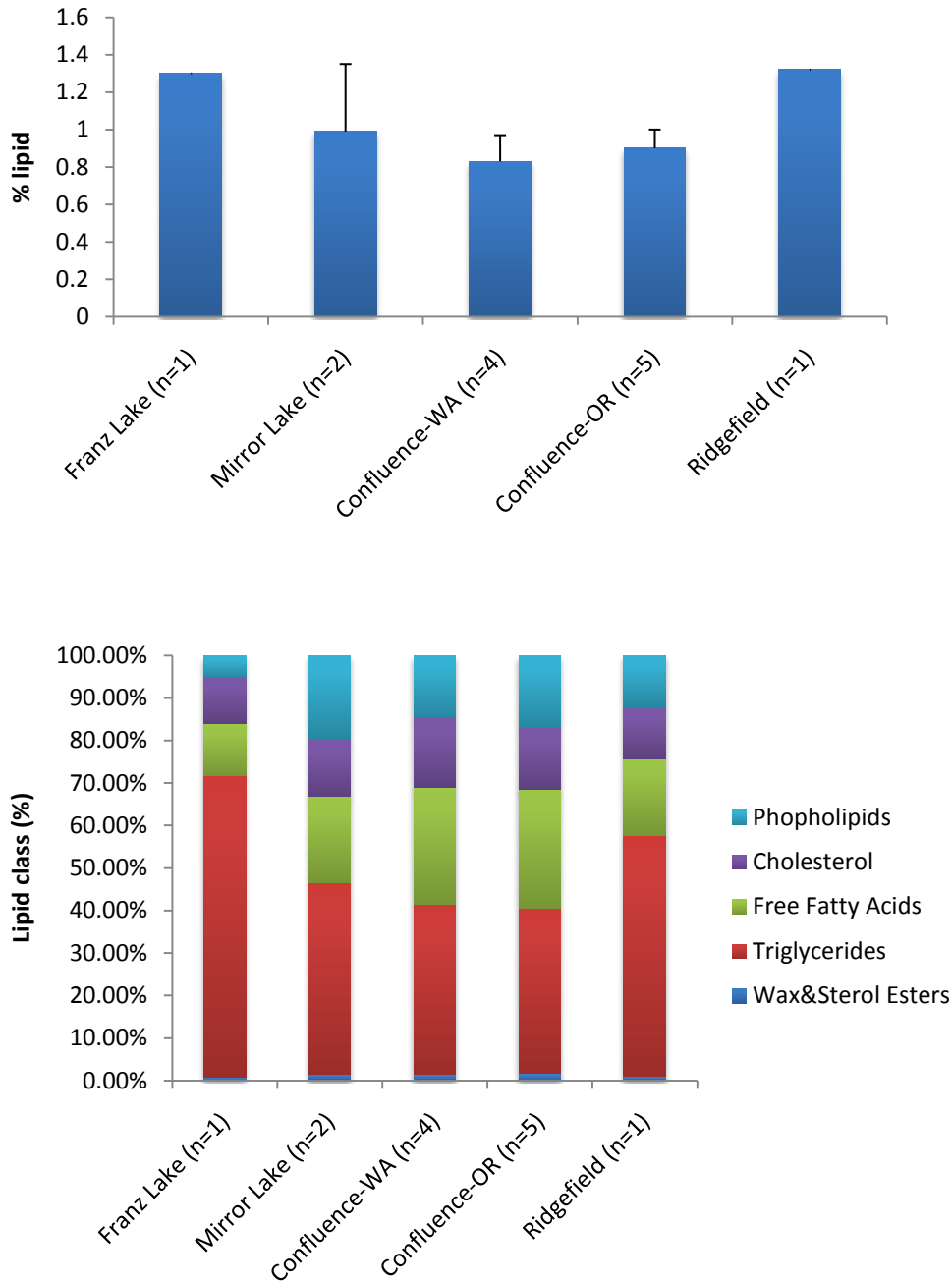


Figure 7. Mean per cent lipid (\pm SE) and percentages of various lipid classes in composite body samples of juvenile Chinook salmon from Lower Columbia River sites. All fish included in these composites are unmarked, presumably wild fish.

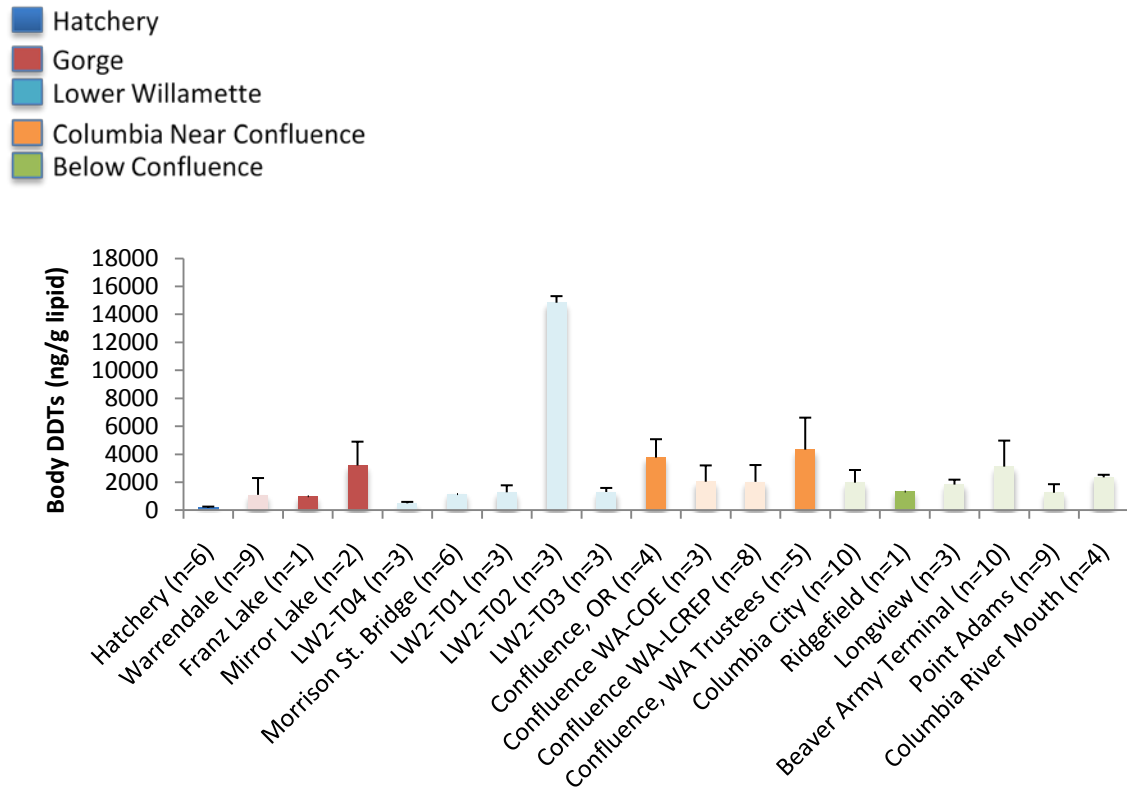


Figure 8. Concentrations of DDTs in salmon from Lower Columbia hatcheries and field sites sampled from 2001-2008. Sites are color-coded by region. Darker coloring indicates that the site was sampled in 2008. All LW-2 sites in the Lower Willamette were sampled by the Lower Willamette Group (LWG 2007). Other sites were sampled in 2005, as part of the LCREP Salmon and Water Quality study (LCREP 2007), or in 2000-2001 as part of a NOAA-Army Corps of Engineers study (Johnson *et al.* 2007).

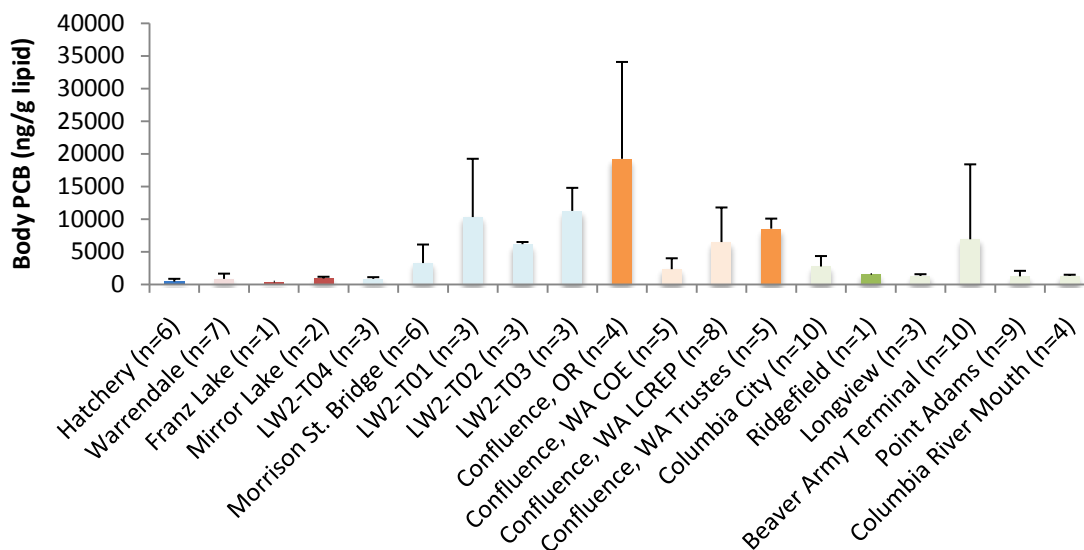
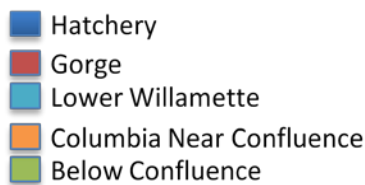


Figure 9. Concentrations of PCBs in salmon from Lower Columbia hatcheries and field sites sampled from 2001-2008. Sites are color-coded by region. All LW-2 sites in the Lower Willamette were sampled by the Lower Willamette Group (LWG 2007). Other sites were sampled in 2005, as part of the LCREP Salmon and Water Quality study (LCREP 2007), or in 2000-2001 as part of a NOAA-Army Corps of Engineers study (Johnson *et al.* 2007).

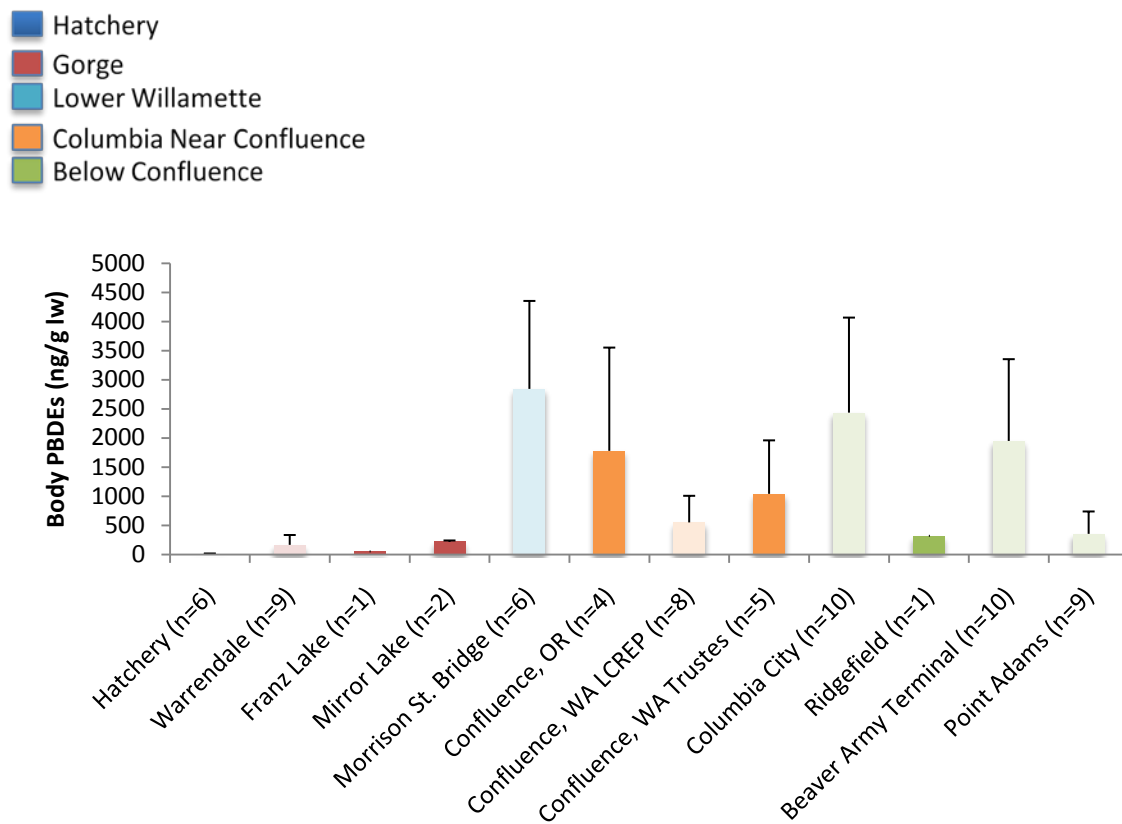


Figure 10. Concentrations of PBDEs in salmon from Lower Columbia hatcheries and field sites sampled from 2005-2008. Sites are color-coded by region. Darker coloring indicates that the site was sampled in 2007-2008; other sites were sampled in 2005, as part of the LCREP Salmon and Water Quality study (LCREP 2007).

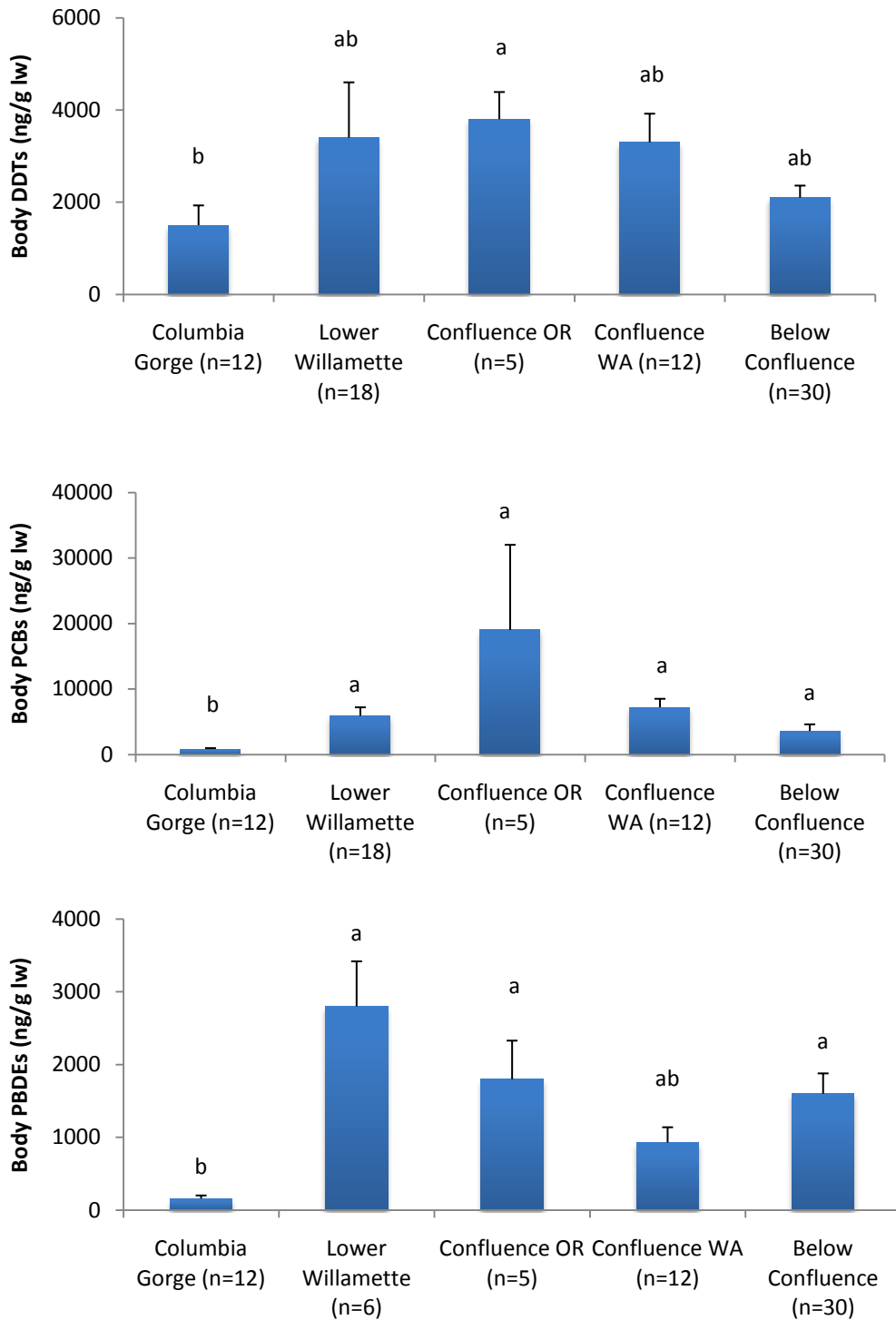


Figure 11. Mean concentrations (\pm SE) of DDTs, PCB, and PBDEs (ng/g lipid) in salmon from Lower Columbia sites by area. Data are from 2008 sampling, as well as Lower Willamette Group sampling (LWG 2007) and LCREP Salmon and Water Quality study (LCREP 2007).

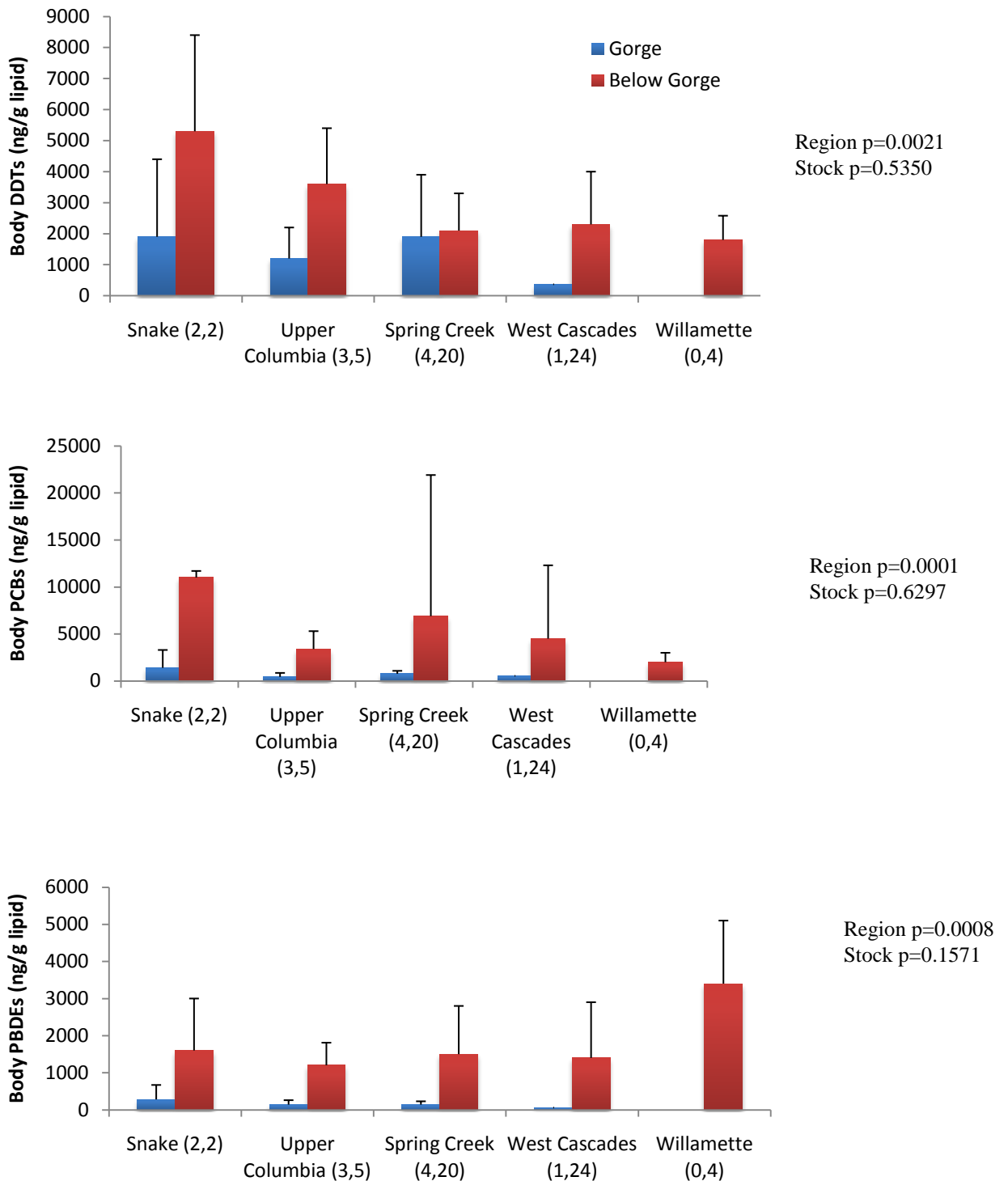


Figure 12. Concentrations of DDTs, PCBs, and PBDEs in salmon from different Columbia River stocks at site within the Columbia Gorge and sites below the Gorge. Samples from 2008 as well as those collected in 2005, as part of the LCREP Salmon and Water Quality study (LCREP 2007) are included in the analysis.

