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Polycyclic Aromatic Hydrocarbons and Fish Health Indicators in the Marine Ecosystem in Kitimat, British Columbia

March 2009

U.S. DEPARTMENT OF COMMERCE
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Polycyclic Aromatic Hydrocarbons and Fish Health Indicators in the Marine Ecosystem in Kitimat, British Columbia

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Executive Summary

The Alcan Primary Metal aluminum smelter, which began operation in the 1950s, is located at the head of Kitimat Arm in Kitimat, British Columbia. Emissions generated by aluminum production at the smelter have been a major anthropogenic source of polycyclic aromatic hydrocarbons (PAHs) in the region. Large releases of these substances, via several routes and over decades, have led to contamination of intertidal and subtidal areas, primarily adjacent to the smelter in the Alcan Inner Harbour.

Several studies have documented high concentrations of PAHs in sediments in the vicinity of the smelter, often well above PAH concentrations associated with health problems in fish exposed to PAHs from other urban and industrial sources. However, it was unknown whether the same effects would be seen in marine organisms exposed to smelter-derived PAHs, as many of the PAHs generated from aluminum smelters are bound to soot particles, thus aquatic animals cannot absorb them. The limited biological availability of the smelter-derived PAHs in Kitimat Arm made their toxicity to marine life uncertain.

Because Kitimat Arm and the Kitimat fjord system are part of the historic fishing grounds of the Haisla First Nation located at Kitamaat Village, the Haisla have been concerned for some time about the potential impacts of PAHs from the smelter, as well as other contaminants, on fisheries resources and the possible effects of consuming fish from this area. A five-year study was initiated to address these concerns, supported by Alcan Inc. (now known as Rio Tinto Alcan Inc.), the Kitamaat Village Council (Haisla First Nation), and the U.S. National Marine Fisheries Service (NMFS) Northwest Fisheries Science Center.

NMFS was asked by the Haisla First Nation and Alcan Inc. to provide an assessment of contamination and biological effects in representative fish from the Kitimat region. This study was intended to provide a more thorough characterization of PAH effects on the marine environment in Kitimat Arm, to resolve questions about the biological availability of smelter-derived PAHs to marine organisms, and to answer outstanding questions about potential impacts to fishery resources. Additionally, this study provides baseline data for measuring future environmental improvement anticipated as a result of changes that Alcan has made to the aluminum smelting process and remediation efforts in Kitimat.

Objectives

The Kitimat Marine Assessment had four objectives:

1. Assess exposure to PAHs in juvenile outmigrant Chinook salmon (*Oncorhynchus tshawytscha*). Specific aims were to a) determine PAH concentrations in sediments at sites utilized by juvenile salmon, b) determine concentrations of PAHs or their metabolites in salmon and their prey, c) assess the bioavailability of smelter-associated PAHs by comparing PAH exposure levels in Kitimat Arm salmon to PAH exposure

levels in salmon from other sites where PAHs from other sources are present, and d) compare PAH exposure levels in Kitimat Arm salmon to PAH exposure levels that have been linked to biological dysfunction in salmon in field or laboratory studies.

2. Assess exposure to PAHs in two species of benthic flatfish, English sole (*Parophrys vetulus*) and yellowfin sole (*Limanda aspera*). Specific aims were to a) determine PAH concentrations in sediments at sites utilized by English sole and yellowfin sole, b) determine concentrations of PAHs or their metabolites in sole and their prey, and c) compare PAH exposure levels in Kitimat Arm sole to PAH exposure levels in sole from other sites where PAHs from other sources are present, to assess the relative bioavailability of smelter-derived PAHs.
3. Assess the extent of PAH-associated biological injury in benthic flatfish from Kitimat Arm. Specific aims were to a) monitor early biochemical responses to PAH exposure (e.g., DNA damage and induction of PAH-metabolizing enzymes) in English or yellowfin sole or both, b) monitor the prevalence of PAH-associated liver lesions in English and yellowfin sole, c) examine reproductively maturing yellowfin and English sole in Kitimat Arm for evidence of inhibited gonadal development and related types of dysfunction that had been observed in English sole from sites in Puget Sound, Washington, contaminated with PAHs, and d) compare data on biological injury in Kitimat sole with observations in other flatfish species from sites contaminated with PAHs from different sources to assess the relative toxicity of smelter-derived PAHs.
4. Assess exposure of salmon and flatfish to organochlorines (OCs) and metals. Specific aims were to a) establish baseline levels of OCs and metals in prey and tissues of Kitimat salmon and flatfish and b) assess the potential for impacts on fish health or seafood safety.

Key Findings

An active program of study has been underway since 2000 to examine the impact of PAHs on living resources in Kitimat Arm. Key findings and conclusions to date are:

- Concentrations of PAHs, especially high molecular weight aromatic hydrocarbons, are elevated in sediments within Kitimat Arm, especially at sites closest to the Alcan plant. The types of individual PAHs in sediments at sites within Kitimat Arm, including Kitamaat Village—the major population center for the Haisla First Nation—are similar to those in Alcan smelter sources. At the reference sites outside Kitimat Arm, PAHs from natural products (wood or decomposing matter) are more prominent. These wood-derived PAHs are also common in sediments near the Eurocan pulp mill.
- Both salmon and flatfish are exposed to PAHs through consumption of prey organisms residing in Kitimat Arm. Stomach contents of salmon and sole from sites within Kitimat Arm, especially those closest to the smelter, contained significant concentrations of PAHs similar to those produced by the smelter. However, concentrations of PAHs in stomach contents of Kitimat salmon and sole were lower than those measured in salmon and sole from other Pacific Northwest sites contaminated with comparable concentrations of nonsmelter-derived PAHs, suggesting reduced biological availability of smelter-derived PAHs. Differences in diet between fish from Kitimat Arm and fish from other

- In juvenile salmon from the Alcan Harbour and Hospital Beach sites nearest the smelter, PAH concentrations in bile and stomach contents were comparable to concentrations found in juvenile salmon in Puget Sound, where reduced disease resistance has been observed in wild populations. In addition to PAHs, however, the Puget Sound fish were exposed to high concentrations of other immunosuppressive contaminants such as PCBs that are not present in Alcan's receiving environment. Therefore, it is uncertain whether PAHs alone would produce the same health impact. PAH exposure levels in juvenile salmon from other Kitimat sites were below toxic concentrations.
- Salmon from sites within Kitimat Arm did not show significant DNA damage, perhaps because of their short residence time in this area. Other biological effects of PAHs were not tested.
- PAHs are having some effects on the health of flatfish in Kitimat Arm. English sole from sites within Kitimat Arm showed increases in DNA damage, typically caused by mutagenic PAHs, as compared to sole from pristine reference sites outside Kitimat Arm. Also, 10–20% of English sole and 5–10% of yellowfin sole from sites within Kitimat Arm had some type of PAH-associated liver disease. These conditions were not generally found in sole from reference sites outside Kitimat Arm, where the natural background prevalence of liver disease was in the 0–2% range.
- Although PAH-associated health effects were found in Kitimat flatfish, liver lesion prevalences and DNA damage in sole from sites closest to the smelter were lower than expected for the sediment PAH levels at these sites, in comparison with levels of these conditions in sole from other west coast sites of North America. Moreover, neither yellowfin sole nor English sole showed significant evidence of PAH-related reproductive dysfunction as is commonly observed in other contaminated industrial areas such as Puget Sound. These findings suggest limited bioavailability of smelter-derived PAHs.
- Concentrations of PCBs, DDTs, and other OCs were very low in stomach contents of English sole and salmon from all sampling sites, and would be unlikely to impact fish health or increase PAH toxicity.
- Concentrations of PAHs, PCBs, OCs, and heavy metals were quite low in the edible flesh of English sole from all sampling sites, well below levels considered by regulatory agencies to be a human health risk.
- The process changes introduced by Alcan appear to be effective at reducing inputs of PAHs into the environment and biota of Kitimat Arm, as PAH concentrations in sediments and fish and fish disease prevalences have remained stable or declined over the past 5 years of sampling. However, additional remedial actions may be needed to reduce PAH levels to baseline concentrations.

In addition to process changes already instituted, Alcan has developed a comprehensive program to continue to reduce PAH releases from the smelting process at Kitimat and is taking steps to address PAH hot spots in sediments in close proximity to the smelter. In view of these

efforts, as well as the fact that sediment PAHs within Kitimat Arm are derived primarily from historical releases, we expect that further decreases in PAH concentrations should be observed over time. The studies conducted thus far serve as a benchmark from which to assess the effectiveness of process changes and remediation efforts in reducing PAH contamination in Kitimat Arm, with periodic monitoring to confirm long-term improvement.

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These studies were supported by the Haisla First Nation and Alcan Primary Metal Group, British Columbia.

Introduction

The Alcan Primary Metal Group, British Columbia, aluminum smelter, which began operation in the 1950s, is located at the head of Kitimat Arm in Kitimat, British Columbia (Figure 1 and Figure 2). Emissions generated by the production of aluminum at the smelter are a major anthropogenic source of polycyclic aromatic hydrocarbons (PAHs) in the region. Releases of these substances via several routes have led to contamination of intertidal and subtidal areas near the smelter in Alcan Inner Harbour, and potentially other portions of Kitimat Arm.

Several studies (Cretney et al. 1983, Simpson et al. 1996, 1998, Paine et al. 1996) were conducted to evaluate the extent of contamination in sediments in Kitimat Arm. Analyses revealed high concentrations of PAHs in sediments in the vicinity of the smelter and at nearby Hospital Beach, with values in the 10,000–100,000 ng/g dry wt range. These concentrations are well above those typically associated with adverse health effects in fish exposed to PAHs in urban sediments (Horness et al. 1998, Johnson et al. 2002). However, there is evidence that soot-associated PAHs, such as those released by the smelter, have limited bioavailability (Paine et al. 1996, Naes et al. 1999, Brion and Pelletier 2005) and, because organisms are unable to absorb them, their toxicity is reduced. Consequently, there was some uncertainty about the effects that smelter-derived PAHs would have on fish and other marine organisms in Kitimat Arm.

Because Kitimat Arm and the Kitimat fjord system are part of the historic fishing grounds of the Haisla First Nation, the Haisla have been concerned for some time about potential impacts of the PAHs from the smelter on fisheries resources in the area and any resultant health impacts from consuming these resources. However, data on PAH uptake and biological effects in fish and shellfish from the site are limited. Clams (*Mya arenaria*) have been sampled from several beaches in the Kitimat fjord system (Simpson 1997). At Hospital Beach (Figure 1), which is relatively close to the smelter, total PAH concentrations in clam tissue ranged from 5,000 to 6,000 ng/g dry wt. At intertidal beach sites near Kitimaat Village and the Eurocan pulp mill, concentrations were approximately 1,100 ng/g dry wt. These levels are within the range of reported values for total PAHs in mussel (*Mytilus* sp.) samples from urban embayments in Puget Sound, Washington (Krishnakumar et al. 1994). At Kildala Beach, in Kildala Arm, which is considered a relatively unimpacted reference area, the mean concentration was much lower (83 ng/g dry wt).

Similarly, analyses of bile from juvenile salmon (*Oncorhynchus* sp.) (Cretney et al. 1997) showed elevated concentrations of PAH metabolites (measured as pyrene-1-glucuronide equivalents) in fish from Hospital Beach, near the smelter, in comparison to salmon from a reference area in Kildala Arm. Another study (Brown et al. 1983) indicated uptake of PAHs and associated declines in condition factor (CF), reproductive output, and other disease conditions in mussels and clams sampled from Kitimat Arm. Hence preliminary data suggested a potential for PAH exposure and biological effects in shellfish residing in areas impacted by the smelter.

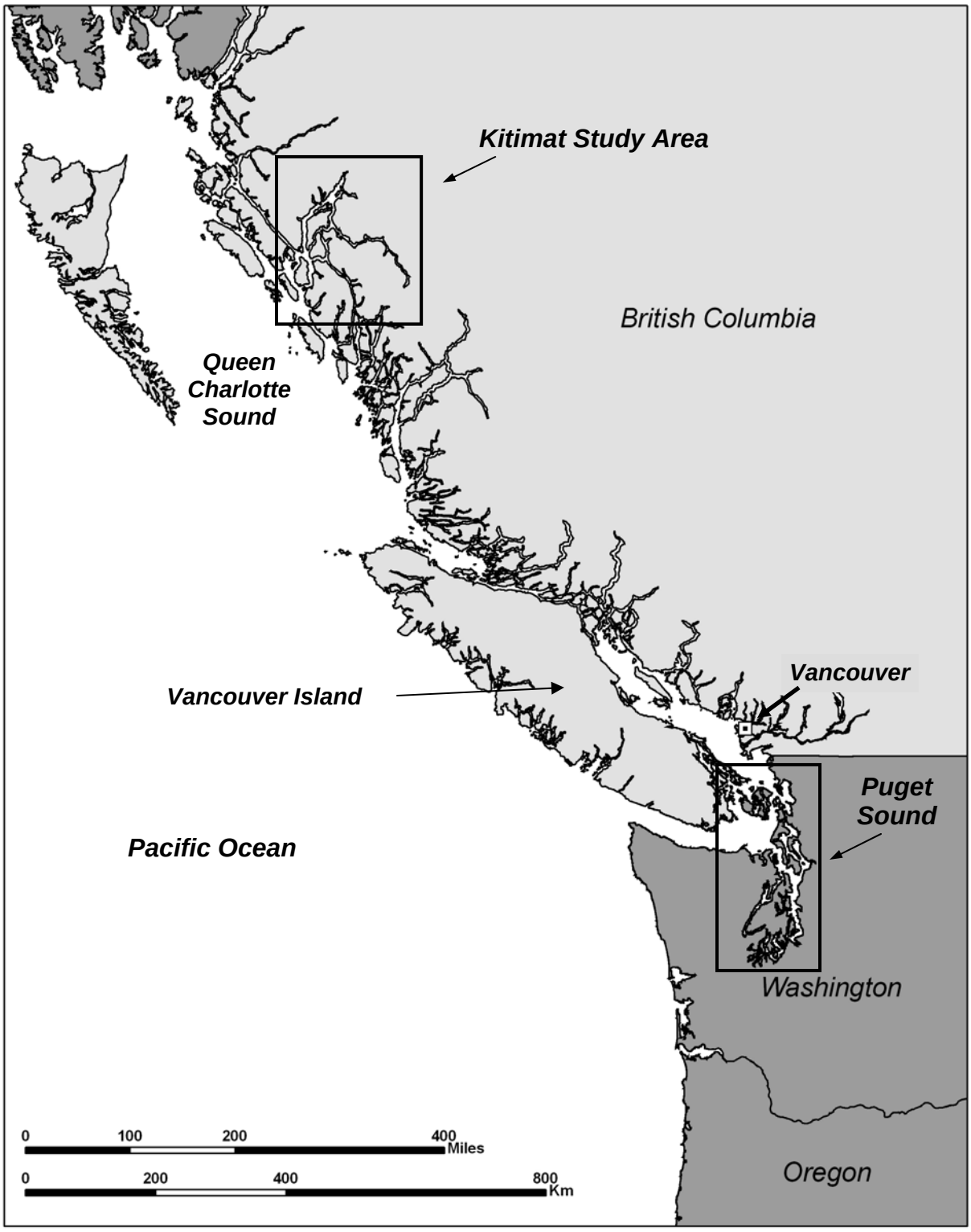

Figure 1. Location of Kitimat study area in relation to Vancouver, British Columbia, and Puget Sound, Washington.

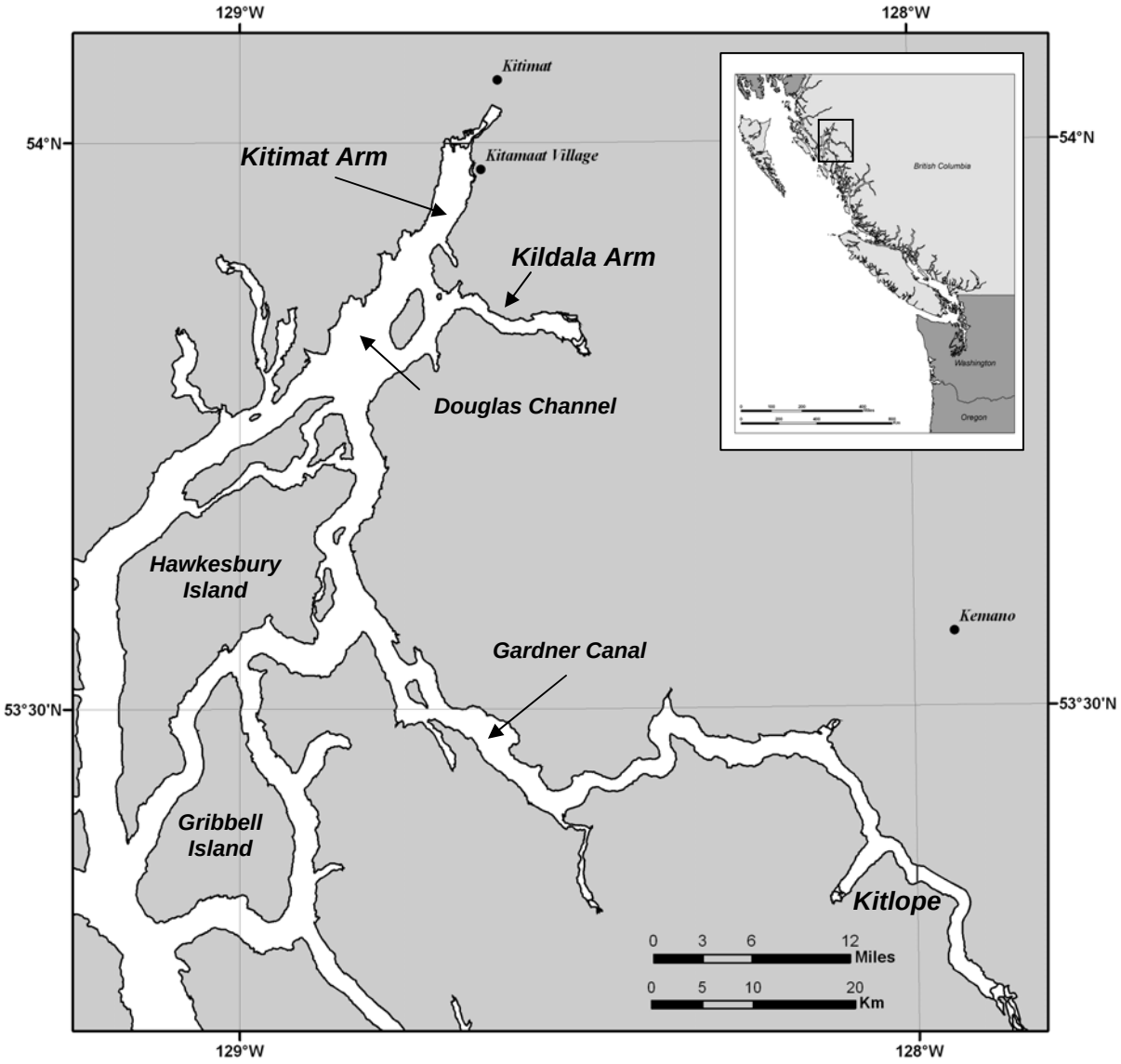

Figure 2. Overview of Kitimat study area showing Kitimat Arm, Kildala Arm, and Kitlope.

The National Marine Fisheries Service's Northwest Fisheries Science Center (NWFSC) was asked by the Haisla First Nation and Alcan Inc. (now known as Rio Tinto Alcan Inc.) to provide an assessment of contamination and biological effects in representative fish from the Kitimat region. The objective of this study was to provide a more comprehensive characterization of PAH contamination and associated biological effects in Kitimat's fishery resources. Our goals were to document the current status of contaminant-associated injuries in fish indigenous to the Kitimat Arm and adjacent areas, quantify the extent of these injuries, and evaluate links between any observed injuries and chemical contaminants in this waterway, specifically PAH contamination associated with the Alcan aluminum smelter.

Evidence for evaluating links included data on concentrations of selected chemical contaminants in sediments from sites where fish were captured and in tissues and bodily fluids of fish. In addition to PAHs, concentrations of other chemical contaminants, including selected organochlorines (OCs), were monitored in fish stomach contents and their potential impacts on fish health were assessed. Metals, OCs, and PAHs were also measured in edible flatfish muscle tissue to determine if levels met current standards for the protection of human health.

The Kitimat Marine Assessment was conceived as a 5-year monitoring plan, establishing a baseline of information against which Alcan could monitor the effectiveness of its process improvements to reduce PAH inputs and levels in the marine environment. These improvements include a number of changes to the smelting process to minimize the release of PAHs, as well as various cleanup and remediation actions on contaminated sites that might lessen the risk of exposure to PAHs in resident biota.

The Kitimat Marine Assessment, conducted from 2000 to 2004, had four major objectives:

1. To assess exposure to PAHs in juvenile outmigrant Chinook salmon (*Oncorhynchus tshawytscha*). The specific aims of the salmon sampling portion of the study were to a) determine PAH concentrations in sediments at sites utilized by juvenile salmon, b) determine concentrations of PAHs or their metabolites in salmon and their prey, c) compare PAH exposure levels in Kitimat Arm salmon to PAH exposure levels in salmon from other sites where PAHs from other sources are present, and d) compare PAH exposure levels in Kitimat Arm salmon to PAH exposure levels that have been linked to biological dysfunction or injury in field or laboratory studies (Arkoosh et al. 2001, Meador et al. 2006). Salmon monitoring was conducted every year from 2000 to 2004.
2. To assess exposure to PAHs in two species of benthic flatfish, English sole (*Parophrys vetulus*) and yellowfin sole (*Limanda aspera*). The specific aims of this component of the study were to a) determine PAH concentrations in sediments at sites utilized by English sole and yellowfin sole, b) determine concentrations of PAHs or their metabolites in sole and their prey, and c) compare PAH exposure levels in Kitimat Arm sole to PAH exposure levels in sole from other sites where PAHs from other sources are present, to assess the relative bioavailability of smelter-derived PAHs. Flatfish exposure monitoring was conducted in 2000, 2002, and 2004 for English sole and 2000 and 2002 for yellowfin sole.
3. To assess the extent of PAH-associated biological injury in benthic flatfish from Kitimat Arm. The specific aims of this component of the study were to a) monitor early biochemical responses to PAH exposure (e.g., DNA adducts and induction of cytochrome P4501A [CYP1A], an enzyme involved in the metabolism of PAHs) in English or yellowfin sole or both, b) monitor the prevalence of PAH-associated liver lesions in English and yellowfin sole, c) examine size and age distributions in English and yellowfin sole for unusual trends that could be associated with PAH exposure, d) examine reproductively maturing yellowfin and English sole in Kitimat Arm for evidence of inhibited gonadal development and related types of reproductive dysfunction that had been observed in English sole from sites in Puget Sound contaminated with PAHs and other industrial contaminants, and e) compare data on biological injury in Kitimat sole

with observations in other flatfish species from sites contaminated with PAHs from different sources to assess the relative toxicity of smelter-derived PAHs. Flatfish biological injury monitoring was conducted in 2000, 2002, and 2004 for English sole and 2000 and 2002 for yellowfin sole.

4. To assess exposure of salmon and flatfish to OCs and metals. The specific aims of this part of the study were to a) establish baseline levels of OCs and metals in prey and tissues of Kitimat salmon and flatfish and b) assess the potential for impacts on fish health or seafood safety. Analyses of OCs and metals in fish muscle and stomach contents samples were conducted in 2000 only.

Juvenile salmon were chosen as a target species because of the importance of salmon as a commercial and subsistence fishery resource, and because previous studies had shown that the health and survival of juvenile outmigrants could be affected by exposure to PAHs. For example, exposure to PAHs—alone and in combination with other environmental contaminants—has been associated with impaired growth and reduced disease resistance in juvenile salmon (Arkoosh et al. 1991, 1994, 2001, 2002, Varanasi et al. 1993, Casillas et al. 1993, 1995, 1997a, 1997b, Stein et al. 1995, Meador et al. 2006, 2008).

We chose to sample English sole because they are a widely used sentinel fish species whose sensitivity to PAH exposure has been established in field surveys in Puget Sound and at other sites throughout the western United States and Canada (Malins et al. 1982, 1984, Rhodes et al. 1987, Myers et al. 1987, 1992, 1994, 2003, Stehr et al. 2004, Johnson et al. 2008a). Yellowfin sole have been used less often in monitoring programs (Sol et al. 2000), but were chosen as a secondary benthic flatfish target species because the timing of their reproductive cycle (Wilderbuer et al. 1992, Nichols 1993) was optimal for a field assessment of reproductive function.

The biological endpoints measured in these flatfish species (presence of liver lesions, DNA damage, enzyme induction, and altered reproductive development) have been established as effects of PAH exposure in both field surveys and controlled laboratory studies (Malins et al. 1982, 1984, Krahn et al. 1986a, Johnson et al. 1988, 1995, 1999, Varanasi et al. 1989a, Casillas et al. 1991, Collier and Varanasi 1991, Schiewe et al. 1991, Stein et al. 1991, 1992, 1993, Collier et al. 1992, 1993a, 1993b, 1995, Myers and Rhodes 1988, 1998a, 1998b, 1993, 2003, Reichert et al. 1998, Sol et al. 1998, 2000). Causative relationships among exposure to PAHs, DNA damage, and liver cancer and related lesions in sole and other fish are especially well documented (Myers et al. 2003). Additionally, using field data collected over a number of years, we have been able to estimate threshold concentrations of PAHs in sediments from urban sites that are associated with the development of liver lesions, DNA damage, and certain kinds of reproductive impairment in English sole and related species (Horness et al. 1998, Johnson et al. 2002). This large body of background data made this suite of endpoints especially useful and appropriate for assessing PAH-related injury in fish from sites potentially impacted by the Alcan smelter.

It is important to note, however, that the PAH sources from the studies described above are different than the PAHs from the Alcan smelter. Smelter PAHs are primarily soot based, while PAHs characteristically found at industrialized urban sites are petroleum based. Soot-based PAHs are known to be less available biologically because they are molecularly bound to

the carbon in soot particles, preventing full uptake by organisms. Therefore, one of the goals of this study was to assess how the biological impacts of soot-derived PAHs might differ from the impacts of PAHs derived from other sources.

In addition, other urban study sites tend to have a "cocktail" or mixture of other contaminants, such as PCBs and heavy metals, which may exacerbate the biological effects of PAHs, acting, for example, as tumor promoters (Myers et al. 2003). Kitimat Arm is somewhat unique in that it is contaminated primarily by soot-associated, smelter-derived PAHs and has very low concentrations of other industrial or agricultural contaminants. This makes it a particular interesting site to study the effects of PAHs without potential confounding factors caused by other contaminants, and provides a unique opportunity to improve our understanding of their toxicity to aquatic organisms.

This report presents the results of the 5-year monitoring program. The research describes the spatial extent and severity of contamination from the smelter, as well as the associated level of fish injury, and will serve as a benchmark from which future reduction in PAH emissions can be measured in the Kitimat area. Additionally, it provides valuable data on how PAH sources can influence PAH bioavailability, exposure, and toxicity in marine biota by comparing biological effects in Kitimat fish with previously documented impacts on fish species from other marine and estuarine areas contaminated with nonsmelter-derived PAHs.

Methods

Field Sampling

Juvenile Salmon Collection

Juvenile Chinook salmon were collected annually from 2000 to 2004 for exposure assessment studies from Alcan Inner Harbour, Hospital Beach, and Wathlsto Creek, within Kitimat Arm, and at Kildala Arm, an undeveloped reference site outside of Kitimat Arm (Table 1, Figure 3). Additionally, fish were sampled from Outer Eurocan Beach in 2000, 2002, and 2003 and from Inner Eurocan Beach in 2002, 2003, and 2004. In 2000 only, salmon were also collected at Minette Bay, within Kitimat Arm, and at Kemano Village, the site of the power plant that provided electricity for the Alcan smelter. Additionally in 2000, juvenile Chinook salmon were collected from the Kitimat River Fish Hatchery, representing separate stocks from lower Kitimat River, upper Kitimat River, and Kildala River. At most sites, salmon sampling was conducted in June, but at Alcan Inner Harbour and Kildala Arm, salmon were collected in both May and June in 2000 and 2002 to examine temporal patterns in contaminant exposure. Sample collection times for all sites are summarized in Table 1.

Salmon were collected from estuarine sites by beach seine, generally following the procedures described in the Puget Sound Protocols (PTI 1990), and by Varanasi et al. (1993). Fish from all sampling sites were a combination of hatchery (as indicated by the presence of fin clips in some fish and a moderate to high amount of adipose tissue associated with the gastrointestinal organs) and wild fish (as strongly suggested by very low adipose tissue associated with the gastrointestinal organs). Of the fish sampled for this study, approximately 15–20% were wild fish; the rest were of hatchery origin. Fish captured from estuarine sites were held alive in aerated seawater and fish from Kitimat Hatchery were maintained in aerated

Table 1. Sampling schedule for juvenile salmon collection during the Kitimat Environmental Assessment, 2000–2004.

	Alcan Inner Harbor	Hospital Beach	Outer Eurocan Beach	Inner Eurocan Beach	Wathlsto Creek	Minette Bay	Kildala Arm	Kemano Village	Kitimat Hatchery
May 2000	X	X	X		X	X	X	X	X
June 2000	X						X		
May 2001	X	X	X		X		X		
May 2002	X	X	X	X	X		X		
June 2002	X						X		
May 2003	X	X		X	X		X		
May 2004	X	X		X	X		X		
June 2004	X						X		

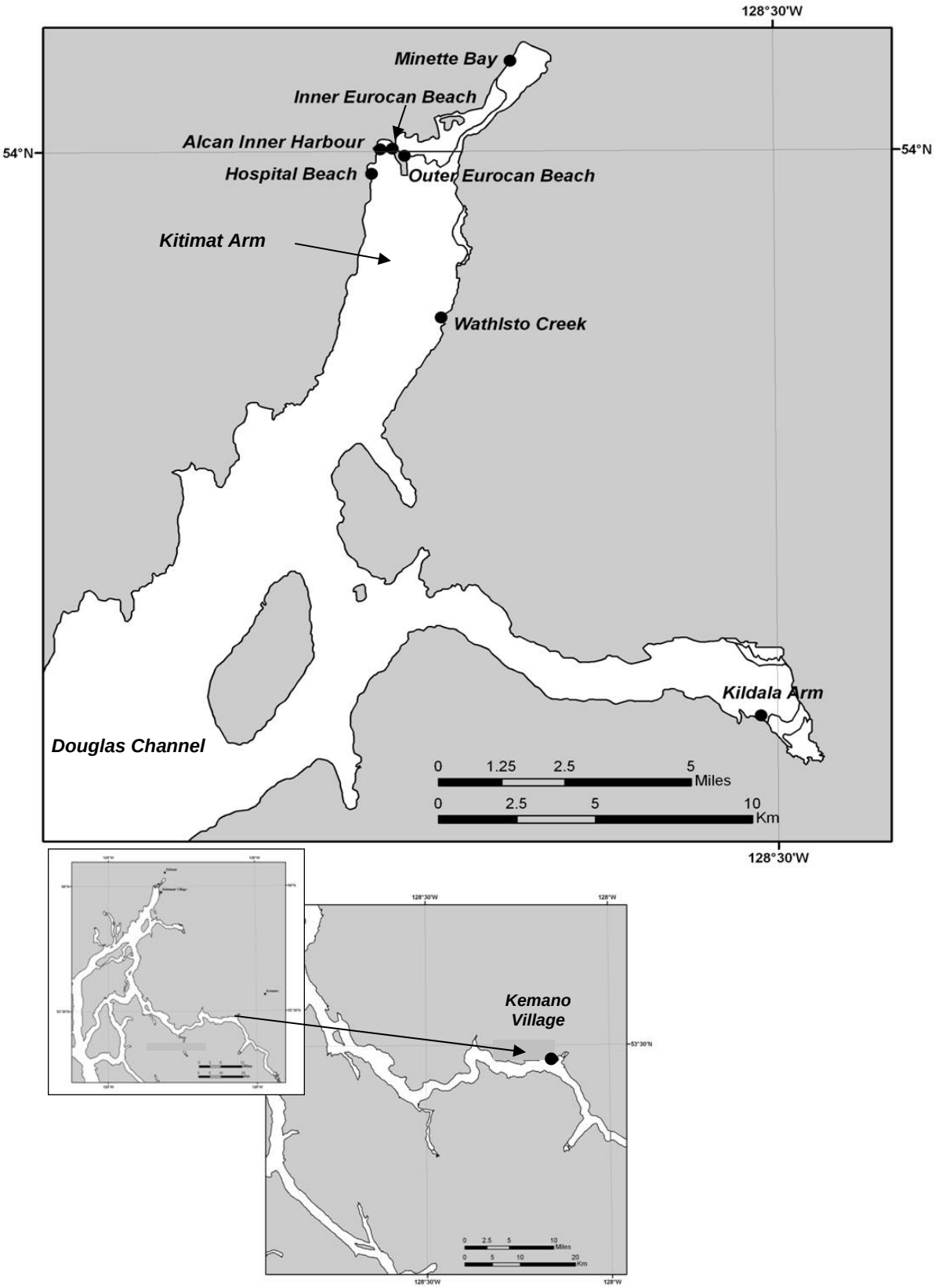

Figure 3. Locations of salmon sampling sites.

freshwater until necropsies could be conducted. Fish processing was done in laboratory facilities at the Department of Fisheries and Oceans Canada Kitimat Hatchery or onboard the 45-foot NWFSC research vessel (RV) *Harold W. Streeter*.

Prior to necropsy, juvenile salmon were weighed to the nearest 0.1 g and measured for fork length to the nearest mm. Tissues collected for these studies included stomach contents and bile for measurements of PAHs and their metabolites; liver for measurement of CYP1A induction as indicated by the activity of aryl hydrocarbon hydroxylase (AHH), an enzyme involved in the metabolism of PAHs (2000 only); and liver for PAH-DNA adducts, an indicator of DNA damage (2000–2003 only). Bile, liver, and stomach contents were collected as described in Varanasi et al. (1993), Stein et al. (1995), and Stehr et al. (2000). Because of the small size of the salmon, tissues were composited for analysis. At each site, or for every collection period, approximately 10–40 juvenile Chinook salmon, depending on the size of fish collected, were sampled for each composite in order to obtain at least 3 g of tissue for stomach contents and whole body analyses, and 3–5 µL of bile for measurement of PAH metabolites. We attempted to collect three composites at each site for every sampling period; however, the number of composites collected was dependent on the number of fish available.

Stomach contents samples were stored in prerinsed 20 ml glass vials. Bile was composited into 4 ml vials containing glass 250-µL inserts. Bile, whole body, and stomach content samples were maintained on ice during the necropsy procedure, then transferred to –20°C or colder freezers for storage. Liver samples for DNA adduct and AHH analyses were composited in plastic cryogenic vials, frozen, and stored in liquid nitrogen. Frozen bile, body, and stomach samples were shipped on ice to Seattle via air, while liver samples were shipped in a dry shipper charged with liquid nitrogen. Samples were distributed to the appropriate laboratories upon return to Seattle. Samples for organic chemical analyses were stored at –20°C, and samples for AHH and DNA adduct analyses were stored at –80°C until analyses could be carried out.

Flatfish Collection

Subadult to adult English sole (length > 150 mm) were collected in June 2000, 2002, and 2004 at six sites (Figure 4): four sites in Kitimat Arm (Hospital Beach, Eurocan, Kitamaat Village, and Emsley Cove south of Alcan on the west side of Kitimat Arm, along the path of the effluent plume from the Alcan plant), one site in Kildala Arm, and one site at Kitlope (a pristine area that is part of the Haisla First Nation's ancestral lands). Adult yellowfin sole (length > 200 mm) were collected in June 2000 and 2002 only from Hospital Beach, Eurocan, Kitamaat Village, Emsley Cove, Kildala Arm, and Kitlope (Figure 4). Up to 20 female and 20 male yellowfin sole were collected at each site.

Flatfish were collected by a 25-foot otter trawl similar to that used by the Southern California Coastal Water Research Project, as described in the Puget Sound Protocols (PTI 1990). The net was deployed from the RV *Harold W. Streeter* and towed for 5–15 minutes at a speed of 1.5–2.5 knots. Fish were identified and sorted by species, and target species (English sole and yellowfin sole) were placed in holding tanks on board the research vessel. Fish were maintained alive for no more than a few hours in tanks with flowing seawater until necropsies could be performed in the shipboard laboratory. Bycatch was released.

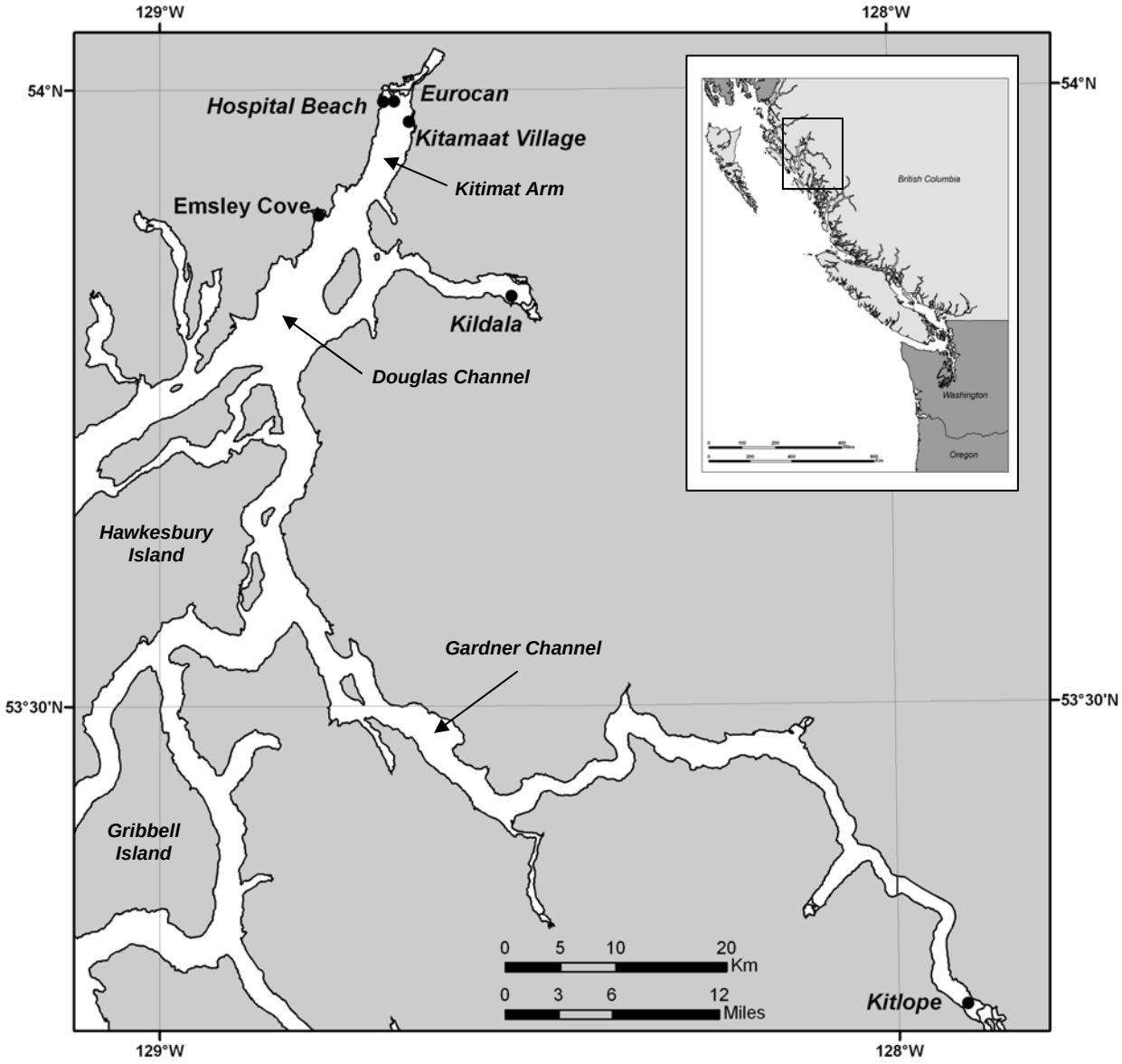

Figure 4. Locations of flatfish sampling sites.

Prior to necropsy, each fish was assigned a unique specimen number and all sample containers were labeled with this number for sample tracking. Fish were weighed (to the nearest gram) and measured (total length to the nearest 1 mm), and otoliths were collected for age determination. Fish were then sacrificed by severing the spinal cord and necropsied. In English sole, tissues collected during necropsy included stomach contents and bile for measurements of PAHs and their metabolites, liver for measurement of DNA adducts, and liver, kidney, and gonad for histopathological examination. In 2000 liver tissue was also collected for measurement of AHH activity, and muscle was collected for organic contaminant and metals analyses. In yellowfin sole, only bile and liver, kidney, and gonad for histopathological examination were collected.

In yellowfin and English sole sampled for reproductive assessment, weights of ovaries, liver, and gutted bodies (determined to the nearest gram) were collected to determine gonadosomatic index (GSI), liver somatic index (LSI), and CF. Samples of liver, bile, and muscle collected for organic chemical analysis were maintained on ice, then transferred to –20°C freezers for temporary storage at the end of each day. Samples of liver for AHH and DNA adduct analyses were immediately placed in liquid nitrogen and stored there until transport to the Seattle laboratory. Frozen samples were shipped on ice to Seattle via air, and liquid nitrogen samples were shipped in a dry shipper charged with liquid nitrogen. Histology samples were preserved in Dietrich's fixative and returned to Seattle onboard the RV *Harold W. Streeter*. Samples were distributed to the appropriate laboratories upon return to Seattle. Prior to analyses, samples for organic chemical analyses were stored at –20°C, and liver samples for CYP1A and DNA adduct analyses and plasma were stored at –80°C. Fixed tissues were transferred to 70% ethanol at the Seattle laboratory and stored in ethanol prior to laboratory processing. Details of necropsy, histology sample collection and fixation, and collection and preservation of tissue samples for chemistry and biomarker analyses are described in Stehr et al. (1993) and Johnson et al. (1988, 1994).

Sediment Collection

At all salmon collection sites sampled in May 2000–2004, sediments were collected using a petite Ponar grab sampler (0.01 m^2). Sediments were collected at all of the salmon sampling sites, shown in Figure 3. Three grab samples were taken at regular intervals across the center of the area encompassed by the beach seine at each salmon collection site. A stainless steel spoon was used to remove the top 2–3 cm of surface sediment from each grab. The surface sediments from the three grab subsamples were then mixed together in an isopropyl alcohol–rinsed stainless steel bowl with an isopropyl alcohol–rinsed stainless steel spoon, then a subsample of this composite was placed in a solvent-rinsed 4-oz jar for chemical analysis.

At flatfish sampling sites (i.e., Hospital Beach, Eurocan, Kitamaat Village, Emsley Cove, Kildala Arm, and Kitlope, Figure 4), grab samples were taken at three stations located at the beginning, middle, and end of the fishing trawl tracks. At each sampling station, surface sediment was collected with a modified Van Veen grab sampler (0.1 m^2). One grab sample was taken at each station. A surface skim (2–3 cm deep) was taken from each grab sample, and sediments from the three subsamples were mixed together in an isopropyl alcohol–rinsed stainless steel bowl with an isopropyl alcohol–rinsed stainless steel spoon. A subsample of this composite was then placed in a solvent-rinsed 4-oz sample for chemical analysis. Sediment samples were frozen and stored at –20°C after collection and returned to Seattle onboard the RV *Harold W. Streeter*. At the Seattle laboratory, the samples were stored at –20°C prior to analysis. During a freezer failure, most of the sediment samples collected from the flatfish sampling sites in 2004 thawed, so could not be analyzed accurately for PAH concentrations. No data are reported for these samples.

Kocso pitch samples

To characterize the types of PAHs generated by smelter processes, two samples of Alcan Kocso pitch (cargo 6/2,000 ppm) from two different batches of pitch were obtained in 2001 from Alcan Inc. for PAH analyses. The pitch is used in the smelting process to form the carbon

anodes used in separating aluminum from bauxite ore. The process produces significant emissions of PAHs. PAHs may also be released to the environment during transport and transfer of the pitch to the locations where it is used in the plant.

Sample Analyses

Analyses of Sediment and Stomach Contents for PAHs using GC/MS

Sediment samples collected from the fishing sites, stomach contents samples from both flatfish and salmon, and Kocso pitch samples were analyzed for individual PAHs by gas chromatography/mass spectrometry (GC/MS) (Sloan et al. 2005). Briefly, each sample was weighed and mixed with drying agents (sodium sulfate and magnesium sulfate), transferred to a 33-ml accelerated solvent extraction (ASE) cell, and the surrogate standard was added to the top of each sample cell. Samples were extracted using ASE at 2,000 psi and 100°C with two cell volumes using dichloromethane, and the combined extract (≈50 ml) was collected in a 60-ml collection tube. A second internal standard was added to measure the percentage of the total extract that was ultimately analyzed by GC/MS after the sample cleanup steps, and an aliquot of the extract was taken and set aside for lipid determination. The remaining sample extract was filtered through a column of silica gel and alumina and concentrated for further cleanup to remove interfering lipid compounds.

For sediment samples, activated copper was added to the sample extracts and allowed to sit overnight to remove interfering sulfur compounds. Size exclusion chromatography with high-performance liquid chromatography (HPLC) was used to collect the fraction containing the analytes of interest. The fraction was concentrated to a minimal volume and a final internal standard was added.

Compounds of interest in the sample fractions were separated on a 60 m DB-5 capillary column (25 μm film thickness) by gas chromatography and analyzed by mass spectrometry (Agilent 5973N Mass Selective Detector, Agilent Technologies), with the instrument operated in the electron impact (EI) scan (sediments and pitch) or single ion monitoring (SIM) mode (stomach contents).

Summed high molecular weight aromatic hydrocarbons (ΣHAHs) included the summed concentrations of fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[e]pyrene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, perylene, dibenz[a,h]anthracene/dibenz[a,c]anthracene, and benzo[ghi]perylene. Perylene was of particular interest in sediments at reference sites as an indication of PAHs derived from natural decomposition of aquatic and terrestrial debris (Venkatesan 1988). Summed low molecular weight aromatic hydrocarbons (ΣLAHs) were calculated by summing concentrations of naphthalene, 2-methylnaphthalene, 1-methylnaphthalene, biphenyl, acenaphthylene, acenaphthene, 1,2,5-trimethylnaphthalene, fluorene, dibenzothiophene, phenanthrene, anthracene, 1-methylphenanthrene, and retene.

Retene concentrations were determined, in addition to the usual suite of PAHs, as an indicator of exposure to wood waste and pulp mill effluent. This low molecular weight PAH is derived from wood products (Zender et al. 1994, Tavendale et al. 1995) and can be toxic to early

life stages of fish (Billiard et al. 1999). Analyte concentrations were calculated against the appropriate surrogate standard, using a multilevel point-to-point calibration. Analyte concentrations for sediment are reported on a dry weight basis, while analyte concentrations for stomach contents are reported on a wet weight basis.

Quality assurance measures adhered to include the analysis of a method blank and an appropriate Standard Reference Material (SRM) certified by the National Institute of Standards and Technology (NIST) with each sample batch. For sediments, SRM 1941a or 1941b was analyzed with each sample batch; for stomach contents, SRM 1947a or 1947b was used. Surrogate recoveries were monitored against quality assurance (QA) criteria. Continuing calibration standards were analyzed on the GC/MS at the beginning, middle, and end of each sample batch, and the variability in the analyte responses was monitored against QA criteria. The method blanks and reference materials analyzed with the samples also met relevant QA criteria (Sloan et al. 2006).

Organochlorine analyses of stomach contents and fish tissues by GC/MS

Composite stomach contents samples from salmon and English sole, salmon whole bodies, and flatfish muscle tissue collected in 2000 were analyzed by GC/MS for OCs (PCBs, DDTs, hexachlorocyclohexanes [HCHs], chlordanes, hexachlorobenzene, aldrin, dieldrin, mirex, and endosulfan) as described in Sloan et al. (2005), with the extraction and cleanup steps as summarized in the subsection above, Analysis of Sediment and Stomach Contents for PAHS using GC/MS, which discusses procedures for PAH analyses.

Compounds of interest in the sample fractions were separated on a 60 m DB-5 capillary column (25 μm film thickness) by gas chromatography and analyzed by mass spectrometry (Agilent 5973N Mass Selective Detector, Agilent Technologies), with the instrument operated in the EI SIM mode for PCBs, DDTs, HCHs, chlordanes, aldrin, dieldrin, mirex, and endosulfan I. Summed PCBs (ΣPCBs) were calculated by adding the concentrations of the following congeners: PCBs 17, 18, 28, 31, 33, 44, 49, 52, 66, 70, 74, 82, 87, 95, 99, 101/90, 105, 110, 118, 128, 138/163/164, 149, 151, 153/132, 156, 158, 170, 171, 177, 180, 183, 187/159/182, 191, 194, 195, 199, 205, 206, 208, and 209. The summed HCHs (ΣHCHs) included α-HCH, β-HCH, and γ-HCH (lindane). Summed DDTs (ΣDDTs) included p,p'-DDD, p,p'-DDE, p,p'-DDT, o,p'-DDD, o,p'-DDE and o,p'-DDT. Summed chlordanes included heptachlor, heptachlor epoxide, γ-chlordane, α-chlordane, oxychlordane, *cis*-nonachlor, *trans*-nonachlor and nonachlor III. Endosulfan I was the only endosulfan measured. Analyte concentrations were calculated against a surrogate standard, using a multilevel point-to-point calibration, and the results were reported on a wet weight basis.

QA measures adhered to include the analysis of a method blank and an appropriate SRM certified by NIST with each sample batch (SRM 1947a or 1947b). Surrogate recoveries in all samples were monitored against QA criteria. Continuing calibration standards were analyzed on the GC/MS at the beginning, middle, and end of each sample batch, and the variability in the analyte responses also met QA criteria. The method blanks and SRMs analyzed with the samples also met the relevant QA criteria (Sloan et al. 2006).

Analyses of Metals in Fish Muscle

Metals analyses on English sole muscle tissue samples collected in 2000 were conducted as described in Meador et al. (1994). Tissue samples were dried and digested with nitric acid using a combination of convective and microwave heating. The digest was initially treated with hydrogen peroxide then diluted with water. Next it was analyzed using an atomic absorption spectrophotometer. Analytes measured included aluminum, silicon, iron, chromium, manganese, nickel, copper, zinc, arsenic, selenium, silver, cadmium, tin, antimony, mercury, and lead.

PAH Metabolites in Bile

Flatfish and salmon bile samples were analyzed by HPLC with fluorescence detection for PAH metabolites as described in Krahn et al. (1986b), with modifications as outlined below.

Fluorescent aromatic compounds (FACs) in bile were analyzed on a Waters (Milford, Massachusetts) HPLC equipped with a Waters WISP model 715 automatic injector, and three Perkin Elmer (Norwalk, Connecticut) model 40 fluorescence detectors connected in series and interfaced to a Waters Millennium data acquisition workstation. A 0.20-cm by 2-cm guard column containing Perisorb 30- to 44-µm reverse-phase C_{18} packing (Upchurch Scientific, Oak Harbor, Washington) was used in series with a Perkin Elmer HC-ODS/PAH 10-µm (0.26-cm by 25-cm) reverse-phase analytical column.

For each sample, 3–5 µL of thawed, untreated bile was injected onto the analytical column and eluted with an HPLC linear gradient (flow rate of 0.7 ml/min) beginning with 100% solvent A (water containing 5 ppm acetic acid) to a final composition of 100% solvent B (methanol) during a period of 15 min. After holding the mobile phase at 100% solvent B for 10 min, solvent conditions were returned to 100% solvent A during a period of 3 min. The system was then allowed to reequilibrate for 10 min at 100% solvent A before the next sample was injected. The total run time, including running of the linear gradient and reequilibration of the system, was 38 min. All solvents were degassed with helium; the column temperature was held at 50°C.

For each set of samples, the HPLC calibration standards (HPLC CS), containing known concentrations of NPH, PHN, and BaP, were analyzed at the beginning of the set and after every six or seven samples. A reference bile sample was analyzed near the beginning and end of each set analyzed by HPLC. Biliary FACs were monitored by fluorescence at excitation/emission (ex/em) wavelength pairs for NPH (ex/em 290/335 nm), PHN (ex/em 260/380 nm) and BaP (ex/em 380/430 nm). The total area for all peaks in the region of the chromatogram where these compounds are known to elute (>9 min) was integrated for each wavelength pair. Quantification of analytes was performed according to Krahn et al. (1986b) using Waters Millenium^{2010} Chromatograpy Manager software. The concentrations (ng PAH equivalents per g of bile, wet wt) of FACs in the samples were calculated according to the response of each PAH in the HPLC CS. If the fluorescence response in a sample was sufficiently high that a detector response reached its maximum (saturated), the sample was reanalyzed using a smaller injection volume.

CYP1A Activity in Liver

CYP1A activity in the liver was measured using a catalytic assay for AHH activity as described in Collier et al. (1995). Hepatic microsomes were prepared from frozen liver samples as described previously (Collier et al. 1995) except that microsomes were resuspended in 0.25 molar sucrose made up in 80/20 v/v water/glycerol. The suspensions were frozen at –80°C until CYP1A assays were performed. AHH activities were assayed in triplicate at 25° using ^{14}C-BaP as the primary substrate (Collier et al. 1995).

DNA Adducts in Liver

Hepatic xenobiotic DNA adducts were measured in livers of English sole and Chinook salmon by the ^{32}P-postlabeling method of Reichert and French (1994). The detection of DNA adducts by ^{32}P-postlabeling was a multistep sequence involving a series of biochemical reactions. Initially, DNA was hydrolyzed enzymatically to 3'-monophosphates. The digest was then enriched in xenobiotic-modified mononucleotides by the selective removal of normal nucleotides. Following the enrichment step, the adducted DNA was enzymatically labeled at the 5'-hydroxyl position with [^{32}P]phosphate to form [5'-^{32}P]deoxyribonucleoside 3',5'-bisphosphates. Separation of the ^{32}P-labeled adducts was accomplished by two-dimensional, thin-layer chromatography on polyethyleneimine-modified cellulose sheets. Autoradiography or storage phosphor imaging (Reichert et al. 1992) was then used to locate the radio-labeled adducts on the chromatogram. The radioactivity on the chromatograms was then quantitated by liquid scintillation spectrometry or storage phosphor imaging.

Age Determination for Flatfish

Fish age for English and yellowfin sole and was determined to the nearest year by counting the number of clearly defined opaque zones of whole otoliths under a binocular dissecting microscope (Chilton and Beamish 1982).

Histology

Liver and gonad samples from English sole and yellowfin sole were preserved in Dietrich's fixative, then transferred to 70% ethanol for storage. Subsequently, the samples were embedded in paraffin, sectioned, and stained with hematoxylin and eosin, as described in Myers et al. (1987) and Stehr et al. (1993), and examined microscopically. Hepatic lesions were classified according to the criteria outlined in Myers et al. (1987), then grouped into the following categories: neoplasms (hepatocellular carcinoma, cholangiocellular carcinoma, adenoma, and cholangioma), foci of cellular alteration or FCA (eosinophilic foci, basophilic foci, clear cell foci), nonneoplastic proliferative lesions (hepatocellular regeneration, oval cell proliferation, chlolangiofibrosis, increased mitotic activity in liver parenchyma), and specific degeneration/necrosis or SDN (nuclear pleomorphism or hepatic megalocytosis).

The developmental stage of ovaries was determined and classified according to criteria described in Johnson et al. (1991). Ovaries were also examined for follicular atresia, hermaphroditism, ovarian macrophage aggregates, and other inflammatory lesions associated with oocyte resorption, including lymphoid or macrophage infiltrates, using criteria described in Johnson et al. (1991). The developmental stages of testes were determined and classified

according to criteria described in Sol et al. (1998) and testes were examined for inflammatory, necrotic, and proliferative lesions.

Determination of Somatic Indices

Fish were weighed (to the nearest gram) and measured (fork length, to the nearest millimeter), and liver and gonads were excised and weighed (to the nearest gram). All other internal organs were then removed and the animal was weighed (to the nearest gram) to determine gutted body weight. GSI (Nikolsky 1963, Shul'man 1974) was calculated according to the formula

$$GSI = (\text{ovary weight (g)}/\text{gutted body weight (g)}) \times 100 \quad (1)$$

LSI (Nikolsky 1963, Shul'man 1974) was calculated according to the formula

$$LSI = (\text{liver weight (g)}/\text{gutted body weight (g)}) \times 100 \quad (2)$$

Because low body weight may be associated with suppressed ovarian development in adult female fish (Burton and Idler 1987) and may generally be a sign of poor health, a CF was determined for all sampled animals. This made it possible to distinguish the influence of emaciation on ovarian development from any potential effects of contaminant exposure, as well as to assess effects of contaminant exposure on fish condition. CF (Ricker 1975) is calculated using the formula

$$CF = \text{gutted body weight (g)}/\text{length}^3\text{ (cm)} \quad (3)$$

Data Analysis and Statistical Methods

Data were stored in a FilemakerPro relational database. Statistical analyses were conducted using Statview and JMP statistical packages. Linear regression analysis (Zar 1999) was used to evaluate relationships between contaminant concentrations in sediments, fish stomach contents, and fish bile. Analysis of variance (ANOVA) and the Tukey-Kramer multiple comparison test (Zar 1999) were used to compare contaminant concentrations in sediments and fish collected from different sites in Kitimat Arm and surrounding areas, as well as with levels from other Pacific Northwest sites. Values used in these analyses were log transformed to conform more closely to a normal distribution as necessary.

Dunnett's test for comparison of proportions (Zar 1999) was used to compare prevalences of liver and gonad lesions in flatfish collected from different sites in Kitimat Arm with reference site prevalences, to compare lesions prevalences in Kitimat flatfish with flatfish from other Pacific Northwest sites, and to compare proportions of reproductively maturing English and yellowfin sole from Kitimat Arm sites and reference sites. Linear regression analysis (Zar 1999) was used to examine the relationships between PAH concentrations in sediment, fish stomach contents, and fish bile. Logistic regression was used to compare proportions of fish with liver and gonad lesions and proportions of fish showing reproductive development, at different sampling sites, while adjusting for potential confounding factors such as fish length and age.

Hockey stick–regression analyses to examine the relationship of sediment PAH concentration with DNA adduct levels and lesion prevalences in fish from Kitimat Arm sites and other Pacific coast sites were carried out as described in Horness et al. (1998) and Johnson et al. (2002).

Results

Sediment Characterization

Contaminants in Sediment Samples

Detailed analysis by GC/MS (Sloan et al. 2005) of sediments collected from 2000 to 2004 showed clearly that PAHs were present in sediments at both the salmon and flatfish sampling sites within Kitimat Arm. Mean concentrations of PAHs in sediment from the salmon sampling sites (i.e., Alcan Inner Harbor, Hospital Beach, Outer Eurocan Beach, Inner Eurocan Beach, Wathlsto Creek, and Minette Bay within Kitimat Arm, and Kemano Village and Kildala Arm outside of Kitimat Arm) are shown in Figure 5.

The mean concentration of ΣHAHs in sediments (Figure 5A) at the Alcan Inner Harbour site (29,000 ng/g dry wt) was more than an order of magnitude greater, and significantly higher, than concentrations at any of the other sites ($p \leq 0.05$, ANOVA and Tukey-Kramer multiple comparison test, performed on log-transformed values). Mean concentrations of HAHs at the Outer Eurocan Beach (1,300 ng/g dry wt) and Inner Eurocan Beach (870 ng/g dry wt) sites were significantly higher than concentrations at the Minette Bay and Wathlsto Creek sites within Kitimat Arm, as well as the Kemano Village and Kildala Arm sites. At Hospital Beach, Minette Bay, and Wathlsto Creek, mean levels of HAHs ranged from 36 to 470 ng/g dry wt, but were not significantly above concentrations measured in Kildala Arm and Kemano Village sediments, which ranged from below the lower limit of quantitation (<LOQ) to 27 ng/g dry wt.

Mean concentrations of ΣLAHs (Figure 5B) at the Alcan Inner Harbour site (2,700 ng/g dry wt) were significantly higher than at any of the other sites except for Outer Eurocan Beach ($p \leq 0.05$, ANOVA and Tukey-Kramer multiple comparison test, performed on log-transformed values). Concentrations of ΣLAHs at the Outer Eurocan Beach site (140 ng/g dry wt) were quite a bit lower than at Alcan Inner Harbor, but still significantly higher than at the Wathlsto Creek and Kemano Village sites, where LAH levels ranged from less than LOQ-2.6 ng/g dry wt. Concentrations of ΣLAHs at the Hospital Beach, Inner Eurocan Beach, Minette Bay, and Kildala Arm sites ranged from 17 to 56 ng/g dry wt and were not significantly different from ΣLAH levels at Wathlsto Creek or Kemano Village.

Mean concentrations of low and high molecular weight aromatic hydrocarbons (AHs) in sediments collected along English sole fishing transects, at sites within Kitimat Arm (Hospital Beach, Eurocan, Kitamaat Village, and Emsley Cove), and outside Kitimat Arm (Kildala Arm and Kitlope), averaged over all sampling years, are shown in Figure 6. Mean concentrations of ΣHAHs (Figure 6A) at the Hospital Beach site near the Alcan plant (24,000 ng/g dry wt), were significantly higher than at any of the other sites (ANOVA and Tukey-Kramer multiple range test on log-transformed values, $p \leq 0.05$). Mean concentrations of ΣHAHs at the three other sites in Kitimat Arm (Eurocan, Kitamaat Village, and Emsley Cove) ranged from 850 to 1,500 ng/g dry wt, and were not significantly different from each other. Kitlope had very low mean

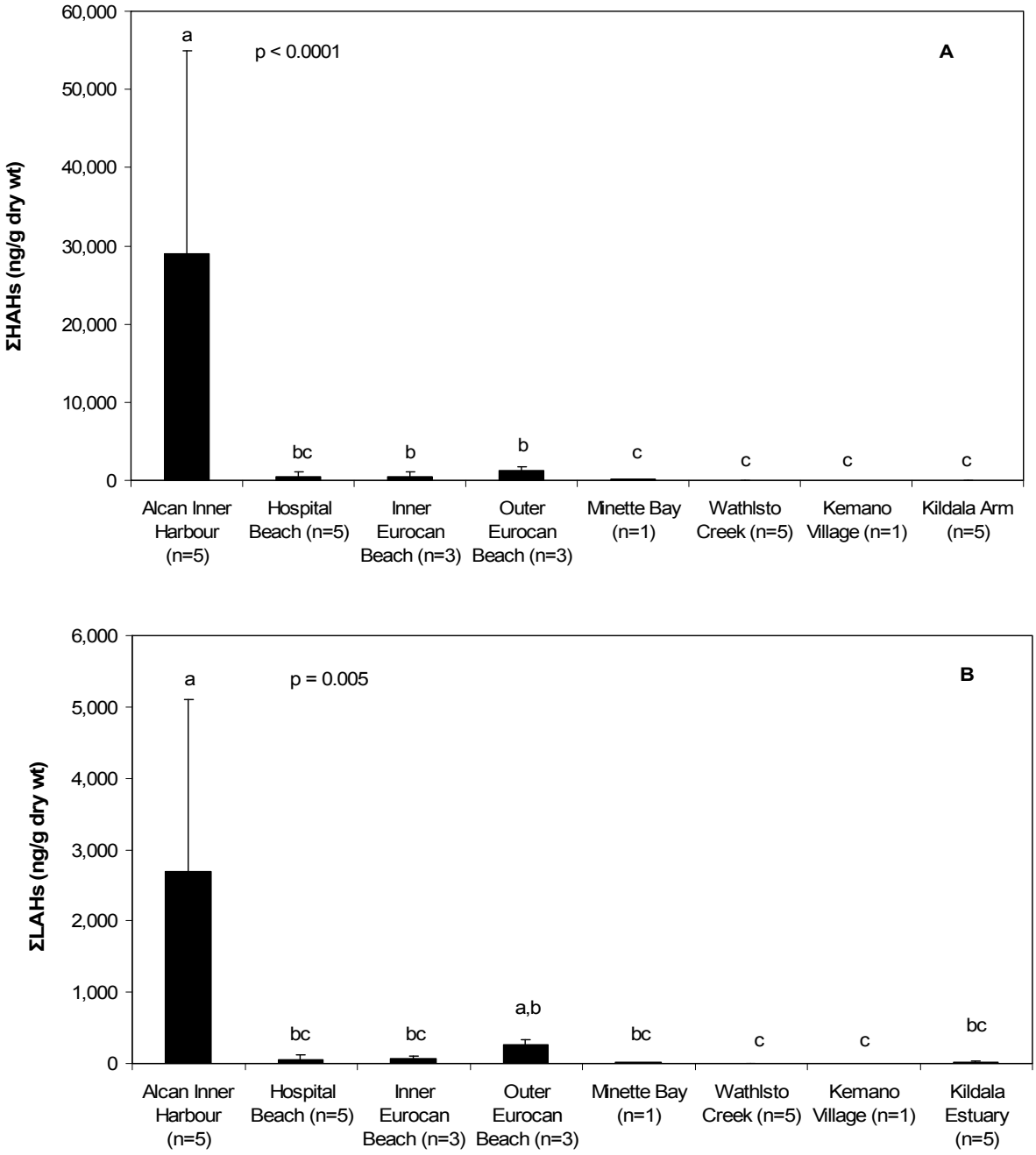

Figure 5. Mean overall concentrations (ng/g dry wt) of (A) high molecular weight aromatic hydrocarbons (ΣHAHs) and (B) low molecular weight aromatic hydrocarbons (ΣLAHs) in sediments from salmon sampling sites within Kitimat Arm (Alcan Inner Harbour, Hospital Beach, Inner Eurocan Beach, Outer Eurocan Beach, Minette Bay, and Wathlsto Creek) and from reference sites outside Kitimat Arm (Kemano Village and Kildala Arm) for the sampling period from 2000 to 2004. Values with different letter superscripts are significantly different ($p < 0.05$).

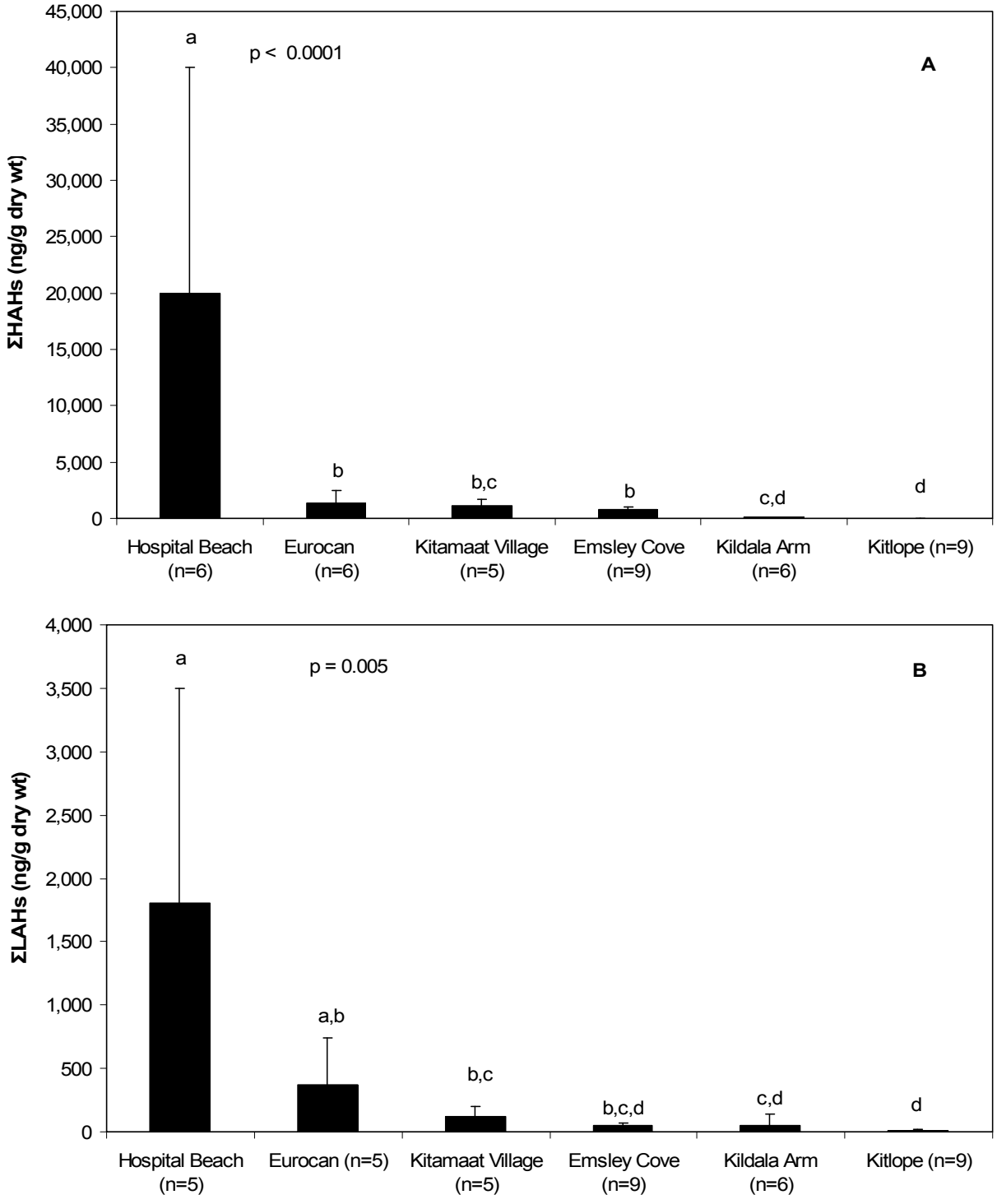

Figure 6. Mean overall concentrations (ng/g dry wt) of (A) ΣHAHs and (B) ΣLAHs in sediments from flatfish sampling sites within Kitimat Arm (Hospital Beach, Eurocan, Kitamaat Village, and Emsley Cove) and from reference sites outside Kitimat Arm (Kildala Arm and Kitlope) for the sampling period from 2000 to 2004. Values with different letter superscripts are significantly different ($p < 0.05$).

concentrations of ΣHAHs (12 ng/g dry wt), significantly lower than any of the Kitimat Arm sites. Levels at the Kildala Arm site were slightly higher (73 ng/g dry wt) and significantly different from levels at Hospital Beach, Eurocan, and Emsley Cove, but not Kitamaat Village.

Mean concentrations of ΣLAHs (Figure 6B) were again highest at Hospital Beach (2,200 ng/g dry wt). Concentrations of ΣLAHs in sediment at Eurocan and Kitamaat Village were considerably lower (120–430 ng/g dry wt) and even lower at Emsley Cove and Kildala Arm (42–62 ng/g dry wt). Concentrations of ΣLAHs were lowest at Kitlope (10 ng/g dry wt). The sediment LAH concentration was significantly higher at Hospital Beach than at any of the sites except Eurocan. Concentrations at Eurocan were significantly higher than at Emsley Cove, Kildala Arm, or Kitlope, and concentrations at Kitamaat Village were significantly higher than at Kitlope (ANOVA and Tukey-Kramer multiple range test on log-transformed values, $p \leq 0.05$).

Characterization of PAH Profiles and Comparison to PAHs in Alcan Pitch

To characterize the types of PAHs found in sediment samples from sites near the smelter and in other locations inside and outside of Kitimat Arm, the distributions of various individual PAHs in these sediments were compared to each other, as well as to the relative PAH distributions of Alcan Kocso pitch samples (cargo 6/2,000 ppm) used in smelter processes that generate PAHs. Kocso pitch samples (Figure 7 and Figure 8) contained a number of high molecular weight PAHs (fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[k]fluoranthene, benzo[e]pyrene, benzo[a]pyrene, ideno[1,2,3-cd]pyrene, and benzo[ghi]perylene) in roughly equal proportions. Perylene and dibenzanthracenes were also present but made up smaller proportions of ΣHAHs. The predominant LAH in the Alcan pitch samples was phenanthrene, although many other compounds were also present.

Sediments from most of the salmon sampling sites showed profiles of HAHs much like those of Alcan pitch (Figure 7A), although sediments from Inner Eurocan Beach had a substantially higher proportion of perylene than the pitch samples. Similarly, LAH profiles in sediments from Alcan Inner Harbour were very similar to those in the pitch samples, and sediments from other sites within Kitimat Arm (i.e., Hospital Beach, Outer Eurocan Beach, Inner Eurocan Beach, Minette Bay, and Wathlsto Creek) all contained substantial proportions of phenanthrene, similar to the pitch samples (Figure 7B). However, all sediment samples also contained some retene, which was absent from the pitch samples. Retene made up a relatively small proportion of sediment ΣLAHs at Alcan Inner Harbour, but was more predominant at other sites within Kitimat Arm, particularly at Outer Eurocan Beach and Minette Bay. At Kildala Arm, sediments had a distinctive LAH profile, with a much lower proportion of phenanthrene than sediments from sites within Kitimat Arm and higher proportions of retene and dimethyl naphthalene. Sediments from Kemano Village were not included in these analyses because concentrations of all PAHs were less than LOQ.

At the flatfish sampling sites, HAH profiles in sediments from Hospital Beach and Emsley Cove were much like those of Alcan pitch, but sediments from Eurocan, Kitamaat Village, Kildala Arm, and Kitlope all contained much higher proportions of perylene than the pitch samples (Figure 8A). Profiles of LAHs in sediments from Hospital Beach and Emsley Cove were also fairly similar to those in the pitch samples, although the sediments also contained

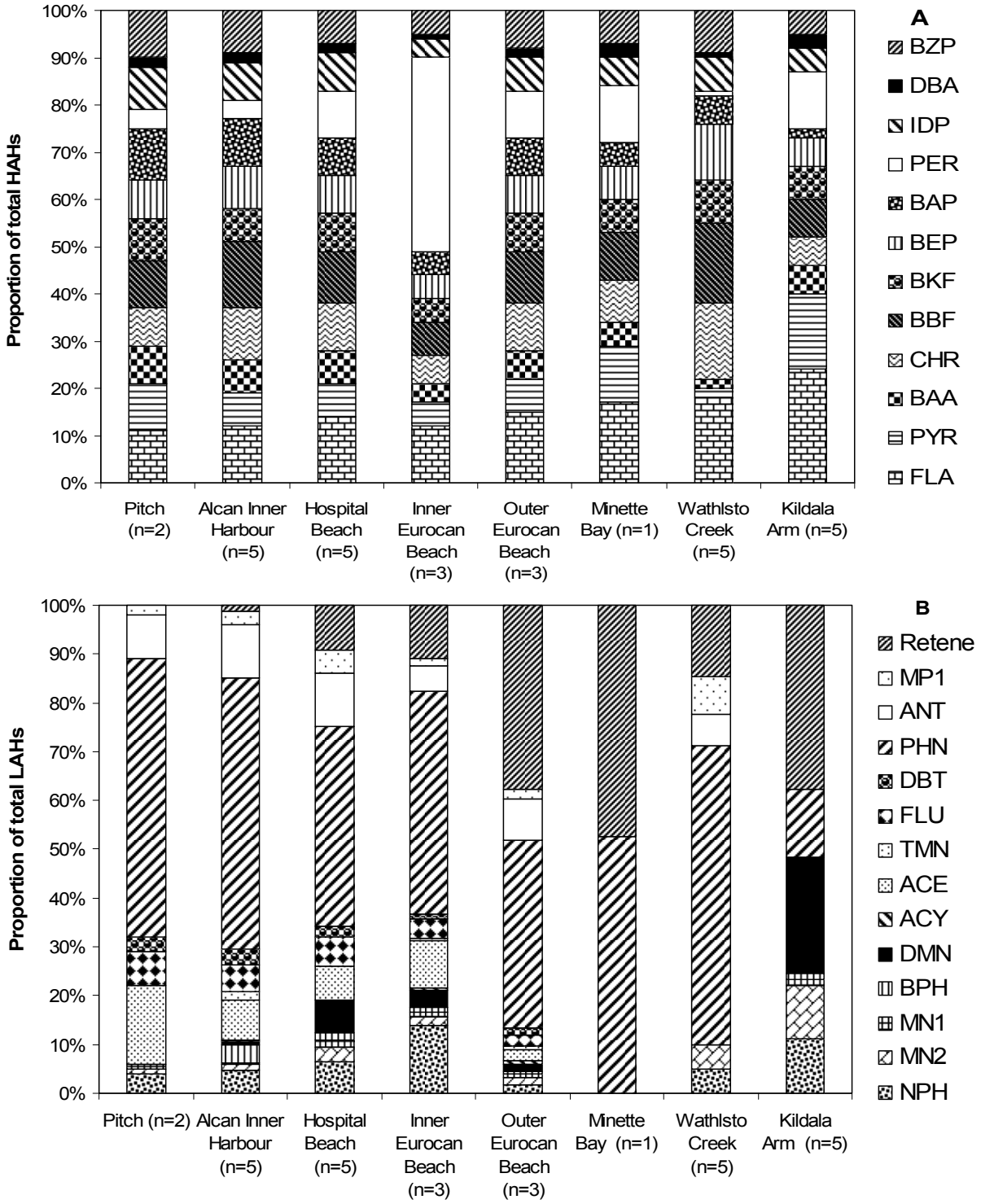

Figure 7. Proportions of (A) HAHs and (B) LAHs in sediments from salmon sampling sites within Kitimat Arm (Alcan Inner Harbour, Hospital Beach, Inner Eurocan Beach, Outer Eurocan Beach, Minette Bay, and Wathlsto Creek) reference sites outside Kitimat Arm (Kemano Village and Kildala Arm), and in pitch samples from the Alcan smelter. Proportions are averages derived from samples collected from 2000 to 2004. (See Glossary under HAH and LAH for a key to abbreviations.)

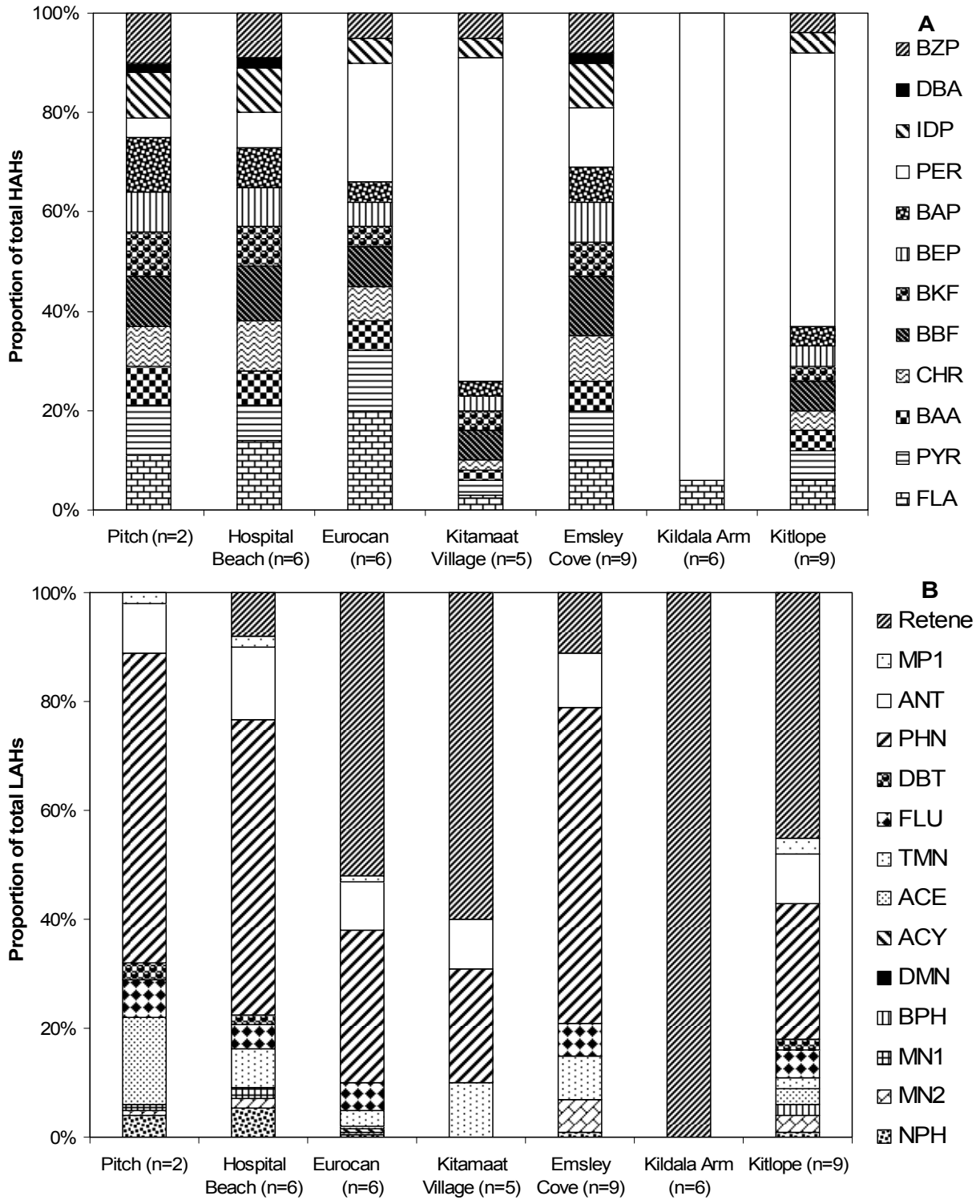

Figure 8. Proportions of (A) individual HAHs and (B) individual LAHs in sediments from flatfish sampling sites within Kitimat Arm (Hospital Beach, Eurocan, Kitamaat Village, and Emsley Cove), from reference sites outside Kitimat Arm (Kildala Arm and Kitlope), and in pitch samples from the Alcan smelter. Proportions are averages derived from samples collected from 2000 to 2004.

retene and trimethyl naphthalene, which were absent from the pitch samples. Sediments from the other sites (Eurocan, Kitamaat Village, Kildala Arm, and Kitlope) all had much higher proportions of retene and lower proportions of phenanthrene than either the pitch samples or sediments from Hospital Beach and Emsley Cove and, except for Kildala Arm, also contained the trimethyl naphthalenes absent from the pitch samples (Figure 8B).

Trends in Sediment PAH Concentrations

Sediments collected from 2000 to 2004 at salmon and flatfish fishing sites were compared to determine if any trends in PAH concentrations could be observed. At most of the salmon sampling sites (Outer Eurocan Beach, Inner Eurocan Beach, Wathlsto Creek, and Kildala Arm), sediment LAH and HAH concentrations showed no clear increasing or decreasing trends from 2000 to 2004 (Table 2). At Outer Eurocan Beach and Inner Eurocan Beach, HAH concentrations ranged from 210 to 1,800 ng/g dry wt, while LAH concentrations ranged from 23 to 310 ng/g dry wt. At Wathlsto Creek and Kildala Arm, HAH concentrations ranged from less than LOQ to 72 ng/g dry wt, while LAH concentrations ranged from less than LOQ to 59 ng/g dry wt. At Alcan Inner Harbour, concentrations of ΣHAHs and ΣLAHs in sediments collected from 2000 to 2003 ranged 10,000–67,000 ng/g dry wt and 770–6,200 ng/g dry wt, respectively, and showed little evidence of increasing or decreasing trends.

In 2004, however, both LAH and HAH levels were much lower than in any other sampling year (110 ng/g dry wt for ΣLAHs and 1,100 ng/g dry wt for ΣHAHs). At Hospital Beach, contaminant concentrations were highest in 2000 (170 ng/g dry wt for ΣLAHs and 1,600 ng/g dry wt for ΣHAHs), but substantially lower in subsequent years (64–350 ng/g dry wt for ΣHAHs and less than LOQ to 66 ng/g dry wt for ΣLAHs). Variation in perylene and retene concentrations, associated with differences in the amount of wood waste and organic material in the samples, contributed to the temporal variation in ΣLAH and ΣHAH concentrations, especially at Inner and Outer Eurocan beaches, but concentrations of these compounds were generally correlated with sediment total ΣLAH and ΣHAH levels.

Similarly, concentrations of ΣLAHs and ΣHAHs in sediments from the flatfish sampling sites showed no clear trends, although our ability to detect temporal changes was limited because of the lack of 2004 data for several of the sites (Table 3). Concentrations of ΣHAHs were consistently in the 10,000–30,000 ng/g dry wt range at Hospital Beach, in the 1,000–2,000 ng/g dry wt range at Eurocan, Kitamaat Village and Emsley Cove, and less than 50 ng/g dry wt at Kildala Arm and Kitlope. Concentrations of LAHs ranged 1,200–2,500 ng/g dry wt at Hospital Beach, 100–500 ng/g dry wt at Eurocan and Kitamaat Village, 25–100 ng/g dry wt at Emsley Cove and Kildala Arm, and less than 20 ng/g dry wt at Kitlope. Similar to sediments collected at salmon sites, sediments collected at flatfish sampling sites had perylene and retene concentrations that varied from year to year, with highest concentrations generally occurring in sampling years in which concentrations of LAHs and ΣHAHs were also highest.

Table 2. Concentrations of ΣLAHs and ΣHAHs in sediments at salmon collection sites within Kitimat Arm (Alcan Inner Harbour, Hospital Beach, Inner Eurocan Beach, and Wathlsto Creek) and the Kildala Arm reference site over time, 2002–2004. Concentrations of retene and perylene are also reported as these represent the concentrations of nonsmelter-derived PAHs present. Their likely origin is from decomposition of organic matter and wood products.

		ng/g dry weight			
Site	**Year collected**	**Retene**	**ΣLAHs**	**Perylene**	**ΣHAHs**
Alcan Inner	2000 (n = 1)	25	3,000	1,100	34,000
Harbor	2001 (n = 1)	67	6,200	2,100	67,000
	2002 (n = 1)	24	770	350	10,000
	2003 (n = 1)	17	3,200	1,100	32,000
	2004 (n = 1)	<LOQ	110	57	1,100
Hospital	2000 (n = 1)	4.8	170	61	1,600
Beach	2001 (n = 1)	3.5	19	10	64
	2002 (n = 1)	4.7	66	29	350
	2003 (n = 1)	2.1	23	18	210
	2004 (n = 1)	<LOQ	<LOQ	20	140
Inner Eurocan	2002 (n = 1)	20	110	810	1,300
Beach	2003 (n = 1)	3.6	23	69	210
	2004 (n = 1)	<LOQ	67	290	1,100
Outer Eurocan	2000 (n = 1)	44	170	82	1,800
Beach	2001 (n = 1)	76	210	130	1,100
	2002 (n = 1)	150	310	120	1,000
Wathlsto	2000 (n = 1)	<LOQ	<4.4	<LOQ	<LOQ
Creek	2001 (n = 1)	3.2	11.0	1.4	40
	2002 (n = 1)	<LOQ	<LOQ	<LOQ	72
	2003 (n = 1)	<LOQ	2.0	<LOQ	28
	2004 (n = 1)	<LOQ	<LOQ	<LOQ	39
Kildala	2000 (n = 1)	<LOQ	<LOQ	<LOQ	<LOQ
Arm	2001 (n = 1)	11	20.0	5.0	32
	2002 (n = 1)	<LOQ	5.5	2.9	25
	2003 (n = 1)	35	59.0	12.0	63
	2004 (n = 1)	<LOQ	<LOQ	<LOQ	13

Table 3. Mean concentrations (±SE) of ΣLAHs and ΣHAHs in sediments at flatfish sampling sites in Kitimat Arm (Hospital Beach, Eurocan, Kitamaat Village, and Emsley Cove) and reference sites outside Kitimat Arm (Kildala Arm and Kitlope) over time, 2000–2004. Concentrations of retene and perylene are also reported along with ΣLAHs and ΣHAHs, as these represent the concentrations of non-smelter derived PAHs present. Their likely origin is from decomposition of organic matter and wood products. Data for 2004 are unavailable for Hospital Beach, Eurocan, Kitamaat Village, and Kildala Arm because samples were compromised during a freezer malfunction and could not be analyzed.

		ng/g, dry weight			
Site	**Year collected**	**Retene**	**ΣLAHs**	**Perylene**	**ΣHAHs**
Hospital Beach	2000 (n = 3)	30 ± 15	1,200 ± 990	590 ± 390	12,000 ± 9,100
	2002 (n = 3)	39 ± 20	2,500 ± 1,500	1,200 ± 770	29,000 ± 18,000
Eurocan	2000 (n = 3)	62 ± 10	220 ± 88	210 ± 150	1,500 ± 820
	2002 (n = 3)	160 ± 120	510 ± 380	92 ± 50	1,000 ± 1,000
Kitamaat Village	2000 (n = 3)	13 ± 6.5	64 ± 38	49 ± 26	870 ± 530
	2002 (n = 3)	43 ± 1.9	180 ± 49	990 ± 19	1,300 ± 270
Emsley Cove	2000 (n = 3)	<LOQ	29 ± 9.6	89 ± 9.5	750 ± 120
	2002 (n = 3)	11 ± 3.9	53 ± 11	84 ± 3.9	890 ± 200
	2004 (n = 3)	3.7 ± 0.3	58 ± 21	78 ± 13	830 ± 180
Kildala Arm	2000 (n = 3)	83 ± 90	83 ± 90	70 ± 19	78 ± 22
	2002 (n = 3)	26 ± 20	26 ± 20	73 ± 39	73 ± 39
Kitlope	2000 (n = 3)	<LOQ	<LOQ	6.5 ± 3.2	8.2 ± 4.0
	2002 (n = 3)	9.3 ± 2.9	12 ± 6.1	26 ± 5.4	26 ± 5.4
	2004 (n = 3)	19 ± 8.6	19 ± 8.6	11 ± 0	11 ± 0

Exposure Assessment of Juvenile Salmon

PAHs in Stomach Contents

Intersite differences in stomach content PAHs

Concentrations of ΣHAHs in stomach contents of juvenile salmon sampled over the 2000–2004 monitoring period (Figure 9A) generally paralleled PAH concentrations in sediment. Like sediment ΣHAH concentrations, mean stomach content ΣHAH concentrations showed significant intersite differences ($p = 0.0001$ for log-transformed values), and were highest in salmon from Alcan Inner Harbour, the site nearest the smelter (950 ± 1,400 ng/g wet wt, mean ± SD). ΣHAH concentrations were also relatively high in stomach contents of salmon from the Hospital Beach and Inner Eurocan Beach sites, with average concentrations of 500 ± 370 ng/g wet wt. and 340 ± 220 ng/g wet wt, respectively. In fish from the other sites within Kitimat Arm (Outer Eurocan Beach, Minette Bay, and Wathlsto Creek), mean ΣHAH concentrations ranged from 43 to 160 ng/g wet wt, and were significantly higher than concentrations of ΣHAHs in stomach contents of salmon from Kildala Arm and Kemano Village, which ranged from 4.3 to

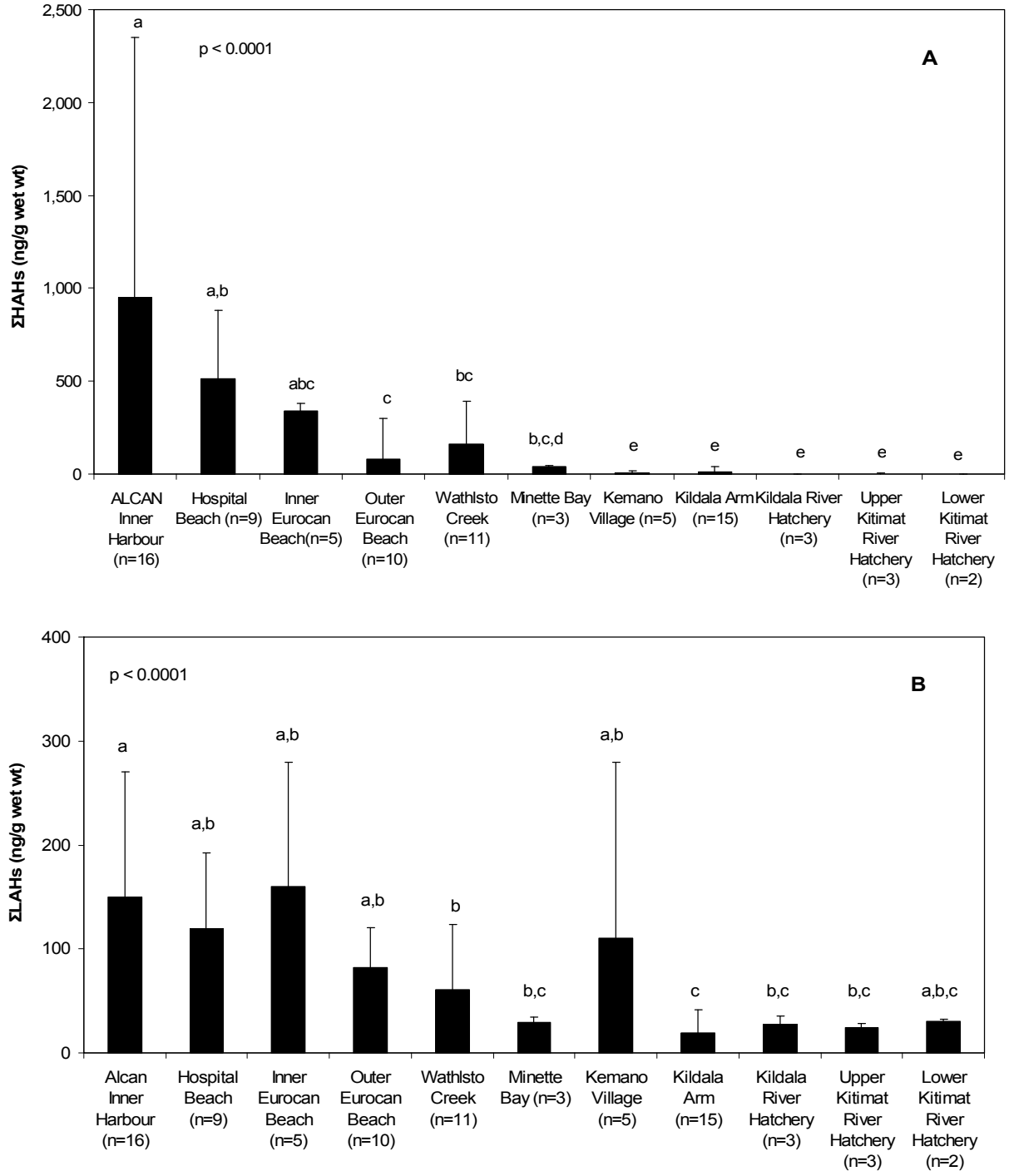

Figure 9. Mean concentrations (ng/g wet wt) of (A) HAHs and (B) LAHs in stomach contents of juvenile Chinook salmon collected from sites within Kitimat Arm (Alcan Inner Harbour, Hospital Beach, Inner Eurocan Beach, Outer Eurocan Beach, Minette Bay, and Wathlsto Creek), from references sites outside Kitimat Arm (Kemano Village and Kildala Arm), and from three hatchery stocks (Kildala River, upper Kitimat River, and lower Kitimat River) from the Kitimat River Fish Hatchery. Letters denote statistically significant differences (ANOVA, $p < 0.05$, log-transformed data).

11 ng/g wet wt. Concentrations of ΣHAHs in stomach contents of salmon from the Kildala River, upper Kitimat River, and lower Kitimat River hatchery stocks sampled in 2000 were similar to those in salmon from Kildala Arm and Kemano Village, with average values of less than 2 ng/g wet wt.

Concentrations of ΣLAHs in salmon stomach contents (Figure 9B) also varied significantly from site to site ($p = 0.0001$ for log-transformed values), though the range of concentrations was narrower than the ΣHAH range. At sites within Kitimat Arm, highest levels (120–160 ng/g wet wt) were seen in salmon from Alcan Inner Harbour, Hospital Beach, and Inner Eurocan Beach. Concentrations of ΣLAHs were somewhat lower in stomach contents of salmon from Outer Eurocan Beach (82 ng/g wet wt), Wathlsto Creek (61 ng/g dry wt), and Minette Bay (29 ng/g wet wt). Concentrations were in a similar range (24–30 ng/g wet wt) in salmon from the hatchery stocks. In salmon from Kildala Arm, stomach content ΣLAH concentrations were lower (19 ng/g wet wt) than in any other groups of fish. Surprisingly, ΣLAH concentrations in stomach contents were quite high in fish from Kemano Village (110 ng/g dry wt), comparable to levels in fish from Hospital Beach, Alcan Inner Harbour, and Inner Eurocan Beach.

PAH composition

Stomach contents of salmon from sites within Kitimat Arm (Alcan Inner Harbour, Hospital Beach, Inner and Outer Eurocan beaches, Minette Bay, and Wathlsto Creek) contained a wide range of HAHs, including compounds similar to those present in Alcan pitch samples (Figure 10A). Pyrene, fluoranthene, and chrysene were especially dominant, accounting for 50–70% of ΣHAHs in samples from most of the sites. Similar HAHs were found in stomach contents of salmon from Kildala Arm. In salmon from Kemano Village and from the Kildala River hatchery stock, fluoranthene and pyrene were the dominant HAHs present, while fluoranthene, pyrene, and chrysene were the major HAHs found in salmon from the upper Kitimat River hatchery stock. However, other HAHs that were present in the pitch samples and stomach contents of salmon from the other sites were not detected in fish from Kemano Village or the hatchery stocks. (Salmon from the upper Kitimat River hatchery stock were not included because all HAHs in their stomach contents samples were less than LOQ.) Perylene made up only a small proportion of ΣHAHs in stomach contents of salmon from any of the sites.

Of the LAHs, phenanthrene dominated in the pitch samples as well as in stomach contents of salmon from most of the sites (Figure 10B). Salmon from Kemano Village and from the three hatchery stocks were exceptions; in these groups of fish, naphthalene and 2-methyl naphthalene were the major LAHs present. Retene, which was not present in the pitch samples, made up a significant proportion (≈30%) of ΣLAHs in stomach contents of juvenile salmon from the Outer Eurocan Beach site and was a minor constituent (≈10%) of stomach content ΣLAHs in salmon from Alcan Inner Harbour, Hospital Beach, Inner Eurocan Beach, Wathlsto Creek, and Kildala Arm. Retene was not analyzed in stomach contents of salmon from Minette Bay, Kemano Village, or the hatchery, which were collected in 2000 only.

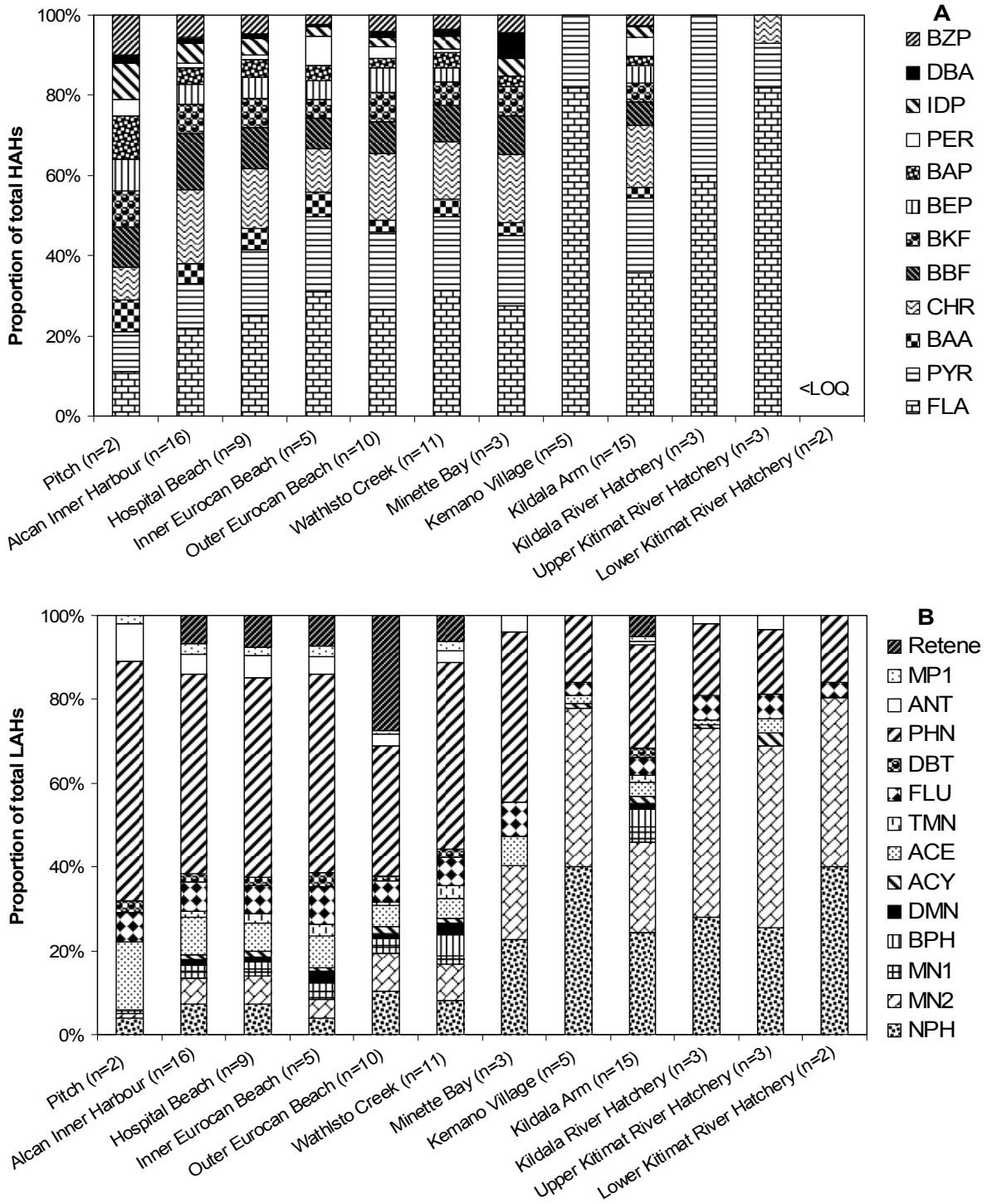

Figure 10. Proportions of (A) individual HAHs and (B) individual LAHs in stomach contents of juvenile Chinook salmon from sites within Kitimat Arm (Alcan Inner Harbour, Hospital Beach, Inner Eurocan Beach, Outer Eurocan Beach, Minette Bay, and Wathlsto Creek), reference sites outside Kitimat Arm (Kemano Village and Kildala Arm), three hatchery stocks (Kildala River, upper Kitimat River, and lower Kitimat River) from the Kitimat River Fish Hatchery, and in pitch samples from the Alcan smelter. Proportions are averages derived from samples collected from 2000 to 2004.

Temporal trends in stomach content PAHs

Concentrations of ΣHAHs in stomach contents of salmon from most of the sampling sites, including Alcan Inner Harbour, Hospital Beach, and Inner and Outer Eurocan beaches, did not vary significantly from year to year (Table 4). Among these sites, ΣHAH concentrations were highest at Alcan Inner Harbour (270–1,600 ng/g wet wt) and Hospital Beach (300–1,200 ng/g wet wt). ΣHAH levels were somewhat lower in stomach contents of salmon from Inner Eurocan Beach (160–400 ng/g wet wt) and Outer Eurocan Beach (66–86 ng/g wet wt). Significant differences in HAH concentrations were observed among sampling years in stomach contents of salmon from Wathlsto Creek and Kildala Arm, although there were no clear declining or increasing trends with time. At Kildala Arm, unusually high HAH concentrations were found in stomach contents of salmon collected in 2000 (56 ng/g wet wt as compared to 0.3–9.8 ng/g wet wt in other sampling years). Stomach content HAH concentrations were especially high in 2001 and 2002 at Wathlsto Creek, 590–650 ng/g wet wt as compared to 23–90 ng/g wet wt in other sampling years.

Patterns were much the same for LAHs in stomach contents (Table 4). LAH levels did not differ significantly from year to year at Alcan Inner Harbour, Hospital Beach, Inner Eurocan Beach, and Outer Eurocan Beach. Concentration ranges (74–270 ng/g wet wt) were similar at Alcan Inner Harbour, Hospital Beach, and Inner Eurocan Beach; at Outer Eurocan Beach, levels were somewhat lower, ranging 57–71 ng/g wet wt. As with ΣHAHs, ΣLAH concentrations in salmon stomach contents differed significantly among sampling years at Wathlsto Creek and Kildala Arm. Concentrations of ΣLAHs were unusually high in stomach contents of Wathlsto Creek salmon collected in 2002 (240 ng/g wet wt as compared to 32–72 ng/g wet wt in other years). ΣLAH levels were also especially high in salmon from Kildala Arm in 2002, 58 ng/g wet wt as compared to 11–18 ng/g wet wt in other years.

Retene and perylene concentrations also showed significant variability from year to year in stomach contents of salmon from a few sampling sites. However, there were no clear trends, and retene and perylene concentrations did not contribute greatly to temporal changes in ΣHAH and ΣLAH concentrations over the sampling period (Table 4).

OCs in Stomach Contents

In addition to PAHs, OCs (aldrin, dieldrin, heptachlor, hexachlorobenzene [HCB], lindane, chlordanes, DDTs, and PCBs) were measured in stomach contents of juvenile salmon collected in 2000 (Figure 11, Table 5). Concentrations of most pesticides (aldrin, dieldrin, HCB, lindane, heptachlor, and chlordanes) were very near or less than LOQ (<2 ng/g wet wt) and did not differ significantly among sites. Both DDTs and PCBs were detected at low but measurable concentrations and some intersite differences were observed (Figure 11). Concentrations of DDTs in field-collected Chinook salmon stomach contents (Figure 11A) ranged from less than 1 ng/g wet wt in fish from Kildala Arm and Kemano Village to approximately 7 ng/g wet wt in fish collected from Hospital Beach and Alcan Inner Harbour. In fish from Outer Eurocan Beach, Minette Bay, and Wathlsto Creek, stomach DDT concentrations ranged 3.2–4.3 ng/g wet wt; Inner Eurocan Beach was not sampled in 2000. In fish from the three hatchery stocks, stomach content DDT concentrations were similar to or slightly higher than in fish from Alcan Inner

Table 4. Mean concentration (±SE) in ng/g wet wt of ΣLAHs and ΣHAHs over time, 2000–2005, in stomach contents of juvenile Chinook salmon from the Kitimat area. Concentrations of retene and perylene are also reported, as these represent the concentrations of nonsmelter-derived PAHs present. Years with different letter superscripts are significantly different ($p \leq 0.05$).

Site	Year collected	Retene	ΣLAHs	Perylene	ΣHAHs
Alcan Inner	2000 (n = 6)	1.6 ± 0.39	170 ± 79	NR*	1,600 ± 970
Harbour	2001 (n = 2)	32 ± 40	150 ± 78	13 ± 12	900 ± 570
	2002 (n = 5)	9.7 ± 2.1	78 ± 8.6	5.1 ± 1.7	270 ± 49
	2003 (n = 2)	4.9 ± 4.2	150 ± 98	10 ± 7.9	670 ± 410
	2004 (n = 1)	4.1	270	15	820
		p = 0.4477	p = 0.2886	p = 0.3413	p = 0.1425
Hospital	2000 (n = 3)	8.4 ± 3.6	120 ± 30	NR	640 ± 210
Beach	2001 (n = 1)	13	80	5.9	300
	2002 (n = 2)	9.5 ± 0.71	90 ± 43	4.8 ± 4.7	290 ± 210
	2003 (n = 2)	2.5 ± 0.21	110 ± 99	2.9 ± 2.0	310 ± 240
	2004 (n = 1)	12	270	25	1,200
		p = 0.4313	p = 0.5752	p = 0.0969	p = 0.4330
Inner Eurocan	2002 (n = 1)	5.0^{b}	74	4.5^{b}	160
Beach	2003 (n = 3)	3.1 ± 0.69^{b}	210 ± 110	8.8 ± 2.7^{b}	400 ± 190
	2004 (n = 1)	27^{a}	130	79^{a}	330
		p = 0.0044	p = 0.8903	p = 0.0070	p = 0.8462
Outer Eurocan	2000 (n = 4)	9.5 ± 4.5	64 ± 17	NR	86 ± 23
Beach	2001 (n = 3)	24 ± 15	57 ± 29	1.0 ± 0.28	66 ± 48
	2002 (n = 3)	31 ± 3.5	71 ± 1.4	3.2 ± 1.8	79 ± 8.4
		p = 0.3395	p = 0.2285	p = 0.2066	p = 0.4799
Wathlsto	2000 (n = 3)	2.3 ± 0.92^{b}	32 ± 5.7^{cd}	NR	77 ± 21^{b}
Creek	2001 (n = 1)	24^{a}	72^{bc}	6.6^{a}	650^{a}
	2002 (n = 1)	5.9^{b}	240^{a}	1.9^{b}	590^{a}
	2003 (n = 3)	0.54 ± 0.16^{b}	25 ± 1.8^{d}	0.27 ± 0.03^{c}	23 ± 2.9^{c}
	2004 (n = 3)	6.0 ± 3.1^{b}	63 ± 11^{b}	1.4 ± 0.33^{b}	90 ± 19^{b}
		p = 0.0140	p = 0.0007	p = 0.0004	p = 0.0004
Kildala Arm	2000 (n = 6)	1.4 ± 0.20	11 ± 3.1	NR	0.3 ± 0.7^{b}
	2001 (n = 1)	0.42	16	0.36	4.6^{ab}
	2002 (n = 1)	0.41	16	0.41	2.8^{a}
	2003 (n = 3)	1.1 ± 0.54	11 ± 1.4	0.35 ± 0.01	7.7 ± 1.1^{a}
	2004 (n = 3)	0.11 ± 0.06	18 ± 3.9	0.21 ± 0.015	9.8 ± 3.0^{a}
		p = 0.1430	p = 00583	p = 0.2756	p = 0.0066

*NR = not reported.

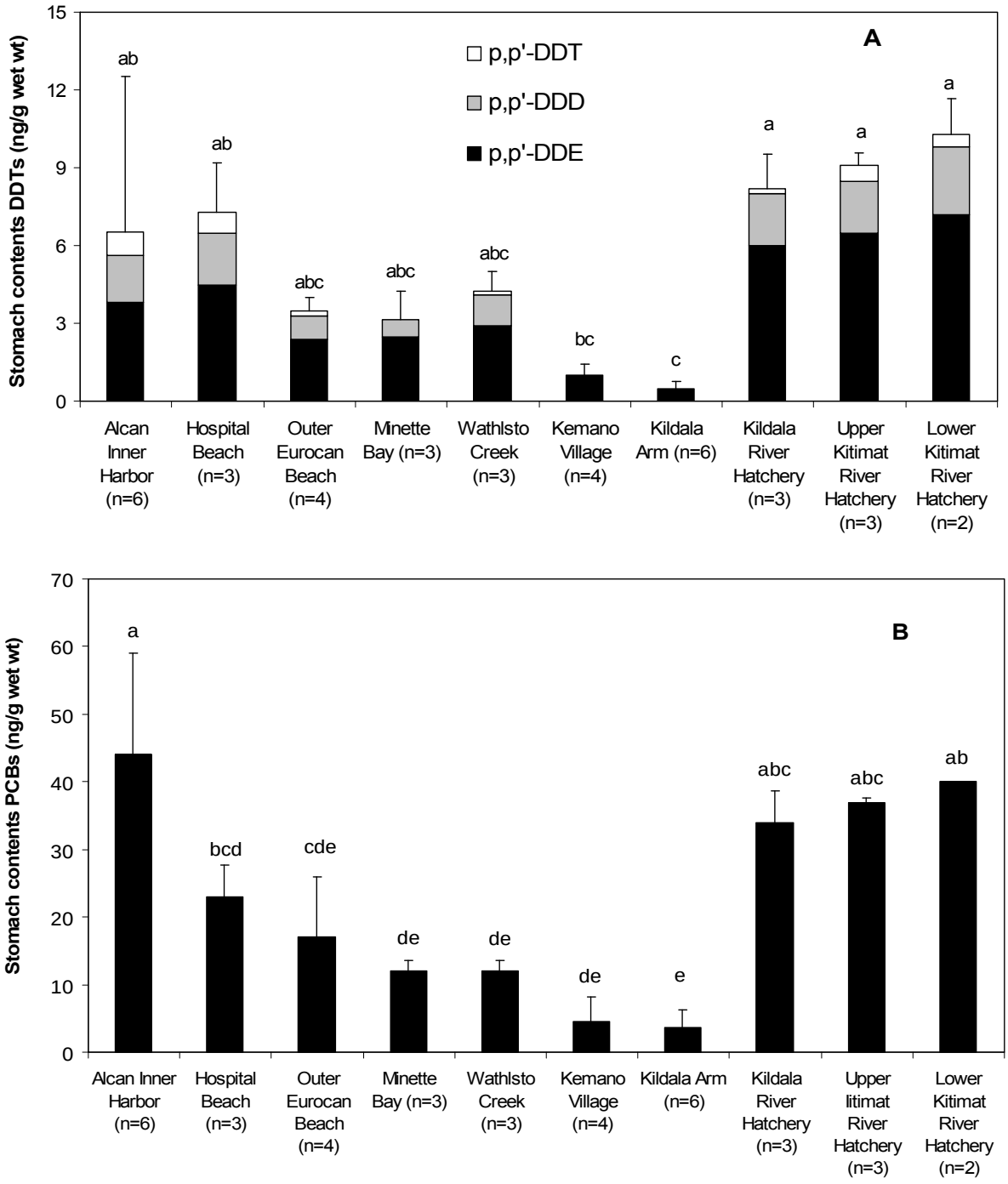

Figure 11. Mean concentrations (ng/g wet wt ± SE) of (A) DDTs and (B) PCBs in stomach contents of juvenile Chinook salmon from sites within Kitimat Arm (Alcan Inner Harbour, Hospital Beach, Outer Eurocan Beach, Minette Bay, and Wathlsto Creek), from references sites outside Kitimat Arm (Kemano Village and Kildala Arm), and from three hatchery stocks (Kildala River, upper Kitimat River, and lower Kitimat River) from the Kitimat River Fish Hatchery. Values with different letter designations are significantly different (ANOVA, $p \leq 0.05$). DDTs and PCBs were analyzed in 2000 samples only; Inner Eurocan Beach was not sampled in 2000.

Table 5. Concentrations (mean ± SE) of selected OCs (ng/g wet wt) in stomach contents of juvenile Chinook salmon from Kitimat Arm (Alcan Inner Harbour, Hospital Beach, Outer Eurocan Beach, Minette Bay, and Wathlsto Creek), reference areas outside of Kitimat Arm (Kemano Village and Kildala Arm), and hatchery stocks (Kildala River, upper Kitimat River, and lower Kitimat River) from the Kitimat River Fish Hatchery. OCs in stomach contents were measured in 2000 only. No data are available for Inner Eurocan because this site was not sampled in 2000.

	HCB*	**Lindane**	**Aldrin**	**Dieldrin**	**Heptachlor**	**α±b-chlordane**	***p,p′*-DDE**	***p,p′*-DDD**	***p,p′*-DDT**
Alcan Inner Harbour May 2000 (n = 3)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	6.3 ± 2.5	2.9 ± 1.2	2.3 ± 0.35
Alcan Inner Harbour June 2000 (n = 3)	<LOQ	0.33 ± 0.03	<LOQ	0.74 ± 0.15	<LOQ	<LOQ	1.9 ± 2.0	0.84 ± 0.37	<LOQ
Hospital Beach (n = 3)	<LOQ	<LOQ	<LOQ	1.6 ± 0.94	<LOQ	<LOQ	4.5 ± 1.0	2.0 ± 0.45	1.2 ± 0.33
Outer Eurocan Beach (n = 4)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.4 ± 0.39	0.88 ± 0.24	0.38 ± 0.11
Minette Bay (n = 3)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.5 ± 0.59	0.96 ± 0.06	<LOQ
Wathlsto Creek (n = 3)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.9 ± 0.72	1.2 ± 0.21	<LOQ
Kemano Village (n = 4)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.0 ± 0.42	<LOQ	<LOQ
Kildala Arm May 2000 (n = 3)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.66 ± 0.19	<LOQ	<LOQ
Kildala Arm June 2000 (n = 3)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.51 ± 0.04	<LOQ	<LOQ
Kildala River Hatchery (n = 3)	<LOQ	<LOQ	<LOQ	1.2 ± 0.30	<LOQ	0.79 ± 0.064	6.0 ± 0.75	2.0 ± 0.25	<LOQ
Upper Kitimat River Hatchery (n = 3)	<LOQ	<LOQ	<LOQ	1.2 ± 0.12	<LOQ	0.80 ± 0.051	6.5 ± 0.35	2.0 ± 0.06	0.61 ± 0.06
Lower Kitimat River Hatchery (n = 2)	<LOQ	<LOQ	<LOQ	1.0 ± 0.57	<LOQ	<LOQ	7.2 ± 0.64	2.6 ± 0.07	0.78 ± 0.25

*HCB = hexachlorobenzene

Harbour and Hospital Beach (8–10 ng/g wet wt). Of the DDT isomers measured, *p,p'*-DDE was the dominant form present, making up about 75% of DDTs in an average sample.

The pattern was similar for PCBs (Figure 11B). In fish collected in the wild, concentrations of PCBs were highest in stomach contents collected from Alcan Inner Harbour (44 ng/g wet wt). Concentrations of PCBs in stomach contents of salmon from other sites within Kitimat Arm ranged 12–23 ng/g wet wt, with highest levels in fish from Hospital Beach and lowest levels in fish from Minette Bay and Walthsto Creek. In fish from Kemano Village and Kildala Arm, PCB concentrations in stomach contents were less than or equal to 5 ng/g wet wt. In the three stocks of salmon sampled from the Kitimat Hatchery, PCB concentrations in stomach contents were similar to those in Alcan Inner Harbour fish (34–40 ng/g wet wt).

PAH Metabolites in Bile

Over the course of the 2000–2004 sampling, bile analyses showed consistently that juvenile salmon from sites near the Alcan smelter were being exposed to PAHs. PAH metabolite levels in composites of bile from juvenile Chinook salmon (Figure 12) were generally correlated with sediment PAH levels at the corresponding sites and showed significant differences for metabolites of both HAHs and LAHs ($p \leq 0.0001$, ANOVA on log-transformed values). Metabolites of HAHs (Figure 12A), represented by compounds fluorescing at benzo[a]pyrene (BaP) wavelengths (BaP equivalents), were highest in bile of juvenile salmon from the Alcan Inner Harbour site (2,800 ng/g bile), significantly higher than levels at any of the other sampling sites. Concentrations of BaP equivalents in salmon from Hospital Beach were also relatively high (1,200 ng/g bile), and were significantly higher than concentrations in salmon from all sites except Inner Eurocan Beach and Minette Bay. Concentrations of BaP equivalents in salmon from Inner and Outer Eurocan beaches, Wathlsto Creek, and Minette Bay were not significantly different, ranging 460–870 ng/g bile. The lowest concentrations of BaP metabolites (130–160 ng/g bile) were observed in fish from Kemano Village and Kildala Arm, levels that were significantly lower than in salmon from any of the sites within Kitimat Arm. Metabolites of HAHs were also found in bile of salmon from three hatchery stocks, with mean concentrations ranging 290–330 ng/g bile. These concentrations were significantly lower than those in salmon from Alcan Inner Harbour and Hospital Beach, but were not significantly different from those in salmon from any other sites.

Like metabolites of HAHs, metabolites of LAHs (PHN equivalents) in bile (Figure 12B) were significantly higher in salmon from Alcan Inner Harbour (140,000 ng/g bile) than in salmon from any other sampling site and were lowest in salmon from Kildala Arm (16,000 ng/g bile) and the hatchery stocks (8,200–11,000 ng/g bile). Levels of PHN equivalents were also relatively high in salmon from Hospital Beach (77,000 ng/g bile), significantly higher than those in salmon from all sites except for Inner Eurocan Beach and Alcan Inner Harbour.

Concentrations of PHN equivalents in bile of salmon from Inner Eurocan Beach, Outer Eurocan Beach, Minette Bay, and Wathlsto Creek, which ranged from 31,000 to 52,000 ng/g bile, were all significantly higher than levels in salmon from Kildala Arm and the hatchery stocks. However, PHN levels in bile of salmon from these sites did not differ significantly from each other, with the exception of salmon from Inner Eurocan Beach, whose PHN equivalent levels were significantly higher than those in salmon from Wathlsto Creek. Levels of PHN

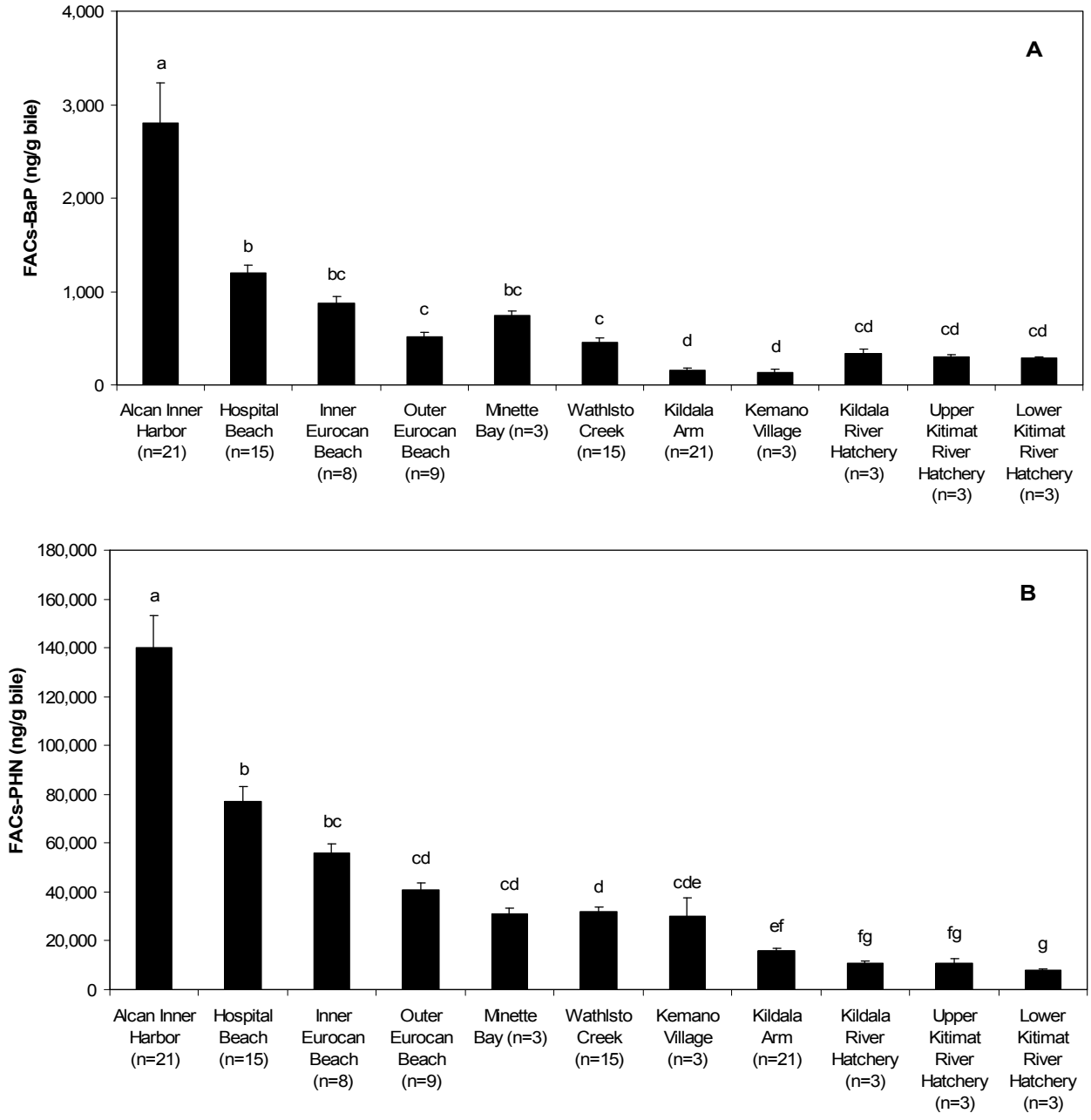

Figure 12. Mean concentrations (ng/g bile ± SE) of (A) fluorescent metabolites of HAHs measured as benzo[a]pyrene equivalents (FACs-BaP) and (B) fluorescent metabolites of LAHs measured as phenanthrene equivalents (FACs-PHN) in bile samples collected between 2000 and 2004 from juvenile Chinook salmon from sites within Kitimat Arm (Alcan Inner Harbour, Hospital Beach, Inner Eurocan Beach, Outer Eurocan Beach, Minette Bay, and Wathlsto Creek), from reference sites outside Kitimat Arm (Kemano Village and Kildala Arm), and from three hatchery stocks (Kildala River, upper Kitimat River, and lower Kitimat River) from the Kitimat River Fish Hatchery. Values with different letter superscripts are significantly different ($p < 0.05$).

equivalents in salmon from Kemano Village (30,000 ng/g bile) were similar to those in salmon from Wathlsto Creek and Minette Bay, but not statistically different from levels in salmon from Kildala Arm. In salmon sampled from the three hatchery stocks, PHN equivalent concentrations were significantly lower than those in salmon from any of the other sites, with the exception of salmon from Kildala Arm.

Concentrations of both HAH and LAH metabolites in juvenile salmon bile showed significant year-to-year variability at all of the sampling sites, but generally there were no strong increasing or decreasing trends (Table 6). Concentrations of PHN equivalents were lowest in 2004 in salmon from Alcan Inner Harbour, Inner and Outer Eurocan beaches, and Wathlsto Creek, but this was not the case for BaP equivalents, which varied from year to year but showed no clear trends. Highest concentrations of PAH metabolites in bile were consistently found in salmon from the Alcan Inner Harbour site, while lowest concentrations were consistently found in salmon from Kildala Arm.

Seasonal Variation in Juvenile Salmon Exposure to AHs and OCs

To examine how contaminant exposure varied over the course of the outmigration season in juvenile salmon in Kitimat Arm and the surrounding area, concentrations of PAHs and OCs in stomach contents and PAH metabolites in bile were measured in fish collected in both May and June of 2000 and 2002 from two sampling sites, Alcan Inner Harbour and Kildala Arm. Concentrations of both ΣLAHs and ΣHAHs in stomach contents (Table 7) tended to be higher in May than in June in salmon from Alcan Inner Harbour, although the differences were not always statistically significant. In salmon from Kildala Arm, on the other hand, concentrations of both ΣLAHs and ΣHAHs were significantly higher in stomach contents of fish collected in June than in May, with the exception of ΣHAHs in salmon collected in 2000. In this group, HAH concentrations were low in both May and June (<0.5 ng/g wet wt) and did not differ significantly.

In juvenile salmon collected from Alcan Inner Harbour, concentrations of DDTs and PCBs in salmon stomach contents tended to be higher in May than in June, but these values were not significantly different (Table 7). In salmon from Kildala Arm, concentrations of PCBs and DDTs were very similar at both time points.

Metabolites of PAHs in salmon bile showed no consistent concentration changes from May to June (Table 7). At Kildala Arm, levels of PHN equivalents in bile were similar in May and June in both 2000 and 2002, with no statistically significant differences ($0.0930 < p < 0.5356$). However, BaP equivalent levels declined significantly ($0.0461 < p < 0.0014$) from May to June in both 2000 and 2002. In salmon from the Alcan Inner Harbour site, concentrations of both BaP and PHN equivalent showed significant to near-significant declines from May to June in 2000 ($0.0485 < p < 0.0575$), but showed no significant change from May to June in 2002 ($0.0955 < p < 0.1848$).

Correlation between PAHs in Sediment, Stomach Contents, and Bile

Levels of both ΣLAHs and ΣHAHs in stomach contents of juvenile salmon were significantly and positively correlated with LAH and HAH concentrations in sediments at the

Table 6. Mean concentrations (±SE) of metabolites of low (PHN equivalents) and high (BaP equivalents) molecular weight AHs in bile of juvenile Chinook salmon from Kitimat Arm (Alcan Inner Harbour, Hospital Beach, Inner and Outer Eurocan beaches, and Wathlsto Creek) and the Kildala Arm reference over time, 2000–2004. Years with different letter superscripts are significantly different ($p \leq 0.05$).

Site	Year Collected	PHN equivalents (ng/g bile)	BaP equivalents (ng/g bile)
Alcan Inner Harbor	2000 (n = 6)	93,000 ± 15,000^{b}	1,600 ± 350^{c}
	2001 (n = 3)	240,000 ± 27,000^{a}	7,100 ± 940^{a}
	2002 (n = 6)	160,000 ± 17,000^{a}	2,600 ± 260^{b}
	2003 (n = 3)	180,000 ± 4,100^{a}	2,100 ± 58b^{c}
	2004 (n = 3)	85,000 ± 7,500^{b}	2,200 ± 210^{bc}
		p = 0.0001	p = 0.0001
Hospital Beach	2000 (n = 3)	77,000 ± 5,600^{ab}	1,200 ± 100^{a}
	2001 (n = 3)	110,000 ± 11,000^{a}	1,400 ± 88^{a}
	2002 (n = 3)	58,000 ± 3,600^{b}	980 ± 67^{ab}
	2003 (n = 3)	77,000 ± 11,000^{ab}	820 ± 120^{b}
	2004 (n = 3)	60,000 ± 8,600^{b}	1,400 ± 220^{a}
		p = 0.0161	p = 0.0386
Inner Eurocan Beach	2002 (n = 2)	69,000 ± 5,000^{a}	970 ± 30^{ab}
	2003 (n = 3)	59,000 ± 2,600^{a}	630 ± 67^{a}
	2004 (n = 3)	45,000 ± 2,500^{b}	1,000 ± 110^{b}
		p = 0.0070	p = 0.0254
Outer Eurocan Beach	2000 (n = 3)	49,000 ± 4,700^{a}	690 ± 49^{a}
	2001 (n = 3)	39,000 ± 1,200^{ab}	490 ± 28^{ab}
	2002 (n = 3)	34,000 ± 2,200^{b}	380 ± 59^{b}
		p = 0.0206	p = 0.0155
Wathlsto Creek	2000 (n = 3)	30,000 ± 670^{ab}	680 ± 23^{a}
	2001 (n = 3)	39,000 ± 2,000^{a}	480 ± 29^{b}
	2002 (n = 3)	37,000 ± 4,200^{a}	420 ± 46^{b}
	2003 (n = 3)	28,000 ± 2,400^{ab}	260 ± 8.5^{c}
	2004 (n = 3)	24,000 ± 1,000^{b}	430 ± 26^{b}
		p = 0.0048	p = 0.0001
Kildala Arm	2000 (n = 6)	22,000 ± 2,400^{a}	160 ± 8.5^{b}
	2001 (n = 3)	13,000 ± 330^{bc}	300 ± 20^{a}
	2002 (n = 6)	17,000 ± 1,200^{ab}	160 ± 34^{ab}
	2003 (n = 3)	9,900 ± 540^{c}	35 ± 1^{c}
	2004 (n = 3)	13,000 ± 330^{bc}	210 ± 3.5^{ab}
		p = 0.0001	p = 0.0001

Table 7. Mean concentrations (±SE), in ng/g wet wt, of ΣLAHs, ΣHAHs, ΣPCBs, and ΣDDTs in stomach contents and metabolites of LAHs, measured as PHN equivalents, and HAHs, measured as BaP equivalents, in bile of juvenile Chinook salmon collected from Alcan Inner Harbour and Kildala Arm in May and June of 2000 and 2002. Values with different letter superscripts are significantly different ($p < 0.05$).

	ΣLAHs	**ΣHAH**	**ΣDDTs**	**ΣPCBs**	**PHN equivalents (ng/g bile, wet wt)**	**BaP equivalents (ng/g bile, wet wt)**
2000						
Alcan Inner Harbour, May (n = 3)	280 ± 140	2,300 ± 1,900	11 ± 5.6	51 ± 17	120,000 ± 18,000	2,100 ± 440
Alcan Inner Harbour, June (n = 3)	55 ± 4.1	430 ± 27	2.3 ± 2.5	36 ± 11	69,000 ± 4,200	1,000 ± 140
	$p = 0.1339$	$p = 0.2058$	$p = 0.0763$	$p = 0.2681$	$p = 0.0485$	$p = 0.0575$
Kildala Arm, May (n = 3)	8.4 ± 0.59	0.85 ± 0.52	0.44 ± 0.41	3.2 ± 2.9	23,000 ± 1,500	160 ± 8.5
Kildala Arm, June (n = 3)	13 ± 1.5	<LOQ	0.51 ± 0.04	4.2 ± 2.6	20,000 ± 4,500	99 ± 17
	$p = 0.0185$	$p = 0.3739$	$p = 0.7816$	$p = 0.6705$	$p = 0.5356$	$p = 0.0461$
2002						
Alcan Inner Harbour, May (n = 4)	84 ± 5.0	280 ± 54	NM*	NM	130,000 ± 10,000	2,300 ± 200
Alcan Inner Harbour, June (n = 1)	51	220	NM	NM	180,000 ± 21,000	3,500 ± 350
	$p = 0.0577$	$p = 0.6561$			$p = 0.0955$	$p = 0.1848$
Kildala Arm, May (n = 2)	17 ± 0.71	4.8 ± 2.8	NM	NM	15,000 ± 880	230 ± 15
Kildala Arm, June (n = 1)	100	110	NM	NM	19,000 ± 1,500	92 ± 8.5
	$p = 0.0066$	$p = 0.0210$			$p = 0.0939$	$p = 0.0014$

*NM = not measured; PCBs and DDTs were only analyzed in samples collected in 2000

sites where juvenile salmon were collected (Figure 13). The correlation was stronger for ΣHAHs ($r^2 = 0.51$) than for ΣLAHs ($r^2 = 0.29$).

Concentrations of ΣLAHs and ΣHAHs in salmon stomach contents were also correlated with concentrations of metabolites of LAHs and HAHs in salmon bile (Figure 14). Again, the correlation was stronger for ΣHAHs; for concentrations of HAHs in stomach contents vs. BaP equivalents in bile, $r^2 = 0.51$, while for ΣLAHs in stomach contents vs. PHN equivalents in bile, $r^2 = 0.42$.

PAH-DNA Adducts

As a measure of more chronic, longer-term PAH exposure in salmon, PAH-DNA adducts were measured in livers of salmon collected in 2000, 2001, and 2002. Low but measurable levels of adducts (<10 nmol/mol bases) were detected in salmon from all sampling sites, as well as in salmon from Kildala River, upper Kitimat River, and lower Kitimat River stocks sampled from the Kitimat Hatchery (Figure 15). Overall, mean adduct levels were highest in salmon from Alcan Inner Harbour and Outer Eurocan Beach and significantly higher than levels in salmon from Minette Bay or the hatchery stocks. However, no other significant differences in adduct levels were observed among the sampling sites.

When temporal changes in adduct levels in salmon were examined (Table 8), few significant differences were observed. Adduct levels showed no significant changes from 2000 to 2002 in salmon from Alcan Inner Harbour, Hospital Beach, Outer Eurocan Beach, or Wathlsto Creek ($0.11 < p < 0.58$). In salmon from Kildala Arm, however, adduct levels increased significantly from 2000 to 2002 ($p = 0.01$). Adduct levels were not measured in salmon collected in 2003 or 2004.

Exposure Assessment in Flatfish

PAHs in Stomach Contents

Intersite differences in stomach content PAHs

Mean concentrations of summed ΣHAHs and ΣLAHs in stomach contents of English sole collected from 2000 to 2004 showed significant intersite differences ($p < 0.0001$, ANOVA on log-transformed values, Figure 16). Concentrations of ΣHAHs in stomach contents of English sole collected at Hospital Beach (1,700 ng/g wet wt) were significantly higher than in sole from any of the other sites. In sole from Eurocan Beach, Kitamaat Village, and Emsley Cove, average levels of ΣHAHs in stomach contents ranged from 240 ng/g wet wt in sole from Emsley Cove to 430 ng/g wet wt in sole from Eurocan Beach. These concentrations were not significantly different in statistical analyses. Concentrations of ΣHAHs in stomach contents of sole from Kildala Arm (59 ng/g wet wt) were significantly lower than in sole from any of the sites within Kitimat Arm but significantly higher than in sole from Kitlope, which had the lowest stomach HAH concentrations of sole from any of the sites (8.7 ng/g wet wt).

Concentrations of ΣLAHs in English sole stomach contents also showed significant intersite differences ($p < 0.001$, ANOVA on log-transformed values), but followed a slightly

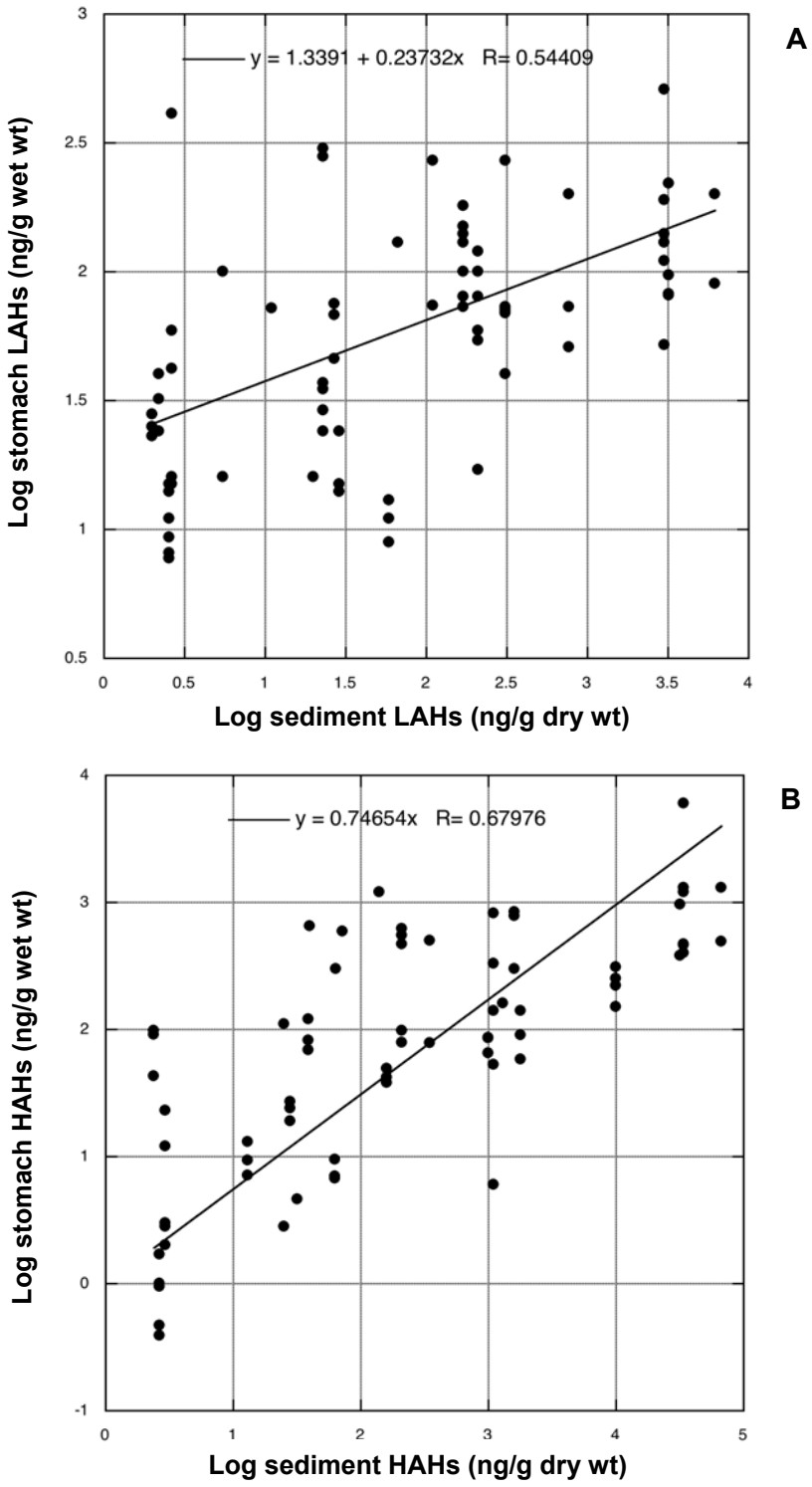

Figure 13. Relationship between (A) concentrations of ΣLAHs in sediment and stomach contents and (B) concentrations of ΣHAHs in sediment and stomach contents for juvenile Chinook salmon from sites within Kitimat Arm (Alcan Inner Harbour, Hospital Beach, Inner Eurocan Beach, Outer Eurocan Beach, Minette Bay, and Wathlsto Creek), and from reference sites outside Kitimat Arm (Kemano Village and Kildala Arm).

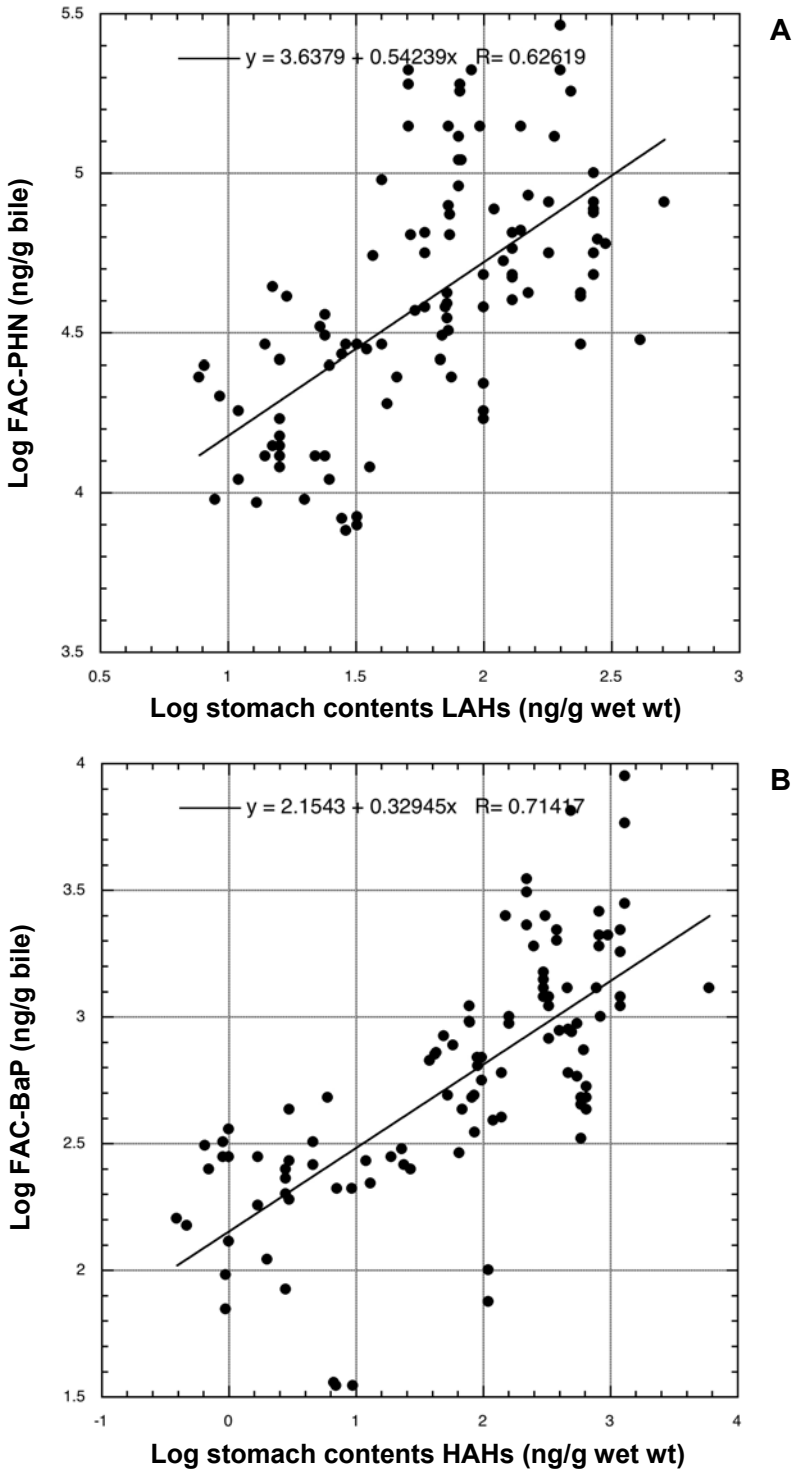

Figure 14. Relationship between (A) concentrations of ΣLAHs in stomach contents and fluorescent metabolites of ΣLAHs in bile, measured as FACs-PHN, and (B) concentrations of ΣHAHs in stomach contents and fluorescent metabolites of ΣHAHs in bile, measured as FACs-BaP, for juvenile Chinook salmon from sites within Kitimat Arm (Alcan Inner Harbour, Hospital Beach, Inner Eurocan Beach, Outer Eurocan Beach, Minette Bay, and Wathlsto Creek) and from reference sites outside Kitimat Arm (Kemano Village and Kildala Arm).

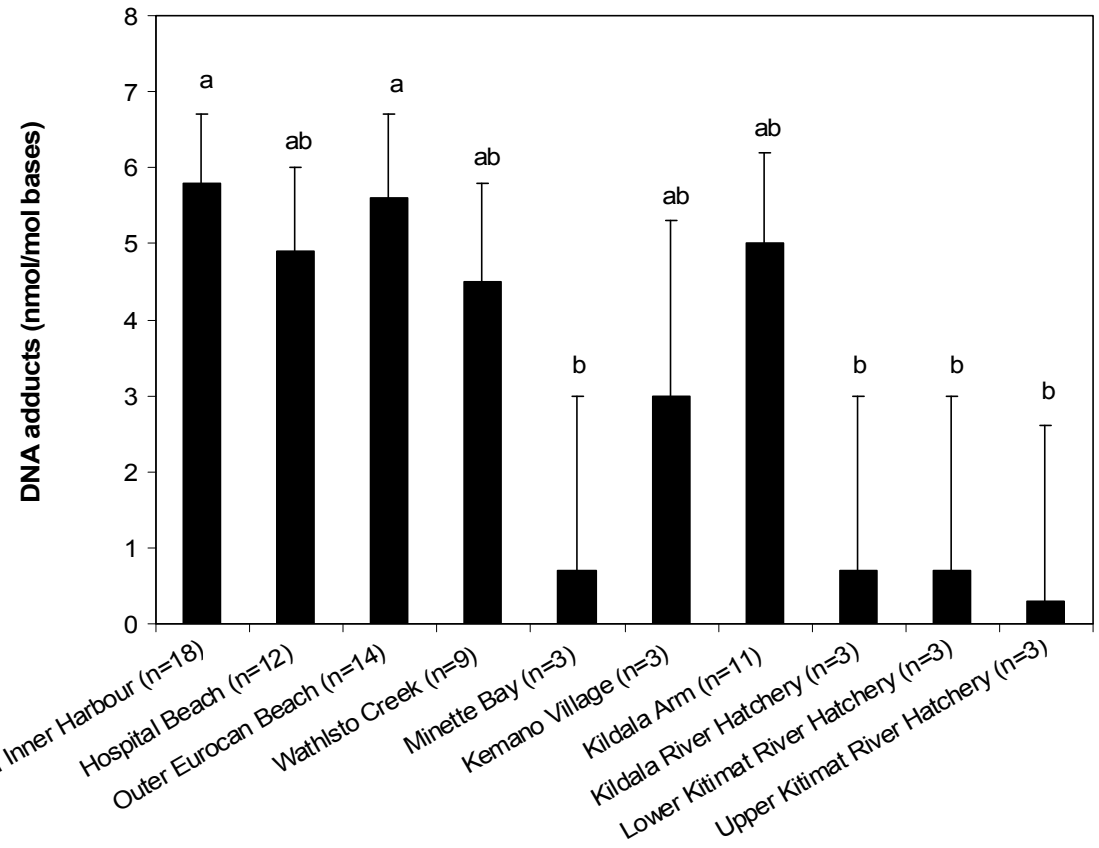

Figure 15. Concentrations of DNA adducts in juvenile Chinook salmon from sites within Kitimat Arm (Alcan Inner Harbour, Hospital Beach, Inner Eurocan Beach, Outer Eurocan Beach, Minette Bay, and Wathlsto Creek), from reference sites outside Kitimat Arm (Kemano Village and Kildala Arm), and from three hatchery stocks (Kildala River, upper Kitimat River, and lower Kitimat River) from the Kitimat River Fish Hatchery. Values are averages of samples from 2000, 2001, and 2002. DNA adducts were not measured in juvenile Chinook salmon in 2003 or 2004. Values with different letter superscripts are significantly different ($p \leq 0.05$).

different pattern. Highest concentrations were seen in fish from Eurocan (110 ng/g wet wt) and Hospital Beach (120 ng/g wet wt), significantly higher than in sole from the other sampling sites. Concentrations in fish from Kitamaat Village (74 ng/g wet wt) were intermediate and significantly higher than in sole from Kitlope. Average concentrations of ΣLAHs in stomach contents of sole from Emsley Cove, Kildala Arm, and Kitlope were comparable, ranging from 19 ng/g wet wt in sole from Kitlope to 37 ng/g wet wt in sole from Kildala Arm.

PAH composition

The composition of HAHs in stomach contents of English sole (Figure 17A) was fairly similar to that of the Alcan pitch samples for fish from sites within Kitimat Arm (Hospital Beach, Eurocan, Kitamaat Village, and Emsley Cove). One major difference in HAH composition between the stomach contents and the pitch samples was the greater proportion of perylene in the fish stomach contents samples. Perylene is a naturally derived PAH that is a byproduct of decaying organic matter (Wakeham et al. 1980, Pichler et al. 1996, Irwin et al.

Table 8. Temporal trends in mean concentrations (±SE) of DNA adducts in livers of juvenile Chinook salmon from sites within Kitimat Arm (Alcan Inner Harbor, Hospital Beach, Outer Eurocan Beach, and Wathlsto Creek) and the Kildala Arm reference site from 2000 to 2002. Sites with different letter superscripts are significantly different ($p \leq 0.05$); years with different number superscripts are significantly different ($p \leq 0.05$). Minette Bay, Kemano Village, and Kitimat hatcheries are not included, as they were sampled only in 2000.

Years	Alcan Inner Harbor	Hospital Beach	Outer Eurocan Beach	Wathlsto Creek	Kildala Arm	*p* values for intersite differences
2000	5.7 ± 1.4^{ab1} (n = 6)	7.7 ± 2.0^{a1} (n = 3)	3.7 ± 2.0^{abc1} (n = 3)	1.3 ± 2.0^{bc1} (n = 3)	1.0 ± 2.0^{bc1} (n = 3)	$p = 0.1213$
2001	70 ± 1.8^{a1} (n = 6)	1.5 ± 1.3^{b1} (n = 6)	7.8 ± 3.7^{a1} (n = 6)	9.3 ± 7.0^{a1} (n = 3)	5.3 ± 2.3^{ab2} (n = 3)	$p = 0.0473$
2002	4.7 ± 0.8^{a1} (n = 6)	5.7 ± 4.2^{a1} (n = 3)	5.4 ± 1.2^{a1} (n = 5)	3.0 ± 0.6^{a1} (n = 3)	7.2 ± 1.2^{a2} (n = 3)	$p = 0.5029$
p values for interannual differences	$p = 0.5777$	$p = 0.2934$	$p = 0.1754$	$p = 0.1153$	$p = 0.0135$	

1998, Yunker et al. 1999). Additionally, stomach contents of sole from Eurocan tended to have higher proportions of fluoranthene and chrysene and lower proportions of BaP than the pitch samples or the stomach contents samples of sole from Hospital Beach, Kitamaat Village, and Emsley Cove. Patterns of HAHs were more distinctive in stomach contents of sole from the two sites outside of Kitimat Arm (Kildala Arm and Kitlope). Perylene was the predominant HAH in stomach contents of fish from both sites, but particularly at Kitlope, where it made up 90% of ΣHAHs. At Kildala Arm, perylene accounted for about 40% of ΣHAHs.

In comparison with HAHs, profiles of LAHs in stomach contents of English sole showed less similarity to the Alcan pitch samples (Figure 17B). In the pitch samples, phenanthrene was the predominant LAH, accounting for 60–70% of ΣLAHs, with acenaphthene accounting for about 20%, and fluorene and anthracene each accounting for up to 10%. The profile for stomach contents of sole from Hospital Beach was fairly similar, except that retene, a low molecular weight PAH derived from wood products (Zender et al. 1994, Tavendale et al. 1995), which was not present in Alcan pitch, made up about 15% of total LAHs. Other differences included a somewhat lower proportion of acenaphthene in Hospital Beach stomach contents and the presence of small proportions of various other LAHs (e.g., 2,6-dimethylnaphthalene, and 2,3,5-trimethylnaphthalene) that were not found in the pitch samples.

The LAH profiles in stomach contents of sole from the other sampling sites within Kitimat Arm (Eurocan, Kitamaat Village, and Emsley Cove) showed less similarity to the pitch samples. Stomach contents samples from all three sites contained substantial proportions of phenanthrene (20–30% of ΣLAHs), but also contained substantial retene (from 20 to 25% of ΣLAHs at Kitamaat Village and Emsley Cove to nearly 60% at Eurocan). Stomach contents of sole from the Kitamaat Village and Emsley Cove sites, in particular, also contained higher

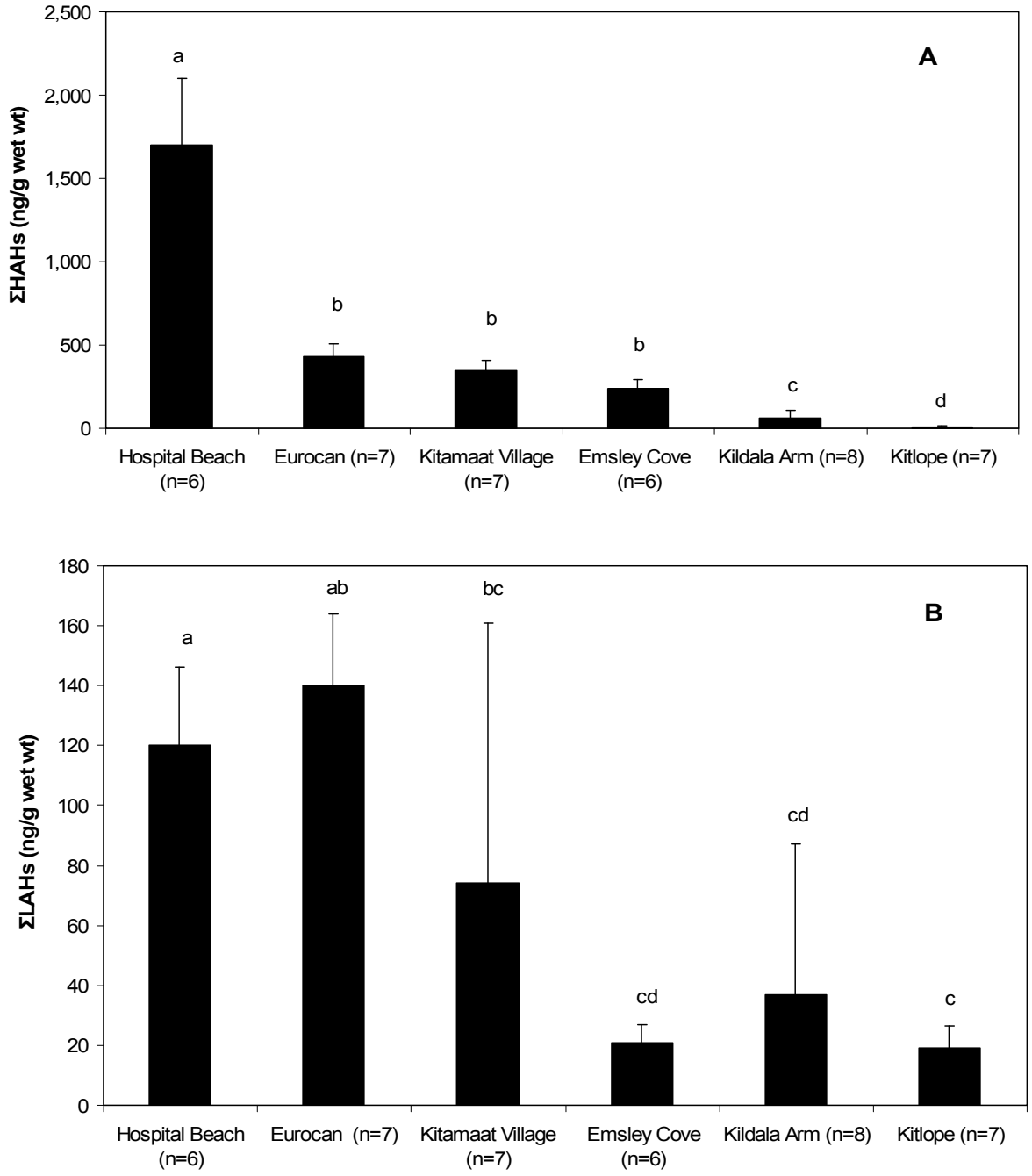

Figure 16. Concentrations of (A) ΣHAHs and (B) ΣLAHs in stomach contents of English sole collected from sites within Kitimat Arm (Hospital Beach, Eurocan, Kitamaat Village, and Emsley Cove) and from reference sites outside Kitimat Arm (Kildala Arm and Kitlope) in 2000, 2002, and 2004. Samples were composites of stomach contents from five fish each. Values with different letter superscripts are significantly different ($p \leq 0.05$).

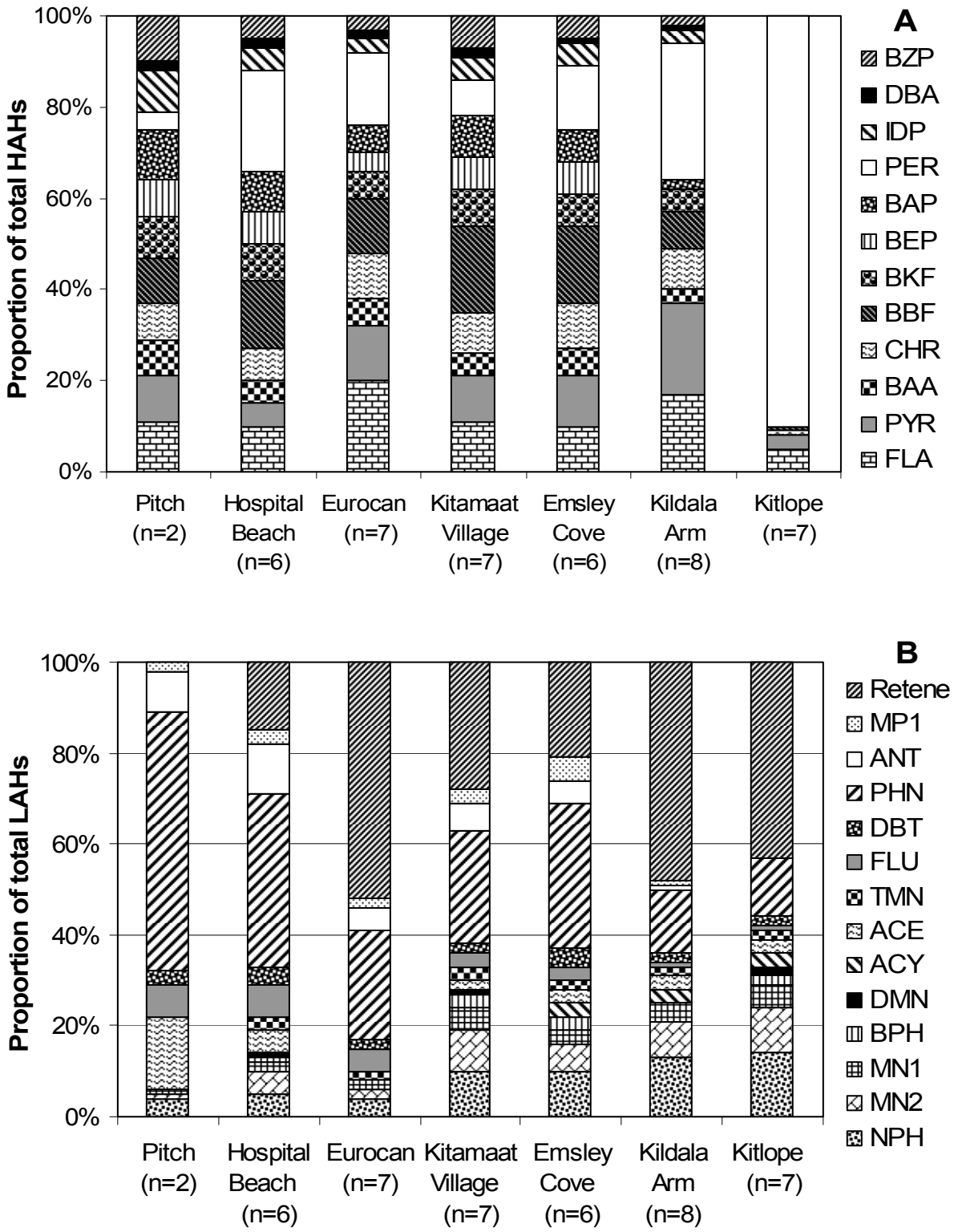

Figure 17. Proportions of (A) individual HAHs and (B) individual LAHs in stomach contents of English sole from sites within Kitimat Arm (Hospital Beach, Eurocan, Kitamaat Village, and Emsley Cove) from reference sites outside Kitimat Arm (Kildala Arm and Kitlope), and in Alcan pitch samples. Proportions are averages derived from samples collected in 2000, 2002, and 2004.

proportions of naphthalene, as well as naphthalene derivatives and other PAHs not found or found only at very low concentrations in the pitch samples (1-methylnaphthalene, 2-methylnaphthalene, 2,6-dimethylnaphthalene, and 2,3,5-trimethylnaphthalene). Profiles of LAHs in stomach contents of sole from Kildala Arm and Kitlope were least like the pitch samples, with lower proportions of phenanthrene (≈10%) and higher proportions of retene and naphthalene and alkylated or substituted naphthalenes.

Temporal trends in AH concentrations in stomach contents

In English sole collected in 2000, 2002, and 2004, concentrations of ΣHAHs in stomach contents were similar from year to year in fish from most of the sampling sites, with no clear increasing or decreasing trends (Table 9). The one exception was for fish from the Kitlope site, where HAH concentrations differed significantly from year to year, with highest concentrations in 2002 and lowest concentrations in 2000. Perylene concentrations were also significantly higher in stomach contents of Kitlope sole in 2002 than in 2004 and may have contributed to the difference observed in total HAHs.

Concentrations of ΣLAHs were comparable in 2000, 2002, and 2004 at most sites. However in sole from Eurocan, concentrations of LAH concentrations in stomach contents were significantly higher in 2002 than in either 2000 or 2004. This difference was primarily due to a difference in retene concentrations in the samples (Table 9). In stomach samples collected in 2002 from sole in Eurocan, for example, the retene concentration was 150 ng/g wet wt, significantly higher than the 49 ng/g wet wt concentration observed in 2004. Concentrations of retene in stomach contents also showed some variability in sole from Kitimat Village in that levels in sole collected in 2002 were significantly higher than those in fish collected in 2000.

Correlation of stomach contents and sediment PAH concentrations

Concentrations of ΣHAHs in stomach contents were significantly and positively correlated with concentrations of ΣHAHs in sediments ($r^2 = 0.62$, $p = 0.0001$), while concentrations of ΣLAHs in stomach contents were significantly and positively correlated with concentrations of LAHs in sediments ($r^2 = 0.33$, $p = 0.0032$).

OCs in Stomach Contents

In 2000 only, several classes of OCs (aldrin, dieldrin, HCB, hexachlorocyclohexanes, chlordanes, DDTs, and PCBs) were measured in stomach contents of English sole. With the exception of the DDTs, concentrations of all pesticides (aldrin, dieldrin, HCB, lindanes, and chlordanes) were below the lower LOQ (data not shown). DDTs were detected only in samples from Hospital Beach and Eurocan. The mean ΣDDT concentration (±SE) was 0.40 + 0.40 ng/g wet wt in stomach contents samples from Eurocan and 0.61 + 0.35 ng/g wet wt in stomach contents samples from Hospital Beach. Concentrations of ΣPCBs in English sole stomach contents ranged from less than 1 ng/g wet wt at Kitlope to 22 ng/g wet wt at Hospital Beach (Figure 18). Concentrations of PCBs were significantly higher in stomach contents of fish from Hospital Beach than at other sites.

Table 9. Temporal trends from 2000 to 2004 in mean concentrations (±SE), in ng/g wet wt, of ΣLAHs and ΣHAHs in stomach contents of English sole from Kitimat Arm (Hospital Beach, Eurocan, Kitamaat Village, and Emsley Cove) and reference areas (Kildala Arm and Kitlope). Concentrations of retene and perylene are also reported, as these represent nonsmelter-derived AHs. Sites with different letter superscripts are significantly different ($p \leq 0.05$); years with different number superscripts are significantly different ($p \leq 0.05$).

Site	Year collected	Retene	ΣLAHs	Perylene	ΣHAHs
Hospital Beach	2000 (n = 3)	12 ± 2.8	130 ± 18	NM	1,600 ± 330
	2002 (n = 1)	24	110	300	1,700
	2004 (n = 3)	13 ± 2.2	130 ± 33	540 ± 150	1,800 ± 280
		p = 0.9095	p = 0.7090	p = 0.6670	p = 0.9095
Eurocan	2000 (n = 3)	92 ± 14[ab]	130 ± 11b	NM*	360 ± 42
	2002 (n = 1)	150[a]	190a	78	430
	2004 (n = 3)	49 ± 5.4[b]	140 ± 4.1b	58 ± 18	480 ± 35
		p = 0.0094	p =0.0219	p = 0.5496	p = 0.1404
Kitamaat Village	2000 (n = 3)	10 ± 2.4[b]	31 ± 2.9	NM	310 ± 11
	2002 (n = 1)	23[a]	59	37	390
	2004 (n = 3)	13 ± 1.8[ab]	120 ± 92	30 ± 1.8	390 ± 47
		p = 0.0498	p = 0.5298	p = 0.1276	p = 0.1869
Emsley Cove	2000 (n = 3)	5.9 ± 1.9	20 ± 2.8	NM	220 ± 27
	2002 (n = 1)	6.9	32	44	280
	2004 (n = 3)	3.1 ± 1.3	21 ± 3.6	37 ± 2.5	270 ± 47
		p = 0.2677	p = 0.1583	p = 0.3000	p = 0.6300
Kildala Arm	2000 (n = 3)	12 ± 8.5	22 ± 11	NM	31 ± 3.5
	2002 (n = 1)	150	160	41	61
	2004 (n = 3)	9.0 ± 6.2	20 ± 6.4	25 ± 7.8	110 ± 43
		p = 0.3306	p = 0.2942	p = 0.8339	p = 0.1507
Kitlope	2000 (n = 3)	15 ± 7.1	21 ± 5.3	NM	1.8 ± 0.28[c]
	2002 (n = 1)	<LOQ	5.4	19[a]	23[a]
	2004 (n = 3)	9.2 ± 1.8	20 ± 2.3	8.7 ± 1.2[b]	12 ± 1.4[b]
		p = 0.3297	p = 0.1547	p = 0.0338	p = 0.0006

*NM = not measured

PAH Metabolites in Flatfish Bile

PAH metabolites in bile of English sole

Mean PAH metabolites in bile of English sole collected from 2000 to 2004 are shown in Figure 19. Metabolites of HAHs (Figure 19A) and LAHs (Figure 19B) were present in bile of fish from all sites but were not necessarily highest at sites where PAH concentrations were highest in sediments or stomach contents. Among the sites within Kitimat Arm, concentrations of BaP equivalents were highest in sole from Hospital Beach (1,200 ng/g bile) and were

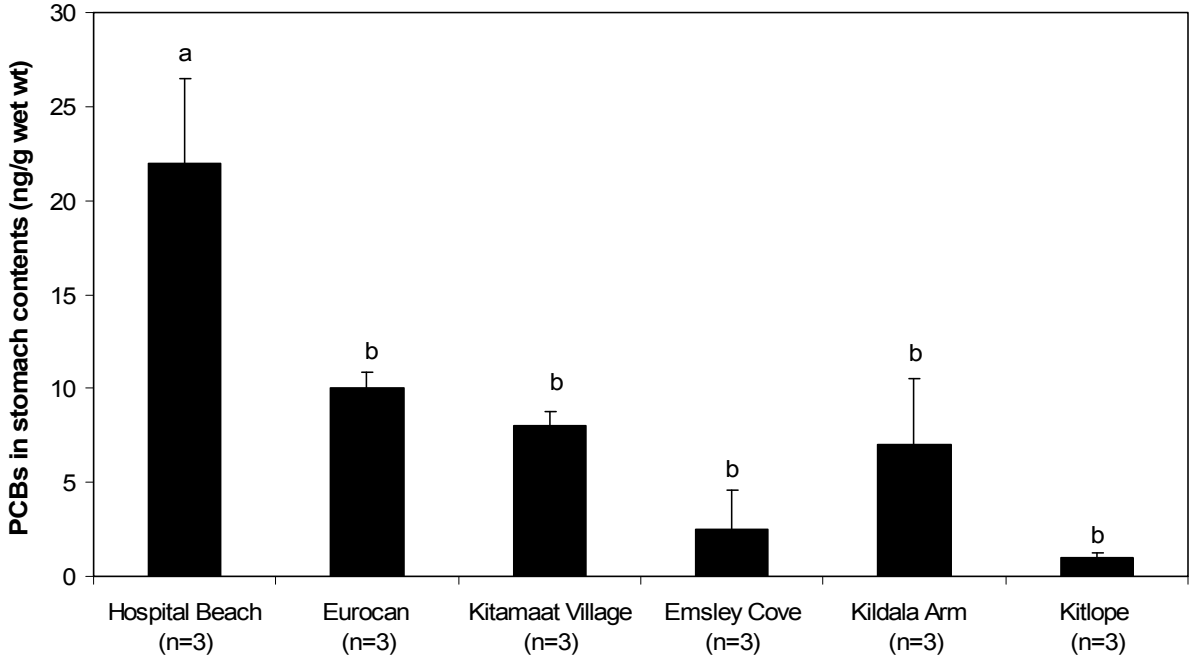

Figure 18. Mean concentrations (ng/g wet wt ± SE) of PCBs in stomach contents of English sole from sites within Kitimat Arm (Hospital Beach, Eurocan, Kitimaat Village, and Emsley Cove) and from reference sites outside Kitimat Arm (Kildala Arm and Kitlope). Values with different letter superscripts are significantly different ($p \leq 0.05$).

somewhat lower (480–700 ng/g bile) in sole from Eurocan, Kitamaat Village, and Emsley Cove. Levels of BaP equivalents in sole from Kildala Arm (600 ng/g bile) were similar to those found in sole from Eurocan, Kitimat Village, and Emsley Cove, but in sole from Kitlope, BaP metabolite levels were quite high (1,400 ng/g bile), comparable to levels in sole from Hospital Beach. Levels of BaP equivalents were significantly higher in sole from Kitlope and Hospital Beach than in sole from Emsley Cove and Eurocan. In sole from Kitamaat Village and Kildala Arm, BaP equivalent levels in bile were intermediate and not significantly different from levels at any of the other sites.

Within Kitimat Arm, concentrations of PHN equivalents were also highest in fish from Hospital Beach (34,000 ng/g bile). At Eurocan and Kitamaat Village, concentrations were comparable and somewhat lower (24,000–26,000 ng/g bile). Lower levels were found in sole from Emsley Cove (14,000 ng/g bile). However, levels of PHN equivalents in bile of fish from Kitlope and Kildala Arm were surprisingly high (23,000–24,000 ng/g bile), comparable to levels in sole from Eurocan and Kitamaat Village. Levels of PHN equivalents in bile of English sole from Emsley Cove were significantly lower than in sole from Hospital Beach, but no other significant intersite differences were observed.

Temporal trends in English sole bile metabolites

Concentrations of metabolites of LAHs in English sole bile (Table 10) showed consistent intersite differences in all sampling years. Levels of PHN equivalents in bile were consistently

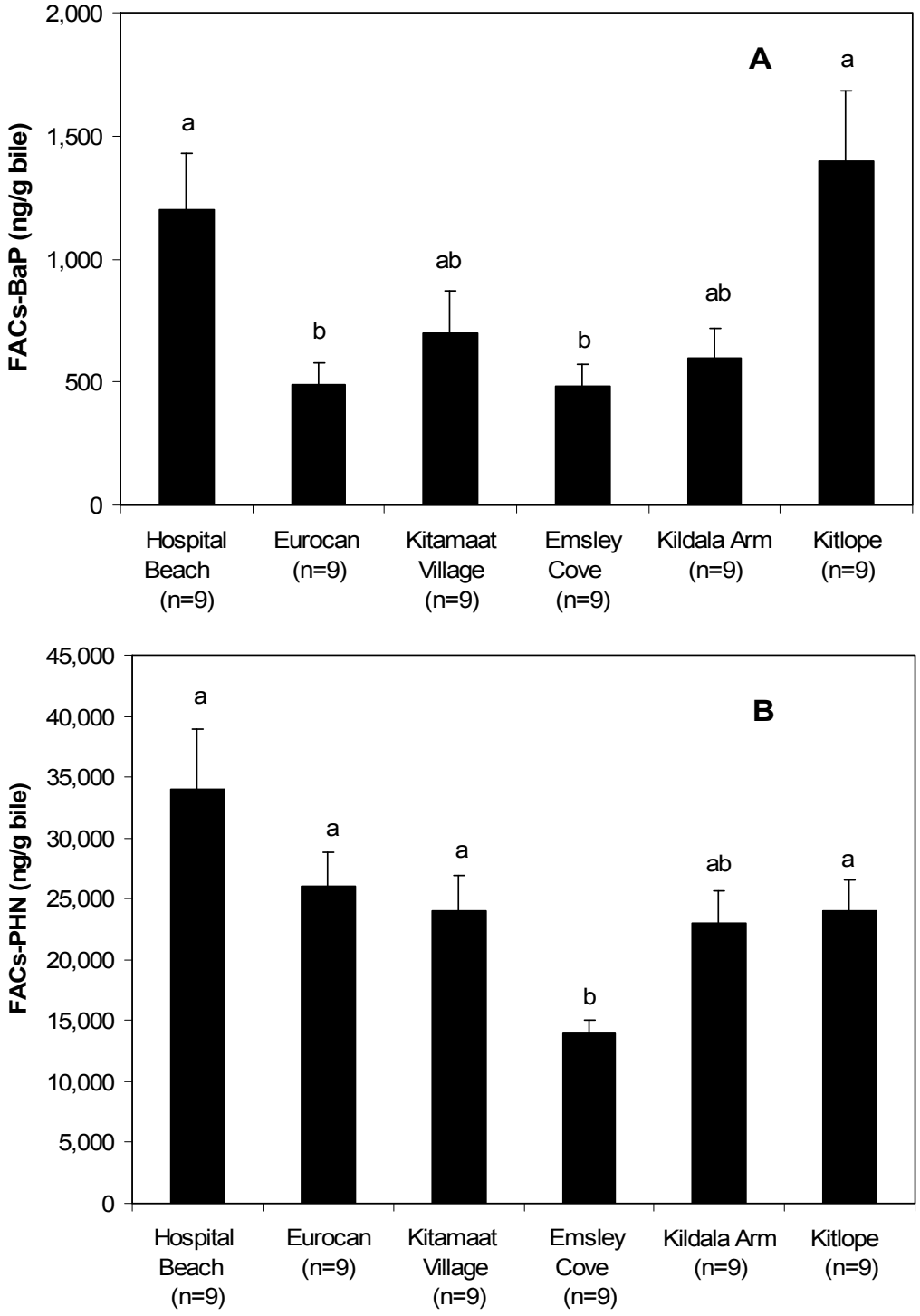

Figure 19. Mean concentrations (ng/g bile ±SE) of (A) fluorescent metabolites of HAHs, measured as (FACs-BaP, and (B) fluorescent metabolites of LAHs, measured as FACs-PHN, in bile samples collected in 2000, 2002, and 2004 from English sole from sites within Kitimat Arm (Hospital Beach, Eurocan, Kitamaat Village, and Emsley Cove) and from reference sites outside Kitimat Arm (Kildala Arm and Kitlope). Values with different letter superscripts are significantly different ($p < 0.05$).

Table 10. Temporal trends in mean concentrations (±SE) of metabolites of LAH and HAH PHN and BaP equivalents, respectively) in bile of English sole from Kitimat Arm sites (Hospital Beach, Eurocan, Kitamaat Village, and Emsley Cove) and reference areas (Kildala Arm and Kitlope) from 2000 to 2004. Sites with different letter superscripts are significantly different ($p \leq 0.05$); years with different number superscripts are significantly different ($p \leq 0.05$).

Year	Hospital Beach	Eurocan	Kitamaat Village	Emsley Cove	Kildala Arm	Kitlope	*p* values for intersite differences
PHN equivalents (ng/g bile, wet wt)							
2000	37,000 ± 6,700^{a12} (n = 3)	19,000 ± 6,000^{bc1} (n = 3)	32,000 ± 5,700^{a1} (n = 3)	11,000 ± 1,200^{c1} (n = 3)	24,000 ±3,400^{ab1} (n = 3)	31,000 ± 4,400^{ab1} (n = 3)	$p = 0.0068$
2002	21,000 ± 4,600^{ab1} (n = 3)	30,000 ± 3,400^{a1} (n = 3)	22,000 ± 3,800^{ab12} (n = 3)	16,000 ± 1,200^{b21} (n = 3)	16,000 ± 1,500^{b2} (n = 3)	17,000 ± 880^{b2} (n = 3)	$p = 0.0308$
2004	45,000 ± 8,700^{a2} (n = 3)	30,000 ± 1,500^{b1} (n = 3)	18,000 ± 1,000^{cd2} (n = 3)	16,000 ± 1,500^{d2} (n = 3)	30,000 ± 4,000^{b1} (n = 3)	25,000 ± 3,000^{bc12} (n = 3)	$p = 0.0006$
p values for interannual differences	$p = 0.0686$	$p = 0.1575$	$p = 0.0882$	$p = 0.0355$	$p = 0.0271$	$p = 0.0252$	
BaP equivalents (ng/g bile, wet wt)							
2000	900 ± 250^{a1} (n = 3)	220 ± 110^{c1} (n = 3)	880 ± 470^{ab1} (n = 3)	170 ± 40^{c1} (n = 3)	230 ± 32^{bc1} (n = 3)	990 ± 110^{a1} (n = 6)	$p = 0.0007$
2002	810 ± 200^{a1} (n = 3)	590 ± 100^{a2} (n = 3)	510 ± 150^{a1} (n = 3)	530 ± 130^{a2} (n = 3)	700 ± 140^{a12} (n = 3)	750 ± 8.5^{a1} (n = 6)	$p = 0.4922$
2004	1,900 ± 400^{a1} (n = 3)	670 ± 69^{b2} (n = 3)	720 ± 290^{b1} (n = 3)	740 ± 82^{b2} (n = 3)	880 ± 160^{b2} (n = 3)	2,400 ± 350^{a2} (n = 3)	$p = 0.0023$
p values for interannual differences	$p = 0.1111$	$p = 0.0313$	$p = 0.8313$	$p = 0.0076$	$p = 0.0037$	$p = 0.0006$	

elevated in fish at Hospital Beach and tended to be relatively higher than those measured in fish from Eurocan and Kitamaat Village. At Emsley Cove, levels were consistently low; however, levels of PHN equivalents were surprisingly high in fish from Kildala Arm and Kitlope and were comparable to levels in sole from Hospital Beach in 2000 and 2002, though they were slightly lower in 2004.

At Hospital Beach, Eurocan, and Kitamaat Village, levels of PHN equivalents in English sole bile did not vary significantly from year to year ($0.0686 < p < 0.1575$), but significant differences over time were observed in sole from Emsley Cove, Kildala Arm, and Kitlope ($0.0252 < p < 0.0355$). In sole from Emsley Cove, levels of PHN equivalents increased from 2000 to 2004, while in sole from Kildala Arm and Kitlope, PHN equivalent levels were lower in 2002 than in the other sampling years.

Concentrations of metabolites of HAHs in English sole bile (Table 10) showed significant intersite differences in 2000 and 2004 but not in 2002 when concentrations of BaP equivalents were similar in sole from all sites. Levels of BaP equivalents in bile were generally among the highest in sole from Hospital Beach and among the lowest in sole from Emsley Cove. Like PHN equivalents, levels of BaP equivalents were surprisingly high in fish from Kildala Arm and especially from Kitlope; these levels were comparable to the values in sole from Hospital Beach in all three sampling years. Significant differences in levels of BaP equivalents in 2000, 2002, and 2004 were observed in sole from Eurocan, Emsley Cove, Kildala Arm, and Kitlope, with concentrations tending to increase with time at all four sites ($0.0006 < p < 0.0313$). In sole from Hospital Beach and Kitamaat Village, levels of BaP equivalents in bile did not differ significantly from 2000 to 2004 ($0.1111 < p < 0.8313$).

PAH metabolites in bile of yellowfin sole

Because the relationship between PAH exposure and reproductive parameters was examined, bile samples from male and female yellowfin sole were composited separately. At sites where both male and female sole could be collected (all sites except Emsley Cove), the sex of the fish had no significant effect on bile metabolite levels. Mean concentrations of PHN and BaP equivalents in composite bile samples from female yellowfin sole (15,000 ± 4,800 ng/g and 600 ± 140 ng/g, respectively; n = 14) and male yellowfin sole (7,200 ± 1,000 ng/g and 450 ± 75 ng/g, respectively; n = 26) were not significantly different ($0.3025 < p < 0.3768$; ANOVA on log-transformed values). Consequently, bile samples from males and females were combined for subsequent analyses of intersite and temporal differences in bile metabolite levels in yellowfin sole. As in English sole, concentrations of PAH metabolites in yellowfin sole bile did not correlate well with sediment PAH concentrations at the sampling sites, especially for the HAHs (Figure 20). Concentrations of bile metabolites fluorescing at BaP wavelengths (Figure 20A) showed no significant intersite differences ($p = 0.69$, ANOVA on log-transformed values); concentrations ranged from 390 ng/g bile in sole from Emsley Cove to 580 ng/g bile in sole from Eurocan.

Concentrations of bile metabolites fluorescing at PHN wavelengths (Figure 20B), on the other hand, differed significantly among the sampling sites ($p = 0.0005$, ANOVA on log-transformed values). Highest LAH metabolite levels were found in yellowfin sole from Eurocan, significantly higher than at any of the sampling sites with the exception of Hospital Beach and

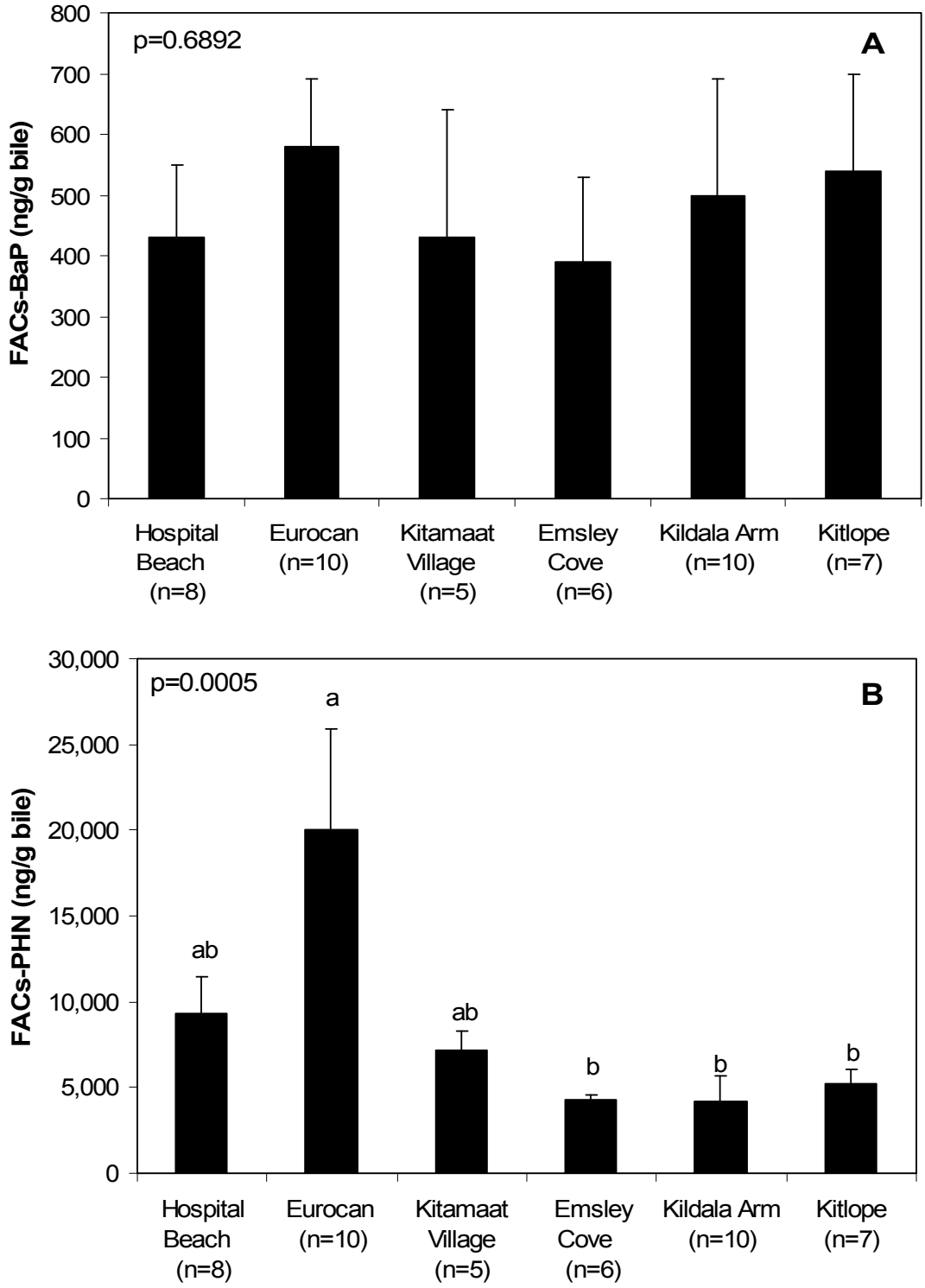

Figure 20. Mean concentrations (ng/g bile ± SE) of (A) fluorescent metabolites of HAHs, measured as FACs-BaP, and (B) fluorescent metabolites of LAHs, measured as FACs-PHN, in bile samples collected in 2000 and 2002 from yellowfin sole from sites within Kitimat Arm (Hospital Beach, Eurocan, Kitimaat Village, and Emsley Cove) and from reference sites outside Kitimat Arm (Kildala Arm and Kitlope). Values with different letter superscripts are significantly different ($p < 0.05$).

Kitamaat Village. Levels were lowest in yellowfin sole from Kildala Arm, with comparable concentrations in sole from Emsley Cove and Kitlope.

Concentrations of LAH metabolites in yellowfin sole bile (Table 11) showed significant intersite differences in both 2000 and 2002 (yellowfin sole were not sampled in 2004). Levels of PHN equivalents in bile were highest in sole from Eurocan and Hospital Beach in 2000 and highest in sole from Eurocan and Kitamaat Village in 2002. Levels of PHN equivalents in sole from Kildala Arm and Kitlope were moderate to low in comparison with other sites in both sampling years. Significant differences in levels of PHN equivalents in 2000 and 2002 were observed in sole from Hospital Beach, Eurocan, and Kildala Arm, where levels decreased, and from Kitamaat Village, where levels increased.

Concentrations of BaP equivalents in yellowfin sole (Table 11) showed significant intersite differences in 2000, with highest levels in sole from Eurocan and Hospital Beach, but in 2002 there were no significant differences among sites. Significant differences in levels of BaP equivalents in 2000 and 2002 were observed in sole from Kitamaat Village, Emsley Cove, Kildala Arm, and Kitlope, with concentrations increasing at all four sites.

PAHs, OCs, and Metals in English Sole Muscle Tissue

Concentrations of PAHs, OCs, and metals in muscle tissue of English sole were measured in 2000 (Tables 12 through 14). Concentrations of both high and low molecular weight PAHs were very low in all samples, far below maximum recommended concentrations in seafood for human consumption and did not differ significantly among sites (Table 12). Concentrations of most pesticides (aldrin, dieldrin, heptachlor, HCB, lindane, chlordanes, and DDTs) were less than 1 ng/g wet wt and did not differ significantly among sites (Table 13). Concentrations of PCBs were also quite low but were significantly higher at Hospital Beach than at the other sites (Table 13).

Concentrations of most metals (silver, chromium, copper, nickel, selenium, tin, and lead) were less than LOQ in all samples (Table 14). Manganese was found only in sole from Emsley Cove, while cadmium was found only in sole from Hospital Beach, in both cases at concentrations less than 2 µg/g dry wt. Measurable quantities of zinc (23–29 µg/g dry wt), iron (6.2–9.0 µg/g dry wt), aluminum (<0.82–3.3 µg/g dry wt), arsenic (21–65 µg/g dry wt), and mercury (0.11–0.23 µg/g dry wt) were detected in muscle samples from all or nearly all sites. With the exception of mercury, no significant intersite differences were observed between metal levels in fish muscle from Kitimat Arm and from reference areas (Table 14). In the case of mercury, levels were significantly higher in muscle of sole from Kitlope than in sole from Kildala Arm and Kitamaat Village.

Biochemical Changes and Liver Lesions in Flatfish

Hepatic AHH Activity

Induction of AHH activity was observed in the liver of English sole collected from all sampling sites, with significant differences in activity among sites (ANOVA, $p = 0.0047$, log-transformed values, Figure 21). Within Kitimat Arm, fish from the Hospital Beach site showed

Table 11. Mean concentrations (±SE) of metabolites of LAH and HAH PHN and BaP equivalents, respectively) in bile of yellowfin sole from Kitimat Arm sites (Hospital Beach, Eurocan, Kitamaat Village, and Emsley Cove) and reference areas (Kildala Arm and Kitlope), sampled in 2000 and 2002. Sites with different letter superscripts are significantly different ($p \leq 0.05$); years with different number superscripts are significantly different ($p \leq 0.05$).

Year	Hospital Beach	Eurocan	Kitamaat Village	Emsley Cove	Kildala Arm	Kitlope	*p* values for intersite differences
PHN equivalents (ng/g bile, wet wt)							
2000	12,000 ± 2,000^{ab1} (n = 6)	29,000 ± 9,200^{a1} (n = 6)	5,500 ± 440^{b1} (n = 3)	4,700 ± 260^{b1} (n = 3)	6,100 ± 2,600^{b1} (n = 6)	7,200 ± 900^{ab1} (n = 3)	$p = 0.0009$
2002	1,800 ± 500^{bc2} (n = 2)	7,900 ± 1,900^{a2} (n = 4)	9,800 ± 350^{a2} (n = 2)	3,800 ± 400^{ab1} (n = 3)	1,500 ± 320^{c2} (n = 4)	3,700 ± 940^{abc1} (n = 4)	$p = 0.0002$
p values for interannual differences	$p = 0.0041$	$p = 0.0135$	$p = 0.0113$	$p = 0.1537$	$p = 0.0367$	$p = 0.0640$	
BaP equivalents (ng/g bile, wet wt)							
2000	370 ± 150^{ab1} (n = 6)	480 ± 200^{a1} (n = 3)	120 ± 21^{abc1} (n = 3)	75 ± 13^{bc1} (n = 3)	89 ± 22^{c1} (n = 6)	100 ± 29^{abc1} (n = 3)	$p = 0.0023$
2002	600 ± 80^{a1} (n = 2)	720 ± 29^{a1} (n = 3)	890 ± 440^{a2} (n = 2)	700 ± 55^{a2} (n = 3)	1,100 ± 310^{a2} (n = 4)	870 ± 100^{a2} (n = 4)	$p = 0.3875$
p values for interannual differences	$p = 0.2428$	$p = 0.1337$	$p = 0.0108$	$p = 0.0003$	$p = 0.0001$	$p = 0.0003$	

Table 12. Concentrations of PAHs (mean ± SE, in ng/g, wet wt) in muscle tissue of English sole from Kitimat Arm sites (Hospital Beach, Eurocan, Kitamaat Village, and Emsley Cove). Samples were collected in 2000 only.

PAHs	Hospital Beach (n = 3)	Eurocan (n = 3)	Kitimaat Village (n = 3)	Emsley Cove (n = 3)	Maximum level in seafood*
Naphthalene	0.60 ± 0.021	0.66 ± 0.16	0.67 ± 0.17	0.56 ± 0.080	90,000
2-methynaphthalene	0.70 ± 0.16	0.83 ± 0.16	0.84 ± 0.16	0.71 ± 0.050	
Acenaphthylene	<LOQ	<LOQ	<LOQ	<LOQ	
Acenaphthene	<LOQ	0.40 ± 0.23	0.3.2 ± 0.099	<LOQ	
Fluorene	<LOQ	<LOQ	<LOQ	<LOQ	90,000
Phenanthrene	0.51 ± 0.030	0.64 ± 0.21	0.53 ± 0.045	0.46 ± 0.074	
Anthracene	<LOQ	<LOQ	<LOQ	<LOQ	
Retene	<LOQ	<LOQ	<LOQ	<LOQ	
Sum LAHs	**1.7 ± 0.61**	**2.5 ± 0.95**	**2.2 ± 0.21**	**1.7 ± 0.20**	
Fluoranthene	0.28 ± 0.001	<LOQ	<LOQ	<LOQ	800
Pyrene	<LOQ	<LOQ	<LOQ	<LOQ	100
Benzo[a]anthracene	<LOQ	<LOQ	<LOQ	<LOQ	1,000
Chrysene	<LOQ	<LOQ	<LOQ	<LOQ	1,000
Benzo[b]fluoranthene	<LOQ	<LOQ	<LOQ	<LOQ	
Benzo[a]pyrene	<LOQ	<LOQ	<LOQ	<LOQ	20
Ideno[1,2,3-cd]pyrene	<LOQ	<LOQ	<LOQ	<LOQ	
Benzo[ghi]perylene	<LOQ	<LOQ	<LOQ	<LOQ	
Sum HAHs	**0.28 ± 0.00**	**<LOQ**	**<LOQ**	**<LOQ**	

*Recommended contaminant level allowed in seafood after North Cape oil spill.

the highest AAH activity (296 pmol/min/mg protein). Average values for fish from the Eurocan, Emnsley Cove, and Kitimaat Village sites were somewhat lower, ranging 179–223 pmol/min/mg protein, but not significantly different from each other or from values for sole from Hospital Beach. The highest activities were in fish from Kildala Arm and Kitlope (404–410 pmol/min/mg protein). AHH activity in sole from Kitlope was significantly higher than in sole from either Eurocan or Kitamaat Village, while AHH activity in sole from Kildala was significantly higher than in sole from Kitamaat Village. AHH activity was only measured in the first year of the study; it was not included in analyses for 2002 and 2004 because it showed no apparent relationship with any other indicator of PAH exposure.

PAH-DNA Adducts

Overall, mean levels of PAH-DNA adducts (measured in 2000, 2002, and 2004) were highest in sole from Hospital Beach and lowest in sole from Kitlope, with intermediate values at the other sampling sites (Figure 22). However, values were quite variable over the three sampling years (Table 15). Significant intersite differences in adduct levels were seen for 2000 and 2002, with highest levels in sole from Eurocan in 2000, in sole from Hospital Beach in 2002 and in sole from Hospital Beach and Eurocan in 2004. However, adduct levels in sole collected in 2004 were very low in fish from all sites, less than 1 mol DNA adducts/mol bases. Significant or near significant differences in adduct levels among the years 2000, 2002, and 2004 were found in sole from all of the sampling sites. With the exception of sole from Kitlope and

Table 13. Concentrations of selected OCs (mean ± SE, in ng/g, wet wt) in muscle tissue of English sole from Kitimat and surrounding areas. Samples collected in 2000 only.

Site	HCB	Lindane	Aldrin	Dieldrin	Heptachlor	α + γ chlordane	*p,p′*-DDE	*p,p′*-DDD	*p,p′*-DDT	ΣPCBs
Hospital Beach (n = 3)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.53 ± 0.06	0.48 ± 0.03	<LOQ	13 ± 4.4 [c]
Eurocan (n = 3)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.55 ± 0.21	<LOQ	<LOQ	5.7 ± 2.8 [b]
Kitimaat Village (n = 3)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.37 ± 0.06	<LOQ	<LOQ	3.7 ± 1.2 [ab]
Emsley Cove (n = 3)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.26 ± 0.04	<LOQ	<LOQ	1.4 ± 0.7 [a]

Table 14. Metals concentrations (mean ± SE, in µg/g dry wt) in muscle tissue of English sole from Kitimat Arm sites (Hospital Beach, Eurocan, Kitamaat Village, and Emsley Cove) and reference areas (Kildala Arm and Kitlope). Values with different letter superscripts are significantly different (ANOVA, Tukey-Kramer multiple range test, $p \leq 0.05$). Metals sampled in English sole muscle tissue in 2000 only.

Site	Silver	Aluminum	Arsenic	Cadmium	Chromium	Copper	Iron
Hospital Beach (n = 3)	<LOQ	3.2 ± 1.20	64 ± 18	1.4 ± 1.30	<LOQ	<LOQ	7.2 ± 1.10
Eurocan (n = 3)	<LOQ	3.3 ± 0.78	35 ± 16	<LOQ	<LOQ	<LOQ	7.9 ± 1.70
Kitimaat Village (n = 3)	<LOQ	2.5 ± 0.28	51 ± 26	<LOQ	<LOQ	<LOQ	6.9 ± 1.10
Emsley Cove (n = 2)	<LOQ	3.1 ± 0.86	39 ± 2.8	<LOQ	<LOQ	<LOQ	9.0 ± 2.60
Kildala Arm (n = 3)	<LOQ	3.1 ± 0.40	35 ± 9.2	<LOQ	<LOQ	<LOQ	6.8 ± 1.00
Kitlope (n = 3)	<LOQ	<LOQ	21 ± 0.91	<LOQ	<LOQ	<LOQ	6.2 ± 0.15
		$p = 0.18$	$p = 0.19$				$p = 0.69$
	Manganese	**Nickel**	**Selenium**	**Tin**	**Zinc**	**Lead**	**Mercury**
Hospital Beach (n = 3)	<LOQ	<LOQ	<LOQ	<LOQ	23 ± 1.10	<LOQ	0.13 ± 0.04 [ab]
Eurocan (n = 3)	<LOQ	<LOQ	<LOQ	<LOQ	23 ± 0.74	<LOQ	0.17 ± 0.05 [ab]
Kitimaat Village (n = 3)	<LOQ	<LOQ	<LOQ	<LOQ	23 ± 0.67	<LOQ	0.11 ± 0.04 [b]
Emsley Cove (n = 2)	0.97 ± 0.32	<LOQ	<LOQ	<LOQ	21 ± 1.60	<LOQ	0.12 ± 0.04 [ab]
Kildala Arm (n = 3)	<LOQ	<LOQ	<LOQ	<LOQ	29 ± 1.00	<LOQ	0.11 ± 0.03 [b]
Kitlope (n = 3)	<LOQ	<LOQ	<LOQ	<LOQ	25 ± 1.30	<LOQ	0.23 ± 0.02 [a]
					$p = 0.56$		$p = 0.021$

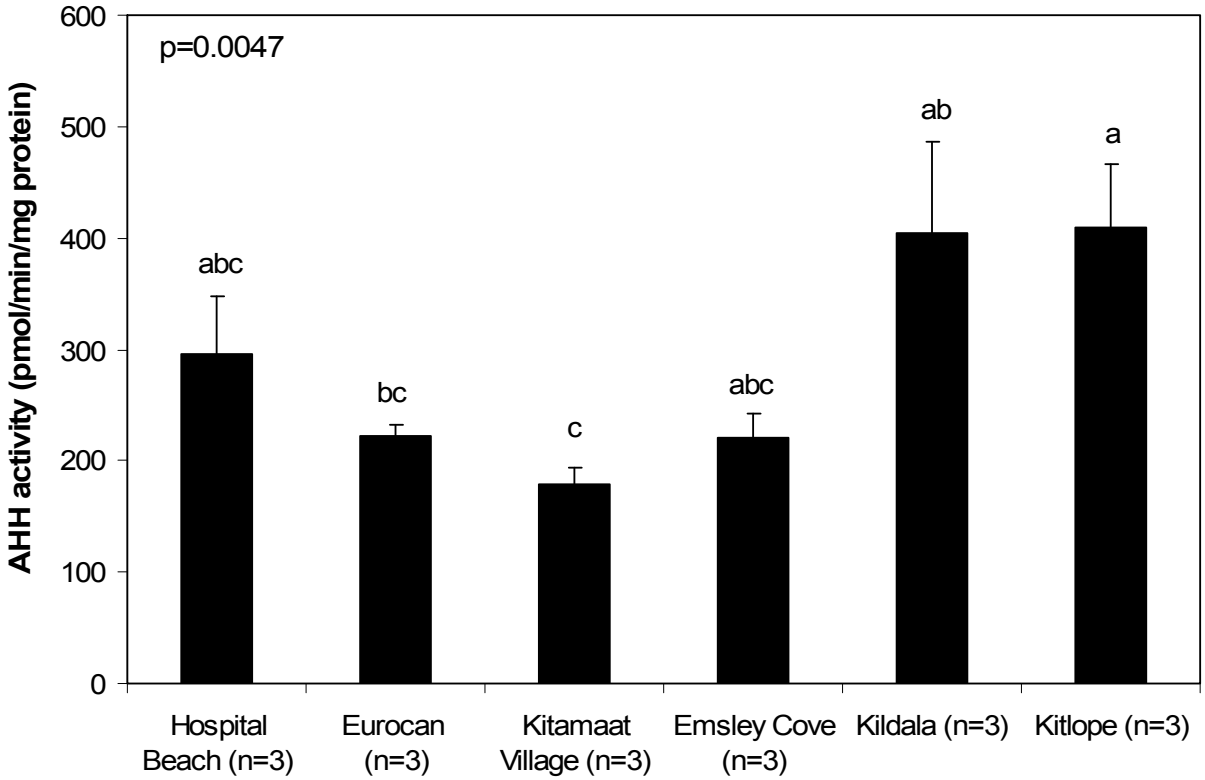

Figure 21. Mean AHH activity (pmol/min/mg protein ± SE) in liver of English sole from Kitimat Arm (Hospital Beach, Eurocan, Kitimat Village, and Emsley Cove) and reference sites in the surrounding area (Kildala and Kitlope). Values with different letter designations are significantly different from each other (ANOVA, $p \leq 0.05$). Fish were sampled in 2000 only.

Eurocan, where adduct levels were below detection limits in both 2002 and 2004, the lowest levels at all sites were observed in 2004.

Liver Pathology in English Sole

Toxicopathic liver lesions were observed in English sole from all sites within Kitimat Arm in 2002, 2002, and 2004, but were found rarely in fish from either the Kildala Arm or Kitlope reference sites (Figure 23). Overall, from 3.7 to 14% of fish from Hospital Beach, Eurocan, Kitamaat Village, and Emsley Cove had one or more toxicopathic lesions (i.e., neoplasms; FCA, which are preneoplasms; proliferative lesions; or SDN, a lesion representing cytotoxicity that is closely associated with exposure to PAHs in English sole and other fish species).

These prevalences were significantly higher than those observed in sole from the Kitlope and Kildala Arm reference areas (<1%). Among the sites within Kitimat Arm, lesion prevalences were generally highest in fish from Kitamaat Village and lowest in fish from Emsley Cove. Of the liver lesions observed in English sole, preneoplasms and SDN were the most common (Figure 23). Neoplasms were less common, occurring only in a small percentage of sole from Hospital Beach and Kitamaat Village. Lesion prevalences tended to decline in sole

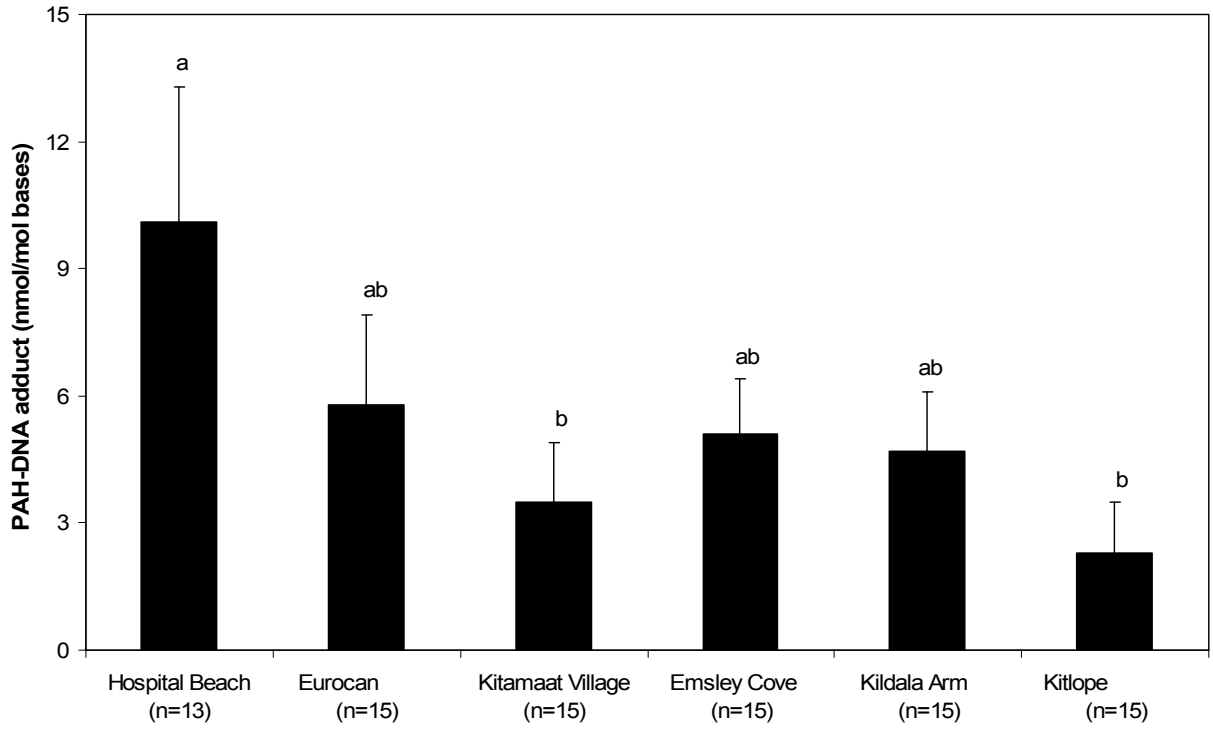

Figure 22. Mean concentrations (±SE) of DNA adducts in pmol/mol bases in livers of English sole from Kitimat Arm (Hospital Beach, Eurocan, Kitimat Village, and Emsley Cove) and reference sites outside Kitimat Arm (Kildala Arm and Kitlope), averaged over 2000, 2002, and 2004. Values with different letter superscripts are significantly different ($p \leq 0.05$).

Table 15. Temporal trends in mean concentrations (±SE) of DNA adducts in liver of English sole from Kitimat Arm sites (Hospital Beach, Eurocan, Kitamaat Village, and Emsley Cove) and reference areas (Kildala Arm and Kitlope) from 2000 to 2004. Sites with different letter superscripts are significantly different ($p \leq 0.05$); years with different number superscripts are significantly different ($p \leq 0.05$).

Year	Hospital Beach	Eurocan	Kitamaat Village	Emsley Cove	Kildala Arm	Kitlope	*p* values for intersite differences
2000	7.0 ± 3.1^{b12} (n = 4)	14.3 ± 2.7^{b1} (n = 6)	2.3 ± 1.6^{b2} (n = 6)	2.7 ± 1.2^{b2} (n = 6)	7.7 ± 2.8^{b1} (n = 6)	5.8 ± 2.3^{b1} (n = 6)	$p = 0.0096$
2002	17 ± 5.4^{b1} (n = 6)	ND^{b2} (n = 6)	6.3 ± 0.7^{b1} (n = 6)	10.0 ± 1.3^{b1} (n = 6)	4.0 ± 1.2^{b12} (n = 6)	ND^{b2} (n = 6)	$p = 0.0001$
2004	<LOQ (n = 3)	<LOQ (n = 3)	<LOQ (n = 3)	<LOQ (n = 3)	<LOQ (n = 3)	<LOQ (n = 3)	*ns**
p values for interannual differences	$p = 0.0980$	$p = 0.0001$	$p = 0.0108$	$p = 0.0004$	$p = 0.1137$	$p = 0.0345$	

*No statistically significant differences among sites.

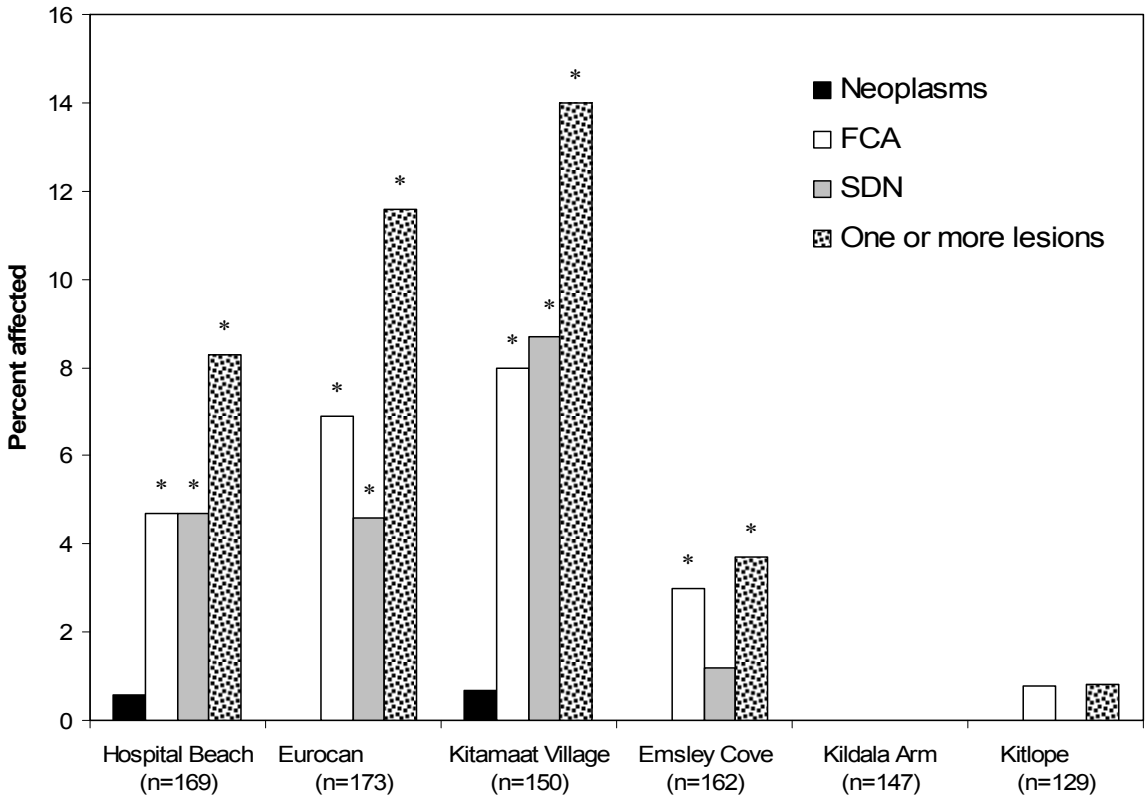

Figure 23. Prevalences of toxicopathic lesions in English sole from sites within Kitimat Arm (Hospital Beach, Eurocan, Kitamaat Village, and Emsley Cove) and from reference sites outside Kitimat Arm (Kildala Arm and Kitlope) sampled in 2000, 2002, and 2004. Values with asterisks (*) are significantly different ($p \leq 0.05$) from values at the Kitlope reference site (Dunnett's compare to control test for proportions). FCA = preneoplasms, SDN = specific degeneration/necrosis, and one or more lesions category indicates the presence of neoplasms, FCA, proliferative lesions, or SDN.

from the Kitimat Arm sites from 2000 to 2004 (Table 16) and were significantly lower ($p \leq 0.05$, Dunnett's compare to control test for proportions) in 2004 than in 2000 at Eurocan, Kitamaat Village, and Emsley Cove. However, lesion prevalences did not change significantly at Hospital Beach or at the Kildala Arm and Kitlope reference sites.

Liver Pathology in Yellowfin Sole

Liver lesion prevalences (Figure 24) were low overall in yellowfin sole (<8%). Several animals with neoplastic or preneoplastic lesions (FCA) were found at Eurocan. Preneoplastic lesions were also observed in one fish from Emsley Cove and two fish from Kildala Arm. Prevalences of lesions at Eurocan were significantly higher than at Kitlope ($p < 0.05$, Dunnett's compare to control test for proportions), but other intersite differences were not statistically significant.

Table 16. Temporal trends from 2000 to 2004 in liver lesion prevalences in English sole from Kitimat Arm sites (Hospital Beach, Eurocan, Kitamaat Village, and Emsley Cove) and reference areas (Kildala Arm and Kitlope). The overall prevalences of toxicopathic lesions (i.e., one or more of the lesions shown below) at each site were tested for increasing or decreasing trends over the sampling period. The asterisk (*) indicates that lesion prevalence declined significantly from 2002 to 2004 (Dunnett's compare to control test for proportions, $p \leq 0.05$).

	Lesion category				
Site	**Neoplasms**	**FCA**	**SDN**	**Proliferative lesions**	**One or more lesions**
Hospital Beach					
2000 (n = 52)	1.9%	3.8%	3.8%	0.0%	9.6%
2002 (n = 56)	1.8%	5.4%	3.6%	0.0%	8.9%
2004 (n = 61)	0.0%	4.9%	1.7%	0.0%	6.6%
Eurocan					
2000 (n = 53)	0.0%	9.4%	11.3%	1.9%	20.8%
2002 (n = 60)	1.7%	10.0%	0.0%	11.7%	11.7%
2004 (n = 60)	0.0%	0.0%	3.3%	0.0%	3.3%*
Kitamaat Village					
2000 (n = 40)	0.0%	12.5%	15.0%	12.5%	20.0%
2002 (n = 47)	2.1%	8.5%	4.3%	12.5%	12.8%
2004 (n = 63)	1.6%	3.2%	7.9%	3.2%	11.1%*
Emsley Cove					
2000 (n = 30)	0.0%	10.0%	3.3%	10.0%	10.0%
2002 (n = 60)	0.0%	3.3%	1.7%	3.3%	5.0%
2004 (n = 72)	0.0%	0.0%	0.0%	0.0%	0.0%*
Kildala Arm					
2000 (n = 40)	0.0%	0.0%	0.0%	0.0%	0.0%
2002 (n = 46)	0.0%	0.0%	0.0%	0.0%	0.0%
2004 (n = 60)	0.0%	0.0%	0.0%	0.0%	0.0%
Kitlope					
2000 (n = 31)	0.0%	0.0%	0.0%	0.0%	0.0%
2002 (n = 49)	0.0%	2.0%	0.0%	2.0%	2.0%
2004 (n = 41)	0.0%	0.0%	0.0%	0.0%	0.0%

Flatfish Size, Age, and Condition

Because exposure to PAHs can alter growth and metabolism, and contribute to increased mortality rates in fish (Rice et al. 2000, Meador et al. 2006, Johnson et al. 2008a), length and age distributions of English and yellowfin sole from Kitimat Arm and from reference sites were examined, as well as somatic indices such as CF and LSI. Size and age information were also needed so their influence on reproductive maturation and liver lesion prevalence could be taken

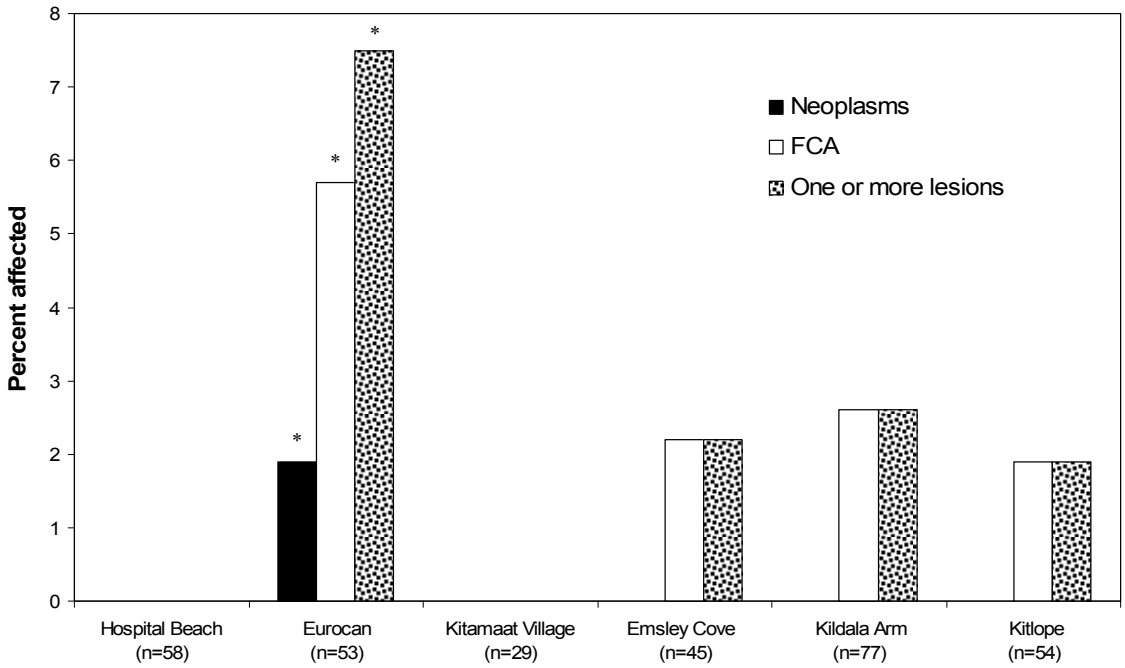

Figure 24. Prevalences of toxicopathic lesions in yellowfin sole from sites within Kitimat Arm (Hospital Beach, Eurocan, Kitimaat Village, and Emsley Cove) and reference sites outside Kitimat Arm (Kildala Arm and Kitlope) sampled in 2000 and 2002. Values with asterisks (*) are significantly difference ($p \leq 0.05$) from values at the Kitlope reference site (Dunnett's compare to control test for proportions). One or more lesions category indicates the presence of neoplasms or FCA. No other toxicopathic liver lesions were found in yellowfin sole.

into account, since both are affected by fish age. Because their size distributions differ, male and female sole were considered separately in analyses of fish length.

Length and Age of Yellowfin Sole and English Sole

In general, female English sole were larger at Hospital Beach, Eurocan, and Kitamaat Village than at Emsley Cove, Kildala Arm, and Kitlope (Table 17). Female English sole, which were sampled in 2000, 2002, and 2004, showed significant variation in fish length from year to year at some of the sampling sites, but this general trend remained the same. A similar relationship was found for male English sole (Table 17).

Female yellowfin sole were generally largest at Kitlope and smallest at Emsley Cove, although significant intersite differences were not detected for individual years due to low sample size (Table 17). At most sites there was little year-to-year variation in length; however, at Eurocan, lengths were significantly different between the two years. The fish collected at this site were among the smallest sampled in 2000, but among the largest collected in 2002. However, the number of fish collected in 2002 was very small (n = 2). Male yellowfin sole were generally smallest at Hospital Beach and Eurocan and largest at Kitamaat Village and Kildala Arm (Table 17). These trends were similar in both 2000 and 2002, with no significant year-to-year variation in length at any of the sites.

Table 17. Mean length in millimeters (±SE) of female and male English sole and yellowfin sole from Kitimat Arm sites (Hospital Beach, Eurocan, Kitamaat Village, and Emsley Cove) and reference areas (Kildala Arm and Kitlope). English sole were monitored in 2000, 2002, and 2004, while yellowfin sole were monitored in 2000 and 2002. Letter superscripts indicate significant differences among sites; number superscripts indicate significant differences between years within a site ($p \leq 0.05$, ANOVA, Tukey HSD test).

Site	Hospital Beach	Eurocan	Kitamaat Beach	Emsley Cove	Kildala Arm	Kitlope
English sole females						
2000	267 ± 7^{bc1} (n = 19)	292 ± 4^{a1} (n = 30)	288 ± 5^{ab1} (n = 30)	269 ± 5^{abc1} (n = 18)	254 ± 6^{c1} (n = 35)	269 ± 11^{abc1} (n = 11)
2002	259 ± 6^{bc1} (n = 30)	296 ± 4^{a1} (n = 46)	270 ± 5^{b12} (n = 29)	239 ± 5^{cd2} (n = 26)	245 ± 6^{cd1} (n = 39)	229 ± 6^{d2} (n = 30)
2004	276 ± 9^{a1} (n = 18)	261 ± 10^{ab2} (n = 25)	261 ± 7^{ab2} (n = 33)	233 ± 5^{c2} (n = 33)	250 ± 3^{abc1} (n = 42)	239 ± 5^{bc12} (n = 11)
English sole males						
2000	240 ± 3^{ab12} (n = 33)	250 ± 5^{a2} (n = 23)	252 ± 8^{a1} (n = 10)	243 ± 5^{ab1} (n = 12)	219 ± 7^{b1} (n = 5)	226 ± 4^{b1} (n = 20)
2002	233 ± 3^{b12} (n = 25)	271 ± 8^{a1} (n = 14)	241 ± 4^{b1} (n = 19)	227 ± 3^{bc2} (n = 30)	227 ± 11^{b1} (n = 6)	225 ± 6^{b1} (n = 19)
2004	243 ± 2^{a1} (n = 41)	226 ± 2^{b3} (n = 35)	240 ± 5^{a1} (n = 29)	218 ± 3^{b2} (n = 40)	213 ± 4^{b1} (n = 18)	197 ± 2^{c2} (n = 30)
Yellowfin sole females						
2000	226 ± 9^{a1} (n = 19)	216 ± 7^{a1} (n = 19)	226 ± 5^{a1} (n = 2)	208 ± 48^{a1} (n = 2)	231 ± 8^{a1} (n = 19)	239 ± 13^{a1} (n = 9)
2002	226 ± 10^{a1} (n = 6)	271 ± 39^{a2} (n = 2)	254 ± 22^{a1} (n = 3)	212 ± 15^{a1} (n = 5)	247 ± 11^{a1} (n = 15)	262 ± 9^{a1} (n = 8)
Yellowfin sole males						
2000	215 ± 3^{ab1} (n = 21)	212 ± 3^{b1} (n = 20)	232 ± 5^{a1} (n = 20)	212 ± 5^{b1} (n = 19)	222 ± 5^{ab1} (n = 18)	220 ± 4^{ab1} (n = 20)
2002	212 ± 3^{c1} (n = 15)	213 ± 3^{bc1} (n = 13)	236 ± 11^{a1} (n = 6)	219 ± 4^{abc1} (n = 17)	228 ± 4^{ab1} (n = 21)	219 ± 2^{abc1} (n = 19)

The average age of English sole females (Table 18) ranged from 3.2 to 6.5 years. Females tended to be younger in 2002 than in 2000, with significant year-to-year differences at Hospital Beach, Kitamaat Village, Emsley Cove, and Kitlope. However, there was no consistent pattern of fish age at the sites. In 2000 the oldest fish were collected at Kitlope and the youngest fish at Kildala Arm, while in 2002 the oldest fish were captured at Eurocan and Emsley Cove and the youngest fish at Kitlope. Males were slightly older than females, with average ages ranging from 5.3 to 9.4 years (Table 18). There were no statistically significant intersite differences in fish age in either 2000 or 2002. Ages of male sole were consistent from year to year at most sites, except at Hospital Beach where fish were younger in 2002 than in 2000 (Table 18).

Table 18. Mean age in years (±SE) of female and male English sole and yellowfin sole from Kitimat Arm sites (Hospital Beach, Eurocan, Kitamaat Village, and Emsley Cove) and reference areas (Kildala Arm and Kitlope). English sole were monitored in 2000, 2002, and 2004, while yellowfin sole were monitored in 2000 and 2002. Letter superscripts indicate significant differences among sites; number superscripts indicate significant differences between years within a site ($p \leq 0.05$, ANOVA, Tukey multiple range test).

Site	Hospital Beach	Eurocan	Kitamaat Beach	Emsley Cove	Kildala Arm	Kitlope
English sole females						
2000	5.1 ± 0.4^{ab1} (n = 19)	5.4 ± 0.3^{ab1} (n = 30)	6.1 ± 0.4^{a1} (n = 30)	5.7 ± 0.4^{ab1} (n = 18)	4.3 ± 0.3^{b1} (n = 35)	6.5 ± 0.7^{a1} (n = 11)
2002	3.8 ± 0.3^{bc2} (n = 30)	5.3 ± 0.3^{a1} (n = 46)	4.9 ± 0.3^{ab12} (n = 29)	5.3 ± 0.4^{a2} (n = 28)	4.7 ± 0.3^{ab1} (n = 40)	3.2 ± 0.3^{c2} (n = 29)
English sole males						
2000	7.0 ± 0.5^{a1} (n = 31)	5.6 ± 0.5^{a1} (n = 23)	6.1 ± 0.9^{a1} (n = 10)	5.7 ± 0.4^{a1} (n = 12)	9.4 ± 2.9^{a1} (n = 5)	5.9 ± 0.6^{a1} (n = 20)
2002	5.0 ± 0.3^{a2} (n = 26)	6.0 ± 0.7^{a1} (n = 14)	5.3 ± 0.6^{a1} (n = 19)	6.0 ± 0.5^{a1} (n = 30)	6.0 ± 0.5^{a1} (n = 6)	6.1 ± 0.8^{a1} (n = 19)
Yellowfin sole females						
2000	5.9 ± 0.6^{a1} (n = 19)	5.2 ± 0.4^{a1} (n = 19)	3.0 ± 0.0^{a1} (n = 2)	4.3 ± 1.5^{a1} (n = 2)	7.9 ± 1.0^{a1} (n = 19)	8.1 ± 0.8^{a1} (n = 9)
2002	4.8 ± 0.2^{a1} (n = 6)	5.5 ± 0.5^{a1} (n = 2)	4.7 ± 0.3^{a2} (n = 3)	5.0 ± 0.8^{a1} (n = 5)	5.8 ± 0.3^{a1} (n = 16)	6.5 ± 0.5^{a1} (n = 8)
Yellowfin sole males						
2000	8.3 ± 0.5^{ab1} (n = 20)	8.1 ± 0.3^{a1} (n = 20)	7.8 ± 0.5^{a1} (n = 20)	5.4 ± 0.3^{b1} (n = 20)	8.4 ± 0.6^{a1} (n = 20)	8.7 ± 0.8^{a1} (n = 20)
2002	7.0 ± 0.4^{ab1} (n = 15)	6.0 ± 0.4^{b1} (n = 14)	6.2 ± 0.7^{ab1} (n = 5)	6.7 ± 0.3^{ab1} (n = 18)	8.0 ± 0.4^{a1} (n = 21)	6.7 ± 0.4^{ab1} (n = 19)

The average age of female yellowfin sole ranged from 4.3 to 8.1 years, with the oldest fish at Kitlope and Kildala Arm and the youngest at Kitamaat Village (Table 18); however, these age differences were not statistically different in either 2000 or 2002 (Tukey Kramer HSD test, $p < 0.05$). Mean ages of females at the sampling sites were similar from year to year. Males were slightly older than females, with average ages ranging from 6.0 to 8.7 years (Table 18). In both 2000 and 2002, the oldest males were collected at Kildala Arm, while in 2000 the youngest males were collected at Emsley Cove. In 2002 the youngest males were collected at Eurocan. For males, there were some significant year-to-year differences in fish age; fish were younger in 2002 than in 2000 at all sites except Emsley Cove (Table 18).

Differences in size at age were examined in both English sole and yellowfin sole using regression analysis. Age and length were highly correlated in males and females of both species ($p < 0.0001$). In female English sole, fish from Eurocan ($p < 0.0001$) and Kitamaat Village ($p = 0.0068$) were significantly larger for their age, and fish from Emsley Cove ($p < 0.0001$) and

Kildala Arm ($p = 0.0005$) were significantly smaller for their age than fish from other sites. In male English sole, Eurocan ($p < 0.0001$) and Kitamaat Village ($p = 0.0077$) fish were significant larger for their age, and Emsley Cove ($p = 0.0301$) and Kildala Arm ($p = 0.0071$) fish were significantly smaller for their age than fish from other sites. Yellowfin sole also showed some intersite differences in length at age. Female yellowfin from Kitamaat Village were significantly larger for their age than fish from other sites ($p = 0.0325$). Male yellowfin from Kitamaat Village ($p = 0.0001$) were significant larger, and male yellowfin sole from Hospital Beach ($p = 0.0015$) and Eurocan ($p = 0.0048$) were significantly smaller, for their age than fish from other sites.

Age distributions (Figure 25) were also examined in English and yellowfin sole from sites within Kitimat Arm (Hospital Beach, Eurocan, Emsley Cove, and Kitamaat Village), and from reference sites outside of Kitimat Arm (Kildala Arm and Kitlope). Age distributions for English sole from the two areas were similar. For yellowfin sole, however, higher proportions of older fish (>10 years of age) were observed at the Kildala Arm and Kitlope reference sites outside of Kitimat Arm, whereas age distributions at sites within Kitimat Arm were truncated at an earlier age; we collected very few fish greater than age 10 (Figure 25).

Somatic Indices

LSI values in English sole (Table 19) showed some variability from year to year at the individual sampling sites, but were consistently among the highest in fish from Hospital Beach and Eurocan. In 2000 and 2002, LSI values in sole from Kitamaat Village, Emsley Cove, Kildala Arm, and Kitlope (measured in 2002 only) were lower than values observed in sole from the other two sites and fairly similar. In 2004, however, LSI values in sole from Kitamaat Village, Emsley Cove, and Kildala Arm were significantly higher than in other years, similar to or even greater than those in fish from Hospital Beach and Eurocan. In yellowfin sole, LSI showed a somewhat similar relationship in that the mean LSI was consistently higher in fish from Hospital Beach (Table 19) compared to fish from the other sites. However, when data from the different sampling years were compared, LSI was more variable at other sites. For example, in 2000, Kitamaat Village had the lowest LSI of all sites, but in 2002 LSI increased significantly to a value comparable to most of the other sites (Table 19).

CF showed significant year-to-year variation in English sole from most of the sampling sites, including Kitamaat Village, Hospital Beach, Eurocan, and Kitlope (Table 20). Eurocan was consistently one of the sites with the highest CF, but relative positions of the other sites varied. In yellowfin sole, CF also tended to vary from year to year at the sampling sites, although the differences were only statistically significant at Hospital Beach (Table 20). There were no consistent patterns among the sampling sites. For example, in 2000, CF was high in fish from Emsley Cove as compared to other sites but not in 2002. The opposite was true of fish from Hospital Beach; CF was low in 2000 but relatively high in 2002.

Flatfish Reproductive Function

Assessment of reproductive function was conducted primarily on yellowfin sole which were close to spawning condition during the summer months in the northern Pacific (Wilderbuer et al. 1992, Nichols 1993). In 2000 female yellowfin sole were successfully collected at three

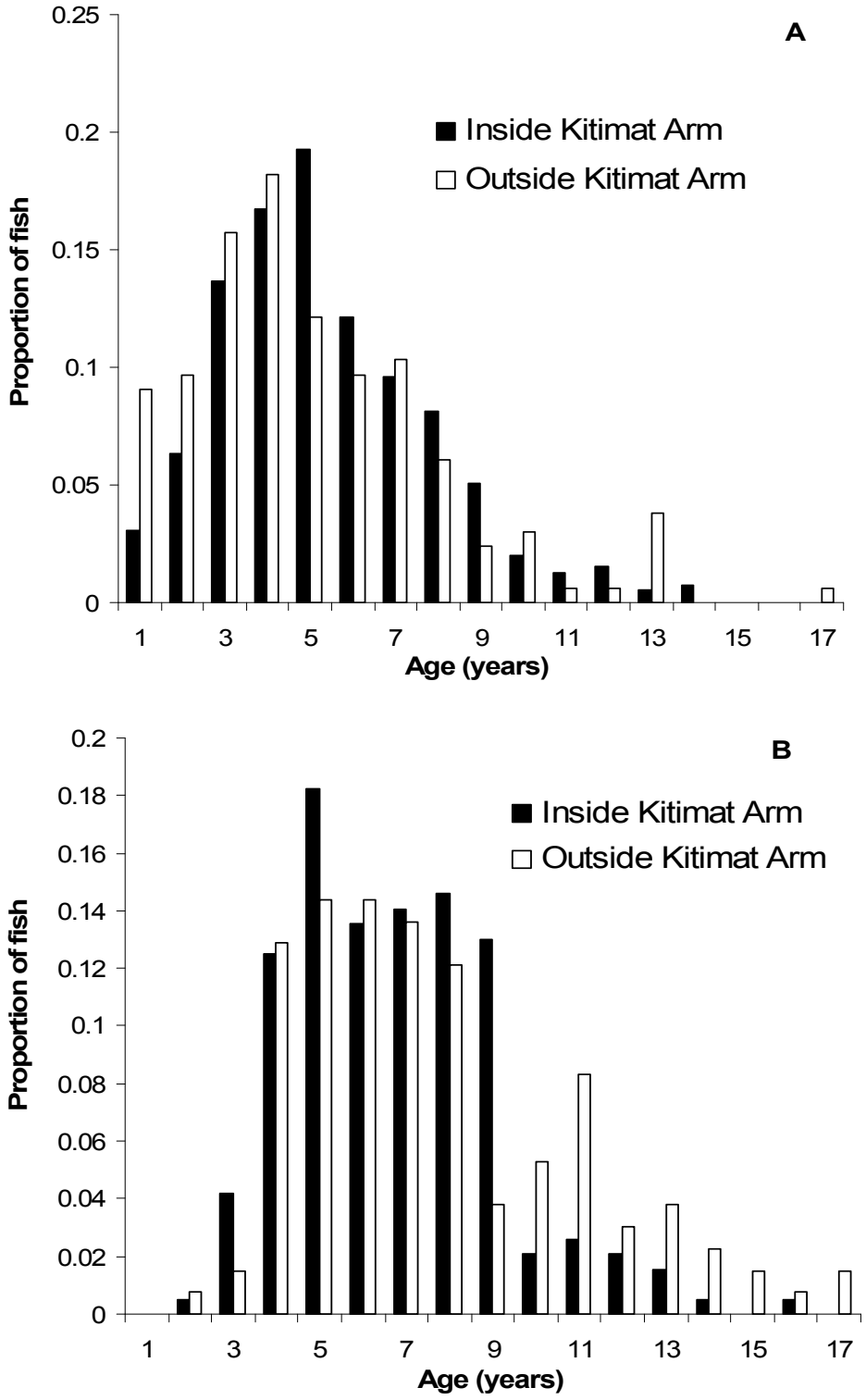

Figure 25. Age distributions for (A) English sole and (B) yellowfin sole from sites within Kitimat Arm (Hospital Beach, Eurocan, Kitamaat Village, and Emsley Cove) and reference sites outside Kitimat Arm (Kildala Arm and Kitlope). For both species, figures show overall distribution of fish collected in 2000 and 2002; English sole collected in 2004 were not included to ensure comparability between the two species.

Table 19. Mean LSI (% weight) ± SE in English sole and yellowfin sole from Kitimat Arm sites (Hospital Beach, Eurocan, Kitamaat Village, and Emsley Cove) and reference areas (Kildala Arm and Kitlope). Yellowfin sole were monitored in 2000 and 2002, while English sole were monitored in 2000, 2002, and 2004. Letter superscripts indicate significant differences among sites; number superscripts indicate significant differences between years within a site ($p \leq 0.05$, ANOVA, Tukey multiple range test). NM = not measured.

Site	Hospital Beach	Eurocan	Kitamaat Beach	Emsley Cove	Kildala Arm	Kitlope
English sole						
2000	2.27 ± 0.32^{ab12} (n = 6)	2.31 ± 0.23^{a1} (n = 10)	1.64 ± 0.08^{bc2} (n = 11)	1.94 ± 0.08^{ab2} (n = 21)	1.21 ± 0.14^{c2} (n = 10)	NM
2002	2.23 ± 0.13^{a2} (n = 55)	2.23 ± 0.11^{a1} (n = 59)	1.58 ± 0.09^{b12} (n = 47)	1.57 ± 0.09^{b2} (n = 55)	1.51 ± 0.10^{b2} (n = 45)	1.55 ± 0.08^{b} (n = 49)
2004	2.93 ± 0.16^{a1} (n = 58)	2.22 ± 0.12^{b1} (n = 60)	3.14 ± 0.23^{a1} (n = 32)	2.70 ± 0.20^{ab1} (n = 13)	2.27 ± 0.14^{b1} (n = 42)	NM
Yellowfin sole						
2000	2.22 ± 0.24^{a1} (n = 37)	2.00 ± 0.14^{ab1} (n = 40)	1.32 ± 0.12^{b2} (n = 2)	1.70 ± 0.15^{ab1} (n = 2)	1.87 ± 0.02^{ab1} (n = 19)	1.89 ± 0.15^{ab1} (n = 9)
2002	2.12 ± 0.17^{a1} (n = 21)	1.96 ± 0.22^{ab1} (n = 15)	1.89 ± 0.18^{bc1} (n = 9)	1.77 ± 0.16^{c1} (n = 22)	1.78 ± 0.19^{c1} (n = 36)	1.99 ± 0.16^{c1} (n = 27)

Table 20. Mean CF (g/cm3) ± SE of English sole and yellowfin sole from Kitimat Arm sites (Hospital Beach, Eurocan, Kitamaat Village, and Emsley Cove) and reference areas (Kildala Arm and Kitlope). Yellowfin sole were monitored in 2000 and 2002; English sole were monitored in 2000, 2002, and 2004. Letter superscripts indicate significant differences among sites; number superscripts indicate significant differences between years within a site ($p \leq 0.05$, ANOVA, Tukey multiple range test).

Site	Hospital Beach	Eurocan	Kitamaat Beach	Emsley Cove	Kildala Arm	Kitlope
English sole						
2000	0.82 ± 0.02^{b2} (n = 52)	0.87 ± 0.01^{ab2} (n = 60)	0.86 ± 0.01^{ab2} (n = 47)	0.89 ± 0.02^{a1} (n = 56)	0.85 ± 0.01^{ab1} (n = 45)	0.76 ± 0.02^{c2} (n = 49)
2002	0.91 ± 0.01^{a1} (n = 55)	0.94 ± 0.01^{a1} (n = 60)	0.91 ± 0.01^{ab1} (n = 47)	0.88 ± 0.01^{ab1} (n = 56)	0.83 ± 0.01^{b1} (n = 45)	0.90 ± 0.04^{ab1} (n = 49)
2004	0.88 ± 0.01^{ab1} (n = 59)	0.90 ± 0.01^{a2} (n = 60)	0.86 ± 0.01^{abc2} (n = 62)	0.87 ± 0.01^{ab1} (n = 73)	0.86 ± 0.01^{bc1} (n = 60)	0.82 ± 0.02^{c12} (n = 40)
Yellowfin sole						
2000	2.22 ± 0.24^{a1} (n = 37)	2.00 ± 0.14^{ab1} (n = 40)	1.32 ± 0.12^{b2} (n = 2)	1.70 ± 0.15^{ab1} (n = 2)	1.87 ± 0.02^{ab1} (n = 19)	1.89 ± 0.15^{ab1} (n = 9)
2002	2.12 ± 0.17^{a1} (n = 21)	1.96 ± 0.22^{ab1} (n = 15)	1.89 ± 0.18^{bc1} (n = 9)	1.77 ± 0.16^{c1} (n = 22)	1.78 ± 0.19^{c1} (n = 36)	1.99 ± 0.16^{c1} (n = 27)

sites, Hospital Beach, Eurocan, and Kildala Arm; only small numbers of fish (<10 individuals) were obtained at the other sites. In 2002 even fewer females were collected, with adequate samples only from the Kildala Arm and Kitlope sites. In both 2000 and 2002 male yellowfin were collected at all six flatfish study sites: Hospital Beach, Eurocan, Kitamaat Village, Emsley Cove, Kildala Arm, and Kitlope. A more limited number of yellowfin sole were collected in 2002, with the largest number of animals captured at Hospital Beach, Kildala Arm, and Kitlope. Because some vitellogenic female English sole were observed during sampling operations in Kitimat Arm, reproductive function and somatic indices were also assessed in English sole in 2000, 2002, and 2004.

In English sole and yellowfin sole, reproductive development was assessed by monitoring the maturation stage of the fish by histology, determining the GSI (a measure of gonad weight as a proportion of body weight), and examining the testes and ovaries for the presence of lesions by histology.

Gonadosomatic Index

In female English sole, values of GSI tended to be higher in fish from Hospital Beach and Eurocan than in fish from the other sampling sites, although this trend was not always consistent (Table 21). There was significant variation in GSI from year to year in sole from Hospital Beach, with high values in 2000 and 2004 and a low value in 2002 but no other significant year-to-year differences at the sampling sites (Table 21). In linear regression analysis, while adjusting for fish length, gonad size remained significantly higher in female sole from Hospital Beach in comparison to other sites ($p = 0073$) and was significantly lower in females from Kitamaat Village ($p = 0009$). In male English sole (Table 21), on the other hand, GSI was highest in fish from Kildala Arm and Kitamaat Village and lowest in fish from Emsley Cove and Kitlope. In fish from Hospital Beach and Eurocan, GSI values were intermediate. These trends were consistent in both 2002 and 2004 (GSI was not measured in male English sole in 2000), although GSI showed some variation from year to year. For example, GSI was significantly higher in 2004 than in 2002 at Hospital Beach and Kitamaat Village. In linear regression analysis, while adjusting for fish length, gonad size remained significantly higher in male sole from Kitamaat Village in comparison to male sole from other sites ($p = 0003$).

In female yellowfin sole, mean GSI varied with site and sampling year, but intersite differences were not significant ($p > 0.05$) either in ANOVA or in linear regression when the effect of fish length was taken into account. GSI values were consistently among the highest at Kitlope in both 2000 and 2002 compared to fish from the other sites but other sites were more variable (Table 22). However, year-to-year differences in GSI values were not significant at any of the sampling sites. In male yellowfin sole, mean GSI for 2000 and 2002 combined was highest in fish from Hospital Beach, but there were no statistically significant differences in GSI among the sites after adjusting for fish size in linear regression analysis, although near-significant differences were observed for Hospital Beach (higher gonad weight for size, $p = 0.0560$), Emsley Cove (lower gonad weight for size, $p = 0769$), and Kitamaat Village (lower gonad weight for size, $p = 0877$). There was no marked intersite difference in GSI when mean GSI values at the sites for individual sampling years were compared (Table 22). Moreover, there was no significant year-to-year variation in GSI at any of the sampling sites.

Table 21. Mean GSI (% body wt) ± SE of female and male English sole from Kitimat Arm sites (Hospital Beach, Eurocan, Kitamaat Village, and Emsley Cove) and reference areas (Kildala Arm and Kitlope). Letter superscripts indicate significant differences among sites; number superscripts indicate significant differences between years within a site ($p \leq 0.05$, ANOVA, Tukey multiple range test). NM = not measured.

Site	Hospital Beach	Eurocan	Kitamaat Beach	Emsley Cove	Kildala Arm	Kitlope
English sole females						
2000	6.07 ± 2.60^{a12} (n = 6)	6.25 ± 1.74^{a1} (n = 10)	3.65 ± 0.54^{a12} (n = 11)	3.16 ± 0.54^{a1} (n = 14)	2.66 ± 0.67^{ab1} (n = 10)	NM
2002	2.66 ± 0.32^{a1} (n = 30)	3.90 ± 0.52^{a1} (n = 46)	3.12 ± 0.44^{b1} (n = 29)	2.72 ± 0.84^{a1} (n = 26)	2.75 ± 0.54^{b1} (n = 39)	2.12 ± 0.15^{a} (n = 30)
2004	5.97 ± 1.14^{a2} (n = 17)	3.78 ± 0.78^{ab1} (n = 25)	3.07 ± 0.35^{ab1} (n = 16)	2.06 ± 0.44^{b1} (n = 2)	3.38 ± 0.27^{bc1} (n = 42)	NM
English sole males						
2002	1.08 ± 0.10^{b1} (n = 25)	1.18 ± 0.13^{a1} (n = 14)	1.46 ± 0.27^{a1} (n = 19)	1.03 ± 0.14^{a1} (n = 30)	1.20 ± 0.32^{a1} (n = 6)	0.87 ± 0.10^{a} (n = 19)
2004	1.60 ± 0.20^{ab2} (n = 41)	1.45 ± 0.18^{b1} (n = 35)	2.57 ± 0.38^{a2} (n = 16)	1.02 ± 0.21^{b1} (n = 11)	1.77 ± 0.34^{ab1} (n = 18)	NM

Table 22. Mean GSI (% body wt) ± SE of female and male yellowfin sole from Kitimat Arm sites (Hospital Beach, Eurocan, Kitamaat Village, and Emsley Cove) and reference areas (Kildala Arm and Kitlope). Letter superscripts indicate significant differences among sites; number superscripts indicate significant differences between years within a site ($p \leq 0.05$, ANOVA, Tukey multiple range test).

Site	Hospital Beach	Eurocan	Kitamaat Beach	Emsley Cove	Kildala Arm	Kitlope
Yellowfin sole females						
2000	9.27 ± 1.00^{a1} (n = 19)	7.09 ± 0.85^{a1} (n = 20)	8.13 ± 3.20^{a1} (n = 21)	9.69 ± 2.83^{a1} (n = 3)	9.60 ± 1.30^{a1} (n = 18)	12.66 ± 1.47^{a1} (n = 9)
2002	5.84 ± 2.00^{a1} (n = 6)	12.31 ± 0.46^{a1} (n = 2)	11.3 ± 1.55^{a1} (n = 3)	4.26 ± 1.46^{a1} (n = 5)	9.83 ± 1.43^{a1} (n = 15)	10.45 ± 1.92^{a1} (n = 13)
Yellowfin sole males						
2000	1.55 ± 0.11^{a1} (n = 17)	1.42 ± 0.09^{a1} (n = 20)	1.31 ± 0.08^{a1} (n = 20)	1.53 ± 0.22^{a1} (n = 18)	1.57 ± 0.08^{a1} (n = 15)	1.56 ± 0.11^{a1} (n = 20)
2002	1.74 ± 0.22^{ab1} (n = 15)	1.45 ± 0.10^{b1} (n = 18)	1.48 ± 0.36^{a1} (n = 4)	1.26 ± 0.11^{b1} (n = 17)	1.59 ± 0.16^{b1} (n = 21)	1.62 ± 0.13^{b1} (n = 19)

Gonad Lesions

Overall, ovarian atresia was observed in 3–16% of female English sole from all sites. Ovarian lesion prevalences were among the highest in fish from Kitlope in all sampling years, although in 2004 the highest lesion prevalence was found in fish from Kitamaat Village (Table 23). In 2002 no fish with atresia were found at Eurocan Beach or Emsley Cove, and in

Table 23. Proportions (%) of female English sole with ovarian lesions (primarily atresia of oocytes) and male English sole with testicular lesions (primarily inflammatory infiltrates) from Kitimat Arm sites (Hospital Beach, Eurocan, Kitamaat Village, and Emsley Cove) and reference areas (Kildala Arm and Kitlope). "L" indicates prevalences that are significantly lower or higher, respectively, than the overall prevalence for that sampling year ($p \leq 0.05$, G-statistic). Different numerical superscripts indicate significant differences between years within a site ($p \leq 0.05$, G-statistic).

Site	Hospital Beach	Eurocan	Kitamaat Beach	Emsley Cove	Kildala Arm	Kitlope
English sole females						
2000	10.5	10	10	17^{1}	8.6	27
	(n = 19)	(n = 30)	(n = 30)	(n = 18)	(n = 35)	(n = 11)
2002	3.3	0^{L}	10.3	0^{L2}	5	13.8
	(n = 30)	(n = 46)	(n = 29)	(n = 37)	(n = 40)	(n = 29)
2004	0	0^{L}	15.6	8.8^{1}	10	11.1
	(n = 18)	(n = 25)	(n = 33)	(n = 34)	(n = 40)	(n = 9)
English sole males						
2000	0	0	0	8.3	0	5.0
	(n = 33)	(n = 21)	(n = 10)	(n = 12)	(n = 5)	(n = 20)
2002	0	0	0	0	0	0
	(n = 26)	(n = 14)	(n = 17)	(n = 31)	(n = 6)	(n = 19)
2004	0	0	0	0	0	0
	(n = 41)	(n = 35)	(n = 30)	(n = 40)	(n = 17)	(n = 29)

2004 no fish with atresia were found at Eurocan or Hospital Beach. Although there was considerable year-to-year variation in lesion prevalence, the differences were significant only in fish from Emsley Cove. In logistic regression analysis, the prevalence of atresia in female English sole from Eurocan was significantly lower than the overall prevalence of 7.3% for all sites (G-statistic, $p \leq 0.05$; logistic regression, chi-square test, $p = 0.045$) but no other significant differences were observed. Testicular lesions were rarely observed in English sole; the only lesions observed were inflammatory infiltrates in one fish from Kitlope and one fish from Emsley Cove (<1.5% of sole sampled from these sites, Table 23). Both fish with lesions were collected in 2000. Sampling site had no significant effect on testicular lesion prevalences in male English sole (logistic regression, likelihood ratio chi-square test, $p = 5{,}504$).

The proportions of female yellowfin sole with ovarian atresia at the sites sampled in 2000 and 2002 varied from 0 to 25% (Table 24). In 2000 ovarian lesion prevalences were significantly lower in yellowfin sole from Kitamaat Village and Emsley Cove than the overall prevalences for fish from all sites, but no significant differences among sites were observed in 2002. Kitamaat Village was the only site where no ovarian atresia was detected in either 2000 or 2002, but the number of fish examined was very small (2–3 individuals). Prevalences of ovarian lesions showed some variance from year to year but no significant differences. In logistic regression analysis, no significant differences among sites were observed in the proportion of females with ovarian atresia ($p = 548$, logistic regression).

Table 24. Proportions (%) of female yellowfin sole with ovarian lesions (primarily atresia of oocytes) and male yellowfin sole with testicular lesions (primarily inflammatory infiltrates) from Kitimat Arm sites (Hospital Beach, Eurocan, Kitamaat Village, and Emsley Cove) and reference areas (Kildala Arm and Kitlope). "L" indicates prevalences that are significantly lower or higher, respectively, than the overall prevalence for that sampling year ($p \leq 0.05$, G-statistic). The numerical superscript indicates significant differences between years within a site ($p \leq 0.05$, G-statistic).

Site	Hospital Beach	Eurocan	Kitamaat Beach	Emsley Cove	Kildala Arm	Kitlope
Yellowfin sole females						
2000	25	10.5	0^{L}	0^{1}	15	14.3
	(n = 20)	(n = 19)	(n = 2)	(n = 3)	(n = 20)	(n = 7)
2002	0	0	0	20	6.3	0
	(n = 6)	(n = 3)	(n = 3)	(n = 5)	(n = 16)	(n = 8)
Yellowfin sole males						
2000	10	5.0	5.0	0	5.0	10
	(n = 20)	(n = 20)	(n = 20)	(n = 20)	(n = 20)	(n = 20)
2002	6.7	0	0	0	0	10.5
	(n = 15)	(n = 14)	(n = 6)	(n = 18)	(n = 21)	(n = 19)

In 2000 minor testicular lesions were observed in 5–10% of male yellowfin sole at all sites except Emsley Cove, where no lesions were detected (Table 24). The lesions observed were primarily inflammatory infiltrates. Although the differences were not statistically significantly, the proportions of male sole with testicular lesions were lower in 2002 than in 2000 at most of the sampling sites. No fish with lesions were found at Eurocan, Emsley Cove, Kitamaat Village, or Kildala Arm; however, 6.7% of fish from Hospital Beach and 10.5% of fish from Kitlope had testicular lesions. Intersite differences in prevalence were not statistically significant in either 2000 or 2002, when prevalences were compared using the G-statistic. Similarly, in logistic regression analysis, no significant site differences in the probability of testicular lesion occurrence were found (logistic regression, $p = 0.574$).

Gonadal Maturation Stage

The proportion of female English sole undergoing ovarian development at our sampling sites was relatively low (39% for all females, 69% for females over 28 cm in length). Fish length was strongly correlated with the likelihood of ovarian development (logistic regression, $p > 0.0001$, n = 502), with larger fish showing a higher probability of being vitellogenic than smaller fish. In all sampling years, the proportions of maturing females tended to be highest at Hospital Beach, Eurocan, and Kitamaat Village and lowest at Emsley Cove, Kitlope, and Kildala Arm, but there was considerable year-to-year variation in the proportions of maturing females at most sampling sites (Table 25). Part of the variability in maturation was related to intersite differences in fish length, with fish generally being smaller at Kildala Arm and Kitlope than at Hospital Beach, Eurocan, or Kitamaat Village. However, logistic regression analysis indicated that there were significant intersite differences in proportions of maturing females, even after adjusting for fish length ($p = 024$, likelihood ratio chi-square test). Once length was taken into

Table 25. Proportions (%) of maturing female and male English sole from Kitimat Arm sites (Hospital Beach, Eurocan, Kitamaat Village, and Emsley Cove) and reference areas (Kildala Arm and Kitlope). "L" and "H" indicate prevalences that are significantly lower or higher, respectively, than the overall prevalence for that sampling year ($p \leq 0.05$, G-statistic). Different numerical superscripts indicate significant differences between years within a site ($p \leq 0.05$, G-statistic).

Site	Hospital Beach	Eurocan	Kitamaat Beach	Emsley Cove	Kildala Arm	Kitlope
English sole females						
2000	63	73^{H1}	57	39^{1}	17^{L1}	18^{L}
	(n = 19)	(n = 30)	(n = 30)	(n = 18)	(n = 35)	(n = 11)
2002	43	67^{H1}	41	15^{L2}	23^{1}	31
	(n = 30)	(n = 46)	(n = 29)	(n = 27)	(n = 40)	(n = 29)
2004	67^{H}	28^{2}	27	12^{L2}	41^{2}	36
	(n = 18)	(n = 25)	(n = 33)	(n = 34)	(n = 41)	(n = 11)
English sole males						
2000	100^{1}	100	100	100	100	95
	(n = 33)	(n = 21)	(n = 10)	(n = 12)	(n = 5)	(n = 19)
2002	85^{2}	100	100	87	100	100
	(n = 26)	(n = 14)	(n = 17)	(n = 31)	(n = 6)	(n = 19)
2004	95^{H2}	94	93	85	82	83
	(n = 41)	(n = 35)	(n = 30)	(n = 40)	(n = 14)	(n = 29)

account, the probability of vitellogenesis was significantly higher for fish from Hospital Beach (p = 007, chi-square test) and lower for fish from Emsley Cove (p = 038, chi-square test) in comparison to other sites.

The proportion of reproductively maturing male English sole was generally higher than for females, with an overall prevalence of 93%. There was some year-to-year and site-to-site variation in the proportion of maturing males at all sites, with prevalences typically ranging 82–100% over the three sampling years (Table 25). However, there were no significant intersite differences in maturation except in 2004, when the proportion of maturing males at Hospital Beach was significantly higher than the overall average for fish collected in that year. At all sites, percent maturation tended to be lower in 2004 than in other years, although Hospital Beach was the only site where year-to-year variation in percent maturation was statistically significant. As with females, length had a significant influence on the likelihood of maturation in male English sole (logistic regression, likelihood ratio chi-square test, (p [length] = 0.0035, n = 402). Once length was taken into account, there was no significant effect of sampling site on the proportions of maturing males (logistic regression, likelihood ratio chi-square test, p [site] = 0.557, n = 402), although of the individual sites, Emsley Cove was borderline significant (p = 0.112).

Assessment of ovarian development in female yellowfin sole (Table 26) revealed that only 50% of female yellowfin sole collected from Hospital Beach in 2002 were mature. Otherwise, the proportion of vitellogenic females was relatively high (80–100%) at nearly all sampling sites and at all sampling times, with an overall prevalence of 93% for all yellowfin

Table 26. Proportions (%) of maturing female and male yellowfin sole from Kitimat Arm sites (Hospital Beach, Eurocan, Kitamaat Village, and Emsley Cove) and reference areas (Kildala Arm and Kitlope). "L" and "H" indicate prevalences that are significantly lower or higher, respectively, than the overall prevalence for that sampling year ($p \leq 0.05$, G-statistic). Different numerical superscripts indicate significant differences between years within a site ($p \leq 0.05$, G-statistic).

Site	Hospital Beach	Eurocan	Kitamaat Beach	Emsley Cove	Kildala Arm	Kitlope
Yellowfin sole females						
2000	95	95	100	100	95	100
	(n = 20)	(n = 19)	(n = 2)	(n = 3)	(n = 20)	(n = 7)
2002	50^{L}	100	100	80	94	100
	(n = 6)	(n = 2)	(n = 3)	(n = 5)	(n = 16)	(n = 8)
Yellowfin sole males						
2000	95	70^{L1}	95	40^{L}	80	90
	(n = 20)	(n = 20)	(n = 20)	(n = 20)	(n = 20)	(n = 20)
2002	100	100^{2}	100	56^{L}	100^{H}	100^{H}
	(n = 15)	(n = 14)	(n = 5)	(n = 18)	(n = 21)	(n = 19)

females sampled. Prevalences of maturing females were similar in 2000 and 2002 at most sampling sites, although the proportion of maturing females at Hospital Beach was significantly lower in 2002 than in 2000. Fish length had a significant effect on the likelihood of ovarian development, with larger fish showing a higher probability of being vitellogenic than smaller fish (logistic regression, likelihood ratio chi-square test, $p = 0.0056$, n = 112). Logistic regression analysis also indicated that there were no significant intersite differences in the prevalence of vitellogenic yellowfin sole after adjusting for fish length (logistic regression, likelihood ratio chi-square test, $p = 0.37$, n = 112).

Male yellowfin sole exhibited some variation among sites in the proportions of fish that were sexually maturing and producing sperm (Table 26), although the overall prevalence of maturation for all fish sampled was relatively high (85%). In 2000 the lowest proportions of maturing fish were found at Eurocan (70%) and Emsley Cove (40%); in 2002 the proportions of maturing males were still low at Emsley Cove (56%) but comparable to other sites at Eurocan (100%). Prevalences of maturing males tended to be higher in 2002 than in 2000, but the difference was significant only in males from Eurocan (Table 23). Logistic regression analysis showed that, for male yellowfin, fish length had no significant effects on the likelihood of maturation ($p = 0.574$); all males were within the adult size range. However, the proportion of fish maturing at Emsley Cove was significantly lower than at any other site ($p = 0.0001$). No other significant intersite differences were observed, although the proportion of maturing males at Eurocan was borderline significant ($p = 0.102$).

Discussion

Although it has been clearly established that PAHs, especially the mutagenic and carcinogenic high molecular weight PAHs, can have serious health effects on aquatic life (e.g., Myers et al. 2003, Johnson et al. 2008a), there has been considerable debate about the risk posed by these same compounds when associated with smelter wastes. Because the majority of PAHs generated from aluminum smelters are bound to oxides and soot particles (Naes and Oug 1998), their bioavailability to marine animals may be greatly restricted (Butler and Crossley 1981, Paine et al. 1996, Naes et al. 1999, Brion and Pelletier 2005). Several studies have indicated that only a small fraction of soot-bound PAHs are desorbed from sediment particles into pore water or into the water column, where they can most readily be taken up by aquatic organisms (Gobas et al. 1993).

After 5 years of monitoring, we have accumulated a substantial body of data on the spatial extent and severity of contamination from the smelter, uptake of PAHs by fish and their prey, and the associated level of fish injury. These data provide a strong basis for further understanding the relationship between the types of biological effects that have previously been shown in other marine and estuarine areas, and factors such as PAH source, bioavailability, exposure, and presence of other contaminants. They also allow us to examine trends in PAH contamination over the past 5 years to see if process changes at the plant or other factors may have altered PAH levels or distribution patterns.

Concentrations and Patterns of PAHs in Kitimat Sediments

The results of the sediment characterization study show that PAHs are present in sediments within Kitimat Arm, with the highest concentrations at the Alcan Inner Harbour site nearest the smelter. The average total PAH concentration in sediment from this site was 26,000 ng/g dry wt, an order of magnitude higher than total PAH concentrations at any of the other sites sampled within or outside Kitimat Arm. At the Hospital Beach site, the average sediment PAH concentration was about 5,000 ng/g dry wt, similar to levels observed at contaminated urban sites on the Pacific coast of Canada and the United States (Brown et al. 1998, McCain et al. 2000, Stehr et al. 2000). At the other sites within Kitimat Arm (Eurocan Beach, Kitamaat Village, and Emsley Cove) concentrations of PAHs were in the 1,000–3,000 ng/g dry wt range, similar to levels found at other Pacific coast sites with moderate levels of urbanization and development (Brown et al. 1998, McCain et al. 2000, Stehr et al. 2000). All PAH compounds were close to detection limits in sediments from the Kildala Arm and Kitlope reference sites.

While some of the compounds present in Kitimat sediments were LAHs that could originate from a variety of petroleum products, the majority of PAHs included HAHs characteristic of the smelting process and most probably produced by Alcan. In sediments from all of the sites within Kitimat Arm, HAHs accounted for 90% or more of the total PAHs present in the samples, similar to the PAH proportions in Alcan pitch samples. The types and proportions of individuals HAHs in sediments from these sites were also similar to those found

in Alcan pitch samples. In sediments from Kildala Arm and Kitlope, on the other hand, the PAH profiles were very different from those of Alcan pitch samples. HAHs accounted for only about 60% of total PAHs, mostly in the form of perylene, a naturally-produced PAH that comes from decomposition of organic matter (Wakeham et al. 1980, Pichler et al. 1996, Irwin et al. 1998, Yunker et al. 1999).

Different sampling sites also showed distinctive profiles for LAHs. Sediments from all of the sites within Kitimat Arm, for example, showed significant proportions of phenanthrene, a major LAH in the Alcan pitch samples, but this compound was absent in sediments from Kildala Arm and Kitlope. Instead, retene, a PAH derived from wood products (Zender et al. 1994, Tavendale et al. 1995), was a predominant LAH at these sites and was not detected in Alcan pitch. Retene was also prominent in sediments from Eurocan Beach and Minette Bay, which would be consistent with pulp mill and logging or log holding and transfer activities in these areas.

Exposure of Juvenile Outmigrant Chinook Salmon to PAHs

Our study showed that PAH compounds were present in food organisms of juvenile salmon. Juvenile salmon from Alcan Inner Harbour, Hospital Beach, Inner Eurocan Beach, Outer Eurocan Beach, and Wathlsto Creek all had significantly higher levels of ΣHAHs in stomach contents than salmon from Minette Bay, Kemano Village, or Kildala Arm, with highest concentrations at Alcan Inner Harbour. While the types of individual HAHs in stomach contents of fish from all sites were similar to those in the Alcan pitch samples, the stomach contents samples typically had higher proportions of fluoranthene and pyrene than the pitch samples.

LAHs were also found in stomach contents of juvenile salmonids from the Kitimat Arm as well as in some other areas including Kemano Village. Concentrations were highest in salmon from Alcan Inner Harbour, Hospital Beach, and Eurocan Beach and lowest in salmon from the Kildala Arm reference site, with intermediate concentrations at Minette Bay, Wathlsto Creek, and Kemano Village. While concentrations of HAHs showed a clear pattern of decline with distance from the smelter, higher concentrations of ΣLAHs were found at several sites, including Inner and Outer Eurocan beaches and Kildala Arm.

This is not surprising, because LAHs may be derived from a variety of sources, including fuel oil, wood products, and boat marinas. In fact, retene, which is a derivative of wood products and often associated with pulp mill effluent (Zender et al. 1994, Tavendale et al. 1995), was found in salmon from all sites, with especially high concentrations at the Inner and Outer Eurocan beaches. Retene accounted for only about 1–2% of ΣLAHs in stomach contents of salmonids from Alcan Inner Harbour but up to 17% at other sites (e.g., Inner and Outer Eurocan beaches, Kildala Arm). If retene was excluded from ΣLAHs, the highest concentrations of ΣLAHs were found at the Alcan Inner Harbour site, similar to the pattern for ΣHAHs.

Concentrations of ΣLAHs and ΣHAHs in stomach contents of juvenile Chinook salmon from Kitimat Arm, Kemano Village, and Kildala Arm were low to moderate when compared with concentrations in Chinook salmon from Oregon and Washington estuaries (Johnson et al. 2007, Figure 26). Even in salmon from the Alcan Inner Harbour sites, ΣPAH concentrations in stomach contents were below concentrations found in salmon from industrial sites in urban

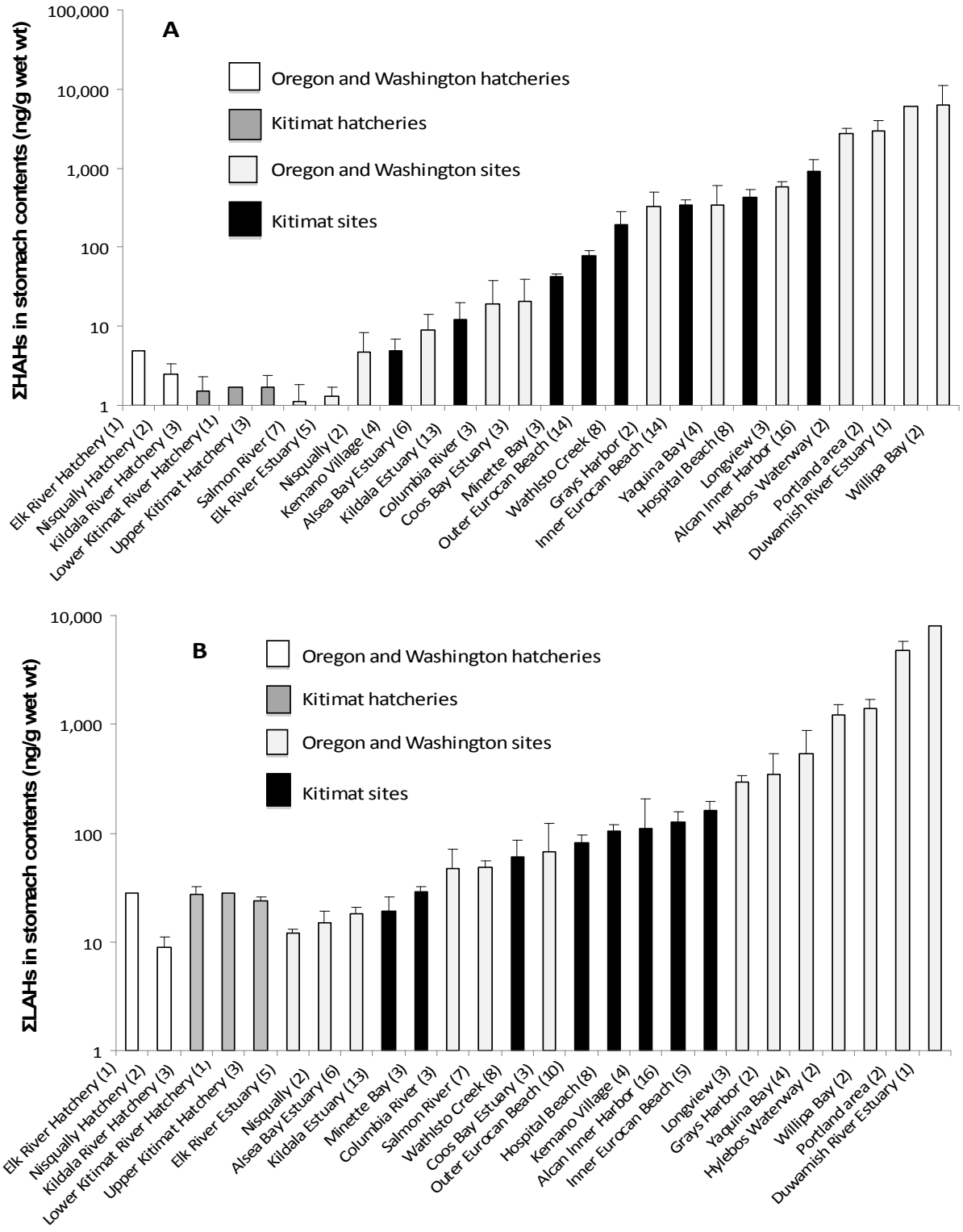

Figure 26. Mean concentrations (ng/g wet wt ± SE) of (A) HAHs and (B) LAHs in stomach contents of juvenile Chinook salmon from Kitimat Hatchery and from sites in the Kitimat region as compared to concentrations of these compounds in stomach contents of juvenile Chinook salmon from sites and hatcheries in Oregon and Washington.

centers (e.g., Seattle, Tacoma, and Portland) in Oregon and Washington (Johnson et al. 2007). The relatively low PAH concentrations in stomach contents of salmon from Kitimat Arm sites, where sediment PAH concentrations are quite high, suggests reduced bioavailability of these smelter-derived PAHs.

Because we did not collect information on the stomach contents taxonomy in this study, we are uncertain whether differences in diet between juvenile salmon from Washington and Oregon and Kitimat might also have influenced PAH concentrations in stomach contents. However, most studies suggest that similar types of food organisms are consumed by juvenile Chinook salmon in nearshore marine environments throughout the Pacific Northwest. Chinook typically consume larval and adult insects and amphipods when they first enter estuaries, with increasing dependence on larval and juvenile fish as they grow larger. Preferred diet items for Chinook salmon include chironomid larvae, dipterans, cladoceans such as Daphnia, amphipods, and other crustaceans (Sasaki 1966, Reimers 1973, Dunford 1975, Levy et al. 1979, Northcote et al. 1979, Healey 1980, 1982, Levy and Northcote 1981, Myers et al. 1981, Kjelson et al. 1982, Levings 1982, Pearce et al. 1982, Birtwell et al. 1984, Gordon and Levings 1984).

We also detected low concentrations of ΣPAHs in stomach contents of salmon from the three hatchery stocks sampled from Kitimat Hatchery; ΣLAH concentrations were similar to levels in salmon collected from the Kildala Arm reference site, while concentrations of ΣHAHs were slightly lower. These concentrations are much the same as those reported in feeds from hatcheries in Washington and Oregon (Johnson et al. 2007, Maule et al. 2007) in the 30 ng/g wet wt range for ΣLAHs and the 1–5 ng/g wet wt range for ΣHAHs (Figure 26). This finding suggests that hatchery feed is not a major source of PAH exposure to juvenile salmon during hatchery rearing, especially for the HAHs characteristic of releases from the smelter.

Because PAHs are metabolized by fish and other vertebrates and do not accumulate in their tissues, PAH uptake is best assessed by measuring concentrations of PAH metabolites in bile (Krahn et al. 1986b). Bile metabolite levels are a good indicator of recent PAH exposure. In the Kitimat Marine Assessment, we found that metabolites of PAHs were present in bile of juvenile salmon. Concentrations were generally highest in fish from sites nearest the smelter (i.e., Alcan Inner Harbour and Hospital Beach) and paralleled PAH concentrations in sediments and stomach contents. Metabolites of LAHs were found in the bile of juvenile salmonids from the Kitimat Arm as well as in some other areas including Kemano Village. Concentrations were highest in fish from Alcan Inner Harbour, Hospital Beach, and Inner and Outer Eurocan beaches, and lowest in fish from the Kildala Arm reference site; concentrations at the other sites (Minette Bay, Wathlsto Creek, and Kemano Village) were intermediate. Like levels of ΣLAHs in stomach contents, concentrations of PHN equivalents in bile did not show a clear pattern of decline with distance from the smelter, again suggesting exposure from other PAH sources, such as fuel oil associated with boat marinas and wood products.

In juvenile salmon from Alcan Harbour and Hospital Beach, levels of BaP equivalents (metabolites of HAHs) in bile were comparable to those observed in juvenile salmon from urban Puget Sound estuaries such as the Hylebos and Duwamish waterways, while levels in salmon from other sampling sites were more comparable to levels previously observed in salmon from rural estuaries (Stehr et al. 2000, Johnson et al. 2007) (Figure 27A). Levels of PHN equivalents (metabolites of LAHs) at all the Kitimat sites were more comparable to levels measured in

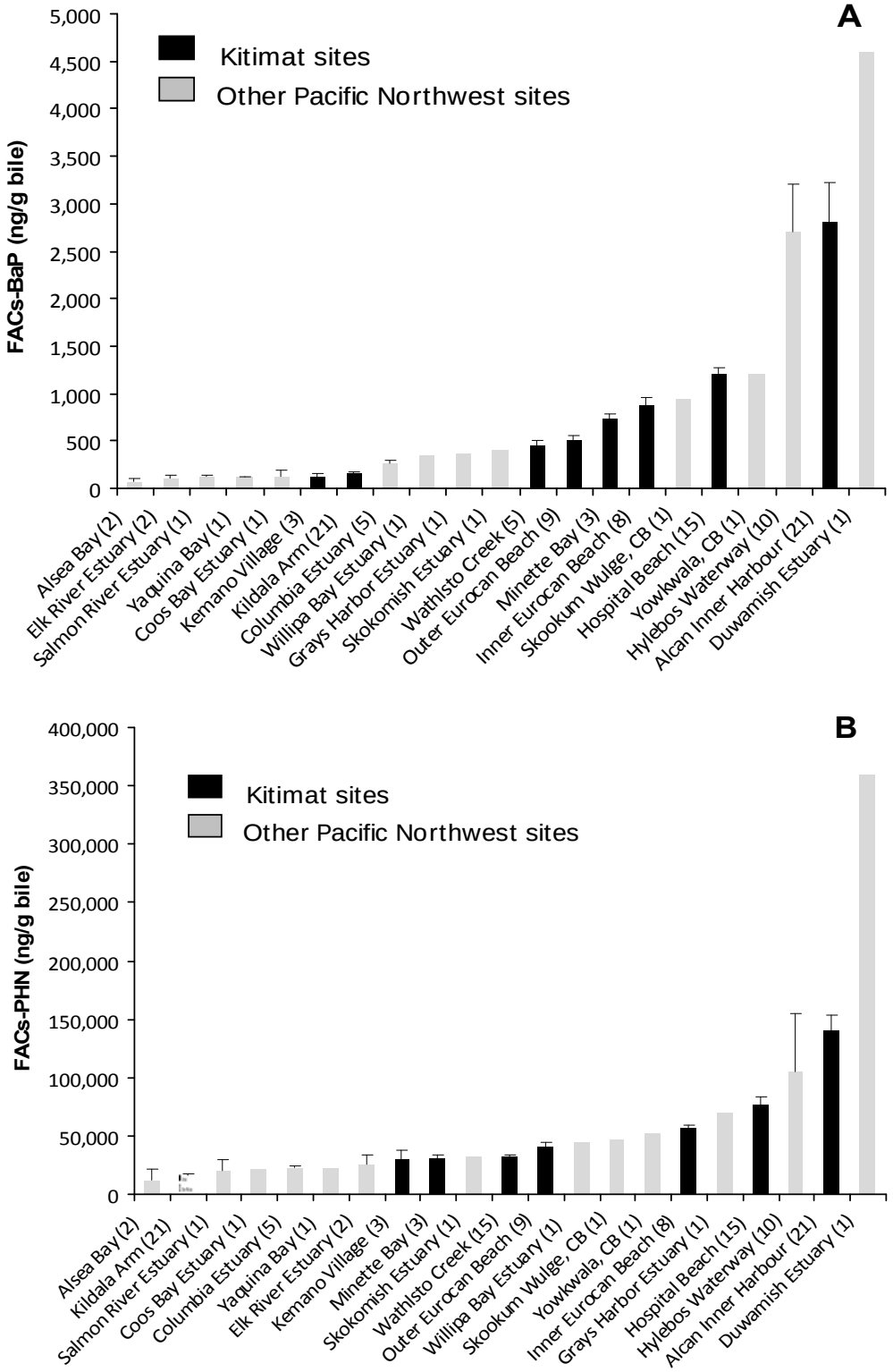

Figure 27. Mean concentrations (ng/g bile ± SE) of (A) fluorescent metabolites of HAHs, measured as FACs-BaP, and (B) fluorescent metabolites of LAHs, measured as FACs-PHN, in bile of juvenile Chinook salmon from sites and hatcheries from the Kitimat area as compared to concentrations in juvenile Chinook salmon from sites and hatcheries in Oregon and Washington.

salmon from rural or semirural estuaries in other areas of the Pacific Northwest (Figure 27B). Moreover, in comparison to Puget Sound sites, concentrations of PAH metabolites in bile of salmon from Kitimat Arm sites were relatively low compared to sediment ΣPAH concentrations (Figure 28). For example, although sediment concentrations of ΣHAHs measured at Alcan Inner Harbour were an order of magnitude higher than at those determined in Elliott Bay or Hylebos Waterway in Puget Sound, biliary concentrations of BaP equivalents in juvenile salmonids from all three sites were essentially the same. Similarly, sediment ΣPAH concentrations at Hospital Beach and Inner and Outer Eurocan beaches were similar to those at Elliott Bay and the Duwamish Waterway, but BaP equivalents in fish bile from the Kitimat sites are only about one-half as high.

Meador et al. (2008) estimated that biliary FACs-PHN concentrations above 2.2 μg/mg bile protein are associated with an increased risk of injury in juvenile salmonids. Concentrations of FACs-PHN in bile of juvenile Chinook salmon from all sampling sites within Kitimat Arm were all above this threshold level, ranging from 3.3 μg/mg bile protein at Minette Bay to 17 μg/mg bile protein at Alcan Inner Harbour.

Chronic exposure to PAHs can be assessed by measuring concentrations of PAH-DNA adducts in the liver. The presence of DNA adducts in the liver is also an early indicator of PAH effects, as this type of DNA alteration is associated with an increased risk of lesions preceding cancer in fish (Myers et al. 2003) and other animals. In contrast to short-term PAH exposure, as indicated by PAH metabolites in bile, we saw little evidence of chronic PAH exposure in salmon based on levels of DNA adducts in liver. DNA adduct levels in salmon from Alcan Inner Harbour and Inner and Outer Eurocan beaches were slightly higher than in salmon from the other sites, but even here were near the limits of detection for this method. Levels of PAH-DNA adducts in hatchery salmon were also very low, reflecting the low PAH concentrations found in hatchery diets. In other studies of juvenile salmon from urban sites contaminated with nonsmelter-derived PAHs at concentrations similar to those at sites within Kitimat Arm (5,000–10,000 ng/g dry wt in sediments), adduct levels ranged from 8 to 14 nmol/mol bases (Varanasi et al. 1993). These results suggest that, because of the lower bioavailability of PAHs from the smelter and relatively short time that juvenile salmon reside in Kitimat Arm, DNA adducts do not accumulate in this species of fish above background levels.

To assess the variability in PAH exposure in juvenile salmon during the time of outmigration, we collected fish in both May and June at Alcan Inner Harbour and at the Kildala Arm reference site. The resulting data indicated that while PAH exposure in salmon from Alcan Inner Harbour tended to be higher in May than in June, the trends were not strong or consistent at either of the sampling sites. Variability in PAH exposure and uptake could be related to changes in the diet of salmon as different prey types become available over the spring and summer (Olson et al. 2008, Cordell et al. 1999, 2001, Roegner et al. 2004).

Potential Health Impacts of PAHs on Salmon

Although we did not measure growth, immune function, or other health indicators in juvenile salmon from Kitimat, we can estimate the likelihood that these effects would occur by comparing their exposure levels with those of juvenile salmon from field and laboratory studies where such effects were found. In juvenile Chinook salmon from Puget Sound, for example,

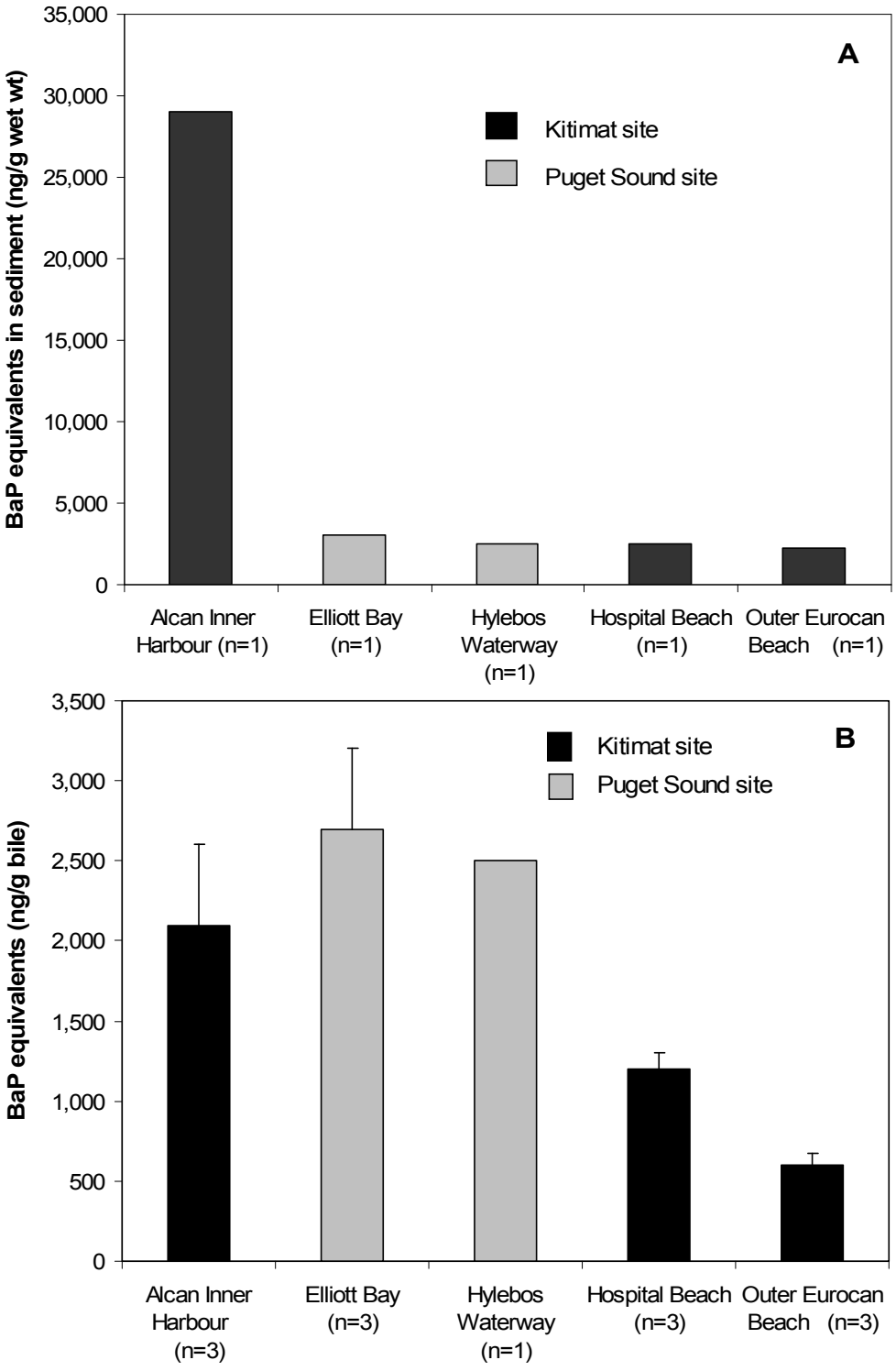

Figure 28. Mean concentrations (ng/g, wet weight ± SE) of (A) HAHs, measured as BaP equivalents in sediments collected from Kitimat and two urban sites in Puget Sound, Washington, and (B) fluorescent metabolites of HAHs, measured as BaP equivalents in bile of juvenile Chinook salmon collected from Kitimat and two urban sites in Puget Sound. Sediments were analyzed by HPLC/UVf, as described in Krahn et al. 1993.

immunosuppression, reduced growth rates, and DNA damage (i.e., PAH-DNA adducts) were observed at two heavily industrialized sites, the Duwamish Hylebos waterways, where fish were exposed to PAHs in combination with other industrial contaminants such as PCBs (Arkoosh et al. 1991, 1994, 1998, 2001, Varanasi et al 1993, Stein et al. 1995, Casillas et al. 1995, 1997a, 1997b, Stehr et al. 2000). Concentrations of ΣPAHs in stomach contents of these fish were in the 4,000 to 15,000 ng/g wet wt range (McCain et al. 1990, Varanasi et al. 1993, Stein et al. 1995, Stehr et al. 2000).

A number of laboratory feeding studies—where fish were exposed to PAHs alone—have reported ΣPAH concentrations associated with immunosuppression and decreased growth in the 16,000 to 40,000 ng/g wet wt range (Palm et al. 2003, Meador et al. 2006, 2008), somewhat higher ΣPAH levels than those typically measured in stomach contents of Duwamish and Hylebos salmon. However, Meador et al. (2006) found changes in plasma chemistry, whole body lipids, and fish weight distribution similar to those in fish exhibiting starvation at threshold ΣPAH concentrations of about 7,600 ng/g wet wt.

At the Kitimat Arm sites, the average ΣPAH concentrations in salmon stomach contents were equal to or less than 1,200 ng/g wet wt, and the highest ΣPAH concentration observed was 6,500 ng/g wet wt at Alcan Inner Harbour. Even this highest concentration is substantially lower than concentrations associated with immunosuppression in laboratory feeding studies (e.g., Palm et al. 2003). However, this ΣPAH level is comparable to doses associated with metabolic changes in juvenile salmon (Meador et al. 2006), and to ΣPAH concentrations observed in stomach contents of salmon from the Hylebos and Duwamish waterways.

Concentrations of PAH metabolites in bile of salmon from Alcan Inner Harbour were also comparable to those observed at two Puget Sound sites and in the feeding study conducted by Meador et al. (2006). These data suggest that exposure levels in some salmon from Alcan Harbour are approaching concentrations where health effects have been observed in wild salmon populations from Puget Sound and in certain laboratory studies. Moreover, based on laboratory exposure studies and extensive field data on juvenile Chinook salmon, Meador et al. (2008) have estimated that biliary FACs-PHN concentrations above 2.2 μg/mg bile protein are associated with an increased risk of injury in juvenile salmonids. Concentrations of FACs-PHN in bile of juvenile Chinook salmon from all sampling sites within Kitimat Arm were all above this threshold level, ranging from 3.3 μg/mg bile protein at Minette Bay to 17 μg/mg bile protein at Alcan Inner Harbour (Figure 29).

It should be kept in mind, however, that field-collected Puget Sound salmon showing immunosuppression and other abnormalities were exposed to contaminants such as PCBs as well as PAHs, so it is uncertain whether PAHs alone would produce the same health impacts at the same exposure concentrations. It is also difficult to know how long the juvenile salmon collected from Alcan Inner Harbour were resident at that site. Although juvenile Chinook salmon may spend several months rearing within the estuary before migrating to the ocean (Fresh et al. 2005), their movements and residence time within Kitimat Arm and the area around the smelter are unknown.

Previous studies have shown that reductions in disease resistance and impaired growth, from contaminant exposure or from other causes, can lead to delayed mortality in outmigrant

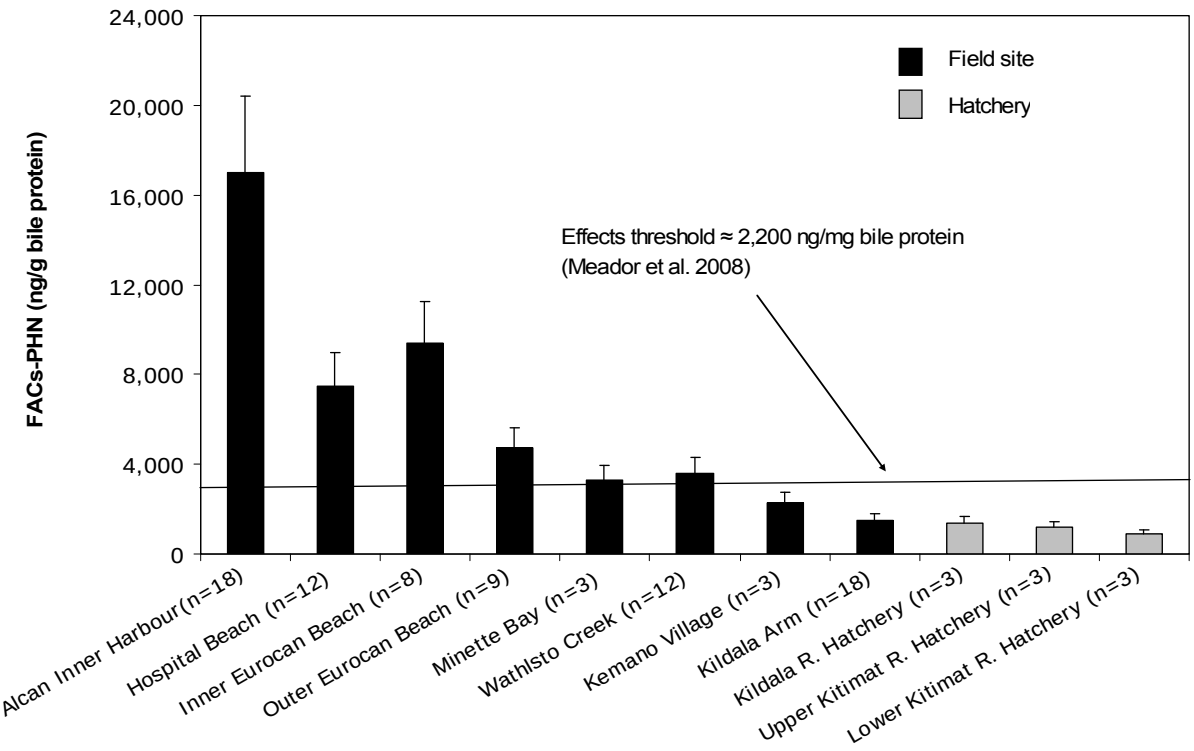

Figure 29. Levels of fluorescent aromatics compounds fluorescing at phenenthrene wavelengths (FACs-PHN) in ng/mg bile protein in juvenile salmon from sites in Kitimat Arm, nearby references areas, and hatcheries as compared to the estimated effects thresholds for bile metabolites (≈2,700 ng/mg bile protein), as developed by Meador et al. (2008). Average bile metabolite levels of juvenile salmon from all sites within Kitimat Arm are above or at the threshold level, while bile metabolite levels in salmon from references areas and hatcheries are lower.

salmon, with associated impacts on the abundance of salmon stocks (Arkoosh and Collier 2002, Loge et al. 2005, Spromberg and Meador 2005). Our data suggest that the proportion of juvenile salmon exposed to high concentrations of PAHs in Douglas Channel is relatively small, so impacts on the salmon fishery would be difficult to detect. Even so, reductions in PAH contamination could only have favorable effects on the survival and productivity of salmon stocks in the region.

PAH Exposure in Flatfish

Like juvenile salmon, flatfish collected from Kitimat Arm showed dietary exposure to smelter-associated PAHs. These compounds were found in stomach contents of resident English sole at concentrations that were highly correlated with ΣPAH concentrations in sediments at the sites where fish were collected. This finding suggests that the PAHs deposited in sediments are being taken up by flatfish through their diet. As expected, HAHs predominated in fish from the Hospital Beach site, nearest the Alcan plant, with LAHs occurring at relatively high concentrations at a wider range of sites. Retene was a major component of ΣLAHs in stomach contents of English sole, more so than in salmonids. At the Eurocan site, retene accounted for up to 70% of ΣLAHs in stomach contents, and was prominent in fish from the Kildala Arm and

Kitlope reference sites as well. As in sediments, a number of HAHs were present, in similar proportions, in stomach contents of English sole from all the Kitimat Arm sites. At Kildala Arm and Kitlope, however, perylene was the major HAH present, accounting for 70–80% of total ΣHAHs.

In general, ΣPAH concentrations in stomach contents tended to be higher in flatfish than in salmon, and correlations between concentrations of ΣPAHs in stomach contents and sediments were stronger in flatfish than in salmon. This probably reflects the longer residence time at the site of capture, the greater exposure to sediments, and the greater reliance on benthic prey by flatfish as compared to juvenile salmon. While benthic invertebrates are an important component of the diet of juvenile salmon, they also feed on pelagic invertebrates and insects of terrestrial origin (Cordell et al. 1999, 2001, Olson et al. 2008). Still, as in salmon, ΣPAH concentrations in stomach contents of English sole were low to moderate (≈200–2,000 ng/g wet wt) at all the Kitimat Arm sampling sites, in comparison to concentrations measured in sole from urban centers in other parts of the United States and Canada where reported ΣPAH concentrations are in the 2,000–8,000 ng/g wet wt range (McCain et al. 2000, Stehr et al. 2004). This is not likely to be due to differences in diet, as studies indicate that English sole consume similar prey items (annelids and mollusks) throughout their range (McCain et al. 2000). Thus the low PAH concentrations in stomach contents of English sole from Kitimat Arm suggest reduced bioavailability of smelter-associated PAHs.

Indicators of PAH Uptake in Flatfish

In flatfish, as in salmon, we measured PAH metabolites as an indicator of short-term exposure to PAHs and DNA adducts as an indicator of chronic exposure to PAHs. Exposure can also be inferred by measuring activity of the liver enzymes that metabolize PAHs, such as CYP1A (Collier et al 1995). However, this indicator is a less accurate indicator of PAH uptake than measuring bile metabolites or DNA adducts because it is not specific to PAH exposure. Other compounds, including dioxins, furans, and dioxin-like PCBs, can also increase liver CYP1A activity. As part of this study, we used all three of these indicators in various ways to evaluate PAH uptake in flatfish.

Metabolites of PAHs in Bile

Metabolites of PAHs were measured in the bile of yellowfin and English sole. In yellowfin sole, bile metabolite levels were highest in fish from the Hospital Beach and Eurocan sites and lowest in fish from Kitlope and Kildala Arm, reflecting ΣPAH concentrations in sediments at these sites. In English sole, on the other hand, PAH metabolites in bile were not as clearly correlated with PAH levels in their prey or sediments. As expected, metabolites of both low and high molecular weight PAHs were relatively high in English sole from Hospital Beach, and lower in sole from other sites within Kitimat Arm. However, metabolites of both HAHs (BaP equivalents) and LAHs (PHN equivalents) in bile were also surprisingly high in English sole from Kitlope and were among the highest levels observed in any English sole sampled. This was anomalous, as concentrations of both low and high molecular weight PAHs were extremely low in sediments from Kitlope, and ΣPAH concentrations in stomach contents of English sole from this site were relatively low as well.

AHH Activity

The activity of PAH-metabolizing enzymes, as indicated by AHH activity, was also measured in English sole. Again, as expected, AHH activity in sole from Hospital Beach was somewhat elevated in comparison to sole from other sites within Kitimat Arm (Kitamaat Village, Eurocan, and Emsley Cove). However, AHH activity was also quite high in sole from Kitlope and Kildala Arm, comparable to activity levels in Hospital Beach sole.

The sources of PAH exposure in English sole from Kitlope and Kildala Arm are not clear. The relatively high levels of LAH metabolites in bile, as well as increased AHH activity, could be accounted for, at least in part, by uptake of retene or related wood product derivatives, which are present in the stomach contents of these animals. Retenes have been shown to be effective inducers of CYP1A enzymes such as AHH (Fragoso et al. 1999) and are also metabolized and are detectable in fish bile (Fragoso et al. 1999, Leppänen and Oikari 1999). Exposure to LAHs in the form of fuel oil or related petroleum products is also a possibility. Such exposure could be transient and related to localized spills or other releases of these substances into the water column. However, the presence of HAH metabolites in bile of Kitlope fish is not easily explained.

The absence of lesions or significant concentrations of DNA adducts in livers of these fish and the low concentrations of ΣHAHs in both sediment and stomach contents suggest that exposure is short-term. However, it appears to be fairly consistent, as elevated bile metabolite levels were observed in Kitlope English sole in 2000, 2002, and 2004. Thus it does not appear to be an anomaly due to a transient, localized spill or to some contamination inadvertently introduced into the water while fish were being held on the sampling vessel. Nor is it likely to be due to analytical problems, both because of its persistence and because the Kitlope samples were recomposited and reanalyzed, and the results obtained were very similar to the original findings.

Effects of Perylene and Kitlope Sediment Extracts on Sole Bile Metabolite Levels

To gain more insight into the possible reasons for the high bile metabolite levels in sole from Kitlope, we injected English sole with extracts of sediments from Hospital Beach and Kitlope, as well as with the model compound, perylene, and measured changes in bile metabolite levels (See Appendix). This naturally derived PAH makes up a high proportion of HAHs in Kitlope sediments and in stomach contents of English sole from Kitlope, and we hypothesized that it might be having an unusual affect on bile fluorescence levels. The experiment was also designed to establish whether or not the source of PAH exposure in Kitlope fish was from compounds in the sediment. In sole injected with extracts of Hospital Beach sediments, there was a strong dose-dependent increase in high molecular weight PAH metabolites levels, but fish receiving perylene or the Kitlope sediment extract showed no change in bile metabolite levels. These results suggest that neither perylene nor compounds found in Kitlope sediments are responsible for elevated bile metabolite concentrations in wild English sole sampled from Kitlope. A logical next step is to identify the specific compounds in English sole bile from Kitlope using bile hydrolysis and GC/MS analysis (e.g., see Krahn and Stein 1997). This may provide clues as to the source of PAH exposure in this isolated fjord.

Liver Lesions and DNA Damage in Flatfish

In English sole, PAH exposure was associated with clear-cut impacts on fish health in the form of toxicopathic liver lesions that are known to be caused, in large part, by exposure to hepatotoxic and hepatocarcinogenic PAHs (Myers et al. 2003). These lesions affected 10–20% of sole at all sites within Kitimat Arm but almost no fish from Kildala or Kitlope, and were consistently observed, at similar prevalences, in all sampling years from 2000 to 2004. Liver lesions were also observed in yellowfin sole at prevalences of 5–10%, but lesion occurrence was more closely associated with increased fish age than with residence at contaminated sampling sites. English sole are known to be especially sensitive to carcinogenic PAHs as compared to other species (Myers et al 1987, 1994, 1998a, 1998b, 2003, Collier et al 1992, 1995), which may explain the differences in lesion prevalences between the two species.

While PAH exposure was associated with liver disease in English sole from Kitimat Arm, the degree of PAH uptake and effects were somewhat lower in Kitimat Arm than would be expected, based on the concentrations of ΣPAHs present in sediments. If liver lesion prevalences at Kitimat Arm sites are compared to those observed in English sole from other sites along the west coast of the United States and Canada, they are similar to those observed at moderately contaminated near-urban sites rather than urban sites with comparable sediment ΣPAH levels (Figure 30).

We also examined the relationships of DNA adduct levels (Figure 31) and liver lesion prevalence (Figure 32) with sediment ΣPAH concentrations in Kitimat area English sole using hockey stick–regression models developed for English sole from other Pacific coast sites (Horness et al. 1998, Johnson et al. 2002). The fitted regression lines in Figure 31 and Figure 32 reflect the relationships for Pacific coast English sole. Examination of these figures shows that both adduct and lesion levels for sole from most of the Kitimat area sites were within the range observed for other Pacific coast sole exposed to sediments with comparable PAH concentrations. However, both adduct and lesion prevalences were much lower than expected in sole from the Hospital Beach site nearest the smelter, where smelter-associated PAHs are most predominant. These results suggest that the bioavailability and biological effects of smelter-associated PAHs may be reduced in comparison to PAHs from other sources, although the compounds are still capable of affecting the health of marine organisms. However, they also suggest that, at sites within Kitimat Arm that are farther from the Alcan plant, the toxic effects of the PAHs present in sediments are comparable to those of PAHs at other urban and industrial sites.

Fish Size, Condition, and Liver Weight

Various studies have shown that exposure to PAHs can interfere with fish metabolism and reduce growth and condition (Johnson et al. 1988, 1998, Rice et al. 2000, Marchand et al. 2004, Dutta et al. 2005, Meador et al. 2006, 2008). In yellowfin sole we did see some differences in growth and condition between sole from sites within Kitimat Arm and sole from the Kitlope and Kildala Arm reference areas. Male yellowfin sole tended to be smaller in size and also smaller for their age at sites near the smelter (i.e., Hospital Beach and Eurocan). Yellowfin sole from Hospital Beach and Emsley Cove also had low CFs in comparison with other sites. However, we did not see this trend in English sole. In general, the English sole we

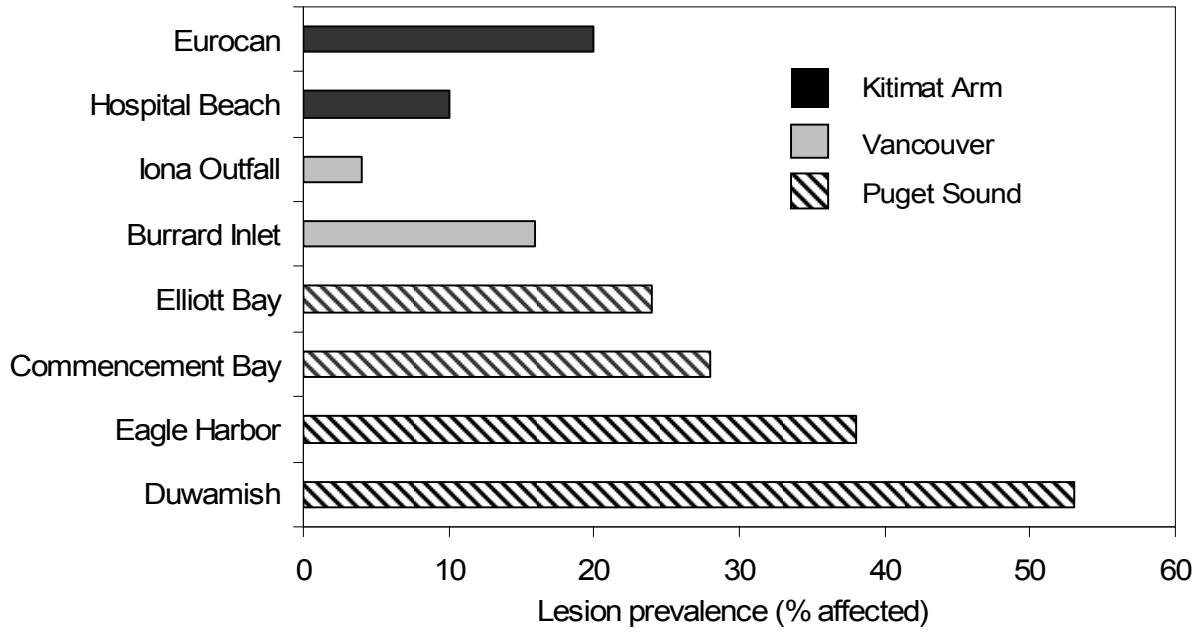

Figure 30. Prevalences of toxicopathic lesions in English sole from sites in Kitimat Arm, as compared to prevalences in English sole from selected sites urban sites in Vancouver, British Columbia, and Puget Sound, Washington.

collected tended to be larger in size and larger for their age at the sites within Kitimat Arm than at the reference areas. Fish from Emsley Cove were the exception; both male and female English sole from this site tended to be smaller for their age than fish from other sites. There was no evidence of reduced condition in English sole from sites within Kitimat Arm.

Overall, these data suggest some intersite differences in fish growth and condition of fish sampled from different areas. However, there was no clear relationship between PAH exposure and these effects, as the sites where ΣPAH levels were highest (e.g., Hospital Beach) did not consistently show the greatest impacts on growth and condition. Also, the concentrations of ΣPAHs in stomach contents of English sole sampled in this study (≈2,000 ng/g wet wt at Hospital Beach and ≈600 ng/g wet wt or less at other sites) were lower than levels that have been associated with impacts on growth in laboratory studies (2,500–10,000 ng/g wet wt total PAHs or above) (Rice et al. 2000, Meador et al. 2006). Although we have no data on PAHs in yellowfin sole stomach contents, it seems unlikely that dietary PAH exposure would be higher in these fish, as their bile metabolite levels were similar or lower than those in English sole.

Interestingly, we found differences in age distributions in yellowfin sole from Kitimat Arm and the reference sites, with higher proportions of older fish (10 years old and older) at the Kildala Arm and Kitlope reference sites than at other areas. This could indicate higher mortality rates for older fish within Kitimat Arm or could be a sign of poor recruitment in the past, so fish year classes 10 years ago or more would have been small. Similar proportions of fish in younger year classes suggest that recruitment may have been improving over the past 10 years. Because lesion prevalences are quite low in yellowfin sole, it seems unlikely that there would be increased adult mortality due to cancer or other liver lesions, although PAH-related declines in

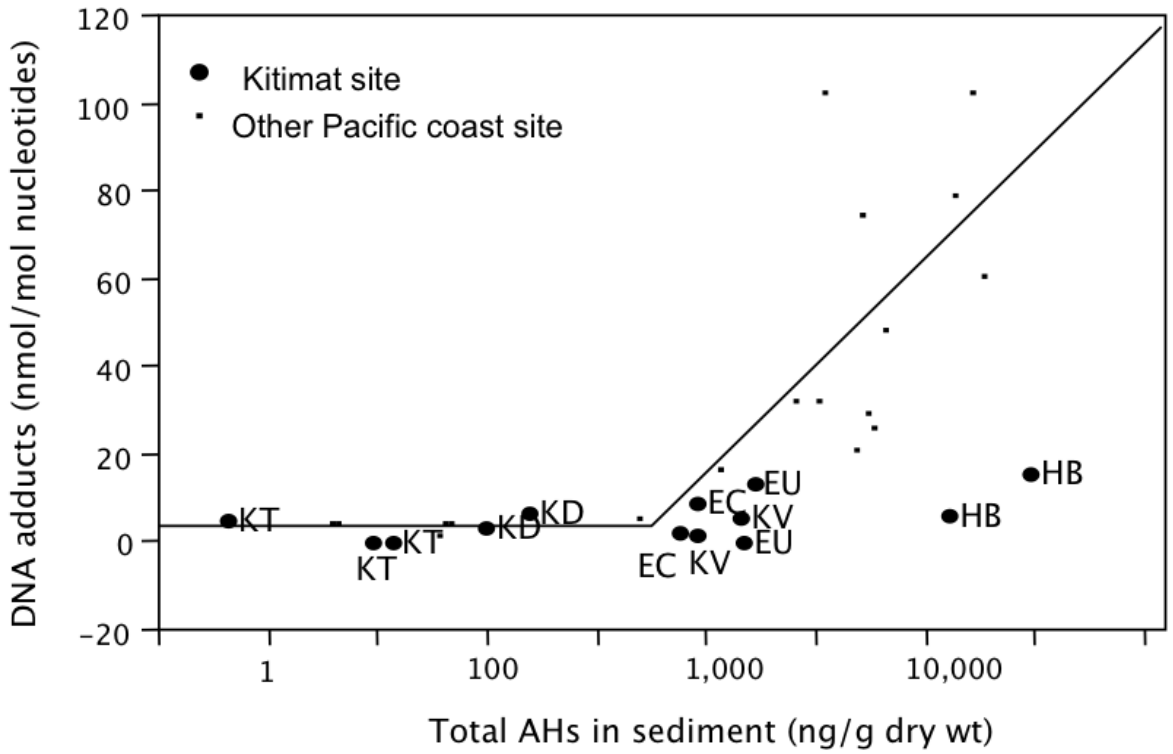

Figure 31. Hockey stick regression of DNA adduct levels vs. sediment total AH concentrations for English sole from the Kitimat area and other Pacific coast sites. The bend in the hockey stick represents the threshold sediment AH value where adduct levels begin to increase. KT = Kitlope, KD = Kildala Arm, EC = Emsley Cove, KV = Kitamaat Village, EU = Eurocan, and HB = Hospital Beach. DNA adduct levels below the regression line are lower than expected for the sediment PAH concentration at that site.

immunocompetence (Arkoosh et al. 1996, Clemons et al. 1999) might contribute to high mortality rates.

Alternatively, it is possible that PAH contamination from Alcan depressed recruitment in yellowfin sole in the past, resulting in a small number of older fish, but that process changes reducing PAH inputs have led to improved productivity. Why this difference in age distribution was seen in yellowfin sole but not English sole is unclear. Both species have pelagic eggs and larvae (Hart 1973), so routes of exposure to PAHs for early life stages would be similar. Whatever the factors causing changes in age distribution may be, it appears that they affect the two species differentially.

Increased liver weight and elevated LSI are common effects of exposure to chemical contaminants, including PAHs (Fletcher et al. 1982, Khan 2003), due in part to the tendency of many toxicants to cause increases in liver cell number and size as a consequence of induction of the mixed-function oxidase system in the liver to detoxify contaminants (Poels et al. 1980). This effect has been observed in previous studies with English sole and other flatfish from contaminated sites (Johnson et al. 1988, 1998, 1999, Sol et al. 2008). In English sole from Kitimat Arm, LSI also appeared to be affected by PAH exposure. LSI was highest in sole from

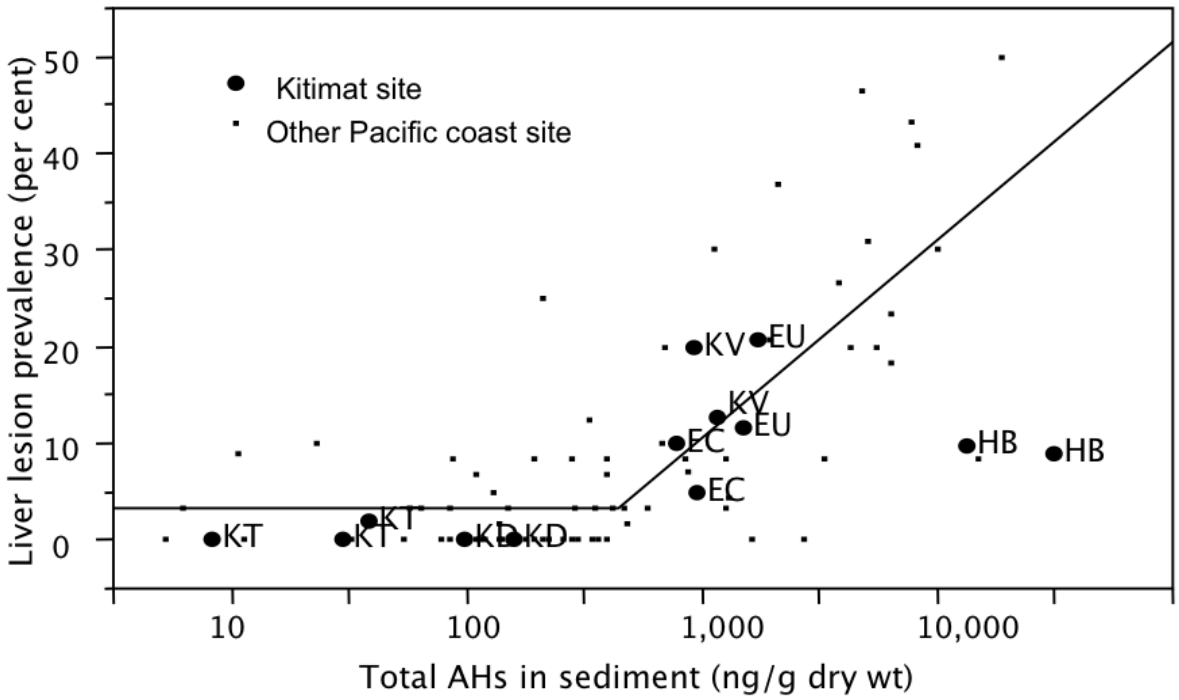

Figure 32. Hockey stick regression of liver lesion prevalences vs. sediment total AH concentrations in sediments for English sole from the Kitimat area and other Pacific coast sites. The bend in the hockey stick represents the threshold sediment AH value where lesion prevalences begin to increase. KT = Kitlope, KD = Kildala Arm, EC = Emsley Cove, KV = Kitamaat Village, EU = Eurocan, and HB = Hospital Beach. Lesion prevalences below the regression line are lower than expected for the sediment PAH concentration at that site.

Hospital Beach, where ΣPAH levels were the highest, followed by sole from Eurocan and Kitamaat Village, which also experienced substantial PAH exposure. LSI was lowest in sole with the lowest levels of PAH exposure from Emsley Cove, Kildala Arm, and Kitlope.

The relationship between PAH exposure and LSI was less clear for yellowfin sole. While LSI tended to be higher in yellowfin from the Hospital Beach and Eurocan sites, it was also fairly high in yellowfin from Kildala and lowest in yellowfin from Kitamaat Village, where PAH levels were significantly above reference levels. In addition to contaminants, liver size can also be affected by reproductive condition and tends to increase in gravid female fish that are producing the yolk protein, vitellogenin, in the liver (Johnson et al. 1991, Rinchard and Kestemont 2003). Since a larger proportion of yellowfin sole were in reproductive condition, this may have influenced LSI in these fish.

Reproductive Effects of PAHs on Flatfish

This survey revealed little evidence of PAH-related reproductive dysfunction in maturing male and female yellowfin and English sole from Kitimat Arm, at least for the limited number of endpoints measured. In female yellowfin sole, there was no evidence of inhibited ovarian development or increased resorption (atresia) of oocytes above baseline levels at any sites within

Kitimat Arm. Gonad size (GSI) was also similar in female yellowfin from all sites, once the effect of fish length or age was taken into account. Female English sole were less likely to be maturing than female yellowfin sole and showed more intersite variation in maturation rates, but showed no signs of inhibited development or increased ovary lesions at sites with the greatest PAH contamination. In fact, English sole from Hospital Beach had a higher likelihood of maturation than fish from other sites, and ovary size and GSI were higher in fish from this site as well. Maturation was least likely in English sole from Emsley Cove, a site where PAH contamination was low to moderate.

In general, both male yellowfin and male English sole showed high levels of maturation in both Kitimat Arm and the reference areas and low prevalences of testicular lesions. The exceptions were the male fish from Emsley Cove, which were less likely to be maturing than fish from any of the other sites, although the difference was statistically significant only for yellowfin sole. GSI also tended to be low in both male yellowfin and male English sole from Emsley Cove. The reason for the low level of maturation in Emsley Cove fish is not known, although their small size and poor condition may have contributed to the problem. It seems unlikely that exposure to PAHs was the major causative factor for the alterations in reproductive development observed in these fish, as PAH levels in sediments, fish stomach contents, and bile were not especially high at the Emsley Cove site. However, there can be considerable variation in the timing of testicular maturation in male flatfish, so the intersite differences observed in this study may fall within the normal range of variation that would be expected in this species.

It is possible that exposure to other chemical contaminants may have influenced reproductive development in sole from Kitimat Arm. A number of studies have shown that pulp mill effluents, even from plants such as the Eurocan pulp mill that use chlorine-free processes, may disrupt fish reproduction (McMaster 2001). Some studies indicate that CYP1A inducing–compounds, such as retene, have antiestrogenic activity (Smeets et al. 1999). Other research suggests that retenes may produce dioxin-like developmental defects in larval fish (Billiard et al. 1999). In contrast, other substances found in pulp mill effluents and wood products, such as plant sterols, may have estrogenic effects (Tremblay and Van der Kraak 1998); estrogenic compounds might also be found in sewage and septic wastewater discharges entering the embayment (Falconer et al. 2006). Exposure to environmental estrogens could alter reproductive timing in sole (Johnson et al. 2008b) or cause other types of reproductive abnormalities (Matthiessen and Sumpter 1998). However, additional study would be needed to document exposure to these types of compounds and to establish that the variations in reproductive development observed in this field survey do not simply reflect the normal variation in reproductive development of flatfish.

It should also be noted that the reproductive data collected in this study were very limited and were restricted to examination of impacts on gonadal growth and gonadal histology. These endpoints are affected by PAHs and are easily measured in the field, but other reproductive endpoints, such as egg and sperm quality, may be more sensitive to PAH exposure (Casillas et al. 1991, Nagler and Cyr 1997, Johnson et al. 2002). Although the level of PAH exposure experienced by flatfish in Kitimat Arm may be too low to reduce gonadal growth, it may still have the potential to lower the productivity of resident flatfish stocks by reducing fertilization success or larval survival. In fact, the altered age distribution of yellowfin sole from Kitimat

Arm suggests there may have been such an impact in the past, and it may be lessening because of process changes that have reduced PAH inputs.

Salmon and Flatfish Exposure to Organochlorine Contaminants

Aside from PAHs, fish appear to be exposed to few contaminants in Kitimat Arm or surrounding areas. Concentrations of PCBs, DDTs, and other OCs were generally low in stomach contents of sole and salmon from all sites (<50 ng/g for PCBs, <10 ng/g for DDTs, <1 ng/g for other pesticides in salmon, <25 ng/g for PCBs, and <1 ng/g for DDTs and other pesticides for flatfish). These levels are in the range reported for sole and salmon from rural estuaries in other studies along the U.S. Pacific Coast (McCain et al. 2000, Johnson et al. 2007) and well below concentrations associated with health effects in fish (Meador et al. 2002, Beckvar et al. 2005). However, OC levels, especially levels of PCBs, tended to be somewhat higher in stomach contents of both sole and salmon from sites within Kitimat Arm (especially in fish from Alcan Inner Harbour and Hospital Beach) than fish from less developed reference areas. This finding is consistent with the industrial activity that has been ongoing in this area over the last 40 years, as PCBs were widely used until the 1970s as paint additives, lubricants, and cooling agents in transformers and in various industrial processes (Eisler 2000). Both PCBs and DDTs can also enter aquatic systems through long-range atmospheric transport from lower latitude regions (e.g., Southeast Asia), where these compounds are still in use, to higher latitude areas such as Kitimat (Iwata et al. 1993, de Wit et al. 2004).

Hatchery feed may also make some contribution to PCB and DDT exposure in juvenile salmon, as concentrations of these contaminants were detected in stomach contents of hatchery salmon at concentrations comparable to those in stomach contents of juveniles collected at Hospital Beach and Alcan Inner Harbour. This is not an unusual finding, as many hatchery feeds have low to moderate levels of PCB and DDT contamination from the fish oil and fish tissues that are used in the manufacture of the feeds (Mac et al. 1979, Jacobs et al. 1998, Maule et al. 2007, Johnson et al. in press). Concentrations of ΣDDTs, ΣPCBs, and ΣPAHs in stomach contents of the three salmon stocks sampled from Kitimat Hatchery were similar to levels found in hatcheries from Washington and Oregon (Johnson et al. 2007, Maule et al. 2007).

Fish Consumption and Human Health

The primary objective of this study was to evaluate the effects of PAHs on fisheries resources within Kitimat Arm, so our sampling was not designed to investigate potential impacts on human health from consuming fish from this area. However, we analyzed fillets from English sole for PAHs, OCs, and selected metals to collect baseline data and screen samples for seafood safety.

Because fish rapidly metabolize PAHs and eliminate them from their bodies, these compounds do not accumulate in very high concentrations in edible tissues (Varanasi et al. 1989b). Consequently, there is little risk of exposure to PAHs for humans consuming fish fillets; concentrations of PAHs added during the cooking process are typically much greater than those found in fish flesh (Hom et al. 1999). As expected, our analyses confirmed that, even in sole collected from sites near the smelter where sediment PAH concentrations are high, PAH levels in edible muscle tissue ranged from low to undetectable. Similar results were obtained in analyses

of PAH concentrations in muscle tissues of freshwater fish in Northern Canada (Braune et al. 1999).

In contrast to PAHs, compounds such as PCBs, DDTs, and other OCs, as well as certain metals, do accumulate in fish tissues and can be taken up by humans who eat the fish. However, even in fish from the Hospital Beach and Eurocan sites with the most industrial development, concentrations of PCBs, DDTs, and metals in English sole muscle samples were well below the action levels recommended by Canada Health and Welfare (Ahmed 1991) and generally in the low lower range of concentrations reported for freshwater and anadromous fish in Northern Canada (Braune et al. 1999). For example, mercury levels in English sole muscle (ranging 0.13–0.49 ppm dry wt or 0.021–0.044 ppm wet wt) were approximately an order of magnitude less than the Canada Health and Welfare action level of 0.5 ppm (wet wt). These findings are consistent with earlier studies, such as Mikkelson et al. (1996), which reported low background levels of PCBs, dioxins, and furans in eulachon (*Thaleichthys pacificus*) from the Kitimat area.

The average English sole we sampled within Kitimat Arm were 5–6 years old and some fish were as old as 13 years, so they had time to accumulate significant concentrations of tissue contaminants if these compounds were present in their habitat. However, accumulation of OC contaminants is also dependent on the fat content of the fish, and lipid levels in English sole and other flatfish are typically quite low (<1%). A fish with a higher fat content would have the potential to accumulate higher concentrations of OCs. While there is nothing in our data to suggest that fish from Kitimat Arm are unsafe for harvest and consumption because of bioaccumulative contaminants, a more comprehensive study would include contaminant measurements in fatty fish and in shellfish such as clams, crabs, and shrimp.

Bioavailability and Effects of Smelter-derived PAHs

Although this study shows consistent uptake of PAHs by salmon and sole within Kitimat Arm, especially at sites nearest the smelter, exposure levels and health effects in fish were consistently lower than those that have been observed in these species at other sites contaminated with PAHs from different sources (e.g., creosote, petroleum products, vehicle emissions, and other combustion processes). This was true for PAH concentrations in salmon and flatfish stomach contents, PAH metabolites in bile, and biological effects such as liver and gonadal lesions and reproductive abnormalities.

Although concentrations of ΣPAHs in sediments from Alcan Inner Harbour were an order of magnitude higher than those typically measured at urban sites in Puget Sound (Long et al. 2003) or at other urban embayments along the U.S. Pacific Coast (Brown et al. 1998, McCain et al. 2000), concentrations of ΣPAHs in stomach contents of salmon from Alcan Harbour were substantially lower than levels reported in salmon from Puget Sound sites. In salmon from Alcan Harbour, the average concentration of ΣPAHs in stomach contents was only 1,500 ng/g wet wt, while in Puget Sound salmon from urban sites, ΣPAH concentrations in stomach contents are in the 10,000–20,000 ng/g wet wt range (Stein et al. 1995, Stehr et al. 2000, Meador et al. 2002). Levels of PAH metabolites in the bile of juvenile salmon from Alcan Inner Harbour were also lower than those in salmon from Puget Sound sites such as the Duwamish and Hylebos waterways, even though ΣPAH concentrations in sediment at the Alcan Harbour site are considerably higher (Johnson et al. 2007). Similarly, although sediment ΣPAH concentrations

are similar in sediments from Hospital Beach and from Seattle's Elliott Bay, ΣPAH concentrations in stomach contents of English sole from Elliott Bay are about 8,000 ng/g wet wt (McCain et al. 2000) as compared to only 2,000 ng/g wet wt in English sole from Hospital Beach.

We also found that there were fewer adverse biological effects in Kitimat fish than in fish from other urban sites contaminated with comparable concentrations of PAHs from different (i.e., nonsmelter) sources. Levels of DNA adducts were low or below detection limits in both salmon and sole from Kitimat sites, and the prevalence of liver lesions in English sole at the site nearest the smelter (Hospital Beach) was only 10% as compared to 30% or higher at Puget Sound sites with comparable sediment PAH contamination. Also, English and yellowfin sole showed little evidence of the types of PAH-related reproductive impairment observed in Puget Sound sole (Johnson et al. 1988, 1998, 1999).

These findings are all consistent with reduced bioavailability of smelter-derived PAHs. While these compounds are being taken up by and causing some injury to marine organisms in Kitimat Arm, their apparent impact is substantially less than the level of reported effects of PAHs from other industrial sources.

Trends in PAH Concentrations, Exposure, and Biological Effects

A review by Alcan Inc. (Lachance et al. 2006) has identified many process changes implemented over the past 20 years that have significantly reduced PAH releases from the smelter to the environment. The primary source of PAHs in the sediments of Kitimat Arm is from losses occurring during historical processes (e.g., pencil pitch off-loading, B-Lagoon discharge, wet scrubbers for pot lines, and an anode paste plant), which have all been discontinued. Releases of PAHs in air emissions have also been reduced. Currently, the expected annual contribution of PAHs to Kitimat Arm is approximately 2% of the annual smelter load release to the air. This translates into about 3 tons of PAH per year, as compared with releases more than 3 times as high 20 years ago. This reduction is substantiated on modeling by Frank Gobas of Simon Fraser University (Harris 1999, Stevenson and Gobas 2005) and the model results are corroborated with similar modelling results in Lac St. Louis, Quebec (MacKay and Hickie 2000).

Our monitoring results suggest that the process changes introduced by Alcan over the last decade have been effective at reducing inputs of PAHs into the environment and biota of Kitimat Arm. Concentrations of PAHs in sediments and fish, as well as fish liver lesion prevalences, have remained stable or declined over the past 5 years of sampling. At the Alcan Inner Harbour site, for example, concentrations of ΣHAHs in sediments and salmon stomach contents in 2004 were both lower than levels measured in the initial assessment in 2000. Also, liver lesions and DNA adducts in flatfish from sites near the smelter have tended to decline with time.

In addition to the process improvements already in place, Alcan has developed a comprehensive program to continue reducing PAH releases from the smelting process at Kitimat. This program will cost millions of dollars over several years, and will realistically result in an approximate 7% decline in releases to the air. In addition to the air release process changes

proposed by Alcan in the coming years, the company is taking steps to address PAH hot spots in sediments close to the smelter through sediment removal, capping, or other forms of remediation.

Given the process changes already made at the smelter and the additional efforts currently underway to reduce PAH loadings, as well as the fact that sediment PAHs within Kitimat Arm are derived primarily from historical releases, we expect that further decreases in PAH concentrations should be observed over time. Based on other studies that have monitored recovery at PAH-contaminated sites after remediation (e.g., Myers et al. 2008), these changes will likely occur slowly, and should be confirmed by intermittent monitoring (e.g., every 3 to 5 years). The studies conducted thus far serve as a benchmark from which to assess improvements due to process changes and remediation efforts that Alcan may conduct in the future.

Conclusions

Overall, the Kitimat Marine Assessment Study was successful in collecting substantial baseline data on sediment chemistry, as well as multiyear sampling of juvenile salmon and marine flatfish. We determined that PAHs similar to those produced by the smelter were present at high concentrations in sediments nearest the Alcan plant and at more moderate levels at other sites within Kitimat Arm (e.g., Kitamaat Village and Emsley Cove). These contaminants were absorbed by fish in their food, and their metabolites were found in bile of flatfish and juvenile salmon.

As expected, given that fish metabolize PAHs and do not accumulate them in their tissues (Varanasi et al. 1989b), we found that levels of PAHs in muscle tissue of flatfish were very low—well below concentrations that would constitute a health risk to humans consuming the fish. The same may not be true, however, of mussels, clams, crabs, or other shellfish that, unlike fish, do accumulate PAHs in edible tissue (Meador 2003). Because these organisms were not sampled in this study, we cannot comment on their safety for human consumption.

Other than PAHs, we found very little contamination in marine organisms from Kitimat Arm. OC compounds and metals were not present at high concentrations in fish stomach contents, and levels in edible flesh of English sole were well below the action levels recommended by Canada Health and Welfare (Ahmed 1991).

Even though fish do not accumulate PAHs, their metabolites can be toxic and we found that the PAHs released from smelter operations were having some effects on the health of flatfish from Kitimat Arm. English sole from this area had PAH-related DNA damage and liver lesions rarely found in sole from the reference sites outside Douglas Channel. However, bile metabolite levels in salmon and flatfish, as well as prevalences of liver disease and DNA damage in flatfish, were lower than would be expected considering the concentrations of high molecular weight PAHs in sediments at sites near the smelter, where PAHs have been discharged in the past. Furthermore, neither yellowfin sole nor English sole showed significant evidence of PAH-related reproductive dysfunction as is commonly observed in other contaminated industrial areas such as Puget Sound (Johnson et al. 1998, 1999). While we found that yellowfin sole from sites within Kitimat Arm tended to be smaller and have reduced condition in comparison to fish from other sites, these effects were not clearly correlated with levels of PAH exposure. Overall, these findings support earlier studies indicating the limited and reduced bioavailability of soot-associated, smelter-derived PAHs.

Because we did not directly measure health effects of PAHs in juvenile salmon, we cannot be certain how PAHs may be affecting them. However, we did find that, with the exception of salmon from the site nearest the smelter (Alcan Inner Harbour), concentrations of PAHs in stomach contents and bile of salmon from Kitimat sites were below the levels typically associated with health effects such as immunosuppression and reduced growth. In salmon from Alcan Inner Harbour, exposure levels were comparable to those causing adverse changes in

growth and metabolism in feeding studies with PAHs (Meador et al. 2006). Immunosuppressive effects have also been found in salmon exposed to similar concentrations of PAHs in combination with other immunosuppressive contaminants such as PCBs (e.g., Arkoosh et al. 2001, Arkoosh and Collier 2002). Since PCBs and other OCs are present only at very low or undetectable concentrations in Alcan's receiving environment, it is uncertain whether PAHs alone would produce the same health impacts.

Our monitoring also yielded some anomalous data on PAHs at the Kildala and Kitlope reference sites. English sole from Kitlope, and to a lesser extent from Kildala, showed exposure to high molecular weight PAHs based on concentrations of metabolites of these compounds in bile and increased AHH activity. However, ΣPAH concentrations in both sediments and stomach contents of English sole from this site were low, and yellowfin sole collected from this site showed no signs of exposure. A laboratory exposure study with English sole indicated that compounds in Kitlope sediments, including perylene, a naturally occurring compound that is one of the main HAHs at Kitlope, are not responsible for high PAH metabolite levels in bile of Kitlope fish. The source of PAH exposure in Kitlope English sole remains unclear but could be from natural product PAHs in the water column. The fish from this site do not appear to be affected, as they showed no signs of liver disease or DNA damage.

Finally, our monitoring results suggest that process changes introduced by Alcan over the last decade have been effective at reducing inputs of PAHs into the environment and biota of Kitimat Arm, as PAH concentrations in sediments and fish, as well as fish liver lesion prevalences, have remained stable or declined during the past five years of sampling. Given the additional process improvements and cleanup efforts to come (Lachance et al. 2006), we would expect continuing improvement over time, which could be confirmed by monitoring on a periodic (e.g., 3–5 year) basis. The studies conducted thus far serve as a benchmark from which to assess improvements due to process changes and remediation efforts that Alcan may conduct in the future.

Glossary

ΣHAH. For *summed HAH*. Summed concentrations of high molecular weight aromatic hydrocarbons that include fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno(1,2,3-cd)pyrene, perylene, and benzo[ghi]perylene.

ΣLAH. For *summed LAH*. Summed concentrations of low molecular weight aromatic hydrocarbons that include naphthalene, 2-methylnaphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, and retene.

adducts. Chemicals (specifically in this report, metabolites of polycyclic aromatic compounds) that are covalently bound to the DNA in the nuclei of liver cells.

AH. For *aromatic hydrocarbon*. Organic compounds consisting of carbon and hydrogen and possessing at least one benzene ring; found in combustion and petroleum products.

AHH. For *aryl hydrocarbon hydroxylase*. An enzyme in fish and mammals associated with the CYP1A xenobiotic metabolizing enzyme system. This enzyme is activated after exposure to a wide range of chemical contaminants, including PAHs. AHH enzyme activity provides a measure of the relative exposure to certain contaminants.

Aldrin. A banned organochlorine pesticide that was used to control termites and grasshoppers in crops.

aliquot. A portion that represents a known quantitative relationship to the whole or to other portions.

analyte. A chemical that is analyzed in order to determine or confirm its presence or measure its concentration.

ANOVA. For *analysis of variance*. A statistical method used to study the effect of independent variables on a continuous dependent variable when the independent variables are nominal (e.g., separate sampling sites) rather than continuous (as in linear regression). For example, analysis of variance could be used to analyze whether there were significant differences in concentrations of a chemical in sediment among the different sampling sites.

anthropogenic. Associated with and generated by human activities.

antiestrogenic. Acting to prevent the effects of an estrogen, as in stopping the production of the female reproductive hormones such as estradiol.

ASE. For *accelerated solvent extraction*.

atomic absorption spectrophotometer. An analytical instrument used to detect low concentrations (parts per million) of metal atoms by measuring the amount of light at various wavelengths absorbed by the atoms or ions vaporized in a flame or an electrical furnace.

autoradiography. A technique in which radioactive compounds on chromatograms make their location known by exposure to photographic films or emulsions.

basophilic focus. A type of preneoplastic focus of cellular alteration in the liver, characterized by a well-circumscribed proliferation of hepatocytes whose cytoplasm stains primarily with the hematoxylin (basic) stain (blue to purple).

beach seine. A type of net consisting of two long (≈50 feet) wings approximately 6-feet deep with an upper float line and a lower lead line connected to a center pocket or bag. The net is swept or pulled in manually from just offshore towards the shoreline by two long lines connected to bridles at the end of each wing, with the fish captured in and sampled from the center bag. This fishing method is commonly used to capture nearshore fishes and juvenile salmonids.

benthic. In this study, living in association with the bottom of the sea; bottom-dwelling.

bile. A liquid secretion of the liver that passes into the common bile duct, then into the gall bladder, and then released into the small intestine to aid in digestion of food, especially fats. Until they can be excreted via the gut, it is also the site of storage of metabolites of PAHs, or fluorescent aromatic compounds (FACs), produced in the liver that are used as a measure of exposure to PAHs.

biliary. Pertaining to the multibranched bile collection system in the liver or to bile itself.

bioavailability. The rate and extent to which a chemical enters the tissues of an animal.

biomarker. A measurement of a biological function or process, often as a biological response to an environmental chemical, which gives a measure of exposure and sometimes also a toxic effect.

biota. Combined and total animal and plant life in an area.

bycatch. The portion of a catch of multiple fish and invertebrate species that is unwanted, not targeted, and typically released back into the area of capture.

carcinogenic. Capable of inducing, producing, or causing cancer.

centrifuge. A device that spins test tubes at high speeds, causing heavy particles in the liquid in the tubes to settle out at the bottom and the lighter liquid to go to the top; used to separate portions of mixtures, such as in blood to separate cellular elements from the plasma.

CF. For *condition factor*. An index of body robustness that relates body weight to body length, typically calculated as Fulton's condition factor, which is body weight (g)/body length^3. In this study we calculated this factor as gutted body weight (g)/length^3 (cm).

chi-square statistic. A statistic used to compare the tallies or counts of categorical responses (e.g., presence/absence data) between two or more independent groups.

chlordanes. A group of organochlorine pesticides, as well as their metabolites, used to control insects and mites. Includes heptachlor, heptachlor epoxide, α-chlordane, γ-chlordane, oxychlordane, *cis*-nonachlor, *trans*-nonachlor, and nonachlor III. These compounds have been banned for use or manufacture in many parts of the world, including Canada and the United States.

cholangiocellular carcinoma. A malignant neoplasm of biliary epithelial cell origin, and characterized by a disorganized proliferation of these cells in the form of abnormal appearing bile ducts.

cholangioma. A benign neoplasm of biliary epithelial cell origin, and characterized by an organized proliferation of these cells in the form of normal appearing bile ducts.

chromatogram. The permanent record produced by chromatography.

chromatography. A method of separating two or more chemical compounds in solution by virtue of their being removed from solution at different rates when percolated down a column of a powdered or coated absorbent or passed across the surface of an absorbent paper.

chrysene. A high molecular weight, fluorescent polycyclic aromatic hydrocarbon that is frequently measured in urban and semiurban marine environments. It occurs in coal tar and petroleum and can be formed during the burning of petroleum, fats, and oils. Chrysene can enter the marine environment by direct (e.g., oil spill) or indirect (e.g., atmospheric deposition) means.

clear cell focus. A type of preneoplastic focus of cellular alteration in the liver, characterized by a well-circumscribed proliferation of hepatocytes whose cytoplasm is unstained and contains primarily glycogen.

composite. A sample of a particular tissue or body component consisting of samples from a combination of several to many individuals.

correlated. The degree to which one variable or factor increases or decreases with respect to another variable or factor.

cryogenic vials. Unique tissue specimen containers capable of enduring extremely low temperatures, such as those associated with freezing tissues in liquid nitrogen (–320°F or –196°C).

CYP1A. For *cytochrome P4501A*. A protein involved in the metabolism of PAHs and other compounds. Aryl hydrocarbon hydroxylase (AHH) is one specific enzyme activity associated with CYP1A.

cytotoxicity. The degree to which something is toxic to living cells.

DDT. For *dichlorodiphenyltrichloroethane* and its metabolites, including *p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, *o,p'*-DDD, *o,p'*-DDE, and *o,p'*-DDT. These are banned organochlorine pesticides that were used to control insects that harm crops, as well as malaria-carrying mosquitoes. DDTs are still used in some parts of the world to control mosquitoes.

dieldrin. An organochlorine pesticide that was used to control for insects and mites. One of the "dirty dozen" compounds that have been banned for use or manufacture in many parts of the world, including Canada and the United States.

dioxins. A class of chemical contaminants that are formed during combustion processes such as waste incineration, forest fires, and backyard trash burning, as well as during some industrial processes such as paper pulp bleaching and herbicide manufacturing. The most toxic chemical in the class is 2,3,7,8-tetrachlorodibenzo-para-dioxin (TCDD). Some PCB congeners are structurally similar to and have toxic effects much like those of dioxins.

DNA. For *deoxyribonucleic acid*. A nucleic acid that is present in chromosomes of the nuclei and is the chemical basis of heredity and the carrier of genetic information for all organisms (except RNA viruses).

Dunnett's Compare to Control Test. A statistical test used to identify groups whose means are significantly different from a reference or control group.

effluent. An outflow of waste, as from a sewer or an industrial process.

EI. For *electron impact*.

elute. To separate one material from another by washing.

endoplasmic reticulum. A network of interconnected tubules, vesicles, and sacs within the cell. It serves specialized functions including protein synthesis, sequestration of calcium, production of steroids, storage and production of glycogen, and insertion of membrane proteins.

endosulfan I. Organochlorine compound used to control insects and preserve wood.

enzyme. An organic catalyst, consisting of complex proteins, that is produced by living cells but is capable of acting independently. An enzyme is capable of inducing chemical changes in other substances without being changed itself.

eosinophilic focus(i). A type of preneoplastic focus of cellular alteration in the liver, characterized by a well-circumscribed proliferation of hepatocytes whose cytoplasm stains primarily with the eosin (acid) stain (pink to red).

epithelial. Composed of cells that line the cavities and surfaces of structures throughout the body.

estrogenic. Acting to produce the effects of an estrogen, as in inducing female reproductive hormones such as estradiol.

estuarine. Relating to an estuary, which is an arm of the sea that extends inland to meet the mouth of a river or several rivers, as in the Kitimat Arm.

FAC. For *fluorescent aromatic compound*. Compounds that contain two or more benzene rings that fluoresce at certain wavelength pairs. Metabolites of these compounds can be measured in fish bile to determine whether the animals have been recently exposed to polycyclic aromatic compounds.

FCA. For *foci of cellular alteration***.** Focal lesions in the liver considered to be preneoplastic or capable of progressing to neoplasms.

fertilization success. The likelihood that eggs will be successfully fertilized by sperm.

Fisher's Exact Test. A statistical method to test whether the occurrence of a binomially distributed outcome (e.g., lesion presence or absence in the liver) of fish from a particular site is significantly different from that in fish from a reference or control site.

fixative. A liquid typically containing alcohol and formaldehyde that is used to preserve tissues for histology.

FLA. For *fluoranthene***.** A high molecular weight polycyclic aromatic hydrocarbon that occurs in coal tar.

flatfish. In the context of this study, flatfish are English sole (*Parophrys vetulus*) or yellowfin sole (*Limanda aspera*), which possess a flattened body symmetry such that the eyes are on the dorsal or upper surface of the body, and the fish lie in or on the sediment in an essentially flat position.

fluorene. A low molecular weight polycyclic aromatic hydrocarbon that occurs in coal tar and coke oven tar.

fluorescence detection. An analytical method to measure the visual emission from molecules that have been excited to higher energy levels by absorption of light energy. Low and high molecular weight polycyclic aromatic compounds that occur in coal tar, coke tar, and crude oil are capable of fluorescing at specific wavelengths.

focal lesion. A localized lesion or injury to tissue.

follicular atresia. Resorption or degeneration of the ovarian follicle or oocyte (maturing egg).

fork length. Length of a fish measured from the tip of the snout to the fork in the tail or caudal fin.

furans. A group of colorless, liquid organochlorine compounds that are prepared from wood tar and used as solvents for resins and plastics. They are toxic to laboratory animals, wildlife, and humans and are suspected to cause cancer.

GC/MS. For *gas chromatography/mass spectrometry*. An analytical method used to measure low levels of chlorinated pesticides and industrial chemicals by first separating them by means of a narrow glass tube or column and eluting the sample components. As each component enters the mass spectroscope, it is bombarded by medium energy electrons, which result in a characteristic fingerprint of its molecular fragments and low energy electrons, which result in an electrical charge picked up by molecules containing halide, sulfur, or oxygen atoms.

gonad. A reproductive organ, specifically either the male reproductive organ (testis) or female reproductive organ (ovary).

GSI. For *gonadosomatic index*. An index of the ratio of total gonad weight to body weight, typically calculated as gonad weight divided by either total body weight or gutted body weight. In this study, the GSI was calculated as (gonad weight (g)/gutted body weight (g)) × 100.

G-statistic. A type of statistic used to test for differences among proportions.

HAH. For *high molecular weight PAH*, including:

BZP	benzo[ghi]perylene
DBA	dibenzanthracenes (dibenz[a,h]anthracene/dibenz[a,c]anthracene)
IDP	indeno[1,2,3-cd]pyrene
PER	perylene
BaP	benzo[a]pyrene
BEP	benzo[e]pyrene
BKF	benzo[k]fluoranthene
BBF	benzo[b]fluoranthene
CHR	chrysene
BAA	benzo[a]anthracene
PYR	pyrene
FLA	fluoranthene

HCHs. For *hexachlorocyclohexanes*, including α-HCH, β-HCH, and γ-HCH (lindane). This group of banned organochlorines was used to control insects and as a pharmaceutical to control head lice and scabies. They are suspected carcinogens.

heavy metals. A group of higher atomic weight elements that have the properties of a metallic substance at room temperature. They include zinc, lead, strontium, cadmium, and copper. Living organisms require trace levels of certain heavy metals whereas other heavy metals are not required and have been shown to be toxic to humans, laboratory animals, or wildlife.

hematoxylin and eosin. A combination of biological stains commonly used in histology to differentially stain the various components of living tissue and cells.

heparinized. Treated with heparin, an anticoagulant that interferes with the process of blood coagulation or thickening, making it possible to collect blood plasma.

hepatic. Related or pertaining to the liver.

hepatic megalocytosis. A liver lesion in the specific degeneration/necrosis category characterized by both enlarged hepatocellular nuclei of various sizes and enlarged cell diameters or cellular hypertrophy. This is considered to be a lesion manifesting cytotoxicity.

hepatocellular adenoma. A benign neoplasm of hepatocyte origin, characterized by an organized proliferation of normal appearing hepatocytes.

hepatocellular carcinoma. A malignant neoplasm, or cancer, of hepatocyte origin, characterized by a disorganized proliferation of abnormal appearing hepatocytes.

hepatocellular nuclei. Nuclei or liver cells.

hepatocyte. Liver cell.

histopathology. The histology or microscopic structure of diseased tissues, as typically assessed with a light microscope.

HPLC. For *high performance liquid chromatography*. An analytical method used to separate chemicals based on shape, size, charge, etc. This method is routinely used to separate chemicals that cannot withstand high temperatures or are polar (water soluble).

hydrolyze. To decompose or break down a chemical into simpler components by the addition or the taking up of the elements of water.

immunocompetence. The ability to mount an immune response to an infectious agent or to develop an antigenic response to stimulation by an antigen. An immune response is the reaction of an organism to substances that are foreign or are interpreted as being foreign (i.e., an antigen).

immunosuppression. Reduction or decrease in various aspects of the cell-mediated or humoral-related branches of the immune response, or the organism's natural response to an antigen or foreign substance.

indigenous. Living or occurring naturally within a specific area or environment; native.

intertidal. The region between the extremes of high and low tide.

isocratic. In liquid chromatography, isocratic flow indicates the movement of a liquid of constant composition (100% water).

LAH. For *low molecular weight PAH*, including:

MP1	1-methylphenanthrene
ANT	anthracene
PHN	phenanthrene
DBT	dibenzothiophene
FLU	fluorene
TMN	2,3,5-trimethylnaphthalene
ACE	acenaphthene

ACY acenaphthylene
DMN 2,6-dimethylnaphthalene
BPH biphenyl
MN1 1-methylnaphthalene
MN2 2-methylnaphthalene
NPH naphthalene

lesion. An area of pathologically altered tissue; an injury or wound.

linear regression analysis. A statistical method of relating and predicting the linear or direct changes in one outcome or dependent variable with respect to changes in one or more independent predictor variables that are continuous in nature (e.g., changes in length as predicted by changes in weight).

liquid scintillation spectrometry. A method for counting radioactivity using liquid scintillators to convert energy from radioactive emissions to photons that can be easily measured.

logistic regression. A statistical method of analyzing epidemiological or epizootiological data that is binomial (e.g., presence/absence of a condition) or proportional (prevalence or percent of animals affected) to examine the influence of multiple risk factors on the probability of disease occurrence, as well as exposure-response relationships. This method allows for the incorporation of risk factors unrelated to contaminant exposure, such as fish age or size, into models examining the relationship between contaminant exposure and disease.

log-transformed. Data that has been transformed by taking the logarithm of the actual number. This technique is often employed to transform data that does not have a normal distribution into one that does, so that parametric statistical methods such as ANOVA can be performed.

LOQ. For *limit of quantitation*.

LSI. For *liver somatic index*. An index of the ratio of liver to body weight, typically calculated as liver weight divided by either total body weight or gutted body weight. In this study LSI was calculated as (liver weight (g)/gutted body weight (g)) × 100.

lymphoid infiltrates. A collection or infiltration in a tissue of cells of the lymphocyte series, representing the cellular component of tissue inflammation.

macrophage. Cell of the immune system having the ability to phagocytose or engulf particulate substances.

macrophage aggregate. Spherical collection of organized, unique macrophages in various organs of fish containing the pigments of hemosiderin, ceroid or lipofuscin, and melanin. Macrophage aggregates are considered the functional equivalent of the reticuloendothelial system, and have been used as nonspecific indicators of exposure to multiple environmental stressors, such as chemicals, poor nutrition, increased heat, and infectious diseases, but are also related to increased fish age.

metabolite. Chemical compound that has been changed from its original structure by biochemical and physical processes—or metabolism—occurring in an organism, such as fish; a breakdown product.

method blank. A quality control sample analyzed with each sample set that is extracted and analyzed exactly as a field sample but which contains no tissue or sediment. This sample helps determine if any interference has occurred during any portion of chemical analysis or by the chemicals or solvents (liquids) used.

microsome. One of the particles derived from the endoplasmic reticulum of cells.

mutagenic. The ability to cause a genetic mutation.

necropsy. The process of examining and dissecting a dead body, in this research for the purpose of collecting biological tissues or fluids for various scientific analyses.

neoplasm. Tumors or growths in living organisms characterized by a new and abnormal formation of tissue. These tumors are classified as benign or malignant, and are typically named after the cell type of origin, such as hepatocytes (parenchymal epithelial cells of the liver). Benign tumors of epithelial cell origin are typically called adenomas and malignant tumors of epithelial cell origin are called carcinomas.

ng/g. For *nanograms per gram*, or parts per billion.

NIST. For *National Institute of Standards and Technology*.

nuclear pleomorphism. A lesion in the specific degeneration/necrosis category, characterized by enlarged hepatocellular nuclei of various shapes. This is considered to be a lesion manifesting cytotoxicity.

nucleotides. A compound formed of phosphoric acid, sugar, and a base (purine or pyrimidine), all of which constitute the structural unit of nucleic acid; a mononucleotide.

OC. For *organochlorine*. Any of a broad group of chemicals that includes chlorinated pesticides (e.g., DDT, lindane) and industrial compounds (e.g., PCBs). Many of these compounds have been banned for use in Canada and the United States due to their toxic effects to laboratory animals and wildlife.

otoliths. Oval-shaped ear bones in the head or cranial cavity of fish that are typically used to age fish much as the age of a tree is determined, by counting the annual rings.

otter trawl. A fish capture device consisting of a large cone-shaped net with two wings attached to two heavy doors (otter boards) opposite each other and between a heavy chain as a foot rope and a head rope with small floats that is dragged along the bottom.

outmigrant. In the context of juvenile salmonids, those fish migrating out from freshwater in streams or rivers to mixed freshwater/saltwater estuaries, then to the ocean, typically

occurring between late April to June or July in Chinook salmon (*Oncorhynchus tshawytscha*).

PAH. For *polycyclic aromatic hydrocarbon*. Chemical compounds that are formed by incomplete combustion of carbon-containing fuels such as wood, coal, diesel, fat, or tobacco. Many of these compounds are known or suspected to cause cancer in laboratory animals, wildlife, and humans.

pathology. The study of the nature and cause of disease, which involves changes in structure and function; commonly used to refer to the presence of disease.

PCB. For *polychlorinated biphenyl*. Any of a group of man-made industrial chemicals used as dielectric fluids in electrical transformers, machining oils, additives to paint, carbonless paper, and other products. These compounds are persistent (do not readily break down) and have been measured in environmental samples worldwide, including pristine regions. As a result of their persistence, PCBs can build up to relatively high levels in marine fish, sediments, and mammals. Some individual PCB congeners have been shown to be toxic to laboratory animals and wildlife. PCBs were banned for most uses and manufacture in Canada and the United States in the 1970s.

PCB congener. A member of the PCBs. There are 209 possible congeners; various congeners are present in the technical mixtures of these industrial compounds.

pelagic. Living in the upper water layers in a body of water.

perylene. A PAH that is formed as a byproduct of decaying organic matter from both aquatic and terrestrial areas.

PHN. For *phenanthrene*. A low molecular weight, (fluorescent) polycyclic aromatic compound that occurs in coal tar and crude oil.

PHN equivalents. Unit of measurement for phenanthrene metabolites in fish bile. These compounds are measured by UV fluorescence detection and calculated based on the ratio of their excitation to that of the phenanthrene standard.

plasma. The liquid part of the blood consisting of serum, proteins, and chemical substances dissolved in water.

polystyrene. A combustible, transparent plastic with high strength and impact resistance. It is an excellent electrical and thermal insulator that is also widely used for packaging, insulation, and lamination.

Ponar grab sampler. A device used to collect bottom sediments, consisting of a clamshell shaped chamber attached to two arms that, when deployed from a line from a boat, captures surface sediments between the two halves of the clamshells that can then be sampled.

preneoplastic. Capable of progressing to a neoplasm or tumor.

prevalence. The proportion (percentage) of animals affected by a certain disease or lesion at a particular point in time.

pyrene. A high molecular weight PAH that occurs in coal tar and crude oils.

pyrene-1-glucuronide equivalent. Unit of measurement for pyrene metabolites in fish bile.

QA. For *quality assurance*.

reequilibrate. To go back to a baseline state.

reference site. A control site chosen for sampling because of its remoteness from contaminant sources and, consequently, low levels of chemical contaminants in sediments and associated organisms such as fish and invertebrates.

replicate. A duplicate sample that contains material from the same sample as a first sample.

retene. A naturally occurring low molecular weight aromatic hydrocarbon associated with wood products and wood debris. Commonly measured in environmental samples collected near pulp mills and other wood processing industries.

reticuloendothelial system. A part of the immune system that consists of the phagocytic cells (e.g., macrophages) located in connective tissue.

reverse-phase analytical column. A high-performance liquid chromatography column used to separate compounds using a nonpolar column packing material and a moderately polar liquid that is passed through the column.

SDN. For *specific degeneration/necrosis*. A category of hepatocellular lesions in the liver consisting of two unique lesions characterized either by enlarged nuclei of various sizes (nuclear pleomorphism) or by both enlarged nuclei of various sizes and enlarged cell diameters or cellular hypertrophy (hepatic megalocytosis).

sediment. The bottom substrate of a river, estuary, lake, or ocean. Typically sediments are either fine gravel, sand, or mud or composed of clay or some combination of these three components.

SIM. For *single ion monitoring*.

size exclusion chromatography. An analytical method that separates compounds based on size and shape.

SRM. For *standard reference material*.

storage phosphor imaging. A technique in which radioactivity on a chromatogram is imaged using a storage phosphor screen. The screen is then scanned to get a digital image of the radioactivity distribution on a chromatogram. Storage phosphor imaging is far more sensitive than autoradiography.

subtidal. In the region below the extreme low tide.

surrogate standard. A compound added at a known concentration to a field sample prior to processing that provides a measure of the overall efficiency of the method (recovery). Surrogate standards have chemical characteristics that are similar to that of the analyte but must provide an analytical response that is distinct from that of the analyte.

toxicopathic. A disease or lesion having an etiology or cause that is related to exposure to toxic chemicals.

transect. In this study, the line or curve describing the route traveled while conducting a fishing trawl or tow.

van Veen grab sampler. A device used to collect bottom sediments, similar in mechanism to that of the Ponar grab sampler but with a larger capacity.

viable. In a living state or condition.

vitellogenic. A stage of maturation in the ovary where the oocytes or developing eggs are accumulating yolk, or vitellin.

xenobiotic. A chemical substance foreign to body; not naturally produced.

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Appendix: English Sole Laboratory Exposure Study

Introduction

As part of the Kitimat Natural Resource Assessment Study, English sole (*Parophrys vetulus*) were sampled from two minimally disturbed reference sites outside Kitimat Arm: Kildala Arm and Kitlope. At both sites, summed polycyclic aromatic hydrocarbons (ΣPAH) concentrations in sediments were typically less than 150 ng/g dry wt, comparable to or lower than ΣPAH concentrations measured in sediments from nonurban references sites along the Pacific coast of the United States and Canada (Brown et al. 1998, Yunker et al. 1999, McCain et al. 2000, Bolton et al. 2004). Moreover, the principal compounds present were perylene and retene, PAHs derived from natural products. However, sole from these sites had relatively high levels of PAH metabolites in bile, as well as increased aryl hydrocarbon hydroxylase (AHH) activity.

We hypothesized that apparent exposure to low molecular weight aromatic hydrocarbons (LAHs) in sole from these pristine sites might be accounted for, at least in part, by uptake of retene or related wood product derivatives, which are present in the stomach contents of these animals. Retenes have been shown to be effective inducers of cytochrome P450 (CYP1A) enzymes such as AHH (Fragoso et al. 1999) and are also metabolized and detectable in fish bile (Fragoso et al. 1999, Leppänen and Oikari 1999). Exposure to LAHs in the form of fuel oil or related petroleum products was also a possibility. Such exposure could be transient and related to localized spills or other releases of these substances into the water column.

However, the presence of high molecular weight polycyclic aromatic hydrocarbon (HAH) metabolites in bile of Kitlope fish was not easily explained. The absence of lesions or significant concentrations of DNA adducts in livers of these fish and the low concentrations of HAHs in both sediment and stomach contents suggested that exposure is short-term. However, it appeared to be fairly consistent, as elevated bile metabolite levels were observed in Kitlope English sole in 2000, 2002, and 2004. Thus it did not appear to be an anomaly due to a transient, localized spill or to some contamination inadvertently introduced into the water while fish were being held on the sampling vessel. Nor was it likely to be due to analytical problems, both because of its persistence and because the Kitlope samples were recomposited and reanalyzed and the results obtained were very similar.

To better understand possible reasons for the elevated bile metabolite levels in sole from the Kitlope sampling site and to characterize the contributions of natural products (e.g., perylene and retene) to biomarker responses in salmon (*Oncorhynchus* sp.) and sole from the Kitimat sites, a laboratory exposure study was conducted on English sole at the Northwest Fisheries Science Center (NWFSC) in Seattle, Washington. English sole from an uncontaminated site in Puget Sound were injected with extracts of sediments from Hospital Beach and Kitlope. The

objective of the study was to evaluate dose response of biomarkers in English sole exposed to sediments collected from Hospital Beach in Kitimat Arm and Kitlope and to establish whether exposure, especially at the Kitlope site, was due to compounds in sediments.

A related objective was to determine how the naturally derived PAH, perylene—which makes up a high proportion of HAHs in Kitlope sediments—may be affecting bile metabolite levels and other biomarkers in English sole. Perylene is derived from the breakdown of organic matter (Wakeham et al. 1980, Pichler et al. 1996, Irwin et al. 1998, Yunker et al. 1999). Due to the presence of wood processing industries, as well as naturally decomposing organic material at our Kitimat Arm, Kildala, and Kitlope sampling sites, perylene is present in sediments from all of these areas. Perylene makes up an especially high proportion of HAHs in Kitlope sediments and in stomach contents of English sole from Kitlope.

Fluorescence of perylene occurs at the same wavelength as other HAHs, such as benzo[a]pyrene (BaP), so we hypothesized that it might be having an unusual affect on bile fluorescence levels, masking or confounding fluorescence signals of metabolites of smelter-derived HAHs present in the bile. In this laboratory exposure study, English sole were exposed to Hospital Beach and Kitlope sediment extracts, as well as perylene, so the effects of these substances on bile metabolite levels and other indicators of PAH exposure could be evaluated in a controlled environment. Because the methodologies used were similar, results could also be compared with previous exposure studies conducted by our laboratory with extracts from other PAH-contaminated urban sediments.

Methods

English sole were collected by otter trawl from an uncontaminated reference site, Pilot Point in Puget Sound, following procedures described in the Puget Sound Protocols (PTI 1990). Fish were then transported to the NWFSC saltwater facility in Mukilteo, Washington, and allowed to acclimate to laboratory conditions for 3 weeks prior to exposures. Bile samples were collected in a subset of fish at the time of capture to determine baseline concentrations of metabolites as described in Krahn et al. (1986) and Stehr et al. (1993).

Sediment extracts were prepared from approximately 500 g each of sediment collected from Hospital Beach and Kitlope, as described in Stein et al. (1991) and Arkoosh et al. (2001). Fish were injected with sediment extracts at the following doses: 2, 10 and 50 g sediment/kg body weight. Perylene was injected at 1.25 mg/kg body weight. Injections were delivered in acetone:emulphor and fish were held for 5 days. Untreated and acetone:emulphor-exposed groups were also included as controls. Each treatment group was made up of 10 sole. Fish were not fed during the exposure period.

At the end of the exposure period, the sole were necropsied and tissues collected for evaluation of PAH exposure and biomarker responses as described in Stehr et al. (1993). Samples collected included bile for analysis of PAH metabolites, liver tissue for measurement of DNA adducts and CYP1A (enzyme) activity, and muscle for measurement of PAH concentrations. Samples were frozen and held at –80°C pending analysis.

Flatfish and salmon bile samples were analyzed by high-performance liquid chromatography with fluorescence detection for PAH metabolites as described in Krahn et al. (1986b); see also the Methods section of the body of our report. Other samples were archived for analysis at a later date.

Results and Discussion

English sole injected with extracts of sediments from Hospital Beach showed a dose-dependent increase in HAH metabolite levels (i.e., fluorescent aromatic compounds measured at BaP wavelengths [BaP equivalents]) (Figure A-1). Final concentrations 5 days after injection ranged from about 175 µg BaP equivalents/mg protein for the 2 g/kg group to 400 µg BaP equivalents/mg protein for the 50 g/kg group. Concentrations of BaP equivalents/mg protein in fish at the time of capture and in acetone:emulphor and untreated controls ranged from 90 to 100 µg BaP/mg protein.

In contrast to sole treated with Hospital Beach sediment extract, BaP equivalent concentrations in fish receiving the Kitlope sediment extract showed no dose-related increase, but remained at concentrations similar to those in control fish for all treatments (Figure A-1). The same was true for PAH metabolites measured at phenanthrene wavelength (PHN equivalents) (Figure A-2). A dose response relationship was observable for fish injected with Hospital Beach sediment extracts, but there was no change in bile metabolite levels in fish

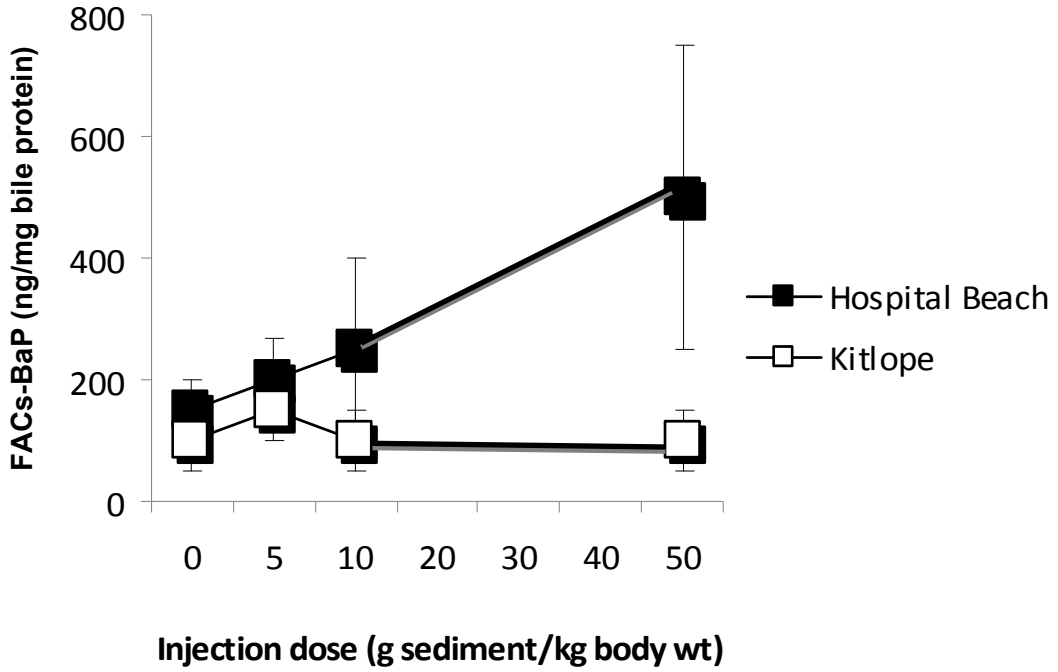

Figure A-1. Levels of fluorescent aromatics compounds (FACs) measured at benzo[a]pyrene (BaP) wavelengths (ng/mg bile protein) in bile of English sole exposed to extracts of sediments from Hospital Beach and Kitlope at doses ranging from 0 to 50 g sediment extract/kg body weight.

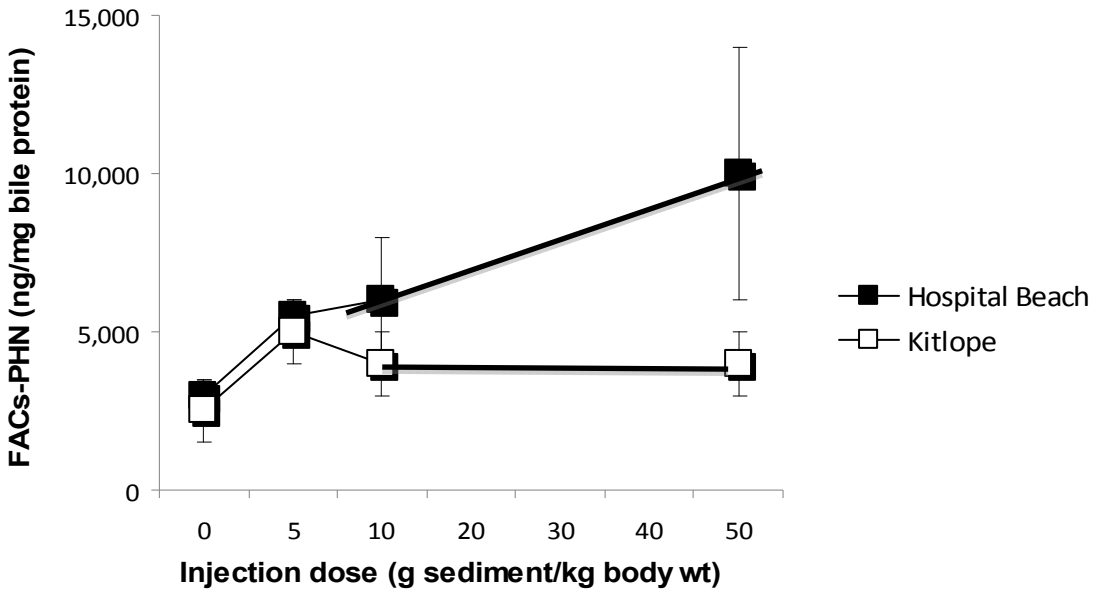

Figure A-2. Levels of FACs measured at phenanthrene (PHN) wavelengths (ng/mg bile protein) in bile of English sole exposed to extracts of sediments from Hospital Beach and Kitlope at doses ranging from 0 to 50 g sediment extract/kg body weight.

injected with Kitlope sediment extracts. Fish injected with perylene responded in much the same way as fish injected with Kitlope sediments (Figure A-3). No significant changes were observed in levels of either BaP equivalents or PHN equivalents in perylene-treated fish as compared to fish at the time of capture, untreated controls, and acetone:emulphor controls.

In summary, English sole injected with extracts of Hospital Beach sediments showed a strong dose-dependent increase in HAH metabolites levels, but sole receiving perylene or the Kitlope sediment extract showed no change in bile metabolite levels. These results suggest that neither perylene nor compounds found in Kitlope sediments are responsible for elevated bile metabolite concentrations in wild English sole sampled from Kitlope. A logical next step is to identify the specific compounds in bile of English sole from Kitlope using bile hydrolysis and gas chromatography/mass spectrometry analysis (e.g., see Krahn and Stein 1997). This may provide clues as to the source of PAH exposure in this isolated fjord.

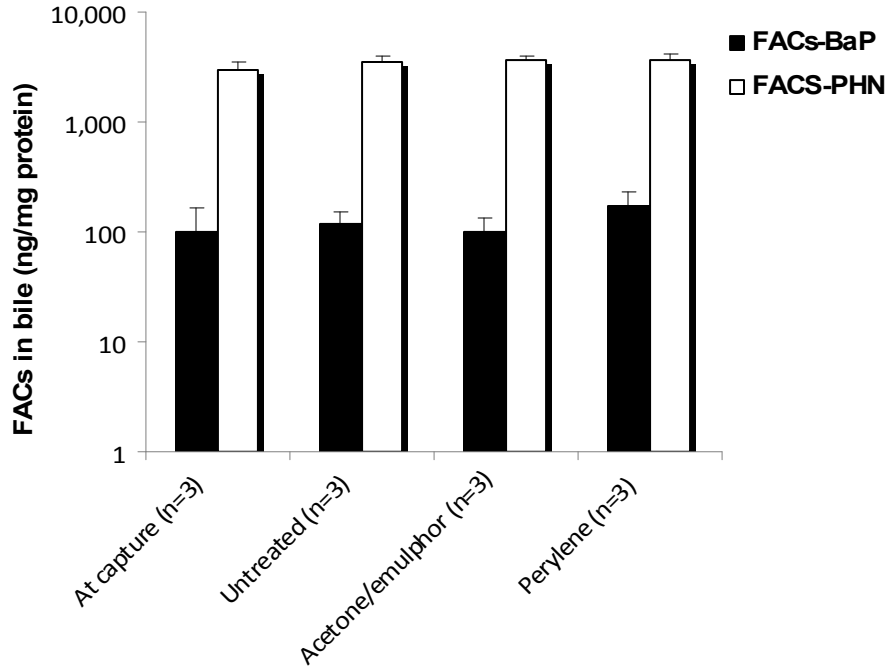

Figure A-3. Mean concentrations (±SE) of FACs in bile of English sole injected with perylene (ng/mg bile protein). Metabolites of high molecular weight aromatic hydrocarbons (HAHs) were measured as BaP equivalents, and metabolites of low molecular weight atomatic hydrocarbons (LAHs) were measured as PHN equivalents. Concentrations of bile metabolites were not significantly higher than levels in recently captured fish, untreated fish, or acetone:emulphor controls. Fish were injected with perylene at a dose of 1.2 mg/kg body wt.

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