

**ASSESSMENT OF CONTAMINANT EXPOSURE, DIET,
AND POPULATION METRICS OF RIVER OTTERS (*Lontra
canadensis*) ALONG THE COAST OF SOUTHERN
VANCOUVER ISLAND**

by

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ABSTRACT

North American river otters (*Lontra canadensis*) are useful indicators of aquatic ecosystem health, but obtaining information on populations is difficult and expensive. By combining non-invasive faecal sampling with DNA genotyping techniques, I investigated: (i) environmental contaminant exposure, (ii) diet, and (iii) population metrics of river otters along the urban coast of southern Vancouver Island, British Columbia, Canada. In Victoria Harbour, mean faecal concentrations of polychlorinated biphenyls (PCBs) exceeded criteria thought to inhibit otter reproduction. However, individual faecal PCB concentrations varied with otter movement and landscape use. Intertidal fish dominated the diet, but otters in the regional harbours consumed proportionally more upper trophic-level fish than otters outside the harbours. Genetic and demographic analyses provided no conclusive evidence of PCB-induced population-level effects. Concurrent monitoring of contaminant exposure and population metrics of river otters is important to detect effects of pollutants on populations, and to guide river otter conservation and ecosystem management.

Keywords: river otter; *Lontra canadensis*; Georgia Basin; non-invasive sampling; faeces; contaminants; PCBs; diet; population genetics; mark-recapture

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CHAPTER 1
GENERAL INTRODUCTION

1.1 Environmental Pollution

Environmental pollution is one of the most serious ways in which humans can influence natural ecosystems. Since the early 1900s, tens of thousands of synthetic chemicals have been mass-produced and released into the environment (Moeller 2005), ultimately contaminating ground water, surface-water, and oceans worldwide. Many of these chemical contaminants are polyhalogenated aromatic hydrocarbons such as organochlorine (OC) pesticides, polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). These structurally related classes of compounds tend to be highly resistant to degradation and, hence, highly persistent in the environment. Because these compounds are also highly lipophilic (fat-soluble), these chemicals accumulate in tissues of animals and biomagnify as they move through the aquatic food web (Ruus et al. 2002; Borgå et al. 2004). Predatory species that feed at the top of the aquatic food chain are, therefore, most susceptible to their toxicological effects, including disruption of the endocrine, reproductive, and immune systems (Ritter et al. 1995). Chronic exposure to these contaminants may ultimately lead to population declines of wildlife species inhabiting contaminated areas (Ritter et al. 1995; Klassen and Watkins 2003).

Widespread use of OC-pesticides and PCB mixtures occurred for more than 40 years, until most were banned or severely restricted in nearly all industrial countries in the 1970s and 1980s (Lallas 2001). Additionally, the use of chlorine in the production of bleached pulp and paper was discontinued in most industrialized countries in the 1990s because of the formation of PCDD/F by-products during the standard chlorine-based

bleaching process (Weber et al. 2008). However, in spite of significant regulations on the production and use of these compounds, chemicals deposited in sediments from historic discharges, as well as industrial, municipal, and agricultural runoff can be a perpetual source of local contamination, particularly in aquatic ecosystems (Larsson 1985).

1.2 The Georgia Basin-Puget Sound Marine Ecosystem

The Georgia Basin-Puget Sound marine ecosystem is a large, complex, and productive estuarial inland sea located in the transboundary region between British Columbia, Canada, and Washington, USA. Nearly seven million people reside along the shores of this ecosystem, where both humans and a diverse array of wildlife share habitat and marine resources (Fraser et al. 2006). However, it is evident that this unique ecosystem is under considerable stress in its current state, with over 60 species listed as threatened, endangered, or candidates to be listed by one or more jurisdictions that share the waters of the Georgia Basin-Puget Sound marine ecosystem (Brown and Gaydos 2007). The impacts of these stressors are likely to increase with a human population expected to double over the next two decades (Fraser et al. 2006).

A primary factor in the decline of species in the Georgia Basin-Puget Sound marine ecosystem may be exposure to toxic chemical contaminants. Studies in the region have identified and quantified several polyhalogenated aromatic hydrocarbon contaminants including OC-pesticides, PCBs, and PCDD/Fs in blubber of harbour seals (*Phoca vitulina*) and killer whales (*Orcinus orca*); eggs of bald eagles (*Haliaeetus leucocephalus*), great blue herons (*Ardea herodias*), double-crested cormorants (*Phalacrocorax auritus*) and pelagic cormorants (*P. pelagicus*); and in fish and invertebrates (Elliott and Norstrom 1998; Ross et al. 2000; Elliott et al. 2001; West 2001;

Ikonomou et al. 2002; Harris et al. 2003; Ross et al. 2004; Harris et al. 2005). Exposure to these compounds has been associated with reproductive impairment, physiological effects, and immunotoxicity in several of these species (Casillas et al. 1991; Sanderson et al. 1994; Elliott et al. 1989; Elliott et al. 1996; Johnson et al. 1998; Mos et al. 2006; Tabuchi et al. 2006; Mos et al. 2007).

1.3 The River Otter as an Indicator Species

River otters are widely distributed in North America, extending across most of Canada and the United States, where they inhabit freshwater rivers and lakes, as well as nearshore marine environments of the Pacific and Atlantic Coasts (Melquist et al. 2003). Throughout their range, river otters feed near the top of the aquatic food web (Towiell 1974; Larsen 1984; Stenson et al. 1984; Reid et al. 1994; Ben-David et al. 2005), and can therefore accumulate high concentrations of persistent contaminants (Henny et al. 1981; Elliott et al. 1999). River otters are relatively long-lived, non-migratory, and do not hibernate (Melquist and Hornocker 1983; Larivière and Walton 1998). Consequently, individuals can be exposed continuously to local sources of pollution, making this mustelid a better indicator of localized environmental degradation than farther ranging avian and mammalian top-predators. Furthermore, home ranges of river otters in the marine environment are relatively small and seasonally constant (20-40 km linear shoreline, Bowyer et al. 2003), with individuals concentrating their movements and activities in the intertidal and subtidal zones (Blundell et al. 2001). This makes river otters well suited for studying the effects of point-source pollution at the scale of the nearshore ecosystem (i.e., closest to human development and coastal activities).

Among Mustelidae, semi-aquatic fish-eating species such as the mink (*Mustela vison*) and Eurasian otter (*Lutra lutra*) have been subjects of numerous toxicological studies. For example, several studies with ranched mink have shown that dioxin-like chemicals cause reproductive dysfunction and reduced offspring survival (Aulerich and Ringer 1977; Jensen et al. 1977; Kihlström et al. 1992; Heaton et al. 1995; Restum et al. 1998; Brunström et al. 2001). Jensen et al. (1977) calculated that reproductive failure in female mink occurs when tissue total-PCB concentrations exceed 50 mg/kg lipid weight. In field studies, declines of some North American mink populations have been tentatively linked to elevated environmental PCB concentrations (Henny et al. 1981, Addison et al. 1991, Henny et al. 1996).

Because mink and otter occupy similar ecological niches and have similar life-history traits (i.e., delayed implantation), a similar sensitivity of otters to the toxicological effects of PCBs has been postulated. Indeed, several authors have suggested that environmental contaminants (especially PCBs) constituted a major factor in the decline of the Eurasian otter (*Lutra lutra*) in Europe during 20th century (Olsson and Sandegren 1983; Mason 1989; Roos et al. 2001). This suggestion is based on correlations between PCB levels in otter tissues and the status of otter populations: high PCB levels in otters were associated with declining or endangered populations, while in thriving populations, PCB levels were low. Populations of river otters in North America were also reduced throughout much of their historic range by the mid-1900s. In addition to habitat destruction and unregulated trapping, aquatic pollution associated with urbanization and industrial activity is considered a major factor contributing to the decline of this species (Larivière and Walton 1998). Thus, river otters have been used throughout Canada and

the United States to monitor contaminants in the environment; including heavy metals, pesticides, petroleum hydrocarbons, and polyhalogenated aromatic hydrocarbons (Clark et al. 1981; Halbrook et al. 1981; Wren 1991; Duffy et al. 1994; Halbrook et al. 1994; Halbrook et al. 1996; Harding et al. 1998; Elliott et al. 1999; Harding et al. 1999; Ben-David et al. 2001a; Ben-David et al. 2001b, Bowyer et al. 2003).

1.4 Faeces as a Monitoring Tool

Monitoring the status of river otter populations is challenging because their life history makes them difficult to survey using conventional techniques. Consequently, no empirically derived estimates of population parameters are currently available for river otters in the Georgia Basin-Puget Sound marine ecosystem. Likewise, studies investigating river otter exposure to environmental pollutants in the region are rare, primarily due to the invasive nature of the sampling process (i.e., trapping and handling). The few studies that have examined river otter contaminant exposure in the region provide no information on population parameters or population status (Elliott et al. 1999; Harding et al. 1999; Grove and Henny 2008).

The inherent challenges associated with studying river otters may be overcome with the sampling of field-collected faeces. Within river otter populations, individuals use specific terrestrial defecation sites with a high degree of site fidelity (Testa et al. 1994). These communal 'latrines' are thought to function as scent marking stations, and thereby play an important role in the social organization of otter populations (Kruuk 1992; Rostain et al. 2004). Consequently, the continuous deposition of faeces at latrines makes sample collection relatively easy and non-invasive to otters. Benefits of non-invasive faecal sampling to study wild otters are three-fold. First, faeces can be used as an

efficient, non-destructive alternative to organ or tissue samples to investigate contaminant threats to otter populations (Mason et al. 1992; Gutleb and Kranz 1998; Van den Brink and Jansman 2006). Second, the identification of prey remains in faeces can provide a good indication of feeding habits in a particular area (Erlinge 1968; Melquist and Hornocker 1983; Larsen 1984; Stenson et al. 1984; Reid et al. 1994; Cote et al. 2008). Third, a researcher can target epithelial cells shed from a defecator's intestinal lining for DNA isolation and analysis (Hoss et al. 1992; Kohn et al. 1995; Reed et al. 1997). Individuals in a population can be distinguished based on their unique genotypes comprised of several microsatellite loci (regions of short-sequence repeats of non-coding nuclear DNA) that can be obtained by polymerase chain reaction (PCR) technology. Once a large sample of faeces is typed, the resulting 'DNA fingerprints' can be used in analyses of population genetics and demography (Dallas et al. 2003; Hung et al. 2004; Prigioni et al. 2006; Arrendal et al. 2007). Thus, field-collected faeces can be an effective means of studying many aspects of river otter ecology, without the need for individual capture.

1.5 Objectives and Contents of Thesis

In a broad regional analysis of contaminants in river otter faeces collected from harbour and industrial sites of the Georgia Basin, Elliott et al. (2008) reported that faeces from Victoria Harbour, British Columbia contained levels of PCBs that exceeded criteria for reproductive impairment developed for the Eurasian otter. Therefore, the impetus for this research was the need to establish a practical, accurate, and reliable method to census and monitor the contamination status of the river otter population potentially affected by elevated contaminant levels in the Victoria region. Because river otters are difficult to

study using direct monitoring techniques, my primary objectives were to use non-invasive faecal sampling to: (i) identify individual otters and investigate patterns of contaminant exposure, (ii) identify the principal prey species of river otters in the study area, and (iii) estimate the genetic diversity and population size of otters inhabiting the region.

My thesis follows the basic structure outlined by the primary objectives. The chapters are written in manuscript format and all are multi-authored reflecting the collaborative approach needed to span field ecology with toxicology and population biology using DNA genotyping techniques. In Chapter 2, we explore a novel approach to contaminant monitoring by combining faecal contaminant analysis with faecal DNA genotyping to investigate spatial and temporal trends in individual river otter exposure to environmental contaminants in the study area. In Chapter 3, we used prey remains recovered from faecal samples to describe the diets of coastal river otters inhabiting the study area, as well as investigate differences in river otter food habits as a result of foraging location. In Chapter 4, we used genetic profiles obtained from faecal DNA genotyping to investigate whether impacts of contaminant exposure in the harbours of southern Vancouver Island, British Columbia were discernable at the population-level. Finally, in Chapter 5, I provide an overall synthesis of the three components of my thesis and suggest possible directions for future research.

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CHAPTER 2
NON-INVASIVE FAECAL SAMPLING AND DNA
GENOTYPING REVEALS EXPOSURE OF INDIVIDUAL
RIVER OTTERS TO PERSISTENT CONTAMINANTS¹

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2.1 Abstract

We investigated contaminant concentrations in faeces of North American river otters (*Lontra canadensis*) inhabiting the urban coast of southern Vancouver Island, British Columbia, Canada. Specifically, we combined faecal DNA genotyping techniques with contaminant analyses to evaluate individual exposure to organochlorine (OC) pesticides and polychlorinated biphenyls (PCBs). Using this individual-based approach, we found that residue concentrations did not differ between winter and summer samples, but varied by location. Pesticide concentrations were generally low throughout the sampling area, whereas PCB concentrations in faeces from the two urban/industrial harbours of the region were significantly higher than in faeces from outside the harbours. Highest PCB concentrations were observed in Victoria Harbour, British Columbia (geometric mean 10.6 mg/kg lipid wt), which exceeded criteria thought to inhibit otter reproduction (9 mg/kg lipid wt). DNA genotyping also revealed that individual faecal PCB concentrations varied with movements and landscape use. Individual otters with the highest PCB concentrations were those inhabiting the inner harbours and those that ventured into the harbour systems. This study demonstrates the suitability of field-collected faeces as a non-invasive tool to assess contaminant threats to river otter populations, and to monitor spatial and temporal trends in individual contaminant exposure.

2.2 Introduction

Wildlife occupying high trophic levels in aquatic food webs are often exposed to industrial and agricultural chemical contaminants, many of which are persistent, bioaccumulative, and toxic. Contamination of the transboundary Georgia Basin-Puget Sound waters of southern British Columbia, Canada and northern Washington, USA is recognized as an international issue of environmental concern (Fraser et al. 2006). Previous studies have identified polyhalogenated aromatic hydrocarbon contaminants such as organochlorine (OC) pesticides, polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) in several top avian and marine mammal predators inhabiting this region, including bald eagles (*Haliaeetus leucocephalus*), great blue herons (*Ardea herodias*), harbour seals (*Phoca vitulina*), and killer whales (*Orcinus orca*) (Elliott and Norstrom 1998; Ross et al. 2000; Elliott et al. 2001; Ross et al. 2004). Evidence suggests that elevated contaminant concentrations have caused reproductive impairment, physiological effects, and immunotoxicity in these species (Sanderson et al. 1994; Elliott et al. 1989; Elliott et al. 1996; Mos et al. 2006; Tabuchi et al. 2006; Mos et al. 2007).

Although not considered true marine mammals, North American river otters (*Lontra canadensis*) are residents along the coast of the Georgia Basin-Puget Sound, and have many traits that make them a useful indicator of nearshore environmental contamination. For instance, river otters have a home range that is relatively small and seasonally constant (Bowyer et al. 2003), they neither migrate nor hibernate (Larivière and Walton 1998), and they primarily feed on intertidal and subtidal fish (Stenson et al.

1984) (see Chapter 3). Consequently, individuals can be exposed continuously to localized sources of pollution.

Although there are no published studies of polyhalogenated aromatic hydrocarbon toxicity to river otters, the mink (*Mustela vison*), another aquatic mustelid that inhabits a similar ecological niche and has similar life-history traits (i.e., delayed implantation) provides a reasonable surrogate. Mink are very sensitive to the toxic effects of dioxin-like chemicals, which adversely impacts the female reproductive system and offspring survival (Aulerich and Ringer 1977; Jensen et al. 1977; Kihlström et al. 1992; Heaton et al. 1995; Restum et al. 1998; Brunström et al. 2001). Several authors postulate a similar sensitivity of otter to dioxin-like chemicals, and it has been suggested that PCB contamination constituted a major factor in the decline of the Eurasian otter (*Lutra lutra*) in Europe during the 20th century (Olsson and Sandegren 1983; Mason 1989; Roos et al. 2001). This suggestion is based on correlations between PCB levels in otter tissues and the status of otter populations: high PCB levels in otters were associated with declining or endangered populations, while in thriving populations, PCB levels were low. Populations of river otters in North America were also reduced throughout much of their historic range by the mid-1900s. In addition to habitat destruction and unregulated trapping, aquatic pollution associated with urbanization and industrial activity is considered a major factor contributing to the decline of this species (Larivière and Walton 1998). Diminished levels of pollution coupled with habitat remediation and otter reintroductions have allowed for re-expansion of both Eurasian otters and North American river otters in recent years (Raesly 2001; Mason and Macdonald 2004). However, monitoring the

contamination status of wild otter populations is challenging due to their elusive nature and the difficulties associated with capture and sampling.

As an alternative to capturing individual animals for toxicant testing, studies have shown that field-collected faeces can be an efficient means of investigating contaminant exposure in mustelids (Mason et al. 1992; Mason and Macdonald 1993b; Henny et al. 1996; Gutleb and Kranz 1998; Van den Brink and Jansman 2006; Zwiernik et al. 2008). Using that approach, we previously showed that faeces collected from river otter communal marking sites (latrines) in Victoria Harbour, British Columbia had concentrations of PCBs exceeding the criteria for reproductive dysfunction developed for the Eurasian otter (Elliott et al. 2008). However, in our earlier field-based study, and those of others, multiple faecal samples from a particular latrine were combined into one composite sample, and as a result, information on individual animals was lost.

The objective of the present study was to investigate the utility of field-collected faeces as a tool for non-invasive biological monitoring of polyhalogenated aromatic hydrocarbon contaminant exposure of individual river otters. Recent developments in molecular techniques now permit researchers to obtain genomic DNA from field-collected faecal samples, thus providing a method of sampling individual animals without the need for capture and restraint (Kohn and Wayne 1997). By genotyping a battery of hypervariable microsatellite loci (regions of short-sequence repeats of non-coding nuclear DNA), a 'genetic fingerprint' can be obtained that can be used to identify unique individual animals. We postulated that field-collected faecal samples could be assigned to individuals through DNA genotyping, and those samples could subsequently be analyzed for contaminant residue concentrations, an approach which could reveal intra- and inter-

individual variation in contaminant exposure. With that technique, many otters could be sampled several times across the landscape. To explore that approach, we further investigated the population of river otters in the Victoria region of British Columbia. Specifically, we examined seasonal and site variation in faecal contaminant concentrations at the population-level, controlling for inter-individual variation. We then combined non-invasive faecal contaminant analysis with faecal DNA genotyping techniques in an effort to evaluate inter- and intra-individual river otter variability in faecal contaminant concentrations over space and time. We hypothesized that otters sampled within the two urban/industrial harbours would have higher concentrations of contaminants in their faeces than otters sampled outside the harbour systems, and that individual otter faecal contaminant concentrations would vary with movement between contaminated and uncontaminated areas.

2.3 Methods

2.3.1 Study area

The study area was located on southern Vancouver Island, British Columbia, Canada (48°25' N, 123°21' W) (Figure 2.1). The urban/industrial harbours of Victoria and Esquimalt, British Columbia were centrally located within the study area. These harbours support (or have supported) shipbuilding, fishing, and forestry industries, as well as other commercial and tourism activities, and have done so for over 75 years. In addition, Esquimalt Harbour is the home to the Canadian Pacific naval fleet. Polyhalogenated aromatic hydrocarbon contamination has been documented in both harbours (Ikonomou et al. 2002; Knapp and Grant 2008). We limited the extent of river otter faecal collection to approximately 40 km east and west of the harbours based on estimates of coastal river

otter linear home range length (20-40 km, Bowyer et al. 2003). We assumed that all otters inhabiting that section of coastline were capable of visiting the harbours.

2.3.2 Sample collection

We conducted a preliminary latrine survey and sampling session along a limited section of coastline from December 13-18, 2005. During that initial survey, we opportunistically collected fresh faeces from latrines and stored individual samples in sterile vials filled with 100% ethanol (EtOH). The main study was conducted during June-August 2006 (for a detailed description of collection methods, see Chapter 4). We collected both faecal material and gelatinous ‘anal jellies’ (a mucus-like deposit expelled through the rectum, which are different from the secretions expelled through the sub-caudal glands) (Kruuk 2006). From hereafter, the term ‘faeces’ refers to both faecal material and anal jellies unless otherwise noted. Prior to collection, we divided each deposit in two: one half of each sample was placed in a sterile vial filled with 100% ethanol (EtOH) for DNA genotyping, while the second half was collected in chemically cleaned jars for chemical residue analyses. We stored samples in EtOH at 4°C before shipping to the University of Wyoming, USA for DNA analyses. The remaining portion was stored at –20°C before shipping to the University of Windsor, Ontario, Canada for chemical analyses.

2.3.3 Individual identification

We collected a total of 893 fresh (< 24 hours old) river otter faeces in winter 2005 and summer 2006. A set of eight polymorphic microsatellite loci were used to genotype each faecal sample, including three tetranucleotide markers (*Rio-19*, *Rio-17*, *Rio-01*) and

one trinucleotide marker (*Rio-05*) developed for the North American river otter (Beheler et al. 2004; Beheler et al. 2005), and four tetranucleotide markers (*Lut-829*, *Lut-733*, *Lut-701*, *Lut-801*) developed for the Eurasian otter (Dallas and Piertney 1998). For complete molecular methods, see Chapter 4. We successfully genotyped 110 of 893 samples (12.3%) at seven or eight loci, resulting in 49 unique individuals (see Appendix 4). We assume our data are accurate and reliable based on a low probability of obtaining an incorrect multilocus genotype after replication at all eight microsatellite loci (0.009). Additionally, the probability that two random individuals share identical genotypes at all typed loci (P_{ID}) was 2.35×10^{-06} . Thus, on average, in a population of 425,532, no two individuals are likely to share the same multilocus genetic profile.

Between one and seven faecal samples per otter were available for chemical residue analysis. A subset of 65 samples representing 23 individuals was used for subsequent chemical analysis. Of those, 45 (69%) were anal jellies, 16 (25%) were faeces, and four (6%) contained a mixture of both faecal and jelly components. We previously analyzed the distinct faecal and jelly fraction of single mixed samples and reported no difference between fractions in total-PCBs and total-OC pesticides concentrations on a lipid weight basis (Elliott et al. 2008). Those samples were collected from latrine sites in the Victoria region during the same time as this study. Therefore, we assume that contaminant concentrations in both sample types are equivalent and comparable in this analysis.

2.3.4 Contaminant residue analysis

River otter faecal samples were analyzed at the Great Lakes Institute of Environmental Research (GLIER), University of Windsor, Ontario, Canada. This

laboratory is accredited for the analysis of OC-pesticides and PCBs in biological samples under the Canadian Association for Laboratory Accreditation (CALA), which adheres to ISO17025 protocols and requires inter-laboratory proficiency testing as well as mandated quality assurance and quality control procedures. Analysis of faecal samples included determination of chlorobenzenes (1,2,4,5-tetrachlorobenzene, 1,2,3,4-tetrachlorobenzene, pentachlorobenzene, and hexachlorobenzene), hexachlorocyclohexanes (α -, β -, and γ -hexachlorocyclohexane), chlordane-related compounds (oxychlordane, *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor, *cis*-nonachlor), dichlorodiphenyltrichloroethane (DDT) and its metabolites (*p,p'*-DDE, *p,p'*-DDD and *p,p'*-DDT), mirex, and for the following PCB congeners identified according to IUPAC numbers: 17/18, 28/31, 33, 44, 49, 52, 70, 74, 82, 87, 95, 99, 101, 105/132, 110, 118, 128, 138, 149, 153, 156/171, 158, 170, 177, 180, 183, 187, 191, 194, 195, 201, 205, 206, 208, and 209). For brevity, the term Σ pesticides is the concentration sum of all pesticides analyzed. Likewise, the term Σ PCBs is the concentration sum of all PCB congeners analyzed.

Chemical extraction and cleanup of pesticides and PCBs followed the procedures of Lazar et al. (1992). Briefly, 1-2 g of sample homogenates were ground and spiked with 1,3,5-tribromobenzene as a surrogate recovery standard and extracted by solid/liquid chromatography using 350 mL of dichloromethane:hexane (50:50% v/v, OmniSolve-Grade, VWR, Ontario, Canada). Extracts were reduced to 10 mL using a rotary evaporator. Ten percent of the extracts (1 mL) were removed to determine neutral lipid content by gravimetric methods (Drouillard et al. 2004). Cleanup of the sample was performed by gel permeation chromatography followed by activated Florisil (VWR, Ontario, Canada) chromatography as described in (Lazar et al. 1992), with the exception

that only fraction one (50 mL hexanes) and fraction two (50 mL hexane:DCM, 85:15% v/v) were collected from Florisil columns and concentrated for chemical analysis.

Chemical analysis was performed using a Hewlett-Packard 5890 gas chromatograph with a ^{63}Ni -electron capture detector (GC-ECD) and 7673 autosampler. The column was a 60 m \times 0.250 mm \times 0.1 μm DB-5 (Chromatographic Specialties, Brockville, Ontario, Canada) with helium as the carrier gas and Ar/Ch₃ (95%, 5%) as the make-up gas. The oven program was initialized at 90°C and held for 0.5 min, ramped at 10°C/min to a temperature of 200°C and then ramped at 2.5°C to 275°C with a final hold time of two minutes. Individual pesticides and PCB congeners were identified by retention time and quantified using a single point concentration in an external standard injected with each batch of samples. Sample responses were checked to ensure that peak areas were within the linear range of the instrument as evaluated on the same instrument using a seven-point standard calibration curve performed on monthly intervals. For every batch of five or six samples injected, the surrogate standard, PCB standard mixture (Quebec Ministry of Environment PCB mixture; Chromatographic Specialties Brockville, Ontario, Canada), organochlorine standard mixture (certified OC-pesticide mixture from AccuStandards; Chromatographic Specialties, Brockville, Ontario, Canada), method blank, and in-house reference sample (Detroit River carp (*Cyprinus carpio*) homogenate) were analyzed. Detection limits ranged from 0.01 to 0.05 ng/g wet weight depending on the chemical of study. Individual analyte recoveries in the in-house reference sample were checked against quality control charts generated by the laboratory. All analytes in the in-house homogenates were within two standard deviations of the quality control chart mean and considered acceptable with regard to analytical precision. Blanks,

quantified during each batch of sample extractions, were in compliance with the normal quality assurance procedures instituted by GLIER. Sample recoveries for the surrogate standard averaged $92 \pm 1\%$ (mean \pm SE). Chemical concentrations were not recovery corrected. Results were initially expressed on a wet weight basis and later converted to a lipid weight basis (i.e., reported as mg/kg lipid wt).

2.3.5 Data analysis

We performed all statistical analyses using SAS 9.0 or JMP 7.0 (SAS Institute Inc., Cary, North Carolina, USA). We log-transformed data to approximate normal distributions prior to statistical evaluation. Where concentrations were below the detection limit, a value equal to one-half the detection limit of that compound was applied to permit statistical analyses. To avoid pseudoreplication, contaminant residue concentrations measured in faeces from the same otter at the same site on the same day were averaged and considered a single observation. Because we were interested in differences between otter contaminant exposure inside and outside the two urban/industrial harbours of the region, we grouped individual otter faecal samples according to collection location (e.g., Victoria Harbour, Esquimalt Harbour (including Esquimalt Lagoon), and non-harbour). Pesticides and Σ PCBs are expressed as geometric means to remove disproportionate effects of outlying values. We analyzed contaminant data using general linear mixed models (PROC MIXED) in SAS with location (Victoria Harbour, Esquimalt Harbour, and non-harbour) and season (winter or summer) as fixed effects and individual otter as a random effect. Our model, therefore, tested the effects of locality and season on the mean residue concentrations, while accounting for multiple observations per otter. Statistical significance was set at $P < 0.05$ in all analyses. Post-hoc

tests for differences between means were corrected for multiple comparisons using Tukey-Kramer adjustment. An insufficient number of faeces collected during the winter (Victoria Harbour: $n = 2$, Esquimalt Harbour: $n = 2$, non-harbour: $n = 6$) precluded further examination of whether the interaction between season and location had an effect on faecal contaminant concentrations.

A principal components analysis was used to evaluate PCB congener pattern differences among the three collection locations. For this analysis, the proportion of each PCB congener was calculated relative to the total PCB concentration. Proportion data were arcsine transformed. Multiple samples from the same individual identified in the same location were averaged prior to analysis. Factor loadings were computed from the correlation matrix following Varimax rotation. The individual factor scores were calculated and plotted in multivariate space.

To assess the toxicological significance of contaminant concentrations in faeces, we compared our results to the hierarchy of effect levels proposed by Mason and Macdonald (1993b):

Critical level: concentrations of total PCBs > 16 mg/kg lipid weight, or concentrations of total organochlorines (OCs) > 20 mg/kg lipid weight;

Levels of concern: total PCBs 9-16 mg/kg lipid weight, or total OCs 16-20 mg/kg lipid weight (conservative critical level);

Maximum allowable concentration: concentrations less than level of concern but greater than no effect level;

No effect level: less than 4 mg/kg lipid weight for all individual contaminants as described above.

Their approach was based on the back-calculation of a single compartment bioaccumulation model relating faecal PCB concentrations to tissue concentrations

(assuming contaminant levels in the faeces to depend largely on the concentrations in recently ingested prey) (Mason et al. 1992, Mason and Macdonald 1993b). According to the model, the threshold value of 16 mg/kg lipid weight Σ PCBs in the faeces is equivalent to 50 mg/kg lipid weight tissue, the level generally assumed to be the critical level for the otter based on reproductive toxicity in mink (Jensen et al. 1977; Mason 1989); Σ PCBs of 9 mg/kg lipid weight faeces is equivalent to 30 mg/kg lipid weight tissue, considered by some to be a more realistic critical level for otter based on data from a declining otter population in Sweden with a geometric mean of 30 mg/kg lipid weight tissue (Mason et al. 1992); and Σ PCBs of 4 mg/kg lipid weight faeces is equivalent to 13 mg/kg lipid weight tissue, the geometric mean in a thriving Eurasian otter population in Norway (Mason et al. 1992). To be conservative, we chose to use the ‘level of concern’ as the critical level in this study. This approach assumes a conserved nature of toxicity between mink and otter and is subject to uncertainty including interspecies differences in sensitivity to dioxin-like compounds, as well as differences between effects observed in a laboratory setting compared to those encountered in the field. Nevertheless, these criteria provide a basis to assess the toxicological significance of contaminant levels in river otter faeces and are valuable for assessing potential risks to natural otter populations.

2.4 Results

2.4.1 Contaminant residues in faeces

All samples from all areas contained detectable amounts of pesticides and PCB congeners. We found no significant seasonal differences in geometric mean concentrations of any individual pesticide, Σ pesticides ($F = 0.84$, $P > 0.05$), or Σ PCBs ($F = 1.05$, $P > 0.05$) throughout the study area (Table 2.1). There was a significant inter-

location difference in geometric mean concentrations of Σ pesticides ($F = 3.97$, $P < 0.05$), with samples from Victoria Harbour having significantly higher concentrations of DDT metabolites and chlordane compounds compared with non-harbour sites only (Table 2.1). Nevertheless, mean concentrations of all pesticides were low in all locations. In contrast, concentrations of PCBs were substantially higher and dominated among the compounds quantified in all areas (Table 2.1).

There were significant inter-location differences in geometric mean concentrations of Σ PCBs ($F = 27.57$, $P < 0.05$) with samples from Victoria Harbour higher than those from both Esquimalt Harbour and non-harbour sites (Table 2.1). Geometric mean concentrations of Σ PCBs in faeces collected from Esquimalt Harbour were also significantly higher than faeces collected from non-harbour sites (Table 2.1). In Victoria Harbour, geometric mean faecal concentrations of Σ PCBs (10.6 mg/kg lipid wt) exceeded criteria thought to inhibit otter reproduction (9 mg/kg lipid wt). Congener-specific PCB profiles for each sampling location (percent contribution to total PCBs) are shown in Figure 2.2. The overall patterns are similar among sampling locations. Congener 153 was the most abundant in all sampling locations, followed by congeners 138 and 180. Together, these three congeners constituted 66%, 64%, and 68% of the Σ PCBs measured in Victoria Harbour, Esquimalt Harbour, and non-harbour samples, respectively. Principal component analysis of congener proportion data showed no discernable differences in PCB congener patterns among sampling locations (Figure 2.3).

Table 2.2 lists the percentage of samples with contaminant concentrations in each of the four effect levels. Victoria Harbour had greater than 20% of samples in effect level one (critical) and greater than 30% of samples in effect level two (conservative critical).

In total, 94% of samples collected from Victoria Harbour had contaminant concentrations above the no effect level. In contrast, the majority of samples collected from Esquimalt Harbour and non-harbour sites had samples in effect level four (no effect), although the number of samples collected from Esquimalt Harbour was relatively low ($n = 9$), with only four samples from inner Esquimalt Harbour. Non-harbour sites had no samples in level one (critical) and only one sample (3%) in level two (conservative critical).

2.4.2 Individual otter PCB exposure

The use of DNA genotyping permitted an evaluation of inter- and intra-individual variability in faecal PCB concentrations across the landscape. Five otters (No. 11, 18, 19, 20, and 23) were identified exclusively in Victoria Harbour. Although faecal samples from those five individuals showed some spatial and temporal variability in Σ PCB concentrations, all but one sample exceeded the no effect level of 4 mg/kg lipid weight (Figure 2.4, Figure 2.5a-d). Nine of the 14 total samples from these five otters (64%) were above the critical level of 9 mg/kg lipid weight. Some individual values were much higher than this threshold. For example, faeces collected from one individual (No. 11) identified on consecutive days at the same latrine in the middle of Victoria Harbour contained Σ PCB levels of 18.7 and 38.2 mg/kg lipid weight, respectively (Figure 2.4, Figure 2.5a).

Three otters (No. 8, 10, and 17) initially sampled at latrines outside Victoria Harbour were re-sampled at latrines inside the harbour. Σ PCBs in the faeces of No. 8 and 10 changed from below the no effect level when sampled outside the harbour to between no effect and critical (4-9 mg/kg lipid wt) inside Victoria harbour (Figure 2.4, Figure 2.5c-d). Otter No. 8 moved from Esquimalt Lagoon to Victoria Harbour and faecal Σ PCB

concentrations increased by 220% (2.5-8.0 mg/kg lipid wt). After leaving Victoria Harbour, Σ PCBs decreased by 76.3% and remained low. Otter No. 10 moved from the outer coast of Victoria to inside Victoria Harbour, and faecal Σ PCB concentrations increased by 3,450% (0.2-7.1 mg/kg lipid wt). In contrast, No. 17 was initially sampled in Esquimalt Harbour with faecal Σ PCB concentrations above the critical level. Two days later, that otter was re-sampled inside Victoria Harbour and Σ PCBs remained elevated. Nearly two months later, No. 17 was re-sampled at the same Victoria Harbour latrine and Σ PCBs remained elevated above the no effect level (Figure 2.5b).

Most faecal samples collected from otters in Esquimalt Harbour and Esquimalt Lagoon had faecal Σ PCB concentrations below the no effect level, with the exception of one sample collected from No. 22, and the previously mentioned sample from No. 17 (Figure 2.5b,c,e). Although we included samples from Esquimalt Lagoon in Esquimalt Harbour for statistical analysis, all samples collected from otters inside inner Esquimalt Harbour had higher PCB concentrations than otters in Esquimalt Lagoon, as well as most otters outside the harbours. However, otters identified in Esquimalt Lagoon also tended to have higher PCB concentrations in their faeces than otters identified at non-harbour latrines. For example, two otters (No. 8 and 34) identified in Esquimalt Lagoon and subsequently re-sampled at latrines further southwest showed a decrease in faecal PCB concentrations.

The majority of otters identified outside Victoria and Esquimalt Harbours had levels of Σ PCBs in their faeces below the no effect level regardless of individual otter, collection location, or time between sample collection, with the notable exception of No. 24 (Figure 2.5b,e-h). The first sample collected from No. 24 at Ogden Point (the closest

non-harbour latrine to Victoria Harbour) had a Σ PCB concentration that exceeded the critical threshold (14.9 mg/kg lipid wt). A second sample collected 0.78 km east had a 50.3% reduction in Σ PCBs to below the critical level, but above the no effect level (7.4 mg/kg lipid wt). In two successive samples collected at a latrine 0.75 km further eastward, Σ PCBs decreased by an additional 56.8% to below the no effect level. Of the remaining otters, one sample obtained from No. 27 also had a Σ PCB concentration that exceeded the no effect level (6.0 mg/kg lipid wt); although two additional samples collected from that animal in the same area did not have elevated PCBs (Figure 2.5h).

2.5 Discussion

Earlier studies that employed faeces to measure contaminant exposure of wild otters pooled multiple samples from a single latrine site for analysis (Smit et al. 1994; Elliott et al. 2008). Pooling of samples is cost-effective and may be generally useful in monitoring contaminant exposure of natural otter populations, but may also result in selection bias due to differences in faecal contaminant concentrations among otters visiting communal latrines. To account for individual variation in contaminant exposure, we included information on otter identity in our analysis as determined by the DNA genotyping of faeces. This individual-based analysis revealed no distinct seasonal variation in contaminant concentrations in faeces. However, contaminant concentrations in otter faeces varied spatially, with otters in Victoria Harbour having the highest concentrations of nearly all compounds measured. Among the compounds analyzed, we found concentrations of Σ PCBs to be highest in river otter faeces at all locations, with concentrations in Victoria Harbour > Esquimalt Harbour > non-harbour sites. In a long-term monitoring study of PCBs in Dungeness crab (*Cancer magister*) and red rock crab

(*Cancer productus*), Knapp and Grant (2008) also report higher Σ PCB concentrations in Victoria Harbour compared to Esquimalt Harbour. When compared to other harbour and industrial sites in coastal British Columbia, Ikonomou et al. (2002) found Victoria Harbour crabs to have the highest levels of Σ PCBs of all sites sampled. In contrast, pesticide concentrations were generally low throughout the study area, which reflect low regional use of those chemicals as previously reported (Elliott et al. 2008). Low levels of pesticides and higher levels of PCBs have also been documented in the eggs of piscivorous birds from southern Vancouver Island, including great blue herons (Harris et al. 2003) and double-crested cormorants (Harris et al. 2005).

The composition of Σ PCBs was dominated by higher chlorinated congeners at all sampling locations, especially PCB-138, -153, and -180. Those congeners are resistant to metabolism due to the position of chlorine atoms around the benzene ring, and are known to bioaccumulate in marine food chains (Kelly et al. 2007). The overall congener patterns observed in our analysis are very similar among sampling locations and similar to the patterns reported in river otter faecal samples collected from harbour and industrial sites in the Strait of Georgia in 1998 and 2004, including Victoria Harbour and Esquimalt Harbour (Elliott et al. 2008). Similar PCB congener patterns have been reported in Eurasian otter faeces (Van den Brink and Jansman 2006; Lemarchand et al. 2007).

Threshold faecal PCB concentrations for possible adverse effects on otters are based on extrapolations of toxicological data obtained from studies of ranched mink (Mason et al. 1992, Mason and Macdonald 1993b). In the current study, mean Σ PCB concentrations in individual river otter faeces from Esquimalt Harbour (2.92 mg/kg lipid wt) are below the proposed effect level of 4 mg/kg lipid weight, which is similar to

sampling data from 1998 (2.11 mg/kg lipid wt) (Elliott et al. 2008). In contrast, mean Σ PCB concentrations in faecal samples from Victoria Harbour (10.6 mg/kg lipid wt) are above the conservative critical level (9 mg/kg lipid wt) for reproductive toxicity. Previously, we reported that mean Σ PCB concentrations in pooled river otter faecal samples from Victoria Harbour in 1998 (12.3 mg/kg lipid wt) and 2004 (9.3 mg/kg lipid wt) also exceeded that threshold (Elliott et al. 2008). Those results collectively indicate consistent long-term exposure of otters to PCBs in Victoria Harbour, and that otters using Victoria Harbour may be accumulating sufficient PCBs to pose a significant hazard to the local population.

Some caution is warranted, however, when extrapolating from faeces to body burden levels, since many biological factors (e.g., age, sex, body condition) can contribute to substantial differences in PCB levels in individual otters (Smit et al. 1996). In addition, the river otter's diet in marine coastal habitats is diverse (Stenson et al. 1984) and faecal samples may vary in chemical residue concentrations due to stochastic variation of concentrations in prey items, prey choice, and the amount of prey consumed. In fact, we documented that otters foraging in the urban/industrial harbours consumed greater proportion of higher trophic level prey compared to outside the harbour systems (see Chapter 3). However, the primary prey items consumed by otters throughout the study area were similar, and it is therefore unclear to what extent differences in diet and trophic level exposure contributes to contaminant concentrations observed in faeces. A detailed investigation of contaminant concentrations in the main prey items may help to elucidate possible food web trophic relationships and location effects on faecal PCB concentrations.

Nevertheless, data from known individuals as revealed by DNA genotyping provides valuable insights into spatial distribution and variance that would otherwise be undetectable with the use of latrine composites. For example, in Victoria Harbour, the geometric mean Σ PCB concentration exceeded the proposed critical threshold of 9 mg/kg lipid weight in both 1998 and 2004, but composites from just two of four latrines sampled in 1998 and three of seven latrines in 2004 actually surpassed that level (Elliott et al. 2008). In the current study, however, we analyzed individual samples from eight known otters at four Victoria Harbour latrines and found concentrations of Σ PCBs in faeces that surpassed the critical level at each latrine. Thus, while pooled samples from some latrines in Victoria Harbour might be well below the critical value, individuals visiting those latrines could still be at risk from the effects of PCBs. Additionally, in Esquimalt Harbour, composite samples from each of the four latrines visited in 1998 were below the effect level of 4 mg/kg lipid weight, resulting in a low overall geometric mean (Elliott et al. 2008). However, in the current study, a closer examination of faeces from individuals within inner Esquimalt Harbour revealed that otters identified at latrines closest to industrial shoreline activity (i.e., No. 17 at Yew Point = 22.3 mg/kg lipid wt; and No. 22 at Ashe Head = 7.6 mg/kg lipid wt) contained concentrations of Σ PCBs in their faeces above the no effect level. Thus, a proportion of the population in that area could still be at risk from the effects of PCBs.

By employing DNA genotyping, some aspects of contaminant exposure associated with otter movements could also be inferred from the collection location of each unique individual's faeces. For example, individual otters sampled multiple times in Victoria Harbour yielded consistently elevated faecal PCB concentrations. Those results

imply that harbour residents will more frequently encounter localized contamination, likely increasing the probability that the critical exposure level is exceeded. In addition, some individuals moved from outside Victoria Harbour to inside (and vice versa). For those individuals, faecal PCB concentrations either increased or decreased with collection location, indicating that a contaminated area might occur in just a portion of an otter's home range. Thus, while the elevated PCB contamination appears to be spatially restricted to the harbours of southern Vancouver Island, the potential for adverse effects on individuals extends beyond the harbour boundaries as a result of individual movements and landscape use. Analyzing samples from known individuals can therefore generate spatio-temporal insights into contaminant exposure that cannot be obtained from analyzing latrine composites.

The pattern of contaminant concentrations in the faeces of individual river otters in the study area begs the question of what, if any, are the population-level effects due to elevated PCB exposure in the harbours of the region. PCB concentrations in faeces collected from the harbours (particularly Victoria Harbour) are comparable to faecal PCB levels reported for endangered or declining Eurasian otter populations inhabiting areas of high human industrial activity (Macdonald and Mason 1988; Mason et al. 1992; Mason and Macdonald 1993a; Mason and Macdonald 1993b; Mason and Madsen 1993). Therefore, we might expect that the PCB-contaminated harbours serve as a functionally suboptimal habitat that results in an ecological 'sink' for river otters (Pulliam 1988; Dias 1996; Delibes et al. 2001). In such a case, the presence of otters would likely be maintained by immigrants from unpolluted habitats. However, additional population-based information obtained from the same faecal DNA dataset (e.g., migration rates and

pairwise relatedness) indicates local reproduction and persistence of otters in the PCB-contaminated areas of the study region (see Chapter 4), thereby supporting the claim that otter populations are able to persist in PCB contaminated environments (Kruuk and Conroy 1996). Nevertheless, PCB concentrations may reach levels causing decreased reproduction or survival of some individuals (particularly in the urban/industrial harbours), and some genetic and demographic observations in that region (e.g., genetic diversity and population density) are also consistent with the hypothesis of contaminant-related effects (see Chapter 4). Hence, it remains unclear to what extent PCB exposure affects population genetic and demographic processes in the region.

In summary, our results illustrate the advantages of individual-based non-invasive faecal sampling techniques in wildlife studies, and underscore the value of this technique across scientific disciplines. We used a previously unexplored combination of faecal DNA genotyping and contaminant analyses that allowed us to assign faeces to individual otters in the study population. Using this combined approach, we showed significant variation in inter- and intra-individual otter exposure to PCB contamination that was not apparent from previous pooling of samples. All individuals identified inside Victoria Harbour produced faeces with elevated PCB concentrations, resulting in an overall geometric mean concentration that exceeded criteria for possible reproductive effects developed for the Eurasian otter. In addition, despite low overall geometric mean PCB levels in Esquimalt Harbour and outside the harbours, some individuals identified in close proximity to industrial shoreline activities produced faeces with elevated PCB concentrations. Future studies using river otters as bioindicators of ecosystem health are expected to emerge for monitoring anthropogenic impacts on aquatic systems,

particularly in areas where river otters have been reintroduced or restocked (Raesly 2001). The comprehensive approach to contaminant monitoring described here allows for a robust assessment of site-contamination, and provides a non-invasive tool for long-term monitoring of populations and individuals, without the need for animal capture and restraint.

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Table 2.1 Geometric mean (range) of select polyhalogenated aromatic hydrocarbon contaminants (mg/kg lipid wt) in faeces from river otters collected along the coast of southern Vancouver Island, British Columbia, Canada (2005-2006) by season and location.

	<i>N</i>	ΣDDT	ΣCIBz	ΣCHLOR	ΣHCH	Mirex	Σpesticides	ΣPCBs
Season								
Winter 2005	10	0.13 ^a (0.01-0.93)	0.14 ^a (0.03-0.63)	0.11 ^a (0.02-0.35)	0.04 ^a (0.01-0.16)	0.03 ^a (0.002-0.14)	0.45 ^a (0.17-1.41)	6.26 ^a (0.24-38.2)
Summer 2006	52	0.14 ^a (0.01-1.45)	0.10 ^a (0.02-0.66)	0.14 ^a (0.03-0.67)	0.05 ^a (0.01-0.48)	0.03 ^a (0.001-0.26)	0.45 ^a (0.08-2.38)	5.17 ^a (0.17-22.2)
Location								
Victoria Harbour	18	0.13 ^a (0.04-0.93)	0.07 ^a (0.02-0.19)	0.19 ^a (0.04-0.35)	0.04 ^a (0.01-0.15)	0.01 ^a (0.002-0.26)	0.51 ^a (0.18-1.41)	10.6 ^a (2.47-38.2)
Esquimalt Harbour	9	0.04 ^{ab} (0.01-0.30)	0.11 ^a (0.03-0.63)	0.08 ^{ab} (0.03-0.25)	0.04 ^a (0.01-0.16)	0.01 ^a (0.001-0.14)	0.32 ^{ab} (0.12-1.13)	2.92 ^b (0.31-22.2)
Non-harbour	35	0.05 ^b (0.01-1.45)	0.07 ^a (0.02-0.66)	0.07 ^b (0.02-0.67)	0.03 ^a (0.01-0.48)	0.01 ^a (0.001-0.17)	0.27 ^b (0.08-2.38)	0.92 ^c (0.17-14.9)

General linear mixed model. Means within a column sharing the same letters are not significantly different at $P < 0.05$ with the Tukey-Kramer adjustment for multiple comparisons.

ΣDDT = *p,p'*-DDE, *p,p'*-DDT, and *p,p'*-DDD.

ΣCIBz = chlorobenzenes (1,2,3,5-tetrachlorobenzene, 1,2,3,4-tetrachlorobenzene, pentachlorobenzene, hexachlorobenzene).

ΣCHLOR = chlordanes (oxychlordanes, *trans*-chlordanes, *cis*-chlordanes, *trans*-nonachlor, and *cis*-nonachlor).

ΣHCH = hexachlorocyclohexanes (α -, β -, and γ -hexachlorocyclohexanes).

Table 2.2 Percentage of river otter faeces collected from the coast of southern Vancouver Island, British Columbia, Canada (2005-2006) in the four effect level categories for organochlorine contaminants: (i) critical; (ii) concern (conservative critical); (iii) maximum allowable; and (iv) no-effect. See text for definition of levels.

Location	<i>n</i>	Effect level			
		(i)	(ii)	(iii)	(iv)
Victoria Harbour	18	22	33	39	6
Esquimalt Harbour	9	11	0	11	78
Non-harbour	35	0	3	6	91

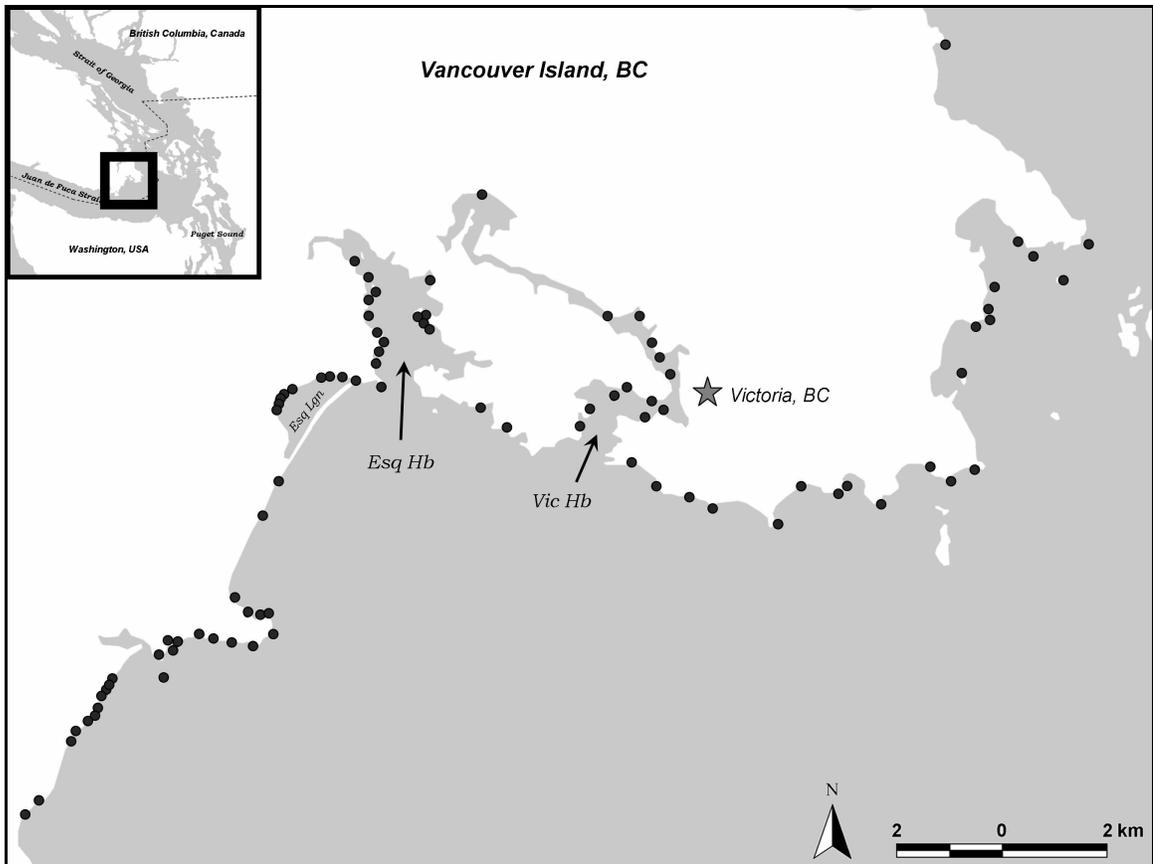


Figure 2.1 Location of active river otter latrine sites (circles) on southern Vancouver Island, British Columbia, Canada where fresh faecal samples were collected for DNA genotyping and contaminant analyses (2005-2006). Vic Hb = Victoria Harbour, Esq Hb = Esquimalt Harbour, Esq Lgn = Esquimalt Lagoon.

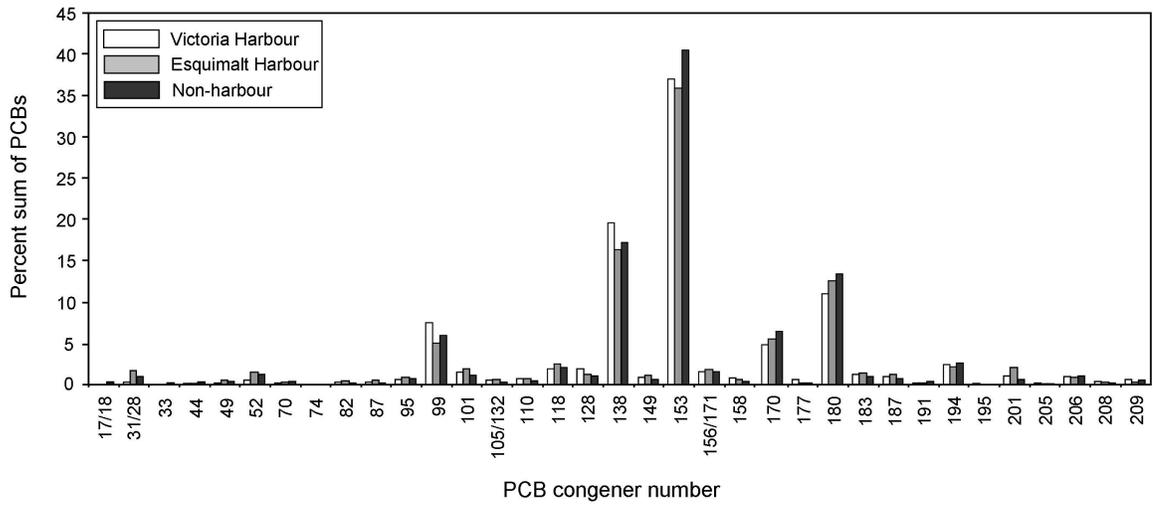


Figure 2.2 PCB congener profiles in river otter faeces as a percent of the sum of all PCB congeners analyzed from the three sampling locations along the coast of southern Vancouver Island, British Columbia, Canada (2005-2006).

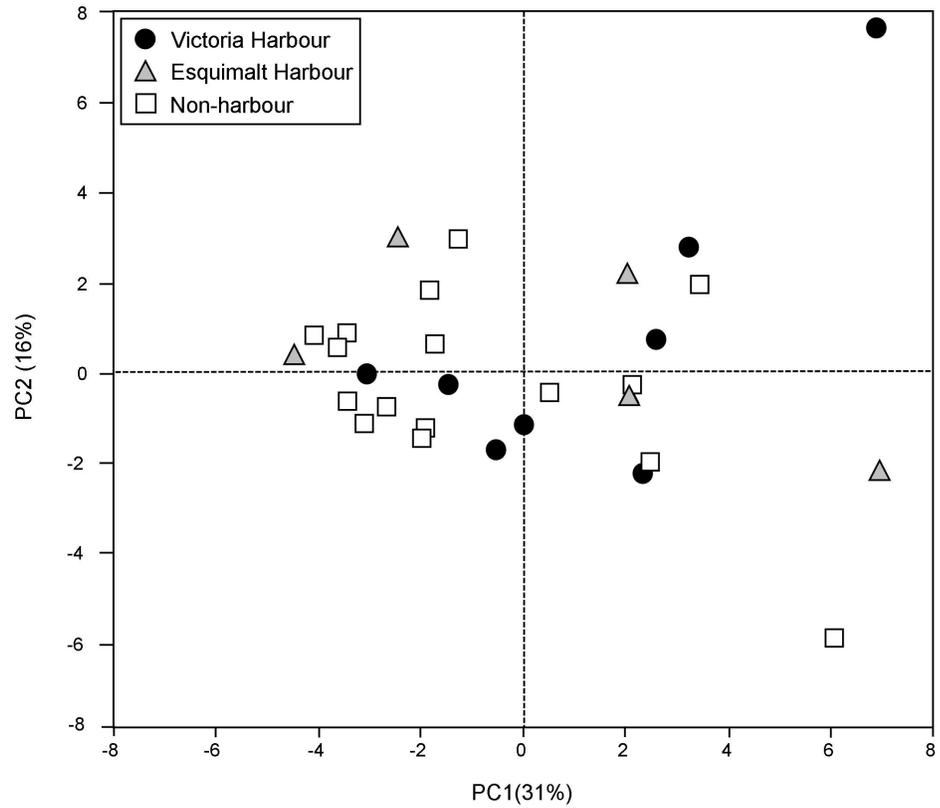


Figure 2.3 Principal component scores for polychlorinated biphenyls (PCBs) in river otter faeces collected from three sampling locations along the coast of southern Vancouver Island, British Columbia, Canada (2005-2006).

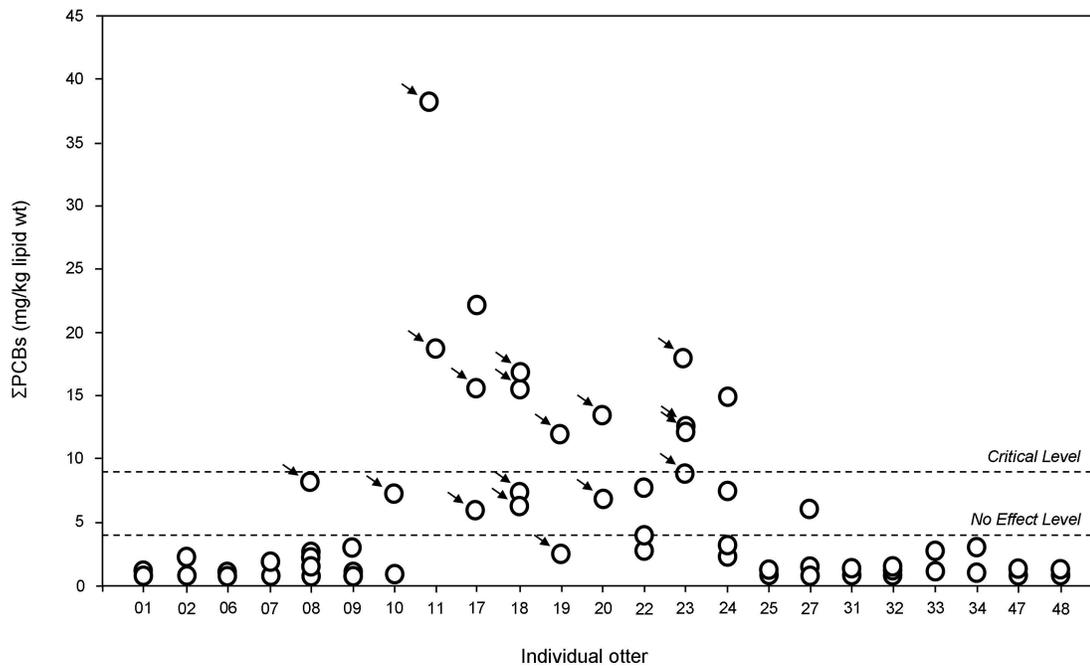


Figure 2.4 ΣPCB concentrations (mg/kg lipid wt) in individual river otter faeces along the coast of southern Vancouver Island, British Columbia, Canada (2005-2006). Arrows indicate samples collected from Victoria Harbour. Hatched lines indicate 9 and 4 mg/kg lipid weight, which are the ‘critical’ and ‘no-effect’ level proposed for otters, respectively (Mason and Macdonald 1993b).

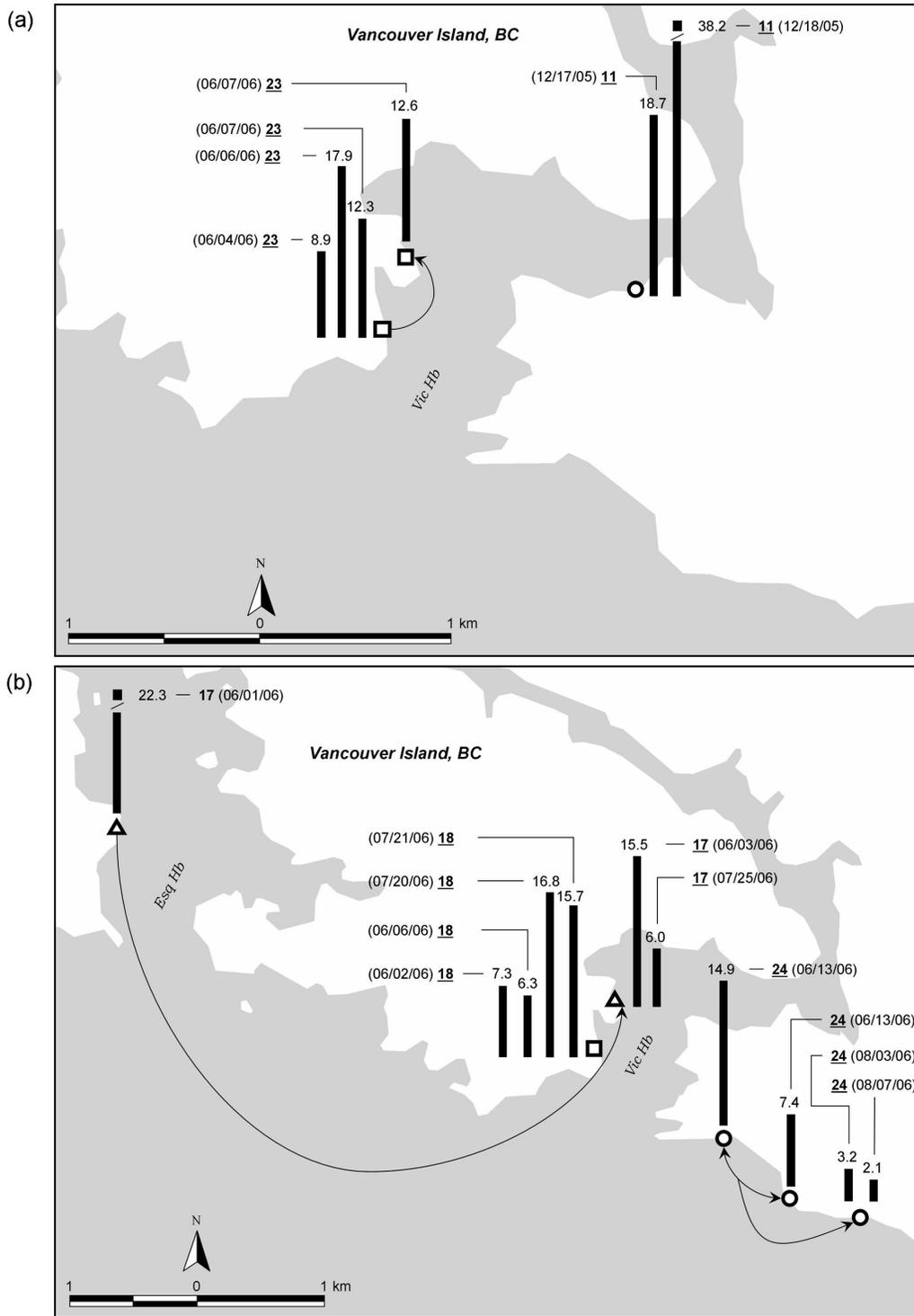


Figure 2.5 EPCB concentrations (mg/kg lipid wt) in individual river otter faeces in relation to spatial and temporal movement along the coast of southern Vancouver Island, British Columbia, Canada (2005-2006). Different symbols in each panel (a-f) represent different otters. Otter identification number (underlined) and collection date (parentheses - mm/dd/yy) are shown. Arrows indicate otter movement between latrines, but do not necessarily represent the route traveled. Vic Hb = Victoria Harbour, Esq Hb = Esquimalt Harbour, Esq Lgn = Esquimalt Lagoon.

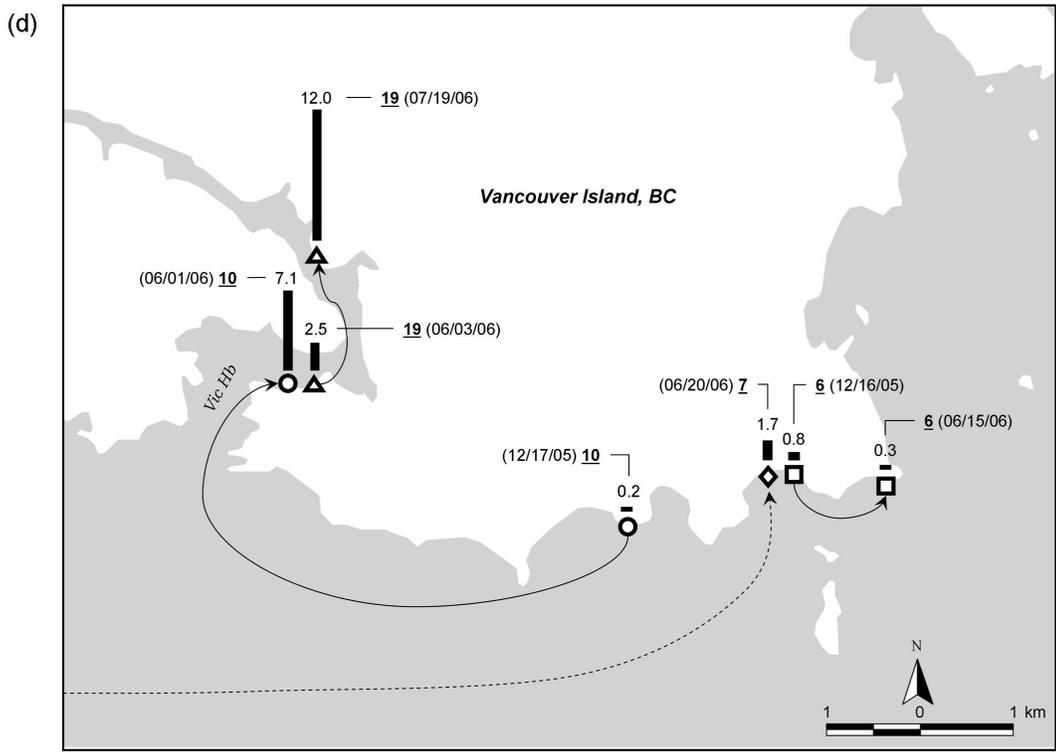
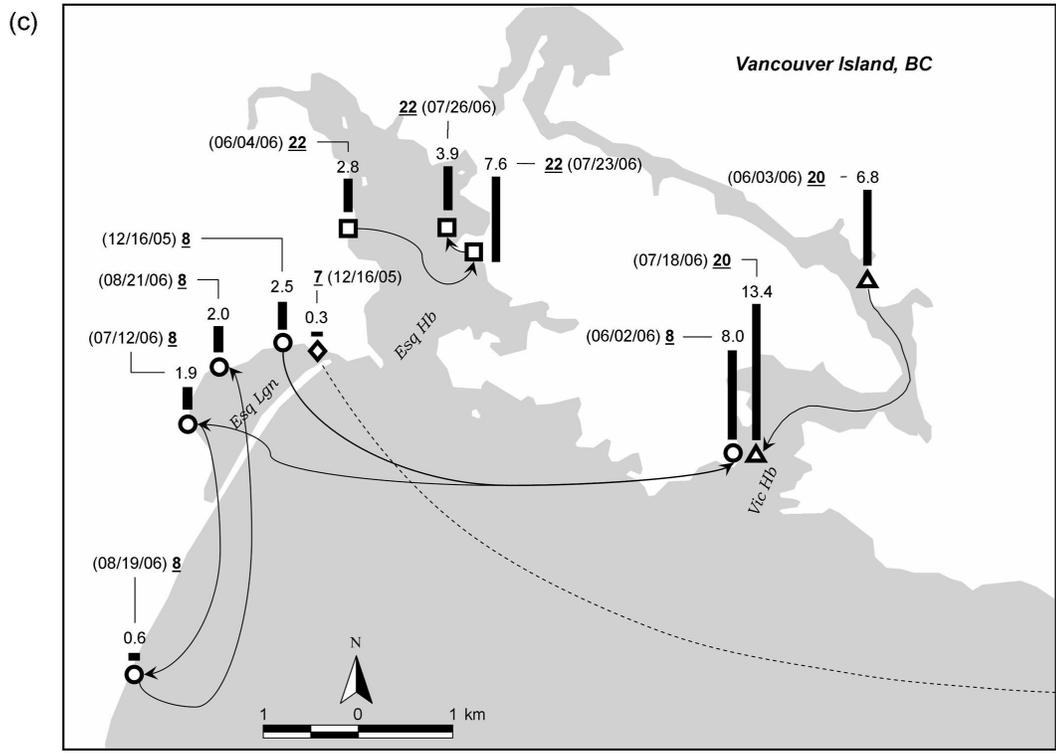


Figure 2.5 (continued)

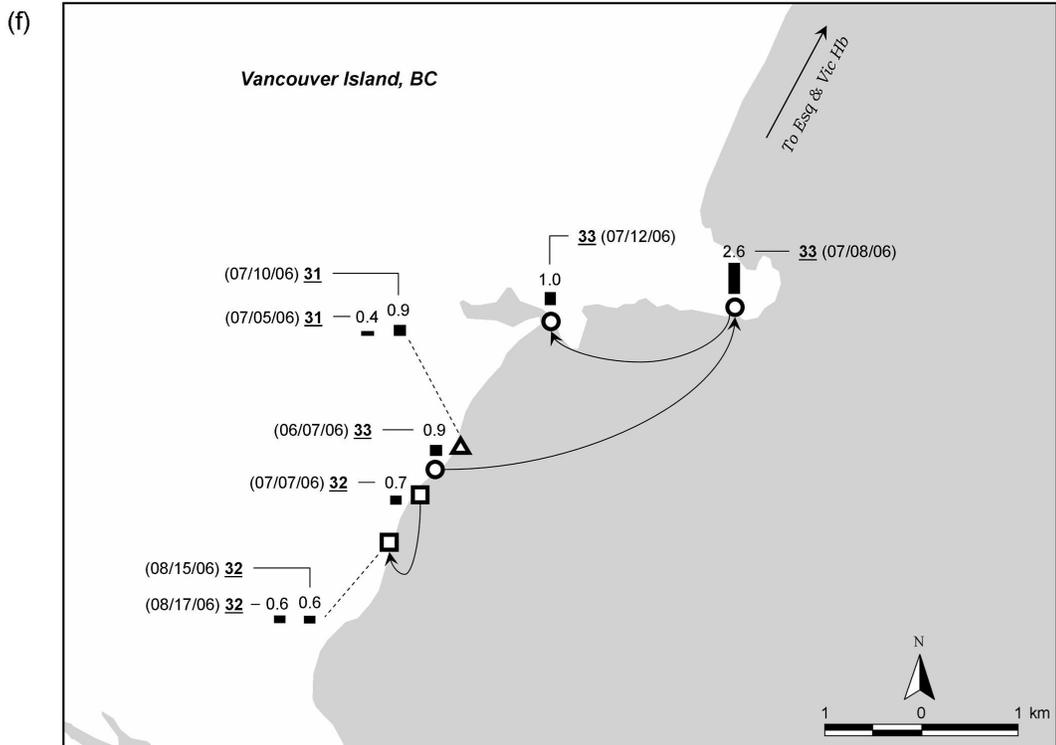
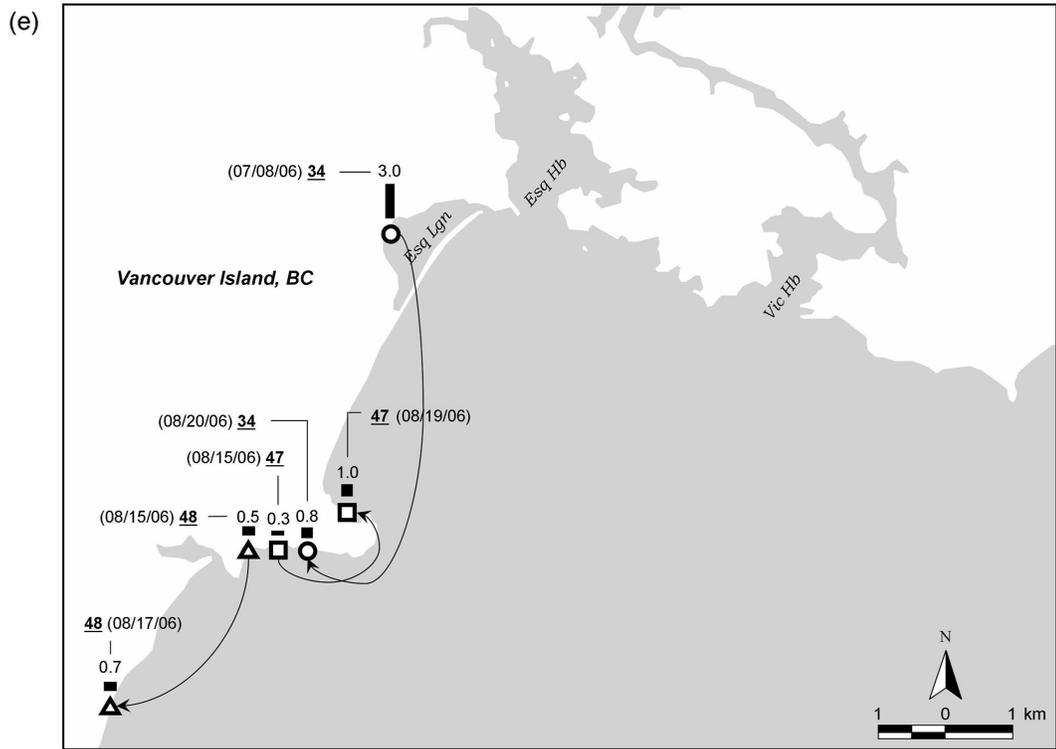


Figure 2.5 (continued)

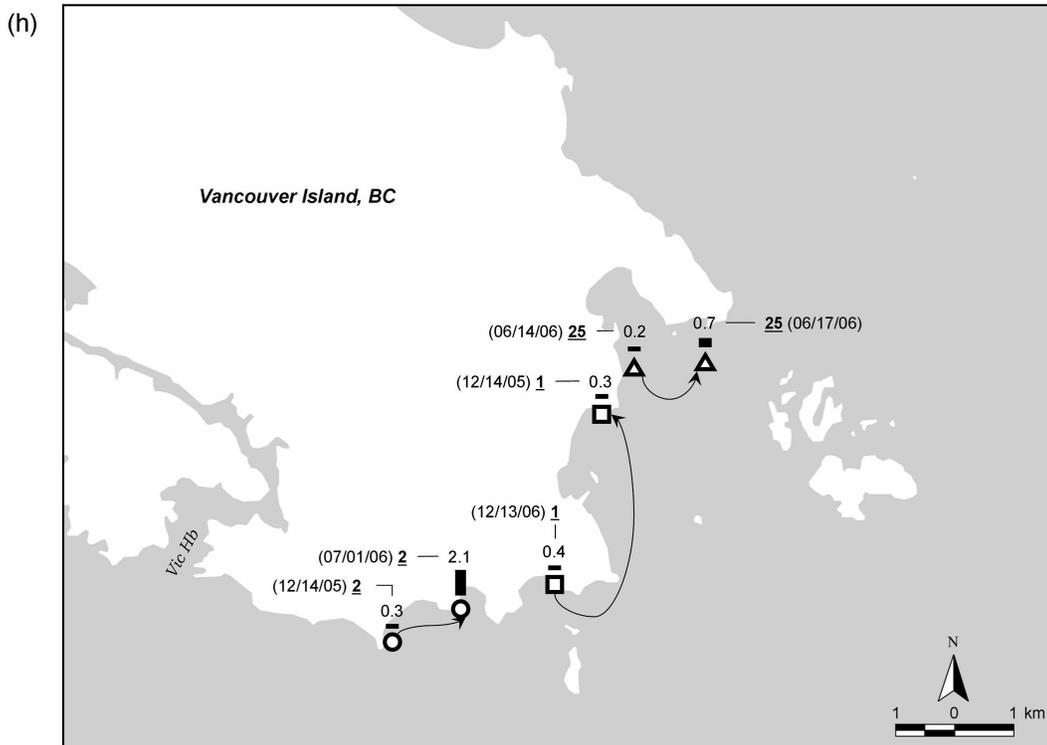
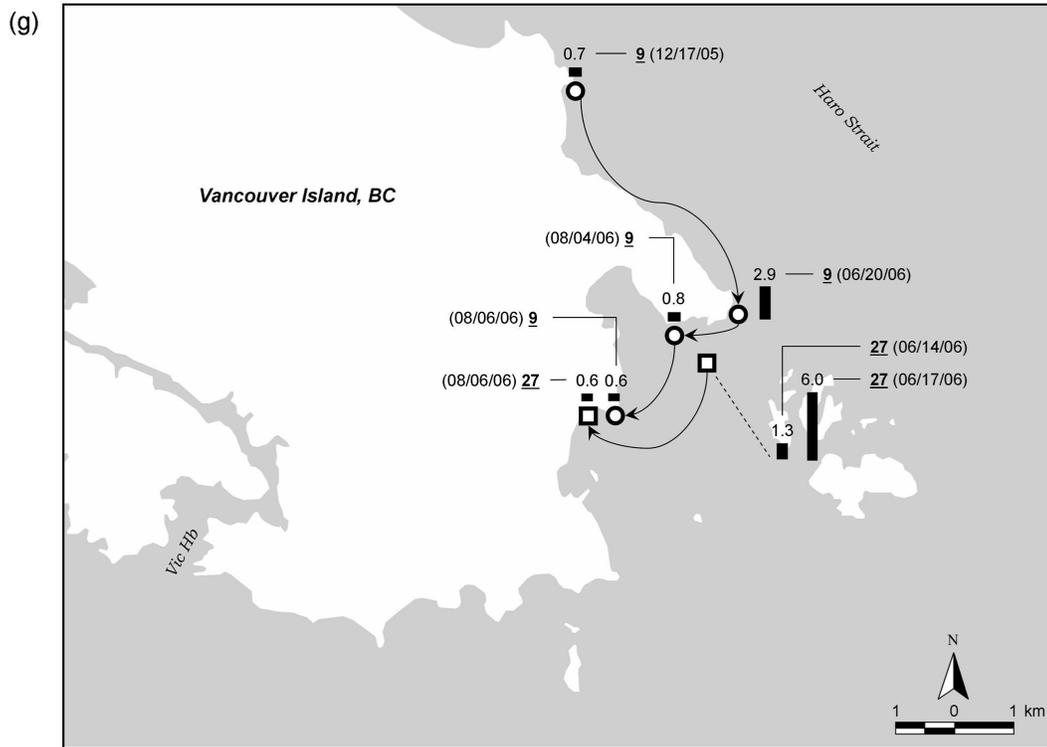


Figure 2.5 (continued)

CHAPTER 3
SUMMER FEEDING HABITS OF RIVER OTTERS ALONG
THE COAST OF SOUTHERN VANCOUVER ISLAND²

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3.1 Abstract

North American river otters (*Lontra canadensis*) foraging along the coast of southern Vancouver Island, British Columbia, Canada may be at risk from aquatic food-web contamination. The aim of this study was to identify prey remains recovered from field-collected faeces to characterize the diet of river otters in the region. Fish occurred in 95.5% of faeces analyzed. Species of gunnels (Pholidae), sculpins (Cottidae), pricklebacks (Stichaeidae), toadfish (Batrachoididae), clingfish (Gobiesocidae), flatfish (Pleuronectiformes), and snailfish (Liparidae) were the most common prey. Species of schooling fish (both pelagic and intertidal) occurred infrequently. Crustaceans were the only non-fish prey identified in the faeces. We also compared the diet of otters foraging inside the two urban/industrial harbours of the region to those foraging outside the harbour systems. Otters consumed proportionally more pricklebacks and clingfish outside the harbour systems, and more toadfish and crustaceans in the harbours. River otters also consumed a higher proportion of upper trophic-level prey species in the harbours. Differences in proportions of some prey items in otter faeces along the coast of southern Vancouver Island likely reflect local habitat heterogeneity and area-related variation in prey abundance and availability.

3.2 Introduction

The Georgia Basin-Puget Sound marine ecosystem is a highly productive inland sea located in the transboundary region between British Columbia, Canada, and Washington, USA. However, disturbance to this ecosystem from human coastal development and water pollution is a significant threat to North American river otters (*Lontra canadensis*) and other wildlife populations of the region (Fraser et al. 2006). Such anthropogenic stressors might influence otters directly in terms of individual health, reproductive ability, and survival, as well as indirectly by affecting their prey base and habitat quality (Mason 1989).

Among mustelids, semi-aquatic piscivorous species such as the river otter and Eurasian otter (*Lutra lutra*) have been used as model species for numerous ecotoxicological studies (Bowyer et al. 2003). As piscivorous top-predators, otters can accumulate high concentrations of persistent contaminants (Henny et al. 1981; Smit et al. 1994; Elliott et al. 1999) and may be sensitive to the toxic effects of some polyhalogenated aromatic hydrocarbon contaminants. Indeed, declines of Eurasian otter populations in large parts of Europe have been attributed to elevated levels of environmental contaminants in the food chain, particularly polychlorinated biphenyls (PCBs) (Olsson and Sandegren 1983; Mason 1989; Roos et al. 2001). Likewise, the distribution of the North American river otter was greatly reduced throughout much of its historic range by the early 1900s primarily due to water pollution associated with urbanization and industrial activity (Larivière and Walton 1998).

Otters are exposed to environmental contaminants primarily through the consumption of contaminated prey (Leonards et al. 1998). Otters are elusive and difficult to sample directly, hence, Mason et al. (1992) used a two-stage bioaccumulation model to estimate potential PCB levels in otters from field-collected faeces. Recently, Elliott et al. (2008) reported that faeces collected from river otter communal marking sites (latrines) in Victoria Harbour, British Columbia contained levels of PCBs that exceeded the criteria for reproductive impairment developed for the Eurasian otter. Using faecal DNA genotyping and contaminant analyses, we examined spatial and temporal patterns of otter PCB exposure in the Victoria region and found that individuals were consistently exposed to elevated levels of PCBs in the urban/industrial harbours of the region (Victoria and Esquimalt Harbour) compared to outside the harbour systems, regardless of individual otter sampled or their movements (see Chapter 2).

It is generally assumed that the PCB concentrations measured in otter faecal samples are largely of dietary origin (i.e., those that are not absorbed through the digestive tract) (Mason et al. 1992, Mason and Macdonald 1993). Therefore, the analysis of prey may reveal sources of PCB contamination, and may be a better tool to track PCBs through the food web in a particular area of concern (Smit et al. 1994). However, river otters are opportunistic predators that usually consume prey species in proportion to their abundance and availability (Ryder 1955; Melquist et al. 2003). Thus, prior to analysing prey, it is necessary to establish the contribution of each species to the overall diet, as some prey species may contain higher contaminant concentrations than others in the same general area (Smit et al. 1994). In that regard, the objectives of this study were to determine (i) the principal prey species consumed by river otters in the Victoria region,

and (ii) whether there are differences in the diets between otters foraging inside the two urban/industrial harbours of the region and outside the harbour systems that may contribute to variation in contaminant exposure. This analysis is part of a broader study of river otter ecology and containment exposure along the coast of southern Vancouver Island, and the results obtained will be used to guide future prey sampling and contaminant analyses.

3.3 Methods

3.3.1 Study area

The study area was located on southern Vancouver Island, British Columbia, Canada (48°25' N, 123°21' W) (Figure 3.1). The urban/industrial harbours of Victoria and Esquimalt, British Columbia were located centrally within the study area. These harbours support (or have supported) shipbuilding, fishing, and forestry industries, as well as other commercial and tourism activities, and have done so for over 75 years. In addition, Esquimalt Harbour is home to the Canadian Pacific naval fleet. Polyhalogenated aromatic hydrocarbon contamination has been documented in both harbours (Ikonomou et al. 2002; Knapp and Grant 2008).

3.3.2 Dietary analysis

We collected fresh faeces ($n = 802$) from river otters at 86 active latrine sites along 91.7 km of shoreline from June to August 2006. Samples were individually collected in 50-mL microcentrifuge tubes and kept cool until examination. Each sample was washed in a fine mesh stainless steel autoclavable sieve to separate faecal and mucilaginous material from larger sized undigested prey remains. All of these hard prey

remains were then spread, air-dried, and stored in individually labelled petri-dishes until further analysis. Because we were interested in otter dietary differences inside the urban/industrial harbours of the region compared to outside the harbour systems, we chose a random subset of 72 faeces from inside the harbours and 62 outside the harbours ($n = 134$) for analysis. Prey remains were transferred to Pacific Identifications, Inc., Victoria, British Columbia, Canada for identification. A multi-structure identification approach was used to compare fish skeletal fragments to those of a reference collection housed on-site (S. Crockford, Pacific Identifications, Victoria, BC, Canada, *personal communication*). Prey remains were identified to the lowest practical taxonomic level (i.e., species when possible). Crustaceans (crab and shrimp) were identified in faeces by the presence of exoskeleton fragments, but were not assigned to a species due to lack of distinguishing characteristics.

We recorded prey items according to the number of faecal samples in which they occurred. We determined the relative percentage of each prey taxa in the diet by dividing the total number of taxon x by the total number of prey taxa recorded. Similarly, we determined the relative frequencies of occurrence of the different prey taxa consumed by dividing the number of faecal samples containing taxon x , by the total number of faeces analyzed (Melquist and Hornocker 1983). To remain conservative in our estimates, when more than one individual of the same species appeared in a sample it was treated as a single occurrence (Melquist and Hornocker 1983). That method has been used in a number of field studies aimed at describing food habits of natural otter populations (Sheldon and Toll 1964; Erlinge 1968; Knudsen and Hale 1968; Modafferi and Yocom 1980; Melquist and Hornocker 1983; Larsen 1984; Stenson et al. 1984; Reid et al. 1994;

Watt 1995; Pardini 1998; Rosas et al. 1999; Anoop and Hussain 2005; Crait and Ben-David 2006; Cote et al. 2008), and gives a reasonable estimate of the rank order of prey consumed by otters (Carss and Parkinson 1996).

The trophic position of prey in each zone was determined using only fish prey items identified to species. Individual prey species were assigned to a single trophic level according to information provided by Allen (2006) for intertidal and subtidal fish assemblages of the Pacific Coast. Fish species present in faeces, but not identified to a trophic level in Allen (2006) were assigned to trophic groups on the basis of reported feeding patterns (Hart 1973; Wheeler 1975; Eschmeyer and Herald 1983). Trophic categories were classified as: (i) herbivore (i.e., species that primarily consume macroalgae and detritus), (ii) primary consumer (i.e., species that feed on gammarid amphipods, polychaetes, and benthic microinvertebrates), (iii) secondary consumer (i.e., species that feed on crabs, benthic macroinvertebrates, and small fish), and (iv) tertiary consumer (i.e., primarily piscivorous fish). Some species can occupy more than one trophic level, and each trophic level usually has many representatives. Consequently, in most marine ecosystems, trophic interactions are described not by simple food chains but as complex food webs (Simenstad et al. 1979). Nonetheless, the four levels used in our analyses provide a reasonable index of prey trophic status and the degree to which they are consumed by otters.

We used Pearson chi-square tests to determine differences in diet based on foraging location. We investigated differences in diet between harbour and non-harbour sites. Differences in the proportion of prey taxa between areas were considered

significant at the $P < 0.05$ level. All statistical analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, Illinois, USA).

3.4 Results

Of the 134 faecal samples analyzed, most contained several prey items (mean of 5.41 ± 2.26 SD). Fish occurred in 95.5% of faecal samples analyzed (Table 3.1). Eighteen families of fish were identified with the most common being gunnels (Pholidae), sculpins (Cottidae), pricklebacks (Stichaeidae), toadfish (Batrachoididae), clingfish (Gobiesocidae), flatfish (Pleuronectiformes), and snailfish (Liparidae). Overall, species of gunnels, sculpins, and pricklebacks accounted for 52.9% of the total prey items identified in faeces (Figure 3.2). Other fish consumed by river otters included poachers (Agonidae), sand lances (Ammodytidae), surfperches (Embiotocidae) cods (Gadidae), sticklebacks (Gasterosteidae), gobies (Gobiidae), sea ravens (Hemitripterae), greenlings (Hexagrammidae), lanternfish (Myctophidae), rockfish (Scorpaenidae), and pipefish (Syngnathidae); however, frequency of occurrence for each of these items was $< 10\%$ (Table 3.1). Crustaceans occurred in 30.6% of otter faeces analyzed, and exoskeleton fragments resembled crab species of the family Cancridae (i.e., Dungeness crab (*Cancer magister*), red rock crab (*Cancer productus*), and slender crab (*Cancer gracilus*)), and shrimp. Bird or mammal remains were not detected in any of the faeces included in our analysis.

There were similar trends in prey frequency of occurrence and prey percentages in faeces collected from harbour and non-harbour sites. Fish occurred in 97% of harbour faeces and 94% non-harbour faeces (Table 3.1). Species of gunnels, sculpins, and pricklebacks accounted for 48.5 % of the total prey items identified in faeces from inside

the harbours, and 58.3% outside the harbours (Figure 3.2). However, there were some significant differences in the proportion of prey taxa in otter faeces between areas. Of the eight main prey taxa identified (i.e., frequency of occurrence > 10%), a significantly higher proportion of pricklebacks (Stichaeidae; $\chi^2 = 12.4$, $P < 0.05$), clingfish (Gobiesocidae; $\chi^2 = 10.8$, $P < 0.05$), and greenlings (Hexagrammidae; $\chi^2 = 5.42$, $P < 0.05$) occurred in faeces from outside the harbours, whereas a significantly higher proportion of toadfish (Batrachoididae; $\chi^2 = 30.1$, $P < 0.05$) and crustaceans (Crustacea; $\chi^2 = 27.6$, $P < 0.05$) occurred in otter faeces collected from inside the harbours (Figure 3.3).

We classified two fish species as herbivores ($n = 5$), 12 as primary consumers ($n = 233$), 13 as secondary consumers ($n = 111$), and one as a tertiary consumer ($n = 1$) (Table 3.1). The contribution of individual prey items identified in each sampling area to the four trophic levels is shown in Figure 3.4. There was a significantly higher proportion of primary consumers in river otter faecal samples collected outside the harbours ($\chi^2 = 19.6$, $P < 0.05$), but a significantly higher proportion of secondary consumers ($\chi^2 = 25.1$, $P < 0.05$) in faecal samples collected inside the harbours. Overall, few herbivores and tertiary consumers were identified in faecal samples.

3.5 Discussion

Marine fish of the intertidal and subtidal zones were the most common prey consumed by river otters foraging during summer along the coast of southern Vancouver Island, British Columbia. The most frequently occurring fish identified included species of gunnels (Pholidae), sculpins (Cottidae), pricklebacks (Stichaeidae), clingfish (Gobiesocidae), flatfish (Pleuronectiformes), snailfish (Liparidae), and toadfish (Batrachoididae). Those species are common to the south coast of British Columbia (Hart

1973; Eschmeyer and Herald 1983; Lamb and Edgell 1986), and are vulnerable to predators because of their poor escape ability (Stenson et al. 1984). A variety of additional fish families, which include the poachers (Agonidae), sand lances (Ammodytidae), surfperches (Embiotocidae) cods (Gadidae), sticklebacks (Gasterosteidae), gobies (Gobiidae), sea ravens (Hemitripterae), greenlings (Hexagrammidae), lanternfish (Myctophidae), rockfish (Scorpaenidae), and pipefish (Syngnathidae) were found in only a few samples. Those fish may represent occasional food items or may have been ingested incidentally. The occurrence of crustaceans was greater in faeces from harbour sites compared to non-harbour sites, but overall crustaceans were less important than fish in the diet of river otters along the coast of southern Vancouver Island.

The preponderance of common intertidal and subtidal fish is consistent with the findings of Stenson (1984) in the southern Gulf Islands, British Columbia, and Jones (2000) in the San Juan Islands, Washington. Intertidal and subtidal fish were also the principal food items in faeces of marine-foraging river otter in coastal southeast Alaska (Larsen 1984), Prince William Sound, Alaska (Bowyer et al. 1994; Ben-David et al. 2005), and coastal Newfoundland, Canada (Cote et al. 2008). Likewise, marine-foraging Eurasian otters consume slow-moving fish of the intertidal and subtidal zones, including many species of the main fish families identified in our study (Herfst 1984; Kruuk and Moorhouse 1990; Watt 1995; McMahon and McCafferty 2006).

It has been reported that the foraging strategy of river otters in coastal habitats may differ with social organization of individuals in the population. For example, in coastal Alaska, solitary river otters typically consumed slow-swimming intertidal fish,

whereas social river otters tended to forage cooperatively on energy-rich schooling pelagic fish such as the Pacific herring (*Clupea pallasii*) and Pacific sand lance (*Ammodytes hexapterus*) that are harder to capture (Blundell et al. 2002; Ben-David et al. 2005). We regularly observed groups of three or more (maximum 11) otters during the course of our study (D.A. Guertin, *personal observations*), but schooling pelagic fish occurred infrequently in the otter faeces that we examined. The absence of those species might be a result of spatial and temporal variability in fish distribution and biomass within the Georgia Basin-Puget Sound marine ecosystem at the time of our study (Lemberg et al. 1997; Stick 2005; Hay and McCarter 2007). However, annual surveys of river otter feeding habits in the region also report a low occurrence of schooling pelagic fish in river otter faeces and stomach contents (Stenson et al. 1984; Jones 2000). When schooling pelagic fish are absent or unavailable to river otters, social groups may alternatively forage on schooling intertidal and subtidal fish such as Pacific cod (*Gadus macrocephalus*) and some rockfish species (Ben-David et al. 2005). Nonetheless, in our study, there was little evidence of river otters foraging on any pelagic or intertidal schooling fish. Thus, it appears that cooperative foraging on schooling fish may not be the sole reason for group formation by river otters.

Crustaceans (crab and shrimp) were the only non-fish prey identified in otter faeces. Crustaceans occurred less frequently in otter faeces than previously reported for San Juan Island, Washington (Jones 2000) and in coastal brackish lakes in California (Modafferi and Yocom 1980), but more frequently than in the Gulf Islands, British Columbia (Stenson et al. 1984), southeast Alaska (Larsen 1984), and Prince William Sound, Alaska (Bowyer et al. 1994). Despite those regional differences, crustaceans

occurred as the second most frequently occurring prey type after fish in all studies. That indicates that crustaceans may be an important secondary food source in coastal marine habitats when fish are less abundant or less available to otters. Other prey such as birds and mammals were not detected in our samples, but have been reported to be minor items in diets of marine-foraging river otters (Larsen 1984; Stenson et al. 1984; Bowyer et al. 1994; Jones 2000). However, unidentified bird remains were in five faecal samples not included in the 134 random samples selected for our detailed analysis, and it has been shown that river otters in the Georgia Basin-Puget Sound marine ecosystem may opportunistically feed on marine birds (particularly nestlings) when foraging near marine bird colonies (Hayward et al. 1975; Footitt and Butler 1977; Verbeek and Morgan 1978; Speich and Pitman 1984).

River otters foraging inside and outside the harbours of southern Vancouver Island consumed several common prey items. Gunnels and sculpins dominated the diet in both areas. Species belonging to those fish families can inhabit a wide range of intertidal habitats of the Pacific Coast, including steep rocky zones that occur outside the harbours, as well as rock rubble and sandy intertidal zones that characterize the inside of the harbours (Hart 1973; Eschmeyer and Herald 1983). Despite those similarities, some differences emerged in the proportional representation of the remaining prey taxa based on foraging location. Among fish taxa, pricklebacks, clingfish, and greenlings occurred more often in faeces collected outside the harbours. Those species primarily inhabit rocky intertidal habitats of the Pacific Coast (Hart 1973; Eschmeyer and Herald 1983; Lamb and Edgell 1986), and their higher occurrence in faeces collected outside the harbours probably reflects their preference for these habitat types. In contrast, plainfin midshipman

(*Porichthys notatus*, Batrachoididae) occurred almost exclusively in faeces collected in the harbours. The plainfin midshipman migrates in early summer from deeper ocean sites into shallow sandy intertidal waters for spawning along the Pacific Coast (Hart 1973; Eschmeyer and Herald 1983; Lamb and Edgell 1986). Therefore, it is not surprising that this species was predominantly recorded in faeces collected inside the harbours. In addition, species of crab and shrimp also occurred more often in faeces collected from the harbours. That a higher proportion of crustaceans occurred in samples from the harbours likely reflects a lower abundance and availability of fish (the preferred prey of otters) in those areas, and indicates that otters foraging in the harbours augment their diet with crustaceans as an alternate prey. Marine-foraging Eurasian otters also supplement their diet with crustaceans when fish abundance and availability are low (Kruuk 2006). Thus, area-related differences in the occurrence of prey items in our study are consistent with the hypothesis that otters adapt to local environmental conditions and take prey in proportion to their abundance and availability (Greer 1955; Ryder 1955; Erlinge 1968; Melquist et al. 2003).

A comparison of fish species belonging to different trophic levels indicates that otters inside the urban/industrial harbours consumed a greater proportion of higher trophic-level prey than outside the harbours. This difference was primarily due to otters consuming more piscivorous plainfin midshipmen in the harbours. Fish-eating sculpins were also more frequent in samples collected from the harbours. Additionally, crab species resembling those of the family Cancridae, which feed on smaller crustaceans, fish, and fish carcasses (Jensen 1995), were almost exclusively found in harbour samples as previously discussed. Given that persistent organic compounds bioaccumulate and

biomagnify with increasing trophic position (Ruus et al. 2002, Borgå et al. 2004), those species may contain higher concentrations of PCBs than non-piscivorous prey; contributing to the higher PCB levels of observed in otter faeces collected from the harbours at the time of our study (Elliott et al. 2008) (see Chapter 2). However, PCB levels within and among local prey populations can vary widely (Smit et al. 1994), and that variation may also be reflected in otter faecal samples (Mason and O'Sullivan 1993). Therefore, some caution is appropriate when faecal PCB concentrations are used as an assessment of otter tissue concentrations. Thus, in addition to faecal sampling, we recommend a detailed analysis of the main otter prey items identified here to accurately characterize PCBs concentrations in the food web. Prey sampling should be location specific (i.e., inside and outside the urban/industrial harbours) to elucidate possible food web trophic relationships and location effects on faecal PCB concentrations. Results from a prey based analysis can also be used to validate the use of faecal sampling as a means of predicting otter tissue levels in the region. In addition, PCB concentrations in the total diet can be extrapolated to otter tissue PCB concentrations based on the advanced age- and gender-specific bioaccumulation model described in Smit et al. (1996). That approach may provide a better indication of the risks to otters posed by foraging in the harbours.

In conclusion, the diet of river otters along the coast of southern Vancouver Island is broadly consistent with previous studies showing that otters consume sedentary species of fish that have poor escape ability and inhabit the nearshore intertidal and subtidal zones. Differences in the proportions of some prey items in otter faeces collected from inside and outside the harbours is likely due to differences in prey abundance and

availability that arise from local habitat heterogeneity. These differences result in a greater frequency of higher trophic level prey consumed by otters using the urban/industrial harbours, potentially exposing those individuals to higher levels of persistent bioaccumulative contaminants. Because the otter diet is diverse and dependent on prey abundance and availability, prey from each of the main families described here should be analyzed to accurately determine dietary exposure to persistent contaminants, particularly PCBs. It would be useful to compare PCB concentrations and congener patterns in the main prey species identified in this study to those in otter faecal samples to test the assumption that faecal PCB concentrations are indeed reflective of levels in their prey at the local scale. Comparing PCB concentrations in major prey taxa inside and outside the harbours may also provide further confirmation of the apparent threat posed by elevated levels of PCBs in the harbour systems (Elliott et al. 2008) (see Chapter 2), as well as aid in determining which species contribute most to the contamination of otters in this particular population. The data presented here provide a basis for such future investigations.

3.6 Acknowledgements

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Table 3.1 Prey items in faeces of river otter collected from the coast of southern Vancouver Island, British Columbia, Canada (summer 2006), the trophic level to which they were assigned (*hb* herbivore, *pc* primary consumer, *sc* secondary consumer, *tc* tertiary consumer), the number of faecal samples in which they occurred (No. of Occur), and their frequency of occurrence based on sampling location.

Prey item	Trophic level	Total (<i>n</i> = 134)		Harbours (<i>n</i> = 72)		Non-harbour (<i>n</i> = 62)	
		No. of Occur	Frequency (%)	No. of Occur	Frequency (%)	No. of Occur	Frequency (%)
FISH		128	95.5	69	95.8	59	95.2
gunnels (Pholidae)		115	85.8	60	83.3	55	88.7
penpoint gunnel (<i>Apodichthys flavidus</i>)	pc	36	26.9	13	18.1	23	37.1
gunnel – unidentified		115	85.8	60	83.3	55	88.7
sculpins (Cottidae)		94	70.1	55	76.4	39	62.9
buffalo sculpin (<i>Enophrys bison</i>)	sc	23	17.2	16	22.2	7	11.3
Pacific staghorn sculpin (<i>Leptocottus armatus</i>)	sc	19	14.2	10	13.9	9	14.5
rosylip sculpin (<i>Ascelichthys rhodorus</i>)	pc	17	12.7	8	11.1	9	14.5
great sculpin (<i>Myoxocephalus polyacanthocephalus</i>)	sc	10	7.5	8	11.1	2	3.2
smoothhead sculpin (<i>Artedius lateralis</i>)	sc	2	1.5	1	1.4	1	1.6
padded sculpin (<i>Artedius fenestralis</i>)	pc	2	1.5			2	3.2
red Irish lord (<i>Hemilepidotus hemilepidotus</i>)	sc	1	< 1			1	1.6
cabezon (<i>Scorpaenichthys marmoratus</i>)	tc	1	< 1			1	1.6
sculpin – unidentified		71	53.0	44	61.1	27	43.5
pricklebacks (Stichaeidae)		92	68.7	40	55.6	52	83.9
high cockscomb (<i>Anoplarchus purpurescens</i>)	pc	71	53.0	28	38.9	42	67.7
snake prickleback (<i>Lumpenus sagitta</i>)	pc	24	17.9	20	27.8	4	6.5
rock prickleback (<i>Xiphister mucosus</i>)	hb	5	10.6	1	1.4	4	6.5
prickleback – unidentified		44	32.8	3	4.2	42	67.7
clingfish (Gobiesocidae)							
northern clingfish (<i>Gobiesox maeandrichs</i>)	pc	66	49.3	26	36.1	40	64.5

(continued)

Table 3.1 (continued)

Prey item	Trophic level	Total (n = 134)		Harbours (n = 72)		Non-harbour (n = 62)	
		No. of Occur	Frequency (%)	No. of Occur	Frequency (%)	No. of Occur	Frequency (%)
FISH							
flatfish (Pleuronectiformes)		47	35.1	27	37.5	20	32.3
starry flounder (<i>Platichthys stellatus</i>)	sc	13	9.7	9	12.5	4	6.5
C-O sole (<i>Pleuronichthys coenosus</i>)	sc	3	2.2	3	4.2		
English sole (<i>Pleuronectes vetulus</i>)	sc	3	2.2			3	4.8
petrale sole (<i>Eopsetta jordani</i>)	sc	1	< 1	1	1.4		
rock sole (<i>Pleuronectes bilineatus</i>)	sc	1	< 1			1	1.6
flatfish – unidentified		37	27.6	22	30.6	15	24.2
snailfish (Liparidae)							
snailfish – unidentified		33	24.6	14	19.4	19	30.6
toadfish (Batrachoididae)							
plainfin midshipmen (<i>Porichthys notatus</i>)	sc	31	23.1	30	41.6	1	1.6
greenlings (Hexagrammidae)							
greenling – unidentified (<i>Hexagrammos</i> spp.)		13	9.7	3	4.2	10	16.1
sea ravens (Hemitripterae)		7	5.2	6	8.3	1	1.6
silverspotted sculpin (<i>Blepsias cirrhosus</i>)	pc	6	4.5	5	6.9	1	1.6
sea raven – unidentified (<i>Nautichthys</i> spp.)		1	< 1	1	1.4		
surfperches (Embiotocidae)		7	5.2	5	6.9	2	3.2
shiner perch (<i>Cymatogaster aggregata</i>)	sc	3	2.2	3	4.2		
white surfperch (<i>Phanerodon furcatus</i>)	sc	1	< 1	1	1.4		
kelp surfperch (<i>Brachyistius frenatus</i>)	pc	1	< 1			1	1.6
perch – unidentified		3	2.2	2	2.8	1	1.6

(continued)

Table 3.1 (continued)

Prey item	Trophic level	Total (<i>n</i> = 134)		Harbours (<i>n</i> = 72)		Non-harbour (<i>n</i> = 62)	
		No. of Occur	Frequency (%)	No. of Occur	Frequency (%)	No. of Occur	Frequency (%)
FISH							
sticklebacks (Gasterosteidae)							
threespine stickleback (<i>Gasterosteus aculeatus</i>)	pc	3	2.2	3	4.2		
pipefish (Syngnathidae)							
bay pipefish (<i>Syngnathus leptorhynchus</i>)	pc	3	2.2	2	2.8	1	1.6
sand lances (Ammodytidae)							
Pacific sand lance (<i>Ammodytes hexapterus</i>)	pc	3	2.2			3	4.8
cods (Gadidae)							
walleye pollock (<i>Theragra chalcogramma</i>)	pc	2	1.5	2	2.8		
rockfish (Scorpaenidae)							
rockfish – unidentified (<i>Sebastes</i> spp.)		2	1.5	2	2.8		
poachers (Agonidae)							
poacher – unidentified (<i>Podothecus</i> spp.)		1	< 1	1	1.4		
gobies (Gobiidae)							
goby – unidentified		1	< 1	1	1.4		
lanternfish (Myctophidae)							
lanternfish – unidentified (<i>Lampanyctus</i> spp.)		1	< 1	1	1.4		
miscellaneous							
unidentified fish		7	5.2	5	6.9	2	3.2
INVERTEBRATES							
Crustacea		41	30.6	36	50.0	5	8.1
crab – unidentified		28	20.9	23	31.9	5	8.1
shrimp – unidentified		14	10.4	14	19.4		

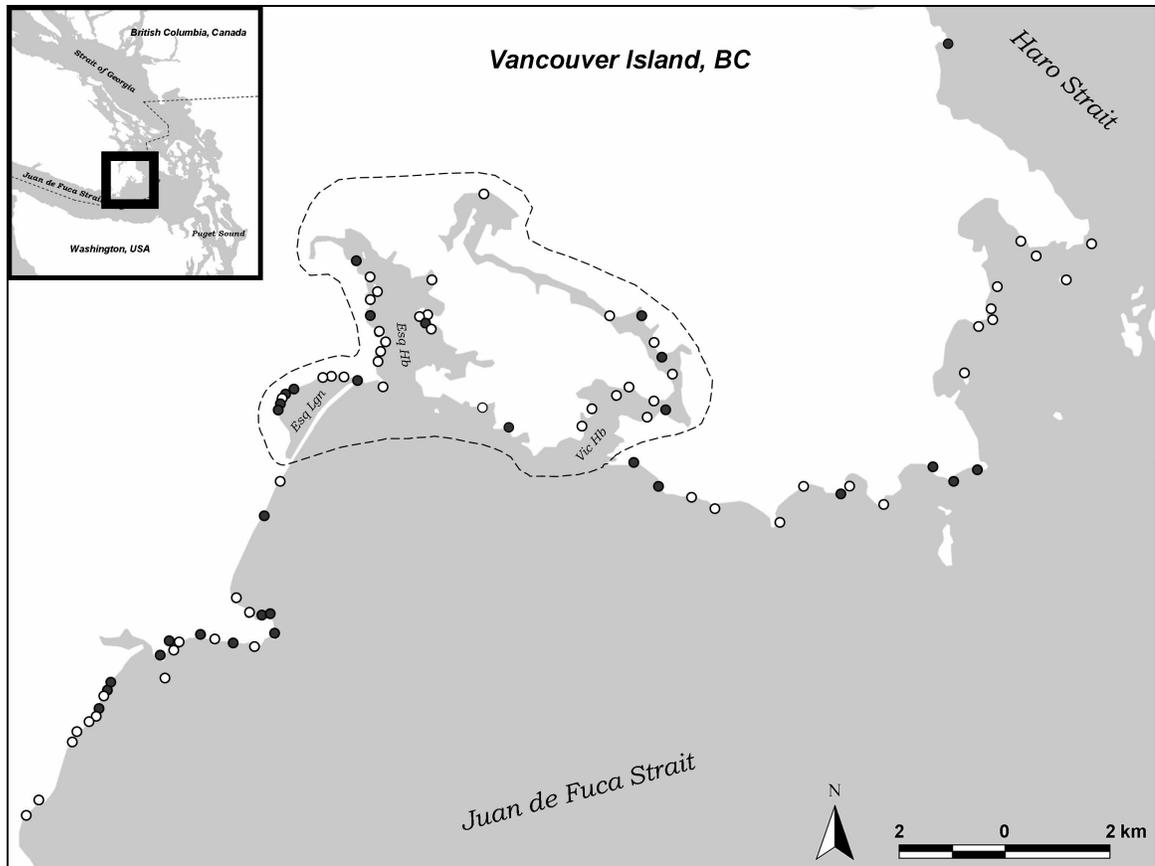


Figure 3.1 Location of river otter latrine sites (circles) sampled during summer 2006 along the southern coast of Vancouver Island, British Columbia, Canada. Differences in prey remains in faeces were compared between samples collected inside the two urban/industrial harbours, and outside the harbours. The dashed line indicates latrines that were included in the 'harbours' sampling area. All other latrines were included in the 'non-harbour' sampling area. Open circles represent latrine sites in which faeces were collected and used in the dietary analysis. Vic Hb = Victoria Harbour, Esq Hb = Esquimalt Harbour, Esq Lgn = Esquimalt Lagoon.

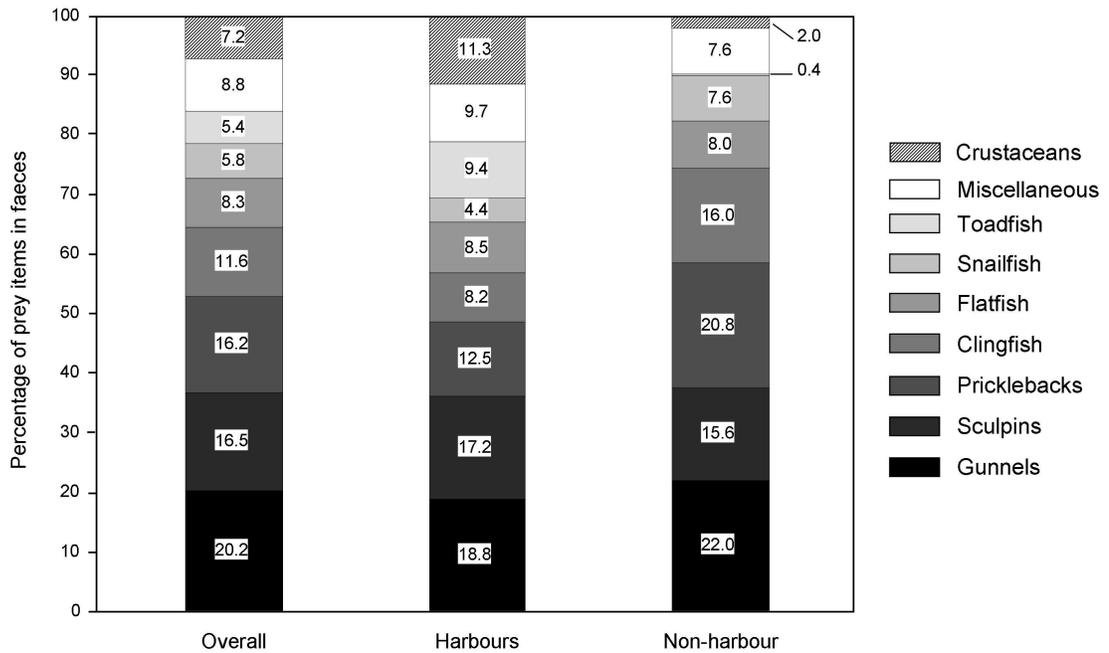


Figure 3.2 Percentage of total prey items recovered from river otter faeces collected from latrine sites inside and outside the harbours of southern Vancouver Island, British Columbia, Canada (summer 2006). Prey items stacked from highest to lowest percentage according to the overall study area, except miscellaneous fish (i.e., frequency of occurrence < 10%) and crustaceans.

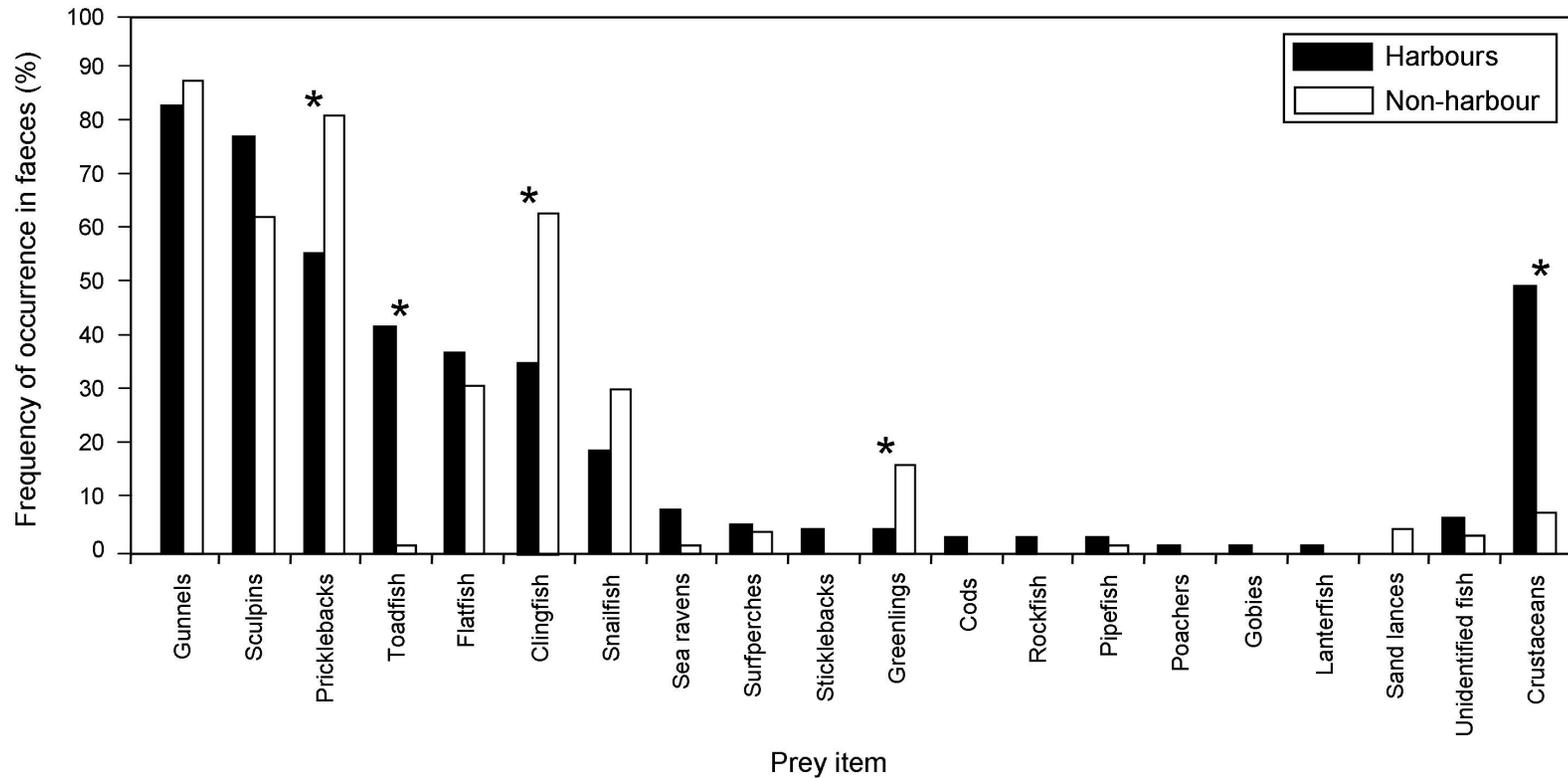


Figure 3.3 Frequency of occurrence of prey items in river otter faeces collected from latrine sites inside and outside the harbours of southern Vancouver Island, British Columbia, Canada (summer 2006). Prey items are arranged from highest to lowest frequency in harbour samples (except unidentified fish and crustaceans). Significant differences between sampling areas (Pearson chi-square) are indicated with an asterisk (*).

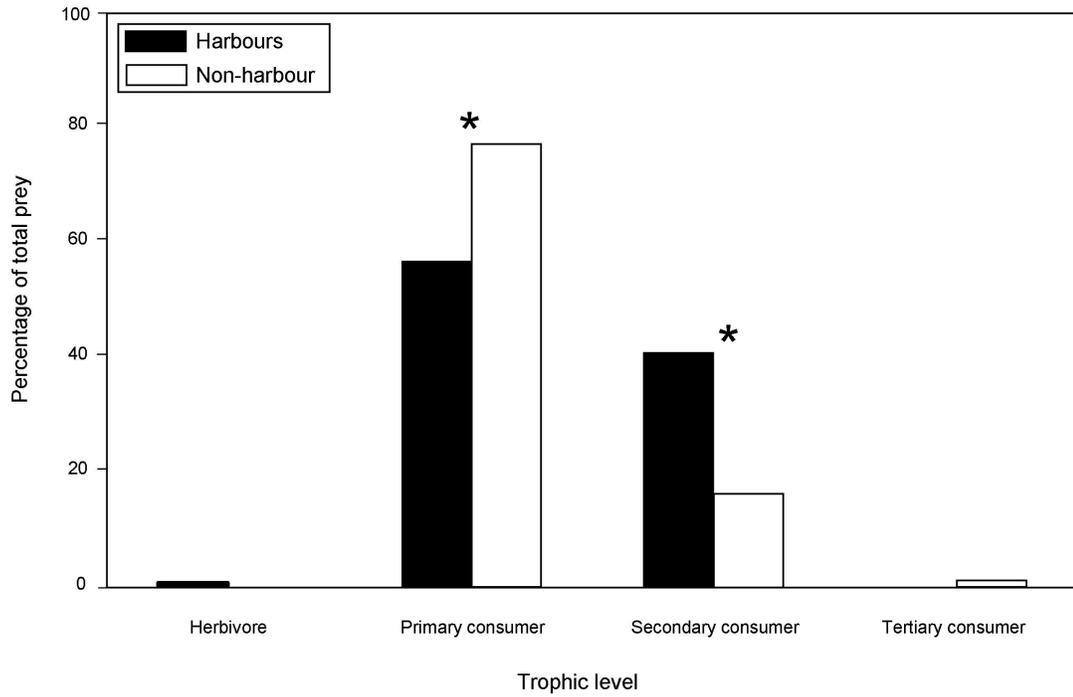


Figure 3.4 Percentage of prey items (identified to species) belonging to four different trophic levels in river otter faeces collected from latrine sites inside and outside the harbours of southern Vancouver Island, British Columbia, Canada (summer 2006). Significant differences between sampling areas (Pearson chi-square) are indicated with an asterisk (*).

CHAPTER 4
FAECAL DNA GENOTYPING REVEALS DEMOGRAPHIC
DIFFERENCES AMONG COASTAL RIVER OTTERS
INHABITING A CONTAMINATED REGION OF
SOUTHERN VANCOUVER ISLAND³

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4.1 Abstract

We used faecal DNA genotyping and population genetic and demographic analyses to study the effect of chronic contaminant exposure in river otters (*Lontra canadensis*) inhabiting the urban coast of southern Vancouver Island, British Columbia, Canada. Previous results documented elevated levels of polychlorinated biphenyls (PCBs) in river otter faeces from the Victoria region of British Columbia. By targeting eight microsatellite DNA loci, we obtained 110 complete genotypes corresponding to 49 unique otters. Genetic analyses suggested small-scale genetic structuring among otters along the coast and estimates of recent migration rates indicated restricted gene flow among inferred subpopulations. Additionally, estimates of population density from mark-recapture modeling were nearly 50% lower in the area associated with PCB contamination. Nonetheless, high relatedness, high self-recruitment, and asymmetrical migration rates indicated successful reproduction among otters inhabiting the contaminated area. We found no conclusive evidence that PCB exposure in the region negatively affects the river otter population. Alternatively, undetected environmental variation and/or urban development may have contributed to the small-scale genetic and demographic patterns observed. Our study demonstrates that faecal DNA genotyping is an effective survey tool to examine the response of river otters to environmental contamination and human-induced environmental degradation.

4.2 Introduction

Wildlife species inhabiting the transboundary Georgia Basin-Puget Sound region of British Columbia, Canada, and Washington, USA face various anthropogenic threats. Of particular concern is exposure of fish-eating birds and marine mammals to persistent, bioaccumulative, and toxic contaminants such as polyhalogenated aromatic hydrocarbons (Elliott et al. 1989; Ross et al. 2000; Gill and Elliott 2003; Ross et al. 2004). Although not considered a true marine mammal, North American river otters (*Lontra canadensis*) are residents of the nearshore marine waters of the region, where they primarily prey on a variety of intertidal and subtidal fish (Stenson et al. 1984) (See Chapter 3). As relatively sedentary piscivorous top-predators of coastal habitats, river otters can accumulate high levels of persistent contaminants, and may be sensitive to dioxin-like chemicals based on studies with another piscivorous aquatic mustelid, the mink (*Mustela vison*).

Although there are no published reports of polyhalogenated aromatic hydrocarbon toxicity to otters, mink are highly susceptible to the toxic effects of dioxin-like chemicals such as polychlorinated biphenyls (PCBs), which adversely impacts the female reproductive system and offspring survival (Aulerich and Ringer 1977; Jensen et al. 1977; Heaton et al. 1995; Restum et al. 1998; Brunström et al. 2001; Beckett et al. 2008). Chronic exposure to these contaminants may therefore lead to local population sinks unable to produce sufficient young necessary to sustain viable populations. In fact, declines of some North American mink populations have been linked to elevated environmental PCB concentrations (Henny et al. 1981, Addison et al. 1991, Henny et al. 1996). Several authors postulate a similar sensitivity of otter to dioxin-like chemicals, and

it has been suggested that the 20th century decline of Eurasian otters (*Lutra lutra*) across Europe was a result of PCB contamination of aquatic systems (Olsson and Sandegren 1983, Mason 1989; Roos et al. 2001). This suggestion is based on correlations between PCB levels in otter tissues and the status of otter populations: high PCB levels in otters were associated with declining or endangered populations, while in thriving populations, PCB levels were low. Likewise, in addition to habitat destruction and unregulated trapping, pollution of aquatic systems is considered to be a major factor in the 20th century decline of river otter populations in North America (Larivière and Walton 1998).

Recently, we reported that PCB concentrations in river otter faeces collected from Victoria Harbour, British Columbia, Canada exceeded the criteria developed for reproductive impairment of Eurasian otters (Elliott et al. 2008) (see Chapter 2), which raised concern about the health status of the local river otter population. Assessing the effects of pollution on wildlife populations is difficult, however, because in many cases before-and-after data are unavailable. Therefore, densities and vital rates of populations subject to pollutant-related stressors are often compared with those free of them as an indication of possible injury (Sellers and Miller 1999; Monson et al. 2000). Previous river otter studies have used methods such as live-trapping and radio-telemetry to obtain information on population dynamics in relation to environmental pollution (Ben-David et al. 2002; Bowyer et al. 2003). Nevertheless, river otters are difficult to capture in the field, and those studies had limited success in generating reliable estimates of population metrics. Consequently, assessing population-level effects of pollution on river otters across North America has been challenging.

Developments in non-invasive genetic sampling techniques now enable researchers to obtain population-level data from several mammalian species where traditional methods were not feasible, thus providing a method of sampling animals without the need for live-capture (Kohn and Wayne 1997). Genomic DNA can be extracted from sources such as faeces and hair follicles, which are easily collected in the field from animal activity sites (Eggert et al. 2003; Wilson et al. 2003; Prugh et al. 2005) or via hair snares (Mowat and Paetkau 2002; Boulanger et al. 2004; DePue and Ben-David 2006). By genotyping a battery of hypervariable microsatellite loci (regions of short-sequence repeats of non-coding nuclear DNA), a ‘genetic fingerprint’ can be obtained that can be used to identify unique individuals, examine genetic diversity (Waits and Paetkau 2005), and develop capture histories that can be incorporated into population estimation procedures (Lukacs and Burnham 2005). Consequently, the development of non-invasive DNA genotyping has made it easier to obtain vital population data from species where traditional methods may not be feasible.

North American river otters are good candidates for non-invasive genetic sampling. Like other mustelids, olfaction plays an important role in river otter communication, resulting in repeated scent-marking at specific terrestrial locations (latrines) with urine, faeces, and anal gland secretions (Melquist and Hornocker 1983). The visitation rate of otters to latrines is high (Testa et al. 1994), and because such sites are easily recognizable, it is possible to collect fresh faecal deposits with minimal effort. Indeed, genetic sampling of field-collected faeces has been used to study genetic diversity, population structure, and population size and density of Eurasian otters (Dallas et al. 2003; Hung et al. 2004; Kalz et al. 2006; Prigioni et al. 2006; Arrendal et al. 2007).

Therefore, the objective of our study was to use DNA recovered from field-collected faeces to estimate genetic and demographic parameters, and to examine whether impacts of pollution associated with the urban/industrial harbours of southern Vancouver Island, British Columbia were discernable at the population-level. Based on findings from other mustelids, we hypothesize that, if PCB exposure is negatively affecting river otters, relatedness between individuals would be low due to reduced fecundity and reduced offspring survival; genetic variability would be high due to the presence of immigrants from unpolluted source populations; and population density would be lower than previously reported for coastal river otters inhabiting other regions of the Pacific Coast.

4.3 Methods

4.3.1 Study area

The study area was located on southern Vancouver Island, British Columbia, Canada (48°25' N, 123°21' W) (Figure 4.1). This area is characterized by rocky headlands, convoluted shorelines, and numerous small islands. The urban/industrial harbours of Victoria and adjacent Esquimalt, British Columbia were centrally located within the study area. These harbours support (or have supported) shipbuilding, fishing, and forestry industries, as well as other commercial and tourism activities, and have done so for over 75 years. Polyhalogenated aromatic hydrocarbon contamination has been documented in both harbours (Ikonomou et al. 2002; Knapp and Grant 2008). We limited the extent of sample collection to approximately 40 km east and west of the harbours based on estimates of coastal river otter linear home range length (20-40 km shoreline, Bowyer et al. 2003). We assumed that all individuals inhabiting that section of coastline were capable of visiting the contaminated harbours.

4.3.2 Sample collection

We conducted a preliminary latrine survey and sampling session along a limited section of coastline from December 13-18, 2005. During this initial survey, we opportunistically collected fresh faeces from latrines. The main study was conducted during June-August 2006. We surveyed the coastline by small inflatable craft and on foot. We defined active latrine sites as locations with at least 10 old otter faeces, or with new faeces. We recorded UTM coordinates of active latrines using Global Positioning Systems (GPS) and plotted them on a digital map of southern Vancouver Island using ArcView 3.3 (Environmental Systems Research Institute, Redlands, California, USA).

We calculated shoreline length surveyed by summing the linear distance between all latrine locations (91.7 km). Because we could not effectively sample the entire 91.7-km section of shoreline in one day, we divided the coastline into three areas to equalize sampling effort across the study area (Figure 1). We designated the two harbours and the connecting shoreline (39.2 km) as one area (Harbours), the outer Victoria and Oak Bay shoreline east of the harbours (34.7 km) as a second sampling area (Oak Bay), and the Colwood shoreline west of the harbours (25.1 km) as a third sampling area (Colwood). We sampled each of the three areas sequentially for a period of eight consecutive days. This process was then repeated resulting in 16 sampling occasions in each area.

To ensure we collected only newly deposited faeces, we marked all old faeces with glitter upon our initial visit to each site during each sampling period. In addition, we visited latrines in early morning to target faeces deposited overnight. We distinguished fresh faeces from old faeces based on moisture content, odour, and lack of glitter. Because river otter sign is easy to distinguish from other species, the chance of collecting non-target faeces was minimal. We collected both faecal material and gelatinous 'anal

jellies' (a mucus-like deposit expelled through the rectum, which is different from the secretions expelled through the sub-caudal glands) (Kruuk 2006). Hereafter, the term 'faeces' refers to both faecal material and anal jellies unless otherwise noted. Prior to collection, we divided each faecal deposit in two: one-half of each sample was placed in a sterile vial filled with 100% ethanol (EtOH), while the second half was collected for chemical contaminant analysis (see Chapter 2). We stored samples preserved in EtOH at 4°C before shipping to the University of Wyoming, USA for DNA genotyping.

4.3.3 DNA extraction

We extracted DNA in a designated area of the laboratory to prevent sample contamination. We used aerosol-resistant pipette tips in all procedures. Initially, we washed all faecal samples with 100% EtOH through a fine-mesh stainless steel autoclavable sieve to remove hard prey remains, foreign material, and parasites. We ensured all faecal matter passed through the wire mesh. We returned washed faecal matter to the original collection vial, resuspended it in 100% EtOH, and allowed it to settle at 4°C before DNA isolation. We then poured off excess EtOH from the vial containing the settled faecal material. We isolated genomic DNA from the faecal material by using a QIAamp DNA Stool Mini Kit (Qiagen Inc., Valencia, California, USA). We targeted river otter epithelial cells for extraction according to the manufacturer's protocol, with slight modifications (see Appendix 1). To monitor for contamination, we included a negative control (no DNA) for each batch of eight sample extractions.

4.3.4 Microsatellite genotyping

We performed DNA amplification by polymerase chain reaction (PCR) in a designated area of the laboratory to prevent sample contamination. We selected a set of eight microsatellite loci based on observed heterozygosities and number of alleles reported in the literature. These included three tetranucleotide markers (*Rio-19*, *Rio-17*, *Rio-01*) and one trinucleotide marker (*Rio-05*) developed for the North American river otter (Beheler et al. 2004; Beheler et al. 2005), and four tetranucleotide markers (*Lut-829*, *Lut-733*, *Lut-701*, *Lut-801*) developed for the Eurasian otter (Dallas and Piertney 1998). We used only tri- and tetranucleotide loci because they produce fewer stutter bands and are easier to interpret than dinucleotide loci (Dallas and Piertney 1998). We amplified microsatellite loci using a PTC-200 Peltier Thermal Cycler (MJ Research, Inc., Waltham, Massachusetts, USA), and following the protocols described in Appendices 2 and 3. We included a positive control (blood sample from a river otter with known genotype) with each plate to confirm PCR success. We resolved amplified PCR products using capillary electrophoresis on a 3130xl DNA Sequencer (ABI, Foster City, California, USA) with a formamide-Liz ladder as the internal lane size standard. We scored products manually using the software PEAK SCANNER 1.0 (ABI, Foster City, California, USA). We calibrated the genotyping process with the genetic profiles of 22 coastal river otters from southern Vancouver Island that had been legally harvested (outside the study area) by local trappers in 2005 and 2006.

4.3.5 Reliability of genotyping results

To reduce the effect of genotyping error in our data set and to test reliability of our data, we followed recommendations of previous studies implementing non-invasive

genotyping techniques. First, we strictly adhered to the comparative multiple tubes approach (see Appendix 5) for assigning consensus genotypes (Frantz et al. 2003; Hansen et al. 2008). We initially screened all samples with the three most reliable primers (*Rio-19*, *Lut-829*, and *Lut-733*) and discarded samples that did not amplify for any primer after three PCR reactions to reduce time and resources spent trying to amplify poor quality samples. For samples that passed initial genotype screening, we scored loci as heterozygous if identical alleles were recorded in two replicate PCRs, and homozygous if identical alleles were reproduced in three replicate PCRs. For ambiguous loci, we performed additional PCRs (for a maximum of seven positive PCRs) until each allele was observed at least twice before assigning a consensus genotype. We classified loci as unknown if they could not be assigned a consensus genotype after seven positive PCRs, or they failed to amplify. We discarded all samples that could not be assigned a consensus genotype at a minimum of seven loci.

Second, we computed genotyping error rates based on the final sample dataset with complete multilocus genotypes according to the formulae in Broquet and Petit (2004) and Prugh et al. (2005). We calculated two types of genotyping errors: (i) false alleles (FA), where an allele-like artefact is erroneously generated during the PCR-amplification process (Goossens et al. 2000), and (ii) allelic dropout (ADO), where one allele of a heterozygous individual fails to amplify in a PCR (Gagneux et al. 1997). We calculated the ADO rate in two different ways: (i) including only heterozygous loci because it is impossible to identify dropouts in homozygotes (referred to as the false homozygote (FH) error rate), and (ii) including both heterozygous and homozygous loci to facilitate the calculation of total error probability (Prugh et al. 2005).

Third, to ensure that we used a sufficient number of loci for individual identification, we calculated the probability of identity (P_{ID} ; the probability that two individuals drawn at random from a given population share identical genotypes at all typed loci) using GIMLET 1.3.2 (Valiere 2002). Examining an insufficient number of microsatellite loci may make close relatives within the population indistinguishable. Therefore, we calculated two theoretical values to estimate the upper and lower limits of P_{ID} . The lower limit, $P_{ID-unbiased}$, assumes a randomly mating population of unrelated individuals in Hardy-Weinberg equilibrium (Paetkau et al. 1998). The upper limit, P_{ID-sib} , assumes the population to be composed only of siblings, and should be 0.01 or lower if data are to be used for population estimation (Mills et al. 2000; Waits et al. 2001). We calculated these values for each locus and across all loci. Waits et al. (2001) showed that the true P_{ID} among the population under investigation is always between theoretical $P_{ID-unbiased}$ and P_{ID-sib} .

Finally, we used a geospatial approach to evaluate reliability of observed ‘recaptures’ in our data set (Smith et al. 2006). We examined distribution of shoreline distance between collection latrines for pairs of faeces with the same multilocus consensus genotypes and randomly chosen (with replacement) pairs of faeces. Because river otter home range in the marine environment is relatively small, the majority of faecal samples ‘recaptured’ from one individual should be within a limited area, and the distribution should be different from random. If one or more samples within a set of identical genotypes were found at the same latrine, we chose only one sample for distance analyses. For sample pairs spanning either of the harbours and/or any large bays or lagoons, we calculated the most conservative distance by assuming animals directly

crossed the feature. We used a Kolmogorov-Smirnov test to compare the mean distance between locations of all scats within a set of identical genotypes ($n = 67$) to distance between scats selected at random ($n = 67$).

4.3.6 Genetic variability

We assessed assumptions of random mating and Hardy-Weinberg equilibrium (HWE) in two ways. First, we used GENEPOP 3.4 (Raymond and Rousset 1995) to measure observed (H_O) and expected (H_E) heterozygosities, and to test for differences in these values using the Markov chain method for estimating P -values (Guo and Thompson 1992). Second, we used FSTAT 2.9.3.2 (Goudet 1995) to test the alternate hypothesis of heterozygote excess or deficit by calculating the inbreeding coefficient (F_{IS} ; Weir and Cockerham 1984). We tested for significant deviations from zero using a randomization approach in FSTAT, adjusting the P -value for multiple comparisons with standard Bonferroni procedure (Rice 1989). We also tested for linkage disequilibrium using FSTAT and Bonferroni corrections.

Before we estimated metrics of this population as if it was a single unit, we evaluated the possibility of cryptic population structure. We used the Bayesian model-based methods implemented in STRUCTURE 2.2 to assign individuals into respective genetic clusters (subpopulations) based on their genotypes at the eight microsatellite loci examined (Pritchard et al. 2000). The model accounts for the presence of Hardy-Weinberg disequilibrium by introducing population structure, and attempts to find population groupings that are not in disequilibrium (Pritchard et al. 2000). We assumed an admixture model with correlated allele frequencies (Falush et al. 2003). To estimate the number of subpopulations (K), we performed 20 independent runs of $K = 1-6$ with a

burn-in period of 100,000 followed by 100,000 Markov chain Monte Carlo (MCMC) repetitions. We determined the most probable number of subpopulations by taking the greatest mean log-likelihood of K ($L(K)$). Simulated data have shown that values of log-likelihood do not always correspond to the true number of K , therefore, we also calculated ΔK , a measure of the second order rate of change in likelihood of K (Evanno et al. 2005). We performed a final run at the inferred K (100,000 burn-in and 500,000 MCMC repetitions) and assigned individuals to a subpopulation based upon their highest proportion of membership (q ; which varies between 0-1 with one indicating full population membership). We chose a threshold value of 0.70 to assign individuals to subpopulations, which indicates that $\geq 70\%$ of ancestry can be attributed to the respective subpopulation (Latch et al. 2008).

We estimated levels of genetic diversity within each inferred subpopulation using FSTAT and GENEPOP as previously described. Population differentiation was analyzed using Wright's F_{ST} (Wright 1965). To investigate gene flow between inferred subpopulations, we estimated the effective number of migrants per generation (Nm) as $Nm = [1/(4F_{ST})] - (1/4)$. We also estimated gene flow between inferred subpopulations using BAYESASS 1.3 (Wilson and Rannala 2003). This Bayesian based program generates estimates of recent migration rates between groups in each direction. The programs default settings were selected for all parameters.

The degree of relatedness between individuals within and among subpopulation clusters is an indicator of the degree of immigration and emigration and can be used to infer local reproductive success (Avisé 2004). We calculated the coefficient of relatedness (r) between otter pairs with KINSHIP 1.2 (Queller and Goodnight 1989).

Values of r range from -1 (no alleles in common) to $+1$ (identical twins) and are calculated by comparing the percentage of shared alleles between two individuals with allele frequencies in the population (Queller and Goodnight 1989). In a randomly mating diploid population, first-degree relatives (full-siblings or parent-offspring) should have $r = 0.5$, second-degree relatives (half-siblings) should have $r = 0.25$, and pairs of randomly chosen individuals should have an $r = 0$ (Queller and Goodnight 1989). We used PERM (Duchesne et al. 2006) to compare differences between observed mean relatedness with a randomized distribution of means generated by reordering the data 1000 times. We randomly reassigned the observed r -values to the two compared groups, and calculated the proportion of randomized differences greater than or equal to the observed difference (with significance at the 5% level).

4.3.7 Population size and density

We estimated otter abundance using only the samples collected during the main summer sampling period. We estimated population parameters for the entire study area, as well as separately for each inferred subpopulation. Because summer sampling occurred over the period when young of the year emerge from birth dens (Melquist and Hornocker 1983), we derived abundance estimates for each of the summer sampling sessions separately (hereafter ‘Early’ and ‘Late’ Summer). We averaged the two estimates to obtain a single abundance estimate for the entire 12-week sampling period. To compare our results among areas (and to those reported for other river otter populations), we translated abundance estimates to density by dividing by the length of the sampled shoreline.

Because our dataset contained a high number of recaptures, we elected to estimate abundance with mark-recapture models. We obtained a single estimate for both Early and Late Summer by pooling capture results from each of the eight collection days in each zone (i.e., Colwood day one + Harbours day one + Oak Bay day one = Early Summer day one, and so on) resulting in a total of eight capture occasions per sampling session. Abundance estimates may be biased upwards if movement occurs between sampling zones, however, we considered this approach acceptable based on the high site fidelity observed throughout the study area (Figure 4.1).

We constructed a capture history for each otter by recording whether or not it was identified (i.e., captured) on each day. We analyzed data using closed-capture models of MARK (White and Burnham 1999). Because our dataset did not allow for the construction of heavily parameterized models, we restricted our analysis to four main candidate models. These models differed in capture probabilities, and included a model with constant capture and recapture probabilities (M_o), constant capture and recapture probabilities with behavioural variation (M_b), temporal variation in capture and recapture probabilities (M_t), and temporal variation in capture and recapture probabilities with behavioural variation (M_{tb}). We did not include individual heterogeneity in candidate models given the overall sparseness of the data. Candidate models were ranked and weighted using Akaike's information criterion (AICc, adjusted for small sample size) in MARK (Burnham and Anderson 2002). We used model-averaging to calculate abundance estimates and standard errors in order to reduce bias resulting from choosing the wrong model (Burnham and Anderson 2002). Over-parameterized models in which parameters were identified as singular, or standard errors of estimates that were zero or very large

were first deleted from the model set. Confidence limits (95%) for model-averaged abundance estimates were calculated while taking into account the minimum number of otters known alive (White et al. 1999).

As a comparison to the estimates produced by the mark-recapture methods, we also employed three accumulation (rarefaction) curve techniques commonly used to estimate population abundance from the faecal DNA datasets (Kohn et al. 1999; Eggert et al. 2003; and Chessel, cited in Valiere 2002). These methods assume population closure and fit a curve to the total number of unique genetic profiles versus the total number of samples typed. The asymptote of the curve represents the estimate of the population size. We obtained a single estimate for Early and Late Summer by following the same approach described for the mark-recapture analysis. Because the order in which samples are analyzed affects the curve (Kohn et al. 1999), we randomized the order of the multilocus profiles 1000 times and used the mean of all iterations as the estimate of population size. Confidence intervals, therefore, reflected variance associated with reordering individual samples and not the precision of the estimate. We used GIMLET and R (Ihaka and Gentleman 1996) for curve fitting and randomizations.

4.4 Results

4.4.1 Sample collection

We identified 86 active river otter latrines in the study area (1.07 latrines/km of linear shoreline). All latrines we identified as active in 2005 remained active in 2006. We counted 7,124 old and 2,716 new (< 24 hours old) faeces at latrine sites. Mean faecal deposition rate was 1.79 (SE = 0.21) fresh faeces/site/day, however the deposition rate

varied considerably among latrine sites (range: 0.11-9.94). We collected 91 faecal samples in 2005 and 802 in 2006 (total 893 samples).

4.4.2 Microsatellite genotyping

Of the 893 faecal samples collected, 598 (67%) yielded river otter DNA. We obtained higher yield (91%) from 2005 samples than from 2006 samples (64%). We successfully genotyped 110 (12.3%) samples at a minimum of seven microsatellite loci. Those corresponded to 49 unique individual profiles (see Appendix 4) and 61 resampled profiles (including both winter and summer samples). Of the 110 samples with complete multilocus genotypes, 87 (79%) were either anal jellies or faeces with anal jelly components. We identified 21 individuals once and 28 individuals multiple times (range: 2-7, Figure 4.1).

4.4.3 Reliability of genotyping results

We conducted an average of 3.0 ± 0.3 (SE) PCR amplifications per sample per locus. The genotyping error rates differed across microsatellite markers. The most common error observed was a false homozygote (Table 4.1). Samples that yielded complete multilocus consensus genotypes had an average per replicate per locus false homozygote error rate of 12%. The average per replicate per locus false allele error rate was 2%. Using the comparative multiple tubes approach, the probability of obtaining an incorrect multilocus genotype after replication at all eight microsatellite loci (P_{ERROR}) was 0.009 (Table 4.1). Therefore, we expect one genetic profile in the data set of 110 samples to be erroneous (or 0.9%).

Theoretical $P_{ID-unbiased}$ and P_{ID-sib} for the eight microsatellite loci were 2.35×10^{-06} and 4.95×10^{-03} , respectively (Figure 4.2). That translates to a one in 425,582 chance of two unrelated individuals in the population sharing the same multilocus genotype, and a one in 202 chance of two siblings in the population sharing the same multilocus genotype. Those results indicate that the selected battery of microsatellite loci was adequate to differentiate between individual otters in the study area (including relatives), and that all samples with identical complete multilocus genotypes likely originated from the same individual.

The distribution of distances between faecal samples with the same multilocus consensus genotype was unimodal with the majority of samples found within a short distance of each other (0-10 km; Figure 4.3a). In contrast, there was no discernable pattern in the distribution of distances between pairs of faeces chosen at random (Figure 4.3b). Mean linear distance (5.46 km; SE = 0.68; range: 0.21-24.9 km) between locations of faecal samples within a set of identical consensus genotypes was three times less than an equal number of sample pairs selected at random (16.7 km; SE = 1.27; range: 0.8-39.3 km) ($Z = 3.54$, $P < 0.05$).

4.4.4 Genetic variability

All microsatellite loci evaluated were polymorphic (Table 4.2). The mean number of alleles per locus was 4.3 (range 2-7). Tests on the overall dataset indicated a significant deviation from Hardy-Weinberg equilibrium ($P < 0.05$) due to disequilibrium at two loci (*Rio-05* and *Lut-801*; Table 4.3). F_{IS} across all loci were significantly different from zero (0.10, $P < 0.05$), indicative of a non-random allelic distribution and likely internal genetic subdivision within the local otter population (Table 4.3). No pairs of loci

exhibited linkage disequilibrium after Bonferroni correction, suggesting the markers were independent.

The STRUCTURE analysis did not reflect a panmictic population and showed evidence of genetic clustering among otters in the study area. The estimated $L(K)$ was greatest at $K = 2$ (Figure 4.4a), and the ΔK measure was strongest at $K = 2$ (Figure 4.4b). Although the ΔK method cannot validate $K = 1$, the ΔK plot would be approximately flat if no substructure exists, because there should be no K around which $L(K)$ rises substantially from $K - 1$ to K and then rises much less from K to $K + 1$ (Evanno et al. 2005). Individual assignments are summarized in Figure 4.5. We assigned most otters ($n = 38$) into one of the two inferred subpopulations. Eleven otters with assignment values below the threshold were not assigned to either of the subpopulations. Based on sample collection location, otters appear to spatially separate into two geographic regions. The majority of otters identified in Oak Bay clustered together (mean $q = 0.92$), whereas the majority of otters identified in Colwood and both Harbours clustered together (mean $q = 0.90$). Of the 49 otters identified, samples from just three were collected at latrines in both clusters. In all, 28 otters were identified in Colwood/Harbours, whereas 21 were identified in Oak Bay. We grouped the three otters identified in both clusters according to their last known location. Of the 28 otters identified in Colwood/Harbours, 20 visited Victoria Harbour (i.e., the most contaminated site in the study area) at least once, while just two of 21 otters identified in Oak Bay visited Victoria Harbour during our study period.

Otters identified in Colwood/Harbours exhibited a significant deviation from HWE ($P < 0.05$) due to disequilibrium at *Rio-05* and *Lut-801* (Table 4.3). Additionally,

we found a significant deficiency of heterozygotes in Colwood/Harbours ($F_{IS} = 0.082$, $P < 0.05$). Conversely, we observed no deviation from HWE in Oak Bay ($P > 0.05$). The estimated pairwise F_{ST} value between inferred subpopulations was 0.0511 ($P < 0.05$), indicating moderate genetic differentiation. The F_{ST} based estimate of the number of effective migrants (Nm) exchanged between inferred subpopulations per generation was 4.6. Results from BAYESASS revealed an asymmetrical migration pattern, as the average migration rate (\pm SD) from Colwood/Harbours to Oak Bay was relatively high (0.18 ± 0.06), whereas migration in the opposite direction was 0.07 ± 0.04 . Self-recruitment rates were 0.93 ± 0.04 for Colwood/Harbours and 0.82 ± 0.06 for Oak Bay.

The overall estimated degree of relatedness (r) between any pair of river otters within the study area ranged from -0.74 to 0.80 with an overall mean of -0.0004 (SD = 0.28 ; Table 4.4). Of the total pairwise relationships, 4.3% were classified as parent-offspring or full-siblings ($r \geq 0.50$). Otters identified in Colwood/Harbours were more related than otters identified in Oak Bay (randomization $P < 0.05$), with a higher percentage of pairwise relationships classified at the parent-offspring, full-sibling, or half-sibling level or closer (Table 4.4). Likewise, otters identified in Victoria Harbour were more highly related than otters in Oak Bay (Table 4.4).

4.4.5 Population size and density

The minimum number of otters identified in the study area was 32 during the Early Summer sampling period and 31 during the Late Summer sampling period. In general, we found good agreement among all methods for estimating the abundance of river otters except for Kohn's rarefaction method, which consistently generated the highest estimates (Figure 4.6). The Eggert and Chessel methods generated estimates that

were slightly higher than estimates obtained from the MARK closed-capture model set, with the main difference being the width of the confidence intervals (Figure 4.6). Estimates of abundance derived from all methods were higher in Colwood/Harbours (64.3 km) than Oak Bay (27.4 km), where a greater distance of the coastline was surveyed (Figure 4.6).

In all analyses, the best approximating model of the MARK closed-capture candidate model set included constant capture probabilities (model M_0 , Table 4.5). Model M_b , which incorporated behavioural variation in capture probability, was also reasonably supported in all analyses (Table 4.5). Both models incorporating time variation in capture probabilities received little support in all analyses (Table 4.5). The overall population size was estimated to be 48.2 with a 95% confidence interval of 34.5-55.7, or 1 otter/1.9 km of linear shoreline (95% CI = 2.7-1.6 km). We estimated a lower density of otters in Colwood/Harbours (1 otter/2.7 km; 95% CI = 3.3-1.1 km) compared to Oak Bay (1 otter/1.4 km; 95% CI = 2.0-0.8 km), although confidence intervals were overlapping.

4.5 Discussion

The microsatellite DNA data obtained from our field-collected faecal samples revealed a deviation from random mating and a genetic pattern consistent with small-scale population structure among river otters inhabiting the coast of southern Vancouver Island, British Columbia. The pattern is best explained by the separation of otters into two local geographic clusters within our study area: (i) those inhabiting both urban/industrial harbours of Victoria and Esquimalt, as well as the Colwood shoreline to the west of the harbours (Colwood/Harbours); and (ii) those inhabiting the outer Victoria and Oak Bay shoreline to the east of the harbours (Oak Bay). This division was further

supported by an asymmetrical migration rate between the inferred genetic clusters, a higher self-recruitment rate in Colwood/Harbours, a higher relatedness among individuals in Colwood/Harbours, and nearly a 50% lower density of otters in Colwood/Harbours. In concert, these data suggest that reproduction occurs along the contaminated coastline, but the Colwood/Harbours area may constitute sub-optimal habitat for river otters compared to Oak Bay.

We base our conclusions on data obtained from non-invasive faecal DNA analyses. For this technique to be an effective tool for estimating river otter population parameters, results must be reliable and accurate. We reduced the effect of genetic errors by targeting tri- and tetranucleotide microsatellite markers, employing a comparative multiple-tubes approach (Frantz et al. 2003; Hansen et al. 2008), and manually verifying genotype results. Consequently, the proportion of faecal samples for which we obtained a complete genotype (12.3%) was below the range (20-65%) reported in other otter faecal DNA studies (Dallas et al. 2003; Hung et al. 2004; Kalz et al. 2006; Prigioni et al. 2006; Arrendal et al. 2007). Nonetheless, by following a strict genotyping protocol and eliminating ambiguous samples from the data set, we obtained a probability of identity low enough to differentiate among all individuals, as well as a low overall genotyping error rate, yielding negligible bias. The close proximity of samples representing recaptures (90% < 11.5 km) provided further assurance that our final data set was relatively error free (Smith et al. 2006).

In spite of the relatively small geographical distance sampled (91.7 km), our results indicated that otters in the study area did not constitute a single panmictic population. We observed slight to moderate differentiation between the two inferred

subpopulations with limited gene flow. Genetic differentiation at such a small spatial scale is surprising given the dispersal capabilities of otters inhabiting the marine environment (60 km, Blundell et al. 2002). According to the one-migrant per-generation rule (Wright 1931), the estimated effective migration rate ($Nm = 4.6$) suggests that there still exists sufficient genetic exchange between the inferred subpopulations to counteract genetic drift. However, more recent analyses have suggested that 5-10 migrants per generation are required to conserve the genetic diversity of many animal populations (Mills and Allendorf 1996), and our estimates of recent migration rates and pairwise relatedness values indicate a possible dispersal bias. For instance, a greater number of effective migrants per generation was detected from Colwood/Harbours to Oak Bay than in the opposite direction, and the proportion of self-recruitment (or retention of individuals) in Colwood/Harbours was higher than Oak Bay. Thus, asymmetrical migration rates may be important to population substructuring in the study area. If the migration rate is low, individuals from the same subpopulations are likely to be more related than individuals from different subpopulations (Gandon 1999), as we observed in Colwood/Harbours. In contrast, otters identified in Oak Bay exhibited low levels of pairwise relatedness, which is indicative of a demographically open population with balanced migration rates, likely with nearby unsampled populations (e.g., Gulf Islands, British Columbia and San Juan Islands, Washington).

By using the DNA microsatellite profiles as individual ‘genetic tags’, we obtained estimates of abundance that were highly comparable among several different analytical techniques previously used with non-invasive genetic sampling (Lukacs and Burnham 2005). The concordance among methods, with the exception of Kohn’s rarefaction

equation, suggests that our estimates are reliable and accurate. Several recent studies have also found Kohn's method to generate highly biased estimates of abundance (Eggert et al. 2003; Wilson et al. 2003; Frantz and Roper 2006; Petit and Valiere 2006). The Eggert and Chessel rarefaction equations generated similar but slightly higher abundance estimates than those obtained from the closed-population mark-recapture estimator. Contrary to the mark-recapture models, however, these estimates were surrounded by very narrow confidence limits. Simulation studies have shown that confidence intervals generated from the Eggert and Chessel methods fail to encompass the true parametric value of abundance in most cases (Petit and Valiere 2006). This is likely because rarefaction indices assume that capture probabilities are constant over time and equal among all individuals (Lukacs and Burnham 2005). In contrast, our mark-recapture candidate model set accounted for possible variation in capture probability, hence the wider confidence limits surrounding the weighted-average estimates obtained from this method. Because of the potential for variation in capture probability, we think that the estimates generated from the mark-recapture estimator are the most reliable. Our resulting estimates of density provide further support for the apparent population subdivision in the study area because a lower density of otters was detected in Colwood/Harbours (1 otter/2.7 km) compared to Oak Bay (1 otter/1.4 km). It is important to stress, however, that if heterogeneity in capture probability among individuals exists, estimates might be biased low. Nevertheless, other otter studies have found that faecal deposition is unbiased by sex and age class (Kruuk 1992; Dallas et al. 2003), and otter pups have been observed defecating at latrine sites during this study (D.A. Guertin, *personal observations*).

The harbours of Victoria and Esquimalt have been industrially active for several decades and are a significant point source of environmental contaminants, including PCBs (Ikonomou et al. 2002; Knapp and Grant 2008). Thus, river otters inhabiting the harbours have been chronically exposed to PCB contamination, as reflected in the consistently elevated PCB concentrations reported in faeces measured over an eight-year monitoring period (Elliott et al. 2008) (see Chapter 3). Contaminated sites often constitute functionally suboptimal habitat 'sinks' (i.e., lower production and persistence of individuals) that attract dispersing animals from less contaminated habitats (Pulliam 1988; Dias 1996; Delibes et al. 2001). If the Colwood/Harbours area acts as population sink compared to Oak Bay, we would expect that area to have a higher immigration rate and a lower self-recruitment rate, resulting in a low degree of pairwise relatedness among individuals due to a lack of local reproduction. However, the trends we observed (asymmetrical migration, high self-recruitment, and high relatedness) are not consistent with that hypothesis. Rather, our results indicate that reproduction is occurring in Colwood/Harbours, but that a large proportion of otters born there emigrate once they reach independence, while relatively few immigrate to this area (at least from Oak Bay).

Some observations, however, are in line with the hypothesis of contamination-related effects. For instance, exposure to high PCB concentrations (particularly in Victoria Harbour) may reduce the effective population size (i.e., the number of breeding females) in Colwood/Harbours, exacerbating the effects of inbreeding and asymmetrical migration, and thereby resulting in higher relatedness and lower genetic variability than Oak Bay. In addition, population density is 50% lower in Colwood/Harbours than Oak Bay, and low population densities of Eurasian otters have correlated well with high PCB

exposure across Europe (Smit et al. 1994). Nevertheless, the genetic variability observed for both inferred subpopulations is similar to that reported for healthy river otter populations in Alaska and Louisiana (Blundell et al. 2002; Latch et al. 2008). Furthermore, density estimates in both areas are similar to those reported in coastal Alaska (1 otter/1.9-2.1 km, Larsen 1983; 1 otter/1.18 km, Woolington 1984; 1 otter/1.25-3.57 km, Testa et al. 1994; 1 otter/ 1.5 km, Bowyer et al. 2003). High PCB levels have also been reported in a relatively dense population of Eurasian otters inhabiting coastal Shetland, Scotland (1 otter/1.2 km, Kruuk et al. 1989), with no evidence of a population-level effect (Kruuk and Conroy 1996).

Alternatively, the genetic variation, spatial organization, and demographic characteristics of the inferred subpopulations might be related to geography and human activities that are unrelated to contamination. For example, Whitehead et al. (2003) found small-scale structuring among geographically proximate fish populations and concluded that watershed geography rather than contaminant exposure history accounted for significant partitioning of genetic variation among these populations. In our study area, there are no obvious physical barriers at the landscape level to restrict coastal river otter movement. Genetic variation and differentiation among river otters in the absence of physical barriers was also documented in Alaska (Blundell et al. 2002), and Louisiana (Latch et al. 2008). However, in those studies, isolation occurred over a broader geographical scale. Nonetheless, river otters inhabiting marine environments generally confine their movements and activities to the shoreline (Larsen 1983; Woolington 1984), and in our study area, that travel corridor is bisected by both natural and anthropogenic obstacles that could limit otter movement and hence gene flow.

One possible explanation for the observed substructure in our study area may be that local habitat heterogeneity encourages otters to coalesce in distinct groups. Habitat selection studies in coastal Alaska revealed that river otters prefer rugged shorelines of bedrock with short and steep intertidal zones (Bowyer et al. 2003), likely related to higher fish prey diversity and biomass (Ben-David et al. 1996). Those habitat features characterize approximately 68% km of the Oak Bay shoreline, which supported nearly twice the density of otters. In contrast, rocky intertidal zones characterize just 29% of the Colwood/Harbours coastline, whereas silt/gravel intertidal flats, fractured rock rubble, and constructed seawall dominate the nearshore of that area. Coastal river otters tend to avoid those habitat types because they generally support a lower abundance of fish prey (Ben-David et al. 1996). Indeed, we found a higher frequency of occurrence of secondary prey items (e.g., crustaceans) in otter faeces collected from Colwood/Harbours, which suggests an overall lower abundance of fish prey in that area (see Chapter 4).

However, given the lack of a 'hard' landscape-level barrier to otter movement, it seems unlikely that the genetic patterns observed could solely result from localized habitat discontinuity. Alternatively, a second explanation is that human development and disturbance to the landscape is responsible for the observed differentiation. The two inferred subpopulations appear to be divided by a major coastal metropolitan area, which may influence river otter movement. For example, Hung et al. (2004) found no evidence of Eurasian otters moving between two rivers separated by just 2 km of urban development. Similarly, Kalz et al. (2006) reported small-scale genetic differentiation among Eurasian otters inhabiting a watershed fragmented by agricultural fields and a major highway. Thus, river otters not habituated to the urban setting of Victoria and

Esquimalt Harbour may avoid those areas, and hence, human-induced changes to the landscape may restrict genetic exchange along the coast.

A third explanation for the observed patterns involves an interaction between the previously mentioned natural and anthropogenic factors, with recent human-induced changes to the landscape superimposed upon a coastline that was already unfavourable for otters. Thus, otter population density could be a function of habitat quality (i.e., a carrying capacity), while coastal development and human disturbance may restrict gene flow. Additional sampling and/or radio-tracking studies in this area could further elucidate the relative effects of, and interactions between, natural and anthropogenic barriers with respect to individual movements and genetic structuring of river otters.

In summary, our study illustrates that non-invasive faecal DNA genotyping offers a valuable and effective tool for studying complicated population processes in river otters, including effects of pollutants. Regardless of the mechanism, our study suggests that river otters do not use the coast of southern Vancouver Island, British Columbia uniformly. We found evidence of limited movement of otters between the two inferred subpopulations during the study period. Although location and movement data are based on a relatively small sample size obtained from just a half a year of sampling, our population delineation is an emergent property of years of dispersal and breeding, which implies that gene flow is (and had been) restricted for an extended period of time. Nonetheless, there is evidence of reproduction and persistence in the area associated with high PCB contamination. Therefore, at present, there is no conclusive evidence that the genetic and demographic patterns observed are directly related to the higher PCB exposure in the urban/industrial harbours of southern Vancouver Island. However, we

caution that because of an asymmetrical migration rate and a lower population density associated with the harbours, the local presence of river otters in that area may be sensitive to additional perturbations to the ecosystem (i.e., coastal development, shoreline modifications, infectious and parasitic disease, poisoning with other pollutants, and the like). Therefore, we recommend investigating temporal stability of both genetic and demographic population-level patterns, continued biomonitoring, and further studies involving other contaminated sites to gain a more complete understanding of the impact of pollution and other stressors on river otter population metrics. Non-invasive faecal sampling offers a valuable and effective tool for such analyses.

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Table 4.1 Summary of genotyping error rates observed in river otter faeces collected from the coast of southern Vancouver Island, British Columbia, Canada (2005-2006). Data are from 110 samples analyzed at eight microsatellite loci. FA is the false allele error rate, ADO is the allelic dropout rate, FH is the false homozygote error rate, and P_{ERROR} is the total estimated error rate when homozygotes were replicated three times and heterozygotes were replicated twice (after Prugh et al. 2005).

Locus	Genotyping error rates					
	(FA)	(ADO)	(FH)	(FA) ²	(ADO) ³	P_{ERROR}
<i>Lut-829</i>	0.023	0.077	0.092	0.001	0.001	0.002
<i>Rio-05</i>	0.033	0.092	0.140	0.001	0.001	0.004
<i>Rio-01</i>	0.055	0.092	0.122	0.003	0.001	0.005
<i>Rio-19</i>	0.032	0.043	0.110	0.001	0.000	0.002
<i>Lut-701</i>	0.011	0.057	0.131	0.000	0.000	0.001
<i>Lut-733</i>	0.020	0.055	0.165	0.000	0.000	0.001
<i>Lut-801</i>	0.007	0.015	0.044	0.000	0.000	0.000
<i>Rio-17</i>	0.008	0.060	0.220	0.000	0.000	0.000
Overall	0.024	0.064	0.122	0.006	0.003	0.009

Table 4.2 Polymorphism characteristics of eight microsatellite loci amplified in river otters from the coast of southern Vancouver Island, British Columbia, Canada (2005-2006). Sample size of individual otters (n), total number of alleles found per locus (N_A), and the size of the smallest and largest allele found in base pairs (bp) are shown.

Locus	n	N_A	Range (bp)
<i>Lut-829</i>	49	6	228 - 248
<i>Rio-05</i>	49	8	318 - 351
<i>Rio-01</i>	49	5	273 - 292
<i>Rio-19</i>	49	5	272 - 288
<i>Lut-701</i>	49	3	202 - 210
<i>Lut-733</i>	49	3	172 - 180
<i>Lut-801</i>	48	2	228 - 232
<i>Rio-17</i>	49	3	171 - 177

Table 4.3 Genetic diversity for river otters along the coast of southern Vancouver Island, British Columbia, Canada (2005-2006). Sample size of individual otters (n), mean number of alleles (A), observed (H_O) and expected (H_E) heterozygosities, and inbreeding coefficient (F_{IS}) are shown.

	n	A	H_O	H_E	F_{IS}
Overall	49	4.3	0.52 ^a	0.57	0.10 ^b
Colwood/Harbours [‡]	28	4.0	0.46 ^a	0.52	0.13 ^b
Oak Bay [‡]	21	4.3	0.60	0.61	0.01

^a Significantly different from the expected heterozygosity value.

^b Significantly different from zero.

[‡] Individuals assigned to area according to last known location.

Table 4.4 Pairwise relatedness (r) of river otters (n) along the coast of southern Vancouver Island, British Columbia, Canada (2005-2006). Percentage of pairwise relationships classified as parent-offspring or full-siblings ($r = 0.5$), or half-siblings ($r = 0.25$) by KINSHIP is also shown.

	n	Coefficient of relatedness (r)				
		Mean [†]	SD [†]	Range	% ≥ 0.5	% ≥ 0.25
Overall	49	0.0004	0.28	-0.74 - 0.80	4.3	19.2
Oak Bay [‡]	21	-0.03	0.30	-0.65 - 0.67	4.2	19.0
Colwood/Harbours [‡]	28	0.11 ^a	0.27	-0.55 - 0.80	9.0	29.6
Victoria Harbour [‡]	26	0.11 ^a	0.28	-0.46 - 0.74	10.0	30.1

[†] Calculated over all pairwise comparisons, with the number of comparisons equal to $n(n-1)/2$.

[‡] Individuals assigned to area according to last known location.

^a Mean r greater than Oak Bay otters (randomization $P < 0.05$).

Table 4.5 Closed-capture candidate models for estimating river otter abundance and density along the coast of southern Vancouver Island, British Columbia, Canada, during summer 2006. Models were ranked according to Akaike's Information Criteria (AIC) in MARK. The number of parameters (K), $\Delta AICc$ value, and $\Delta AICc$ weight (w) for each model for each area during each sampling period is shown. See text for description of models.

	Model	K	Early Summer 2006		Late Summer 2006	
			$\Delta AICc$	$AICc$ w	$\Delta AICc$	$AICc$ w
Overall	M_o	2	0.00	0.58	0.00	0.62
	M_b	3	0.07	0.42	1.19	0.34
	M_t	9	11.5	0.00	6.11	0.03
	M_{tb}	15	14.6	0.00	8.31	0.01
Colwood/Hbs	M_o	2	0.00	0.66	0.00	0.66
	M_b	3	1.31	0.34	1.38	0.33
	M_t	9	13.3	0.00	11.4	0.00
	M_{tb}	15	14.7	0.00	17.6	0.00
Oak Bay	M_o	2	0.00	0.70	0.00	0.69
	M_b	3	2.02	0.26	1.97	0.26
	M_t	9	5.79	0.04	5.47	0.04
	M_{tb}	15	10.6	0.00	8.47	0.01

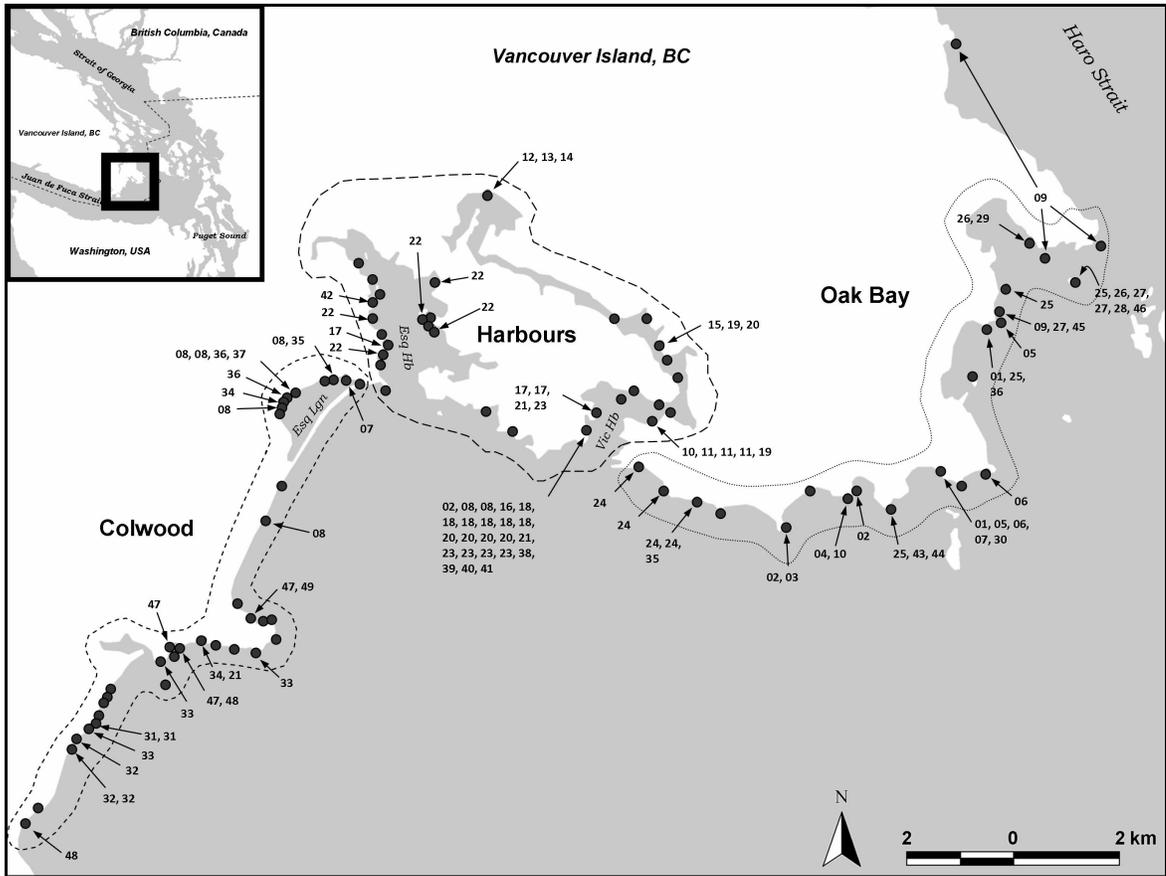


Figure 4.1 Location of active river otter latrine sites (circles) along the coast of southern Vancouver Island, British Columbia, Canada where fresh faeces were collected for DNA genotyping (2005-2006). The different dashed lines outline designated sampling areas in 2006. These areas had not been established in 2005, and faeces were opportunistically collected from latrines located in a portion of each sampling area during that initial survey. Unique otters identified by faecal DNA genotyping are shown (1-49). Numbers displayed more than once indicate ‘recaptures’. Vic Hb = Victoria Harbour, Esq Hb = Esquimalt Harbour, Esq Lgn = Esquimalt Lagoon.

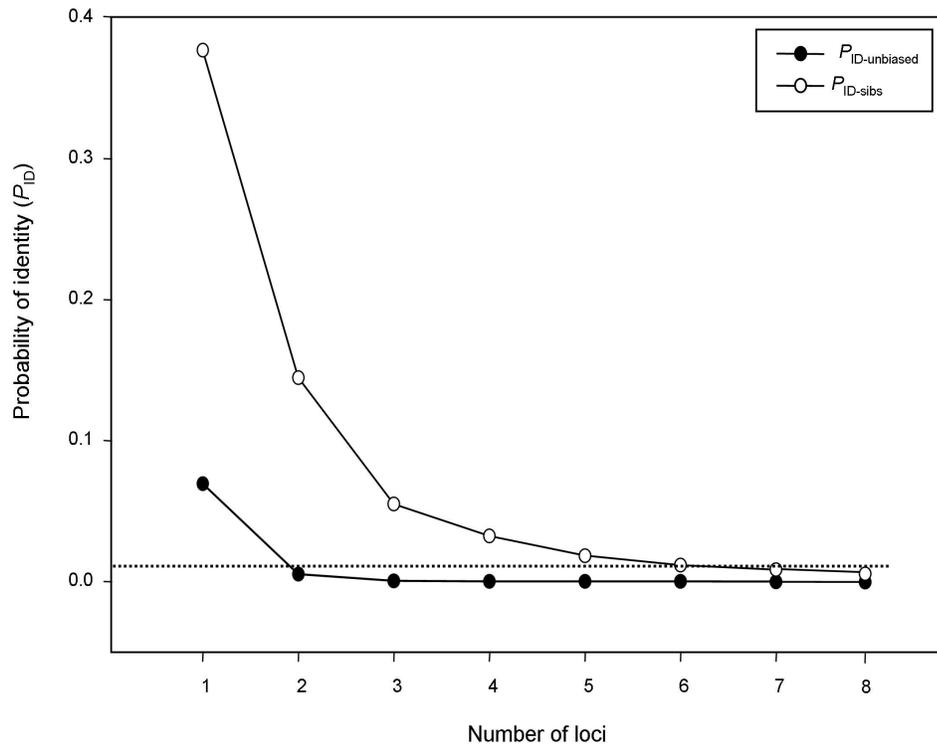


Figure 4.2 The cumulative probability of identity (P_{ID}) values based on the eight microsatellite loci used in this study for randomly chosen unrelated individuals ($P_{ID-unbiased}$) and siblings (P_{ID-sib}). The dashed line identifies a $P_{ID} \leq 0.01$ (sufficient for population size estimation) (Mills et al. 2000). Loci are ordered from least to most informative.

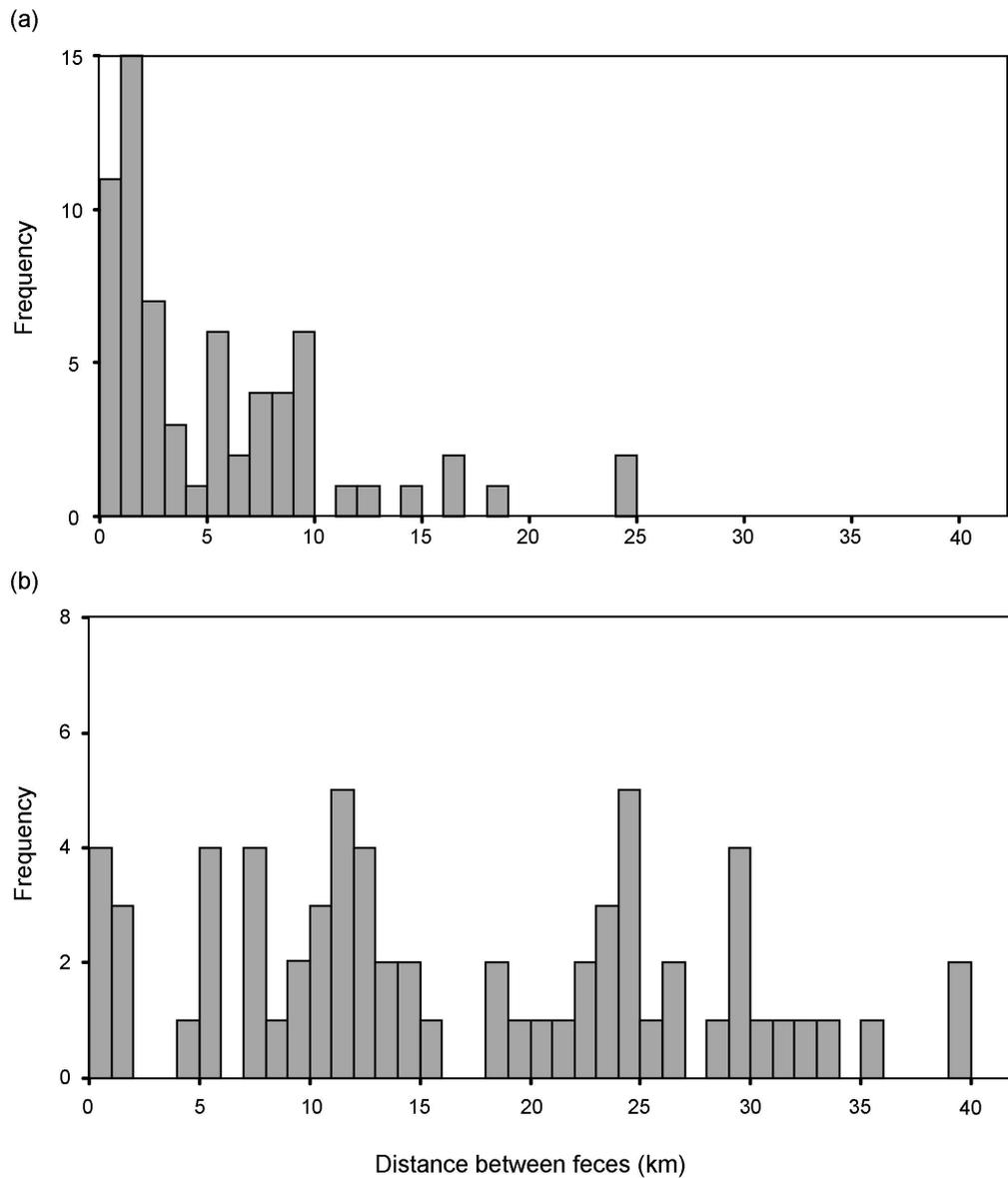


Figure 4.3 Distances between collection location for pairs of river otter faecal samples (a) with the same multilocus consensus genotype ($n = 67$), and (b) chosen at random ($n = 67$) along the coast of southern Vancouver Island, British Columbia, Canada (2005-2006).

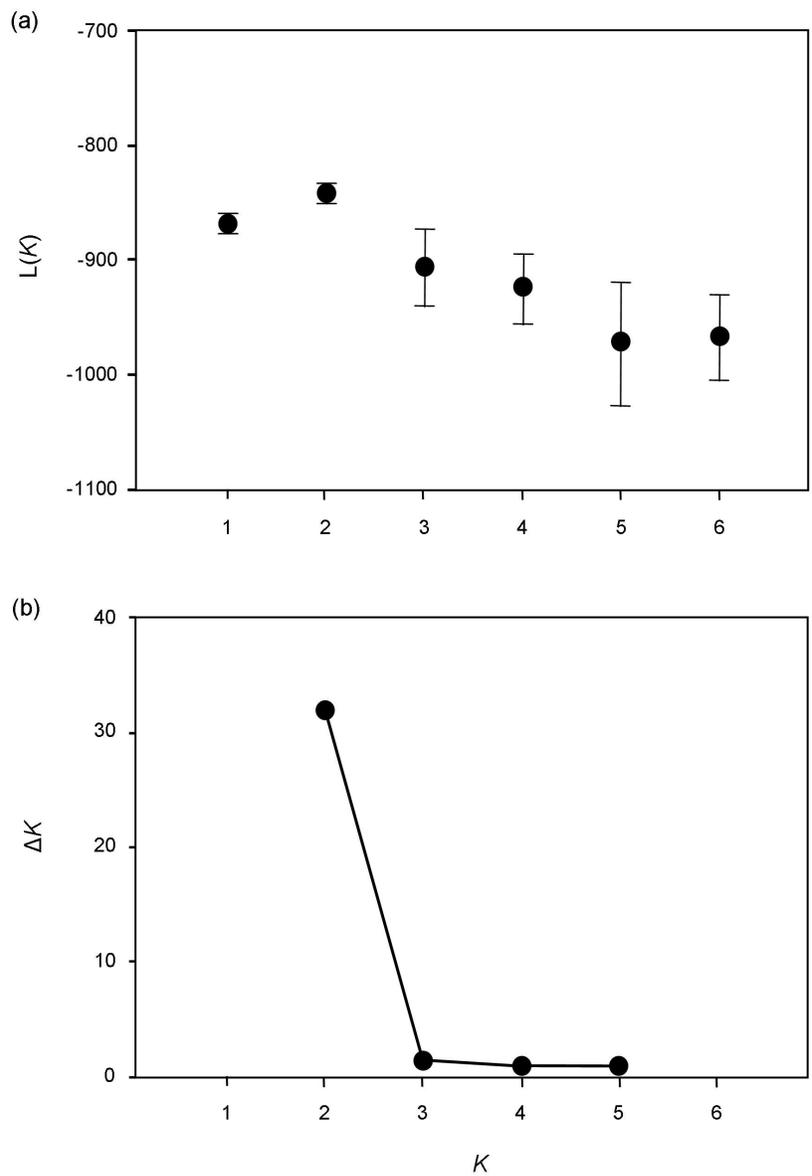


Figure 4.4 Plot of (a) mean log-likelihood ($L(K)$) values ($\pm 95\%$ CI, averaged across 20 runs), and (b) estimate of ΔK for each possible value of K using data obtained from STRUCTURE.

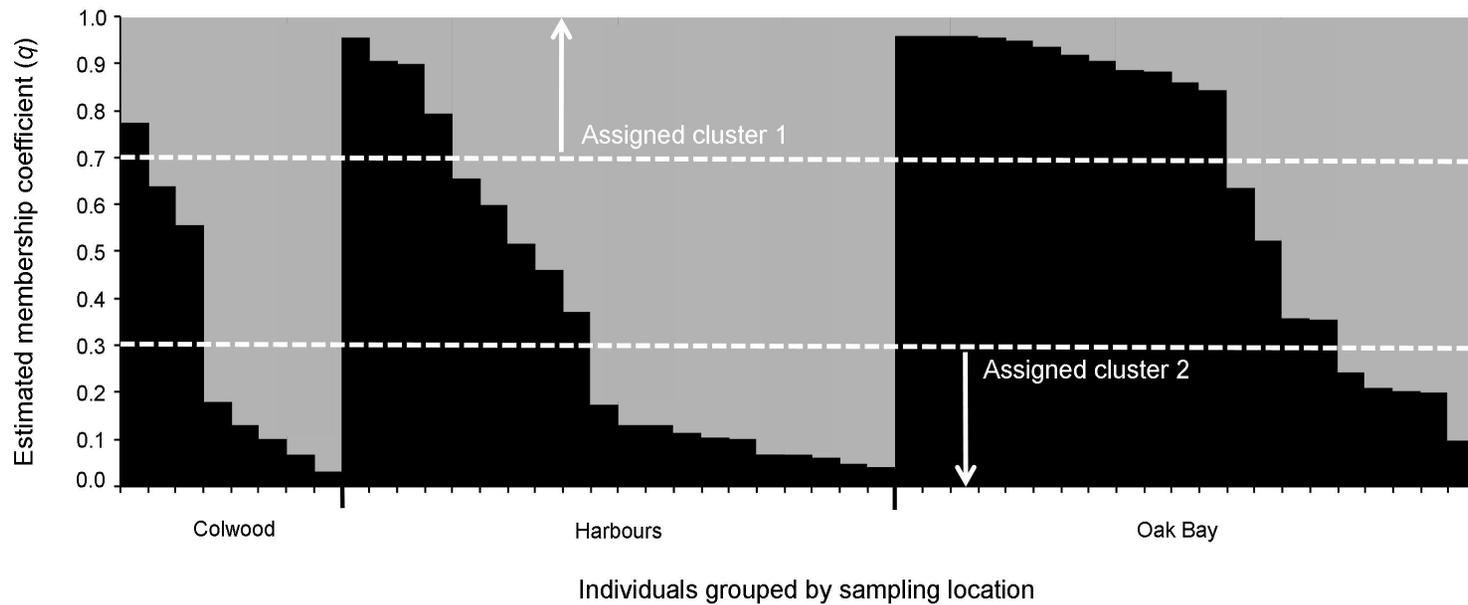


Figure 4.5 Posterior probability assignments of river otters to two genetic clusters inferred by STRUCTURE along the coast of southern Vancouver Island, British Columbia, Canada (2005-2006). Each otter is represented by a vertical bar fragmented in K sections of specific length (black and gray), according to their membership proportion (q) in both genetic clusters. Individuals with black bars ≥ 0.70 were assigned to cluster one (Oak Bay). Individuals with black bars ≤ 0.3 (gray bar ≥ 0.70) were assigned to cluster two (Colwood/Harbours). Individuals with black bars between 0.3 and 0.7 were unassigned.

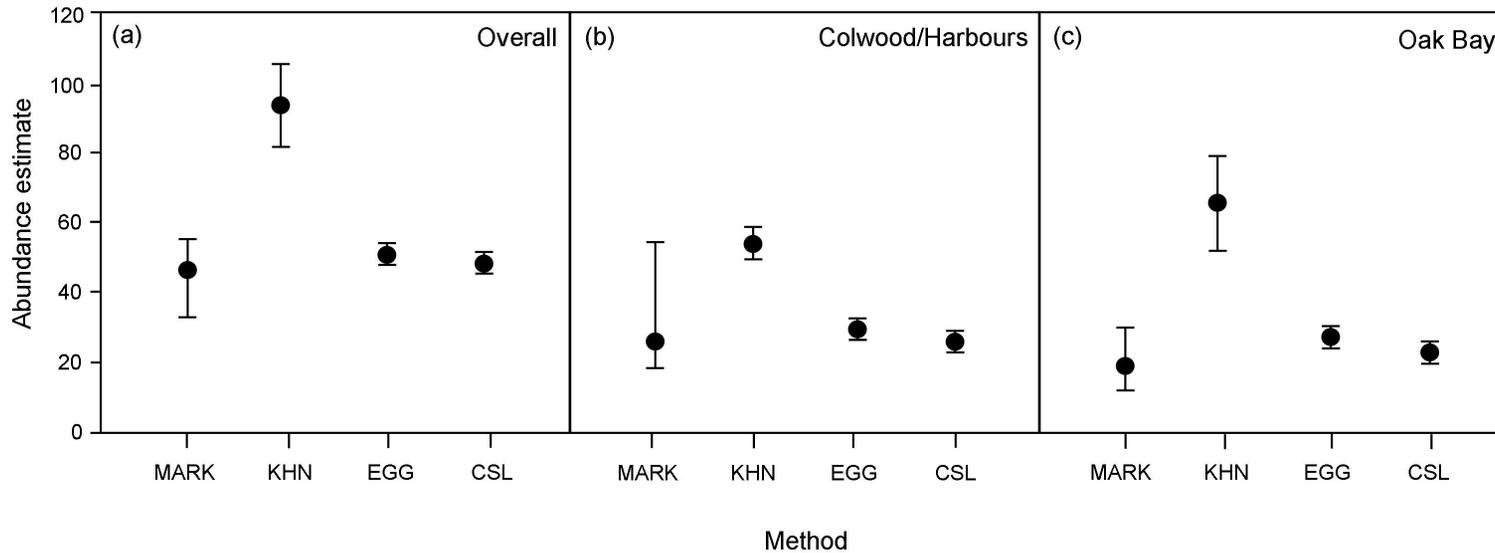


Figure 4.6 Estimates of river otter abundance (\pm 95% CI) along the coast of southern Vancouver Island, British Columbia, Canada (2006). Estimates were generated using closed-capture mark-recapture models implemented in MARK (MRK), as well as three common rarefaction curve equations (Kohn – KHN; Eggert – EGG; and Chessel – CSL). Estimates were obtained for (a) the overall study area, (b) Colwood/Harbours, and (c) Oak Bay.

CHAPTER 5
GENERAL CONCLUSIONS

5.1 Summary and Future Directions

Semi-aquatic, piscivorous mammals, especially river otters (*Lontra canadensis*), are ideal sentinel species to characterize effects of contaminants on ecosystem health (Bowyer et al. 2003). However, obtaining information on river otter populations has been a long-standing logistical problem because they are secretive, difficult to capture, and if radio-tagged, expensive to monitor (Blundell et al. 1999; Bowyer et al. 2003). Nevertheless, the ability to deduce information on animal populations (particularly mammals) from the analysis of field-collected faeces is an integral part of wildlife conservation and management (Putman 1984; Kohn and Wayne 1997). These methods offer advantages over more direct techniques in that they are logistically simple and non-invasive to wildlife populations. Consequently, faecal sampling has been widely used to study various aspects of river otter ecology, including determining food habits (Larsen 1984, Stenson et al. 1984, Ben-David et al. 2005), and more recently, as a source of genomic DNA for individual identification and population genetic and demographic analyses (Dallas et al. 2003, Hung et al. 2004, Kalz et al. 2006; Prigioni et al. 2006). Faeces are also valuable for monitoring exposure of otter populations to environmental pollutants at both spatially broad and site-specific scales (Mason et al. 1992, Mason and Macdonald 1993, Elliott et al. 2008). Hence, integrating these analyses may provide valuable insight to contaminant studies.

The main objective of my thesis was to apply non-invasive faecal sampling techniques to questions relating to river otter ecology, with particular emphasis on the population of river otters in the Victoria region of southern Vancouver Island, British

Columbia, Canada, an area of known environmental contamination. Specifically, I aimed to: (i) identify individual otters and investigate patterns of contaminant exposure, (ii) identify the principal prey species of river otters in the study area, and (iii) estimate the genetic diversity and population size of otters inhabiting the region.

Prior to my study, a survey of harbour and industrial sites in the Georgia Basin, was conducted by Elliott et al. (2008), who reported that mean concentrations of polychlorinated biphenyls (PCBs) in otter faeces collected from Victoria Harbour, British Columbia were above criteria for reproductive toxicity based on the Eurasian otter (*Lutra lutra*) (Mason et al. 1992; Mason and Macdonald 1993). However, the geographical extent of the contamination, factors contributing to contaminant concentrations in river otters, and its effects on the river otter population were unknown. Therefore, in Chapter 2, we combined faecal DNA genotyping with contaminant residue analyses of the same faeces to investigate exposure of individual river otters to environmental contaminants in the study area. Using that approach, a number of different individuals were sampled several times, and across a range of ecological conditions. We found that river otter exposure to organochlorine (OC) pesticides was low throughout the study area. However, concentrations of total-PCBs varied by sampling location, with the highest levels recorded in faeces collected in both industrial harbours of the region.

We chose to focus on quantifying total-PCBs in this analysis because the majority of studies using faeces to investigate contaminant exposure in natural otter populations have been conducted using that approach. Therefore, to assess the toxicological significance of total-PCB concentrations in river otter faeces, we compared our results to a hierarchy of effect levels established for Eurasian otter faeces based on reproductive

toxicity in the closely related mink (Mason et al. 1992; Mason and Macdonald 1993). We found that the geometric mean concentration of total-PCBs in faecal samples collected from Victoria Harbour exceeded the threshold for reproductive impairment and population-level effects according to the back-calculation of a one-compartment bioaccumulation model developed for the Eurasian otter (Mason and Macdonald 1993). However, we also found that an individual's faecal PCB levels can vary with movements to and from areas of high- and low-contamination. Hence, our findings indicate that the potential effects of elevated PCB levels in the industrial harbours are not strictly limited to otters that are permanent residents of the harbours, but also extend to individuals that periodically use the contaminated harbour systems. However, caution is appropriate when faecal PCB levels are extrapolated to otter tissue levels, because tissue levels are likely to be related to the size and location of an individual's home range, as well as the time an individual spends foraging at contaminated sites; neither of which were quantified in this analysis.

In Chapter 3, we used prey items recovered from field-collected faecal samples to describe the composition of the otter diet in the study area. We analyzed faeces and determined the frequency of occurrence of each different prey species or genera. We found that intertidal and subtidal fish species such as gunnels (Pholidae), sculpins (Cottidae), pricklebacks (Stichaeidae), and clingfish (Gobiesocidae) dominated the diet of river otters throughout the study area, although the proportional representation of particular prey taxa varied based on foraging location. We also found that otters inside the harbours consumed a higher proportion of upper trophic level prey species, which may be a factor contributing to the higher contaminant concentrations measured in faecal

samples collected inside the harbours compared to outside during the time of our study. Because contaminant residue concentrations measured in river otter faecal samples are largely derived from concentrations in recently ingested prey, we suggest measuring contaminant residue concentrations in the main prey items identified in our diet analysis to better characterize river otter dietary exposure to environmental contaminants. Smit et al. (1996) established an advanced bioaccumulation model to estimate PCB levels in otter tissue from prey levels. Using that approach, it may be possible to validate the use of faecal PCB levels to predict otter tissue levels in a particular area of concern (Mason et al. 1992; Mason and Macdonald 1993). Furthermore, concentrations of contaminants in river otter prey, faeces, and tissue samples from the sampling area can be used to elucidate food chain accumulation and biomagnification, as well as to examine the capacity of river otters to metabolize various compounds.

Chapter 4 illustrates the utility of non-invasive faecal sampling and individual identification as an approach for collecting population data on river otters. An added benefit of identifying individuals genetically is that the resulting genetic database can provide information for analyses of genetic variation, population structure, migration rates, and genetic relatedness. The 110 useful faecal DNA extracts obtained in our analysis were attributed to 49 unique individuals. Using those genetic profiles and population genetics analyses, we identified moderate genetic differentiation and support for population substructure among river otters in the study area. Otters identified in the harbours and along the shoreline to the west of the harbours constituted one subpopulation, whereas otters identified along the shoreline to the east of the harbours constituted the other. We then used individual DNA profiles as ‘genetic tags’ to estimate

abundance and density of otters in the two areas. Mark-recapture modeling estimated a nearly 50% lower density of otters inhabiting the harbours and the shoreline to the west compared to the eastern shoreline. This difference is consistent with the hypothesis of contaminant induced population-level effects, but also the habitat type and the degree of coastal development varied greatly. Hence, the difference in density cannot be attributed to contaminants alone. Additionally, pairwise relatedness values and migration rates indicated successful reproduction among otters inhabiting the contaminated area. Therefore, our results provide no conclusive evidence that PCB levels in the harbours adversely affect population performance.

It is unclear why otters in the region cluster into two local subpopulations. Perhaps natural habitat heterogeneity and/or anthropogenic changes to the landscape contribute to the genetic and demographic patterns that we detected. Regardless of the mechanism, because of an asymmetrical migration rate and a relatively low local density of otters associated with the urban/industrial harbours, river otters inhabiting those areas may be particularly vulnerable to additional landscape change induced by anthropogenic activities (e.g., coastal development, shoreline modifications, disease transmission, additional pollutant discharge, and the like). Faecal sampling and DNA genotyping over a larger geographical scale combined with live-capture and radio-tracking studies could further elucidate the relative effects of natural and anthropogenic disturbances on population metrics, as well as provide important insights into factors driving population dynamics in the region, such as home-range size, movement patterns, and post-natal dispersal.

5.2 Management Implications

The research presented in my thesis makes several contributions to the ecology and conservation of North American river otters. This study demonstrates that faecal sampling can be used effectively to reveal contaminant exposure, movements, diet, abundance, spatial organization, migration rates, and genetic relatedness in natural river otter populations. In addition, this study provides the first empirically derived estimate of population size and density for river otters in British Columbia based on statistical mark-recapture modeling techniques. Although river otter populations in British Columbia are considered stable (Hatler and Beal 2006), periodic monitoring across the province is essential to ensure that any significant reverse of this situation does not go undetected. Although too costly to conduct on a provincial-wide scale, the methods described here may be useful to study populations inhabiting specific areas of concern, or in validating other index-based measures of abundance that can be used over larger areas (i.e., observation, sign, and track surveys).

It is clear from our analyses that river otters do not use the landscape uniformly, and that habitat use could play an important role in small-scale population structuring. Therefore, we recommend that wildlife managers consider the possibility of small-scale genetic differentiation among river otters when setting future management strategies (particularly in fragmented and/or urbanized environments) to minimize the loss of genetic variation and to ensure stable, harvestable populations. Non-invasive faecal sampling provides a relatively fast and easy means of addressing questions regarding such population processes. Furthermore, future studies using river otters as bioindicators of water quality are expected to emerge following the various reintroduction and

restocking projects undertaken across North America over the last several decades (Raesly 2001, Melquist et al. 2003). Continued monitoring is therefore essential to evaluate the success of those projects, and to ensure long-term population persistence. Thus, information gained from faecal sampling (including population genetic and demographic estimates) may be useful in designing appropriate management and conservation strategies for restored populations.

In conclusion, the unique contribution of individual-based and population-level studies, such as the ones described in my thesis, can help to understand population processes and responses to environmental pollution. We successfully linked the use of faecal sampling for contaminant analyses, dietary analysis, and DNA genotyping to study the ecology and behaviour of river otters along the coast of southern Vancouver Island. To our knowledge, such an integration of disciplines to investigate contaminant exposure and population-level effects is rare. Our non-invasive study design allowed us to sample and re-sample individual river otters from the area of concern while avoiding the challenges associated with capture and handling of study animals. Therefore, we recommend this approach for monitoring river otters, as well as other elusive species in which a large number of fresh faecal samples can be obtained easily in the field.

5.3 References

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APPENDICES

Appendix 1: Faecal DNA Extraction Protocol Using QIAamp DNA Stool Mini Kit.

1. Arrange all 50 mL collection vials containing faecal samples in ascending order.
2. Take each tube in turn. Pour off excess EtOH from 50 mL sample vial.
3. Trim approximately 2 cm off a 1000 μ L pipette tip with a pair of sterile scissors.
4. Pipette approximately 500 μ L faeces into a 2 mL microcentrifuge tube (careful not to touch the sides of the sample vial).
5. Centrifuge tubes briefly at 5,000 rpm to pellet stool particles.
6. Pour off excess EtOH, there should be approximately 250-300 μ L of faeces remaining in the tube.
7. With a 1000 μ L pipette tip, add 1.6 mL Buffer ASL to each sample.
8. Vortex continuously for 1 minute or until the faecal sample is thoroughly resuspended and homogenized.
9. Spin tubes at 13,200 rpm for 1.5 minutes to pellet the faecal particles.
10. Pipette 1.4 mL of the lysis supernatant into a new 2 mL safe-lock microcentrifuge tube and discard the pellet.
11. Add one InhibitEX tablet to each sample and vortex immediately and continuously for 1 minute or until the tablet is completely suspended.
12. Let sit at room temperature for 1 minute.
13. Spin tubes at 13,200 rpm for 7 minutes.
14. Immediately transfer all supernatant into a new 1.5 mL tube and discard the pellet.
15. Spin tubes at 13,200 rpm for 7 minutes.
16. Pipette 25 μ L of Proteinase K into a new 2 mL microcentrifuge tube.
17. Pipette 600 μ L of supernatant from step 15 to the new 2 mL tube containing Proteinase K.
18. Add 600 μ L Buffer AL and vortex for 15 seconds to mix thoroughly.
19. Incubate in heat block at 70°C for 10 minutes, and then spin for 0.5 minutes.
20. Add 600 μ L EtOH, vortex to mix, and spin for 0.5 minutes.
21. Add 600 μ L of the lysate from step 20 to spin column without moistening the rim (save the rest).
22. Spin at 13,200 rpm for 1.5 minutes.
23. Place the spin column in a new 2 mL collection tube. Discard the tube containing the filtrate.
24. Add another 600 μ L of the lysate to the new spin column and repeat steps 22 and 23.
25. Add the remaining 600 μ L of the lysate and repeat steps 22 and 23.
26. Add 500 μ L of Buffer AW1 to the spin column, spin at 13,200 rpm for 1.5 minutes.
27. Place the spin column in a new collection tube and discard the filtrate.
28. Add 500 μ L of Buffer AW2 to the spin column, spin at 13,200 rpm for 3.5 minutes.
29. Transfer the spin column to a new 1.5 mL microcentrifuge tube.
30. Pipette 200 μ L Buffer AE directly into the spin column.
31. Let sit at room temperature for 1 minute.

32. Spin at 13,200 rpm for 1.5 minutes to elute the DNA.
33. Discard the spin column and save the filtrate.
34. Label the microcentrifuge tubes with initials, sample type, sample ID, date, and store at -20°C to -40°C .

Appendix 2: PCR Reaction Mixture Protocols Used to Amplify Microsatellite Loci.

PCR reaction mixture	Amount (μL) per microsatellite locus							
	<i>Lut-829</i>	<i>Rio-05</i>	<i>Rio-01</i>	<i>Rio-19</i>	<i>Lut-701</i>	<i>Lut-733</i>	<i>Lut-801</i>	<i>Rio-17</i>
Sample	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
ddH ₂ O	5.4	5.4	5.2	5.4	2.7	4.7	4.7	5.4
Buffer	2	2.0 ^a	1.0 ^b	2.0 ^a	1.0 ^b	1.0 ^b	1.0 ^b	2.0 ^a
dNTP mix (2 mM)	0 ^c	0 ^c	1	0 ^c	1.0	1	1.0	0 ^c
Q solution	0	0	0	0	2.0	0	0	0
MgCl ₂	0 ^c	0 ^c	0 ^c	0 ^c	0.5	0.5	0.5	0 ^c
Forward unlabeled primer	0.16 ^d	0.16 ^d	0.2 ^e	0.16 ^d	0.2 ^e	0.2 ^e	0.2 ^e	0.16 ^d
Forward labelled primer	0.04 ^d	0.04 ^d	0	0.04 ^d	0	0	0	0.04
Reverse primer	0.2 ^d	0.2 ^d	0.2 ^e	0.2 ^d	0.2 ^e	0.2 ^e	0.2 ^e	0.2 ^e
M13 dye (FAM, NED, or PET)	0	0	0.2 ^e	0	0.2 ^e	0.2 ^e	0.2 ^e	0
<i>Taq</i> DNA polymerase	0.2 ^f	0.2 ^f	0.2	0.2 ^f	0.2	0.2	0.2	0.2 ^f
Total volume (μL)	10	10	10	10	10	10	10	10

^a = 5x buffer

^b = 10x buffer

^c = dNTP mix and MgCl₂ included in 5x buffer

^d = 100 μM concentration

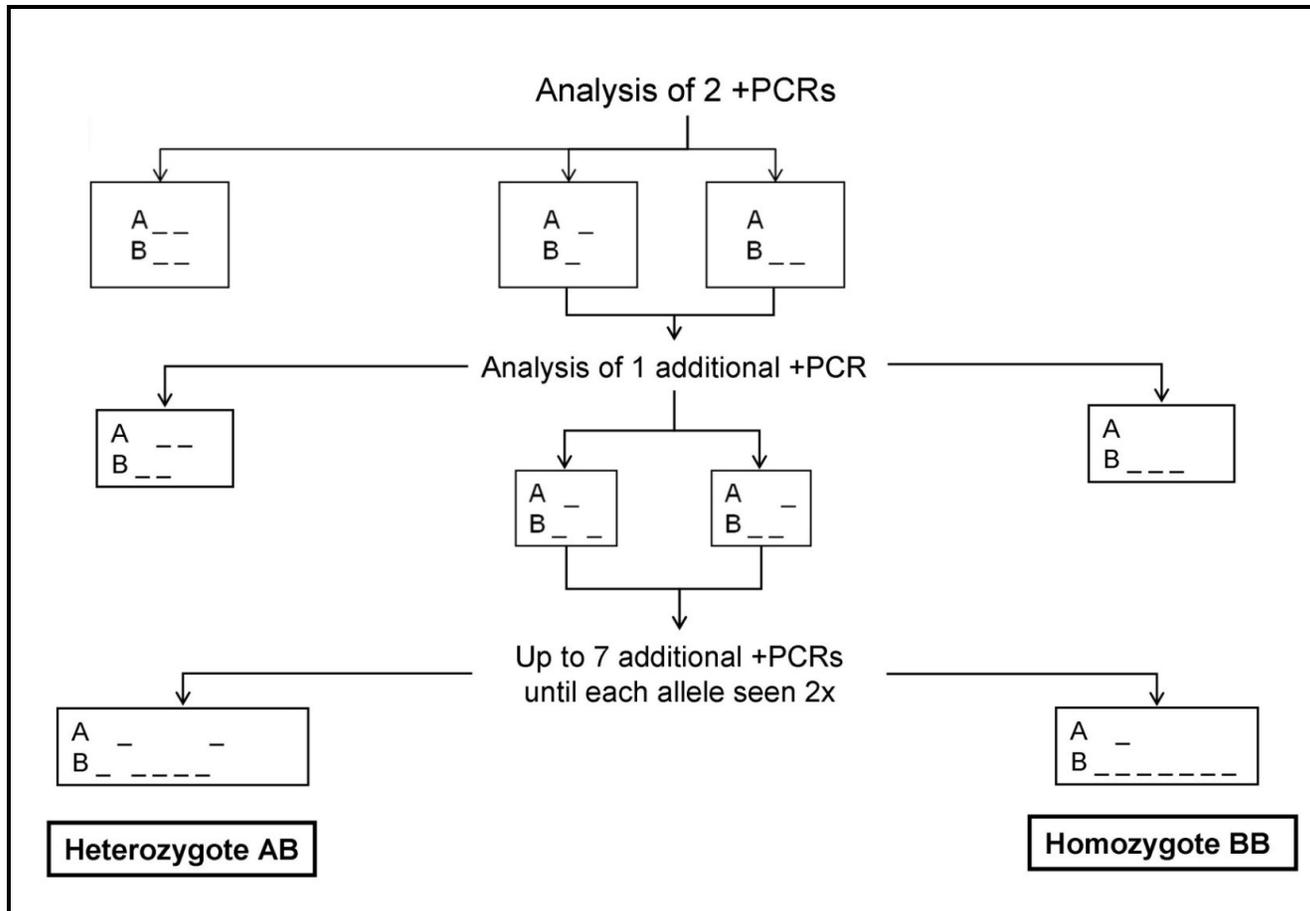
^e = 10 μM concentration

^f = HotStar HiFidelity *Taq* DNA Polymerase

Appendix 3: PCR Reaction Thermal Cycler Programs Used to Amplify Microsatellite Loci.

PCR step	Microsatellite locus							
	<i>Lut-829</i>	<i>Rio-05</i>	<i>Rio-01</i>	<i>Rio-19</i>	<i>Lut-701</i>	<i>Lut-733</i>	<i>Lut-801</i>	<i>Rio-17</i>
1. Activation	95°C for 5:00	95°C for 5:00	95°C for 15:00	95°C for 5:00	95°C for 15:00	95°C for 15:00	95°C for 15:00	95°C for 5:00
2. Denaturation	95°C for 0:20	95°C for 0:20	95°C for 0:30	95°C for 0:20	95°C for 0:30	95°C for 0:30	95°C for 0:30	95°C for 0:20
3. Annealing	58°C for 0:30	63°C for 0:30	48°C for 0:45	55°C for 0:30	48°C for 0:45	45.5°C for 0:45	47.5°C for 0:45	52°C for 0:30
4. Extension	72°C for 0:30	72°C for 0:10	72°C for 0:30	72°C for 0:10	72°C for 0:30	72°C for 0:30	72°C for 0:30	72°C for 0:10
5. Cycles	Goto 2, 40 times	Goto 2, 40 times	Goto 2, 40 times	Goto 2, 40 times	Goto 2, 35 times	Goto 2, 35 times	Goto 2, 35 times	Goto 2, 40 times
6. Final extension	72°C for 10:00	72°C for 10:00	72°C for 10:00	72°C for 10:00	72°C for 10:00	72°C for 10:00	72°C for 10:00	72°C for 10:00
7. End of cycling	4°C Forever	4°C Forever	4°C Forever	4°C Forever	4°C forever	4°C forever	4°C forever	4°C forever

Appendix 4: Comparative Multiple Tubes Approach (adapted from Hansen et al. 2008, *Molecular Ecology Resources* 8:282-289).



Appendix 5: Consensus Genotypes at Microsatellite Loci.

Otter No.	Consensus alleles at microsatellite loci							
	<i>Rio-19</i>	<i>Rio-05</i>	<i>Rio-01</i>	<i>Lut-829</i>	<i>Lut-733</i>	<i>Rio-17</i>	<i>Lut-701</i>	<i>Lut-801</i>
BC01	280/288	342/345	288/288	240/244	172/180	171/177	202/206	232/232
BC02	280/280	339/348	288/288	228/228	172/172	171/171	202/206	228/232
BC03	280/288	342/342	288/292	240/244	172/176	171/177	202/206	232/232
BC04	280/284	339/348	284/288	228/228	172/172	171/175	202/210	232/232
BC05	280/280	342/342	288/288	236/244	172/172	171/175	202/206	232/232
BC06	276/276	333/336	280/288	228/240	172/172	171/175	202/202	232/232
BC07	280/284	345/348	288/288	240/248	172/180	171/171	202/202	228/232
BC08	280/280	348/348	280/284	240/248	172/172	171/171	202/206	228/232
BC09	280/280	348/348	284/288	240/244	172/180	171/175	206/206	228/232
BC10	272/280	342/342	288/288	244/244	172/176	171/171	202/202	232/232
BC11	280/284	339/348	280/292	232/240	172/172	171/171	206/206	228/232
BC12	280/280	339/348	284/292	240/248	172/172	171/171	202/206	232/232
BC13	280/280	345/345	284/288	228/240	172/176	171/171	206/206	232/232
BC14	280/280	339/345	288/288	240/244	172/180	171/177	202/202	232/232
BC15	280/280	345/348	284/288	240/244	172/172	171/171	202/206	228/232
BC16	280/284	342/345	273/292	232/236	172/172	171/177	202/202	232/232
BC17	280/280	345/345	288/288	236/244	172/180	171/171	202/206	228/228
BC18	280/280	339/348	280/284	228/248	172/172	171/171	206/210	228/232
BC19	276/280	348/348	280/288	228/228	172/176	171/171	202/206	228/232
BC20	280/280	339/348	284/284	232/232	172/180	171/171	206/206	228/228

continued

Consensus alleles at microsatellite loci								
Otter No.	<i>Rio-19</i>	<i>Rio-05</i>	<i>Rio-01</i>	<i>Lut-829</i>	<i>Lut-733</i>	<i>Rio-17</i>	<i>Lut-701</i>	<i>Lut-801</i>
BC21	280/284	339/348	284/284	228/248	172/172	171/171	206/206	228/232
BC22	280/280	339/348	280/280	240/240	172/172	171/171	202/206	228/228
BC23	280/280	345/345	284/288	232/244	172/172	171/171	202/206	228/228
BC24	280/284	345/348	280/292	228/232	172/172	171/175	206/206	228/228
BC25	276/280	348/351	288/292	240/240	172/180	171/175	202/202	232/232
BC26	276/280	345/348	273/288	240/240	172/180	171/171	202/202	228/232
BC27	280/280	348/351	273/292	228/244	172/180	171/175	202/206	228/228
BC28	280/280	339/348	292/292	228/232	172/176	171/175	206/206	228/228
BC29	280/280	345/348	273/292	228/240	176/180	171/175	202/202	228/232
BC30	276/280	333/336	273/280	240/240	172/172	175/175	202/202	0
BC31	280/284	336/345	280/292	228/240	172/172	171/171	202/206	232/232
BC32	284/284	348/348	288/292	232/240	172/172	171/171	202/202	228/228
BC33	284/284	348/351	273/280	240/248	172/172	171/171	202/206	228/228
BC34	284/284	345/345	280/280	244/248	172/180	171/171	206/206	228/232
BC35	276/280	339/342	288/288	228/240	172/172	171/171	202/206	232/232
BC36	272/280	339/348	284/288	228/240	172/180	171/171	202/206	228/232
BC37	276/280	345/348	280/288	240/240	172/172	171/171	202/202	228/228
BC38	280/280	339/348	284/288	228/244	172/172	171/171	206/206	232/232
BC39	280/280	345/345	280/288	240/248	176/176	171/171	202/206	232/232
BC40	280/280	345/348	288/292	232/240	172/176	171/175	206/206	228/232
BC41	280/284	345/345	284/288	244/248	172/172	171/171	206/210	228/228
BC42	280/280	345/351	284/288	240/244	172/172	171/171	202/206	228/228
BC43	276/280	333/336	280/292	232/240	176/176	171/175	206/206	228/232

continued

Otter No.	Consensus alleles at microsatellite loci							
	<i>Rio-19</i>	<i>Rio-05</i>	<i>Rio-01</i>	<i>Lut-829</i>	<i>Lut-733</i>	<i>Rio-17</i>	<i>Lut-701</i>	<i>Lut-801</i>
BC44	280/280	345/345	288/288	228/240	172/176	175/175	202/202	228/228
BC45	276/280	333/336	273/292	228/244	172/180	171/175	202/202	228/232
BC46	280/280	339/342	284/292	228/240	176/180	171/171	202/206	228/228
BC47	276/280	345/345	273/292	228/240	172/172	171/171	206/206	232/232
BC48	280/284	348/351	280/284	248/248	172/172	171/171	206/206	232/232
BC49	280/280	345/345	280/288	232/236	172/180	171/171	206/206	232/232

Note: A '0' indicates an insufficient number of positive PCR runs to determine the consensus genotype at that particular locus.