Potential Effects on Fraser River Salmon from an oil spill by the Trans Mountain Expansion Project

Prepared for the National Energy Board (NEB) hearings reviewing Kinder Morgan's proposed Trans Mountain Expansion project

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ABBREVIATIONS

ANSC Alaska North Slope crude oil

BSD Blue sac disease

BTEX Benzene, toluene, ethylbenzene, and xylenes

CU Conservation unit

CYP1A Cytochrome P450 1A1 protein

EROD Ethoxyresorufin-O-deethylase

HFO Heavy fuel oil

HMW High molecular weight

Kow Octanol-water partition coefficient

LMW Low molecular weight

OSA Oil-sediment aggregates

PAH Polycyclic aromatic hydrocarbon

RoW Right-of-Way

SARA Species at Risk Act

SCAT Shoreline cleanup assessment technique

TLM Target lipid model

TPAH Sum of alkyl substituted and unsubstituted PAH

EXECUTIVE SUMMARY

This report examines the potential consequences to Fraser River salmon populations from exposure to crude oil spilled from a pipeline rupture in the lower Fraser River or from an oil tanker spill in the waters of Georgia Strait. This report also identifies risks to salmon populations outside the Fraser River and non-salmonid fish, including SARA and COSEWIC listed species, within the Fraser River.

Methodology and Approach

The literature on the toxicity of oil to fish was reviewed to provide a detailed analysis of the potential environmental effects of a petroleum product spill from the Trans Mountain Expansion pipeline. These effects are described by reviewing the nature of the Lower Fraser River, its estuary, and nearshore waters as fish habitat, the properties and constituents of the products that may be shipped via the proposed pipeline (primarily diluted bitumen), the fate and distribution of oil following a spill, and how different life stages of fish might be exposed to and affected by the constituents of oil. We provide an overview of the fish species that inhabit the Lower Fraser River, including the time of year that various life stages may be present and the habitat they would occupy. We examine the toxic effects of oil on different life stages of fish, the constituents of oil associated with those effects, concentrations that cause effects, and the consequences of oil toxicity to their survival. Much of the toxicity literature was originally reviewed in Hodson, Collier, and Martin in 2011. We have used their original work on hydrocarbon toxicity, with updates to the literature where available and specific references to the Lower Fraser River where appropriate.

Fraser River and Salish Sea fish populations

The Fraser River is one of the most productive salmon rivers in the world, supporting 56 different Conservation Units[1] of five species harvested in commercial, recreational and First Nation food, social and ceremonial fisheries. The Fraser River and its tributaries are home to a total of 42 different species of fish including 9 species of salmonids, 2 species of sturgeon, eulachon, and numerous important forage fish species. In addition to the Fraser, another 48 unique Conservation Units of salmon use the surrounding rivers, estuaries and nearshore waters of Georgia Strait, Haro Strait and the Juan de Fuca within the Canadian portion of the Salish Sea. US salmon populations and other salmonid species are in addition to this.

Critical habitat

The Fraser estuary, which is the largest on the west coast of North America at 21 703 ha, provides critical nursery habitats for juvenile salmon from throughout the watershed. As noted by Trans Mountain in their application, the Fraser River is home to a number of 1) federally

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[1] Evolutionarily distinct population of salmon that, if lost, is not likely replacable in human lifetimes

listed Species at Risk Act (SARA) species, including white sturgeon (Upper Fraser River Population), green sturgeon, salish sucker, and nooksack dace; 2) provincially Red-listed white sturgeon (Middle and Lower Fraser River Population); Blue-listed mountain sucker, bull trout, eulachon, and chiselmouth; and 3) Committee on the Status of Endangered Wildlife in Canada (COSEWIC) at-risk populations including Interior Fraser coho salmon, Cultus Lake sockeye salmon, Lower Fraser white sturgeon, and Fraser River eulachon populations. The Fraser and its tributaries are used by these fish species throughout the year either for incubating eggs and embryos, as juveniles for rearing and overwintering, and as adults for migration and spawning. For salmon, the Lower Fraser River acts as a bottleneck through which the entire diversity of Fraser River salmon populations must pass twice during their lifetime. Many depend heavily on Lower Fraser habitats for spawning, incubation and rearing. There is no safe time of the year when the impacts of a spill would be low. A spill during peak migration of economically important or at risk species could be devastating to these populations.

While the focus of this report is the Lower Fraser River and its estuary, we include information on Georgia Strait and the Salish Sea. Oil spilled in these locations has the potential to contaminate nearshore rearing habitat for juvenile salmon from the Fraser River, as well as dozens of other important salmon populations that rear and migrate in the waters of Georgia Strait and the Salish Sea.

Diluted bitumen

Kinder Morgan's proposed pipeline expansion is intended to carry primarily diluted bitumen products, which would be shipped from Alberta's tar sands by pipeline and exported from Vancouver by oil tanker. Bitumen differs from other petroleum products in that it has undergone a greater degree of microbial degradation. Because low molecular weight (LMW) hydrocarbons are preferentially degraded, bitumen is composed primarily of high molecular weight (HMW) hydrocarbons and as such is 'heavier' than crude oil. In order to be of sufficiently low viscosity to be pumped through a pipeline, bitumen must be diluted with condensate or another solvent. Because of the presence of LMW compounds in the diluent, the combined diluted bitumen product may be expected to cause fish kills similar in severity to those caused by spilled crude oil in the first hours and days following a spill. However, the bitumen remaining after the diluent weathered away would be expected to submerge or sink and potentially cause chronic toxicity[2] to aquatic species.

Bitumen (and other petroleum products such as crude oil) contains a wide array of hydrocarbons (including both low and high molecular weight compounds), but only some are sufficiently small to be soluble in water and bioavailable to fish. Low molecular weight linear (alkanes) and cyclic (aromatic) hydrocarbons are lost during weathering, but are sufficiently soluble in water to cause fish kills immediately after a spill. Because they are readily diluted and are also volatile, acutely lethal conditions do not generally persist beyond 24 to 48 hours, unless fresh oil continues to be added. Compounds with multiple rings (polycyclic aromatic

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[2] Any effects on exposed individuals that develop over a long period of time, either due to brief exposure (i.e. delayed effects) or to a prolonged or chronic exposure

hydrocarbons or PAHs) are less soluble, more persistent, and more slowly accumulated by fish. While they are less likely to cause acute lethality than smaller compounds, they cause chronic toxicity to fish as they partition back and forth between stranded oil, interstitial sediment, and water.

Condensate differs from bitumen and crude oil in that it is composed primarily of LMW hydrocarbons. If spilled, a higher proportion would be lost due to weathering than would be typical of a crude oil, and it would likely cause a greater fish kill than bitumen or crude oil. However, it would be less persistent and less likely to cause chronic toxicity, depending on what compounds are in the residue.

Fate and behaviour of spilled oil

An oil spill to a tributary or to the main stem of the lower Fraser River would spread quickly over water and move downstream, with the amount of oil stranding on shorelines varying with the time of year and the flow of the river. In the case of a winter spill, most spilled oil (>80%) is predicted to strand due to lower flows, while in summer, stranding would be less likely (<60% in the first three days). However, in summer oil would be more likely to flow into side channels, wetlands, and sloughs and become stranded.

An oil spill from a tanker transiting the marine waters of Georgia Strait could quickly arrive on the shoreline of the outer delta. Oil could potentially be carried into the river /estuary as far as New Westminster, depending on the season. Oil could arrive in near shore and estuarine habitats of the Fraser in hours depending on the spill location.

Acutely toxic conditions would likely occur in any area covered with an oil slick, and would last about 24-48 h, or until oil weathered significantly or cleared from the surface, unless fresh inputs of oil continued. The size and impacts of a fish kill are difficult to predict: very large fish kills are generally observed following spills of petroleum products that are lighter and which have a higher proportion of acutely toxic LMW compounds than does dilbit (e.g. gasoline, diesel fuel). Conversely, following a spill of dilbit into the Kalamazoo River, 42 dead fish were recovered. The presence of diluent (e.g. condensate) in dilbit raises concerns about the potential for a fish kill, particularly in areas with dense aggregations of fish (e.g. the spring outmigration of hundreds of thousands of juvenile Fraser salmon) but the acute toxicity of the bitumen itself is unknown.

Spilled oil would be subject to multiple weathering processes that would affect its distribution in the environment. As noted, most LMW compounds would be lost in the first 48 hours due to evaporation and dissolution, in addition to some biodegradation and photo-oxidation. As oil weathers further and mixes with water, water-in-oil emulsions can form, which can persist long after the initial spill. While the outer shell weathers, the oil inside emulsions can remain much fresher and thus pose an ongoing risk of toxicity. Both decreasing salinity and increasing particulate matter, conditions that would be expected in the lower Fraser River, have been found to stabilize water-in-oil emulsions. Also of concern as weathering progresses is the potential for oil to submerge and/or sink. This makes recovery of spilled oil much more difficult, if not impossible, and creates new exposure routes and toxic potential. Oil can sink when its density is greater than that of water, which can occur by various mechanisms. As oil weathers, the relative proportion of HMW compounds increases, thereby increasing the viscosity and the potential for oil to submerge or sink. Alternatively, suspended particulate can adhere to oil droplets (creating oil-sediment aggregates, or OSAs), decreasing their buoyancy and increasing the likelihood that they will submerge or sink.

In the event that oil is submerged in the water column or sinks to the river bottom, it can be entrained within bed sediments and pose a serious risk to developing salmonids. Entrainment could occur via hyporheic flows, in which negative pressure results in the flow of water through substrates (particularly gravel). As hydrocarbons partitioned from oil to water, they would be mobilized by hyporheic flow, and at low flow rates, hydrocarbon concentrations in interstitial waters of spawning shoals could reach concentrations toxic to fish.

Toxicity of oil to salmon

Salmon and other fish species that are most vulnerable to population-level impacts of spilled oil are those whose embryos would be chronically exposed to oil entrained in bed sediments. The immobility of embryos, coupled with extended incubation times in substrate, would result in extended exposure and an increased risk of toxicity. The range of water-borne TPAH concentrations that cause embryo toxicity (1 to 100 μg/L) are associated with concentrations of oil on substrate of approximately 2 to 8 000 mg/kg of gravel.

It is also important to consider that multiple life stages (e.g. resident juveniles and adults in addition to newly fertilized and developing embryos) may experience toxic effects as the result of a single spill event, the result of which would likely be multi-year class effects. There are many measureable biochemical and physiological responses of fish to oil exposure that allow for ongoing monitoring and assessment following an oil spill. However, behavioural responses, such as impacts on normal behaviours including migration, feeding, nest building and mating, are not well characterized. The ability of fish to avoid oil is also poorly understood, with studies presenting conflicting evidence. For obvious reasons, avoidance would be positive in that fish could avoid acutely toxic habitat and find refuge in clean areas upstream or in tributaries. However, avoidance may also mean that reproductive potential is lost if there is no suitable alternate habitat.

Concentrations of waterborne TPAH associated with chronic toxicity of oil to the early life stages of fish range from about 1 to 100 μg/L (parts per billion), with effects such as decreased growth rates and increased incidence of larval deformities observed at the low end of the range. Other effects of chronic toxicity include increased mortality rates, increased frequencies and severities of deformities and pathology, and increased rates of hydrocarbon metabolism and excretion. While toxicity can be expected to increase with TPAH concentrations in spilled oil, the amount that dissolves into water and is thus bioavailable may be determined more by the physical and chemical characteristics of the oil. Ultimately, it is the concentration of TPAH in water that determines toxicity, and existing data do not demonstrate wide differences in toxicity among oil types. Based on multiple studies, the individual PAHs of greatest concern include the 3- to 5-

ringed alkyl PAHs. However, recent work has also observed toxicity that could not be attributed to known and conventionally measured hydrocarbons, underscoring the significant knowledge gaps with respect to the toxicity of petroleum products.

Oil toxicity can be exacerbated by factors including weathering and photomodification (induced in the presence of sunlight). Weathering results in the enrichment of larger 3- to 6-ringed PAHs in the remaining oil, causing an apparent increase in chronic toxicity to the early life stages of fish. Photomodification can result in significant increases in the toxicity of some individual PAHs (e.g. anthracene) that may be accumulated by fish embryos. Because embryos are nearly transparent, photomodification can occur within the tissues, resulting in oxidative stress, cell death, and acute mortality.

Oil entrained in substrate would be expected to remain for a time period ranging from months to years, depending on flow rates through the substrate and the amount of oil trapped during the spill. Unless all contaminated substrate was physically removed and cleaned, hydrocarbons from stranded oil would partition into interstitial water, creating a chronic exposure regime for nearby spawning shoals and for aquatic life downstream of the stranded oil. Removal of contaminated substrate poses its own set of risks to aquatic life, including disruption and/or destruction of spawning habitat, siltation of the water column and subsequent smothering of fish embryos, and remobilization of contaminants.

The effects on fish reproduction of sediment contamination can also last from months to years, reflecting the residence time of oil in spawning shoals. Because all species in the lower Fraser River are sediment spawners (surface or sub-surface), runs may show multiple weak or missing year classes following an oil spill, depending on the extent to which their primary spawning areas were affected by the spill. There is a potential for reductions in returns of migrating adult salmon because of the delayed effects of exposure to oil as eggs and embryos that would reduce survival during growth and maturation at sea.

The toxic effects of spilled oil in the lower Fraser River may combine with other environmental and anthropogenic stressors to cause additional, unexpected effects, and/or effects of unexpected severity. Environmental stressors could include physiological changes associated with migration (saltwater and freshwater adaptation) and reproduction (sexual maturation, energy depletion), as well as seasonal changes in temperature, while anthropogenic stressors include habitat changes to water quality, water quantity, and physical features impacted by urban, industrial, and agricultural land uses and practices. The combined effects of oil toxicity (both acute and chronic) to multiple species and life stages, increased susceptibility to disease facilitated by increased stress and decreased immune function, and a potential period of reduced recruitment due to fewer spawners and contaminated spawning grounds could reduce overall fisheries productivity in the river for years to decades. Due to the site-specific (and spill-specific) nature of potential effects, it is extremely difficult to accurately predict the timing and severity of effects, but we have no reason to believe that they will not occur.

1 INTRODUCTION

On December 16, 2013, Kinder Morgan Trans Mountain filed an application with the National Energy Board (NEB) to expand their existing pipeline, which runs for 1150 km from Strathcona County, Alberta to Burnaby, British Columbia. The application states that the existing pipeline would be twinned, where possible, within the existing right-of-way (RoW). The proposed expansion would consist of approximately 994 km of new pipeline, reactivation of 193 km of pipeline, as well as the construction of 12 new pump stations, 20 new tanks at existing storage terminals, and three new berths at the Westridge Marine Terminal in Burnaby. In addition, more than 500 watercourses would be crossed. The new line is slated to carry ‘heavier oils’ (e.g. dilbit). The existing line will continue to carry refined products, such as synthetic and light crude oils.

The purpose of this report is to assess the potential risks associated with oil contamination in the Lower Fraser River and estuary (either as the result of a spill directly into the Fraser River or as the result of a spill into Georgia Strait) and the implications for salmon populations.

This report reviews and synthesizes published and unpublished literature on the toxicity of oil and its components to fish and the behaviour, fate, and effects of oil following a spill to aquatic ecosystems. Much of this information is drawn from the peer-reviewed literature, with some additional information from supporting documents prepared for Trans Mountain. In particular, Chapter 5 expands on a detailed review of oil toxicity to fish prepared for the Joint Review Panel of the Northern Gateway Pipeline proposal by Hodson, Collier, and Martin (2011). The risks posed to fish by spilled oil is a function of the sensitivity of each life stage, the extent of exposure to petroleum hydrocarbons, the toxicity of those hydrocarbons, and site-specific interactions among these factors.

Where possible and relevant, we will refer to the scant literature on the behaviour and fate of diluted bitumen, and to real world experience (e.g. the Enbridge spill into the Kalamazoo River in 2010).

This report is comprised of the following chapters:

- “Fish Habitat” (Chapter 2), which describes the hydrogeology of the Lower Fraser River and its tributaries, and the nature of the Lower Fraser River, the Fraser River estuary and delta, and the Salish Sea as salmon habitat;
- “Oil properties, behaviour and fate in the aquatic environment” (Chapter 3), which reviews the properties and constituents of crude oil, diluted bitumen, condensate, and synthetic crude oil; and assesses the fate and distribution of oil following a spill; and reviews the pathways by which fish at different life stages might be exposed to the constituents of oil;
- “Salmon and other fish species at risk in the Lower Fraser River and Salish Sea” (Chapter 4), which describes the salmonids and eulachon that utilize the Lower Fraser

River and the Salish Sea, the times of year at which various life stages may be present, the habitat they would occupy, the routes by which they might be exposed to spilled oil, and the consequences of oil toxicity to their survival and to the abundance and characteristics of fish populations;

- “Toxicity of oil to fish” (Chapter 5), which describes the toxic effects of oil products on different life stages of fish, the constituents of oil that are associated with toxicity, the concentrations required to cause effects, and factors that may modify toxicity;
- “Cumulative effects” (Chapter 6), which describes the historic and current use of the Fraser River and the potential implications of those uses for salmon populations; and
- “Concerns with Trans Mountain’s Submission to the National Energy Board” (Chapter 7), which discusses omissions and failures on the part of Trans Mountain in their application.

2 FISH HABITAT

The Fraser River is the largest river on Canada's west coast. The Lower Fraser River comprises the section of 180 km downstream of Hope. This section is a gravel bed reach until Mission, where the bed becomes predominantly sand. Saline intrusion occurs as far upstream as New Westminster. Human activity has significantly altered habitat in the Lower Fraser, with highly developed areas and floodplains isolated by dikes and floodgates. The Fraser River has historically been one of most productive salmon rivers in the world, and all Fraser salmon utilize the lower river and estuary to varying degrees to complete their lifecycle. These salmon species would all be at risk in the event of an oil spill in either the mainstem or tributaries of the Lower Fraser River.

Historically, the Fraser River was one of the world's most productive salmon rivers. Today it continues to support large runs of all five economically important salmon species (*Oncorhynchus spp.* Chinook, chum, coho, pink, and sockeye), producing over 50% of Canada's wild Pacific salmon (Levy and Northcote 1982; Northcote and Atagi 1997; FRAP 1999). The Fraser's five commercial salmon species come from more than 1070 spawning populations distributed through the mainstem and tributaries of the watershed. These populations are grouped into 56 distinct lineages known as Conservation Units (CUs; Holtby and Ciruna 2007; Grant et al. 2011; DFO 2013). A Conservation Unit is a level of genetic, ecological and geographical uniqueness that if lost, is not likely to be replaced by natural recolonization within human timeframes (Holtby and Ciruna 2007). All of these salmon species and populations migrate in and out of the Lower Fraser River through the estuary. They utilize the estuary to varying degrees as juveniles and adults to complete their lifecycle (Slaney et al. 1996; Healey 1982).

The Lower Fraser is also home to anadromous steelhead and cutthroat salmon (traditionally referred to as trout), resident salmonids (coastal cutthroat, rainbow, Dolly Varden, and bull trout) and 32 other ecologically important fish species. The Fraser is also home to COSEWIC designated at-risk populations of endangered Interior Fraser coho salmon, Cultus Lake sockeye salmon, and Fraser River eulachon populations. These fish species would all be at risk in the event of an oil spill in either the mainstem or tributaries of the Lower Fraser River, and many would also be at risk if spilled oil were to reach the outer Fraser estuary from Georgia Strait (discussed in Chapter 4).

In addition to their economic and cultural importance, adult salmon returning to these waters are important prey items in the diets of local cetaceans (Ford et al. 2005), pinnipeds (Lance & Jefferies 2007, Stansell et al. 2010), and terrestrial mammals (Darimont et al. 2010), as well as broader food webs of the Northeast Pacific coast. The federally endangered southern resident killer whales are an obligate predator on salmon with Chinook from the Fraser River comprising an important component of their diet for many months over the spring and summer (Ford et al. 2010). Juvenile salmon out-migrating from the Fraser River depend on the estuary's high biological productivity for food and its intertidal shoreline structure for rearing and protection.

These juvenile salmon in turn provide food for hundreds of thousands of resident and migratory birds and other fish species whose survival depends on the food and resources provided from the Fraser River delta.

2.1 LOWER FRASER RIVER AND ITS TRIBUTARIES

The Fraser River is the largest river on the west coast of Canada with a total length of over 1 375 km, and a watershed exceeding 230 000 km^2 (Milliman 1980; Richardson et al. 2000). The Fraser drains over one quarter of the province of British Columbia, passing through a variety of ecoregions ranging from coastal temperate rainforest to cold desert to alpine areas (Hall and Schreier 1996; Richardson et al. 2000). The headwaters originate in the Rocky Mountains, and flow west with high gradients in the first 100 km (McLean et al. 1999). The river then flows southward, passing through canyons and gorges with moderate gradients for approximately 900 km (McLean et al. 1999). It finally turns west towards the Pacific Ocean, reaching an extensive low gradient alluvial floodplain before discharging into the Salish Sea (Milliman 1980; McLean et al. 1999). The main stem of the river contains no dams or diversions, however flows are affected by reservoirs on some of its major tributaries (Richardson et al. 2000).

The Fraser River is a snowmelt driven system with considerable variation in flows, from peak flows of up to 15 200 $m^3\ s^{-1}$ (measured at Mission) during the freshet in late spring and early summer, to low flows of 680 $m^3\ s^{-1}$ in late summer, with a mean annual discharge of 3 410 $m^3\ s^{-1}$ (McLean et al. 1999; McLean and Church 1999; Richardson et al. 2000). Sediment discharge in the Fraser is sand-dominated, characterized by high sediment discharge in late spring and early summer, and low sediment discharge values from late summer through autumn and early spring (Milliman 1980; McLean et al. 1999). The average annual total suspended load in the lower Fraser is approximately 17 x 10^6 tonnes/year, of which the suspended sand load comprises about one third, approximately 5.5 x 10^6 tonnes/year (McLean et al. 1999). The river also transports a significant amount of gravel (averaging 2.0 x 10^5 tonnes/year of primarily 25-30 mm diameter gravel), the majority of which is deposited in the Lower Fraser reach between Hope and Mission (McLean and Church 1999). During the spring freshet, the sediment discharged is slightly over 50% sand, while during the remainder of the year sediment discharge is lower and primarily consists of silt and clay (Milliman 1980). The majority of sediment is suspended in the water column until it reaches the Lower Fraser, where approximately 20% settles in the upper estuary and much of the remainder settles before reaching the lower estuary (Milliman 1980).

The Lower Fraser River is the final 180 km section of river flowing from Hope, past Mission, through Metro Vancouver, and into the lower estuary where it meets the ocean. Near Hope, the river emerges from its rock canyon, flowing into a 55 km section that is comprised of a single-channel wandering gravel-bed with mid-channel islands and gravel bars that become exposed at low water levels (McLean and Church 1999; McLean et al. 1999). The majority of gravel is deposited in this section, and once the river reaches Mission, the gradient decreases significantly and the river becomes predominantly a sand-bed channel (Table 2.1.; McLean et al. 1999). Tidal influence extends upstream as far as Chilliwack during low discharge, but only to Mission during peak freshet, with the largest tidal range of 4.8 m at the mouth of the river

(Thomson 1981). During low flow periods, flood tides can cause reversal of river flows upstream to Mission, with water backing up and accumulating in delta and tributary channels (Drinnan and Clark 1980). The tendency of the river to reverse flow complicates interpretations of the fate and effects of point source discharges into the river (Drinnan and Clark 1980). Saline intrusion can extend up to 22 km upstream to New Westminster during low flows, but during the freshet does not generally extend past the Oak Street Bridge on the North Arm or past Steveston on the main arm (Ages 1979; Hall and Schreier 1996). At New Westminster the river splits into the South Arm, which carries approximately 88% of the total flow, and the North Arm, carrying the remaining 12% (Milliman 1980). Major tributaries which contribute substantially to flows in the Lower Fraser include the Pitt, Stave, Sumas-Chilliwack, and Harrison Rivers.

Table 2.1. Hydrological parameters of the Lower Fraser River at gauging stations within the main reaches. Different conditions will affect the fate and behavior of spilled oil and exposure pathways for salmonids and other fish species.

Gauging station (Surface slope)	**River bed sediment type**	**Flow condition**	**Mean discharge ($m^3 s^{-1}$)**	**Velocity ($m s^{-1}$)**
Hope ($6.0x10^{-4}$)	*Cobble-Gravel Bed*	LTM	2,830	1.5
		MAF	8,766	3.2
		Peak	12,900	4.0
Agassiz ($4.8x10^{-4}$)	*Gravel Bed*	LTM	2,880	1.4
		MAF	8,760	2.6
		Peak	13,100	3.2
Mission ($5.5x10^{-5}$)	*Sand Bed*	LTM	3,410	0.7
		MAF	9,790	1.5
		Peak	14,400	1.9

LTM: long term mean; MAF: mean annual flood; Peak: peak value over the study period which occurred in 1972. Reproduced from McLean et al. 1999. Based on 20 year dataset collected by the Water Survey of Canada between 1965 and 1986.

Historically, the Fraser River delta was an intricate floodplain of tidally influenced freshwater and estuarine creeks (Levings et al. 1995). However, human activity beginning in the late 19th century has significantly altered the Lower Fraser and its floodplain. More than 80% of foreshore wetlands, marshes and riparian forests in have been logged, diked, drained and/or converted for urban/agricultural uses (FRAP 1999). The Lower Fraser Valley (3 062 km^2), of which 700 km^2 is directly on the river's floodplain, comprises agricultural land, which is responsible for 50% of BC's farming output (Milliman 1980; Hall and Schreier 1996). Human encroachment along the river intensifies toward the estuary. It begins with light agriculture around Hope, that hosts natural floodplains and setback or absent dikes, to highly urbanized and agriculturally dominated areas downstream of Mission that are protected by flood control dikes along much of the river (Richardson et al. 2000). The Lower Fraser experienced significant flooding events in 1894 and 1948, which led to a flood control program consisting of main stem dikes, along with floodgates and pumping stations (Healey and Richardson 1996; Thomson et al. 1999). This engineering enabled continued urban and agricultural development on the floodplain and surrounding area. The region is now home to the greater urban area of Metro Vancouver, with approximately 2.6 million people (300,000 living on the floodplain itself) and structures with a total value of 13 billion dollars (Fraser Basin Council 2010a; Lyle and McLean 2008).

Historically, the Lower Fraser was connected to a network of large and small watercourses that supported 150 significant salmon spawning tributaries (FRAP 1999, Figure 2.1). Dense urbanization and agricultural intensification, with associated impacts including loss of riparian vegetation, channelization, diversions and poor water quality, has led most streams in the Lower Fraser Valley to be considered lost (20%), endangered (63%), or threatened (13%), with comparatively few considered wild (5%) (PIBC 1997). The loss of these streams would carry accompanying loss of salmonid productivity and genetic diversity.

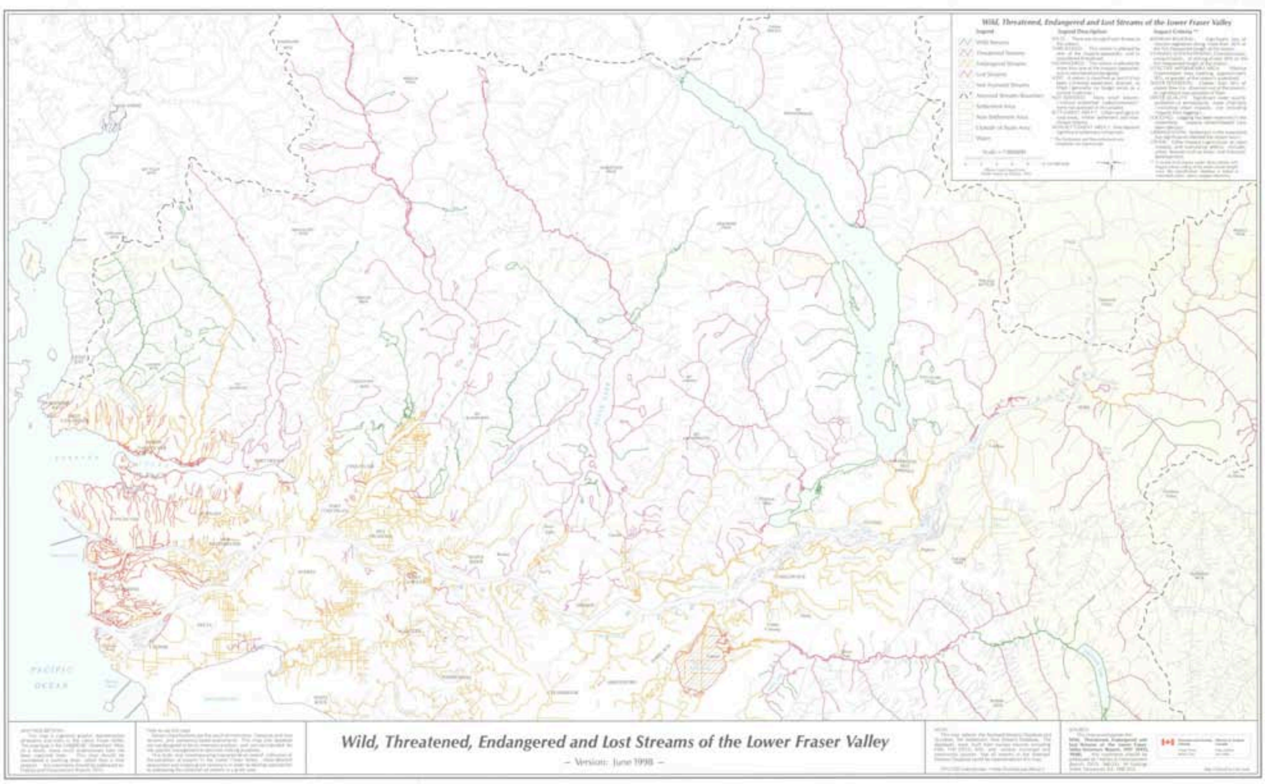

Figure 2.1. Most streams in the Lower Fraser Valley are considered lost (20%), endangered (63%), or threatened (13%), with comparatively few considered wild (5%). Source: PIBC 1997.

2.2 FRASER RIVER ESTUARY AND DELTA

The near-shore marine environment is a unique ecological zone that hosts consistently higher species diversity, density, and productivity than deep-water marine habitats. Kelp, saltmarsh, and eelgrass vegetation that colonize near-shore habitats in the Fraser delta and surrounding shorelines serve as nurseries for young salmon by offering shelter, food, and protection from predators. The U.S. federal government has recognized the physical and structural features within these zones as being *Essential Fish Habitat* for salmon (NOAA 2009). The relevant geo-physical, biological, ecological and bio-chemical attributes of these habitats are identified and mapped under the U.S. Magnuson-Stevens Act. In coastal marine waters, U.S. definitions mean virtually every estuary, river mouth, slough, bay, foreshore and extended shoreline on the US coastline is classified as Essential Fish Habitat for Salmon. The mudflat/intertidal region of estuary deltas is often the most ecologically important of these coastal habitats (Elliott and Taylor 1989).

As one of the most productive estuaries on the Pacific coast, the Fraser estuary is no exception. 52 species of fish have been reported on the delta (Levings 2004). It supports bird populations from three continents (Schaefer 2004) and is the major stopover point along the Pacific Flyway for millions of birds migrating from Canada's Arctic and the eastern tip of Russia to wintering grounds in Central and South America (Butler and Campbell 1987). The delta is a vital link in a chain that extends from wetlands in California to the Copper River delta in Alaska (Schafer 2004).

The intertidal mudflats of the Fraser River delta are the substrate for a range of invertebrates (macro and meio infauna) (Otte and Levings 1975, Harrington et al. 1999) that support the hundreds of millions of out-migrating juvenile salmon that move into the lower estuary to feed and undergo osmoregulation changes. These mudflats serve as the entry and exit point for all Fraser populations of Chinook, sockeye, coho, pink and chum.

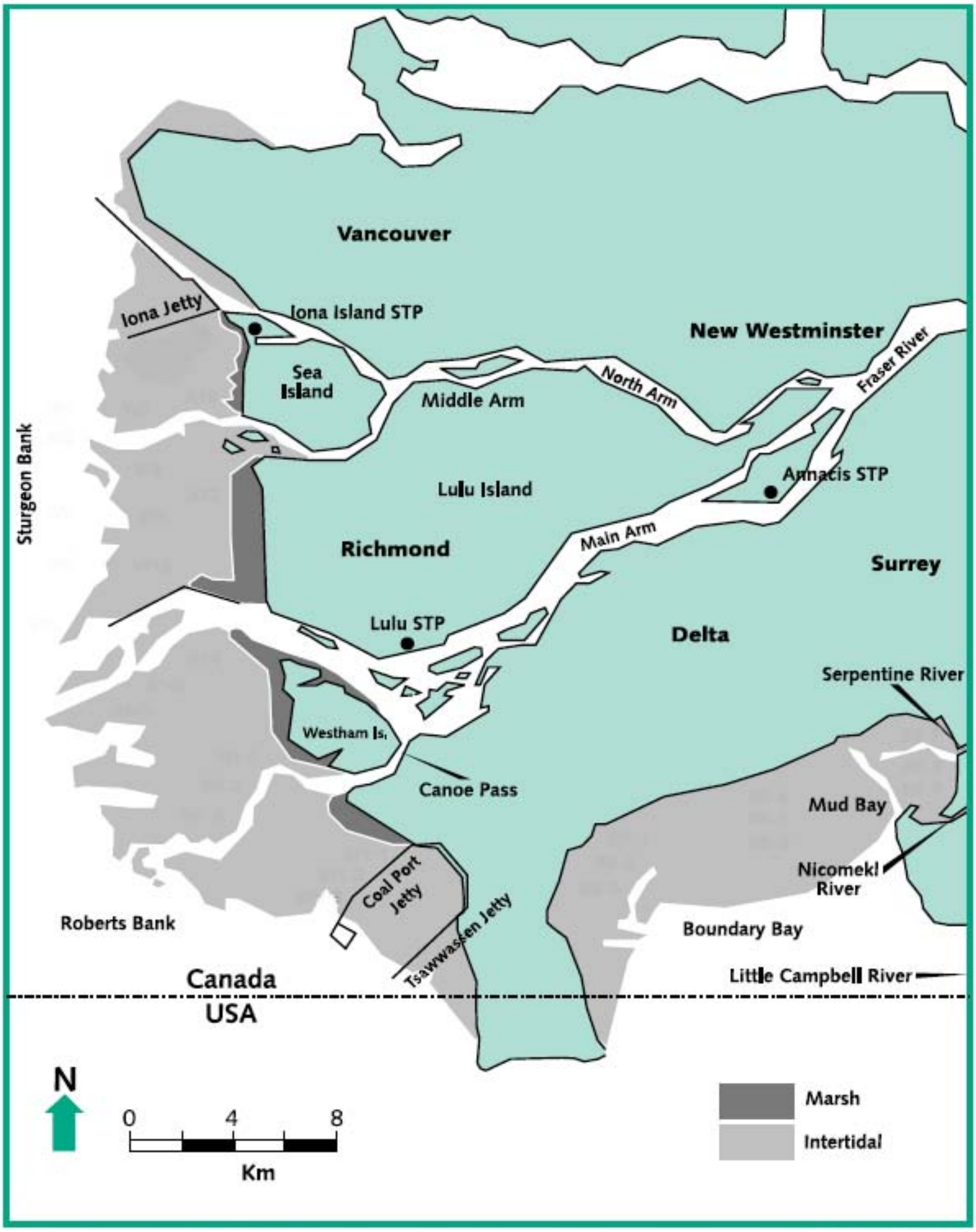

Figure 2.2. Primary sections of the Fraser River's outer delta showing productive marsh and intertidal mudflats on Sturgeon and Roberts Banks and adjacent rivers (Serpentine, Nicomekl, Little Campbell) that flow into Boundary Bay. Figure modified from Harrington et al. 1999.

The Fraser River delta can be divided into six habitat zones that are differentiated by variable salinity, sediment type and supply (Harrison et al 1999). These six zones (1) Iona Island sand flats between the Iona Island Jetty and the North Arm; (2) Sturgeon Bank; (3) Roberts Bank; (4) the inter-causeway, between the coal port terminal and the ferry terminal; (5) south of the ferry terminal to Point Roberts peninsula; and (6) Boundary Bay (Harrison et al. 1999) are shown in Figure 2.2.

The main primary producers on the mudflats are benthic microalgae (e.g. diatoms) on top of the sediment, but benthic macroalgae (kelp) such as *Ulva* /*Enteromorpha* are occasionally present (Harrison et al. 1999). Invertebrate infauna can be comprised from up to 300 species (Rosenau 2012), but the dominate taxonomic groups on Sturgeon and Roberts Banks are from 35 species of polychaete worms, bivalves, amphipods, isopods, decopods, harpacticoid (benthic) copepods, nematodes and oligochaetes (Otte and Levings 1975).

The presence and functions of burrowing animals and benthic organisms have major effects on the structure, movement and chemistry of marine sediments (McCall and Tevesz 1982) and ultimately productivity. By circulating water through their burrows, benthic animals transport oxygen to otherwise anoxic layers of sediment. They affect the types and rates of chemical reactions occurring at the sediment-water interface, in particular the recycling of nutrients such as nitrate and phosphate, and metals such as manganese (Elliott et al. 1998).

Importantly, these invertebrates are eaten by forage fish such as herring *(Clupea harengus pallasi),* sand lance *(Ammodytes hexapterus),* eulachon *(Thaleichthys pacijicus),* longfin smelt *(Spirinchus thaleichthys)* and surf smelt *(Hypomesus pretiosus)* (Levings 2004). These fish, along with juvenile salmon, are in turn prey for larger vertebrate predators such as marine mammals, thousands of migratory, resident and overwintering waterfowl, shorebirds and raptors, and other fish such as adult salmonids.

2.3 SALISH SEA

The coastal waters and watersheds of the Salish Sea (broadly Puget Sound, Haro Strait, Juan de Fuca and Georgia Strait) support five species of anadromous commercial salmon (*Oncorhynchus spp.)* that originate in watersheds within British Columbia and Washington State. As a unit of assessment, the Trans Mountain Regional Study Area (RSA) captures only 58% of the Salish Sea (Figure 2.3), but the Salish Sea is a more appropriate assessment unit because of its oceanographic basis, particularly in northern Georgia Strait.

Generally, between 5 and 20 million adult salmon return each year to the Salish Sea's Canadian rivers, (mostly to the Fraser), but annual fluctuations are large. Not including the Fraser River, 380 salmon-bearing streams and rivers drain from Vancouver Island, the Gulf Islands and BC's mainland coast into the Canadian side of the Salish Sea (DFO NUSEDS). These waterways support more than 900 spawning populations of salmon that are grouped into 48 distinct lineages of Conservation Units, (CUs; Holtby and Ciruna 2007, Grant et al. 2011, DFO 2013).

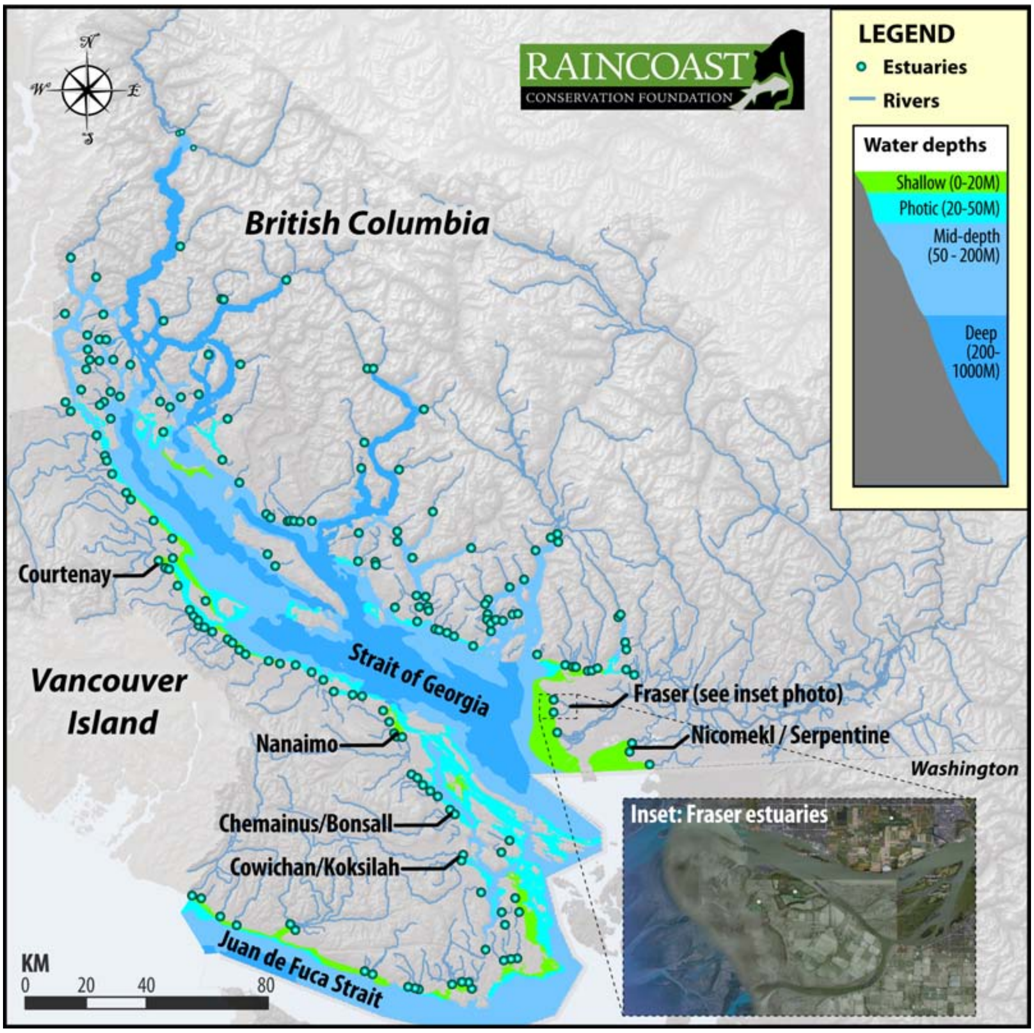

Figure 2.3 Essential rearing habitat for juvenile salmon in the Canadian waters of the Salish Sea. Critical nearshore and estuary rearing habitat is depicted in green (<20 m) and circles (estuaries). Larger immature and migrating young salmon will also occupy the deeper photic and mid-depth zones. Only 43% of the estuaries and 58% of the rearing habitat shown is included within Trans Mountain's marine Regional Study Area.

These 48 Conservation Units are comprised of 13 Chinook CUs, 13 sockeye CUs (9 lake-type; 4 river-type), 7 coho CUs, 6 chum CUs, and 9 pink CUs (6 odd-year; 3 even year). When added to the Fraser's 56 Conservation Units, a total of 104 unique lineages of salmon rely on the Salish Sea and its stream and rivers for spawning, rearing, staging and migration (Table 2.2). Appendix C (Figures C1 – C11) shows the geographic locations of all Conservation Units within the Salish Sea and the Fraser River watershed. Steelhead and cutthroat salmon (traditionally referred to as trout), bull trout, and dozens of unique populations (Evolutionarily Significant Units) of these species from US watersheds, are additional to these commercially managed Canadian populations. In Canadian waters, most of these salmon bearing streams flow to Georgia Strait.

Table 2.2 Summary of the 104 unique salmon Conservation Units within the five species (7 types) of commercially managed salmon returning to the Fraser River watershed and the surrounding watersheds (non-Fraser) that drain to the Canadian waters of the Salish Sea. See Appendix C for a full alpha-numeric list of CUs.

Species	Fraser River	Non-Fraser River	Total
Chinook	21	13	34
Coho	8	7	15
Chum	2	6	8
Sockeye (Lake-type	22	9	31
Sockeye (river-type)	2	4	6
Pink (odd-year)	1	6	7
Pink (even-year)	0	3	3
Total	56	48	104

While Canada’s largest runs of sockeye, Chinook and pink salmon return to the Fraser River, the populations that return to the hundreds of streams and rivers of the Salish Sea contribute to the region’s extensive genetic diversity and biological complexity. In addition, these smaller runs play key roles in natural ecosystems, providing food and nutrients to a web of interconnected species in marine, estuarine and terrestrial environments (Cedarholm et al. 2000; Piccolo et al. 2009; Hocking and Reynolds 2011). In addition to the naturally produced Pacific salmon, more than 50 hatcheries produce hundreds of millions of hatchery fry that enter the Salish Sea every year (Beamish et al. 2000; USFW undated).

3 OIL PROPERTIES, BEHAVIOUR AND FATE IN THE AQUATIC ENVIRONMENT

The expanded Trans Mountain pipeline would carry diluted bitumen (‘dilbit’), although the specific characteristics of the product would vary between batches and between seasons.

Kinder Morgan Trans Mountain (‘Trans Mountain’) proposes to expand the capacity of its current pipeline, which extends 1 150 km from Strathcona County, AB to Burnaby, BC, from 300 000 barrels per day (bpd) to 890 000 bpd (Trans Mountain ULC 2013d). The existing pipeline carries products including heavy crude, light crude, distillates, and gasoline, which often travel together in the same line in a process known as ‘batching’ (Kinder Morgan website). The proposed expansion calls for approximately 994 km of new pipeline and the reactivation of 193 km of existing pipeline. The new line will carry primarily diluted bitumen (‘dilbit’; Trans Mountain Pipeline ULC 2013b); as such, this report will, where possible with existing knowledge, deal primarily with the behaviour, fate, and potential effects of dilbit. The existing line will continue to carry refined products, such as synthetic and light crude oils.

Diluted bitumen does not have a fixed composition insofar as the constituents and their concentrations may vary from batch to batch and between seasons. It is also unclear from Trans Mountain’s submission to the National Energy Board (NEB) which dilbit product would be carried in the proposed pipeline. The study commissioned by Trans Mountain to study the behaviour and fate of dilbit (carried out in Gainford, AB, hence ‘the Gainford study’) used Access Western Blend (AWB) and Cold Lake Winter Blend (CLWB) as representative dilbit products. The properties and composition of these two dilbits has also been described in recent work by Environment Canada (Environment Canada 2013).

3.1 PRODUCTS THAT MAY BE CARRIED IN THE PIPELINE

3.1.1 Crude Oil

Crude oil is composed primarily of hydrocarbons. These compounds range in size and complexity, properties which impact their behaviour in the environment. The contribution of polycyclic aromatic hydrocarbons (PAHs) to the total hydrocarbon content varies from near zero in gasoline to 1.5% in heavy crude oils. PAHs exert toxicity by varying mechanisms depending on their size and structure.

Crude oil is composed primarily of hydrocarbons, although most crude oils include small amounts (<1%) of compounds containing sulphur, nitrogen, or oxygen, and trace elements (e.g. metals), including cadmium and mercury. The hydrocarbons in oil vary widely in their molecular size, shape and properties (Table E.1), but can be broadly classified into saturates (compounds without double bonds), including aliphatics (linear or branched chains of carbon atoms, including waxes) and cyclo-alkanes (rings of carbon atoms); aromatics (one or more benzene rings, with or without alkyl side chains); and resins and asphaltenes (very large and complex molecules that combine aromatic rings and aliphatic structures). The majority of compounds in oil are non-polar and have relatively low water solubilities. The structure of these compounds influences their behaviour, fate, and persistence in the environment and their bioavailability and toxicity to fish and other aquatic species.

Aromatics include monoaromatic hydrocarbons (single benzene rings) with and without alkyl substituents, polycyclic aromatic hydrocarbons (PAH; two or more benzene rings fused together in flat plates, with and without alkyl substituents), and heterocycles (PAH containing one or more atoms of nitrogen, sulphur, or oxygen). Because the potential toxicity of heterocycles to aquatic organisms, and to fish in particular, is poorly characterized, this report will focus on monoaromatics and PAHs. The most abundant aromatics are the monoaromatics (e.g 1.62% by weight in Alaska North Slope Crude (ANSC) oil), and the most abundant of these are benzene, toluene, ethylbenzene, and xylenes (BTEX). The monoaromatics are highly volatile, relatively water soluble, and relatively easily biodegraded, and as such they are lost quickly (on a timescale of hours to days) after an oil spill as they are exposed to air, water washing, and bacterial degradation.

The most abundant PAHs in crude oil are the two-ringed naphthalenes, which are lost relatively quickly to evaporation and dissolution following an oil spill. As PAHs increase in size (e.g. 3 to 8 rings) and have increasing numbers of alkyl substituents, they will persist longer in the environment (Barron and Holder 2003), will have increased lipophilicity and decreased volatility, and in some cases, can be up to 10 times more toxic than their parent compounds (e.g. Turcotte et al. 2011). In general, alkyl-substituted PAHs account for more than 90% of PAHs in oil. In crude and refined petroleum products, total PAH (TPAH; sum of alkyl substituted and unsubstituted PAH) vary from almost absent in gasoline to 0.1-1.5% by weight in light to heavy crude oils (e.g. 1.1% in ANSC), to 3- 6% by weight in heavy fuel oils (Martin 2011), and from 0.01-1.1% in various samples of dilbits and synthetic crude oils.

Linear and branch-chained aliphatic compounds exhibit similar behaviour to aromatics, with short-chain LMW compounds lost quickly after a spill, and persistence increasing in proportion to chain length and molecular size. Very large aliphatics are classified as waxes, and have extremely low water solubility. Similarly, the resins and asphaltenes are generally so large that they are not volatile, are insoluble in water, and are thus persistent, forming the tarry materials that remain following extensive weathering of spilled oil.

Crude oils can be classified according to their relative proportions of saturates, aromatics, resins, and asphaltenes. Light crude oils contain higher proportions of LMW aliphatics and aromatics and lower proportions of waxes, resins, and asphaltenes than a medium or heavy crude oil. As a consequence, light crude oils have a lower viscosity (flowing more easily, even at low temperatures) and a lower density or specific gravity than heavy crude oils. When fresh, films of lighter oils have a greater propensity to be broken up into small droplets than heavy oils, so they are more easily dispersed by mixing or by chemical dispersion.

The LMW constituents of oil are highly and acutely toxic to aquatic organisms, causing mortality within 12 to 96 h of exposure (Chapter 5). Concentrations that will cause mortality can be predicted with reasonable accuracy by a model that sums the contributions to toxicity of the LMW aromatic compounds (French-McCay 2002). The higher molecular weight 3 to 5-ring PAH are chronically toxic to fish embryos, causing sublethal developmental defects and often death of embryos that do not develop sufficiently to begin feeding (Carls et al. 1999). Developmental toxicity to newly fertilized fish embryos can be caused by exposure to oil as brief as one hour at concentrations typical of oil spills, or at concentrations 100-fold less if exposure occurs throughout development (McIntosh et al. 2010).

3.1.2 Diluted bitumen

Bitumen deposits in Canada are found primarily in Alberta. Because bitumen formed close enough to the earth's surface to allow for degradation via microbial activity, the lighter components of the oil are absent, such that the remaining oil has a much higher viscosity than conventional crude. For this reason, bitumen must be diluted with a solvent (condensate or synthetic crude oil) for it to be pumped through pipelines. Diluted bitumen ('dilbit') differs from crude oil in important ways, including its composition, density, viscosity, and PAH profile. All of these characteristics would affect the way dilbit behaves in the environment, and how it would affect salmonids and other fish.

Bitumen deposits in Canada are found primarily in the Western Canadian Sedimentary Basin (WCSB), mostly in Alberta. The largest reserves of bitumen are found in three regions (Athabasca, Peace River, and Cold Lake), and total approximately 140 000 square kilometers (TRB 2013).

Bitumen formation occurred when oil came close enough to the earth's surface to cool sufficiently to allow for microbial activity, resulting in the biodegradation of the lighter components of the oil (Environment Canada 2013). The remaining oil - 'bitumen' - has higher organic acid content, higher sulfur content (3-6% by weight; it would thus be classified as 'sour' (TRB 2013; Bakker 2011)) and a higher viscosity than conventional crude oil (Environment Canada 2013), accounted for in large part by its 14-17% asphaltene content (reviewed in TRB 2013). For this reason, bitumen must be diluted with a lower molecular weight solvent in order to allow it to be pumped through pipelines. In Alberta, diluents include naphtha-based oils called condensate, typically mixed at a ratio of 25:75 (Bakker 2011; Environment Canada 2013), or synthetic crude oil, mixed at a ratio of 1:1 (Environment Canada 2013). When synthetic crude is used as the diluent, the resulting compound is known as 'synbit' or 'dilsynbit'.

In some ways, diluted bitumen ('dilbit') resembles and behaves like a very heavy crude oil. However, there are also some important differences that will affect the fate and behavior of dilbit in the environment. As dilbit weathers, the lower molecular weight compounds in the diluent are lost relatively quickly (i.e. within approximately 24-48 hours), with the residue resembling the original bitumen. Bitumen itself has very low concentrations of LMW compounds (e.g. alkanes, BTEX, naphthalenes) relative to crude oils, but has a much higher proportion of 3-5 ring alkyl PAHs (Yang et al. 2011). The increased proportion of alkyl PAHs has critical implications for the toxicity of dilbit to fish, particularly early life stages (discussed in detail in Chapter 5). Diluted bitumen has API gravity values between 7 and 13 degrees; liquids with values lower than 10 degrees will sink in fresh water (TRB 2013). Thus, in the absence of the LMW compounds found in the diluent, bitumen would be expected to submerge or sink. Additionally, due to its high viscosity, dilbit is less easily dispersed than crude oil (Environment Canada 2013). Finally, bitumen has the following PAH profile: $C_0<C_1<C_2<C_3<C_4$, whereas conventional crude has a bell-shaped alkyl PAH distribution (Environment Canada 2013; Yang et al. 2011). The bitumen profile is a reflection of the biodegradation that took place as the bitumen was formed: the smaller compounds (e.g. C_0, C_1) were preferentially biodegraded, resulting in a greater proportion of larger compounds (e.g. C_3, C_4) remaining. This may have implications for the persistence of dilbit. Dilbit in the environment may take longer to degrade than would conventional crude oil, given that a substantial part of the biodegradation has already taken place.

3.1.3 Condensate

Condensate, a product used to dilute bitumen, is comprised primarily of LMW compounds that would be lost rapidly to evaporation and dissolution in the event of a spill. Condensate would be expected to exert acute toxicity due to its lower viscosity and its relatively high percentage of LMW PAHs, including the BTEX compounds (benzene, toluene, ethylbenzene, and xylene).

Condensates differ from crude oil and bitumen in that they are comprised of variable mixtures of LMW, highly volatile compounds recovered from oil and gas wells. BTEX comprises between 2 and 10% of condensate by volume (Bakker 2011).

Following a spill, a large portion of the condensate would be lost to evaporation and a smaller amount would dissolve in water, depending on temperature and the extent of mixing in the water. Because of its lower viscosity, condensate would spread and form droplets more quickly than crude oil or diluted bitumen. Condensate would also be likely to cause a larger fish kill than crude oil due to the acute toxicity of LMW PAHs (Chapter 5.1). However, it would be less persistent and perhaps cause less chronic toxicity, depending on the chemical composition of the residue.

3.2 BEHAVIOUR AND FATE OF SPILLED OIL

Despite its ongoing extraction and transport, there is very little known about the behaviour and fate of dilbit spilled in freshwater or estuarine environments. This section of the report will discuss a modeled potential spill in the Lower Fraser River near the Port Mann Bridge.

Despite ongoing transport of dilbit from Alberta's oil sands, there is very little information available on the behaviour and fate of dilbit spilled in freshwater environments. For example, a recent report by Fisheries and Oceans Canada examining the state of knowledge with respect to the aquatic toxicology of petroleum products lists seven main research recommendations, the first two of which address dilbit (toxicity and fate/behaviour; Dupuis and Ucan-Marin 2015). Much of what is known comes from wave tank studies in laboratories (e.g. Environment Canada 2013) and a single known spill of dilbit into a freshwater environment (i.e. the Enbridge oil spill into the Kalamazoo River near Marshall, Michigan in 2010). Research has also been carried out on products that may behave similarly to dilbit (e.g. Orimulsion; Boudreau et al. 2009) and on the toxicity of natural oil sands deposits to fish (e.g. Colavecchia et al. 2004, 2006, 2007). These studies will be discussed in cases where they may help shed light on the potential behaviour and fate of dilbit spilled in a freshwater environment.

In general, the behaviour and fate of spilled oil in a freshwater environment, and the coincident exposure of and effects on fish, will depend on the interactions among the physical and chemical characteristics of the oil, the physical and hydrogeological characteristics of the receiving environment, the season and weather conditions at the time of the spill, and the weathering of the oil.

The fate of dilbit spilled as a consequence of a full-bore pipe rupture was modeled by Stantec Consulting Ltd. (2013) at four locations along the proposed pipeline expansion. The purpose was to examine the path oil might take following entry into fresh water, as well as how the physical and chemical characteristics of the dilbit might be expected to change. Each spill was modeled for summer, winter, and spring/fall conditions (Stantec Consulting Ltd. 2013).

As the purpose of this report is to examine the potential effects of an oil spill on salmon populations in the Lower Fraser River, particular emphasis will be placed on the fourth scenario: a full-bore pipeline rupture in the rail yards approximately 500 m west of the Port Mann Bridge, 400 m from the Fraser River, and approximately 30 km upstream from the mouth of the Fraser River Estuary (Stantec Consulting Ltd. 2013).

3.2.1 Modeling a Potential Spill

A pipeline rupture could cause contamination of the Fraser River either directly or via overland transport. The proposed pipeline would parallel the Fraser River as it travels through the Fraser Canyon, and again west of the Salmon River, creating multiple possible points of direct entry in the event of a pipeline rupture.

The duration of a pipeline spill would be a function of the size of the break and the time needed to detect it and close adjacent valves. Estimates of this time frame assume that valves remain undamaged at either end of the damaged section of pipeline.

Trans Mountain has indicated that the worst case scenario for an oil spill into the lower Fraser River is a full-bore rupture resulting in the release of approximately 1 250 m^3 of dilbit. This estimate assumes perfect functioning of leak detection systems and the ability to shut off the valves.

The timing of a potential spill has implications not just in terms of the duration of the spill, but in terms of the impacts of seasons on the behaviour of spilled oil and in terms of the presence or absence of various salmonid life stages and their relative sensitivities.

3.2.1.1 Source of petroleum spill

Potential spill scenarios include pipeline breaks that would contaminate the Fraser River through direct discharge to the watercourse (e.g., breaks at tributary crossings) or overland transport from a rupture away from the river.

South of Hope, BC, the proposed pipeline corridor would parallel the Fraser River for approximately 140 km through the Fraser Canyon, Chilliwack/Vedder River, and the Lower Fraser and Squamish watersheds (Trans Mountain ULC 2013a). West of Chilliwack, the corridor travels through largely private and agricultural land as far as Abbotsford. Between Abbotsford and Burnaby, the corridor travels through urban, agricultural, and industrial land on the north side of Highway 1. West of the Salmon River however, the proposed corridor would travel parallel to the Lower Fraser River itself for approximately 17 km, crossing the river just west of the Port Mann Bridge (Trans Mountain ULC 2013a).

Spills that may occur as a consequence of terminal operations or as small, chronic inputs resulting from regular, day-to-day operations are beyond the scope of this report.

3.2.1.2 Duration of the spill

The duration of a pipeline spill would be a function of the size of the break and the time needed to detect it and close adjacent valves. In the case of a full-bore pipeline rupture, Trans Mountain's submission to the NEB indicated that the leak would be detected at the control center and the line would be shut down within 15 minutes (Stantec Consulting Ltd. 2013). However, this time frame assumes that alarms are synonymous with shut down, that the valves at each end of the section are undamaged and can be closed, and that small drops in pressure are detected. Potential failures in these systems, along with other human error, would likely result in a much longer time frame between detection and line shut down.

3.2.1.3 Volume of oil

Where a break occurs at a river or stream crossing, the majority of oil would likely be discharged directly to the watercourse. For breaks between stream crossings, the oil would travel some distance over land before encountering a stream. The amount that entered the watercourse would depend on the intervening terrain (i.e. flow over vegetated terrain would likely result in a smaller volume of oil being discharged to a watercourse than would flow over impervious surfaces (e.g. roads, culverts)).

The Trans Mountain application to the NEB indicates that the worst case scenario for an oil spill into the lower Fraser River at the Port Mann Bridge is a full-bore rupture resulting in the release of approximately 1 250 m^3 of dilbit. The assumption is that due to the presence of culverts and drainage systems, the majority of the spilled oil would be transported directly into the Fraser River (Stantec Consulting Ltd. 2013). In the case of a smaller puncture in the pipeline, it was estimated that the spill volume would be 65% of the full-bore rupture scenario, or 812.5 m^3.

As stated above, these assessments assume that leak detection systems function perfectly, and that the cause of a break is not the destruction of the valve itself.

3.2.1.4 Timing of the spill

All aspects of the timing of an oil spill, from the season to the time of day, to the duration of exposure to the toxic components of oil are critically important in terms of the consequences for salmon and other fish species.

The influence of timing begins immediately upon rupture of the pipeline, in terms of whether the event is a single, short-term spill or a prolonged leak. This in turn affects the duration of exposure of various receptors. For example, in the event of a spill near the Port Mann Bridge, it was estimated that under winter and spring/fall conditions, oil would take one to two days to reach the Fraser River estuary, and only one day in the high flows of summer (Stantec Consulting Ltd. 2013). Chapter 4 discusses the potential implications of this timing to fish populations.

As mentioned above, the seasonality of the spill is also important, as this impacts the transit time of oil to the estuary, the rates of weathering (which depend on many factors, including sunlight, and air and water temperatures), and the presence and life stage of receptors at, and downstream of, the spill site (Chapter 4). Even the time of day of an oil spill is important, as it will dictate the early effects of tidal currents and salinity on the behaviour and fate of the spilled oil. The following sections will discuss in greater detail the impact of season on the behaviour and fate of spilled oil.

Timing is also important to the receptors themselves in terms of life stages. For example, the incubation time of fish embryos in sub-surface gravel (e.g. from fertilization to emergence of swimming fry) is crucial in that the embryos are immobile and would be vulnerable to oil exposure for periods as long as four to six months. Sensitivity to oil toxicity varies with the age of the embryo, with highly sensitive stages in development include fertilization, gastrulation, organogenesis, and the development of the capacity to oxygenate and excrete PAH, which can modify the response of fish to oil exposure (e.g., Brinkworth et al. 2003).

3.2.2 The densities of oil and water: Float, sink, or submerge

There are two circumstances under which oil will submerge and/or sink (Michel 2010):

1) When oil is less dense than water, it will initially float and will only sink if mixed with sediment. Sediment can be added to oil when it strands and mixes with shoreline sediments, or in the presence of waves when sediment is present in the water column.

2) When oil is more dense than water (density > 1.0 g/mL or API gravity < 10 for fresh water). The density of water is influenced by temperature and salinity. Densities typical of open ocean seawater are around 1,025 kg/m^3; densities typical of freshwater are around 1,000 kg/m^3. As such, the threshold for submergence/sinking in fresh water is much lower than for submergence in seawater. Estuarine waters lie in the spectrum between fresh and salt, decreasing in density with greater distance up the river. Water temperature also influences density. At 20^{0}C, the density of freshwater is 998.20 kg/m^3; at 4^{0}C it is 999.97 kg/m^3. As such, the potential for submergence/sinking is generally greater at warmer temperatures than at colder temperatures.

3.2.3 Weathering

Evaporation of the LMW components of oil begins immediately following a spill; however, the effects of evaporation are proportionately less on heavier oils. Trans Mountain's projections indicate loss to evaporation of 10-11% within the first three days of a spill, meaning that evaporation would be likely be ongoing as oil reaches the outer estuary (in 1-2 days). Loss of diluent would lead to increased density and an increased likelihood of oil submerging and/or sinking.

As oil weathers and mixes with water, water-in-oil mixtures called emulsions can form. These can be of varying stability and longevity, but are of concern from a toxicological perspective because they can persist long after the spill event is over. Some properties of diluted bitumen appear to favour the formation of emulsions.

As weathering progresses, the relative proportion of high molecular weight compounds increases, increasing the viscosity and increasing the potential for oil to submerge and/or sink. The adsorption of suspended particulate matter to oil slicks and droplets can result in the formation of oil-sediment aggregates (OSAs), decreasing the buoyancy of the oil and increasing its tendency to submerge in the water column. OSAs can create chronic exposure routes and can hinder efforts to recover spilled oil. Despite the significant formation of OSAs following the 2010 spill of dilbit into the Kalamazoo River, and despite the fact that sediment addition was not included in their studies of dilbit fate

and behaviour, Trans Mountain indicated repeatedly that OSA formation is not expected to play a significant role in the fate of spilled oil.

Tar ball formation is of concern because they can deliver oil directly to sediments, where it will continue to be released over time.

Oil spilled on land will accumulate terrestrial debris (e.g. soil, vegetation) that will raise its specific gravity and increase the likelihood of sinking when it enters the river.

3.2.3.1 Evaporation

When oil is spilled, the process of weathering begins immediately, with significant amounts of LMW aliphatics (i.e. < 12 carbons) and aromatics (e.g. BTEX, naphthalenes) lost within the first 24-48 hours to evaporation, dissolution, biodegradation, and photo-oxidation (McAuliffe 1977; George et al. 1995). Evaporation is considered to be the most important behavioural characteristic of oil (Fingas and Fieldhouse 2006). The proportionately greater effect of weathering (evaporation in particular) on lighter oils was reviewed in Fingas (1999), who reported that while light and medium oils can lose up to 75% and 40% of their volume, respectively, in the first few days following an oil spill, heavy oils lost only 5% of their volume. This value for heavy oils is slightly less than the values predicted for dilbit in the Trans Mountain models of a spill at the Port Mann Bridge: in winter it was estimated that 11% of the oil would be lost to evaporation within three days of the spill, 10% in summer, and 10% in spring/fall (Trans Mountain ULC 2013b). Thus, in the brief time it is predicted to take for the dilbit to reach the Fraser River estuary (one to two days, as discussed above), the composition will have changed only slightly, and the evaporation and dissolution of LMW hydrocarbons would be expected to be ongoing as the oil moves into the estuary.

If oil were to flow into and be stranded in side channels, wetlands, and/or sloughs, as is predicted to occur in the lower Fraser River in the event of a spill in the summer and spring/fall months, the ongoing loss of the diluent from diluted bitumen and subsequent increase in density would increase the likelihood that the residue would submerge or sink and accumulate in bed sediments. The adverse consequences of this occurrence for salmon are discussed in Chapter 5.

3.2.3.2 Emulsions

As oil weathers and mixes with water, water-in-oil mixtures can form. These mixtures can be grouped into four categories, of which only the first two can be classified as emulsions, also referred to as “mousse” as a result of their stiff, foam-like appearance (Fingas and Fieldhouse 2009, 2011):

> 1) stable: contain 60-80% water, original volume of spilled material increases 2-5 times, viscosity of oil increases by an average factor of 400 on the day of formation, and by an average factor of 850 one week later, difficult to recover with spill recovery equipment (Fingas 2014), can persist indefinitely

2) meso-stable: contain 60-65% water when formed, <30% water one week after formation, viscosity of oil increases by average factor of 7 on day of formation, and by average factor of 2 one week later (Fingas 2014), half-lives of hours to days (most water lost within a few days)

3) entrained water-in-oil: lower water content (40-50% when formed, <30% one week later), viscosity increases by an average factor of two, loses water very slowly over about a year

4) no-emulsion: oil and water remain apart

Asphaltene content has been identified as the most important factor in the formation of emulsions (reviewed in Fingas 2014). Asphaltene is an important component of dilbit. As weathering continues, asphaltenes migrate to the oil-water interface, forming a stable film that provides a source of stability for emulsions (Lobato et al. 2007). Because this process is thermodynamically favoured (i.e. moving toward a more stable state), it proceeds for at least a year and possibly longer (Fingas and Fieldhouse 2006). However, very high asphaltene content (>10%; undiluted bitumen contains approximately 14-17% asphaltenes) appears to inhibit the formation of stable emulsions by increasing the viscosity of the oil such that water cannot penetrate the oil mass (Fingas 2014). It is possible that the presence of diluent, at least for the first few days following a spill, would decrease the viscosity and lower the percent contribution of asphaltenes sufficiently to allow for the formation of stable emulsions.

This possibility is supported by Environment Canada (2013), who examined the behaviour of dilbit in the marine environment. While they found that weathered dilbit formed only entrained water-in-oil mixtures, fresh (unweathered) Cold Lake Blend dilbit formed a meso-stable emulsion (Environment Canada 2013). While mixing with water alone is not enough to cause oil to sink, the formation of emulsions may change the properties of dilbit sufficiently to increase the likelihood of sinking by other processes (Environment Canada 2013).

Similarly, Belore (2010) examined the emulsion formation tendencies of diluted bitumens (Cold Lake bitumen (CLB) and Mackay River Heavy bitumen (MKH)) and found that at 1°C, fresh CLB was likely to form entrained water-in-oil mixtures while at 14°C, fresh CLB was very likely to form meso-stable emulsions. The likelihood of emulsion formation decreased as the dilbit weathered. Similarly, fresh MKH was very likely to form meso-stable emulsions at 1°C and 15°C, and lightly weathered MKH was likely to form water-in-oil mixtures at 15°C (Belore 2010).

While these studies were carried out in the marine environment, recent research has demonstrated that decreasing water salinity appears to increase the stability of water-in-oil emulsions (Moradi et al.2011, Wang and Alvarado 2009). The presence of particulate matter (e.g. sediments) has also been found to stabilize or enhance the stability of emulsions (Sztukowski and Yarranton 2004), which is of potential concern in the turbid Lower Fraser River (McLean et al. 1999).

Emulsions, or ‘mousse’, are of concern from an ecotoxicological standpoint because they have been found to persist long after the spill event is over (e.g., Irvine et al. 1999, 2006; Short et al. 2007; Nixon et al. 2013). For example, following the *Exxon Valdez* oil spill in 1989, oil mousse originating from the spill was found on distant beaches (500 km from the spill site) as late as 2005 (Short et al. 2007). In most cases, while the outer shells of the emulsions were heavily weathered, oil inside the emulsions was found to be compositionally similar to 11-day old *Exxon Valdez* crude (Irvine et al. 2006). The oil emulsions were able to persist due to a phenomenon known as ‘boulder-armouring’ (Irvine et al. 1999). The shorelines studied have been subject to centuries or millennia of wave erosion, which selectively removes material smaller than boulders from the beach. The remaining rocks are large enough to form an ‘armour’ that is not moved by typically occurring waves, thereby protecting the sediment and anything else underneath them, including oil emulsions.

While these processes do not occur naturally in the Fraser River, industrial development has created stretches of artificial boulder-armouring (riprap) along shorelines as a means of bank stabilization. If emulsions formed and were deposited in the riprap following a spill in summer (at the highest water level of the Fraser River), the falling water level throughout the rest of the year could strand the oil at the high water mark, creating the potential for release the following year.

3.2.3.3 Submergence, Sinking, and the Formation of Oil-Sediment Aggregates (OSA)

As weathering progresses, the relative proportion of HMW compounds (e.g., PAH with greater than 3 rings, waxes, resins, and asphaltenes) in the residual oil increases, thereby increasing the viscosity and specific gravity, and increasing the potential for the oil to sink or be entrained in the water column. In some cases, evaporation can be enough to cause sinking of oils that are already approaching the density of water (Michel 2010). However, recent laboratory studies by Environment Canada (2013) found that in the marine environment, evaporation alone was not enough to cause dilbit to sink.

Laboratory studies on Orimulsion, a Venezuelan product containing 70% bitumen and 30% water, found that the most important factor determining whether the product would float or sink was its density in relation to the density of the receiving water (i.e. increased salinity resulted in more floating oil; Stout 1999). A second important factor was the energy of the receiving environment: in a low energy, freshwater environment, the majority of the oil (65.5%) formed particles and sank to the bottom of the tank, while in a very high energy environment, the majority of the oil (54.7%) was suspended in the water column (Stout 1999).

Oil slicks, emulsions, and droplets can accumulate suspended particles in the water column, decreasing their buoyancy and increasing the likelihood that they will sink or be submerged. Known as oil-sediment aggregates (OSAs), these small particles can be stable in water over periods of weeks (Lee et al. 2001). For example, following a spill of approximately 4 600 m^3 of mixed crude oil and condensate into the Rio Desaguadero, Brazil in January 2000, more than 40% of the oil was reported lost to evaporation, 3-13% was recovered, and it was thought that remaining 27-37% likely formed OSAs (Lee et al. 2001). OSAs in the water column can release oil droplets over time (Lee et al. 2002), and sunken OSAs can shed their sediment burdens and refloat (Environment Canada 2013), creating chronic exposure routes. Such was the case following the Wabamun Lake, AB spill, when persistent oil slicks were thought to be the result of resurfacing of oil (Birtwell 2008).

The formation of OSAs can also hinder efforts to recover spilled oil by causing it to sink or submerge (Lee 2002; Lee et al. 2003a). Following the release of dilbit into Michigan's Kalamazoo River in 2010, OSAs were readily formed from native river sediments and the spilled dilbit. Of the 843 000 gallons (3.2 million L) of oil that spilled, 180 000 ± 100 000 gallons (680 000 ± 379 000 L) were estimated to remain in benthic sediments by 2013, indicating that OSAs formed from dilbit are both stable and persistent (Lee et al. 2012). Of this amount, 12 000 – 18 000 gallons (45 000 – 68 000 L) were recovered; however, because the environmental costs of further clean up were deemed to outweigh the benefits, between 162 000 and 168 000 gallons (613 000 – 636 000 L) remains in the river (US EPA 2013). Despite this experience, Trans Mountain's quantitative environmental risk assessment repeatedly indicates that OSA formation is not expected to be a 'dominant fate' or a 'significant factor in the fate' of spilled oil. Trans Mountain holds that OSA formation will be limited by factors like salinity and suspended sediment concentration (Stantec Consulting Ltd. 2013).

There are many factors affecting OSA formation, including the physical properties of the oil (e.g. viscosity, density, and degree of weathering), sediment concentration in the water column, sediment grain size, organic content, salinity, and temperature (reviewed in Stantec Consulting Ltd. 2013). In the case of heavy oils such as bitumen, relatively low mixing energy is all that is required to cause submersion in the water column; however, the higher viscosity of dilbit may make it resistant to droplet formation.

The potential for OSA formation also increases with increasing sediment concentration, decreasing grain size, and increasing organic content (Ajijolaiya et al. 2006, Khelifa et al. 2008). Le Floch et al. (2002) reported that if salinity is below 1‰ (parts per thousand), OSA formation is inhibited; values greater than 2‰ are typically required. Due to estuarine circulation in the lower Fraser River, this threshold of 2‰ is reached at 22 km upstream (New Westminster) in bottom waters, to Annacis Island at depths of 10 m, and throughout the water column as far as the Oak Street Bridge on the North Arm and to Steveston on the south arm (Ages 1979; Hall and Schreier 1996).

In Trans Mountain's 'Gainford study', where the fate and behaviour of dilbit was modeled under a variety of conditions, the authors noted that in no instance was any oil observed to have sunk or submerged in the water column, although density increased to only slightly positive buoyancy within 24-48 hours. However, none of the trials included freshwater or the addition of sediment of any grain size (Witt O'Brien's et al. 2013). Thus, it is difficult to apply the results to a spill in a freshwater or even estuarine environment. However, based on other work (e.g. Environment Canada 2013) and experience from the Kalamazoo River, in the event of a spill in the Lower Fraser River it is likely that in the day or two it is speculated to take for oil to reach the high suspended sediment loads of the Fraser River delta (Attard et al. 2014), the effects of evaporation and dissolution, interaction with suspended particulate, and the formation of OSAs would result in neutral or negative buoyancy and submergence or sinking of oil would occur. Higher suspended sediment loads would also be expected during rain and high water events, as well as during the annual melt cycle (Attard et al. 2014). Despite the importance of suspended sediments and particulate matter in the behaviour of spilled oil, it appears that the only information provided to establish baseline turbidity and total suspended sediment (TSS) values by Trans Mountain in support of their submission to the NEB was qualitative (i.e. based on visual observations alone; Trans Mountain ULC 2013a).

Of further concern is that sunken oil can be very difficult to find. Following the Wabamun Lake, AB spill, crews searched for sunken oil using numerous methods, including visual, drag sampling, Ekman dredging, SCUBA divers, and underwater cameras. None of these methods proved effective in detecting or mapping the sunken oil except in very shallow waters where the lake bed was visible to the eye (Birtwell 2008). Due to the turbidity of the Lower Fraser River, similar challenges would likely exist. Trans Mountain's NEB submission does not address the possibility of submerged or sunken oil in its clean up or environmental protection plans (Trans Mountain ULC 2013c).

3.2.3.4 Tar balls

Extensive weathering of heavy oil can cause a thick outer shell of waxy or asphalt-like material to form, which would hinder the transfer of PAH to water, even though the oil in the interior of the tar ball may remain in liquid form (Goodman et al. 2003).

Trans Mountain's Gainford Study found that weathered dilbit surpassed viscosities of 10 000 centiStokes within 48 hours and exhibited a strong tendency to form continuous thick mats on the surface of the water (at 20‰ salinity). It was predicted that this tendency, in combination with continued weathering and agitation, would be expected to produce tar balls (Witt O'Brien's et al. 2013).

The formation of tar balls is critically important because they deliver oil directly to sediments, where liquid oil and PAH may be released to habitats essential for benthic species and fish reproduction. For example, Bunker C fuel oil was stranded on a Nova Scotia shoreline following the 1970 sinking of the tanker *Arrow* in Chedabucto Bay. Within five years, the oil had weathered to tar balls and hard pavements, but the undersides of tar deposits at the upper high tide mark were still liquid, resembling freshly-spilled oil (Vandermeulen and Gordon 1976). The liquid oil remained as a reservoir to periodically re-oil the beach when storms disturbed the sediment. In 1997, 27 years after the spill, sufficient oil was still present in the beach to release oily sheens when sediments were disturbed, and concentrations were still acutely toxic to marine amphipods (small crustaceans), although other test species were unaffected (Lee et al. 2003b).

More recently, tar balls washing up on beaches in Spain have been traced back to the 2002 spill of heavy fuel oil from the tanker *Prestige* (Bernabeu et al. 2013). The authors determined that tar balls at varying degrees of degradation continue to accumulate in the subtidal zone and wash up on the beach when suitable wave conditions occur.

Tar balls formed within hours following the spill of crude oil into Wabamun Lake, AB in 2005, and exhibited neutrally buoyant behaviour (i.e. moved within the water column) (Birtwell 2008). Because they formed so quickly, they were relatively unweathered and were observed with associated sheens months later (Birtwell 2008), causing the potential for ongoing exposure for aquatic organisms. The rapidity with which tar balls formed was thought to be due to adsorption of terrestrial materials as the oil flowed overland, and to the further addition of particulate once the oil entered freshwater (Birtwell 2008). Small tar balls (1 mm to 2 cm in diameter) were still being recovered three years after the spill, as were empty 'skins', the weathered exteriors of tar balls that had cracks or holes in them through which relatively unweathered oil had leaked out (Birtwell 2008; Short 2008).

The observation of rapidly forming tar balls would be of concern in the event of a pipeline rupture along the Lower Fraser River, in which oil would flow overland before entering the river. The formation of tar balls would create the potential for chronic release of oil and ongoing exposure for salmon and other aquatic organisms.

3.2.3.5 Overland flow

Oil spilled on land may weather significantly before overland flows reach the river. Further, during overland flow, the oil will accumulate soil particles, vegetation and other debris that will raise the overall specific gravity. The experience at Wabamun Lake, AB in 2005 (Parker-Hall and Owens 2006), demonstrated that a large portion of spilled heavy fuel oil, which had a nominal specific gravity of less than 1.0 and should have floated, sank due its contact with soil during a short (<100 m) overland flow. As a consequence, a large part of clean-up costs and environmental damage were associated with the removal of oil and contaminated vegetation from shoreline reed beds where pike and other fish species spawn (Evans 2008). The oil was present as small (<5 mL) to large (>1 L) masses of liquid oil contained within a weathered, waxy exterior (i.e. tar balls); significant amounts were also found offshore at whitefish spawning shoals (Evans 2008).

Overland flow also occurred following the Enbridge pipeline rupture near Marshall, MI in 2010. Before entering Talmadge Creek (and subsequently the Kalamazoo River), dilbit flowed approximately 600 feet overland through forested scrub-shrub wetland area (Enbridge Energy 2011a). While the effect of this contact was not quantified, the adsorption of terrestrial materials would almost certainly have increased the propensity of the dilbit to sink.

Despite ongoing research, there is still very little known about the behaviour and fate of spilled dilbit, particularly in fresh water environments. These gaps are encapsulated in the list of recommended research at the end of Trans Mountain's Gainford Study. The authors suggest that further work must be done to understand the effect on oil retention of everything from different sediment types, different hydraulic conditions (e.g. water level drops and rises, tidal flushing), grain size, and temperature – in other words, all the conditions that might be encountered in the Fraser River.

3.2.4 Stranding

The extent to which spilled oil strands along shorelines, mid-stream islands and bars depends on several factors, many of which were not included by Trans Mountain in their modeling effort, likely resulting in an underestimate of the potential volume of stranded oil.

As the Fraser River flows toward its mouth, it becomes increasingly complex, with sandflats, mudflats, and marshes. Seasonality is projected to impact the extent of shoreline oiling, with a greater probability in the spring, fall, and winter months, and a lower probability in summer due to high flows.

The likelihood of recovering stranded oil is affected by several factors, including substrate type, depth of oil penetration, and ocean and weather conditions. The extensive development along much of the Lower Fraser River could complicate efforts to recover stranded oil. In winter, when flows are low, stranding would occur on sandy or muddy beaches, creating the possibility that oil could percolate into sediments and be protected from further weathering and that reoiling events could occur if stranded oil is disturbed.

Stranding may also add terrestrial materials to oil, increasing the likelihood that it will submerge and/or sink if it is reintroduced to the water column.

Spilled oil (both fresh and weathered) can become stranded along the shorelines of rivers and their tributaries and on mid-stream islands and bars (sand or gravel). The extent to which stranding will occur depends on a variety of factors, including channel sinuosity, channel braiding, emergent bars, the amount of debris (e.g. log jams), and the nature of surfaces coated. Interestingly, the oil spill modeling carried out in support of the Trans Mountain proposal did not factor in "any braiding, debris, backwater, log jams or other impediments to waterborne travel but simply [assumed] a straight channel". The model assumes that "the only product entrainment [i.e. oils stranding] is along the simulated banks" (Trans Mountain ULC 2013b), likely resulting in an underestimate of the volume of stranded oil. In reality, the Lower Fraser shoreline is not a straight channel, and exhibits the very conditions omitted from the model.

Approximately 25 km upstream of the mouth of the Fraser River and 8 km downstream of the site of Trans Mountain's hypothetical oil spill at the Port Mann Bridge, the river becomes increasingly complex and begins to split into multiple channels. The main river channel flows south of Annacis Island as far as the George Massey tunnel, at which point it begins to divide again and flows through a series of sandflats, mudflats, and marshes before entering the Strait of Georgia (Harrison et al. 1999). There are also two large intertidal mudflats in the delta: Sturgeon Bank (west of Richmond) and Roberts Bank (west of Delta).

The river banks along the main channel between the Port Mann Bridge and the George Massey tunnel are highly developed industrial lands, with parallel dikes running along either side

(Richardson et al. 2000; Richmond Chamber of Commerce 2014). The substrate in this area is primarily sand and mud, while the banks are dominated by dikes consisting of rip rap (Attard et al. 2014; Grout et al. 1997).

The extent of oiling will also be determined by whether a spill occurs during a flood or falling stage. In both cases, the length of shoreline changes, and during a falling stage, much larger areas will be contaminated as receding water strands oil at the high water mark, resulting in a 'bathtub ring' effect. During a flood stage, increasing turbulence due to higher flow rates may re-mobilize oil stranded at a low stage.

As discussed previously, Trans Mountain carried out several oil spill modeling exercises, with one hypothetical spill taking place near the Port Mann Bridge adjacent to the lower Fraser River. Chronic effects were evaluated assuming that Shoreline Cleanup Assessment Techniques (SCAT) and other remedial measures would be applied until they were deemed to cause more harm than good (Trans Mountain ULC 2013b). They assume that most visible oil would be removed from shorelines and riparian zone soils, but acknowledge that a residual load of approximately 1 kg/m^3 might be expected (Trans Mountain ULC 2013b).

In the case of a spill in the winter, modeling results estimated a very high probability of shoreline oiling (60-100% between the Port Mann Bridge and the upstream end of Annacis Island and 60-90% along the western and southern shores of Annacis Island). Most spilled oil (>80%) was predicted to strand along the shoreline within three days of the spill. Again, this estimate was made without consideration of channel sinuosity, emergent bars, or debris in or along the river, any of which may increase the percentage of stranded oil. Because the river level is relatively low in winter, it is expected that most exposed shoreline would be sand and/or mud. A relatively lower river flow in the winter means that tidal influence on spilled oil would be greater.

In the case of a spill in the summer months, modeling results estimated that because of higher summer flows, there would be much less stranding in the main channel (<60% in the first three days). However, modeling indicated that more oil would likely flow into side channels, wetlands, and sloughs near Ladner and Port Guichon, and become stranded.

Finally, in the case of a spill in the spring or fall months, modeling results estimated a very high probability of shoreline oiling between the Port Mann Bridge and the upstream end of Annacis Island (60-100%) and a moderate probability (40-60%) along the western and southern shores of Annacis Island and along the north shoreline of the Fraser River as far as the George Massey Tunnel. Approximately 70% of the spilled oil was predicted to strand within three days of the spill. A smaller percentage (<10%) is predicted to flow into side channels and wetlands near Ladner and Port Guichon.

The extent to which stranded oil could be recovered depends on several factors (Fingas 2013):

1. the type of substrate
2. the depth to which oil has penetrated the sediments
3. the amount and type of oil and the degree to which it has weathered

4. the ability of the shoreline to support traffic and other infrastructure required for clean up
5. the sensitivity of the shoreline (e.g. human, environmental)
6. ocean and weather conditions

In much of the lower Fraser River, the shorelines are highly developed. Extensive lengths of shoreline between Mission and the Strait of Georgia are taken up by moored log booms (Richmond Chamber of Commerce 2014), as well as rail yards, wharves, docks, and rip rap. In particular, oil stranded in log booms and rip rap would be extremely challenging to recover. As discussed above (Section 3.2.2), oil stranded in rip rap and not successfully recovered could be protected from significant weathering and retain some toxicity.

In the case of a winter oil spill, much of the stranding would occur on sandy or muddy beaches due to seasonally low water levels in the river (Stantec Consulting Ltd. 2013). Depending on the height of the water table and the viscosity of the oil at the time of stranding, it may percolate into sediments and be protected from weathering by factors including:

1. limited nutrient availability to allow for biodegradation;
2. low oxygen and light levels, which would decrease photo- and chemical oxidation; and
3. low surface area to volume ratios, which would decrease evaporation and dissolution (Short et al. 2006).

Following the *Exxon Valdez* oil spill, slightly weathered crude oil stranded on sandy beaches percolated into the sand over several hours and was largely prevented from refloating on the incoming tide by capillary forces (Short et al. 2006). This oil was encountered by researchers as recently as 2003 (Short et al. 2006). After losses of 30% of the initial mass of the oil due to evaporation, the stranded crude had a viscosity of <1000 centipoise (cP).

Environment Canada (2013) provided viscosity measurements for fresh AWB and CLB at 0°C of 1300 cP and 803 cP respectively. At 15°C, values were 347 cP for AWB and 285 cP for CLB. Values for lightly weathered dilbit ranged from 1 300 cP to 9 800 cP. These values suggest that fresh or very lightly weathered dilbit could penetrate sandy beaches to some extent, leaving it protected from continued weathering and creating the potential for reoiling in the event that it was disturbed by dredging, development, and/or biological activity.

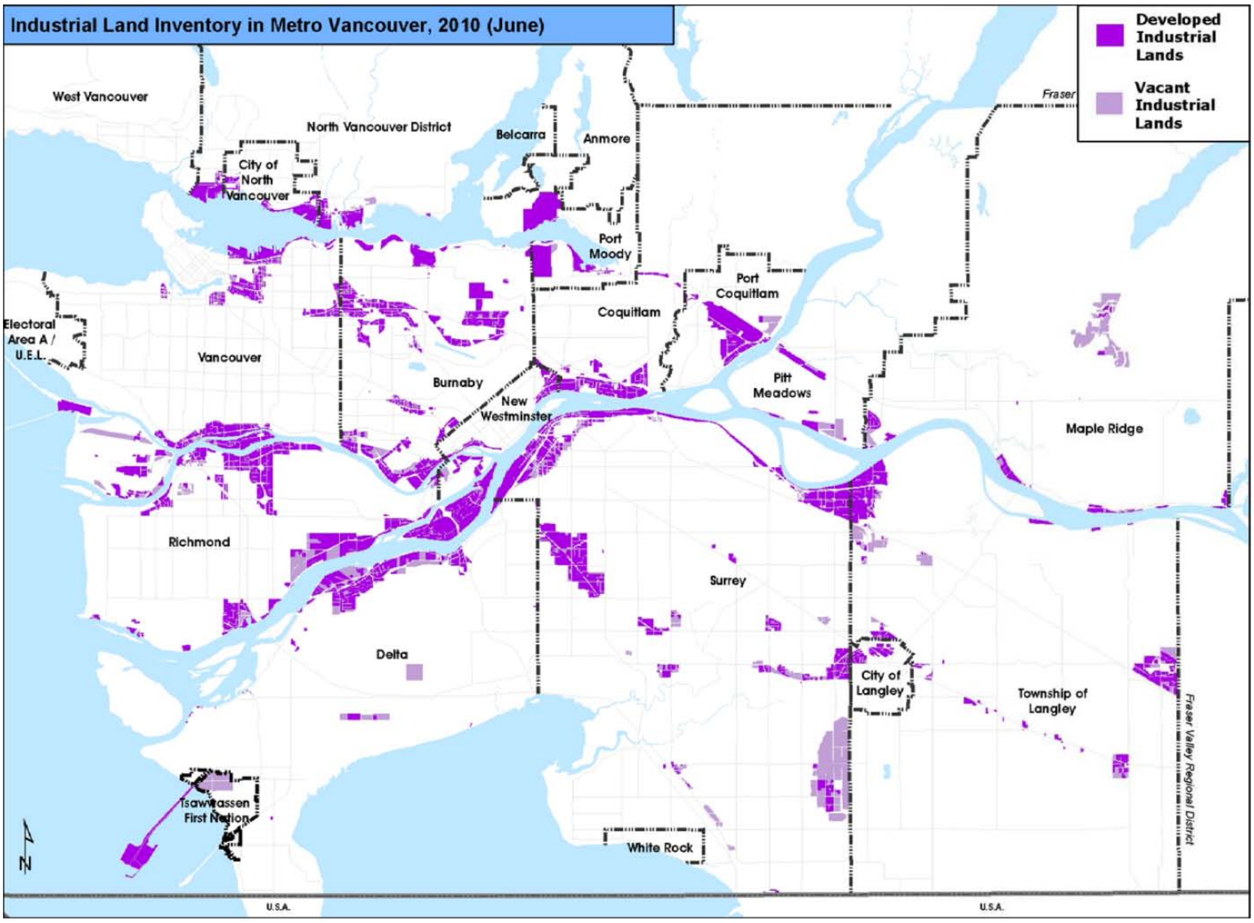

Figure 3.2. Much of the shoreline length along the lower Fraser River is occupied by developed and vacant industrial lands (Metro Vancouver 2011).

Another important consequence of stranding is the addition of supra- or intertidal particulate matter (e.g. mud, sand) to oil. If this oil were then reintroduced to the water column by wave action, rising water levels, and/or clean-up activities, the addition of sediment would make it more likely that the oil would sink. This is an important consideration, given that modeling results suggested that >80% of spilled oil would strand in the event of a spill in the winter months. This pathway has the potential to greatly increase the amount of oil that ends up in bed sediments of the main channel and side channels and suspended in the water column.

3.2.5 Dispersion

Chemical dispersion of spilled oil is intended to increase its surface area and thereby the rate of weathering as well as dilution by mixing the oil into a larger water mass (i.e. into the water column). Chemical dispersion increases exposure risk in areas where aquatic species are present.

The efficacy of chemical dispersants on diluted bitumen in freshwater are not well understood, particularly considering that the composition of dilbit can change depending on the season and the diluent used.

Due to the increased risk of exposure and toxicity to aquatic organisms, chemical dispersants are not usually considered for use in small or confined water bodies. It is unclear whether Trans Mountain would consider the use of dispersants in the event of a spill in the Lower Fraser River.

Regardless of the use of chemical dispersants, oil may still be dispersed in the Lower Fraser River as a consequence of water turbulence, particularly during flood conditions.

In spill response, oil is often chemically dispersed to increase its surface area, thereby enhancing the loss of hydrocarbons by volatilization, dilution in water, and biodegradation. Dispersion rapidly dilutes surface oil into a much larger water mass by allowing downward mixing into near-surface waters (McAuliffe 1977). The extent of dilution will be a function of mixing energy, where the net effect, in addition to increasing the rate of degradation, is to increase the volume of water containing measurable and potentially toxic concentrations of oil (McAuliffe 1977). In the open ocean, where animal density is often lower, oil dispersion can be highly successful in reducing impacts on aquatic resources and clean-up costs. However, in areas close to shore, bird, marine mammal, and fish populations may be abundant. Because the impacts of oil on aquatic species are associated with the toxicity of petroleum compounds dissolved in water, the exposure of fish to hydrocarbons, and the risks of toxicity, increase when oil is chemically dispersed.

The extent to which oil is dissolved in the water column is a major determinant of exposure. When oil is dispersed as fine droplets (1 – 100 μm), the surface-to-volume ratio increases, and the rate of partitioning of hydrocarbons to water is enhanced. The extent of droplet formation may be reduced for oils with high viscosity and density (e.g. dilbit, heavy fuel oil) due to a) the formation of stable emulsions with water, b) the difficulty of delivering the dispersant to the oil-water interface, and c) the high asphaltene and wax content (Environment Canada 2013; Chapman et al. 2007). Wave tank studies carried out by Environment Canada found that chemical dispersant application to a dilbit spill in sea water was only effective in breaking wave conditions, which are rare in the Lower Fraser River, and even then, the majority of spilled oil was not dispersed but persisted as a slick on the surface of the water (Environment Canada 2013). Despite all this, the efficacy of chemical dispersants on a dilbit spill in a freshwater environment is unknown (Environment Canada 2013). Further, depending on the particular bitumen, the diluent, and the season, chemical composition of dilbit can change, thereby making it difficult to prescribe the correct dispersant.

Droplet dispersion in and of itself does not increase hydrocarbon solubility; rather, it facilitates the transport of oil droplets into the water column where the droplets, with their high surface area to volume ratio, allow hydrocarbon partitioning into the water phase. Laboratory tests of oil dispersed in fresh water have demonstrated that the exposure of fish to PAHs, and thus the risk of toxicity, was increased by 6 to 1100-fold, depending on the nature of the oil being tested (heavier oils generally resulted in greater exposure; Ramachandran et al. 2004). During the process of dispersion, the concentrations of soluble components may exceed the threshold for toxicity to aquatic organisms for hours or days (McIntosh et al. 2010). For this reason, chemical dispersants are not usually considered for spill management in small or confined water bodies where the opportunities for dilution are limited, and where sensitive receptors may be abundant. It is unclear whether Trans Mountain would consider the use of dispersants in the event of a spill in the freshwater environment. While they indicate their intention to seek pre-approval for the use of chemical dispersants in the event of a spill, the Risk Assessment submitted to the NEB discussed dispersant use only in the marine environment (Trans Mountain ULC 2013b).

Regardless of whether a chemical dispersant is used, oil dispersion may be a risk factor in the Lower Fraser River because oil can also be physically dispersed by mechanical energy associated with standing waves and water turbulence in rivers and streams, particularly during flood conditions. The entrainment of droplets and water-in-oil emulsions into the water column increases the potential for oil transfer to sediments, where it may be toxic to benthic organisms and those that feed on them. Filter-feeding species can accumulate particulate oil (e.g. Meador et al. 1995; Meador 2003), making it bioavailable to the benthic food web and to salmonids and other fish that feed on drifting invertebrates.

3.2.6 Entrainment of oil into sediments

Entrainment of oil in benthic sediments is of greater concern with respect to fish health than contamination of the shoreline. Ecological impacts of oil spills in rivers have been associated primarily with sediment contamination, which persists longer than contamination of the water column.

In addition to sinking directly to the river bed, mechanisms by which bed sediments can be contaminated with oil include transport to the river bottom during channel restructuring and hyporheic flows.

In the Lower Fraser River, an ongoing dredging program means that sunken oil entrained in benthic sediments would almost certainly be disturbed, creating an ongoing exposure route.

Species that would be particularly vulnerable to sediment contamination in the mainstem Lower Fraser River include eulachon and pink salmon, but all species of juvenile salmon could be affected if sediment contamination impacted benthic food resources.

The entrainment of oil within bed sediments of the river is of more concern for fish health than contamination of the shoreline. Oil in contaminated sediments persists longer than in the water column, because it is immobile and thus not subject to dispersion and dilution (Fingas et al. 2005; Hollebone et al. 2011). In virtually all cases of oil spills to rivers, there has been oil contamination of sediments, with measurable residual oil detected months and years following the spill (e.g. Pine River, B.C., Goldberg, 2006; Kalamazoo River, MI, US EPA 2011). In addition to the fish kills that occurred immediately following these spills (e.g. Baccante 2000), the ecological impacts of spilled oil were associated primarily with sediment contamination, oil toxicity to benthic invertebrates, a major source of fish food, and oil toxicity to fish species that deposit eggs in nests created in bed sediments.

Following a spill of approximately 475 000 L of heavy sour crude oil to the Pine River in August 2000, about 29% of the oil was mechanically removed. However, sediments were significantly contaminated in months following the spill (AMEC 2001), and oily sediments were still evident after 5 years, though oil concentrations were greatly reduced (Goldberg 2006). In the case of Enbridge's 2010 spill of dilbit into the Kalamazoo River, of the 843 000 gallons that spilled from the pipeline, approximately 180 000 ± 100 000 gallons remained in river bottom sediments by 2013. Only 12 000 – 18 000 gallons of this was deemed to be recoverable, leaving 162 000 – 168 000 gallons on the river bed (US EPA 2013).

In addition to the sinking of oil directly to the river bed, there are other mechanisms by which bed sediments may be contaminated with oil. If an oil spill is followed by heavy flooding, as can occur on the Fraser, contaminated surface sediments on shorelines and gravel bars can be eroded and mixed with clean sediments, carrying oil-coated sediments deep within new bars formed downstream as the river channel is re-structured. Residual hydrocarbons could then partition (i.e. allocate) into interstitial water flowing through the bed sediments.

Oiled groundwater can also be transported from shores to surface and subsurface waters, and subsequently into sediments where salmonids spawn and where their eggs incubate. As river currents encounter gravel bars or the downstream side of pools, they exert hydrostatic pressure that drives water into the bar. At the downstream edge of gravel bars and below pools, there is a negative pressure, resulting in a variable but predictable flow of water through the gravel substrate ('hyporheic' flow), with measurable currents as deep as several meters below the bed of the river (Tonina and Buffington 2007, 2009a, 2009b; Buffington and Tonina 2009). The pool-riffle and braided channel reaches of many of the tributaries of the Lower Fraser River facilitate hyporheic flows, which transport dissolved oxygen into gravel bed sediments and remove metabolic waste products such as ammonia from eggs that have been buried by spawning salmon and trout.

However, the characteristics that create good spawning habitat can also entrain pollutants such as dissolved and particulate oil into bed sediments (Tonina and Buffington 2009b). Following the 1989 *Exxon Valdez* oil spill, statistically significant mortality of pink salmon was observed despite the apparent lack of oil in stream gravel (Carls et al. 2003). At the time of the spill, researchers believed that spawning channels were protected because flowing freshwater seemed to prevent the deposition of oil in stream channels (Carls et al. 2003). However, alevins (larval stage salmon) from streams in oiled areas had elevated cytochrome P450 activity (detoxification enzymes that indicate oil exposure) and elevated embryo mortality through 1997 as compared with alevins from streams in reference areas (Carls et al. 2003). Researchers introduced tracer dyes to beaches at ebb tide to establish the mechanism by which oil contamination might occur. The dyes spread throughout the watershed, including both surface and hyporheic stream water, and in less than 30 minutes, the dyes had upwelled from streambed gravel back into stream water (Carls et al. 2003). The mechanism for this movement involved tidal cycles, which allowed stranded oil to be contacted by water, and the resultant hydraulic gradients, which transported dissolved PAHs into the stream channel and the hyporheic zone (Carls et al. 2003). These conditions could occur in the intertidal interface of all rivers and streams. Oscillating flows along river shorelines can also result in contaminant transport from groundwater to river water (Li et al. 1999). Thus, as water flows over and through stranded oil, the constituents of oil that are toxic to the early developmental stages of fish (eggs, post-hatch embryo, larvae) partition from oil to water to the limits of their water solubility, creating conditions that may reduce embryo survival (Chapter 5) and fisheries productivity in areas that have been oiled. With nine salmonid species spawning in tributaries of the Lower Fraser River and utilizing the main channel at various times of year, it is likely that the full range of available spawning and rearing habitat is used, and as such, a spill of dilbit may make many of those areas unsuitable for embryo growth and survival (e.g. Chapter 5).

Of particular concern in the south arm of the Lower Fraser River is the ongoing dredging program (June 15 to March 1 of the following year), to maintain the river's status as a shipping channel (Det Norske Veritas 2012). The main deep-sea shipping facilities are located 35 km upstream at New Westminster (Det Norske Veritas 2012), necessitating dredging for that entire distance. In the event that oil submerges or sinks and is entrained in benthic sediments, it would almost certainly be disturbed and redistributed in the water column on a continuous basis by dredging operations. While salmonids do not spawn in the main channel of the Lower Fraser River below Mission, it is utilized for spawning by eulachon, which could face ongoing oil exposure as a consequence of dredging.

The persistence of oil in sediment could threaten the continued production of fish species that are particularly vulnerable to oil spills by virtue of their long residence time in river sediments (which increases the likelihood and severity of oil exposure) or a short life cycle (vulnerable to repeated weak year classes due to on-going recruitment failure). Pink salmon would be most vulnerable to recruitment failure because they have a fixed two-year spawning cycle and are only abundant in even years. There are no 'reserve' year classes that spend a prolonged time at sea while the river recovers from oil pollution.

4 FISH SPECIES AT RISK FROM OIL SPILLS IN THE LOWER FRASER RIVER AND SALISH SEA

4.1 FISH SPECIES IN THE LOWER FRASER RIVER

There are at least 42 species of fish that use the Lower Fraser River and estuary for part or all of their lifecycles. All species of salmon migrate through the lower river and estuary twice a year (once as juveniles and once as adults), with some juveniles spending extended time feeding, rearing, and adapting for ocean life. Ecological effects of an oil spill in the lower river, estuary, or one of its tributaries would vary depending on the species and life stages present. Fraser River salmon and their important commercial, recreational, and First Nations fisheries are of high value to Canadians. The effects of an oil spill could be compounded by the presence of "at risk" populations and species. Due to the large diversity of populations and their variable life histories and use of the lower river, there is no time of year when salmon are not vulnerable to an oil spill.

Along with resident fish species, which are present year round, all anadromous (migrating between freshwater and the ocean) fish populations of the Fraser River must use the Lower Fraser and estuary to some degree, depending on their life history characteristics (development, growth, mobility, habitat utilization, feeding, etc.), species, and populations. The ecological severity of an oil spill in the Lower Fraser River or one of its tributaries would vary depending on the season (species, populations and life stages present) and the year (high versus low abundance return years for various species and populations).

There are at least 42 species of fish that use habitats in the Lower Fraser for part or all of their lifecycles (Richardson et al. 2000). Historically, the Fraser River watershed produced some of the world's largest salmon runs, supporting economically and culturally important commercial, recreational and First Nations fisheries (Northcote and Levy 1982; Northcote and Larkin 1989; Healey 2009). Many of these runs continue today, but are diminished relative to their former levels of abundance (Grant et al. 2011; Riddell et al. 2013; DFO 2005).

Fish harvested commercially and by First Nations include Chinook salmon (*Oncorhynchus tshawytscha*), chum salmon (*O. keta*), coho salmon (*O. kisutch*), sockeye salmon (*O. nerka*), and pink salmon (*O. gorbuscha*). Recreational fishing occurs in tidal and freshwater areas of the Lower Fraser, where anglers target salmon along with coastal cutthroat trout (*O. clarkii clarkii*), steelhead and rainbow trout (*O. mykiss;* steelhead are anadromous rainbow trout), bull trout (*Salvelinus confluentus*) and Dolly Varden char (*S. malma*). Eulachon, a species of smelt, were traditionally harvested by First Nations but currently exist at 2% of their historic abundance (COSEWIC 2011). Other important forage fish in the Lower Fraser include Pacific lamprey (*Entosphenus tridentatus*), peamouth chub (*Mylocheilus caurinus*), northern pikeminnow (*Ptychocheilus oregonensis*), largescale sucker (*Catostomus macrocheilus*), starry flounder

(*Platichthys stellatus*), threespine stickleback (*Gasterosteus aculeatus*), and prickly sculpin (*Cottus asper*) (Richardson et al. 2000).

Due to their economic and cultural importance, much is known about Fraser River salmon and the timing, location and extent of their utilization of the Lower Fraser River and estuary during different life stages. Fraser River salmon are of high value to Canadians as they support valuable commercial, recreational, and First Nations food, social and ceremonial (FSC) and economic opportunity fisheries (Northcote and Levy 1982; Northcote and Larkin 1989; Healey 2009). Fraser salmon are harvested in Canadian and American waters, and are actively managed by Fisheries and Oceans Canada and the Pacific Salmon Commission under the Pacific Salmon Treaty of 1985. There are 78 Fraser watershed and marine approach First Nations which are "Member Delegates" to the Fraser Salmon Management Council (Fraser River Aboriginal Fisheries Secretariat 2014). These First Nations depend on the Fraser River salmon for food, social and ceremonial purposes and have constitutionally protected access to this resource (R v Sparrow, [1990] 1 S.C.R. 1075). The landed value for all Fraser commercial salmon fisheries in the past has been over $250 million (1985-88); however, more recently, the combined total of all South Coast landings has been only $23 - $74 million (2003 - 2012), depending largely on Fraser sockeye abundance (DFO 1993; DFO 2014). Recreational fishing occurs along the mainstem and throughout tributaries of the Lower Fraser, contributing between $604 and $705 million annually (2000 – 2010) in expenditures and investments in BC. Approximately 63% of this total is associated with salmon fishing (DFO 2013).

Regardless of spawning location, all species of Fraser salmon must pass through the Lower Fraser twice to complete their lifecycle. While some populations use the Lower Fraser only as a migratory corridor, pink and chum rely heavily on the Lower Fraser for spawning grounds, and Chinook and coho spend a significant amount of time in the lower river and estuary as juveniles (Table 4.1; also Tables 4.2 and A.1). Due to the large diversity of populations and their variable life histories, there is no period of time during the year when the Lower Fraser is least sensitive.

To preserve the locally adapted diversity of salmon populations, the five commercially harvested Pacific salmon species are managed as Conservation Units (CUs; Table A.1) in accordance with Canada's Wild Salmon Policy. CUs are composed of one or more populations based on their unique ecology, life history and genetics (Holtby and Ciruna 2007). The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) assesses the health of at-risk species and populations. Endangered or threatened salmon populations may be listed at their CU level (e.g. Cultus Lake sockeye) or grouped together (e.g. Interior Fraser coho) for listing by COSEWIC. The Fraser River has 56 unique CUs of commercially managed salmon, including 16 in the Lower Fraser (Table A.1). The current status and recent spawning escapements for each CU are outlined in Table C.1. While an oil spill may not have significant impacts on all Fraser salmon, individual CUs are much more likely to be adversely affected, particularly those that already exist at reduced abundance, such as DFO's 'stocks of concern' or those with formal COSEWIC designations. Equally, a large spill that occurred during the juvenile outmigration of the Fraser's most abundant runs, such as lake-type sockeye or ocean-type Chinook, could be disastrous for the cultural, commercial, recreational and ecological users of those salmon.

The Lower Fraser and its tributaries are habitat for the federally SARA listed green sturgeon, Salish sucker, and nooksack dace, as well as COSEWIC endangered Interior Fraser coho salmon, Cultus Lake sockeye salmon, Lower Fraser white sturgeon, and Fraser River eulachon (SARA registry). The Lower Fraser is also home to provincially Red-listed white sturgeon (Middle and Lower Fraser River populations), and provincially Blue-listed mountain sucker, coastal cutthroat trout, bull trout, Dolly Varden, eulachon, and chiselmouth (BC MoE). Provincially, any indigenous species or subspecies considered special concern (particularly sensitive to human activities or to natural events) is blue listed, while any indigenous species or subspecies that is extirpated, endangered, or threatened is red listed (BC MoE 2011).

The consequences of an oil spill into the Lower Fraser would likely be compounded for “at risk” species, populations, and Conservation Units. By definition, at-risk populations are already at lower abundance and heightened risk of extinction because of pre-existing stressors. Further, the original cause of the population decline might be very different from current threats. This is true for salmon populations that often suffer from the ghosts of past overfishing and habitat loss, and now face new threats at every stage of their life cycle.

In some cases (e.g. Cultus Lake sockeye), endangered status is accompanied by the dual challenge of reduction to very low numbers. Small populations behave differently from larger ones, making them more vulnerable to extinction. There are three main reasons for this (Paquet et al. 2006). First is the role of “chance variability”, which occurs when there is a random drop in birth rate, an increase in death rate, or repeated offspring of the same sex in a generation. All of these can lead to extinction. Secondly, when small populations experience random events such as food shortages or oil spills, the loss of individuals can have dire consequences. This concept underscores the importance of numbers to maintain the resilience and adaptive abilities of populations faced with disturbances. Finally, small populations are vulnerable due to reduced genetic variation. By their very nature, small populations are a narrow subset of individuals from what was once a much larger population. As small populations breed, the role of chance error in genetic makeup becomes much higher. For populations to adapt and evolve with changing conditions, genetic variability is key. Hence, reductions in genetic variation result in decreased survival (i.e. increased mortality). In a negative feedback loop, increased mortality leads to further reduction in genetic variation resulting in what is known as an “extinction vortex.” Loss of genetic diversity through random genetic drift is the most commonly invoked evolutionary concern in conservation biology (Picket et al. 2012).

Stressors, like climate change and habitat loss that push salmon toward their thermal limits or reduce food supply, can impart strong physical distress (Mantua et al. 2010). In a weakened state, the presence of low exposure to pollution that would otherwise be tolerated or overcome, becomes fatal. Salmon Conservation Units that have experienced loss of genetic diversity as their numbers or range declines are less resilient to stressors and are at greater risk of extinction (Healey 2009).

4.2 FISH SPECIES IN THE SALISH SEA

The coastal waters and watersheds of the Salish Sea (Puget Sound, Haro Strait, Juan de Fuca and Georgia Strait) support five species of anadromous commercial salmon (*Oncorhynchus spp.)* that originate in watersheds within British Columbia and Washington State. As a unit of assessment, the Salish Sea region overlaps about 58% of TMX Regional Study Area (RSA), but is deemed a more appropriate assessment unit because of its natural oceanographic basis, particularly in northern Georgia Strait.

There are 48 Conservation Units, which include 13 Chinook CUs, 13 sockeye CUs (9 lake-type; 4 river-type), 7 coho CUs, and 9 pink CUs (6 odd-year; 3 even year) that return to Salish Sea waters outside the Fraser River. When added to the Fraser's 56 Conservation Units, a total of 104 unique lineages of salmon rely on the Salish Sea and its stream and rivers for spawning, rearing, staging and migration. Figures C1 – C11 show the geographic locations of all Conservation Units within the Salish Sea and the Fraser River watershed. Steelhead and cutthroat salmon (commonly referred to as trout), bull trout, and dozens of unique populations (Evolutionary Significant Units) of these species from US watersheds, are additional to these commercially managed Canadian populations. 68% of these streams drain into the RSA. In Canadian waters, many of these salmon bearing streams drain to Georgia Strait.

Juvenile Chinook, chum, pink, and coho salmon are known to be widespread over both Sturgeon and Roberts Banks and have been found at low, middle, and high intertidal habitats (Levings 2004). Studies conducted by Levings (2004) found that when Sturgeon and Roberts Banks are almost completely dry during tides less than 0.5 m in the spring and summer, juvenile salmon moved into habitats which remain watered during these periods. Juvenile Chinook appear to concentrate in low-tide refuges during extremely low tides.

Most returning adult salmon migrate upstream through the main (South arm) of the river (Levings 2004). However before the main channel was trained and diked around the estuary islands, adults likely moved through the numerous channels at high tide that lead onto Sturgeon and Roberts Banks. Channels that are still open, such as Canoe Passage, are used by returning adults as migratory corridors (Levings 2004). Most returning adult salmon migrate upstream through the main (south arm) of the river (Levings 2004). However before the main channel was trained and dyked around the estuary islands, adults likely moved through the numerous channels at high tide that lead onto Sturgeon and Roberts banks. Channels that are still open, such as Canoe Passage, are used by returning adults as migratory corridors (Levings 2004).

4.3 FRASER RIVER HABITAT USE AND VULNERABILITY OF SALMONIDS AND EULACHON TO OIL EXPOSURE

The Lower Fraser is an active migration corridor for salmon virtually year round, creating a constant risk of exposure to oil from a pipeline rupture. Salmon embryos developing in redds are particularly susceptible to dissolved oil constituents. Species which have few

populations, shorter life cycles and residence time in the river and/or its tributaries are the most vulnerable to significant adverse effects.

The Fraser River is home to both resident and anadromous salmonids and other valuable fish species such as sturgeon and eulachon. Resident species of fish utilize the Lower Fraser River and tributaries for all aspects of their life history. Anadromous species must all use the Lower Fraser as a migratory corridor twice during their lifecycle and some rely heavily on freshwater spawning and rearing habitats within the Lower Fraser. Table 4.1 provides an overview of the timing of fish presence at various life stages in the Lower Fraser River and tributaries. Table A.1 details spawning behaviour and life history characteristics of Fraser River salmon, trout and eulachon. This overview captures the extent of unique life history types within species.

Table 4.1. Timing of use of the Lower Fraser River and tributaries throughout the year by various fish species and life stages (see Appendix Table A.1 for detailed information on spawning behaviour and life history characteristics).

Embryos	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
Chinook salmon	■	■	■			■	■	■	■	■	■	■
Chum salmon	■	■	■	■	■					■	■	■
Coho salmon	■	■	■	■	■					■	■	■
Pink salmon	■	■	■	■	■				■	■	■	■
Sockeye salmon	■	■							■	■	■	■
Steelhead		■	■	■	■	■	■					
Rainbow trout			■	■	■	■	■	■	■	■		
Cutthroat trout	■	■	■							■	■	■
Bull trout	■	■	■	■	■	■			■	■	■	■
Dolly Varden	■	■	■	■	■	■			■	■	■	■
Eulachon				■	■	■	■					
Juveniles	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
Chinook salmon	Stream-type spend 1+ years in freshwater, ocean-type use LFR tributaries, tidal creeks, and estuary from Mar-Aug	■	■	■	■	■	■	■	■	■	■	■
Chum salmon			Rely on estuary rearing	■	■	■						
Coho salmon	Juvenile coho spend at least one year in freshwater tributaries of the Lower Fraser before migrating to sea	■	■	■	■	■	■	■	■	■	■	■
Pink salmon		■	■	■	■	■						
Sockeye salmon			River type sockeye use sloughs and estuary for several months	■	■	■						
Steelhead	Juveniles live 1 to 4 years in LFR tributaries or utilize tidal creeks and the estuary before migrating to the sea	■	■	■	■	■	■	■	■	■	■	■
Rainbow trout	Rainbow trout spend their entire lives in freshwater.	■	■	■	■	■	■	■	■	■	■	■
Cutthroat trout	Juveniles live 1 to 4 years in LFR tributaries before anadromous individuals migrate to sea	■	■	■	■	■	■	■	■	■	■	■

Bull trout	Juveniles live 2 to 4 years in LFR tributaries before anadromous individuals migrate to sea											
Dolly Varden	Juveniles live 3 to 4 years in LFR tributaries before anadromous individuals migrate to sea											
Eulachon												
Adults	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
Chinook salmon												
Chum salmon												
Coho salmon												
Pink salmon												
Sockeye salmon												
Steelhead	Winter steelhead migrate Nov – Apr, summer run migrate May – Sept. and can spend 8 months in freshwater before spawning											
Rainbow trout	Resident in freshwater areas of the LFR year-round											
Cutthroat trout	Resident in freshwater areas of the LFR year-round											
Bull trout	Resident in freshwater areas of the LFR year-round											
Dolly Varden	Resident in freshwater areas of the LFR year-round											
Eulachon												

The Lower Fraser is a year-round active migration corridor for outgoing juveniles and incoming adult salmon from throughout the watershed due to the diversity of run-timing groups and populations (Table A.1). Juvenile salmon migrate to the estuary or to the Strait of Georgia generally starting in March and continuing through the spring and summer. The duration of this transit depends on fish size and the length of time already spent in freshwater. After spending generally one to four years at sea mature salmon return to the Fraser throughout the spring, summer, fall and even winter for some populations. At this time they migrate to spawning grounds in the Lower Fraser and throughout the system. Due to this life history all Fraser salmon are susceptible to exposure to a potential spill in the Lower Fraser, with timing and extent of vulnerable periods related to juvenile life histories and adult run timing groups which vary with species, CU, and population (Table 4.1). These variations in run timing and use of the Lower Fraser and estuary is described below.

4.3.1 Interior Fraser coho salmon

The endangered Interior Fraser coho salmon are vulnerable to exposure to a potential spill during migration through the Lower Fraser. These juvenile coho are present in the Lower Fraser during the spring from April through June as they migrate to sea (Table A.1). Adults must again migrate through the Lower Fraser when they return to spawn, primarily from October to December. Interior coho are particularly vulnerable to the potential effects of a spill as they are considered to be at serious risk of extinction due to factors including over-exploitation, habitat loss, and degradation in the watershed (COSEWIC 2002). Furthermore 50% of the subpopulations which make up the Interior coho population spawn (as adults) and rear (as juveniles) in the Thompson River (COSEWIC 2002), a Fraser tributary also vulnerable to a pipeline rupture due to its proximity to the proposed pipeline route.

4.3.2 Lower and other Fraser River coho

Coho salmon that rear as juveniles in Lower Fraser tributaries are present year-round and thus always vulnerable to exposure to a spill. Some juvenile coho (nomads) also move between freshwater and the estuary (Koski 2009). Populations from other areas of the watershed are vulnerable primarily during migration periods in the spring and fall. Mature coho return to the Fraser from August through February depending on the population, migrating upstream and spawning in at least 92 large and small tributaries of the Lower Fraser (Holtby and Ciruna 2007). Spawning populations occur in numerous tributaries on the south side of the river which are directly crossed by the proposed pipeline route including the Salmon River (largest of the indicator stocks), Coghlan Creek, Nathan Creek, and West Creek (Trans Mountain B8-1 – V5C TR 5C7 1 FISH BC A3S2C1; Grant et al. 2007). Embryos are then present in these areas primarily from October through May, and after emergence juveniles then spend at least one full year rearing in freshwater streams (DFO 1996). Juvenile coho from throughout the watershed then migrate through the Lower Fraser to sea typically in their second spring from April to June.

4.3.3 Cultus Lake sockeye salmon

The endangered Fraser River Cultus Lake sockeye salmon population also faces the risk of exposure to a potential spill along the Lower Fraser. Cultus Lake sockeye utilize the Lower Fraser including the Vedder River on their way downstream as juveniles and upstream as adults (COSEWIC 2003). Cultus sockeye primarily migrate upstream from August to December and downstream from late March to June (Table A.1). During these life periods Cultus sockeye are vulnerable to exposure to a potential pipeline rupture in the Vedder River, which intersects the proposed pipeline route, as well as the Lower Fraser and estuary. Cultus sockeye are genetically distinct from all other sockeye populations and considered irreplaceable, as efforts to stock non-native sockeye in the lake were unsuccessful (COSEWIC 2003).

4.3.4 Fraser River sockeye salmon

Fraser River sockeye salmon populations exist throughout the watershed, all of which are vulnerable to exposure to a spill during migration through the bottleneck of the Lower Fraser. Sockeye typically return in four-year life cycles, and can make up the large majority of commercial catch in high abundance years such as 2010 (DFO 2014). Mature sockeye return to the Lower Fraser in four distinct run timing groups spanning from June until December (Cohen 2012). The majority of sockeye are lake-type, spending at least one year in a nursery lake, before seaward migration through the Lower Fraser typically from March until July (Cohen 2012).

River-type sockeye CUs are present in two Lower Fraser tributaries, the Harrison River and Widgeon Creek (Grant et al. 2011). Their spawning grounds are less susceptible to exposure to a spill as they are on the north side of the river; however, river-type juveniles are known to rear in the Lower Fraser river and estuary until June/July before out-migration (Johannes et al. 2011), so their risk of spill exposure is far greater than that for lake-type sockeye. Hence, the risk of a potential spill to Fraser sockeye varies between river and lake types, but is highest during peak migration windows which occur throughout much of the spring, summer and fall due to the diversity of populations which spawn throughout the watershed.

4.3.5 Fraser River Chinook salmon

Chinook salmon populations from throughout the Fraser are vulnerable to exposure to a potential spill during much of the year depending on population, CU, and life stage. Mature Chinook migrate through the Lower Fraser to their natal streams to spawn in three run timing groups spanning from February to November (DFO 1995). The Harrison River Chinook population (Lower Fraser River Fall CU) is one of the largest runs in North America, often making up the majority of Fraser Chinook returns (CTC 2014; DFO 1995). This population has a unique life history that makes them vulnerable to exposure to a spill in the Lower Fraser during the spring and summer (Table A.1). Harrison River Chinook fry migrate downstream immediately after emergence to the Lower Fraser and estuary where they rear in tidal creeks, feeding and growing primarily from April to June (can be up to 6 months) before ocean entry (DFO 1995; Levy and Northcote 1982; Murray and Rosenau 1989). Other populations of ocean-type Chinook from throughout the watershed also utilize the Lower Fraser and estuary in the spring months as juveniles (DFO 1995). There are also three CUs of stream-type Chinook populations which occur in Lower Fraser tributaries where they are present as juveniles throughout the year (DFO 2013). Stream-type Chinook from upper and middle parts of the watershed are also vulnerable during their downstream migration which occurs from April to June (Table A.1). The most vulnerable populations to exposure to a spill in the Lower Fraser are likely stream-type populations in tributaries intersected by the proposed pipeline route (Trans Mountain ULC 2013a; DFO 2013), and ocean-type Chinook due to their extensive use of Lower Fraser habitats as juveniles.

4.3.6 Fraser River chum salmon

Chum salmon (*O. keta*) are vulnerable to exposure to a pipeline rupture on the Lower Fraser during upstream migration in fall, and in winter and spring as spawning and incubation areas. Chum spawn at 78 different sites in the Lower Fraser including the mainstem.

Chum begin depositing eggs in the gravel in September, leaving embryos and alevin potentially vulnerable until they emerge in June (Holtby and Ciruna 2007; Grant and Pestal 2009). After emergence juveniles migrate downstream and have been shown to utilize tidal marshes of the Lower Fraser from late-March until early-June (Levy and Northcote 1982), then moving to the Fraser estuary where they are known to rear for up to 6 months before migrating to sea (Groot and Margolis 1991). Chum salmon return to spawning grounds in the Lower

Fraser and throughout the Salish Sea after 3, 4, or 5 years at sea from September through January (Holtby and Ciruna 2007).

Outside the Fraser River, chum salmon spawn in approximately 227 locations in rivers that drain to the Salish Sea (DFO 2014). Upon their return as adults, chum will spawn in the intertidal portions of these streams, a very vulnerable location in the event of tanker based oil spills because as eggs, alevins, and fry are present in the intertidal gravel as early as September and as late as June of the following year. Hence, there is only a very narrow window in the summer when chum are not present in the intertidal zone in hundreds of Salish Sea streams.

4.3.7 Fraser River pink salmon

The vulnerability of Fraser pink salmon is highly dependent on the year, as they follow a strict two year cycle with high and low abundance returns during odd and even years respectively. During odd years an average of 5.8 million pink return to the Fraser, and approximately 70% spawn in the Lower Fraser and tributaries including along the mainstem from Mission to Hope (DFO 1999). Incubating embryos and alevin are vulnerable to exposure while present in the gravel from September until as late as June, before emerging and migrating to the estuary soon after (DFO 1999). During even years pink exist in much lower abundance spawning in only a few locations in the Lower Fraser and Fraser Canyon (Holtby and Ciruna 2007). Therefore it is primarily during the period from August of odd-years until the following June that Fraser pink are vulnerable to exposure to a potential spill.

4.3.8 Provincially managed salmonids

Provincially managed rainbow trout (including steelhead), coastal cutthroat trout, bull trout and Dolly Varden all exist in stream resident, freshwater-migratory, and anadromous forms in the Lower Fraser and tributaries (Table A.1). Steelhead (anadromous rainbow trout) are in decline in the Fraser (MELP and DFO 1998), bull trout were designated as special concern by COSEWIC in 2012 (COSEWIC 2012), and coastal cutthroat trout and bull trout are blue-listed species in BC (BC MoE). While not harvested commercially, all are prized by recreational and First Nations fishers. Stream resident and freshwater migratory populations of these species are present in the Lower Fraser and tributaries year round and therefore always vulnerable to exposure to a potential spill. Anadromous forms of each of these species exist in low abundances relative to salmon, and migrate through the Lower Fraser at various times during the year.

Mature steelhead return to the Fraser in three distinct groups Coastal Winter, Coastal Summer, and Interior Summer (Table A.1) with different vulnerabilities to exposure to a potential spill. Coastal Winter steelhead migrate to twenty-one Lower Fraser tributaries from November to April where they spawn typically from February until April (MoELP and DFO 1998). Coastal Summer steelhead, which spawn only in the Coquihalla, Chehalis and Silverhope rivers, migrate through the Lower Fraser from mid-April to July (MELP and DFO 1998). Coastal Summer steelhead migrate to spawning grounds while still immature, completing their maturation before spawning from April to June. Interior Summer steelhead migrate through the Lower Fraser from September to early November (MELP and DFO 1998). A small percentage of spawning steelhead, the majority females, survive and migrate immediately back to the sea, with less than 10% returning to spawn a second time (MELP and DFO 1998). Coastal steelhead are the most vulnerable to exposure to a spill as they spawn in Lower Fraser tributaries, particularly summer run which rear for up to eight months prior to spawning.

Much less is known about the timing and location of spawning for anadromous coastal cutthroat trout, bull trout and Dolly Varden populations in the Lower Fraser. Mature coastal cutthroat trout are known return to the Lower

Fraser to spawn in sloughs and backwater channels of the mainstem and in many of the major tributaries, returning primarily during August and September (Costello 2008; McPhail 2007). Bull trout and Dolly Varden return to spawn in the fall beginning in August (BC MOE 2004; McPhail 2007). Juvenile of these species all spend at least one year in freshwater, with the majority spending two to three years before migrating to sea. Due to this prolonged use of freshwater tributaries as juveniles these species are all vulnerable year round to exposure to a potential spill in the Lower Fraser.

4.3.9 Fraser River eulachon

Eulachon are an anadromous, semelparous (die after spawning) fish species which spend the majority of their life at sea, but spawn in the mainstem of the Lower Fraser at which time they are vulnerable to exposure to a spill. Eulachon were historically harvested in the Lower Fraser yet this population has declined by 98% to current levels and is currently considered endangered by COSEWIC (COSEWIC 2011). Mature eulachon migrate to the Lower Fraser from February through April, and spawn in areas of medium gravel, pebbles and sands in mainstem reaches of the North and South Arms and upstream to Chilliwack (COSEWIC 2011; LGL and TRSI 2009). Embryos then incubate for less than two months before emerging from April to June, and are immediately swept downstream into the estuary (COSEWIC 2011). As they spawn directly in the Lower Fraser mainstem and currently exist at severely diminished levels, exposure to a potential spill could lead to serious effects on this culturally important fish.

4.3.10 Impacts to fish habitat

Habitat requirements are one of many factors that must be considered when predicting the relative sensitivities of fish to oil exposure after a spill. For example, for all Pacific salmon species, adult fish prepare benthic nesting areas called redds, through which water flow must be high enough to create sufficient oxygen exchange for developing embryos (Geist and Dauble 1998). Thus, embryos developing in redds from fertilization to hatch, and from incubation post- hatch until the onset of feeding, are particularly susceptible to dissolved oil constituents flowing through redds during these life stages (Carls et al. 2005). Oil that becomes entrained in sediments does not wash out quickly, but rather releases its constituents (e.g. PAHs) slowly over time (Chapter 3.2; Marty et al. 1997b; Carls et al. 1999; Martin 2011).

The portion of oil that does not readily evaporate can become stranded (Chapter 3.2.3), causing serious harm to fish that are part of commercial, recreational or Aboriginal fisheries, or to fish that support these fisheries. Shoreline cleanup assessment techniques (SCAT) surveys of shorelines affected by spilled oil can be used in conjunction with mapping of important fish habitat to indicate those habitats susceptible to serious harm. For example, after the release of approximately 149 000 L of Bunker C crude oil into Wabamun Lake, Alberta in 2005, nearly 64% of shoreline representing fish habitat was coated in oil, resulting in harmful alteration, disruption, and/or disruption (HADD) of spawning, rearing, nursery, and migration areas for eight fish species in the Lake (Evans 2008). As a combined result of oiling and subsequent cleanup efforts, which resulted in the release of toxic hydrocarbons (Birtwell 2008), near-shore fish habitat, including spawning habitat for northern pike (*Esox lucius*), was adversely affected and could not be compensated (Evans 2008).

Without cleanup, oil can persist in sediments for many years. For example, by 2005, oiled sediments were still evident in the Pine River, BC following the August 1, 2000 spill of 475 000 L of heavy sour crude (Goldberg 2006), and geospatial models indicate the possibility that there may be areas of Prince William Sound, Alaska that still contain subsurface oil more than two decades after the 1989 *Exxon Valdez* oil spill (Michel et al. 2011). Oil persistence after a spill may be exacerbated by the fact that clean up can take several years. Two years after the Wabamun Lake spill, oil sheens were still visible in reed beds occupied by juvenile fish, and by spring 2008, PAH concentrations toxic to developing fish were still measurable in lake water (Birtwell 2008). Trans Mountain indicated in their submission to the NEB that restoration and recovery may take up to five years following a spill in the lower Fraser River (Stantec Consulting Ltd. 2013). This time frame increases the likelihood that various life stages would experience chronic exposure to oil. Species with life cycles of five years or less (i.e. most Fraser populations) could face exposure in each year class (Chapter 5.2).

Importantly, Trans Mountain does not appear to have a plan in place to clean up oil that sinks to the river bottom. They acknowledge that "weathering of the diluted bitumen combined with specific environmental conditions can increase the potential of some portion of the oil becoming submerged, reducing the effectiveness of a conventional spill response" but go on to discuss methods for recovery of surface oil only (e.g. skimmers, booms, dispersant, in situ burning) (Trans Mountain ULC 2013b). It is also important to note that during freshet, sediment deposition and build-up intensifies to the point that there can be up to 1 metre of sediment deposited in the river in a week (Det Norske Veritas 2012), likely complicating efforts to recover spilled oil.

The most relevant example of the possible consequences of a dilbit spill in the lower Fraser River is the Enbridge pipeline spill near Marshall, MI. In July 2010, diluted bitumen flowed overland into Talmadge Creek and then into the Kalamazoo River. Clean-up of the river bottom and sediments began after the spill and extended into 2014 (EPA 2014). Cleanup operations included, but were not limited to, dredging of oil-contaminated sediments using either excavators or industrial vacuum systems, as well as agitation ('poling') of sediments coupled with oil collection (Enbridge Energy 2013, EPA 2013). Clean up efforts also left 180 000 ± 100 000 gallons of oil on the river bottom. Given the expected widespread distribution of oil in the main river channel, these methods of sediment remediation likely caused extensive damage to fish habitat.

Fish that spawn in tributaries upstream of pipelines and crossings may be less susceptible to oil exposure from a pipeline rupture than fish that spawn in the main stem lower Fraser River downstream of pipeline crossings and proximity. Unaffected tributaries could provide a refuge for these species if they avoided detectable oil, but there is little evidence to suggest this would be the case. Fish habitats most at risk from an oil spill include tributaries crossed by the pipeline, main stem reaches downstream of tributaries at risk, and main stem reaches in close proximity to the pipeline right of way (e.g. much of the lower Fraser River).

4.3.11 Comparing the vulnerability of salmon species

It is difficult to compare salmon vulnerability at a species level because factors such as con-specific life history (within species) and geographic location of spawning populations are more relevant to oil spill risk. A large spill is more likely to have significant adverse effects on specific Conservation Units, specific life history types, and/or specific run timing groups. The exception to this might be Fraser River pink and chum salmon, most of which spawn and rear in the lower sections of the river. All Fraser ocean-type Chinook and river-type sockeye could also be significantly impacted by a spill in the lower river or estuary. These are also important commercial, recreational and First Nations fisheries that could be closed, and their closures could constrain fisheries on healthy populations that co-migrated with these affected populations, extending those consequences beyond just the Fraser River. At regional scale, the loss or decline of any Conservation Unit of Fraser salmon has the potential for significant adverse impacts on First Nations. Wildlife populations are also linked to the local presence and timing of Conservation Units, hence these ecological communities can also be adversely impacted.

There is great uncertainty about the fate and behaviour of spilled diluted bitumen. This translates to speculation about salmon vulnerability. In early stages of a spill, large amounts of dilbit in confined sloughs and channels of the Fraser River during the spring outmigration (of juveniles) could be disastrous for hundreds of thousands of juvenile salmon present. If spilled oil sinks or is not recovered, it could remain in rearing and/or spawning habitats for extended periods, providing ongoing or repeat exposure of embryos to toxic oil residues in spawning gravels, and contaminating food supply and rearing habitat for juveniles. It is likely that spilled dilbit would severely damage the food web on which juvenile salmon depend, leaving young salmon without adequate resources at a time when food availability dictates their survival.

The scale of potential effects on salmon abundance from oil spilled in the Lower Fraser is related to life-history, the number and diversity of spawning populations and locations, and the lifespan for each species. We have calculated the relative sensitivities for each species and life stage based on the number of months they are present in the Lower Fraser and tributaries (Table 4.2). The greater proportion of the year that each species spends in the lower river and estuary, the more susceptible they are to exposure and consequences from an oil spill. Species that have few populations, shorter life cycles and longer residence time in the lower river and/or its tributaries (e.g. rely heavily on spawning areas in the Lower Fraser) are the most likely to be adversely and significantly affected. In this case, chum and pink salmon that spawn and rear in the Lower Fraser River main stem are at high risk. Coho and Chinook are at high risk because of their reliance on the estuary, and the streams downstream of the pipeline crossing. These are discussed below.

Fraser River pink salmon have only one Conservation Unit. They are abundant only in odd-years, during which over 70% of spawning occurs in the mainstem of the Lower Fraser and tributaries (DFO 1999). Their spawning grounds are vulnerable to effects from residual oil, they have a fixed two year life cycle, and spawn in relatively few locations (Grant and Pastel 2009b). Regardless of the timing of a spill, the persistence of oil in spawning grounds of the Lower Fraser could have serious adverse impacts on Fraser River pink salmon.

Similar to pink, the majority of spawning locations for chum salmon are in the Lower Fraser mainstem and tributaries, and the Lower Fraser is one of only two CUs for Fraser chum (Holtby and Ciruna 2007). However, unlike pink, chum typically spend between two and five winters at sea. This could lower the overall risk for a given year class if toxic residual oil was present only in the short term.

Juvenile salmon of species and life history types that spend extended time feeding, rearing and undergoing osmoregualtory changes in the lower river and estuary prior to migrating would also be at risk. For these salmon (specifically chum, ocean-type Chinook, river-type sockeye, and nomadic coho), a spill could result in significant adverse effects on commercial, recreational and First Nations fisheries. Conversely, species with a large diversity of populations that spawn throughout the watershed, have longer life cycles, and have less reliance on the lower river and estuary (i.e. Lake-type sockeye, stream-type Chinook, some coho), are less likely to experience significant abundance reductions.

Many coho populations spawn in tributaries of the Lower Fraser; these populations account for two of the eight Fraser CUs (Holtby and Ciruna 2007). Populations that spawn in the Lower Fraser are susceptible to residual oil in their natal streams where juveniles are present year round (Table 4.2). Coho also spend two to four years at sea, which reduces the overall risk for a given year class in the short term.

Fraser Chinook also face a similar level of risk, as a significant portion of juveniles rely heavily on the Lower Fraser and estuary. Chinook salmon spawn throughout the Fraser system, and five out of eighteen Fraser CUs spawn in Lower Fraser tributaries (DFO 2013). The Harrison River CU, which often makes up the majority of

Fraser Chinook returns, extensively utilizes Lower Fraser tributaries as primary rearing habitats (DFO 1995). Stream-type Chinook populations are also present in several Lower Fraser tributaries year round. Chinook typically spend one to four years at sea, which reduces the risk for an individual year class if residual oil is present only in the short term (Tables 4.2 and B.1).

Sockeye have a large diversity of populations distributed throughout the watershed and most CUs use the Lower Fraser only as a migration corridor. Sockeye also typically return after more than four years at sea, increasing the number of brood years and lowering their overall risk. Of the 24 sockeye CUs in the Fraser watershed, seven (two river-type and five lake-type) spawn in the Lower watershed (Grant et al. 2011). Spawning locations for lake-type populations are unlikely to be exposed to residual oil as they are upstream of associated nursery lakes and the proposed pipeline corridor. However river-type sockeye are known to spawn in tributaries such as the Salmon River, which is crossed by the proposed pipeline corridor (Grant et al. 2011; PIBC 1999). The river-type sockeye also rely on the lower river and estuary for rearing. One of these CUs (Harrison) is very important to commercial, recreation and First Nations fisheries.

Table 4.2. Summary of timing, habitat utilization, and relative sensitivities of all life stages of salmon and eulachon in the Lower Fraser River (See Table A.1 for detailed review of spawning behaviour and life history characteristics).

Species	Life stages in the lower Fraser River	Habitat used in the Lower Fraser River	Total time spent at sea (years)	Time spent in Lower Fraser and tributaries per year (months)	Relative vulnerability to exposure to a spill (# months in Lower Fraser River/ 12 months per year
Chinook Salmon	**Embryo**	Large and Medium sized gravel redds		**10**	**1**
	Juvenile	Small tributary streams and marshes	**2-5**	**6**	**.50**
	Adult	Migrate through mainstem to tributaries		**6**	**.50**
Chum Salmon	**Embryo**	Medium sized gravel redd		**9**	**.75**
	Juvenile	Small tributary streams and marshes	**2-5**	**4**	**.33**
	Adult	Spawn in main stem and tributaries		**6**	**.50**
Coho Salmon	**Embryo**	Medium sized gravel redd		**8**	**.67**
	Juvenile	Small tributary streams and marshes	**1-3**	**12**	**1**
	Adult	Migrate through main stem to tributaries		**5**	**.42**
Pink Salmon	**Embryo**	Medium sized gravel redd		**9**	**.75**
	Juvenile	Small tributary streams and marshes	**1**	**5**	**.42**
	Adult	Spawn in main stem and tributaries		**5**	**.42**
Sockeye salmon	**Embryo**	River-type- medium sized gravel		**6**	**.5**
	Juvenile	Small tributary streams and marshes	**1-4**	**4**	**.33**
	Adult	Migration through main stem to tributaries		**4**	**.33**
Steelhead/	**Embryo**	Medium sized gravel redd		**6**	**0.5**

rainbow trout	**Juvenile**	Small tributary streams and marshes	**2-3**	**12**	**1**
	Adult	Migrate through main stem to tributaries		**12**	**1**
Coastal cutthroat trout	**Embryo**	Medium sized gravel redd		**6**	**0.5**
	Juvenile	Small tributary streams and marshes	**1**	**12**	**1**
	Adult	Spawn in main stem and tributaries		**12**	**1**
Bull trout	**Embryo**	Small to Medium sized gravel redd		**6**	**0.5**
	Juvenile	Small tributary streams and marshes	**1**	**12**	**1**
	Adult	Spawn in small tributaries		**12**	**1**
Dolly Varden	**Embryo**	Small to Medium sized gravel redd		**6**	**0.5**
	Juvenile	Small tributary streams and marshes	**1**	**12**	**1**
	Adult	Spawn in main stem and tributaries		**12**	**1**
Eulachon	**Embryo**	Mixed gravel and sand, no redd		**4**	**.33**
	Juvenile	Migrate downstream soon after emergence	**2-3**	**0**	**.0**
	Adult	Spawn mostly in main stem		**3**	**.25**

Rainbow trout (steelhead), coastal cutthroat trout, bull trout and Dolly Varden are all vulnerable to exposure to residual oil as they spend extended periods (in many cases two to three years) in the Lower Fraser and tributaries as juveniles. These species all have long life cycles, including various levels of repeat spawning, which could buffer their populations from the effects of residual oil. Eulachon are vulnerable to exposure to residual oil during incubation periods in the Lower Fraser mainstem, but are somewhat buffered by a two to four year life cycle.

Overall, a large spill could affect salmon broadly across species and the watershed, but it is more likely to have significant adverse effects on specific Conservation Units, specific life history types, and/or specific run timing groups; the exception to this being Fraser River pink salmon and Fraser River chum salmon. Fraser River First Nations cultures are often closely tied to individual CUs. The loss or degradation of any Conservation Unit of Fraser salmon thus has the potential for significant adverse impacts on First Nations. Wildlife populations are also linked more tightly to the local presence and timing of Conservation Units, hence these ecological communities can also be adversely impacted. If the spill was to impact the Fraser's ocean-type Chinook or river-type sockeye, both commercial and recreational fisheries could be severely impacted. These economic and FSC impacts could extend to healthy populations of Pacific salmon that co-migrate with these impacted populations, and are constrained.

4.4 SALISH SEA SALMON HABITAT USE AND VULNERABILITY TO EXPOSURE TO AN OIL SPILL

Important spawing and rearing habitats for all five species of commercially managed salmon lie within the Salish Sea (Figure 2.3). Trans Mountain's marine Regional Study Area (RSA) does not capture the full extent of the Salish Sea habitats that could be exposed to a spill, representing 58% of the Salish Sea's marine waters and 68% of its Canadian salmon bearing streams (259 out of 380). In addition to the Fraser, 190 large and small estuaries critical for salmon rearing, staging and migration also lie within the Salish Sea. Five of these estuaries- the Nicomekl/Serpentine River, Cowichan River, Chemainus River/Bonsall Creek, Nanaimo River, and the Courtenay River, lie within Georgia Strait and rank in the top 10 of British Columbia's 442 estuaries for their important ecological value (CWS 2007). With the exception of the Courtney River, all these estuaries lie with Trans Mountains' RSA.

Juvenile salmon rely on estuaries and near-shore environments largely in accordance with their body size (Koski 2009; Table 4.3). Generally, larger fry and fingerlings that have been rearing in freshwater depend on estuaries for shorter periods; yet even these less dependent types will use intertidal habitats for feeding as they migrate towards the open ocean. More dependent types of juvenile salmon can spend several months feeding, growing, and transitioning to marine waters. Chum, pink, ocean-type Chinook, ocean-type and nomadic coho (Koski 2009), and river-type sockeye use the stream-estuary ecotone, sloughs, estuaries, and near-shore waters for weeks to several months (Langer 2010) before undertaking further coastal migrations.

Traditional perspectives on the timing of fry out-migration (i.e., that most species leave near-shore habitats by early summer) are being revised as more research shows some species and life history types utilize near-shore and estuarine habitats for much longer than previously believed (PERS 2004). These early life history stages are very important in determining ultimate survival rates of salmon (Greene et al. 2004, Beamish et al. 2004, Bottom et al. 2005).

Table 4.3. Use of local estuaries and near-shore habitats by different species and life-history types

More estuary use	Less estuary use
Pink – few weeks to few months [a]	Stream-type Chinook
Chum – 1 to 3 months [b]	Stream-type coho
Ocean-type Chinook – few months to a year [c]	Lake-type sockeye
River-type sockeye - weeks to months [d]	
Ocean-type /nomadic coho – several months to a year [e]	

[a] NOAA 2005; [b] Dunford 1975, NOAA 2005; [c] Levings et al 1986; [d] Langer 2010; Johannes et al. 2011 [e] Koski 2009.

The presence, timing and use of the Salish Sea, by juvenile, immature and adult salmon varies with each species and life-history type. Pacific salmon have developed highly variable rearing strategies to take advantage of diverse freshwater, estuarine and marine habitats as juveniles. This general behavior allows salmon to take advantage of more productive habitats downstream resulting in an adaptive capacity to be more ecologically resilient (Healey 2009). Within the Salish Sea, the Strait of Georgia (the Fraser estuary) is considered the most important rearing area for juvenile Pacific salmon on Canada's Pacific coast (Beamish et al. 2005). The majority of juvenile salmon enter Georgia Strait from their natal streams and rivers between April and June, with juvenile coho and Chinook salmon generally entering later than juvenile chum (Beamish et al. 2005). Many of these juvenile salmon stay in Georgia Strait and surrounding waters until the fall (Beamish et al. 2000). In addition, hatchery and wild salmon from Puget Sound are also present in Canadian waters of Georgia Strait and other parts of the Salish Sea (Beamish et al. 2000; Trudel et al. 2000).

Chinook salmon stand apart with the most atypical life history types and the greatest year round presence. Upon leaving their natal rivers, Chinook that migrate as subyearling can rear for extensive periods in estuaries. Irrespective of their freshwater life history (i.e., whether migrating as sub yearlings or yearlings) and run timing, juvenile Chinook salmon from Salish Sea remain within 200–400 km of their natal rivers until their second year at sea (Trudel et al. 2009). Adult Chinook return to estuaries in Georgia Strait prior to river entry. Spring-run timing Chinook such as those returning to the Nanaimo and Fraser Rivers, enter their natal streams from May to July. Summer-run adults, such as those returning to the Puntledge River (PSRCC 2009), Cowichan River (Hyatt 2013), Squamish River, the Fraser River and the mainland Inlets (DFO 1999) enter their natal streams from June to August. Fall-run adults (Fraser, Cowichan, Nanaimo, Chemanius, Goldstream) enter their natal streams August to September. These strategies dictate a year-round presence of Chinook as juvenile, immature or adult salmon within Georgia Strait and the Salish Sea (DFO 1999, Trudel et al. 2009).

Young chum salmon rely heavily on the shallow intertidal waters of estuaries and the head of bays upon their marine entry (NOAA 2005). They will spend several months over the spring and summer in these shallow, protected areas. As they grow, juvenile chum salmon will increase their presence in deeper coastal waters from July through October, as they move north along the coast. As adults, summer runs of chum salmon are present in the Salish Sea, migrating back to inside waters from June to August (DFO 1999), but most adult chum spawners return to inside waters of the Salish Sea in late summer, and enter streams from October to December. There is a very narrow window in the summer when the intertidal zone in hundreds of Salish Sea chum streams would be free of chum salmon in their egg, alevin, fry or adult life stage.

While most coho salmon (*O. kisutch*) fry will remain in freshwater for up to two years, more extensive rearing periods in estuarine habitats are typical of ocean-type and nomadic coho, which exploit the higher productivity of the estuarine environment by migrating downstream as subyearlings (Koski 2009). Ocean-type coho will rear in the estuary for several months (May to August) while they acclimatize and reach minimum sizes (around 9 cm) before undertaking further marine migration. Nomadic coho also migrate to brackish water as subyearlings but use the stream estuary ecotone for rearing from May to October (Koski 2009), occupying glides and pools during low tide, and the freshwater lens during high tide. Instead of migrating farther to the ocean, they return upstream into freshwater to overwinter before migrating to sea as smolts the following year. This unique use of overwintering and estuarine habitats has enabled coho to develop a life strategy that promotes their resilience. The loss or decline of these nomads affects adversely the diversity and abundance of coho populations.

There are 55 known even- year spawning sites and 155 odd-year spawning sites for pink salmon in the Canadian waters of the Salish Sea (Holtby and Ciruna 2007). Upon ocean entry from their natal streams, pink salmon fry tend to stay near shorelines during the first weeks at sea, spending much of their time in shallow water often only a few centimeters deep (NOAA 2005). Bays, inlets and kelp beds are typically utilized over the following two to three months as they grow. Juvenile pink salmon will continue to utilize the upper portion of the water column for up to six months as they transit into coastal and shelf migrations (NOAA 2005). Pink and chum salmon fry of similar age and size often co-mingle in early sea life and can travel in schools as large as tens or hundreds of thousands of fish (NOAA 2005).

Lake-type sockeye move relatively quickly through near shore marine waters to begin their north coastal migration. River-type (also called ocean-type) sockeye on the other hand, migrate seaward at a much younger age and rely on estuary and intertidal river habitats for rearing for the first 3 - 4 months (NOAA 2005). The largest river-type populations in the Salish Sea are from the Lower Fraser River and are known to utilize estuary habitats primarily in June and July before out-migration (Johannes et al. 2011). River-type sockeye generally have small populations compared with their lake-rearing counterparts, however from an evolutionary perspective, river-type sockeye are colonizers of vacant habitats (Holtby and Ciruna 2007) – an unlikely trait in the lake-specific ecotype; hence the importance of river-type sockeye.

4.5 EXPOSURE SCENARIOS BY RECEPTOR: MECHANISMS FOR TOXICITY TO SALMON

There are several mechanisms by which oil can injure salmon, including direct contact, contamination of food supply, and toxicity of hydrocarbons dissolved in water. Juvenile salmon can also be indirectly affected by the loss of food resources and the inability to feed at key life stages and locations.

Oil can harm salmon by fouling them directly, by coating substrates on which they rear and feed (e.g., benthic or inter-tidal habitat), by direct toxicity of oil dissolved in water, by contaminating and tainting their food supply, and/or by loss of food supply and resources impacted by oil exposure. Salmon may face oil exposure in their diet if they consume benthic invertebrate species, particularly filter-feeders. Many invertebrate species cannot effectively metabolize hydrocarbons and as such, accumulate them in their tissues (e.g. Meador et al. 1995; Meador 2003). Filter feeders may even accumulate droplets of oil (Neff and Burns 1996). Juvenile salmon are also susceptible to the loss of food resources. Benthic fish species and/or those with a benthic life stage or feeding stage (e.g., eggs and embryos that are deposited in sediments or juvenile salmon that feed on benthic invertebrates), could also be exposed to oil that coats substrates. Because of gill respiration, all fish would be exposed to oil droplets in water, or to individual hydrocarbons dissolved in water.

4.5.1 Embryos

The embryonic and larval life stages of salmon development are particularly susceptible to oil toxicity. This is due to their immobility, their ongoing development, and the potential for PAHs to partition into the lipid membranes of embryos. However, exposure to oil lasting only a matter of hours can be enough to decrease the survival of embryos or compromise development. The extent to which dilbit sank following the Kalamazoo River spill suggests a risk for long-term toxicity to developing embryos.

Embryonic stages of fish are particularly susceptible to oil toxicity (both acute and chronic) in part because they are immobile. The most sensitive stages of development are as gametes immediately following fertilization, and as embryos immediately following hatch. Of particular importance are the blastula and gastrula stages within 24-48 hours of fertilization (McIntosh et al. 2010; Greer et al. 2012). Exposure within this time frame decreased hatch success by 40%, whereas exposure 72 hours post-fertilization did not affect hatch success (Kocan et al. 1996a).

Embryos before hatch appear to be more resistant to exposure to oil components (e.g., PAH), because the chorion surrounding the egg creates some resistance to uptake and excretion of toxic components. In contrast, embryos after hatch (larva, or alevins in salmon) are the most sensitive life stage when exposed to alkyl PAHs found in oil (Brinkworth et al. 2003).

If oil becomes entrained in bottom substrate (e.g. gravel or sediment), the eggs would be continuously exposed to hydrocarbons partitioning from oil into the interstitial water and then into lipid-rich embryos. As demonstrated by Carls et al. (2005) using simulated gravel redds, water moving through oil-coated gravel can pick up hydrocarbons at concentrations that are embryo toxic. Salmon redds (nests) are purposely created in the hyporheic zone of stream beds so that fish embryos will receive an adequate oxygen supply (Carls et al. 2005; Tonina and Buffington 2009b).

Salmon and eulachon eggs deposited in sediments are immobile, and can thus be exposed to toxic levels of dissolved PAHs for their entire developmental period as a consequence of PAHs partitioning into interstitial water from oil entrained in sediments, and then into the eggs themselves (Marty et al. 1997b; Carls et al. 1999; Heintz et al. 1999). However, even short exposures can be enough to impact the viability of larva. A 2.4 hour exposure to chemically dispersed crude oil was sufficient to induce Blue Sac Disease (BSD) in herring embryos and lead to fewer normal embryos at hatch (Greer et al. 2012). If oil is deposited in spawning substrates, exposure to its toxic constituents will be greatest when the oil is relatively fresh and each constituent can partition from the oil into water and from the water to the embryo (Carls et al. 2005). Uptake of hydrocarbons may also occur in cases where eggs are in direct contact with oiled substrate (Couillard 2002). Similarly, oil droplets generated by chemical dispersion appear to stick to gill surfaces of larva and absorb into the tissue (Ramachandran 2005). In these cases, the constituents of oil may partition from the oil phase directly into the lipid phase of biological membranes, such that uptake and toxicity are rapid. These findings indicate that oil droplets can act as PAH reservoirs that may cause uptake and toxicity as a result of direct contact; however, toxicity can be explained on the basis of uptake from water alone, without requiring direct contact with oil droplets (Carls et al. 2008).

Recent work suggested that “the propensity of HFO [heavy fuel oil] to sink and strand in spawning shoals creates a long-term risk to developing fish because of the sustained release of PAHs from HFO to interstitial waters” (Martin et al. 2014). Observations from the Enbridge spill into the Kalamazoo River, MI, suggest that the same concern exists with respect to dilbit.

4.5.2 Juveniles and adults

The extent to which juvenile and/or adult salmon can or would avoid spilled oil is unclear. Regardless, juvenile salmon must spend considerable time in the Fraser River estuary to complete smoltification, during which time they are particularly vulnerable to additional stressors. Adult salmon must also migrate through the Lower Fraser River to reach their natal streams. Studies offer conflicting evidence as to whether salmon will avoid spilled oil, but even successful avoidance of contaminated areas may result in failed spawning and subsequent population reductions.

Immature and adult stages of salmon and other anadromous fish may face less risk than embryos and larva when they are at sea. It is unclear whether juvenile and adult stages of resident fish species are able to or would avoid areas affected by an oil spill.

Although juveniles leaving the spawning gravels are mobile and may be able to avoid direct contact with spilled oil, they must, at some point, complete smoltification in the Fraser River estuary. Smoltification occurs in brackish water, and is the metamorphic stage of development for anadromous fish, consisting of a series of morphological, physiological, and behavioural changes that facilitate transition to seawater (summarized in Folmar and Dickhoff 1980). Physiological changes facilitate osmoregulation (maintenance of internal water/salt balance) in higher salinity environments (seawater), while behavioural changes prepare the fish for migration to sea and a pelagic lifestyle. During smoltification, salmon are under physiological stress and are highly sensitive to additional stressors, including disease and degraded habitat conditions (Folmar and Dickhoff 1980). Further, fish are essentially ‘stuck’ in the estuary while they undergo this process, such that avoidance of spilled oil may be impossible. Due to predicted rapid transit times of spilled oil (e.g. 1-2 days from the Port Mann Bridge to the estuary; Trans Mountain ULC 2013b) and similar time frames for oil spilled in Georgia Strait to reach the delta (Trans Mountain 2013d), it is possible that the oil will still contain acutely toxic LMW components, such as BTEX and naphthalenes. In the shallower waters of the estuary, phototoxicity (sunlight enhanced toxicity) may present an additional risk factor (Chapter 5.4.3).

Adult salmon spawning migrations to reaches of the Lower Fraser River are critical events that occur mainly from April to December every year (Table 4.1). There is limited research on the impacts of an oil spill on adult salmon migration behaviour. In laboratory studies, juvenile coho salmon avoided a mixture of hydrocarbons similar to a slick from an oil spill (Maynard and Weber 1981), and there is evidence to suggest that adult salmon can detect and will avoid an oil slick (reviewed in Birtwell 2008). However, in both cases, the authors pointed out that unmotivated behavior in the laboratory may not reflect motivated wild behavior, such that they could not say with certainty that mature salmon migrating upstream during the peak of a spawning run would avoid an oil slick, given the strength of the migration instinct at spawning. Other research indicated that juvenile salmonids do not always avoid oil and will feed on oiled prey and strike at oil droplets (Willette et al. 1996). Further, their innate surface water orientation behavior may actually result in increased contact with oil (Willette et al. 1996). For Pacific salmon species, attempts to avoid certain areas (e.g. migratory routes and home spawning streams) where oil is present may result in failure to spawn successfully, causing reductions in spawning adults in future years.

Adult salmon that do not avoid contaminated areas and continue with their migration may not be able to arrive at their natal streams if the olfactory cues that assist with homing are affected (Hara et al. 1976). Adult Chinook salmon exposed to both undispersed and dispersed Prudhoe Bay crude oil for one hour in fresh water were still able to return to their natal stream (72% overall homing frequency), indicating that olfactory cues were unaffected (Brannon et al.1986). Similar results were obtained by Birtwell et al. (1999), who concluded that there were no effects on homing ability 17-18 months after a ten day exposure of pink salmon fry to the water-soluble fraction (WSF) of North Slope crude oil. However, the WSF consisted primarily of monoaromatics, and thus reflects a minor temporal component of likely exposure scenarios, and does not consider the effects of larger, more chronically toxic PAHs.

4.6 INVERTEBRATES

Invertebrates are an important food source for salmonids inhabiting the Lower Fraser River. Contamination of invertebrate species is of concern for two reasons: 1) dietary exposure to PAHs can impact survival, growth, and development in fish, and 2) toxicity-induced mortality of invertebrates may reduce populations and limit prey availability for salmonids.

The health of invertebrate communities and their vulnerability to the effects of an oil spill are beyond the scope of this report, and will not be discussed in detail here. However, due to their importance as a food source for juvenile fish, some general comments are provided.

Invertebrate communities, including those in pelagic and benthic areas, are the primary food source for juvenile salmon and other fish. As indicated in Table A.1, fish species that utilize the Lower Fraser River and its tributaries feed on a variety of invertebrates, including, but not limited to: amphipods, chironomids, cladocerans, copepods, decapods, dipterans, euphausiids, mysids, oligochaetes, snails, and trichopterans. For example, Figure 4.1 illustrates the complexity of the food web in the Fraser River estuary.

To examine the effects of dietary exposure to PAHs, zebra fish were fed a diet spiked with PAH fractions derived from heavy fuel oil (HFO) and light crude oil (LCO) from 5 days post-fertilization until they became reproducing adults (6 months later) (Vignet et al. 2014a). Exposure to both HFO and LCO resulted in inhibited growth in males and exposure to HFO reduced survival in both sexes. Larvae also exhibited disrupted jaw growth, resulting in jaw malformations in adults, and 2 month old fish exhibited abnormal intestinal and pancreatic enzyme activities (Vignet et al. 2014a). These results indicate that chronic consumption of oil-contaminated invertebrates may result in adverse effects in developing fish, including salmon.

Unlike vertebrates, many invertebrate species have a very limited ability to metabolize hydrocarbons, resulting in bioaccumulation in their tissues (e.g. Meador 2003). This is of concern for two reasons. First, hydrocarbons may be directly toxic to invertebrate species. Following the Pine River spill in 2000, both the abundance and diversity of benthic invertebrates was depleted for up to three years, with an apparent correlation between sediment TPAH concentrations and invertebrate abundance (de Pennart et al. 2004). A depleted invertebrate community would negatively impact the aquatic ecosystem of the Lower Fraser River by limiting the food source for some fish species at various life stages. Second, by bioaccumulating hydrocarbons in their tissues, invertebrates can act as a hydrocarbon source to their predators.

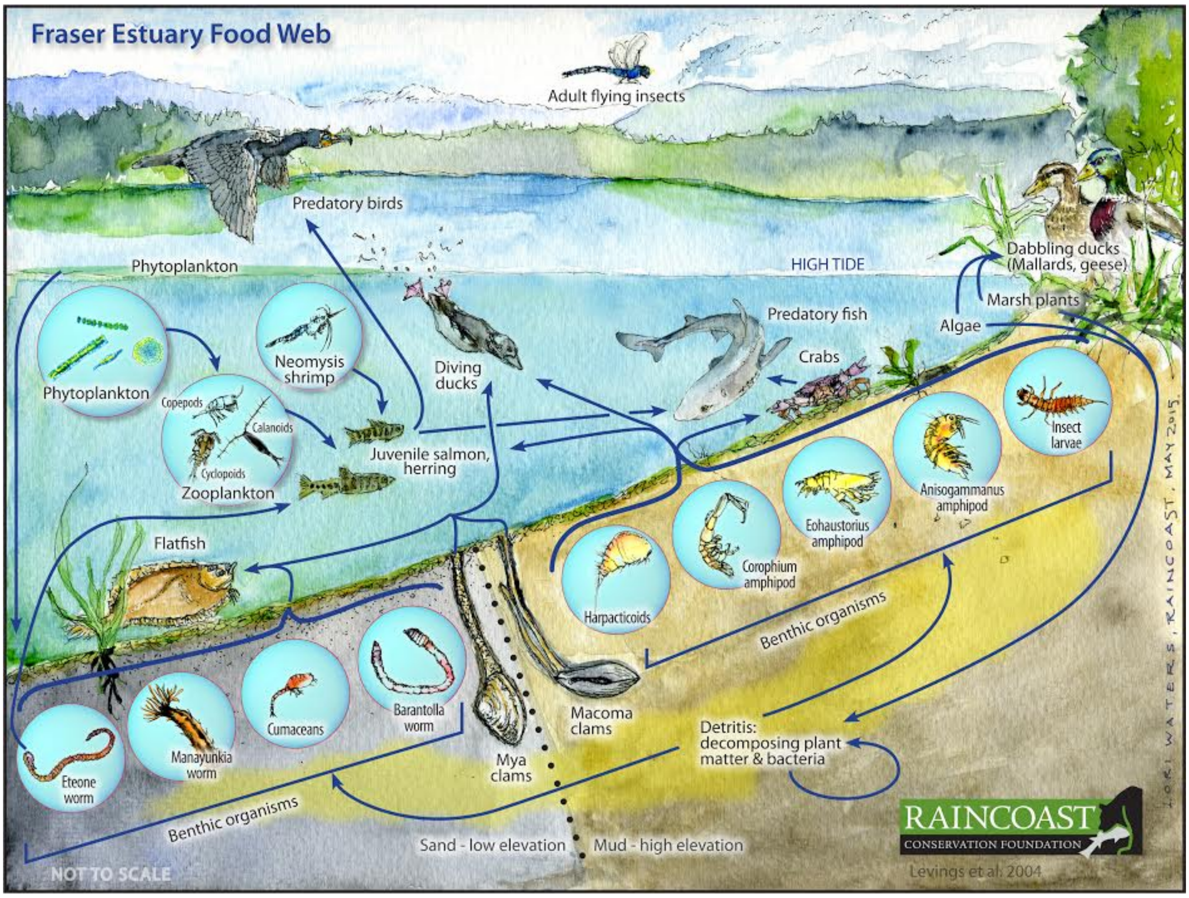

Figure 4.1. A stylized version of the food web at the Fraser River estuary. Typical flora and fauna found along a vertical gradient from high to low tide and offshore and their trophic interactions. Detritus is the combination of decomposing plant material and bacteria fed upon by invertebrates such as bivalves, amphipods, and other crustaceans. These in turn are fed upon by fish, crabs and birds (Levings 2004). Figure after Levings 2004.

5 TOXICITY OF OIL TO FISH

It should be noted that this chapter is an update and expansion of Chapter 4 in Hodson, Collier, and Martin (2011), with updates to the literature where available, and specific references to the Lower Fraser River where appropriate.

Oil toxicity to fish stems either from direct action of hydrocarbons on tissues, or from indirect effects of hydrocarbon degradation products created by oxygenation or photoactivation either in the environment or in fish tissues. Indirect effects may also include suffocation if there is sufficient microbial activity to rapidly biodegrade oil and reduce waterborne oxygen concentrations. This chapter describes direct and indirect effects on fish of exposure to oil products observed in both laboratory and field settings.

This chapter reviews toxicity studies on both freshwater and marine fish species (and freshwater and marine life stages). Despite the fact that PAHs are more soluble in freshwater than in salt water, PAH toxicity to fish embryos has not demonstrated a systematic dependence on salinity (Hodson 2008). Further, only small differences in toxicity appear to exist among marine, estuarine, and freshwater fish species, and as such, it is judged appropriate to use data derived from both freshwater and marine species to examine potential oil toxicity (Hodson 2008; Birtwell 2008).

5.1 ACUTE TOXICITY

Acute toxicity generally refers to toxicity observed within a short period of time following exposure or after a short exposure period. Following an oil spill, this is often expressed as fish kills within 24-48 hours. Acutely toxic PAHs are primarily the low molecular weight (LMW) compounds, which exert toxicity via narcosis by several potential mechanisms. Acute toxicity is strongly correlated with the octanol-water partitioning coefficient (Kow), such that it is ultimately limited by molecular size. Acute toxicity would be expected to vary based on the sensitivity of the life stage and the type of oil spilled.

Acute toxicity refers to any effect observed after a short exposure period, and/or an effect that becomes apparent within a short period of time following exposure. Acute is a relative term: what is acute for an adult salmon (e.g., 1 or 2 days in a 6 year life span) would be considered chronic for a bacterium whose life span is 1 or 2 days. In this report, 'acute' will refer to exposures or toxic effects that occur within 1 to 4 days, and 'chronic' to refer to exposures or responses that may occur or develop over weeks, months, or years.

These categories can overlap. For example, the concentrations of oil required to cause toxicity decrease as exposure duration increases. Acute exposures (1-2 hours) of Atlantic herring (*Clupea harengus*) embryos to dispersed oil immediately following egg fertilization caused toxicity that was evident in embryos when they hatched 12 days later (a chronic or delayed effect) (McIntosh et al. 2010). However, as exposure time increased, the cumulative exposure to hydrocarbons also increased, such that the median effective concentration (EC50) lethal to herring embryos decreased. For 24 hour exposures, the concentrations causing long-term embryo toxicity decreased 3-fold compared to the 1-hour exposure, and when the exposure was prolonged throughout the entire 12 day period of embryonic development, these concentrations decreased 40-fold (McIntosh et al. 2010). Essentially, the longer the period of exposure, the lower the concentrations required to cause toxicity.

While McIntosh et al. (2010) measured the chronic toxicity of acute exposures to oil, most acute toxicity refers to acute or short-term mortality. In the environment, this is typically expressed as fish kills in the 24-48 hours following a spill. For example, in August 1994, a spill of 30 m^3 (30 000 L) of gasoline and 24 m^3 of diesel fuel in the Pine River, BC caused the direct mortality of 150 mature and 1000 juvenile fish (Goldberg 2011). On August 1, 2000, a second spill resulting from a pipeline rupture leaked of 952 m^3 of crude oil, half of which (475 m^3) entered the river. About 1600 dead fish were collected downstream of the rupture (Goldberg 2011), but the river was difficult to survey and estimates of total fish mortality varied widely, from 15 000 to 250 000 individuals (Goldberg 2011; Baccante 2000).

The components of oil that are acutely toxic include LMW alkanes, monoaromatics (benzene, toluene, ethylbenzene, xylenes; BTEX), and LMW PAH (2-ringed naphthalenes). These compounds are sufficiently water soluble to rapidly reach lethal concentrations in water contaminated with fresh oil. While their concentrations may diminish rapidly due to dilution, volatilization, and biodegradation, lethal concentrations may persist if conditions do not favour weathering (e.g., cold, cloudy conditions with little wind), or if there is a continuous input of fresh oil.

Acute toxicity is due to narcosis, a reversible, non-specific effect similarly to over-anaesthesia. While narcosis implies one mode of action, a review by Campagna et al. (2003) indicated several molecular interactions between lipid-soluble organic compounds and different membrane receptors. Despite multiple modes of action, the toxicity of lipid-soluble petroleum hydrocarbons is strongly correlated with their octanol-water partitioning coefficient (Kow), calculated as the ratio of solubility in n-octanol (a surrogate for lipid) to solubility in water. The larger the molecular size, the more lipid soluble (lipophilic) and the less water soluble (hydrophobic), such that it can be taken up via the gills. Ultimately, the acute toxicity of hydrocarbons is limited by their molecular size. Hydrocarbons with a log Kow greater than 6.0 are too large for rapid passage through complex cell membranes. However, this upper limit applies only to acute toxicity, specifically the Target Lipid Model (TLM) (Di Toro et al. 2000). While this model was later updated in an attempt to capture chronic toxicity (McGrath and Di Toro 2009), the upper limit of log Kow values encompassed by the model was still below the values of most 3-5 ring alkyl PAHs. Chronic toxicity, which occurs by different mechanisms, can occur at log Kow values surpassing 6.0 and is discussed in Chapter 5.2.

For chemicals with a log Kow less than 6.0, log Kow can be used in the TLM, which predicts the concentration of a chemical in water that will bioaccumulate to a lethal dose (critical body burden) in fish lipids (Di Toro et al. 2000; McGrath and Di Toro 2009). Compounds whose acute toxicity varies with Kow and which share a common mode of action (e.g. LMW hydrocarbons acting by narcosis), appear to act additively in mixtures. The rate of uptake of hydrocarbons by fish also varies with Kow; small molecules are taken up more rapidly than large. Thus, models of oil spills can be used to integrate Kow, rates of uptake, bioconcentration factors, and exposure time to estimate the acute lethality to fish of complex mixtures of hydrocarbons measured in water following oil spills (French-McCay 2002). Narcotic effects are reversible if the exposure is reduced or removed before lethality occurs, because the hydrocarbons can partition from fish tissue back into water, thereby reducing tissue concentrations below a lethal level. Thus, the application of these models during an oil spill can help to predict the extent of acute impacts on fish populations and help to direct spill-response measures.

The exposure time required to induce acute toxicity can also vary with the sensitivity of the life stage. As discussed previously, the most sensitive life stages for herring are when they are gametes immediately following fertilization, as embryos immediately following hatch, and the blastula and gastrula stages within 24-48 hours of fertilization (reviewed in Greer et al. 2012). Exposure to environmentally relevant concentration of chemically dispersed crude oil for a duration of only 2.4 hours from fertilization was sufficient to cause symptoms of BSD and to decrease the number of normal embryos at hatch (Greer et al. 2012).

In the case of the Pine River spills, only small amounts of gasoline and diesel fuel were required to cause a fish kill compared to the spill of crude oil, likely because these products contained a much higher proportion of the LMW components associated with acute lethality. To the best of our knowledge, in-depth studies on the potential for chronic exposure and effects after the Pine River oil spill are unavailable. However, as will be reviewed below, the residual heavy oil would likely cause a much greater chronic toxicity to fish embryos. Following the Kalamazoo River spill, 42 dead fish were recovered (Enbridge Energy 2011b). Again however, to the best of our knowledge, peer-reviewed literature on this spill, and specifically on the acute and chronic toxicity to fish and other aquatic organisms is unavailable. Without a more in-depth understanding of acute exposure to fish and potential ongoing toxicity as a consequence of sunken dilbit, it is difficult to apply lessons learned in this spill to a potential spill in the Lower Fraser River.

5.2 CHRONIC TOXICITY

Chronic toxicity refers to effects that develop over a long period of time resulting either from brief or prolonged exposure. Of the compounds in oil, PAHs are considered to exert the greatest chronic toxicity, particularly the 3- to 5-ringed alkyl PAHs. However, chronic toxicity has been observed that cannot be attributed to identified PAHs, underscoring the amount of research still required to provide toxicity data on the thousands of individual PAHs present in oil. Models to estimate acute toxicity cannot be applied with certainty to many of the compounds known to cause chronic toxicity, largely due to the uncertainty of the relationship between Kow and toxicity for larger compounds and to the many mechanisms by which chronic toxicity may occur.

As indicated, chronic toxicity refers to any effects on exposed individuals that develop over a long period of time, resulting either from a brief exposure (i.e., delayed effects), or from a prolonged or chronic exposure. Compounds associated with chronic toxicity will be those that persist in the days, weeks, months, and years following a spill and that are bioavailable (i.e., that can cross biological membranes sufficiently quickly to reach toxic concentrations in fish tissues). These could include intermediate-sized linear or branch-chained alkanes with 12 to 24 carbon atoms and 3- to 6-ringed PAHs and heterocycles. Of these, PAH are considered to exert the greatest chronic toxicity (Tuvikene 1995). The 3- to 5-ringed alkyl PAH are the most likely PAH to cause chronic toxicity to fish embryos (e.g. Le Bihanic et al. 2014; Brette et al. 2014), based on studies of single compounds (e.g., Turcotte et al. 2011), on similarities between the toxic effects of whole oil and of reference PAH such as retene (e.g., Schein et al. 2009), on the correlation between concentrations of specific families of PAHs and chronic toxicity in studies of weathered oil (e.g., Carls et al. 1999), and on effects-driven chemical fractionation (EDCF) (Hodson et al. 2007a, Adams et al.2014).

In fractionation studies with Alaska North Slope Crude (ANSC) and Scotian Light (SCOT) oils, fractions rich in alkyl phenanthrenes, fluorenes, chrysenes, pyrenes, dibenzothiophenes, and naphthobenzothiophenes were toxic to Japanese medaka embryos and caused CYP1A induction in rainbow trout (Hodson et al. 2007a). The induction of these enzymes is a detoxification response and a signal of exposure. Non-toxic fractions contained LMW aliphatics and aromatics (including alkyl naphthalenes) and the very high molecular weight waxes, resins, asphaltenes and PAHs (Hodson et al. 2007a, Adams et al. 2014). Alkyl PAH concentrations were much higher in ANSC than in SCOT fractions, and ANSC fractions containing alkyl PAH were more toxic than equivalent SCOT fractions. Similarly, recent work has found that heavy fuel oils can be up to 14 times more toxic to rainbow trout embryos than crude oil. Toxicity did not vary with exposure method; rather, differences in toxicity were explained primarily to a greater percentage of 3- and 4-ring alkyl PAHs in HFO (Martin et al. 2014; Adams et al. 2014).

A similar result was obtained by Brette et al. (2014), who found that the cardiotoxic potential of crude oil from the Deepwater Horizon spill to the cardiomyocytes of juvenile bluefin and yellowfin tuna was correlated with the concentrations of 3-ringed PAHs, rather that with total PAH concentrations. Whereas alkyl PAHs comprise only 0.5-1.5% by weight of crude oil, they can contribute up to 6% by weight of heavy fuel oils (Hollebone et al. 2011, Wu et al. 2012, Martin et al. 2014). As previously mentioned, dilbit has an even greater relative proportion of 3- to 5-ringed unsubstituted and alkylated PAHs (Yang et al. 2011).

Although the chronic toxicity of complex hydrocarbon mixtures such as crude oil is attributed to their PAH content, predicting the specific toxicities of crude oil and dilbit is difficult because there is a paucity of data on the toxicity of the thousands of individual PAH present. For example, in a recent EDCF study of the effects of heavy fuel oil (HFO) 7102 on rainbow trout embryos, the most toxic fractions exerted a toxicity that could not be accounted for by 3-4 ring alkyl PAH concentrations alone, suggested that additional unidentified compounds may be contributing to embryotoxicity (Adams et al. 2014). Other recent work (e.g. Incardona et al. 2012) has similarly documented toxicity to Pacific herring embryos that could not be accounted for by known and conventionally measured hydrocarbons. Taken together, these findings underscore the amount of research still required identify all the chemical constituents found in products like crude oil, HFO, and dilbit, and perhaps more importantly, to understand their toxic potential and the consequences of a spill in the aquatic environment.

As described in Chapter 5.1, the acute toxic potentials of PAHs can be calculated by comparing the concentration of compounds in water to critical concentrations that cause toxicity (Di Toro et al. 2000). While the TLM was developed to estimate acute lethality (usually 96-h LC50s), McGrath and Di Toro (2009) applied the model to published data on chronic PAH toxicity using an average acute to chronic ratio, and concluded that it could also predict concentrations that would represent chronic toxicity to fish embryos. However, there were too few chronic toxicity data published to validate the model with statistical strength, and the model includes a wide array of assumptions. Further, the PAH included in the model had a log Kow range of 1.9 (benzene) to 6.4 (perylene), and of the 33 compounds only nine were alkyl PAHs, and only four were 3- to 5-ringed alkyl PAHs. 3- to 5-ringed PAHs with more than one alkyl substitution have, for the most part, log Kow values greater than 6.0 and because the relationship between toxicity and log Kow becomes increasingly uncertain past a log Kow of approximately 5.5, there is a great deal of uncertainty in trying to model chronic toxicity of these higher alkylated compounds, likely due in large part to differences in the mechanisms of chronic toxicity. Indeed, recent work observed that the toxicity of alkyl chrysenes and alkyl benz(α)anthracenes to Japanese medaka increased with increasing alkylation (log Kow values ranged from 5.7 to 6.9), and the researchers concluded that upper limit of toxicity is likely greater than the value of 6.4 proposed by McGrath and Di Toro (2009; Lin et al. 2015).

The need for model validation reflects the different modes of action and toxic effects of various PAHs. Unsubstituted phenanthrene, pyrene, and chrysene exert direct toxic effects on the development of the heart (Incardona et al. 2004), and cardiotoxicity may be responsible for edema and other deformities seen in fish embryos (Incardona et al. 2005). For some alkyl PAH, however, metabolism by cytochrome P450 (CYP1A) enzymes also plays a role. These enzymes add oxygen to the double bonds of the aromatic rings of PAH as the first step in their degradation or excretion. Experiments on inhibitors of CYP1A metabolism suggested that specific metabolites might be more toxic to fish embryos than the parent compounds (Hodson et al. 2007b; Scott and Hodson 2008; Scott et al. 2009). Subsequent tests of hydroxylated derivatives of 1-methylphananthrene demonstrated that some were four times more toxic than the parent compound, and that toxicity was not due to narcosis (Fallahtafti et al. 2011). In addition, photodegradation of PAHs to toxic reactive oxygen species can occur in the environment and/or in the organism itself (e.g. Incardona et al. 2012).

5.2.1 Embryotoxicity

As discussed previously, the embryonic stage of fish is particularly vulnerable to the effects of oil contamination. Different PAHs appear to exert toxicity by different mechanisms, including cardiotoxicity, toxicity via reactive metabolites, and phototoxicity. Chronic exposure results in blue sac disease, a syndrome with several symptoms including cardiac dysfunction, deformities, reduced growth, and increased mortality. Delayed adverse effects have also been observed in fish that survive exposure and appear to exhibit no sublethal effects at the time of their release.

Embryotoxicity can be caused by light and medium crude oils (McIntosh et al. 2010; Wu et al. 2012), refined oils such as diesel (Schein et al. 2009), and by heavy fuel oils (Martin 2011; Martin et al. 2014; Adams et al. 2014), bitumen emulsions (e.g. Orimulsion; Boudreau et al. 2009), bituminous sediments (Colavecchia et al. 2004), and extracts of solids from oil sands tailings ponds (Farwell et al. 2006).

The most embryotoxic PAHs are the 3 to 5-ring compounds, with and without alkyl substitutions. They include PAHs that appear to act directly on cardiac development (e.g., chrysenes, pyrenes; Incardona et al. 2004, 2005; Incardona et al. 2012), by indirect effects due to enzymatic metabolism in fish tissues to reactive intermediates (e.g., retene; Hodson et al. 2007b; Scott and Hodson 2008; Scott et al. 2009), or indirectly by photoactivation of PAH accumulated in tissues to reactive intermediates (e.g., anthracene, Oris and Giesy 1987; retene, Vehniainen et al. 2003; complex mixtures of PAH, Farwell et al. 2006; Incardona et al. 2012). As indicated in Chapter 3, PAHs can comprise up to 6% of heavy fuel oil by weight, with alkyl PAH the predominant form (85 to 95% of TPAH). In dilbit, this percentage is less, with TPAH comprising only up to about 1.1% by weight (similar to crude oil). However, the relative proportion of toxic 3- to 5-ringed unsubstituted and alkylated PAHs is much greater in dilbit (Yang et al. 2011). For example, the Cold Lake Winter Blend (CLWB) tested by Trans Mountain as part of their submission to the NEB contained 96% alkyl PAHs, and 3-5 ring PAHs contributed 72% of TPAH (Stantec Consulting Ltd. 2013).

In terms of acute toxicity, as mentioned above, there is a fundamental lack of data of dilbit toxicity. However, some research has been done on similar products. For example, while Orimulsion (a 70:30 bitumen-in-water emulsion) produced abnormalities in herring and mummichog embryos similar to those produced by crude oil and heavy fuel oil (e.g. pericardial edema and spinal deformities), Orimulsion was approximately 300 fold more toxic than heavy fuel oil (Boudreau et al. 2009). The results discussed in the remainder of this section will focus on the effects of PAHs themselves, rather than on whole oil.

There is a large database of literature that describes how the chronic exposure of fish embryos to PAHs causes blue sac disease (BSD), a syndrome that stops embryonic development and impairs population level recruitment. The association of BSD with PAHs in oil has been demonstrated by tests of single PAH found in crude and refined oils (e.g. Fallahtafti et al. 2012, Turcotte et al. 2011), effects-driven fractionation of crude oil (Hodson et al. 2007a; Adams et al. 2014), and statistical correlations between toxicity and the concentrations of PAH in test solutions (Carls et al. 1999, 2005).

Blue sac disease may include induction of CYP1A enzymes, CYP1A metabolism of PAH to toxic metabolites, and disruption of normal development via hemorrhaging, edemas, cardiac dysfunction, craniofacial, spinal, and ocular deformities, fin erosion, reduced growth, and increased mortality rates (Table D.1; e.g., Birtwell and

McAllister 2002; Brinkworth et al. 2003; Carls et al. 1999; Carls and Rice 2007; Colavecchia et al. 2004, 2006; Incardona et al. 2004, 2009; Fallahtafti et al. 2011; Marty et al. 1997a,b; Turcotte et al. 2011; Adams et al. 2014). The relative extent of each response varies among different species of fish and the specific PAH tested. Where there is photomodification of the PAH accumulated in embryo tissues, acute embryo mortality is associated with tissue necrosis, without the signs of BSD (e.g. Incardona et al. 2012).

The consequences of BSD may range in severity from a complete cessation of embryo development and death before feeding begins, to minor reductions in growth. Embryos affected by BSD die because of circulatory failure or because they fail to develop the structures needed for swimming and feeding and ultimately starve to death. Mortality rates increase with increasing frequency and severity of signs of BSD, and after an oil spill it is predicted that a high proportion of impaired embryos would be consumed by predators (Heintz et al. 2000).

The overarching consequence of chronic oil embryotoxicity is a reduction in recruitment (i.e., a reduction in the number of young that survive to reproduce). However, even fish that survive exposure and appear to exhibit no sublethal effects may have been adversely affected. Pink salmon that survived embryonic exposure to ANSC were tagged and released along with control salmon. Although the oil-exposed fish appeared normal at release, they returned to spawn in far fewer numbers than control fish, suggesting a sublethal effect on their capacity to swim, feed, escape predation, or migrate back to their release point (Heintz et al. 2000). Such delayed effects of oil exposure are difficult to measure with statistical certainty, but increased mortality may be a function of reduced growth and increased susceptibility to disease and/or predation. These data also indicate that exposures to oil previously considered to be “safe” might actually be harmful through delayed impacts on growth and survival at sea.

5.2.2 Effects on sexual maturation and reproduction

PAH exposure can affect reproduction in aquatic species through effects on gonadal development, hormone disruption, physiological function, all of which can affect spawning success. Overall however, the impacts of PAH exposure on sexual maturation and reproduction are poorly characterized, representing an important knowledge gap.

PAHs can affect reproduction in aquatic organisms (Hall and Oris 1981; Casillas et al. 1991; Johnson et al. 1998; Thomas and Budiantara 1995; Lotufo et al.1997), specifically by exerting effects on gonadal development and spawning success (i.e. offspring production), and/or by mimicking or altering the concentrations of hormones such as estradiol.

The exposure of sexually maturing fish to oil can also impair physiological function (e.g., by disrupting the regulation of reproductive hormones; Truscott et al. 1983). The extent of reproductive impacts (in terms of hormone concentrations, fecundity, spawning behaviour, gamete production, and fertilization success) caused by exposures of fish to oil from the *Exxon Valdez* was estimated by Sol et al. (2000). Because no measurements were made immediately following the spill, the authors projected backward using results of sampling in subsequent years and predicted that the spill had caused a significant decline in the estradiol concentrations of mature Dolly Varden (*Salvelinus malma*).

Mature Atlantic cod (*Gadus morhua*) exhibited decreased weight gain as well as irreversible disruption of gonadal development and delayed spawning and spermiation following chronic exposure to the water-accommodated fraction (WAF) of Hibernia crude oil (Khan 2013).

If reproductive impairment of sexually maturing or mature fish occurs only as a result of chronic exposure, it would be of concern primarily for resident species such as rainbow trout that would face ongoing exposure to PAHs released from oil trapped in benthic sediments.

Overall, the impacts of chronic PAH exposure on the sexual maturation and reproductive capacity of fish are poorly characterized. This represents an important omission, as decreased reproductive capacity could have population-level impacts on lower Fraser River salmonid populations facing several challenges to continued success (see also Chapter 6).

5.2.3 Effects on immune function and disease

PAHs have been shown to compromise immune function in fish, resulting in increased susceptibility to pathogens and disease and subsequent increases in mortality.

Some PAHs are known immunosuppressants in fish and other vertebrates (Khan 1991, 1995; Faisal and Huggett 1993; Tuvikene 1995; Kiceniuk and Khan 1987; Moles and Norcross 1998; Carls et al. 1999; Reynaud and Deschaux 2006; Kennedy and Farrell 2008; Bado-Nilles et al. 2009; Hogan et al. 2010). Visible signs of disease in fish, including skin ulceration, fin rot, and tumours can be direct consequences of PAH exposure, and immunosuppression resulting from PAH exposure has been linked to increased mortality following subsequent exposure to pathogens (Khan 1991; Arkoosh et al. 2001; Bravo et al. 2011). Thus, while exposure to PAHs may not always result directly in measurably increases in mortality, the combination of PAH exposure and the presence of naturally occurring pathogens has the potential to reduce survival of a wide range of fish species.

It can be difficult to link causes and effects with certainty in studies of wild fish populations. For example, the Pacific herring (*Clupea pallasi*) population in Prince William Sound crashed in the winter of 1992-1993, more than 3 years after the 1989 *Exxon Valdez* oil spill (EVOS). Some authors attribute the decline to the combined effects of oil toxicity experienced by herring embryos hatched in 1989 resulted in poor growth in the summer of 1992, which led to poor body condition in the spring of 1993 and subsequent susceptibility to naturally occurring viral hemorrhagic septicemia virus (VHSV; Hose and Brown 1998; Carls et al. 2002; Marty et al. 2003). Others argued that oil toxicity played no role, and that overpopulation resulted in poor fish condition, which increased susceptibility to VHSV (Pearson et al. 1999). Proof-of-concept laboratory tests demonstrated an interactive effect between oil exposure and VHSV, in which wild-caught adult herring developed acute signs of VHSV during exposure to low concentrations of oil in water (4-13 µg/L of TPAH). Control fish from the same group of wild stock held under the same conditions were virtually free of signs of disease (Carls et al. 1998). The authors hypothesized that herring could carry the virus but remain asymptomatic until a stressor (in this case oil exposure) reduced their immunity. While this does not constitute proof that the EVOS caused a disease epidemic in Pacific herring, these studies underline the risk of complex interactions between oil exposure, immune function, and natural environmental stressors.

5.2.4 Genotoxicity and carcinogenicity

Genotoxicity is often associated with metabolites of higher molecular weight PAHs than those which are generally responsible for embryotoxicity. Metabolites of these compounds form adducts with macromolecules, resulting in cellular damage and genotoxicity. While these large PAHs are primarily associated with combustion products, genotoxicity, including chromosomal damage and altered

regulation of genes responsible for multiple processes and products, has been observed in fish following oil spills. Carcinogenicity, like genotoxicity, is generally associated with higher molecular weight PAHs, and has not been explicitly linked to oil exposure.

While embryotoxicity is associated with 3- to 5-ringed PAHs, some higher molecular weight PAHs (e.g., benzo(a)pyrene (BaP), indeno(1,2,3-cd)pyrene), exert toxicity via different mechanisms. Metabolism of these PAHs, often by the aryl hydrocarbon receptor (AHR) and CYP1A enzymes, creates highly reactive metabolites, some of which form covalent bonds with fatty acids, proteins, DNA, and other macromolecules. These 'adducts' to macromolecules can produce cellular damage (e.g., loss of membrane integrity) and genotoxicity, leading to mutagenesis, teratogenesis, and carcinogenesis (Tuvikene 1995), so that mutations, deformities, and cancers might be expected in fish exposed to heavier fractions of oil. Because the reactive PAH metabolites that bind with DNA also readily attack RNA, proteins, and other cell constituents, DNA adducts can be used not just as a direct measure of toxicity, but as indicators of a wider range of toxic exposure (Balk et al. 2011).

Genotoxicity has been reported in fish following oil spills (e.g. Hose and Brown 1998; Aas et al. 2000; Whitehead et al. 2012). Larval Pacific herring were found to have elevated rates of chromosomal damage in the 1-4 months following the EVOS, although this was not observed in subsequent years (Hose and Brown 1998). Laboratory testing confirmed these observations, finding elevated rates of chromosomal damage in Pacific herring at exposure concentrations of 0.24 mg/L HMW PAHs (Kocan et al. 1996a). In a field study following the Deepwater Horizon drilling disaster in the Gulf of Mexico in April 2011, adult killifish collected from an area that experience direct oiling exhibited divergent genetic expression with respect to fish from uncontaminated areas. For example, both AHR activity and CYP1A protein levels were upregulated in gill tissue, indicating sufficient PAH exposure (in both concentration and duration) to elicit biological responses (Whitehead et al. 2012). Gene ontology categories were also enriched in exposed fish, indicating activation of the system responsible for cellular responses to stress, regulation of DNA repair, apoptosis, and immune responses (Whitehead et al. 2012). Several possible genotoxic impacts to reproduction were also measured, including down-regulation of transcripts for egg envelope proteins and choriogenin (Whitehead et al. 2012). Finally, several changes in regulation of ion transport genes were observed in fish from the oiled site, including those responsible for osmotic regulation. The authors note that this particular gene group is critical in maintaining homeostasis in the face of changes in parameters such as salinity, and that alteration due to oil exposure may compromise the ability of fish to physiologically adapt to changing conditions. This may be of particular concern for salmonids in the Lower Fraser River, which face multiple natural stressors at all life stages (e.g. developing embryos, out migrating juveniles, return migrating adults).

In terms of carcinogenicity, there are a variety of PAHs that have been identified as potentially carcinogenic by the USEPA (1993), including: benz[a]anthracene; benzo[a]pyrenes; benzo[b]fluoranthene; benzo[j]fluoranthene; benzo[k]fluoranthene; chrysene; 7, 12-dimethylbenz[a]anthracene, indeno[1,2,3-cd]pyrenes, and 5-methylchrysene. While these compounds are found in oil, they are present at very low levels. They are present at much higher concentrations in combustion products. Hence, liver, skin, and bile duct tumours associated with PAH carcinogenicity are most often found in benthic fish species such as brown bullheads (*Ictalurus nebulosus*) that are in continuous contact with sediments contaminated by pyrogenic PAHs from heavy industry (e.g. Baumann et al. 1996).

While genotoxicity has been observed, we are unaware of reports linking carcinogenicity in fish to oil exposure. This may be due in part to the long time periods required for these effects to manifest.

5.2.5 Physiological biomarkers

Biomarkers act as indicators of exposure to and/or effects of contaminants. Effective biomarkers in fish include increased CYP1A synthesis (detoxification enzymes), indicators of oxidative stress (inability to counter the effects of free radicals), PAH metabolites in bile and/or urine, and DNA fragmentation and adducts. All of these indicators are associated with the metabolism of PAHs that occurs following oil exposure. In general, biomarker analysis and interpretation is most effective where baseline values have been established, particularly in areas of existing contaminants sources.

Physiological biomarkers are useful indicators of exposure and effects resulting from exposure to the toxic constituents of petroleum products. Specifically, a biomarker of exposure is defined as “the detection and measurement of an exogenous substance or its metabolite or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism” (Douben 2003). Consequently, a biomarker of effect is defined as “measurable biochemical, physiological, or other alterations within tissues or body fluids of an organism that can be recognized as associated with an established or possible health impairment or disease” (Douben 2003).

In fish, biomarkers have been established with sufficient understanding of their physiological meaning (Douben 2003). These include, but are not limited to:

- Increased synthesis (induction) of CYP1A proteins as indicated by CYP1a mRNA concentrations, CYP1A protein concentrations, and ethoxyresorufin-O-deethylase (EROD) or aryl hydrocarbon hydroxylase (AHH) activity (Collier et al. 1996; Ramachandran et al. 2004)
- Oxidative stress as indicated by increased activity of anti-oxidant enzymes (Balk et al. 2011), decreased concentrations of anti-oxidants (e.g., Vitamin E, Bauder et al. 2005), and/or increased concentrations of the products of oxidative stress (e.g., fatty acid methyl esters, Balk et al. 2011)
- PAH metabolites in bile and/or urine (glucuronic acid conjugates of PAH, Aas et al. 2000; Collier et al. 1996; Balk et al. 2011) - these are specific and sensitive to recent PAH exposure
- Genotoxicity (assays of DNA fragmentation and adducts): Hose and Brown 1998; Aas et al. 2000)

All of these biomarkers are associated with the enhanced metabolism of PAH (CYP1A induction) and the production of reactive metabolites and reactive oxygen species that follows exposure to oil. In natural fish populations located around known sources of oil, measured biomarker responses have indicated that oil exposure has occurred and that there is an increased likelihood of adverse effects. For example, there is significant contamination in both oil producing and non-oil producing areas of the North Sea as a consequence of produced water, drill cuttings, muds, and accidental spills from offshore oil production (Balk et al. 2011). In haddock (*Melanogrammus aeglefinus*) and Atlantic cod (*Gadus morhua*) exposure to oil-contaminated drilling muds resulted in exposure to and uptake of PAHs as measured by the presence of PAH metabolites in bile. Biomarker analyses revealed induction of biotransformation enzymes (e.g. CYP1A, EROD), oxidative stress, altered fatty acid composition, and genotoxicity (e.g. hepatic DNA adducts) (Balk et al. 2011). Chronic exposure to environmental pollution has also been demonstrated in Atlantic cod by measuring PAH concentrations in tissue, PAH metabolites in bile, and DNA adducts (Aas et al. 2000).

Because these biomarkers respond rapidly to oil exposure, and because they indicate both exposure and effects, they are very useful indicators of contamination of fish by petroleum products, the potential ecological effects of spills, and cause-effect relationships that facilitate natural resource damage assessment (P. Hodson, pers. comm. 2011).

Within the context of the proposed Trans Mountain pipeline expansion, the capacity to use and interpret biomarker responses would be greatest if pre-construction surveys were carried out in order to establish base lines. This is particularly important in the lower Fraser River, where multiple inputs to the river exist from varying land uses (including, but not limited to, industry, agriculture, forestry, and water treatment). If this were the case, biomarkers could be a valuable tool for assessing future fisheries impacts of oil spills.

5.2.6 Effects on behaviour

As discussed previously, the extent to which fish can or will avoid oil is unclear. However, depending on the rate at which spilled oil moves downstream, fish may not have time to avoid the oil even if they could. While tributaries may offer uncontaminated habitat and survival in the short term, the inability or unwillingness of fish to return to contaminated natal streams to spawn could have negative consequences at a population level. Other behavioural effects observed as a result of oil exposure include increased mobility, decreased exploratory activity, and increased anxiety. As with other forms of toxicity, the effects of oil exposure on fish behaviour are in general poorly characterized.

Fish behaviour during and after an oil spill can result in an increased or decreased likelihood of exposure. While behavioural changes that result in avoidance of oil can be beneficial in the long term, these behavioural disruptions can be detrimental to population health.

As discussed above (Chapter 4.3.2), preference/avoidance behaviour of fish with respect to oil is not well understood, with early studies offering conflicting results. Some work suggested that juvenile salmonids may avoid acutely lethal concentrations of some constituents of spilled oil (Maynard and Weber 1981); however, it was unclear whether fish would avoid sublethal, but still toxic, concentrations. Additionally, this study examined only monoaromatic hydrocarbons (e.g. benzene, toluene, xylene). The extent to which fish would avoid heavier toxic compounds (e.g. 3-5 ring parent and alkyl PAH) is unknown. Similar results were obtained for adult salmon, which avoided concentrations of monoaromatics at concentrations of approximately 3 mg/L and higher (Weber et al. 1981). However, other studies found no avoidance behaviour when juvenile salmonids were exposed to slicks of whole oil (reviewed in Maynard and Weber 1981). In the Lower Fraser River, there are many tributaries with suitable habitat for fish reproduction and development (Grant et al. 2007) that may provide uncontaminated habitat should a spill occur in one tributary or in the main stem. However, given the predicted rate at which spilled oil would move downstream with the flow of the river and the propensity of acutely lethal LMW compounds to partition from oil to water (Chapter 3.2), fish inhabiting the spill zone may not have sufficient time to avoid the spill, even if they could.

From a life history perspective, avoidance of spilled oil may have negative consequences if it deters salmonids from completing their spawning migration. While adult Chinook salmon that survived an oil exposure were able to migrate to their natal stream (Brannon et al. 1986), they may avoid oil spills that block access to spawning habitat. In the case of semelparous salmon, their entire reproductive potential may be lost due to avoidance; however, this may also be a concern for iteroparous species. In New Brunswick's Miramichi River watershed, sexually mature Atlantic salmon (*Salmo salar*) were tagged as they migrated upstream through a counting fence over a four-year period. However, each year the tagged fish were observed moving downstream through the fence without spawning when concentrations of copper and zinc (known to be avoided by salmon) increased due to the presence of an upstream mine (Saunders and Sprague 1967). In each year's run, only a small percentage of salmon re-ascended the river when concentrations declined, and an estimated 62% of those fish failed to complete their spawning migration.

Avoidance is not the only potential fish behaviour resulting from oil exposure. Zebra fish were chronically exposed to heavy fuel oil (HFO) and a light crude oil (LO) in their diets from five days post-fertilization until adulthood (six months later) (Vignet et al. 2014a). Heavy fuel oil was found to be the most toxic, followed by light crude oil, with exposed fish exhibiting increased mobility (resulting in shorter resting times and a greater demand for energy), decreased exploratory activity, and increased anxiety (as measured by delayed exits from tank refuge zones, increased time in 'safe' areas of the tank, erratic bouts of high activity, and lower exploration levels) (Vignet et al. 2014b). Interestingly, exposure of fish to HMW PAH such as benzo(α)pyrene (BaP) has been associated with increased cortisol concentrations (e.g. Gesto et al. 2008, Knag and Taugbol 2013). Cortisol is considered an indicator of stress (reviewed in Vignet et al. 2014b), and these findings may confirm a stress response in fish exposed to PAHs. In the wild, behavioural disruptions of this type may impact the ability and/or willingness of fish to explore their environment, their ability to spawn, and perhaps even their ability to survive.

The mechanisms underlying behavioural changes as a consequence of PAH exposure are poorly understood, although some authors have hypothesized that endocrine disruption may play a role (Vignet et al. 2014b).

5.3 QUANTITATIVE MEASURES OF OIL AND PAH TOXICITY TO FISH

The concentrations of TPAH in water that have been shown to cause toxicity in fish species are relatively consistent, ranging from approximately 1 to 100 µg/L. Observed effects ranged from malformations and developmental defects to decreased reproductive success to mortality. Trans Mountain's study of dilbit behaviour and fate measured concentrations in the water column that were orders of magnitude above those at which effects on fish have been observed.

Laboratory testing using oiled gravel desorption columns (columns of oil-contaminated gravel over which water flows, allowing partitioning of PAHs from the oil into the water prior to exposure of test species) has indicated that toxic effects may be estimated based on concentrations measured on contaminated gravel, as these concentrations correlated with TPAH concentrations in water. These studies are important because they mimic the way in which stranded and/or submerged oil may be transported to spawning shoals.

While studies of oil toxicity to fish are numerous, those reporting measured concentrations of oil in water, or, more importantly, TPAH concentrations in water, are much more limited. Nevertheless, the studies that are available provide a good perspective on toxicity. TPAH concentrations that were found to cause chronic toxicity are summarized in Figure 5.1, and range from approximately 1 µg/L to more than 100 µg/L, corresponding to 100to 1000 µg/L of whole oil, assuming 1% PAH in whole oil. The studies summarized in Figure 5.1 are those in which the TPAH concentrations of test solutions were measured and reported, and represent only a small subset of studies of oil toxicity to fish. While the ranges of toxic concentrations reported in Figure 5.1 may appear broad, they are remarkably consistent, considering the wide range of fish species (including marine herring and freshwater salmonids), oil types, and water qualities reported. This consistency likely reflects a relatively minor effect of water quality and fish species on oil toxicity.

Mortality

Reduced growth

Embryo deformities

Immunosuppression

CYP1A Induction

0.1 1 10 100 1000

Concentration of total PAH in water (µg/L)

Figure 5.1. Range of total PAH concentrations (µg/L) observed to cause chronic toxicity (lethal and sublethal) in early life stages of fish (data from 27 studies reporting TPAH concentrations in exposure solutions: Carls et al. 1996; Kocan et al. 1996a; Marty et al. 1997b; Moles and Norcross 1998; Adams et al. 1999; Birtwell et al. 1999; Carls et al. 1999; Heintz et al. 1999, 2000; Gulec and Holdway 2000; Little et al. 2000; Barron et al. 2003; Fuller et al. 2004; Kazlauskiene et al. 2004, 2008; Koyama and Kakuno 2004; Ramachandran et al. 2004; Colavecchia et al. 2004, 2006; Carls et al. 2005; Brannon et al. 2006; Carls et al. 2008; McIntosh et al. 2010; Ndimele et al. 2010; Shen et al. 2010; Martin 2011; Martin 2014). X = studies with pink salmon, O = studies with rainbow trout. Figure adapted from Hodson et al. 2011.

Mortality of pink salmon embryos was observed at TPAH concentrations in water ranging from 5.2 – 18 µg/L (Heintz et al. 1999, 2000), while Pacific herring exhibited increased mortality at concentrations as low as 0.7 µg/L TPAH (Carls et al. 1999). In rainbow trout embryos, exposure to effluent from oiled gravel columns caused malformations and mortality at concentrations as low as 1 µg/L TPAH (Adams et al. 2014), and developmental defects (BSD, DNA damage) were observed in rainbow trout embryos exposed to heavy fuel oil at concentrations as low as 0.7 µg/g (Le Bihanic et al. 2014).

More recently, cardiotoxicity was observed in herring embryos at TPAH concentrations of 0.5 ug/L (Incardona et al. 2012). While this is a sublethal effect, edema in embryos results in delayed mortality as a consequence of poor feeding (Hicken et al. 2011). Of note is that this TPAH concentration in water corresponded to very lightly oiled gravel (0.3 g oil/kg gravel), which in post-spill shoreline surveys, would be unlikely to be characterized as 'visibly oiled' (Incardona et al. 2012). Doses of 1 g bunker oil /kg gravel resulted in TPAH concentrations in water of 1.8 µg/L, which resulted in measured TPAH concentrations in tissues of 225 ± 111 ng/g (UV-exposed embryos) and 175 ± 4 ng/g (UV-protected embryos) (Incardona et al. 2012).

Other sublethal responses to relatively low TPAH concentrations include decreased reproductive success, compromised larval health, disruption of cellular membrane integrity and function, disruption of endocrine function, increased occurrence of physical abnormalities, increased MFO and EROD enzyme activity, and increased DNA adduct formation (Carls et al. 1999; Aas et al. 2000; Deb et al. 2000; Hodson et al. 2002). The variations in test conditions and species sensitivity are reflected by concentrations that caused acute lethality in some studies, but caused only sublethal growth effects in others.

Overall, CYP1A induction was the most sensitive endpoint reviewed, but this sublethal biomarker response indicates primarily that fish have been exposed to PAHs, that PAH bioaccumulation has occurred, and that the fish has responded to PAH tissue burdens at the molecular and cellular levels. While there are potential roles for CYP1A activity in mechanisms of toxicity (e.g. activation of pro-carcinogens such as BaP to carcinogenic derivatives), these mechanisms are complex and there are currently no direct correlations between oil or TPAH concentrations causing CYP1A induction and those causing toxicity. Further, while CYP1A induction reflects bioavailability, it has been found not to be an accurate predictor of some types of toxicity (e.g. embryotoxicity), because compounds that are potent CYP1A inducers are not necessarily those that are most toxic to fish (Adams et al. 2014). Nevertheless, increasing CYP1A induction indicates increased PAH exposure, leading to increased risk of toxicity by some pathways (e.g. carcinogenesis).

The majority of effects were elicited at exposure concentrations between 1 and 100 µg TPAH/L (Figure 5.1). Following the application of dispersant to spilled oil, waterborne hydrocarbon concentrations in a marine system ranged from 40- 60 mg/L in the upper 10 metres of water, but declined to 1 mg/L within 5 hours due to dilution (Lessard and DeMarco 2000). Assuming a PAH content of 1%, these concentrations would correspond to 10 to 600 µg/L of TPAH, well within the range of concentrations causing toxicity.

Figure 5.1 includes data for both rainbow trout and pink salmon, species that are found in the Lower Fraser River (Grant and Pestal 2009; McPhail 2007). CYP1A induction, indicative of PAH exposure, occurs at concentrations of < 3.7 µg TPAH/L in pink salmon, and as low as 5.0 µg TPAH/L in rainbow trout. Due to a lack of data for other species, it is difficult to assess the relative risks of oil toxicity to all fish species in the lower Fraser River. Therefore, for the purpose of this report, information on reproductive strategies (time spent in the lower Fraser River and time spent at sea) and habitat utilization were used to assess the relative sensitivities of the different life stages of each species to an oil based on the likelihood of exposure (Table 4.2).

Trans Mountain's Gainford study examined the fate and behaviour of dilbit in wave tank systems, and found that in tanks with moderate agitation, waterborne TPAH concentrations of up to 120 mg/L were measured at 1 m below the surface. This value is five orders of magnitude above concentrations at which lethal and sublethal effects have been observed in fish. Further, the study states that TPAH measured in the water column were below detection thresholds 'in nearly all cases'. However, detection limits in this study were 2 mg/L; again, three full orders of magnitude above levels at which effects on fish have been observed.

	Scotian Light[a]	Federated Crude[a]	Alaska North Slope Crude[a]	Medium South American Crude[a]	Bunker C (Heavy Fuel Oil)[b]	Diluted oil sands bitumen[c]	Albian Heavy Synthetic Oil[c]	Cold Lake Winter Blend (dilbit)[d]
Total PAH (µg/g oil)	3 502	9 956	11 242	17 941	61 568	4 558	5 755	9 517
Total alkyl PAH (µg/g oil)	3 333	9 370	10 232	16 293	57 692	4 467	5 131	9 241
Total EPA Priority PAH (µg/g oil)	169	586	1 011	1 648	3 877	91	624	206
EC50 for embryo toxicity[e,f] (µg TPAH/L)	3.0	3.0	7.0	2.0	14-17	n/a	n/a	n/a

Table 5.1 Concentrations of total PAH, total alkyl PAH, total EPA priority PAH in four crude oils ([a]Wang et al. 2003), Bunker C heavy fuel oil ([b]Hollebone et al. 2011), diluted crude oil sands bitumen, Albian Heavy Synthetic Crude ([c]Yang et al. 2011), and in Cold Lake Winter Blend dilbit, which Trans Mountain proposes to ship in the expanded pipeline ([d]Trans Mountain Pipeline ULC 2013b). Where available, EC50s for fish embryos are provided ([e]Martin et al. 2011; [f]Wu et al. 2012). Table adapted from Hodson et al. 2011.

Research has also provided some perspective on oil concentrations in sediments and on gravel that are associated with chronic toxicity (Figure 5.2). Investigators commonly use oiled-gravel desorption columns supplied with a continuous flow of water to simulate the transfer of hydrocarbons from oil stranded on gravel into interstitial waters of a spawning shoal (Marty et al. 1997; Carls et al. 1999; Heintz et al. 1999; Martin, 2011; Incardona et al. 2012; Martin et al. 2014; Adams et al. 2014; Le Bihanic et al. 2014). By directing outflow to a test chamber containing fish embryos, toxicity testing of the contaminated water can be conducted without direct contact of the embryos with oil. The water quality of these systems is generally characterized by an initial sharp decline in hydrocarbon concentrations over the first 24-48 hours, as LMW constituents are lost to evaporation and dissolution, and excess oil droplets break free from the gravel and are washed out. However, following this exponential decline, PAH concentrations can remain in a quasi-steady state for up to three months.

In a study comparing the toxicity of heavy fuel oils (HFO) and crude oils using multiple exposure scenarios (e.g. WAF, chemically enhanced WAF, and effluent from oiled gravel columns), Martin et al. (2014) observed that the greatest toxicity was caused by HFO stranded on gravel substrates, and that toxicity was explained by alkyl PAHs (particularly 3-4 ring compounds) regardless of exposure method. They concluded that risks to aquatic life were greatest when surface area: volume ratios were high, such that stranded HFO posed the greatest risk (Martin et al. 2014).

The regressions in Figure 5.2 demonstrate that TPAH concentrations in water increase with increasing oil concentrations on gravel, such that toxic effects in fish embryos can be estimated based on the dose of oil applied to gravel. The data fall into two groupings: one includes four studies of pink salmon and rainbow trout, and the other includes studies of pink salmon and herring. The correspondence among the first group of three studies is remarkable, given that two types of oil (ANSC and Bunker C or heavy fuel oil) and different sized systems (1.8 kg of gravel in a 10 cm diameter tube (Martin 2011; Martin et al. 2014), and 10.8 kg of gravel in a

15 cm diameter tube (Marty et al.1997b; Heintz et al. 1999)) were used. While other group (Brannon et al. 2006; Incardona et al. 2012) diverged in terms of the dose required to obtain the same concentration in water, the trend remained the same, such that toxicity to early life stages of fish can be predicted from similar doses of oil on gravel. The cause of the divergence is unclear, but may relate to the surface area of oil exposed to water flow.

The results of studies using oiled gravel demonstrate that prolonged exposure of fish embryos to waterborne PAHs resulted in chronic toxicity and population declines. Based on data in Figure 5.1, the range of water borne concentrations of TPAH that caused embryo toxicity (approximately 1 to 100 µg/L) would be associated with concentrations of oil on gravel of about 4 to 14 000 mg/kg of gravel. These systems provide an important perspective on the potential for impacts of oil on salmonid reproductive success in the lower Fraser River because they mimic oil transport to and stranding in spawning shoals. This is a critical consideration because stranded oil (e.g. crude, HFO, or dilbit) may provide a long-term source of PAHs in the aquatic environment, and when the stranding occurs in or near spawning areas, concentrations in the water may reach levels sufficient to cause embryotoxicity for months or years (Martin et al. 2014).

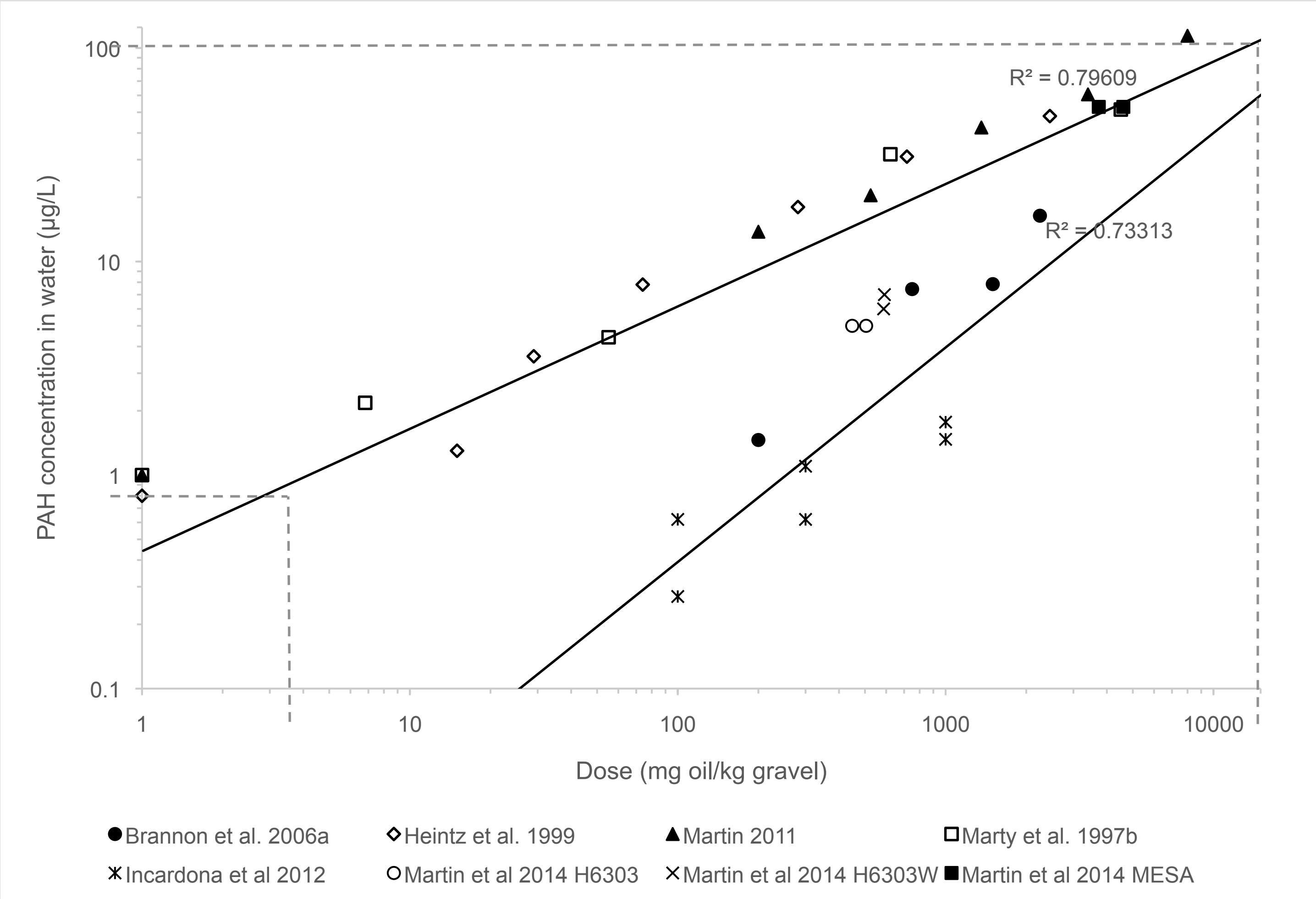

Figure 5.2. Concentrations of TPAH in water discharged from model spawning beds of gravel contaminated oil (Marty et al. 1997b, Heintz et al. 1999, Brannon et al. 2006: pink salmon embryo exposures to Exxon Valdez oil loaded on 10.8 kg gravel (0.5-5.0 cm) in 10.6 L capacity column at 7.5-12 L water/hour flow rate; Martin 2011, Martin et al. 2014: rainbow trout embryo exposures to heavy fuel oil and MESA crude oil loaded on 1.8 kg gravel (0.5-2.0 cm) in 2.4 L capacity column at 1.2 L water/hour flow rate; Incardona et al. 2012: herring embryo exposures to Bunker C oil loaded on 1.8 kg gravel in a 1.0 L capacity column at 0.8 to 1.0 L water/hour flow rate). The dashed lines indicate the range of concentrations of water borne PAH, and the corresponding concentrations of oil on gravel, that caused toxicity to fish embryos (from Figure 5.1). Much of the data included in Figure 5.1 was derived by experiments with oiled gravel columns. Figure adapted from Hodson et al. 2011.

The effects of chronic toxicity include higher rates of mortality, reductions in growth, increased frequency of characteristic deformities and pathology, and increased rates of hydrocarbon metabolism and excretion. Sublethal biochemical responses, such as increased rates of CYP1A enzyme activity associated with chemical metabolism, are the most sensitive responses (e.g. occur at the lowest PAH concentrations), but effects on growth rates and incidence of deformities are observed at concentrations as low as approximately 1.0 -5.0 µg/L TPAH.

5.4 Factors That Affect Toxicity

5.4.1 Oil type

The primary determinant of oil toxicity appears to be the relative contribution of total PAHs (TPAH) and the rate at which they are released to the environment. Thus, oil type is an important factor in determining potential toxicity, not only because various oil types contain different TPAH percentages, but also because oil type determines behaviour and fate of the oil.

Total PAH concentrations (the sum of all un-substituted and alkyl-substituted homologs) vary considerably among petroleum products (Table 5.1). Thus, if PAH are the cause of chronic toxicity, concentrations of whole oil that cause toxicity should vary in proportion to their TPAH content, assuming that exposure methods are consistent. The toxicity to trout embryos of four chemically dispersed oils varied only two-fold when expressed as the dilution of the chemically-enhanced water accommodated fraction (% v/v; Wu et al. 2012). However, TPAH concentrations in whole oil varied 5-fold (Table 5.1), indicating that the lightest oil (Scotian Light) was more toxic than predicted. When toxicity was compared among the four oils using waterborne TPAH concentrations estimated from measured TPAH concentrations in 0.1% (v/v) CEWAF and the EC50s expressed as dilutions (% v/v), there was little difference in toxicity among the oils. In other words, no matter what the type of oil, the amount of PAH that was toxic was virtually constant (Table 5.1). The higher-than-expected toxicity of Scotian Light when expressed on a dilution basis may have been due to a more efficient chemical dispersion due to its lower viscosity and density compared to the heavier oils. In toxicity tests of heavy fuel oils coated on gravel in simulated spawning shoals, HFO 6303 was more nearly 2.5-fold more toxic than MESA crude oil. In this case, the HFO 6303 contained nearly 2.5-fold more TPAH than MESA (Martin 2011). Toxicity was predicted by TPAH concentrations on gravel because the concentration gradient from oil to gravel controlled the TPAH concentrations in test solutions.

Compared to the crude oils tested by Wu et al. (2012) and Martin (2011), diluted bitumen contains about 2-fold lower TPAH concentrations (Yang et al. 2011; Table 5.1). However, as discussed, relative proportions of toxic 3- to 5-ringed PAHs are higher in dilbit. Further, if the primary determinant of toxicity is the rate at which PAH are released from oil rather than their absolute concentrations, toxicity will be governed primarily by the issues of environmental fate and distribution discussed in Chapter 3.

5.4.2 Weathering

Weathering of oil has varying effects with respect to toxicity. As low molecular weight compounds are lost, the acute lethality of the oil decreases. Conversely, as weathering progresses, oil residues become enriched in higher molecular weight compounds, thereby increasing the potential for chronic toxicity, particularly to early life stages of fish.

Weathered oil retains the less soluble but more persistent PAHs that exert toxic effects in the parts per billion concentration range (Carls et al. 1999, 2000; Heintz et al. 2000; Middaugh et al. 2002; Albers and Loughlin 2003; Payne et al. 2003; Heintz 2007; Logan 2007; Incardona et al. 2012; Adams et al. 2014). The LMW components are lost to evaporation, dissolution, biodegradation, and photodegradation; loss rates decrease as the degree of weathering increases and as the molecular weight of the residual compounds increases (McAuliffe 1977). As a consequence, the acute lethality of oil also decreases with weathering (Di Toro et al. 2007), such that fish kills occur less frequently under conditions that accelerate weathering. Thus, if weathering of spilled oil occurs without acute lethality to fish in the first 24 hours, the overall impact of the oil will be greatly reduced.

As petroleum products weather, they become progressively enriched in higher molecular weight compounds such as 3- to 6-ringed parent PAHs, alkyl PAHs, waxes, resins, and asphaltenes as the total volume of oil decreases. This causes an apparent increase in chronic toxicity to early life stages of fish (in the ppb range; Carls et al. 1999), but a decrease in the total amount of toxic material (Di Toro et al. 2007). In examining chronic embryo toxicity, Heintz et al. (1999) observed that highly weathered ANSC oil coated on gravel in desorption columns was more toxic to pink salmon embryos than fresh oil due to a higher proportion of 3- to 5-ringed PAHs (e.g. phenanthrenes and chrysenes).

5.4.3 Photomodification of PAH

Photomodification of some hydrocarbons, particularly those with four rings, can result in increased toxicity to aquatic species. Photomodification of PAHs in translucent fish embryos causes a suite of symptoms different from those of blue sac disease, including rapid and extensive tissue necrosis. Interestingly, known and commonly measured PAHs cannot always account for observed toxicity, underscoring the need for further research, particularly in the case of dilbit.

The UV wavelengths of sunlight can photomodify many of the components of oil, especially those with double bonds (photolysis). In many cases, the derivatives formed are more polar, more water soluble, and less bioavailable and toxic to aquatic species. However, for some hydrocarbons, toxicity is enhanced by exposure to UV-A and UV-B radiation. The most phototoxic PAHs are those with four rings: anthracene, fluoranthene, and pyrene (Incardona et al. 2012). In these compounds, UV photomodification creates oxygenated derivatives such as quinones and anthroquinones, which are more water soluble but also more reactive and much more toxic than the parent compound. The products of these reactions will depend on the structure of each PAH and on its specific light absorption characteristics (Diamond et al. 2000).

In nearly transparent fish embryos, photomodification of PAH accumulated by the embryos can occur in tissues, where the photo-products and associated reactive oxygen species cause oxidative stress, cell death, and acute mortality (Arfsten et al. 1996; Duesterloh et al. 2002). In Pacific herring embryos exposed to the water-accommodated fraction (WAF) of ANSC oil for 96 h and then to weak sunlight, toxicity was increased by up to 50-fold in comparison to embryos exposed to WAF only (Barron et al. 2003). Similarly, juvenile tidewater silversides (*Menidia beryllina*) were unaffected by exposure to UV radiation alone, but the acute lethality of the WAF of a weathered Californian oil increased fivefold in combination with UV irradiance (Little et al. 2000). The same result was found for larval whitefish (*Coregonus lavaretus*) exposed to UV-B and to retene; on their own, neither was lethal, but the combination caused acute lethality (Vehnianen et al. 2003). Photosensitive PAHs exhibit enhanced toxicity to fish at concentrations as low as 1.0 µg/L (Dong et al. 2000; Little et al. 2000; Barron and Ka'Aihue 2001).

Toxic photoproducts can react directly with cellular components (Hatlen et al. 2010), causing rapid tissue necrosis and signs of toxicity quite different from those of BSD. Most recently, Pacific herring spawned three months after the Cosco Busan spill in San Francisco Bay where found to be extraordinarily sensitive to the combination of weathered oil and UV exposure (Incardona et al. 2012).   Observations in the field revealed that embryos in the intertidal zone reached the hatching stage and then suffered extensive tissue deterioration and death, while embryos in the subtidal zone (in > 1 m of water) exhibited sublethal exposure (cardiotoxicity) but very low mortality (Incardona et al. 2012). To test their hypothesis that increased UV levels in the intertidal zone were the additional stressor responsible for the enhanced toxicity, the authors exposed herring embryos to weathered Cosco Busan oil and sunlight, with and without UV protection. They found that oil and UV co-exposure was both required *and* sufficient to induce the acutely lethal necrotic syndrome observed in hatching embryos in the field.

One of the most interesting findings of this work was that tissue concentrations of PAHs known to be phototoxic were too low to explain the observed toxicity, leading the authors to speculate that unidentified or unmeasured compounds were the cause of toxicity (Incardona et al. 2012). In similar studies with Pacific herring and zebra fish, concentrations of the most highly phototoxic and commonly measured PAHs (e.g. anthracene, fluoranthene, and pyrene) could not account for observed phototoxicity (Barron et al. 2003; Hatlen et al. 2010). These findings again serve to underline the lack of toxicity data available for most petroleum products, as well as a lack of understanding regarding toxic constituents and mechanisms of toxicity. This is of particular concern in the case of dilbit, of which so little is known in terms of behaviour and fate in the environment, let alone the toxic potential of the whole oil and of individual constituents.

Overall, the risk of increased toxicity of PAH due to photomodification will be site-specific and a function of turbidity, dissolved organic carbon (affecting water colour and light quenching), the time of year (solar radiation intensity, shading), and the fish species present. For each species, the location of spawning shoals and the extent of protective pigmentation in developing embryos will determine the exposure to UV-A and UV-B (reviewed in Little et al. 2000). While the Lower Fraser River is generally relatively turbid, the shallow tributaries where chum, coho, and pink salmon spawn have relatively clear water outside of freshet and high runoff periods.

6 CUMULATIVE EFFECTS

While an oil spill would undoubtedly affect multiple individuals of a given species, our primary concern is how these individual effects could translate into population-level effects (abundance, growth rate, size and age distribution, size and age at first maturity, fecundity), community-level effects (salmon and other fish species present or absent; diversity; indicator species and runs), ecosystem-level effects (productivity, biomass, energy flow, resilience, interactions between terrestrial and marine systems), and socioeconomic effects (subsistence, sports, and commercial fishery production, fisheries closures, tainting,). Due to the site-specific nature of each river ecosystem and the variable nature of products that may be spilled, the nature and extent of those effects in the Lower Fraser River may be partially predicted by experience elsewhere (e.g. the dilbit spill into the Kalamazoo River, MI), but will only be fully understood in the event of a spill.

The difficulty in predicting real-world impacts is made greater by the vast number of natural and anthropogenic stressors that act on individual fish species and on the river as a whole. These cumulative effects can have unexpected and serious consequences for aquatic ecosystems (e.g. Arkoosh and Collier 2002, Jacobson et al. 2003). Effects on salmon following oil spills may not coincide exactly with the spill, and may vary in intensity according to existing stress levels associated with annual cycles of migration, reproduction, feeding, and growth, in addition to environmental factors such as temperature, salinity, disease, prey availability and quality, and pollutants.

6.1 HISTORIC USE OF THE LOWER FRASER RIVER

Along the Lower Fraser River and its floodplain, much of the landscape has been converted for agriculture, dense urban settlements and industrial uses, with negative consequences for salmonid communities. Prior to colonization, nearly two-thirds of the land base of the Lower Fraser was forested, with the remainder comprised of wetlands (Healey and Richardson 1996). Colonization began as early as 1850, and over the next one hundred years, the vast majority of the forest was harvested and cleared (Healey and Richardson 1996). To enable the drainage of wetlands and to protect developments from flooding, dikes were constructed, such that by the mid-20th century approximately 70% of the floodplain had been isolated from the river (Healey and Richardson 1996). By the beginning of the 21st century, the forests and wetlands had been reduced to approximately one-tenth of the land base, with agricultural and urban land uses dominating the landscape (Healey and Richardson 1996). Intact river-floodplain connectivity is critical as it facilitates the mobilization of nutrients, the influx of detritus, and it creates nursery habitat for young salmon, other fishes, and invertebrates (Junk et al. 1989). Thus, the loss of floodplain connectivity in the Lower Fraser has resulted not only in enormous habitat loss for salmon, but in a steep decline in organic carbon production from the floodplain.

Flood control structures in the Lower Fraser have been an important stressor to fish populations since agricultural practices began, reducing the passage of juvenile and adult salmon and leading to reductions in habitat quality. Major flooding events (in 1894 and in 1948) resulted in significant damage to developments located on the floodplain (e.g. $20 million 1948 dollars in damages) (NHC 2008; Fraser Basin Council 2010a). This led to the widespread use of dikes, floodgates and pump stations, reducing connectivity between the Lower Fraser mainstem and many of its tributary streams (Thomson 2005). Floodgates create a barrier to migratory salmonids, particularly during spring freshet when gates are closed for extended periods due to high water levels, isolating large sections of quality rearing habitat (Fraser Basin Council 2010b; Thompson 2005). Freshet can also coincide with peak smolt out-migration periods, resulting in out-migrating smolts becoming caught in pumps, resulting in high levels of mortality (Thompson 2005). Dams for hydroelectricity production also occur in at least 10 systems throughout the Fraser watershed (Hirst 1991), including those constructed in the early 20th century on Lower Fraser tributaries such as the Stave River and the Alouette and Coquitlam rivers. In the case of the latter two rivers, dam construction resulted in the extirpation of sockeye salmon populations that spawned upstream of the dams (Godbout et al. 2011). Many small streams have been culverted, paved over, and converted to storm sewers, likely resulting in the loss of many small runs of coho and chum salmon in the Lower Fraser (PIBC 1999). Overall, the availability of salmon habitat in the Lower Fraser has been substantially reduced by a variety of anthropogenic modifications and barriers to fish passage.

The Fraser River also has a long history of industrial developments along its banks. One of the most notable was the construction of the Canadian Northern Railway near Hell's Gate, which was completed in 1912 (Ricker 1947). Reports indicate that construction led to a subsequent rock slide in 1914, obstructing passage of many of the salmon attempting to migrate upstream to spawn (Ricker 1947). While much of the rock was removed the following year, many of the affected salmon populations were thought to have remained at greatly reduced abundances (Ricker 1947).

More recently, the Lower Fraser has been affected by sand and gravel removal, an annual activity with the potential to damage both habitat and incubating salmon and eulachon eggs. This potential is exemplified by the loss of up to 2.25 million pink salmon in 2006 due to improper construction of a causeway to access one gravel removal site (Auditor General of Canada 2009). The ongoing effects of decades of sand removal and industrial activity has likely contributed to the decline of Fraser eulachon populations (COSEWIC 2011). Over many years, the effects of human activities throughout the watershed have put many stressors on salmonids and eulachon in the Lower Fraser and tributaries.

6.2 CURRENT USE

6.2.1 Fraser Headwaters to Hope

The Fraser watershed is a diverse landscape, comprising a variety of economic activities and land uses. Cumulative effects of these activities place stress on local salmon populations, and can also flow downstream to contribute to cumulative effects in the lower river.

Agriculture is important throughout the watershed (Nener and Wernick 1997), but is associated with impacts to salmon habitat. In the semiarid Thompson region, water withdrawals can result in low flows and high temperatures in coho streams (Bradford and Irvine 2000). The use of chemical fertilizers and pesticides creates water quality concerns, including low dissolved oxygen concentrations and the presence of substances toxic to fish (Nener and Wernick 1997). In the north and central portions of the watershed, there are water quality concerns including total suspended solids, nutrient release associated with fertilizer and manure use, and pesticide release as a consequence of livestock and hay production (Verrin et al. 2004). Further south in the Thompson River area, the use of herbicides, insecticides, and fungicides contribute to water quality concerns (Verrin et al. 2004).

Logging on 9.6 million ha of crown forest is also a prominent land use in the watershed (Nener and Wernick 1997). The effects of logging on streams are varied, and include effects associate with road building, logging itself, silviculture activities, log handling, and waste generation. Logging is also the basis for the five pulp mills (two at Prince George, two at Quesnel, and one at Kamloops) which discharge effluent into the Fraser River (MacDonald et al. 2011). Discharge from these five mills contributes 75% of the permitted waste discharge upstream of Hope (McGreer and Belzer 1999; Nener and Wernick 1997), with an estimated total effluent of 410

000 m^3/day; however, significant reductions in the toxicity of pulp and paper mill effluent have been achieved since 1993 (McGreer and Belzer 1999). Regulated parameters include biological oxygen demand (BOD), total suspended solids (TSS), and acute lethality, with lethality testing based on 96-hour effluent toxicity tests with rainbow trout and/or 48-hour effluent toxicity tests with *Daphnia magna* (MacDonald et al. 2011). Some sub-lethal toxicity testing may also occur, although typically only for 7 days (MacDonald et al. 2011). However, there are still concerns about the toxicity of pulp mill effluent. For example, while endocrine disruption and sex-reversal is a known effect of pulp mill effluent, the compounds causing this effect have not been positively identified (e.g. Johannessen and Ross 2002). Other compounds of ongoing concern include ammonia, chlorides, metals (e.g. copper, cadmium, and mercury), PAHs, benzene, toluene, and chlorophenols (reviewed in MacDonald et al. 2011; Johannessen and Ross 2002).

The watershed also includes saw mills (14 at Prince George alone), plywood mills, and wood preservation facilities (Calbick et al. 2004; McGreer and Belzer 1999). Prior to 1987, there were about 70 of these facilities in the watershed, discharging over 100 million m^3 /year of acutely toxic stormwater. As of 1999, the volume had dropped provincially by 90% to approximately 1.6 million m^3/year (McGreer and Belzer 1999). Contaminants of concern released from these facilities include ammonia, phosphorus, sulphides, sulphates, and formaldehyde, as well as wood preservation and anti-sapstain chemicals such as creosote, chromated copper arsenate (CCA), and pentachlorophenol (PCP) (MacDonald et al. 2011).

Wastewater treatment plants upstream of Langley (n = 87) discharge approximately 150 000 m^3 of sewage effluent to the Fraser River each day (McGreer and Belzer 1999).

Mining is also of concern for water quality in the Fraser watershed. There are seven active metal mines in the Fraser River watershed, and all but one (an underground gold mine) are open-pit mines. Contaminants typically associated with mine effluent include nutrients (e.g. ammonia, phosphorus), metals (e.g. arsenic, copper, mercury, selenium), cyanides, PAHs, and monoaromatics (e.g. BTEX) (MacDonald et al. 2011).

6.2.2 Lower Fraser

The Lower Fraser is a focal point of industry in Metro Vancouver and in the province of British Columbia, hosting a variety of economic activities along with the millions of salmon that return each year. The Lower Mainland urban region is home to 2.6 million people (over 50% of BC's population), as well as $50 billion worth of development (Richmond Chamber of Commerce 2014). The Lower Fraser is also home to Port Metro Vancouver, one of the largest ports in North America (Richmond Chamber of Commerce 2014). As the deposition of river sediment creates a major barrier to the transit of large ships, an annual dredging program now removes 2 million tons of sediment and gravel from the lower reaches (Milliman 1980). There are also nine federal small craft harbours used by the commercial fishing sector and recreational boaters (Richmond Chamber of Commerce 2014). In addition to the pulp and paper mills in the upper Fraser, there are five smaller mills in the lower Fraser Basin near Vancouver (MacDonald et al. 2011).

Salmon and eulachon habitat in the Lower Fraser River is highly altered, and experiences a variety of stressors related to the large human population. As such, the majority of streams are now considered lost (20%), endangered (63%), or threatened (13%), while comparatively few are considered wild (5%) (PIBC 1997). Threatened and endangered streams face multiple stressors, including loss of riparian vegetation channelization and diking, impermeable surfaces (e.g. roads, buildings, pavement), flow diversions, water quality issues, logging, urbanization, and barriers like dams (PIBC 1997). It has been estimated that total carbon production was historically 2.5 times greater than at present, a decrease which has resulted in a reduced base of production for food webs in the river and estuary (Healey and Richardson 1996). In general, tributary sloughs and streams are most susceptible to contaminant inputs due to their low flows, and can experience episodic fish kills during urban runoff events (Nener and Wernick 1997).

As discussed, much of the Lower Fraser is affected by flood protection structures, including over 600 km of dikes with an associated 400 floodgates and 100 pumping stations to control tributaries (Fraser Basin Council 2010b). Floodgates in the Lower Fraser isolated significant amounts of tributary stream habitat (Thompson 2005), and were recently shown to result in upstream "dead zones", where impounded water exhibits oxygen concentrations that have dropped below provincial safe minimum standards (Gordon et al. 2015). These are the existing stressors to salmon in the Lower Fraser; juveniles of species such as Chinook and coho also face extensive stress in the search for suitable rearing habitats, increasing their susceptibility to the effects of an oil spill.

The Fraser Valley is also farmed intensively, generating 62% of BC's farm revenues annually (Richmond Chamber of Commerce 2014) with livestock and fruit and vegetable production. Subsequently, pesticide releases to waterways include multiple herbicides, insecticides, and fungicides. Also of concern is the proximity of many of these crops to waterways (e.g. cranberries, ginseng).

Discharge of municipal waste from the Lower Mainland enters the river via two main facilities located at Annacis and Lulu Islands. These two stations are responsible for processing nearly 90% of the total volume of sewage discharged into the Fraser River each day: of the 90 municipal sewage treatment plants draining into the Fraser River, 87 are upstream of Langley but together account for only 13% of the total volume of discharge (approximately 150 000 m^3/day; EC 1997; McGreer and Belzer 1999). There is also a smaller secondary treatment plant located in Langley. The Annacis and Lulu plants were upgraded from primary to secondary treatment in 1996. These upgrades resulted in decreases in biological oxygen demand (by 85%) and total suspended solids (by 70%), as well as concentrations of copper, lead, manganese, zinc, chromium, and oil and grease (McGreer and Belzer 1999; BC MoE 2007); however, these decreases may not consistently reduce effluent toxicity due primarily to the increasing population pressure on WWTPs (McGreer and Belzer 1999). While secondary treatment removes more solids and therefore the contaminants that have adsorbed onto them (e.g. lipophilic contaminants such PCBs), more water soluble contaminants (e.g. newer generation pesticides, pharmaceutical products) are unlikely to be affected (Johannessen and Ross 2002). In addition to sewage treatment plants, there are also more than 50 combined sewer overflows (CSOs) in Lower Basin (35 in Vancouver, five in Burnaby, and 13 in New Westminster; Norecol, Dames and Moore Inc. 1996). In dry weather, when volumes are low, combined sewer overflow is directed to and treated at sewage treatment plants. However, during storm events when pipe capacities are exceeded, an untreated mixture of sewage and stormwater is discharged at designated 'relief points' (Norecol, Dames and Moore Inc. 1996).

It has also been estimated that there are approximately 1 700 storm water outfalls in the Greater Vancouver Regional District, 925 of which discharge directly to fish-bearing waters (Norecol, Dames and Moore Inc. 1996). Urban and suburban runoff may contains contaminants originating primarily from residential and recreational activities (e.g. pesticides, fertilizers) and roadways (e.g. PAHs, metals, salts). As noted above, this runoff would affect smaller tributaries more than the main stem of the Fraser River due to dilution in the main stem.

Climate change has already begun to affect flows and temperatures in the Fraser River. Spring freshet is arriving earlier in the year and reaching half of its annual cumulative flow an average of nine days earlier than a century ago (Fraser Basin Council 2010c). Summer water temperatures have increased over the past 50 years, equivalent to 2.2°C per century (Fraser Basin Council 2010c; Gallaugher and Wood 2010). Climate change and associated sea-level rise will also require further expansion and upgrades to flood protection structures, with an estimated $8.8 billion in projected costs just along the tidal portion of the Lower Fraser (Delcan 2012). Human populations in the Lower Fraser area have increased by 150% over the last twenty years, and will continue to grow (Johannes et al. 2011).

6.3 IMPLICATIONS FOR SALMON

The ongoing challenges faced by salmon populations in the Fraser River are well documented, and while there have been successful years for various species (e.g. 20 million pink salmon returned to the Fraser in 2009, 30 million sockeye returned in 2010), the overall trend across species over the last two decades has been one of decline (e.g. Gallaugher and Wood 2010; Grant et al. 2011; Riddell et al. 2014). Salmon attempting to migrate and spawn in the lower Fraser River and its tributaries face both physical and chemical challenges to their success and survival. This is of critical importance, as it is believed that approximately half of the variability in recruitment success is related to conditions in the freshwater phase of the salmon lifecycle (Bradford 1995). As mentioned above, flood protection structures isolate habitat and can result in ‘dead zones’ in which oxygen concentrations are below acceptable concentrations. Unlike the impacts of physical structures, the impacts of complex contaminant mixtures derived from a multitude of sources are almost impossible to quantify.

Much of the land along the lower Fraser River is used for agriculture. In addition to the introduction of pesticides into waterways, agriculture can result in increased biochemical oxygen demand, introduced pathogens, increased total suspended solids and nutrient concentrations in water, water extraction, damage to riparian areas caused by livestock, impediments to fish passage, and ditching, diking, and stream channelization (Cohen et al. 2011a).

Municipal effluents also introduce many compounds of concern for salmonids, including endocrine disruptors (e.g. Bisphenol A, triclosan, and even some PAHs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and nanoparticles. The treatment plant at Annacis Island, which is the largest plant discharging effluent into the Fraser River, uses what is called ‘trickling filter’ treatment, which has been shown to be ineffective at removing endocrine disrupting compounds (Gallaugher and Wood 2010). Research has demonstrated sex-reversal in salmonids exposed to wastewater treatment plant effluent plumes, and endocrine disrupting compounds may also interfere with the olfactory imprinting process during early life stages (Gallaugher and Wood 2010). PAHs have been observed to have anti-estrogenic effects on female fish, with exposure resulting in decreased production of estrogens and vitellogenin (Pait and Nelson 2002). Other effects observed in vertebrates include abnormal thyroid function, decreased fertility, decreased hatching success, and alteration of immune function (Crisp et al. 1998). Wastewater treatment plants also discharge ammonia, which, in its NH_3 form, is lethal to fish. Ammonia toxicity increases with pH as well as with increasing temperature, which is of concern given warming trend in the Fraser River. In 2008, final effluent from the Annacis wastewater treatment plant failed the 96 hour LD50 standard bioassay tests for ammonia in 7 of the 12 months (Gallaugher and Wood 2010).

While PCBs have been banned in Canada since 1977, their persistent and bioaccumulative nature means that they are still found at measureable levels in several matrices, including sediment and biota (e.g. Cullon et al. 2009). Although it appears that in at least some salmon populations, much of the PCB burden is acquired during their time at sea (Cullon et al. 2009), this must still be taken into account when considering cumulative effects, particularly as salmon can lose more than 80% of their lipid stores during their return migration (Brett

1995). This means that contaminant burdens are mobilized and redistributed into other fatty tissues such as the gonads (O'Neill and West 2009). It has been estimated that PCB concentrations in the muscle tissues of sockeye salmon could increase by as much as 9.7 times during the return migration (DeBruyn et al. 2004). PCBs are toxic to salmon, causing effects including compromised immune function and increased susceptibility to disease (e.g. Arkoosh and Collier 2002; Meador et al. 2002).

PBDEs (flame retardants used prolifically in manufacturing), have been banned in North America; however, they are expected to persist in the environment for decades due to existing levels in and release from soils, sediments, and existing consumer products (De Wit 2002). Studies have found that while municipal processes successfully reduce PBDE concentrations in effluent (Rayne and Ikonomou 2005), discharge from wastewater treatment plants can still act as an important source of these compounds (Song et al. 2006; Rayne and Ikonomou 2005), and their resistance to degradation allows for accumulation the environment (Arnold et al. 2008). Similarly to PCBs, PBDEs have been shown to compromise immune function and increase disease susceptibility in salmonids (Arkoosh et al. 2010).

In terms of urban and suburban runoff, the combination of contaminants in this untreated effluent has been shown to be highly toxic to salmonids (e.g. Scholz et al. 2011). As vehicle traffic and the percentage of impervious surface area increase, a proportionate increase is observed in contaminant loading in runoff (Hall et al. 1999), including PAHs from oil, gas, and greases, metals, PCBs, antifreeze, solvents, pesticides, herbicides, fertilizer, paint, detergents, road salt, and animal feces (Cohen 2012). Pesticides are of concern due to their ubiquity and their toxicity. They are prevalent in both urban and agricultural runoff (e.g. Harris et al. 2008), and in 1999, over 8 million kg of pesticide active ingredients were purchased or used in BC (Enkon Environmental Ltd. 2001; Johannessen and Ross 2002). Current-use pesticides were expected to be less bioaccumulative, persistent, and toxic than their organochlorine predecessors; however, it is now clear that they can have significant effects on salmon, including interference with olfaction (Tierney et al. 2006; Tierney et al.2007; Scholz et al. 2000), physiological stress and mortality (Waring and Moore 2004), and declines in the number of spawning adults (Fairchild et al. 1999).

PAHs are a compound class of concern in the Fraser River basin. Juvenile Chinook salmon in the upper Fraser River exhibiting sublethal toxic effects appeared to be affected by PAHs, rather than by other contaminants such as PCBs (Wilson et al. 2000). The entire Fraser Basin exhibits PAH levels derived from industrial and urban activities (e.g. pulp mills, municipal waste water, vehicular emissions) that in many cases exceed Canadian federal and provincial guidelines for the protection of aquatic life (Yunker et al. 2002). Anthropogenic PAH sources to the Fraser River basin include petroleum products spills, urban runoff, industrial combustion, wood burning, and vehicle exhaust (Brewer et al. 1998). Guideline exceedances occur primarily at urban sites subject to inputs from storm sewers and combined sewage outflows, boat traffic, and atmospheric deposition. The North Arm and mainstem have source signatures consistent with both pyrogenic (combustion derived) and petrogenic (petroleum derived) PAHs (Brewer et al. 1998; Yunker et al. 2002), while petrogenic sources predominate in the delta (Yunker et al. 2002). While concentrations in the North Arm are generally higher, likely due to the large number of stormwater outfalls combined with lower flows, concentrations in both arms of the river exceeded guidelines for LMW and HMW PAHs (Brewer et al. 1998). Interestingly, measured PAH concentrations in the main stem were higher within the zone of salt-wedge formation relative to sites upstream of the limit of saline intrusion (Yunker et al. 1999). The authors suggested that the difference may reflect differences in hydrological processes: salt-wedge intrusions have been found to alter suspended sediment concentrations through processes including interference of sediment exchange between the flow and the bed, reduced turbulence, and flocculation of sediment (reviewed in Yunker et al. 1999). These observations may have implications for oil spills in the main stem of the Fraser River: in the case of a spill within the limit of saline intrusion, suspended sediment concentrations may be higher and may thus impact the formation of oil-sediment aggregates and the potential for oil to submerge and sink.

While it is possible to identify individual compound classes that pose a toxic risk, the combined effects of the hundreds of compounds in urban and suburban runoff are much more difficult to understand. For example, coho salmon returning to spawn in streams around Puget Sound exhibited abnormal behaviours including erratic surface swimming, gaping, fin splaying, and loss of orientation and equilibrium (Scholz et al. 2011). Fish exhibited these behaviours died within hours of first observation, with female carcasses showing high egg retention rates (>90%; Scholz et al. 2011). No relationship between affected fish and water quality parameters, pesticide exposure, disease, or condition was observed, leading the authors to conclude that fish were vulnerable to an unknown contaminant or contaminant mixture, likely delivered to waterways via stormwater runoff (Scholz et al. 2011).

In addition to immediate toxic effects, contaminants can also negatively impact salmon during their transition to the marine environment. For example, impaired survival and compromised ability to adapt to saline conditions were also observed during the parr-smolt transformation in Atlantic salmon exposed to environmentally relevant atrazine, 4-nonylphenol, and estrogen levels (Fairchild et al. 1999; 2002). Chinook salmon from contaminated freshwater systems (evidenced by PAH, PCB, and OC pesticides in stomach contents, PAH metabolites in bile, and CYP450 activity) exhibited significantly lower survival rates than those from relatively uncontaminated systems when held in saltwater for 40 days (Varanasi et al. 1993).

Increasing temperatures also have negative effects on salmon survival (e.g. Martins et al. 2011). Sockeye salmon returns have been below expected numbers (by an average of 32%) in all recorded years in which river temperatures were anomalously high (Gallaugher and Wood 2010). This means that in years in which river temperatures are warmer than usual, returning salmon are at a competitive disadvantage even before they face physical barriers and contaminant inputs as they make their way up the river.

Since 1994, many Fraser River late-run sockeye populations have also been entering the Fraser River to begin their up-river migration 2-6 weeks early in relation to historic average dates (Mathes et al. 2010). While the reasons behind this shift are not fully understood, the result is increased mortality en route to their spawning grounds. Past migration mortality was generally under 20%, it is now over 60% and in some years 90% (Cooke et al. 2004). Because migration now occurs in the summer rather than in early fall, sockeye encounter temperatures that are several degrees higher than their historical norms. Further, because actual spawning times remain the same (only the migration is earlier), salmon must spend longer in these warmer environments (Mathes et al. 2010). Sockeye that migrate in higher than normal temperatures and that die during migration have been found to have impaired ionoregulatory systems, advanced senescence, and symptoms of physiological stress (Cooke et al. 2006; Young et al. 2006; Mathes et al. 2010).

Increases in temperature also increase the likelihood of parasitic, bacterial, and fungal infections (Gallaugher and Wood 2010). For example, the bacterium *Flexibacter* spp. causes gill damage in Fraser River sockeye and is more virulent at high temperatures, and the kidney parasite *Parvicapsula minibicornis* is associated with reduced swimming performance and increased migration mortality in sockeye, and develops more rapidly at warm temperatures (reviewed in Mathes et al. 2010).

Salmon, particularly larval life stages, are also extremely sensitive to UV exposure. While UV exposure can exacerbate hydrocarbon toxicity via photomodification (Chapter 5.4.3), UV-B radiation alone has been observed to result in DNA and macromolecular damage, impaired larval development and decreased recruitment, sunburn, and lesions in the brain and retina (reviewed in Hakkinen et al. 2004). Finally, UV-B exposure can cause severe and irreversible neurobehavioural disorders (e.g. inability to swim straight, uncontrollable spinning), leading to substantial late mortality (Hakkinen et al. 2004). Increases in UV exposure in spring coincide with the most intensive and vulnerable reproductive phases of several salmon species.

Acoustic disturbance by development and marine traffic also pose risks to salmon. Little is known about the effects of anthropogenic sound on fish and even less is known about the impacts to developing eggs and embryos (Popper 2003). However, artificial underwater noise may be harmful (Slabbekoorn et al. 2010). Although the harm caused by short-term intense sounds like sonar, pile driving and explosions have attracted the most attention, the greater impact on salmon will likely be from less intense sounds that are of longer duration (Popper and Hasting 2009; Slabbekoorn et al. 2010). Sublethal physiological responses to underwater noise generated by vessel traffic such as increased heart rate (Graham and Cooke 2008), increased metabolism and motility (Buscaino et al. 2010), and the secretion of stress hormones (Slabbekoorn et al. 2010) are all documented responses in fish exposed to noise.

Salmon also face potential competition and in some cases, an increased risk of predation, from non-native species in the Lower Fraser River. There are nine fish species that have established populations in freshwater areas, including smallmouth bass and yellow perch, which may prey upon salmon during early life stages (Johannes et al. 2011). The threat of further invasions remains despite recent changes in legislation (Scott et al. 2013). Non-native plant species are also common, and include purple loosestrife, which has recently invaded marsh areas of the estuary and altered the dynamics of detritus availability to the food webs upon which juvenile salmon rely (Grout et al. 1997).

The cumulative impact of physical structures, invasive species, and contaminant inputs from the multiple sources discussed above is unknown. These anthropogenic stressors are present in addition to natural stressors associated with the physical stresses of both out- and return migrations, osmotic and ionic stress during adaptation to saline and fresh water environments, respectively, UV radiation, temperature fluctuations, feeding, growth, and sexual maturation. Because much of the lower Fraser River is industrialized, there are multiple point and non-point pollution sources along its length. This, combined with the dilution provided by the river, makes it difficult to pinpoint the direct impacts of contaminants and other stressors. Further, multiple contaminants can combine to form complex mixtures, which can interact and behave in unpredictable ways (e.g. additively, synergistically, and/or antagonistically).

Cumulative effects are extremely difficult to test and prove in natural ecosystems. However, interactive stressors were suggested as the underlying cause of the collapse of Pacific herring in Prince William Sound following the *Exxon Valdez* oil spill (Carls et al. 1998). In Lake Winona, MN, a massive spring die-off of bluegill sunfish (*Lepomis macrochirus*) was attributed to the combined effects of a winter under-ice spill of Bunker C fuel oil, rapid temperature shifts, the stress of spring spawning, and a loss of condition over the winter (Fremling 1981). As such, the impacts of oil spills into the lower Fraser River, estuary, and/or nearshore marine environments may prove to be very difficult to understand and manage when combined with other environmental factors and stresses.

7 CONCERNS WITH TRANS MOUNTAIN'S SUBMISSION TO THE NATIONAL ENERGY BOARD

While these concerns are discussed in greater detail in the main body of this report, they are summarized here for the convenience of the reader.

7.1 TRANS MOUNTAIN DOES NOT INDICATE PRECISELY WHAT WILL BE CARRIED IN THE PIPELINE

7.1.1. While the submission indicates that dilbit is expected to be carried in the new pipeline, the composition of the dilbit and thus its behaviour and fate in the environment, as well as its potential effects, will change with the season and with the diluent used (e.g. condensate or synthetic crude oil).

7.2 TRANS MOUNTAIN INDICATED IN THEIR SUBMISSION THAT OSA FORMATION WILL NOT BE A DOMINANT FATE OF SPILLED OIL

7.2.1. This assertion was made despite the findings of Environment Canada (2013), who found that various types of sediment material resulted in the formation and subsequent sinking of OSAs. While Trans Mountain asserts that sediment concentrations in the Fraser River do not reach levels high enough to result in OSA formation, this does not take into account potential overland flow of oil, which would add terrestrial materials to the oil even before it reaches the river, increasing the likelihood that the oil will sink.

7.3 TRANS MOUNTAIN'S ASSESSMENTS OF TURBIDITY/TSS WERE QUALITATIVE

7.3.1. Trans Mountain carried out these measurements using only visual assessments, despite the importance of these parameters in determining the behaviour of spilled oil.

7.4 TRANS MOUNTAIN DID NOT ADDRESS THE POSSIBILITY OF SUBMERGED AND/OR SUNKEN OIL

7.4.1. The possibility that oil may be submerged in the water column and/or sink to the river bed was not addressed in either the clean up or environmental protection plans. Again, this was despite experience following the Kalamazoo River spill (US EPA 2013) as well information in the literature (Short 2013; Dupuis and Ucan-Marin 2015).

7.5 IN MODELLING THE POTENTIAL FOR OIL TO STRAND, TRANS MOUNTAIN ASSUMED A STRAIGHT CHANNEL

7.5.1. In the models of stranding potential for oil, Trans Mountain assumed that "the only product entrainment is along the simulated banks". They did not account for "any braiding, debris, backwater, log jams or other impediments to waterborne travel". These omissions likely resulted in a significantly underestimate projection of the potential volume of stranded oil.

7.6 IT IS UNCLEAR WHETHER TRANS MOUNTAIN WOULD CONSIDER THE USE OF CHEMICAL DISPERSANTS IN FRESHWATER/ENCLOSED ENVIRONMENTS

7.6.1. In their submission to the NEB, Trans Mountain limited explicit discussion of dispersant use to the marine environment, and expressed their intention to seek pre-approval for the use of dispersants. Whether this application would encompass use in the freshwater environment is unclear.

7.7 DETECTION LIMITS IN THE GAINFORD STUDY WERE VERY HIGH

7.7.1. Trans Mountain used the Gainford study to inform their modeling efforts around the behaviour and fate of dilbit.

The detection limit of oil in water of 2 mg/L is three orders of magnitude higher than concentrations at which effects on fish have been observed. Thus, the conclusion of the Gainford study that “concentrations in the water column were below the detection limit” is relatively meaningless in terms of implications for fish health.

7.8 THE GAINFORD STUDY DID NOT INCLUDE FRESHWATER OR THE ADDITION OF ANY KIND OF SEDIMENT OR PARTICULATE MATTER

7.8.1 Despite these omissions, the study still concluded definitively that in no instance was oil observed to sink or submerge. This conclusion is misleading in that it ignores some fundamental factors governing the behaviour of oil.

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9 APPENDICES

9.1 APPENDIX A

Table A.1 Detailed review of spawning behaviour and life history characteristics of Fraser River salmon and trout species and eulachon. (BC MOE 2004; Beacham and Murray 1993; COSEWIC 2002, 2003, 2012; Costello 2008; DFO 1995, 1996, 1999; English et al. 2011; Grant and Pestal 2009 a, b; Grant et al. 2011; Groot and Margolis 1991; Holtby and Ciruna 2007; Johannes et al. 2011; Levy and Northcote 1982; Levy and Parkinson 2014; Murray and Rosenau 1989; MELP and DFO 1998; McPhail 2007)

	Spawning				**Embryos and larvae**	**Juveniles**	**Adults**		
Species	Conservation Units	Timing of Spawning in Lower Fraser and Tributaries	Habitat & Redd Description	Egg, Redd, & Female Traits	Development	Rearing	Maturity Traits	Migration timing groups	Harvest

Chinook Salmon (*Oncorhynchus tshawytscha*)	**5 LFR CU's** / 21 Total FR CU's **CU Names** (# of sites): **LFR fall white – ocean type** (1 *Harrison River*) **LFR spring – stream type** (7) **LFR Upper Pitt – stream type** (2) **LFR summer** – **stream type**(9) **Maria Slough – ocean type** (1)	Mature Chinook spawn from **Jun–Nov** (Peaks range from Late spring mean Jun.11th; mid-fall mean Nov.9th)	**Habitat**: Large and small tributaries **Sediment Size:** Large cobbles to fine gravels **Water Depth:** 5–700cm (mean ~30cm) **Water Velocity:** 10–150 cm/s (mean ~50cm/s) **Redd Area:** 4.0–15.0m^2 **Egg Burial Depth:** mean 18–28cm (typically in areas with high subsurface flows) **Female Behavior:** Diggs nest, buries eggs after deposition, remains over redd until death.	**Egg Size:** Largest eggs of the Pacific salmon species at 6-10mm diameter **# Eggs/ Redd:** Fecundity can range from 2,000–17,000 in Fraser averages 3,200-4,000.	**Incubation Time:** 32 days (16^{o}C) – 159 days (3^{o}C) **Alevin Behavior:** Once alevin reach maximum size, they emerge as fry to be swept/swim downstream (typically occurs at night)	**Migration:** Fry emerge **Mar – May** **Ocean-Type (**66% of stock) juveniles migrate in spring or summer after emergence. Includes "immediate" type which migrate directly to estuary after emergence. **Stream-Type** (34% of stock) juveniles spend at least one year in freshwater. **LFR / FRE Use:** Ocean-type juveniles, particularly Harrison River stock, migrate to the LFR and estuary soon after emergence where they utilize tributary sloughs, side channels and tidal marshes as their primary rearing habitat in early summer (**Apr-Jul**), growing from 40mm to 60-68mm in fork length, before moving to estuary until Aug/Sept. **Prey:** Freshwater – variety of larval and adult insects (dipterans, trichopterans, notonectids, copepods, cladocerans and chironomids) Estuary – chironomids, *Daphnia, Eogammarus, Corophium* and *Noemysis*.	**Age:** 2–7 years (mean 4 years) for ocean and stream type, **Weight:** Mean 7-9 kg (can reach 45 kg). **Length:** Mean 740 mm	Migration in the Fraser is divided into 3 run timing groups that occur over the spring, summer and fall from **Feb – Nov** **Spring Run:** Before July 15th, Mean combined spawning escapement of 12,000 (1951–1993). **Summer Run:** July 15th to August 31, Mean combined spawning escapement of 18,000 (1951–1993) **Fall Run:** Harrison River population, which makes up the majority of LFR Chinook migrate from **Sept–Nov,** with mean escapement of 116,000 (1984–1993).	**Overall Harvest:** Largest Canadian producer of Chinook with 200,000 historical production rate and 240,000 target escapement **Commercial:** Mixed-stock marine fisheries from Washington–Alaska **Recreational:** Mean catch 5,000 annual prior to 1984 **Aboriginal:** First Nations FSC catch, mean 41,000 (1990) **Enhancement:** Numerous hatcheries including the Chehalis and Chilliwack River hatcheries. Contribute little to overall abundance, producing an estimated 10,000 (1983) to 41,000 (1990) adults.

Chum Salmon (*Oncorhynchus keta*)	**1 LFR CU** / 2 Total FR CU's **Lower Fraser CU** (78 sites LFR and Lillooet River 3 sites)	Mature chum spawn from **mid-Sept – Jan** (Peaks range Oct. 9th–Jan. 29th) Spawning lasts 2-3 weeks in small streams but 2-3 months in large rivers such as the Harrison)	**Habitat:** Mainstem FR, as well as small and large tributaries. 110 spawning streams but ~90% spawn in mainstem and Harrison/ Chehalis/ Weaver, Chilliwack/ Vedder, and Stave watersheds. **Sediment Size:** Gravel mostly less than 15 mm diameter **Water Depth:** 13.4–49.7cm **Water Velocity:** 21.3–83.8cm/s **Egg Burial Depth:** 7.5–50cm **Female Behavior:** Females dig nests (4-6 nests) in a row, after eggs are deposited the female buries them, and guards redd until they die.	**Egg Size:** BC mean 8.5mm diameter **# Eggs/ Redd:** 2000–4,000 (4-6 nests per redd)	**Incubation Time:** Mean 52–61 days (9.75°C) **Alevin Behavior**: After hatching, alevins remain in gravel for an average of 30 days, after which fry begin to emerge at night starting in Feb.	**Migration:** Juveniles migrate directly to the estuary from **Feb–Jun** (peak Mar–Apr) after emergence. **LFR / FRE Use:** Juveniles remain in estuary for up to 6 months. Known to utilize tidal marshes in the LFR from late March until early June. **Prey:** Feed on detritus based food webs that rely on high carbon output from freshwater discharge. Diet dominated by harpacticoid copepods, chironomid larvae and gammarid amphipods. **Predators:** Coho and trout, various species of birds and mammals.	**Age:** Return after 2 – 5 winters at age 3–6 (mean 4 years). **Weight:** Mean 5.1kg (can reach 20.8kg) **Length:** Mean 59.6cm (can reach 108cm)	Chum salmon migrate primarily during the fall from **Sept–Jan** Adults only migrate as far as Fraser Canyon with no spawning occurring in Middle and Upper Fraser or Thompson River.	**Overall Harvest:** Largest Canadian producer with average run size 2.3 million (1995-2007), peak 3.9 million (1998). 800,000 escapement target since 1999. **Commercial:** Fisheries in marine and FR areas. Chum intercepted in mixed-stock fisheries of SOG and JS. **Recreational:** Sports fisheries concentrated in Mainstem FR, Harrison and Chilliwack Rivers. **Aboriginal:** First Nations FSC and economic opportunity fisheries mostly in Lower Fraser, 75,000 target for Lower Nations; 500 for Middle and Upper Nations. **Enhancement:** 3 main hatcheries, including the Chehalis River, Chilliwack River, Inch Creek. Peak operation from 1980-1998. Reduced output currently.

Coho Salmon (*Oncorhynchus kisutch*)	**2 LFR CU's** / 8 Total FR CU's **CU Names** (# Sites): **LFRA** (77) **LFRB** (15) 69% of 187 stocks reported in FR spawn in the LFR, and the largest LFR stocks spawn in Vedder-Chilliwack, Harrison, Upper Pitt and Salmon River systems. Interior Fraser River coho population designated as endangered; COSEWIC, 2002.	Mature coho spawn from **Oct - Feb** Peaks from Nov.6th–Jan.29th (Spawning can continue until late Feb. Latest spawning populations in BC occur in the LFR)	**Habitat:** Prefer most coastal, small (<1m wide), low elevation streams with low–moderate gradients, but can be found in large rivers **Sediment Size:** Gravel <15cm diameter **Water Depth:** 4–33cm **Water Velocity:** 30–55cm/s **Redd Area:** 1.5–2.8m^2 **Egg Burial Depth:** 17–40cm **Female Behavior:** After eggs are deposited, the female moves upstream and begins digging a new nest while burying the eggs	**Egg Size:** 4.9 to 8.4 mm diameter **# Eggs/ Redd**: 2000–3000 (300–1200 eggs/nest; mean 800–900)	**Incubation Time:** 38–137 days (4–11°C optimum) **Alevin Behavior:** After hatching, alevin remain in the gravel ~20–40 days before they reach 30mm fork length and emerge in April and May as fry.	**Migration:** Juveniles migrate to the estuary from **Apr - Jun** **LFR / FRE Use:** Juveniles rear and overwinter in freshwater streams for at least one year after emergence. They remain in natal streams and nearby ponds, marshes and backwater channels until migration. Juveniles take cover under large stones and banks, and aggressively defend territories. **Prey:** Feed on terrestrial and aquatic insects (primarily chironomids of varying life stages, and stoneflies), along with crustaceans, and small fish (coho, chum and pink fry). **Predators:** Fish (trout, charr, and northern pikeminnow), mammals (river otters and mink), and birds (dippers, robins, crows, herons and fish-eating ducks).	**Age:** 2–4 years (Typically 3 years). Return after 18 months at sea. **Weight:** Mean 3.22kg (can be as large as 14.0kg) **Length:** Mean 52.7±3.2cm for 3 year-olds.	Migrate primarily during the fall from late **Aug–late Dec** Migrate further upriver than chum and pink, found throughout the Fraser except for the Upper Fraser.	**Overall Harvest:** Harvest substantially reduced following steep declines in Coho starting in late 1970's. Escapement averaged 40,000 for LFR and 18,000 TR (1980–1993). **Commercial:** Mixed-stock marine fisheries, with majority caught in WCVI and SOG troll fisheries. **Recreational:** Largest individual contributor to coho harvest (39%), mostly occurs in SOG. Freshwater harvest primarily on hatchery stocks. **Aboriginal:** First Nations FSC fishing occurs throughout LFR. **Enhancement:** Hatcheries responsible for production of ~400,000 adults in late 1980's. Primarily from Chehalis, Chilliwack and Inch Creek, although many small local hatcheries contribute.

Pink Salmon (*Oncorhynchus gorbuscha*)	Pink salmon have a fixed 2-year life cycle, even and odd years are distinct. **Odd-Year** **1 LFR CU** (35 sites) only FR CU Approximately 70% of production occurs in LFR mainstem and tributaries. **Even-Year:** **No FR CU's** (low abundance during even years with only 2 spawning populations both occurring in the MFR).	Mature pink spawn from **Sept - Dec** Odd-Year peaks range from Sept.27th–Oct.22nd	**Habitat:** Spawn in the Mainstem of the LFR, primarily in the gravel reach from Hope to Mission. 35 of 69 populations spawn in the LFR. **Sediment Size**: Clean coarse gravel **Water Depth**: 30–100cm (15cm on crowded spawning grounds). **Water Velocity:** 30–140cm/s **Redd Area**: 1.1–2.0m^2 **Egg Burial Depth:** 15–50cm **Female Behavior:** Like other salmon, female digs nests, defends redd	**Egg Size:** Smaller eggs than other pacific salmon, weigh 150-250 mg **#Eggs/ Redd:** 1500–1900 (mean 500 eggs/nest)	**Incubation Time**: Mean 100–120 days (Fertilized eggs incubate over winter for 5–8 months) **Alevin Behavior:** Alevin remain in gravel for 13–35 days until fry emergence at night.	**Migration:** Juveniles spend least time in freshwater of the five species. Once juveniles become neutrally buoyant, they migrate to the estuary between **Feb–June** (peak mid-April to early May). Mean 28–35mm fork length and 1.30–2.60g during migration. **LFR / FRE Use:** Juveniles are present in the LFR and estuary from Mar.–May, and remain in protected areas of the estuary for 2–3 months before migrating to the open ocean (some remain in SOG until Sept.) **Prey:** Estuary – feed primarily on calanoid, cyclopoid, and hapactioid copepods. SOG – feed on fish, insects, decapods, euphasiids, amphipods, and copepods, cirripeds, and cladocerans from July to Sept. **Predators:** Trout, charr, sculpin, coho, drippers, crows, ducks, and muskrat.	**Age:** 2 years; return after one winter at sea. **Weight:** Mean 1.0–2.5kg **Length:** Males – mean 52.5 – 57.6cm Females – mean 50.3–53.7cm	There are early run and late run timing groups which migrate through LFR from **Aug–Dec** Most pink spawn within 100 km of the ocean, some Fraser populations including Thompson migrate as far as 300 km.	**Overall Harvest:** Harvest only occurs for Odd-Year Pink. Average Escapement 5.8 million (1959-2007), with a peak of 23 million in 2003. Target escapement is 6 million. **Commercial:** Harvest primarily takes place in FR Panel Area and LFR below Mission, also captured in U.S. waters and mixed-stock fisheries in JS, JDFS, and SOG. **Recreational:** Harvested primarily along Fraser mainstem, Stave, Harrison and Chilliwack Rivers. **Aboriginal:** FN harvest Pink in FSC and economic opportunity fisheries in marine areas and throughout the FR and tributaries. 125,000 target for Lower Nations; 500 for Middle and Upper Nations. **Enhancement:** Minimal enhancement at Weaver Creek and Chehalis River hatchery contribute <5% to total production.

Sockeye salmon (*Oncorhynchus nerka*)	Anadromous sockeye have two distinct life history types: lake-type and river-type. Lake type populations are divided into CU's based on nursery lake. Freshwater sockeye (kokanee) are also present in lakes throughout the Fraser watershed. **River-Type:** **2 LFR CUs** (4 sites) (only river-type CUs) **CUs**: Harrison River Widgeon Creek **Lake-Type:** **5 LFR CU's** / 22 Total FR CU's **LFR CUs:** **Chilliwack** (Early summer), **Cultus** (Late timing), **Harrison** (Downstream – Late), **Harrison** (Upstream-Late), **Pitt** (Early summer) Cultus Lake population designated as endangered; COSEWIC, 2003	Mature sockeye spawn from **Sept - Dec** Peaks: River-Type: Nov.7^{th} Lake-Type: Sept.22^{nd}	**Habitat:** prefer habitats associated with nursery lakes including tributary streams, streams between lakes, and lake outlets **Sediment Size:** Gravels between 10-25 mm. **Water Depth:** 6 to 37 cm in small tributaries, some lake spawners up to 30m deep. **Water Velocity:** 15 – 85 cm/s **Egg Burial Depth:** 15-23cm **Female Behavior:** After spawning, female quickly buries eggs and stays with nest for several hours before excavating next nest (3-7 nests). Once complete, female stays on redd until death	**Egg Size:** Eggs are 5.3 -6.6 mm in diameter **#Eggs/ Redd:** 3,000 – 4,000 (500 – 1,100 eggs/nest)	**Incubation Time:** ~135–290 days (2–8°C) **Alevin Behavior:** Remain below gravel surface until yolk supply has been utilized (6 – 10 weeks), then emerge as fry (typically at night).	**Migration:** River-Type – migrate immediately after emergence (e.g., Harrison River rapids stock). Lake-Type – migrate one year after emergence. Smolts in Fraser system average between 74-90mm and 4.6-7.8 g. **LFR / FRE Use:** River-Type – utilize LFR tidal sloughs and estuary for 2 – 6 months, peaking in **Jun-Jul,** before entering the SOG. Harrison stock use LFR sloughs and side channels as their primary nursery areas for 4 to 6 months. Lake-Type – rear in nursery lakes before using LFR primarily as migration corridor, typically migrating through in 7 to 10 days before reaching the estuary and SOG. **Prey:** Freshwater – littoral dipteran insects, zooplankton LFR and Estuary – Cladocerans including *Daphnia*, copepods, amphipods, oligochaetes, Euphausiids, decapods and insects. **Predators:** Fish including coho, trout, Dolly Varden, sculpin, and northern pikeminnow, birds, mammals.	**Age:** Sockeye typically return after 2 (range 1-4) winters at sea with most following 4 year life cycles. **Weight:** Range from 2.3 – 7 kg **Length:** Range from 60- 84 cm, males slightly larger than females	Adult migration is in 4 distinct run timing groups occurring from **June - Nov**: Early Stuart Run, Early Summer Run, Summer Run, Late-Run. Late Run includes SARA-listed Cultus Lake population.	**Overall Harvest:** Significantly declined over the last several decades from an average of 10.08 million (1986–1991), to 6.41 million (1991–2000), continuing down to 2.09 million (2001–2009). Although it rebounded substantially in 2010. **Commercial:** Average harvest in Canadian and U.S. waters was 889,000 (42% total) and 18,000 (15% total) respectively (2001–2009). Peak harvests were 13.7 and 2.9 million for Canadian and U.S. waters respectively (1993). **Recreational:** Accounts for 3% of total harvest, much of which takes place along the mainstem of the LFR. 59,400 average catch (2001–2009), with peak of 171,000 (2006). **Aboriginal:** FN FSC accounts for 29% of total harvest, and 6% for economic opportunity fisheries. 723,900 average catch (2001–2009), with peak of 1.2 million (1997).

	Spawning				Embryos	Juveniles	Adults		
Species	Conservation	Timing of Peak Spawning in LFR	Habitat & Redd Description	Egg, Redd, & Female Traits	Early Devo	Rearing	Maturity Traits	Migration timing groups	Harvest

Rainbow Trout/ Steelhead (*Oncorhynchus mykiss*)	Steelhead and rainbow trout are managed by the BC Ministry of Environment. Occur in two genetically indistinguishable forms; freshwater residents known as rainbow trout and an anadromous form known as steelhead. Fraser River steelhead occur in three distinct groups; **Coastal Winter, Coastal Summer, and Interior Summer**. Coastal Winter spawn in 21 streams all in the Lower Fraser, majority production (70%) from Chilliwack River. Coastal summer Steelhead spawn in 3 streams in the LFR.	Resident forms spawn typically in spring with rising stream temps, can continue from **Apr-July**. Steelhead: ~10% of spawners (generally females) survive to spawn again (iteroparous), though very few spawn >2 times. Winter run spawn from **Feb – Apr** Summer run spawn from **Apr - Jun**	**Habitat:** Spawn mostly in small streams but also in lakes, and the Thompson River. **Sediment Size:** Related to body size with larger females preferring larger gravels. **Water Depth:** 0.15 – 2.5 m **Water Velocity:** 30 – 90 cm/s **Egg Burial Depth:** 8 – 6 inches deep **Female Behavior:** Following egg deposition, the female digs another nest upstream creating a series of nests (redd). Primarily spawn at water temps between 4 – 10°C	**Egg Size:** Range from 3 – 7 mm diameter **#Eggs/ Redd:** 200 for small stream resident females; 1000 – 3000 for lake inhabiting females; 5,900 – 18,400 (mean 12,600) for . Thompson River steelhead females.	**Incubation Time:** 6 – 8 weeks (8 – 10°C optimal temp.) **Alevin Behavior:** Remain in gravel for 3 – 4 weeks while absorbing yolk sack. Fry emerge from gravel around 18 – 21 mm fork length. Peak emergence occurs in during May for coastal stocks and mid-June to early July in interior.	**Migration:** Juvenile steelhead spend between 1 and 4 years in freshwater before migrating to the sea in the spring and early summer. **LFR / FRE Use:** Juveniles of Coastal Winter population are present in 21 tributaries of the Lower Fraser year round and typically spend 2-3 years in freshwater before migrating to sea. **Prey:** Begin feeding on insects and plankton as fry. Depending on habitat, feed on benthic organisms (e.g., amphipods and snails), and pelagic organisms (e.g., chironmids, cladocerans and other zooplankton). **Predators:** Fish, mergansers, herons, kingfishers, garter snakes and racoons.	**Age:** Stream-resident populations can mature after one year; large-bodied lake residents can mature as late as their fifth year; Steelhead typically spend 2 or 3 (range 1-5) years at sea before returning to freshwater to spawn. **Length:** Length at maturity can range from 150 mm for stream-residents to over 400 mm for piscivorous adults.	Winter steelhead migrate from **Nov – Apr** while summer run migrate **May – Sept.** Summer steelhead migrate into rivers while still immature and spend up to 8 months in freshwater before spawning; fall and winter run fish are mature when they enter freshwater and spawn shortly after.	**Overall Harvest:** Rainbow trout are common in freshwaters throughout BC where they are fished recreationally and are not of conservation concern. Steelhead populations in the SOG and FR Interior are of significant management concern following declines in abundance during the 1990's. Target steelhead escapement of 8,000 -12,000 for coastal winter, 1,000-1,500 for coastal winter, and 8,000 for interior summer runs. **Commercial:** Intercepted as bycatch in commercial bet fisheries **Recreational:** Steelhead are prized by recreational fisherman in catch and release fishing and targeted along the mainstem of the LFR and along the Chilliwack and Chehalis rivers. **Aboriginal:** Targeted by FN fisheries throughout much of their range, historically captured 1,000-5,000 fish per year but reduced currently. **Enhancement:** Hatchery steelhead are produced Chilliwack, Alouette, and Chehalis River hatcheries, along with InchCreek, Bell Irving amd Fraser Valley trout hatcheries. Hatcheries release up to 260,000 smolts which are thought to produce from 3,100 – 11,900 in adult returns annually.

Coastal Cutthroat Trout (*Oncorhynchus clarkii clarkii)*	Are iteroparous and occur in stream- resident, freshwater-migratory and anadromous forms. Blue-listed species in BC, considered vulnerable, several South Coast populations in decline. Residents present year-round throughout tributaries of the LFR in resident Sea-run forms (adults) range in habitats from large side channels and sloughs of the Fraser to small headwater streams.	**Feb – May** with ~40% of spawners surviving to spawn again (some individuals spawn up to 4 or 5 times).	**Habitat:** Generally prefer small spawning streams but known to spawn in side channels of the gravel reach of the LFR. **Sediment Size:** Gravel from 5 – 50 mm diameter. **Water Depth:** 15 – 50 cm **Water Velocity:** 300-400 cm/s. **Egg Burial Depth:** 5 – 50 cm deep **Female Behavior:** Similar to other salmon and trout, female digs redd typically in tai-outs of pools.	**Egg Size:** 5 to 6 mm diameter **#Eggs/ Redd:** 50 – 150 for stream-resident females; 500 – 1,500 for sea-run and lake-resident females	**Incubation Time:** 20 days ($14^{\circ}C$) – 123 days ($2^{\circ}C$). **Alevin Behavior:** Alevin and fry emerge from gravel 35 – 190 days after fertilization, typically from **Mar - Jun**	**Migration:** Sea-run populations spend 1 to 4 summers in freshwater before seaward migration from **Mar-Jun** **LFR / FRE Use:** Juveniles prefer small streams < 5m wide, low to moderate gradients and gravel substrates. Compete with juvenile coho for quality habitats. **Prey:** Juveniles in streams eat chironomid larvae and drifting insects; Lake-type eats zooplankton, surface insects, and a variety of fish species including juvenile salmon. Adults feed primarily on salmon fry and eggs along with three-spine stickleback.	**Age:** Anadromous southern populations return at age 4, but can also return at age 5 and 6. Males reach maturity around their 3rd year and typically live between 4-5 years. **Weight:** Sea-run less than 3.6 kg while lake residents can reach 7.7 kg. **Length:** <15 cm (stream-resident females). 20 – 50 cm (sea-run and lake-resident females); 68 cm (sea-run males); 76 cm (resident males);	Anadromous populations migrate in early run (**Aug – Sept)** and late run **(Feb – Apr)** spawning groups**.** Exist in sea-run forms in at least 19 streams in the Lower Fraser including West and Nathan Creek's. Salmon River, Nicomen Slough, Hatzic Slough, Alouette River, DeBovile Slough, Coquitlam River.	**Overall Harvest:** Targeted primarily by recreational fisherman along mainstem of the Lower Fraser and in many tributary streams, including the LFR. Can amount to over 100,000 angler days per year and an economic value of $4 million. Harvest high relative to total number of adults, production in Lower Fraser has been estimated at 8,300 adults annually. **Enhancement:** Hatchery stocking occurs throughout the Lower Fraser with peak production of 100,000 smolts in 2000, reduced since. Can account for 75% of fish captured in the mainstem.

Bull trout (*Salvelinus confluentus)*	Bull trout are iteroparous and occur in stream-resident, river-migratory, lake-migratory, and anadromous forms throughout the LFR. Long considered to be same species as Dolly Varden before being recognized as distinct in the early 1980's. The South Coast BC bull trout Designable Unit were listed as special concern by COSEWIC in 2012. Bull trout and are blue listed in BC. Anadromous life history form is unique to the South Coast British Columbia population.	Bull trout spawn in their natal streams primarily in **Sept - Oct**	**Habitat:** Prefer cold water, spawn in small low gradient streams with cover form debris, vegetation and undercut banks. **Sediment Size:** Gravel less than 20 mm in diameter **Water Depth:** 30 – 40 cm **Water Velocity:** 3 – 80 cm/s **Egg Burial Depth:** Nests are typically 10 to 20 cm deep in gravel **Female Behavior:** Dig large redds of greater than 2 m squared and spawn in water temperatures < 9^oC. Typically move back downstream soon after spawning.	**Egg Size:** Typically 5.0-6.2 mm diameter **#Eggs/ Redd:** Females of stream resident populations produce ~500 eggs while large migratory females ~2-5,000 eggs.	**Incubation Time:** Incubation occurs at an optimum temp between 2 – 4 degrees Celsius, incubation time depends on temp can range from 50-126 days. **Alevin Behavior:** Emergence primarily occurs from **mid-April to mid-May.** Fry emerge approximately 220 days after initial deposition and take refuge in pools with instream cover and in gravels.	**Migration:** Juvenile bull trout all typically spend 2 – 4 years in freshwater before anadromous migrate to sea throughout the spring and summer. **LFR / FRE Use:** Typically rear in small streams where they prefer benthic areas and areas with instream overhead cover. **Prey:** Juvenile Bull trout primarily prey on benthic and drift insects and amphipods before reaching ~11cm at which point they become piscivorous and begin feeding on small fish such as sculpin, mountain whitefish, and trout fry.	**Age:** Mature bull trout range from 3- 8 years old **Length:** Range in size from stream residents around 20 - 33 cm to lake and river residents from 60 to 73 cm, while anadromous forms can be larger. Adult bull trout are piscivorous consuming prey such as trout, whitefish, kokanee, whitefish, grayling, minnows, suckers and sculpins.	Migrate from the ocean and large rivers to small tributaries to spawn (average migration 50-400km) where they spend ~1 month maturing. Spawning migration occurs from **May to Aug** Little is known about anadromous Bull trout. Tagged individuals from Pitt Lake have been regularly captured in Fraser estuary.	**Harvest:** Recreational fisherman target adult bull trout throughout tributaries of the LFR. Bull trout are distributed throughout the majority of BC. Little is known about the status of most populations, Lower Fraser stocks are thought to have declined significantly.

Dolly Varden (Salvelinus malma)	Dolly Varden are iteroparous and occur in stream-resident, lake-migratory, and anadromous forms throughout coastal BC including the LFR. Dolly Varden are a blue listed species in BC.	Dolly Varden stream to spawn typically in the Fall from **Sept - early Nov.**	**Habitat:** Typically spawn in shallow slow moving headwater streams. **Sediment Size:** Typically gravels mostly less than 20 mm **Water Depth:** Average 15 cm **Water Velocity:** Less than 0.40 m/s) **Female Behavior:** Females select site and dig redd, redd characteristics vary with female size and life history type.	**Egg Size:** Resident females produce smaller eggs (3.5 mm diameter) while sea-run larger typically 4.5 mm diameter **#Eggs/ Redd:** Varies considerably with body size and life history, stream resident produce 70 – 500 eggs, anadromous 100 – 6,000.	**Incubation Time:** Eggs hatch usually in spring, 4.5 months after spawning. Fry emerge late **April to mid-May** after about 18 days in gravel.	**Migration:** Typically remain in freshwater for first 3 years before anadromous forms migrate to sea. **LFR / FRE Use:** Present year-round in freshwater tributaries of the LFR **Prey:** Juveniles feed primarily on chironomid larvae as well as mayflies, caddisflies, stoneflies and amphipods.	**Age:** Mature at age 3 to 6 after which they spawn each fall. **Weight:** Range in size from up to 1 kg for residents, and up to 2.3kg for anadromous forms. **Length:** Range in size from 10 to 25 cm for stream-residents, and 30 – 60 cm for anadromous forms.	Mature Dolly Varden of anadromous populations migrate to spawning areas **May – Dec**. Adult Dolly Varden typically feed on aquatic insects, fish eggs, and small fish including stickleback, sculpin and salmon.	**Overall Harvest:** Dolly Varden are found mostly in coastal drainages throughout BC where they are targeted by recreational anglers. Little is known about the current status of Dolly Varden, areas where their range overlaps with bull trout are of particular conservation concern.

Eulachon (*Thaleichthys pacificus*)	Eulachon are an anadromous semelparous species of smelt of significant management concern. Fraser stocks are at all-time lows and designated as endangered by COSEWIC in 2011.	Eulachon spawn in **Mar - May** in the LFR. Eulachon spawn in the LFR including the lower reaches of the north and south arms and upstream to Chilliwack.	**Habitat:** Spawn in areas of mixed gravel, pebbles and sand, avoid areas with a mobile sand layer. **Water Depth:** Can spawn at depths up to 7m **Water Velocity:** Slow water velocities (less than 0.6m/s)**.** **Female Behavior:** Females do not bury eggs as do salmon, rely on adhesive eggs sticking to sediments for anchoring.	**Egg Size:** Small less than 1 mm in diameter. **#Eggs/ Redd:** Females lay an avg of 30,00 eggs, fecundity can reach up to 60,000 eggs per female in the Fraser.	**Incubation Time:** Eulachon eggs are small and incubation time is much shorter and ranges from 3 – 5 weeks at temperatures ranging from 3 to 10 degrees Celsius.	**Migration:** After eulachon hatch they are immediately flushed downstream into the estuary or marine waters. **LFR / FRE Use:** Juvenile eulachon spend little time in freshwater before entering the estuary.	**Age:** Eulachon spend two to four years at sea. **Weight:** Mature eulachon typically between 20-100 grams **Length:** Between 11 to 22 cm.	Eulachon migrate in the LFR from late **Feb to April**. During up-river migration predators include gulls, eagles, sea lions.	**Overall Harvest:** Eulachon are a culturally important species and were historically prized by First Nations for their high fat content. Eulachon in the Fraser River have been in decline since the 1950's (98% decline in spawning biomass) and commercial fishing on the LFR is closed.

FN = First Nations, FSC = Food Social Ceremonial, LFR = Lower Fraser River, JDFS = Juan de Fuca Strait, SARA = Species at Risk Act, SOG = Strait of Georgia, WCVI = West Coast Vancouver Island

9.2 APPENDIX B

Table B.1. Description of historical salmonid presence in Lower Fraser River tributary creeks intersected by the proposed pipeline route. (Trans Mountain - B8-1-V5C TR 5C7 01of45 FISH BC-A3S2C1; DFO 1999).

Watercourse	Salmonids Present.	Size
[1]Stoney Creek	*Chum, coho, Dolly Varden, cutthroat*	NA
Como Creek	*Coho, cutthroat, rainbow*	7.1 km^2
Nelson Creek	*Coho, cutthroat*	NA
Bon Accord Creek	*Chum, coho*	5.3 km^2
Center Creek	*Chinook, chum, coho, cutthroat*	4.4 km^2
Yorkson Creek	*Chum, coho, pink, cutthroat*	17.9 km^2
Salmon River	*Chinook, Coho, sockeye, cutthroat, steelhead, rainbow*	76.9 km^2
West Creek	*Chinook, chum, coho, steelhead, cutthroat*	14.5 km^2
Nathan Creek	*Coho, pink, steelhead, cutthroat, rainbow*	33.8 km^2
McLennan Creek	*Chum, coho, steelhead, rainbow, cutthroat*	30.9 km^2
Clayburn Creek	**Cutthroat, Chinook, coho, Dolly Varden, Bull trout, rainbow, sockeye, steelhead*	NA
Sumas River	*Chinook, chum, coho, pink, steelhead, cutthroat*	1284.5 km^2
[2]Sumas Lake Canal	*Chinook, chum, coho, pink, steelhead, cutthroat*	NA
[2]Stewart Slough	**Cutthroat, chum, coho, rainbow*	NA
[3]Street Creek	**Cutthroat, chum, coho, pink steelhead, rainbow*	NA

Chilliwack River	*Chinook, chum, coho, pink, sockeye, steelhead, cutthroat, Dolly Varden, rainbow*	1260 km^2
Chilliwack Creek	*Chum, coho, cutthroat*	78.4 km^2
[4]Semmihault Creek	*Chum, coho, cutthroat*	NA
[5]Elk Creek	*Chum, coho, steelhead, cutthroat*	NA
[5]Nevin Creek	*Chum, coho, steelhead, cutthroat*	NA
[5]Dunville Creek	*Chum, coho, steelhead, cutthroat*	NA
Bridal Creek	*Chum, coho, pink, cutthroat*	20.4 km^2
[6]Anderson Creek	*Chinook, chum, coho, pink, cutthroat*	NA
Wahleach Creek	*Chum, coho, pink*	114 km^2
Lorenzetta Creek	*Chum, coho, pink, steelhead, cutthroat*	11 km^2
Hunter Creek	*Chum, coho, pink, steelhead, cutthroat, rainbow*	41.6 km^2
Chawuthen Creek	*Cutthroat*	NA
Silverhope Creek	*Chum, coho, pink, steelhead, cutthroat, Dolly Varden, bull trout*	328 km^2
Coquihalla River	*Chum, coho, pink, sockeye, steelhead, cutthroat, bull trout, Dolly Varden, rainbow*	932 km^2

[1]Stoney Creek is a tributary of Brunette River, [2] Sumas Lake Canal and Stewart Slough are a tributaries of Sumas River, [3]Street Creek is tributary to Chilliwack River, [4]Semmihault Creek is a tributary to Chilliwack creek, [5] Elk Creek, Dunville Creek, and Nevin Creek are tributaries to Hope Slough, [6] Anderson Creek is tributary to Bridal Creek, *Salmonid presence data from Trans Mountain B8-1-V5C TR 5C7 FISH BC-A3S2C1.

9.3 APPENDIX C

Table C.1 Alpha numeric names of the 104 unique Conservation Units within the five species (7 types) of commercially managed salmon returning to the Fraser River watershed and the surrounding watersheds (non-Fraser) that drain to the Canadian waters of the Salish Sea.

Species	Fraser River Conservation Units	Non-Fraser Conservation Units	Total
Chinook	**21**	**13**	**34**
Chinook CU Name and Number	03-Lower Fraser River_FA_0.3, 04-Lower Fraser River_SP_1.3, 05-Lower Fraser River-Upper Pitt_SU_1.3, 06-Lower Fraser River_SU_1.3, 07-Maria Slough_SU_0.3, 08-Middle Fraser-Fraser Canyon_SP_1.3, 09-Middle Fraser River-Portage_FA_1.3, 10-Middle Fraser River_SP_1.3, 11-Middle Fraser River_SU_1.3, 12-Upper Fraser River_SP_1.3, 13-South Thompson_SU_0.3, 14-South Thompson_SU_1.3, 15-Shuswap River_SU_0.3, 16-Suth Thompson-Bessette Creek_SU_1.2, 17-Lower Thompson_SP_1.2, 18-North Thompson_SP_1.3, 19-North Thompson_SU_1.3, 82-Upper Adams River_SU_1.x, 9004-Fraser-Miscellaneous, 9006-Fraser-Cross-CU Supplementation Exclusion, 9008-Fraser-Harrison fall transplant_FA_0.3.	01-East Vancouver Island Goldstream, 02- Boundary Bay, 20-Southern Mainland-Georgia Strait, 22-Eat Vancouver Island-Cowichan & Koksilah, 23-East Vancouver Island-Nanaimo, 25-East Vancouver Island-Nanaimo & Chemainus, 27-East Vancouver Island-Qualicum & Puntledge, 28-Southern Mainland-Southern Fjords, 29-East Vancouver Island-North, 31-West Vancouver Island-South, 34-Homathko, 83-Vancouver Island-Georgia Strait, 9007-Southern BC-Cross-CU Supplementation Exclusion.	
Coho	**8**	**7**	**15**

Coho CU Name and Number	09-Fraser Canyon, 17-Lower Fraser-B, 15-Lillooet, 16-Lower Fraser-A, 21-Lower Thompson, 23-Middle Fraser, 27-North Thompson, 35-South Thompson.	03-Boundary Bay, 7-East Vancouver Island-Georgia Strait, 12-Homathko-Klinaklini Rivers, 13-Howe Sound-Burrard Inlet, 14-Juan de Fuca-Pachena, 36-Georgia Strait Mainland, 37-Southern Coastal Streams-Queen Charlotte Strait-Johnstone Strait-Southern Fjords	
Chum	**2**	**6**	**8**
	06-Fraser Canyon 12-Lower Fraser	03-Bute Inlet, 05-Northeast Vancouver Island, 07-Georgia Strait, 10-Howe Sound-Burrard Inlet, 11-Loughborough, 38-Southwest Vancouver Island	
Sockeye (All sub-types)	**24**	**13**	**37**
Sockeye sub-type: Lake	22	9	31
Sockeye: Lake CU Name and Number	01-Cultus-L, 02-Harrison-(D/S)-L, 03-Harrison-(U/S)-L, 04-Pitt-ES, 05-Taseko-ES, 06-Bowron-ES, 07-Nahatlatch-ES, 08-Chilko-ES,	37-Phillips, 38-Sakinaw, 40-Tzoonie, 41-Village Bay, 53-Cheewat, 56-Fairy, 60-Hobiton, 68-Nitinat,	

	09-Chilko-S, 10-Quesnel-S, 11-Seton-L (de novo), 12-Takla-Trembleur-EStu, 13-Takla-Trembleur-Stuart-S, 14-Shuswap Complex-L, 15-Kamloops-ES, 16-North Barriere-ES (de novo), 17-Anderson-Seton-ES, 18-Francois-Fraser-S, 19-Shuswap-ES, 20-Lillooet-Harrison-L, 21-Nadina-Francois-ES, 22-Chilliwack-ES.	73-Sooke	
Sockeye Sub-type: River	2	4	6
Sockeye: River CU Name and Number	24-Widgeon 25-Harrison.	02-Boundary Bay, 05-East Vancouver Island & Georgia Strait, 15-Southern Fjords, 23-West Vancouver Island.	
Pink (All sub-types)	**1**	**9**	**10**
Pink sub type odd-year	1	6	7
Pink: odd year CU Name and Number	03-Fraser River	01- East Vancouver Island-Johnstone Strait 02-East Howe Sound-Burrard Inlet, 04-Georgia Strait, 05-Homathko-Klinaklini-Smith-Rivers-Bella Coola-Dean,	

		16-Southern Fjords, 19-West Vancouver Island.	
Pink sub type even-year	0	3	3
Pink: even-year CU Name and Number	None	01-Georgia Strait, 05-Southern Fjords, 12-Northwest Vancouver Island.	
Total CUs	56	48	104

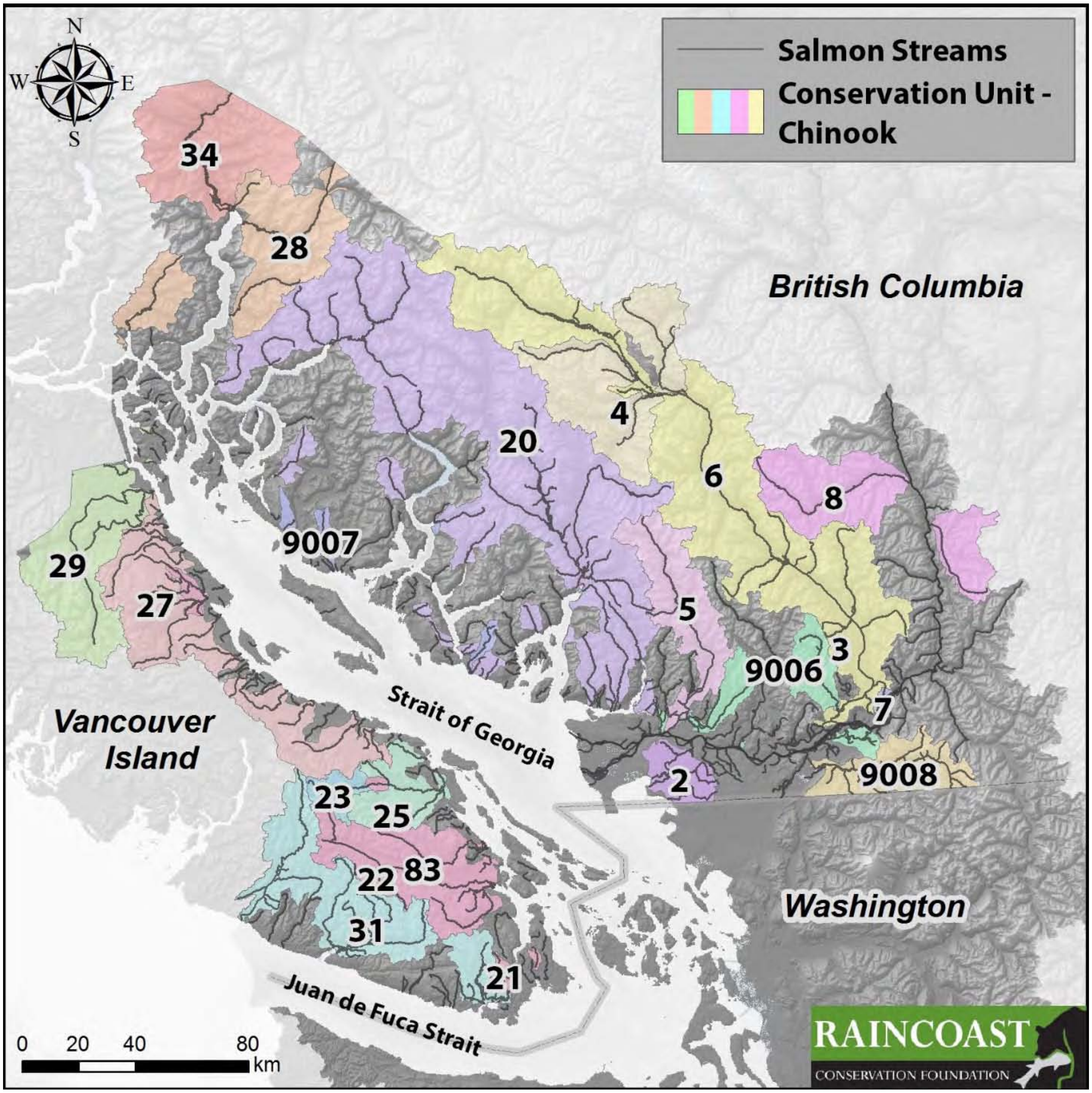


Figure C.1 21 Conservation Units (CUs) of Chinook salmon within the Canadian region of the Salish Sea including six from the Lower Fraser River. Primary watersheds draining to the Salish Sea are coloured (Canada) and dark (US). Chinook CUs are as follows: 2-Boundary Bay, 20-Southern Mainland-Georgia Strait, 21-East Vancouver Island-Goldstream, 22-East Vancouver Island-Cowichan & Koksilah, 23-East Vancouver Island-Nanaimo, 25-East Vancouver Island-Nanaimo & Chemainus, 27-East Vancouver Island-Qualicum & Puntledge, 28-Southern Mainland-Southern Fjords, 29-East Vancouver Island-North, 31-West Vancouver Island-South, 34-Homathko, 83-Vancouver Island-Georgia Strait, 9007-Southern BC-Cross-CU Supplementation Exclusion. Eight Lower Fraser Chinook CUs are: 3-Lower Fraser Fall, 4-Lower Fraser Spring, 5-Lower Fraser-Upper Pitt, 6-Lower Fraser Summer, 7-Maria Slough, 8-Fraser Canyon–Nahatlach, 9006-Fraser-Cross-CU Supplementation Exclusion, 9008-Fraser-Harrison fall transplant.

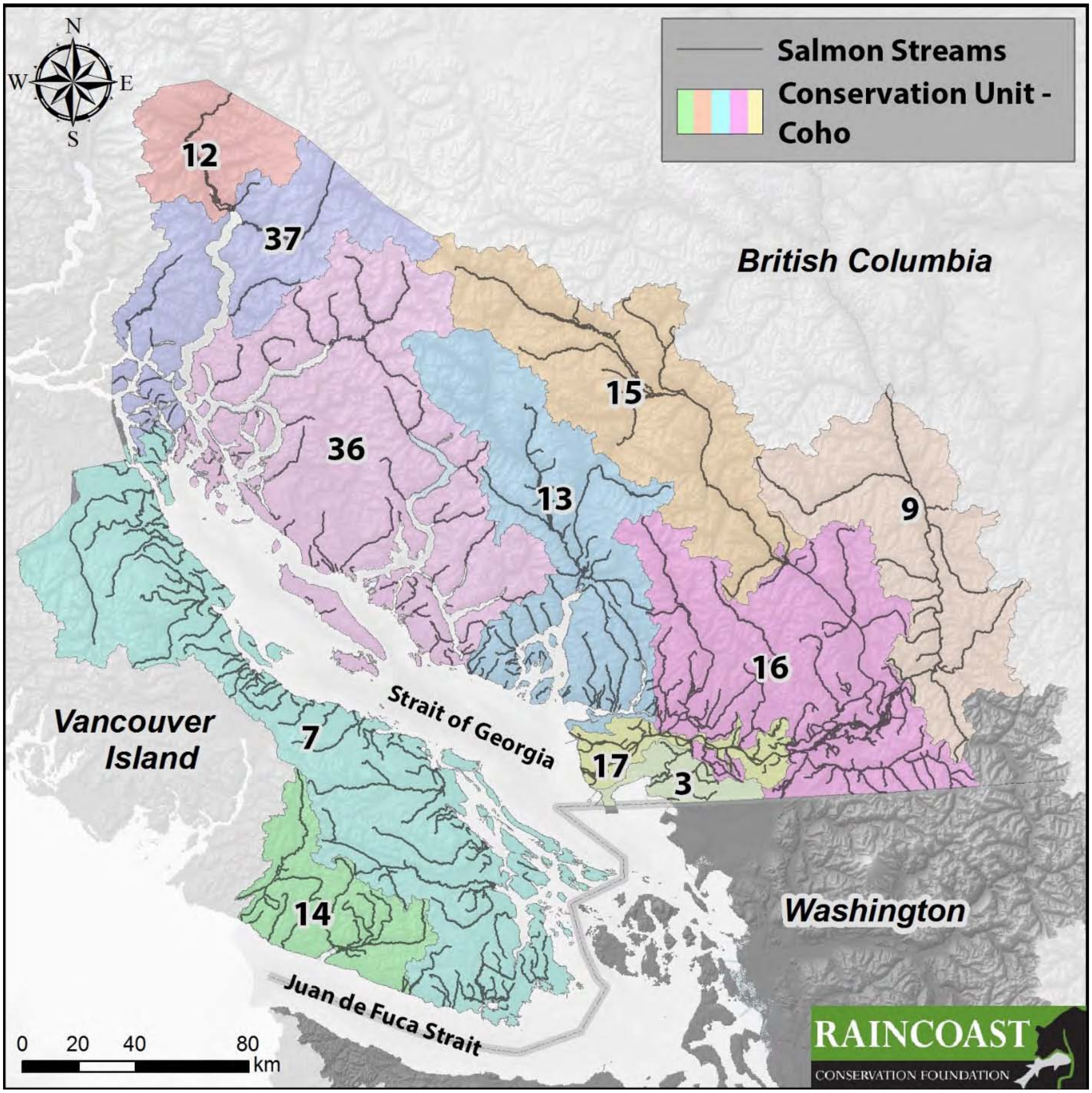


Figure C.2. Eleven Conservation Units (CUs) of coho salmon within the Canadian region of the Salish Sea including 4 from the Lower Fraser River. Primary watersheds draining to the Salish Sea are coloured (Canada) and dark (US). CUs are as follows: 3-Boundary Bay, 7-East Vancouver Island-Georgia Strait, 12-Homathko-Klinaklini Rivers, 13-Howe Sound-Burrard Inlet, 14-Juan de Fuca-Pachena, 36-Georgia Strait Mainland, 37-Southern Coastal Streams-Queen Charlotte Strait-Johnstone Strait-Southern Fjords. Four Lower Fraser coho CUs are: 9-Fraser Canyon, 15-Lillooet, 16-Lower Fraser-A, 17-Lower Fraser-B,

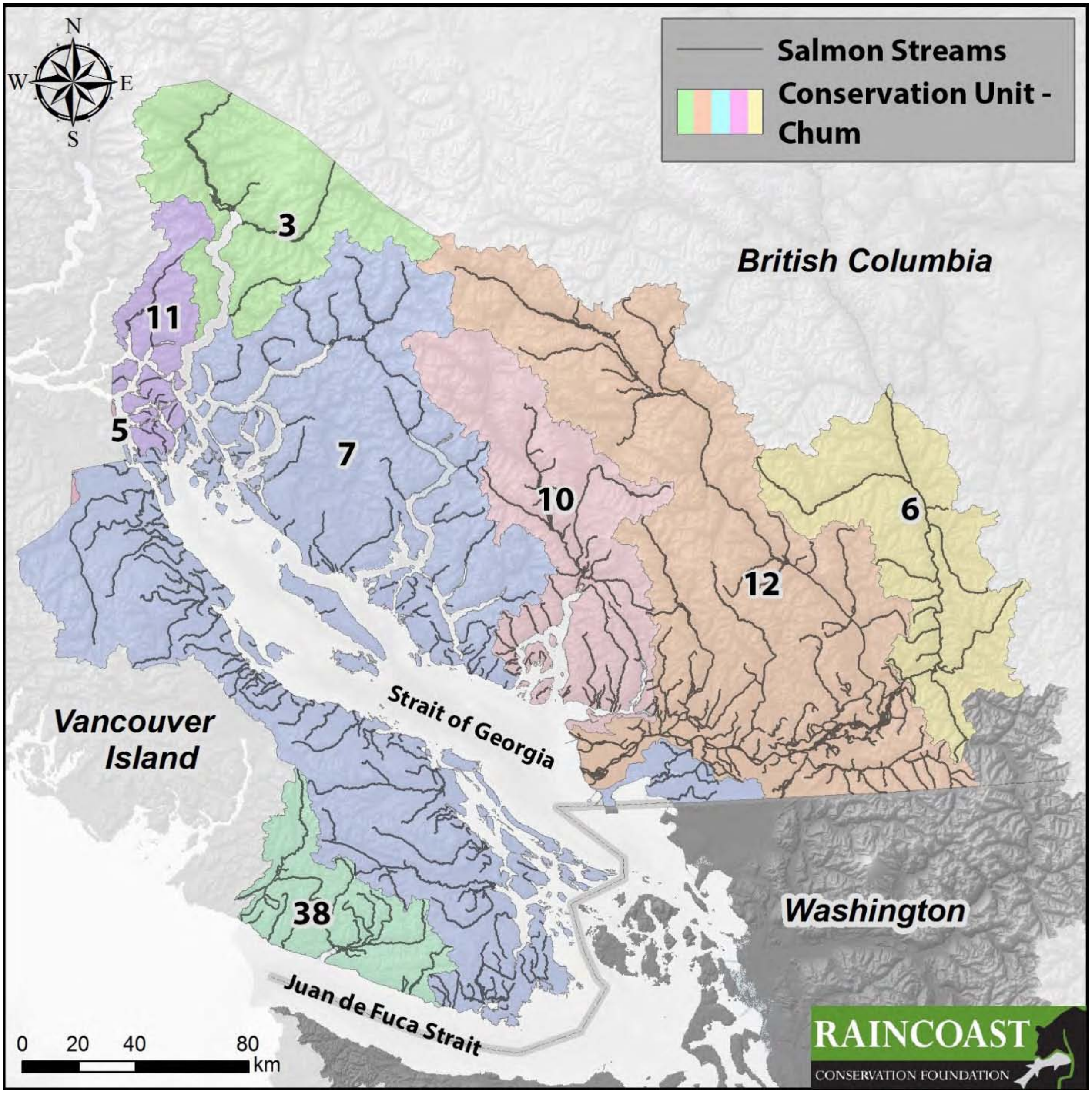


Figure C.3 Eight Conservation Units (CUs) of chum salmon within the Canadian region of the Salish Sea including 2 from the Lower Fraser River. Primary watersheds draining to the Salish Sea are coloured (Canada) and dark (US). CUs are as follows: 3-Bute Inlet, 5-Northeast Vancouver Island, 7-Georgia Strait, 10-Howe Sound-Burrard Inlet, 11-Loughborough, 38-Southwest Vancouver Island. Two Lower Fraser chum CUs are: 6-Fraser Canyon, 12-Lower Fraser.

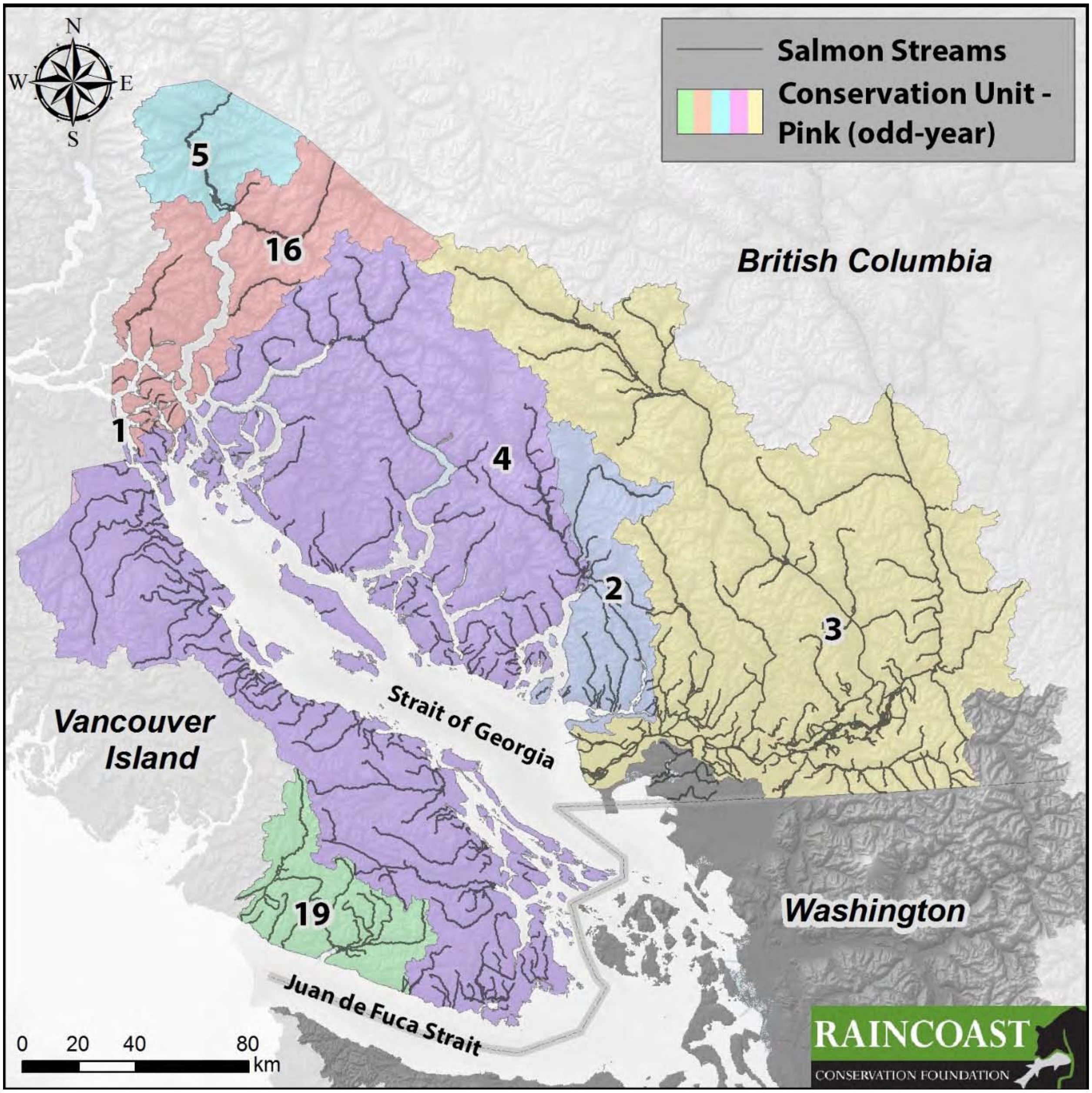


Figure C.4 Seven Conservation Units (CUs) of odd-year pink salmon within the Canadian region of the Salish Sea including one from the Lower Fraser River. Primary watersheds draining to the Salish Sea are coloured (Canada) and dark (US). CUs are as follows: 1-East Vancouver Island-Johnstone Strait, 2-East Howe Sound-Burrard Inlet, 4-Georgia Strait, 5-Homathko-Klinaklini-Smith-Rivers-Bella Coola-Dean, 16-Southern Fjords, 19-West Vancouver Island. The one Lower Fraser CU is 3-Fraser River

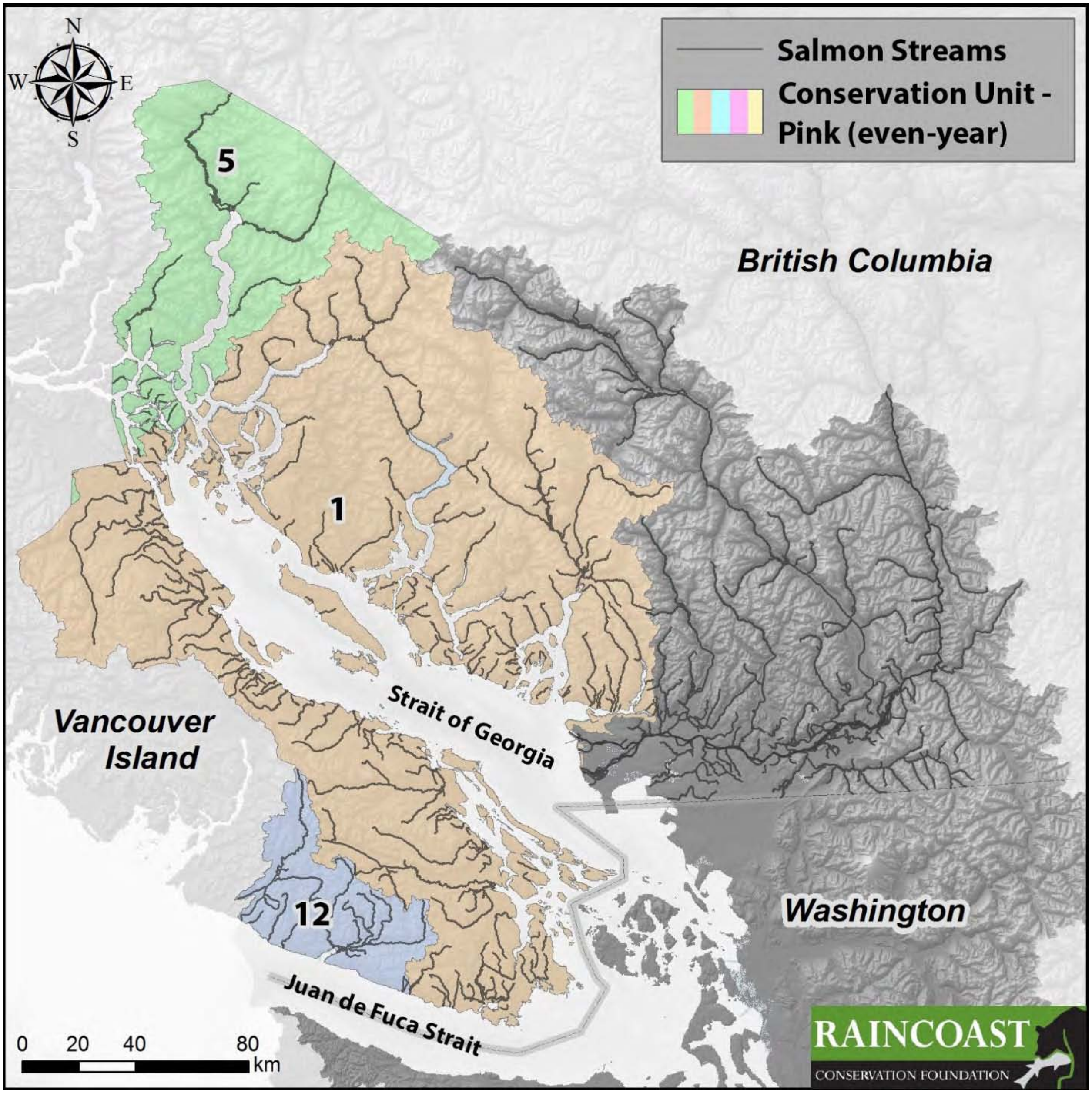


Figure C.5. Three Conservation Units (CUs) of even-year pink salmon within the Canadian region of the Salish Sea. Dark and coloured areas drain to the Salish Sea. Conservation Units are as follows: 1-Georgia Strait, 5-Southern Fjords, 12-Northwest Vancouver Island. There is no Fraser River CU for even year pink salmon.

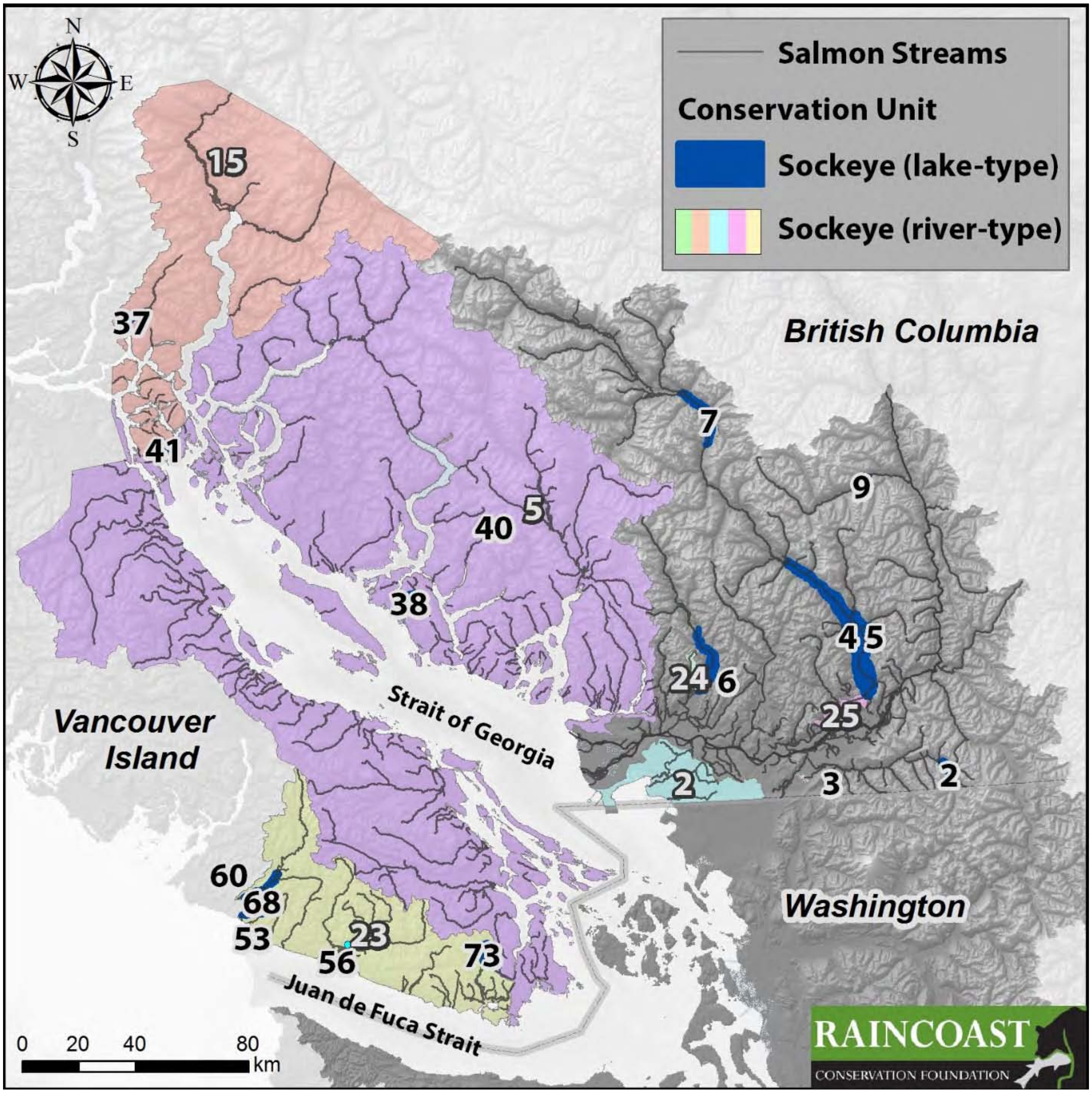


Figure C.6. 22 Conservation Units (CUs) of two types of sockeye salmon within the Canadian region of the Salish Sea. Blue shapes represent rearing lakes of lake-type sockeye (dark numbers). There are 16 lake-type CUs within the Salish Sea including seven from the Lower Fraser River. Conservation Units are as follows: 37-Phillips, 38-Sakinaw, 40-Tzoonie, 41-Village Bay, 53-Cheewat, 56-Fairy, 60-Hobiton, 68-Nitinat, 73-Sooke. Seven lake-type sockeye CUs within the lower Fraser are: 2-Chilliwack-Early Summer timing, 3-Cultus-Late timing, 4-Harrison - downstream migrating-Late timing, 5-Harrison -upstream migrating-Late timing, 6-Pitt-Early Summer timing, 7-Lillooet-Late timing, 9-Nahatlatch-Early.

There are six river-type sockeye salmon CUs within the Canadian region of the Salish Sea (light numbers) including two from the Lower Fraser River. CUs are as follows: 2-Boundary Bay, 5-East Vancouver Island & Georgia Strait, 15-Southern Fjords, 23-West Vancouver Island. Two Fraser river-type CUs are 24-Widgeon and 25-Harrison Summer.

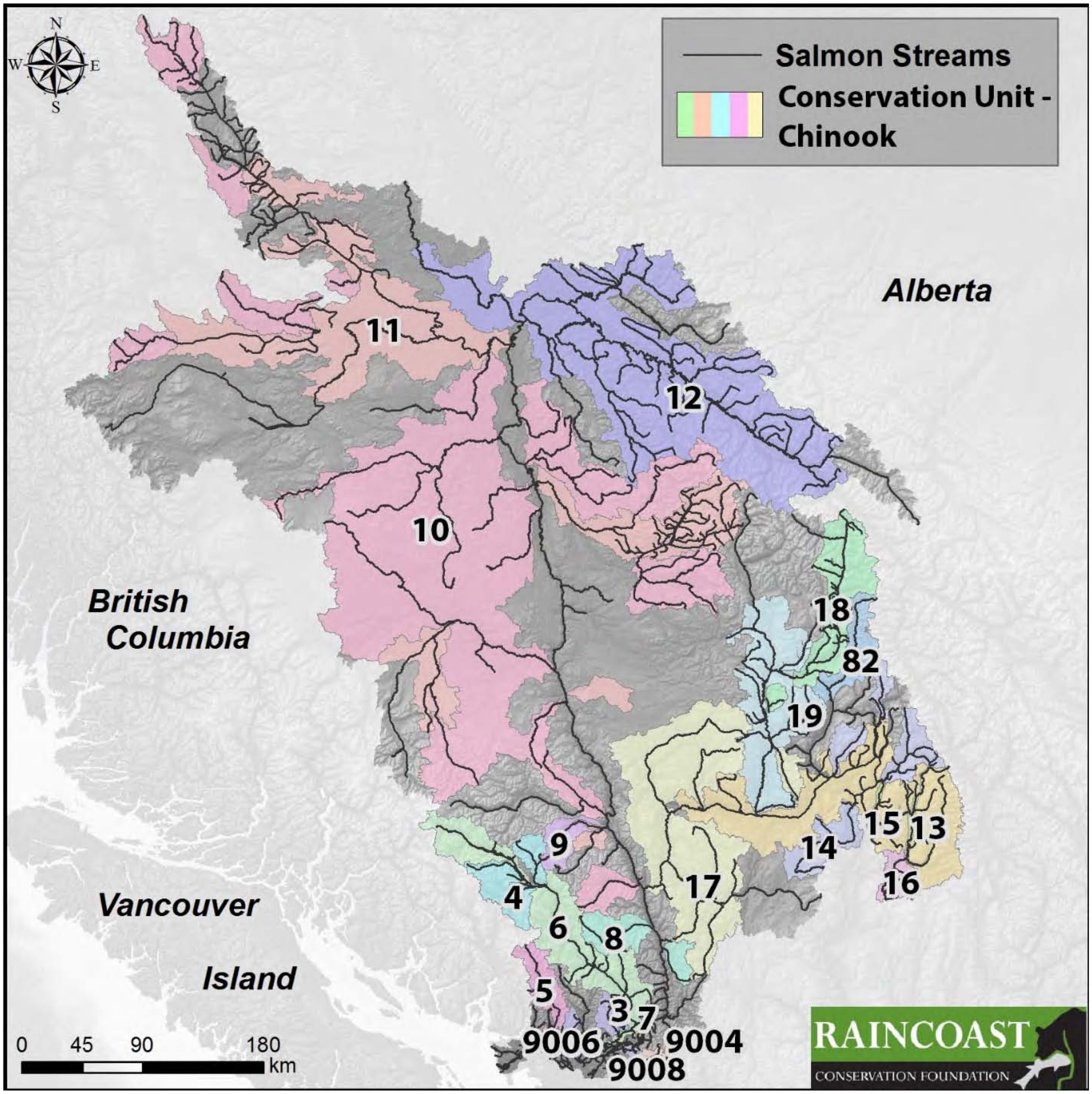


Figure C.7. 21 Conservation Units (CUs) of Chinook salmon in the Fraser River watershed (dark shaded/ coloured area). Conservation units are as follows: 3-Lower Fraser River_FA_0.3, 4-Lower Fraser River_SP_1.3, 5-Lower Fraser River-Upper Pitt_SU_1.3, 6-Lower Fraser River_SU_1.3, 7-Maria Slough_SU_0.3, 8-Middle Fraser-Fraser Canyon_SP_1.3, 9-Middle Fraser River-Portage_FA_1.3, 10-Middle Fraser River_SP_1.3, 11-Middle Fraser River_SU_1.3, 12-Upper Fraser River_SP_1.3, 13-South Thompson_SU_0.3, 14-South Thompson_SU_1.3, 15-Shuswap River_SU_0.3, 16-South Thompson-Bessette Creek_SU_1.2, 17-Lower Thompson_SP_1.2, 18-North Thompson_SP_1.3, 19-North Thompson_SU_1.3, 82-Upper Adams River_SU_1.x, 9004-Fraser-Miscellaneous, 9006-Fraser-Cross-CU Supplementation Exclusion, 9008-Fraser-Harrison fall transplant_FA_0.3.

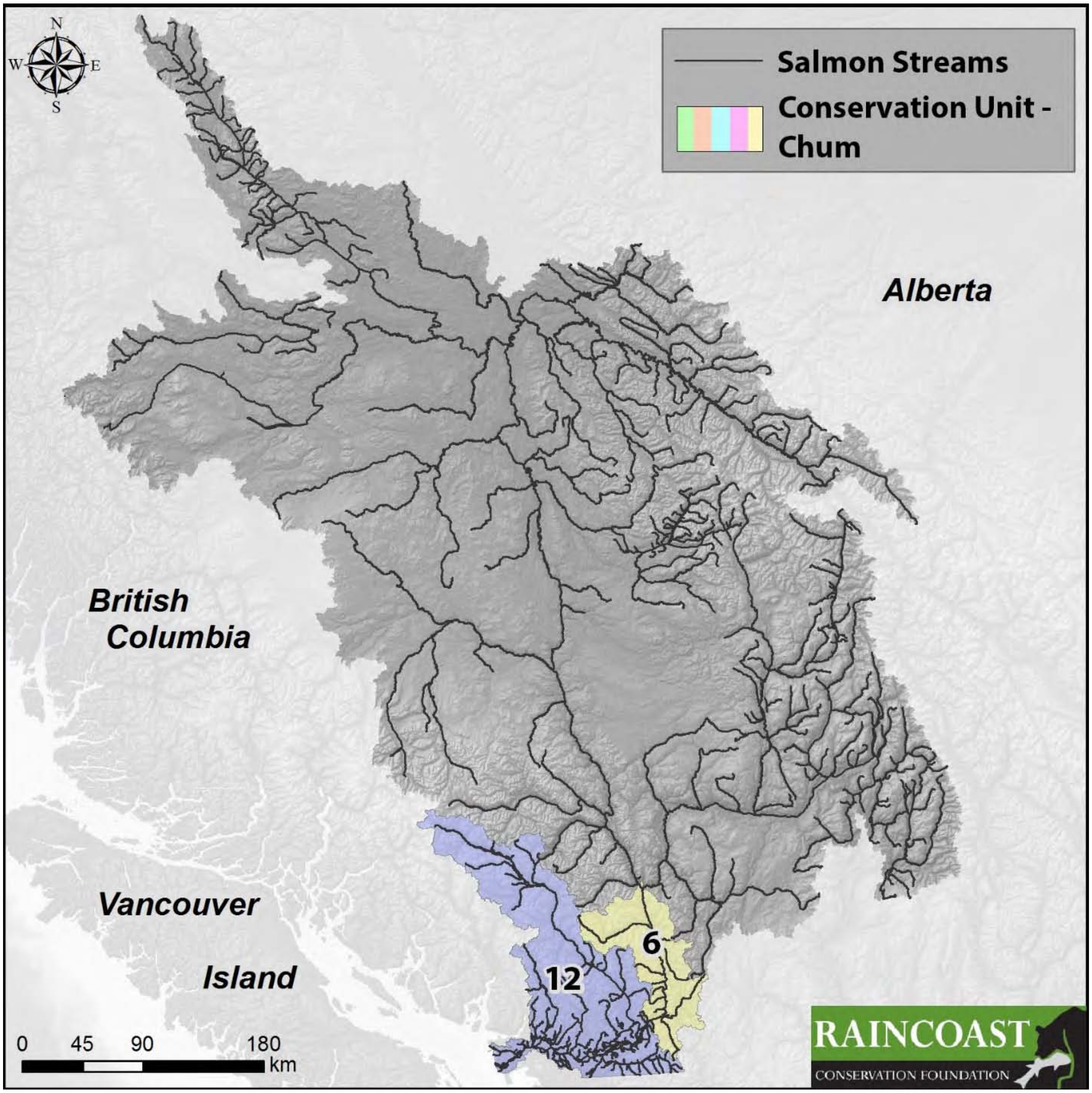


Figure C.8. Two Conservation Units (CUs) of chum salmon in the Fraser River watershed (dark shaded/colour area). CUs are: 6-Fraser Canyon and 12-Lower Fraser.

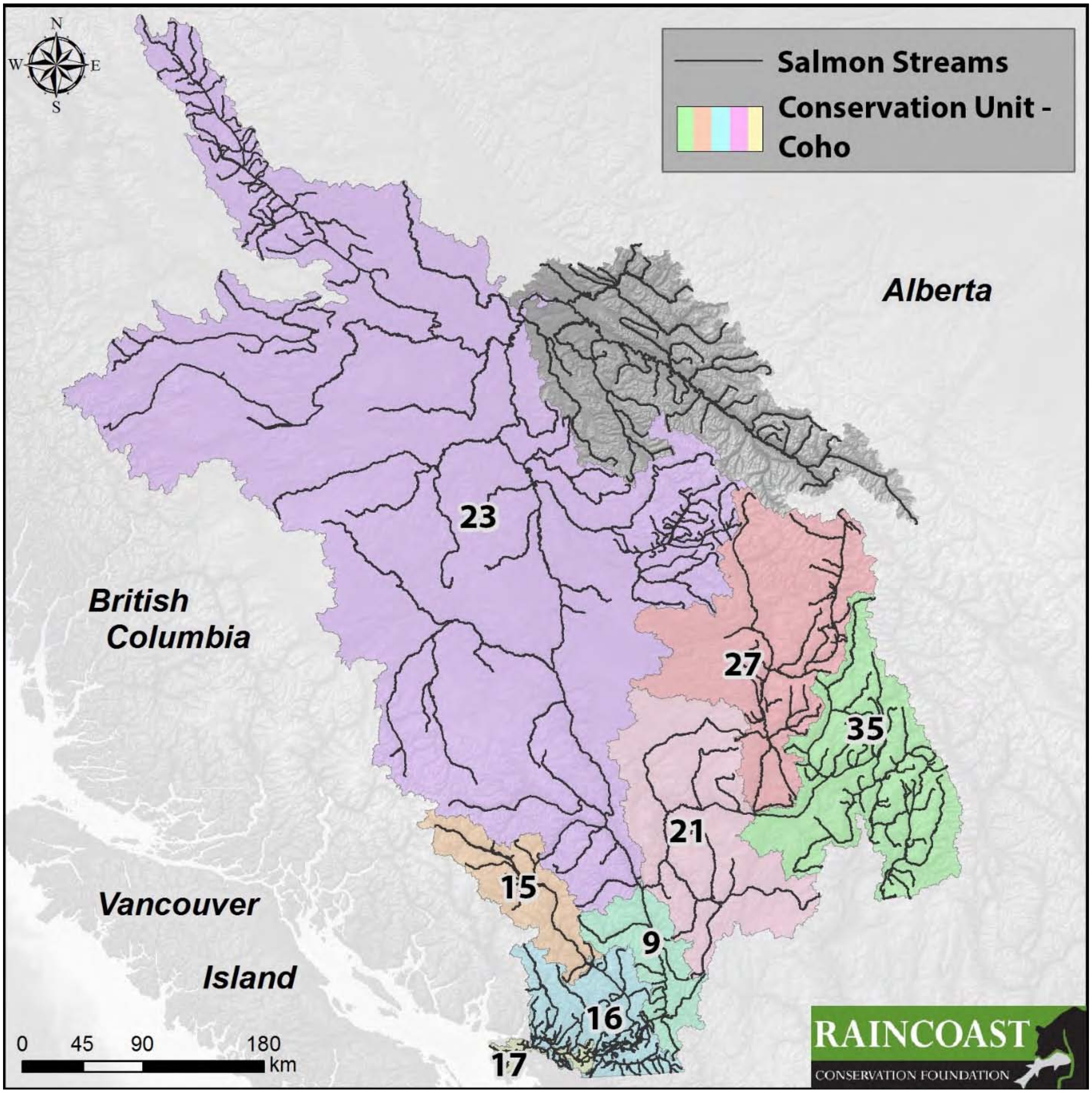


Figure C.9. Eight Conservation Units (CUs) of coho salmon in the Fraser River watershed (dark shaded/colour area). CUs are as follows: 9-Fraser Canyon, 15-Lillooet, 16-Lower Fraser-A, 17-Lower Fraser-B, 21-Lower Thompson, 23-Middle Fraser, 27-North Thompson, 35-South Thompson.

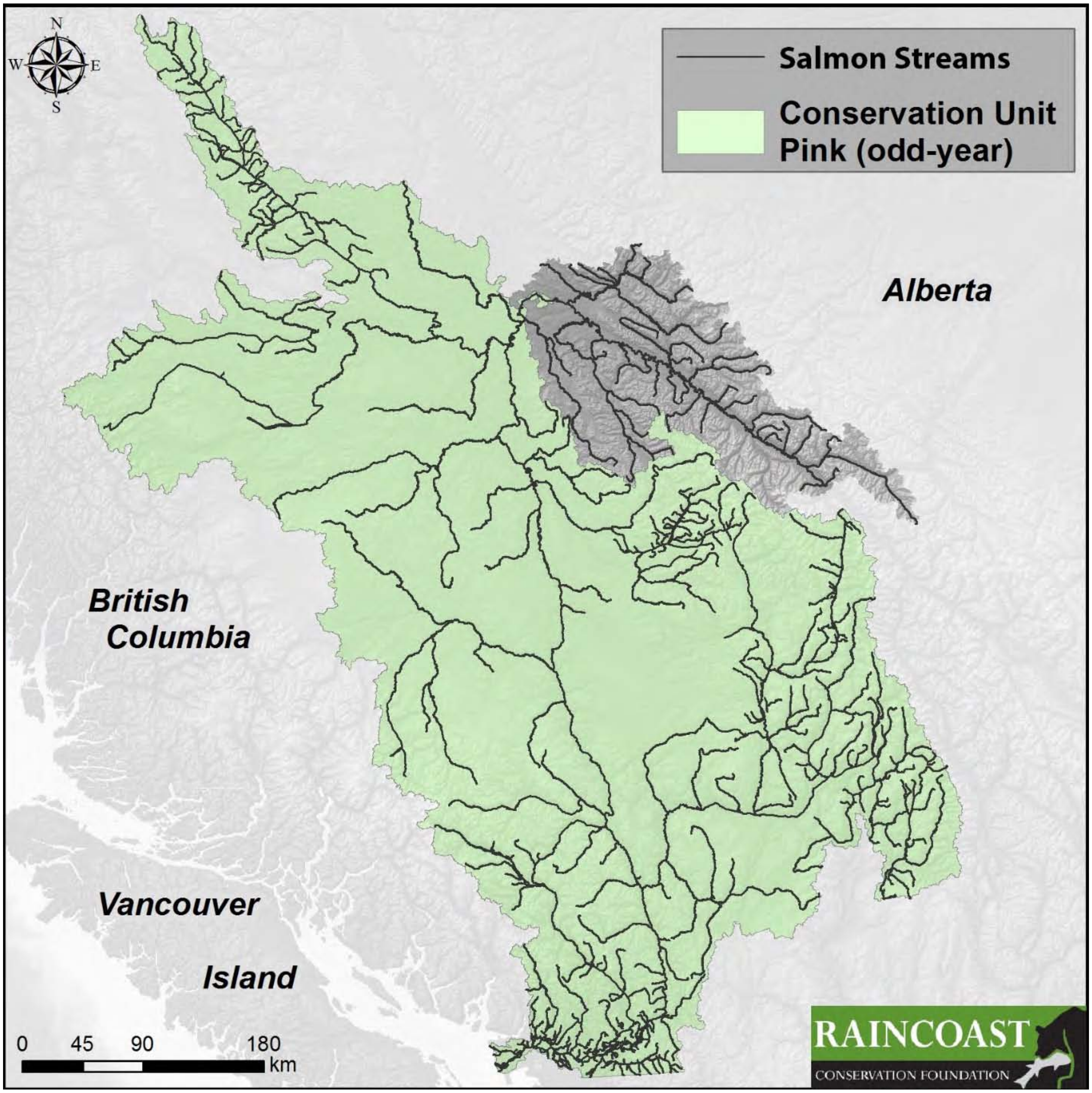


Figure C.10 The Conservation Unit (CU) for pink (odd-year) salmon in the Fraser River watershed (dark shaded / Coloured area). There is only one CU, the Fraser River. There are currently no even-year pink salmon CUs in the Fraser River watershed.

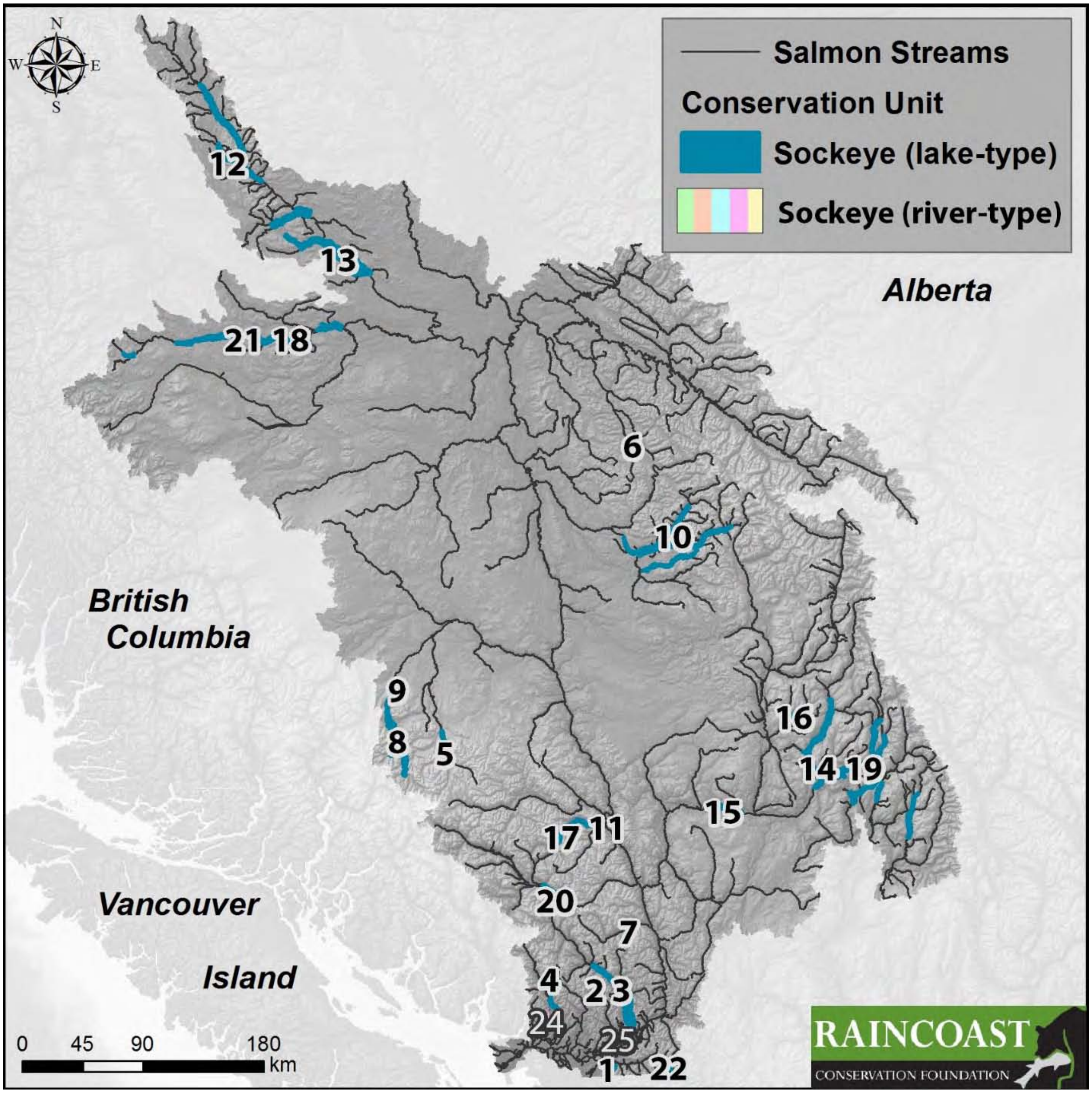


Figure C.11. 24 Conservation Units (CUs) of sockeye salmon in the Fraser River watershed (dark shaded area). Blue shapes are rearing lakes for lake-type sockeye. 22 lake-type sockeye CUs (dark numbers) are as follows: 1-Cultus-L, 2-Harrison-(D/S)-L, 3-Harrison-(U/S)-L, 4-Pitt-ES, 5-Taseko-ES, 6-Bowron-ES, 7-Nahatlatch-ES, 8-Chilko-ES, 9-Chilko-S, 10-Quesnel-S, 11-Seton-L (de novo), 12-Takla-Trembleur-EStu, 13-Takla-Trembleur-Stuart-S, 14-Shuswap Complex-L, 15-Kamloops-ES, 16-North Barriere-ES (de novo), 17-Anderson-Seton-ES, 18-Francois-Fraser-S, 19-Shuswap-ES, 20-Lillooet-Harrison-L, 21-Nadina-Francois-ES, 22-Chilliwack-ES. Two river-type sockeye CUs (light numbers) are 24-Widgeon and 25-Harrison.

9.4 APPENDIX D

Table D.1. Summary of effects of petroleum products (crude oil, refined/residual oil, synthetic oil, oil sands bitumen) on fish. Table updated from Hodson et al. (2011).

Oil type	Weathered?	Species	Life stage	Response	Type	Time	Method	Fresh/ Seawater	Lab/ Field	Nominal	Measured	Notes	Reference
Crude oil (Source unknown)	Unknown	Nile tilapia (*Oreochromis niloticus*)	Adult	Blood (RBCC and hematocrit value)	Acute	72 h	WAF	Fresh	Lab	Hemoglobin dropped at 2000 ppm after 48 h		Nuclear swelling of erythrocytes did not recover after removal of crude oil exposure, unlike hemoglobin, hematocrit	Al-Ayed 2001
Hungary crude (from Szeged-Algyo, Hungary)	No	Common carp (*Cyprinus carpio*), Silver carp (*Hyphothalmichtys molitrix*), European eel (*Anquilla anquilla*)	Adult	EROD, ECOD induction, antioxidant parameters (lipid peroxidation (LP) only affected)	Acute and chronic	3d and 8d	IP injection of 2 mL/kg	Fresh	Lab			2mL/kg for 3d: EROD elevated 6-fold over control in carp and 4-fold over control in eel. LP reduced in carp. 2mL/kg for 8d (Carp): Drop in EROD, but still higher than controls. 4mL/kg for 3d or 8d: Same increase in EROD as 2mL/kg	Deer et al. 2010
ANS crude	Yes; 70 °C until 20% loss	Pacific herring (*Clupea pallasi*)	Adult	TPAH in tissue, VHSV prevalence, mortality	Chronic	16-18 d	WAF from oiled gravel columns	Seawater	Lab			Significant correlations between PAH exposure and VHSV prevalence, cumulative motality	Carls et al. 1998

ANS crude	Yes; 70 °C until 20% loss	Pacific herring (*Clupea pallasi*)	Adult	BSD, mortality	Chronic	16 d	WAF from oiled gravel columns	Seawater	Lab	No effects on ova from 16 d exposure of adult females to 58 ug PAH/L		82-94% of PAH accumulated to ova in exposed adults were naphthalenes, indicating that HMW and alkyl PAH were metabolised and/or exposure occurred before cell differentiation; indicates that embryos are more sensitive than gametes	Carls et al. 2000
ANS crude	Yes; heated to 70 °C until 20% loss	Pacific herring (*Clupea pallasi*)	Adult	EROD induction	Chronic	16 d	WAF from oiled gravel columns	Seawater	Lab			Prespawn fish more vulnerable (less EROD activity)	Thomas et al. 1997

Oil type	Weathered?	Species	Life stage	Response	Type	Time	Method	Fresh/ Seawater	Lab/ Field	Nominal	Measured	Notes	Reference
Exxon Valdez oil	Yes, naturally in field	Dolly varden (*Salvelinus malma malma*), yellowfin sole (*Limanda aspera*), pollock (*Pollachius* sp.)	Adult	Reproductive parameters (plasma estradiol, GSI, GTH-1), biliary fluorescent aromatic compounds (FACs)	Chronic	Unknown (1-3 y)	Stranded oil in field	Seawater	Field			Elevated biliary FAC (naph and phen) in 1989, decreased by 1991; reproductive parameters were consistently low at high FAC	Sol et al. 2000
Exxon Valdez oil	Yes, naturally in field	Pacific herring (*Clupea pallasi*)	Adult	Histopathology (hepatic necrosis, spleen, kidney), tissue concentrations of PAH	Chronic	Unknown	Unknown	Seawater	Field	Hepatic necrosis in oiled sites in 1989 only		Hepatic necrosis in herring from oiled sites in 1989 may have been a result of viral hemorrhagic septicemia (VHS); see Carls et al. 1998.	Marty et al. 1999
Exxon Valdez oil	Yes (in field)	Salmonids	Adult	Population density	Chronic	Unknown	Unknown	Seawater	Field			Mean fish densities were low in 1990; same as control (unoiled sites) by 1991, suggests recovery of PWS	Barber et al. 1995

Oil type	Weathered?	Species	Life stage	Response	Type	Time	Method	Fresh/ Seawater	Lab/ Field	Nominal	Measured	Notes	Reference
Campos Bay crude, Brazil	No	(*Sebastes* / (*Astyanax* sp.) *schlegeli*), marbled flounder (*Pseudopleuronectes yokohamae*)	Adult	Histopatholog / CYP1A induction / y (gill, liver) biliary PAH metabolite concentrations	Acute	12, 24, 96 h	WAF / oil from Hebei Spirit oil spill (2007)	Fresh	Lab	Liver: necrotic areas and loss of hepatocyte cell limits at 15% WAF,; Gill: aneurysms at 33% WAF	PAH = 284 ng/g dw (5 d post-spill)	Also measured acetylcholinesterase activity (neurotoxicity): / concentrations decreased to background by 11 m post-spill. decreased activity at 15% WAF for 96 h and 33% for 24 h	Akaishi et al. 2004
ANS crude	Yes; heated to 70 °C until 20% loss	Zebrafish (*Danio rerio*)	Adult	Heart shape, reduced swimming performance (reduced cardiac output)	Chronic	96 h	Static WAF and WAF from stranded oil columns	Fresh	Lab		Subtle changes in heart shape and reduced swimming ability, indicative of reduced cardiac output	See Incardona 2005 for embryo exposures	Hicken et al. 2011
	No	Crimson-spotted rainbowfish (*Melanotaenia fluviatillis*)	Adult	Reproductive performance (egg production) / histopatholog y	Acute	3 d oil exposure, 14 d (clean water)	WAF, CEWAF	Fresh	Lab		BSD EC50 = 0.43 mg HC/L; genotox LOEC = 0.24 mg/L		Pollino and Holdway 2002
Prudhoe Bay crude	Yes (in field)	Pacific herring (*Clupea pallasi*)	Adult	Reproductive success and	Chronic	Unknown (field collected)	Field collected adults: artificial fertilization and rearing of embryos	Seawater	Field		Unknown	Artificial fertilization of embryos from "exposed" adults in PWS: oiled - reduced hatching success, increased deformities	Kocan et al. 1996
		Japanese flounder (*Paralichthys olivaceus*)	Adult	Immune system gene expression	Acute	3 d	3.8 g/L oil in water	Seawater	Lab			Downregulation of immune response genes; may be used as biomarkers?	Nakayama et al. 2008
Prestigelike heavy fuel oil (IFO 380)	No	Rainbow trout gill cell line	Cells	Cytotoxicity, EROD	Acute	24 h	5 - 45 g oil per 100 ml growth medium (produced WAF)	Fresh	Lab		LOEC = >70 µg TPAH/L	No cytotoxicity in RTG-2 cells, but dose-dependent increases in EROD were observed.	Navas et al. 2006

Oil type	Weathered?	Species	Life stage	Response	Type	Time	Method	Fresh/ Seawater	Lab/ Field	Nominal	Measured	Notes	Reference
Arabian medium crude	Yes; sparged with air at room T, until 25% loss	Sheepshead minnow (*Pimephales* (*Cyprinodon* *promelas*) *variegatus*), White sucker Inland silverside (*Catastomus* *commersoni*) (*Menidia* *beryllina*)	Embryo	Mortality CYP1A induction, eye pathology	Acute	96 h	WAF, exposure to CEWAF contaminated from sediments stirred (renewed aspirator daily) flasks - both	Seawater	Lab	Sheepshead LC50 = 0.06 minnow: Continuous (WWP) - 0.91 (S- NOEC (mortality) Nat) g = 10 mg oil/L, sediments/L declining NOEC = White sucker: 36 mg/L LC50 = 0.38 (E- Silverside Nat) - 1.15 minnow: (SNat) g/L continuous NOEC (mortality) = <1.2 mg/L, declining NOEC = 26 mg/L	minnow: LC50 = 1 - 29 ng TPAH/L water (calculated) White sucker: LC50 = 14 - 36 ng TPAH/L water (calculated)	kidney) was more sensitive than eye pathology and values are available	Fuller et al. et al. 2007 2004
Bass Strait crude	No	Australian bass (*Macquaria novemaculeata*)	Embryo	Mortality	Acute	96 h	WAF, CEWAF (24 h stir, 1 h settle - no renewal)	Seawater	Lab		CEWAF: LOEC (mortality) = 3.13 mg/L		Gulec and Holdway 2000
MESA	Yes; sparged with air for 24-48 h at room T, until 20% loss	Mummichog (*Fundulus heteroclitus*)	Embryo	Survival, body length, EROD	Acute	96 h	WAF, CEWAF	Seawater	Lab	(Estimated) LC50: WAF >1 g oil/L, CEWAF = 0.35 g/L Body length EC50: WAF = 0.1 g/L, CEWAF = 0.025 g/L	(Estimated) EC50 (body length and EROD) = 150 ng PAH/mL (Estimated) LC50 = 200- 400 ng PAH/mL		Couillard et al. 2005

MESA	Yes; sparged with air for 24-48 h at room T, until 20% loss	Mummichog (*Fundulus heteroclitus*)	Embryo	Survival, body length, EROD	Acute	96 h	WAF, CEWAF	Seawater	Lab	(Estimated) LC50: WAF >1 g oil/L, CEWAF = 0.35 g/L Body length EC50: WAF = 0.1 g/L, CEWAF = 0.025 g/L EROD EC50: WAF = 0.1 g/L, CEWAF = 0.05 g/L	(Estimated) EC50 (body length and EROD) = 150 ng PAH/mL (Estimated) LC50 = 200- 400 ng PAH/mL		Couillard et al. 2005
No 2 fuel oil + Omni- Clean dispersant	No	Sheepshead minnow (*Cyprinodon variegatus*)	Embryo	Mortality	Acute	96 h	WAF from 20-30 s electric stirring	Seawater (20 ppt)	Lab	LC50 (WAF) = 80 mg/L, (CEWAF) = 80 mg/L (estimated)			Adams et al. 1999
	17.5 h mixing with deionized water, 10 or												

Oil type	Weathered?	Species	Life stage	Response	Type	Time	Method	Fresh/ Seawater	Lab/ Field	Nominal	Measured	Notes	Reference
Alberta oil sands bitumen and tailings pond sediments	No	Fathead minnow (*Pimephales promelas*)	Embryo	Mortality, BSD, growth	Chronic	12 d (5 d to hatch, 7 posthatch)	Indirect exposure to contaminated sediments (renewed daily)	Fresh	Lab	LOEC: 0.05 to 0.8 g sediment/L	65 (WWP) - 176 (A-Nat) µg TPAH/L (Calculated from TPAH of stock sediments)	Sediments were dominated by HMW 3- to 6-ringed alkyl PAH, very little alkyl naph - most sensitive response was mortality	Colavecchia et al. 2004
Alberta oil sands bitumen and tailings pond sediments	No	White sucker (*Catastomus commersoni*)	Embryo	Mortality, BSD, growth	Chronic	35 d (14 d to hatch, 21 posthatch)	Indirect exposure to contaminated sediments (renewed daily)	Fresh	Lab	LOEC: 0.1 (weight) to 0.4 (edema, hemorrhage) g sediment/L; LOEC (EROD activity) = 0.002 g/L (WWP)	36 (S-Nat) - 100 (E-Nat) µg TPAH/L (Calculated from stock sediments - LOEC across all endpoints	Most sensitive response was growth and BSD and EROD activity; premature hatch occurred at all expsoures; pericardial edema responsible for observed mortality	Colavecchia et al. 2006
ANS crude	"Yes"; biodegraded 14d using Phe#6, Hexaco#2, EI2V	Inland silversides (*Menidia beryllina*)	Embryo	Teratogenicity (abnormalities)	Chronic	7-10 d	WAF recovered after degradation	Seawater	Lab	LOEC (developmental) = 0.75 mg/L WAF			Middaugh et al. 2002
ANS crude	Yes; and biodegraded	Inland silversides (*Menidia beryllina*)	Embryo	Teratogenicity (abnormalities)	Chronic	7-10 d	WAF recovered after degradation	Seawater	Lab	LOEC (developmental) = 1% WAF			Middaugh et al. 1996

ANS crude	No	Pacific herring (*Clupea pallasi*)	Embryo	Hatching success and timing, vitality, abnormalities	Chronic	4 d	WAF, CEWAF	Seawater	Lab		96-h NOEC (yolk sac edema embryos) = 17 µg TPAH/L 96-h NOEC (oil and oil+disp) = 9.2 µg TPAH/L 96-h NOEC (oil+disp+UV) = 0.2 µg TPAH/L	Oil + dispersant (high energy WAF) exposures were followed by exposure to UVA. Exposure to UVA caused 50-fold increase in toxicity.	Barron et al. 2003
ANS crude	Yes; 70 °C until 20% loss	Pacific herring (*Clupea pallasi*)	Embryo	LOEC: mortality, BSD	Chronic	16 d	WAF from oiled gravel columns	Seawater	Lab		LWO: LOEC = 9.1 µg TPAH/L, EC50 = 18.4 µg TPAH/L (swimming ability); MWO: LOEC = 0.41 µg TPAH/L, EC50 = 0.33 (craniofacial)	Based on initial or peak concentrations, which declined with time. MWO = more weathered oil - same oiled gravel as originally used in LWO; LWO = less weathered oil	Carls et al. 1999

Oil type	Weathered?	Species	Life stage	Response	Type	Time	Method	Fresh/ Seawater	Lab/ Field	Nominal	Measured	Notes	Reference
ANS crude	No	Pink salmon (*Oncorhynchus gorbuscha*)	Embryo	Growth	Chronic	10 d	WAF (glass extraction column; Moles et al. 1985)	Seawater	Lab		0.18-0.35 mg HC/L		Birtwell et al. 1999
ANS crude	No	Pink salmon (*Oncorhynchus gorbuscha*)	Embryo	Histology (hepatic, head kidney, gill tissues); OR released and recaptured 1 or 2 years later	Chronic	10 d	WAF (glass extraction column; Moles et al. 1985)	Seawater	Lab		LOEC (histopathology) = 25-54 µg HC/L, 96 h LC50 = 1-3 mg/L	Morphological and stress-induced lesions in their hepatic, head kidney, and gill tissue; most effects are consistent over 3 years	Brand et al. 2001
ANS crude	Yes; 70 °C until 20% loss; and natural weathering	Pink salmon (*Oncorhynchus gorbuscha*)	Embryo	Mortality, BSD	Chronic	83 d	WAF from oiled gravel columns	Fresh	Lab	1500-2250 mg oil/kg gravel	7.8 - 16.4 µg TPAH/L, 8.3 - 12.1 mg TPAH/kg gravel	50% mortality achieved using artificially weathered oil (not naturally weathered)	Brannon et al. 2006a

ANS crude	Yes; 70 °C until 20% loss	Pink salmon (*Oncorhynchus gorbuscha*)	Embryo	Mortality, BSD, CYP1A induction, and effects on growtha nd survival 6 mo later	Chronic	6 mo	WAF from oiled gravel columns	Seawater	Lab		LOEC (mortality) <16.5 µg TPAH/L; LOEC (CYP1A induction; reduced length) < 3.7 µg TPAH/L; LOEC (reduced mass) < 0.94 µg TPAH/L		Carls et al. 2005
ANS crude	Yes; heated to 70 °C until 20% loss	Pink salmon (*Oncorhynchus gorbuscha*)	Embryo	Mortality, visible lesions, size at emergence	Chronic	Hatch to emergence (up to 7 mo)	WAF from stranded oil columns, either fresh or 1 y W	Seawater	Lab	LOEC (mortality) = 281 mg oil/kg gravel (dose) - mortality of 35% 3.8 mg TPAH/kg on gravel	LOEC (mortality) = 18 µg TPAH/L	Direct and indirect exposed embryos showed similar effects	Heintz et al. 1999
ANS crude	Yes; heated to 70 °C until 20% loss	Pink salmon (*Oncorhynchus gorbuscha*)	Embryo	Mortality and growth 2 y after embryo exposure	Chronic	Up to 8 mo	WAF from oiled gravel columns	Seawater	Lab		LOEC (mortality) = 5.4 µg TPAH/L (15% mortality in returning fish) LOEC (growth) = 18 µg TPAH/L (may	See Heintz et al. 1999 for effects of oil exposure on embryos. This paper describes delayed effects (on returning adults 2y later)	Heintz et al. 2000

Oil type	Weathered?	Species	Life stage	Response	Type	Time	Method	Fresh/ Seawater	Lab/ Field	Nominal	Measured	Notes	Reference
ANS crude	No	Pink salmon (*Oncorhynchus gorbuscha*)	Embryo	Mortality, growth, condition, feeding	Chronic	6 d	Contaminated food	Seawater	Lab		LC50 = 29 mg account for mortality) HC/L, LOEC = 2.2 mg/g		Carls et al. 1996
ANS crude (from EVOS oiled areas)	Yes; naturally in field (1 - 3 y)	Pacific herring (*Clupea pallasi*)	Embryo	Morphologica l deformities, cytogenetic abnormalities (anaphasetelephase aberations), and histopathologi cal lesions	Chronic	Unknown	Oiled beaches in PWS - 1989, 1990, 1991	Seawater	Field			No oil-related histopathological lesions observed. Also, oil-related developmental and genetic effects were undetectable in 1990 and 1991	Hose et al. 1996
ANS crude	Yes; heated to 70 °C until 20% loss	Mummichog (*Fundulus heteroclitus*)	Embryo	Mortality, BSD	Chronic	11 d	Direct exposure to contaminated sediments (renewed daily)	Seawater	Lab	LOEC (body length) = 12.7 µg oil/g sand	LOEC (calculated) = 270 ng TPAH/g sand		Couillard 2002
Exxon Valdez oil	Yes, naturally in field	Pacific herring (*Clupea pallasi*)	Embryo	Embryo density, mortality, length, growth	Chronic	Unknown	Unknown	Seawater	Field	Oil effects on growth rates		Growth rates of larval herring in PWS were 0.10-0.17 mm/d, half the rate of wild populations (0.21-0.41 mm/d). Evidence of oil damage? See Norcross et al. 1996	McGurk and Brown 1996

Iranian crude (TJ014), No. -20 diesel oil	No	Large yellow croaker (*Larmichthys crocea*)	Embryo	Mortality, hatch rate, BSD	Chronic	Unknown	WAF (stirred 0.5 h, emulsified 8 h)	Seawater	Lab	TJ014: EC50 = 112.4 mg/L, LC50 = 8.5 mg/L -20: EC50 = 510.8 mg/L, LC50 = 156.2 mg/L	(Calculated) TJ014: EC50 = 0.66 mg TPH/L, LC50 = 3.01 mg/L; -20: EC50 = 0.05 mg/L, LC50 = 0.92 mg/L		Shen et al. 2010

Oil type	Weathered?	Species	Life stage	Response	Type	Time	Method	Fresh/ Seawater	Lab/ Field	Nominal	Measured	Notes	Reference
MESA	No	Atlantic herring (*Clupea harengus*)	Embryo	Mortality, BSD, fertilization success	Chronic	1-144 h	CEWAF	Seawater	Lab	1 h EC50 (fertilization inhibition) = 0.19 %v/v 3 h LC50 (freeswimming embryos) = 0.35 %v/v 10 h LC50 (freeswimming embryos) = 0.18 %v/v 24 h EC50 (0dpf embryos - BSD) = 0.08 %v/v	1 h EC50 = 79 mg TPH/L 3 h LC50 = 146 mg TPH/L 10 h LC50 = 75 mg TPH/L 24 h LC50 = 32 mg TPH/L	Also reported ET50s using exposure times 1 - 144 h.	McIntosh et al. 2010
MESA	Yes; heated to 70 °C until 20% loss	Mummichog (*Fundulus heteroclitus*)	Embryo	Mortality, BSD	Chronic	11 d	Direct exposure to contaminated sediments (renewed daily)	Seawater	Lab	LOEC (body length) = 4.5 µg oil/g sand	LOEC (calculated) = 230 ng TPAH/g sand		Couillard 2002
No 2 fuel oil + Omni-Clean dispersant	No	Sheepshead minnow (*Cyprinodon variegatus*)	Embryo	Mortality, fish biomass	Chronic	7 d	WAF from 20-30 s electric	Seawater (20 ppt)	Lab	LC50 (WAF) = 130 mg/L, (CEWAF) = 70 mg/L			Adams et al. 1999

Oil type	Weathered?	Species	Life stage	Response	Type	Time	Method	Fresh/ Seawater	Lab/ Field	Nominal	Measured	Notes	Reference
No 2 fuel oil + Omni-Clean dispersant	No	Sheepshead minnow (*Cyprinodon variegatus*)	Embryo	Mortality, fish biomass	Chronic	7 d	WAF from 20-30 s electric stirring	Seawater (20 ppt)	Lab	LC50 (WAF) = 130 mg/L, (CEWAF) = 70 mg/L (estimated)			Adams et al. 1999
Prudhoe Bay crude	Yes (in field)	Pacific herring (*Clupea pallasi*)	Embryo	Mortality, hatching success, growth	Chronic	Field: 8-12 d Lab: 18 d	FIELD: Caged in situ at PWS oiled and unoiled sites LAB: 36 h exposures to oil-water dispersions (OWD)	Seawater	Lab and field		LOEC (deformities) = 0.24 mg TPH/L LOEC (hatch) = 0.24 mg/L LOEC (genotoxicity - # mitotic cells per fin) = 0.24 mg/L	Embryos exposed in field to oiled sites were consistently of lower weight; confounding variable effects on mortality and hatching success	Kocan et al. 1996a
Prudhoe Bay crude	Field - yes, lab - no (evidently)	Pacific herring (*Clupea pallasi*)	Embryo	Histopathology	Chronic	14-20 d (at hatch - differed by dose)	Oil-water dispersions (OWDs), or collected from "oiled" and "unoiled"	Seawater	Lab and field		LOEC (ascites) = 0.48 mg/L	Larvae from oiled sites were shorter, had ingested less food, had slower growth, high prevalence of cytogenetic damage, ascites	Marty et al. 1997a

Prudhoe Bay crude	Yes; heated to 70 °C until 20% loss	Pink salmon (*Oncorhynchus gorbuscha*)	Embryo	Sampled 4 weeks pre-, at, and 13 d postemergence	Chronic	Up to 18 d	WAF from oiled gravel columns	Fresh	Lab	LOEC (developmental) = 55.2 mg oil/kg gravel LOEC (CYP1A induction; histopathology - epidermal thickness) = 622 mg/kg	LOEC (developmental) = 4.42 µg TPAH/L (peak)		Marty et al. 1997b
Ultralow sulfur Diesel No. 2	No	Rainbow trout (*Oncorhynchus mykiss*)	Embryo	BSD, mortality	Chronic	24 d	WAF, CEWAF	Fresh	Lab	CEWAF BSD EC50: 0.04 % v/v CEWAF mortality LC50: 0.05 % v/v		WAF BSD EC50 and mortality LC50 > 1 % v/v	Schein et al. 2009
ANS crude	Yes; heated to 70 °C until 20% loss	Pacific herring (*Clupea pallasi*)	Embryo	PAH uptake, immunofluorescence, heart rate	Chronic	6-7 d	WAF from oiled gravel columns	Seawater	Lab			Significant bradycardia at 12.5% oiled gravel dose	Incardona et al. 2009
ANS crude	No	Zebrafish (*Danio rerio*)	Embryo	Pericardial edema, abnormal heart looping, intracranial hemorrhaging	Chronic	2 d	Mechanically dispersed oil (WAF); direct and indirect contact	Fresh	Lab		(Estimated) EC50 (abnormal heart looping) = 25 µg TPAH/L; EC50 (pericardial edema) = 45 µg	Overlapping toxicity curves for embryos exposed to mechanically dispersed oil and for embryos embedded in agarose and only exposed to dissolved fraction	Carls et al. 2008

ANS crude	No	Zebrafish (*Danio rerio*)	Embryo	Pericardial edema, abnormal heart looping, intracranial hemorrhaging	Chronic	2 d	Mechanically dispersed oil (WAF); direct and indirect contact	Fresh	Lab		(Estimated) EC50 (abnormal heart looping) = 25 µg TPAH/L; EC50 (pericardial edema) = 45 µg TPAH/L	Overlapping toxicity curves for embryos exposed to mechanically dispersed oil and for embryos embedded in agarose and only exposed to dissolved fraction	Carls et al. 2008
Norman Wells crude	No	Rainbow trout (*Oncorhynchus mykiss*)	Embryo	Mortality, water content, growth	Chronic	55 d	WAF, CEWAF	Fresh	Lab	30 uL oil/L: LT50 (estimated): oil alone = 55 d, oil+disp = 20-45 d		After 55 d exposure, fry from oi, disp, and oil+disp mixtures had greater water content than controls (edema?) Corexit 7664 was more toxic than Corexit 9600	Lockhart et al. 1996
Lithuanian crude	No	Rainbow trout (*Oncorhynchus mykiss*)	Embryo, adult	Gill ventilation frequency (GVF), body mass, heart	Acute and chronic	25 d (chroni c) and	Injection of 1610 mg oil/L to	Fresh	Lab			Reduced GVF, heart rate, and body mass in oil-exposed fish	Vosyliene et al. 2005

Oil type	Weathered?	Species	Life stage	Response	Type	Time	Method	Fresh/ Seawater	Lab/ Field	Nominal	Measured	Notes	Reference
Lithuanian crude	No	Rainbow trout (*Oncorhynchus mykiss*)	Embryo, juvenile	Mortality, physiological (heart rate, gill ventilation)	Acute and chronic	Embryo (7-8d), larvae (42-44d), juvenile (4d and 14d)	WAF (stirred and aerated for 24 h)	Fresh	Lab	Embryo LOEC (mortality) = 3.46 g oil/L, Larvae LOEC (mortality) = 0.43 g oil/L Embryo and larvae LOEC (developmental and heart rate) = 0.87 g/L			Kazlauskiene et al. 2008
Heavy fuel oil (Lithuanian thermal plant)	No	Rainbow trout (*Oncorhynchus mykiss*)	Embryo, juvenile, adult	Mortality	Acute and chronic	96h (LC50), 60d (embryos, larvae LC50), 28d (adult LC50)	WAF (stirred and aerated for 24 h)	Fresh	Lab	Embryo LC50 = 1.55 g HFO/L Larvae LC50 = 0.33 g HFO/L Adult LC50 = 2.97 g HFO/L			Kazlauskiene et al. 2004
ANS crude	Yes; 70 °C until 20% loss; and natural weathering	Pink salmon (*Oncorhynchus gorbuscha*)	Gamete	Mortality, BSD	Chronic	8 h	WAF (effluent) - exposure of eggs during sperm activation, fertilization, and water	Fresh	Lab	> 2250 mg oil/kg gravel	> 16.4 µg TPAH/L	Observed mortality in gametes not statistically greater than controls	Brannon et al. 2006a

ANS crude	Yes; 70 °C until 20% loss; and natural weathering	Pink salmon (*Oncorhynchus gorbuscha*)	Gamete	Mortality, BSD	Chronic	8 h	WAF (effluent) - exposure of eggs during sperm activation, fertilization, and water hardening	Fresh	Lab	> 2250 mg oil/kg gravel	> 16.4 µg TPAH/L	Observed mortality in gametes not statistically greater than controls	Brannon et al. 2006a
ANS crude	No	Gulf killifish (*Fundulus grandis*)	Juvenile	Mortality	Acute	96 h	Flowthrough canal with caged organisms - simulated oil spill	Seawater	Lab	At least 83% survival @ 30 mg oil/L dose (HC concentration at t 0 = 14-24 mg/L water)		One time addition of oil+disp in a continuous flow system. HC concentrations declined to background after 3 h	Liu et al. 2006
ANS crude	No	Pink salmon (*Oncorhynchus gorbuscha*)	Juvenile	Mortality	Acute	96 h	WAF (glass extraction column; Moles et al. 1985)	Seawater	Lab		96-h LC50 = 2.2 mg/L (1990), 2.8 (1991), 1.0 (1992)		Birtwell et al. 1999
Arabian crude oil (54% saturates,	Yes; evaporated for 24 h using natural UV	Golden grey mullet (*Liza aurata*)	Juvenile	Biliary PAH metabolites, glutathione live content, EROD,	Acute	48 h	CEWAF	Seawater	Lab			PAH metabolites and total glutathione content of liver were found with CEWAF exposures (44	Milinkovitch et al. 2011b
Oil type	**Weathered?**	**Species**	**Life stage**	**Response**	**Type**	**Time**	**Method**	**Fresh/ Seawater**	**Lab/ Field**	**Nominal**	**Measured**	**Notes**	**Reference**

Australian crude oils: condensate, light, medium; Australian diesel from Apache	Yes, artificial weathering by distillation (topping) at 150-250 °C to simulate 1-15 d at sea	Clownfish (*Amphiprion clarkii*), silverside minnows (*Menidia beryllina*)	Juvenile	Mortality	Acute	96 h	WAF (28 g/L stock - 0,8,16,64,100%)	Seawater	Lab	LC50 (Clownfish): Wonnich F = 35% 100%, light crudes = >100% -LC50 (Minnow): Wonnich F = 32% - 79% W, overall more sensitive than clownfish		Overall, acute toxicity is attributed to MAHs; toxicity of crude oils also attributed to PAHs (about 58%), moreso with weathering - where MAH are lost and PAH persist	Neff et al. 2000
Bombay high oil field (India) crude	Yes; sparged with air for 130 h at room T, until 14% loss	Tilapia (*Tilapia mossambica*)	Juvenile	PAH uptake	Acute	96 h	WAF (Singer)	Fresh and seawater (15, 30 ppt)	Lab			Increased salinity reduced PAH uptake to liver, gills, muscle, gonad; due to reduced solubility or uptake rates? Only measured 5 parent PAH in water, tissue	Shukla et al. 2007
Brut Arabian Light oil	No	Thinlip grey mullet (*Liza ramada*)	Juvenile	Mortality	Acute	24 h	WSF, WAF, CEWAF (0-40%)	Seawater	Lab			CEWAF was more toxic than MDO and WSF (mortality), correlating with high PAH, TPH concentration.	Milinkovitch et al. 2011a
Bunker C	No	Red sea bream (*Pagrus major*)	Juvenile	Mortality	Acute	48 h	Static WAF (1-2 min mixing, 5 min settling; no renewal)	Seawater (34.5 ppt)	Lab		0.325 mg total hydrocarbons (?)/L		Koyama and Kakuno 2004

Prestigelike heavy fuel oil (IFO 380)	Fresh and weathered (2.5 h water washing + aeration)	Thicklip grey mullet (*Chelon labrosus*)	Juvenile	Gene expression	Acute and chronic	2 d and 16 d	Oil mixed with sediments and placed in static system (no renewal) + fish	Seawater (35 ppt)	Lab			cyp1a and gst transcription values of biotransformation enzymes elevated in F and W treatments	Bilbao et al. 2010
ANS crude	Yes; heated to 80 °C for 8 h	Pacific halibut (*Hippoglossoides stenolepis*)	Juvenile	LOEC: growth, histopatholog y	Chronic	90 d	1 and 2 % v/v oil mixed in sediments	Seawater	Lab	1600 ug/g in sediment			Moles and Norcross 1998
ANS crude	Yes; heated to 80 °C for 8 h	Rock sole (*P. bilineatus*)	Juvenile	LOEC: growth, histopatholog y	Chronic	90 d	1 and 2 % v/v oil mixed in sediments	Seawater	Lab	1600 ug/g in sediment			Moles and Norcross 1998

Oil type	Weathered?	Species	Life stage	Response	Type	Time	Method	Fresh/ Seawater	Lab/ Field	Nominal	Measured	Notes	Reference
ANS crude	Yes; heated to 80 °C for 8 h	Yellowfin sole (*Pleuronectes asper*)	Juvenile	LOEC: growth, histopathology	Chronic	90 d	1 and 2 % v/v oil mixed in sediments	Seawater	Lab	1600 ug/g in sediment		34-56% reduced growth in all flatfishes, occurring at sediment concentrations of 1600 ug/g	Moles and Norcross 1998
Bunker C	Yes, naturally in field over 30 y	Winter flounder (*Pleuronectes americanus*)	Juvenile	Hepatic MFO activity	Chronic	1-14 d	Contaminated sediments collected after Arrow oil spill	Seawater	Lab		LOET (time for EROD induction) = 14 d	Overall, MFO induction was limited, non-toxic by Microtox, very toxic by amphipod survival tests.	Lee et al. 2003
California crude	Yes	Tidewater silverside (*Menidia beryllina*)	Juvenile	Mortality	Chronic	7 d	WAF + UV	Seawater	Lab		7d LC50 = 0.5 - 2.8 mg TPH/L 4d LC50 = 1.09 - 1.77 mg/L LOEC (4d or 7d) = 1.50 mg/L		Little et al. 2000
Erika tanker fuel	Yes (minimum 2 months at sea)	Sole (*Solea solea*)	Juvenile	DNA adducts	Chronic	Unknown	Field exposure to spilled oil	Seawater	Field	DNA adducts present two months after spill in juvenile sole from contaminated sites		Fish collected along French Brittany coasts (oiled beaches from 1999 Erika oil spill)	Amat et al. 2006

Oil type	Weathered?	Species	Life stage	Response	Type	Time	Method	Fresh/ Seawater	Lab/ Field	Nominal	Measured	Notes	Reference
Heavy crude from Victory Oil Field (Shandong Province, China)	Unknown	Goldfish (*Carassius auratus*)	Juvenile	LOEC (calculated) from SOD, CAT, GST, MDA	Chronic	20 d	Field collected oil-contaminated soils (stirred daily)	Fresh	Lab	SOD activity LOEC = 10 g oiled sediment/L, CAT activity LOEC = 0.5 g/L(?), GST activity LOEC = 0.5 g/L, MDA content LOEC = 50 g/L		SOD, CAT, GST in the hepatic system are sensitive to crude oil and may serve as a monitoring index	Wang et al. 2009
Nigeria crude oil (Bonny Light)	No	Guinean tilapia (*Tilapia guineensis*)	Juvenile	Mortality	Chronic	96 h	WAF	Fresh	Lab	crude oil LC50 = 125.89 mg oil/L			Ndimele et al. 2010
MESA	Yes; sparged with air for 130 h at room T, until 14% loss	Mummichog (*Fundulus heteroclitus*)	Juvenile	EROD induction	Acute	48 h	WAF, CEWAF	Seawater (15 and 30 ppt)	Lab	15 ppt - WAF: 7.56 % v/v; CEWAF: 0.022 % v/v 30 ppt - WAF: 1.10 % v/v; CEWAF: 0.115 % v/v		EC50 WAF/EC50 CEWAF (induction potential) = 15 ppt: 343; 30 ppt: 9.56	Ramachandran et al. 2006

MESA	Yes; sparged with air for 130 h at room T, until 14% loss	Rainbow trout (*Oncorhynchus mykiss*)	Juvenile	EROD induction	Acute	48 h	WAF, CEWAF	Fresh	Lab	WAF: 0.106 % v/v, CEWAF: 0.001 % v/v	WAF: 0.72 µg TPAH/L, CEWAF: 0.60 µg TPAH/L	EC50 WAF/EC50 CEWAF (induction potential) = 106	Ramachandran et al. 2004
MESA	Yes; sparged with air for 130 h at room T, until 14% loss	Rainbow trout (*Oncorhynchus mykiss*)	Juvenile	EROD induction	Acute	48 h	WAF, CEWAF	Fresh and seawater (15 ppt)	Lab	Fresh - WAF: 0.088 % v/v; CEWAF: 0.00034 % v/v 15 ppt - WAF: 4.98 % v/v; CEWAF: 0.018 % v/v		EC50 WAF/EC50 CEWAF (induction potential) = Fresh: 258; 15 ppt: 276	Ramachandran et al. 2006
Scotian Light	No	Rainbow trout (*Oncorhynchus mykiss*)	Juvenile	EROD induction	Acute	48 h	WAF, CEWAF	Fresh	Lab	WAF: 0.390 % v/v, CEWAF: 0.066 % v/v	WAF: 1.56 µg TPAH/L, CEWAF: 2.00 µg TPAH/L	EC50 WAF/EC50 CEWAF (induction potential) = 5.91	Ramachandran et al. 2004
Terra Nova	No	Rainbow trout (*Oncorhynchus mykiss*)	Juvenile	EROD induction	Acute	48 h	WAF, CEWAF	Fresh	Lab	WAF: 3.350 % v/v, CEWAF: 0.003 % v/v	WAF: 1.80 µg TPAH/L, CEWAF: 1.50 µg TPAH/L	EC50 WAF/EC50 CEWAF (induction potential) = 1116	Ramachandran et al. 2004
Ultralow sulfur	No	Rainbow trout (*Oncorhynchus*	Juvenile	EROD induction	Acute	24 h	WAF, CEWAF	Fresh	Lab	CEWAF EC50: <			Schein et al. 2009

Diesel No. 2		*mykiss*)								0.01 % v/v WAF EC50: 6.17 % v/v			
Diesel	No	Atlantic croaker (*Micropogonias undulatus*)	Juvenile	Histology of pituitary/hypo thalamus and ovarian tissues	Chronic	5 to 8 w	WAF (Anderson 1974; dosed every two days)	Seawater	Lab			30-56% of oilexposed fish failed to reach sexual maturation; ovarian growth was impaired, slowed development of ovarian follicles and widespread atresia; few oocytes.	Thomas and Budiantara 1995
Grand Banks crude	No	Winter flounder (*Pleuronectes americanus*)	Juvenile, adult	Mortality, histopatholog y of gills, reproduction	Chronic	8 w	Artificially contaminat ed sediments	Seawater	Lab	300 mg/kg sediment			Khan 1995

Oil type	Weathered?	Species	Life stage	Response	Type	Time	Method	Fresh/ Seawater	Lab/ Field	Nominal	Measured	Notes	Reference
Prudhoe Bay crude	Unknown	High cockscomb (*Anoplarchus purpurescens*)	Juvenile, adult	CYP1A induction	Chronic	28 d	Caged in field at oiled sites, or exposed in lab to fieldcollected oil-contaminated sediments and/or oiled food	Seawater	Lab and field			6-fold greater induction in field, 49-fold greater induction in lab	Woodin et al. 1997
Scotian Light	No	Rainbow trout (*Oncorhynchus mykiss*)	Embryo	Embryo deformities	Chronic	25 d	WAF, CEWAF	Fresh	Lab	0.03 %v/v	(Calculated) 2.1 µg TPAH/L		Wu et al. 2012
Federated crude	No	Rainbow trout (*Oncorhynchus mykiss*)	Embryo	Embryo deformities	Chronic	25 d	WAF, CEWAF	Fresh	Lab	0.015 %v/v	(Calculated) 2.7 µg TPAH/L		Wu et al. 2012
ANS crude	No	Rainbow trout (*Oncorhynchus mykiss*)	Embryo	Embryo deformities	Chronic	25 d	WAF, CEWAF	Fresh	Lab	0.02 %v/v	(Calculated) 3.4 µg TPAH/L		Wu et al. 2012
MESA	No	Rainbow trout (*Oncorhynchus mykiss*)	Embryo	Embryo deformities	Chronic	25 d	WAF, CEWAF	Fresh	Lab	0.015 %v/v	(Calculated) 2.1 µg TPAH/L		Wu et al. 2012
Louisiana crude	Yes, mixed with clean brackish water for 30days, water fraction isolated	Gulf killifish (*Fundulus grandis*)	Adult males	Mortality	Acute	7 day	WAF 1:10 oil:water, 100%	Brackish	Lab			Range finding experiment	Pilcher et al. 2014

Louisiana crude	Yes, mixed with clean brackish water for 30 days, water fraction isolated	Gulf killifish (*Fundulus grandis*)	Adult males	Mortality	Acute	7 day	WAF, 75%	Brackish	Lab			Range finding experiment	Pilcher et al. 2014
Louisiana crude	Ye s, mixed with clean brackish water for 30 days, water fraction isolated	Gulf killifish (*Fundulus grandis*)	Adult males	Mortality	Acute	7 day	WAF, 50%	Brackish	Lab			Range finding experiment	Pilcher et al. 2014
Louisiana crude	Yes, mixed with clean brackish water for 30-40 days, water fraction isolated	Gulf killifish (*Fundulus grandis*)	Adult males	No Mortality	Acute	7 day	WAF, 25%	Brackish	Lab			Range finding experiment	Pilcher et al. 2014
Louisiana crude	Yes, mixed with clean brackish water for 40 days, water fraction isolated	Gulf killifish (*Fundulus grandis*)	Adult males	DNA strand breakage elevated for, high and moderate-low damage categories	Acute	7 day	WAF, 25%	Brackish	Lab			Definitive exposure experiment	Pilcher et al. 2014
Louisiana crude	Yes, mixed with clean brackish water for 40 days, water fraction isolated	Gulf killifish (*Fundulus grandis*)	Adult males	Non statistically significant effects	Acute	7 day	WAF, 2.5%	Brackish	Lab			Definitive exposure experiment	Pilcher et al. 2014

Phenanthrene	Used Phenanthrene (98% purity, Aldrich Chemical Co.)	Harpacticoid copepod (*Schizopera knabeni*)	Adult female	Sublethal effects: significantly fewer realized offspring	Acute	10 day bioassay	Phenanthrene was added to sediment slurry	25 ‰ artificial seawater	Lab		LC50 345 $\mu g\ g^{-1}$ dry wet (291 - 407)		Lotufo and Fleeger 1997
Phenanthrene	Used Phenanthrene (98% purity, Aldrich Chemical Co.)	Harpacticoid copepod (*Schizopera knabeni*)	Adult Male		Acute	10 day bioassay	Phenanthrene was added to sediment slurry	25 ‰ artificial seawater	Lab		LC50 349 $\mu g\ g^{-1}$ dry wet (291 - 417)		Lotufo and Fleeger 1997
Phenanthrene	Used Phenanthrene (98% purity, Aldrich Chemical Co.)	Harpacticoid copepod (*Schizopera knabeni*)	Copepodite	Sublethal effects: reduced mean fraction of realized offspring composed of copepodites	Acute	10 day bioassay	Phenanthrene was added to sediment slurry	25 ‰ artificial seawater	Lab		LC50 172 $\mu g\ g^{-1}$ dry wet (155 – 190)		Lotufo and Fleeger 1997
Phenanthrene	Used Phenanthrene (98% purity, Aldrich Chemical Co.)	Harpacticoid copepod (*Schizopera knabeni*)	Nauplius	Reduced mean hatching number at 90 $\mu g\ g^{-1}$ dry wt	Acute	10 day bioassay	Phenanthrene was added to sediment slurry	25 ‰ artificial seawater	Lab		LC50 84 $\mu g\ g^{-1}$ dry wet (74 – 96)		Lotufo and Fleeger 1997
Phenanthrene	Used Phenanthrene (98% purity, Aldrich Chemical Co.)	Harpacticoid copepod (*Nitocra lacustris*)	Adult female	Survival lower at >177 µg g-1 dry weight concentration, reduced proportional realized offspring and fraction of realized	Acute	10 day bioassay	Phenanthrene was added to sediment slurry	25 ‰ artificial seawater	Lab		LC50 105 $\mu g\ g^{-1}$ dry wet (95 – 116)		Lotufo and Fleeger 1997

				offspring comprised of copepodites at 45 and 90 μg g^{-1} dry wt									
Phenanthrene	Used Phenanthrene (98% purity, Aldrich Chemical Co.)	Harpacticoid copepod (*Nitocra lacustris*)	Adult male	Survival lower at > 90 μg g^{-1} dry weight concentration	Acute	10 day bioassay	Phenanthrene was added to sediment slurry	25 ‰ artificial seawater	Lab		LC50 72 μg g^{-1} dry wet (62 – 83)		Lotufo and Fleeger 1997
Phenanthrene	Used Phenanthrene (98% purity, Aldrich Chemical Co.)	Harpacticoid copepod (*Nitocra lacustris*)	Copepodite	Survival lower at >22 μg g-1 dry weight concentration	Acute	10 day bioassay	Phenanthrene was added to sediment slurry	25 ‰ artificial seawater	Lab		LC50 43 μg g^{-1} dry wet (36-52)		Lotufo and Fleeger 1997
Phenanthrene	Used Phenanthrene (98% purity, Aldrich Chemical Co.)	Harpacticoid copepod (*Nitocra lacustris*)	Nauplius	Survival lower at >45 μg g-1 dry weight concentration	Acute	10 day bioassay	Phenanthrene was added to sediment slurry	25 ‰ artificial seawater	Lab		LC50 71 μg g^{-1} dry wet (65-77)		Lotufo and Fleeger 1997
Hibernia crude oil	200mL of crude in an 80L tank with inflow 2L/min, WAF drawn off bottom	Atlantic cod (*Gadus morhua*)	Adult male	Significant changes in gonadal somatic index	Chronic	89 days	WAF July – October, 9-14 degrees Celsius	Seawater	Lab			Mean total hydrocarbon (ppb) concentration (THC) = 49 ± 16	Khan 2012
Hibernia crude oil	200mL of crude in an 80L tank with inflow 2L/min, WAF drawn	Atlantic cod (*Gadus morhua*)	Adult female	Significantly less weight gain, significant changes in gonadal somatic	Chronic	89 days	WAF July – October, 9-14 degrees Celsius	Seawater	Lab			Mean total hydrocarbon (ppb) concentration (THC) = 49 ± 16	Khan 2012

	off bottom			index									
Hibernia crude oil	200mL of crude in an 80L tank with inflow 2L/min, WAF drawn off bottom	Atlantic cod (*Gadus morhua*)	Adult male	Significant changes in gonadal somatic index	Chronic	86 days	WAF August – November 10-14 degrees Celsius	Seawater	Lab			Mean total hydrocarbon (ppb) concentration (THC) = 42 ± 19	Khan 2012
Hibernia crude oil	200mL of crude in an 80L tank with inflow 2L/min, WAF drawn off bottom	Atlantic cod (*Gadus morhua*)	Adult female	Significantly less weight gain, significant changes in gonadal somatic index	Chronic	86 days	WAF August – November 10-14 degrees Celsius	Seawater	Lab			Mean total hydrocarbon (ppb) concentration (THC) = 42 ± 19	Khan 2012
Hibernia crude oil	200mL of crude in an 80L tank with inflow 2L/min, WAF drawn off bottom	Atlantic cod (*Gadus morhua*)	Adult male	Significant changes in gonadal somatic index, Immature testes small spermatocytes, 90% external lesions	Chronic	92 days	WAF, September – November 10-14 degrees Celsius	Seawater	Lab			Mean total hydrocarbon (ppb) concentration (THC) = 40 ± 12	Khan 2012
Hibernia crude oil	200mL of crude in an 80L tank with inflow 2L/min, WAF drawn off bottom	Atlantic cod (*Gadus morhua*)	Adult female	Immature ovaries, small oocytes, 60% external lesions	Chronic	92 days	WAF, September – November 10-14 degrees Celsius	Seawater	Lab			Mean total hydrocarbon (ppb) concentration (THC) = 40 ± 12	Khan 2012
Hibernia crude oil	200mL of crude in an 80L tank with inflow 2L/min,	Atlantic cod (*Gadus morhua*)	Adult male	Significant changes in gonadal somatic index, small	Chronic	86 days	WAF, November – February 0-2 degrees	Seawater	Lab			Mean total hydrocarbon (ppb) concentration (THC) = 31 ± 14	Khan 2012

Hibernia crude oil	200mL of crude in an 80L tank with inflow 2L/min, WAF drawn off bottom	Atlantic cod (*Gadus morhua*)	Adult male	Significant changes in gonadal somatic index, small spermatocyte s , disrupted development, 40% external lesions	Chronic	86 days	WAF, November – February 0-2 degrees Celsius	Seawater	Lab			Mean total hydrocarbon (ppb) concentration (THC) = 31 ± 14	Khan 2012
Hibernia crude oil	200mL of crude in an 80L tank with inflow 2L/min, WAF drawn off bottom	Atlantic cod (*Gadus morhua*)	Adult female	Significant changes in gonadal somatic index, small oocytes disrupted development, 33% external lesions	Chronic	86 days	WAF November – February 0-2 degrees Celsius	Seawater	Lab			Mean total hydrocarbon (ppb) concentration (THC) = 31 ± 14	Khan 2012
Hibernia crude oil	200mL of crude in an 80L tank with inflow 2L/min, WAF drawn off bottom	Atlantic cod (*Gadus morhua*)	Adult male	Significant changes in gonadal somatic index, small spermatocyte s , disrupted development, 53 % external lesions	Chronic	86 days	WAF December – February 0-2 degrees Celsius	Seawater	Lab		5	Mean total hydrocarbon (ppb) concentration (THC) = 15 ± 10	Khan 2012
Hibernia crude oil	200mL of crude in an 80L tank	Atlantic cod (*Gadus morhua*)	Adult female	Small oocytes disrupted	Chronic	86days	WAF December – February 0-2	Seawater	Lab			Mean total hydrocarbon (ppb) concentration (THC)	Khan 2012

Hibernia crude oil	200mL of crude in an 80L tank with inflow 2L/min, WAF drawn off bottom	Atlantic cod (*Gadus morhua*)	Adult male	50 % external lesions	Chronic	38 days	WAF, April – May 1-3 degrees Celsius	Seawater	Lab			Mean total hydrocarbon (ppb) concentration (THC) = 22 ± 12	Khan 2012
Hibernia crude oil	200mL of crude in an 80L tank with inflow 2L/min, WAF drawn off bottom	Atlantic cod (*Gadus morhua*)	Adult female	40 % external lesions	Chronic	38 days	WAF April – May 1-3 degrees Celsius	Seawater	Lab			Mean total hydrocarbon (ppb) concentration (THC) = 22 ± 12	Khan 2012
Hibernia crude oil	200mL of crude in an 80L tank with inflow 2L/min, WAF drawn off bottom	Atlantic cod (*Gadus morhua*)	Adult female	Atretic oocytes, post-ovulatory follicles, disrupted development 57 % external lesions	Chronic	90 days	WAF , June – June 6-12 degrees Celsius	Seawater	Lab			Mean total hydrocarbon (ppb) concentration (THC) = 28 ± 9	Khan 2012
Hibernia crude oil	200mL of crude in an 80L tank with inflow 2L/min, WAF drawn off bottom	Atlantic cod (*Gadus morhua*)	Adult female	Significant changes in gonadal somatic index, disrupted development, small oocytes 56% external lesions	Chronic	4 days	WAF , October – March 0-10 degrees Celsius	Seawater	Lab			Mean total hydrocarbon (ppb) concentration (THC) = 21 ± 8	Khan 2012
Heavy fuel oil 6303	WAF	Rainbow trout (*Oncorhynchus mykiss*)	Embryo	PAH concentration to cause toxicity	Chronic	25 days	WAF 1:9 water: oil ratio	Fresh	Lab	WAF: >0.01% v/v, LC 50 >45 µg /L TPH-F, >5 µg /L TPAH-F			Martin et al. 2014

Heavy fuel oil 6303	Chemically enhanced	Rainbow trout (*Oncorhynchus mykiss*)	Embryo	PAH concentration to cause toxicity	Chronic	25 days	Chemically enhanced WAF (1:9 water:oil ratio then surface application of dispersant at dispersant: oil ratio 1:10)	Fresh	Lab	CEWAF: 0.0037 % v/v, LC 50 473 µg /L TPH-F, EC50 338 µg /L			Martin et al. 2014
Heavy fuel oil 6303	Oil poured on gravel,	Rainbow trout (*Oncorhynchus mykiss*)	Embryo		Chronic	25 days	Contaminated gravel in columns from which effluent taken as WAF	Fresh	Lab	LC50 505 µg oil/g gravel, EC50 49 µg /			Martin et al. 2014
Heavy fuel oil 6303	Artificially weathered-stranded on gravel	Rainbow trout (*Oncorhynchus mykiss*)	Embryo		Chronic	25 days	Contaminated gravel in columns from which effluent taken as WAF	Fresh	Lab	LC50 591 µg oil/g gravel, EC50 66 µg /L			Martin et al. 2014
Heavy fuel oil 7102	Oil stranded on gravel	Rainbow trout (*Oncorhynchus mykiss*)	Embryo		Chronic	25 days	Contaminated gravel in columns from which effluent taken as WAF	Fresh	Lab	LC 50 1031 µg oil/g gravel, EC50 120 µg /L			Martin et al. 2014
MESA crude	Oil stranded on gravel	Rainbow trout (*Oncorhynchus mykiss*)	Embryo		Chronic	25 days	Contaminated gravel in columns from which effluent taken as WAF	Fresh	Lab	LC 50 4607 µg oil/g gravel, EC50 1076 µg /L			Martin et al. 2014
Heavy fuel oil 7102	WAF produced from flow of water through oiled gravel	Rainbow trout (*Oncorhynchus mykiss*)	Embryo	Induced liver EROD activity to a level equivalent to that of bnaphthoflav	Chronic	24 days	High energy, chemically enhanced WAF	Fresh	Lab	LC 115 µg /L, 48-h EC50 6.03 µg TPH-F/L			Adams et al. 2014

				one									
Pyrolytic PAH fraction from contaminated sediment	No	Rainbow trout (*Oncorhynchus mykiss*)	Embryo	Significantly more half hatched embryos and abnormal larvae, DNA damage	Acute	22 days	WAF: Embryos placed in gravel spiked with PAH fraction for first ten days	Freeh	Lab				Le Bihanic et al. 2014
Erika heavy oil	No	Rainbow trout (*Oncorhynchus mykiss*)	Embryo	Embryo mortality and decreased hatching success, abnormal larvae, DNA damage	Acute	22 days	WAF: Embryos placed in gravel spiked with PAH fraction for first ten days	Fresh	Lab				Le Bihanic et al. 2014
Arabian Light oil	No	Rainbow trout (*Oncorhynchus mykiss*)	Embryo	Reduced larval size, abnormal larvae, hemorrhages, DNA damage	Acute	22 days	WAF: Embryos placed in gravel spiked with PAH fraction for first ten days	Fresh	Lab				Le Bihanic et al. 2014
PAH	No	Haddock (*Melanogrammus aeglefinus*)	Adult	Elevated concentration of bile PAH metabolites with 2,3 and 4 rings, and 2 hydroxynaphthalene. Altered Enzyme activity, increased hepatic DNA	Chronic		Fish sampled from natural populations in areas with extensive oil production	Seawater	Field – North Sea				Balk et al 2011

PAH	No	Haddock (*Melanogrammus aeglefinus*)	Adult	Elevated concentration of bile PAH metabolites with 2,3 and 4 rings, and 2 hydroxynaphthalene. Altered Enzyme activity, increased hepatic DNA adducts	Chronic		Fish sampled from natural populations in areas with extensive oil production	Seawater	Field – North Sea				Balk et al 2011
PAH	No	Atlantic cod (*Gadus morhua*)	Adult	Elevated concentration of bile PAH metabolites Enzyme activity induced by oxidative stress caused by petroleum pollutants	Chronic		Fish sampled from natural populations in areas with extensive oil production	Seawater	Field – North Sea		Significantly elevated EROD activity		Balk et al 2011
Orimulsion-400 (PDVSA-BITOR)	No	Atlantic herring (*Clupea harengus*)	Embryo	Survival reduced at 0.0032% concentration	Acute	24h	WAF	Artificial seawater	Lab	LC50 0.00259-0.0375%			Boudreau et al. 2009
Orimulsion-400 (PDVSA-BITOR)	No	Mummichog (*Fundulus heteroclitus*)	Embryo	Survival reduced at 0.1 and 0.032% concentration, significant increase in	Acute	24h	WAF	Artificial seawater	Lab	LC50 0.0421-0.0478, EC50 .0082-0.0157%			Boudreau et al. 2009

PAHs (C3-naphthalene and phenanthrene) – Wabamun Lake	Spill of bunker C oil and Imperial pole treating oil, plus constant inputs from coal plant	Lake Whitefish (*Coregonus clupeaformis*)	Larvae	Significantly increased level of kyphosis and skeletal and fin deformities	Chronic		Wabamun Lake water	Fresh	Field – in situ			Comparing oil exposed vs. reference sites, PAH's detected at oil-exposed sites	Debruyn et al. 2007
Pyrene	No	*Daphnia magna*		Highly toxic in presence of UV radiation	Acute	24h in solution, 15 min UV exposure	1 and 10 µg /L	Fresh	Lab	EC50 0.06 uM		Accumulation of chemical prior to exposure important to toxicity	Huovinen et al. 2001
Anthracene	No	*Daphnia magna*		Photo induced toxicity	Acute	24h in solution, 15 min UV exposure	10 µg /L	Fresh	Lab	EC50 0.22uM		Accumulation of chemical prior to exposure important to toxicity	Huovinen et al. 2001
Retene	No	*Daphnia magna*		Photo induced toxicity	Acute	24h in solution, 15 min UV exposure	10 and 32 µg /L	Fresh	Lab	EC50 1.54 uM		Accumulation of chemical prior to exposure important to toxicity	Huovinen et al. 2001
Phenanthrene	No	*Daphnia magna*		Not phototoxic	Acute	24h in solution, 15 min UV exposure	10, 32, 100, 320 µg /L	Fresh	Lab	EC50 >1.80 uM		Accumulation of chemical prior to exposure important to toxicity	Huovinen et al. 2001
Dehydroabietic acid	No	*Daphnia magna*	Embryo	Not phototoxic	Acute	24h in solution, 15 min UV exposure	100 and 320 µg /L	Fresh	Lab				Huovinen et al. 2001

Napthalene	No	Zebrafish (*Danio rerio*)	Embryo	100% mortality 24h exposure	Acute	24 – 48h	Passive dosing via PDMS silicone	Fresh	Lab	PAC 27193.2 ± 936			Seiler et al. 2014
Acenaphthene	No	Zebrafish (*Danio rerio*)	Embryo	100% mortality 24h exposure	Acute	24 – 48h	Passive dosing via PDMS silicone	Fresh	Lab				Seiler et al. 2014
Fluorene	No	Zebrafish (*Danio rerio*)	Embryo	100% mortality 24h exposure	Acute	24 – 48h	Passive dosing via PDMS silicone	Fresh	Lab				Seiler et al. 2014
Phenanthrene	No	Zebrafish (*Danio rerio*)	Embryo	100% mortality 24h exposure	Acute	24 – 48h	Passive dosing via PDMS silicone	Fresh	Lab				Seiler et al. 2014
Fluoranthene	No	Zebrafish (*Danio rerio*)	Embryo	100% mortality 24h exposure	Acute	24 – 48h	Passive dosing via PDMS silicone	Fresh	Lab				Seiler et al. 2014
Pyrene	No	Zebrafish (*Danio rerio*)	Embryo	50% mortality 24h exposure	Acute	24 – 48h	Passive dosing via PDMS silicone	Fresh	Lab				Seiler et al. 2014
Cosco Busan bunker oil	Artificial	Pacific herring (*Clupea pallasi*)	Embryo	Pericardial edema after exposure to 1.0 g/kg gravel effluent, phototoxicity in 0.3 and 1.0g/kg UV-t	Chronic	8 days	WAF: Embryos placed in oiled gravel weathered with continuous seawater	Seawater mix 22 psu salinity	Lab	LC50 11 – 138, EC20 for cardiotoxicity 300-1000 ng/L PACs		High losses of herring following spill in San Fransisco Bay, CBBO interacts with sunlight to produce acutely lethal necrosis at low water and tissue PAC concentrations	Incardona et al 2012
Alaskan North Slope crude	Artificial	Pacific herring (*Clupea pallasi*)	Embryo	Pericardial edema after exposure to 0.3 and 1.0 g/kg gravel	Chronic	8 days	WAF: Embryos placed in oiled gravel weathered	Seawater mix 22 psu salinity	Lab				Incardona et al 2012

Alaskan North Slope crude	Artificial	Pacific herring (*Clupea pallasi*)	Embryo	Pericardial edema after exposure to 0.3 and 1.0 g/kg gravel effluent treatments phototoxicity in 1.0g/kg UV-t	Chronic	8 days	WAF: Embryos placed in oiled gravel weathered with continuous seawater	Seawater mix 22 psu salinity	Lab				Incardona et al 2012
Cosco Busan bunker oil	Artificial	Zebrafish (*Danio rerio*)	Embryo	Cytotoxicity, cellular membrane damage, mortality	Chronic	24 h	WAF: Embryos placed in oiled gravel weathered with continuous seawater	Seawater mix 22 psu salinity	Lab			Pyrene, fluorathene, and chrysene contribute to toxicity	Incardona et al 2012
Alaska North Slope crude oil	Artificial: water passed through columns containing 6 g/kg of oil and gravel	Zebrafish (*Danio rerio*)	Embryo - Adult	Reduced larval survival, (5 to 17% increase mortality), changes in ventricular shape correlated with reduced adult swimming performance, reduced cardiac output	Chronic	48h	WAF: Oiled gravel effluent	Artificial seawater	Lab			Exposed as embryos then reared for 10-11months to detect effects on adults, small group exposed again at day 97 and found CYP1A induction played protective role.	Hicken et al. 2011

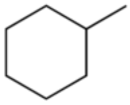
Pyrolitic fraction	No	Zebrafish (*Danio rerio*)	Embryo - Adults	Slower growth in mass and length, females with lower body mass. Disruption of jaw growth in larvae and malformation in adults. Specific activities of digestive enzymes reduced. Adult behaviour disrupted.	Chronic	9 months	Food spiked with complex mixture of PAHs	Fresh	Lab				Vignet et al. 2014a/b
Erika heavy oil	No	Zebrafish (*Danio rerio*)	Embryo – Adults	Slower growth in mass and length, males with smaller body mass. Disruption of jaw growth in larvae and malformation in adults. Specific activities of digestive enzymes reduced, Adult behaviour disrupted, most effected relative to light oil and	Chronic	9 months	Food spiked with complex mixture of PAHs	Fresh	Lab				Vignet et al. 2014a/b

Erika heavy oil	No	Zebrafish (*Danio rerio*)	Embryo – Adults	Slower growth in mass and length, males with smaller body mass. Disruption of jaw growth in larvae and malformation in adults. Specific activities of digestive enzymes reduced, Adult behaviour disrupted, most effected	Chronic	9 months	Food spiked with complex mixture of PAHs	Fresh	Lab				Vignet et al. 2014a/b

9.5 APPENDIX E.

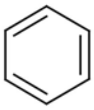
Saturates

Heptane
(linear alkane)

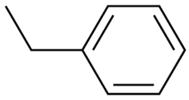


Methylcyclohexane
(branched cycloalkane)

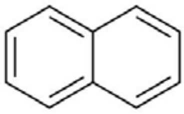
Aromatics



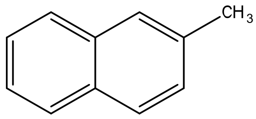
Benzene
(monoaromatic)



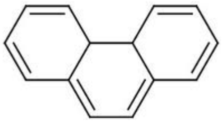
Ethylbenzene
(monoaromatic)



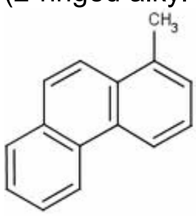
Naphthalene
(2-ringed PAH)



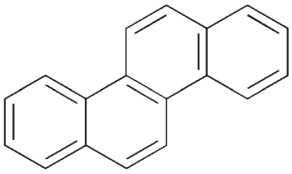
2-methylnaphthalene
(2-ringed alkyl PAH)



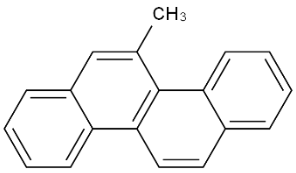
Phenanthrene
(3-ringed PAH)



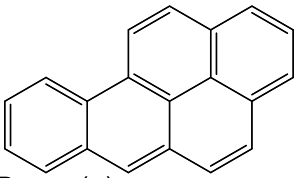
9-methylphenanthrene
(3-ringed alkyl PAH)



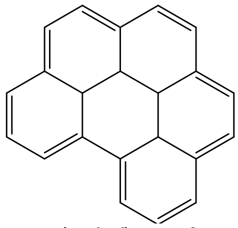
Chrysene
(4-ringed PAH)



5-methylchrysene
(4-ringed alkyl PAH)

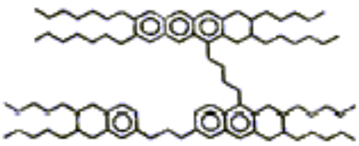


Benzo(*a*)pyrene
(5-ringed PAH)

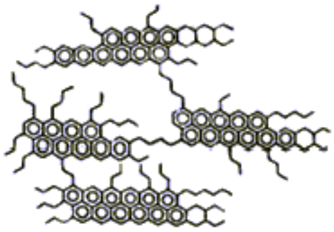


Benzo(*g,h,i*)perylene
(6-ringed PAH)

Resins and Asphaltenes



Resin



	Asphaltene

Table E.1. Representative structures of saturates, aromatics (e.g. PAHs), resins, and asphaltenes. The compounds shown in this figure represent only a tiny fraction of the compounds found in petroleum products. Figure adapted from Hodson et al. 2011