Heavy Metals, Selenium, and Pacific Dunlin: Patterns of Accumulation, Exposure from Prey and Toxicity Risks

by

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Abstract

My research objectives for the thesis were: 1) Investigate factors that contribute to heavy metal and selenium dietary exposure and accumulation in Dunlin (*Calidris alpina*). 2) Examine the potential for adverse effects to Dunlin from such elements. To pursue these objectives I examined elements in feather and kidney tissues and analyzed ingested items in gizzard contents. Habitat preference (terrestrial vs estuarine), trophic level, age, sex, bill length, and size were investigated as factors potentially influencing element accumulation. Toxicity risks associated with accumulated element burdens and dietary exposure were assessed with comparisons to levels of demonstrated adverse effects for avian species.

I report concentrations of cadmium, copper, and zinc in kidneys as well as copper, mercury, selenium, lead, and zinc in feathers. Cadmium concentrations in kidneys increased logarithmically with age. Cadmium accumulated to a greater degree in Dunlin that foraged in estuarine habitat as compared to more terrestrial feeders. Cadmium, copper and zinc in kidneys did not occur at concentrations known to incur deleterious health or reproductive effects. Copper, lead, and zinc concentrations in feathers were within documented ranges for sandpipers and/or below levels associated with adverse effects. Mercury in feathers of some Dunlin nearly reached concentrations associated with risks of adverse effects. Selenium in feathers of most individuals exceeded the threshold above which toxicity risks are present.

Daily exposure to cadmium, copper, and zinc was determined for six diet types. Diets from agricultural fields were lower in all metals than terrestrial diets from birds collected at Vancouver International Airport (YVR). Diets containing mostly sediment exposed Dunlin to low amounts of metals compared to other estuarine diet types. Cadmium exposure was greatest in the diet type containing mostly mud snails (*Batillaria attramentaria)*, and copper and zinc exposures were greatest in YVR diets. Exposure was concerning as eight of eighteen assessments predicted probable toxic effects. Exposure risk is mitigated by co-abundance of metals and Dunlin's tendency to feed in both estuarine and terrestrial habitats. Potential issues with applying daily exposure

models to estuarine feeding sandpipers with relatively high metabolisms as compared to other avian species of similar size are discussed.

Keywords: Dunlin; Fraser River Delta; exposure routes; risk assessment; heavy metals; selenium

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Chapter 1:

General Introduction

Rationale for the Study

Living organisms can tolerate heavy metal and selenium exposure to varying degrees (Stanton et al. 2010; Wayland and Scheuhammer 2011). In fact, many trace elements (e.g. copper, magnesium, zinc) are essential for optimal growth and health in plants and animals. However, when exposure exceeds organisms' capacities to utilize excrete, or immobilize such elements, toxicity can result in the form of negative impacts on health, development, and reproduction, or even mortality (Scheuhammer 1987; Levengood et al. 1999; Sileo et al. 2003). With anthropogenic emissions now exceeding those from natural sources (Nriagu 1989; Pacyna and Pacyna 2001), many trace elements are increasing in abundance and concentration in natural systems (Eisler 2000). Metals present within natural mineral deposits and those emitted by human activities are often leached or washed into aquatic systems where metal ions have a greater affinity for suspended fine grain silts, clays and organic matter than for water (Tessier and Campbell 1987; Grant and Middleton 1990; Allison and Allison 2005). When water moves from rivers to estuarine systems, flow rates slow and such suspended materials deposit into sediments. Estuarine systems, thus, receive high inputs of heavy metals relative to other habitat types and act as long term sinks for these potential toxicants (Cundy et al. 1997).

Sediment-dwelling organisms in estuarine habitats often accumulate trace elements in concentrations corresponding to those in sediments (Tessier et al. 1994; Ferns and Anderson 1997; Thomas and Bendell-Young 1998) functioning as exposure vectors to their consumers. Some elements are even bio-concentrated in the tissues of such organisms relative to the waters and sediments in which they live (e.g. cadmium in

shellfish; Burger 2008). Birds feeding in shoreline habitats accumulate higher levels of heavy metals and other trace elements (e.g. cadmium, selenium) than those in terrestrial environments (Burger 1993; Lucia et al. 2010; Wayland and Scheuhammer 2011). Numerous industrial, agricultural, and residential areas along the North American Pacific Coast increase the potential for deleterious heavy metal exposure to estuarine biota. Some coastal ducks and estuarine feeding shorebirds in this region have already been documented with trace element tissue concentrations above levels associated with impaired avian health and reproduction (e.g. selenium, mercury, cadmium: Hui 1998; Hui et al. 2001; Barjaktarovic et al. 2002).

Dunlin (*Calidris alpina*) are migratory sandpipers with a circumpolar distribution. They are an appealing subject for toxicology as their behaviour and physiology have been studied throughout the world resulting in an extensive knowledge-base that can be used to help identify and explain patterns of toxicant exposure and accumulation. Pacific Dunlin feed in intertidal and agricultural habitat (Shepherd 2001) on bivalves, gastropods, and crustaceans in estuaries, as well as on terrestrial insects and larvae, and even some vegetation (Evans Ogden et al. 2008; this study). Dunlin have also recently been shown to feed on biofilm (Elner et al. 2005; Mathot et al. 2010; Kuwae et al. 2012): a muscelagenous layer of sediment, microphytobenthos, bacteria, and extracellular polymeric substances (EPS) produced by those organisms (Decho 1990). The EPS and sediment ingested with biofilm are potential exposure vectors for cadmium, lead, and other metals (Schlekat et al. 1998; Franson and Pain 2011).

The Fraser River Delta (FRD), located in southwest British Columbia, contains the largest estuary on Canada's pacific coast. It has been designated an Important Bird Area (BirdLife International) and a site of hemispheric importance to shorebirds (Western Hemisphere Shorebird Reserve Network) due to its use by nationally threatened species and as many as 1.4 million migrant and resident birds anually (Butler and Campbell 1987). The delta stands adjacent to the Vancouver metropolitan area and is, consequently, subject to industrial, agricultural, and other anthropogenic effluents. Additionally, upwelling ocean currents bring cadmium-rich waters to the surrounding Salish Sea and have been linked to cadmium concentrations in bivalves (Shiel et al. 2012) that often exceed levels fit for human consumption (Kruzynski 2004; Bendell 2009). The effects of upwelling have also been observed in avifauna at pristine sites on

British Columbia's coast where cadmium levels in seabirds exceed those of the same species on Canada's Atlantic coast (Elliott and Scheuhammer 1996). Considering oceanic cadmium sources and the potential for additional anthropogenic inputs, heavy metal and trace element exposure to wildlife in the FRD may be of concern.

The Dunlin is the most common winter resident sandpiper in the FRD with 25,000 to 40,000 individuals living on FRD habitat each fall and winter (Butler and Vermeer 1994; Shepherd 2001). In spring, their northward migration follows a coastal route that brings most of the population through the FRD (Butler and Campbell 1987; Warnock and Gill 1996) thereby increasing the impact of FRD habitat on the Pacific Dunlin population.

Previous studies have measured accumulated metals and trace elements in Dunlin and other sandpipers (e.g. Ferns and Anderson 1994, 1997; McFarland et al. 2002; Braune and Noble 2009). However, to my knowledge, no study has investigated metal exposure to sandpipers in the Fraser River Delta. Additionally, most toxicological studies report only tissue concentrations while research on the influence of habitat preference, prey choice, and morphological characteristics on toxicant exposure and accumulation is lacking. By pairing trace metal and selenium measures with information on Dunlin's ecology and morphology, the thesis provides novel knowledge of exposure pathways and sources. The eco-toxicological approach also broadens the relevance of results to other sandpiper species that share habitat with Dunlin and consume some of the same food items. Information on tissue concentrations in Dunlin also supplements a limited knowledge base of heavy metal and trace element levels in shorebirds of the Pacific American coast.

Research Objectives

The primary research goals for the thesis are two-fold:

1) Investigate factors that contribute to heavy metal and selenium exposure and accumulation in Dunlin.
2) Examine the potential for adverse effects to Dunlin from such elements.

Outline of Thesis

The thesis is introduced here, in the first chapter, with the rationale for study, research objectives, and the following general outline to give the reader context and to clarify the purpose of the work.

The second chapter approaches research objectives using measurements of Dunlin characteristics and their tissues. Habitat preference (estuarine vs. terrestrial), trophic feeding level, age, sex, bill length, and body size are considered as factors potentially influencing element accumulation. Accumulated burdens are compared with toxicity thresholds set by laboratory studies of other avian species and, for additional context, are also compared with previously documented element levels in Dunlin and related sandpipers.

Chapter three examines exposure routes at the prey item level via a case study of gizzard contents from Dunlin collected in the Fraser River Delta. Comparisons of dietary exposure to heavy metals were made across various diet types and sites in the FRD, as well as across specific prey items, including biofilm. Chapter three also assesses the risk of adverse effects from dietary exposure to cadmium, copper, and zinc in the FRD. The risk assessment incorporates estimation of site-specific energy expenditure, energy content and metal concentrations in prey items, as well as toxicity thresholds that are, again, based on laboratory studies of other avian species.

The thesis is concluded with a summary of the most important exposure routes and toxicity risks to Pacific Dunlin from the elements investigated. Comparisons between conclusions of the Dunlin level (i.e. accumulated elements) and prey level (i.e. dietary exposure) risk analyses are also made with discussion of discrepancies between the two.

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Chapter 2:

Heavy Metals and Selenium in Pacific Coast Dunlin (*Calidris alpina*): Patterns of Accumulation and Concentrations in Kidneys and Feathers

Abstract

Estuarine systems are reservoirs for heavy metals and the metalloid selenium. Accumulation of such elements in biota can have adverse effects even at low concentrations. Dunlin (*Calidris alpina*) are sandpipers that feed in both estuarine and agricultural habitats. I investigated concentrations of cadmium, copper, and zinc in kidney tissues as well as copper, lead, manganese, mercury, selenium and zinc in feathers of Pacific Dunlin (*C. a. pacifica*) collected in the Fraser River Delta of British Columbia and Skagit Bay, Washington to: 1) Investigate factors influencing accumulated element concentrations. 2) Determine if accumulated elements exceed concentrations observed to have adverse effects on avian species. I analyzed the influence of age, sex, bill length, sample group, body size, habitat preference, and trophic feeding level on element concentrations in Dunlin using morphometrics, dissections, and stable isotope analyses as investigative tools. Cadmium, copper and zinc in kidneys did not occur at concentrations known to cause deleterious health or reproductive effects. Bill length, sex, and body size were not significantly related to concentrations of any of the listed elements. Kidney cadmium concentrations increased logarithmically with age reaching an apparently asymptotic stable state after ten months from hatching suggesting concentration dependent excretion rates. A concentration-dependent half-life of approximately 7 months was estimated for kidney cadmium in the sampled population. Cadmium concentrations in Dunlin kidneys were significantly related to foraging habitat as described by stable isotope signatures in muscle tissue. Estuarine feeding birds had the greatest cadmium burdens. Although stable isotope

signatures indicated no significant relationship between trophic level of, and metal concentrations in Dunlin, comparisons with published data on Western Sandpipers (*C. mauri*) suggest biofilm may be an important exposure vector for cadmium. Copper lead and zinc concentrations in feathers were below levels associated with deleterious effects and/or within documented ranges for sandpipers. Mercury in feathers of some Dunlin nearly reached concentrations associated with risks of adverse effects and selenium in feathers of most individuals exceeded the threshold above which such risks are present. This research improves understanding of metal exposure pathways for sandpipers and suggests further research on sub-lethal and developmental effects of trace elements in wild populations, especially selenium, should be a priority for sandpiper ecotoxicology.

Introduction

The dose, stability, and form of a substance determine its toxicity. When quantities of trace elements in an organism's diet exceed its capacities to utilize, excrete, or immobilize such elements, toxicity can result in the form of negative impacts on health, development, and reproduction, or in extreme cases can even lead to premature death (Scheuhammer 1987; Burger and Gochfeld 1994; Levengood et al. 1999; Sileo et al. 2003). Trace elements that induce toxic effects at low concentrations (i.e. below 1 mg/g) include a number metals (e.g. copper, manganese, zinc, cadmium, lead, mercury) and other elements (e.g. selenium). Many of these elements have been elevated above natural levels since the industrial revolution (Eisler 2000) with anthropogenic emissions now exceeding those from natural sources (Nriagu 1989; Pacyna and Pacyna 2001). Metals and other elements present in natural mineral deposits and those emitted by human activities are often washed or leeched into aquatic systems where element ions have a greater affinity for suspended fine grain silts, clays and organic matter than for water (Tessier and Campbell 1987; Grant and Middleton 1990; Allison and Allison 2005). When water moves from rivers to estuarine systems flow rates slow and such suspended materials deposit into sediments. Estuarine systems, thus, receive high inputs of trace elements and act as long term sinks for these potential toxicants (Cundy et al. 1997).

Sediment-dwelling organisms in estuarine habitats often accumulate elements in concentrations corresponding to those in sediments (Tessier et al. 1994; Ferns and Anderson 1997; Thomas and Bendell-Young 1998) functioning as exposure vectors to their consumers. Some elements are even bio-concentrated in the tissues of such organisms relative to the waters and sediments in which they live (e.g. cadmium in shellfish: Burger 2008). Birds feeding in near shore and shoreline habitats accumulate higher levels of trace elements and metals (e.g. cadmium, selenium) than birds that forage terrestrially (Burger 1993; Lucia et al. 2010; Wayland and Scheuhammer 2011). Numerous developed areas along the North American Pacific Coast increase the potential for deleterious heavy metal exposure as a result of industrial, agricultural, and human effluents. Some coastal ducks and estuarine-feeding shorebirds in this region have already been documented with tissue concentrations of trace elements above levels associated with impaired avian health and reproduction (e.g. selenium, mercury, cadmium: Hui 1998; Hui et al. 2001; Barjaktarovic et al. 2002).

Dunlin (*Calidris alpina*) are migratory sandpipers with a circumpolar distribution. They are an appealing study subject for ecotoxicology, as their behaviour and physiology have been studied throughout the world resulting in an extensive knowledge-base, which can be utilized to interpret patterns in contaminant accumulation. The Pacific sub-species, *C. alpina pacifica,* breeds in western Alaska. Following summer breeding, Pacific Dunlin spend August and September completing feather moult at staging areas in southwest Alaskan estuaries (Holmes 1971). Migration from Alaska occurs from late September through October when Dunlin fly across the Pacific Ocean to winter residence sites primarily from southern British Columbia through Mexico (Warnock and Gill 1996). Body feathers are moulted again in March and April into breeding plumage (Holmes 1966). The northward spring migration occurs predominately in April and May along a coastal route (Butler and Campbell 1987; Warnock and Gill 1996).

Pacific Dunlin feed in intertidal and agricultural habitat (Shepherd 2001) on bivalves, gastropods, and crustaceans in the upper three to five centimeters of estuarine sediments, as well as on terrestrial insects, insect larvae, and vegetation (Evans Ogden et al. 2008; Chapter 3). Dunlin have also recently been shown to feed on biofilm (Elner et al. 2005; Mathot et al. 2010; Kuwae et al. 2012): a muscelagenous layer of sediment,

microphytobenthos (e.g. diatoms), bacteria, and extracellular polymeric substances (EPS) produced by those organisms (Decho 1990). Sediment ingestion in avian species is a known exposure pathway for lead (Franson and Pain 2011). Furthermore, EPS associated with biofilm significantly elevates the adsorption of many metals into the aerobic silica matrices of estuarine surface sediments (Schlekat et al. 1998) and cadmium can be incorporated as a component of enzymes in some marine diatoms (Lane et al. 2005).

The goals of this study were to investigate factors that contribute to heavy metal and selenium accumulation and to identify the potential for toxic effects to Dunlin from such elements. While I analyzed a wide variety of elements in Dunlin tissues, I was only able to verify and report on measurements for cadmium, copper, manganese, mercury, lead, selenium, and zinc. Habitat preference (estuarine vs. terrestrial), trophic level, sample group (i.e. date and location of capture), age, sex, bill length, and size were all investigated as factors potentially influencing accumulated trace element concentrations in tissues. Additionally, I reviewed previously published element concentrations in Dunlin and related sandpipers to put my observations in the context of other populations and examine any overarching patterns of metal accumulation in this group of waders.

Methods

Study site and sample collection: Located in southern British Columbia, the Fraser River Delta (FRD) is the largest estuary on Canada's pacific coast. During migration, the FRD hosts Dunlin and many other avian species that utilize the Pacific Flyway. Dunlin were first collected in November 2009 from the Vancouver International Airport at Sea Island (49° 12' N, 123° 12' W) in the FRD after a collision with aircraft. Others were donated from a study in Skagit Bay, Washington (48° 16' N, 122° 22' W) where captures during December 2007 through February 2008 resulted in occasional accidental mortalities (U.S. Fish & Wildlife Service Scientific Collection/Import/Export Permit # MB004887-0). These initial samples were mostly juvenile and stable isotope analyses indicated little variation in habitat preferences among individuals. To determine the full range of accumulated metal concentrations and improve the capacity to investigate the effect of habitat preference as well as age, additional samples were

warranted. Dunlin were collected by shotgun at Boundary Bay in the FRD (49° 04' N, 122° 57' W) on April 17 and 18, 2010 under Simon Fraser University Animal Care Committee protocol # 946B-09 and Environment Canada scientific permit # BC-10-0034. Boundary Bay collections were made on each day during the hour following sunrise, as the tide began to fall and birds were coming to the shoreline to feed on newly exposed mudflat. Individuals were collected from a total of eleven flocks. A few days later, I salvaged a second group of Dunlin that collided with aircraft at the Vancouver airport (YVR). Permissions for holding and possession of birds collected at YVR were granted under the following salvage permits: BC-09-0141, BC-SA-0046-10, BC-SA-0046-11. By the dates of collection in April 2010, increases in Dunlin numbers and the arrival of other migratory species indicated that migration was underway. Therefore, collections were composed of migrant and FRD winter resident birds in unknown proportions. To sample tissues representing FRD exposure, Dunlin were captured by net gun for feathers in early April 2011 (i.e. before migration) at Robert's Bank of the FRD (49° 03' N, 123° 09' W) and at Boundary Bay. Concurrent bird surveys at Boundary Bay in 2011 confirmed that feather collections were made before the arrival of migrants (HPJ van Veelen, unpublished data). These Dunlin were tagged with Canadian Wildlife Service steel bands and released after feather collection. Sample sizes, tissues analyzed, and collection dates are presented in Table 2.1. Capture and collection locations are presented in Figure 2.1.

Table 2.1 Pacific Dunlin (Calidris alpina pacifica) sample groups and tissues analyzed for heavy metal and selenium concentrations

Date	Location	Sample Size	Tissues Analyzed
Dec 2007 – Feb 2008	Skagit Bay, WA	5	Kidney
Dec 2008	Skagit Bay, WA	2	Kidney
Nov 5, 2009	YVR Airport, BC	12	Kidney
Nov 24, 2009	Boundary Bay, BC	1	Kidney
Apr 17-18, 2010	Boundary Bay, BC	44	Kidney, Feathers
Apr 28, 2010	YVR Airport, BC	20	Kidney
Apr 5-6, 2011	Boundary Bay, BC	9	Feathers
Apr 7, 2011	Robert's Bank, BC	6	Feathers

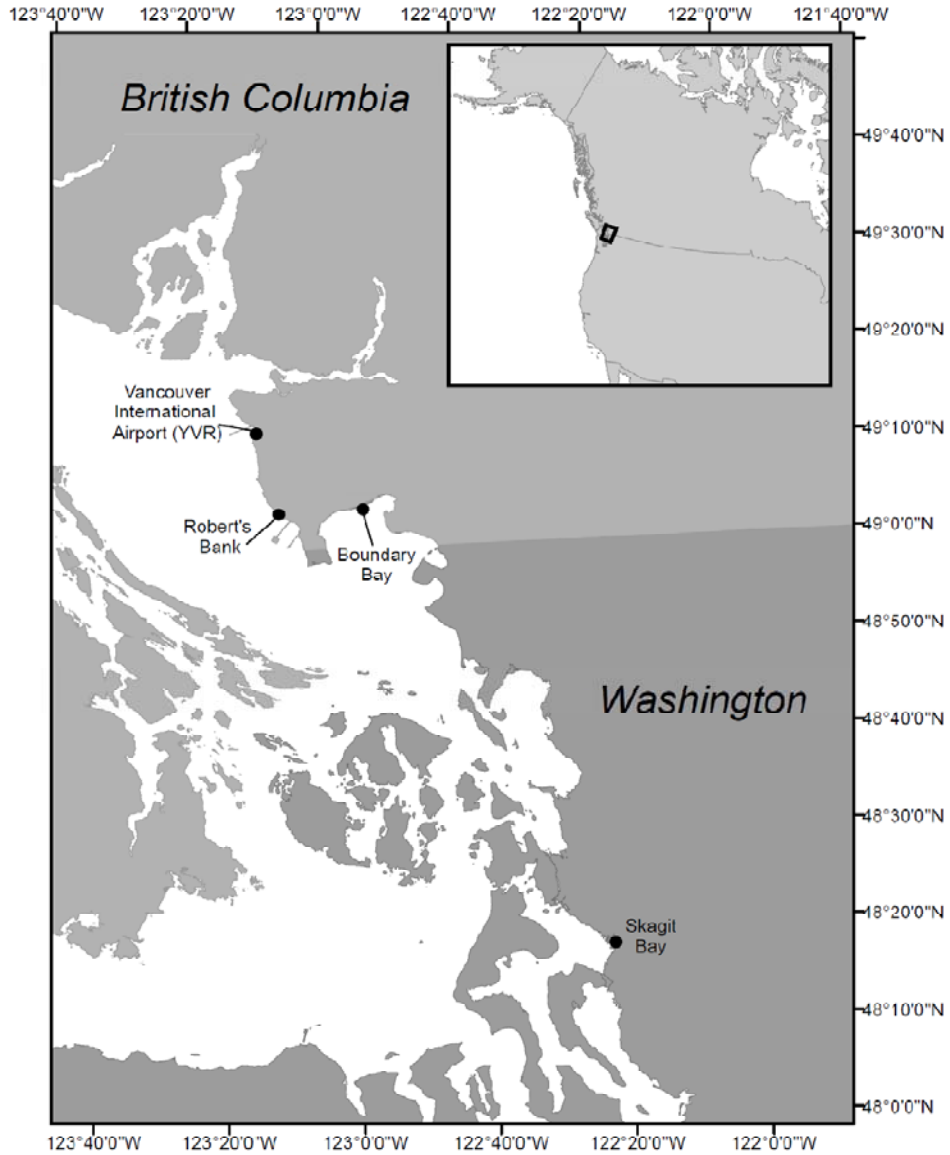

Figure 2.1 ***Capture and collection sites of Dunlin (Calidris alpina) for tissue sampling and investigation of trace element accumulation***

Measures of Dunlin characteristics: Tarsus length, bill length, and age class as determined by plumage (Prater 1977), along with gender based on bill length (males < 37.7; females > 39.8: Prater 1977) were recorded for all sample groups. Tarsus length was used as a proxy for body size. Tarsus measurements included all joint articulations and bill length was measured from the tip of the bill to the base, where the bill meets facial feathers. Weight was also recorded for Dunlin captured and released in April 2011. Stable isotope ratios of muscle tissue were used to indicate

habitat preference and trophic level (see explanation below). Juveniles caught in their first calendar year (i.e. before January 1) were classified as “hatch-years” (HY), individuals in their second calendar year as “second-years” (SY), and adults in their third calendar year or older as “after-second-years” (ASY). Dunlin age classes relative to migratory history and range are presented in Figure 2.2. Dunlin from 2010 were measured within one hour of collection then frozen at -20°C until dissection.

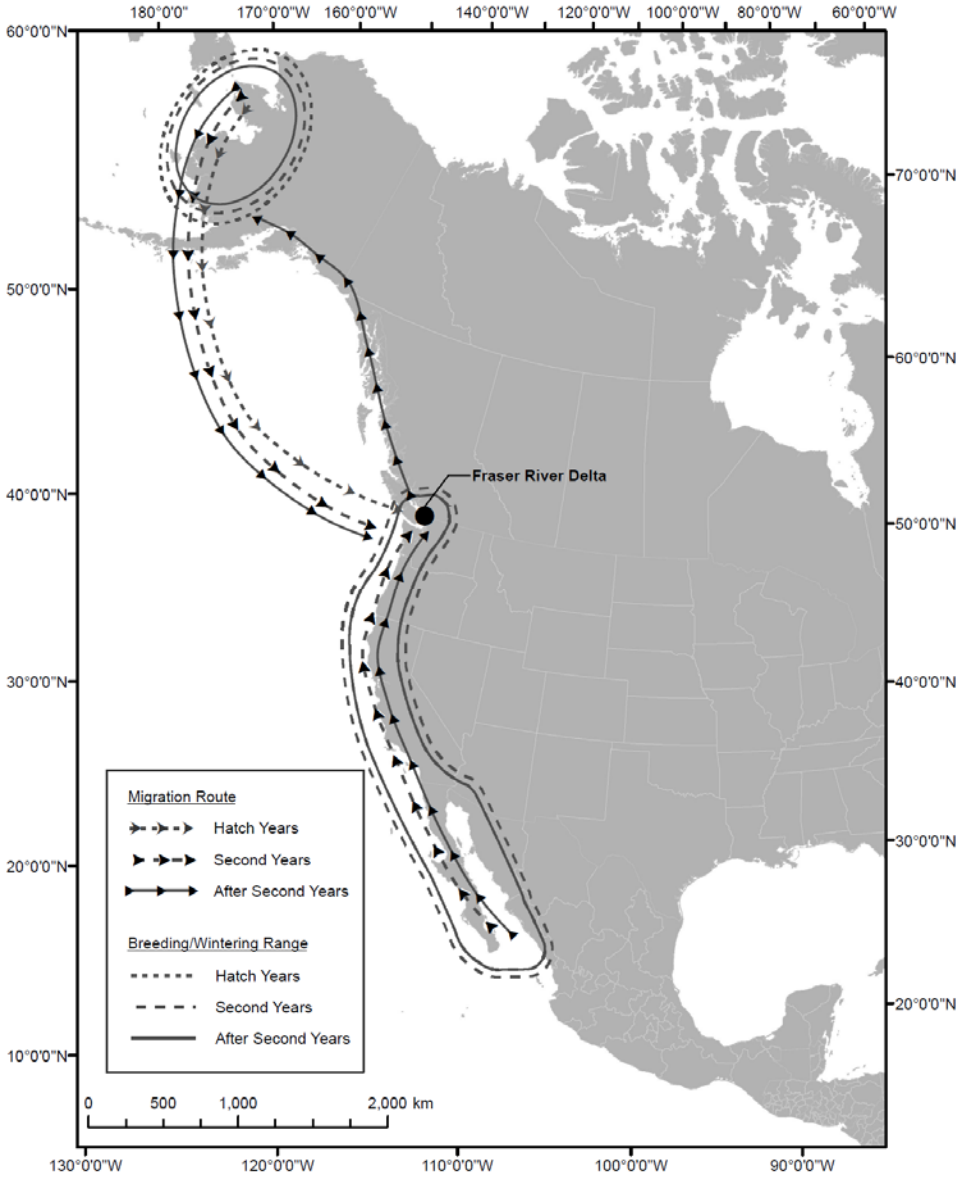

Figure 2.2 ***Migration routes, wintering range and breeding range of Dunlin (Calidris alpina pacifica) collected in the Fraser River Delta, BC during fall 2009 and spring 2010.***

Note: Hatch-years were collected on their first fall southward migration, second-years were collected during their first spring, and after-second-years were captured during spring after having already completed the full migration cycle at least once. Parallel arrows indicate overlapping migration pathways.

Dissections and tissue samples: Dissections were performed in a class II bio-safety cabinet in accordance with bio-safety protocols. Sex was determined via dissection for all spring collections in which testes were developed sufficiently to be

distinguished. Final sex designations were made with dissection observations rather than bill measurements except when sexing by dissection was inconclusive. If birds were unable to be sexed by dissection and had intermediate bill lengths between the known ranges for males and females, they were designated unknown and excluded from analyses involving sex. During dissections, separate scalpel blades were used for extraction of each tissue type. Kidneys generally have the highest concentrations of cadmium in birds (Wayland and Scheuhammer 2011) and are therefore the best indicators of accumulation for this element. Other metals such as copper and zinc also accumulate in sandpiper kidneys to a similar degree as in the liver (Blomqvist et al. 1987; Lucia et al. 2010), so kidneys were selected as the most appropriate tissue in which to investigate patterns of accumulation. Mercury occurs in a number of inorganic and organic forms of which organic methyl-mercury is the most toxic (Thompson 1996). Mercury in feathers is almost entirely comprised of methyl-mercury (Thompson and Furness 1989) and typically represents a large percentage of total mercury body burdens in birds (e.g. 70-93%: Burger 1993). Consequently, feathers are an appropriate tissue for assessing mercury toxicity risks. Feathers offer an indication of exposure during and preceding growth for a variety of other elements as well (Burger 1993). Contour feathers grown on the back, breast, rump and belly were chosen for analyses since they have less variable metal concentrations than flight feathers (Furness et al. 1986). Tissue was extracted from breast muscles of Dunlin for analyses of stable isotope ratios (see explanation below).

Invertebrate sampling: Invertebrates were collected at Robert's Bank and Boundary Bay from estuarine mudflats and agricultural fields within one km from intertidal areas. Collections were taken in triplicate at randomly selected locations in each of those four areas and were made with sediment cores 10 cm in diameter and 5 cm in depth. Three sets of cores were taken during winter (Dec-Jan) and spring (Apr-May) of 2010. Additional cores were taken in 2011 to supplement samples for invertebrates that were not sufficiently abundant in 2010 samples. Invertebrates were separated from sediments with deionised water and a 2 mm sieve followed by a 1 mm sieve, then placed into separate scintillation vials according to prey type (e.g. polychaetes, mud snails, small crustaceans, worm larvae, fly larvae, etc.), then dried at 60°C until weights were stable.

Stable isotope analysis: Advances in the ecological applications of stable isotope ratios in the environment have provided tools to investigate exposure pathways and sources of accumulation. The ratio of ^{15}N to ^{14}N isotopes in biota is generally indicative of trophic level while ratios of ^{13}C and ^{12}C are relatively stable across trophic levels but often distinct across habitat types (Michener and Lajitha 2007). Thus, one can use stable carbon isotope ratios to infer the habitat where an organism has fed. For comparative purposes, stable isotope ratios are described with delta notation ($\delta^{13}C$ and $\delta^{15}N$ in the case of carbon and nitrogen) where the ratio of heavy to light isotopes is multiplied by 1000 and subtracted by the isotope ratio of a standard material unique to each element. Delta values for all standard materials are zero and serve as a reference point. Ratios greater or less than standards have positive or negative values, or "signatures", respectively and are described as "enriched" or "depleted" when more positive or negative relative to other values. As the ratio is multiplied by a factor of 1000, delta notation describes values in units of per mil (‰).

Due to variation in isotopic turnover rates across tissues, stable isotope ratios in muscle tissues represent habitat use and trophic feeding level over a greater period of time than other soft tissues (e.g. liver, blood), but a shorter period than bone (Hobson and Clark 1992a). Since bone signatures integrate signatures from prey items from over a year, and the interests of this study lied in exposure to elements over the previous 6-8 months (i.e. the wintering period for SY and ASY birds, and the entire life of HY birds), muscle was selected as the most appropriate tissue for stable isotope analyses. Stable isotope ratios for $\delta^{13}C$ and $\delta^{15}N$ in breast muscle were determined at the Stable Isotope Facility of the University of California (UC), Davis. Before analysis, dried and homogenated muscle tissue was treated with two lipid rinses involving 10 ml of a two to one chloroform:methanol solvent which was agitated and allowed to sit for twelve hours. After each rinse the solvent was decanted off and after the second rinse samples were air dried in scintillation vials for 48 hours. Approximately one mg of the dried, lipid rinsed muscle tissue from each bird was used for analyses. I analyzed two replicates of each muscle sample to examine variation within the homogenated tissue. One mg of cleaned (see cleaning methods below for elemental analysis) contour feathers from April 2011 Dunlin were cut and weighed into tin capsules for analysis of $\delta^{13}C$ and $\delta^{15}N$ at UC Davis.

Dunlin prey items collected from the FRD were identified, dried, ground, and weighed (1 mg) for $\delta^{13}C$ and $\delta^{15}N$ analysis at UC Davis as well.

Sample preparation for element analysis: Feather and kidney samples were analyzed for trace element concentrations. All glassware used to handle samples was soaked in a 10% HCl acid bath for a minimum of 24 hours then rinsed six times with ultrapure water before use. Following dissection, kidneys were placed in vials and dried at 60°C for 48 hours then ground and homogenized using a mortar and pestle. Approximately 0.07 g of each homogenized sample was weighed into 50 ml flasks into which 4 ml of nitric acid (70%) was added (adapted from Burger and Gochfeld 1990; McFarland et al. 2002). For validation of concentration measurements (i.e. quality control) all digestions included at least two certified reference materials (e.g. TORT-2, DOLT-2; National Research Council Canada, Ottawa, ON) with known metal concentrations, at least one pair of samples from the same kidney, and a procedural blank. Samples and the quality controls were digested together in open flasks on a hot plate at 200°C until ca. 0.5 ml of acid digestate remained. The remaining sample was diluted with 4 ml ultrapure water and transferred into polypropylene tubes. Flasks were rinsed twice with 4 ml of 2% nitric acid solution and rinses were added to the tubes which were closed and refrigerated until analysis. Kidney digestates were analyzed via Flame Atomic Absorption Spectrometry (AAS) for cadmium, copper, and zinc at Simon Fraser University, in Burnaby, British Columbia. Time and funding restraints prevented analyses of additional elements in kidney tissues.

After collection, recently grown contour feathers from the back and breast of all birds were cleaned of external debris with a jet of air and ten minutes of rinsing in an ultrasonic bath. For sonication, feathers were placed in 20 ml scintillation vials, then vials were filled with ultra pure 18MΩ H_20 and placed in an ultrasonic bath for two, five minute rinses (adapted from Norris et al. 2007). Water was replaced between rinses. I decided against a solvent rinse because feathers had grown in within two weeks of collection or shortly thereafter, so the potential for metal deposition onto feathers from external sources to significantly alter concentration was minimal. Additionally, when used to remove external deposition, solvent rinses can remove some internally deposited metals (Edwards and Smith 1984; Weyers and Gluk 1988; Burger 1993).

After rinsing, feathers were dried at 60°C for 48 hours. All digests were again accompanied by the quality controls listed in the kidney metal analysis procedure. Feathers from 2010 (15-20 per individual) were digested with 9 ml nitric acid (70%) via microwave in closed, acid washed, Teflon vessels in accordance with EPA method 3052 to prevent mercury loss by volatilization. Feathers from 2011 (10-25 per individual) were digested on a hot plate at 200°C in flasks with 5 ml nitric acid. Once the volume fell below 1 ml another 2 ml of acid (1 ml hydrochloric acid (35%), 1 ml nitric acid) was added and samples were left to digest until < 1 ml remained. The remaining sample was treated in the same manner as kidney hot-plate digestions. After dilution, digestates were spiked with iridium, thulium, and rhodium standards, and analyzed alongside additional lab blanks for concentrations of 24 trace elements (Ag, Al, As, Au, Ba, Ca, Cd, Cr, Cu, Co, Fe, Kr, Mn, Mo, Ni, Pb, Sb, Se, Sn, Sr, Ti, Tl, V, Zn) at the Institute for Integrated Research in Materials, Environments, and Society (IIRMES) of California State University at Long Beach via Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Digestates from 2010 collections were analyzed for mercury concentrations via cold vapour atomic fluorescence spectroscopy (CVAFS). Mercury was not measured in feathers collected during 2011 because of financial limitations. For quality assurance testing of the laboratory methods and instruments employed, measurements of element concentrations in certified reference materials and spikes were compared to their reported concentrations. Results are reported for elements where measured concentrations of reference materials were within one standard error of certified values and sample concentrations were above detection limits. For elements with no certified values in reference materials, concentrations were reported if sample replicates from the same individuals had similar concentrations and differences in total element quantities were proportional to differences in sample weights.

Measurement precision, adjustments, and variation across replicate samples: Analyses of element concentrations in kidney tissues were performed over the course of eight months. To account for variation in instrument readings on separate days over this period of time, I applied correction coefficients determined from the average differences between concentration measurements of certified reference materials on the day of analysis and their certified concentrations. Variance in element concentrations across replicate samples from the same individuals

was quantified with a standard deviation for each element. Mean concentrations for replicate samples vary across individuals, so overall standard deviations for each metal were calculated with the set of differences between replicate samples from their respective means. Variation in isotopic signatures across replicate samples were calculated by the same method. Standard deviations of reference isotope samples from their certified value were also determined.

Elemental turnover rates in kidneys: Elements in internal tissues such as the liver and kidney have varying turnover rates that are important for interpreting metal concentrations. Turnover rates of copper and zinc in kidneys were estimated from studies of other avian species. Data on kidney cadmium, however, were sufficient to calculate a half-life for Pacific Dunlin. Rapid increases in kidney cadmium concentration between HY and SY age classes, followed by an absence of significant accumulation between SY and ASY age classes (Figure 2.6) indicates the rate of cadmium excretion increases linearly with kidney concentration. Therefore, a 1st order reaction model should describe the rate at which Dunlin process kidney cadmium. In 1st order reactions half-life is defined as follows:

$$t_{1/2} = 0.693/k$$

where k is the fraction of kidney cadmium reacting per unit time

Since cadmium appears to reach a steady-state (i.e. constant concentration) in kidneys, reaction (k) can be defined as input or output because the two are the same at equilibrium. I used accumulation over the ten-month period from June when juveniles hatch, through April when SY Dunlin were captured, to define input per unit of time. Input is more accurately defined by the sum of accumulation and excretion, but the high rate of accumulation in juvenile Dunlin relative to adults suggests excretion from kidneys is relatively low in the younger birds and an assessment of excretion rates was beyond the scope of this research. I assumed initial cadmium concentrations in hatchlings to be zero as the true concentration was unknown, but likely very low considering previously documented levels in juvenile Western Sandpipers collected during August in the FRD (ca. 1 µg/g mean; McFarland et al. 2002) and juvenile Dunlin collected during early fall in Sweden (ca. 0.3 µg/g mean; Blomqvist 1987). Also, since cadmium in kidneys of Dunlin

in fall reflect exposure from breeding and staging sites whereas spring levels are more related to exposure at wintering sites (see discussion on metal turnover rates) there was potential for season of collection to influence differences in age class cadmium levels because HY birds were collected in November while SY and ASY Dunlin were collected in April. However, I am confident that relatively low concentrations in HY Dunlin were a result of age because previous analyses of Dunlin collected during November at Boundary Bay also showed high cadmium in SY and ASY age classes relative to HY birds (Braune and Noble 2009).

Data transformations: Element concentration distributions were tested for normality with Shapiro-Wilks goodness of fit tests. Log transformations were applied to all element concentrations for statistical analyses except normally-distributed mercury in feathers.

Risk assessment: The range and means of investigated elements in Dunlin tissues are presented in the results and compared to guidelines identifying tissue concentrations of potential and likely toxicity in the discussion. For additional context, results are also compared with previously documented element levels in Dunlin and related sandpipers.

Statistical analyses of kidney metals: I examined the influence of the following factors on metal concentrations in Dunlin kidneys: age, sex, size (as indicated by tarsus length), bill length, habitat preference (estuarine vs. terrestrial), trophic level, and sample group. To investigate the influence of age class, HY Dunlin from November 2009 Vancouver airport (YVR) collections and one Boundary Bay Dunlin salvaged the same month were compared to SY and ASY Dunlin obtained during spring migration in 2010 at Boundary Bay and YVR. The proportions of SY and ASY Dunlin in 2010 collections from Boundary Bay and YVR were very similar, eliminating the potential for sample group effects to influence metal levels across those age classes. Consequently, data from the two locations were pooled. Washington birds from winter captures were excluded from age class comparisons because they were obtained in different months from the other sample groups, so the birds were not the same age as those caught in April 2010 or November 2009 and there were too few samples to compare such distinct

age classes. Age class was not correlated with other Dunlin characteristics (e.g. habitat preference, size, trophic level) so these factors were not considered in age class comparisons. ANOVA and Tukey's pair-wise analyses were used to test the significance of differences across age classes.

Analyses investigating other factors' influence on kidney metal concentrations excluded HY and Washington samples because they were obtained earlier than most of the other collections. HY and Washington Dunlin metal concentrations were, therefore, potentially more influenced by breeding site and staging sites than were birds caught in spring. Restricting analyses to spring sample groups removed that potential source of variation and, thus, reduced noise in the data that would have otherwise reduced power to detect influences of the factors for which data was collected.

Forward stepwise regressions were used to determine which Dunlin characteristics were significantly related to cadmium, copper, and zinc in kidneys of April 2010 collections. Collinearity was checked with variance inflation factors (VIF) calculated for each independent variable. Any variables with a VIF greater than $1/(1 - r^2)$ were considered sufficiently correlated with other variables to cause misleading results in the multiple regression analysis (Farrar and Glauber 1967). To avoid this, correlated independent variables were input into the regression separately. ANCOVAs were used to investigate the influence of correlated variables bill length and sex on kidney cadmium, copper, and zinc separate from multiple regression analyses. Analyses of covariance (ANCOVA) were also used to check for interaction effects among sample groups, sexes and age classes.

Nitrogen stable isotope ratios typically vary with trophic level, not habitat type. However, isotope signatures of invertebrate sandpiper prey reported from previous studies in the FRD demonstrated enriched $\delta^{15}N$ signatures in estuarine invertebrates relative to terrestrial invertebrates and a corresponding correlation in $\delta^{15}N$ and $\delta^{13}C$ in Dunlin blood samples (Evans Ogden et al. 2005; Beninger et al 2011). To determine whether variation in $\delta^{15}N$ was still correlated with habitat, isotope signatures of Dunlin prey items collected from the FRD in 2010/2011 were compared to those from previous publications. Since habitat was again found influence $\delta^{15}N$ invertebrate signatures, analyses examining the influence of trophic level on elements in Dunlin controlled for the

effect of habitat by limiting tests to within estuarine feeding Dunlin. Low numbers of terrestrial Dunlin samples prevented a similar analysis within terrestrial foragers. Mean $\delta^{13}C$ signatures associated with an estuarine prey range from -11.0‰ to -16.0‰ while terrestrial invertebrates and seeds ranged from -24.9‰ to -27.4‰ (Appendix A). I, therefore, considered Dunlin with $\delta^{13}C$ tissue signatures greater than -15‰ to be estuarine specialists and used bivariate linear regressions to test if $\delta^{15}N$ had an effect on metal concentrations within this group. All estuarine specialists were from the Boundary Bay 2010 sample group. Also, since all individuals in this group were SY or ASY age classes and no significant differences were found between these classes or between sexes, all data from the sample group were pooled for these analyses.

Analyses of element concentrations in feathers: The ICP-MS and CVAFS analyses provided quality control validated measures of copper, mercury, and selenium in feathers from 2010 collections. A small sample size (n=13) for 2010 feathers limited statistical analyses to tests on the influence of habitat preference, sex, bill length, and size on element concentrations. To analyze the effect of habitat preference, Dunlin were categorized as either terrestrial ($\delta^{13}C$ < -20‰) or estuarine ($\delta^{13}C$ > -15‰) based on stable isotope signatures in muscle tissue. Forward stepwise multiple regressions were used to test the significance of these factors' relationships with the listed elements in feathers. Variance inflation factors were determined to test for collinearity among variables and collinear variables were treated as described for statistical analyses of kidney metals. As noted above, sexual dimorphism in Dunlin drives a strong correlation between sex and bill length. Consequently, the relationships of these characteristics with element concentrations in feathers from 2010 were examined with ANCOVAs to control for differences in bill length between sexes and also to test for interaction effects associated with bill length, habitat preference and size.

ICP-MS analyses provided quality control validated measures of copper, lead, selenium, and zinc from 2011 collections. For 2011 feathers, (n=16), statistical analyses on the effects of collection site (e.g. Robert's Bank, Boundary Bay), age, bill length, and tarsus on element concentrations were restricted to comparisons within males due to a lack of female samples. Variance inflation factors revealed no collinearity among those factors so multiple regressions considering all variables were used to test the

significance of their relationships with element concentrations. ANCOVAs were used to test for interaction effects between different sample groups and age classes.

T-tests were used to analyze across year differences in feather concentrations of copper and selenium in Dunlin as these were the only elements for which I obtained verified measurements in samples from both years. Data was pooled across sexes, age classes, collection sites, and habitat preferences for these across year analyses since multiple regression analyses revealed the variables were not significantly related to metal concentrations in feathers. Reference material measurements for manganese were above 95% confidence limits of the certified value indicating measured concentrations in feathers were potentially elevated as well. As a result, I do not present manganese concentrations or compare them to other studies. However, in element concentration analyses of feathers from 2010 and 2011 collections, manganese readings across distinct masses of certified reference material yielded very similar concentrations and replicate samples from the same individuals with different sample weights were also very similar to each other. Thus, differences between measured manganese concentrations were considered accurate so samples were compared relative to each other to test for differences associated with Dunlin characteristics in each year (sex, size, age class, collection site (e.g. Boundary Bay vs. Robert's Bank) and habitat preference). Forward stepwise regressions were again used for these analyses along with the same tests for collinearity and interaction effects. A t-test was used to compare feather manganese concentrations of estuarine specialist Dunlin from 2010 and 2011, pooling data for sex, age class, and collection site, but not habitat preference.

Statistical analyses were performed with JMP (ver. 8.2), graphs were constructed with SigmaPlot (ver. 12.3), and maps with ArcGIS (ArcMap 10.0). The level of statistical significance for all analyses was set at 0.05, thus all p-values above 0.05 are reported as non-significant.

Results

Stable isotopes signatures in Dunlin and their prey: Stable carbon and nitrogen isotope signatures of marine and terrestrial prey items collected for this study and reported in previous publications are presented in Appendix A. Averages for $\delta^{15}N$ signatures of invertebrate prey in the estuarine environment were 10.94, 9.68 and 12.11‰ relative to 6.12, 5.76, and 7.63‰ in terrestrial habitats (Evans Ogden et al. 2005; Beninger et al. 2011; this study). Benthic estuarine diatoms analyzed by Beninger et al. (2011) were also enriched in nitrogen relative to terrestrial seeds collected from Dunlin gizzards (Appendix A).

Dunlin muscle and feather $\delta^{13}C$ and $\delta^{15}N$ signatures showed a similar positive correlation to that found in their prey (Figures 2.3, 2.4, 2.5). By comparing Dunlin tissue signatures with those of their food items, and considering trophic enrichment factors of +3.1‰ for $\delta^{15}N$ and +1.9‰ for $\delta^{13}C$ from food to muscle tissue (Evans Ogden et al. 2004), I found that Dunlin stable isotope signatures spanned a gradient from mostly terrestrial to predominantly marine (Figures 2.4 and 2.5). I also found a strong effect of sample group on $\delta^{13}C$ and $\delta^{15}N$ signatures. Among the Dunlin collected at Boundary Bay in 2010 (SY and ASY) most birds had either estuarine or terrestrial $\delta^{13}C$, $\delta^{15}N$ signatures (Figure 2.4). In contrast, birds collected from Vancouver International Airport (YVR) in 2010 (SY and ASY) were mostly terrestrial, and HY birds from 2009 YVR collections had intermediate signatures (Figure 2.5). Dunlin feathers from spring 2011 captures had isotope signatures that suggest a primarily estuarine diet and were enriched in $\delta^{15}N$ relative to muscle isotope signatures to a similar degree (+1‰) as found by Hobson and Clark (1992b) in a range of other species.

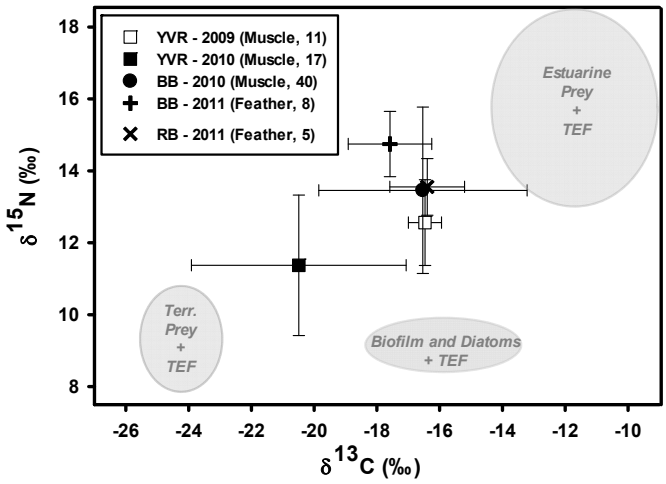

Figure 2.3 Muscle and feather stable isotope signatures in Dunlin (Calidris alpina) sample groups collected from Vancouver International Airport (YVR), Boundary Bay (BB), and Robert's Bank (RB)

Note: Symbols and whiskers represent means and standard deviations of stable isotope signatures for groups of Dunlin collected at the same location on the same day or one day apart. Sample size and type are presented in the legend for each sample group. Ellipses describe the range of mean isotopic signatures reported for sandpiper food items (Appendix A) adjusted by prey-muscle enrichment factors (TEF) of +1.9‰ for δ^{13}C and +3.1‰ for δ^{15}N (Evans Ogden et al. 2004).

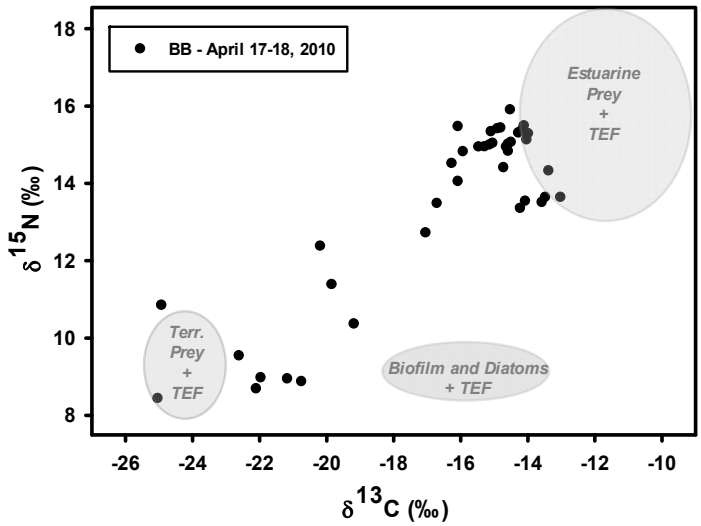

Figure 2.4 Stable isotope signatures of muscle tissue from individual Dunlin collected at Boundary Bay (BB), in BC, Canada

Note: Date of collection in legend. Ellipses describe the range of mean isotopic signatures reported for sandpiper food items (Appendix A) adjusted by prey-muscle enrichment factors (TEF) of +1.9‰ for δ^{13}C and +3.1‰ for δ^{15}N (Evans Ogden et al. 2004).

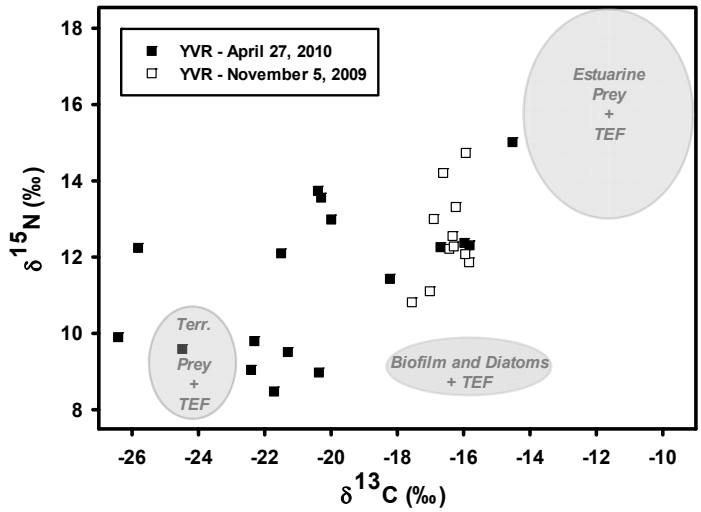

Figure 2.5 Stable isotope signatures of muscle tissue from individual Dunlin collected at Vancouver International Airport (YVR) in BC, Canada

Note: Dates of collection in legend. Ellipses describe the range of mean isotopic signatures reported for sandpiper food items (Appendix A) adjusted by prey-muscle enrichment factors (TEF) of +1.9‰ for $\delta^{13}C$ and +3.1‰ for $\delta^{15}N$ (Evans Ogden et al. 2004).

Cadmium turnover rate in Dunlin kidneys: It takes approximately ten months for Dunlin to acquire their apparently steady-state concentration of cadmium (as demonstrated by April SY Dunlin). Half-life was calculated in years, so the unit of time for Dunlin to acquire their maximum body burden is:

10 months /12 months year $^{-1}$ = 0.83 years

The kidney cadmium reacting (i.e. the input) in that time is equal to the entire kidney cadmium burden so the fraction reacting is 100% or 1. Thus:

k = 1/0.83 = 1.20

and the half-life is calculated as:

Cd $t_{1/2}$ = 0.693/1.20 = 0.58 years = 7.0 months

Kidney metal concentrations by age class, relative to toxicity thresholds: Metal concentrations in kidneys of all age classes were below levels found to have adverse effects in field or laboratory studies (see discussion). Significant

differences in kidney metal concentrations across age classes were found for cadmium (F = 12.067, df = 2, $p < 0.001$) and zinc (F = 6.868, df = 2, $p = 0.002$), but not copper (Figures 2.6, 2.7, 2.8). Tukey's pair-wise comparisons found lower cadmium concentrations in HY relative to SY ($p = 0.003$) and ASY ($p < 0.001$) Dunlin. Zinc concentrations in kidneys were higher in HY than in SY ($p = 0.004$) and ASY ($p = 0.005$) Dunlin. Averages were calculated as geometric means to account for the log-normal distribution of metal concentrations since arithmetic means would be higher than the majority of concentrations and would, thus, misrepresent the data (Figures 2.6, 2.7, 2.8).

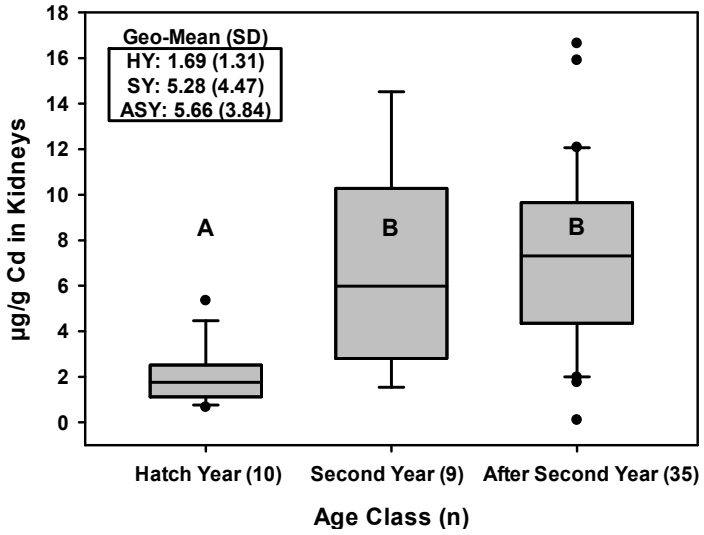

Figure 2.6 Kidney cadmium concentrations in Dunlin (Calidris alpina) age classes

Note: Boxes reflect the median concentration and 25 quartile ranges. Whiskers represent 75 quartile ranges and all samples outside such ranges are shown as points. Different bolded capital letterss represent significant differences between age classes.

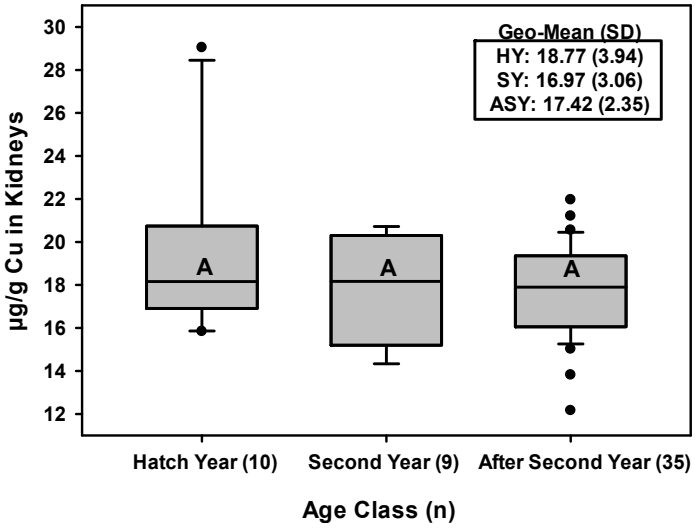

Figure 2.7 Kidney copper concentrations in Dunlin (Calidris alpina) age classes

Note: Boxes reflect the median concentration and 25 quartile ranges. Whiskers represent 75 quartile ranges and all samples outside such ranges are shown as points. Different bolded capital letters represent significant differences between age classes.

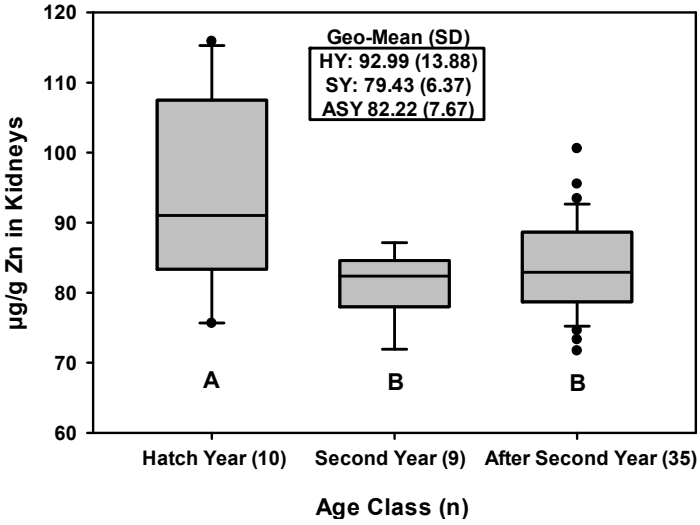

Figure 2.8 Kidney zinc concentrations in Dunlin (Calidris alpina) age classes

Note: Boxes reflect the median concentration and 25 quartile ranges. Whiskers represent 75 quartile ranges and all samples outside such ranges are shown as points. Different bolded capital letters represent significant differences between age classes.

Kidney metal concentrations by sex and bill length: Analyses of covariance (ANCOVA) demonstrated that cadmium, copper, and zinc kidney concentrations showed no effect of either sex (Cd: F = 0.443, df = 1, p = 0.509; Cu: F = 0.134, df = 1, p = 0.716; Zn: F = 0.322, df = 1, p = 0.573) or bill length (Cd: F = 0.418, df = 1, p = 0.521; Cu: F = 1.122, df = 1, p = 0.295; Zn: F = 0.137, df = 1, p = 0.713) and no interaction effect between the two factors.

Kidney metal concentrations by trophic level: Slopes ± 95% confidence intervals relating δ^{15}N to metal concentrations in kidneys of estuarine specialists (n = 21) were as follows: Cd: -0.06 ± 0.32; Cu: 0.06 ± 0.08; Zn: 0.02 ± 0.05. Since 95% confidence intervals of slopes include zero, there is no evidence of any significant relationships between δ^{15}N and kidney metal concentrations.

Other factors influencing kidney metal concentrations: Results for collinearity tests among factors with potential influence on kidney metal levels are presented with variance inflation factors (VIF) in Table 2.2. Sex and bill length as well as δ^{13}C and δ^{15}N were collinear. Because δ^{15}N was analyzed separately, controlling for correlated variation in δ^{13}C, it was not included in multiple regressions. No interaction effects were observed among independent variables. δ^{13}C, age class, sample group (e.g. YVR fall 2009, YVR spring 2010, Boundary Bay spring 2010), and tarsus length were included in stepwise regression models analyzing variation in cadmium, copper, and zinc kidney concentrations. For cadmium, δ^{13}C was found to be significantly correlated ($p < 0.001$, $r^2 = 0.409$) while sample group was either just above or just below the significance level depending on which stage it was added to the model. Including sample group in the model after δ^{13}C significantly improved the model ($p = 0.045$, $r^2 = 0.466$). Sample group was significantly related to copper and zinc concentrations in kidneys (Cu: $p < 0.001$; Zn: $p < 0.001$) with higher concentrations of zinc and copper in SY/ASY collections from Boundary Bay than in SY/ASY collections from YVR in April 2010. Adding sex and bill length separately and subsequent to other factors that described significant variation in metal levels showed they still had no effect on metal levels when considering the influence of other variables

Table 2.2 Variance inflation factors (VIF) testing for correlations in characteristics of Dunlin (Calidris alpina pacifica) collected from the Fraser River Delta, British Columbia in April 2010

Factor of Interest	VIF
δ^{13}C (‰)	***4.26***
δ^{15}N (‰)	***4.46***
Age Class (SY/ASY)	1.32
Bill Length	***2.66***
Sample group	1.29
Tarsus Length (Size)	1.49
Sex	***2.52***

Note: Bolded italics indicate correlated variables (VIF > $1/(1-r^2)$ = 1.89)

Measurement precision, adjustments, and variation across replicate samples: Precision for isotope measurements was very high with average standard deviations of reference samples at 0.2‰ for δ^{13}C (‰) and 0.3‰ for δ^{15}N (‰). With the exception of three samples which were removed from analyses, differences across replicate muscle isotope measurements from their respective means were very small at 0.1‰ for δ^{13}C and 0.1‰ for δ^{15}N within a δ^{13}C range of -12.7‰ to -26.4‰ and a δ^{15}N range of 8.5‰ to 25.0‰. There were insufficient replicates to calculated standard deviations for prey and feather isotope values, but variation across samples was always greater than variation between replicates from the same sample. Kidney sample concentrations were all detected above background levels in lab blanks. Standard deviations of metal concentrations across replicated kidney samples were as follows (with average percent deviation from replicate means in parentheses): Cd: 0.75 μg/g (11.4%); Cu: 0.94 μg/g (3.7%); Zn 14.84 μg/g (3.3%). Measured reference material metal concentrations relative to certified concentrations are presented in Table 2.3. Correction coefficients based on deviation from certified concentrations were as follows for flame AAS measurements: Cd: 0.938; Cu: 0.947; Zn: 0.945.

Table 2.3 Standard reference material certified and measured concentrations

	Cd (µg/g)	Cu (µg/g)	Zn (µg/g)	Std. Ref. Material
Cert. Value ± 95% CL	26.7 ± 0.6	106 ± 10	180 ± 6	TORT – 2
Measured Value ± SD	29.7 ± 2.1	108.1 ± 4.8	189.6 ± 12.5	(Lobster Hepatopancreas)
Cert. Value ± 95% CL	20.8 ± 0.5	25.8 ± 1.1	85.8 ± 2.5	DOLT – 2
Measured Value ± SD	21.4 ± 1.5	27.1 ± 1.8	90.1 ± 7.4	(Dogfish Liver)

Element concentrations in feathers: Element concentrations in feathers from both 2010 and 2011 are presented in Table 2.4 and a summary of factors tested for influence over feather metal concentrations and significant results is presented in Table 2.5. Selenium in feathers exceeded adverse effect levels, but was the only element to do so. Mercury was the only other element for which measured feather concentrations even approached such thresholds (see discussion). In analyses on the relationships between Dunlin characteristics and element concentrations in feathers, no interaction effects were significant among independent variables in either 2010 or 2011.

In feathers collected in 2010 at Boundary Bay, mercury concentrations in Dunlin feathers (n = 13) ranged from 1.61-4.38 µg/g with a normal distribution (arithmetic mean: 2.91 µg/g ± 0.91(SD)). While neither size, sex, bill length, nor habitat preference was found to significantly influence feather mercury levels in ANCOVAs (sex and bill length) or multiple regressions, all females (n = 4) had feather concentrations above the mean (2.93-4.38 µg/g) while only two of nine male samples (range: 1.61-3.83 µg/g) were above the mean. No significant effect of sex or bill length on copper, manganese, or selenium feather concentrations in 2010 samples was observed in analyses of covariance (ANCOVA), and they were similarly insignificant when separately included in multiple regression analyses. The effects of habitat preference and size were also insignificant for copper and selenium, however manganese concentrations were significantly higher in terrestrially feeding birds ($F = 6.290$, df = 11, $p = 0.029$).

For feathers collected in 2011, neither site (Boundary Bay vs. Robert's Bank), age, bill length, nor size significantly influenced feather concentrations of copper, lead, manganese, selenium, or zinc. Comparisons of copper, and selenium concentrations

between 2010 and 2011 feathers showed significantly higher concentrations of copper in 2010 captures (Cu: F = 5.876, df = 1,25, p = 0.023) and higher concentrations of selenium in 2011 samples (F = 34.740, df = 1,26, p < 0.001) (Table 1.4). Feather manganese concentrations of estuarine specialists were not significantly different across years.

Table 2.4 Concentrations (µg/g dry weight) of selected elements in contour feathers of calidrid sandpipers

Species	Location (Season, Year)	Se Mean (SE); *n* Geomean	Hg Mean (SE); *n* Geomean	Cu Mean (SE); *n* Geomean	Pb Mean (SE); *n* Geomean	Zn Mean (SE); *n* Geomean	Source
Dunlin *C. alpina*	Boundary Bay, BC (Spring 2010)	4.7 (0.5); *13* 4.4	2.9 (0.9); *13* -	12.2 (0.5); *13* 12.1	- -	- -	This study
	Fraser River Delta, BC (Spring 2011)	10.4 (0.9); *16* 9.7	- -	10.1 (0.5); *15* 9.9	0.9 (0.1); *15* 0.8	197 (12); *16* 191	
	Yeongjong Island, Korea (Fall 1994)	- -	- -	- 8.2; *6*	- 14.8; *6*	- 103; *6*	Kim et al. 2008
	West Wadden Sea (Oct 1982)	6 (1.5) -	3.3-6.8†; *7* -	- -	- -	- -	Goede et al. 1989
	East Wadden Sea (Oct 1982)	9-11 -	4.3 (0.9); *9* -	- -	- -	- -	
	East Wadden Sea (Apr, May 1985, 1986)	10-11 -	4.3; *26* -	- -	- -	- -	
Red Knot *C. canatus*	Southwest France (Winter 2005-2007)	8.7(3.4); *3* -	2.1 (0.6); *3* -	21.6 (3.1); *3* -	- -	189 (63); *3* -	Lucia et al. 2010
	Delaware Bay (Spring 1991)	6.2 (0.6); *16* -	1.2 (0.2); *16* -	- -	1.7 (0.8); *16* -	- -	Burger et al. 1993
	Dutch Wadden Sea (Fall 1979-1982)	15.7* (5.3); *20* -	5.8*(1.7); *20* -	- -	- -	100-400 -	Goede 1985
Great Knot *C. tenuirostris*	Yeongjong Island, Korea (Fall 1994)	- -	- -	- 4.08; *10*	- 20.8; *10*	- 71.5; *10*	Kim et al. 2008
Sanderling *C. alba*	Delaware Bay (Spring 1991)	1.3 (0.1); *13* -	2.8 (0.3); *13* -	- -	2.7 (1.0); *13* -	- -	Burger et al. 1993
Semipalmated Sandpiper *C. pusilla*	Delaware Bay (Spring 1991)	6.0 (0.9); *11* -	- -	- -	2.3 (0.5); *11* -	- -	
	Delaware Bay (Spring 1992)	5.4 (0.8); *15* -	0.02 (0.0); *15* -	- -	2.0 (0.2); *15* -	- -	

*Vane of primary, †Range of means

Summary: For ease of interpretation, elements reported in tissues and significant influences of Dunlin characteristics on element concentrations are summarized in Table 2.5.

Table 2.5 Elements analyzed in tissues of Dunlin collected in the Fraser River Delta, BC (2009-2011), Dunlin characteristics analyzed for influence on element accumulation, and significant relationships between characteristics and element concentrations.

Dunlin Characteristics	Elements in Kidneys	Elements in Feathers (2010)	Elements in Feathers (2011)	Elements Significantly Related to Dunlin Characteristics
Age	Cd, Cu, Zn	-	Cu, Pb, Mn, Se, Zn	Cd_k, Zn_k*
Size	Cd, Cu, Zn	Cu, Hg, Mn, Se	Cu, Pb, Mn, Se, Zn	none
Sex	Cd, Cu, Zn	Cu, Hg, Mn, Se	-	none
Bill Length	Cd, Cu, Zn	Cu, Hg, Mn, Se	Cu, Pb, Mn, Se, Zn	none
Sample Group	Cd, Cu, Zn	Cu, Mn, Se	Cu, Pb, Mn, Se, Zn	Zn_k*, Cd_k, Se_f, Cu_f
Habitat Preference	Cd, Cu, Zn	Cu, Hg, Mn, Se	-	Cd, Mn_f
Trophic level	Cd, Cu, Zn	-	-	none

Note: Dashes represent factors not analyzed for a particular group of samples. Significant relationships from kidney and feather concentrations denoted with subscript k and f after elements in the last column. *Zinc levels were significantly different across age classes, but the age classes with significant differences were collected in separate sample groups so the difference may be a result of age class and/or sample group effects.

Discussion

Stable isotopes signatures in Dunlin and their prey: Average stable isotope signatures from sample groups of this study were similar in range and disparity to those reported by Evans Ogden et al. (2005) from wintering Fraser River Delta (FRD) Dunlin sampled in 1997-2001. Overall, $\delta^{13}C$ signatures from both studies suggest individual Dunlin diets ranged from almost exclusively estuarine to predominantly terrestrial and the majority of individuals had signatures that were more estuarine than terrestrial.

While $\delta^{15}N$ signatures are known to change as a function of trophic level, baseline isotopic ratios can also be affected by differences in nitrogen inputs to those systems (McClelland et al. 1997). In systems with different $\delta^{15}N$ baselines, the nitrogen incorporated at the base of a food chain will have distinct values and organisms at the same trophic level will have distinct signatures across systems corresponding to the differences in the baseline nitrogen source. FRD invertebrates of the same trophic level were consistently more $\delta^{15}N$ depleted in terrestrial systems relative to estuarine systems (Appendix A). Thus, enriched Dunlin $\delta^{15}N$ signatures in estuarine feeding birds relative to terrestrial feeders were a product of distinct baselines and not higher trophic level prey consumption in estuarine habitats.

Element concentrations relative to migration, site fidelity, and element turnover in tissues: To determine site or sites of exposure relevant to any substances' concentration in migratory organisms, one must consider both the organisms' migratory habits and the time period of accumulation in the tissues sampled. Collections from YVR and Boundary Bay in 2010 were made during spring migration and are, thus, a sample of individuals that spent the previous several months at unknown wintering sites, potentially including the FRD. HY Dunlin collected during November at YVR had similarly migrated from an unknown combination of hatch sites and staging areas. During spring migration, Pacific Dunlin only spend one to four days at stopover sites (Warnock et al. 2004), so element levels in tissues of migrants would represent little cadmium exposure from the collection site and an uncertain proportion of local copper and zinc exposure (see turnover rates below). Additionally, one needs evidence of site fidelity during winter months to have confidence that individuals sampled before migration are representative of the capture site. Shepherd (2001) found that approximately 65% of the Dunlin tracked by radio-telemetry during winter months in the FRD were re-located in the region either 29 or 30 days out of 30 total days of tracking; however, other individuals moved down to Washington and returned during that time demonstrating that while the majority of individuals are highly site faithful, some mixing with nearby wintering site populations does occur so even samples from birds taken before migration will be influenced by exposure from other sites.

Research on White Leghorn and Hubbard broiler chicks (*Gallus gallus domesticus*) suggests that half-lives of essential elements copper and zinc are short in birds as tissue concentrations return to normal levels within a week after removal from elevated dietary exposure (Oh et al. 1979; Chiou et al. 1997). Non-essential cadmium has a longer half-life. Blomqvist et al. (1987) estimated a half-life for cadmium in Dunlin at 1-2.5 years based on logarithmic increases in kidney concentrations observed between HY, SY, and ASY fall captures. Cadmium half-life in Pacific Dunlin (ca. 7 months) was estimated to be shorter than reported by Blomqvist et al. (1987) for Dunlin in Sweden as a result of a more rapid rate of accumulation in Pacific juveniles.

Considering excretion was not included in the estimate of reacting cadmium, cadmium half-life in Pacific Dunlin is likely some degree shorter than the calculated estimate. Rapid cadmium accumulation in juveniles relative to adults suggests excretion is lowest in this life stage, but the extent to which it is reduced is unknown. Thus, the degree to which excretion should reduce the half-life estimate is also unknown so the true cadmium half-life in kidneys should be similar to or somewhat shorter than estimated. Thus, kidney cadmium in April collections, (i.e. those used to examine the relationships between metal accumulation and sex, bill length, size, habitat preference, and trophic level), primarily reflect exposure at wintering sites (7 months: October-April) and is influenced to a lesser extent by exposure at staging areas and fall migration sites.

Logarithmic increases in cadmium tissue concentrations with age were also reported in Mallards (*Anas platyrynchops*) dosed with cadmium (White and Finley 1978). Juvenile Western Sandpipers (*Calidris mauri*) that follow the same migration pathway as *pacifica* Dunlin have also been found with low levels in the fall, on wintering grounds, and increasing levels in the winter leading to spring concentrations in SY birds that are similar to those of adult ASY individuals (McFarland et al. 2002). The same pattern has been documented in Oystercatchers (*Haematopus ostralegus*) (Stock et al. 1989) and also in Willow Ptargmigan (*Lagopus lagopus*) in which even higher cadmium concentrations (100-200 μg/g) accumulate in kidneys (Myklebust and Pedersen 1999) indicating that the ability to regulate cadmium, perhaps through a concentration-dependent excretion rate, may be widespread across avian species. The only other species for which cadmium half-lives have been reported are the Chipping Sparrow (*Spizzela passerine*) and Japanese Quail (*Coturnix coturnix japonica*). Those half-lives

were 99 days (Anderson and Von Hook, Jr. 1973) and 116 days (Jacobs et al. 1978) respectively. However, if excretion rates are concentration-dependent in birds, direct comparisons are an over-simplification since the half-life may increase with dose or exposure. In either case, the relatively short cadmium half-lives in sparrows and quails show Dunlin are not unique in their short kidney half-lives relative to those in mammals which are generally on the order of years and result in distinctly continuous increasing cadmium levels with age (Eisler 2000).

Because trace elements diffuse into feathers from the bloodstream (Burger and Gochfeld 1999; Bortolloti 2010) and some elements (e.g. mercury) are transported from internal tissues to feathers during growth (Lewis and Furness 1991) feather samples represent a combination of accumulation since the last moult, and exposure during feather growth. Feathers from April 2010 and 2011 were collected during or shortly after growth (pre-alternate moult). The previous, pre-basic moult in Dunlin is typically completed in September, directly before migration to wintering sites. Thus, 2011 feather samples represent exposure at wintering sites between September and April.

Stable isotope turnover relative to metal half-lives: Stable isotope ratios in biota have specific turnover rates corresponding to the rate at which molecules are replaced in different tissues. As a result, each tissue type is representative of diet sources for a particular length of time which must be considered when attempting to relate element accumulation to isotope signatures. Hobson and Clark (1992a) investigated turnover rates in different tissues of Japanese Quails (*Coturnix coturnix japonica*) and found whole blood as well as muscle tissue have ^{13}C half-lives of 11.4 and 12.4 days respectively. Evans Ogden et al. (2004) showed Dunlin have a similar whole blood ^{13}C half-life: 11.2 days ± 0.8(SE), and that the ^{15}N half-life: 10.0 days ± 0.6(SE) was significantly correlated with ^{13}C. Since the turnover rates appear to be the same across these species and elements, I estimated half-life of ^{13}C and ^{15}N in Dunlin muscle tissue to be similar to that of ^{13}C in quails (ca.12 days). Thus, Dunlin diet sources over the previous 12, 24 and 50 days represent approximately 50%, 75% and 90% of isotope signatures reported for muscle isotope signatures respectively. Feather isotope signatures more simply reflect the time required for growth. Observations of plumage during captures from late March through early April 2011 suggest contour feather growth in FRD Dunlin took place over the course of one and a half to two weeks.

Metal half-lives in kidneys are longer for cadmium than for stable isotopes in muscle tissue, but those of zinc and copper are shorter. Individuals with muscle isotopes indicating diet and habitat use over 24-50 days (ca. 75-90% of isotope signature) carry cadmium accumulated over the course of several months. Thus, variation in diet over the course of the winter or between the winter residence period and spring migration could potentially result in misleading relationships between habitat preference and cadmium in kidneys of Dunlin collected in the spring. There is no previous evidence that speaks to how consistently Dunlin maintain their habitat preference beyond the period described by stable isotope ratios in tissues, but given the significant relationship of $\delta^{13}C$ with kidney cadmium concentrations, random diet shifts have a less than 5% chance of leading to the described trend. If birds with relatively high cadmium burdens concentrated their feeding in estuarine habitats during migration and shortly before, this could also result in the relationship observed with stable isotope signatures. However, cadmium heavy Dunlin would not benefit from a diet shift to estuarine habitats which are generally associated with elevated cadmium levels (Bryan and Langston 1992). Thus, it seems likely that the stable isotope signatures in muscle tissue gave a proper representation of the relationship between cadmium in kidneys and habitat preference. Alternatively, FRD resident Dunlin and migrant groups may have distinct isotopic signatures, and, since collections were made during migration, the relationship between those signatures and cadmium burden could be influenced by different burdens across groups from various wintering sites. However, FRD winter residents demonstrate that Dunlins have a wide variety of both estuarine and terrestrial signatures within sites (Evans Ogden et al. 2005) and the spring collections from this study that suggest a relationship between habitat preference and kidney cadmium were from three separate days, two sites, and twelve separate flocks. While this does not rule out the possibility of a migrant group effect, it strongly supports the idea that habitat preference is a real source of variation in kidney cadmium.

Dunlin may switch habitat preferences over the course of a few days, so exposure over the previous week (i.e. the time period that zinc and copper in kidneys reflect) may not be accurately related to the more long term habitat preferences reflected in isotope signatures of muscle tissue. As a result, unless there is evidence of stable habitat preferences over the course of a month, such variation could obscure trends in

zinc and copper accumulation across the habitat preference gradient leading to my failure to demonstrate such an effect with stable isotope data. Short half-lives of zinc and copper in avian kidneys are more comparable to isotopic turnover rates in the liver (2.6 day half-life: Hobson and Clark 1992a). Thus, the relationship between habitat preference and copper and zinc exposure in birds could be better assessed using isotopic signatures of liver tissue.

Kidney metal concentrations by age class: Cadmium has regularly been shown to be higher in adults than juvenile birds (Blomqvist et al. 1987; Burger 1993; Ferns and Anderson 1996; Dailey et al. 2008) so it is fitting to find the lowest concentrations in HY Dunlin. As described earlier, Braune and Noble (2009) sampled HY and ASY Dunlin at Boundary Bay and found higher levels in ASY birds confirming that the observed difference was not an effect of time of year. Significant increases in cadmium burdens of juvenile Dunlin followed by insignificant differences between SY and ASY individuals appears to be the result of concentration-dependent excretion rates and low natal cadmium burdens.

While I found higher levels of zinc in HY juveniles than in SY or ASY captures, Blomqvist et al. (1987) found similar levels of zinc across ages in fall caught Dunlin and Curlew Sandpipers (*C. ferruginea*). Additionally, Hogstad (1996) found higher zinc concentrations in livers of adult birds than in those of juveniles across five species of passerines. Considering the short half-life of zinc in kidney tissues, a lack of corresponding trends in other studies, and entirely HY collections at YVR in November, the difference is likely more an effect of recent exposure at different sites than of age class. Surprisingly, salvaged Dunlin from YVR in April had the lowest zinc concentrations of all sample groups. Variation in the amount of time spent at YVR relative to other migratory stopover sites in the days before collisions may explain this discrepancy.

Kidney metal concentrations by sex and bill length: Neither sex nor bill length were significantly related to cadmium, copper, or zinc concentrations in kidneys or other elements in feathers. In contrast, Ferns and Anderson (1994) found cadmium concentrations in female kidneys was 24% lower than in male Dunlin from the Bristol Channel, UK (Appendix C). Adult Western Sandpiper males also have markedly

higher cadmium burdens than females (McFarland et al. 2002). What factors might contribute to the disparity in gender effects on cadmium accumulation in Pacific Dunlin relative to other sandpiper populations? McFarland et al. (2002) suggested gender effects may be a result of excretion into eggs; however this does not explain the lack of sexual variation in cadmium found in this study. Moreover, the rate of cadmium accumulation demonstrated by juvenile Dunlin and Western Sandpipers indicates any offloading of cadmium that occurs during egg production would have little or no effect on spring body concentrations. Two alternative explanations for the effect of sex are: 1) females excrete cadmium at a higher rate than males relative to kidney concentration; 2) diet differences between sexes effect exposure. The first scenario also fails to explain similar concentrations observed in both sexes of Pacific Dunlin relative to the differences observed in Bristol Channel Dunlin and in Western Sandpipers on the Pacific Coast. Alternatively, the diet explanation is plausible given sexual dimorphism of bill lengths in Western Sandpipers and Dunlin and the potential for diet to vary across sites. Differences in diet will have varying effects on metal exposure depending on the available prey items at each site and may be quite similar for both sexes at some sites, thus potentially explaining inconsistent differences between sexes across different sandpiper populations.

Trophic level and kidney metal concentrations: The lack of significant relationships between trophic level and metal concentrations suggests that metals do not bioaccumulate in the estuarine food chain before they are consumed by Dunlin. It also appears to suggest that biofilm, a low trophic level food, does not expose Dunlin to higher levels of metals than do invertebrate prey. However, as figures 2.3, 2.4, and 2.5 demonstrate, estuarine-feeding Dunlin $\delta^{15}N$ signatures (14.8‰ ± 0.7(SD)) were more enriched than they would be if they were consuming large amounts of benthic diatoms and biofilm (6-8‰; Kuwae et al. 2008; Beninger et al. 2010) given their food-muscle tissue enrichment factor (+3.1‰; Evans Ogden et al. 2004). Since, Dunlin analyzed for this study were not feeding heavily on biofilm, the absence of a trophic level effect does not rule out biofilm as a potent exposure vector for other birds or Dunlin feeding at other sites where they may consume relatively more biofilm.

Other factors influencing kidney metal concentrations: Of all variables, kidney cadmium concentrations were best described by variation in $\delta^{13}C$ indicating that habitat preference plays the greatest role in cadmium accumulation. The relationship between Dunlin kidney cadmium concentrations and sample group may be an artifact of sampling: a result of fewer estuarine-feeding individuals in the YVR Airport group (Figure 2.9). Variation in stable carbon isotope ratios describes 40.9% of the variation in kidney cadmium levels of Dunlin collected in April 2010. Distinct metal accumulation in prey items with similar $\delta^{13}C$ signatures along with physiological differences between individual Dunlin must contribute to some of the remaining variation in concentrations. Additionally, because Dunlin with $\delta^{13}C$ signatures more enriched than -15‰ all feed in estuarine habitat, $\delta^{13}C$ does not describe a habitat gradient past this point. As a result, the slope of $\delta^{13}C$ by cadmium concentrations is smaller than would be a regression of truly quantified habitat preference by cadmium.

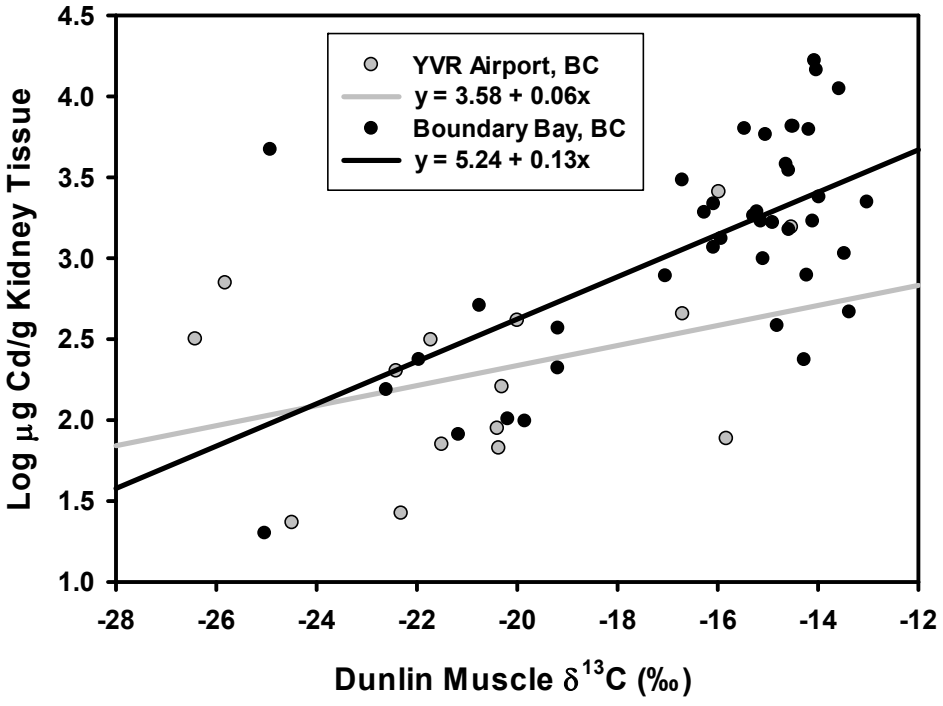

Figure 2.9 ***Cadmium concentrations (µg/g dry weight) in kidneys relative to muscle tissue $\delta^{13}C$ isotope values of individual Dunlin (Calidris alpina) collected during spring migration at Boundary Bay and Vancouver International Airport (YVR) in the Fraser River Delta, BC***

The conclusion that estuarine feeding Dunlin accumulate more cadmium than terrestrial feeders begs the following question: What is it that drives the difference in metal accumulation? Potential explanations are: 1) higher concentrations of (or more bio-available) cadmium in estuarine prey; 2) higher energetic costs associated with processing a more saline estuarine diet demanding greater quantities of prey intake. Chapter three investigates the first question via analysis of gizzard contents of the same Dunlin analyzed in this study. Research by Gutierrez et al. (2011) observed higher basal metabolic rate and energy consumption in Dunlin (*Calidris alpina alpina*) residing in highly saline environments both in the wild and under controlled laboratory conditions, supporting the second hypothesis.

Kidney metal concentrations by sample group: SY/ASY Dunlin collected from Boundary Bay had significantly higher concentrations of copper and zinc in kidneys than SY/ASY collections from YVR in April 2010. Dunlin from Skagit Bay, Washington had zinc levels more similar to those in Boundary Bay collections, and intermediate between the two FRD sample groups for copper. Metal exposure varies across winter residence sites and differences in the winter residence sites of the two sample groups are probably responsible for differences in accumulated zinc and copper. Fall 2009 YVR collections were predominantly HY Dunlin and were consequently not included in analyses of sample group effects due to potential interaction effects with age class. However, as described in the discussion of age class effects, the significant difference in zinc levels between HY Dunlin collected at YVR and SY/ASY Dunlin from Boundary Bay is most likely an effect of variation in recent exposure, reflected in differences across sample groups.

Element concentrations in feathers: Surveys of shorebird abundances in Boundary Bay and Robert's Bank during March and April 2011 showed no increase in Dunlin numbers until after the dates of capture (HPJ van Veelen, unpublished data) suggesting 2011 feather samples were from FRD winter residents. Abundances during mid-April in 2010 were elevated well above typical winter numbers and other species that are usually only present during migration were observed in the area during the dates of capture. Thus, 2010 samples were from unknown proportions of migrants and winter residents. Consequently, significant differences in concentrations of copper and

selenium between the 2010 and 2011 sample groups could result from exposure at distinct wintering sites. If 2011 captures were primarily migrants from other wintering sites the results indicate relatively high selenium and low copper exposure in the FRD. Alternatively, it is possible that some or many of the 2011 samples were resident birds in which case the differences would reflect variation in exposure in the FRD across years.

Gizzard and intestine content analyses in the FRD indicate that Dunlin at Robert's Bank ingest more sediment and biofilm than at Boundary Bay: Robert's Bank diet: 42% sediment (Mathot et al. 2010); Boundary Bay diet: 29% sediment (This study). However, no differences were found in concentrations of copper, lead, manganese, selenium, or zinc between feathers from birds at these two sites.

Standard deviations of feather metal concentrations were similar in 2010 and 2011 feathers indicating, perhaps, that 2010 captures were not from a wide variety of sites where differences in exposure would likely result in greater variation among accumulated levels in Dunlin. While most Dunlin are known to maintain home ranges restricted to 20-30 km^2 in the FRD through March, some do move back and forth between sites in Washington and movements may increase as the migration period approaches (Shepherd 2001). Since copper, manganese, and selenium in feathers reflect exposure shortly before and during the time of growth (Burger 1993), movement across sites may partially explain the similar levels of these metals observed in feathers from birds sampled at Robert's Bank and Boundary Bay, as well as the similarity in variance across 2010 and 2011 samples.

While toxicity thresholds for concentrations of manganese in feathers are not documented and the true concentrations of the feather samples could not be determined due to discrepancies in measurements obtained for certified reference materials, this micro-nutrient was higher in Dunlin feeding in terrestrial habitats. Agricultural inputs are a potential source of elevated terrestrial manganese exposure as the element is essential in plants for a number of biological functions and is, consequently, a common fertilizer component. Fertilizer products such as manganese sulphate, manganese oxide, and manganese chelate are all potential sources for elevated accumulation in avian tissues.

None of the factors measured in this study had a significant effect on mercury concentrations in Dunlin feathers. Although other studies have shown higher mercury levels in female birds (Burger et al. 2007; Hoffman and Curnow 1979) and the results from this study showed a similar, albeit non-significant, trend, a summary of studies addressing sex differences in metal accumulation presented by Burger (1993) demonstrates sex typically has no effect on mercury levels in birds.

Comparisons to Western Sandpipers: ASY and SY Western Sandpipers collected from various sites on the American Pacific coast typically had mean kidney cadmium concentrations between 15-20 μg/g (McFarland et al. 2002). Four Western Sandpipers caught incidentally with Dunlin in Boundary Bay in April 2010 had similarly elevated levels (15.9, 14.0, 5.4, and 13.5 μg/g) relative to Dunlin (Arithmetic mean of all ASY and SY: 6.25 μg/g ± 0.55 SE, 4.33 SD). Western Sandpiper $\delta^{13}C$ signatures (Beninger et al. 2010: -15.7‰ ± 1.6(SD)) indicate they feed more exclusively in estuarine habitat than Dunlin (This study: -17.7‰ ± 3.7(SD); Evans Ogden et al. 2005: -16.7‰ ± 3 (SD)). While a more estuarine diet explains some degree of the higher concentrations of cadmium in Western Sandpipers, even estuarine specialist Dunlin have lower mean kidney cadmium concentrations (9.0 μg/g ± 3.7(SD)) than Western Sandpipers. Although trophic feeding level was not correlated with kidney cadmium concentrations in Dunlin, a typical Western Sandpiper has a $\delta^{15}N$ signature of ca. 11‰ (Beninger et al. 2010) whereas estuarine specialist Dunlin from this study had a mean of 14.8‰ ± 0.7(SD). Considering the $\delta^{15}N$ signature of diatoms (6-7‰: Kuwae et al. 2008, Beninger et al. 2011) and a trophic enrichment factor of approximately +3‰, it appears that biofilm makes up a large proportion of the Western Sandpiper diet and relatively little of the Dunlin's. Gut content analyses of both species also indicate Western Sandpipers feed more heavily on biofilm (Mathot et al. 2010). Thus, despite the lack of trophic level effects found in this study, higher rates of biofilm feeding may still be responsible for higher cadmium burdens in Western Sandpipers. Another important difference between Western Sandpipers and Dunlin that may contribute to differences in cadmium burdens is wintering residence sites. Western Sandpipers primarily inhabit sites south of and including San Francisco Bay down to Peru whereas Dunlin winter from the FRD to Mexico and no further. If there is more cadmium exposure at wintering sites south of Mexico that would explain some of the differences in kidney levels. Future

analyses of cadmium exposure in the Bay of Panama and other wintering sites with abundant populations of Western Sandpipers would aid in understanding this issue.

Observed element concentrations relative to adverse effect levels: Cadmium concentrations in Dunlin kidneys from FRD collections ranged broadly from 0.6 to 16.6 μg/g. Avian cadmium toxicity studies suggest potential adverse effects when kidney concentrations exceed 40 μg/g (Eisler 2000) and effects are more than 50% probable above 264 μg/g (66 μg/g wet weight: Wayland and Scheuhammer 2011). Although high inter-specific variation in metal tolerance makes risk of toxicity uncertain, Ferns and Anderson (1996) reported no signs of histological damage in Dunlin with kidney cadmium concentrations reaching as high as 60.8 μg/g. Sub-lethal effects can certainly occur without apparent physical damage to tissues, but the mean concentration measured in this study was within the range reported in Dunlin from a number of other sites (Appendix B) indicating no particular cause for concern.

Background zinc kidney concentrations range from 50-90 μg/g and toxic effects have been observed in wild Mallards (*Anas platyrynchos*) and Canada Geese (*Branta canadensis*) at concentrations from 220-970 μg/g (Sileo et al. 2003) and also over 1000 μg/g (Gasaway and Buss 1972; Levengood et al. 1999). While observed zinc concentrations were on the high end of levels in other avian species, they were well below levels with known toxic effects and normal relative to other sandpipers (Appendix C). Observed copper concentrations were also in the middle range of concentrations reported in other studies of calidrid sandpipers (Appendix C). Kidney copper concentrations may not be a valid measure of toxic stress, however, as concentrations in the kidney have been found to remain stable, even at toxic doses (Jackson et al. 1979).

Copper concentrations in feathers were also within the range commonly documented for sandpipers (Kim and Koo 2008; Lucia et al. 2010) and other species (Burger 1993). Zinc is an element required in feather formation; consequently, high concentrations relative to other elements are common across species. Findings of zinc feather concentrations near 200 μg/g are similar to other sandpiper studies (Table 2.4) and agree with reports from Goede (1995) that shorebirds have higher zinc concentrations in feathers than other birds. Observed lead levels in 2011 feathers were

below adverse effect levels (4 μg/g: Burger and Gochfeld 1994, Burger 1995) and concentrations reported in all other sandpiper studies (Table 2.4).

The mean mercury concentration in feather samples analyzed for this study was 2.9 μg/g and concentrations from individual Dunlin reached as high as 4.4 μg/g. Feather mercury concentrations of 5 μg/g and higher are associated with a range of adverse effects including lowered reproductive success (Eisler 1987; Burger 1997). While feather samples from this study were not so high, documented levels in feathers from a number of other sandpiper populations exceed that concentration demonstrating risk of toxic effects (Table 2.4). Combined monitoring of mercury levels in sandpiper feathers and measures of reproductive success (e.g. mating success, hatching success, and fledging success) would be informative regarding the potential impacts of such concentrations. Importantly, concurrent exposure to selenium has been shown to mitigate toxic effects of mercury in adult birds and other animals (Burger 1997). Many studies have demonstrated increasing proportions of inorganic mercury relative to methyl mercury with increasing total mercury accumulation in avian livers (Kim et al. 1996; Eagles-Smith 2009; Ohlendorf and Heinz 2011). Positively correlated increases of selenium and mercury in birds with high total mercury levels combined with a lack of relationship between the two metals when total mercury levels are low, suggest that selenium acts as a receptor for de-methylated mercury (Scheuhammer et al. 2008; Eagles-Smith et al. 2009). The inorganic compound, mercuric selenide (HgSe), that results from selenium binding to de-methylated mercury is relatively non-reactive and, therefore, less toxic than methyl-mercury and other organic forms of selenium, but may result in increased retention of selenium overall. Although mercuric selenide has the potential to make up a significant portion of total mercury in birds, mercury in feathers is comprised of methyl mercury and may, therefore, be less affected by this interaction.

The most common toxic effects of selenium in birds are reduced egg hatchability and embryo deformities resulting from exposure to its organic forms, especially selenomethionine (Spallholz and Hoffman 2002). However, accumulated concentrations of total selenium are generally used as the indicators of toxicity risks when observing tissues, and little information is available on the forms of selenium present in different tissues (e.g. Ohlendorf and Hienz 2011). Feather selenium concentrations with likely

adverse effects have been calculated at 3.8-26 μg/g based on feather to liver ratios of 1:5 and adverse effects at 19-130 μg/g in liver (Burger and Gochfeld 2000). Additionally, a summary of studies by Ohlendorf and Heinz (2011) suggests a tentative threshold of 5 μg/g for selenium concentrations in feathers. Feather selenium levels were at or above this threshold in many of the Dunlin sampled for this study as well as in sandpipers of most other studies reporting feather concentrations (Table 2.4). Similar feather levels in Dunlin captured in the Dutch Wadden Sea corresponded to kidney concentrations ranging from 20-35 μg/g (Goede 1985). High selenium levels in livers and kidneys were also found in Dunlin (9.6-20.0 μg/g) and other sandpipers (6.4-40.8 μg/g) captured on the gulf shore of Texas (White et al. 1980). Additionally, Braune and Noble (2009) reported mean kidney selenium concentrations in *pacifica* Dunlin as high as 16.7 μg/g, higher than a number of other shorebird species sampled throughout Canada. Studies on Mallards (*Anas platyrynchos*) and chickens describe kidney and liver concentrations above 22 μg/g as likely to reduce reproductive success (Ohlendorf and Heinz 2011).

Goede et al. (1989) found selenium concentrations in kidneys of migrating Dunlin in the Dutch Wadden Sea were far higher than adult birds at breeding grounds and more northerly stopover sites. The authors suggested rapid depletion of selenium body concentrations due to reduced exposure on the breeding grounds was probably the reason for the difference and concluded that elevated levels in the Wadden Sea were not likely to hinder reproduction. Dramatic decreases in selenium concentrations in tissues and eggs over the course of 10-20 days after exposure have since been documented in other species (Heinz 1993; USDI 1998). Additionally, sea birds appear to tolerate high selenium burdens (i.e. tissue concentrations) relative to terrestrial birds by producing eggs without correspondingly high selenium content (Ohlendorf and Heinz 2011), so estuarine feeding birds such as sandpipers might similarly tolerate higher levels with reduced adverse effects on reproduction. However, apart from the research by Goede et al. (1989), there is little direct evidence that sandpipers can mitigate the effects of high selenium burdens. Considering selenium in sandpipers is widely present at concentrations associated with adverse effects on reproductive success, the subject warrants further investigation.

When interpreting the tissue concentrations in wildlife, we must keep in mind the limitations of toxicity thresholds used to quantify risk. Toxicity thresholds based on element concentrations that cause visible tissue damage or observable impacts under laboratory conditions may not be sufficiently protective to prevent adverse impacts under the stresses of life in the wild. For example, survival rates under predation pressures or reproductive success under the pressure of sexual selection are likely more sensitive than the endpoints typically observed in lab studies and used to set thresholds. For elements that are potentially toxic at low levels and have variable tissue concentrations across populations (e.g. cadmium, mercury, and selenium), field-based research examining potential sub-lethal effects such as those listed above, failure to reach breeding grounds, delayed/prolonged migration, or other behavioural effects that would negatively impact reproductive success could be conducted to determine more realistic thresholds.

Since many element measures reported in this study are from Dunlin collected during migration with unknown wintering origins, I cannot say to what extent the findings hold true across the *pacifica* population or if they are representative of Dunlin exposure in the Fraser River Delta. Instead, the observations offer a case study of Pacific coast Dunlin and enhance understanding of exposure pathways for sandpipers. Feather concentrations demonstrate selenium and mercury pose a risk to Dunlin and other shorebirds, thus warranting further investigation. I found no evidence of deleterious levels of metals in kidneys; however, as knowledge increases on sub-lethal effects of contaminants on wildlife, and other contamination issues become apparent, understanding of exposure routes will certainly be of importance.

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Appendix A.

Stable Isotope Values for Estuarine and Terrestrial Food Items of Dunlin (Calidris alpina pacifica) in the Fraser River Delta, BC Canada

Habitat	Prey Type	Location (Year)	n	$\delta^{13}C$ (‰) Mean (SD)	$\delta^{15}N$ (‰) Mean (SD)	Source
Estuarine	Clam (*Macoma, Mya* Sp.)	Boundary Bay (1996)	4*	-17.21 (3.35)	9.75 (2.73)	Evans Ogden et al. 2005
		Robert's Bank (2004)	-	-13.8† (0.5)	8.25† (0.7)	Beninger et al. 2011
	Mud Snails (*Battilaria attramentaria*)	Boundary Bay (1996)	8	-11.00 (1.67)	9.90 (1.50)	Evans Ogden et al. 2005
		Boundary Bay (2010)	4*	-11.75 (0.37)	10.17 (0.88)	This study
	Benthic Crustacean (*Corophium* Sp.)	Boundary Bay (2010)	2*	-16.21, -15.82	11.78, 11.52	This study
		Robert's Bank (2010)	2*	-14.73, -14.87	9.99, 10.69	This study
	Meio-fauna	Robert's Bank (2004)	-	-15.2† (0.8)	10.3† (2)	Beninger et al. 2011
	Polychaete Annaelids	Boundary Bay (1996)	9*	-15.19 (1.88)	12.38 (0.79)	Evans Ogden et al. 2005
		Boundary Bay (2010)	1*	-13.70	14.94	This study
		Robert's Bank (2004)	-	-13.5† (0.5)	10.5† (0.5)	Beninger et al. 2011
		Robert's Bank (2011)	2*	-14.37, -14.45	15.17, 15.40	This study
	Benthic Diatoms	Robert's Bank (2004)	-	-15.6† (0.6)	6.8† (0.6)	Beninger et al. 2011
Terrestrial	Terrestrial Seeds	Boundary Bay (2010)	4*	-27.41 (0.91)	4.76 (1.29)	This study
	Oligochaete Annaelids	Boundary Bay (2010)	3	-25.02 (1.89)	7.63 (2.8)	This study
		Robert's Bank (2002)	13	-24.94 (1.01)	6.12 (1.36)	*Evans Ogden et al. 2005*
	Insects (Coleoptera: Carabidae; Diptera: Tipulidae)	Robert's Bank (2002)	3*	-27.18 (0.89)	5.76 (0.81)	*Evans Ogden et al. 2005*

*Composite samples of multiple individuals, †Estimated from graph in publication, "-" sample size unknown

Appendix B.

Kidney Cadmium Concentrations (µg/g, dry weight) in Calidrid Sandpipers

Species	Location (Season, Year)	Hatch Year Mean; *n* Geomean	After Second Year Mean; *n* Geomean	Overall Mean; *n* Geomean	Source
Dunlin *C. alpina*	Ottenby, Sweden (Fall, 1981-1983)	0.28*; *17* -	4.92*; *26** 0.96*	3.04*; *70** -	Blomqvist et al. 1987
	Bristol Channel, United Kingdom (1979-1982)	- 3.0†	- 12.5†	- -	Ferns and Anderson 1994
	Boundary Bay, BC (Winter 1991-1992)	1.43††; *6* -	4.31††; *8* -	3.08††; *14* -	Braune and Noble 2009
	Tofino, BC (Winter 1991-1992)	- -	1.10††; *3* -	- -	
	Bay of Fundy, ON (Fall 1990, 1991)	1.07††; *5* -	- -	- -	
	Churchill, MB (Summer 1991)	- -	2.16††; *4* -	- -	
	Boundary Bay and Sea Island, BC (HY Fall, ASY Spring 2010)	1.99; *11* 1.69	7.00; *36* 5.10	5.51; *69* 3.80	This study
	Skagit Bay, WA (Winter 2008)	1.01, 2.63 -	2.48; *4* 2.20	2.26; *6* 1.99	
	Corpus Christie, TX (Winter 1976-1977)	- -	- -	- 7.76*; *10*	White et al. 1980
	Yeongjong Island, Korea (Fall 1994)	- -	- -	9.60*; *6* -	Kim et al. 2007
	Dutch Wadden Sea (Spring 1985,1986)	All < 5 -	Most <5, 5.1-40.1 -	<5-40.1 -	Goede et al. 1989
Western Sandpiper *C. mauri*	Corpus Christie, TX (Winter 1976-1977)	- -	- -	- 10.96*; *15*	White et al. 1980
	Various Sites, Central/N. America (1995-1996)	1-5 -	10-22 -	- -	McFarland et al. 2002
Least Sandpiper *C. minutilla*	Corpus Christie, TX (Winter 1976-1977)	- -	- -	- 17.24*; *16*	White et al. 1980
Red Knot *C. canatus*	Southwest France (Winter 2005-2007)	- -	- -	7.1; *3* -	Lucia et al. 2010
Great Knot *C. tenuirostris*	Yeongjong Island, Korea (1994)	- -	- -	8.48*; *10* -	Kim et al. 2007
Curlew Sandpiper *C. ferruginea*	Ottenby, Sweden (Fall, 1981-1983)	0.52*; *12* 0.48*	15.04*; *16* 9.32*	8.84*; *28* 2.64*	Blomqvist et al. 1987
Sanderling *C. alba*	Corpus Christie, TX (Winter 1976-1977)	- -	- -	- 5.52*; *15*	White et al. 1980
	Northern Chile (March 1982)	- -	- -	29.3*†; *12* -	Vermeer and Castilla 1991

†Mean of means, ††Pooled samples, *[wet weight] x 4 (Goede et al. 1989; Clark and Scheuhammer 2003)

Appendix C.

Kidney Copper and Zinc Concentrations (dry weight, µg/g) in Calidrid Sandpipers

Species	Location (Season, Year)	Cu in HY Mean; *n* Geomean	Cu in ASY Mean; *n* Geomean	Cu Overall Mean; *n* Geomean	Zn in HY Mean; *n* Geomean	Zn in ASY Mean; *n* Geomean	Zn Overall Mean; *n* Geomean	Reference
Dunlin *Calidris alpina*	Ottenby, Sweden (Fall, 1981-1983)	17.2*; *17* 17.0*	13.3*; *26* 13.1*	14.2*; *70* 13.8*	84*; *17* 84*	80*; *26* 80*	80*; *70* 80*	Blomqvist et al. 1987
	Boundary Bay and YVR, BC (HY Fall, ASY Spring 2010)	19.1; *11* 18.8	17.6; *37* 17.4	17.6; *70* 17.4	93.9; *10* 93.0	82.6; *36* 82.2	82.5; *68* 81.8	This study
	Skagit Bay, WA (Winter 2007, 2008)	19.2, 17.3	15.7; *4* 15.49	16.5; *6* 16.4	80.9, 128.3 -	80.0; *4* 79.8	88.2; *6* 86.5	This study
Red Knot *C. canutus*	Southwest France (Winter 2005-2007)	- -	- -	29.9; *3* -	- -	- -	104.6; *3* -	Lucia et al. 2010
Curlew Sandpiper *C. ferruginea*	Ottenby, Sweden (Fall, 1981-1983)	19.7*; *12* 19.5*	16.2*; *16* 16.0*	17.7*; *28* 17.4*	84*; *12* 84*	84*; *16* 84*	84*; *28* 84*	Blomqvist et al. 1987
Sanderling *C. alba*	Northern Chile (March 1982)	- -	- -	42.4*†; *12* -	- -	- -	- -	Vermeer and Castilla 1991

*[wet weight] x 4 (Clark and Scheuhammer 2003, Goede et al. 1989), †Mean of means

Chapter 3:

Sources and Risks of Cadmium, Copper, and Zinc Exposure to Dunlin (*Calidris alpina*) in the Fraser River Delta, British Columbia

Abstract

Estuaries receive deposition of suspended sediments and organic matter from aquatic systems. Metals bound to such deposits accumulate in estuarine sediments. For coastal biota, risk of exposure to toxic quantities of metals can be elevated by other natural inputs and anthropogenic emissions. The Fraser River Delta (FRD), adjacent to Vancouver, British Columbia, provides habitat for over one million migratory and resident birds annually and receives industrial, agricultural and human effluents as well as metal inputs from upwelling oceanic currents. Dunlin (*Calidris alpina*) are migratory sandpipers that utilize FRD estuarine and shore-side agricultural habitat as migration stopover and winter residence sites. I examined cadmium, copper, and zinc exposure to Dunlin via analyses of ingested prey within gizzards of birds collected in the FRD to investigate the contribution of metals from different prey and habitat types, and to assess toxicity risks. Specifically, I measured metal and energy concentrations in three terrestrial and three estuarine diet types. Risk was assessed for each diet type by modelling daily metal exposure under the energetic needs of winter resident and spring migrant Dunlin in the FRD and then comparing daily exposures to no-observed-adverse-effect-level (NOAEL) toxicity thresholds. Terrestrial samples from agricultural habitat in the FRD exposed Dunlin to significantly less metals than terrestrial samples from the Vancouver Airport (YVR). Ingested sediment was not associated with high exposures for cadmium, copper, or zinc relative to other ingested items. Some level of risk was predicted from all three metals in all diet types. Probable adverse effects were predicted from cadmium in

most diet categories, with mud snails presenting the greatest toxic exposure risk. Probable effects were also predicted from copper in YVR terrestrial diets and one estuarine diet, as well as from zinc in one of two winter resident YVR diets. Exposure risk is likely mitigated to some degree by co-abundance of the metals and Dunlin's tendency to feed in both estuarine and terrestrial habitats. Potential issues with applying daily exposure models to estuarine feeding sandpipers with relatively high metabolisms as compared to other avian species of similar size are discussed.

Introduction

Heavy metals can have adverse effects on wildlife when exposure exceeds capacities to utilize, excrete, or immobilize such elements. Anthropogenic emissions have led to widespread increasing abundance of heavy metals in natural systems since the industrial revolution (Eisler 2000). Metals present in natural mineral deposits and emitted by human activities are often washed into aquatic systems where metal ions have a greater affinity for suspended fine grain silts, clays and organic matter than for water (Tessier and Campbell 1987; Grant and Middleton 1990; Allison and Allison, 2005). When water moves from rivers to estuarine systems flow rates slow and such suspended matter deposits into sediments. Estuarine systems, thus, receive high inputs of metals relative to terrestrial systems and act as long term sinks for these potential toxicants (Cundy et al. 1997).

Located in southern British Columbia, the Fraser River Delta (FRD) contains the largest estuary on Canada's Pacific coast. The delta stands adjacent to the Vancouver metropolitan area and is, consequently, subject to industrial, agricultural, and human effluents. Additionally, upwelling ocean currents bring cadmium rich waters to the Pacific Coast region (Shiel et al. 2012). The effects of upwelling have been observed at various sites in the surrounding Salish Sea where cadmium concentrations in bivalves often exceed levels fit for human consumption (Kruzynski 2004; Bendell 2009) as well as at pristine sites on British Columbia's coast where cadmium levels in seabirds have been found to exceed those of the same species from Canada's Atlantic coast (Elliott and Scheuhammer 1996). Considering oceanic cadmium sources and the likelihood of additional anthropogenic inputs, exposure to wildlife in the FRD may be of concern.

The FRD hosts many resident bird populations and provides stopover habitat for migratory species from throughout the North American Pacific Flyway. The Dunlin (*Calidris alpina pacifica*) is the most common winter resident sandpiper in the delta with 25,000 to 40,000 individuals wintering on FRD habitat annually (Butler and Vermeer 1994; Warnock and Gill 1996; Shepherd 2001). Pacific Dunlin breed in northern Alaska and migrate to wintering sites primarily from British Columbia through Mexico. In spring, their northward migration follows a coastal route that brings most of the population through the FRD (Butler and Campbell 1987; Warnock and Gill 1996). FRD Dunlin feed in intertidal and shore-side agricultural habitat (Shepherd 2001) on small benthic bivalves, gastropods, and crustaceans, as well as terrestrial insects, larvae, and vegetation (Evans Ogden et al. 2005). They also graze on biofilm (Elner et al. 2005; Mathot et al. 2010), a mucilaginous surface layer of sediment, diatoms, other microphytobenthos, bacteria, and extracellular polymeric substances (EPS) produced by bacteria and diatoms (Decho 1990). Sediment ingestion in avian species is a known exposure pathway for lead (Franson and Pain 2011) and EPS associated with biofilm significantly elevates the adsorption of many metals into the aerobic silica matrices of estuarine surface sediments (Schlekat et al. 1998). Furthermore, cadmium can be incorporated as a component of enzymes in some marine diatoms (Lane et al. 2005).

Dunlin are useful study subjects for investigating toxicological threats in the FRD because they are common, their habits are well studied, and they utilize agricultural as well as estuarine habitats. The objectives of this study are to determine the risk of toxic metal exposure for Dunlin in the delta and to examine the relative contribution of different diet items and foraging habitats to such exposure. The research has implications for Dunlin and other shorebird species that utilize FRD habitat and contributes to the understanding of heavy metal exposure pathways for shorebirds in general.

Specifically, I examined dietary exposure from cadmium, copper, and zinc. Copper and zinc are essential elements necessary for haemoglobin formation, collagen and connective tissues, cellular division and a range of other functions in plants and animals. In contrast, cadmium has no demonstrated function apart from in a recently described marine diatom enzyme (Lane et al. 2005), and typically causes toxic effects at far lower tissue concentrations. Cadmium also has a much longer half-life in organisms

than copper and zinc. However, there remains potential for zinc and copper toxicity in high metal environments and the relative abundances of zinc, copper, and cadmium influence their respective bioavailabilities and, subsequently, the risk of adverse effects at any given exposure level (Fox et al. 1984; Mckenna et al. 1992). Thus, it is appropriate to consider these metals together when interpreting exposure.

Methods

Ingestion is typically the greatest source of exposure for relatively non-volatile metals such as cadmium, copper, and zinc (Leeson 2009; Wayland and Scheuhammer 2010). Exposure to these metals via the respiratory route is generally less and is not considered in this study. I assessed diet exposure by sampling ingested prey which offers some advantages over collecting prey samples from the environment. Many organisms have specified feeding mechanisms and preferences for prey that are difficult to replicate or are unknown. Biofilm feeding, for example, requires sophisticated morphological adaptations to the tongue aided by saliva in the mouth (Elner et al. 2005). Ingested prey offered a sample selected by the study organism and collected with the exact mechanism used by the organism, eliminating some potential sampling biases.

Dunlin were collected from the Vancouver International Airport (YVR; 49° 12' N, 123° 12' W) in the FRD after a collision with aircraft (Figure 3.1). The highest concentrations of cadmium observed in sediments and benthic invertebrates of the FRD have been reported from Boundary Bay (Figure 3.1) (Thomas and Bendell-Young 1998). Additional collections were made by shotgun at Boundary Bay (49° 04' N, 122° 57' W) under Simon Fraser University Animal Care Committee protocol # 946B-09 and Environment Canada scientific permit # BC-10-0034. Boundary Bay collections were made just after dawn, as the tide began to fall and birds were densely feeding on newly exposed mudflats. These conditions were selected to obtain birds carrying samples of both estuarine and terrestrial prey and birds of various general habitat preferences for a related study on accumulated metals. Further, birds were likely more exposed to the habitat at the collection site than most other areas in the bay since, in addition to hosting dense flocks during high tides, the area remains exposed and available for foraging throughout a greater proportion of the tide cycle than most other intertidal habitat. Ten

days after Boundary Bay collections, a second group of Dunlin was collected from YVR following another aircraft collision. Permissions for holding and possession of birds collected at YVR and Boundary Bay were granted under the following salvage permits: BC-09-0141, BC-SA-0046-10, BC-SA-0046-11. Sample sizes from each of the collection dates and locations are presented in Table 3.1.

Table 3.1 Sample groups of Pacific Dunlin (Calidris alpina pacifica)

Date	Location	Sample Size
Nov 5, 2009	YVR Airport, BC	12
Nov 24, 2009	Boundary Bay, BC	1
Apr 17-18, 2010	Boundary Bay, BC	44
Apr 28, 2010	YVR Airport, BC	20

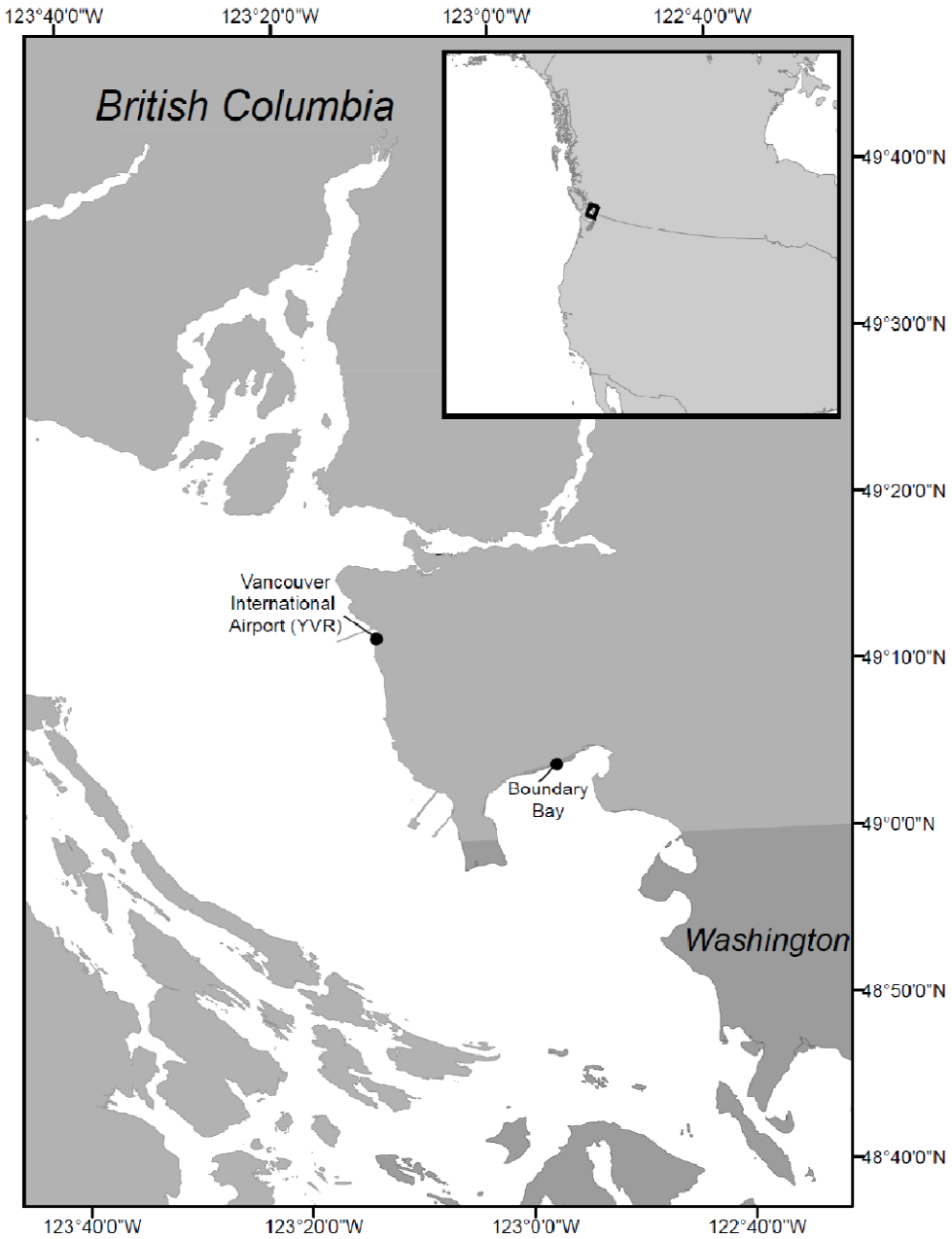

Figure 3.1 ***Sites in the Fraser River Delta of British Columbia where Dunlin (Calidris alpina) were collected for heavy metal dietary exposure investigations and risk assessment via gizzard content analyses***

All birds were frozen at -20°C after collection. Dissections, including extraction of gizzards and intestines, were performed in a class II bio-safety cabinet in accordance with bio-safety protocols. All materials used in the processing of gizzard contents were soaked in a 10% HCl acid bath for 24 hours, rinsed 6 times with deionised water and

once with ultrapure (18MΩ H_20) water before use. After dissections, gizzards and intestines were placed in vials and frozen again until analysis.

Gizzard content composition analyses: Dissections revealed that within the stomach of the sampled Dunlin, the proventriculus was small and often not discernable from the esophagus, while the gizzard was quite large and contained the vast majority of stored food. Gizzard contents, therefore, offered the best sample ingested prey. Following thaw, each gizzard was cut open and contents were spread across a Petri dish and mixed until contents appeared homogenously distributed for microscope aided composition analysis. Petri dishes were placed over a 16 cell grid and six randomly selected cells were analyzed for percent cover of prey items (adapted from Mathot et al. 2010). Prey was identified when whole or otherwise by indicator fragments such as polychaete jaws, snail opercula, and crustacean exoskeleton fragments. Diet components that could not confidently be identified were recorded as "Unidentified Detritus". To estimate the proportion of each prey item in a sample (i.e. composition), percent cover of each prey item encountered was summed from the six cells and taken as a fraction of total recorded components.

Stable isotope signatures in Dunlin demonstrate that they consume a variety of prey items and individuals can specialize on certain diet types (e.g. terrestrial prey, estuarine prey) (Evans Ogden et al. 2005). Variations in Dunlin isotope signatures across individuals suggest some Dunlin may even specialize their diets on specific food items within estuarine and terrestrial habitats. To model and compare exposure from different diet types and sites, I grouped gizzard content samples into categories defined by collection site and similar compositions. Metal exposure was estimated with average metal and energy concentrations of samples in each diet category. To minimize differences in uncertainty of exposure estimates across categories, exposure was only modelled for diet categories with at least five samples. Six diet categories met the five sample requirement and are presented in Table 3.2 along with the descriptions of compositions used to define these groups.

Table 3.2 Diet categories defined for gizzard content samples of Dunlin (Calidris alpina) collected in the Fraser River Delta, BC at Boundary Bay and Vancouver International Airport

Diet Category Title	Collection Site	Description
Mud Snail	Boundary Bay	>50% *Batillaria attramentaria*
Sediment	Boundary Bay	>50% sediment
Marine Mix	Boundary Bay	Similar proportions of mud snail, clam (*Macoma balthica*), sand, and benthic crustaceans (i.e. <50% of any)
Terrestrial Mix-1	Boundary Bay	Mix of mostly terrestrial insects, seeds, and vegetation
Terrestrial Mix-2	Vancouver Airport	>33% grit, >25% unidentified detritus, 25-50% vegetation
Terrestrial Mix-3	Vancouver Airport	>33% vegetation, 20-40% grit, 10-30% invertebrates

Many of the gizzard samples collected from YVR in November 2009 and April 2010 had similar compositions described in the Terrestrial-2 and Terrestrial-3 diet categories, respectively. Gizzard contents from Boundary Bay collections were more variable, but most could be grouped into four categories, three of which were dominated by unique combinations of estuarine food items, and another that encompassed a wider range of terrestrial food.

Fine grain marine sediment in the gizzard, found predominantly in the Sediment diet category, was considered a proxy for biofilm as the EPS matrix of biofilm often forms around and adheres to sediment particles, making them an obligate ingestate for biofilm consumers (Mathot et al. 2010). Investigation of intestine contents found proportional quantities of sediment passing through the digestive tract as found in the gizzard, supporting the assumption that sediments in the gizzard were ingested for digestive purposes and not for storage in the gizzard to assist in mechanical breakdown of other food items.

Sample preparation: Following composition analyses, gizzard contents were dried until weights were stable. Samples were then homogenized with mortar and pestle. Grit large enough to collect with forceps was separated from samples and analyzed independently for metals because it interfered with homogenization. Sub-samples of homogenate were taken for metal, stable isotope, and energetic analyses (described below).

Stable isotope analyses: Stable isotope ratios were measured in gizzard contents to provide an alternative measure of composition. The ratio of ^{15}N to ^{14}N isotopes in biota is generally indicative of trophic level while ratios of ^{13}C to ^{12}C are relatively stable across trophic levels but are often distinct for organisms feeding in different habitat types. For comparative purposes, stable isotope ratios are described with delta notation ($\delta^{13}C$ and $\delta^{15}N$ for carbon and nitrogen) where the ratio of heavy to light isotopes is multiplied by 1000 and subtracted by the isotope ratio of a standard material unique to each element. Delta values for all standard materials are zero and, thus, set the scale used for comparisons. Ratios greater or less than standards have positive or negative values, or "signatures", respectively and are described as "enriched" or "depleted" when more positive or negative relative to other values. As the ratio is multiplied by a factor of 1000, delta notation describes values in per mil (‰).

Values of $\delta^{13}C$ and $\delta^{15}N$ were determined for gizzard content samples at the Stable Isotope Facility at University California, Davis. Three to eight mg of dried, homogenized gizzard contents from each bird was weighed into a tin capsule. Variation in weight reflects efforts to maintain similar amounts of organic material across samples of differing organic/inorganic content. Every tenth sample was replicated (i.e. analyzed in two separate samples) for quality assurance testing and to test the homogeneity of samples. Average stable isotope signatures were determined for each diet category and were compared to the results of microscope aided composition analyses.

Exposure model: Daily metal exposures from diet categories were calculated with the average concentration of metals for each category and intake rate (i.e. consumption) relative to the average Dunlin weight:

$$\textbf{Dose } (\mu\textbf{g metal / g body weight / d}) = \frac{\textbf{Metal (ug/g)} \times \textbf{Consumption (g/d)}}{\textbf{Body Weight (g)}}$$

Where:

$$\textbf{Consumption } (\textbf{g / d}) = \frac{\textbf{Daily Energy Consumption (kJ/d)}}{\textbf{Energy Density (kJ/g)}}$$

Daily consumption was estimated from average energy concentrations measured in each category and daily energetic requirements. Confidence intervals for daily dose

were calculated as 1.96*SE of exposure estimates. Standard error of exposure estimates was derived from propagated error from measured energy and metal concentration averages:

$$\mathbf{SE\ dose/dose} = \sqrt{\left(\left(\mathbf{SE[energy]}/\mathbf{[energy]}\right)^2 + \left(\mathbf{SE[metal]}/\mathbf{[metal]}\right)^2\right)}$$

Thus:

$$\mathbf{SE\ dose} = \sqrt{\left(\left(\mathbf{SE[energy]}/\mathbf{[energy]}\right)^2 + \left(\mathbf{SE[metal]}/\mathbf{[metal]}\right)^2\right)} \times \mathbf{dose}$$

Variation in body weight and consumption are correlated and were, therefore, not included in the estimation of error for confidence intervals.

Energy density analyses: Energetic concentrations of homogenized gizzard contents were determined as kilojoules per gram of homogenate using a Parr 1341 plain jacket oxygen bomb calorimeter. Each gizzard content sample was analyzed independently for energy density. Samples were mixed with benzoic acid of a known energy concentration to ensure complete combustion.

Daily energy consumption: Digestive inefficiencies require organisms to consume more energy than they expend; thus, Daily Energy Consumption (DEC) > Daily Energy Expenditure (DEE). Energy assimilation efficiency (AE) describes the proportion of energy that an organism is able to use out of total energy in an ingested food type and can therefore be used to determine DEC from DEE or vice versa (DEE * 1/AE = DEC). After estimating DEEs of temperate, over-wintering Dunlin (see below), I estimated DECs for each diet category using composition data and AEs for the different food types as determined by Castro et al. (1989).

Specifically:

$$\mathbf{Diet\ Category\ AE} = \sum(\mathbf{F_i} \times \mathbf{AE_i})$$

Where "*F*" is the average proportion of prey type "$_i$" relative to other prey types in a specific diet category, and "*AE*" is the energy assimilation efficiency for prey type "$_i$" or, if unknown, the AE of a similar prey type described by Castro et al. (1989). The sum of all *F* values total 100% and the resulting diet category AE is an average value weighted by the relative abundance of the different diet items in each diet category. Unidentified detritus was not included in these calculations, and was thus assumed to be made up of the other diet components in their relative proportions for AE estimations.

For species such as the Dunlin, with geographic ranges that extend across climatic zones, variation in DEE, and consequently DEC, can be large due to metabolic costs of thermoregulation. As exposure estimates are based on DEEs, it is important to estimate the influence of such variation, especially at sites at the extreme ends of a species' climatic range like the FRD. While I am not aware of any studies that have quantified DEEs for Dunlin in temperate winter climates, information is available on the effects of temperature change on DEE for Dunlin and a congener, the Sanderling (*Calidris alba*). Considering their similar masses, basal metabolic rates (BMR) and Arctic DEEs, I assumed similar changes in energy expenditure relative to temperature for Dunlin and Sanderling. Based on this assumption, I used the ratio of Sanderling BMR (Castro 1987) to winter DEE at a site in coastal New Jersey (Castro et al. 1992) to estimate a temperate winter DEE for Dunlin from BMR (Gutierrez et al. 2011):

$$\frac{\text{Sanderling BMR (kJ/d)}}{\text{Sanderling Temperate DEE (kJ/d)}} \approx \frac{\text{Dunlin Arctic BMR (kJ/d)}}{\boldsymbol{\text{Dunlin Temperate DEE (kJ/d)}}}$$

To validate the method, I also used arctic DEEs of Sanderling and Dunlin (Piersma et al. 2003; Tulp et al. 2009) in place of BMRs to estimate temperate Dunlin DEE:

$$\frac{\text{Sanderling Arctic DEE (kJ/d)}}{\text{Sanderling Temperate DEE (kJ/d)}} \approx \frac{\text{Dunlin Arctic DEE (kJ/d)}}{\boldsymbol{\text{Dunlin Temperate DEE (kJ/d)}}}$$

I calculated separate DEEs for terrestrial and estuarine feeding Dunlin since saline diets require greater energy expenditure due to the costs of osmoregulation (Guttierez et al. 2011). Finally, I factored in the average energetic cost of over-ocean flocking (OOF), a predator evasion behaviour that takes place in the FRD and other

wintering sites during high tide. Body weight was taken from Dunlin captured in March and April of 2011 as part of a related investigation of feather metal levels.

While the DEE estimates incorporate important climatic and behavioural factors, they must also contain error as a result of site-specific behaviours not considered and uncertainties in predicting the influence of temperature. Ideally, DEE estimates for a particular species, site, and season would be presented with an estimate of uncertainty, but studies on the effects of climate on shorebird metabolism are insufficient to confidently quantify such variation. Considering the uncertainty in DEE, it is prudent to examine the sensitivity of the exposure model to variation in DEE. The FRD is heavily utilized by Dunlin during spring migration so I also modeled exposure under a spring migrant energy budget to examine the degree to which variation in DEE alters daily metal consumption and risk of toxic exposure. Differences in temperature and fattening rate were factored into the calculation of spring DEE.

Metal concentration analyses: Approximately 0.07 g of each homogenized sample was weighed into 50 ml flasks to which 12 ml nitric acid (70%) was added for metals extraction. Samples were digested with acids by heating the open flasks on hot plates at 200°C until ca. 0.5 ml of acid digestate remained. For validation of concentration measurements, samples were digested alongside duplicates of at least one sample, two certified reference materials with known metal concentrations (Tort-2, Dolt-2; National Research Council Canada, Ottawa, ON), and a procedural blank. Concentrated acid solutions remaining after digestion were diluted with 4 ml ultra-pure water and transferred into polypropylene tubes. Flasks were rinsed twice with 4 ml of 2% nitric acid solution and rinses were also added to the tubes. Tubes were left for 12 hours to allow any remaining sediment to settle to the bottom. Diluted acid solutions were then decanted off into a second tube and refrigerated until analysis. I analyzed gizzard content digestates with a Perkin Elmer AAnalyst 100, Flame Atomic Absorption Spectrometer (AAS) for cadmium, copper, and zinc at Simon Fraser University, in Burnaby, British Columbia. Grit removed from samples was digested by the same method except with no weight restriction so all grit was subjected to digestions and a measure of metals associated with the total amount of grit could be obtained.

Metal concentrations in gizzard contents with no grit were calculated based on the weight of digested homogenate and metals measured in that sample. Otherwise, to account for the contribution of metals associated with grit, gizzard content metal concentrations were calculated as metals from grit plus those in homogenate relative to the total weight of homogenate (i.e. total metals (µg) / total homogenate mass (g)). All metal measurements are reported as dry weight concentrations.

Measurement precision, adjustments, and variation between replicate samples: Acid digestions of gizzard contents and analyses by flame AAS were carried out over the course of eight months. To account for variation in instrument readings across separate analyses, correction coefficients were calculated based on the average difference between standard reference material readings on the day of analysis and the standards' certified values. Variance in element concentrations across replicate gizzard content samples from the same individuals was quantified with a standard deviation for each element. Mean concentrations for replicate samples vary across individuals, so overall standard deviations for each metal were calculated with the set of differences between replicate samples and their respective means. Variation in isotopic signatures across replicate samples was quantified by the same method. Standard deviations of reference isotope samples from their certified value were also determined.

Snail shell adjustments: Field observations of Dunlin excreta revealed mostly undigested fragments of shells indicating that metals in mud snail shells contribute little to heavy metal exposure. To determine the ratio of metals in mud snail tissues relative to metals in tissues and shells together (as present in gizzard contents) and subsequently calculate a correction factor that could be applied to measured values from mud snails in gizzard contents, separate analyses of metal concentrations in mud snail tissues and shells collected from the FRD were carried out using the same laboratory methods described for gizzard content metal analyses. Correction factors for cadmium, copper, and zinc were calculated based on the concentration of metals in tissues and shells as well as the average proportion of shell mass relative to tissue mass in mud snails:

$$\mathbf{CF} = \mathbf{tissue\ metal}\ (\mu\mathbf{g/g}) \Big/ \left(\mathbf{tissue\ metal}\ (\mu\mathbf{g/g}) + \left(\mathbf{shell\ metal}\ (\mu\mathbf{g/g}) \times \frac{\mathbf{shell\ mass\ (g)}}{\mathbf{tissue\ mass\ (g)}}\right)\right)$$

Correction factors were applied to all gizzard content metal measurements with mud snails. Given observations of shell fragments in excreta, the maximum percentage of digested shell was estimated at 30%. Thus, correction factors were applied to 70% of total metals from mud snails. Adjusted concentrations were calculated as follows for Mud Snail and Marine Mix diet category samples with mud snails:

$$\textbf{Shell adjusted metal exposure } (\mathbf{ug/g}) = \left(([\mathbf{MS}] \times \mathbf{MS}\% \times \mathbf{0.7}) \times \mathbf{CF}\right) + ([\mathbf{MS}] \times \mathbf{MS}\% \times \mathbf{0.3}) + ([\mathbf{MM}] \times \mathbf{MM}\%)$$

Where [*MS*] is Mud Snail metal concentration (average of samples with over 80% mud snail composition), *MS*% is the proportion of mud snail in the sample, *CF* is the correction factor, [*MM*] is Marine Mix metal concentration (average for all Marine Mix samples without snails), and *MM*% is the proportion of sample that was not mud snail.

Toxicity thresholds and risk assessment: Estimated daily metal exposures for the FRD Dunlin diet categories were compared to toxicity thresholds to assess risks of toxic effects. Toxicity benchmarks developed from laboratory dosing studies of other avian species were used to determine risk associated with measured dietary exposures. Two common toxicity benchmarks are the no-observed-adverse-effect-level (NOAEL) and the lowest-observed-adverse-effect-level (LOAEL). In laboratory studies that expose organisms to multiple concentrations or doses of a potentially toxic substance, the NOAEL is the highest exposure level without significant adverse effects for the species and endpoint observed while the LOAEL is the lowest exposure that does have negative effects. Tolerance of metal exposure and resulting toxicity thresholds vary across avian taxa and no experimental data known to me is available for shorebirds. To account for such variation, I used NOAELs of daily element consumption relative to body size (Sample et al. 1996). Additionally, I compared measured metal exposures to low NOAELs from sensitive species or endpoints (hereafter: conservative NOAEL), as well as with the highest NOAEL I could find in the literature (hereafter: non-conservative NOAEL), thereby putting an upper and lower bound on toxicity risk. The conservative NOAEL selected for cadmium was the toxicity reference value chosen by Stanton et al. (2010) after a review of the larger body of work on cadmium toxicity for birds. Selection of conservative NOAELs for copper and zinc was done with consideration of LOAELs to ensure the conservative NOAEL was the

result of adverse effects found at low exposure levels and not just large differences between dosages. For copper the lowest NOAEL found in the literature had a LOAEL only slightly larger (Appendix A) and was employed directly. There was a large dosage gap in the study reporting the lowest zinc NOAEL (Stahl et al. 1989), so it was not considered a relevant threshold. Instead, I used the geometric mean of the study's NOAEL and LOAEL as a conservative zinc toxicity threshold (adapted from the Canadian Tissue Residue Guidelines for the Protection of Wildlife Consumers of Aquatic Biota protocol: CCME 1998).

Risk assessments often compare toxicity thresholds to a distribution of exposures derived from variation across prey samples. However, Dunlin feed at a variety of sites over the course of a day and season within intertidal areas (e.g. Boundary Bay) so daily exposure is more appropriately described by the average exposure across individual gizzard contents. Variation in DEC and body weight are highly correlated (Castro et al. 1992; Nagy et al. 1999). Thus, since exposure is described as metals ingested per unit of body weight, most of the variation in Dunlin consumption across a population is accounted for by variation in body weight. Consequently, exposure per unit of body weight should be similar across the population, so metal exposures that place the average individual (i.e. 50% of the population) at risk were considered appropriate thresholds for the entire population. For essential metals copper and zinc, exposures were similarly compared to levels at which 50% of the population would fail to meet dietary requirements for optimal health and productivity. Daily nutritional zinc and copper requirements for Dunlin were estimated from weight specific nutritional needs of laying hens (*Gallus gallus domesticus*) (NRC 1994; Huang et al. 2007).

Log transformations were applied to metal data to normalize concentration distributions. To allow comparison between exposure and nutritional/toxicity thresholds, the same transformation was applied to all other exposure parameters (metal concentration, energy density, body weight, DEC) as well as threshold NOAELs. Metal tolerance in Dunlin relative to the species tested in studies that set threshold dosages is uncertain. Consequently, risk of toxic exposure begins at conservative NOAELs and increases as exposure approaches and exceeds non-conservative thresholds. I used three risk classifications to describe this gradient of risk. Risk of adverse effects was

deemed present if 95% confidence intervals (CI) around exposure estimates exceeded conservative NOAELs or if they extended below nutritional thresholds. Risk of toxic exposure was considered high if exposure CIs exceeded non-conservative NOAELs because these levels would represent exposure potentially higher than any level measured with no observed effects. Exposure estimates exceeding non-conservative NOAELs indicate at least 50% risk of adverse effects, thereby making adverse effects probable.

Relative influence of diet components on metal exposure:

Exposure from the different diet categories were compared using 95% confidence intervals to indicate significant differences. Exposure from mud snails and sediment were directly compared by this method, but there were insufficient quantities of other estuarine food items to conduct further analyses on specific diet items. Mixed composition samples from YVR, however, necessitated multiple regression analyses to elucidate the relative contribution of different diet items to metal exposure. Multiple regression analyses were conducted for each metal considering the effect of log-normalized quantities of inorganic matter (i.e. grit and sand), vegetation, and terrestrial invertebrates on log-normalized total metal quantities in gizzard contents. Samples with more than 5% unidentified detritus diet components were not included in the analysis as they were uninformative in describing which prey items contribute to exposure, and quantities were bi-modally distributed (usually either high quantities or zero). Restricting analyses to samples with little or no unidentified detritus left only three samples from YVR 2009 collections for which metal data was obtained. Since YVR 2009 samples had generally higher metal levels, even in samples without unidentifiable material, they were not pooled with data from YVR 2010 collections. Consequently multiple regression analyses were only conducted on data from 2010 collections. Collinearity of the different diet item quantities was checked with variance inflation factors (VIF). No VIF was greater than $1 / (1 - r^2)$ so I concluded that no problematic collinearity was present between quantities of the three prey items of interest (Farrar and Glauber 1967). Forward step-wise regressions were used to investigate cadmium, copper, and zinc, adding the different diet item variables only if they significantly improved the model's description of variance in total metal levels.

Cadmium, copper, and zinc correlations: Correlations among cadmium, copper, and zinc were examined individually with linear regressions to identify significant relationships (Bonferroni-corrected: $\alpha=0.05/3$) and quantify the correlation strength of metals across the three areas where collected Dunlin had been feeding (Boundary Bay Marine, Boundary Bay Terrestrial, and YVR Airport Terrestrial). Log normalized total metal masses from gizzard contents were used for these analyses and a habitat covariate was included to test for interaction effects across feeding areas and to account for any differences among them.

Metal levels in Fraser River Delta sediments: Finally, I compared documented sediment concentrations in the FRD to other estuaries and to Canadian sediment quality guidelines (CCME 1998). The guidelines are set based on field studies observing the biological effects of metals at varying concentrations in estuarine and marine sediments under relevant conditions with other typically co-occurring chemicals (CCME 1998).

All statistical analyses were performed with JMP (Ver. 9.0) and all significance levels were set at 0.05. Figures made for data presentation were created with SigmaPlot (Ver. 12.3). The study site map was created using ArcGIS (Ver. 10.0).

Results

Diet category composition: Approximately 65% of samples were grouped into the listed diet categories. Gizzard contents with more uncommon or uncertain compositions were not included in the daily exposure calculations. Diet category compositions are presented in Table 3.3. The most common estuarine diet components were sediment and mud snails. Crustaceans were common across samples, but generally not abundant, with no more than several small individuals in any given gizzard. Grit, sand, and unidentified detritus dominated Terrestrial Mix-2 samples from 2009 collections at YVR while vegetation and grit/sand were the two most common components of Terrestrial Mix-3 gizzard contents from 2010 collections. Estimated energy assimilation efficiencies and resulting DECs for each diet category are also

presented in Table 3.3. Low assimilation efficiencies of plant material resulted in high DECs for samples collected from YVR.

Table 3.3 Composition of gizzard contents, energy assimilation efficiencies, and resulting daily energy consumption for six diet categories of Dunlin (Calidris alpina) collected at Boundary Bay and Vancouver International Airport (YVR) in British Columbia, Canada.

Diet Category (n)	Diet Composition (Mean ± SD)		Energy Assimilation Efficiency	Daily Energy Consumption Spring, Winter
Mud Snail Boundary Bay (6)	Mud Snail Sediment Other Invertebrates	85% ± 8% 8% ± 5% 4% ± 5%	74%	239, 300 kJ/d
Marine Mix Boundary Bay (5)	Sediment Unidentified Detritus Mud Snail Other Invertebrates	39% ± 6% 22% ± 23% 21% ±16% 15% ± 18%	74%	239, 300 kJ/d
Sediment Boundary Bay (6)	Sediment Vegetation Unidentified Detritus Mud Snail	68% ± 15% 17% ± 15% 7% ± 7% 6% ± 6%	68%	260, 326 kJ/d
Terrestrial Mix-1 Boundary Bay (5)	Terrestrial Inverts. Vegetation Grit and Sand Unidentified Detritus	55% ± 33% 22% ± 15% 9% ± 10% 8% ± 6%	64%	222, 278 kJ/d
Terrestrial Mix-2 Vancouver Airport (7)	Grit and Sand Unidentified Detritus Vegetation Terrestrial Inverts.	41% ± 17% 39% ± 10% 13% ± 7% 6% ± 7%	48%	296, 371 kJ/d
Terrestrial Mix-3 Vancouver Airport (11)	Vegetation Grit and Sand Terrestrial Inverts. Unidentified Detritus	49% ± 12% 30% ± 11% 19% ± 6% 3% ± 6%	48%	296, 371 kJ/d

Energy assimilation efficiencies (AEs) calculated from composition data excluding grit/sand and unidentified detritus based on AEs of plant material-37%, invertebrates-74% (Castro et al. 1989), and biofilm-75% (Kuwae et al. 2008). Daily energy consumption calculated as DEE*(1/AE) for estuarine and terrestrial DEEs under spring and winter energy budgets.

Measurement precision, adjustments, and variation across replicate samples: Precision for isotope measurements was very high with standard deviations of reference samples at 0.1‰ for $\delta^{13}C$ and 0.2‰ for $\delta^{15}N$. Precision across replicated gizzard content isotope samples was not as high, but still low in

comparison to differences across diet categories. Standard deviations of isotope signatures in gizzard content duplicates relative to normalized means were 0.9‰ $\delta^{13}C$ and 0.1‰ $\delta^{15}N$ within a $\delta^{13}C$ range of -7.8‰ to -26.0‰ and a $\delta^{15}N$ range of 4.0‰ to 10.3‰. Standard deviations across replicates of metal concentrations from homogenated gizzard contents were as follows (with average percent deviation from replicate means in parentheses): Cd: 0.49ug/g (3.5%); Cu: 0.49ug/g (4.2%); Zn: 20.58ug/g (10.9%). Standard reference material measured metal concentrations relative to certified concentrations are presented in Table 3.4. Correction coefficients, based on deviation from certified concentrations, were as follows for flame AAS measurements: Cd: 0.938; Cu: 0.947; Zn: 0.945.

Table 3.4 Standard reference material certified and measured concentrations

	Cd (µg/g)	Cu (µg/g)	Zn (µg/g)	Std. Ref. Material
Cert. Value ± 95% CL	26.7 ± 0.6	106 ± 10	180 ± 6	TORT – 2
Measured Value ± SD	29.7 ± 2.1	108.1 ± 4.8	189.6 ± 12.5	(Lobster Hepatopancreas)
Cert. Value ± 95% CL	20.8 ± 0.5	25.8 ± 1.1	85.8 ± 2.5	DOLT – 2
Measured Value ± SD	21.4 ± 1.5	27.1 ± 1.8	90.1 ± 7.4	(Dogfish Liver)

Stable isotope signatures of diet categories: Isotopic variation in diet categories showed a wide gradient of $\delta^{13}C$ signatures (Figure 3.2). Mud Snails had the most enriched signature while a more estuarine signature characterized Sediment and Marine Mix samples. Terrestrial samples were $\delta^{13}C$-depleted relative to estuarine samples, as is typically the case. Nitrogen signatures for Boundary Bay Terrestrial Mix were enriched relative to reported values of other terrestrial invertebrates from the region. Sediment $\delta^{15}N$ values were unexpectedly similar to invertebrate values suggesting similar trophic level.

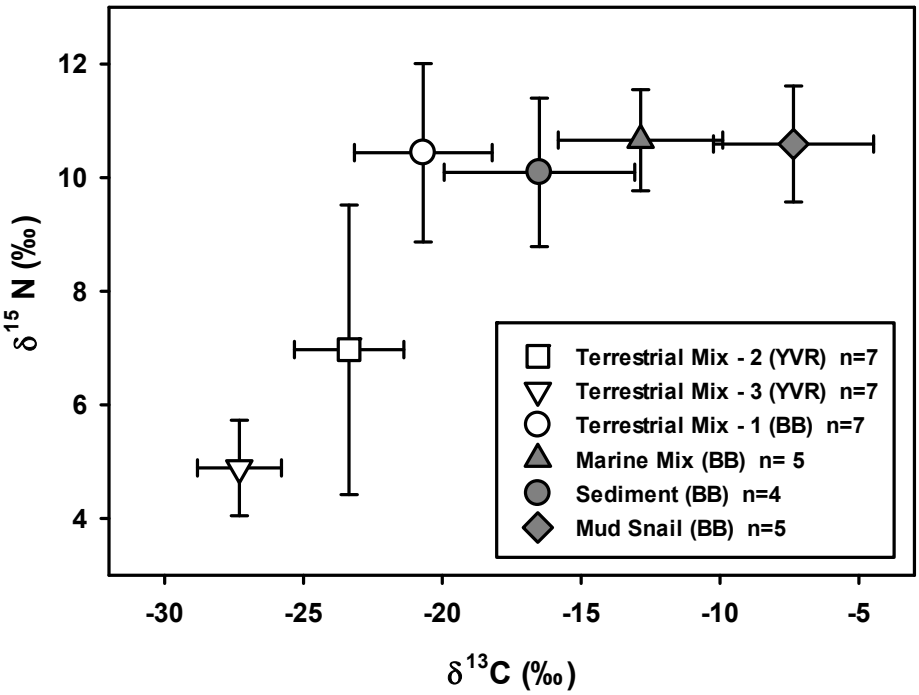

Figure 3.2 Stable isotope ratio variation in diet types of Dunlin (Calidris alpina) feeding in the Fraser River Delta, British Columbia, Canada

Symbols and whiskers represent arithmetic means and one standard deviation respectively. Shaded and white symbols were those with expected estuarine and terrestrial signatures, respectively, as predicted from composition analyses.

Estimation of daily energy expenditure: Average body weight of FRD Dunlin was 56 grams. Dunlin and Sanderling BMRs were estimated at 49 kJ/d (Kelly and Weathers 2002; Gutierrez et al. 2010) and 48 kJ/d (Castro 1987) respectively. Coastal New Jersey winter DEE was calculated at 200 kJ/d (Castro et al. 1992). The BMR to Temperate DEE ratio method consequently estimates saline habitat, temperate winter Dunlin DEE at 204 kJ/d:

$$\frac{\text{Sanderling BMR (kJ/d)}}{\text{Sanderling Temperate DEE (kJ/d)}} \approx \frac{\text{Dunlin BMR (kJ/d)}}{\boldsymbol{Dunlin\ Temperate\ DEE\ (kJ/d)}}$$

$$\frac{48\ \text{kJ/d}}{200\ \text{kJ/d}} \approx \frac{49\ \text{kJ/d}}{\mathbf{204}\ \boldsymbol{kJ/d}}$$

With two distinct estimates of Dunlin arctic DEE (Piersma et al. 2003: 193 kJ/d; Tulp et al. 2009: 231 kJ/d) the Arctic DEE to Temperate DEE ratio method yielded two estimates for saline habitat, temperate winter Dunlin DEE:

$$\frac{\text{Sanderling Arctic DEE (kJ/d)}}{\text{Sanderling Temperate DEE (kJ/d)}} \approx \frac{\text{Dunlin Arctic DEE (kJ/d)}}{\boldsymbol{\text{Dunlin Temperate DEE (kJ/d)}}}$$

$$\frac{229\ \text{kJ/d}}{200\ \text{kJ/d}} \approx \frac{231\ \text{kJ/d}}{\mathbf{202\ kJ/d}}$$

$$\frac{229\ \text{kJ/d}}{200\ \text{kJ/d}} \approx \frac{193\ \text{kJ/d}}{\mathbf{169\ kJ/d}}$$

I took the average of the more similar DEE calculations as my estimate of Dunlin saline DEE (203 kJ/d), added 19 kJ/d (± 8 kJ/d (SD)) to account for additional energetic costs of over-ocean flocking (178 minutes/d; N. Hentze et al. unpublished data), and used the findings of Gutierrez et al. (2011) to calculate energetic savings of terrestrial feeders (20%). The final DEE estimate was 222 kJ/d for marine feeding Dunlin, and 178 kJ/d for terrestrial specialists.

Using the methods employed for winter DEE determination, warmer spring FRD DEE was estimated at 2.8 times BMR (coastal Texas winter climate: Castro et al. 1992). However, the energetic costs of migration necessitate increased feeding at stopover sites. Sandpipers such as Dunlin typically have fattenings rate of 1-2% lean body weight per day (Zwarts et al. 1990). In order to assess the impact of the largest potential difference in energy budgets relative to more expensive winter budgets on toxicity risks, I calculated the spring DEE with a 1% fattening rate. The consequent net DEE increase is approximately 22 kJ/d (lean body weight 54 g x 0.01 = 0.54 g/d equal to ca. 21 kJ/d at 39 kJ/g of fat (Castro et al. 1992, Kuwae et al. 2008)). Over-ocean flocking occurs only occasionally in the FRD during spring (HPJ van Veelen, unpublished observations) so it was not included into the DEE calculation. Migratory spring Dunlin DEE was, therefore, estimated at 177 kJ/d (49 kJ/d x 2.8 + 19 kJ/d (OOF) + 21 kJ/d (fattening rate)) for estuarine feeders. Energy expenditure for spring terrestrial diets were, again, calculated at a 20% reduction in energy (142 kJ/d).

Snail shell correction factor: Cadmium in mud snails was similarly concentrated in both the tissues and shell (Table 3.5); however the mean shell to tissue mass ratio was 8.2 resulting in the large majority of cadmium being in the shell. Copper and zinc were much more concentrated in mud snail tissues than in shells (Table 3.5). Thus, even considering the greater weight of shells, the majority of copper and zinc were in tissues. Measured exposures in Mud Snail and Marine Mix diet categories were adjusted according to the correction factors presented in Table 3.5.

Table 3.5 Cadmium copper and zinc concentrations of mud snail (Batillaria attramentaria) soft tissue and shell for determination of correction factors for mud snail tissues relevant to sandpiper dietary exposure

	µg Cd /g Mean (SE)	µg Cu / g Mean (SE)	µg Zn / g Mean (SE)
Tissues	7.3 (0.7)	157.1 (26.2)	155.6 (15.7)
Shell	6.3 (0.3)	11.1 (0.7)	9.0 (0.3)
Correction Factor	0.11	0.59	0.64

Note: Correction factors represent the quantities of metals in tissues relative to metals in tissues + shells. Shell concentrations were multiplied by the ratio of shell mass to tissue mass to account for the larger mass of shell relative to tissue in mud snails (mean shell mass (g) / mean tissue mass (g) = 8.2).

Measured exposures and risk threshold comparisons: Average homogenate metal and energy concentration measurements for gizzard contents of each diet category as well as the number of samples used for their determination are listed in Appendix B. Exposure relative to toxicity thresholds are presented for cadmium, copper, and zinc in Figures 3.3, 3.4, and 3.5 respectively. Risk of adverse effects from metal exposure was present for all diet categories and all metals in both spring migrants and winter residents. Projected daily cadmium exposures indicate adverse effects are probable for all diet categories except Terrestrial Mix-1 from Boundary Bay (BB) collections. Cadmium exposure risk classification was the same for spring and winter Dunlin with the exception of the BB Terrestrial Mix-1 diet category which was not high risk for spring Dunlin. Winter resident and spring migrant copper exposure estimates from YVR Terrestrial Mix-2 and 3 indicate probable adverse effects (Figure 3.4). Copper exposures in winter Mud Snails and both winter and spring Marine Mix diets were high risk with risk classification reduced for Mud Snail diets in the spring. Zinc measures

found Terrestrial Mix-2 diets from fall YVR collections exposed winter Dunlin to levels associated with probable toxic effects, but risk classification for the diet category was reduced to high risk for spring migrants as it was for the Marine Mix and Terrestrial Mix - 3 diets from BB in both winter and spring.

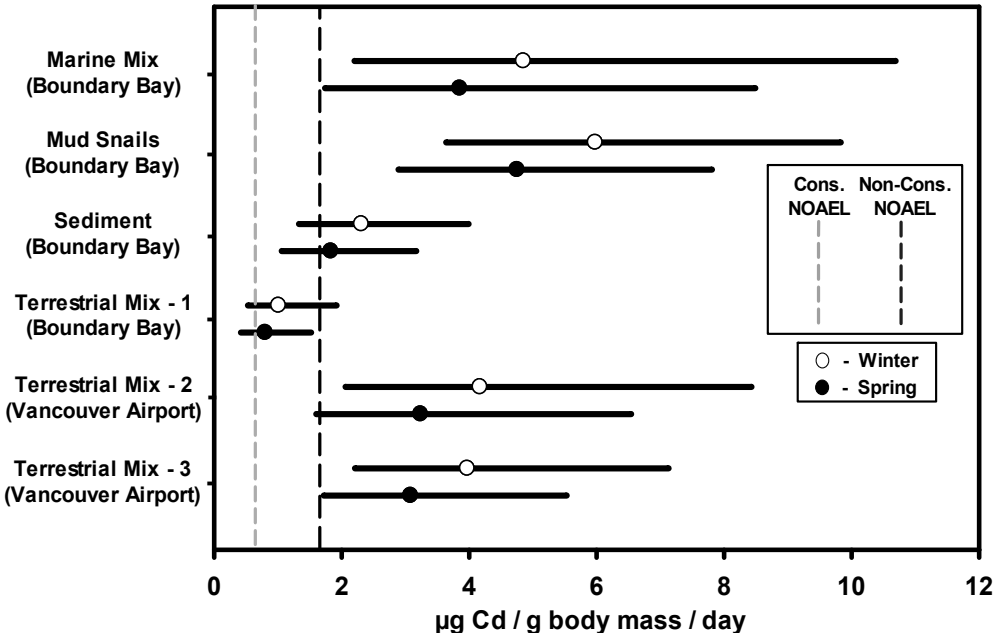

Figure 3.3 Daily cadmium exposures to Dunlin (Calidris alpina) from diet categories under spring and winter energy budgets relative to no-observed-adverse-effect-levels (NOAEL)

Note: Symbols represent back transformed log mean exposure (node) and 95% confidence intervals of the mean (whiskers). Whiskers exceeding conservative and non-conservative NOAELs indicate risk and high risk of toxic effects respectively; nodes exceeding non-conservative NOAELs suggest toxic exposure is probable.

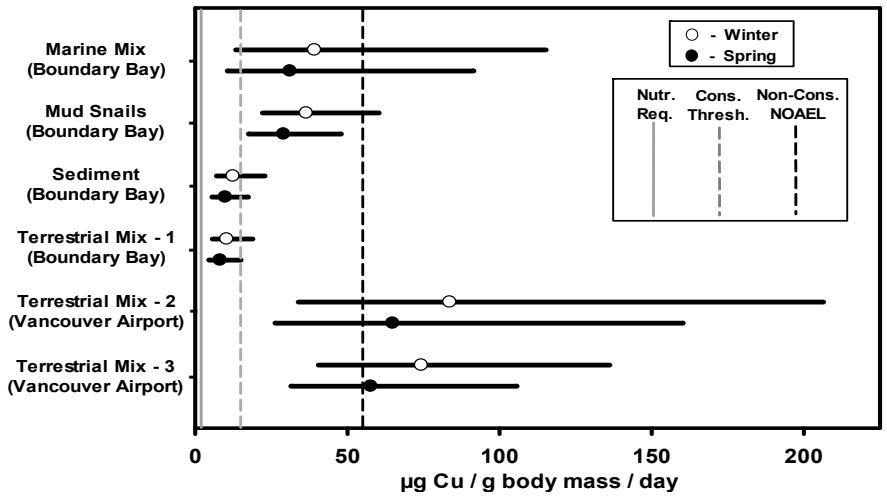

Figure 3.4 Daily copper exposures to Dunlin (Calidris alpina) from diet categories under spring and winter energy budgets relative to no-observed-adverse-effect-levels (NOAEL)

Note: Symbols represent back transformed log mean exposure (node) and 95% confidence intervals of the mean (whiskers). Whiskers exceeding conservative and non-conservative NOAELs indicate risk and high risk of toxic effects respectively; nodes exceeding non-conservative NOAELs suggest toxic exposure is probable.

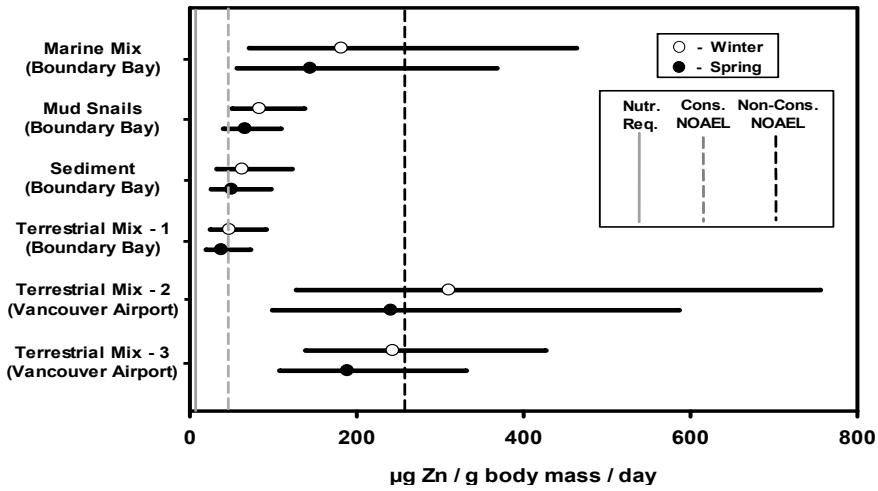

Figure 3.5 Daily zinc exposures to Dunlin (Calidris alpina) from diet categories under spring and winter energy budgets relative to no-observed-adverse-effect-levels (NOAEL)

Note: Symbols represent back transformed log mean exposure (node) and 95% confidence intervals of the mean (whiskers). Whiskers exceeding conservative and non-conservative NOAELs indicate risk and high risk of toxic effects respectively; nodes exceeding non-conservative NOAELs suggest toxic exposure is probable.

Relative influence of diet components on metal exposure: Significant differences in exposure across diet categories are shown by lack of overlap in 95% confidence intervals in Figures 3.3, 3.4, and 3.5. The BB Terrestrial Mix-1 diet exposed Dunlin to significantly less metals than YVR Terrestrial Mix diets for all metals and significantly less cadmium exposure than all other diet categories except Sediment (Figure 3.3). Copper and zinc exposure estimates from Marine Mix and Sediment diet categories were significantly less than YVR diets. The BB Terrestrial Mix diet also had significantly less zinc exposure than Mud Snail samples. While cadmium and copper exposure appear less in Sediment samples than in Mud Snail samples, the difference is not significant and risk classification was the same for all metals.

Multiple regressions of cadmium, copper, and zinc revealed none of the investigated diet components (e.g. inorganic matter, invertebrates, vegetation) significantly influenced metals in gizzard contents from 2010 collections at YVR.

Cadmium, copper, and zinc correlations: No significant interaction effects were observed in metal correlations across feeding areas (BB Terrestrial, BB Estuarine, YVR Terrestrial). Cadmium concentrations were significantly and positively related with zinc ($n = 48$, $df = 3$ and 41, $p = 0.004$) and copper ($n = 45$, $df = 3$ and 41, $p = 0.011$) although not particularly well correlated ($r^2 = 0.29$ and $r^2 = 0.21$, respectively). Copper and zinc were also significantly positively associated ($n = 44$, $df = 3$ and 40, $p < 0.001$) and, in contrast, were very tightly correlated ($r^2 = 0.80$).

Metal levels of Fraser River Delta sediments: Heavy metals have been monitored in sediments across a range of pristine to highly contaminated sites in UK estuaries (Bryan and Langston 1992). Reports of metal concentrations in FRD sediments are presented in Table 3.6 relative to UK estuaries, Canadian interim sediment quality guidelines (ISQGs), and probable effect levels (PELs) determined by the Canadian Council of Ministers of the Environment (CCME) for comparison. The ISQGs define sediment concentrations below which biological effects rarely occur, whereas sediment levels between the ISQGs and PELs and above PELs are associated with occasional and frequent effects, respectively (CCME 1998).

Table 3.6 Sediment metal concentrations (dry weight) in the Fraser River Delta relative ranges reported across estuaries in the United Kingdom, Canadian sediment quality guidelines, and probable effect levels

Site/Guidelines	Cadmium (µg/g)	Copper (µg/g)	Zinc (µg/g)	Author/Study
FRD, Sturgeon Bank	0.033-0.432	10.7-54.1	46.6-95.8	
FRD, Robert's Bank	0.038-0.177	17.3-52.8	14.3-100.8	Thomas 1998
FRD, Boundary Bay	*0.063-0.253*	*4.4-23.4*	*21.2-53.0*	
FRD, Robert's Bank	<0.50-0.91	9.3-38.2	0.60-22.3	Hemmera Envirochem Inc. et al. 2010
UK sites	0.2-10	10-2000	<100-3000	Bryan and Langston 1992
Sediment Quality Guidelines	0.7	18.7	124	CCME 1998
Probable Effect Level	4.2	108	271	CCME 1998

Discussion

Diet composition and isotopic signatures: Variation in diet components was greatest in Marine Mix and BB Terrestrial Mix-1 categories reflecting their loosely defined composition criteria. Stable isotope ratio variation was similar across samples with the exception of high variance in Marine Mix $\delta^{13}C$ and YVR-1 $\delta^{15}N$. While Sediment $\delta^{15}N$ signatures averaged around 10‰, Kuwae et al. (2008) reported ingested biofilm signatures under 6‰ in Western Sandpipers (*Calidris mauri*) collected at another site in the FRD. The enriched Sediment signature indicates higher trophic level components than the microphytobenthos-dominated biofilm ingested by Western Sandpipers. Considering how distinct the Sediment signature was from previously reported biofilm signatures, it may indicate that there was little biofilm in Sediment gizzard contents and that the Dunlin were consuming some form of higher trophic level detritus that was mixed in with the sandy sediments. Biofilm is generally associated with silt and clay sediments as opposed to more sandy sediments (R. Elner, personal communication) typically found in Boundary Bay and in the gizzard contents of the sampled Dunlin offering further indications that the Sediment diet category was not representative of biofilm consumption. Terrestrial Mix-2 gizzard content samples from YVR were isotopically similar to terrestrial invertebrates sampled in agricultural fields of

the FRD (Evans Ogden et al. 2005; this study: see Chapter 2, Appendix A) in which terrestrial prey $\delta^{13}C$ and $\delta^{15}N$ signatures ranged from -27.2‰ to -24.9‰ and 5.8‰ to 7.6‰ respectively. Terrestrial Mix-3 samples from YVR had signatures more similar to seeds (see Chapter 2, Appendix A), reflecting the higher proportion of vegetation described by composition analyses (Table 3.2). BB Terrestrial Mix-1 samples were relatively enriched in $\delta^{13}C$ and $\delta^{15}N$ suggesting samples included some marine prey items. Composition data confirmed this as two samples had a small number of shell fragments. Stable isotope analyses of field sampled mud snails revealed that enriched Mud Snail $\delta^{13}C$ signatures reflect signatures in shells. When analyzed separately, snail tissue and shells had mean $\delta^{13}C$ signatures of -11.8‰ ± 0.4(SD) and -3.0‰ ± 2.0(SD) respectively.

Estimation of daily energy expenditure: Metabolic rate calculations based on mass alone predict a Dunlin DEE of 165 kJ/day (Nagy et al. 1999), substantially lower than the winter DEE estimate but similar to that of spring. Risk classifications for spring migrant Dunlin were reduced for three of the 18 diet category exposure assessments relative to winter residents. While this represents some change, overall risk across diet types remains generally high for both winter and spring Dunlin suggesting the risk assessment is not very sensitive to variation in DEE at this scale. Lack of sensitivity to DEE also suggests the model is robust relative to estimation error of AE. The Terrestrial Mix-2 diet category AE appears to be the most likely to suffer from such error since the estimate considers the diet to be primarily composed of plant matter whereas stable isotope signatures suggest a higher trophic level, likely resulting from higher trophic level prey in “unidentified detritus”. If the “unidentified detritus” is considered invertebrate terrestrial prey, as the stable isotope data suggest, overall absorption increases from 48% to 66% and daily consumption is reduced by 27%. This has the effect of reducing probable risk for zinc exposure from the Terrestrial Mix-2 diet category to high risk under winter energy budgets and the same risk reduction for copper under spring energy budgets. However, risk classifications are again still generally high, regardless of these seemingly large differences in DEE.

Sources of metal exposure: Metal exposure from Sediment and Terrestrial Mix-1 diet categories from BB was lower than all other diet categories for all

metals. Sediment diet category exposure had very little potential for toxicity in this study. As discussed above, isotope signatures suggest Sediment samples either contained a distinct biofilm composition than has been observed previously in the FRD or did not contain quantities of biofilm sufficient to influence isotope or metal measurements. Biofilm, therefore, cannot be ruled out as a potential vector for metal exposure. Of all diet items, mud snails presented the greatest risk of toxic cadmium exposure to sandpipers even after accounting for metals not absorbed from shells. The low energy density (15 kJ/g) and relatively high cadmium concentrations in their tissues make them a potent exposure vector to consumers. Marine Mix samples, which included mud snails, had similar levels of exposure, indicating that other benthic invertebrates may also be important vectors of cadmium exposure. High risk from exposure in YVR diets was, in part, due to a small number of samples with particularly high metal concentrations which increased estimates of average exposure and widened confidence intervals around the estimates (Figures 3.3, 3.4, and 3.5). For example, the copper concentration from one Terrestrial Mix-2 gizzard content sample exceeded 380 μg/g. In addition, a number of man-made objects such as plastic beads (3 mm in diameter) and brightly coloured material resembling painted tarmac were observed in YVR gizzard contents. Such debris and high metal exposure may have come from contamination from plane traffic at the airport (e.g. copper in brake pads) and/or chemicals and other materials used for maintenance of facilities on the runways adjacent to mudflats. Boundary Bay samples suggest lower exposure to all metals from terrestrial diet types than estuarine diets. Accumulated cadmium levels in Dunlin are higher for estuarine feeding birds than in terrestrial feeders (Chapter 2) suggesting that such is the norm. High cadmium exposures in the terrestrial diet categories from YVR collections are an exception to this trend, further indicating that plane and other runway activity result in elevated metal levels at the airport. Dunlin and other shorebirds are already actively discouraged from using YVR lands as foraging habitat by airport staff (David Ball, YVR Wildlife Control and Landscaping, personal communication) and the planes themselves (e.g. aircraft collisions).

In analyses of the contributions of invertebrates, inorganic matter, and vegetation to gizzard content metal levels in YVR collections from 2010, I found no significant description of metal variation by any diet item. Gizzard content samples from 2009

collections at YVR could not be included in these analyses, but generally had greater metal quantities than those of 2010 and also had higher proportions of inorganic matter (mostly grit) on average (Table 3.3). Although not significant, slopes of YVR 2010 inorganic content regressed against copper and zinc levels were positive. Additionally, the terrestrial YVR diet categories had the highest zinc and copper exposure levels and contained far more grit than any other diet categories. While the multiple regression analyses of YVR gizzard content items' metal content offer no conclusive evidence, copper and zinc exposure from grit is worthy of further investigation, especially considering the high levels of risk associated with exposure from YVR diet categories.

Risk assessment model: Risk and high risk were classified using 95% confidence intervals (CI) for exposure estimates which were quite wide for some diet categories resulting in uneven and sometimes high probabilities of risk designation. Variation in composition and isotopic signatures within diet categories were not strongly related to uncertainties in exposure estimates (i.e. CIs) so the likelihood of risk was not a result of diet category composition criteria. For example, the relatively loosely defined BB Terrestrial Mix category had the shortest 95% CIs, and terrestrial diet categories from YVR with very similar composistion across samples had some of the broadest CIs. Variation in metal and energy concentrations within similar samples, as described above for YVR samples, were the greatest sources of uncertainty in daily exposure estimates. Greater sample sizes would reduce this uncertainty and thereby reduce the number of risk and high risk classifications because some exposure estimates fell below risk thresholds. However, almost half the estimates were above non-conservative NOAELs and no amount of CI reduction would change such probable adverse effect classifications.

The toxicity risks projected from cadmium copper and zinc in Dunlin's ingested prey (i.e. dietary exposure) were dramatically greater than risks associated with metal concentrations in the same birds' kidney and feather tissues (Chapter 2). This discrepancy may be explained by the fact that collections for kidney samples were made during migratory periods and tissue levels in migrants often reflect exposure from other sites. However, some of the sampled birds could just as easily have been residents that had not yet migrated. In either case, the risk assessment methods require scrutiny

before concluding metal exposure to Dunlin in the FRD has such high risks of toxicity as described by my analyses.

Risk assessments typically use uncertainty or safety factors that reduce toxicity thresholds by some factor (often 10) to account for uncertainties associated with cross-species comparisons. Additionally, many risk assessments set toxicity thresholds with only conservative NOAELs. The model employed here does not include a safety factor, and results project exposure above the highest documented NOAELs for avian species in many cases. Risk described by the model is, therefore, not a result of a conservative model.

Daily exposure predicted by the model is dependent on the following factors: Consumption (DEE, AE, and energy densities in food items), metal concentrations in food, and the ratio of exposure to body weight of the study species. Estimation of those parameters is a potential source of risk miss-classification. Risk classification was not very sensitive to DEC, so inaccuracies in DEE or AE estimations are unlikely to significantly influence risk. Energy measurements were validated as diet categories yielded averages around 20 kJ/g organic mass, similar to other studies that estimated sandpiper prey energy concentrations (Masero and Perez-Hurtado 2001). Metal concentrations were validated with analyses of certified concentration reference standards alongside samples.

Metabolism in sandpipers is 20-40% higher than in most avian species of similar size and mass (Kersten and Piersma 1987). As a result, the metal consumption to body weight ratio for Dunlin is quite different from those of the reference test species from which toxicity thresholds are determined. If DEE is conservatively estimated at 165 kJ/d (Nagy et al. 1999) under conditions similar to lab-based dosing studies, and food has a typical AE of 75% and energy density of 20 kJ/g, then Dunlin eat approximately 11 grams of food per day (165 kJ/d * (1/0.75) / 20 kJ/g), equal to 20% of lean body weight (ca. 54 g). Starlings (*Sturnus vulgaris*), used for determination of the non-conservative cadmium threshold, consume 17% (11 g / 74 g bw; Stanton et al. 2010) of their body weight per day. Waterfowl chicks and poultry chicks, used for determination of all other thresholds, consume ca. 11% (13 g / 126 g bw; Sample et al. 1996) of their body weight per day. Since daily exposures and thresholds are calculated as consumption relative to

body mass, according to the exposure model, Dunlin have elevated risk relative to waterfowl/poultry chicks and starlings that eat foods with the same metal concentration. However, relatively low metal tolerance in Dunlin seems unlikely considering they inhabit estuarine environments that are generally elevated metal concentrations relative to other habitats. Another important consideration is that field-based energy expenditure and food consumption, which daily exposure models were based on, are higher than metabolic rates under typical conditions of dosing studies. Under winter FRD conditions, estuarine feeding Dunlin DEE is closer to 220 kJ/d necessitating a consumption rate of ca. 15 g/d, equal to 27% of body weight (54 g). To examine the impact of Dunlin's high metabolism relative to body size and the use of field based consumption rates on risk predicted by the daily exposure model, I adjusted Dunlin mass to obtain a 14% mass consumption to body weight ratio (still greater than that of most reference test species). The adjusted model projected a reduction of 10 out of 18 risk classifications for winter resident exposure, 11 of 18 for spring migrants, and maintained no nutritional risk classifications.

Bioavailability*:* Toxicity is dependent not only on a substance's concentration in food items and daily consumption, but also on bioavailability (i.e. what proportion of a substance is absorbed after consumption). A wide variety of factors influence metal bioavailability such as fibre content and type, protein content, and other characteristics of the food matrix in which it is delivered (e.g. shells, exoskeletons, bones) as well as the chemical form of the metal, the quantity and proportion of other metals in the diet, and the digestive physiology of the study species (Reinfelder and Fisher 1994; Asagba 2009). Toxicity thresholds are generally set using diets supplemented with metals in the form of highly soluble salts which, although they are only absorbed at a rate of 0.5-8.0% (Friberg et al. 1974; Jacobs et al. 1978), are usually more bio-available than metals within diet items. Toxicity thresholds like NOAELs, as well as estimated exposure in this study, are based on the total dosage, not the quantity of metals absorbed. However, reduced bioavailability of metals in FRD prey relative to the salt forms used to determine NOAELs would reduce toxicity risks from what the exposure model describes.

Sample digestion method plays a large role in determining what proportion of metals measured are bio-available. Nitric acid does not release metals from all sample

matrices, but it is stronger than sandpiper stomach acids and likely overestimates metal exposure by dissolving all organic components and releasing a fraction of inorganically bound zinc and cadmium into solution as well. I utilized a correction factor to account for how this disparity in digestion strengths affected measures of Mud Snail metal as a result of shells dissolving in nitric acid, but not in sandpiper digestion. Invertebrate exoskeletons in Marine Mix and Terrestrial Mix samples from Boundary Bay may have also digested more in metal analyses than they would in sandpiper digestion, and this was not accounted for. Additionally, inorganic metals likely contribute to some of the metals measured in YVR samples containing grit. Sediment samples would potentially have a similar digestion method issue considering their high proportions of inorganic material, but metal levels for this diet category were low, even with nitric acid digestions.

Elemental interactions: Calcium, copper, and zinc, along with iron, selenium, vitamin D and ascorbic acid are all known to decrease dietary cadmium absorption when they are consumed in quantities that meet or exceed nutritional requirements (Jacobs et al. 1978; Fox et al. 1984; Mckenna et al. 1992). Measures of copper and zinc exposure from this study are tightly correlated and observed ingestion of shells containing calcium carbonate imply these metals are abundant in the FRD diet. Intestinal absorption of cadmium may, therefore, be reduced in FRD-feeding Dunlin relative to reference test species from which thresholds were set. Zinc and copper bind to the same protein carrier that facilitates absorption in the intestines (Leeson 2009). Consequently, high dietary exposure to one of these elements can harmfully reduce absorption of the other, but high exposure to both avoids this issue. The positively correlated concentrations of these metals in FRD diets are, thus, beneficial.

Risk of adverse effects: Cadmium, copper, and zinc exposure in most diet types were clearly high relative to daily dietary exposure NOAELs. However, the extent to which Dunlin can consume metals without adverse effects relative to species that have been tested to set toxicity thresholds (e.g. NOAELs) is unknown. Consequently the high estimates of risk described by the daily exposure model must be interpreted with caution. Other indicators of risk were investigated to aid in this interpretation.

Sediment metal levels measured in previous studies of FRD intertidal habitat were low relative to a range of sites in the UK (Table 3.5). Cadmium, copper and zinc

levels in FRD sediments were also below sediment quality guidelines, and lower than all levels associated with probable toxicity risks. However, a number of factors (e.g. other metals, sulphide levels, pH) can influence bioavailability of metals in sediments (CCME 1998). Furthermore, benthic marine invertebrates are sometimes influenced more by dissolved metals in the water column than by those in sediments (Bryan and Langston 1992). As a testament to this, cadmium concentrations in FRD diet categories and mud snail tissues had similar averages as those found in sandpiper prey from a number of sites in the Bristol Channel, UK that had far higher cadmium concentrations (1-3 µg/g) in sediments (Ferns and Anderson 1994).

Copper and zinc concentrations in feathers from FRD Dunlin captured in early April 2011 were within the range reported from other sandpiper populations (Chapter 2) offering some indication that exposure to these metals did not result in toxic levels of accumulation. However, no studies that I'm aware of have reported threshold levels of copper or zinc in feathers that are associated with toxic effects. Concentrations of metals observed in kidneys of Dunlin sampled for this study were all lower than toxicity thresholds (Chapter 2). However, as discussed above, it is possible that those Dunlins were all migrants from other sites and do not reflect FRD exposure. In summary, while tissue levels seem to suggest low risks of local toxicity, they offer no concrete evidence.

Since other indicators of risk were inconclusive, what can be concluded from the information on dietary exposure alone? Although all diet categories had some level of risk associated with daily metal exposure, estimates of mean exposure were below conservative NOAELs for copper exposure in Sediment and Terrestrial Mix diet categories from BB under both winter and spring energy budgets. Zinc exposure for the BB Terrestrial Mix - 1 diet category was also below conservative NOAELs for Dunlin in both seasons, so these categories, at least, can be considered low risk. As discussed above, elemental interactions should reduce dietary absorption and toxicity, and factors affecting bioavailability may further reduce absorption and, thus, risk. There is also a toxicological advantage in Dunlin's utilization of varying food and habitat types. Studies in the FRD have shown that more than 80% of Dunlin utilize terrestrial foraging habitat (Shepherd and Lank 2004) and, on average, approximately 38% of Dunlin's diet comes from terrestrial food items (Evans Ogden et al. 2005). Foraging in terrestrial habitat at Boundary Bay appears to offer a low metal diet source that reduces overall exposure,

potentially allowing Dunlin to take advantage of abundant estuarine prey such as mud snails that, on their own, present considerable toxicity risk. These factors certainly reduce the risk of toxicity to some extent; however, improved understanding of elemental interactions, metal bioavailability would be required to quantify the risk reduction.

Risk to Dunlin is also greatly affected by their capacity to utilize, excrete, or immobilize metals into an innocuous state (Oh et al. 1979; Wayland and Scheuhammer 2011). The risk described by comparing daily exposure to NOAELs assumes that these capacities are the same across species of similar size. While size logically influences capacities for metal storage and nutritional utilization of elements like copper and zinc, other interspecific differences must also have important influences on toxicity risks (Sample et al. 1996). For example, many species of seabirds across a broad geographic range have been found with cadmium burdens (i.e. tissue concentrations) that are demonstratedly toxic to other species (Elliot et al. 1992; Elliott and Scheuhammer 1997). While population monitoring data is sparse for many of these species, exposure is often of natural origin and populations appear to persist without any obvious adverse effects (Elliott and Scheuhammer 1997; Burger 2008). Metallothionein (Mt), a metal binding protein that functions in the storage and transportation of cadmium, copper, zinc, and mercury, is known to form a stable complex with cadmium, and is highly correlated with cadmium in seabird kidneys where complexed Mt-Cd is predominantly stored (Elliot et al. 1992; Elliott and Scheuhammer 1997; Wayland and Scheuhammer 2011). Seabirds' capacity to produce Mt appears to be great enough to match high dietary cadmium exposure, thereby allowing them to persist under conditions that would be toxic to other species (Burger 2008). Higher than average capacities to excrete or otherwise manage metals have not been demonstrated in Dunlin, but would make sense considering they often inhabit naturally high metal estuarine environments and considering their consumption rates are greater than most other bird species of the same size.

In conclusion, risk assessment using NOAELs of daily exposure relative to body weight suggest cadmium copper and zinc exposure in the FRD generally pose toxicity risks to Dunlin and potentially other sandpipers using the habitat during winter and spring. The most concerning levels of risk were found for cadmium exposure, which was classified as probable for most diet categories. However, due to the potential for

overestimation of toxicity risks, I suggest that risk not be considered “probable” until better understanding of metal toxicokinetics in sandpipers is achieved, or more conclusive evidence is obtained from FRD Dunlin tissue samples. Other studies assessing exposure risk of animals with high metabolism relative to body size should take similar caution when interpreting risk classifications based on daily exposure relative to body weight. Additionally, site-specific and season-specific energy expenditure should be considered in any risk assessment involving such parameters since exposure to toxicants in the wild often occurs under conditions requiring greatly increased activity and dietary consumption relative to energetic requirements predicted from body mass and taxonomic group alone.

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Appendix A:

Toxicity benchmarks of cadmium, copper, and zinc ingestion for sensitive and non-sensitive avifauna. Also, copper and zinc nutritional requirements determined for poultry.

Metal (Threshold Type)	NOAEL, LOAEL *NOAEC, LOAEC* µg/g bw/day *µg/g in food*	Reference Threshold Study Species and Effect Measured	Reference Threshold Source
Cadmium (Conservative Tox.)	0.7, 1.0 *7.61, 14.6*	Wood Duck, Mallard Gen. Histology, Nephrosis	Mayack et al. 1981* Cain et al. 1983*
Cadmium (Non- Cons. Tox.)	1.64, 8.22 *10, 50*	Starling NADPH Cyt. C reductase	Pilastro et al. 1993*
Copper (Conservative Tox.)	13.0, 26.0 *125, 250*	1 Day Old Chicks Gizzard Lining Erosion	Poupoulis and Jensen 1976
Copper (Non- Cons. Tox.)	55.4, 65.6 *676, 800*	Turkey Poults Growth Rate	Vohra and Kratzer 1968
Copper (Nutritional Req.)	0.5† 6†	Various Poultry Species	Leeson 2009
Zinc (Conservative Tox.)	14.5**, 130.9 *100**, 2000*	New Hamp. x Leghorn Chicks Growth Rate	Stahl et al. 1989
Zinc (Non-Cons. Tox.)	256.2, 322.8 *4000, 8000*	Hubbard Broiler Chicks Weight + Consumption Rate	Oh et al. 1979
Zinc (Nutritional Req.)	4.0† 63†	Broiler Chicks	Huang et al. 2007

† Highest nutritional requirement for optimal health

* As cited in Stanton et al. 2010

**Value unrealistically low for toxicity threshold. NOAEL threshold substituted with the geometric mean of NOAEL and LOAEL (43.6 µg/g bw/d).

Appendix B:

Energy and metal quantities per gram of homogenate from diet categories of Dunlin (*Calidris alpina*) gizzard samples collected from the Fraser River Delta of British Columbia, Canada

Diet Category	Energy (kJ/g) Mean *SD, SE (n)*	Cd (µg/g) Geo-Mean Mean *SD, SE (n)*	Cu (µg/g) Geo-Mean Mean *SD, SE (n)*	Zn (µg/g) Geo-Mean Mean *SD, SE (n)*
Mud Snail Boundary Bay	1.3 *0.6, 0.3 (5)*	1.3 1.4 *0.2, 0.1 (5)*	8.2 8.3 *1.5, 0.8 (4)*	18.8 19.1 *3.9, 1.8 (5)*
Marine Mix Boundary Bay	1.8 *1.2, 0.6 (4)*	1.3 1.4 *0.5, 0.2 (5)*	10.7 13.1 *7.5, 3.7 (4)*	49.6 56.5 *30.1, 15.1 (4)*
Sediment Boundary Bay	2.4 *0.6, 0.2 (5)*	0.9 1.0 *0.6, 0.3 (5)*	4.9 5.5 *2.3, 1.0 (5)*	24.9 29.1 *13.9, 6.2 (5)*
Terrestrial Mix-1 Boundary Bay	6.6 *3.5, 1.8 (4)*	1.1 1.1 *0.7, 0.3 (6)*	12.5 13.2 *4.9, 2.0 (6)*	57.0 63.4 *31.1, 12.7 (6)*
Terrestrial Mix-2 Vancouver Airport	4.2 *1.8, 0.8 (4)*	2.3 2.7 *1.8, 0.7 (6)*	47.1 70.1 *78.4, 32.0 (6)*	174.9 248.1 *251.3, 102.6 (6)*
Terrestrial Mix-3 Vancouver Airport	4.4 *1.7, 0.8 (5)*	2.4 2.7 *1.2, 0.4 (9)*	45.0 51.7 *33.7, 11.9 (9)*	147.3 162.2 *84.9, 28.3 (9)*

Chapter 4:

Summary, Synthesis, and Conclusions

Introduction

My research goals for the thesis were to: 1) Investigate factors contributing to heavy metal and selenium exposure and accumulation in Dunlin; 2) Examine the potential for adverse effects to Dunlin from such elements. To conclude the thesis I first summarize and discuss the observed patterns of accumulation and exposure. Second, I compare conclusions from toxicity risks associated with heavy metal and selenium tissue levels and cadmium, copper, and zinc dietary exposure in the Fraser River Delta (FRD). Finally, I discuss the conservation implications of the research and future priorities given my results.

Factors Affecting Accumulation and Exposure

The influences of age, sex, bill length, size, sample group, prey items, habitat preference, and trophic level on heavy metal and selenium exposure and accumulation are reviewed here:

Age: Cadmium in kidneys was found at significantly higher concentrations in second-year (SY) and after-second-year (ASY) birds than in hatch-years (HY). Logarithmic increases of cadmium concentrations in kidneys with age has been observed in other Dunlin populations (e.g. Blomqvist et al. 1987) and appears to be the result of concentration-dependent excretion rates (Chapter 1) and low natal cadmium burdens. Zinc concentrations in kidneys were significantly higher in HY than in SY or ASY Dunlin. Considering that high zinc levels in HY relative to SY or ASY birds have not

previously been found in Dunlin or other sandpipers (Blomqvist et al. 1987) and tests on chickens suggest concentrations are quickly regulated and return to normal levels within a week after atypical exposure (Oh et al. 1979), differences in recent exposure are the most likely reason for apparent age class effects on zinc burdens. No age class effect was found for copper concentrations in kidneys and no significant differences in feather concentrations of copper, lead, manganese, selenium, or zinc were found between SY and ASY Dunlin.

Sex and bill length: Bill length and sex had no significant influence on metal accumulation in the observed Dunlin.

Size: Tarsus length, used as a proxy for size, had no significant impact on metal accumulation in the observed birds.

Trophic level: Trophic level of prey items as indicated by $\delta^{15}N$ stable isotope signatures in Dunlin muscle tissue showed no significant impact on metal or selenium accumulation.

Habitat preference: Kidney cadmium concentrations in Dunlin were significantly correlated with $\delta^{13}C$ stable isotope signatures. Dunlin with estuarine signatures had the highest cadmium burdens. Shorebirds foraging in saline environments must consume more food to support the increased costs of osmo-regulation associated with saline diets and will, therefore, consume a greater amount of metals and selenium or any other substance in their diet. In addition, modelled daily exposures from terrestrial and estuarine diets in Boundary Bay indicate cadmium, copper, and zinc exposure is greater in estuarine environments. Cadmium exposure from terrestrial diets at Vancouver International Airport (YVR) was also high, but high copper and zinc exposure at YVR indicate this is a site specific effect unique to the airport and is not representative of terrestrial diets in general. High metal exposure from YVR samples could be due to metal inputs from aircraft traffic (e.g. copper in brake pads, jet fuel) and other activities such as painting and paving on the runways.

Manganese was significantly higher in terrestrially feeding birds. Agricultural inputs are a potential source of elevated terrestrial manganese exposure as the element

is essential in plants for a number of biological functions and is, consequently, a common fertilizer component.

Sample group: Including sample group in a stepwise regression model after $\delta^{13}C$ (i.e. habitat preference) significantly improved the model describing kidney cadmium concentrations. SY and ASY Dunlin collected in during spring migration at YVR had lower cadmium burdens than those collected at Boundary Bay. However, the difference between groups may have resulted from an absence of $\delta^{13}C$ signatures greater than -15.5‰ in YVR samples since all the highest burdens were in Dunlin with such enriched estuarine signatures. Sample group was significantly related to copper and zinc concentrations in kidneys with higher concentrations of zinc and copper in captures from Boundary Bay than in Dunlin collected at YVR in Spring 2010. Fall 2009 YVR collections were almost entirely HY Dunlin and were consequently not included in analyses of sample group effects due to the potential for interference from age effects. However, as described above, the significant differences in zinc between HY and older age classes is most likely an effect of recent exposure and, therefore, sample group. A significant difference between zinc burdens in the two YVR sample groups highlights the importance of migration influences on accumulated metal levels in birds at stopover sites. HY Dunlin were collected from YVR in November 2009 during the peak of fall migration in the Fraser River Delta (Butler and Vermeer 1994) and April 2010 collections from YVR occurred during spring migration. Since these migrants spent an unknown period of time at YVR and may have arrived from another site within the previous few days, it is not surprising that their cadmium burdens were different. Unfortunately, since I don't know where the different sample groups migrated from, how recently they arrived, or some were winter residents from the FRD, I cannot make any concrete conclusions as to the source of differences in metal levels.

The exposure model indicated that diets from YVR had the highest levels of copper and zinc exposure of all diet categories. While kidney zinc concentrations in migratory Dunlin cannot be directly linked to exposure at the site of capture, the combination of high levels of zinc in one group of migrants from YVR together with findings of high exposure from gizzard content samples in Dunlin collected at that site increase its likelihood of being a potential source of high zinc tissue burdens in Dunlin.

Boundary Bay estuarine diets exposed Dunlin to more cadmium than YVR or Boundary Bay terrestrial diets. Gizzard content analyses indicate terrestrial Boundary Bay diets expose Dunlin to lower copper and zinc levels than estuarine diets from adjacent habitat. Metal inputs from airport traffic may explain the high copper and zinc exposure in YVR diets relative to terrestrial diet samples from Boundary Bay.

Prey items: Of all prey types analysed in Dunlin gizzard contents, mud snails (*Batillaria attramentaria*) presented the greatest cadmium toxicity threat. Estuarine prey samples, including mud snails, sediment, and other invertebrates, exposed Dunlin to similar levels of metals indicating that other benthic invertebrates also function as vectors for cadmium exposure. The Terrestrial Mix-1 diet from Boundary Bay presented the lowest exposure levels to Dunlin for all metals. Copper and zinc exposure was greatest in YVR terrestrial diets with uniquely high quantities of grit. Analyses of ingested sediments indicate low cadmium, copper and zinc exposure from sediment consumption at Boundary Bay. Lack of a relationship between trophic level of Dunlin prey and metal accumulation appears to suggest biofilm is not a potent exposure vector. However, stable isotope signatures of sediment and muscle tissue indicate the Dunlin sampled for this study did not consume much biofilm. Additionally, Western Sandpipers, which feed more heavily on biofilm than Dunlin (Mathot et al. 2010), generally have higher cadmium burdens than Dunlin (McFarland et al. 2002; Chapter 2). Other potential sources of higher cadmium burden in Western Sandpipers are the use of different wintering sites and greater dependence on estuarine habitat relative to terrestrial habitat (Beninger et al. 2010; Franks 2012).

Toxicity Risks

I obtained element concentration measurements verified by certified reference materials and quality controls for copper, mercury, lead, selenium and zinc in feathers, and for cadmium, copper and zinc in kidneys. Selenium in feathers was the only instance of tissue concentrations exceeding toxicity guidelines, and mercury in feathers was the only other element to occur at concentrations approaching such levels. Similar concentrations in feathers of Dunlin captured in the Dutch Wadden Sea corresponded to kidney concentrations ranging from 20-35 μg/g (Goede 1985) and Braune and Noble

(2009) reported mean kidney selenium concentrations in *pacifica* Dunlin as high as 16.7 μg/g. Studies on Mallards (*Anas platyrynchos*) and chickens describe kidney and liver concentrations above 22 μg/g as likely to reduce reproductive success (Ohlendorf and Heinz 2011). Thus, it seems likely that selenium levels in kidneys would have also been at levels associated with toxicity risks had they been analyzed. Mercury is deposited into feathers from internal tissues (Burger 1993) so kidney and liver levels will vary relative to those in feathers depending on moult stage.

Analyses of gizzard content cadmium, copper, zinc and energy concentrations permitted modeling of daily exposure across a range of diet types. In contrast to the lack of risk associated with these metals in tissues, some level of toxicity risk was predicted from metal exposure of all three metals in all diet types analyzed and high risk was predicted for a number of diet types including zinc and copper exposure at YVR and cadmium exposure for mud snail diets. Toxicity risks were universally greater for winter resident than for spring migrant Dunlin due to differences in energy requirements, but risk classifications were very similar for both groups. Differences in energy expenditure also contributed to higher exposure for estuarine foraging Dunlin relative to those using primarily terrestrial habitat.

Toxicity risks may be partially mitigated by the co-abundance of metals which reduces dietary absorption. Risk may also be reduced by Dunlin's tendency to supplement their diet with terrestrial prey that is relatively low in metal content. However, the dramatic difference in risk associated with element concentrations accumulated in kidneys and feathers (Chapter 2) and projected daily exposure levels (Chapter 3) necessitates further explanation. One potential explanation is that sampled birds were migrants from other locations where they were exposed to lower metal levels and FRD residents are exposed to adverse quantities of these metals. It is also possible that the collected birds did include local residents in which case either tissue concentration adverse effect thresholds should be set lower for Dunlin and similar species or, conversely, daily exposure thresholds are set too low for these birds. The latter explanation suggests that Dunlin can accommodate greater doses of metals relative to their body size than birds used to set toxicity thresholds. The fact that Dunlin inhabit environments that are generally higher in metals lends some rational support to this explanation, but it remains to be proven. High metabolism in sandpipers relative to

species of similar size may have also artificially inflated risk predictions considering that body size to consumption ratios have a large impact on risk in the exposure model used for the risk assessment.

Conservation Implications and Future Research Priorities

Evans Ogden et al. (2008) highlighted the importance of agricultural habitat in supporting Dunlin populations that winter and migrate through the FRD. The results of this study suggest that such terrestrial habitat also provides Dunlin with low cadmium diet items relative to estuarine habitat. Other shorebird species like the Black-bellied Plover (*Pluvialis squatarola*) and Western Sandpiper, also utilize both estuarine and terrestrial habitat in the FRD and stand to benefit from this aspect of agricultural habitat as well. Terrestrial exposure from Boundary Bay prey was also found to be low in copper and zinc relative to estuarine exposure. Manganese accumulated more in terrestrial foraging Dunlin but, again, use and availability of multiple habitat types mitigates toxicity risks.

Feather concentrations demonstrate that selenium and mercury approach adverse effect levels in Dunlin and other shorebirds, warranting further investigation of the impacts of these elements on sandpipers in the future. Although I found no direct evidence of deleterious levels of metals in kidneys, relatively high zinc levels in YVR food items and in tissues of some Dunlin using the site raise concern that foraging at the airport elevates risk of adverse zinc exposure to shorebirds. Due to obvious risks of collisions with aircraft, shorebirds are already actively discouraged from using YVR habitat by wildlife managers at the airport.

There is much to consider when assessing and interpreting toxicity risks. One issue, which became apparent in the dietary exposure study, is that variation in consumption to body mass ratios across species can greatly impact the levels of predicted risk. Additionally, exposure to toxicants in the wild often occurs under energetically demanding conditions whereas toxicity thresholds are generally determined by exposing organisms under conditions of moderate temperature and few energetic demands. Increases in consumption required to meet elevated energy budgets bring

proportional increases in exposure to any substance in food items. Consumption to body mass ratios and site-specific energy expenditure should be considered in any risk assessment involving such parameters.

Risk assessments based on tissue concentrations may avoid the complications of determining bioavailability, but they are still limited to comparisons across species and are usually restricted to comparisons to endpoints determined under laboratory conditions where important ecological pressures are not applied. Studies that monitor alternative endpoints in wild populations (e.g. migration success, reproductive success, predator evasion) in relation to metal exposure or tissue levels of metals can examine important sub-lethal effects that might not be observable using lab-based endpoints. Investigation of such sub-lethal effects would be particularly appropriate for mercury and selenium that approach and exceed toxicity thresholds in a number of sandpiper populations and can be analyzed non-invasively in feathers.

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