Nutrient linkages between freshwater and marine ecosystems: Uptake of salmon-derived nutrients in estuaries

by

Jennifer Kristine Chow
B.Sc., University of British Columbia, 2004

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**Abstract**

Anadromous Pacific salmon (*Oncorhynchus* spp.) return annually from marine ecosystems to their natal freshwater habitat to spawn and die. Runs of spawning salmon provide an important source of nutrients and energy to watersheds. However, in coastal systems, substantial amounts of salmon-derived nutrients can be exported back to estuaries. Human land use, including agriculture and urban development, also contribute substantial nutrients to coastal ecosystems, and have the potential to confound results from salmon-derived nutrient studies.

This thesis examines the influences of spawning salmon and human land use on stream nutrient and particulate dynamics, including export to estuaries. It also investigates the use of the stable isotope composition ($\delta^{13}C$ and $\delta^{15}N$) of estuarine clams, the varnish clam (*Nuttalia obscurata*: Reeve, 1857) and the manila clam (*Tapes philippinarum*), and their food sources, as indices of the freshwater export of salmon-derived nutrients to estuaries. Samples were collected from three nearby river-estuary systems along Southeast Vancouver Island, British Columbia. Study systems had either a large number of returning salmon and little human land use (Goldstream), few returning...
salmon and extensive human land use (Shawnigan), or few returning salmon and little human land use (Holland).

In Goldstream River, high abundance of salmon carcasses increased concentrations of total nitrogen and total phosphorus stream water below a barrier to upstream salmon migration. Carcasses also contributed substantial amounts of organic matter to the stream, as indicated by high $\delta^{13}C$ and $\delta^{15}N$, and corresponding low C:N ratios in suspended particulate organic matter. My calculations indicate that between 51-77% of the phosphorus transported upstream by migrating salmon, was exported back to the estuary. Human land use also increased downstream nutrient concentrations and raised baseline $\delta^{15}N$ in stream ecosystems, which is cause for concern and caution for salmon-derived nutrient studies in land use-affected watersheds, or in the reverse situation, for anthropogenic nutrient studies in watersheds that support runs of anadromous salmon.

The high $\delta^{15}N$ of anthropogenic nitrogen was not evident in the Shawnigan Estuary. In the Goldstream Estuary salmon-derived nutrients appeared to increase the $\delta^{15}N$ of clams, and both the $\delta^{13}C$ and $\delta^{15}N$ of sedimentary organic matter (SOM), with more enrichment in the high intertidal zone near the river mouth, than in the mid-intertidal zone. The stable isotope composition of clams and SOM was relatively constant across the period of salmon spawning and carcass decay, indicating that they may reflect a legacy salmon-derived nutrient input into estuaries.

This study demonstrates that substantial amounts of salmon-derived nutrients are exported back downstream to the Goldstream Estuary where they appear to become integrated into the estuarine food web. Data from a series of estuaries receiving a range of nutrients inputs from salmon is needed to confirm indices of salmon-derived nutrients in estuaries. There is also need for more extensive examination regarding the downstream effects of salmon-derived nutrients in areas such as estuarine productivity, community composition, and positive feedback mechanisms that influence salmon populations. This last area of research is of particular importance considering the high number of salmon stocks at risk in B.C.
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Chapter 1  General Introduction

Every year, millions of Pacific salmon migrate from the marine ecosystems to accessible freshwater streams, rivers, and lakes to spawn. In the Pacific Northwest, five species of the genus *Oncorhynchus* are both anadromous – meaning they return from the ocean to spawn in their natal streams, and semelparous – meaning they die after spawning once. After fertilization, female salmon incubate their eggs in gravel redds (nests), and fry emerge the following spring. Some species migrate downstream soon after emergence, while others spend up to two years rearing in freshwater before migrating towards marine ecosystems as smolts (Groot and Margolis 1991). Depending on the species, juvenile salmon spend the next one to five years feeding and growing in the ocean before returning to freshwater to spawn (Groot and Margolis 1991). It is during their spawning migration that Pacific salmon become important vectors for the transfer of nutrients and organic matter between marine and freshwater ecosystems (for reviews see Willson et al. 1998; Cederholm 1999; Gende et al. 2002; Naiman et al. 2002).

Runs of spawning salmon provide an important source of nutrients and energy to a wide array of consumers (Cederholm et al. 1999). Salmon are captured and consumed by terrestrial predators, including many species of mammals and birds that benefit from the spawning fish at a time when other food resources are becoming less abundant (for a review see Willson and Halupka 1995). Terrestrial insects, such as larval blowflies (Calliphoridae) feed on carcass materials left by predators, and on carcasses that are swept onto stream banks during high flows (Reimchen et al. 2002). Aquatic insects and fish also feed on carcasses and eggs that are held in shallow pools or amongst woody debris in the stream channel (Bilby et al. 1996; Chaloner et al 2002a).

Primary producers also take advantage of the nutrient subsidy provided by Pacific salmon (eg. Helfield and Naiman 2001; Johnston et al. 2004; Mathewson et al 2003). Riparian plants access salmon-derived nutrients through multiple pathways, including bear-mediated salmon carcass transfer (Reimchen 1994), bear urine and feces deposition (Hilderbrand et al. 1999), flooding events (Ben-David et al. 1998), and transfer through
the hyporheic zone (O'Keefe and Edwards 2002), which is the area that extends immediately below the water-substratum interface and laterally to the wetted margins of the stream (Cummins et al. 1995). In streams, microbial and invertebrate processing releases dissolved nutrients from carcasses, which can then be taken up by aquatic plants and algae (Johnston et al. 2004; Wipfli et al. 1998).

The upstream transfer of marine organic matter by spawning salmon has drawn much attention, however, these fish also mediate the transfer of nutrients out of freshwater ecosystems. Female salmon re-work the streambed gravel to build their redds (Montgomery et al. 1996). Redd construction re-suspends benthic sediment, which is exported downstream by the stream current (McConnachie and Petticrew 2006; Petticrew and Arocena 2003). Gravel cleaning by female salmon reduces hydraulic resistance time within the streambed, which reduces storage of nutrients and particulate organic matter in the hyporheic zone (Johnston et al. 2004). Species of salmon that spend time rearing in freshwater provide another means of salmon mediated nutrient export when smolts migrate downstream. Moore and Schindler (2004) calculated that, under certain circumstances, smolts could even export more nitrogen and phosphorus than their parents transported to freshwater from the ocean.

Salmon spawn in freshwater, but ultimately their carcasses are distributed among terrestrial, freshwater, and estuarine ecosystems (Cederholm and Peterson 1985, Cederholm et al. 1989). Scavenging carnivores deposit carcasses in riparian forest, while woody debris in the stream channel retains some carcasses in streams (Cederholm and Peterson 1985). Low scavenging by carnivores and high flows increase the downstream transport of carcasses (Brickell and Goering 1970; Cederholm et al. 1989; Richey et al. 1975), and in coastal streams many carcasses are exported to estuaries (Brickell and Goering 1970; Gende et al. 2004), along with carcass tissue fragments (McConnachie and Petticrew 2006) and dissolved nutrients (Sugai and Burrell 1984). Previous studies have estimated nutrient mass transport of salmon-derived nutrients, and indicated that between one and two thirds are exported downstream (Johnston et al. 2004; Mitchell and Lamberti 2005). These estimates suggest that salmon may play an important role in the flux of energy and nutrients to estuaries.
Salmon carcasses that are exported to estuaries could provide a valuable source of organic matter and nutrients to estuarine scavengers. Reimchen (1994) observed a number of marine invertebrate scavengers including whelks, starfish, shrimp, and crabs, feeding on salmon carcasses. He also performed field experiments where he anchored carcasses to the bottom of an estuary, and subsequently measured weight loss over time. Based on this study, he estimated that carcasses were completely processed within a week, which suggests that carcasses are readily processed in estuaries.

Salmon-derived nutrients can also stimulate primary production in estuaries, where nitrogen and phosphorus are often limiting nutrients (Rice and Ferguson 1975). Salmon contain large amounts of both of these nutrients (Robbins 1993), and depending on the species, adult salmon can contain up to 3.3% nitrogen and 0.48% phosphorus (Gende et al. 2004). Fujiwara and Highsmith (1997) linked salmon-derived nutrient inputs with increased production in Ulva sp., an estuarine macroalga, in Seldovia Bay, Alaska, using stable isotope data to provide nutrient tracer information.

Currently, in the southern part of their range, many salmon stocks are depressed or at risk due to large scale climatic forcing (Finney et al. 2002), over-utilization by commercial and recreational fisheries, and habitat degradation (Slaney et al. 1996). The decline of salmon populations is an even more widespread concern if coastal watersheds are adapted to the seasonal nutrient subsidy provided by carcasses, consistent with independent studies carried out by Larkin and Slaney (1997), Michael (1998), and Gresh et al. (2000). These studies estimated that streams in the Pacific Northwest currently receive as little as one tenth of the nutrients historically delivered by spawning anadromous salmon. Other studies have postulated that diminishing numbers of returning salmon may lead to decreased watershed productivity, further diminishing the likelihood of recovery for salmon populations (Bilby et al. 1996; Gresh et al. 2000). Mitigating actions such as fertilizing streams with inorganic nitrogen and phosphorus can increase algal standing stock, salmonid fry weights, and production (Stockner and Maclsaac 1996; Ashley and Slaney 1997; Perrin and Richardson 1997). However, anthropogenic nutrient additions do not replace the biomass-related flows of salmon and carcass tissue that are critical for many stream and riparian consumers (Gende et al. 2002).
Stable isotopes are the main tool used in this study, and so the following will briefly describe the theory behind their application in ecological studies and their particular use in salmon-derived nutrient studies. Stable isotope composition, denoted by ‘delta’ (δ), is expressed as the ratio of the abundance of heavy isotope and light isotope relative to a standard that is specific to each element. For carbon and nitrogen, the ratios $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ correspond with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. The unit for δ is parts per thousand or per mil (denoted as %o). Increases in this value indicate increases in the amount of heavy isotope with corresponding decreases in light isotope content. Conversely, decreases in the value of δ indicate decreases in the amount of heavy isotope with corresponding increases in light isotope content. For more detailed reviews see Peterson and Fry (1987) and Lajtha and Michener (1994).

Distinct differences exist in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of organic matter from freshwater, terrestrial ecosystems, and marine ecosystems. These differences are due to the sources of carbon and nitrogen available to primary producers, and to isotopic discrimination, also known as fractionation, during uptake and processing. Marine derived materials tend to be enriched in $^{15}\text{N}$ and $^{13}\text{C}$ relative to freshwater or terrestrially derived materials, with the exception of C4 plants that are also enriched in $^{13}\text{C}$ (Michener and Schell 1994; Peterson and Fry 1987). These differences allow stable isotopes to act as tracers for nutrient flow between freshwater/terrestrial and marine ecosystems.

Stable isotope composition changes in predictable ways as elements cycle through food webs. In consumers, metabolic processes favour particular isotopes, altering the stable isotope composition of organic matter. The $\delta^{13}\text{C}$ of an organism reflects that of its diet within ~ 1%o (Rau et al.1983; Fry and Sherr 1984). The $\delta^{15}\text{N}$ of an organism also reflects that of its diet, but with an increase of ~ 3.4%o at each trophic step (Deniro and Epstein 1981).

Pacific salmon gain most of their adult biomass in the ocean where they occupy a high trophic position in these food webs (Groot and Margolis 1991). Consequently, they are high in $^{13}\text{C}$ and $^{15}\text{N}$ relative to freshwater or terrestrial food sources (Kline et al. 1990). This difference allows stable isotope data to provide tracer information for the
flow of salmon-derived nutrients into terrestrial, freshwater, and estuarine ecosystems (Chaloner et al. 2002b; Kline et al. 1990; Mathewson et al. 2003).

When $\delta^{15}N$ is used to provide salmon-derived nutrient tracer information, many assumptions are made about alternate isotopic pools and competing factors that affect the abundance of $^{15}N$ (Kline et al. 1997). Examples of these factors include nitrogen availability, denitrification, and trophic enrichment. In aquatic habitats, nitrogen pool depletion can lead to an increase in $^{15}N$ in the remaining nitrogen, resulting from a preference by primary producers for $^{14}NO_3^-$ and $^{14}NH_4^+$ (Kline et al. 1997). Denitrification is generally mediated by heterotrophic bacteria under anoxic or suboxic conditions. This process results in the reduction of nitrite and nitrate to gaseous nitrogen forms, which significantly increases the concentration of $^{15}N$ in the remaining nitrogen pool (Kreitler 1979; Heaton 1986). Trophic enrichment increases the $^{15}N$ in organisms at higher trophic levels, and will vary depending on food chain length (Cabana and Rasmussen 1994). Among the published salmon-derived nutrient studies, most research is descriptive, not experimental, and confounding factors between sites are poorly quantified. Stable isotope data in ecological studies are further complicated by a lack of standardized sample treatment protocols which can make comparisons between studies difficult (Jacob et al. 2005).

Many salmon streams along Southeast Vancouver Island drain land use-affected watersheds, and thereby receive allochthonous nutrient inputs from anthropogenic sources in addition to those from returning salmon. Nitrogen derived from anthropogenic sources such as livestock and human effluent tends to have higher $\delta^{15}N$ than that of terrestrial organic matter (Kreitler 1979; Heaton 1986). This observation has allowed researchers to identify anthropogenic nitrogen inputs to aquatic ecosystems (Aravena et al. 1993; Cabana and Rasmussen 1996; McClelland and Valiela 1998; Anderson and Cabana 2006). Habitat degradation caused by human land and water use poses one of the greatest threats to salmon stocks in B.C. (Slaney et al. 1996; Bradford and Irvine 2000). Human population growth across Southeast Vancouver Island is projected to increase by more than 25% over the next 24 years (BC STATS 2006), with corresponding increases in human land use. Nutrient inputs from anthropogenic sources are a concern for most of the world (Vitousek et al. 1997), and in coastal ecosystems, it is likely that human land
use and spawning salmon have confounding influences in stream ecosystems. However, this topic has not been addressed by previous studies.

The stable isotope composition of consumers can provide a long-term, integrated perspective on the carbon and nitrogen sources that are important for secondary production in estuaries (Fry 1999). Estuaries are supplied with a variety of potentially important sources of organic matter including marsh grasses, plankton, benthic algae, eelgrass, chemosynthetic and photosynthetic bacteria, and organic matter from river inputs (Peterson et al. 1985), the latter of which includes salmon carcasses and anthropogenic nutrient inputs. The stable isotope composition of bivalves may be a useful index of the organic matter supplying estuaries because bivalves are widely distributed geographically, they are sedentary, and they often have stable local populations that can be sampled repeatedly (Farrington et al. 1983).

In this thesis I used the stable isotope composition of clams and their potential food sources to evaluate the importance of freshwater export of salmon-derived nutrients to estuaries. The varnish clam (*Nuttalia obscurata*: Reeve, 1857) and the manila clam (*Tapes philippinarum*) are not native to B.C., but have become well established in bays and estuaries throughout the Straight of Georgia (Bourne 1982; Gillespie and Kronlund 1999). Both species grow at similar rates, achieving approximately 38 mm in length after four years (DFO 2001). They also have similar diets (Kanaya et al. 2005), but generally occupy different portions of the intertidal zone (DFO 2001). Competition studies indicate that the two species do compete for resources; however, varnish clams have the advantage in the high intertidal zone, and manila clams have the advantage in the mid-intertidal zone (DFO 2001).

The varnish clam is originally native to Korea, China, and southern Japan (Gillespie and Kronlund 1999), and was first reported in the Pacific Northwest in Semiahmoo Bay, Washington, in 1991 (Forsyth 1993). Varnish clams inhabit the high intertidal zone, and are capable of suspension feeding – meaning they selectively consume particulate organic matter from the water column, and deposit feeding – meaning they collect organic matter from the sediment using the foot (Parker and Reid *unpublished manuscript*).
The manila clam was first found in Ladysmith Harbour in 1936, and has since become the most common bivalve in some areas of the Strait of Georgia (Bourne 1982). Manila clams inhabit the mid- to high intertidal zone, and rely solely on suspension feeding, although research by Kanaya et al. (2005) indicates that manila clams also consume organic matter from the sediment when particles become re-suspended in the water column.

The effects of salmon-derived nutrients in estuaries have received limited investigation, although it is evident that in some systems large amounts of carcasses and nutrients are flushed downstream during high flows. The goals of this research were to (1) evaluate the potential influence of human land use in salmon-derived nutrient studies; (2) estimate the percent of salmon-derived nutrients that are exported from freshwater; (3) measure whether salmon-derived nutrients exported from freshwater ecosystems become integrated into estuarine ecosystems, and (4) test if salmon-derived nutrients are an important subsidy to estuaries. In Chapter 2, I examine the nutrient and particulate dynamics, including export to estuaries, in streams with varying numbers of spawning salmon, and varying amounts of human land use. In Chapter 3, I use stable isotopes of carbon and nitrogen, in attempt to trace the flow of salmon-derived nutrients into estuarine clams and their food sources. In Chapter 4, I conclude with a synthesis of the research presented in this thesis, and discuss the possible implications of salmon-derived nutrient subsidies to estuaries (i.e. community structure, productivity, feedback mechanisms).
Chapter 2  The confounding influences of spawning Pacific salmon (*Oncorhynchus* spp.) and human land use on nutrient and particulate dynamics in coastal streams of Southeast Vancouver Island, British Columbia

2.1 Abstract

Anadromous Pacific salmon (*Oncorhynchus* spp.) transport substantial amounts of nutrients into coastal watersheds, as do human land use activities such as agriculture and urban development. These sources of allochthonous nutrient have gained much attention in the past few decades. However, their combined influences in stream ecosystems have not been addressed. This study compares the effects of spawning salmon and human land use on stream nutrient and particulate dynamics. Samples were collected from three nearby watersheds with (1) a large number of returning salmon and little human land use, (2) few returning salmon and extensive human land use, and (3) few returning salmon and little human land use (reference system). Spawning salmon increased the amount of suspended particulate organic matter (SPOM) in stream water, except in streams where few salmon returned to spawn. Elevated downstream total phosphorus (TP) and total nitrogen (TN) concentrations were associated with both salmon carcasses and human land use; however, temporal patterns depended on the nature of the nutrient input. Anthropogenic and salmonid nutrient inputs appeared to more than double the estimated export of TN and TP (kg · km$^2$), compared to a reference system, and my calculations indicate that between 51-77% of phosphorus transported to freshwater by returning salmon, was exported back downstream to the estuary. Salmon carcasses also contributed substantial quantities of particulate organic matter to the stream water, as indicated by high SPOM $\delta^{13}$C and $\delta^{15}$N and corresponding low C:N ratios; however, high $\delta^{15}$N values were also associated with human land use. These findings highlight the complexity of allochthonous nutrient fluxes into and out of coastal watersheds, and suggest a possible role for salmon-derived nutrients in estuarine nutrient cycling.
2.2 Introduction

Research from over four decades has revealed the importance of anadromous Pacific salmon (*Oncorhynchus* spp.) as a source of food and nutrients for watersheds of the Pacific Northwest (for reviews see: Willson et al. 1998; Cederholm 1999; Gende et al. 2002; Naiman et al. 2002). Salmon-derived nutrients are incorporated into freshwater and terrestrial ecosystems through multiple pathways including autotrophic uptake, uptake of dissolved organic matter by stream biofilm, and direct consumption (Cederholm et al. 1999; Chaloner et al. 2002). The waterborne nutrients and tissue fragments from carcasses that are not incorporated into watersheds are exported to downstream reaches, lakes, and estuaries (Wipfli et al. 1998; McConnachie and Petticrew 2006).

The decomposition of salmon carcasses in stream channels releases significant amounts of nutrients into the water column. Johnson et al. (2004) observed that the abundance of salmon carcasses was directly related to stream nutrient concentrations. Additional studies also observed increased nutrient concentrations while salmon carcasses decomposed in streams (Brickell and Goering 1970; Richey et al. 1975; Mitchell and Lamberti 2005), with reaches downstream of barriers to salmon migration having significantly higher concentrations of nitrogen and phosphorus relative to upstream reaches. Trends reported for organic carbon concentrations were not consistently related to the presence of salmon carcasses.

Stable isotope analysis provides a more direct method for measuring the contributions of salmon-derived nutrients to watersheds (eg. Kline et al. 1990; Bilby et al. 1996; Chaloner et al. 2002). The carbon and nitrogen stable isotope composition of marine organic matter reflect enrichment with the heavier isotopes of carbon (\(^{13}\)C) and nitrogen (\(^{15}\)N) relative to freshwater and terrestrial organic material (Peterson and Fry 1987). Pacific salmon gain most of their adult biomass in marine ecosystems; consequently, the \(\delta^{13}\)C and \(\delta^{15}\)N of their tissues are elevated when they return to their natal streams to spawn. The returning adult salmon stop feeding once they enter freshwater, and thus remain isotopically distinct from freshwater and terrestrial sources of organic matter (Kline et al. 1990).
Stable isotope data and the associated carbon to nitrogen (C:N) ratios were used by McConnachie and Petticrew (2006) to assess the dominance of salmon-derived nutrients in stream suspended particulate organic matter (SPOM). Salmon muscle has a low C:N ratio (3.4:1) relative to terrestrially derived (36:1) and freshwater derived (10.2:1) sources of organic matter (Elser et al. 2000; McConnachie and Petticrew 2006), which can help to differentiate salmon tissue in the SPOM. Lower C:N ratios also imply better quality food resources for suspension feeders because nitrogenous materials are often limiting to consumer organisms (Sterner and Hessen 1994; Bouillon et al. 2000; Elser et al. 2000). Organic matter from salmon that is not consumed is readily exported to downstream habitats by the stream current.

Habitat degradation caused by human land use poses one of the greatest threats to salmon stocks in B.C. (Slaney et al. 1996; Bradford and Irvine 2000), and can confound the influences of salmon carcasses on nutrient and particulate dynamics in streams. Urbanization has proceeded rapidly along the east coast of Vancouver Island, causing direct salmon habitat losses, changes in river and riparian habitats, pollution from sewage, storm-water, and landfills, and changes in water tables or run-off patterns (Slaney et al. 1996). Agriculture and urban development often contribute large amounts of nitrogen and phosphorus to watersheds (Vitousek et al. 1997; Carpenter et al. 1998). Consequently, stream nitrogen and phosphorus concentrations are useful indicators of the prevalence of these types of land use (Gergel et al. 2002). Stable isotopes of nitrogen can also be used to identify the contribution of anthropogenic nitrogen to watersheds (Aravena et al. 1993; Cabana and Rasmussen 1996; McClelland and Valiela 1998; Lake et al. 2001; Anderson and Cabana 2006) because livestock manure and human wastewater are enriched in $^{15}$N relative to freshwater and terrestrial organic materials (Kreitler 1979; Heaton 1986).

This study compares the effects of spawning salmon and human land use on stream nutrient and particulate dynamics. I predict that both salmon streams and land use-affected streams will receive substantial allochthonous nutrient inputs resulting in increased downstream nutrient concentrations and high $\delta^{15}$N values relative to a reference system. The specific objectives of this study are to (1) determine if salmon carcasses alter stream nutrient and particulate dynamics; (2) estimate freshwater export of salmon-
derived nutrients to estuaries; and (3) assess the extent to which human land use can confound the interpretation of data normally used to identify the influence of spawning salmon.

2.3 Methods

2.3.1 Site Description

This study examines Goldstream, Shawnigan, and Holland Rivers located in three separate watersheds along Southeast Vancouver Island, British Columbia (Figure 2.1). This area is located in the Pacific southwest of Canada, in the coastal douglas-fir biogeoclimatic zone, and has a relatively mild climate with wet winters and drier summers. Mean monthly air temperature across all watersheds ranges from 2.7°C to 17.9°C, with a mean annual precipitation of 116 cm (Environment Canada 2004). In 2005, all the rivers had similar precipitation and discharge trends with mean annual discharge for ranging between 1-2 m$^3$·s$^{-1}$, with a period of low precipitation and discharge from late November to early December (Figure 2.2). Three species of salmon spawn in these watersheds: Oncorhynchus keta (chum), O. kisutch (coho), and O. tshawytscha (chinook) (Ministry of Environment 2001), and all study rivers have been influenced to some extent by hatchery or salmon enhancement programs.

2.3.1.1 Goldstream River

Goldstream River drains a 47.5 km$^2$ forested watershed, that is subject to low intensity forest management practices, some urban development, and controlled water through dams upstream (Figure 2.3 e). The majority of the watershed is protected in Goldstream Provincial Park, and the nearby protected drinking water reservoir located upstream. Goldstream River receives annual runs of salmon that are generally in the tens of thousands, with chum making up the majority of the returning salmon, along with small populations of coho and chinook that are supplemented by hatchery fry. A small waterfall approximately 2.2 km from the river mouth hinders the upstream migration of chum. Further upstream, approximately 5.5 km from the river mouth, Japan Gulch dam
halts the upstream migration of coho and chinook. Over the last 10 years Goldstream received a mean annual escapement of over 33,000 salmon. In 2005, ~10,000 salmon returned to spawn, but this escapement size was still orders of magnitude greater than either of the other two rivers in this study (Figure 2.4).

2.3.1.2 Shawnigan River

Shawnigan River drains a 113.2 km$^2$ watershed, subject to extensive forest management practices, with agriculture and urban development using most of the remaining land base (Figure 2.6a). Shawnigan Lake is a dominant feature in the landscape and the main draw for urban development. The residential population living around the lake has experienced considerable growth over the past 15 years, nearly doubling from 1986 to 2001 (Statistics Canada 2004). Residential density is highest at the north end of the lake by the outlet to Shawnigan River. Septic systems are the main method of disposing of household effluent around the lake, and septic contamination is a concern during periods of heavy rains and the fall freshet (Rieberger et al. 2004). Anderson and Cabana (2006) found that the $\delta^{15}$N of aquatic consumers started to increase noticeably in a lightly developed watershed with less than 5% of the land base devoted to agriculture or fewer than 19 inhabitants per km$^2$. Shawnigan Lake watershed has approximately 10% of its land base devoted to agriculture, and greater than 60 inhabitants per km$^2$ (Statistics Canada 2004). An impassable waterfall in the tidal area prevents upstream migration of salmon. However, since the late 1970’s, the stream has been stocked with hatchery coho fry that are able swim over the waterfall as smolts. When the mature adults return to spawn, local volunteers transport the salmon above the waterfall to upstream spawning habitat. In 2005, only 11 coho returned to Shawnigan River to spawn, so I expect human land use to be the dominant source of allochthonous nutrients in this system.

2.3.1.3 Holland River

Holland River drains a 32.2 km$^2$ watershed that was reforested during the 1960s and 70s to restore the watershed from extensive forest management practices (Figure 2.3b). The watershed has since recovered hydrologically (Pommen 1996), and is presently mostly forested with some urban development. Historically, Holland River supported
major runs of chum and coho salmon, with spawners returning in the thousands; however, the number of returning salmon has steadily declined since the early nineties (Figure 2.4). The mean escapement from 1995 to 2003 was less than 200, and Fisheries and Oceans Canada (DFO) data indicated that no surveys were conducted in 2004 and 2005. Judging by historical trends and my own estimates derived from walking the stream bank every two weeks, I am confident that fewer than 200 salmon returned to spawn in Holland River in 2005.

2.3.2 Sample Collection and Analysis

Water samples from all systems were collected from stream surface water, from September 2005 to February 2006. Water samples from Goldstream River were also collected from September 2004 to February 2005 for total nitrogen and total phosphorus measurements. Salmon dorsal muscle tissue was collected by volunteers from the Goldstream Volunteer Salmonid Enhancement Association in 2005 for analysis of $\delta^{13}$C and $\delta^{15}$N, as well as C:N ratio.

2.3.2.1 Nutrient Analysis

Water samples were collected every two weeks from upper reaches and lower reaches, located near the river mouths (Figure 2.5). In Goldstream and Holland Rivers the upper reaches were above barriers to salmon migration, and in Shawnigan River, Shawnigan Lake was sampled at the upper reach. Stream water concentrations of total nitrogen (TN), total phosphorus (TP), and total organic carbon (TOC) concentrations were measured for all water samples.

River water was collected for TN and TP analysis in acid-washed 250 ml plastic bottles that had been immersed in 10% HCl for 24 hours, and then rinsed 6 times with distilled deionized water. Samples were stored in a cooler shortly after collection, and then frozen at $-20^\circ$C within 8 hours. TN and TP were determined colourimetrically within a month of collection by flow injection analysis on a Lachat autoanalyzer, Lachat QuickChem® FIA+ 8000 series, following QuickChem® Methods 10-107-04-1-C and 10-115-01-1-B respectively.
Additional river water samples were collected for TOC analysis in ashed (500°C for 6 hours) 30 ml glass vials that had thick silicone rubber backed TFE septa with open ring caps. These caps produce a positive seal and reduce exposure to atmosphere. Samples were stored in a cooler shortly after collection and then transferred to a dark fridge at 5°C within 8 hours. TOC concentration was determined from these samples within a week of sample collection by oxidative combustion-infrared analysis on a Shimadzu Total Organic Carbon Analyzer, TOC-V CPH.

2.3.2.2 Suspended Particulate Organic Matter (SPOM) Analysis

Suspended particulate matter was collected on a monthly basis from the lower reaches to measure the concentration of suspended particulate organic matter (SPOM), and for stable isotope analysis. Surface water samples were collected in acid-washed 4L plastic containers, and pre-filtered through a 200 µm mesh filter to remove large debris. River water was then filtered through two replicate, pre-combusted (500°C for 1 hour), pre-weighed 25 mm Whatman GF/F filters until the filters were clogged (500 – 2500 mL).

Filters were stored in petri dishes at –20°C prior to freeze drying. One filter was exposed to concentrated HCl fumes to remove inorganic carbon, and then oven dried (60°C for 24 hours) prior to δ¹³C analysis. The other filter was not acid-fumed, in order to prevent the loss of particulate nitrogen and/or alteration of the δ¹⁵N values of the SPOM (Lorrain 2003). Samples were analyzed for δ¹³C and δ¹⁵N, as well as C:N ratio on a Thermo Delta Plus continuous flow isotope ratio mass spectrophotometer coupled to a Costech elemental analyzer at the Water and Watershed Laboratory, University of Victoria, British Columbia, Canada (see Matthews and Mazumder 2003 for details).

SPOM concentration was estimated by determining ash free dry mass of the suspended particulates per litre of river water filtered. Filters were dried (60°C for 72 hours) and then weighed to determine the dry weight of the filter and particulate matter. Subsequently, filters were ashed at 500°C, and reweighed to determine the ashed weight of the remaining inorganic matter and the filter. Ash free dry mass was calculated by subtracting the weight of the ashed filter from that of the dried filter.
2.3.2.3 Statistical Analysis

The objective of this work was to determine the effects of salmon carcasses on stream nutrient concentrations, and on the concentration and stable isotope composition of SPOM in Goldstream River. To do so, carcass abundance was estimated using an exponential mass loss model modified from Johnston et al. (2004):

$$\text{Carcasses}_t = \text{Carcasses}_{t-1} \times e^{-kT} + \text{Salmon}_{t-1}$$

Where $\text{Carcasses}_t$ is the number of carcasses in the river at time $t$, $\text{Carcasses}_{t-1}$ is the number of carcasses in the previous time step, $k$ is the daily loss rate, $T$ is the elapsed times in days from $t-1$ to $t$, and $\text{Salmon}_{t-1}$ is the number of live salmon observed in the river in the previous time step. During salmon spawning carcass abundance was calculated in weekly time steps because live salmon abundance was measured on a weekly basis. The daily loss rate of 0.0338 was calculated by Johnston et al. (2004) using data on the temporal changes in the abundance of sockeye carcasses from Bivouac and Forfar Creeks, both of which flow into Takla Lake in interior B.C.. This value is similar to the daily loss rate of 0.033, calculated by Chaloner et al. (2002) using data from pink carcasses in south-eastern Alaska streams. Daily loss rate includes decomposition, fragmentation, downstream transport, and consumption by scavengers. Live spawning salmon were counted during weekly stream bank walks by the Goldstream Volunteer Salmonid Enhancement Association and Fisheries and Fisheries and Oceans Canada staff. These values were added to the carcass total the week following data collection because salmon are generally moribund or dead a week following spawning (Groot and Margolis 1991), at which point they become easy prey to predators, while also releasing nutrients and carcass fragments into streams.

Scatter plots and Pearson’s correlation coefficient were used to explore the relationships between carcass abundance and concentrations of TN, TP, and TOC. For Goldstream River, analysis of covariance (ANCOVA) was used to determine if separate regressions were necessary to describe the relationships in 2004 and 2005. Subsequently, simple linear regression was used to fit lines to the significant relationships.
Concentrations of TN, TP, and TOC in lower reaches were compared with upper reaches using paired t-tests for each variable in each river (as per Mitchell and Lamberti 2005). Multiple paired t-tests increase the chance of Type 1 error (finding a significant correlation when none exists) because as more tests are performed the greater the chance of finding a significant result when none exists. The sequentially rejective multiple test procedure prescribed by Holm (1979) was used to correct for multiple testing so that the overall alpha level remained near 0.05.

Total nutrient and particulate export was calculated based on mean monthly nutrient and particulate concentrations in lower reaches, and mean monthly discharge values. Discharge values for Shawnigan River were obtained from Environment Canada (2006). Daily discharge values were estimated for Holland River using a computer model adapted from Arp and Yin (1992) that predicts discharge based on daily precipitation and air temperature in watersheds. Daily discharge values for Goldstream River were calculated using upstream discharge data collected in 2001 by the Capital regional District (CRD) of Victoria, and downstream discharge data collected by the Ministry of Environment. For Goldstream River, the simple linear regression of downstream discharge on upstream discharge showed a strong, significant relationship ($F_{1, 4339} = 115 \, 004, P < 0.001, R^2 = 0.964$):

\[
\text{Downstream discharge} = 2.666 \times \text{Upstream Discharge} - 0.069
\]

Downstream discharge data were collected approximately 3 km upstream from the mouth of the stream, so it is a conservative estimate of total discharge. Additional water from runoff, groundwater, and other smaller streams likely increased the total discharge at the river mouth.

Analysis of variance (ANOVA) was used to compare upstream nutrient concentrations among streams. Tukey’s honestly significant difference (HSD) pairwise comparisons were used to identify specific differences among streams. Levene’s test was used to test homogeneity of variance. Normality was assessed for all statistical tests using Shapiro-Wilk’s test.
For all statistical tests, dependent variables were natural log transformed to correct for normality and serial autocorrelation, thereby minimizing the influence of time series trends. All statistics were carried out using SPSS version 14.0.

2.4 Results

2.4.1 Carcass Abundance

Salmon abundance in Goldstream River reached its peak in mid-November (Figure 2.6 a). The exponential mass loss model predicts that the instantaneous carcass abundance reached its peak approximately two weeks afterwards (Figure 2.6 b), which corroborates stream bank walk observations (Arthur Inglis, Goldstream Volunteer Salmonid Enhancement Association, unpublished data). Based on the model, I calculated that by the end of December approximately 75% of all carcasses were processed, and by the end of February less than 5% of carcass materials remained in the stream.

2.4.2 Nutrient Concentrations

Among all streams, upstream and downstream TOC concentrations varied similarly over time, whereas TN concentrations varied less at upper reaches than at lower reaches (Figure 2.7 a-i and Figure 2.8). Upstream TP concentrations were relatively constant and in all streams; however, downstream TP concentrations increased dramatically during distinct time periods.

Goldstream and Shawnigan Rivers had significantly higher concentrations of TN and TP at lower reaches relative to upper reaches (Paired t-tests, see Table 2.1). None of the streams had significant downstream enrichment in TOC, nor did concentrations of TN and TP vary significantly between upper and lower reaches in Holland River.

TP and TN concentrations at the mouth of Goldstream River increased in late November during peak carcass abundance (Figure 2.7 a, d and Figure 2.8 a, b). TP concentrations were significantly correlated with carcass abundance in 2004 ($r_{\text{Pearson}} = 0.764, N = 10, P = 0.010$), but not in 2005 ($r_{\text{Pearson}} = 0.640, N = 9, P = 0.063$). TN
concentrations were not correlated with carcass abundance (2004: $r_{\text{Pearson}} = 0.285$, N = 10, $P = 0.425$; 2005: $r_{\text{Pearson}} = 0.328$, N = 9, $P = 0.389$). High concentrations before the arrival of salmon in September, and in late February, indicate that lower reaches of Goldstream River received nitrogen inputs from other sources besides salmon. TOC concentrations in Goldstream River had small peaks that were not correlated with carcass abundance ($r_{\text{Pearson}} = 0.355$, N = 9, $P = 0.348$). Rather, TOC concentrations appear to be directly correlated with precipitation and discharge since low TOC concentrations occurred in late November early December during the period of low precipitation and discharge (Figure 2.2 b and Figure 2.7 g). Downstream concentrations of TN, TP, and TOC at Holland and Shawnigan Rivers also appear to be correlated with precipitation (Figure 2.2 c and Figure 2.7); but with less extreme variation in Holland River relative to Shawnigan River.

TP concentrations in Goldstream River were strongly correlated with carcass abundance, and peak concentrations between years were proportional to the number of returning salmon. Simple linear regression of TP concentrations on estimated carcass abundance showed a significant positive relationship for both 2004 and 2005. Full factorial ANCOVA showed that there was no significant interaction between year and TP concentration ($F_{1, 15} = 0.263$, $P = 0.615$). In the subsequent ANCOVA, without the interaction, year was not significant ($F_{1, 16} = .759$, $P = 0.397$), consequently data from 2004 and 2005 was pooled into a single simple linear regression, which describes the relationship between carcass abundance and TP concentration ($F_{1, 18} = 33.64$, $P < 0.001$, $r^2 = 0.66$):

$$TP = 8.69 \times \text{Carcass Abundance} + 0.002$$

Export of TP (kg·km$^{-2}$) from Goldstream River was greater in 2004 than in 2005 (Table 2.2), and this difference was proportional to the number of spawning salmon. TN export from Goldstream River was similar between years, and did not reflect differences in salmon escapement. Compared to Holland River where few salmon returned to spawn, Goldstream River exported about twice as much TN, TP and SPOM, but similar amounts of TOC. Shawnigan River exported similar amounts of TN and TP as Goldstream River,
intermediate amounts of SPOM, and the highest amount of TOC. For all streams, nutrient and particulate export was highest in January, which was the period of highest precipitation and discharge for all systems (Figure 2.2).

2.4.3 Suspended Particulate Organic Matter (SPOM)

SPOM concentrations were relatively constant in all streams except Goldstream River, where shortly after the return of salmon, SPOM concentrations more than tripled, reaching a mean of 2407 µg/L (Figure 2.9 a). Near the end of salmon spawning, in late November, SPOM concentrations were back down to pre-salmon levels.

Salmon muscle had lower a C:N ratio, and heavier stable isotope composition than SPOM from all stream in all months (Table 2.3). In Goldstream River, the stable isotope composition and associated C:N ratios of SPOM exhibited seasonal patterns that were distinct from the SPOM in Holland and Shawnigan Rivers. During high carcass abundance in November and December (Figure 2.6 b), the SPOM δ¹³C and δ¹⁵N increased to peak values of -24.0‰ and 10.4‰, respectively (Figure 2.9 b, c and d). During the same two months, the SPOM δ¹³C and δ¹⁵N from Holland and Shawnigan Rivers were relatively constant, with mean values of -28.9‰ and 3.1‰, respectively. C:N ratios in these two streams increased to approximately 19.4 during this same time, in contrasts with the decrease to 8.4 in Goldstream River.

SPOM collected in September from Shawnigan River had peak δ¹³C and δ¹⁵N values of -25.4‰ and 7.9‰, respectively. These values were nearly as high as the peak values in Goldstream River in December; however, SPOM δ¹³C and δ¹⁵N in Shawnigan River subsequently decreased in October and remained low for the duration of the study. The SPOM δ¹³C dropped below values observed in Holland River, and SPOM δ¹⁵N remained intermediate between values observed in Goldstream and Holland Rivers. Holland River had the lowest SPOM δ¹⁵N throughout the study, with values that varied between -2.1‰ and 3.8‰.
2.5 Discussion

In the Goldstream River, salmon carcasses were associated with elevated downstream nutrient concentrations, higher SPOM δ¹³C and δ¹⁵N, and lower C:N ratios relative to conditions before the return of spawning salmon. Human land use in the Shawnigan River was also associated with higher downstream nutrient concentrations, and higher baseline δ¹⁵N values compared to Holland River where there was little human land use. Coastal streams that received nutrients subsidies from either anthropogenic or salmonid sources appear to export more total nutrients and particulates per square kilometre of watershed compared to a stream that received less external nutrients. In the following sections I begin by discussing the factors that influence retention and export of salmon-derived nutrients in watersheds. I then compare the effects of human-land use and spawning salmon on nutrient and particulate dynamics in streams.

2.5.1 Salmon-Derived Nutrient and Particulate Inputs

2.5.1.1 Nutrient Concentrations

The accumulation of salmon carcasses in Goldstream River appeared to create a pulse of waterborne nutrients. Goldstream and Holland Rivers had similar upstream nutrient concentrations in reaches above barriers to salmon migration, and both streams support annual runs of Pacific salmon. However, in 2005 Goldstream River received over ten thousand returning salmon, whereas historical trends suggest that Holland River received less than two hundred. Higher downstream concentrations of TN and TP were associated with high carcass abundance in Goldstream River, whereas nutrient concentrations in Holland River showed little difference between upper and lower reaches during salmon spawning and carcass decomposition. Neither stream had any significant downstream enrichment in TOC. Rather than being linked to carcass abundance, TOC concentrations were associated with precipitation and discharge patterns, potentially reflecting inputs of terrestrial organic carbon carried to streams in groundwater and/or runoff.

The presence of salmon carcasses in stream channels is known to increase stream water nitrogen concentrations (Chaloner et al. 2002; Johnston et al. 2004). Downstream
TN concentrations peaked during the peak abundance of carcasses in Goldstream River in both 2004 and 2005. However, TN concentrations and monthly TN export was not significantly related to salmon escapement between years. Ammonium (NH$_4^+$) is released from salmon and gametes during spawning (Gende et al. 2002), and from decomposing carcasses (Hargreaves 1998). Previous studies correlated stream NH$_4^+$ concentrations with carcass biomass (Brickell and Goering 1970; Sugai and Burrell 1984; Mitchell and Lamberti 2005; Chaloner et al. 2007), thus NH$_4^+$ concentrations might have been a better index of nitrogen release by salmon carcasses. High TN concentrations were also observed in Goldstream River in the absence of salmon carcasses, which indicates that lower reaches in Goldstream River received nitrogen inputs from additional sources besides salmon carcasses. TN concentrations were not an accurate means of identifying nitrogen inputs from salmon carcasses in Goldstream River; however, NH$_4^+$ concentrations may provide a more salmon-specific alternative for future studies.

In Goldstream River, the abundance of salmon carcasses was strongly correlated with downstream TP concentrations. Previous studies also observed that stream phosphorus concentrations varied predictably with the number of salmon carcasses (O'Keefe and Edwards 2002; Johnston et al. 2004). Johnston et al. (2004) modeled the rate at which phosphorus was lost from salmon carcasses, and the rate of loss was negligible after four to six weeks. At this time only refractory phosphorus remained, mostly in the salmon skin and bones. In this study, TP concentrations returned to pre-spawning levels approximately five weeks after the end of spawning, in early January. High flows observed in the early winter in Goldstream River might have contributed to the downstream export of salmon carcasses at this time, thereby removing the source of phosphorus in the lower reaches.

If productivity in coastal ecosystems is nutrient limited, then nitrogen and phosphorus from carcasses can provide a valuable nutrient subsidy. Phosphorus generally limits primary production in streams (Bothwell 1985; Schlesinger WH 1997); however, nitrogen is also important depending on the geological substrate (Rier and Stevenson 2006). In some freshwater ecosystems salmon are the dominant source of nitrogen supplying food webs (Kline et al. 1990), and spawning salmon can significantly increase stream biofilm and macroinvertebrate abundance (Helfield et al. 1998). Nutrient
subsidies from carcasses can increase primary productivity in streams. However, low light levels with the onset of fall and winter conditions limit autotrophic nutrient uptake in the late summer and fall (Bothwell 1988; Bilby et al. 1996).

In Goldstream River, higher concentrations of TN and TP below salmon spawning reaches compared to upstream reaches were observed in the presence of salmon carcasses, which suggests substantial water-borne export of salmon-derived nitrogen and phosphorus. Whole carcasses were also exported into the estuary and can be added to the proportion of water-borne salmon-derived nutrients exported downstream. Based on the values in Table 2.2 and Table 2.4 I estimate that between 51% and 77% of salmon-derived phosphorus was exported from Goldstream River to the estuary during the fall months. These estimates are likely affected by variation in escapement between years, environmental conditions such as temperature, which regulates decomposition (Minshall et al 1991), and discharge patterns, which regulate transport of whole carcasses into estuaries (Brickell and Goering 1970; Cederholm et al. 1989; Richey et al. 1975). Large variation in downstream TN concentrations in the absence of salmon, made it impossible to accurately estimate the amount of salmon-derived nitrogen that was exported to the estuary. Previous studies that examined the fate of salmon-derived nitrogen and phosphorus estimated similar ranges values for export to a downstream lake (N: 61-74%, P: 47-55%; Johnston et al. 2004) and to an estuary (N: 46%, P: 60%; Mitchell and Lamberti 2005). Variation in the relative export of salmon-derived nitrogen and phosphorus could be due to differences in the N:P composition among salmon species, as well as to the methods used to estimate percent export between the two studies. Substantial amounts of both salmon-derived nitrogen and phosphorus appear to be exported downstream in both studies however, which may also be the case in the Goldstream system even though the TN data collected for this study did not reveal distinct trends related to salmon carcass.

### 2.5.1.2 Suspended Particulate Organic Matter (SPOM)

In Goldstream River, salmon carcasses contributed substantial amounts of organic matter to the SPOM pool. Salmon muscle tissue had high stable isotope values and low C:N ratios (Table 2.3) compared stream SPOM. As salmon carcasses accumulated in
Godlstream River, SPOM $\delta^{13}$C and $\delta^{15}$N increased, with a corresponding decrease in C:N ratio. During salmon spawning and carcass decomposition in Holland River, stable isotope composition of SPOM remained low and relatively constant, and C:N ratios increased. These data suggest that the dominant source of organic matter to Holland River was riparian vegetation entering the river through litterfall and runoff (McConnachie and Petticrew 2006). Approximately five weeks after the end of spawning, SPOM from Goldstream and Holland Rivers had similar stable isotope compositions and C:N ratios, corroborating TP data, indicating that the majority of carcass materials were processed by early January.

2.5.2 Comparison of Spawning Salmon and Human Land Use

2.5.2.1 Nutrient Concentrations

Goldstream and Shawnigan Rivers both receive large external nutrient inputs compared to Holland River. Goldstream River receives large annual runs of spawning salmon, and Shawnigan River drains a land use-affected watershed, which my data indicate, contributes substantial anthropogenic nutrient inputs to the stream. Lower reaches in both streams had greater and more variable enrichment in TN and TP than Holland River, but the pattern in the nutrient peaks differed between the two streams. This difference can potentially be explained by the nature of their respective nutrient inputs. The abundance of decomposing carcasses appeared to be an important factor determining downstream nutrient enrichment in Goldstream River. Salmon carcasses contribute nutrients to the stream water as a result of microbial and invertebrate processing (Wipfli et al. 1998), and downstream TN and TP concentrations were highest during peak carcass abundance as expected. Nutrient concentrations in Shawnigan River were associated with precipitation and discharge patterns (Figure 2.2 and Figure 2.2 c, f, and i). Nutrient concentrations in Holland River were also associated with precipitation and discharge patterns, but with less downstream nutrient enrichment than Shawnigan River. This is similar to observations from previous studies comparing forested and human impacted watersheds (Ellison and Brett 2006; Poor and McDonnell 2007). Thus it
appears that in streams where few salmon return to spawn, hydrological processes and human land use are more important determinants of stream nutrient concentrations.

Previous studies also observed that watersheds with extensive human land use exported more nutrients than more pristine watersheds (Groffman et al. 2004; Poor and McDonnell 2007). Both Shawnigan and Goldstream Rivers exported more nutrients than Holland River. In Shawnigan River this is attributed to anthropogenic nutrient inputs from human land use, whereas in the Goldstream River, higher nutrient export is attributed to salmon-derived nutrient inputs.

2.5.2.2 Suspended Particulate Organic Matter (SPOM)

Previous research has traced anthropogenic nutrients into aquatic environments using stable isotopes of nitrogen to identify nitrogen loading from agricultural and urban sources (Aravena et al. 1993; Cabana and Rasmussen 1996; Harrington et al. 1998; McClelland and Valiela 1998; Lake et al. 2001; Cole et al. 2004; Anderson and Cabana 2005; Anderson and Cabana 2006). These studies correlated agricultural and urban effluent with elevated $\delta^{15}N$ in aquatic ecosystems. SPOM $\delta^{15}N$ in Shawnigan River was enriched in $^{15}N$ compared to Holland River, which suggests that human land use contributed significant amounts of anthropogenic nitrogen to this watershed. Matthews and Mazumder (2003) found that zooplankton collected from Shawnigan Lake, had higher $\delta^{15}N$ compared to more pristine lakes, which also reflects higher baseline $\delta^{15}N$ values. SPOM $\delta^{15}N$ was also used to trace anthropogenic nitrogen loading into freshwater systems by Cole et al. (2004) and into estuarine systems by McClelland and Valiela (1998).

In Shawnigan River, there was a steep drop in the SPOM $\delta^{15}N$ from September to October coinciding with the first rainfall. The drop in $\delta^{15}N$ may reflect faster flushing rates of groundwater from agricultural and septic fields to the stream, with the influx of rainwater. Nitrogen transformations such as volatilization and denitrification leave the remaining nitrogen enriched with $^{15}N$ (Kreitler 1979; Heaton 1986). These processes occur after nitrogen has been applied to fields or while it is transported through sewage systems (Anderson and Cabana 2006). When heavy rainfall causes groundwater to move more quickly into the stream, there is less time for nitrogen transformation, and therefore less $^{14}N$ is removed resulting in lower $\delta^{15}N$. Alternatively, the decrease in the effective
population size around Shawnigan Lake in October could also explain the drop in SPOM $\delta^{15}N$. The majority of residences are occupied throughout the year, but there are a number of campsites and resorts that receive less use in the fall and winter (Rieberger et al. 2004). Fewer individuals produce less urban effluent, which could reduce the enrichment of SPOM $\delta^{15}N$.

Cabana and Rasmussen (1994) proposed that the stable isotope composition of organic matter can provide an integrated index of the nutrient sources supporting production in food webs. Pacific salmon have $\delta^{15}N$ ranging from 10‰ to 16‰ (Satterfield and Finney 2002), consequently, their contribution to stream food webs can be easily confounded with the nitrogen inputs from human and animal waste (10‰ to 22‰) (Heaton 1986; Kendall 2006). SPOM $\delta^{15}N$ from Goldstream and Shawnigan Rivers was consistently higher relative to Holland River. However, in all streams, SPOM $\delta^{15}N$ was quite variable, with differences as great as 5‰ among months. The isotopic composition of primary consumers is less temporally and spatially variable than among primary producers (Cabana and Rasmussen 1996). June collections of aquatic invertebrate grazers (Ephemeroptera) from streams, suggest that baseline nitrogen isotope compositions in the Goldstream and Shawnigan Rivers are similarly enriched in $^{15}N$ relative to Holland River (Figure 4.1). Patterns in the concentration, stable isotope composition, and C:N ratio of SPOM helped to distinguish between the influence of salmon, and that from anthropogenic nutrients. In Goldstream River, the composition of the SPOM reflected the seasonal inputs of carcass materials, whereas in Shawnigan River it reflected more continuous inputs of anthropogenic nitrogen.

Kline et al. (1997) emphasized that heavy isotopes can only be traced through food webs by making many assumptions about competing processes and alternative isotopic pool values. Stable isotopes of nitrogen have been used extensively to study the incorporation of salmon-derived nutrients into stream food webs (Kline et al. 1990; Chaloner et al. 2002; Mathewson et al. 2003), however, this study indicates that stable isotopes of nitrogen must be used with caution if other $^{15}N$-enriched nitrogen sources, such as anthropogenic nitrogen, are present, and that they are best paired with additional measures such as $\delta^{13}C$ and C:N ratios.
2.6 Conclusions

Salmon carcasses and human land use can both provide substantial nutrient inputs to streams. Goldstream River annually receives large numbers of returning salmon, and Shawnigan River has extensive human land use in its surrounding watershed, and both of these streams had higher downstream concentrations of nitrogen and phosphorus, and enriched baseline $\delta^{15}$N. However, the pattern of enrichment in both nutrients and $^{15}$N depended on the nature of the nutrient input. Salmon transport both nutrients and organic matter directly into Goldstream River during spawning and carcass decay, whereas anthropogenic nutrients in Shawnigan watershed appear to rely on waterborne transport from terrestrial ecosystems, which is potentially related to climate and periods of higher rainfall.

Anthropogenic nutrient subsidies to the Shawnigan River met and exceeded salmon nutrient subsidies in Goldstream River in terms of water-borne nitrogen and phosphorus. Globally, human activities have affected biogeochemical cycling through natural systems by doubling the flux of nitrogen, and quadrupling the flux of phosphorus, causing undesirable eutrophication in many aquatic systems (Falkowski et al. 2000). In this study the Goldstream and Shawnigan Rivers had a similar range of nutrient concentration, and exported comparable amounts of nutrients per square kilometer. Future studies need to include samples from the spring and summer, in the absence of carcasses. This would provide a full comparison of the effects of the seasonal nutrient input provided by salmon, and the more continuous input of anthropogenic nutrients. It would also provide valuable insight regarding the legacy of salmon-derived nutrients throughout the year, as suggested by the elevated baseline $\delta^{15}$N of stream Ephemeroptera in June.

Substantial amounts of salmon-derived nutrients were exported to the Goldstream Estuary, where they may provide an important subsidy to the estuarine food web. Primary production in estuaries is often limited by nitrogen and phosphorus (Rice and Ferguson 1975), and coastal streams that receive annual runs of spawning salmon can export more nutrients and organic matter to estuaries, as described for the Goldstream system. However, as salmon populations decline, this could cause a corresponding
decline in the freshwater export of salmon-derived nutrients to estuaries, the consequences of which are currently unknown.
2.7 Figures

Figure 2.1 Location of study streams (shown in bold) along Southeastern Vancouver Island.
Figure 2.2 Running average precipitation — and mean weekly discharge — in three stream watersheds: Goldstream in (a) 2004 and (b) 2005, (c) Holland in 2005, and (d) Shawnigan in 2005. Running average precipitation is based on a 7-day window.
Figure 2.3 Land use in three nearby watersheds: (a) Shawnigan, (b) Holland, and (c) Goldstream. Images adapted from Land use Zone maps created by Environment Canada (2002).
Figure 2.4 Total annual salmon escapement from 1995 to 2005 in three streams: Goldstream ——; Holland ---; and Shawnigan ——-. Data were collected by the Department of Fisheries and Oceans. Returning salmon were not counted in Holland River in 2004 and 2005.
Figure 2.5 Location of sample collection in the upper and lower reaches of streams: (a) Shawnigan, (b) Holland, and (c) Goldstream. Images adapted from Fish Wizard maps created by the Freshwater Fisheries Society of British Columbia (2005).
Figure 2.6 (a) Live salmon abundance, estimated from stream bank walks, and (b) calculated carcass abundance in Goldstream River in 2004 and 2005.
Figure 2.7 Stream water concentrations of (a, b, c) total nitrogen (TN), (d, e, f) total phosphorus (TP), and (g, h, i) total organic carbon (TOC) in 2005: upper reach \(\triangledown\); and lower reach \(\bullet\).

Figure 2.8 Stream water concentrations of (a) total nitrogen (TN), and (b) total phosphorus (TP) in Goldstream River in 2004: upper reach \(\triangledown\); and lower reach \(\bullet\).
Figure 2.9  Suspended particulate organic matter (SPOM) concentrations (a), C:N ratios (b), and $\delta^{13}\text{C}$ (c), and $\delta^{15}\text{N}$ (d) from the three streams in 2005: Goldstream — ; Shawnigan — ; and Holland — .
2.8 Tables

Table 2.1 Results from paired t-tests comparing upstream and downstream nutrient concentrations from the three streams. Variables are stream water total nitrogen (TN), total phosphorus (TP), and total organic carbon (TOC) concentrations. Samples were collected from September 2005 to February 2006, which encompasses the period of salmon spawning (October-November). All statistical tests were carried out on natural log transformed data. P critical is the value below which indicates statistically significant result, based on an alpha = 0.05, when carrying out multiple statistical tests related to one hypothesis (Holm 1979).

<table>
<thead>
<tr>
<th>Stream</th>
<th>Variable</th>
<th>t</th>
<th>df</th>
<th>P</th>
<th>P critical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goldstream</td>
<td>TN</td>
<td>7.159</td>
<td>8</td>
<td>&lt;0.001</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>TP</td>
<td>8.028</td>
<td>8</td>
<td>&lt;0.001</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>TOC</td>
<td>-1.505</td>
<td>8</td>
<td>0.171</td>
<td>0.050</td>
</tr>
<tr>
<td>Holland</td>
<td>TN</td>
<td>0.783</td>
<td>7</td>
<td>0.460</td>
<td>0.050</td>
</tr>
<tr>
<td></td>
<td>TP</td>
<td>1.517</td>
<td>8</td>
<td>0.168</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>TOC</td>
<td>0.781</td>
<td>8</td>
<td>0.457</td>
<td>0.025</td>
</tr>
<tr>
<td>Shawnigan</td>
<td>TN</td>
<td>4.495</td>
<td>8</td>
<td>0.002</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>TP</td>
<td>4.189</td>
<td>8</td>
<td>0.003</td>
<td>0.025</td>
</tr>
<tr>
<td></td>
<td>TOC</td>
<td>1.738</td>
<td>8</td>
<td>0.120</td>
<td>0.050</td>
</tr>
</tbody>
</table>
Table 2.2 Calculated total monthly nutrient and particulate export (kg · km$^2$) from three streams. The export products are total nitrogen (TN), total phosphorus (TP), total organic carbon (TOC), and suspended particulate organic matter (SPOM). Samples were collected from September 2005 to February 2006, which encompasses the period of salmon spawning (October-November).

<table>
<thead>
<tr>
<th>Stream</th>
<th>Export Product</th>
<th>Sept</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
<th>Jan</th>
<th>Feb</th>
<th>Total Export Sept - Feb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goldstream</td>
<td>TN</td>
<td>14.6</td>
<td>7.8</td>
<td>38.9</td>
<td>32.5</td>
<td>102.7</td>
<td>46.2</td>
<td>242.6</td>
</tr>
<tr>
<td>2005-2006</td>
<td>TP</td>
<td>0.2</td>
<td>0.1</td>
<td>1.1</td>
<td>0.8</td>
<td>2.9</td>
<td>1.0</td>
<td>6.1</td>
</tr>
<tr>
<td></td>
<td>TOC</td>
<td>32.5</td>
<td>27.2</td>
<td>185.4</td>
<td>104.5</td>
<td>941.6</td>
<td>267.2</td>
<td>1558.3</td>
</tr>
<tr>
<td></td>
<td>SPOM</td>
<td>11.9</td>
<td>32.5</td>
<td>41.4</td>
<td>19.5</td>
<td>165.2</td>
<td>133.3</td>
<td>403.8</td>
</tr>
<tr>
<td>2004-2005</td>
<td>TN</td>
<td>16.6</td>
<td>12.9</td>
<td>38.7</td>
<td>36.6</td>
<td>87.3</td>
<td>41.7</td>
<td>233.8</td>
</tr>
<tr>
<td></td>
<td>TP</td>
<td>0.2</td>
<td>0.4</td>
<td>2.1</td>
<td>1.4</td>
<td>4.9</td>
<td>0.6</td>
<td>9.7</td>
</tr>
<tr>
<td>Holland</td>
<td>TN</td>
<td>1.3</td>
<td>17.7</td>
<td>18.3</td>
<td>20.8</td>
<td>41.6</td>
<td>13.4</td>
<td>113.1</td>
</tr>
<tr>
<td>2005-2006</td>
<td>TP</td>
<td>0.1</td>
<td>0.6</td>
<td>0.3</td>
<td>0.9</td>
<td>1.0</td>
<td>0.4</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>TOC</td>
<td>35.9</td>
<td>148.0</td>
<td>293.4</td>
<td>240.1</td>
<td>683.6</td>
<td>232.7</td>
<td>1633.7</td>
</tr>
<tr>
<td></td>
<td>SPOM</td>
<td>3.2</td>
<td>13.9</td>
<td>31.8</td>
<td>12.7</td>
<td>97.8</td>
<td>46.7</td>
<td>206.1</td>
</tr>
<tr>
<td>Shawnigan</td>
<td>TN</td>
<td>0.2</td>
<td>2.9</td>
<td>53.0</td>
<td>20.8</td>
<td>153.6</td>
<td>37.8</td>
<td>268.2</td>
</tr>
<tr>
<td>2005-2006</td>
<td>TP</td>
<td>0.0</td>
<td>0.4</td>
<td>1.4</td>
<td>0.7</td>
<td>4.2</td>
<td>1.2</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>TOC</td>
<td>1.2</td>
<td>24.4</td>
<td>361.2</td>
<td>221.7</td>
<td>1090.7</td>
<td>522.8</td>
<td>2222.0</td>
</tr>
<tr>
<td></td>
<td>SPOM</td>
<td>0.3</td>
<td>4.7</td>
<td>36.6</td>
<td>47.4</td>
<td>100.5</td>
<td>111.0</td>
<td>300.5</td>
</tr>
</tbody>
</table>
Table 2.3 $\delta^{13}$C and $\delta^{15}$N, and C:N ratios of non-lipid extracted salmon muscle tissue. All values are means ± standard deviations.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Chum $\delta^{13}$C (‰)</th>
<th>Coho $\delta^{13}$C (‰)</th>
<th>Chinook $\delta^{13}$C (‰)</th>
<th>Chum $\delta^{15}$N (‰)</th>
<th>Coho $\delta^{15}$N (‰)</th>
<th>Chinook $\delta^{15}$N (‰)</th>
<th>Chum C:N</th>
<th>Coho C:N</th>
<th>Chinook C:N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-19.4 ± 0.5</td>
<td>-17.3 ± 0.5</td>
<td>-17.2 ± 0.9</td>
<td>12.4 ± 1.1</td>
<td>14.5 ± 0.4</td>
<td>14.8 ± 0.5</td>
<td>3.7 ± 0.2</td>
<td>4.0 ± 0.1</td>
<td>4.1 ± 0.5</td>
</tr>
</tbody>
</table>

Table 2.4 Mean weights (kg), elemental composition (%N and %P), and escapement estimates for Pacific salmon spawning in Goldstream River in 2004 and 2005. Escapement estimates are the sum of the individuals enumerated during weekly stream bank walks.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Chum</th>
<th>Coho</th>
<th>Chinook</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>4.63</td>
<td>2.52</td>
<td>6.04</td>
<td>(Bigler et al. 1996)</td>
</tr>
<tr>
<td>%N</td>
<td>3.30</td>
<td>-</td>
<td>-</td>
<td>(Moore and Schindler 2004)</td>
</tr>
<tr>
<td>%P</td>
<td>0.48</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Escapement Estimates</th>
<th>Chum</th>
<th>Coho</th>
<th>Chinook</th>
<th>% Exported to Estuary</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>18,804</td>
<td>642</td>
<td>11</td>
<td>39</td>
</tr>
<tr>
<td>2005</td>
<td>10,746</td>
<td>74</td>
<td>124</td>
<td>27</td>
</tr>
</tbody>
</table>
Chapter 3  Tracing salmon-derived nutrients into estuarine food webs using stable isotopes of carbon ($\delta^{13}$C) and nitrogen ($\delta^{15}$N)

3.1 Abstract

Anadromous Pacific salmon (Onchorhynchus spp.) transport large quantities of nutrients into watersheds along the west coast of North America during their annual spawning migration. Streams carry substantial amounts of these nutrients back downstream; however, the freshwater export of salmon-derived nutrients to estuaries has received limited investigation. I measured the effects of salmon-derived nutrients on the $\delta^{13}$C and $\delta^{15}$N of two species of estuarine clam, the varnish clam (Nuttalia obscurata: Reeve, 1857) and the manila clam (Tapes philippinarum), and their food sources, the suspended particulate matter (SPOM) and sedimentary organic matter (SOM). Samples were collected from Goldstream Estuary, where the adjacent river receives large numbers of returning salmon, and from two nearby systems with few returning salmon. Salmon were enriched in $^{15}$N and $^{13}$C relative to estuarine SPOM and SOM, with higher $\delta^{15}$N than estuarine clams, but comparable $\delta^{13}$C. The stable isotope composition of estuarine SPOM was variable among months, and did not reflect nutrient inputs from salmon. Clams and SOM had relatively constant stable isotope compositions from September to February; however, samples from Goldstream Estuary were significantly enriched in $^{15}$N and $^{13}$C compared to nearby systems with few returning salmon. Clams were significantly enriched in $^{15}$N, and SOM was significantly enriched in both $^{13}$C and $^{15}$N. More enrichment appeared to occur in the high intertidal zone near the river mouth, than in the mid-intertidal zone. These results suggest that the stable isotope composition of clams and SOM can potentially provide long-term integrated indices of the freshwater export of salmon-derived nutrients into estuarine ecosystems.
3.2 Introduction

It is well documented that the nutrients from spawning Pacific salmon are integrated into watersheds of the Pacific Northwest (for reviews see Willson et al. 1998; Gende et al. 2002; Naiman et al. 2002). In coastal streams, salmon-derived nutrients and organic matter that are not incorporated into freshwater and terrestrial ecosystems are exported downstream to estuaries (Wipfli et al. 1998; Cederholm 1999; Gende et al. 2004; McConnachie and Petticrew 2006). Previous studies attributed elevated stream nutrient concentrations to salmon carcasses (Richey et al. 1975; Johnston et al. 2004), which also increases freshwater nutrient export to estuaries (Brickell and Goering 1970; Sugai and Burrell 1984; Mitchell and Lamberti 2005). Periodic high flows can also wash large numbers of salmon carcasses into downstream lakes and estuaries (Richey et al. 1975, Brickell and Goering 1970; Gende et al. 2004), supplying these environments with substantial amounts of high quality organic matter.

The seasonal influx of salmon-derived nutrients has the potential to influence the community structure and productivity of estuaries; however, investigation into this mechanism remains limited. Reimchen (1992; 1994) observed that many species feed on tissue from spawned out salmon carcasses that were deposited in estuaries. Fugiwara and Highsmith (1997) used stable isotopes to connect estuarine macroalgae production to salmon-derived nutrients, and hypothesized a link to juvenile salmon with harpacticoid copepods as an intermediary. These studies represent the extent of our understanding on the downstream influences of spawning salmon.

Stable isotope compositions of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) have been used to trace the transport of salmon-derived nutrients into terrestrial and aquatic food webs (Kline et al. 1990; Bilby et al. 1996; Chaloner et al. 2002; Mathewson et al. 2003). Most ecological applications are based on the observation that the stable isotope composition of consumers reflects their food sources with predictable enrichment due to metabolic fractionation. At each trophic step $\delta^{13}C$ increases by ~ 1‰ (Rau et al. 1983; Fry and Sherr 1984), and $\delta^{15}N$ increases by ~ 3.4‰ (Michener and Schell 1994). Salmon consist
mainly of marine-derived C and N that are enriched in $^{13}\text{C}$ and $^{15}\text{N}$ relative to freshwater and terrestrial food sources (Kline et al. 1990). This difference has allowed researchers to trace the uptake of salmon-derived nutrients into terrestrial and freshwater food webs.

Estuaries are characterized by diverse sources of nutrients and organic matter, including inputs from marine, freshwater, and terrestrial sources (Canuel et al. 1995). The stable isotope compositions of estuarine consumers can provide an integrated index of the nutrient sources supporting production in estuaries (Fry 1999; McKinney et al. 2001). Bivalves are useful index organisms because they are widely distributed, generally sedentary, and have many populations that can be sampled repeatedly (Farrington et al. 1983). Estuarine bivalves feed on particulate organic matter from both in situ production and stream runoff (Cifuentes et al. 1988). In situ production includes phytoplankton, benthic macroalgae and periphyton, bacterioplankton and protist grazers, and detritus from adjacent salt marshes. Stream runoff includes terrestrial-derived plant and soil materials, freshwater phytoplankton (Martineau et al. 2004), and organic matter from salmon carcasses.

The varnish clam (*Nuttalia obscurata*: Reeve, 1857) and the manila clam (*Tapes philippinarum*) are not native to B.C., but have become well established in bays and estuaries throughout the Strait of Georgia (Bourne 1982; Gillespie and Kronlund 1999). Varnish clams inhabit the high intertidal zone and are capable of selective suspension feeding and deposit feeding. Manila clams inhabit the mid- to high intertidal zone and rely solely on selective suspension feeding. Each species feeds from the SPOM and the SOM pools. Varnish clams obtain more than 70% of their carbon from the SOM, while manila clams obtain approximately 60% of their carbon from the SPOM (Kanaya 2005).

The goal of this study was to observe whether the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of estuarine clams and their food sources, the SPOM and SOM, reflect the integration of salmon-derived nutrients in an estuarine ecosystem. Samples were collected from the Goldstream Estuary, where the adjacent stream receives large annual runs of returning salmon, and from two nearby estuaries with few returning salmon. To test this objective, temporal trends were compared among estuaries, and spatial trends were examined within estuaries. While this study is limited to examining the importance of salmon-derived
nutrients for these two clam species, the results also might enhance understanding of freshwater-marine nutrient linkages and their importance to estuarine productivity.

3.3 Methods

3.3.1 Site Description

Vancouver Island is located off the Canadian Pacific Southwest, and has a relatively mild climate with wet winters and drier summers. Mean monthly air temperature among all sites ranges from 2.7°C to 17.9°C, with a mean annual precipitation of 116 cm (Environment Canada 2004). Saanich Inlet is located along Southeast Vancouver Island, British Columbia, Canada. The Goldstream and Shawnigan Rivers are the main sources of freshwater discharging directly into the inlet; however, the Fraser River, which discharges from the mainland, is the most significant source of freshwater. Goldstream River discharges at the head of the inlet, and Shawnigan River discharges into Mill Bay near the mouth of the inlet. Samples were collected from estuaries adjacent to these streams and from a third nearby estuary where Holland River discharges into Ladysmith Harbour. Three species of salmon spawn in the area: *Oncorhyncus keta* (chum salmon), *O. kisutch* (coho salmon), and *O. tshawytscha* (chinook salmon); however chum salmon make up the majority of returning salmon. All study streams have been influenced to some extent by hatchery or salmon enhancement programs, but only Goldstream River currently supports a large abundance of salmon (>10,000 salmon annually). For detailed sites descriptions and historical escapement see Chapter 2.

3.3.2 Sample Collection and Analysis

Samples were collected monthly from September 2005 to February 2006. Tissue samples were analyzed for δ¹³C, δ¹⁵N, and C:N ratio on a Thermo Delta Plus continuous flow isotope ratio mass spectrophotometer coupled to a Costech elemental analyzer at the Water and Watershed Laboratory, University of Victoria (see Matthews and Mazumder 2003 for details).
3.3.2.1 Suspended Particulate Organic Matter (SPOM)

Bivalves generally feed when immersed in water (Gosling 2003), so water samples were collected within one hour of high tide, such that samples were representative of water from which both the high and mid-intertidal zone clams were feeding. An integrated water column sampler was used in water ~ 3 m deep within the intertidal zone of estuaries. This device consisted of soft plastic tubing, 5 cm in diameter that was weighted at one end, with both ends connected by a rope. The weighted end was lowered into the water to the desired depth, effectively sampling the water column, while keeping the opposite end above the water. The weighted end was then drawn up using the rope and the water was collected in three acid-washed 4 L plastic containers, and stored in a cooler for a maximum of three hours prior to filtration. All water was pre-filtered through a 200 μm mesh filter to remove large debris, and then filtered through four pre-combusted (500°C for 1 hour), pre-weighed, 25 mm Whatman GF/F filters until the filters were clogged (700 – 1800 mL).

Replicate filters were stored in petri dishes at –20°C prior to freeze drying. Two replicates were exposed to concentrated HCl fumes to remove inorganic carbon, and then oven dried (60°C for 24 hours), prior to δ¹³C analysis. The other two replicates were not acid-fumed to prevent loss of particulate nitrogen and alteration of the δ¹⁵N of the suspended particulate organic matter (Lorrain et al. 2003). C:N ratios were determined from the acid-fumed samples.

3.3.2.2 Sedimentary Organic Matter (SOM)

Sediment samples were collected during low tide using a cylindrical corer, 4 cm in diameter x 10 cm long, from high and mid-intertidal zone sites, located near the clam collection sites (Figure 3.1). Samples were stored at -80°C prior to being freeze dried and sifted through a 200 μm sieve. The < 200 μm size fraction was kept for stable isotope analysis. Inorganic carbonates were removed by treating the sediment with a 10% HCl solution for 24 hours, followed by a twofold rinse with distilled de-ionized water. Rinsing with distilled de-ionized water reduces the harm done to the elemental analyzer and mass spectrometer due to HCl fumes (method B: Schubert and Nielsen
Decarbonated samples were subsequently dried at 60°C for three days, and then ground with a mortar and pestle before being packaged for stable isotope analysis.

### 3.3.2.3 Clams and Salmon

All clam samples were collected from estuaries during low tide. Varnish clams were collected from the high intertidal zone near the river mouths, and manila clams were collected from mid-intertidal zone (Figure 3.1). Clamshell length (Figure 3.2) was measured and foot muscle tissue was removed and kept for stable isotope analysis. Preliminary analysis revealed significant positive correlations between clamshell length and stable isotope composition, so only clams with shell lengths between 30.0 mm and 39.9 mm were used to minimize any effects of size or age. Clamshell length ranged from 20.6 mm to 54.2 mm, but those that were 30.0 mm to 39.9 mm long were the most common across all estuaries. A minimum of three clams was used to calculate mean monthly δ¹³C and δ¹⁵N.

Salmon dorsal muscle tissue was also collected for stable isotope analysis by volunteers from the Goldstream Volunteer Salmonid Enhancement Association.

### 3.3.2.4 Statistical Analysis

There is an increase in the relative abundance of the heavier isotopes of carbon and nitrogen in organic matter when moving from terrestrial and freshwater ecosystems to marine ecosystems. Consequently, in estuaries, where these two sources of organic matter mix, the may exist a correlation between the δ¹³C and δ¹⁵N of organic matter. Paired t-tests were used to compare the monthly δ¹³C and δ¹⁵N of high and mid-intertidal zone sediment, and varnish clams and manila clams, within each estuary. Analysis of variance (ANOVA) was used to compare stable isotope ratios of clam foot tissue and SOM among sites, and Tukey’s honestly significant difference (HSD) pair-wise comparisons identified specific differences among sites. The assumptions of homogeneity of variance and normality of distribution were tested using Levene’s test of homogeneity of variance and Shapiro-Wilks W, respectively. Spearman’s correlation was used to examine the relationship between the δ¹³C and δ¹⁵N of SOM. All statistics were carried out using SPSS version 14.0.
3.4 Results

Chum salmon $\delta^{15}\text{N} (12.4 \pm 1.1\%\text{o})$ and $\delta^{13}\text{C} (-19.4 \pm 0.5\%\text{o})$ were high compared to other samples of estuarine organic matter (Figure 3.3); however, clam $\delta^{13}\text{C}$ was similarly enriched in $^{13}\text{C}$ with some individuals ranging up to $-15.8\%\text{o}$.

3.4.1 Suspended Particulate Organic Matter (SPOM)

The $\delta^{13}\text{C}$ of riverine SPOM varied between $-29.5\%\text{o}$ and $-24.0\%\text{o}$, and the $\delta^{15}\text{N}$ varied between $-2.1\%\text{o}$ and $10.4\%\text{o}$ (Figure 3.4). Riverine SPOM had the lowest $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of all the samples collected, but the range of values largely overlapped with those of estuarine SPOM and SOM (Figure 3.3). In a given month, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of riverine SPOM were generally within $2\%\text{o}$ of the estuarine SPOM. The most distinct exception was in December in Goldstream River, coinciding with peak carcass abundance in the stream. During that time riverine SPOM $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values exceeded estuarine SPOM by $4.6\%\text{o}$ and $3.2\%\text{o}$ respectively.

The $\delta^{13}\text{C}$ of estuarine SPOM varied between $-30.4\%\text{o}$ and $-20.6\%\text{o}$, and had similar values and trends among estuaries from September to February (Figure 3.4, 1a-e). Among all sites the mean $\delta^{13}\text{C}$ of estuarine SPOM declined from a mean of $-22.8\%\text{o}$ in September to a mean of $-28.8\%\text{o}$ in January. During this same time, the C:N ratio of estuarine SPOM more than doubled in all estuaries (Figure 3.5). Adjacent rivers had similar trends and values in C:N ratio, except for the Goldstream River, where the C:N ratio of SPOM declined from October to December (Chapter 2). However, this trend was not observed in the Goldstream Estuary.

The $\delta^{15}\text{N}$ of estuarine SPOM varied between $2.7\%\text{o}$ and $9.6\%\text{o}$ (Figure 3.4, 2a-c). In Goldstream and Shawnigan Estuaries SPOM $\delta^{15}\text{N}$ decreased dramatically in October, and then rose again in November. SPOM $\delta^{15}\text{N}$ from Holland Estuary also peaked in November, but subsequently all estuaries had variable values from December to February.

3.4.2 Sedimentary Organic Matter (SOM)

SOM had relatively constant monthly $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ from September to February (Figure 3.4). SOM was generally more enriched in $^{13}\text{C}$ than the SPOM, whereas SOM
\[\delta^{15}N\] was generally within the range of values of the SPOM \[\delta^{15}N\] (Figure 3.3). There was a strong correlation between the \[\delta^{13}C\] and \[\delta^{15}N\] of SOM among the estuaries (\(r_{\text{Spearman}} = 0.909, \text{df} = 36, P < 0.001\); Figure 3.6).

SOM \[\delta^{13}C\] in Goldstream Estuary was significantly enriched relative to Holland and Shawnigan Estuaries (Table 3.2, Tukey HSD: \(P < 0.05\) for all comparisons with Goldstream Estuary). High and mid-intertidal zone SOM \[\delta^{13}C\] in Goldstream Estuary were similar (Paired t-test: \(P > 0.05\)); whereas, in Holland and Shawnigan Estuaries, high intertidal zone SOM \[\delta^{13}C\] was significantly higher than mid-intertidal zone SOM \[\delta^{13}C\] (Paired t-test: \(P < 0.05\); Table 3.3).

The \[\delta^{15}N\] of the SOM was significantly different among all three estuaries (Table 3.2, Tukey HSD: \(P < 0.05\) for all comparisons). The highest SOM \[\delta^{15}N\] values were observed in Goldstream Estuary, and the lowest were observed in the high intertidal of Shawnigan Estuary (Table 3.3). High and mid-intertidal zone SOM in Goldstream Estuary had similar \[\delta^{15}N\] (Paired t-test: \(P > 0.05\)), whereas, in Holland and Shawnigan Estuaries, high intertidal zone SOM \[\delta^{15}N\] was significantly lower than mid-intertidal zone SOM \[\delta^{15}N\] (Paired t-test: \(P < 0.05\)).

3.4.3 Clams

Clams had relatively constant \[\delta^{13}C\] and \[\delta^{15}N\] from September to February (Figure 3.4), and were more enriched in \(^{13}C\) and \(^{15}N\) than the SOM and SPOM (Figure 3.3). Clams were depleted in \(^{15}N\) relative to chum salmon, but had equal or greater \[\delta^{13}C\]. The \[\delta^{13}C\] of clams was significantly different among all three estuaries (Table 3.4, Tukey HSD: \(P < 0.05\) for all comparisons). The highest \[\delta^{13}C\] values were observed in Shawnigan Estuary, and the lowest in Holland Estuary. In all three estuaries manila clams were significantly more enriched in \(^{13}C\) than varnish clams (Paired t-test: \(P < 0.05\); Table 3.3).

The \[\delta^{15}N\] of clams from Goldstream Estuary shows that clams were significantly enriched in \(^{15}N\) relative to Holland and Shawnigan Estuaries (Table 3.4; Tukey HSD: \(P < 0.05\) for comparisons with Goldstream Estuary). In Holland and Shawnigan Estuaries, \[\delta^{15}N\] of both clam species were similar (Table 3.3; Paired t-test: \(P > 0.05\)), but in
Goldstream Estuary, manila clams had significantly lower $\delta^{15}$N than varnish clams (Paired t-test: $P < 0.05$).

3.5 Discussion

Estuaries occur at the confluence of terrestrial, freshwater, and marine ecosystems, which can obscure the marine stable isotope signature of salmon-derived nutrients. In this study, the stable isotope values of clams and SOM from Goldstream Estuary were significantly enriched relative to nearby estuaries with few returning salmon. This difference may reflect the freshwater export of salmon-derived nutrients to the estuary. In the following sections I discuss how salmon-derived nutrients can become integrated into estuarine ecosystems, and conclude with a discussion of possible indices for measuring the influence of this nutrient subsidy in estuaries.

3.5.1 Suspended Particulate Organic Matter (SPOM)

The abundance of salmon carcasses in Goldstream River was correlated with high $\delta^{13}$C and $\delta^{15}$N for riverine SPOM (Chapter 2), and these values exceeded the $\delta^{13}$C and $\delta^{15}$N of estuarine SPOM at that time. When salmon carcasses were not present in the stream in large abundance, the $\delta^{13}$C and $\delta^{15}$N of riverine SPOM were equal to or less than estuarine SPOM, consistent with a greater proportion of terrestrial detritus with low $\delta^{13}$C and $\delta^{15}$N (Incze et al. 1982; Stephenson and Lyon 1982; Fichez et al. 1993; Canuel et al. 1995; Riera and Richard 1997; Riera 1998; Martineau et al. 2004). Elevated stable isotope values corresponded to low C:N ratios, and suggests that salmon carcasses made significant contributions to the SPOM pool of Goldstream River (McConnachie and Petticrew 2006; Chapter 2, this thesis). However, contributions to estuaries remain unclear.

Salmon tissue fragments that are carried downstream may become well mixed into the estuarine SPOM pool, which might explain why the freshwater export of salmon-derived nutrients was not apparent in the $\delta^{13}$C and $\delta^{15}$N of estuarine SPOM. Alternatively, the enriched stable isotope compositions of Goldstream Estuary SOM and clams suggest that salmon tissue fragments might settle out of the water column in the
upper estuary, closer to the river mouth, and become integrated into the SOM or be consumed by suspension and deposit feeders.

During the fall the $\delta^{13}C$ of estuarine SPOM decreased, and C:N ratios increased, coinciding with increasing river discharge and influx of riverine SPOM (Chapter 2). Similar trends were also observed by Fichez et al. (1993) in the Great Ouse Estuary, England, and by Riera and Richard (1997) in Marennes-Oléron Bay, France. These trends were attributed to increased influx of $^{13}C$-depleted terrestrial detritus.

Seasonal trends in $\delta^{15}N$ varied among estuaries, and did not reflect the increase in terrestrial detritus as stream discharge increased, indicating that variation in $\delta^{15}N$ within and among estuaries may be caused by system specific biogeochemical processes more so than by physical mixing (Cifuentes et al. 1988).

3.5.2 Sedimentary Organic Matter (SOM)

Salmon-derived nutrients can be integrated into estuarine food webs and SOM through (1) autotrophic uptake by benthic diatoms and microalgae in the sediment, or by macroalgae and marsh plants that subsequently fragment or die providing detritus to the SOM pool, (2) uptake by the benthic microfauna of dissolved organic matter released by carcasses, or (3) direct consumption of salmon tissue by estuarine consumers (Cederholm et al. 1999). Reimchen (1994) observed that whole salmon carcasses were rapidly eaten by estuarine invertebrate scavengers, including whelks, starfish, shrimp, and crabs. Salmon tissue fragments that settle out of the water column could also be consumed by benthic suspension and deposit feeders. Deposit feeders can process tens to hundreds of times their body weight in sediment each day (Miller 1996), which may minimise variation in the $\delta^{13}C$ and $\delta^{15}N$ of estuarine SOM relative to SPOM by buffering changes in nutrient inputs throughout the entire benthic community.

In all three estuaries, there was a strong correlation between the $\delta^{13}C$ and $\delta^{15}N$ of SOM, particularly in the high intertidal zone near the river mouths. Previous studies on marine SOM also observed strong correlations between these two parameters (e.g. Peters et al. 1978, Wada et al. 1987), and these were interpreted as the result of mixing of terrestrial and marine organic matter (Rau et al. 1990).
The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of SOM in Goldstream Estuary were greater than in systems without large runs of salmon. This may reflect the long-term integration of salmon-derived nutrients, which is corroborated by spatial trends within estuaries. In estuaries without large runs of salmon, high intertidal zone SOM, near the river mouth, was depleted in $^{13}\text{C}$ and $^{15}\text{N}$ relative to mid-intertidal zone SOM. These values are consistent with increased concentrations of $^{13}\text{C}$- and $^{15}\text{N}$-depleted terrestrial detritus closer to the river mouth. In Goldstream Estuary, high intertidal zone SOM had similar $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ as mid-intertidal zone SOM, consistent with increased concentrations of salmon-derived organic matter closer to the river mouth. Anthropogenic nutrients are another source of $^{15}\text{N}$- enriched nitrogen to estuaries (McClelland et al. 1997; McClelland and Valiela 1998; Cole 2004). Using nitrogen stable isotopes, Savage et al. (2004) observed that anthropogenic nitrogen was sequestered in SOM nearest the wastewater outfall of a coastal bay. Shawnigan Estuary received high inputs of anthropogenic nutrients (Chapter 2); however, the $\delta^{15}\text{N}$ of SOM did not reflect $^{15}\text{N}$-enriched nitrogen influx.

Previous studies correlated historical sockeye salmon abundance to $\delta^{15}\text{N}$ in sockeye lake sediment (Finney et al. 2000; Finney et al. 2002; Brock et al. 2006). In Little Port Walter estuary, Alaska, Brickell and Goering (1970) noted a gelatinous quality to estuarine sediment samples taken even prior to the arrival of salmon, and postulated that estuarine SOM might be a long-term nutrient sink for salmon carcasses. This study supports previous research that indicates that the composition estuarine SOM may be correlated with salmon abundance in nearby streams.

### 3.5.3 Clams

Varnish clams and manila clams feed from both the SPOM and the SOM pools; and from the SOM pool they are known to feed selectively on benthic diatoms (Kanaya et al. 2005). Benthic diatoms are generally enriched in $^{13}\text{C}$ relative to other sources of organic matter found in estuaries, such as higher plants, SOM, and SPOM (Riera and Richard 1997; Riera et al. 2002; Doi et al. 2005) and can account for the higher $\delta^{13}\text{C}$ of clams. Salmon-derived nutrients could be integrated into clam diets both directly through the consumption of salmon tissue fragments, and indirectly through the consumption of
benthic diatoms, phytoplankton, and fragments of plants and algae that had previously
taken up nutrients released by salmon carcasses.

The ability of stable isotopes to trace salmon-derived nutrients into clams depends
on a sufficient difference in $\delta^{13}$C and $\delta^{15}$N between salmon and the other food sources
consumed by clams. On average, clam $\delta^{13}$C was $\sim 1.6\%$, and $\delta^{15}$N was $\sim 3.5\%$
less than the mean stable isotope composition of chum salmon. At each trophic step
$\delta^{13}$C of organic matter generally increases by $\sim 1\%$ (Rau et al. 1983; Fry and Sherr
1984), and $\delta^{15}$N increases by $\sim 3.4\%$ (Michener and Schell 1994). This limited the
usefulness of $\delta^{13}$C as a tracer for salmon, but $\delta^{15}$N could still be used because consumers
have higher $\delta^{15}$N than their food sources, and clams were depleted in $^{15}$N relative to chum
salmon.

The $\delta^{15}$N of clams in Goldstream Estuary was greater than in systems without
large runs of salmon, which may reflect the long-term integration of salmon-derived
nutrients. This is corroborated by trends in $\delta^{15}$N between varnish clams located in the
high intertidal zone near river mouths, and manila clams located in the mid-intertidal
zone. In estuaries without large runs of salmon, the differences in $\delta^{15}$N were not
significantly different. In Goldstream Estuary, however, varnish clams were significantly
enriched in $^{15}$N relative to manila clams, which could reflect a gradient of enrichment in
salmon-derived nutrients from river mouth out into the estuary. In all three estuaries, the
mean differences in $\delta^{15}$N between clam species were small ($\leq 0.5\%$), and can be
attributed to slight differences in food sources or differences in metabolism (Stephenson
and Lyon 1982).

Fry (1999) suggested that the isotopic compositions of animal consumers could be
a useful index of the carbon and nitrogen sources supporting aquatic ecosystems. He
found higher $\delta^{15}$N in the clam *Potamocorbula amurensis* in a coastal bay that received
greater influxes of anthropogenic nitrogen. Higher $\delta^{15}$N were not found in clams in
Shawnigan Estuary, where the adjacent watershed had the most human land use among
the study sites. Salmon also provide a source of $^{15}$N-enriched nitrogen, however, and
could become integrated into clams based on the same mechanism as anthropogenic
nitrogen.
Small monthly variation in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of clams, may be the result of low metabolic rates resulting in long turnover times of stable isotopes in clam tissue (Tieszen et al. 1983). Laboratory experiments with the marine mussel *Geukensia demissa* indicate that it can take up to a year for bivalves to come into equilibrium with the $\delta^{15}\text{N}$ of their food source (McKinney et al. 2001). Smaller individuals may need a shorter time to reach equilibrium; however, $\delta^{15}\text{N}$ of clams less than 30.0 mm long also showed small monthly variation, with no significant correlation with carcass abundance (*unpublished data*). The pulse of terrestrial and salmon-derived nutrients during the fall may be too short to manifest as a distinct change in clam foot muscle tissue. Tieszen et al. (1983) observed that if an animal’s diet varies over time, then the relative contributions of isotopically distinct dietary components were obscured. Alternatively, if clams feed selectively on benthic or planktonic microalgae from within the SOM and SPOM, they could effectively minimize variation in diet across seasons (Riera and Richard 1997; Riera et al. 2002).

Generally, consumers become enriched in $^{13}\text{C}$ along the freshwater-marine continuum present in estuaries (Stephenson and Lyon 1982; Canuel et al. 1995; Deegan and Garritt 1997; Doi et al. 2005), and manila clams were consistently more enriched than varnish clams, as predicted by their location in estuaries. Stephenson and Lyon (1982) and Peterson et al. (1985; 1986) also observed small seasonal shifts in estuarine bivalves, with more variation among locations indicating that location is an important determinant of $\delta^{13}\text{C}$ in estuaries.

### 3.6 Conclusions

The $\delta^{15}\text{N}$ of clams, and the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of SOM in Goldstream Estuary were significantly greater than in estuaries with few returning salmon. No seasonality was identified, which would indicate a direct consumption of salmon by clams, rather, this enrichment appears to reflect a legacy of salmon-derived nutrients in the Goldstream Estuary ecosystem. Isotopic enrichment was greater in the upper estuary near the river mouth than in the mid-intertidal zone. This may be due to (1) dilution of salmon-derived nutrients in the estuarine nutrient and particulate pool further from the river mouth, (2)
selective consumption by suspension and detritus feeders, or (3) nutrient sequestration into the SOM nearest the river mouth.

Previous studies found a strong correlation between $\delta^{15}$N of estuarine organisms and anthropogenic nitrogen inputs, and suggested that stable isotopes could be used to identify wastewater inputs in coastal waters (McClelland et al. 1997; McClelland and Valiela 1998; Cole et al. 2004). Using a similar approach, this study indicates that $\delta^{13}$C and $\delta^{15}$N of estuarine clams and SOM may provide a means to predict salmon-derived nutrient inputs to estuaries. A more complete understanding of the influence of spawning salmon in estuaries will involve collecting stable isotope data from a series of estuaries receiving a range of nutrient inputs from spawning salmon and carcasses. Future studies should also endeavor to differentiate between the various nutrient sources and sinks through more detailed pathway and mass balance model analysis.
Figure 3.1 Samples were collected from high intertidal zone sites located near the river mouth, and mid-intertidal zone sites (both indicated by stars) in three estuaries: (a) Shawnigan, (b) Holland, and (c) Goldstream. Images adapted from Fish Wizard maps created by the Freshwater Fisheries Society of British Columbia (2005).
Figure 3.2 Length measurement for (a) varnish and (b) manila clams (as per Gillespie and Kronlund 1999).
Figure 3.3 Combined monthly $\delta^{13}C$ and $\delta^{15}N$ of: varnish $\bullet$ and manila clams $\circ$, chum salmon $\square$, high intertidal zone sedimentary organic matter (SOM) $\triangledown$, mid-intertidal zone SOM $\triangledown$, riverine suspended particulate organic matter (SPOM) $\otimes$, and estuarine SPOM $\otimes$, with mean benthic diatoms values taken from the literature $\star$ (Table 3.1).
Figure 3.4 Mean monthly (1) δ¹³C and (2) δ¹⁵N of varnish • and manila clams ○, high intertidal zone sedimentary organic matter (SOM) ---, mid-intertidal zone SOM ⌗, riverine suspended particulate organic matter (SPOM) – and estuarine SPOM ⌂ from three estuaries: (a) Goldstream, (b) Holland, and (c) Shawnigan. Data points for SOM and SPOM are means of two replicates, those for clams are means of three or more individuals.
Figure 3.5 Monthly C:N ratios of suspended particulate organic matter from three estuaries: Goldstream — , Holland — , Shawnigan — . Data points are means of two replicates.
Figure 3.6 Combined monthly $\delta^{13}C$ and $\delta^{15}N$ of sedimentary organic matter (SOM) from three estuaries: Goldstream $\bigcirc$, Holland $\triangledown$, and Shawnigan $\square$. Dark fills indicate high intertidal zone SOM, and no fill indicates mid-intertidal zone SOM.
3.8 Tables

Table 3.1 Published $\delta^{13}$C and $\delta^{15}$N for benthic diatoms.

<table>
<thead>
<tr>
<th>Sub categories</th>
<th>$\delta^{13}$C (%o)</th>
<th>$\delta^{15}$N (%o)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>-18.8 ± 0.4</td>
<td>-</td>
<td>(Doi 2005)</td>
<td></td>
</tr>
<tr>
<td>-16.2 to -17.9</td>
<td>-</td>
<td>(Haines 1979)</td>
<td></td>
</tr>
<tr>
<td>-19.4 to -15.2</td>
<td>5.3 to 7.6</td>
<td>(Kanaya 2005)</td>
<td></td>
</tr>
<tr>
<td>winter</td>
<td>-11.3 ± 0.1</td>
<td>9.1 +/- 0.2</td>
<td>(Riera 2002)</td>
</tr>
<tr>
<td>spring</td>
<td>-14.5 ± 0.2</td>
<td>7.2 +/- 0.3</td>
<td>(Riera 2002)</td>
</tr>
<tr>
<td></td>
<td>-16.1 ± 0.7</td>
<td>-</td>
<td>(Riera 1997)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>4.1 to 6.9</td>
<td>(Riera 1998)</td>
</tr>
<tr>
<td>&lt; 5 psu</td>
<td>-20.2 to -19.8</td>
<td>6.1 to 7.0</td>
<td>(Cloern 2002)</td>
</tr>
<tr>
<td>&gt; 10 psu</td>
<td>-27.4 to -19.6</td>
<td>2.8 to 10.9</td>
<td>(Cloern 2002)</td>
</tr>
</tbody>
</table>

Table 3.2 Analysis of variance (ANOVA) of $\delta^{13}$C and $\delta^{15}$N of high and mid-intertidal zone sedimentary organic matter from three estuaries.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$\delta^{13}$C (%o)</th>
<th>$\delta^{15}$N (%o)</th>
<th>df</th>
<th>F</th>
<th>Sig.</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estuary</td>
<td>3</td>
<td>242.3</td>
<td>98.9</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intertidal zone height</td>
<td>1</td>
<td>64.8</td>
<td>0.2</td>
<td>0.014</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Est. x Intertid. h.</td>
<td>3</td>
<td>11.5</td>
<td>10.5</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Error df, MSE)</td>
<td>(30, 0.071)</td>
<td>(30, 0.065)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R²</td>
<td>0.88</td>
<td>0.91</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
Table 3.3 Means ± standard deviation of the δ^{13}C and δ^{15}N (‰) of riverine and estuarine suspended particulate organic matter (SPOM), high and mid-intertidal zone sedimentary organic matter (SOM), and varnish clams from the high intertidal zone near river mouths, and manila clams from the mid-intertidal zone, from three estuaries.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Goldstream</th>
<th>Holland</th>
<th>Shawnigan</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>δ^{13}C</td>
<td>δ^{15}N</td>
<td>δ^{13}C</td>
</tr>
<tr>
<td>River</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPOM</td>
<td>-26.6 ± 1.4</td>
<td>6.7 ± 2.6</td>
<td>-28.1 ± 0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estuary</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPOM</td>
<td>-26.7 ± 2.4</td>
<td>6.8 ± 1.7</td>
<td>-25.3 ± 2.9</td>
</tr>
<tr>
<td>High intertidal zone SOM</td>
<td>-23.6 ± 0.2</td>
<td>6.5 ± 0.1</td>
<td>-25.0 ± 0.2</td>
</tr>
<tr>
<td>Mid-intertidal zone SOM</td>
<td>-23.6 ± 0.4</td>
<td>6.2 ± 0.3</td>
<td>-24.4 ± 0.3</td>
</tr>
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<td>Varnish clams</td>
<td>-18.0 ± 0.3</td>
<td>10.5 ± 0.2</td>
<td>-18.8 ± 0.3</td>
</tr>
<tr>
<td>Manila clams</td>
<td>-17.6 ± 0.2</td>
<td>10.0 ± 0.3</td>
<td>-18.5 ± 0.2</td>
</tr>
</tbody>
</table>

Table 3.4 Analysis of variance (ANOVA) of the δ^{13}C and δ^{15}N of varnish clams from the high intertidal zone near river mouths, and manila clams from the mid-intertidal zone, from three estuaries.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>df</th>
<th>δ^{13}C (%)</th>
<th>F</th>
<th>Sig.</th>
<th>δ^{15}N (%)</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estuary</td>
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<td>28.5</td>
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<tr>
<td>Species</td>
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<td>&lt; 0.001</td>
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</tr>
<tr>
<td>Est. * Sp.</td>
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<td>2</td>
<td>0.156</td>
<td></td>
<td>8.3</td>
<td>0.001</td>
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</tr>
<tr>
<td>(Error df, MSE)</td>
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<td>(30, 0.075)</td>
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<td>(20, 0.048)</td>
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</tr>
<tr>
<td>R^2</td>
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<td>0.900</td>
<td></td>
<td></td>
<td>0.741</td>
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<td></td>
</tr>
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</table>
Chapter 4  General Conclusions

Downstream nutrient transport has received little attention among the studies examining uptake of nutrients from spawning salmon and carcasses. In the Goldstream system, I estimated that the majority of carcasses materials were processed five weeks after the end of spawning (Chapter 2), during which time substantial amounts of organic matter, more than half of the phosphorus and nitrogen carried upstream by spawning salmon was exported back downstream to the estuary (Chapter 2). In the estuary some of these nutrients appear to be integrated into the benthic food web, which retains the isotopic signature of salmon throughout the year (Chapter 3).

Watersheds can also retain the isotopic signature of Pacific salmon throughout the year. After the end of spawning, salmon-derived nutrients can be stored in the hyporheic zone of stream channels or in adjacent floodplains (O'Keefe and Edwards 2002). The following spring, these nutrients are either re-released into the stream or taken up by primary producers in which the isotopic signature of salmon can then be measured. Riparian forests can store salmon-derived nutrients in soils (Reimchen et al. 2002; Mathewson et al. 2003) and in vegetation (Bilby et al. 1996; Helfield and Naiman 2002) adjacent to salmon streams, as indicated by high $\delta^{15}$N in samples collected prior to the return of salmon. Field experiments by Drake et al. (2006) corroborate these observations. They applied $^{15}$N-tracer to riparian plots during salmon spawning, and found that ~ 20% of the nitrogen was lost from the plots after one year, and that trees retained at least 28% of the tracer. Mathewson et al. (2003) also observed that nitrogen-rich soil indicators were only present below a waterfall barrier to salmon migration. These findings may reflect an isotopic legacy, created by thousands of years of accumulated nitrogen from the annual influx of salmon, and suggest a role for salmon in structuring and supporting riparian communities.

Nutrients from spawning salmon can also persist in aquatic ecosystems. For example, in early autumn, prior to the return of coho salmon, aquatic grazers, invertebrate predators, and resident cutthroat trout had high $\delta^{13}$C and $\delta^{15}$N compared to organisms from stream reaches that were inaccessible to spawning salmon (Bilby et al. 1996).
Aquatic invertebrate grazers (Ephemeroptera) were also collected from the streams examined in this thesis, during June 2006. Grazers from the Goldstream River, which annually receives tens of thousands of salmon, had high $\delta^{13}$C and $\delta^{15}$N compared to Holland River, where relatively few salmon return to spawn (Figure 4.1). Ephemeroptera from the land use-affected Shawnigan River also had high $\delta^{15}$N compared to Holland River, which may reflect anthropogenic nitrogen inputs. Shawnigan and Goldstream Rivers had similar grazer $\delta^{15}$N, which is another example of how human land use can confound isotopic trends that are often attributed to salmon-derived nutrients (Chapter 2).

Positive feedback cycles have been proposed whereby nutrients from salmon carcasses enhance stream habitat for subsequent generations in systems that otherwise are nutrient limited. Helfield and Naiman (2001) observed that nutrients from spawning salmon fertilized trees and shrubs in riparian forests, which appeared to increase growth rates of Sitka spruce ($Picea sitchensis$). Increased riparian production may benefit subsequent salmon generations by enhancing spawning and rearing habitat through shading, sediment and nutrient filtration, and production of large woody debris. Spawning salmon have also been associated with increased stream biofilm and macroinvertebrate abundance, which may benefit juvenile salmon by providing increased food resources (Helfield et al. 1998).

Similar mechanisms may be present in estuaries, where nitrogen and phosphorus often limit primary production (Rice and Ferguson 1975). Mass balance calculations for the Goldstream system, based on data from Chapter 2, suggest that salmon-derived nutrients can account for up to 90% of the total riverine phosphorus exported to the estuary during the period of salmon spawning and carcass decomposition (Oct-Dec). If a similar proportion of salmon-derived nitrogen was exported it would make up to 50% of the total riverine nitrogen exported to the estuary. In Goldstream Estuary, however, water column chlorophyll $a$ concentration did not reflect increased primary production during carcass decay in November and December (Figure 4.2). In a previous study, Fugiwara and Highsmith (1997) showed that estuarine macroalgae production increased as a result of salmon-derived nutrient inputs. In estuaries, emergent vegetation such as sedges and rushes, may also benefit, and thus provide better habitat and food sources for
juvenile salmon as they migrate toward the open ocean (Levings et al. 1991). Increased primary production can also lead to higher secondary production, which may provide more prey for juvenile salmon while they are in estuaries (Fujiwara and Highsmith 1997).

Growth rate of estuarine clams was also measured as part of this thesis, although I did not observe a response to inputs of salmon-derived nutrients in the Goldstream Estuary. Growth rate was based on the change in shell length from August 2005 to February 2006, and both the varnish clam and the manila clam had similar results. Growth rate was related to initial shell length, with smaller clams growing more in length than larger clams (Figure 4.3). Overall, the mean increase in shell length from clams 30 mm – 39.9 mm in length was small (< 1 mm), and large variance made differences that may exist between estuaries difficult to detect. Annual growth rates, rather than just fall/winter growth rates, may have encompassed more of the potential variation between systems. Alternatively, smaller and thus younger individuals would have been better suited to this study because they have higher growth rates (Dudas 2005). Unfortunately, due to patchy recruitment for intertidal zone bivalves (Gillespie and Kronlund 1999), smaller size classes were not available in sufficient abundance in all estuaries. Clams < 35 mm initial shell length showed the greatest variation in growth rate both within and among sites, and smaller varnish clams from Shawnigan Estuary had some of the highest growth rates. Substrate differences may account for the differences in growth rate for smaller varnish clams between estuaries. Summerson et al. (1995) observed that the hardshell clam (*Mercenaria mercenaria*) had higher growth rates in natural sand substrate compared to when a layer of gravel was added. Varnish clams from Shawnigan Estuary were collected from a sand substrate, whereas in the Goldstream and Holland Estuaries, varnish clams were collected from mixed sand and gravel substrates that may have had indirect interference effects on growth rate (Summerson et al. 1995).

It is highly probable that the food and nutrient subsidy provided by salmon affect the community structure of coastal ecosystems. Large numbers of predators are drawn to runs of spawning salmon in both freshwater and marine habitats (Reimchen 1994; Willson et al. 1998). In Southeast Alaska, over forty species of mammals and birds forage on salmon and their carcasses, and on eggs and juveniles (Willson and Halupka 1995). In the Goldstream watershed, I observed gulls, eagles, and bears feeding directly
on carcass tissue and eggs. Invertebrate populations often increase during salmon spawning (Hocking and Reimchen 2002), and prolific communities of algae, fungi, and bacteria develop on carcasses (Wipfli et al. 1998). Estimates for the Goldstream system suggest that up to half of the total salmon carcasses are deposited into estuaries (Johannes and Chow in prep), while anecdotal evidence suggests that these carcasses attract higher fall and winter densities of Dungeness crab, and that seasonal ‘fishy’ taste differences are observed in these crab (Mark Aitken, Goldstream Boathouse Marina, Victoria, B.C., pers. comm.). Other marine species might also migrate to estuaries during salmon spawning, since crab and other marine invertebrate predators, in addition to many species of mammals and birds, are known to feed on salmon carcasses (Reimchen 1994).

Stable isotopes of carbon and nitrogen have been used to trace the movement of salmon-derived nutrients through estuarine food webs. For instance, Fujiwara and Highsmith (1997) used stable isotope data to infer the uptake of salmon-derived nutrients by Ulva sp, an estuarine macroalga, which they then traced into harpacticoid copepods. The δ13C of varnish clams and manila clams in this study did not reflect the carbon stable isotope composition of the suspended particulate organic matter (SPOM) or the sedimentary organic matter (SOM) (Chapter 3). Rather, the high δ13C of clams indicates that they feed selectively on benthic diatoms, which make up varying proportions of each species’ diet. For future research the stable isotope composition of estuarine primary producers such as benthic diatoms and algae may provide more information on uptake pathways of salmon-derived nutrients into estuarine food webs.

Previous studies that used stable isotopes of nitrogen to quantify anthropogenic nitrogen input to estuaries may also provide a model for salmon-derived nutrient input to estuaries. In land use-affected estuaries the δ15N of producers and consumers can be a reliable indicator of anthropogenic nitrogen inputs within a particular region (McClelland et al. 1997; McClelland and Valiela 1998; Cole et al. 2004). Anthropogenic nitrogen and spawning salmon are both enriched in 15N. Consequently, the δ15N of estuarine biota may respond in a similar manner to both nutrient inputs. In support of this hypothesis, this thesis showed that clams and SOM from Goldstream Estuary had high δ15N compared to systems with smaller abundance of salmon (Chapter 3). Enrichment in 15N appears to reflect nutrient inputs from salmon in the Goldstream estuary. Thus by
sampling a series of estuaries receiving a range of nutrient loads, future studies can confirm whether the stable isotope composition of estuarine biota records increases in the freshwater export of salmon-derived nutrients.

Implications of this research for the Goldstream system are that the freshwater export of salmon-derived nutrients is substantial, and that these nutrients may be important for the estuary ecosystem. The enriched stable isotope composition of estuarine clams and sediment indicate that salmon-derived nutrients become integrated into the benthic ecosystem of the Goldstream estuary. Consequently, estuarine clams and sediment can potentially be used to provide long-term indices of the relative contribution of salmon-derived nutrients to estuarine food webs. Samples from a variety of estuaries that receive a range of nutrient input from salmon carcasses are needed to confirm this hypothesis, however. The effects of salmon-derived nutrients on estuarine productivity remain unconfirmed, and warrant more extensive examination. Nutrients from salmon carcasses may fertilize estuaries, thereby enhancing an important rearing environment for out-migrating juvenile salmon during the potentially critical early marine residence period (eg. Levings et al. 1991; Fujiwara and Highsmith 1997). Future studies should target systems with a range of escapement sizes, and include organisms from nearshore habitats frequented by juvenile salmon and their prey. As we gain a greater understanding of the many aspects of salmon-derived nutrient subsidies, escapement targets for salmon stocks may need to be reevaluated to include sufficient spawners for stream, riparian, and estuarine productivity.
4.1 Figures

Figure 4.1 Combined monthly $\delta^{13}$C and $\delta^{15}$N of stream Ephemeroptera from the families Baetidae (●), Ephemerellidae (احة), and Heptageniidae (◊) from three rivers: Goldstream (black fill), Holland (white fill), and Shawnigan (grey fill).
**Figure 4.2** Monthly chlorophyll $a$ concentrations from integrated water column samples collected during high tide from three estuaries: Goldstream $\bullet$, Holland $\triangleright$, and Shawnigan $\blacksquare$.

**Figure 4.3** Relationship between initial shell length and growth rate over 6 months (August-February) for (a) varnish and (b) manila clams in three estuaries: Goldstream $\bullet$, Holland $\triangleright$, and Shawnigan $\blacksquare$. 
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