NOAA Technical Memorandum NMFS-NWFSC-8

Contaminant Exposure and Associated Biological Effects in Juvenile Chinook Salmon *(Oncorhynchus tshawytscha)* from Urban and Nonurban Estuaries of Puget Sound

April 1993

U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Marine Fisheries Service

NOAA Technical Memorandum NMFS

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This document should be cited as follows:

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. Dep. Commer., NOAA Tech. Memo. NMFS-NWFSC-8, 112 p.

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by
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April 1993

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EXECUTIVE SUMMARY

This report presents and interprets the results of chemical, biochemical, and biological studies on juvenile chinook salmon (*Oncorhynchus tshawytscha*) outmigrating from urban and nonurban estuaries of Puget Sound, Washington. These studies were conducted between 1989 and 1991 by the National Marine Fisheries Service (NMFS) of the National Oceanic and Atmospheric Administration (NOAA) with sponsorship from the Environmental Protection Agency, Region 10 and NMFS/NOAA. The objective of these studies was to determine the degree of chemical exposure to juvenile chinook salmon as they migrate through urban-associated compared to nonurban estuaries and to evaluate the effects of chemical contaminant exposure on these animals.

Urban estuaries studied included the Duwamish Waterway entering Elliott Bay near Seattle, Washington, the Puyallup River entering Commencement Bay near Tacoma, Washington, and the Snohomish River entering Port Gardner Bay near Everett, Washington. Sediments in these aquatic urban environments are known to be highly contaminated, although the sediments from the Duwamish Waterway and the Puyallup estuary, particularly in the waterways, are significantly more contaminated than sediments in the Snohomish estuary. Additionally, juvenile chinook salmon from the Nisqually River estuary, a minimally contaminated nonurban estuary, and from the respective hatcheries of each of the waterways and rivers mentioned above were sampled as reference fish.

The chemical indicators of contaminant exposure include levels of hepatic polychlorinated biphenyls (PCBs) and biliary levels of fluorescent aromatic compounds (FACs), which are semiquantitative measures of exposure to aromatic hydrocarbons (AHs). Stomach contents of juvenile salmon were also analyzed for selected AHs and chlorinated hydrocarbons (CHs) to assess the importance of diet as a possible route of uptake of xenobiotics from polluted estuaries. The study also included measurement of early physiological and biochemical (bioindicator) responses to chemical contaminant exposure. These bioindicators have been shown to reflect the degree of exposure to particular contaminants as well as to indicate some of

the biological consequences of chemical exposure. In addition to the chemical indicators, biochemical measures of contaminant-induced responses of the hepatic enzyme system and genotoxic damage were assessed. Measures of the hepatic cytochrome P-450 system included hepatic aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin-O-deethylase (EROD) activities. Hepatic levels of DNA-xenobiotic adducts, detected by ^{32}P-postlabeling, were measured as a bioindicator of genetic damage. Biological parameters that demonstrate the effects of chemical contaminant exposure were also measured. Initially, considerable effort was expended in understanding and enhancing our fish maintenance and husbandry practices. This allowed us to maintain healthy fish in saltwater for periods of months, which was critical for studies on depuration and measurement of biological effects. Biological effects that were monitored included effects on immune function and effects on growth and long-term survival of juvenile salmon. Alterations in immune function have been shown to be a sensitive index on the effects of contaminants. Suppression of immune function can seriously affect the ability of salmon to face the stresses posed in the saltwater environment. Similarly, effects on growth and survival are more classical measurements on the effects of chemical contaminants; negative effects are more easily interpreted as detrimental to the individual and thus eventually to the population.

Significant Findings

- Concentrations of chemical contaminants (AHs and PCBs) in stomach contents of juvenile chinook salmon from the Duwamish Waterway and the Puyallup estuary were significantly higher than those in stomach contents of fish from the Nisqually River estuary or the hatcheries. However, concentration of chemical contaminants in stomach contents of salmon from the Snohomish estuary were not significantly higher than those in stomach contents of fish from the Nisqually estuary or the hatcheries. These results indicate that diet serves as a potential route of uptake of chemical contaminants in juvenile salmon from polluted estuaries.

- Levels of PCBs and FACs in liver and bile, respectively, were consistently higher in salmon from the Duwamish Waterway and the Puyallup estuary than those in fish from the Nisqually estuary or the hatcheries, thus demonstrating exposure of juvenile salmon from polluted urban sites to potentially toxic chemicals. However, levels of PCBs and FACs were not higher in salmon from the Snohomish estuary, another urban-associated estuary, than in fish from the hatcheries. This is consistent with the lower concentration of contaminants in sediments from this estuary.
- Body burden of PCBs were found to persist for at least 3 months after removing fish from a contaminated environment and holding them in the laboratory. This suggested that exposure to some anthropogenic contaminants could potentially continue for at least some time after juvenile salmon migrate to an ocean environment.
- The activity of an enzyme (hepatic AHH), which plays a critical role in activation of several chemical contaminants (including AHs) to toxic intermediates, was increased in juvenile salmon from the Duwamish Waterway and the Puyallup estuary. A consequence of the activation of toxic chemicals to reactive forms is their binding to DNA; this event is believed to be an early step in the process of chemical carcinogenesis and other toxic effects. In the present study, the levels of binding of chemical contaminants to DNA in liver were higher in fish from the Duwamish Waterway and the Puyallup estuary than in fish from the Nisqually estuary and the hatcheries. These results demonstrated that increased exposure to chemical contaminants in outmigrant juvenile salmon during their brief residency in urban estuaries was sufficient to elicit certain biological responses. These responses, commonly referred to as biomarkers, indicate the potential for other serious biological effects to ensue.
- Immune competence of juvenile salmon from the Duwamish Waterway was found to be suppressed when compared to juvenile salmon from the nonurban Nisqually estuary or the hatcheries. Immune competence was assessed by measuring the primary and secondary in vitro B-cell response of splenic and anterior kidney lymphocytes, using the

plaque assay, or by measuring their in vivo primary antibody response to an antigen. An altered ability of these cells to produce antibodies to an antigen could be indicative of an animal's potential for increased susceptibility to infections.

- To causally link the relationship between contaminants and altered immune function, juvenile chinook salmon were injected with an organic extract of a contaminated sediment from the Duwamish Waterway. As in the field-exposed juvenile salmon, suppression of immune function was observed in this laboratory study. These findings suggest that the immunosuppression observed with juvenile salmon from the Duwamish Waterway was most likely due to chemical contaminants and not due to other environmental variables.
- Survival of juvenile chinook salmon from the Duwamish Waterway, the only urban estuary tested, was also significantly lower than that of fish from the Nisqually estuary or from the hatcheries, when held in the laboratory for up to 80 days. Similarly, growth, determined by measuring changes in length and weight of individually tagged juvenile salmon over an 80-day period, was lower in juveniles from the urban estuary than in juveniles from its respective hatchery.

Overall, these results demonstrate that increased chemical contaminant exposure in juvenile chinook salmon during their brief residency in urban estuaries of Puget Sound, Washington, was sufficient to elicit reponses at the chemical, biochemical, and biological level. Measurements of this type provide evidence of linkage between complex mixtures of chemical contaminants in the environment and effects on health and survival of fish. This information will be critical in increasing our capabilities for assessing the full spectrum of effects on salmon resulting from exposure to the myriad anthropogenic chemicals that can be present in the near coastal environment. The detection of chronic effects requires sensitive and reliable tools that cover a broad range of important biological functions and that are both cost-effective in their application and amenable for use in studies as described here. The availability of such tools

would allow generation of sufficient data to identify and statistically quantify potential risk factors in the etiology of effects observed in fish from contaminated coastal environments.

CONTENTS

PREFACE

Several U.S. West Coast populations of Pacific salmon are dwindling and, in some cases, declining so seriously that they have been listed under the Endangered Species Act. A number of factors, such as dramatic habitat loss and overfishing, are believed to be the major contributors to the problem. However, other environmental factors may also play a role. One of these is the chemical contamination of urban estuaries through which some juveniles pass on their migration to sea. To address this pollution factor, scientists within the Environmental Conservation (EC) Division of the Northwest Fisheries Science Center initiated a multidisciplinary project to assess the levels of contaminant exposure and signs of adverse biological effects in migrating juvenile chinook salmon *(Oncorhynchus tshawytscha)* from polluted urban estuaries of Puget Sound, Washington.

Scientists in the EC Division scientists previously documented that certain bottom-dwelling fish species, such as English sole *(Pleuronectes vetulus)*, feeding from and living on contaminant-laden sediments in polluted areas of Puget Sound bioaccumulate or biotransform toxic contaminants and show a number of adverse biological effects (e.g., reproductive impairment and a variety of liver lesions, including neoplasms). Moreover, healthy English sole exposed in the laboratory to toxic chemicals extracted from urban sediments develop, over a period of 18 months, many of the same lesions found in the livers of fish sampled from polluted areas. These field and laboratory studies have helped establish cause-and-effect relationships between chemical contaminant exposure and certain adverse biological effects in benthic marine fish.

Because juvenile salmon generally reside in urban estuaries for a brief time (several weeks to months), they seemed at little risk compared to chronically exposed resident species. Furthermore, being primarily water-column inhabitants, it did not seem likely that salmon would be as directly or as significantly exposed to contaminant-laden bottom sediments as benthic fish.

Over the past few years, however, a deeper understanding has been gained of the subtle biochemical and biological responses occurring in marine organisms exposed to contaminants. These early responses (bioindicators) are believed to serve as harbingers of later, more serious effects. Such understanding has led EC Division scientists to develop more sophisticated analytical, biochemical, and immunological techniques that should lead to a better assessment of whether the brief residency of juvenile salmon in polluted estuaries results in long-term consequences for their health and survival.

Funding for this study was jointly provided by the Environmental Protection Agency (EPA) and the National Oceanic and Atmospheric Administration (NOAA) in 1989 and 1990. NOAA carried on the the study reported herein in 1991.

INTRODUCTION

Estuaries serve as important habitats for salmon during the juvenile stage of their life cycle (Healy 1982). Outmigrating juvenile salmon use estuaries as an area of refuge from predators, as an environment with a rich food supply to support rapid growth, and as a transition area to adjust to a marine habitat (Dorcey et al. 1978, Simenstad et al. 1982). However, estuaries located near urban centers also serve as depositories for both point and nonpoint sources of chemical contaminants from municipal and industrial activities. The chemicals from these sources are known to accumulate in the bottom sediments (Dexter et al. 1985). Several estuaries within Puget Sound, Washington, are located near urban centers and are used by migrating salmonids as areas of residence. These include the Duwamish Waterway entering Elliott Bay in Seattle, the Puyallup River entering Commencement Bay in Tacoma, and the Snohomish River entering Port Gardner Bay in Everett (Fig. 1). During their outmigration period, juvenile salmon undergo numerous physiological adaptations and adjustments. Thus, additional stresses, such as exposure to toxic chemicals while in the estuarine environment, could prove harmful to these juvenile salmon.

Of the five species of Pacific salmon, chinook salmon (*Oncorhynchus tshawytscha*) are most dependent upon estuaries during the early stages of their life cycle. Smolting chinook salmon reside in estuaries for a longer period than other species of salmonids (Thom 1987, Healy 1982). The residence time of juvenile chinook salmon in estuaries is variable and depends upon many factors, such as their age when they enter an estuary. Juvenile chinook have been reported to reside in some estuaries for up to 6 months (Reimers 1973, Levy and Northcote 1982, Simenstad et al. 1982). One to two months, however, appears to be an average duration of residence of juvenile chinook salmon in Puget Sound estuaries (Simenstad et al. 1982).

The presence of chemically contaminated sediments in the Duwamish Waterway coupled with the extended residence time of juvenile chinook salmon in estuaries prompted a study by McCain et al. (1990) to examine the degree to which juvenile fall chinook salmon are exposed to toxic chemicals such as aromatic hydrocarbons (AHs) and chlorinated hydrocarbons (CHs). Stomach contents and livers of juvenile salmon captured from the Duwamish Waterway were

analyzed for levels of polychlorinated biphenyls (PCBs), and stomach contents and bile were analyzed for parent AHs and polar metabolites of AHs, respectively. These findings were compared to concentrations of chemicals in chinook salmon from the Nisqually estuary, a nonurban area known to be minimally contaminated, and the Kalama Creek Hatchery, which releases salmon smolts into the Nisqually River (Fig. 1). The findings showed that juvenile chinook salmon from the Duwamish Waterway were exposed to substantially higher concentrations of contaminants than juvenile chinook from the Nisqually River system. However, juvenile salmon specifically from the Green River Hatchery (the Green River is the upper reach of the Duwamish Waterway) were not examined in this study, thus making a direct comparison of increased contaminant levels in fish from the Duwamish somewhat difficult. Additionally, it was not clear if these findings could be extended to salmon outmigrating through other urban estuaries in Puget Sound.

In 1989, a cooperative study initiated by the National Marine Fisheries Service (NMFS) and the Environmental Protection Agency (EPA) was designed to confirm the findings by McCain et al. (1990) and to determine if juvenile chinook salmon, migrating through estuaries located near other Puget Sound urban centers, including the Puyallup River estuary and the Snohomish River estuary, were similarly exposed to chemical contaminants. Juvenile chinook salmon from the Nisqually River estuary and the hatcheries for each of the sampled rivers were considered reference fish. The study was also expanded to include measurement of early biochemical responses to contaminant exposure and measurement of biological effects that represent significant physiological processes. These biochemical responses, known as bioindicators, have been shown to reflect the degree of exposure of particular contaminants by marine organisms in a polluted environment as well as to indicate some of the early biological consequences of chemical exposure. The indicators of contaminant exposure (Stein et al. 1992) include levels of hepatic PCBs and biliary levels of fluorescent aromatic compounds (FACs), a semiquantitative measure of exposure to AHs. Biochemical responses to chemical exposure include hepatic cytochrome P-450 activities, measured by changes in aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin-O-deethylase (EROD) activities; and hepatic DNA-xenobiotic adducts, detected by ^{32}P-postlabeling. Aryl hydrocarbon hydroxylase and EROD activities represent inducible enzymes whose activities

increase rapidly after exposure to AHs, whereas adduct levels represent the long-term cumulative effects of exposure to AHs and damage to the DNA. Biological parameters that were measured included effects on immune function and effects on growth and long-term survival of juvenile salmon. An effective immune system is a critical physiological process in fish that has been shown to become impaired due to contaminant exposure (McLeay and Gordon 1977, Arkoosh and Kaattari 1987, Bengtsson et al. 1988). Similarly, exposure to contaminants has been shown to significantly reduce survival and growth in a number of marine species (Swartz et al. 1985, Barron and Adelman 1984, Casillas et al. 1992).

The results to date show that outmigrating juvenile chinook salmon from both the Duwamish Waterway and from the Puyallup estuary in Commencement Bay exhibited consistent evidence of exposure to contaminants. Juvenile chinook salmon from the Snohomish estuary, another urban estuary, also appeared to be exposed to contaminants, but to a much lesser degree than salmon from the Duwamish Waterway or Puyallup estuary. In addition, when held in tanks with flow-through seawater for a period of several months, juvenile salmon from the Duwamish Waterway suffered significantly more mortalities, exhibited reduced growth, and showed evidence of impaired immune function when compared to salmon from either the Green River Hatchery (the primary source of salmon for the Duwamish Waterway) or to salmon from the nonurban Nisqually system. The biological consequences of chemical exposure to juvenile chinook salmon in the estuary may place additional stresses on these fish that may affect their long-term health and survival as they enter the marine environment.

METHODS

Field Sampling

In 1989, juvenile chinook salmon *(Oncorhynchus tshawytscha)* were sampled from hatcheries and the respective estuaries of four river systems in Puget Sound. These included the Green-Duwamish, Puyallup, Nisqually, and Snohomish River systems (Fig. 1). In 1990, the hatcheries and estuaries of the Green-Duwamish River, Puyallup River, and the Nisqually River were sampled for juvenile chinook salmon; fish from the Snohomish system were not sampled. In

1991, fish were sampled only from the hatchery and estuary of the Green-Duwamish River system.

Fish Collections

Juvenile chinook salmon were collected from the hatcheries just prior to their release into the river and collected from their respective estuaries a minimum of 2 weeks after the initial hatchery release. This schedule allowed time for transit of juveniles to the estuaries. Weitkamp and Campbell (1980) estimated that the transit time from hatchery to estuary for chinook salmon was 15-19 days in the Green River-Duwamish system. Sampling dates at the hatcheries and at all estuarine sites and the number of fish collected during each sampling period are shown in Table 1. Juvenile chinook salmon were collected at the hatcheries from late May to early June and at the estuaries from June through early July of each year. Fish at the estuarine sites were captured using a 30-m beach seine. It was necessary to find sites where the beach seine could be successfully deployed. Typically, these sites were areas with little debris and with moderate currents. Further, if sites were not productive, alternative sites were sampled. In the various estuaries, the number of juvenile salmon captured ranged from 4 to approximately 550 fish per sampling day.

Juvenile chinook salmon collected at the hatcheries and in the estuaries were placed in 94-L ice chests filled with fresh or salt water, respectively. Water was continuously aerated using air stones and small battery-powered portable aerators. Salmon were transported to the NMFS's Montlake facility in Seattle for immediate tissue and fluid sampling, as described below, or to the NMFS's Mukilteo Marine Laboratory, a salt water facility. Mortalities of salmon during transportion were minimal. Fish held at our Mukilteo Marine Laboratory were used for depuration, survival, growth, or immune function studies as described below.

Tissue Sampling

Tissue and fluids of juvenile chinook salmon were sampled for chemical and biochemical analyses after each collection period. Five to ten fish were randomly selected and immediately stored at -20°C for whole body analysis of CH content. Because of the small size of fish, individual samples of bile and liver were pooled as a single composite (usually 60 fish per

composite) in order to obtain sufficient quantities of tissues and fluids for biochemical and chemical analyses. A minimum of two composite tissue samples per hatchery and three composite tissue samples per estuary were obtained, whenever possible. Fish were randomly selected, individually euthanized by a quick blow to the head, measured for length, weighed, and examined for adipose fin clip (these were fish containing a coded wire nose tag) before the liver and gall bladder were removed. Approximately one-third of the liver from each fish was excised and placed in a chilled tared glass vial. The remaining two-thirds of the liver sample was placed in a second tared glass vial and stored on ice. The vials containing liver composite samples were weighed to determine net weight of tissue The liver composite was then frozen in liquid nitrogen and stored at -80°C until analyzed, as described below. Blood samples were taken from selected fish as described later. Bile was collected by excising the gall bladder and emptying the contents into a 4-mL vial containing a glass limited-volume insert and stored at -20°C until analyzed. Stomach contents were removed from 10 randomly selected juveniles from each composite and frozen for chemical analysis. A portion of the stomach contents were examined with a dissecting microscope for taxonomic compositon and relative volume of each taxonomic group.

Laboratory Holding

Juvenile salmon collected at the hatcheries for the depuration, immune function, growth and survival studies were acclimated to full-strength seawater at the Mukilteo Marine Laboratory over a 5-day period. Juvenile salmon were placed in 750-L circular fiberglass tanks, initially containing seawater adjusted to 5 parts per thousand (ppt), and held for 24 hours. Salinity was adjusted daily in increasing 5 ppt increments until the fifth day, when juveniles were adjusted to full-strength salt water (28-30 ppt). Flow was maintained at 12 L/minute. Juvenile chinook salmon captured in the estuaries were held initially in 15 ppt seawater the first day. Salinity was adjusted daily in increasing 5 ppt increments until the third day, when juveniles were adjusted to full strength seawater (28-30 ppt). Salmon were fed daily at 3% of their body weight with Oregon Moist Pellet (OMP; Moore-Clark, La Conner, WA) for a 10-day period and then fed a prophylactic diet for control of disease at 3% of their body weight with OMP supplemented with oxytetracycline

(4 g $100g^{-1}$ of diet) for another 10-day period. This prophylactic feeding regime was repeated every 20 days.

Chemical Analyses of Tissues and Stomach Contents

Analysis for organic chemicals in livers, whole bodies (minus gastrointestinal tract), and stomach contents were done according to procedures described by MacLeod et al. (1985) and Krahn et al. (1988). The AHs determined by gas chromotography with mass spectrometric detection (GC/MS) are listed in Table 2. The CHs (Table 2) were analyzed using gas chromotography (GC) with electron capture detection (ECD) and GC/MS for chemical confirmation on selected samples. The steps of the chemical analysis are briefly described below.

Tissue samples were extracted according to the procedures of MacLeod et al. (1985). Briefly, 3 g of tissue or stomach contents were added to a centrifuge tube containing sodium sulfate, methylene chloride, and the surrogate standards for analyzing AHs and CHs. The mixture was then macerated with a Tekmar® Tissumizer and the resulting extract filtered through a column of silica and alumina. The organic extract was then concentrated to 1 mL.

Cleanup of the concentrated 1-mL organic extracts was done using a high-performance liquid chromatograph (HPLC). Samples were injected on a Spectra-Physics Model 8800 HPLC equipped with two preparatory size Phenomenex (containing Phenogel,100-Å size-exclusion packing) columns in series and the analytes monitored by ultraviolet (UV) detection. The mobile phase consisted of helium-gassed, methylene chloride solvent, run at a flow rate of 7 mL/minute for 20 minute at ambient temperature. The fraction containing the AHs and CHs were collected according to Krahn et. al. (1988). The methylene chloride fraction volume, reduced by evaporation to about 0.1 mL, was then exchanged into hexane. Standards were then added before analysis by GC.

The organic solvent extracts of stomach contents were analyzed for AHs by GC/MS according to MacLeod et al. (1985). The organic extracts of stomach contents and liver were analyzed for CHs (PCBs and pesticides) by capillary column GC with electron capture detection

(ECD). Representative samples were also analyzed by GC/MS to confirm the identifications of the CHs.

The concentrations of AHs in stomach contents are reported as sums of low molecular weight AHs (LAHs, 2-3 rings) and high molecular weight AHs (HAHs, > 3 rings) and their alkylated counterparts (Table 2). The concentrations of PCBs in stomach contents and liver are reported as the sum of PCB homolog classes, from trichlorobiphenyls to decachlorobiphenyls (Table 2). Concentrations of other CHs in stomach contents are also reported as the sum of selected pesticides. Concentrations of individual AHs, classes of PCBs, and other CHs determined in stomach contents are reported in the Appendix. The quality assurance procedures using method blanks and percent recoveries of surrogate standards of a National Institute of Science and Technology (NIST) control material are also reported in the Appendix.

Analysis of liver tissue for butyltins was conducted according to the method described by Krone et al. (1989 a,b). Briefly, the organotin chlorides are extracted from liver tissue (by homogenization with a Tissumizer) using methylene chloride with 0.1% tropolone as a complexing agent. The extracted organotins are converted to their n-hexyl derivatives through the Grignard reaction (Morrison and Boyd 1973) and then cleaned up by two chromatographic steps: 1) eluting the sample extracts through a glass column containing 4.5 g each of alumina and silica, with 20 mL pentane, and 2) loading the eluate onto an amino Sep-Pak® and eluting with 3 mL of pentane. The pentane eluate is then concentrated to 1 mL and transferred to a GC vial for GC/MS analysis. Quality assurance procedures using blanks and recovery of tripropyltin showed no detection of butyltins in blanks, and percent recoveries (98 ± 19) for tripropyltin were above acceptable levels.

Biochemical Analyses of Fish Liver and Bile

Bile was analyzed by the HPLC/UV method of Krahn et al. (1986) to estimate the exposure of juvenile salmon to fluorescent aromatic compounds (FACs) such as AHs with 2-5 benzenoid rings. Bile is injected directly into a HPLC equipped with a reverse-phase analytical column. The polar analytes (primarily metabolites of AHs) in bile were separated using a water/methanol gradient (100% water containing 5 µL acetic acid/L, to 100% methanol) and monitored by two

fluorescence detectors in series. The excitation/emission wavelengths of one detector was set to 290/335 nm (where metabolites of naphthalene (NPH) fluoresce) and the other set to 380/430 nm (where the metabolites of benzo[a]pyrene (BaP), pyrene and fluoranthene fluoresce). The levels of biliary FACs are reported as equivalents of known concentrations of BaP or NPH standards on the basis of biliary protein, because recent sudies (Collier and Varanasi 1991) have shown that such a normalization can, to a large extent, account for changes in the levels of FACs due to differences in the feeding status of some fish. The concentrations of biliary protein were measured by the method of Lowry *et al.* (1951) using bovine serum albumin as the standard.

Quality assurance of BaP and NPH calibration standards run at the start of the analysis set had a relative standard deviation of 3.4% in 1989 and 2.2% in 1990. The number of bile composites for each sampling year was small enough that all samples were analyzed in a single set. A "bile pool" reference material was analyzed with each set in 1989 and 1990. Concentrations of FACs in the bile pool reference material during these analyses were within 10% of the mean concentration results of the previously determined FAC concentrations in the bile pool reference material. Analysis of blanks and replicate analyses showed that quality assurance tests were passed.

Hepatic microsomes for AHH and EROD activity were prepared by a slight modification of the procedure of Collier et al. (1986). Composite liver samples were homogenized with a Potter-Elvehjem homogenizer, using 4 mL of 0.25 M sucrose per gram wet weight. The homogenate was then centrifuged at 10,000 g for 20 minutes, and the resulting supernatant centrifuged at 100,000 g for 60 minutes. The resulting supernatant was discarded and the surface of the microsomal pellet rinsed with 1 mL of 0.25 M sucrose, and the pellet resuspended, using a Dounce homogenizer, in 1 mL of 0.25 M sucrose in 20% glycerol per gram of original liver weight. Protein was measured by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

Aryl hydrocarbon hydroxylase activity was assayed by a method described by Collier et al. (1986). The reaction mixture consisted of the following: 450 µL 0.05 M Tris buffer, pH 7.5, 5 mM $MgCl_2$, 1 mM NADPH, and 37.5 µL microsomal suspension (0.2-0.8 mg protein). The

mixture was incubated at 25°C for 15 minutes, and the reaction started by adding 40 nmole ^{14}C benzo[a]pyrene, 4.6 μ Ci/mole, in 12.5 μL acetone. Assays were run at 25°C for 15 minutes, and the reactions stopped by the addition of 1.0 mL of 0.15 M KOH in 85:15 volume:volume (v:v) DMSO:H_2O. The reaction mixture was extracted with 15 mL hexane in triplicate to remove the unreacted substrate. A 150-μL aliquot of the DMSO: aqueous phase was then neutralized by adding 0.5 mL of 0.1 N HCL. After the addition of 5 mL liquid scintillation cocktail, the ^{14}C associated with the reaction products was determined by liquid scintillation spectroscopy. Quality assurance procedures included duplicate zero-time and boiled enzyme blanks for each set of assays. Each sample was run in duplicate and those samples showing more than 10% difference between duplicates were repeated.

Hepatic EROD activity was measured according to the method of Prough et al. (1978). Briefly, the assay (2 mL total volume) contained 0.1 M Tris buffer, pH 8.0; 0.5 μM ethoxyresorufin; 60 μM NADPH, and 0.1 to 0.5 mg microsomal protein. The reaction was initiated by the addition of the NADPH, and the formation of resorufin was continuously monitored by fluorescence at 530 nm (excitation) and 585 nm (emission) for 1 to 3 minutes. A blank (minus NADPH) was run with each set of samples.

Hepatic DNA for DNA-xenobiotic adduct determination was isolated by a chloroform/phenol extraction procedure of Gupta (1984), which included treatment with Proteinase K and both RNase A and T1 and precipitation of DNA with ethanol. The levels of hydrophobic xenobiotic-DNA adducts were determined using ^{32}P-postlabeling assay after adduct enrichment using the n-butanol procedure as described in Varanasi et al. (1989a). Briefly, 10 μg of DNA, determined spectrophotometrically (1 absorbance unit = 20 μg DNA/μL), was hydrolyzed to 3'-mononucleotides using micrococcal endonuclease and spleen phosphodiesterase (Gupta and Randerath 1988). The adducts were extracted with n-butanol and labeled in the 5' position by T4 polynucleotide kinase-catalyzed transfer of ^{32}phosphate from ^{32}P-labelled adenosine triphophate (ATP) (5 to 6 x 10^3 Ci/mmol). The remaining normal nucleotides and adducts were separated by anion-exchange thin-layer chromotography (TLC) on polyethyleneimine (PEI) cellulose sheets, and the ^{32}P-labeled adducts were detected by screen-enhanced autoradiography. Areas of the

TLCs corresponding to DNA-xenobiotic adducts were excised and levels of ^{32}P measured by liquid scintillation spectroscopy. Salmon sperm DNA from Sigma Chemical Co. (St. Louis, MO) was carried through the ^{32}P-postlabeling assay and used to correct for background. Adduct levels are reported as nanomoles of adducts per mole of DNA bases. Total DNA bases in a sample were determined according to Gupta et al. (1982). In 1990, the n-butanol enhancement procedure was replaced by the nuclease P1 enhancement method according to Gupta and Randerath (1988). Studies have shown that the n-butanol and nuclease T1 methods give comparable results for several carcinogenic aromatic hydrocarbons (Gupta and Early 1988).

Immunological methods

Antigen Preparation

The T-independent antigen, trinitrophenylated lipopolysaccharide (TNP-LPS), was prepared as described by Jacobs and Morrison (1975). Briefly, *Escherichia coli* lipopolysaccharide (LPS), serotype 0111.B4 (Difco, Detroit, MI) was dissolved in 0.28 M cacodylate buffer and adjusted to a pH of 11.5. Picrylsulfonic acid (Sigma Chemical Co., St. Louis, MO) was added dropwise to a test tube containing the LPS solution. Upon coupling, the TNP-LPS solution was exhaustively dialyzed against 0.077 M phosphate buffered saline (PBS), pH 7.4, with a final dialysis against the media (Rosewell Park Memorial Institute (RPMI)-1640; Gibco, Grand Island, NY). The solution was then pasteurized for 30 minutes at 70°C and stored at 4°C in a sterile stoppered serum bottle.

The T-dependent antigen, TNP-keyhole limpet hemocyanin (TNP-KLH), was prepared as described by Rittenberg and Amkraut (1966). Briefly, KLH (Sigma Chemical Co., St. Louis, MO) was added to 0.28 M cacodylate buffer and mixed for 1 hour at room temperature. This mixture was added to a foil-wrapped tube and picrylsulfonic acid was added. The mixture was then dialyzed against PBS and a final change against RPMI-1640. The conjugate was filtered, sterilized, and stored in a sterile stoppered serum bottle. The conjugation ratio of TNP to KLH was determined to be 16 mmoles of TNP to 100 g KLH.

Trinitrophenylated-bovine serum albumin (TNP-BSA), used in the enzyme-linked immunosorbent assay (ELISA) for the quantification of anti-TNP antibodies, was prepared according to the method described by Garvey et al. (1977). Trinitrophenylation was achieved by first mixing picrylsulfonic acid in borate acid. The picrylsulfonic acid solution was added dropwise into a BSA solution under constant mixing. The solution was dialyzed extensively against 0.17 M borate buffered saline. At the end of dialysis, the mixture was sterilized by filtration and stored in a sterile stoppered serum bottle at 4°C.

Measurement of Total Immunoglobulin

Total plasma immunoglobulin concentrations were determined for juvenile chinook salmon collected during May to July, 1989. Blood samples from juvenile salmon from the Green River Hatchery, Kalama Creek Hatchery, Puyallup Hatchery, Skykomish Hatchery, Duwamish Waterway, Nisqually estuary, Puyallup estuary and Snohomish estuary were sampled at the Montlake facility. Upon arrival of the fish, blood samples were immediately collected from the caudal blood vessels after removal of the caudal peduncle (Hesser 1960). Blood samples were collected in heparinized micro-hematocrit tubes (Van Waters and Rogers, Seattle, WA) and one end of the tube was plugged in seal-ease (Cray Adams, Parsippany, NM). The samples were then spun down in a hematocrit centrifuge (International Equipment Company, Needham Heights, MA.) for 5 minutes. The hematocrit tubes were broken above the cell pellet, the plasma was removed and stored in microfuges tube at -20°C.

An ELISA was used to determine the relative concentration of plasma immunoglobulin as modified from Kaattari and Yui (1987). Briefly, wells of a 96-well ELISA plate (Costar, Cambridge, MA) were coated overnight with a monoclonal antibody against rainbow trout *(Oncorhynchus mykiss)* immunoglobulin. This monoclonal antibody (1-14) is known to cross react with salmonid antibodies (Kaattari and Yui 1987). The monoclonal antibodies were MAPS (monoclonal antibody purification system) purified (Bio-Rad, Richmond, CA) and the protein content of the solution was determined by the Lowry method (Lowry et al. 1951). The coating agent was removed and 50 µL of various dilution of the standard rainbow trout sera and the chinook plasma samples were added to the wells. The standard sera allowed for normalization of

the data from day to day and was also used to assign immunoglobulin units per microliter of plasma for each sample tested. Also for quality assurance, another source of pooled rainbow trout sera was added to the wells. Although this method does not allow for an absolute quantification of total plasma immunoglobulin concentration, it does allow for relative quantification of immunoglobulin concentration of unknown samples. The plasma and sera were incubated and washed from the wells, and 100 µL of strepavidin-horseradish peroxidase was added for 30 minutes at room temperature. Substrate was then added as described by Arkoosh et al. (1991). A 10-minute kinetic-based ELISA was measured with a Titertek Multiskan® spectrophotometer (Flow Laboratories, McLean, VA). Results were reported as immunoglobulin units per µL of plasma.

Primary in vivo Anti-TNP Response

The primary in vivo anti-TNP response of juvenile chinook salmon was determined for fish collected from the Green-Duwamish and the Nisqually systems during the spring of 1990. Both estuary and hatchery chinook salmon were allowed to acclimate at the Mukilteo Marine Laboratory for a minimum 2-week period prior to injection with TNP-KLH as described in Arkoosh et al. 1991. To minimize stress during sampling and injecting, which can result in immunosuppression (Maule et al. 1989), salmon were anesthetized by adding 20 mg/L of tricaine methanesulphonate (MS-222; Sigma Chemical Co, St. Louis, MO) directly to an aerated tank with the seawater flow turned off. After the salmon were quiescent, they were injected intraperitoneally with either 100 µg of TNP-KLH (16 mmoles TNP/100 g of KLH) emulsified in Freund's complete adjuvant (FCA; Difco Laboratories, Detroit, MI) or with PBS emulsified in FCA. After the injection was completed, the seawater flow was reinstated, allowing the salmon to recover from the anesthetic.

Plasma samples were collected from 10 primed and 10 unprimed fish from the Green-Duwamish system at 2, 4, 6, 8, and 10 weeks post-injection for the detection of specific antibodies to TNP. Plasma samples were collected from primed and unprimed fish from the Nisqually system at 4 and 9 weeks post-injection for the detection of specific antibodies to TNP. Four weeks had been previously determined to be the peak of the in vivo anti-TNP response in juvenile chinook salmon (M. Arkoosh, NMFS. Pers. observ., June 1990). Plasma samples from five

salmon were taken from the hatcheries and estuaries of each system before injection of the antigen to determine the presence of naturally occurring anti-TNP antibodies. These samples represent time zero.

An ELISA was used to determine anti-TNP activity per microliter in the chinook's plasma. This method was modified from Arkoosh and Kaattari (1990) and described in Arkoosh et al. 1991. Results were reported as units of anti-TNP activity per microliter of plasma. If the ELISA did not detect any anti-TNP antibody in the plasma sample, we assigned these samples one half the detection limit of this method for statistical analyses, which was 72 units of anti-TNP activity per microliter of plasma.

In Vitro Immune Response: Field-Exposed Salmon

The primary and secondary in vitro anti-TNP response of juvenile chinook salmon was determined for fish collected from the Green-Duwamish and the Nisqually systems during the spring of 1990. Both estuary and hatchery chinook salmon were injected intraperitoneally with 100 μg of TNP-KLH at least 2 weeks after they were brought to the Mukilteo Marine Laboratory as previously described. Both primed and unprimed fish were netted from their tanks after immobilization with MS-222 as described above at 9 weeks post-primary injection for the Nisqually River system and at 12 weeks post-primary injection for the Green River-Duwamish system. Ten salmon from each treatment were sampled.

After the salmon were euthanized with MS-222 (100mg/L), the tail was severed at the caudal peduncle and the fish were transported on ice from the Mukilteo Marine Laboratory to the Montlake facility within 2 hours. Standard leukocyte culturing and plaque assay techniques as described by Kaattari et al. (1986) and Arkoosh et al. (1991) were used. Briefly, sterile single-cell suspensions were made of the spleen and anterior kidney in tissue culture media (TCM; Kaattari et al. 1986). Leukocytes from the anterior kidney and spleen single-cell suspensions were enumerated by a hemocytometer and cell viability was determined by trypan blue exclusion. Cells were adjusted to a concentration of $2x10^7$ cells per milliliter in TCM. Fifty microliters of this single-cell suspension was added to each well of a 96-well flat-bottom plate (Corning, Cambridge, MA) in triplicate with 50.0 μL of the appropriate dilution of TNP-KLH, TNP-LPS, or TCM.

Leukocytes from the anterior kidney were exposed to various concentrations of the T-independent antigen, TNP-LPS (0.04, 0.4, 4.0 µg/mL), and the T-dependent antigen, TNP-KLH (0.4, 4.0, 40.0 µg/mL). Leukocytes from the spleen were exposed in vitro only to TNP-LPS (0.04, 0.4 µg/mL) and not to TNP-KLH due to limited cell numbers. These concentrations of this batch of TNP-LPS provided for optimal in vitro responses (data not shown). The cells were incubated at 17°C in a CO_2 (10%) incubator (Forma Scientific, Marietta, OH). The cultures were fed on alternate days with 0.01 mL of nutritional supplement (Kaattari et al. 1986).

After 7 days of culture, the Cunningham modification of the Jerne plaque-forming cell assay (Cunningham and Szenberg 1968) was performed to determine the number of specific B cells which produce antibody to trinitrophenol (Kaattari et al. 1986). The cells were harvested on day 7 of culture, which was determined to be the peak day of their plaque-forming cell (PFC) response (data not shown). The plates were centrifuged at 1400 X g with plate carriers at 17°C in a Beckman TJ-6 centrifuge for 10 minutes. The spent media were removed from the wells and 50 µL of fresh TCM, 10 mL TNP-sheep red blood cells (Rittenberg and Pratt 1969), and 10 µL of chinook serum (complement source) at the appropriate dilution were added to the wells. These components were gently mixed together and added to a Cunningham slide chamber. The slides were incubated for 1.5-3 hours at 17°C and the number of anti-TNP PFC were determined with the aid of a dissecting microscope.

In Vitro Immune Response: Laboratory-Exposed Salmon

Juvenile chinook salmon collected from the Green River Hatchery were used to examine the effects of a contaminated sediment extract from the Duwamish Waterway delivered by ip injection on the primary and secondary in vitro anti-TNP response. Salmon transferred to the Mukilteo Marine Laboratory were allowed to acclimate for a minimum 2-week period prior to the beginning of the immunological tests. Two sediment extract solutions were prepared according to Krahn et al. (1988) for the injections: solution 1 contained 614 µg/mL polycyclic aromatic hydrocarbons (PAHs) and 90.2 µg/mL PCBs; solution 2 contained 793 µg/mL PAHs and 97.7 µg/mL PCBs. Salmon were injected with 12 µL of either 200 mg equivalents of Duwamish Waterway sediment (DWSE) (extract solution 1), in emulphor 620/g of fish (1:1 volume/volume)

or with 12 µL of the carrier (acetone: emulphor 620, 1:1 volume/volume) as a control. One week after the initial injection, the fish were injected for a second time with either the same concentration of sediment extract (test fish; solution 2) or acetone/emulphor 620 solution (control fish). Ten days after the second injection with sediment extractor carrier, bile from 10 fish of the control group and 20 fish of the sediment extract group were collected for analysis for FACs by HPLC/UV (Krahn et al., 1986). Five days after we collected bile samples for FAC analysis, salmon were injected with either 100 µg of the antigen TNP-KLH emulsified in Freund's complete adjuvent, or with PBS emulsified in Freund's complete adjuvent. Twelve weeks after the antigen or PBS injection, the Mishell Dutton and the Cunningham modification of the Jerne plaque assays were performed, as described above.

Measurement of Rates of Growth and Survival

Growth and survival studies were begun approximately 10-14 days after juvenile chinook salmon were collected. This delay allowed time for fish to be acclimated to full-strength seawater and for the cessation of any mortalities due to the stress of capture and transport. During 1990, juvenile chinook salmon from the Kalama Creek Hatchery, Green River Hatchery, Nisqually estuary, the Puyallup estuary and the Duwamish Waterway were evaluated for differences in growth and survival. During 1991, only chinook salmon from the Green River Hatchery and the Duwamish Waterway were evaluated for differences in growth and survival.

For studies of growth rate we utilized PIT (passive integrated transponder) tags as described by Prentice et al. (1990). Groups of 50 or 100 chinook received PIT tags; these groups were also used to obtain estimates of survival rates. Use of individual PIT tags and an electronic readout of tag numbers allowed us to identify and calculate growth on individual fish, thus greatly reducing statistical variability. In 1990, juveniles were anesthetized with MS-222 solution in seawater for tag insertion. In 1991, we tested a relatively new anesthetic, Metomidate Wildlife® (metomidate hydrochloride, Wildlife Pharmaceuticals Inc. Fort Collins CO), in an attempt to further reduce the stress of handling. After juvenile chinook were anesthetized, the glass-encased PIT tags were loaded into a 12-gauge needle and inserted with a 20 cc syringe into the right ventral

abdominal body cavity. Tag numbers were read with a standard reader (BioSonics Inc., Seattle, WA), initial fork lengths were measured to the nearest millimeter, and fish were then weighed to the nearest 0.1 gram. Length and weight measurements were made at monthly intervals during the 1990 study and at 6-week intervals during the 1991 study. Survival and growth measurements were intended to be conducted over a 4-month period; however, due to large increases in mortality, the maximum period was 40 days in 1990 and 84 days in 1991.

Statistical Methods

Analysis of variance (ANOVA) was used to determine the significance of differences among means for each of the parameters or endpoints. Statistical analyses of data for tissue contaminants, bioindicators, immunology, growth, and survival were performed using the StatView II and SuperANOVA statistical packages (Feldman and Gagnon 1986). Multiple means obtained for contaminants in tissues and bioindicators of chemical contaminant exposure were compared using Fisher's protected least significant difference test (Zar 1974). Tissue contaminant data for the hatcheries were combined in 1989 and 1990 due to low sample sizes for composites from some of the hatcheries and because there were generally no significant differences in concentrations of contaminants in fish tissues from the hatcheries. Net growth in length and weight of individual juvenile salmon were calculated by subtracting initial from final lengths and weights. Means of the parameters obtained from immunological and growth studies for fish from contaminated estuaries were compared to means of the parameters obtained for fish from hatcheries and the reference (Nisqually) estuary using Dunnett's multiple comparison test or Student's t-test (Zar 1974). The proportion of surviving juvenile chinook salmon was calculated as the final number of juveniles in the growth studies from each hatchery or estuary divided by the number of salmon at the initiation of the growth studies. Differences in percent survival for fish from the estuaries and hatcheries were evaluated using Chi-square analysis. Results of all statistical tests in this study were considered significant at $P \leq 0.05$.

RESULTS

Tag Recoveries and Size of Juvenile Salmon

The recovery of hatchery-released juvenile salmon tagged with coded wire tags and comparison of the length and weight of juvenile salmon from the hatchery and estuaries was used to estimate the proportion of hatchery salmon collected from the estuary. The percent of the salmon tagged and released at the hatcheries, the estimated number of natural and hatchery-derived salmon, and the percent of tagged salmon recovered from all estuaries in 1989 are shown in Table 3. Additionally, the length and weight of fish sampled in 1989 and 1990 to assess contaminant exposure in their tissues are shown in Tables 4 and 5, respectively.

Hatchery fish were frequently sampled in some of the estuaries using our sampling design. This sampling was based on the proportion of recovered adipose-clipped juvenile chinook salmon and the similarity in the average length and weight of fish sampled in the hatcheries and the estuaries. For example, recovery of tagged fish in 1989 (Table 3) from the Duwamish Waterway (6.4%) was similar to the percentage of tagged juvenile salmon released in a large group from the Green River Hatchery (5.3% tagged of 4 million) just prior to estuary sampling. Additionally, in 1989 and 1990 the average length and weight of salmon sampled from the Green River Hatchery and the Duwamish Waterway were similar (Tables 4 and 5, respectively). In 1989, 7.5% of all the salmon released from the Kalama Creek Hatchery were tagged. In our particular sample, 21.7% of the Kalama Creek Hatchery fish were tagged, indicating that tagged fish were not uniformly distributed between the ponds at this hatchery at the time of sampling. Recovery of tagged salmon in the estuary was 11.8%, which indicates that a large proportion of hatchery salmon were being sampled in the Nisqually estuary. Those tags that were recovered were identified by the Washington Department of Fisheries to be from the Kalama Creek Hatchery. However, the average lengths and weights of Nisqually estuary juveniles were not statistically similar to the average lengths and weights of juvenile salmon sampled from Kalama Creek Hatchery in both

1989 and 1990 (Table 4 and 5). This dissimilarity may reflect the mixing and sampling of juvenile salmon from other sources in this estuary.

Chinook outmigrants from the Puyallup River system were captured primarily in the Milwaukee and City Waterways of Commencement Bay. No tagged fish were released from the Puyallup Hatchery, thus the sampling of hatchery-derived fish in the estuary could not be confirmed by recovery of tagged salmon. However, the average lengths and weights of juvenile salmon captured in 1989 and 1990 in Commencment Bay were similar to the size of salmon released from the hatcheries (Tables 4 and 5, respectively). It should be noted that a large proportion of tagged fish were recovered from Commencement Bay in 1990. The source of these fish sampled in the estuary is unknown at this time, but it is likely that salmon from the Nisqually River system were appearing in the Commencement Bay area.

Many of the salmon captured in the Snohomish estuary may not have been released from the Skykomish Hatchery. Tagged salmon were not released from the Skykomish Hatchery, thus the sampling of hatchery-derived fish in the estuary could not be confirmed. However, 20.5% of the fish released from the Tulalip Hatchery were tagged, and 6.0% of the fish captured in the Snohomish estuary were tagged, indicating that some fish of Tulalip Hatchery origin may have been captured during sampling in the Snohomish estuary. Tulalip Hatchery is located near Tulalip Bay which is approximately 5 miles north of the Snohomish River estuary. The average length and weight also indicates that fish of an origin other than the Skykomish Hatchery were also being sampled in the Snohomish estuary. Salmon captured in the Snohomish estuary were approximately 30 mm longer and weighed approximately 9 g more on average than fish released from the Skykomish Hatchery. The larger (>110 mm) juvenile salmon captured in the Snohomish estuary were not used for any of our investigations.

Contaminant Exposure in Juvenile Chinook Salmon

Stomach Contents

Aromatic hydrocarbons--Chemical analyses of stomach contents for selected aromatic hydrocarbons (AHs), including primarily 2-5 benzenoid ring aromatic compounds, and chlorinated

hydrocarbons (CHs), including primarily polychlorinated biphenyls (PCBs), were done to assess possible dietary exposure of juvenile salmon to anthropogenic chemicals. The results showed significantly higher concentrations of total AHs, reported as low and high molecular weight AHs (LAHs and HAHs and their alkylated counterparts), in stomach contents of juvenile salmon from the Duwamish Waterway and Puyallup River estuary compared to fish from the Nisqually River estuary or hatcheries in both sampling years (Table 6). In contrast, the concentrations of LAHs in stomach contents of juvenile salmon from the Snohomish estuary, also an urban estuary, were not significantly different from concentrations of LAHs in stomach contents of fish found in the Nisqually estuary or corresponding hatchery fish. The concentrations of HAHs in stomach contents of salmon from the Snohomish estuary were significantly higher than concentrations of HAHs in stomach contents of fish found in the Nisqually estuary or corresponding hatchery fish. However, the concentration of HAHs in stomach contents of salmon caught in the Snohomish estuary were considerably less than the concentrations of HAHs in stomachs of fish captured from the Duwamish Waterway or Puyallup River estuary. Results for individual AHs found in stomach contents of juvenile salmon sampled in 1989 and 1990 by GC/MS are reported in Appendix Tables A-1 and A-2, respectively.

Chlorinated hydrocarbons--Concentrations of PCBs in stomach contents of juvenile salmon from the Duwamish Waterway and Puyallup River estuary were significantly higher than in fish from the Nisqually estuary in 1989 and 1990 (Table 6). Concentrations of PCBs in stomach contents of juvenile salmon from the Duwamish Waterway and Puyallup River estuary were also significantly higher than for fish from the hatcheries in 1989 but were not significantly higher than PCBs in stomach contents for hatchery fish in 1990. No significant differences were observed in PCB concentrations in stomach contents of salmon sampled from the Snohomish River estuary compared to fish from the hatcheries or the Nisqually River estuary in 1989.

Results of analysis for CHs other than PCBs such as the pesticides 4,4'-DDE, 4,4'-DDD and 4,4'-DDT generally showed low concentrations in stomach contents of salmon at all sites in 1989 and 1990 (Table 6). No significant difference in summed concentrations of other selected CHs (see Table 2 for a listing of the selected pesticides) was observed for fish sampled from the

estuaries compared to fish from the hatcheries. Results for individual CHs found in stomach contents of juvenile salmon sampled in 1989 and 1990 are reported in Appendix Tables A-3 and A-4, respectively.

Bile

Because fish metabolize AHs extensively, the AHs don't accumulate in tissues but are found in the bile as metabolites (Varanasi et al. 1989b). Bile was analyzed for FACs by HPLC/fluorescence at wavelengths specific for benzo[a]pyrene to estimate exposure of juvenile chinook salmon to AHs. In 1989, concentrations of FACs in bile were significantly higher in fish from the Duwamish Waterway and Puyallup estuary compared to concentrations in fish from the Nisqually estuary and the hatcheries (Fig. 2). Moreover, levels of biliary FACs in salmon from the Snohomish estuary were significantly higher than FACs in fish from the Nisqually estuary, a nonurban area. In 1990, the concentrations of FACs in bile of salmon from both the Duwamish Waterway and Puyallup estuary were significantly greater than the concentrations of FACs in salmon from the Nisqually estuary and the hatcheries (Fig. 2).

Liver

Chlorinated hydrocarbons, such as PCBs, are commonly found in sediments of highly industrialized urban waterways and bioaccumulate in tissues of fish inhabiting these areas, because they are not effectively metabolized by fish to excretable polar metabolites (Stein et al. 1984). The concentrations of hepatic PCBs in liver of juvenile salmon from the Duwamish Waterway, the Puyallup estuary, the Snohomish estuary, and the Nisqually estuary were significantly higher than the concentrations in liver of juvenile salmon from the hatcheries for both sampling years (Fig. 3). In addition, the concentrations of hepatic PCBs in juvenile salmon from the Duwamish Waterway and the Puyallup estuary were significantly higher than concentrations found in livers of juvenile salmon from the Nisqually estuary for both years. Results for individual CHs found in liver of juvenile salmon sampled in 1989 and 1990 are reported in Appendix Tables A-5 and A-6, respectively.

Exposure of fish to organotins is of concern because of the high toxicity of these compounds to marine species (Lee 1985, Snoeij et al. 1987). Recently, methods have been

developed in our laboratories that allow for accurate measurement of mono-, di-, and tributyltins in tissues along with appropriate quality assurance procedures (Krone et al. 1989) . Results of the analysis of selected liver tissue composites from juvenile salmon sampled in 1989 for butyltins are shown in Table 7. Mono-, di-, tri-, and tetrabutyltins were not detected in most samples of juvenile salmon analyzed. Butyltins were detected only in fish from the Duwamish Waterway; however, the concentrations were near the limit of detection. Additionally, the concentrations of butyltins detected were low compared to the concentrations in adult English sole (*Pleuronectes vetulus*), a benthic species from the Duwamish Waterway, which had concentrations of butyltins that were up to three times as great as those in the juvenile chinook salmon from the Duwamish Waterway (Krone et al. 1989 a,b). Because of the low concentrations of butyltins found in liver of salmon in 1989, additional analyses were not conducted in 1990.

Biochemical Measures of Contaminant Exposure

Hepatic Cytochrome P450 Activity

In the present study, the catalytic assays for AHH and EROD, were used to measure hepatic cytochrome P4501A (CYP1A) activity, the major inducible cytochrome P450 in fish (Goksøyr et al. 1991). Because hepatic AHH and EROD were highly correlated in juvenile salmon sampled in 1989 ($r = 0.933$, $P = 0.0001$) and 1990 ($r = 0.809$, $P = 0.0001$), the results for only hepatic AHH activity are shown (Fig. 4). Significantly higher hepatic AHH activities were observed for fish from the Duwamish Waterway and the Puyallup River estuary when compared to AHH activity in salmon sampled from the Nisqually estuary or from the hatcheries during both years of the study. Hepatic AHH activity in salmon from another urban area, the Snohomish estuary, was similar to the mean hepatic AHH activity for salmon from the Nisqually estuary or the hatcheries.

Hepatic Xenobiotic-DNA Adducts

The levels of hepatic xenobiotic-DNA adducts are a measure of the exposure, metabolism, and binding of hydrophobic contaminants, such as AHs, to a critical cellular macromolecule, DNA. The covalent binding of a chemical carcinogen to DNA is believed to be a critical step in the

multistep process of chemical carcinogenesis (Swenberg et al. 1985, Poirier et al. 1991). The levels of hepatic xenobiotic-DNA adducts in juvenile chinook salmon sampled in 1989 and 1990 are shown in Figure 5. In 1989, hepatic DNA adduct levels were significantly higher in salmon from the Duwamish Waterway than adduct levels in fish from the Nisqually estuary and the hatcheries. However, the concentrations of DNA adducts in fish from the Puyallup estuary and the Snohomish estuary were not significantly different from concentrations of adducts in salmon from the hatcheries or the Nisqually estuary (Fig. 5). In 1990, the levels of hepatic xenobiotic-DNA adducts were significantly higher in salmon from the Duwamish Waterway and the Puyallup estuary than adduct levels in fish from the hatcheries and the Nisqually estuary.

Depuration Studies

In a preliminary laboratory experiment, juvenile chinook salmon from the Duwamish Waterway and the Green River Hatchery were held at our seawater research facility to assess the rate and extent of removal of PCBs in salmon from a PCB-contaminated site. The results showed no significant decline in the concentration of PCBs in the whole body (i.e., body burden) of juvenile chinook salmon from the Duwamish Waterway over 3 months of holding in filtered flowing seawater in the laboratory (Fig. 6). The PCB body burden of Duwamish fish at the termination of a 90-day holding period was still significantly higher than body burden of PCBs for juvenile chinook salmon from the Green River Hatchery. Results for individual classes of PCBs found in whole bodies of juvenile salmon sampled in 1989 by GC/MS are reported in Appendix Table A-7.

Immunological Analysis

Primary in vivo Anti-TNP Response

The concentration of total immunoglobulin in the plasma of juvenile chinook salmon collected from the hatcheries and the respective estuaries was determined for fish sampled in 1989. The concentration ranged from a high of approximately 36,000 immunoglobulin units/μL of plasma to a low of approximately 10,000 immunoglobulin units/μL of plasma (Fig. 7). However, the amount of total immunoglobulin in the plasma of salmon from each hatchery did not differ

significantly from the amount of immunoglobulin in the plasma of salmon collected from their corresponding estuary.

To establish when the peak of a specific antibody response in the plasma of juvenile salmon occurred, the kinetics of the primary in vivo response to a standard hapten, TNP, were monitored over time (10 weeks) with a quantitative ELISA (Arkoosh and Kaattari 1990) using juvenile salmon captured from the Green River and Kalama Creek Hatchery and the Duwamish Waterway and Nisqually estuary. A significant increase (peak response) in the anti-TNP titer (1860 ± 900 units of anti-TNP activity/μL of plasma) in the plasma of the Green River Hatchery salmon occurred at 4 weeks post primary injection. In contrast, the significant increase (peak response) in the anti-TNP titer (850 ± 390 units of anti-TNP activity/mL of plasma) in the plasma of the Duwamish Waterway salmon occurred between 6 and 8 weeks post primary injection (Fig. 8). Because of a limited number of salmon available to sample, salmon from the Kalama Creek Hatchery and the Nisqually estuary were sampled for plasma levels of anti-TNP antibodies only at 4 and 9 weeks after exposure to the protein-conjugated hapten (antigen). Although the levels in the anti-TNP titer were comparable to concentrations observed for salmon from the Green River-Duwamish Waterway system, there were no statistical differences in the anti-TNP titer response in the Kalama Creek Hatchery and Nisqually estuary juvenile salmon at 4 and 9 weeks post primary injection (Fig. 9).

In Vitro Immune Response: Field-Exposed Salmon

To determine if juvenile chinook salmon leukocytes could generate a primary and secondary in vitro B cell response to TNP-KLH and TNP-LPS, a modified Mishell-Dutton (1967) culture system and the Cunningham modification of the Jerne hemolytic plaque assay (Jerne et al. 1963) was used (Kaattari et al. 1986). Various doses near the optimal antigen concentrations were used in the event that one of the antigen doses became suboptimal during the experimental period. However, there were no significant differences in the number of PFCs per culture produced with respect to the doses of antigen used in the assays. Leukocytes from the anterior kidney of juvenile salmon collected from the Green River Hatchery, Kalama Creek Hatchery and the Nisqually estuary were able to generate a significantly higher secondary response with TNP-KLH (Fig. 10)

than that produced during the primary response. The number of primary PFCs per culture generated with TNP-KLH in salmon from the Green River Hatchery, Kalama Creek Hatchery and Nisqually estuary ranged from 1 to 5 PFCs per culture. Upon a second exposure to TNP-KLH, the PFC response increased significantly (9 to 12 times more PFCs per culture) for the juvenile chinook salmon from all three sites. However, this heightened secondary PFC response relative to the number of primary PFCs per culture to TNP-KLH did not occur with leukocytes from the anterior kidney of primed Duwamish Waterway juvenile salmon (Fig. 10).

The number of PFCs per culture generated during the secondary response in anterior kidney leukocytes with TNP-LPS was significantly higher than the primary response in juvenile salmon from all four locations (Fig. 11). However, there was a statistical difference between the secondary PFC response per culture with the anterior kidney leukocytes from the Green River Hatchery and Duwamish Waterway chinook salmon. Salmon from the hatchery produced a significantly higher secondary PFC response per culture than juvenile salmon captured from the Duwamish Waterway. However, the secondary in vitro PFC response elicited in salmon from the Kalama Creek Hatchery and Nisqually estuary with TNP-LPS was not significantly different (Fig. 11) from each other.

Similarly, leukocytes from the spleen of juvenile salmon collected from the Green-Duwamish system and the Nisqually system were able to produce a significantly higher secondary response to TNP-LPS compared to the primary response (Fig. 12). Unlike the anterior kidney's PFC response, suppression of immunological memory was not found in the splenic PFC response of juvenile chinook salmon from an urban estuary when compared to juvenile salmon from the respective hatchery or salmon from the Nisqually River system. Because an insufficient number of cells could be harvested, the splenic primary and secondary PFC response to TNP-KLH was not performed.

In Vitro Immune Response: Laboratory-Exposed Salmon

To substantiate that chemical contaminants were in part responsible for the alterations of immune function observed in Duwamish Waterway juvenile salmon in 1990, Green River Hatchery juvenile chinook were exposed directly to organic solvent extracts of DWSE by

intraperitoneal injection in 1991. These sediment extracts contain AHs and PCBs, contaminants known to alter immune function in vertebrates. Lymphocytes from the anterior kidney of chinook salmon, exposed to either the DWSE or the carrier control, were able to produce an enhanced in vitro secondary PFC response (Fig. 13). An approximate 115 to 129% increase in the number of PFCs per culture was observed in the secondary response when compared to the primary response in these juvenile salmon. However, lymphocytes from the spleen of chinook salmon exposed to DWSE were unable to produce an enhanced in vitro secondary PFC response, while the splenic lymphocytes from the control group of salmon were able to produce a secondary in vitro response (Fig. 13). The level of stimulation of the secondary response in the control group increased approximately 250% when compared to the primary response of splenic lymphocytes of juvenile chinook salmon.

Survival

Survival of juvenile chinook salmon from hatcheries and estuaries sampled in 1990 and held for 40 days is shown in Table 8. No significant difference in survival was observed between fish from the Kalama Creek Hatchery and the relatively uncontaminated reference Nisqually estuary. Survival of fish from the Kalama Creek Hatchery was 88% (n = 50) while survival of fish from the Nisqually estuary group was 81% (n = 100). In contrast, survival of fish from the Green River Hatchery was significantly higher (86%, n = 100) after 40 days than survival of fish from the Duwamish Waterway (56%, n = 50). Survival of salmon from the Puyallup estuary (58%, n = 50) was comparable to survival of juveniles from the Duwamish Waterway and significantly lower than for fish from the Green River Hatchery. Survival studies in 1990 were terminated after 40 days because of increasing mortality in all groups. The salmon in all groups showed a high incidence of bloating with unknown etiology associated with the increased mortality.

Because of the high mortality observed in 1990, observations on survival of juvenile salmon in 1991 were made only with fish from the Green River Hatchery and the Duwamish Waterway. A study was conducted to confirm the effects of an urban estuary on survival of

juveniles observed in 1990 and to evaluate the effect of changes in our protocols of fish husbandry. Modifications to the protocol included placing fish in a reduced light environment, minimizing handling, using a different anaesthetic, and feeding at a reduced frequency. The proportion of surviving fish was significantly improved in 1991. With these changed protocols, survival of fish from the Green River Hatchery was 77% (n = 100) over a period of 84 days. With respect to survival of salmon from an urban estuary and the respective hatchery, there was no significant difference in the percent survival of juvenile salmon from the Duwamish Waterway and the Green River Hatchery after 40 days (Fig. 14). However, under these improved holding conditions we were able to prolong the experiment and noted that the survival of Duwamish Waterway juvenile chinook salmon was significantly lower (59%, n = 100) than the survival of fish from the Green River Hatchery (77%, n = 100) over a period of 84 days (Table 8).

Growth

Growth of juvenile fall chinook from the different hatcheries and estuaries sampled in 1990 and 1991 are summarized in Table 9. Growth was assessed as the net increase in fork length and weight of individual juvenile chinook salmon. During 1990, a significantly smaller increase in length, but not weight, was observed for juvenile salmon captured in the Duwamish Waterway compared to fish taken from the Green River Hatchery during the 40-day holding period. However, a significantly smaller increase in length, as well as weight, was observed for juvenile salmon captured in the nonurban Nisqually estuary compared to salmon taken from the Kalama Creek Hatchery (the corresponding reference hatchery) during the 40-day holding period. No difference in the net increase in length or weight was observed for fish sampled from the Puyallup estuary compared to juveniles sampled from the Kalama Creek Hatchery. During 1990, growth of salmon from all the hatcheries and from the Duwamish Waterway and Puyallup estuary ranged from a 3.9 to 7.9% increase in length and a 31 to 38% in weight. However, during 1990, salmon from the Nisqually estuary increased only 1.5% in length and 14% in weight over the 40-day period. Juvenile salmon from the Nisqually system, in particular the estuary, were found to harbor a high infestation of the kidney fluke, *Nanophaetes* sp. (M. Myers, NMFS, pers. commun., July

1990) which may have reduced the growth of these salmon. Further studies will be needed to determine the effect of the fluke infestation on growth of juvenile chinook salmon.

In 1991, growth of juvenile salmon was significantly improved relative to growth of salmon studied in 1990. This was principally attributed to the improved holding conditions during the course of this study. Daily growth rates for length increased on average from 0.23 mm/day in 1990 to 0.46 mm/day in 1991 for Green River Hatchery fish. Similarly for fish from the Duwamish Waterway daily growth rates in length increased on average from 0.18 mm per day in 1990 to 0.37 mm per day in 1991. The increase in survival and the increases in rate of growth indicated that changes in the holding conditions instituted in 1991 improved our ability to evaluate the effect of contaminant exposure on growth and survival of these fish.

In 1991, only growth in the Green-Duwamish River system was studied, partly as a result of the difficulties in holding fish for extended periods of time as previously described. Juvenile salmon from the Green River Hatchery increased an average of 44% in length and 253% in weight over 84 days, whereas salmon from the Duwamish Waterway grew 34% in length and 242% in weight. Green River Hatchery fish grew significantly more in length than juveniles captured from the Duwamish Waterway. Green River Hatchery salmon (87.0 ± 5.3 mm, $n = 100$) at the beginning of the growth study were approximately 4 mm smaller in length compared to salmon captured from the Duwamish Waterway (90.9 ± 5.2 mm, $n = 100$); however, final lengths for Green River and Duwamish salmon were 125.6 ± 10.5 mm ($n = 77$) and 121.9 ± 14.0 mm ($n = 59$), respectively (Table 9).

DISCUSSION

The results of the present study demonstrate increased chemical contaminant exposure in outmigrant juvenile chinook salmon during their relatively brief residence in polluted urban estuaries when compared to juvenile chinook salmon that reside in minimally contaminated estuaries. Chemical analyses showed concentrations of anthropogenic contaminants in stomach contents of salmon from two urban estuaries, the Duwamish Waterway and the Puyallup River estuary, were much greater than the concentrations in stomach contents of fish from the hatcheries

or the nonurban Nisqually estuary. Moreover, there were consistently higher concentrations of hepatic PCBs and biliary FACs (an indicator of exposure to AHs) in juvenile salmon collected from the Duwamish Waterway and the Puyallup estuary than in salmon from the hatcheries of the Nisqually estuary. The exposure of juvenile salmon to chemical contaminants was sufficient to elicit responses at the biochemical level. For example, significantly higher hepatic P450 monooxygenase activities and xenobiotic-DNA adduct levels were observed in salmon collected from the Duwamish Waterway and the Puyallup estuary than in fish from the hatcheries or the Nisqually estuary. However, evidence for chemical contaminant exposure was not found in fish from all three urban estuaries studied. For example, fish from the Snohomish estuary showed very little evidence of chemical contaminant exposure. This finding is consistent with the documented differences in sediment contamination in these aquatic urban environments (i.e., the urban sediments of the Duwamish Waterway and the Puyallup estuary, particularly in the waterways, are significantly more contaminated than those in the Snohomish estuary (Stein et al. 1992)). Moreover, the level of chemical exposure in fish persisted for months for some contaminants. Specifically, the elevated body burden of PCBs were found to remain stable for at least 3 months after exposure in contaminated urban estuaries. Concomitant with the increased exposure of juvenile chinook salmon to contaminants in some of these urban estuaries was evidence of immune dysfunction, reduced survival, and possibly reduced growth relative to animals taken directly from the hatcheries or from the Nisqually estuary. Thus, brief exposure to chemical contaminants in urban estuaries appears to be of sufficent magnitude to impair significant biological processes in juvenile chinook salmon. The long-term consequences of these effects warrant future studies.

Success of Capturing Hatchery Fish in the Estuary

In the present study, fish from the Duwamish Waterway and the Puyallup estuary were of comparable size to fish sampled from their respective hatcheries. Moreover, there was often close agreement between the percent of tagged salmon from the hatchery and the percent of tagged salmon recovered in the estuary, suggesting that hatchery fish constituted the major portion of our

sample from the estuary. In the Nisqually system, tagged juvenile salmon taken from the Nisqually estuary were confirmed to be from the Kalama Creek Hatchery. However, juvenile salmon from the estuary were much larger than juveniles from the Kalama Creek Hatchery, suggesting that juvenile salmon other than hatchery fish were also being sampled in the estuary. This finding, when coupled with allowances for mixing with juvenile salmon from the adjacent McAllister Creek system, suggests that chinook smolts captured in the Nisqually estuary reflect a more uniform mixture of natural and hatchery-produced salmon in the estuary. In contrast, it appears that many of the fish captured in the Snohomish estuary were not of Skykomish Hatchery origin. Beauchamps et al. (1987) report additional mixing of juvenile chinook from the Stillaguamish River, 25 km to the north of the Snohomish River estuary, and also had difficulty identifying the source of juvenile salmon captured in the Snohomish estuary.

Chemical Exposure and Biochemical Responses

Results of chemical analyses of stomach contents indicated diet as a possible route of xenobiotic exposure of juvenile salmon residing in polluted urban estuaries. The diet of juvenile chinook salmon from the Duwamish Waterway consisted of copepods, amphipods, insects, annelids and small fish; while chinook from the Puyallup River estuary (Milwaukee, Blair, and City Waterways) consumed similar prey, except annelids, and small fish were not found. In the Snohomish River estuary, salmon fed primarily on crustaceans and small fish and salmon from the Nisqually estuary preferred insects and annelids (P. Plesha, NMFS. Pers. commun., Sept. 1990). The results of taxonomic examination of stomach contents of juvenile salmon from the estuaries in the present study are supported by results of earlier studies (Meyer et al. 1981a,b; Weitkamp and Campbell 1980; Shreffler 1989), which showed preferences for copepods, chironomid insects, and gammarid amphipods by juvenile salmon in a number of Puget Sound estuaries. Many of these food organisms have been shown to be a potential source of contaminants in the food chain. For example, Brown et al. (1985) reported that amphipods collected in the Duwamish Waterway contained elevated concentrations of AHs (1.3 ± 0.20 µg/g dry wt.) and PCBs (0.24 ± 0.01 µg/g dry wt.), whereas AH and PCB concentrations in

amphipods from a reference site were only 0.12 ± 0.04 and 0.01 ± 0.0006 µg per gram dry wt., respectively, indicating that amphipods can bioaccumulate anthropogenic contaminants from their environment. Moreover, uptake of AHs and PCBs from sediment by amphipods was demonstrated when two amphipod species, *Rhepoxynius abronius* and *Eohaustorius washingtonianus*, from clean sites were exposed to Duwamish Waterway sediments for 7 days (Reichert et al. 1985, Varanasi and Stein 1991). For salmon feeding on these and similar organisms in urban estuaries, diet may represent a significant route of exposure to sediment-associated AHs and CHs.

Accordingly, stomach contents of salmon collected in the present study were analyzed for selected chemicals to determine the potential for uptake of contaminants through the diet. Concentrations of AHs in stomach contents showed a very marked increase (>16,000-fold greater) in salmon from the Duwamish Waterway and sites in the Puyallup estuary compared to salmon from the hatcheries or the Nisqually estuary. Interestingly, the broad spectrum of AHs analyzed in salmon from the Duwamish Waterway and the Puyallup estuary included, in addition to unsubstituted AHs, a substantial contribution of alkylated AHs in both sampling years (Table 6). A single analysis of stomach contents of juvenile salmon from contaminated urban estuaries for all the alkylated AHs listed in Table 2 can result in the quantitation of hundreds of individual analytes (not shown). For example, analysis of chromatographic results of stomach contents of juvenile salmon from the Duwamish Waterway or the Puyallup estuary identified about 30 alyklated C3-phenanthrenes (Appendix Fig. A-1-2). Moreover, a comparison of analyses of stomach contents, which consist primarily of benthic and epibenthic invertebrates, in salmon from the urban sites and a sediment sample from the Duwamish Waterway showed marked similarities in profiles of the aklkylated C3-phenanthrenes (Appendix Fig. A-3). These results support findings of earlier studies (Varanasi and Stein 1991, Stein et al. 1992) that showed benthic organisms inhabiting contaminated urban estuaries accumulate many of the same chemicals associated with the sediment. This similarity in profiles of contaminants in sediment and stomach contents again emphasizes the importance of diet as a route of exposure to sediment-associated contaminants in pelagic fish.

Salmon from the Duwamish Waterway were held to assess the depuration of PCBs in fish from a PCB-contaminated site. The results showed little evidence for a substantial decline in the level of PCBs in the whole body (i.e., body burden) during 3 months of holding in filtered seawater in the laboratory. These results are consistent with an earlier study with rainbow trout showing that the body burden of PCBs did not change with increasing size or after prolonged starvation (Lieb et al. 1974) and indicating that the majority of PCBs are not readily excreted. The results suggest that PCBs accumulated by juvenile salmon during their residency in a contaminated estuary are retained after the salmon leave for the open ocean. Moreover, because PCBs are associated with numerous significant biological effects, such as immunological dysfunction, reproductive impairment, and cancer (Safe 1984), the retention of PCBs and potential for such chronic effects warrant further investigation.

In the present study, a suite of biochemical (hepatic monooxygenase activities and xenobiotic-DNA adduct levels) and chemical (hepatic PCBs and biliary FACs) indicators of xenobiotic exposure and sublethal effects were used to evaluate exposure of juvenile chinook salmon as they migrate out into urban estuaries. The use of these bioindicators to assess xenobiotic exposure in marine species is well documented (Stein et al. 1992, Collier and Varanasi 1991, Krahn et al. 1986, Varanasi et al. in press). The results of the present study showed that these bioindicators were generally responsive to differences in the level of contamination in salmon from the various urban and nonurban estuaries within Puget Sound. The findings also show that these indices gave consistent information regarding the amount of exposure juvenile chinook salmon were undergoing. For example, the bioindicators of chemical contaminant exposure for fish sampled from the Duwamish Waterway and the Puyallup estuary were consistently higher than for fish taken from the Nisqually estuary, whereas the bioindicators of exposure for salmon from the Snohomish estuary, another urban estuary, were only occasionally higher (biliary FACs) than for salmon from the Nisqually estuary. This is consistent with the documented differences in sediment contamination in these aquatic urban environments, (i.e., the urban environments of the Duwamish Waterway and the Puyallup estuary, particularly in the waterways, are significantly more contaminated than the Snohomish estuary (Stein et al. 1992)). In addition, some of the

bioindicators of exposure (bile FACs and contaminants in stomach contents) showed that juvenile salmon sampled in the Nisqually estuary were, in general, less exposed to chemical contaminants than juveniles from the hatchery. This is interesting in that hatchery practices may also contribute to the exposure of juvenile salmon to contaminants, albeit to much lower levels than seen in the urban estuaries. However, the significance of this background exposure to the future well being of juvenile chinook salmon is not clear at this time.

Several studies (reviewed in Buhler and Williams 1989) have shown that exposure of fish to certain chemical contaminants, such as 4-5 ring AHs and certain PCB congeners, substantially induces (increases) hepatic cytochrome P-450-dependent monooxygenases. The monooxygenases are enzymes that catalyze biotransformation of anthropogenic chemicals to more polar compounds to enhance their excretion. However, in addition to the detoxication of toxic chemicals, the monooxygenases also activate some chemicals to more toxic metabolites that readily and covalently bind to cellular macromolecules (Lutz 1979, Conney 1982). Our results showing enhanced hepatic AHH activity in juvenile outmigrant chinook salmon from the Duwamish Waterway and the Puyallup estuary demonstrate that these fish were exposed to inducers, presumably the AHs and PCBs, in their environment. The biological consequences of induction of hepatic monooxygenases are not fully delineated; however, the induction of certain monooxygenases, such as the one measured by the AHH assay, are linked to the currently accepted mechanism for the toxic effects induced by environmental contaminants including coplanar PCBs, dibenz-p-dioxins, and dibenzofurans (Safe 1990).

The measurement of hepatic xenobiotic-DNA adducts to evaluate contaminant exposure was useful, but the levels were not consistent for each year of the study. The covalent binding of a chemical carcinogen to DNA is believed to be a critical step in the multistep process of chemical carcinogenesis (Swenberg et al. 1985, Poirier et al. 1991). Previous results with fish have shown that some of the adducts detected by the ^{32}P-postlabeling assay, used here to measure DNA adducts, may be due primarily to AHs, some of which are known to be carcinogenic in fish (Hendricks et al. 1985, Schiewe et al. 1991). Additionally, our previous (Varanasi et al. 1989b, Stein et al. 1992) results demonstrate that hepatic xenobiotic-DNA adducts also provide a measure

of cumulative exposure to compounds (e.g., AHs) which are not accumulated in tissues of fish because of their extensive metabolism to more easily excreted metabolites. Levels of hepatic xenobiotic-DNA adducts are unique because they not only are a measure of the exposure and binding of genotoxic agents to DNA, a critical cellular macromolecule, but they also persist in the tissues and thus can serve as a long-term and cumulative indicator of exposure to contaminants. Thus, while biliary FACs provide an estimate of recent exposure to AHs and their biotransformation, DNA adducts provide a long-term cumulative estimate of exposure to certain major classes of environmental contaminants.

Salmon from the Duwamish Waterway in 1989 had adduct levels that were 1.8 times greater than levels in salmon from the Duwamish Waterway in 1990. This is consistent with the lower hepatic PCB levels in fish sampled from the Duwamish in 1990, compared to 1989, indicating a lower magnitude or duration of exposure to this class of contaminants in fish in 1990 compared to fish in 1989. In contrast, hepatic DNA adduct concentrations in salmon from the Puyallup estuary were significantly higher than adduct concentrations in salmon from the hatcheries in 1990, but not in 1989 (Fig. 5). The higher levels of hepatic xenobiotic-DNA adducts in fish from the Puyallup estuary in 1990, compared to 1989, appears to be at least in part due to greater exposure to AHs, as reflected in contaminants in stomach contents and bile FACs. Differences in chemical contaminant exposure may be a result of where the fish were sampled. Salmon in 1989 were predominantly from the Milwaukee Waterway whereas in 1990 a majority of the fish were from the City Waterway.

Another factor that may contribute to differences in hepatic DNA-adduct levels in fish in 1989 and 1990 may be related to the sensitivity of the technique to document chemical contaminant exposure after a relative short exposure period. Based on our studies, benthic fish that reside in these contaminated environments for longer periods of time than salmon have hepatic xenobiotic-DNA adduct and PCB levels that are approximately 10 times higher than those observed in salmon from these same contaminated environments (Stein et al. 1992). Differences in adduct levels after long-term exposure periods in benthic resident fish are more easily discernable than adduct levels in migratory fish that reside in these urban environments for a shorter time span. Although benthic

fish and salmon represent different species, species differences among adduct levels for animals from the same environment or exposed to the same contaminants are very small (Stein et al. 1992). The fact that we can observe significant differences in hepatic xenobiotic-DNA adduct levels in fish from the contaminated urban estuaries after the relatively short exposure period reflects the strength of the method to serve as bioindicator of exposure to contaminants, as well as to confirm that significant exposure has occurred to salmon in these urban environments.

Biological Effects

The biological effects that were monitored in this study demonstrated that salmon exposed to contaminants in an urban estuary, specifically the Duwamish Waterway, suffered greater mortality and exhibited greater immune dysfunction than salmon from the hatcheries or a minimally contaminated estuary. We also demonstrated that salmon from the Duwamish Waterway grew less than fish from the hatchery. A similar effect was observed for fish from the Nisqually estuary compared to fish from the hatchery; thus we could not separate an estuary from a contaminant-related effect on growth at this time. Although it is premature to extrapolate the significance of these findings to the long-term consequences, the implications are that juvenile salmon that must outmigrate through an urban estuary may be less equipped to face the multiple challenges of survival as they enter the ocean environment.

The immune system is responsible for protecting an organism against infectious diseases and neoplastic cells. The three main components of the immune system are cell-mediated immunity, humoral immunity, and macrophage function. All of these components have been shown to be perturbed by interaction with environmental contaminants (Dean et al. 1986). In the present study, we examined the effect of contaminant exposure on humoral immunity of juvenile chinook salmon. Alterations in the humoral immunity may result in a suppressed host ability to fight diseases. This is the first field study to examine the primary and secondary humoral immune response of juvenile chinook salmon from an urban estuary. Although there were no differences in the concentrations of naturally occurring immunoglobulins between juvenile chinook salmon from an urban estuary and juveniles from its respective hatchery, there was significant evidence of

altered in vivo production of primary antibodies to the specific antigen TNP-KLH and in the secondary in vitro response of plaque-forming cells (B cells) to TNP-KLH and TNP-LPS. The altered primary in vivo response and suppressed secondary in vitro PFC response may be linked to differences in exposure to contaminants, as described previously.

The differences observed in the immune response of chinook salmon from the Duwamish Waterway and the Nisqually estuary may be due to factors other than contaminants. For example, genetic differences between salmon from these two river systems could account for the differences in their immune responses. However, salmon from the Green River Hatchery are cultured at the Kalama Creek Hatchery for release; therefore, strain differences are unlikely to be a factor. Another factor which could account for the differences in immune response is that the composition of salmon caught from the estuary is not representative of the salmon released from the hatchery and this results in population differences between the hatchery and estuary. However, we have shown that a large portion of hatchery salmon are captured in the estuary. It appears, therefore, that contaminant exposure plays an important role in bringing about a differential reponse in the immune system of juvenile salmon.

To more causally link the relationship between contaminants and altered immune function, salmon from the Green River Hatchery acclimated to saltwater in the laboratory were injected with an organic solvent extract of a contaminated sediment from the Duwamish Waterway (DWSE). As we observed in the field-exposed juvenile salmon, the ability of plaque-forming cells (B-cells) to produce specific antibodies to TNP-LP was suppressed. These findings (M. Arkoosh E. Clemons, and E. Casillas, NMFS, Unpubl. manuscr.) suggest that the immunosuppression observed with salmon from the Duwamish Waterway was most likely due to chemical contaminants and not due to other environmental variables. The concentrations of biliary FACs from DWSE-injected salmon were comparable to the values of biliary FACs in salmon sampled from urban estuaries, suggesting that the dose of DWSE administered to salmon in the laboratory study was environmentally relevant. Interestingly, the lymphoid organs affected in the laboratory and field studies were different. This difference may reflect differences in length of exposure of contaminants or differences in route of exposure (Ward et al. 1985). In general it appears that both

field and laboratory exposure of juvenile salmon to contaminants have produced similar results, in that immunomodulation (suppression) of the in vitro secondary response was observed. Suppression of immunological memory after exposure to chemical contaminants is not without precedent. For example, a previous laboratory study demonstrated that rainbow trout exposed to aflatoxin B_1 were also found to have a suppressed humoral secondary response (Arkoosh and Kaattari 1987). The consequences of a suppressed immune system are not clear at this time. Results of an earlier laboratory study where investigators injected a commercial PCB mixture into channel catfish (*Ictalurus punctatus*) showed that disease resistance to *Aeromonas hydrophilia* (Jones et al. 1979) was much reduced. We have yet to undertake this type of disease challenge with salmon passing through an urban estuary or exposed to selected environmentally relevant contaminants in the laboratory. But, because of the stresses encountered by these salmon entering a marine environment, perturbation of this important physiological system may have significant implications on their long-term health and survival.

During each year of the study, survival was lower in juvenile salmon from the urban estuaries compared to the survival of fish from the hatcheries or from the reference Nisqually estuary. Although extensive mortalities were found in all groups during the first year (1989) of the study (data not shown), including fish sampled from the hatcheries, mortalities of fish from two of the urban estuaries (the Duwamish Waterway and the Puyallup estuary) were always higher than for fish from the reference estuary (the Nisqually estuary) or the hatcheries. However, confidence in the interpretation of the data for 1989 was reduced because of the high mortality in all groups, including fish from the hatcheries (controls). During this first year, salmon were held for less than 30 days before mortalities reduced the survivorship to less than 30% in all groups. Mortalities during 1989 were, in part, attributed to mechanical problems with the seawater system leading to reduced water flows to the tanks and to an air leak that resulted in extensive gas bubble disease in all groups of salmon held for the survival and growth portion of the study. In 1990 and 1991, improvements in the holding facilities and care increased survival for all groups. For example, survival rates of juvenile salmon from the hatcheries and the Nisqually estuary were greater than 80% over an approximate 40- and 80-day period, respectively. Moreover, the mortality rates of

fish from the Duwamish Waterway and Puyallup estuary in 1990 and Duwamish Waterway in 1991 were consistently higher than for fish from the Nisqually estuary or the hatcheries. The cause of the higher mortalities of salmon from the urban estuaries is unclear at this time, but it may be linked to increased or greater physiological stress (e.g.,immune dysfunction) in fish exposed to chemical contaminants in the urban environments.

The rate of growth of juvenile salmon from urban environments also appeared to be lower than that of fish from the hatcheries. However, the significance of this finding is uncertain because the growth rate of fish from the reference Nisqually estuary in 1990 was also lower than that of fish from the hatchery. Overall salmon from the Kalama Creek Hatchery and the Nisqually estuary grew much less than salmon from the Green River Hatchery and the Duwamish Waterway. One possible explanation for the reduced growth of fish from the Nisqually River was the presence of the parasite, *Nanophyetus salmincola* (Lee Harrell, NMFS. Pers. commun., July 1990). *Nanophyetus salmincola* is a digenic trematode that infects salmonids via the freshwater snails *Juga plicifera* (Bennington and Pratt 1960). This parasite infects the kidney of salmonids and may affect their survival in seawater. *Nanophyetus salmincola* was found to infect juvenile coho salmon (*Oncorhynchus kisutch*) in the Chehalis River system and cause extensive mortalites as they entered the estuarine environment (Schroder and Fresh 1992). The prevalence of this parasite in fish from the Nisqually system, particularly for juveniles from the estuary, was extremely high (M. Myers, NMFS, Pers. commun., July 1990). Thus, the reduced growth in fish from the Nisqually system may have been in part attributable to the high parasitic infection, although this has yet to be confirmed.

In addition, the growth of juvenile chinook salmon appeared to be affected by the number of fish in each tank. In the beginning of the studies, distributions of lengths and weights of fish were more uniform. However, toward the end of the studies, variability in length and weight between individuals was dramatically increased. A few of the individual salmon were very large at the conclusion of the studies while growth of many other fish was almost negligible. Salmon in tanks with high mortality of juveniles may actually experience higher rates of growth because of decreased fish densities. Fish densities would necessarily need to be continually adjusted to

correct this problem. In this study, maintaining a constant number of fish was not possible, because the number of fish in the growth study was also used to determine survival. Reimers (1973) observed little change in size of chinook salmon in the Sixes River estuary from June through August, which he attributed to high densities. This problem was also recognized by Schroder and Fresh (1992) in a study of salmonid growth in the Chehalis estuary. Future studies should consider the impact of both stocking densities and parasitic infections on the measurement of growth of juvenile salmonids in order to more accurately assess the impact of xenobiotic exposure.

Because of the difficulties of holding juvenile chinook salmon for extended periods of time, much of our effort was spent in trying to improve our husbandry techniques and to identify other reference sites for accurate evaluation of the growth impairment observed in chinook salmon from the Duwamish Waterway. Improved survival was accomplished by using metomidate as an anaesthetic, placing the juvenile salmon in darkened tanks, and minimizing their handling. Using these modifications, the survival over a 90-day period was improved from less than 10% in 1989 to greater than 85% in 1991. Despite these difficulties, growth of fish from the Green River Hatchery was found to be always significantly greater than that of fish from the Duwamish Waterway. But because of a lack of growth in fish from the reference estuary, we are not able to unequivocally state that the rate of growth was less in fish as a result of exposure to contaminants in an urban estuary. Further studies are ongoing to measure growth rates in salmon from other reference estuaries in Puget Sound.

CONCLUSION

The data presented in this 3-year study represent new information evaluating chemical contaminant exposure and associated effects in juvenile chinook salmon in urban estuaries of Puget Sound, Washington. On the basis of this study, juvenile chinook salmon that outmigrate through several urban estuaries show extensive evidence of exposure to chemical contaminants. The level of exposure not only persists for months for some contaminants, but also the magnitude of the exposure is concordant with the severity of the contamination in the estuaries studied. This was

supported by the concentration of chemical contaminants measured in the stomach contents and tissues as well as in the increased levels of the bioindicators of exposure (bile FACs, xenobiotic-DNA adducts, and AHH activity) in urban-exposed juvenile salmon. The bioindicators provided information about the exposure of juvenile chinook salmon to chemicals and provided a measure of sublethal biological effects, some of which may be early signs of more serious effects. Concomitant with the increased chemical exposure, juvenile chinook salmon inhabiting these urban estuaries exhibited evidence of immune alterations, reduced survival, and possibly impaired growth relative to juveniles taken directly from the hatcheries or from the nonurban estuary. In particular, the observed immunosuppression could weaken the fishes resistence to pathogens and increase vulnerability to a wide variety of diseases. Suppression of immune function could, in part, account for the decreased survival of juvenile salmon from the urban estuaries. Because it is difficult to accurately assess the impact of chemical contaminant exposure on the proportion of salmon returning from contaminated and minimally contaminated environments, the long-term consequences of xenobiotic exposure and the subsequent effects to the health and well-being of the salmon populations can not yet be precisely stated. What is needed now are laboratory studies using controlled exposures of fish to sediment contaminants either by using model compounds or mixtures of contaminants extracted from sediments. This controlled exposure will enable us to more closely link the contaminants in the environment to effects in juvenile salmon and to evaluate the longer term potential for survival, either by using disease challenges or behavioral studies. The effects of chemical contaminant exposure clearly occur after a brief residency in polluted estuaries and should be evaluated as a contributing factor affecting future salmon returns.

ACKNOWLEDGMENTS

The work reported here was a result of efforts by an interdisciplinary team of NMFS scientists. Participants in the study included Nicolaus Adams, Bernie Anulacion, Luara Berggren, Ethel Blood, Lisa Bogatski, Richard Bogar, Daryle Boyd, Jennie Bolton, Cristin Bryant, Katherine Dana, Tho Dang, Tara Felix-Slinn, Barbara French, Bill Gronlund, Mark Gustafson, Rebecca Hastings, Deborah Holstad, Anna Kagley, Lyndal Johnson, Thomas Merculief, Hannah

Morris, Mark Myers, Paul Olson, Ronald Pierce, Herb Sanborn, Eunice Schnell, Robert Snider, Sean Sol, Dana Whitney, and Gladys Yanagida. Thanks also are extended to Drs. Walt Dickoff and Orlay Johnson of the Coastal Zone and Estuarine Studies Division of the NMFS for providing PIT tags and the loan of the tag detector. Jim Peacock provided the map illustration. We would also like to thank Dr. Mike Watson and Jerry Larrance of the U.S. EPA for providing thoughtful and critical reviews of this document.

We are indebted to the following: Dr. S. L. Kaattari, Oregon State University for providing standard anti-TNP rainbow trout sera;.Dr. G. Warr, University of South Carolina for giving us the monoclonal cell line (1-14 monoclonal cell line); and Washington State Department of Fisheries for providing unpublished data and granting us authorization to obtain juvenile fall chinook salmon from the state-operated hatcheries. We also thank the following hatchery managers for their cooperation: Dutch Henderson and Don Peterson of the Green River Hatchery, Bob Jateff and Ron Warren of the Puyallup Hatchery, Don Rerdnick of the Skykomish Hatchery, and Bill Thomas and the Nisqually Tribe of the Kalama Creek Hatchery.

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TABLES

Table 1. Summary of sampling dates and numbers of juvenile fall chinook salmon collected at four hatcheries and four estuaries* during the 1989, 1990, and 1991 sampling periods.

Year	1989			1990			1991		
Source	Date	Number collected	Number of seine sets	Date	Number collected	Number of seine sets	Date	Number collected	Number of seine sets
Green River Hatchery	May 1	75	NA	May 8	1400	NA	May 21	1500	NA
	May 8	75	NA	May 22	175	NA			
	May 31	300	NA						
Duwamish Waterway	May 23	149	3	May 24	364	5	June 4	379	4
	June 1	201	8	May 31	271	6	June 5	153	9
	June 7	194	7	June 1	218	8	June 11	203	7
	June 8	99	3	June 18	87	6	June 17	104	8
Puyallup Hatchery	May 24	75	NA	June 4	170	NA	Not sampled		
	May 31	75	NA	June 6	250	NA			
Commencement Bay	June 14 (MLW)	275	6	June 25 (MLW)	86	7	Not sampled		
	June 19 (MLW)	111	9	June 26 (SP,MID,CTY)	92	7			
	June 26 (BLR)	24	11	June 28 (CTY)	138	3			
				July 3 (CTY)	274	5			
Kalama Hatchery (Nisqually)	May 22	200	NA	May 25	90	NA	Not sampled		
	June 12	70	NA	May 29	880	NA			
				July 9	300	NA			
Nisqually Estuary	June 20	71	8	June 13	553	2	Not sampled		
	June 27	188	3	June 14	270	5			
	July 7	138	9	July 5	8	4			
	July 10	145	11	July 12	72	7			
Skykomish Hatchery (Snohomish)	May 30	200	NA	Not sampled			Not sampled		
	June 6	75	NA						
Snohomish Estuary	June 22	29	8	Not sampled			Not sampled		
	June 28	4	8						
	June 29	6	4						
	June 30	11	3						
	July 11	10	7						

* Estuary site abbreviations: MLW = Milwaukee, BLR = Blair, SP = St. Paul, MID = Middle, CTY = City.

Table 2. Aromatic hydrocarbons (AHs) and chlorinated hydrocarbons (CHs) determined in tissues of juvenile chinook salmon.

Low molecular weight AHs (LAHs, 2-3 rings)	High molecular weight AHs (HAHs, > 3 rings)
naphthalene	fluoranthene
2-methylnaphthalene *	pyrene
1-methylnaphthalene *	benz[a]anthracene
biphenyl *	chrysene
2,6-dimethylnaphthalene *	benzo[b]fluoranthene
acenaphthylene	benzo[k]fluoranthene*
acenaphthene	benzo[e]pyrene *
2,3,5-trimethylnaphthalene *	benzo[a]pyrene
fluorene	perylene *
phenanthrene	indeno[1,2,3-c,d]pyrene
1-methylphenanthrene *	dibenz[a,h]anthracene
	benzo[g,h,i]perylene
Alkylated LAHs	**Alkylated HAHs**
C1-C4 naphthalenes	C1-fluoranthenes/pyrenes
C1-C3 fluorenes	C1-C4 chrysenes/benz[a]anthracenes
C1-C4phenanthrenes/anthracenes	
Polychlorinated biphenyls (PCBs)	**Other CHs**
trichlorobiphenyls	hexachlorobenzene
tetrachlorobiphenyls	lindane
pentachlorobiphenyls	heptachlor
hexachlorobiphenyls	aldrin-e
heptachlorobiphenyls	alpha-chlordane
octachlorobiphenyls	dieldrin
nonachlorobiphenyls	p,p'-DDE
decachlorobiphenyl	p,p'-DDD
	p,p'-DDT

* Compounds not analyzed in 1989.

Table 3. Hatchery production, estimates of natural production, percent of hatchery salmon tagged and percent tag recoveries of fall chinook in four river systems in Puget Sound during 1989.

River system	Number of juveniles	Percent of hatchery fish tagged and released prior to sampling[b]	Percent tagged fish recovered
GREEN-DUWAMISH			
Green River Hatchery	8,670,000	5.3	6.5
Natural production[a]	2,467,000		
Estimated total	11,137,000		
PUYALLUP RIVER			
Puyallup Hatchery	2,500,000	0	1
Natural production[a]	523,300		
Estimated total	3,023,300		
NISQUALLY RIVER			
Kalama Creek Hatchery	900,000	7.5	11.8
Nisqually natural production[a]	500,000		
McAllister Creek Hatchery	1,900,000	0	0
McAllister Creek natural production[a]	94,500		
Estimated total	3,394,500		
SNOHOMISH RIVER			
Skykomish Hatchery	370,000	0	0
Tulalip Hatchery	672,000	20.5	6.0
Natural production[a]	672,400		
Estimated total	1,667,400		

[a] Estimates of natural production were calculated from adult escapement data provided by the Washington State Department of Fisheries.

[b] Calculated as the percent of juveniles which were tagged and released from the hatchery prior to estuarine sampling.

Table 4. Length and weight of juvenile fall chinook sampled from four hatcheries and four estuaries in 1989. Tissues were composited for use in measuring exposure to contaminants.

Site	Sample size (n)	Length (mm)*	Weight (g)*	Percent salmon tagged
Green River System				
Green River Hatchery	122	85 ± 7	5.2 ± 1.3	5.5
Duwamish Waterway	215	89 ± 12	5.5 ± 2.5	6.5
Nisqually River System				
Kalama Creek Hatchery	120	96 ± 9	7.2 ± 2.0	21.7
Nisqually River estuary	172	109 ± 13	10.0 ± 3.9	11.8
Puyallup River System				
Puyallup Hatchery	115	89 ± 12	6.4 ± 1.7	0
Puyallup River estuary	181	90 ± 7	5.4 ± 1.3	1.0
Snohomish River System				
Skykomish Hatchery	125	81 ± 7	4.3 ± 1.1	0
Snohomish River estuary	55	116 ± 26	13.8 ± 10.5	6.0

* Values represent the mean ± standard deviation

Table 5. Length and weight of juvenile fall chinook at three hatcheries and three estuaries in 1990. Tissues were composited for use in measuring exposure to contaminants.

Site	Sample size (n)	Length (mm)	Weight (g)	Percent salmon tagged
Green River System				
Green River Hatchery	234	92 ± 11*	6.7 ± 2.1*	5.1
Duwamish Waterway	282	95 ± 12	7.2 ± 5.8	6.4
Nisqually River System				
Kalama Creek Hatchery	191	97 ± 6	7.8 ± 5.5	17.8
Nisqually River estuary	200	105 ± 8	8.9 ± 2.1	10.5
Puyallup River System				
Puyallup Hatchery	190	98 ± 6	8.4 ± 1.7	0
Puyallup River estuary	336	102 ± 18	8.7 ± 5.6	16.7

* Values represent the mean ± standard deviation.

Table 6. Concentrations of aromatic hydrocarbons (AHs reported as low-molecular weight AHs, LAHs, alkylated LAHs, high-molecular weight AHs, HAHs, and alkylated HAHs) and chlorinated hydrocarbons (PCBs and other CHs) in composites of stomach contents of juvenile salmon sampled in 1989 and 1990.[a]

Year/Site	n	LAHs	Alkylated LAHs	HAHs	Alkylated HAHs	PCBs	Other CHs
1989							
Hatcheries	7	31 ± 5.0	31 ± 12	3.4 ± 2.8	0.4 ± 0.4	86 ± 13	29 ± 6.0
Duwamish Waterway	3	5300 ± 4400[c]	116000 ± 115000[c]	18400 ± 14000[c]	3000 ± 1500[c]	300 ± 40[c]	30 ± 10
Puyallup River estuary[b]	3	640 ± 270[c]	2500 ± 400[c]	2500 ± 900[c]	350 ± 20[c]	190 ± 50[c]	30 ± 10
Snohomish River estuary	2	37 ± 3.0	15 ± 6.0	48 ± 10[c]	nd	59 ± 20	3.0 ± 1.0[e]
Nisqually River estuary	3	18 ± 4.0	12 ± 5.0	4.0 ± 2.0	nd	22 ± 10[e]	2.0 ± 1.0[e]
1990							
Hatcheries	4	59 ± 11	270 ± 50	22 ± 0.5	20 ± 10	120 ± 20	42 ± 10
DuwamishWaterway	3	15000 ± 14000[c]	19000 ± 8000[c]	14000 ± 9700[c]	3300 ± 1400[c]	260 ± 90[d]	41 ± 20
Puyallup River estuary[b]	2	16000 ± 15000[c]	23000 ± 18000[c]	30000 ± 27000[c]	6500 ± 5400[c]	260 ± 70[d]	51 ± 30
Nisqually River estuary	2	6.2 ± 1.5[e]	12 ± 2.0[e]	4.8 ± 1.5[e]	2.0 ± 1.0[e]	33 ± 8.0[e]	11 ± 4.0[e]

[a] See Table 2 for list of analytes analyzed. Values are expressed as ng/g, wet weight (mean ± SE).

[b] In Commencement Bay, the sites sampled in 1989 were the Milwaukee and Blair Waterways and in 1990 the site sampled was the City Waterway.

[c] Significantly different than for juvenile salmon from the hatcheries or for juvenile salmon from the Nisqually River estuary.

[d] Significantly different than for juvenile salmon from the Nisqually River estuary.

[e] Significantly different than for juvenile salmon from the hatcheries.

Table 7. Concentrations of butyltins in composite liver samples of juvenile chinook salmon in 1989.

Site	Tetrabutyl	Tributyl	Dibutyl	Monobutyl
Duwamish Waterway				
Composite 1	< 5[a]	< 5	< 5	< 5
Composite 2	< 5	< 5	< 5	< 5
Composite 3	< 5	36	72	< 5
Commencement Bay	< 5	25[b]	32	< 5
Nisqually Estuary				
Composite 1	< 5	25[b]	32	< 5
Composite 2	< 5	< 5	< 5	< 5

[a] The "less than" symbol (<) indicates that the analyte was not detected in concentrations above the stated value.

[b] The analyte was detected, but below the limit of quantitation of 25 ng/g.

Table 8. Survival of juvenile chinook salmon (*Oncorhynchus tshawytscha*) over a 40-day period in 1990 and an 84-day period in 1991.

Site	Percent survival	
	1990	1991
Green River System		
Green River Hatchery	86 (n = 100)	77 (n = 100)
Duwamish Waterway	56[a] (n = 50)	59[a] (n = 100)
Nisqually River System		
Kalama Creek Hatchery	88 (n = 50)	---
Nisqually River estuary	81 (n = 100)	---
Puyallup River System		
Puyallup Hatchery	---[b]	---
Puyallup River estuary	58[a,b] (n = 50)	---

[a] Survival is significantly different than for salmon from their respective hatchery ($p \leq 0.05$).
[b] Comparison is made to fish from the Kalama Hatchery.

Table 9. Growth of juvenile chinook salmon over a 40-day period in 1990 and an 84-day period in 1991.

Site	Mean initial weight (g)	Mean final weight (g)	Mean weight increase (g)	Mean initial length (mm)	Mean final length (mm)	Mean length increase (mm)
1990						
Green River System						
Green River Hatchery	9.4 ± 1.9	13.1 ± 2.6	3.6±1.3	94 ± 6	104 ± 7	9 ± 3
Duwamish Waterway	7.6 ± 1.6	11.3 ± 3.9	3.0 ± 2.3	91 ± 6	100 ± 9	7 ± 4*
Nisqually River System						
Kalama Creek Hatchery	13.4 ± 2.4	18.2 ± 3.1	4.6 ± 1.6	111 ± 7	117 ± 7	5 ± 3
Nisqually River estuary	9.3 ± 2.2	10.7 ± 3.1	1.9 ± 1.8*	99 ± 8	100 ± 7	2 ± 4*
Puyallup River System						
Puyallup Hatchery		---			---	
Puyallup River estuary	8.8 ± 1.2	12.0 ± 2.2	3.0 ± 1.7*	96 ± 5	101 ± 6	4 ± 4
1991						
Green River System						
Green River Hatchery	7.5 ± 1.4	26.3 ± 7.2	19.0 ± 5.1	87 ± 5	126 ± 11	38 ± 8
Duwamish Waterway	7.3 ± 1.3	25.3 ± 9.6	17.6± 7.4	91 ± 5	122 ± 14	31 ± 11*

* Significantly different than juvenile salmon from their respective reference hatchery ($p \leq 0.05$).

FIGURES

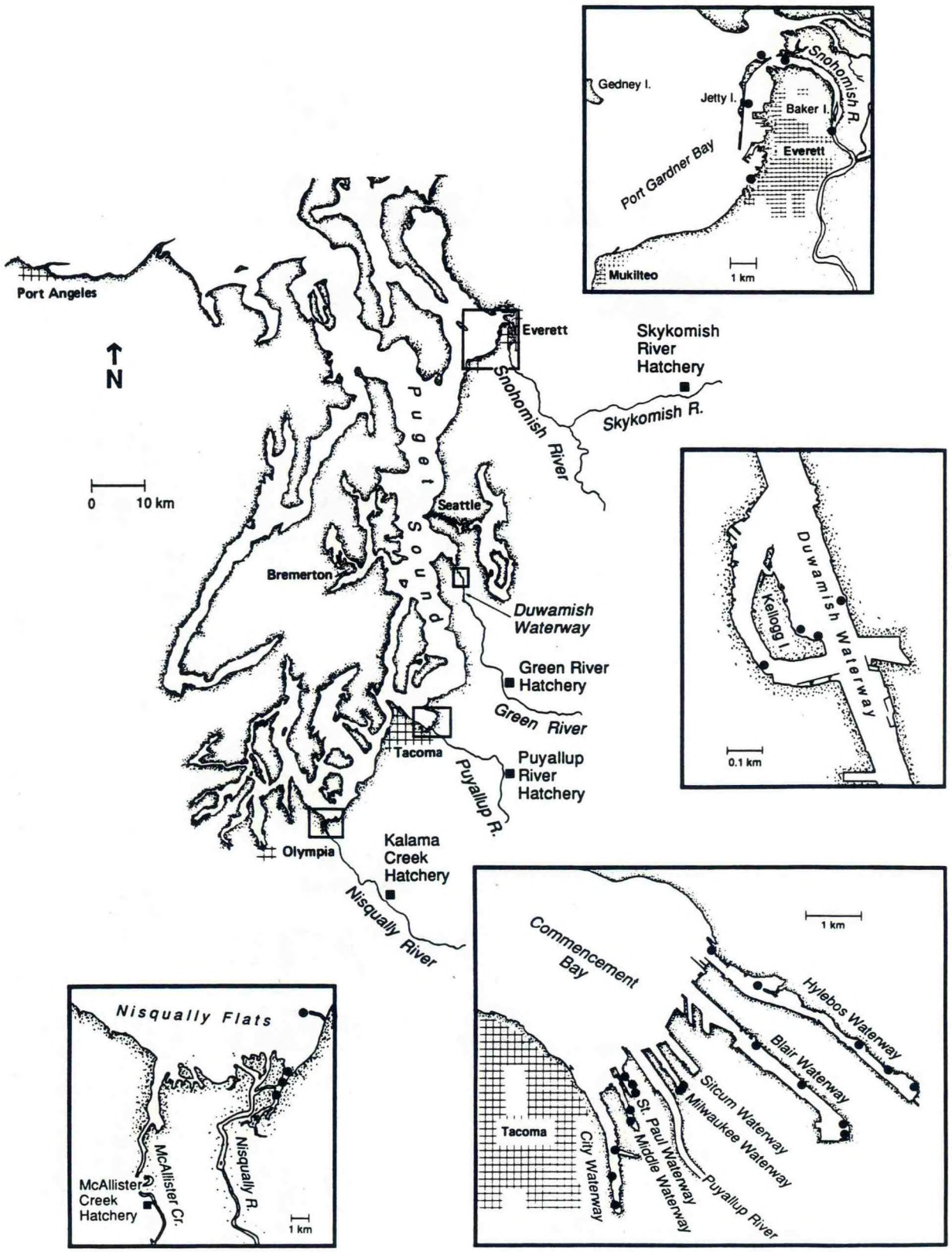

Figure 1. Map of Puget Sound, WA showing the location of rivers, hatcheries, and sampling sites (•) in estuaries studied to determine uptake and effects of contaminants on juvenile chinook salmon (*Oncorhynchus tshawytscha*).

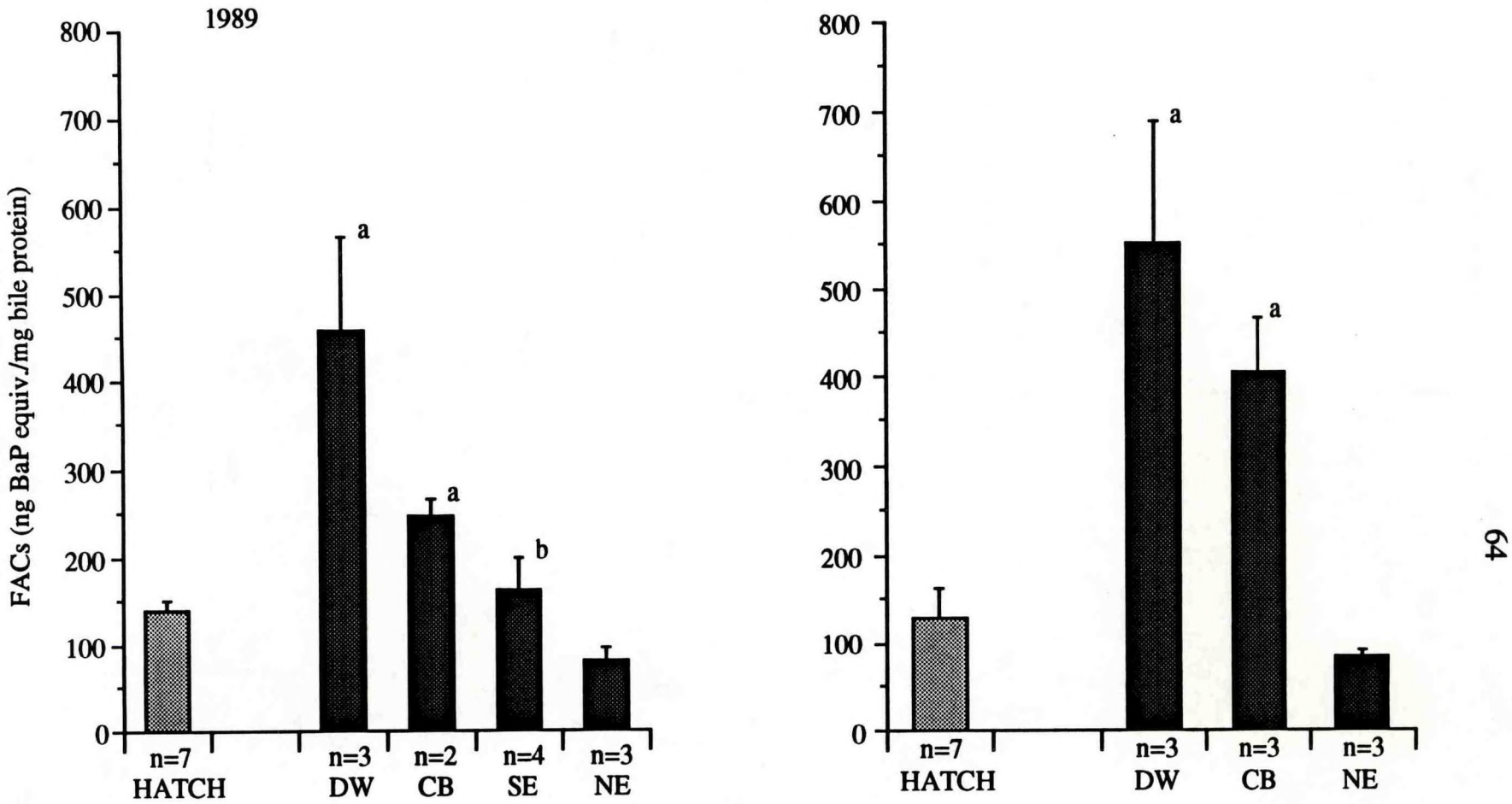

Figure 2. Biliary fluorescent aromatic compounds (FACs) (ng BaP equiv/mg bile protein) in juvenile salmon from estuaries and hatcheries sampled from Puget Sound, Washington, in 1989 and 1990, BaP = benzo[a]pyrene. The bars represent the mean ± standard error. The sample size (n) equals the number of bile composites that were analyzed. Site abbreviations are: Hatch=Combined hatcheries of the estuarine systems sampled in 1989 or 1990, DW=Duwamish Waterway, CB=Commencement Bay, SE=Snohomish River Estuary, and NE=Nisqually River Estuary. The letter (a) indicates values that are significantly different than for salmon from the combined hatcheries and the NE, and (b) indicates values that are significantly different than for salmon from the NE only. The results (mean ± standard error) for the individual hatcheries is presented in Appendix Table A-8.

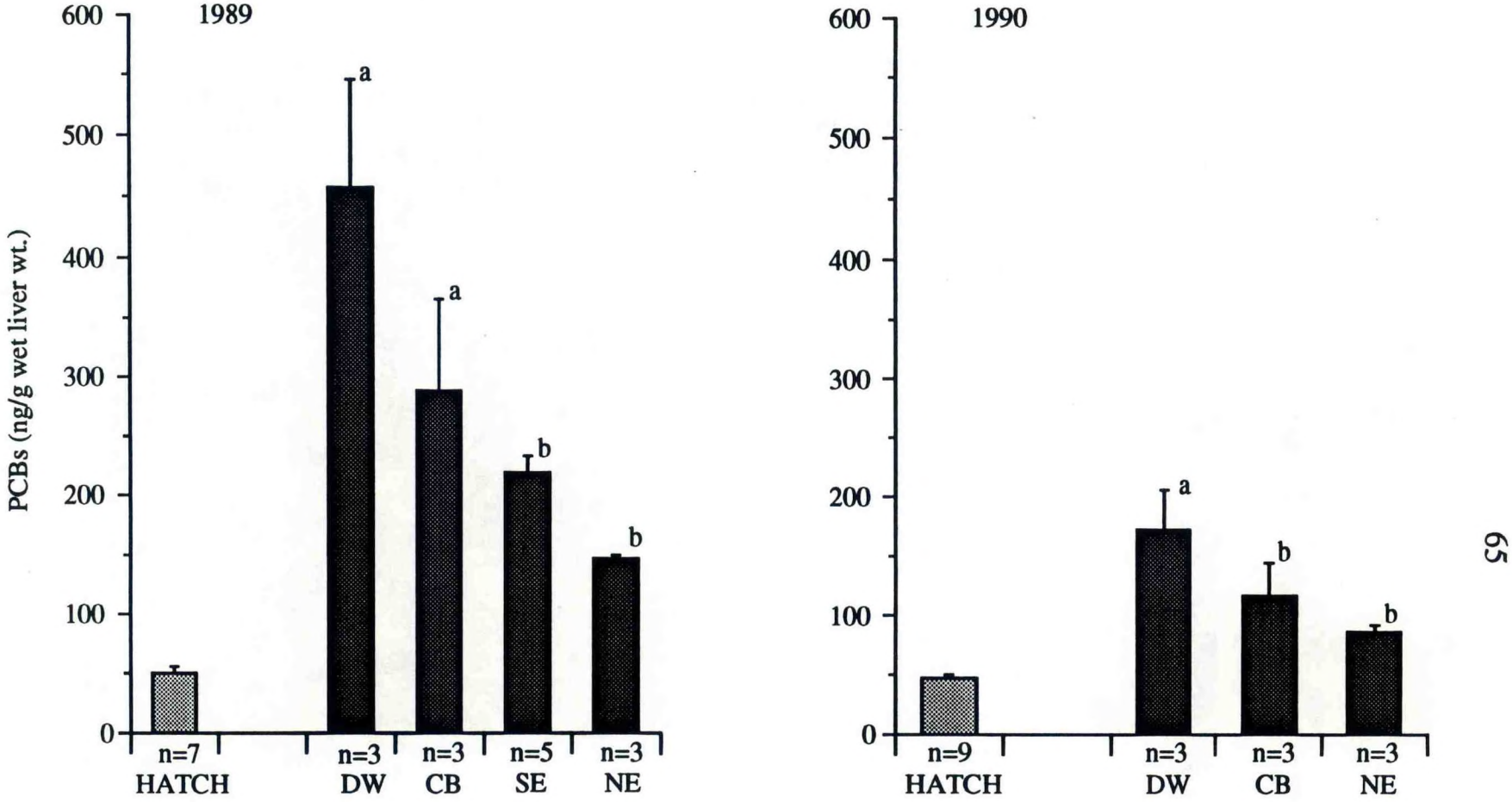

Figure 3. Hepatic polychlorinated biphenyls (PCBs) (ng/g wet liver wt.) in juvenile salmon from estuaries and hatcheries sampled from Puget Sound, Washington, in 1989 and 1990. The bars represent the mean ± standard error. The sample size (n) equals the number of tissue composites that were analyzed. Site designations are as described in Figure 2. The letter (a) indicates values that are significantly different than salmon from the combined hatcheries and the NE, and (b) indicates values that are significantly different than for salmon from the combined hatcheries only. The results (mean ± standard error) for the individual hatcheries are presented in Appendix Table A-8.

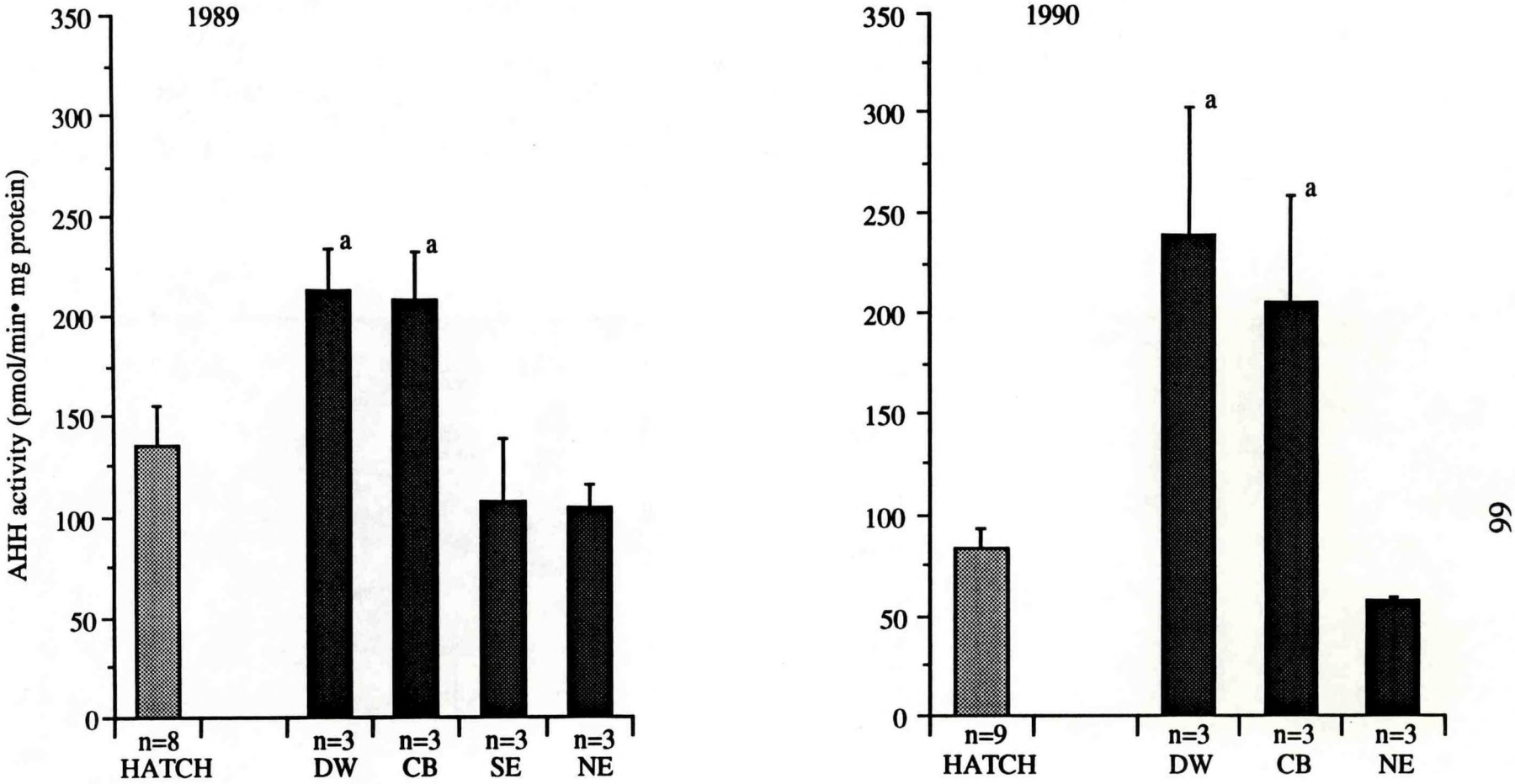

Figure 4. Hepatic aryl hydrocarbon hydroxylase (AHH) activity (pmol/min• mg protein) in juvenile salmon from estuaries and hatcheries sampled from Puget Sound, Washington, in 1989 and 1990. The bars represent the mean ± standard error. The sample size (n) equals the number of tissue composites that were analyzed. Site designations are as described in Figure 2. The letter (a) indicates values that are significantly different than for salmon from the combined hatcheries and the NE. The results (mean ± standard error) for the individual hatcheries is presented in Appendix Table A-8.

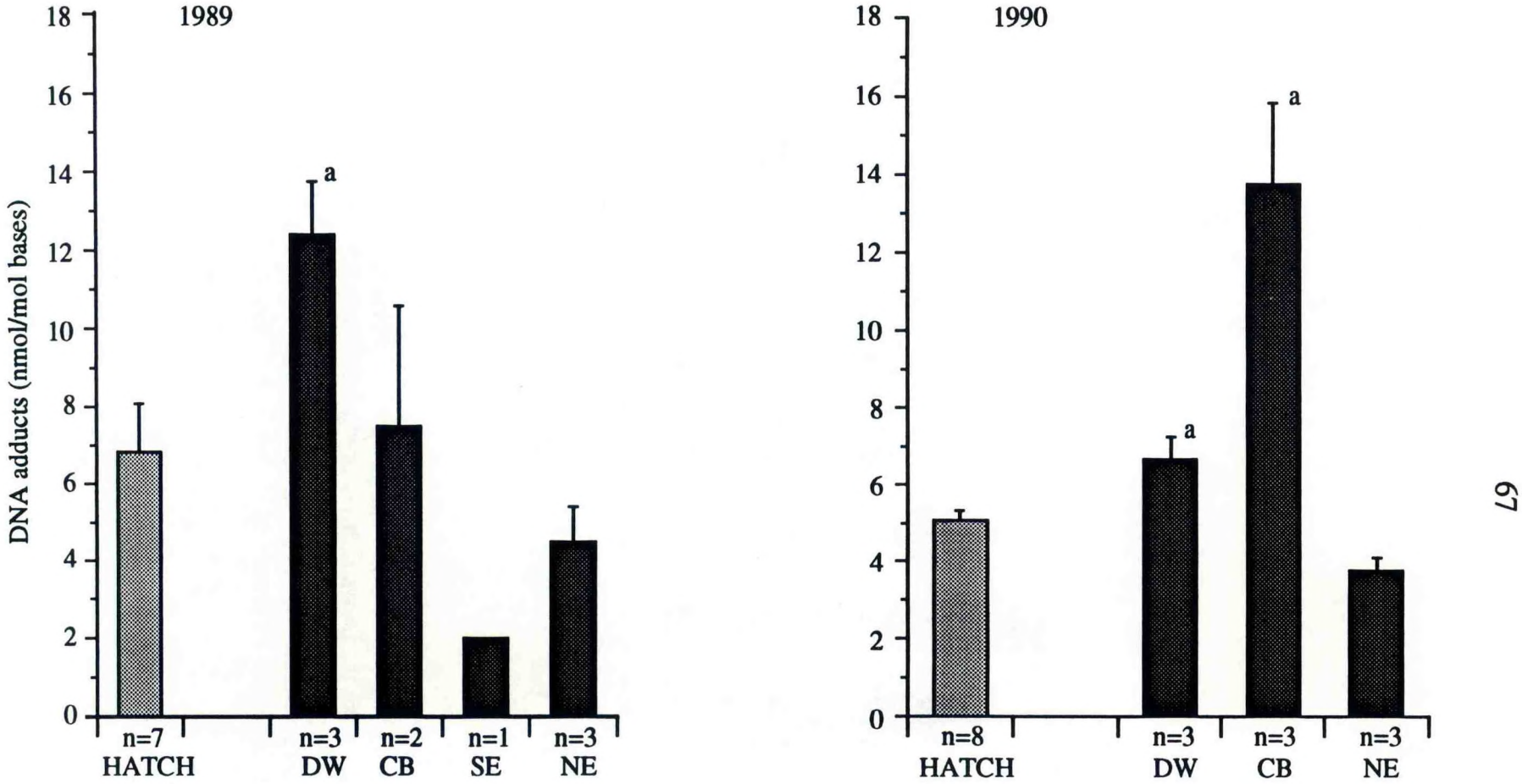

Figure 5. Hepatic DNA adducts (nmol/mol bases) in juvenile salmon from estuaries and hatcheries sampled from Puget Sound, Washington, in 1989 and 1990. The bars represent the mean ± standard error. The sample size (n) equals the number of tissue composites that were analyzed. Site designations are as described in Figure 2. The letter (a) indicates values that are significantly different than for salmon from the combined hatcheries and the NE. The results (mean ± standard error) for the individual hatcheries is presented in Appendix Table A-8.

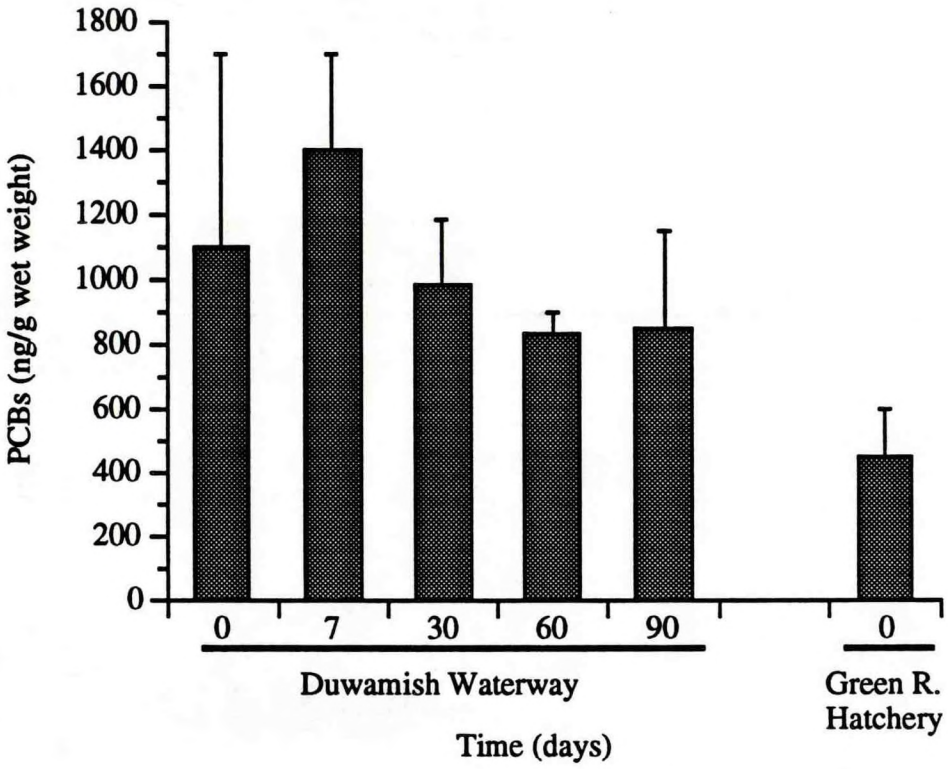

Figure 6. Total body burden of PCBs (ng/g wet weight) in juvenile chinook salmon sampled from the Duwamish Waterway and Green River Hatchery in 1989. The bars represent the mean ± standard error for 3 composite samples consisting of 2 to 3 fish per composite. The concentrations of individual PCBs are presented in Appendix Table A-7.

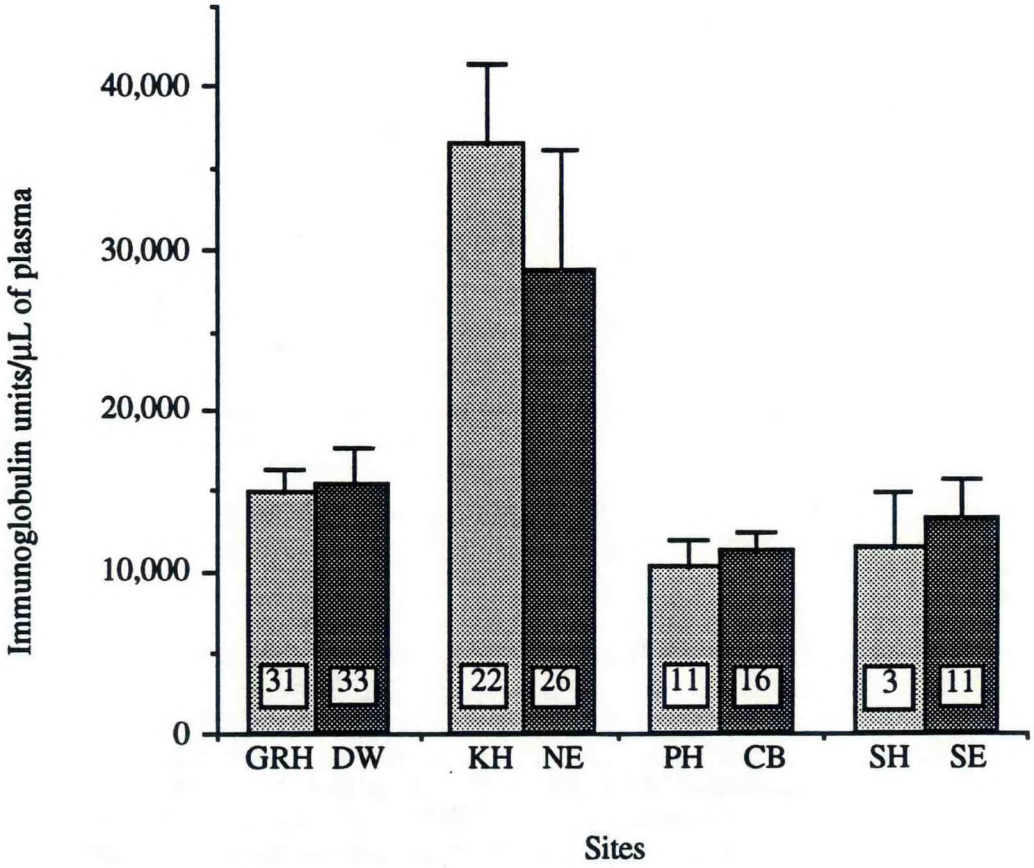

Figure 7. Total immunoglobulin as determined by an ELISA. The immunoglobulin units/µL of plasma were determined for chinook salmon collected from the various hatcheries (▨) and the corresponding estuarine systems (■), GRH = Green River Hatchery, KH = Kalama Creek Hatchery, PH = Puyallup River Hatchery, and SH = Skykomish River Hatchery. Other site designations are as described in Figure 2. Data are expressed as mean ± standard error. The numbers overlaying the histograms represent the number of salmon from that area sampled.

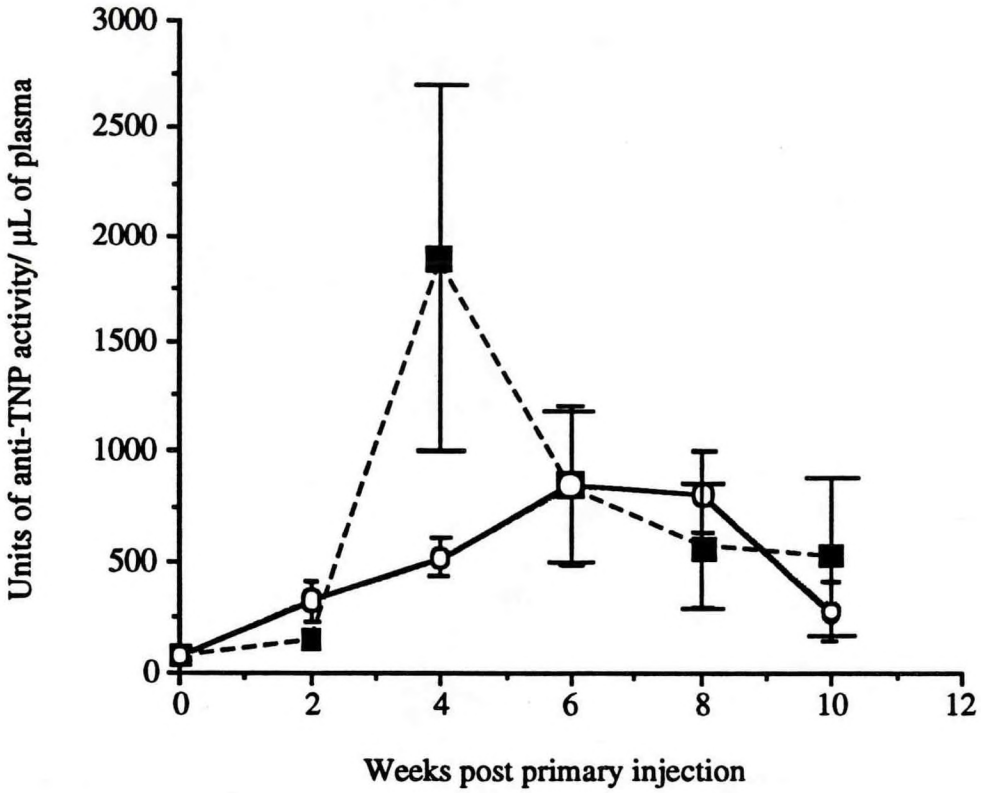

Figure 8. The primary in vivo antibody titer and kinetics to the TNP hapten as determined by an ELISA. The units of anti-TNP activity/µL of plasma were determined for chinook salmon collected from the Green River Hatchery (■) and from the Duwamish Waterway (○) at various time points after injection with the antigen TNP-KLH. Data are expressed as mean ± standard error. Plasma from 10 salmon were analyzed at each time point tested except at time 0, when 5 plasma samples were analyzed. (Adapted from Arkoosh et al. 1991.)

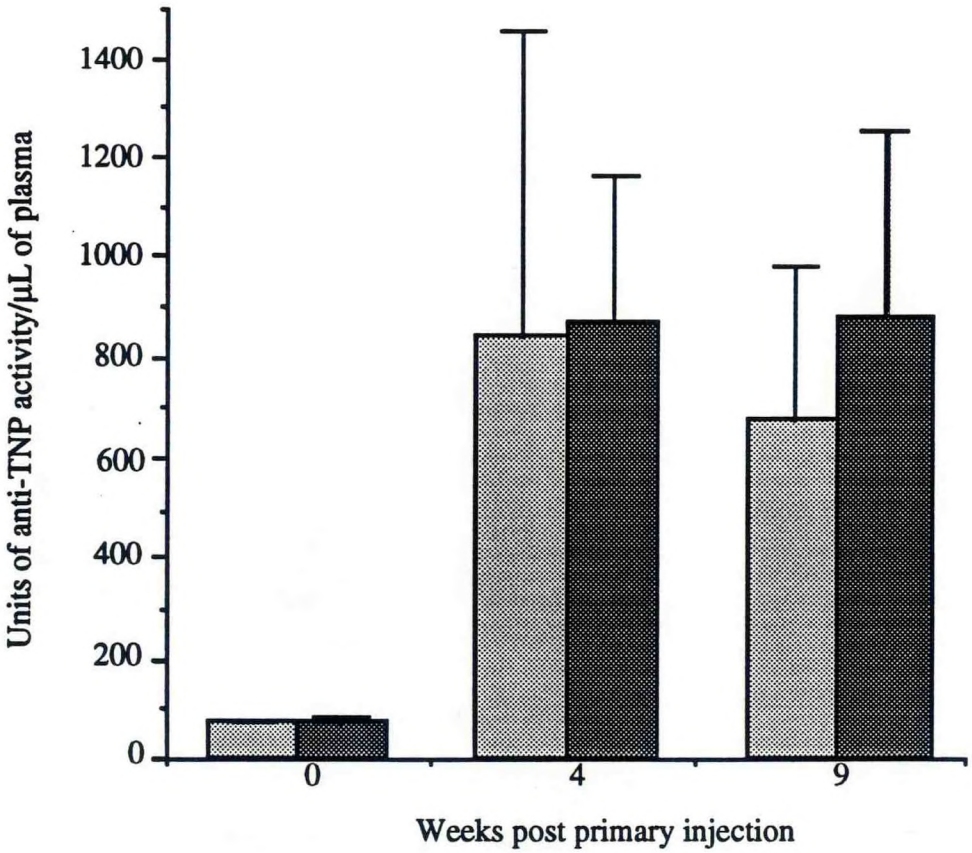

Figure 9. The primary in vivo antibody titer to the TNP hapten at 0,4,9 weeks post primary injection as determined by an ELISA. The units of anti-TNP activity/µL of plasma were determined for chinook salmon collected from the Kalama Creek Hatchery (▨) and from the Nisqually River Estuary (■). The vertical bars represent one standard error above the mean. An average of 8 salmon were examined per time point. (Adapted from Arkoosh et al. 1991.)

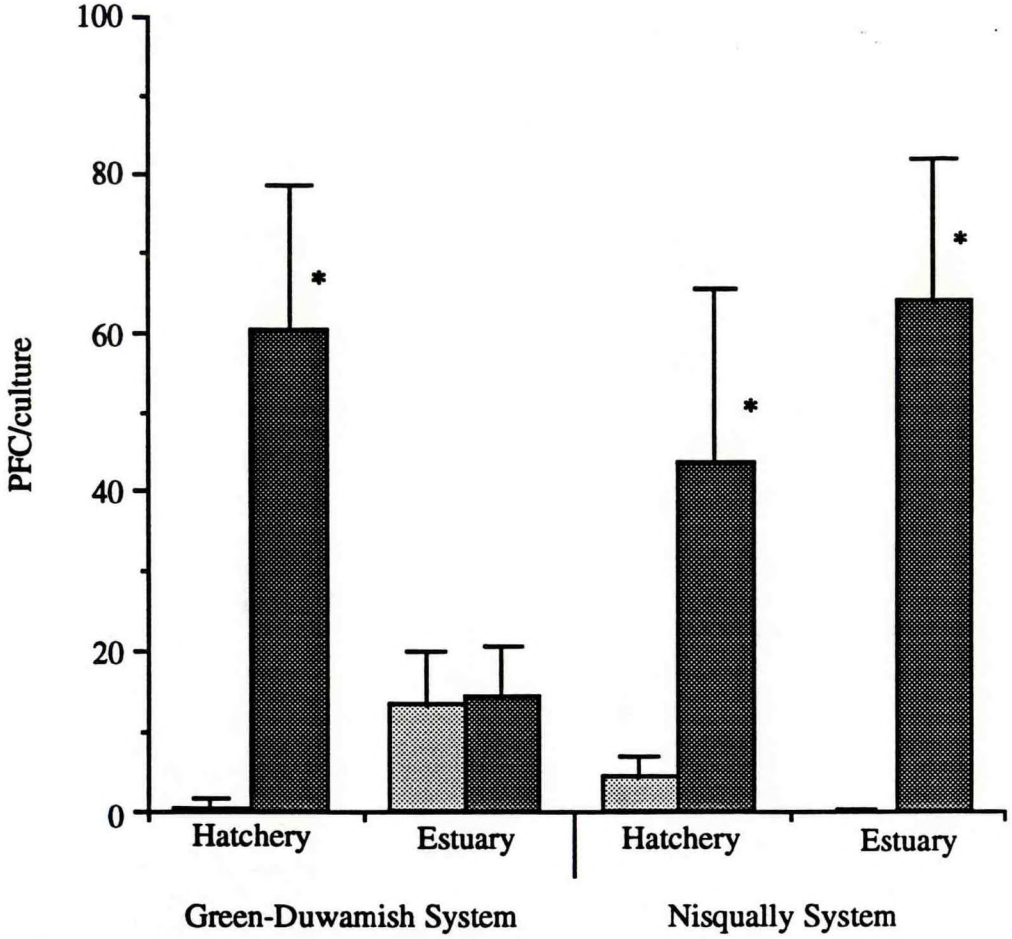

Figure 10. The primary (▩) and secondary (■) in vitro plaque forming cell response per culture (PFC/culture) to TNP-KLH generated in the anterior kidney. The vertical bars represent one standard deviation above the mean. The mean PFC response was analyzed in chinook salmon from the Green-Duwamish system and the Nisqually system. Anterior kidneys from 10 chinook salmon were examined from each of the four locations. Anterior kidney cells cultured only with tissue culture medium (background) did not produce more than a mean of 2 PFC/culture. *Indicates PFC/culture significantly higher than observed in the primary response. (Adapted from Arkoosh et al. 1991.)

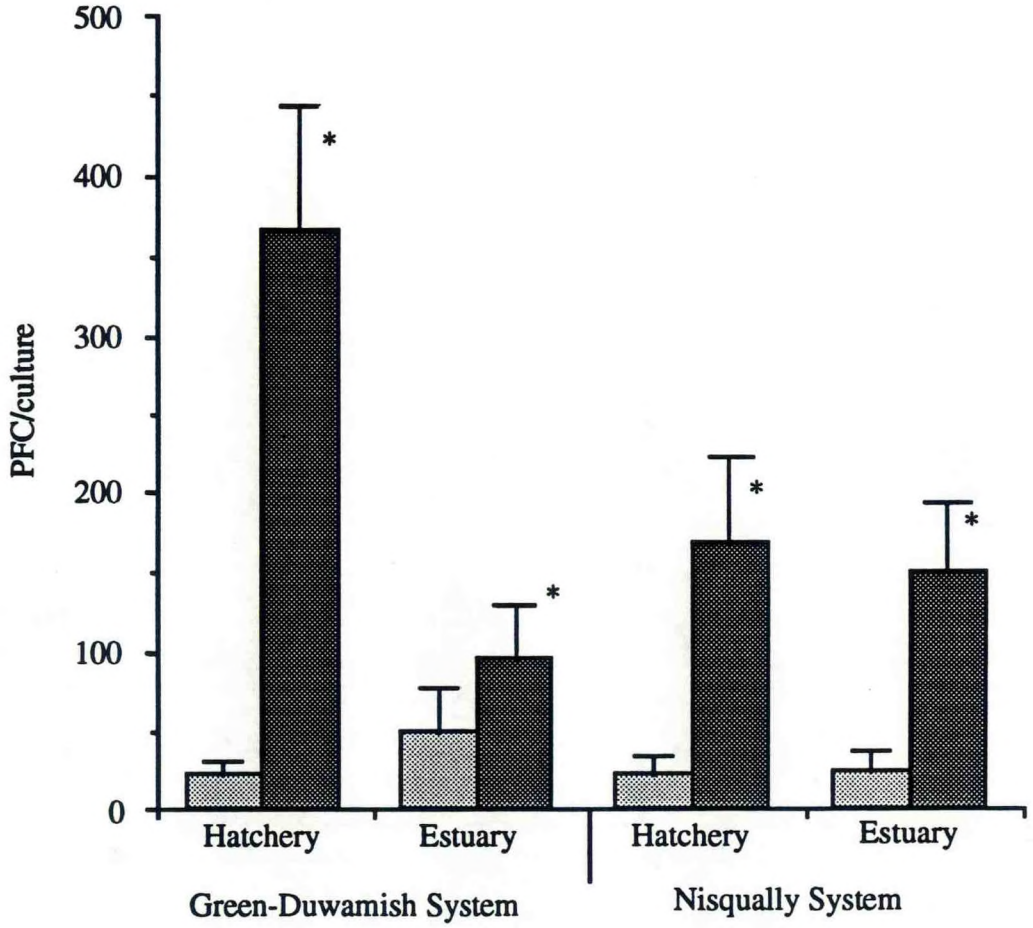

Figure 11. The primary (▩) and secondary (■) in vitro plaque forming cell response per culture (PFC/culture) to TNP-LPS generated in the anterior kidney. The vertical bars represent one standard deviation above the mean. The mean PFC response was analyzed in chinook salmon from the Green-Duwamish System and the Nisqually system. Anterior kidneys from 10 chinook salmon were examined from each of the four locations. Anterior kidney cells cultured only with tissue culture medium (background) did not produce more than a mean of 2 PFC/culture. *Indicates PFC/culture significantly higher than observed in the primary response. (Adapted from Arkoosh et al. 1991.)

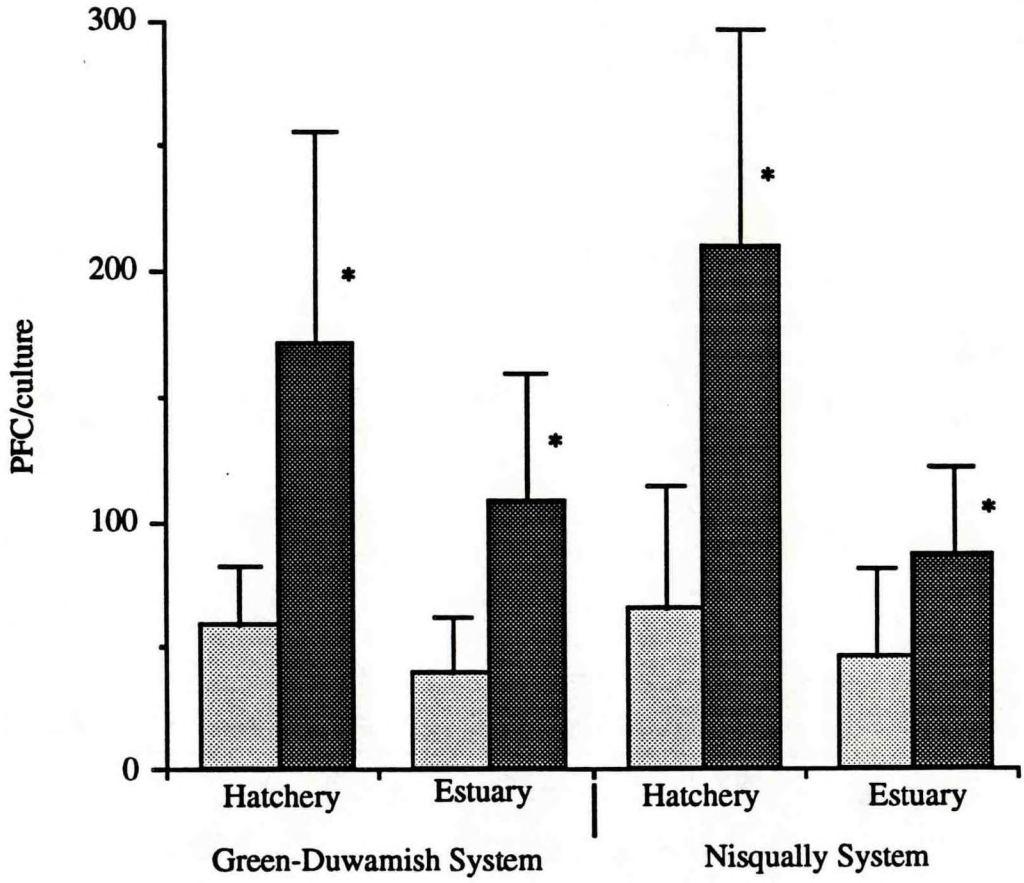

Figure 12. The primary (▨) and secondary (■) in vitro plaque forming cell response per culture (PFC/culture) to TNP-LPS generated in the spleen. The vertical bars represent one standard deviation above the mean. The mean PFC response was analyzed in chinook salmon from the Green-Duwamish system and the Nisqually system. Spleens from 10 chinook salmon were examined from each of the four locations. Spleen cells cultured only with tissue culture medium (background) did not produce more than a mean of 4 PFC/culture. *Indicate PFC/culture significantly higher than observed in the primary response. (Adapted from Arkoosh et al. 1991.)

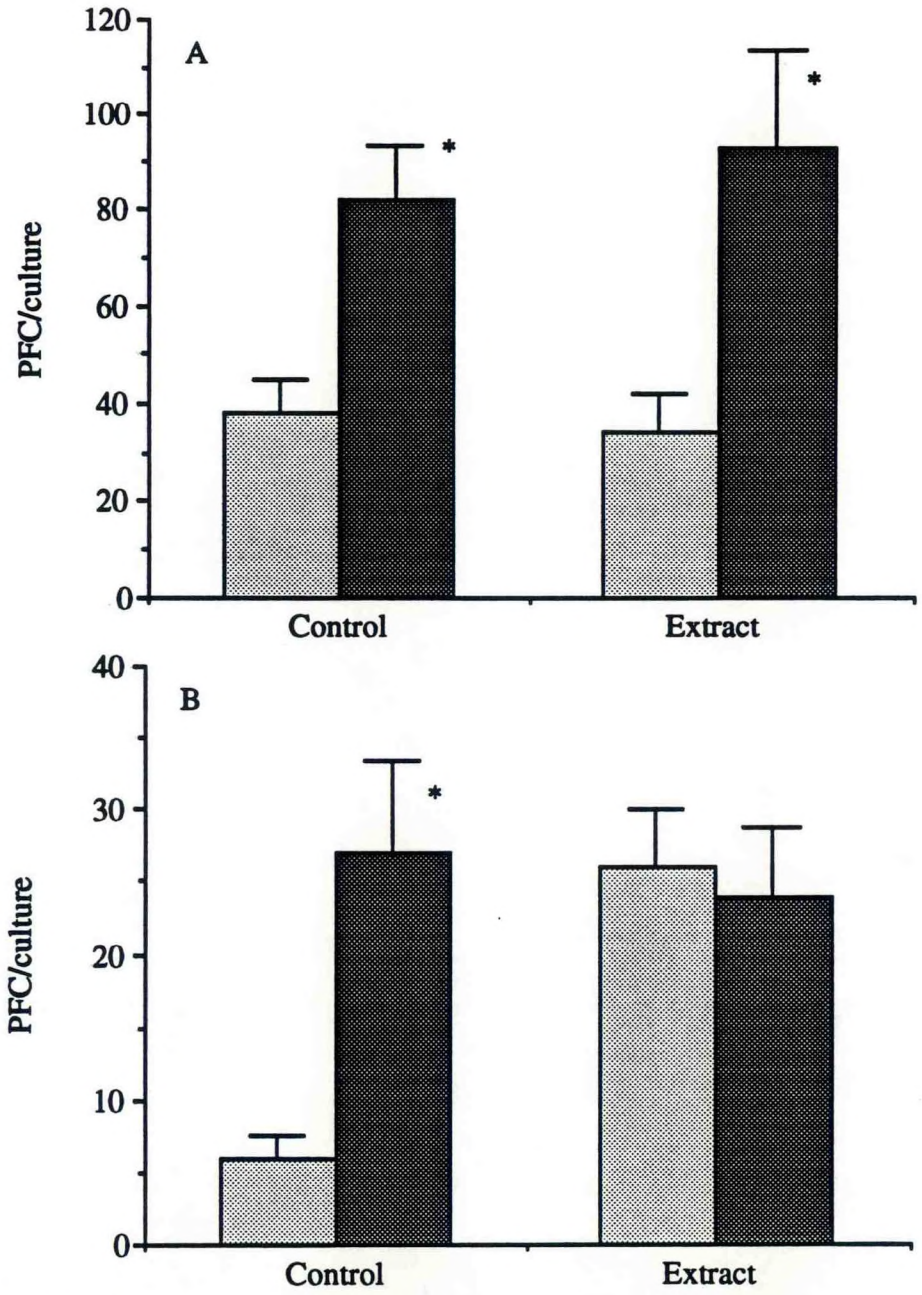

Figure 13. The primary (□) and secondary (■) in vitro plaque forming cell response per culture (PFC/culture) to TNP-LPS generated in the anterior kidney (A) and spleen (B). The salmon were either injected with Duwamish Waterway sediment extract with an emulphor carrier or with acetone and emulphor control. *Indicates PFC/culture significantly higher than observed in the primary response.

APPENDIX A

TABLES AND FIGURES

Table A-1. Concentrations, ng/g (ppb) wet weight, of aromatic contaminants in stomach contents of juvenile chinook salmon sampled in 1989. - [a,b,c,d,e,f,g,j]

Site name	Green River Hatchery	Duwamish River estuary			Nisqually Hatchery		Nisqually River estuary			Puyallup Hatchery
Date collected	5/8/89	5/23/89	6/1/89	6/7/89	5/22/89	6/12/89	6/20/89	6/27/89	7/7/89	5/24/89
Aromatic contaminants / Lab no.	63-283	63-279	63-280	63-281	63-284	63-285	63-336	63-337	63-338	63-286
naphthalene	17	26	1100	21	21	29	19	6	15	12
C1-naphthalenes	2	19	9800	29	23	5	7	5	7	17
C2-naphthalenes	< 0.7	11	37000	16	21	< 0.6	< 0.2	7	< 0.3	< 0.2
C3-naphthalenes	< 0.7	2	55000	0.6	7	< 0.6	< 0.2	3	< 0.3	< 0.2
C4-naphthalenes	< 0.7	< 0.4	40000	< 0.4	< 0.1	< 0.6	< 0.2	0.6	< 0.3	< 0.2
acenaphthylene	< 1	22	510	< 0.6	0.5	< 1	< 0.3	< 0.2	< 0.5	< 0.3
acenaphthene	< 2	65	1400	100	< 0.2	< 1	3	< 0.2	< 0.7	< 0.4
fluorene	< 2	82	33	170	2	< 1	< 0.4	< 0.2	< 0.6	< 0.4
C1-fluorenes	< 2	9	17000	< 0.8	< 0.2	< 1	< 0.4	< 0.2	< 0.6	< 0.4
C2-fluorenes	< 2	3	34000	< 0.8	< 0.2	< 1	< 0.4	< 0.2	< 0.6	< 0.4
C3-fluorenes	< 2	1	16000	< 0.8	< 0.2	< 1	< 0.4	1	< 0.6	< 0.4
phenanthrene	4	550	11000	850	29	11	3	4	5	11
C1-phenanthrenes/anthracenes	< 0.7	30	26000	< 0.3	19	< 0.6	< 0.2	2	< 0.3	< 0.2
C2-phenanthrenes/anthracenes	< 0.7	580	23000	200	14	< 0.6	< 0.2	3	< 0.3	< 0.2
C3-phenanthrenes/anthracenes	< 0.7	220	4700	56	< 0.1	< 0.6	< 0.2	0.5	< 0.3	< 0.2
C4-phenanthrenes/anthracenes	< 0.7	160	1800	< 0.3	< 0.1	< 0.6	< 0.2	< 0.09	< 0.3	< 0.2
dibenzothiophene	< 1	29	9500	79	0.7	< 0.8	< 0.3	0.3	< 0.4	< 0.2
C1-dibenzothiophenes	< 1	15	23000	56	< 0.1	< 0.8	< 0.3	< 0.1	< 0.4	< 0.2
C2-dibenzothiophenes	< 1	41	29000	94	< 0.1	< 0.8	< 0.3	< 0.1	< 0.4	< 0.2
C3-dibenzothiophenes	< 1	11	17000	93	< 0.1	< 0.8	< 0.3	< 0.1	< 0.4	< 0.2
Sum of LACs	23	1900	360000	1800	140	45	32	32	27	40
fluoranthene	< 0.8	11000	500	1700	9	< 0.7	1	3	0.9	0.8
pyrene	< 0.7	15000	91	990	8	< 0.6	1	4	0.6	1
C1-fluoranthenes/pyrenes	< 0.8	4300	2900	250	3	< 0.7	< 0.2	< 0.1	< 0.4	< 0.2
benz[a]anthracene	< 0.8	3300	910	250	0.7	< 0.7	< 0.3	< 0.1	< 0.4	< 0.2
chrysene	< 0.6	7500	1600	260	2	< 0.5	< 0.2	0.9	< 0.3	< 0.1
C1-chrysenes/benz[a]anthracenes	< 0.6	900	< 0.1	38	< 0.09	< 0.5	< 0.2	< 0.09	< 0.3	< 0.1
C2-chrysenes/benz[a]anthracenes	< 0.6	180	330	< 0.3	< 0.09	< 0.5	< 0.2	< 0.09	< 0.3	< 0.1
C3-chrysenes/benz[a]anthracenes	< 0.6	16	< 0.1	< 0.3	< 0.09	< 0.5	< 0.2	< 0.09	< 0.3	< 0.1
C4-chrysenes/benz[a]anthracenes	< 0.6	32	< 0.1	< 0.3	< 0.09	< 0.5	< 0.2	< 0.09	< 0.3	< 0.1
benzo[b]fluoranthene	< 0.6	4600	780	170	< 0.09	< 0.6	< 0.2	< 0.1	< 0.4	< 0.1
benzo[k]fluoranthene[i]	< 0.6	2000	320	71	< 0.08	< 0.5	< 0.2	< 0.08	< 0.3	< 0.1
benzo[a]pyrene	< 0.6	180	240	56	0.3	< 0.5	< 0.2	< 0.09	< 0.3	< 0.1
indeno[1,2,3-cd]pyrene	< 0.8	1500	320	24	< 0.1	< 0.7	< 0.3	< 0.1	< 0.4	< 0.2
dibenz[a,h]anthracene	< 0.9	83	35	2	< 0.1	< 0.8	< 0.2	< 0.1	< 0.4	< 0.2
benzo[ghi]perylene	< 0.6	1200	250	21	< 0.09	< 0.5	< 0.2	< 0.08	< 0.3	< 0.1
Sum of HACs	-	52000	8300	3800	23	-	2	8	2	2
Sample weight, grams	0.51	0.80	1.27	0.75	2.46	0.57	1.01	3.01	1.11	1.68

Footnotes follow

Table A-1. Continued. Concentrations, ng/g (ppb) wet weight, of aromatic contaminants in stomach contents of juvenile chinook salmon sampled in 1989. - [a,b,c,d,e,f,g,j]

Site name	Puyallup Hatchery		Commencement Bay			Skykomish Hatchery		Snohomish River estuary[h]		
Date collected	5/31/89	5/31/89	6/14/89	6/19/89	6/26/89	5/30/89	6/6/89	6/22/89	6/28/89	6/29/89
Aromatic contaminants / Lab no.	63-286[k]	63-287[k]	63-339	63-340	63-341	63-299	63-300	63-342	63-343	63-344
naphthalene	10	12	21	24	55	23	21	[39]	[96]	15
C1-naphthalenes	9	17	56	40	110	26	37	[17]	[31]	6
C2-naphthalenes	< 0.6	< 0.1	270	110	170	9	18	[31]	[30]	5
C3-naphthalenes	< 0.6	< 0.1	550	170	210	< 0.2	< 0.3	[12]	< 2	< 0.3
C4-naphthalenes	< 0.6	< 0.1	510	220	190	< 0.2	< 0.3	[6]	[2]	< 0.3
acenaphthylene	< 0.9	< 0.2	14	4	11	< 0.2	< 0.5	< 2	< 3	< 0.5
acenaphthene	< 1	< 0.3	4	16	100	< 0.3	< 0.7	< 2	[56]	3
fluorene	< 1	< 0.3	110	28	170	< 0.3	< 0.7	[3]	[90]	2
C1-fluorenes	< 1	< 0.3	170	74	83	< 0.3	< 0.7	[7]	[20]	< 0.6
C2-fluorenes	< 1	< 0.3	440	330	220	< 0.3	< 0.7	< 2	[45]	7
C3-fluorenes	< 1	< 0.3	190	180	110	< 0.3	< 0.7	[16]	< 4	< 0.6
phenanthrene	1	5	470	120	780	11	8	[43]	[600]	15
C1-phenanthrenes/anthracenes	< 0.5	< 0.1	210	150	160	4	< 0.3	[7]	[40]	0.5
C2-phenanthrenes/anthracenes	< 0.5	< 0.1	380	330	270	< 0.1	< 0.3	[19]	[13]	2
C3-phenanthrenes/anthracenes	< 0.5	< 0.1	120	140	130	< 0.1	< 0.3	[4]	[3]	< 0.3
C4-phenanthrenes/anthracenes	< 0.5	< 0.1	65	72	54	< 0.1	< 0.3	< 0.9	< 2	< 0.3
dibenzothiophene	< 0.8	< 0.2	86	40	86	< 0.2	< 0.4	[4]	[45]	0.7
C1-dibenzothiophenes	< 0.8	< 0.2	29	14	14	< 0.2	< 0.4	< 1	< 3	< 0.4
C2-dibenzothiophenes	< 0.8	< 0.2	130	170	95	< 0.2	< 0.4	[4]	< 3	< 0.4
C3-dibenzothiophenes	< 0.8	< 0.2	130	160	100	< 0.2	< 0.4	[6]	< 3	< 0.4
Sum of LACs	20	34	4000	2400	3100	73	84	[220]	[1100]	56
fluoranthene	< 0.6	< 0.2	720	260	1600	0.7	< 0.3	[53]	[340]	17
pyrene	< 0.6	< 0.2	490	220	930	1	< 0.3	[38]	[170]	11
C1-fluoranthenes/pyrenes	< 0.6	< 0.2	160	94	180	< 0.2	< 0.3	[3]	< 2	< 0.3
benz[a]anthracene	< 0.7	< 0.2	230	65	320	< 0.2	< 0.3	[1]	< 2	2
chrysene	< 0.5	< 0.1	400	100	370	< 0.1	< 0.3	[14]	[6]	3
C1-chrysenes/benz[a]anthracenes	< 0.5	< 0.1	120	50	110	< 0.1	< 0.3	[1]	< 2	< 0.3
C2-chrysenes/benz[a]anthracenes	< 0.5	< 0.1	54	80	48	< 0.1	< 0.3	< 0.9	< 2	< 0.3
C3-chrysenes/benz[a]anthracenes	< 0.5	< 0.1	32	77	22	< 0.1	< 0.3	< 0.9	< 2	< 0.3
C4-chrysenes/benz[a]anthracenes	< 0.5	< 0.1	7	5	2	< 0.1	< 0.3	< 0.9	< 2	< 0.3
benzo[b]fluoranthene	< 0.5	< 0.1	260	50	260	< 0.1	< 0.3	[9]	[3]	4
benzo[k]fluoranthene[i]	< 0.5	< 0.1	200	43	170	< 0.1	< 0.2	[2]	< 2	0.6
benzo[a]pyrene	< 0.5	< 0.1	130	35	90	< 0.1	< 0.2	< 0.9	< 2	< 0.2
indeno[1,2,3-cd]pyrene	< 0.7	< 0.2	120	41	69	< 0.2	< 0.4	< 1	< 2	< 0.3
dibenz[a,h]anthracene	< 0.8	< 0.2	23	5	14	< 0.2	< 0.4	< 1	< 2	< 0.3
benzo[ghi]perylene	< 0.5	< 0.1	98	28	54	< 0.1	< 0.3	< 0.9	< 1	< 0.2
Sum of HACs	-	-	3000	1200	4200	2	-	[120]	[520]	38
Sample weight, grams	1.50	1.94	1.56	0.69	0.90	2.30	1.20	0.28	0.13	0.87

Footnotes follow

Table A-1. Continued. Concentrations, ng/g (ppb) wet weight, of aromatic contaminants in stomach contents of juvenile chinook salmon sampled in 1989. - [a,b,c,d,e,f,g,j]

	Site name	Snohomish River estuary	Mukilteo Hatchery	Method blanks				NIST reference tissue (Mytilus edulis)[l]	Previously analyzed NIST reference tissue (n = 3)	
	Date collected	5/31/89	7/6/89							
Aromatic contaminants	Lab no.	63-286	63-301	63-290	63-304	63-326	63-348	63-327	mean	RSD[m]
naphthalene		13	8	1	3	4	9	3		
C1-naphthalenes		7	0.8	< 0.4	< 0.3	3	1			
C2-naphthalenes		4	< 0.4	< 0.4	< 0.3	< 0.06	4			
C3-naphthalenes		< 0.2	< 0.4	< 0.4	< 0.3	< 0.06	< 0.2			
C4-naphthalenes		0.4	< 0.4	< 0.4	< 0.3	< 0.06	< 0.2			
acenaphthylene		< 0.4	< 0.6	< 0.6	< 0.4	< 0.09	< 0.4	1		
acenaphthene		0.8	< 0.8	< 0.8	< 0.6	< 0.1	< 0.5	0.4		
fluorene		1	< 0.8	< 0.8	< 0.6	< 0.1	< 0.5	2		
C1-fluorenes		< 0.5	< 0.8	< 0.8	< 0.6	< 0.1	< 0.5			
C2-fluorenes		< 0.5	< 0.8	< 0.8	< 0.6	< 0.1	< 0.5			
C3-fluorenes		2	< 0.8	< 0.8	< 0.6	< 0.1	< 0.5			
phenanthrene		16	35	< 0.3	6	1	1	5	1	28
C1-phenanthrenes/anthracenes		1	34	< 0.3	< 0.3	< 0.06	< 0.2			
C2-phenanthrenes/anthracenes		3	46	< 0.3	< 0.3	< 0.06	< 0.2			
C3-phenanthrenes/anthracenes		< 0.2	10	< 0.3	< 0.3	< 0.06	< 0.2			
C4-phenanthrenes/anthracenes		< 0.2	< 0.3	< 0.3	< 0.3	< 0.06	< 0.2			
dibenzothiophene		1	< 0.5	< 0.5	< 0.4	0.3	< 0.3	1		
C1-dibenzothiophenes		< 0.3	7	< 0.5	< 0.4	< 0.1	< 0.3			
C2-dibenzothiophenes		< 0.3	23	< 0.5	< 0.4	< 0.1	< 0.3			
C3-dibenzothiophenes		< 0.3	2	< 0.5	< 0.4	< 0.1	< 0.3			
Sum of LACs		49	170	1	9	8	15			
fluoranthene		23	74	< 0.4	9	0.6	0.3	39	18	12
pyrene		21	38	< 0.4	6	0.5	< 0.3	37	17	24
C1-fluoranthenes/pyrenes		< 0.3	< 0.4	< 0.4	< 0.3	< 0.06	< 0.3			
benz[a]anthracene		2	2	< 0.4	< 0.3	< 0.08	< 0.3	10		
chrysene		11	11	< 0.3	< 0.2	< 0.06	< 0.2	20	1	0
C1-chrysenes/benz[a]anthracenes		< 0.2	< 0.3	< 0.3	< 0.2	< 0.06	< 0.2			
C2-chrysenes/benz[a]anthracenes		< 0.2	< 0.3	< 0.3	< 0.2	< 0.06	< 0.2			
C3-chrysenes/benz[a]anthracenes		< 0.2	< 0.3	< 0.3	< 0.2	< 0.06	< 0.2			
C4-chrysenes/benz[a]anthracenes		< 0.2	< 0.3	< 0.3	< 0.2	< 0.06	< 0.2			
benzo[b]fluoranthene		0.9	1	< 0.3	< 0.2	0.1	< 0.2	6		
benzo[k]fluoranthene[i]		1	< 0.3	< 0.3	< 0.2	0.09	< 0.2	3		
benzo[a]pyrene		< 0.5	< 0.3	< 0.3	< 0.2	0.07	< 0.2	2		
indeno[1,2,3-cd]pyrene		< 0.2	< 0.4	< 0.4	< 0.3	< 0.08	< 0.3	0.8		
dibenz[a,h]anthracene		< 0.2	< 0.5	< 0.5	< 0.3	< 0.08	< 0.2	< 0.7		
benzo[ghi]perylene		< 0.2	< 0.3	< 0.3	< 0.2	< 0.06	< 0.2	2		
Sum of HACs		59	130	-	15	1	0.3			
Sample weight, grams		1.09	0.99					3.02		

Footnotes follow

Table A-1. Continued. Concentrations, ng/g (ppb) wet weight, of aromatic contaminants in stomach contents of juvenile chinook salmon sampled in 1989.

Footnotes:

[a] The concentrations of the analytes and the percent recoveries of the surrogate standards were calculated using hexamethylbenzene as the internal standard.

[b] The alkylated compounds are not in the calibration solution. The response factors of the parent compound, e.g., naphthalene, phenanthrene are used to calculate the alkylated compounds, e.g., C1, C2, C3, C4 - naphthalenes and C1, C2, C3, C4 - phenanthrenes, respectively.

[c] Concentrations and initial identifications were determined using gas chromatography/mass spectrometry (GC/MS) - multiple ion detection.

[d] The "less than" symbol (<) indicates that the analyte was not detected in concentrations above the stated value.

[e] Low molecular weight aromatic contaminants (LACs) and high molecular weight aromatic contaminants (HACs) are 2,3-ring aromatic compounds and 4,7-ring aromatic compounds, respectively.

[f] Concentrations less than 10 are rounded to one significant figure; concentrations greater than 10 are rounded to two significant figures.

[g] Percent recoveries for the internal standards (surrogates) averaged 93%, CV = 16%, n = 81.

[h] The brackets indicate that due to the very small sample size the values should be considered semiquantitative.

[i] Benzo[k]fluoranthene and benzo[j]fluoranthene often coelute. This combined concentration is reported.

[j] The concentration of naphthalene was calculated using the surrogate standard naphthalene-d8; analytes from acenaphthylene through pyrene were calculated using acenaphthene-d10; analytes from benz[a]anthracene through benzo[ghi]perylene were calculated using benzo[a]pyrene-d12. The surrogate standards were used to calculate the concentrations of analytes so that the data can be compared to previously analyzed data.

[k] These samples are duplicate analyses. The data for the duplicate samples were somewhat different which implies that the samples were not homogeneous.

[l] Standard reference material from the National Institute of Science and Technology.

[m] RSD is the relative standard deviation which is the standard deviation divided by the mean and expressed as a percent.

Table A-2. Concentrations, ng/g (ppb) wet weight, of aromatic contaminants in stomach contents of juvenile chinook salmon sampled in 1990. - [a,b,c,d,e]

Aromatic contaminants	Site name	Green River Hatchery	Duwamish River estuary			Nisqually Hatchery	Nisqually River estuary[g]		Puyallup Hatchery	
	Date collected	5/8/90	5/24/90	6/1/90	6/18/90	5/29/90	6/13/90	6/14/90	6/4/90	6/6/90
	Lab no.	63-411	63-413	63-414	63-415	63-417	63-428	63-429	63-418	63-419
naphthalene		26	51	230	2900	60	1	3	21	21
C1-naphthalenes		29	100	920	4200	42	3	5	18	28
C2-naphthalenes		34	120	1600	3500	35	1	2	25	54
C3-naphthalenes		40	170	3500	1900	47	0.8	1	30	53
C4-naphthalenes		43	170	3900	770	10	< 0.2	< 0.2	12	31
acenaphthylene		3	29	12	98	6	0.8	0.8	3	3
acenaphthene		2	76	86	8500	5	0.7	0.9	2	3
fluorene		4	99	220	9100	8	0.8	0.9	4	5
C1-fluorenes		9	75	990	1500	13	< 0.3	< 0.3	5	10
C2-fluorenes		20	210	3000	930	7	< 0.3	< 0.3	15	25
C3-fluorenes		< 0.3	240	2800	120	< 2	< 0.3	< 0.3	7	< 0.2
phenanthrene		18	440	870	20000	13	1	2	11	17
C1-phenanthrenes/anthracenes		37	360	2300	4700	11	1	1	15	25
C2-phenanthrenes/anthracenes		50	480	2500	2100	9	1	1	17	30
C3-phenanthrenes/anthracenes		65	380	2400	750	15	0.7	1	7	13
C4-phenanthrenes/anthracenes		17	150	990	210	< 1	< 0.1	< 0.1	< 0.3	8
dibenzothiophene		5	41	270	2300	2	< 0.3	< 0.3	2	4
C1-dibenzothiophenes		14	61	1200	690	3	0.5	0.3	6	12
C2-dibenzothiophenes		19	150	2600	450	3	1	0.4	8	15
C3-dibenzothiophenes		18	150	2300	230	< 1	0.8	0.8	7	13
Sum of LACs		450	3600	33000	65000	290	14	20	220	370
fluoranthene		4	1200	910	17000	7	2	1	6	7
pyrene		10	660	730	9200	7	1	1	6	7
C1-fluoranthenes/pyrenes		25	1200	1100	4900	9	2	0.6	4	7
benz[a]anthracene		2	520	210	2500	1	0.3	0.3	1	2
chrysene		4	1200	320	1900	2	2	0.6	2	3
C1-chrysenes/benz[a]anthracenes		10	340	410	810	1	0.4	0.3	1	5
C2-chrysenes/benz[a]anthracenes		10	100	350	220	< 0.6	< 0.1	< 0.1	< 0.2	1
C3-chrysenes/benz[a]anthracenes		7	36	260	55	< 0.6	< 0.1	< 0.1	< 0.2	1
C4-chrysenes/benz[a]anthracenes		0.6	< 0.2	110	< 0.5	< 0.6	< 0.1	< 0.1	< 0.2	< 0.1
benzo[b]fluoranthene		0.6	360	200	780	1	< 0.1	< 0.1	1	2
benzo[k]fluoranthene[f]		0.4	310	130	670	0.8	0.2	0.2	0.8	0.7
benzo[a]pyrene		0.6	150	82	390	0.8	0.2	< 0.1	0.8	0.9
indeno[1,2,3-cd]pyrene		0.3	100	83	160	0.9	0.2	< 0.1	0.7	0.6
dibenz[a,h]anthracene		0.2	30	23	52	0.6	< 0.1	< 0.1	0.2	0.2
benzo[ghi]perylene		0.7	110	150	100	1	0.3	0.2	2	0.6
Sum of HACs		75	6300	5100	39000	32	9	4	26	38
Sample weight, grams		2.99	1.39	0.75	0.56	0.60	2.84	2.69	1.34	3.02

Footnotes follow

Table A-2. Continued. Concentrations, ng/g (ppb) wet weight, of aromatic contaminants in stomach contents of juvenile chinook salmon sampled in 1990. - [a,b,c,d,e]

	Site name	Commencement (City Waterway)			Method blanks				NIST reference tissue (Mytilus edulis)[j]		Previously analyzed NIST tissue SRM 1974 (n = 124)	
Aromatic contaminants	Date collected / Lab no.	6/26/90 63-430	6/28/90 63-431[h]	6/28/90 63-432[h]	63-421	63-422	63-434	63-435	63-420	63-433	mean	RSD[i]
naphthalene		55	130	180	10	12	6	5	6	5	4	38
C1-naphthalenes		120	1200	1100	4	5	2	2	4	3	3	44
C2-naphthalenes		290	2700	3000	2	2	< 0.3	< 0.3	3	4	4	50
C3-naphthalenes		390	4500	5300	< 0.5	< 0.4	< 0.3	< 0.3	6	9	8	39
C4-naphthalenes		370	3600	4200	< 0.5	< 0.4	< 0.3	< 0.3	27	24	24	41
acenaphthylene		20	190	210	1	1	< 0.4	0.7	2	1	0.7	54
acenaphthene		78	4500	4500	< 0.7	< 0.6	< 0.5	< 0.5	0.9	1	0.8	37
fluorene		130	6800	7100	< 0.6	0.9	< 0.4	< 0.4	2	2	1	43
C1-fluorenes		140	2300	2500	< 0.6	< 0.5	< 0.4	< 0.4	6	6	4	31
C2-fluorenes		320	3100	3500	< 0.6	< 0.5	< 0.4	< 0.4	31	31	21	36
C3-fluorenes		360	2600	2600	< 0.6	< 0.5	< 0.4	< 0.4	65	42	24	61
phenanthrene		540	18000	18000	2	2	0.9	< 0.2	5	8	6	26
C1-phenanthrenes/anthracenes		470	5500	7200	< 0.3	1	< 0.2	< 0.2	9	9	11	33
C2-phenanthrenes/anthracenes		560	3500	4700	< 0.3	< 0.2	< 0.2	< 0.2	46	40	45	33
C3-phenanthrenes/anthracenes		670	2000	2800	< 0.3	< 0.2	< 0.2	< 0.2	60	52	49	35
C4-phenanthrenes/anthracenes		690	600	940	< 0.3	< 0.2	< 0.2	< 0.2	31	26	12	72
dibenzothiophene		49	2100	2200	< 0.3	0.4	< 0.2	< 0.2	1	1	0.9	38
C1-dibenzothiophenes		87	1700	1900	< 0.3	< 0.3	< 0.2	< 0.2	7	5	6	35
C2-dibenzothiophenes		250	2400	2500	< 0.3	< 0.3	< 0.2	< 0.2	39	35	30	34
C3-dibenzothiophenes		270	1800	1900	< 0.3	< 0.3	< 0.2	< 0.2	47	43	35	37
Sum of LACs		5900	69000	76000	19	24	9	8	400	350	270	40
fluoranthene		950	20000	24000	1	0.8	0.8	0.7	46	47	46	16
pyrene		770	11000	18000	0.8	0.6	0.7	0.5	44	46	44	16
C1-fluoranthenes/pyrenes		550	5100	9500	< 0.3	< 0.3	< 0.2	< 0.2	34	33	25	26
benz[a]anthracene		150	2900	7800	< 0.2	< 0.2	< 0.2	< 0.2	6	7	5	23
chrysene		330	2100	6800	< 0.2	< 0.2	0.4	0.4	18	20	18	14
C1-chrysenes/benz[a]anthracenes		220	900	4800	< 0.2	< 0.2	0.3	< 0.1	11	11	7	34
C2-chrysenes/benz[a]anthracenes		130	320	2000	< 0.2	< 0.2	< 0.1	< 0.1	5	6	3	70
C3-chrysenes/benz[a]anthracenes		110	130	810	< 0.2	< 0.2	< 0.1	< 0.1	3	3	1	84
C4-chrysenes/benz[a]anthracenes		31	30	140	< 0.2	< 0.2	< 0.1	< 0.1	< 0.1	0.3	0.6	89
benzo[b]fluoranthene		190	1400	4700	< 0.1	< 0.1	0.3	< 0.2	7	8	7	31
benzo[k]fluoranthene[f]		170	1000	3900	< 0.1	< 0.1	0.3	< 0.2	5	6	4	28
benzo[a]pyrene		66	730	4100	< 0.1	< 0.1	< 0.2	< 0.1	2	3	2	51
indeno[1,2,3-cd]pyrene		76	420	3100	< 0.1	< 0.2	< 0.2	< 0.1	2	3	2	52
dibenz[a,h]anthracene		17	55	910	< 0.1	< 0.2	< 0.1	< 0.1	0.5	0.9	0.4	64
benzo[ghi]perylene		75	270	2200	< 0.1	< 0.2	< 0.2	< 0.1	3	6	2	44
Sum of HACs		3800	46000	93000	2	1	3	2	190	200	160	17
Sample weight, grams		0.71	1.52	2.33					3.02	3.00		

Footnotes follow

Table A-2. Continued. Concentrations, ng/g (ppb) wet weight, of aromatic contaminants in stomach contents of juvenile chinook salmon sampled in 1990.

Footnotes:

[a] The concentrations of the analytes and the percent recoveries of the surrogate standards were calculated using hexamethylbenzene as the internal standard.

[b] Concentrations and initial identifications were determined using gas chromatography/mass spectrometry (GC/MS) - multiple ion detection.

[c] The "less than" symbol (<) indicates that the analyte was not detected in concentrations above the stated value.

[d] Concentrations less than 10 are rounded to one significant figure; concentrations greater than 10 are rounded to two significant figures.

[e] Percent recoveries for the internal standards (surrogates) averaged 91%, CV = 18%, n = 58. Percent recoveries of the surrogates include quality control samples.

[f] Benzo[k]fluoranthene and benzo[j]fluoranthene often coelute. This combined concentration is reported.

[g] Blank data were subtracted from the sample, and the results are reported here.

[h] These samples are duplicate analyses. The data for the duplicate samples were somewhat different which implies that the samples were not homogeneous.

[i] RSD is the relative standard deviation which is the standard deviation divided by the mean and expressed as a percent.

[j] Standard reference material from the National Institute of Science and Technology.

Table A-3. Concentrations, ng/g (ppb) wet weight, of chlorinated contaminants in stomach contents of juvenile chinook salmon sampled in 1989. - [a,b,c,d,e,g,j]

Site name	Green River Hatchery	Duwamish River estuary			Nisqually Hatchery		Nisqually River estuary			Puyallup Hatchery
Date collected	5/8/89	5/23/89	6/1/89	6/7/89	5/22/89	6/12/89	6/20/89	6/27/89	7/7/89	5/24/89
Chlorinated contaminants Lab no.	63-283	63-279	63-280	63-281	63-284	63-285	63-336	63-337	63-338	63-286
hexachlorobenzene	3	< 0.4	< 0.2	0.8	4	2	< 0.4	< 0.2	< 0.6	2
lindane (gamma-BHC)	< 1	< 0.6	< 0.2	< 0.6	< 0.2	< 0.9	< 0.7	< 0.3	< 1	< 0.2
heptachlor	< 0.9	< 0.5	< 0.2	< 0.5	< 0.1	< 0.7	< 0.6	< 0.3	< 0.8	< 0.2
aldrin[f]	< 1	< 0.6	< 0.2	< 0.6	< 0.2	< 0.8	< 0.7	< 0.3	< 1	< 0.2
alpha-chlordane	3	3	1	1	4	2	< 0.5	< 0.2	< 0.7	2
dieldrin	< 0.9	< 0.5	< 0.2	< 0.5	< 0.1	< 0.7	< 0.6	< 0.3	< 0.8	< 0.2
p,p'-DDE	19	38	14	11	47	17	3	2	1	16
p,p'-DDD	5	7	4	2	6	3	< 0.9	< 0.4	< 1	2
p,p'-DDT	3	5	4	1	3	2	< 0.7	< 0.3	< 1	1
trichlorobiphenyls	5	15	23	10	2	4	4	1	2	2
tetrachlorobiphenyls	35	89	89	63	29	21	11	3	5	17
pentachlorobiphenyls	47	110	96	73	48	27	12	4	6	20
hexachlorobiphenyls	32	69	85	59	36	27	7	7	2	10
heptachlorobiphenyls	15	31	52	23	16	7	2	1	< 0.4	5
octachlorobiphenyls	3	4	8	3	1	1	< 0.2	< 0.1	< 0.4	0.4
nonachlorobiphenyls	1	< 0.3	< 0.09	0.9	0.3	< 0.4	< 0.2	< 0.1	< 0.3	0.3
decachlorobiphenyls	3	< 0.3	< 0.1	0.6	0.4	< 0.5	< 0.3	< 0.1	< 0.4	< 0.1
Sum of PCBs	140	320	350	230	130	87	36	16	15	55
trichlorobutadienes	< 2	< 0.9	< 0.3	< 0.9	< 0.3	< 1	< 1	< 0.5	< 2	< 0.4
tetrachlorobutadienes	< 2	< 1	< 0.4	< 1	< 0.3	< 2	< 1	< 0.6	< 2	< 0.5
pentachlorobutadienes	< 1	< 0.7	< 0.3	< 0.7	< 0.2	< 1	< 0.8	< 0.4	< 1	< 0.3
hexachlorobutadiene	< 0.8	< 0.4	< 0.1	< 0.4	< 0.1	< 0.6	< 0.5	< 0.2	< 0.7	< 0.2
Sample weight, grams	0.51	0.80	1.27	0.75	2.46	0.57	1.01	3.01	1.11	1.68

Footnotes follow

Table A-3. Continued. Concentrations, ng/g (ppb) wet weight, of chlorinated contaminants in stomach contents of juvenile chinook salmon sampled in 1989. - [a,b,c,d,e,g,j]

Chlorinated contaminants	Site name	Puyallup Hatchery		Commencement Bay			Skykomish Hatchery		Snohomish River estuary[h]		
	Date collected	5/31/89	5/31/89	6/14/89	6/19/89	6/26/89	5/30/89	6/6/89	6/22/89	6/28/89	6/29/89
	Lab no.	63-286[i]	63-287[i]	63-339	63-340	63-341	63-299	63-300	63-342	63-343	63-344
hexachlorobenzene		1	2	2	2	1	2	2	< 2	< 4	< 0.6
lindane (gamma-BHC)		< 0.7	< 0.3	< 0.6	< 1	< 1	< 0.3	< 0.3	< 3	< 6	< 1
heptachlor		< 0.6	< 0.2	< 0.5	< 0.9	< 0.9	< 0.2	< 0.3	< 3	< 5	< 0.8
aldrin[f]		< 0.7	< 0.2	< 2	< 4	< 1	< 0.2	< 0.3	< 4	< 6	< 1
alpha-chlordane		1	1	2	3	1	2	2	< 2	< 4	< 0.6
dieldrin		< 0.6	< 0.2	< 0.5	< 0.9	< 0.9	< 0.2	< 0.3	< 3	< 5	< 0.8
p,p'-DDE		9	15	24	25	8	13	16	< 3	< 5	2
p,p'-DDD		2	2	5	7	< 1	2	3	< 4	< 8	< 1
p,p'-DDT		0.9	2	7	5	1	1	1	< 3	< 6	< 0.9
trichlorobiphenyls		2	2	4	16	7	3	7	[14]	[16]	4
tetrachlorobiphenyls		6	13	42	56	28	13	15	[44]	[41]	12
pentachlorobiphenyls		17	43	55	62	31	16	23	[63]	[33]	14
hexachlorobiphenyls		12	15	78	54	20	16	23	[33]	[12]	8
heptachlorobiphenyls		3	6	53	26	7	6	8	[10]	[6]	4
octachlorobiphenyls		< 0.4	0.5	7	4	1	0.4	0.6	< 1	< 2	0.4
nonachlorobiphenyls		< 0.3	0.7	0.5	0.5	< 0.4	0.1	0.4	< 1	< 2	< 0.3
decachlorobiphenyls		< 0.4	< 0.1	0.6	< 0.4	< 0.4	< 0.1	< 0.2	< 1	< 2	< 0.4
Sum of PCBs		40	80	240	220	94	54	77	[160]	[110]	42
trichlorobutadienes		< 1	< 0.4	< 1	< 2	< 2	< 0.2	< 0.3	< 6	< 10	< 2
tetrachlorobutadienes		< 1	< 0.5	< 1	< 2	< 2	< 0.2	< 0.4	< 7	< 10	< 2
pentachlorobutadienes		< 0.9	< 0.3	< 0.7	< 1	< 1	< 0.1	< 0.3	< 4	< 8	< 1
hexachlorobutadiene		< 0.5	< 0.2	< 0.4	< 0.8	< 0.8	< 0.09	< 0.2	< 3	< 5	< 0.7
Sample weight, grams		1.50	1.94	1.56	0.69	0.90	2.30	1.20	0.28	0.13	0.87

Footnotes follow

Table A-3. Continued. Concentrations, ng/g (ppb) wet weight, of chlorinated contaminants in stomach contents of juvenile chinook salmon sampled in 1989. - [a,b,c,d,e,g,j]

	Site name	Snohomish River estuary	Mukilteo Hatchery	Method blanks				NIST reference tissue (Mytilus edulis)[k]	Previously analyzed NIST reference tissue (n = 21)	
	Date collected	5/31/89	7/6/89							
Chlorinated contaminants	Lab no.	63-286	63-301	63-290	63-304	63-326	63-348	63-327	mean	RSD[i]
hexachlorobenzene		< 0.4	0.8	< 0.3	< 0.3	< 0.2	< 0.6	< 0.1	0.6	38
lindane (gamma-BHC)		< 0.8	< 0.3	< 0.4	< 0.3	< 0.3	< 1	< 0.2	0.3	34
heptachlor		< 0.6	< 0.2	< 0.3	< 0.3	< 0.2	< 0.8	< 0.1	0.6	13
aldrin[f]		< 0.8	< 0.3	< 0.3	< 0.4	< 0.3	< 1	< 0.2	2	46
alpha-chlordane		< 0.5	0.6	< 0.2	< 0.3	< 0.2	< 0.7	3	3	11
dieldrin		< 0.6	< 0.2	< 0.3	< 0.3	< 0.2	< 0.8	3	4	28
p,p'-DDE		4	6	< 0.3	< 0.3	< 0.2	< 0.8	6	8	23
p,p'-DDD		< 1	1	< 0.4	< 0.4	< 0.3	< 1	6	8	20
p,p'-DDT		< 0.7	0.7	< 0.4	< 0.4	< 0.2	< 1	0.4	0.6	16
trichlorobiphenyls		2	4	1	3	2	2	17	21	6
tetrachlorobiphenyls		21	26	6	1	4	8	130	170	11
pentachlorobiphenyls		28	22	4	7	2	6	97	120	12
hexachlorobiphenyls		18	18	2	3	2	0.6	44	56	20
heptachlorobiphenyls		6	5	0.8	1	0.6	< 0.4	8	12	23
octachlorobiphenyls		0.4	0.9	< 0.2	< 0.2	< 0.09	< 0.4	0.2	0.4	85
nonachlorobiphenyls		< 0.3	0.3	< 0.2	< 0.2	< 0.08	< 0.3	< 0.06	0.6	80
decachlorobiphenyls		< 0.3	< 0.2	< 0.2	< 0.2	0.1	< 0.4	< 0.06	0.6	92
Sum of PCBs		75	76	14	15	11	17			
trichlorobutadienes		< 1	< 0.2	< 0.6	< 0.4	< 0.3	< 2			
tetrachlorobutadienes		< 1	< 0.3	< 0.7	< 0.5	< 0.4	< 2			
pentachlorobutadienes		< 0.9	< 0.2	< 0.4	< 0.3	< 0.3	< 1			
hexachlorobutadiene		< 0.6	< 0.1	< 0.3	< 0.2	< 0.1	< 0.7			
Sample weight, grams		1.09	0.99					3.02		

Footnotes follow

Table A-3. Continued. Concentrations, ng/g (ppb) wet weight, of chlorinated contaminants in stomach contents of juvenile chinook salmon sampled in 1989.

Footnotes:

[a] The concentrations of the analytes and the percent recoveries of the surrogate standards were calculated using terachloro-m-xylene as the internal standard.

[b] Concentrations and initial identifications were determined using gas chromatography/mass spectrometry (GC/MS) with electron capture detection (ECD).

[c] The "less than" symbol (<) indicates that the analyte was not detected in concentrations above the stated value.

[d] Concentrations less than 10 are rounded to one significant figure; concentrations greater than 10 are rounded to two significant figures.

[e] Percent recoveries for the internal standards (surrogates) averaged 120%, CV = 8%, n = 27.

[f] Aldrin coelutes with unknown compound. The concentration would not be higher than stated value.

[g] Identification of analyte was confirmed by gas chromatography/mass spectrometry.

[h] The brackets indicate that due to the very small sample size the values should be considered semiquantitative.

[i] These samples are duplicate analyses. The data for the duplicate samples were somewhat different which implies that the samples were not homogeneous.

[j] The percent recovery of the surrogate standard was calculated using tetrachloro-m-xylene as the internal standard.

[k] Standard reference material from the National Institute of Science and Technology.

[l] RSD is the relative standard deviation which is the standard deviation divided by the mean and expressed as a percent.

Table A-4. Concentrations, ng/g (ppb) wet weight, of chlorinated contaminants in stomach contents of juvenile chinook salmon sampled in 1990. - [a,b,c,d,e]

	Site name	Green River Hatchery	Duwamish River estuary			Nisqually Hatchery	Nisqually River estuary[g]		Puyallup Hatchery	
Chlorinated contaminants	Date collected / Lab no.	5/8/90 63-411	5/24/90 63-413	6/1/90 63-414	6/18/90 63-415	5/29/90 63-417	6/13/90 63-428	6/14/90 63-429	6/4/90 63-418	6/6/90 63-419
hexachlorobenzene		1	0.8	4	0.3	3	< 0.1	0.5	3	3
lindane (gamma-BHC)		< 0.1	< 0.1	5	< 0.3	0.7	< 0.1	< 0.1	< 0.1	< 0.1
heptachlor		< 0.1	< 0.1	< 0.2	< 0.3	< 0.2	< 0.1	< 0.1	< 0.2	< 0.1
aldrin[f]		< 0.1	< 0.1	6	< 0.3	< 0.2	< 0.1	< 0.1	< 0.1	< 0.1
alpha-chlordane		1	0.7	3	1	3	1	0.4	4	3
dieldrin		0.8	0.7	11	1	2	0.9	0.3	2	1
p,p'-DDE		11	3	20	6	37	11	5	48	27
p,p'-DDD		1	0.3	4	2	2	1	0.6	5	3
p,p'-DDT		0.7	0.7	2	1	2	0.5	0.2	2	1
trichlorobiphenyls		2	20	13	7	5	0.3	0.7	1	0.9
tetrachlorobiphenyls		16[g]	31	95[h]	94	55	3	7	52	70
pentachlorobiphenyls		14	18	130	65	54	17	8	37	24
hexachlorobiphenyls		9	16	75	69	28	14	7	33	21
heptachlorobiphenyls		6	6	83	39	13	5	2	13	12
octachlorobiphenyls		< 0.1	0.2	1	0.8	< 0.2	< 0.1	< 0.1	0.2	< 0.1
nonachlorobiphenyls		0.2	< 0.1	1	0.4	0.5	< 0.1	< 0.1	0.5	< 0.1
decachlorobiphenyls		< 0.1	0.3	1	0.8	0.5	0.2	< 0.1	< 0.1	< 0.1
Sum of PCBs		47	92	400	280	160	40	25	140	130
trichlorobutadienes		< 0.1	< 0.2	< 0.5	< 0.5	< 0.5	< 0.1	< 0.1	< 0.2	< 0.1
tetrachlorobutadienes		< 0.1	< 0.4	< 0.5	< 1	< 0.5	< 0.1	< 0.1	< 0.4	< 0.2
pentachlorobutadienes		< 0.1	< 0.2	< 0.4	< 0.4	< 0.4	< 0.1	< 0.1	< 0.2	< 0.1
hexachlorobutadiene		< 0.1	< 0.1	< 0.2	< 0.3	< 0.2	< 0.1	< 0.1	< 0.1	< 0.1
Sample weight, grams		2.99	1.39	0.75	0.56	0.60	2.84	2.69	1.34	3.02

Footnotes follow

Table A-4. Continued. Concentrations, ng/g (ppb) wet weight, of chlorinated contaminants in stomach contents of juvenile chinook salmon sampled in 1990. - [a,b,c,d,e]

	Site name	Commencement (City Waterway)			Method blanks				NIST reference tissue (Mytilus edulis)[j]		Previously analyzed NIST reference tissue (n = 32)	
Chlorinated contaminants	Date collected / Lab no.	6/26/90 63-430	6/28/90 63-431[i]	6/28/90 63-432[i]	63-421	63-422	63-434	63-435	63-420	63-433	mean	RSD[i]
hexachlorobenzene		1	3	3	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.3	160
lindane (gamma-BHC)		0.4	0.2	0.8	< 0.1	< 0.2	< 0.1	< 0.1	< 0.1	< 0.1	0.2	98
heptachlor		< 0.2	< 0.1	2	< 0.2	< 0.2	< 0.1	< 0.1	0.2	< 0.1	0.4	130
aldrin[f]		< 0.1	< 0.1	< 0.1	< 0.1	< 0.2	< 0.1	< 0.1	< 0.1	< 0.1	2	58
alpha-chlordane		2	4	13	< 0.1	< 0.1	< 0.1	< 0.1	3	3	3	26
dieldrin		7	15	18	< 0.1	< 0.1	< 0.1	< 0.1	2	2	2	60
p,p'-DDE		5	35	34	< 0.1	< 0.1	< 0.1	< 0.1	5	7	5	29
p,p'-DDD		2	3	6	< 0.2	< 0.2	< 0.1	< 0.1	5	7	5	41
p,p'-DDT		0.8	2	12	< 0.1	< 0.2	< 0.1	< 0.1	0.7	0.4	0.5	77
trichlorobiphenyls		38	15	13	1	1	0.5	0.6	18	15	22	25
tetrachlorobiphenyls		38	42	170	8	6	4	4	190	160	180	16
pentachlorobiphenyls		64	48	130	0.5	1	1	1	75	110	120	19
hexachlorobiphenyls		28	40	51	2	2	1	1	49	51	52	20
heptachlorobiphenyls		17	38	97	0.2	0.3	< 0.1	0.3	10	10	10	30
octachlorobiphenyls		0.4	0.5	1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.4	110
nonachlorobiphenyls		1	0.5	1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.3	95
decachlorobiphenyls		1	2	16	< 0.1	< 0.2	< 0.1	< 0.1	< 0.1	< 0.1	0.3	160
Sum of PCBs		190	190	480	12	10	6	7	340	350	350	17
trichlorobutadienes		< 0.6	< 0.3	< 0.3	< 0.2	< 0.2	< 0.2	< 0.2	< 0.1	< 0.1	-	-
tetrachlorobutadienes		< 0.8	< 0.4	< 0.3	< 0.4	< 0.4	< 0.2	< 0.2	< 0.2	< 0.1	-	-
pentachlorobutadienes		< 0.5	< 0.2	< 0.2	< 0.2	< 0.2	< 0.1	< 0.1	< 0.1	< 0.1	-	-
hexachlorobutadiene		< 0.4	< 0.2	< 0.1	< 0.1	< 0.2	< 0.1	< 0.1	< 0.1	< 0.1	-	-
Sample weight, grams		0.71	1.52	2.33					3.02	3.00		

Footnotes follow

Table A-4. Continued. Concentrations, ng/g (ppb) wet weight, of chlorinated contaminants in stomach contents of juvenile chinook salmon sampled in 1990. - [a,b,c,d,e].

Footnotes:

[a] The concentrations of the analytes and the percent recovery of the surrogate standard were calculated using tetrachloro-m-xylene as the internal standard.

[b] The "less than" symbol (<) indicates that the analyte was not detected in concentrations above the stated value.

[c] Concentrations and initial identifications were determined using gas chromatography (GC) with electron capture detection (ECD).

[d] Concentrations less than 10 are rounded to one significant figure; concentrations greater than 10 are rounded to two significant figures.

[e] Percent recovery for the internal standard (surrogate) averaged 94%, CV = 11%, n = 18. Percent recovery of the surrogate includes quality control samples.

[f] Aldrin coelutes with an unknown compound. The concentration would not be higher than the stated value.

[g] A large unidentified peak appeared at 45.03 minutes on the GC/ECD chromatogram.

[h] A large unidentified peak appeared at 47.28 minutes on the GC/ECD chromatogram.

[i] These samples are duplicate analyses. The data for the duplicate samples are not identical which implies that the samples were not completely homogeneous.

[j] Standard reference material from the National Institute of Science and Technology.

[k] RSD is the relative standard deviation which is the standard deviation divided by the mean and expressed as a percent.

Table A-5. Concentrations, ng/g (ppb) wet weight, of chlorinated contaminants in livers of juvenile chinook salmon sampled in 1989. - [a,b,c,d,e,g,h]

Chlorinated contaminants	Site name	Green River Hatchery		Duwamish River estuary			Nisqually Hatchery	Nisqually River estuary				Puyallup Hatchery
	Date collected	5/1/89	5/8/89	5/23/89	6/1/89	6/7/89	6/12/89	6/20/89	6/27/89	7/7/89	7/7/89	5/24/89
	Lab no.	63-138	63-139	63-177	63-172	63-178	63-142	63-173	63-174	63-140	63-179	63-195
hexachlorobenzene		< 0.2	0.9	2	2	1	1	0.8	1	0.7	0.6	0.8
lindane (gamma-BHC)		< 0.3	< 0.2	< 0.5	< 0.3	< 0.3	< 0.1	< 0.3	< 0.2	< 0.1	< 0.2	< 0.2
heptachlor		< 0.2	< 0.1	< 0.6	< 0.5	< 0.3	< 0.09	< 0.2	< 0.2	< 0.1	< 0.2	< 0.2
aldrin[f]		< 0.3	< 0.2	< 0.6	< 0.4	< 0.3	< 0.1	< 0.3	< 0.2	< 0.1	< 0.2	< 0.2
alpha-chlordane		0.5	0.7	3	3	1	1	0.7	1	0.7	0.3	0.5
dieldrin		1	0.7	2	0.9	1	0.3	0.3	0.6	0.2	0.3	< 0.2
p,p'-DDE		9	8	43	46	23	10	18	26	17	20	8
p,p'-DDD		5	2	6	7	3	2	2	3	2	2	1
p,p'-DDT		0.4	0.3	3	9	2	0.3	0.7	1	0.4	0.4	0.3
trichlorobiphenyls		6	5	15	25	12	4	5	6	5	8	4
tetrachlorobiphenyls		13	10	87	130	51	9	26	20	16	22	5
pentachlorobiphenyls		14	11	140	180	87	11	43	38	30	39	6
hexachlorobiphenyls		10	8	110	160	86	8	37	34	41	32	4
heptachlorobiphenyls		4	3	57	81	40	4	14	17	10	13	2
octachlorobiphenyls		0.7	0.6	8	17	7	0.4	4	3	3	3	0.5
nonachlorobiphenyls		0.2	0.4	2	2	0.6	0.08	0.6	0.5	0.3	0.4	< 0.1
decachlorobiphenyls		0.3	0.2	2	3	0.7	0.2	1	0.5	0.4	0.6	0.2
Sum of PCBs		48	38	420	600	280	37	130	120	110	120	22
trichlorobutadienes		< 0.4	< 0.2	< 0.9	< 0.6	< 0.5	< 0.2	< 0.5	< 0.3	< 0.2	< 0.3	< 0.4
tetrachlorobutadienes		< 0.5	< 0.3	< 1	< 0.7	< 0.6	< 0.2	< 0.6	< 0.4	< 0.3	< 0.4	< 0.4
pentachlorobutadienes		< 0.3	< 0.2	< 0.7	< 0.4	< 0.4	< 0.1	< 0.4	< 0.2	< 0.2	< 0.2	< 0.3
hexachlorobutadiene		< 0.2	< 0.1	< 0.4	< 0.3	< 0.2	< 0.07	< 0.2	< 0.1	< 0.1	< 0.1	< 0.2
Sample weight, grams		1.72	2.82	0.70	1.05	1.23	3.22	1.19	1.56	3.08	1.78	2.28

Footnotes follow

Table A-5. Continued. Concentrations, ng/g (ppb) wet weight, of chlorinated contaminants in livers of juvenile chinook salmon sampled in 1989. - [a,b,c,d,e,g,h]

Chlorinated contaminants	Site name	Puyallup Hatchery	Puyallup River estuary			Skykomish Hatchery		Snohomish River estuary				
	Date collected	5/31/89	6/14/89	6/19/89	6/26/89	5/30/89	6/6/89	6/22/89	6/28/89	6/29/89	6/30/89	7/11/89
	Lab no.	63-196	63-175	63-143	63-144	63-176	63-136	63-197	63-198	63-199	63-200	63-137
hexachlorobenzene		0.9	2	10	7	0.8	0.8	1	0.7	1	0.8	1
lindane (gamma-BHC)		< 0.1	< 0.3	< 0.3	< 0.6	< 0.4	< 0.4	< 0.7	< 0.4	< 0.2	< 0.6	< 0.5
heptachlor		< 0.1	< 0.3	< 0.2	< 0.5	< 0.4	< 0.3	< 0.6	< 0.4	< 0.2	< 0.6	< 0.4
aldrin[f]		< 0.1	< 0.4	< 0.3	< 0.6	< 0.5	< 0.4	< 0.7	< 0.4	< 0.2	< 0.6	< 0.5
alpha-chlordane		0.4	1	2	2	0.7	0.7	2	0.8	1	1	2
dieldrin		< 0.1	2	3	< 0.5	< 0.4	< 0.3	< 0.6	< 0.4	< 0.2	< 0.6	1
p,p'-DDE		7	14	54	25	7	6	14	11	25	10	18
p,p'-DDD		1	3	7	4	0.9	2	3	2	2	3	6
p,p'-DDT		0.2	1	2	2	< 0.4	< 0.4	1	0.5	1	< 0.7	0.9
trichlorobiphenyls		3	11	9	15	7	9	16	7	5	16	12
tetrachlorobiphenyls		7	28	42	55	17	20	44	33	27	59	66
pentachlorobiphenyls		6	28	89	120	18	16	66	54	53	79	85
hexachlorobiphenyls		3	24	90	82	12	10	44	40	46	45	47
heptachlorobiphenyls		4	17	55	31	5	3	26	21	31	24	21
octachlorobiphenyls		0.5	5	18	9	0.8	< 0.2	4	3	4	3	3
nonachlorobiphenyls		0.08	0.6	3	2	0.4	< 0.2	2	0.5	0.6	0.6	0.4
decachlorobiphenyls		0.4	< 0.2	3	2	< 0.2	< 0.2	2	0.6	0.4	0.5	< 0.2
Sum of PCBs		24	110	310	320	60	58	200	160	170	230	230
trichlorobutadienes		< 0.2	< 0.5	< 0.4	< 1	< 0.7	< 0.6	< 1	< 0.7	< 0.3	< 1	< 0.8
tetrachlorobutadienes		< 0.3	< 0.6	< 0.5	< 1	< 0.9	< 0.7	< 1	< 0.8	< 0.4	< 1	< 0.9
pentachlorobutadienes		< 0.2	< 0.4	< 0.3	< 0.7	< 0.6	< 0.4	< 0.9	< 0.5	< 0.3	< 0.8	< 0.6
hexachlorobutadiene		< 0.1	< 0.2	< 0.2	< 0.4	< 0.3	< 0.3	< 0.5	< 0.3	< 0.1	< 0.5	< 0.3
Sample weight, grams		3.45	1.07	1.35	0.61	0.68	1.18	0.68	1.20	2.59	0.67	0.94

Footnotes follow

Table A-5. Continued. Concentrations, ng/g (ppb) wet weight, of chlorinated contaminants in livers of juvenile chinook salmon sampled in 1989. - [a,b,c,d,e,g,h]

	Site name	Mukilteo Hatchery	Methods blanks						NIST reference tissue (Mytilus edulis)[j]		Previously analyzed NIST reference tissue (n = 40)	
Chlorinated contaminants	Date collected / Lab no.	7/6/89 63-201	63-146	63-147	63-188	63-183	63-203	63-204	63-202	63-145	mean	RSD[k]
hexachlorobenzene		2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	5	4	4	28
lindane (gamma-BHC)		< 0.2	< 0.3	< 0.3	< 0.2	< 0.2	< 0.3	< 0.3	4	3	5	27
heptachlor		< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	5	5	5	32
aldrin[f]		< 0.2	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	4	8	5	23
alpha-chlordane		0.9	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	6	6	7	21
dieldrin		0.3	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	2	5	6	26
p,p'-DDE		12	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	6	6	8	34
p,p'-DDD		2	< 0.3	< 0.3	< 0.3	< 0.3	< 0.4	< 0.3	5	5	7	24
p,p'-DDT		0.6	< 0.3	< 0.2	< 0.2	< 0.2	< 0.3	< 0.3	4	4	6	34
trichlorobiphenyls		6	< 0.4	1	1	1	2	4	3	2	4	47
tetrachlorobiphenyls		21	< 0.5	8	2	5	2	< 0.5	33	37	48	44
pentachlorobiphenyls		28	1	2	2	2	1	0.6	81	91	100	27
hexachlorobiphenyls		20	0.4	0.9	2	0.2	0.9	0.4	33	39	62	31
heptachlorobiphenyls		10	0.2	0.2	2	4	< 0.2	< 0.2	9	10	16	42
octachlorobiphenyls		1	< 0.1	< 0.1	< 0.1	0.3	< 0.2	< 0.2	0.2	0.4	0.4	60
nonachlorobiphenyls		0.2	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.2	0.2	0.2	53
decachlorobiphenyls		0.3	< 0.1	< 0.1	< 0.1	< 0.1	< 0.2	< 0.2	0.4	0.4	0.4	0
Sum of PCBs		86	2	12	9	12	6	5				
trichlorobutadienes		< 0.3	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4				
tetrachlorobutadienes		< 0.4	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5				
pentachlorobutadienes		< 0.2	< 0.3	< 0.3	< 0.3	< 0.3	< 0.4	< 0.3				
hexachlorobutadiene		< 0.1	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2				
Sample weight, grams		2.18							3.02	3.13		

Footnotes follow

Table A-5. Continued. Concentrations, ng/g (ppb) wet weight, of chlorinated contaminants in livers of juvenile chinook salmon sampled in 1989. - [a,b,c,d,e].

Footnotes:

[a] The concentrations of the analytes and the percent recovery of the surrogate standard were calculated using tetrachloro-m-xylene as the internal standard.

[b] The "less than" symbol (<) indicates that the analyte was not detected in concentrations above the stated value.

[c] Concentrations and initial identifications were determined using gas chromatography (GC) with electron capture detection (ECD).

[d] Concentrations less than 10 are rounded to one significant figure; concentrations greater than 10 are rounded to two significant figures.

[e] Percent recovery for the internal standard (surrogate) averaged 100%, CV = 5%, n = 31. Percent recovery of the surrogate includes quality control samples.

[f] Aldrin coelutes with an unknown compound. The concentration would not be higher than the stated value.

[g] The concentrations of the analytes were calculated using the surrogate standard 4,4'-dibromooctafluorobiphenyl so that the data can be compared to previously analyzed data.

[h] The percent recovery of the surrogate standard was calculated using the GC internal standard tetrachloro-m-xylene.

[i] Standard reference material from the National Institute of Science and Technology.

[j] RSD is the relative standard deviation which is the standard deviation divided by the mean and expressed as a percent.

Table A-6. Concentrations, ng/g (ppb) wet weight, of chlorinated contaminants in livers of juvenile chinook salmon sampled in 1990. - [a,b,c,d,e,g,h]

	Site name	Green River Hatchery			Duwamish River estuary				Nisqually Hatchery		
Chlorinated contaminants	Date collected Lab no.	5/8/90 63-380	5/22/90 63-381	5/22/90 63-404	5/22/90 63-382	5/24/89 63-383	6/1/90 63-384	6/18/90 63-385	5/25/90 63-386	5/29/90 63-387	5/29/90 63-388
hexachlorobenzene		0.8	0.8	0.7	1	2	2	1	1	1	1
lindane (gamma-BHC)		0.9	0.4	0.5	0.4	0.4	0.5	0.2	0.4	< 0.1	< 0.3
heptachlor		< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
aldrin - f		0.4	< 0.2	< 0.2	< 0.3	0.7	< 0.1	0.6	< 0.3	< 0.2	< 0.2
alpha-chlordane		0.6	0.5	0.5	0.6	2	1	1	0.7	0.6	0.6
dieldrin		0.9	0.9	1	1	2	2	1	0.4	< 0.3	< 0.3
p,p'-DDE		5	5	5	7	25	17	24	10	8	9
p,p'-DDD		0.5	0.5	0.5	0.6	3	2	2	0.8	0.6	0.7
p,p'-DDT		< 0.1	< 0.1	< 0.1	< 0.1	1	0.6	1	< 0.1	< 0.1	< 0.1
trichlorobiphenyls		3	3	2	3	7	5	6	5	4	3
tetrachlorobiphenyls		16	11	16	14	54	26	43	19	13	14
pentachlorobiphenyls		15	11	14	13	68	36	71	18	13	14
hexachlorobiphenyls		8	6	8	7	49	25	59	10	7	8
heptachlorobiphenyls		3	2	3	3	21	11	27	4	3	3
octachlorobiphenyls		< 0.1	< 0.1	< 0.2	< 0.1	0.9	0.5	1	< 0.3	< 0.2	< 0.2
nonachlorobiphenyls		< 0.1	< 0.2	< 0.3	< 0.3	0.9	0.5	0.9	0.7	0.6	0.4
decachlorobiphenyls		0.5	0.5	2	1	1	0.9	0.9	2	2	1
Sum of PCBs		46	34	45	41	200	100	210	59	43	43
trichlorobutadienes		< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
tetrachlorobutadienes		< 0.2	< 0.1	< 0.2	< 0.1	< 0.2	< 0.1	< 0.2	< 0.1	< 0.1	< 0.1
pentachlorobutadienes		< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
hexachlorobutadiene		< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Sample weight, grams		2.00	3.00	1.48	3.01	2.00	3.00	2.01	2.00	3.02	3.02

Footnotes follow

Table A-6. Continued. Concentrations, ng/g (ppb) wet weight, of chlorinated contaminants in livers of juvenile chinook salmon sampled in 1990. - [a,b,c,d,e,g,h]

	Site name	Nisqually River estuary			Puyallup Hatchery			Puyallup River estuary		
	Date collected	6/13/90	6/13/90	6/14/89	6/4/90	6/4/90	6/6/90	6/26/90	6/28/90	6/28/90
Chlorinated contaminants	Lab no.	63-398	63-399	63-400	63-389	63-396	63-397	63-300	63-342	63-343
hexachlorobenzene		2	2	2	1	1	1	2	1	1
lindane (gamma-BHC)		< 0.3	0.4	0.4	< 0.2	< 0.2	< 0.1	< 0.2	< 0.1	< 0.1
heptachlor		< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
aldrin[f]		< 0.1	< 0.1	0.5	0.4	0.4	< 0.1	0.8	0.9	0.9
alpha-chlordane		2	1	2	0.6	0.6	0.5	2	1	1
dieldrin		2	1	2	0.4	0.4	0.4	3	6	4
p,p'-DDE		28	22	27	8	8	7	20	12	18
p,p'-DDD		2	1	2	0.5	0.6	0.5	3	1	2
p,p'-DDT		0.7	0.4	0.8	< 0.1	< 0.1	< 0.1	0.9	0.6	0.7
trichlorobiphenyls		2	2	3	5	4	3	5	2	3
tetrachlorobiphenyls		21	17	19	19	16	12	40	19	24
pentachlorobiphenyls		31	28	34	15	13	9	58	26	34
hexachlorobiphenyls		23	18	23	10	8	7	40	17	24
heptachlorobiphenyls		9	7	9	4	4	5	18	10	14
octachlorobiphenyls		0.4	< 0.3	0.5	< 0.2	< 0.2	< 0.1	0.9	0.4	0.7
nonachlorobiphenyls		0.7	0.5	0.8	0.4	0.9	0.6	2	0.7	1
decachlorobiphenyls		2	2	2	2	3	2	4	1	2
Sum of PCBs		89	74	91	55	49	39	170	76	100
trichlorobutadienes		< 0.1	< 0.1	< 0.1	< 0.2	< 0.1	< 0.1	< 0.2	< 0.2	< 0.1
tetrachlorobutadienes		< 0.1	< 0.1	< 0.1	< 0.2	< 0.1	< 0.1	< 0.3	< 0.2	< 0.1
pentachlorobutadienes		< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.2	< 0.1	< 0.1
hexachlorobutadiene		< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Sample weight, grams		3.04	3.04	3.03	2.04	2.00	3.00	0.99	1.99	2.01

Footnotes follow

Table A-6. Continued. Concentrations, ng/g (ppb) wet weight, of chlorinated contaminants in livers of juvenile chinook salmon sampled in 1990. - [a,b,c,d,e,g,h]

	Site name	Method blanks				NIST reference tissue (Mytilus edulis)[j]		Previously analyzed NIST reference tissue (n = 20)	
Chlorinated contaminants	Date collected Lab no.	63-391	63-392	63-406	63-407	63-390	63-405	mean	RSD[k]
hexachlorobenzene		< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.6	140
lindane (gamma-BHC)		< 0.1	< 0.1	< 0.1	< 0.1	< 0.2	< 0.2	0.4	100
heptachlor		< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	1	77
aldrin[f]		< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	2	74
alpha-chlordane		< 0.1	< 0.1	< 0.2	< 0.1	3	3	3	27
dieldrin		< 0.1	< 0.1	< 0.2	< 0.1	2	2	2	72
p,p'-DDE		< 0.1	< 0.1	< 0.2	< 0.1	7	6	5	22
p,p'-DDD		< 0.1	< 0.1	< 0.1	< 0.1	5	6	5	38
p,p'-DDT		< 0.1	< 0.1	< 0.1	< 0.1	0.4	0.4	0.5	0
trichlorobiphenyls		0.7	0.6	1	0.7	27	21	21	28
tetrachlorobiphenyls		5	4	5	5	200	180	160	18
pentachlorobiphenyls		2	1	3	1	140	120	110	21
hexachlorobiphenyls		1	0.7	2	0.9	58	53	47	21
heptachlorobiphenyls		< 0.1	< 0.1	0.9	< 0.2	14	11	9	35
octachlorobiphenyls		< 0.1	< 0.1	0.4	< 0.1	< 0.3	< 0.3	0.5	110
nonachlorobiphenyls		< 0.1	< 0.1	0.4	< 0.1	< 0.3	0.4	0.4	65
decachlorobiphenyls		< 0.1	< 0.1	0.5	< 0.1	< 0.2	< 0.2	0.5	150
Sum of PCBs		9	6	11	8	440	380	350	19
trichlorobutadienes		< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	-	-
tetrachlorobutadienes		< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	-	-
pentachlorobutadienes		< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	-	-
hexachlorobutadiene		< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	-	-
Sample weight, grams						3.12	3.00		

Footnotes follow

Table A-6. Continued. Concentrations, ng/g (ppb) wet weight, of chlorinated contaminants in livers of juvenile chinook salmon sampled in 1990.

Footnotes:

[a] The concentrations of the analytes and the percent recovery of the surrogate standard were calculated using the surrogate standard tetrachloro-m-xylene as the internal standard.

[b] The "less than" symbol (<) indicates that the analyte was not detected in concentrations above the stated value.

[c] Concentrations and initial identifications were determined using gas chromatography (GC) with electron capture detection (ECD).

[d] Concentrations less than 10 are rounded to one significant figure; concentrations greater than 10 are rounded to two significant figures.

[e] Percent recovery for the internal standard (surrogate) averaged 99%, CV = 6%, n = 25. Percent recovery of the surrogate includes quality control samples.

[f] Aldrin coelutes with an unknown compound. The concentration would not be higher than the stated value.

[g] The concentrations of the analytes were calculated using the surrogate standard 4,4'-dibromooctafluorobiphenyl so that the data can be compared to previously analyzed data.

[h] The percent recovery of the surrogate standard was calculated using tetrachloro-m-xylene as the internal standard.

[i] Standard reference material from the National Institute of Science and Technology.

[j] RSD is the relative standard deviation which is the standard deviation divided by the mean and expressed as a percent.

Table A-7. Concentrations, ng/g (ppb) wet weight, of chlorinated contaminants in whole juvenile chinook salmon sampled in 1989. - [a,b,c,d,e,g,h,i]

	Site name	Green River Hatchery				Duwamish River estuary			
Chlorinated contaminants	Date collected / Lab no.	5/1/89 63-251	5/1/89 63-252	5/8/89 63-253	5/8/89 63-254	5/23/89 63-226	5/23/89 63-227	6/15/89 63-228	6/15/89 63-229	6/15/89 63-230
hexachlorobenzene		2	2	2	2	0.6	2	2	2	2
lindane (gamma-BHC)		< 0.5	< 0.8	< 0.5	< 0.4	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
heptachlor		< 0.2	< 0.5	< 0.3	< 0.3	< 0.1	< 0.2	< 0.1	< 0.1	< 0.1
aldrin[f]		< 0.3	< 0.2	< 0.2	< 0.3	< 0.2	< 0.2	< 0.1	< 0.2	< 0.1
alpha-chlordane		3	3	3	2	1	2	2	2	2
dieldrin		2	3	3	3	0.3	1	2	2	2
p,p'-DDE		28	27	23	22	27	24	26	25	25
p,p'-DDD		6	6	6	5	4	5	6	5	5
p,p'-DDT		3	5	4	3	3	3	4	3	4
trichlorobiphenyls		8	6	7	8	10	16	13	12	12
tetrachlorobiphenyls		27	26	24	28	41	30	46	48	26
pentachlorobiphenyls		49	45	46	38	75	39	62	77	39
hexachlorobiphenyls		39	35	35	32	75	27	52	63	34
heptachlorobiphenyls		23	19	20	15	48	15	28	27	18
octachlorobiphenyls		3	4	4	3	11	3	6	3	3
nonachlorobiphenyls		0.4	0.4	0.4	< 0.2	1	0.3	0.5	0.5	0.6
decachlorobiphenyls		< 0.2	< 0.1	0.3	< 0.2	0.3	< 0.1	0.4	0.2	< 0.08
Sum of PCBs		150	140	140	120	260	130	210	230	130
trichlorobutadienes		< 0.4	< 0.3	< 0.4	< 0.5	< 0.3	< 0.3	< 0.2	< 0.3	< 0.2
tetrachlorobutadienes		< 0.6	< 0.4	< 0.5	< 0.7	< 0.4	< 0.4	< 0.3	< 0.3	< 0.3
pentachlorobutadienes		< 0.4	< 0.3	< 0.3	< 0.4	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
hexachlorobutadiene		< 0.2	< 0.2	< 0.2	< 0.2	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Sample weight, grams		5.01	5.06	5.03	5.05	5.02	5.03	5.07	5.05	5.07

Footnotes follow

Table A-7. Continued. Concentrations, ng/g (ppb) wet weight, of chlorinated contaminants in whole juvenile chinook salmon sampled in 1989. - [a,b,c,d,e,g,h,i]

Chlorinated contaminants	Site name	Duwamish River estuary								
	Date collected	7/14/89	7/14/89	7/14/89	8/15/89	8/15/89	8/15/89	9/14/89	9/14/89	9/14/89
	Lab no.	63-231	63-232	63-233	63-234	63-246	63-247	63-248	63-249	63-250
hexachlorobenzene		2	1	2	1	1	1	0.8	0.7	0.8
lindane (gamma-BHC)		< 0.1	< 0.1	< 0.1	< 0.1	< 0.3	< 0.2	< 0.4	< 0.2	< 0.2
heptachlor		< 0.1	< 0.1	< 0.1	< 0.1	< 0.3	< 0.2	< 0.3	< 0.2	< 0.3
aldrin[f]		< 0.1	< 0.1	< 0.1	< 0.2	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
alpha-chlordane		2	1	2	1	1	1	0.6	0.5	0.7
dieldrin		1	0.5	1	0.6	0.5	0.4	< 0.3	0.3	< 0.3
p,p'-DDE		26	19	26	40	28	38	43	40	43
p,p'-DDD		5	3	5	4	3	4	2	2	3
p,p'-DDT		3	2	3	3	2	3	2	2	3
trichlorobiphenyls		8	7	7	5	4	3	3	2	2
tetrachlorobiphenyls		28	30	36	16	11	12	11	6	9
pentachlorobiphenyls		37	46	52	29	22	27	35	22	29
hexachlorobiphenyls		26	38	45	23	15	19	38	21	29
heptachlorobiphenyls		15	20	23	13	9	12	22	16	18
octachlorobiphenyls		4	4	5	2	2	3	4	4	4
nonachlorobiphenyls		0.4	0.3	0.7	0.5	< 0.2	0.4	0.7	0.8	0.3
decachlorobiphenyls		0.09	0.2	0.3	< 0.1	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
Sum of PCBs		120	150	170	89	63	76	110	72	91
trichlorobutadienes		< 0.2	< 0.2	< 0.3	< 0.3	< 0.5	< 0.4	< 0.5	< 0.5	< 0.5
tetrachlorobutadienes		< 0.3	< 0.3	< 0.3	< 0.3	< 0.7	< 0.6	< 0.6	< 0.6	< 0.6
pentachlorobutadienes		< 0.2	< 0.2	< 0.2	< 0.2	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4
hexachlorobutadiene		< 0.1	< 0.1	< 0.1	< 0.1	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
Sample weight, grams		5.03	5.07	5.00	5.06	5.08	5.05	5.02	5.01	5.04

Footnotes follow

Table A-7. Continued. Concentrations, ng/g (ppb) wet weight, of chlorinated contaminants in whole juvenile chinook salmon sampled in 1989. - [a,b,c,d,e,g,h,i]

	Site name	Method blanks				NIST reference tissue (Mytilus edulis)[j]	Previously analyzed NIST reference tissue (n = 40)	
Chlorinated contaminants	Date collected / Lab no.	63-391	63-392	63-406	63-407	63-235	mean	RSD[k]
hexachlorobenzene		< 0.1	< 0.1	< 0.2	< 0.2	6	4	28
lindane (gamma-BHC)		< 0.1	< 0.1	< 0.3	< 0.2	8	5	27
heptachlor		< 0.1	< 0.1	< 0.3	< 0.2	8	5	32
aldrin[f]		< 0.1	< 0.1	< 0.3	< 0.3	7	5	23
alpha-chlordane		< 0.1	< 0.1	< 0.2	< 0.2	7	7	21
dieldrin		< 0.2	< 0.1	< 0.3	< 0.2	5	6	26
p,p'-DDE		< 0.2	< 0.1	< 0.2	< 0.2	6	8	34
p,p'-DDD		< 0.2	< 0.2	< 0.3	< 0.3	9	7	24
p,p'-DDT		< 0.1	< 0.1	< 0.3	< 0.3	6	6	34
trichlorobiphenyls		1	1	0.7	1	5	4	47
tetrachlorobiphenyls		3	3	2	0.8	60	48	44
pentachlorobiphenyls		0.5	0.7	3	2	110	100	27
hexachlorobiphenyls		1	0.7	0.9	0.4	50	62	31
heptachlorobiphenyls		< 0.09	< 0.08	< 0.2	< 0.2	10	16	42
octachlorobiphenyls		< 0.08	< 0.08	< 0.2	< 0.1	0.7	0.4	60
nonachlorobiphenyls		< 0.08	< 0.07	< 0.2	< 0.1	0.5	0.2	53
decachlorobiphenyls		< 0.1	< 0.09	< 0.2	< 0.2	0.9	0.4	0
Sum of PCBs		6	5	7	4			
trichlorobutadienes		< 0.3	< 0.2	< 0.5	< 0.4			
tetrachlorobutadienes		< 0.3	< 0.3	< 0.7	< 0.6			
pentachlorobutadienes		< 0.2	< 0.2	< 0.4	< 0.4			
hexachlorobutadiene		< 0.1	< 0.1	< 0.3	< 0.2			
Sample weight, grams						5.08		

Footnotes follow

Table A-7. Continued. Concentrations, ng/g (ppb) wet weight, of chlorinated contaminants in whole juvenile chinook salmon sampled in 1990.

Footnotes:

[a] The concentrations of the analytes and the percent recovery of the surrogate standard were calculated using tetrachloro-m-xylene as the internal standard.

[b] The "less than" symbol (<) indicates that the analyte was not detected in concentrations above the stated value.

[c] Concentrations and initial identifications were determined using gas chromatography (GC) with electron capture detection (ECD).

[d] Concentrations less than 10 are rounded to one significant figure; concentrations greater than 10 are rounded to two significant figures.

[e] Percent recovery for the internal standard (surrogate) averaged 110%, CV = 9%, n = 23. Percent recovery of the surrogate includes quality control samples.

[f] Aldrin coelutes with an unknown compound. The concentration would not be higher than the stated value.

[g] Identification of analytes was confirmed by gas chromatography/mass spectrometry.

[h] The concentrations of the analytes were calculated using the surrogate standard 4,4'-dibromooctafluorobiphenyl so that the data can be compared to previously analyzed data.

[i] The percent recovery of the surrogate standard was calculated using the tetrachloro-m-xylene as the internal standard.

[k] Standard reference material from the National Institute of Science and Technology.

[l] RSD is the relative standard deviation which is the standard deviation divided by the mean and expressed as a percent.

Table A-8. The results (mean ± standard error) of chemical and biochemical measures of chemical contaminant exposure in juvenile chinook salmon from the individual hatcheries sampled from Puget Sound rivers in Washington during 1989 and 1990.

Hatchery	Year	Biliary $FACs_{pro}$[a]	PCBs	Hepatic AHH	DNA adducts
Green River Hatchery	1989	160 ± 10 (2)[b]	54 ± 45 (2)	190 ± 9 (2)	8.8 ± 4.2 (2)
	1990	250 ± 15 (2)	42 ± 1 (3)	95 ± 14 3)	4.9 ± 0.3 (2)
Puyallup River Hatchery	1989	130 ± 7 (2)	32 ± 2 (2)	170 ± 4 (2)	6.9 ± 1.7 2)
	1990	90 ± 12 (3)	48 ± 5 (3)	91 ± 19 (3)	5.5 ± 0.4 (3)
Skykomish River Hatchery	1989	114 (1)	64 ± 2 (2)	120 ± 5 (2)	5.7 ± 2.0 (2)
	1990	NS[c]	NS	NS	NS
Kalama Creek Hatchery	1989	150 ± 26 (2)	50 (1)	62 ±25 (2)	4.9 (1)
	1990	59 ± 18 (2)	48 ±5 (3)	66 ±9 (3)	4.6 ± 0.4 3)

[a] Biliary fluorescent compounds (ng benzo[a]pyrene equivalents per gram biliary protein).
[b] Value in the parentheses represents the number of sample composites that were analyzed. Each composite was composed of tissue or bile from generally 60 juvenile chinook salmon.
[c] Not sampled.

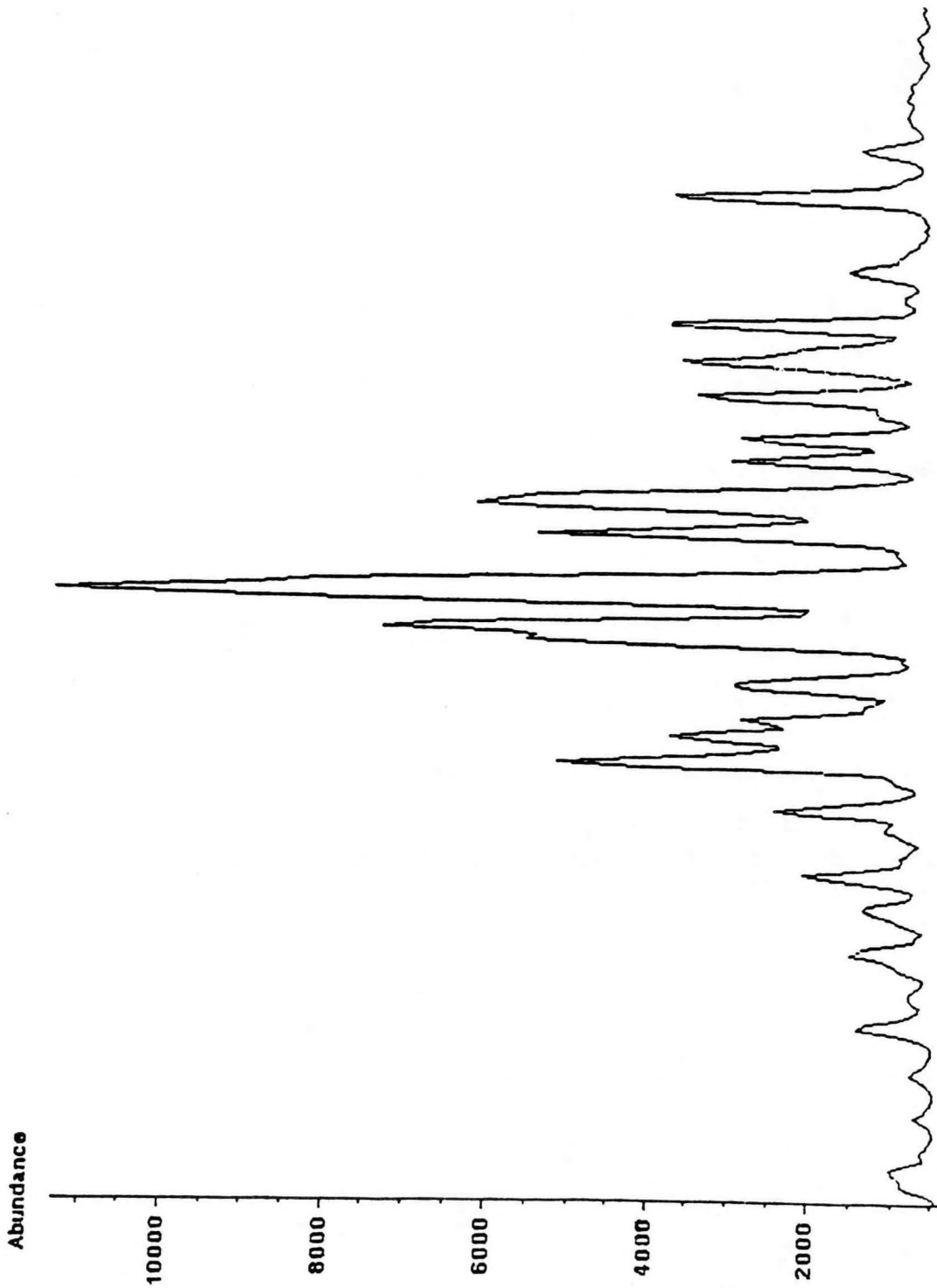

Figure A-1. Mass chromatogram of the C_3-phenanthrenes measured in stomach contents of juvenile chinook salmon from the Duwamish Waterway.

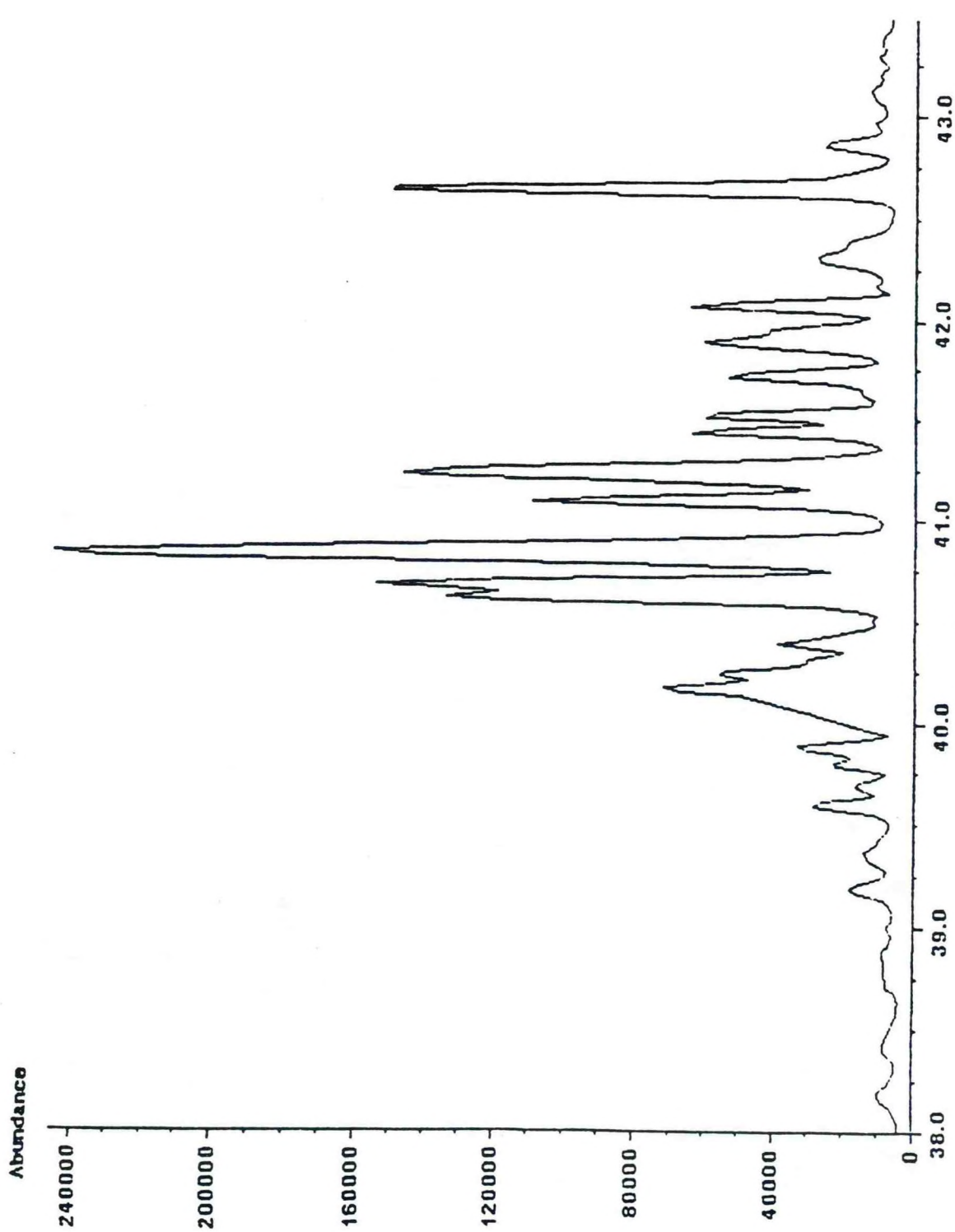

Figure A-2. Mass chromatogram of the C_3-phenanthrenes measured in stomach contents of juvenile chinook salmon from the Commencement Bay.

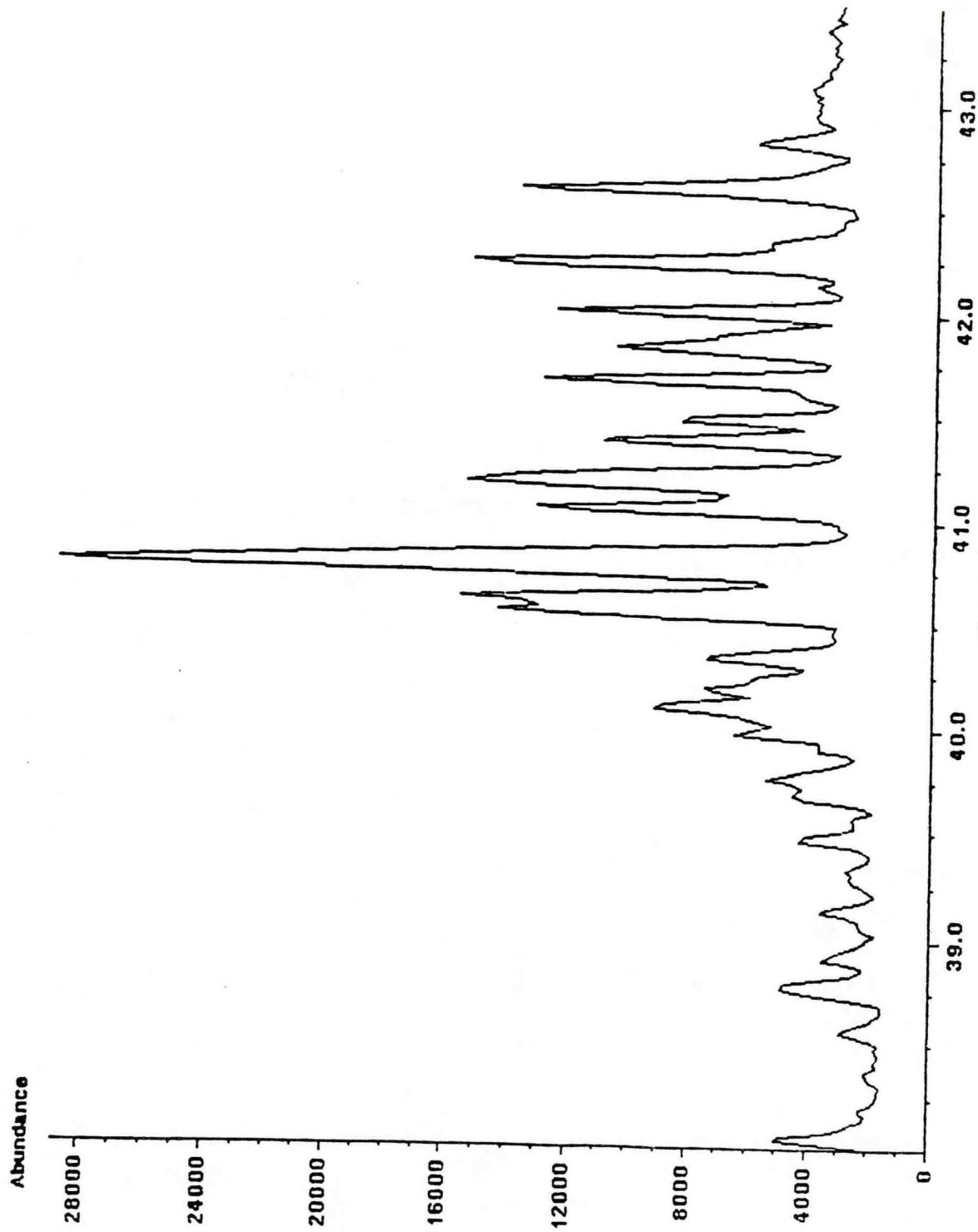

Figure A-3. Mass chromatogram of the C_3-phenanthrenes measured in sediment from the Duwamish Waterway.

APPENDIX B

GLOSSARY OF ABBREVIATIONS

Glossary of Abbreviations

AHs	aromatic hydrocarbons
AHH	aryl hydrocarbon hydroxylase
ANOVA	analysis of variance
ATP	adenosine triphosphate
BaP	benzo[a]pyrene
BSA	bovine serum albumin
CHs	chlorinated hydrocarbons
CYP1A	cytochrome P4501A
DWSE	Duwamish Waterway sediment extract
ECD	electron capture detector
ELISA	enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
EROD	ethoxyresorufin-O-deethylase
FACs	fluorescent aromatic compounds
GC/MS	gas chromatography/mass spectrometry
HAHs	high molecular weight AHs
HPLC	high-performance liquid chromatograph
KLH	keyhole limpet hemocyanin
KOH	potassium hydroxide
LAHs	low moleculr weight AHs
LPS	lipopolysaccharide
MAPS	monoclonal antibody purification system
NADPH	nicotinimide adenosine diphosphate
N HCL	normal hydrochloric acid
NIST	National Institute of Science and Technology

NMFS	National Marine Fisheries Service
NPH	napthalene
PAHs	polycyclic aromatic hydrocarbons
PBS	phosphate buffered saline
PCBs	polychlorinated biphenyls
PEI	polyethyleneimine
PFC	plaque-forming cells
PIT tags	passive integrated transponder tags
RPMI	Roswell Park Memorial Institute
TCM	tissue culture media
TLC	thin-layer chromatography
TNP	trinitrophenylated
UV	ultraviolet
UVF	ultraviolet frequency

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