CONTAMINANT EXPOSURE IN MARINE FORAGING RIVER OTTERS FROM VICTORIA, BRITISH COLUMBIA, CANADA

by

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Abstract

Past industrial activities on Southern Vancouver Island, British Columbia, Canada have resulted in localized polychlorinated biphenyl (PCB) contamination in the near shore marine environment. The ecological impacts of new and residual contaminants on wildlife species in this area are unknown. North American river otters (*Lontra canadensis)* are ideal biological indicators for aquatic ecosystem health and can be useful monitors for environmental and anthropogenic stressors on wildlife. Non-invasive scat sampling is an effective tool for studying aspects of river otter ecology without disrupting their natural behavior. Interpretation of river otter data derived from scat however can be limited without validation with live animal data. By combining scat sampling with live animal sampling I was able to compare the two sources of data to assess the effectiveness of non-invasive techniques. I investigated (i) home range analysis and spatial patterns through radio-telemetry to inform (ii) an assessment of environmental contaminant exposure and potential adverse health effects. Fixed kernel home range estimates revealed limited ranges, localized exposure and potential small scale population structuring. This indicates that only the river otters inhabiting the contaminated sites are being exposed to high levels of PCBs. Mean PCB concentrations in river otter blood and feces were significantly higher in harbour sites relative to the rest of the study area. Contaminant patterns between the two sample types were comparable and support the use of non-invasive sampling for investigating environmental contamination. Non-invasive hormone measures were used as indicators for contaminant related effects. Although there were differences between harbour and non-harbour sites, it is not clear the patterns were associated with contaminants.

Preface

The capture, sampling, chemical immobilization and transmitter implant surgeries were done by Dr. Helen Schwantje, wildlife veterinarian with the Ministry of Environment, under provincially approved animal care methods. Once the tagged river otters were released, the radio tracking and non-invasive scat collections were carried out under the UBC animal care certificate #3757-09. The primary research team that contributed to my thesis consisted of myself, as principal investigator, my co-supervisors, Dr. Kim Cheng (UBC) and Dr. John Elliott (Environment Canada) as well as thesis advisor Dr. Tom Sullivan (UBC). Field work, data collection and sample processing was led by myself, with technical and logistical support from the BC Ministry of Environment in Victoria, British Columbia. Scat samples were sent to the Great Lakes Institute for Environmental Research at the University of Windsor, Windsor, Ontario, Canada for sample preparation and chemical analysis under the supervision of Dr. Ken Drouillard. Blood samples were sent to Environment Canada's National Wildlife Research Center, Ottawa, Ontario, Canada for sample processing and chemical analysis under the supervision of Dr. Adbe Idrissi. Scat samples for hormone analysis were prepared at the University of British Columbia, Vancouver, by myself. Prepared scat samples were then taken to the University of Guelph, Guelph, Ontario, Canada where hormone analysis was carried out by myself under the supervision of Dr. Laura Graham. Home range analysis and all other data analyses were conducted by myself.

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CHAPTER 1 – GENERAL INTRODUCTION

The Salish Sea in the Pacific Northwest is home to diverse wildlife species and a productive marine ecosystem. This region has had a long history of surrounding agricultural and industrial activities whereby contaminants have accumulated in the adjacent waterways and near shore marine environment. Many of those contaminants are persistent polyhalogenated aromatic hydrocarbons (PAHs) that bioaccumulate in the tissues of animals as they ascend the food web. These persistent organic pollutants (POPs) are highly resistant to degradation and although their use has been restricted for decades, high levels have persisted in coastal ecosystems. The impacts of POPs in the Salish Sea have been studied in a number of top predator wildlife species, including bald eagles (*Haliaeetus leucocephalus)* (Elliott & Norstrum 1998), great blue herons (*Ardea herodias)* (Elliott et al 2001), cormorants (*Phalacrocorax sp).* (Harris et al 2005), harbor seals (*Phoca vitulina)* (Ross et al 2004) and killer whales (*Orcinus orca)* (Ross et al 2000). There are ongoing concerns that chronic POP contamination may impact health of important wildlife species.

Victoria and Esquimalt Harbours on southern Vancouver Island have had a long history of industrial activities including forestry, ship building and metal processing. These sites are known hot spots for POPs, particularly polychlorinated biphenyls (PCBs) that have accumulated in nearshore marine sediment through industrial effluent (Elliott et al 2008). A river otter (*Lontra canadensis*, Lariviere and Walton, 1998*)* population inhabiting the area was studied to investigate the finer scale effects of new and residual POPs in the environment.

River otters are piscivorous predators in the weasel family (Mustelidae) that inhabit rivers, lake and near shore marine environments across North America. Coastal river otters forage on a variety of prey species, including intertidal fish and crustaceans (Guertin et al 2010a). River otters are considered sentinel species for overall ecosystem health in aquatic environments because of their sensitivity to pollutants (Bowyer et al 2003). Elliott et al (2008) reported that otters inhabiting the urban/industrialized areas on southern Vancouver Island are being exposed to relatively elevated levels of PCBs.

The river otter, among other wildlife species, have been studied to assess toxic pollutants in the environment (Harding et al 1999; Ross et al 2000; Ross et al 2004; Elliott & Norstrum 1998). Like other top predator species, otters are exposed to POPs through their diet. River otters are well suited for monitoring local sources of contamination as they have relatively small and seasonally constant home ranges and do not hibernate or migrate over long distances. If their home range is positioned close to a source of contamination, exposure could be chronic. River otters inhabiting areas of high industrial and anthropogenic activities have been reported to have contaminant levels "comparable" to coastal cetaceans (Kannan et al. 1999) which are thought to be among the most highly contaminated marine mammals in the world.

Wildlife exposed to chlorinated hydrocarbons are susceptible to various physiological effects as a result of hormonally active chemicals in the environment. Endocrine disrupting chemicals (EDCs) can mimic hormones and alter endocrine signaling by interacting with various nuclear receptors (Cheek et al 1998) and lead to compromised reproductive, immune and endocrine system function (Colborn et al 1993). EDCs can affect hormone function by inhibiting synthesis,

altering serum transport and increasing catabolism (the breakdown of complex molecules) (Devito et al 1999). Halogenated hydrocarbon chemicals, such as organochlorine (OC) pesticides and PCBs, can alter thyroid hormone levels in vertebrates (Cheek et al 1999; Simms et al 2000), disrupting development and differentiation of cells (Vos et al 2000). Interference of EDCs with steroid hormone receptors has been linked to reduced immune function and reproductive success in polar bears (*Ursus maritimes),* harbor seals and cetaceans (Fossi and Marsili 2003; Mos et al. 1996).

The sensitivity of mink (*Mustela vison)* to PCBs was recognized in the 1970s when reproductive failure in ranch mink was linked to their diet of contaminated fish from the Great Lakes (Aulerich et al 1971). Food trials later confirmed that PCB toxicity was proportional to the total intake of the compound (Aulerich and Ringer 1977). Long term exposure to PCBs, even in low doses, has caused reproductive impairment, including developmental abnormalities, fetal deaths and decreased kit survival (Brunstrom et al 2001).

PCB toxicity has also been investigated in other mustelid species. Bleavins et al (1980) carried out food trials comparing PCB toxicity in mink and ferrets (*Mustela putorius furo)*. It was concluded that mink were far more sensitive to the effects. The same level of PCB that caused complete reproductive failure in ferrets, caused 100% mortality in mink. Henny et al (1981) measured PCB residues in wild mink and river otter from the Lower Columbia River. PCB levels in mink livers were as high as levels associated with reproductive failure, and PCB levels in river otter livers were even higher. Due to the similarities in diet and ecology, it has been suggested that river otters would exhibit similar vulnerability to physiological impacts as mink (Leonards et

al 1998). Harding et al (1999) found that reproductive abnormalities in mink were correlated with PCB concentrations, even though the mink had lower contaminant body burdens overall relative to river otters from the same region. The relative sensitivity of river otters and mink to PCB exposure is unknown.

In Western Europe, otter (*Lutra lutra)* populations declined significantly in the 20th century (Mason & MacDonald 1993). The disappearance of otters from much of their historic range was thought to be caused by a number of different factors, but exposure to PCBs appeared to be involved in population declines around industrialized areas (Mason 1989[a], Mateo et al 1999). Kruuk and Conroy (1996) found that there was a strong negative correlation between PCB concentration in otter liver tissue and body condition. Interestingly, some of the highest values were measured in otters with good body condition. They also found that there was no correlation between PCB concentration and age. This implies that significant amounts of PCBs do not accumulate in the tissues of otters over the long term.

There is a marked increase in PCB concentration as compounds are transferred from prey to predator. A diet specific biomagnification factor developed by Leonards et al (1997) indicates that even in areas of low or moderate contamination, top predators can be exposed to high concentrations of these contaminants. An apparent shift in the congener patterns occurs as PCBs are transferred up the food chain. Lower chlorinated PCBs are predominant at lower trophic levels, while higher chlorinated PCBs are predominant at higher trophic levels. This suggests that otters can metabolize the lower chlorinated congeners. Pattern analyses have shown PCB

138 and PCB153 to be the most predominant congeners observed in European otters (Kruuk & Conroy 1996, Leonards et al 1997, Van den Brink & Jansman 2006).

River otters use communal latrine sites for communication and territorial scent marking (Melquist & Dronkert 1987). River otters deposit feces, anal jelly and/or a mixture of both at latrine sites. Anal jellies are mucous-like substances produced in the intestinal tract of the otter, likely to facilitate the passing of bones and shells from their prey. These biological materials can serve as indicators, allowing for non-invasive animal sampling without disrupting natural behaviours. Field collected fecal material can be an effective tool in studying multiple aspects in river otter ecology. The technique provides a method to assess spatial trends in new and residual contaminants and potential impacts to otter populations as well as multidisciplinary approaches to investigate toxicological, population and health parameters.

Elliott et al (2008) surveyed harbours and industrialised areas along the southern British Columbia (BC) coast for chlorinated hydrocarbon contaminants in river otter feces. Feces were collected from latrine sites and samples from the same latrine were pooled for a suite of chemical analyses. PCBs were present in all samples (pooled) and were highest in Victoria Harbour, on Southern Vancouver Island. Similar trends in PCB contamination were observed around the same time when Dungeness crabs, *Cancer magister*, were analyzed from harbours and industrialised areas on the BC Coast (Ikonomou et al 2002).

The geometric mean concentrations of sum PCBs in Victoria Harbour in 1998 (12.3 mg/kg lw) and 2004 (9.5 mg.kg lw) (Elliott et al 2008) were above the level of concern (9 mg/kg lw) for

adverse reproductive effects in the European otter (Mason & O'Sullivan 1992). Similar to European otters, the most prominent PCBs in the Victoria river otter feces were higher chlorinated congeners, PCB138 and PCB153 (Elliott et al 2008). This work demonstrated the effectiveness of sampling otter feces to investigate spatial trends in contamination and in population level exposure. There was concern, however that because of the pooled sample approach, individual samples may have driven the high PCB levels.

Guertin et al (2010b) sampled river otter latrines and assessed both fecal DNA genotyping and contaminant levels to assess site and individual specific contaminant levels in Victoria and Esquimalt Harbors. River otter feces collected from the harbor sites had the highest concentrations of PCBs relative to the surrounding area. The individual-based approach confirmed high individual PCB exposure within the harbour and allowed for re-sampling of genetically identified individuals. Fecal PCB levels were variable over time, space and individuals (Guertin et al 2010[b]). This variability in the fecal contaminant levels were likely representative of contaminant load in the prey species rather than the contaminant levels in the tissue of the otter.

This study will investigate the relationship between contaminant levels in otter feces and actual toxic burden of the animal. Although the contaminants are point source in nature the impacts to river otters and their ecosystem could be extending beyond the harbour boundaries. Fecal sampling effectively tells us where the contaminants are coming from, but spatial and geographical extent of the contamination and its effects on the ecosystem are unknown.

Based on the principles of bioaccumulation, toxins from dietary sources accumulate in the liver and fat deposits of the animal (Ruus et al 2002). The degree of contaminant exposure will be influenced by the time spent foraging within contaminated sites. Characterizing the spatial patterns of foraging behaviour will be important parameters in quantifying contaminant exposure in this population of river otters.

The distribution of a river otter population is related to several factors, including habitat and prey availability. Due to the abundance of prey in productive marine environments river otters typically have smaller home ranges than those in fresh water systems (Blundell et al 1999). Blundell et al (2002) identified resource related special relationships and home range sizes. That study found that relatively larger home ranges of males were dependant on female distribution (during breeding season), whereas, female home ranges were dependant on food availability (Blundell et al 2002). Alternatively, when females are raising young, males will sometimes form social or "bachelor" groups. During that time the drivers affecting distribution may be altered. This indicates that demographics could also play a significant role in distribution and contaminant exposure within a population.

The Kernel density technique defines a utilization distribution by assessing the probability that an animal will occur at a particular point in space (Blundell et al 2002). Kernel estimates supply a third dimension representing the amount of time an animal spends in any given area (Seaman et al 1999) and is useful for examining core areas of use that may be important for foraging and habitat use (Blundell et al 2002).

This is the first comprehensive study of coastal river otter distribution and spatial ecology in the Salish Sea. An animal's home range has fundamental consequences for many ecological processes such as, population regulation (Wang & Grimm 2007), habitat selection (Rhodes et al 2005) and predator-prey dynamics (Lewis and Murray 1993). The resulting home range and movement patterns are important for the management and conservation of river otters in an urban and industrialized area.

Live animal sampling can be challenging due to the costly and invasive nature of trapping, handling and chemical immobilization. Non-invasive scat sampling at latrine sites can be an alternative approach to studying river otters and their interactions with the environment. In order to support further non-invasive studies on contaminant exposure in river otters, the data derived from river otter scat requires more rigorous assessment, particularly the relationship between contaminant levels in otter scat and actual toxin burden in the animal. Mason & O'Sullivan (1993) found that PCB levels in scat from the European otter (*Lutra lutra*) were significantly correlated to PCB levels of liver tissue (representing body burden). Van den Brink and Jansmen (2006) showed that fecal PCB levels, particularly non-metabolized congeners, reflect the internal PCB concentrations of the European otter. Although fecal PCB levels mainly reflect the contaminant load of their recently ingested prey, fecal materials can be used to estimate the body burden of the animal and then compared to the levels measured in blood or tissue.

Concentrations of PCBs, OC Pesticides and polybrominated diethyl ethers (PBDEs) in river otter scat samples from Victoria Harbour and the surrounding area have been presented at multiple time periods (Elliott et al 2008, Guertin et al 2010[b]) and will be presented again here. This study

is the first to measure contaminants in blood from river otters in this population. The contaminant analysis of river otter blood and scat has been carried out concurrently so that relative contaminant patterns between the two sample types can be assessed.

Fecal hormone data can be complementary to contaminant data by providing information on physiological responses to the animal's environment. Multiple assays of the same samples, combining thyroid, glucocorticoid (GC) and reproductive hormone measures, can help to characterize general endocrine function (Wasser et al 2010). Steroid and thyroid hormones will respond differently to various environmental cues or stressors. Thyroid hormones are particularly responsive to environmental stressors (Eales 1988), but appear unaffected by psychological stress (Geris et al. 1999). By contrast, GC levels increase in circulation (Sapolsky et al 2000) and feces (Wasser et al 1997, 2000) in response to both psychological and nutritional stress. Reproductive hormone data can further complement this analysis by providing information on reproductive function (Bateman et al. 2009). Investigating multiple hormone profiles will help to decipher the various causes and effects in terms of stress, reproductive physiology and effects associated specially with PCBs.

This study is part of an ongoing initiative by Environment Canada, in support of a Transport Canada led risk assessment of the Federal contaminated site of Victoria Harbour, to investigate the impact of persistent pollutants on top predatory wildlife species in the Salish Sea. The impacts of new and residual contaminants to marine foraging river otters and their ecosystem were investigated using the river otter as a biological monitor. River otter scat sampling and

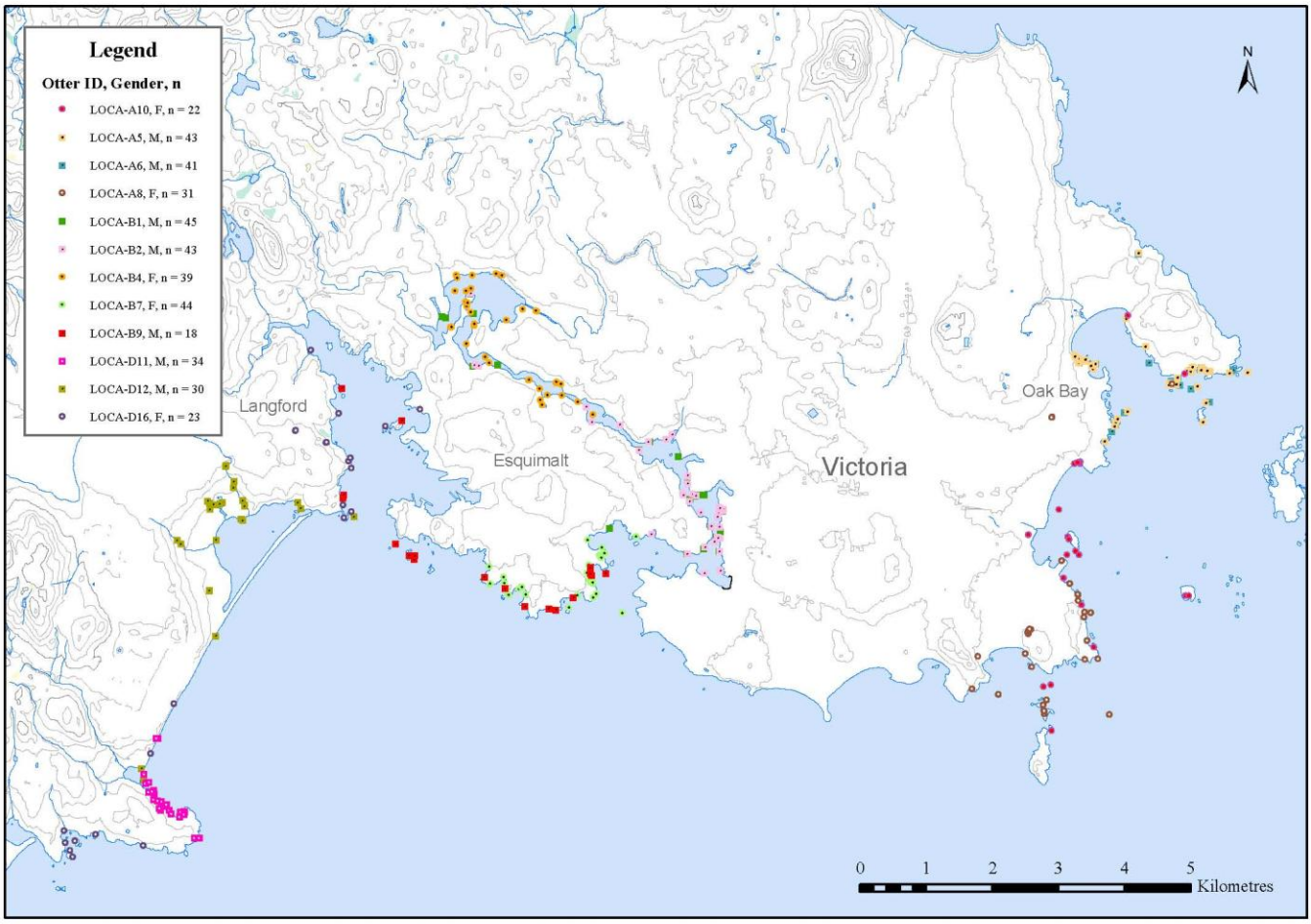
radio-tracking was carried out concurrently to examine otter distribution, spatial trends in contaminant exposure and indicators of effects. The study area is illustrated in Figure 1.

Chapter two will describe home ranges of radio tagged river otters, defined to characterize contaminant exposure and population dynamics. Guertin et al (In press) proposed that there are two local subpopulations of marine foraging river otters. That supposition was based on fecal genetic data and implied that only one subpopulation was being exposed to contaminants within the harbour. Another implication of population structuring is that if contaminant exposure leads to population declines within the harbour, healthy individuals could migrate from the adjacent population. Population structuring based on movement patterns and home range analysis was examined in relation to the proposed subpopulations.

Chapter three will describe the results of fecal and blood contaminant levels and fecal hormone levels were investigated. Contaminant levels in blood (plasma) from known (radio tagged) otters was compared to contaminant levels of field collected scat to determine the relationship between the different sample types. Individual home range and movement patterns from chapter two were incorporated into the interpretation of site specific contaminant and hormone data. A number of fecal hormones were assessed as potential indicators of physiological effects. Although difficult to determine without long term survival and recruitment data (not possible in this study), stress-related hormones may give some indication of population level impacts associated with contaminants or other stressors.

Telemetry data and various analyses of fecal data were compared to validate the effectiveness of non-invasive fecal sampling in river otters as a method of evaluating the ecological impacts of environmental contaminants. Characterizing population dynamics, exposure and anthropogenic stressors affecting this species will be important to ensure their continued productivity around urban and industrial areas.

Figure 1.1: Map of study area and river otter observations on Southern Vancouver Island from December 2009 to September 2011.



CHAPTER 2 - HOME RANGE ANALYSIS IN COASTAL RIVER OTTERS TO CHARACTERIZE CONTAMINANT EXPOSURE AND POPULATION DYNAMICS

River otters (*Lontra canadensis*) are semi-aquatic piscivorous predators of the weasel family (Mustelidae) and are found across North America. River otter movement, distribution and habitat use is driven by the availability of food, fresh water and shelter (Melquist and Dronkert 1987). The geographical area where an animal spends its time needs to encompass the resources required for survival and reproduction. The population of river otters under this study inhabit a coastal environment and forage almost exclusively on a marine-based diet. Due to the abundance of prey in productive marine environments, river otters typically have smaller home ranges than those in fresh water systems (Blundell et al 1999).

While river otters use near shore coastal waters to forage, travel and socialize, they rely on terrestrial habitat along the marine-terrestrial interface for rest, denning and refuge (Melquist and Dronkert 1987). Fixed kernel methods (Blundell et al 2001), from home range measurements, can overestimate the range of a coastal river otter because unused spaces of water and land are incorporated (Sauer et al 1999). River otter homes ranges have therefore been characterized as linear lengths of shoreline within a kernel estimate (Sauer et al 1999). In general, males have larger home ranges than females, but females utilize a larger proportion of their range for foraging (Blundell et al 1999). Home range estimates have been reported for individual coastal river otters in Alaska between 10-40 km of coastline (Sauer et al 1999; Bowyer et al 2003) with mating or natal dispersal distances ranging from 35 km in males and 58 km in females (Blundell et al 2002a).

It has been proposed that spatial relationships in river otter populations are resource driven for females and mate driven (at least in the breeding season) for males (Blundell et al 1999). In the breeding season males expand or even shift their range, while females travel outside of their ranges less often but for greater distances for natal dispersal (Blundell et al. 2002a).

Victoria and Esquimalt Harbours on Southern Vancouver Island, British Columbia, are known hot spots for persistent organic pollutants (POPs), particularly polychlorinated biphenyls (PCBs) that have accumulated in the marine ecosystem through industrial effluent (Elliott et al. 2008). River otters are exposed to POPs through their diet (Clark et al 1981, Henny et al 1981, Harding et al 1999) and as a top-predator they are vulnerable to the effects of these toxins as they biomagnify at higher trophic levels of a marine based food web (Ruus et al 2002). There are ongoing concerns that chronic POP contamination in the Pacific Northwest can impact the health of wildlife species. Relatively elevated levels of POPs have been reported in top-predators inhabiting the near shore marine environment of the Salish Sea (Ross et al 2000, Ross et al 2004, Elliott and Norstrum 1998). POPs are known to reduce reproductive fitness (reduced kit survival, reduce male reproductive development) in the closely related mink (*Mustela vison*) (Aulerich and Ringer 1977, Bleavins et al 1980, Brunstrom et al 2001). The concern is that, if the same effect is seen in river otters, chronic exposure could affect population stability as has been reported in populations from various areas of the U.S.A. (Raesly 2001).

This population of river otters was previously studied through non-invasive scat sampling in 2005 and 2006 to investigate population metrics and potential impacts of POPs in Victoria and

Esquimalt Harbours (Elliott et al 2008, Guertin et al 2010[b], Guertin et al In press). River otter feces collected from Victoria harbour had the highest concentrations of PCBs relative to other sites on southern Vancouver Island and the adjacent mainland (Elliott et al 2008, Guertin et al 2010 [b]). Fecal contaminant concentrations, however, were variable when genetically identified individuals were re-sampled at different times and locations (Guertin et al. 2010 [b]). Fecal DNA data from this population, combined with basic spatial data, revealed two genetic clusters of individuals within the population (Guertin et al. In press). One group of otters was identified on the west side of the study area (including the harbours), and the other group on the east side. This sub-population structuring suggests that only certain individuals are being exposed to contaminants and other habitat related stresses within the harbour. In this study, we investigated whether radio telemetry data revealed the same patterns of population structuring (from scat derived data). Previous studies have combined genetic and telemetry data to investigate river otter population dynamics in a marine environment (Blundell et al 2002a, Bowyer et al 2003, Blundell et al 2004).

Characterizing a river otter's use of space is important in understanding and quantifying their exposure to contaminants. The extent of individual exposure would depend on where the animals spend their time and how far they travel from the source of contamination. There has been a wealth of information on contaminants (Elliott et al 2008), diet (Guertin et al 2010b) and genetics (Guertin et al 2010a) of this population derived from non-invasive scat sampling. However, the details of home range and movement patterns are still unknown. Distribution, spatial relationships and use of the landscape are all important factors in the conservation and management of a wildlife species (Schonewald-Cox et al 1991).

The goal of this study was to build on work of Guertin et al (In press) derived from scat sampling and fecal genetics. River otters were identified and radio tracked to characterize movement and distribution with respect to the harbours. Home range size and core areas of use were determined to investigate potential population structuring. The implications of population structuring would influence the extent and impacts of contaminant exposure, based on where the otters spend their time foraging. Localized exposure and limited movement could affect whether or not river otters are able to sustain a population in a contaminated industrialized environment, such as Victoria Harbour.

I tested the hypothesis that, because there are no natural barriers between the proposed subpopulations, limited movement and small range size have resulted in population structuring. If ranges of the two groups do not overlap, limited, if any, mixing of otters from the west and east sides of the study area could explain the genetic clustering reported by Guertin et al (Guertin et al, In Press). Thus, the objectives of this study were to (1) define home range and movement patterns, (2) evaluate population structuring based on telemetry data and (3) determine otter distribution in relation to contaminated sites.

STUDY AREA

The study area spans approximately 80 km of coastline along Southern Vancouver Island 48°25′43″N 123°21′56″W and consists of 4 distinct areas along a continuous coastline; from east to west (A) Oak Bay and Cadboro Bay, (B) Victoria Harbour, (C) Esquimalt Harbour and (D) Colwood/Methcosin. The habitat characteristics of each area are distinctive. Victoria and Esquimalt Harbours have a long history of industrial activities including ship building and metal processing. The harbours have primarily industrialized foreshore, many docks and heavy boat traffic. Oak Bay and Cadboro Bay have relatively natural foreshore with rocky intertidal ecosystems and a number of near shore islands as well as many waterfront homes and a significant amount of boat traffic. Colwood/Metchosin has mainly natural foreshore with pebble beaches, some waterfront homes and minimal boat traffic.

METHODS

From December 2009 to September 2011, radio locations were collected from 12 adult river otters. Based on a previously reported population estimate of 57 otters (Guertin In press) these 12 animals made up approximately 20% of the population. Each otter was assigned identification (ID) which corresponded to the area it was captured from. Radio tracking was conducted by boat, car or foot (depending on weather conditions and access) between 0800 and 2000. River otter location data were recorded on a handheld Garmin GPS unit. Triangulation was not required because animal locations were confirmed visually or to within 5 m if the otter was in a den or under cover (rock, vegetation, dock). Transmitter signals were only heard when otters were above water.

Home range estimates were only calculated for otters that had 30 or more radiolocation points (based on methods from Seaman et al 1999). Fixed kernel estimates were calculated using the 95% and 50% isopleths of the minimized extent utilization distribution (UD) with the reference smoothing parameter (h_{ref}) (Blundell et al 2001). Home range (95%) and core areas of use (50%) are reported as the length of shoreline (km) within the contours of fixed kernel analyses. Shoreline length was calculated in GIS (Arcview 9.3) along the marine-terrestrial interface and included islands, lagoons and both sides of marine channels, but did not include freshwater channels, such as streams that flow into the ocean.

RESULTS

The number of radiolocations for each individual ranged from 18 to 45 observations. Two of the river otters went missing 11 months into the study, either because of transmitter failure, unconfirmed mortality or movement out of range. Adequate data were not collected in these two cases. A third otter inhabited an area that was out of telemetry range from land, therefore locations were limited to days when boat searches were possible. River otters in this study were observed to travel from one end of their range to the other in only a couple of hours, therefore all radiolocations were considered to be temporally independent.

There was no evidence of seasonal shifts in range with the exception of females moving outside of their core areas temporarily to have their offspring in natal dens. The natal den areas were included in the range calculations.

Fixed kernel estimates were calculated for 9 of the 12 river otters (6 males, 3 females) (Table 1). Home range (95%) size ranged from 9.91 to 33.04 km of shoreline for males (average = 16.84

km, SE = 4.66 km) and 10.05 to 24.07 km of shoreline for females (average = 16.22 km, SE = 4.13 km). Core areas of use (50%) ranged from 1.07 to 8.18 km of shoreline for males (average = 4.73 km, SE = 1.07 km) and 3.72 to 8.64 km for females (average = 6.69 km, SE = 1.51 km). There was overlap in ranges between river otters inhabiting the same designated area (i.e. within Victoria Harbour) and some overlap between individuals from the Harbours (B & C) and the Colwood/Metchosin (D) areas. There was no overlap between the individuals from the Oak Bay / Cadboro Bay area (A) with those from the west side of the study area (B, C, & D) (Figure 2).

We examined the core areas of use (50%) in the context of contaminant exposure. There appeared to be three categories of individual exposure; (1) full time, (2) part time and (3) none. The biological implications of these categories will be explored in chapter 3 through fecal and blood measures of contaminants. The river otters in the Oak Bay / Cadboro Bay area (A5, A6, & A8) did not venture anywhere near the Harbours at any point during the study. We conclude, based on these observations, that those otters had no exposure to the contaminated areas. Three of the Victoria Harbour otters (B1, B2, & B4) never left the Victoria Harbour Boundaries. These otters appeared to have full time exposure to the contaminated areas. The 4th Victoria Harbour otter, B7, appeared to move in and out of the Harbour. In this case the exposure to contaminants would be considered part time. The river otters from the Colwood/Metchosin area (D11 & D12) did not venture into either Esquimalt or Victoria Harbour. One of the female river otters (D16) that moved outside of her normal range for natal denning travelled from the Colwood/Methchosin area into Esquimalt Harbour (for 1-2 months).

DISCUSSION

Different geographical features of each area would have influenced the calculated range and core area of use. There are a number of small islands in the Oak Bay / Cadboro Bay area that were included in the calculation of shoreline length. These features were not present in the Coldwood/Metchosin area and are present to a much lesser degree in the Harbours. The Victoria Harbour area, which includes the Gorge Waterway and Portage Inlet, is made up of a series of long narrow channels. Both sides of these channels were included in the calculation of shoreline length and would have resulted in a greater length relative to forage (marine) area. With this consideration, home range size and core areas of use varied between individuals but did not seem to differ between the harbour and non-harbour areas. This is interesting given the differences in habitat and environmental characteristics between sites.

A number of factors will influence the space utilised by individual otters, mainly prey abundance, habitat availability and spatial relationships with other otters (Blundell et al 1999, Crowley et al 2012, Ben-David et al 2005). The productive rocky intertidal ecosystems and island shelters of Oak Bay and Cadboro Bay contrasts with the industrialised foreshore and anthropogenic activities of the Harbours. It might be assumed that since the habitat quality and presumably prey abundance would be better in the Oak Bay / Cadboro Bay area, that otter ranges would be smaller than those in the Harbour. A river otter only needs to maintain a range that encompasses the needed resources of prey and habitat but when those resource requirements are met, a river otter's range might expand or shift temporarily in response to other factors, such as distance to mates (Blundell et al 1999). Another possibility is that the high density of river otters in Oak Bay / Cadboro Bay relative to the Harbours (Guertin et al, In Press) translates to

increased competition and a need to expand the forage area. In the Harbours, although the resources might be limited there were fewer animals to compete with so the ranges remained relative small.

Sociality may also have an effect on river otter distribution. It is known that otters will communicate through scent marking to establish mutual avoidance, defence of territory or intra-group signals of food resources (Ben-David et al 2005, Kruuk 1992). Otters will sometimes form social groups, which could impact foraging behaviour and use of space. Males tend to be more social than females and will carry out cooperative foraging to gain access to larger pelagic fish (Blundell et al 2002b). Females tend to be solitary or maintain small family groups throughout the year (Blundell et al 2002b), limiting their diet to small intertidal prey. Females will therefore utilize a larger proportion of their range (core area), relative to males, for foraging (Blundell et al 1999).

Coastal river otters are opportunistic in prey selection; therefore, I did not anticipate that fluctuations in a particular prey species would affect their distribution (Gallant et al 2009). Unlike thatseen by Blundell et al (2002a) with a small number of individuals travelling great distances from the original capture location, none of the otters in this study exhibited patterns of natal or breeding dispersal. In the late spring (April-May) two female river otters (D16 & A10) left their normal ranges to have offspring in natal dens located 2-6 km away. Those were the only observable seasonal shifts in river otter ranges during this study. After this movement both females returned to their original range (late June); however the home ranges were not calculated as insufficient radiolocations were collected.

The small home range of D11 is located on a headland section of undeveloped forested coastline (Albert Head), which is unlike the rest of the Colwood/Metchosin area. That protected and productive environment presents excellent river otter habitat. River otters depend on such sheltered habitats in the presence of anthropogenic disturbance (Crowley et al 2012, Gallant et al 2009) which could explain the limited movement exhibited by this individual. This study included telemetry data from adult river otters only. The spatial behaviour of different age classes might have revealed different patterns.

The home range of D12 was a special case as this individual was the only river otter that ventured into fresh water systems. This male also foraged in the marine environment, as well as the fresh water system, therefore his home range and core area of use was calculated in the same manner as the others, however the fresh water channels were not included in the calculation of shoreline length. Other river otters in this study had the opportunity (by proximity) to use fresh water channels, but they did not. Crowley et al (2012) found that habitat selection at the fine scale (based on latrine activity) was influenced by characteristics of conifer trees and cover rather than access to food. The space inhabited by D12 offered a relatively undisturbed forest and stream network that would have provided shelter primarily, rather than an abundant supply of prey.

Individual river otters were radio tracked consistently over a full year to allow assessment of seasonal shifts and spatial relationships among otters. Aside from a couple of examples of dispersal, river otter ranges were found to be seasonally constant suggesting that the animals in this study were residents, rather than transient river otters.

The hypothesis of limited movement and small range sizes, resulting in population structuring of otters, was supported. Telemetry data revealed limited, if any, mixing between the river otters from the west side (B, C, & D) and the east side (A) of the study area. These findings agree with the proposed partitioning of two local subpopulations, based on genetic data, and demonstrate that the two sources of data (fecal DNA and radio-telemetry) bring us to the same general conclusions.

MANAGEMENT IMPLICATIONS

This study showed that coastal river otter home ranges on Southern Vancouver Island are confined to relatively small areas. This has implications for river otter conservation and management because certain otters in this population were using the contaminated sites within the harbour and potentially being exposed to contaminants, while others were not.

The amount of time spent foraging in contaminated sites will influence the extent to which an individual is exposed. Environmental contamination as well as anthropogenic disturbance has been shown to have adverse effects on river otters (Mason and MacDonald 1993, Bowyer et al 1994). The localized population of otters inhabiting Victoria Harbour full time have the highest risk of adverse effects associated with contaminant exposure. It is unlikely that these river otters are aware of the toxins present at these contaminated sites (Delibes et al 2009), although they may inadvertently mitigate their exposure by avoiding areas of higher human activity (Guertin et al In Press). The presence of otters in Victoria Harbour only signifies that the basic resources of food and shelter are available, but whether or not this environment can sustain a healthy otter population is yet to be determined.

The contaminant exposure to river otters living outside the harbour is minimal, particularly the population in Oak Bay/ Cadboro Bay because it is unlikely that they will travel the distance to the harbour. If the Victoria Harbour otter population is in decline, the population of otters adjacent to the harbours could be an important source population. It would appear that otters across the study area have become adapted to their localized environments and are dependent on relatively small stretches of coastline. If the required resources of food and shelter are available within the harbour, otters will continue to use this space regardless of anthropogenic disturbance or pollution (Gallant et al 2009, Delibes et al 2009). Limited movement and restricted landscape use might therefore make these otters more vulnerable to future habitat degradation and changes in their environment.

TABLES

Table 2.1: Summary of fixed kernel estimates for home ranges (95%) and core area of use (50%) for river otters on southern Vancouver Island between December 2009 and September 2012 (n = # of observations, href = smoothing parameter). Represented in length (km) of shoreline.

Males				
Otter ID	n	href	95% (km)	50% (km)
LOCA-A5	43	339.395	13.46	5.05
LOCA-A6	41	329.262	12.81	4.62
LOCA-B1	45	614.026	33.04	8.18
LOCA-B2	43	502.934	28.38	6.84
LOCA-D11	34	159.788	3.42	1.07
LOCA-D12	30	539.839	9.91	2.62

Females				
Otter ID	n	href	95% (km)	50% (km)
LOCA-A8	31	532.507	24.07	7.70
LOCA-B4	39	355.475	14.54	8.64
LOCA-B7	44	234.302	10.05	3.72

FIGURES

Figure 2.1: Summary of Radiolocations for river otters on southern Vancouver Island between December 2009 and September 2011.

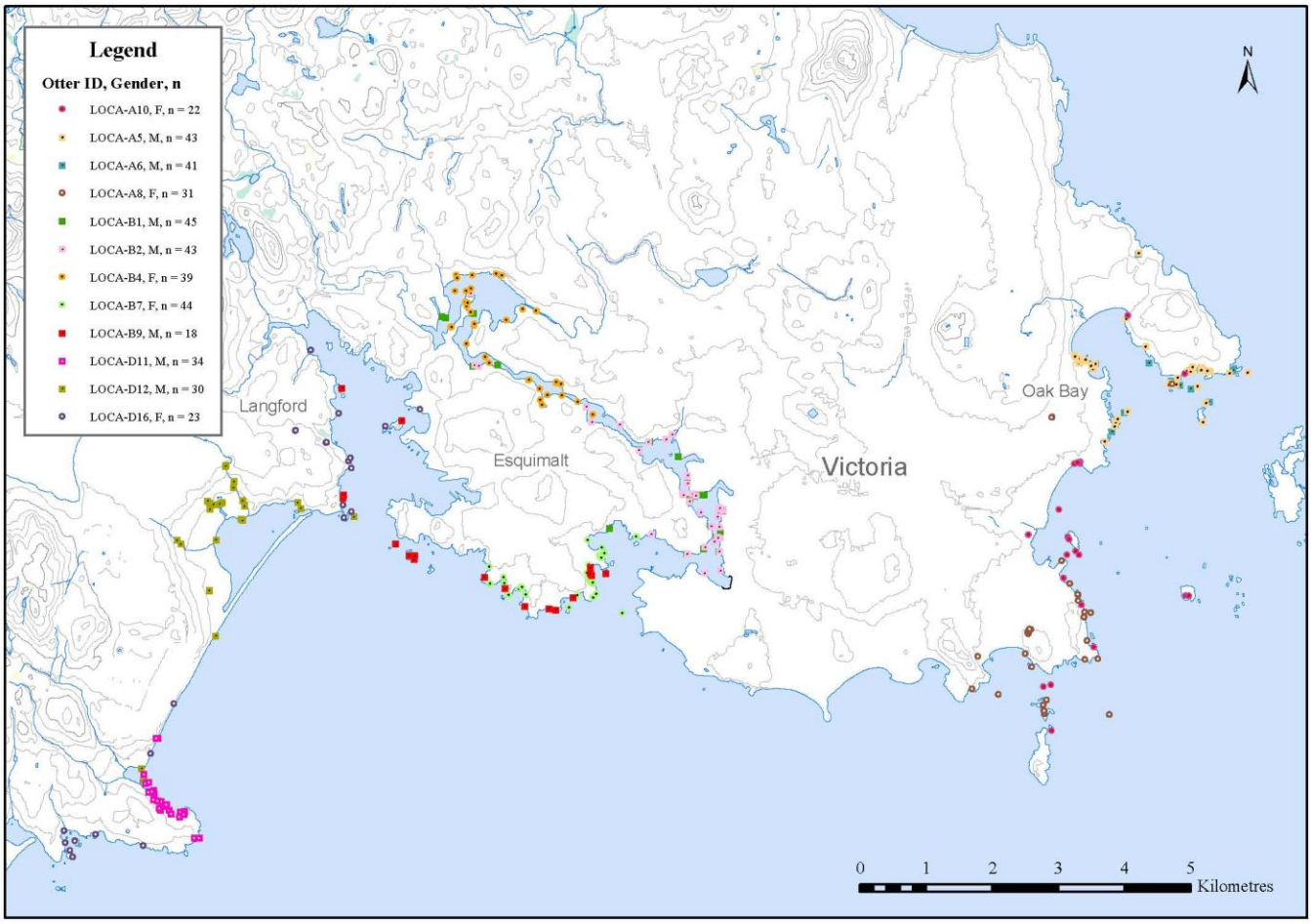
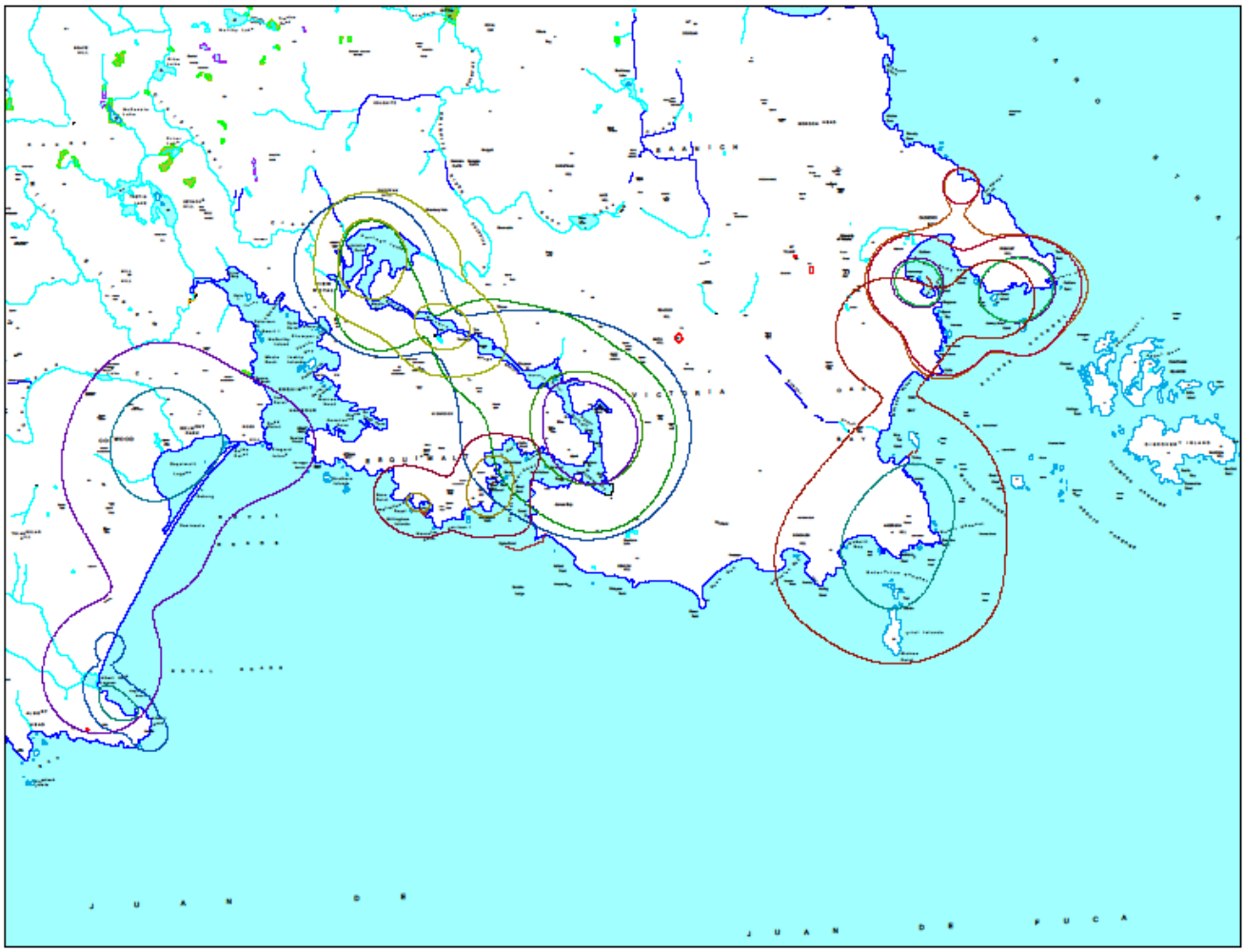


Figure 2.2: Summary of Fixed Kernel Analyses for home Range (95% contour) and Core areas of use (50% contour) for river otters on southern Vancouver Island between December 2009 and September 2011.



CHAPTER 3 - CONTAMINANT EXPOSURE AND NON-INVASIVE HORMONE MEASURES IN COASTAL RIVER OTTERS

Historical anthropogenic activities in the Pacific Northwest have resulted in the accumulation of toxic pollutants in the marine environment through industrial effluent and agricultural run-off. These toxic compounds include polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs) and certain organochlorine (OC) pesticides. Chlorinated hydrocarbons are highly resistant to degradation and although their use has been restricted for several years, high levels have persisted in coastal ecosystems. These lipophilic (fat-soluble) compounds tend to accumulate in animal tissue, biomagnify as they ascend the aquatic food web (Ruus et al 2002) and could pose a threat to wildlife, particularly top predators that inhabit these ecosystems.

In the Georgia Basin-Puget Sound area, known now as the Salish Sea, a number of avian and mammalian species have been studied to monitor for impacts of environmental contamination in the marine environment. Significant levels of persistent organic pollutants (POPs) have been reported in harbor seals (Ross et al 2004), killer whales (Ross et al 2000) and bald eagles (Elliott and Norstrum 1998). There are ongoing concerns that chronic exposure to POPs could impact health of important wildlife populations.

Victoria and Esquimalt Harbours, on southern Vancouver Island, are contaminated sites where a variety of toxic xenobiotic chemicals, for example, polychlorinated biphenyls (PCBs), have accumulated in the marine ecosystem from past industrial activities (Ikonomou et al 2002, Elliott

et al 2008). These localized contaminant sources are not amenable to investigate using wider ranging avian and marine mammal predators. The river otter, in part because of their smaller home range (Melquist & Dronkert 1987), was selected as a sentinel top predator to investigate the finer scale dynamics of new and residual POPs in and around Victoria, BC.

Many POPs, including PCBs, are proven or suspected endocrine disrupting chemicals (EDCs) and can affect hormone function by inhibiting synthesis, altering serum transport and increasing catabolism (Devito et al 1999). Interference of EDCs with steroid hormone receptors has been linked to reduced immune function and reproductive impairment in polar bears, harbor seals and cetaceans (Reijnders 1986, Fossi and Marsili 2003; Mos et al. 1996). Certain compounds have a similar chemical structure to thyroid hormone and therefore interfere with binding to receptors and transport proteins (Boas et al 2006). Chronic PCB exposure can affect thyroid hormone transport and function (thyroxine, T4, and triiodothyronine, T3) in vertebrates (Cheek et al 1999, Rolland 2000) and disrupt the development and differentiation of cells (Vos et al 2000).
River otters are exposed to contaminants primarily through their diet (Clark et al 1981, Henny et al 1981, Harding et al 1999) and as a top-predator they are particularly prone to accumulation of toxicants which biomagnify with trophic level (Ruus et al 2002). Elliott et al (2008) reported that otters inhabiting Victoria and Esquimalt Harbours were exposed to elevated levels of PCBs but potential toxicological consequences were not clear.

In a number of studies chronic PCB exposure has been shown to affect reproductive fitness in mink (*Mustela vison*) (Aulerich and Ringer 1977, Bleavins et al 1980, Brunstrom et al 2001), a closely related species to the river otter and one that shares the same general habitat

requirements. Population declines of river otters have been associated with contaminated environments in various regions of the USA (Raesly 2001) and in Europe (Mason and MacDonald 1993). The disappearance of otters from much of their historic range in Europe was thought to be caused by a number of different factors, but exposure to PCBs appeared to be involved in population declines around industrialized areas (Mason 1989[a], Mateo et al 1999). Kruuk and Conroy (1996) found that there was a strong negative correlation between PCB concentration in otter liver tissue and body condition.

River otters tend to be elusive and difficult to capture, therefore contaminant exposure in this species has typically been studied through scat (feces) or carcass sampling (Mason and O'Sullivan 1992, Kruuk and Conroy 1996, van den Brink and Jansmen 2006, Elliott et al. 1999, Grove & Henny 2008, Harding et al 1999, Elliott et al. 2008, Guertin et al 2010a, b). Mason & O'Sullivan (1993) found that PCB levels in scat from the Eurasian otter (*Lutra lutra*) were significantly correlated to PCB levels of liver tissue (representing body burden). Although fecal PCB levels mainly reflect the contaminant load of their recently ingested prey, scat can be used to estimate the body burden of the animal.

Scat sampling at latrine sites is an effective, non-invasive technique used to study multiple individual at specific geographic and temporal scales. This type of sampling also allows for multidisciplinary approaches by combining toxicological, genetic and hormone data (to name a few) that provide a more complete picture of the potential ecological impacts. For example, fecal hormone data can complement contaminant data by providing information on potential

physiological responses to the animal's environment (Bateman et al. 2009, Douyon and Schteingart, 2002, Wasser et al. 2010).

Guertin et al (2010a) combined fecal DNA genotyping with toxicological and diet analysis to assess site and individual specific contaminant levels in river otters from Victoria and Esquimalt Harbours. This technique allowed for re-sampling of known otters and revealed fecal PCB levels were variable over time, space and individual. The river otter diet is very diverse in a marine environment (Guertin et al 2010b) therefore different prey species could be contributing different proportions of contaminants at various times and locations.

In order to determine the extent and implications of contaminant exposure within this population, the relationship between contaminant levels in otter scat and actual toxin burden in the animal requires more rigorous assessment. The concurrent radio telemetry study (Chapter 2) in Victoria allowed for blood to be collected from 16 river otters that were then radio tagged and tracked for one year. Radio telemetry data collected from animals in this population indicated limited movement and seasonally constant home ranges and implied that certain animals inhabiting the harbor were being exposed to contaminants year-round. A marine foraging river otter on southern Vancouver Island used a home range (95% contours) of approximately 16 km of coastline (Chapter 2). Because I now have a better understanding of the space an animal uses and how far they travel, these measures of landscape use can be applied to the interpretation of spatial contaminant patterns.

I tested the hypothesis that data derived from river otter scat will reveal localized areas of exposure, relative toxic burden on the animal, consistently elevated PCB levels within the Harbour sites and hormone responses to environmental and anthropogenic stressors. The objectives of this study were to (1) relate contaminant levels to spatially explicit movement of otters (2) compare fecal and blood contaminant data (3) assess fecal contaminant levels over multiple years of sampling and (4) investigate fecal hormones as indicators of effect of multiple stress factors, particularly contaminant exposure.

STUDY AREA

The study area spans approximately 80 km of coastline along Southern Vancouver Island 48°25′43″N 123°21′56″W. The study area consists of 4 distinct sections along an urban-industrial gradient of continuous coastline; From East to West (A) Oak Bay and Cadboro Bay, (B) Victoria Harbour, (C) Esquimalt Harbour and (D) Colwood/Methcosin. Based on the home range analysis from Chapter 2, an individual otter will use most of the space within these defined sections, making them biologically relevant for spatial comparisons. The habitat characteristics of each area are distinctive. The harbours have primarily industrialized foreshore, many docks and heavy small boat and seaplane traffic. Oak Bay and Cadboro Bay have a relatively natural foreshore with rocky intertidal ecosystems and a number of near shore islands, as well as many waterfront homes and a significant amount of boat traffic. Colwood/Methosin has mainly natural foreshore with pebble beaches, some waterfront homes and minimal boat traffic.

METHODS

River otter samples were field collected between November 2009 and October 2010 at latrine sites along the marine-terrestrial interface. River otters will use multiple latrine sites within their range and tend to show high site fidelity. River otters deposit feces, anal jelly and/or a mixture of both at latrine sites. Anal jellies are a mucous-like substance produced in the intestinal tract of the otter, likely to facilitate the passing of bones and shells from their prey. Elliott et al (2008) showed that jellies contain the same concentration of contaminants relative to scat or mixed feces/jelly samples. Guertin et al (2010a) reported a higher success rate for genotyping in jellies relative to scat. With this information it was determined that fresh anal jellies would be the most suitable sample type for this study, therefore sampling focused on the collection of jellies. This sample type would not be suitable if the objective was to determine diet, as jellies contain minimal prey material. These samples will be referred to as scat here after. Each sample was processed in the field and subsampled for chemical, genetic and hormone analysis. Contaminant samples were placed in chemically-rinsed (acetone/hexane) amber glass jars and stored at -20ºC for several months until shipment to the Great Lakes Institute for Environmental Research, Windsor, ON, where they were stored at -40ºC until analyzed. Hormone samples were placed in plastic bags and stored at -20ºC until analyzed. Samples for genetic analysis were stored at -4ºC until analyzed. The results of genetic analysis will not be presented at this time.

Blood samples werepreviously collected by the provincial wildlife veterinarian from 16 river otters during the capture of animals for the related telemetry study. Blood was centrifuged and the plasma portion was stored in chemically-rinsed amber glass vials at -20ºC until shipment to

the National Wildlife Research Centre, Ottawa, ON, where they were stored at -20ºC until analyzed.

Chemical Analysis

Sixteenblood samples and 77 field collected scat samples were analyzed for a suite of chemical compounds. Chemical analysis of scat was carried out at the Great Lakes Institute for Environmental Research at the University of Windsor, 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 river otter scat included determination of total chlorobenzenes (ΣCBz represents 1,2,4,3-tetrachlorobenzene, 1,2,3,4-tetrachlorobenzene, pentachlorobenzene, and hexachlorobenzene), hexachlorocyclohexanes (ΣHCH represents α-, β-, and γ-hexachlorocyclohexane), chlordane-related compounds (ΣCHLOR represents oxychlordane, *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor, *cis*-nonachlor), dichlorodiphenyltrichloroethane (DDT) and its metabolites [ΣDDT represents *p,p′*-dichlorodiphenyltrichloroethane (*p,p′*-DDT), *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE), and *p,p′*-dichlorodiphenyldichloroethane (*p,p′*-DDD)], mirex, PCBs (ΣPCBs represents 39 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), and polybrominated diethyl ethers (PBDEs) (ΣPBDEs represents 12 congeners according to IUPAC numbers: 17, 28, 49, 47, 66, 100, 99, 85, 154, 153,

138, and 183). Chemical extraction and cleanup of OCPs, PCBs, and PBDEs followed the procedures of Lazar et al. (1992).

Chemical analysis of blood was carried out at Environment Canada's National Wildlife Research Center, Ottawa, Ontario, Canada. Analysis of river otter blood included determination of selected organochlorine pesticides (OCs), polychlorobiphenyls (PCBs), brominated diphenyl ethers (BDEs) and brominated flame retardants (BFRs), measured by gas chromatograph with mass selective detector (GC/MSD). Plasma samples were denatured with formic acid at aone-to-one ratio. The extraction of OCs/PCBs/BDEs/BFRs was done with activated C18 Cartridges and elution with dichloromethane (DCM)/Hexane (1:1). The DCM/Hexane extracts were cleaned up by Florisil column chromatography. The purified sample extracts were analyzed for OCs, PCBs and BDE/BFRs using a capillary gas chromatograph (Agilent 6890N), coupled with a mass selective detector (Agilent 5973N) operated in selected ion monitoring mode. The analysis of contaminants followed standard procedures described in the Laboratory Service Methods Manual (Environment Canada 2003).

Hormone Analysis

Two hundred field collected scat samples were analyzed for reproductive (testosterone and progesterone), stress (Corticosterone) and thyroid (T4, T3) hormones at the University of Guelph, Guelph, Ontario, Canada. Fecal hormone levels were grouped by season and location to investigate population level patterns. This lab was selected because they developed in-house Enzyme Immunoassays (EIA) for thyroid and reproductive hormones that had been validated for North American river otters (Bateman et al 2009). Fecal hormone extractions were carried out by first drying 1.0 g of wet scat in a 60ºC oven for 24 hours, then grinding dried scat into a powder,

then mixing 0.2 g of powder with 5ml of 80% EtOH in a glass vial. The vials were left on a shaker (1 pulse/second) at room temperature for 16 hours, then centrifuged for 10 minutes at 500 x g. Supernatant was then decanted and stored at 4ºC.

Serial dilutions of fecal extracts were assayed with the following enzyme-immunoassays (EIA) and gave displacement curves parallel to that of the respective standard curves.

Fecal progesterone metabolites were quantified using a progestagen EIA previously described (Graham et al. 2001). In brief, microtitre plates (Nunc; Fisher Scientific) were coated with affinity purified goat anti-mouse gamma globulin (50 ug/plate; Sigma Chemicals, St. Louis, MI) dissolved in coating buffer (0.015M Na_2CO_3, 0.035M $NaHCO_3$; pH 9.6) and incubated overnight at room temperature. Wells were emptied and refilled with trizma assay buffer (0.02M Trizma, 0.300M NaCl, 0.1% BSA; pH 7.5) and stored at room temperature for at least 30 minutes prior to use. Coated plates were washed (0.04% Tween 20) and 50 ul of diluted sample and standards were dispensed. Biotinylated progesterone was dispensed followed by 100 ul of primary antibody (Quidel clone #425; final purification by C. Munro, Davis, CA). Plates were incubated overnight at room temperature. Following incubation, plates were washed and 200 ul of streptavidin-peroxidase conjugate (1ul in 30 mls trizma assay buffer; Roche Molecular Biochemicals, Indianapolis, IN) were added to each well. Following incubation (45 min; room temperature) plates were washed and 200 ul of substrate solution (0.5 ml of 0.016M tetramethylbenzidine in dimethylsulphoxide and 100 ul of 0.175M H_2O_2 diluted in 24 ml of 0.01M $C_2H_3O_2Na$; pH 5.0) was added to each well. After incubation (45 min, room temperature) the enzyme reaction was stopped with 50 ul of stop solution (3M H_2SO_4). The

optical density was measured at 450 nm (reference 595 nm). The standard curve of progesterone ranged from 3.9 – 500 pg/50 ul

Fecal testosterone metabolites were quantified using a testosterone EIA. In brief, microtitre plates (Nunc; Fisher Scientific) were coated with affinity purified goat anti-rabbit gamma globulin (25 ug/plate; Sigma Chemicals, St. Louis, MI) dissolved in coating buffer (0.015M Na_2CO_3, 0.035M $NaHCO_3$; pH 9.6) and incubated overnight at room temperature. Wells were emptied and refilled with trizma assay buffer (0.02M Trizma, 0.300M NaCl, 0.1% BSA; pH 7.5) and stored at room temperature for at least 30 minutes prior to use to block non-specific binding. Coated plates were washed (0.04% Tween 20) and 50 ul of diluted sample and standards were dispensed. Horse-radish peroxidase-labeled testosterone (supplied by CJ Munro) was dispensed followed by anti-testosterone antibody. Plates were incubated overnight at 4C. Following incubation, plates were washed, incubated with substrate and optical density was measured as described above. The standard curve of testosterone ranged from 4.9 – 625 pg/50ul.

The T3 and T4 enzyme-immunoassay protocols were a modification of previously published protocols (Graham et al., 2001). The optimum concentrations and dilutions of antibodies and biotinylated T3 and T4 were determined by checkerboard titration. In brief, microtiter plates (Nunc; Fisher Scientific) were coated with T3 antibody or T4 antibody (Sigma Chemicals, St. Louis, MO) dissolved in coating buffer (0.015 mol/L Na2CO3, 0.035 mol/L NaHCO3; pH 9.6) and incubated overnight at room temperature. Coated plates were washed (0.04% Tween 20), and 100 ul of assay buffer added to each well and incubated at RT for 1 hr. Then 50 ul of diluted sample and standards were dispensed. Immediately, 100 ul of biotinylated T3 or T4 was

dispensed to each well. Plates were incubated overnight at room temperature. After incubation, plates were washed and 200 ul of streptavidin-peroxidase conjugate (1 uL in 22 ml assay buffer; Roche Molecular Biochemicals, Indianapolis, IN) were added to each well. After incubation (45 minutes; room temperature) plates were washed and 200 ul of substrate solution (0.5ul of 0.016 mol/L tetramethylbenzidine in dimethyl sulfoxide and 100 ul 0.175 mol/L H2O2 diluted in 24 ul 0.01 mol/L C2H3O2Na; pH 5.0) was added to each well. After incubation (45 minutes, room temperature) the enzyme reaction was stopped with 50 ul of stop solution (3 mol/L H2SO4). The optical density was measured at 450 nm. T3 and T4 were used as standards and serial dilutions of fecal extracts gave displacement curves parallel to that of the standard curve.

Fecal Corticosterone was quantified using a commercially available EIA kit, validated in river otters by Rothschild et al (2008). We used the Correlate-EIATM Corticosterone EIA Kit (Enzo Life, Inc., Ann Arbor, MI) to determine glucocorticoid values. Manufacturer instructions were followed without modification.

Data analysis

Statistical analyses were performed using R version 2.15.0 (The R foundation for Statistical Computing). Contaminants data were log transformed, as chlorinated hydrocarbon data are commonly log normally distributed (Guertin et al 2010a). Sum PCBs data were was log-transformed (log +1 for all values) for statistical analysis to approximate normal distribution. To determine if scat contaminant patterns reflect contaminant patterns in circulation, the relationship between PCB patterns in blood (expected) and scat (observed) was tested with a Chi-square Test.

Effects of location on contaminant levels in blood and scat were tested by Analysis of Variance (ANOVA). Effects of location and season on hormone levels were also tested by ANOVA. When an ANOVA was significant, mean separation wasdone using Tukey's Honest Significant Difference (HSD) Test. Matrix Correlation calculations were performed to test the response of hormone (dependent) levels in relation to contaminant (independent) levels. Statistical significance was set at $p < 0.05$.

RESULTS

Summary of all contaminants for blood and scat

Sixteen blood samples and 77 field-collected scat samples were analyzed for a suite of chemical compounds. A summary of all contaminant concentrations are presented in Figures 3.1a-c and 3.2a-c (Detailed data presented in Appendix 1). Contaminant concentrations are expressed as geometric means to remove disproportionate effects of outlying values.

Overall levels of OC pesticides in river otter samples (scat and blood) were low across the study area. PBDEs in river otter samples were low across the study area, with the exception of two scat samples with high levels of BDE-209 (162.5 and 956.1 mg/kg/lw). Sum PCBs, OC pesticides and PBDEs are presented as geometric means (mg/kg lipid weight) for scat and blood in Table 1.

Selected PCB congeners by Zone (Objective 1)

Concentrations (mg/kg lipid weight) for selected PCB congeners for blood and scat are presented in Figures 3.3a and 3.3b, respectively. The five main PCB congeners were CB-99, CB-153, CB-138, CB-180 and CB-170. Patterns (ratios to sum-PCBs) of these congeners do not differ

significantly between blood and scat samples (Chi-square Test, X^2=0.727, DF=4, p=0.9479). Although not significantly different, scat ratios appear to be higher than blood ratios in the higher chlorinated congeners. This is contrary to what has been seen in similar studies where tissue ratios were higher than prey ratios (van den Brink and Jansmen 2006).

The highest levels for a specific congener (e.g. PCB 153) were observed in Victoria Harbour (B) for both blood (Geometric mean, 10.33 mg/kg lw) and scat (Geometric mean, 1.712 mg/kg lw).

Sum PCBs for blood vs. Scat by Zone (Objective 2)

Sum PCB concentrations for blood and scat, by zone, are combined in Figure 3.4. The highest levels for Sum PCBs were observed in Victoria Harbour (B) for both blood (Geometric mean, 38.94 mg/kg lw) and scat (Geometric mean, 6.35 mg/kg lw). In Victoria Harbour individual samples ranged from 1.23-61.26 mg/kg lw for scat and 14.50-75.48 mg/kg lw for blood.

There were significant differences between zones for both blood (DF=2/13, F=33.62, p<0.01) and scat (DF=3/73, F=20.79, p<0.01). Mean separation for multiple comparisons (Tukey HSD) are summarized in Table 3.2. Sum PCB levels in scat and blood from Victoria Harbour (B) were significantly higher than Oak Bay (A) (p=0.00000, p=0.00001) and Colwood-Metchosin (p=0.00000, p=0.0001). Sum PCB levels in scat from Esquimalt Harbour (B) were also significantly higher than Oak Bay (A) (p=0.01) and Colwood-Metchosin (p=0.02). There was no blood sampled from Esquimalt Harbour.

Sum PCBs in scat over multiple years (Objective 3)

The Sum PCBs in scat from this study (geometric mean, 6.35 mg/kg lw) were combined with reported levels from previous work in this area (Elliott et al 1998, Guertin et al 2010b). in Figure 3.6. There appeared to be a decreasing trend in mean value, however levels were within range of previous years. Statistical comparisons between years were not possible due to differences in sampling design.

Fecal Hormones as indicators of effects (Objective 4)

Geometric mean hormone concentrations are summarized in table 3.4. There was a significant difference between hormone levels in winter (breeding) and summer (non-breeding) for testosterone (ANOVA, DF=1, F=33.401, $p<0.01$), progesterone (ANOVA, DF=1, F=15.496, $p<0.01$) and thyroid, T3 (ANOVA, DF=1, F=7.598, $p<0.01$). Winter levels were consistently higher, relative to summer, for all 4 zones. There was a significant difference between zones for testosterone (ANOVA, DF=3, F=3.645, p=0.0137). Fecal testosterone levels from Esquimalt Harbour (C) were significantly lower than Oak Bay (A) (Tukey's HSD, p=0.01685). In winter, Oak Bay (A) presented the highest geometric mean fecal testosterone concentration overall (743.25 ng/g). Geometric mean concentrations of fecal testosterone by season and zone are presented in Figure 3.7.

Individual samples that were analyzed for both hormone and contaminant levels were compared to investigate individual level patterns. Matrix correlation calculations were performed to determine if there were changes in hormone levels in response to multiple contaminants. There were no significant results from the correlation calculation, summarized in table 3.5.

DISCUSSION

Home range analyses from otters in this population indicate limited movement and therefore suggest localized exposure and potentially chronic exposure for individuals inhabiting Victoria Harbor (Chapter Two). Based on home range analysis, it is unlikely that otters inhabiting non-harbour sites will be exposed to toxicologically significant levels of contaminants. PCB levels in river otter blood and scat from Victoria and Esquimalt Harbours were significantly higher than levels observed at non-harbour sites. Mean PCB levels (blood and scat) were highest within Victoria Harbour (B) followed by Esquimalt Harbour (C). The data indicated localized exposure within the harbours and minimal exposure at non-harbour sites.

Results for specific PCB congeners revealed the same patterns (ratio of sum PCB) between blood and scat samples, indicating that PCBs observed in scat reflect PCBs in circulation. The data indicated that scat derived contaminants data was representative of body burden. Van den Brink (2006) experimentally validated a finding in the European otter, whereby fecal concentrations of non-metabolized PCBs reflected internal concentrations. Similarly to the van den Brink and Jansmen (2006) study, the most significant PCB congeners here, in both blood and scat, were higher chlorinated PCB-138 and PCB-153. This suggests that otters could be metabolizing the lower chlorinated congeners.

The lower sum PCB concentration in scat relative to past studies (Elliott et al 2008, Guertin et al 2010a) is probably an artifact of the variability in fecal PCB levels rather than an actual trend. Mason and O'Sullivan (1992) developed effects criteria for PCB exposure in European otter scat resulting in reproductive toxicity ([PCB] >16 mg/kg = critical, 16-9 mg.kg = of concern, 4-9

mg/kg = maximum allowable, <4 mg/kg = no effects). The number of scat samples within each of these effects criteria has been summarized in Table 3.3. From a total of 78 scat samples, 7 (9%) were above the critical effects level, and 5 (6%) were within the level of concern. Within Victoria harbour, 4 of 30 scat samples (13%) were above the critical level and 6 of 30 scat samples (20%) were within the level of concern. The data indicated that there continued to be elevated levels of PCB exposure among the river otters inhabiting Victoria Harbour. Comparable PCBs levels in European otters have been negatively correlated to population status (Mason 1989[a]) and Vitamin A levels in tissue (Murk et al 1998).

With the exception of PCBs, other measured chlorinated-hydrocarbon contaminants were generally low across the study area. There was however two river otter scat samples with high levels of BDE-209 (162.5 and 956.1 mg/kg/lw). These high BDE-209 levels were measured in samples collected from the same site in inner Victoria Harbour. River otters are known to chew through the material holding marine fish in an underwater aquarium at this site. This specific congener (BDE-209) is used as a flame retardant in high impact (hard) plastics (Hites 2004) so it is possible that the material containing this aquarium was the source of the BDE-209, however, I was not able to confirm the actual source.

Fecal Hormone levels were grouped by season and location to investigate population level patterns. These measures from field collected scat will only provide a snap shot of an individual's endocrine profile. Hormone levels in river otters will vary depending on a number of physiological and environmental factors (Bateman et al. 2009) that we were not able to measure. Reduced fecal testosterone levels in Equimalt Harbour and Victoria Harbour relative to non-

harbour sites could be indicative of population levels effects of chronic contaminant exposure. The significant location effect was seen between Oak Bay and Esquimalt Harbour, rather than Victoria Harbour where the highest levels of PCBs were observed. This suggests that other factors, such as otter density and demographics, are influencing hormone levels. Guertin et al (In Press) reported that the otter population density in the harbours and west side of the study area was one-half that of Oak Bay. An additional parameter that is still unknown is the demographic structure of the population across the study area. The lower testosterone levels observed in harbour sites could be due to a higher percentage of young males inhabiting these lower quality habitats, while dominant males preferentially select high quality habitat outside the harbour. Contaminant and hormone concentrations, measured in the same scat sample, were compared to investigate hormone response to contaminants in an individual otter at a particular location and time. There was no significant correlation (response) in fecal hormone levels relative to fecal contaminant levels. This analysis was limited as the source animals were unknown. It is difficult therefore to interpret these results without knowing the animal's gender, age and activity at that time. It would have been useful to have incorporated a genetic component so that the individuals could be identified and re-sampled. It remains unclear whether hormone levels are indicative of a response to contaminants or other stressors.

MANAGEMENT IMPLICATIONS

This study validated some aspects of non-invasive scat sampling by comparing blood and scat data by demonstrating consistent patterns and relative levels in the sample types. This supported the use of scat sampling to further investigate contaminant exposure in river otters non-invasively. The approaches to measuring hormones as indicators of physiological effects to

contaminant exposure and other stressors will require further development. Combining contaminant, hormone and genetic approaches would help to reveal individual patterns over time and space.

Within this population of river otters there was localized exposure to contaminants. Only the animals inhabiting the Harbours, particularly Victoria Harbour, are being exposed to high levels of PCBs. If individuals were exposed to contaminants year round (full time) there is potential for reproductive toxicity and population declines. River otters cannot detect the dangers of contaminant exposure (Delibes et al. 2009) so individuals from the surrounding areas could immigrate unknowingly. Although it has been demonstrated over multiple sampling years that Victoria Harbour otters are being exposed to high levels of PCBs (Elliott et al 2008, Guertin et al 2010a), there is no evidence at this time of population level effects of chronic contaminant exposure.

TABLES

Table 3.1: Sum Polychlorinated Biphenyl (PCB), Organochlorine (OC) Pesticide and Brominated Diphenyl Ether (BDE) concentrations (geometric means) from river otter samples (blood ad scat) collected on Southern Vancouver Island between November 2009 and October 2010.

Sample	**n**	**Sum PCBs**	**Sum BDEs**	**Sum OC Pests.**
Scat	78	2.2246	0.1785	0.26662
Blood	16	6.0640	0.4777	0.6895

Table 3.2: Multiple comparisons of sum Polychlorinated Biphenyl (PCB) concentrations between zones; Oak Bay (A), Victoria Harbour (B), Esquimalt Harbour (C) and Colwood-Metchosin (D). Summary of Tukey Honest Significant Difference (HSD) Results.

Zone	**Scat**	**Blood**
B-A	p=0.00000*	p=0.0000125*
C-A	p=0.00960*	
D-A	p=0.99928	p=0.5334035
C-B	p=0.25649	
D-B	p=0.00000*	p=0.0000693*
D-C	p=0.01570*	

Table 3.3: The number of river otter scat samples, collected on Southern Vancouver Island between November 2009 and October 2010, which were assigned to effects categories from Mason & O'Sullivan (1992) based on PCB concentration.

Effects Level	Oak Bay (A)	Victoria Hb (B)	Esquimalt Hb (C)	Colwood (D)
Sample size	23	30	10	15
Critical	0	6	1	0
Of Concern	1	4	0	0
Max. Allowable	0	10	3	1
No Effects	22	10	7	14

Table 3.4: Mean fecal hormone concentrations (ng/g) from river otter scat colleceted from Southern Vancouver Island between November 2009 and October 2010. By Season; Summer/non-breeding (S) and Winter/breeding (W). By Hormone; Progesterone (PROG), Testosterone (TEST), Triidothyronine (T3), Thyroxine (T4) and Corticosterone (CORT). By zone ; Oak Bay (A), Victoria Harbour (B), Esquimalt Harbour (C) and Colwood-Metchosin (D).

Zone	**Season**	**PROG**	**TEST**	**T3**	**T4**	**CORT**
A	S	264.67	151.30	352.85	1458.22	417.66
B	S	190.66	72.11	234.08	1194.56	281.97
C	S	217.53	75.56	236.85	1112.48	168.03
D	S	204.11	86.33	311.44	1394.89	256.14
A	W	460.22	463.43	414.50	1449.17	575.68
B	W	331.20	256.87	369.10	1484.28	474.24
C	W	378.61	209.39	308.92	1066.16	334.77
D	W	362.78	443.35	402.00	1338.93	484.96

Table 3.5: Correlation matrix calculations between fecal hormone concentrations (Progesterone (PROG), Testosterone (TEST), Triidothyronine (T3), Thyroxine (T4) and Corticosterone (CORT)) and fecal contaminant concentrations (Sum Polychlorinated biphenyl (PCB), Sum Organochlorine pesticide (OCP), Sum Brominated Diphenyl ether (BDE) and specific PCB congeners PCB-153 and PCB-138) from river otter scat collected from active latrine sites on Southern Vancouver Island between November 2009 and October 2010.

Progesterone	Sum PCB	Sum OCP	Sum BDE	PCB 153	PCB 138
rcorr	0.09	0.07	0.01	0.08	0.08
P value	0.577	0.659	0.929	0.61	0.624
Testosterone					
rcorr	0.06	0.02	0.26	0.12	0.14
P value	0.676	0.918	0.08	0.403	0.354
T3					
rcorr	0.12	0.13	0.1	0.2	0.19
P value	0.414	0.382	0.484	0.179	0.21
T4					
rcorr	0.1	0.06	0.1	0.03	0.03
P value	0.525	0.71	0.522	0.825	0.817
Corticosterone					
rcorr	0.24	0.12	0.25	0.09	0.11
P value	0.161	0.503	0.152	0.617	0.536

FIGURES

Figure 3.1a: Geometric means of Organochlorine (OC) Pesticides (mg/kg lw) in river otter scat collected from active latrine sites on Southern Vancouver Island, BC, Canada between November 2009 and October 2010.

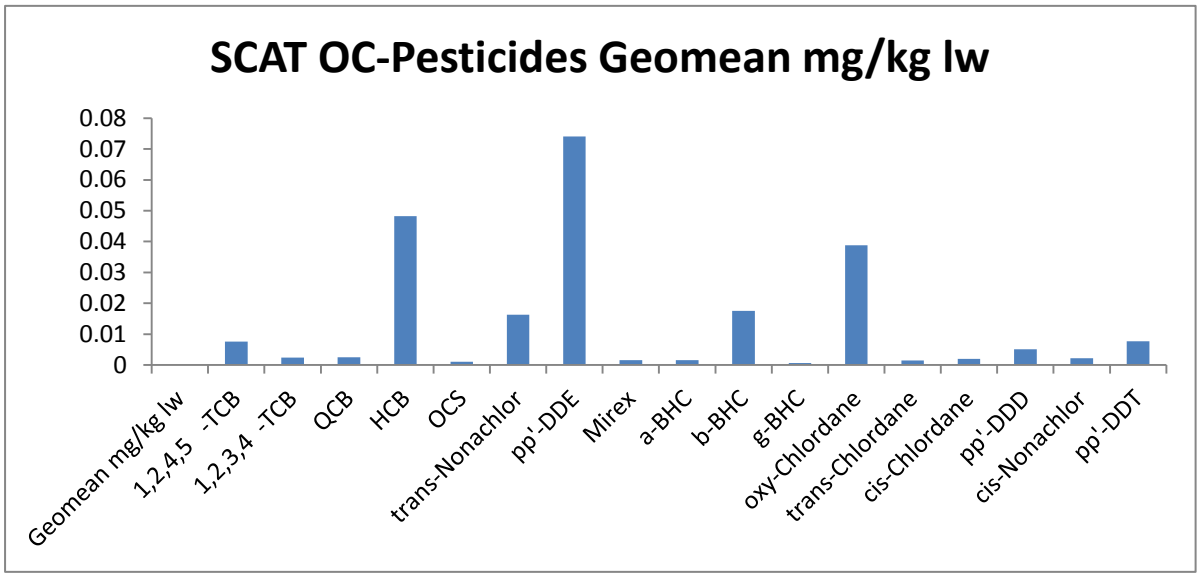


Figure 3.1b: Geometric mean of Brominated Diphenyl Ethers (BDEs) (mg/kg lw) in river otter scat collected from active latrine sites on Southern Vancouver Island, BC, Canada between November 2009 and October 2010.

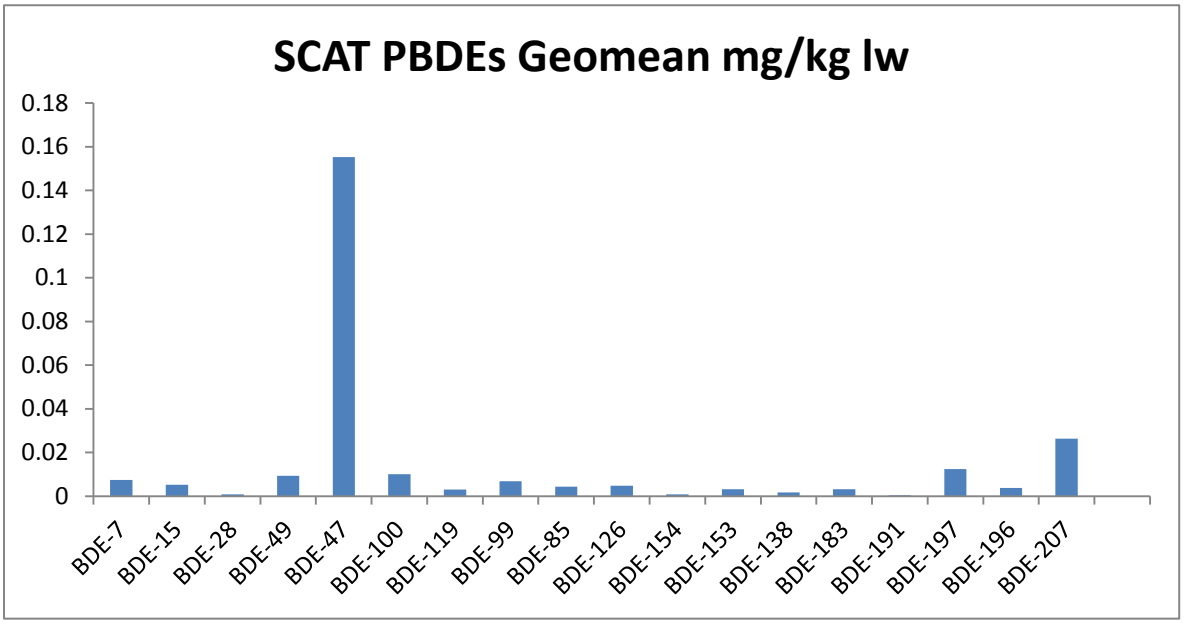


Figure 3.1c: Geometric mean of Polychlorinated Biphenyls (PCBs) (mg/kg lw) in river otter scat collected from active latrine sites on Southern Vancouver Island, BC, Canada between November 2009 and October 2010.

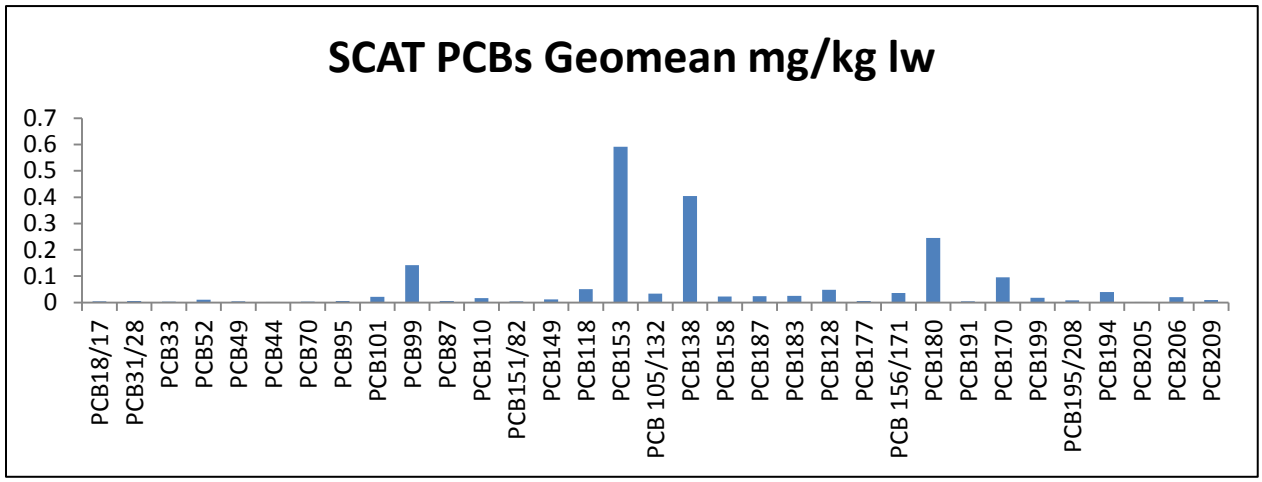


Figure 3.2a: Geometric mean of Organochlorine (OC) Pesticides (mg/kg lw) in river otter blood (plasma) collected from live captured river otters on Southern Vancouver Island, BC, Canada between December 2009 and March 2010.

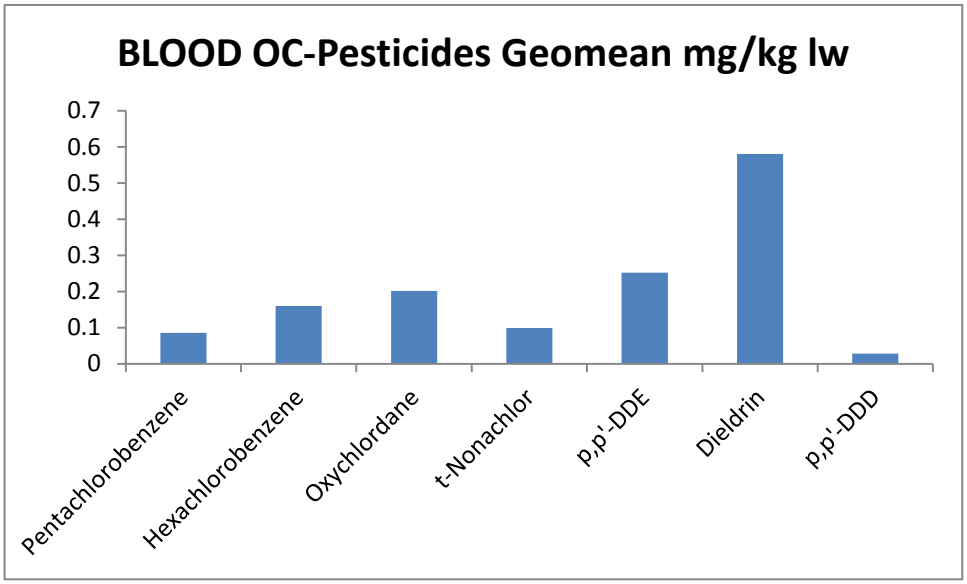


Figure 3.2b: Geometric mean of Brominated Diphenyl Ethers (BDEs) (mg/kg lw) in river otter blood (plasma) collected from live captured river otters on Southern Vancouver Island, BC, Canada between December 2009 and March 2010.

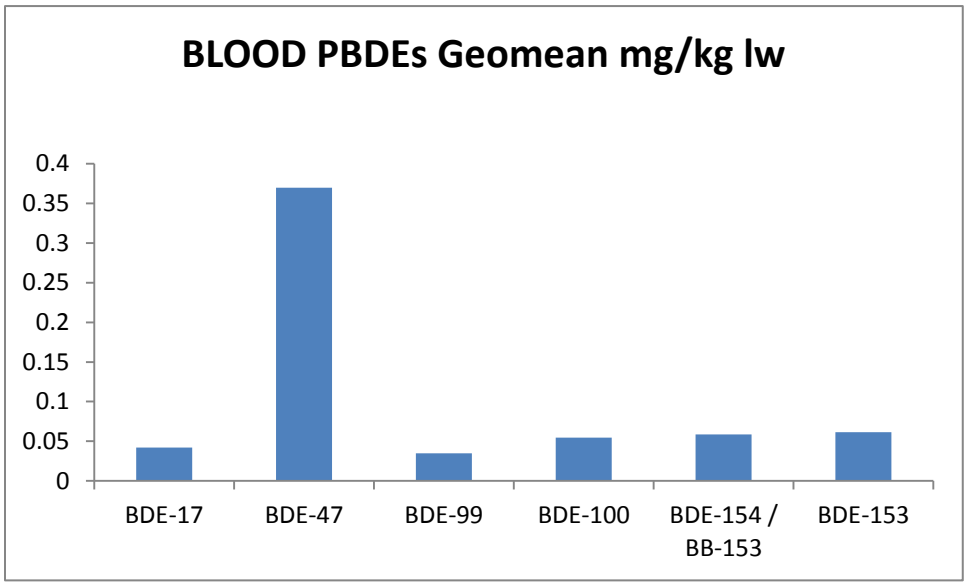


Figure 3.2c: Geometric mean of Polychlorinated Biphenyls (PCBs) (mg/kg lw) in river otter blood (plasma) collected from live captured river otters on Southern Vancouver Island, BC, Canada between December 2009 and March 2010.

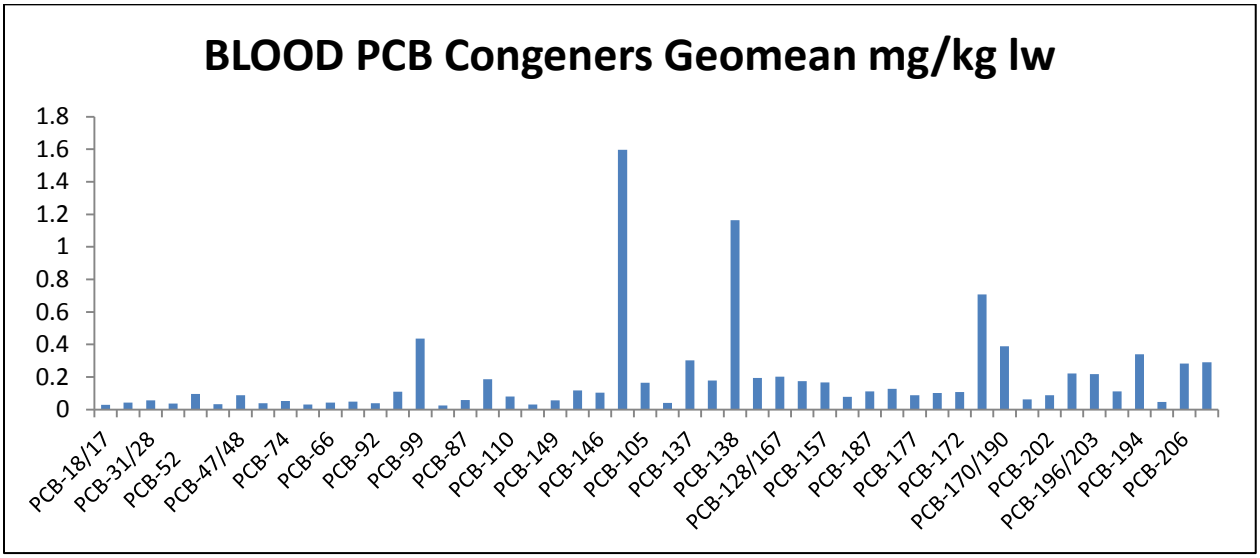


Figure 3.3a: Geometric mean for specific Polychlorinated Biphenyl (PCB) congeners (mg/kg lw) from river otter blood (plasma) collected from live captured river otters on Southern Vancouver Island, BC, Canada between December 2009 and March 2010. Presented by zone; Oak Bay (A), Victoria Harbour (B), Colwood-Metchosin (D). No blood samples from Esquimalt Harbour (C).

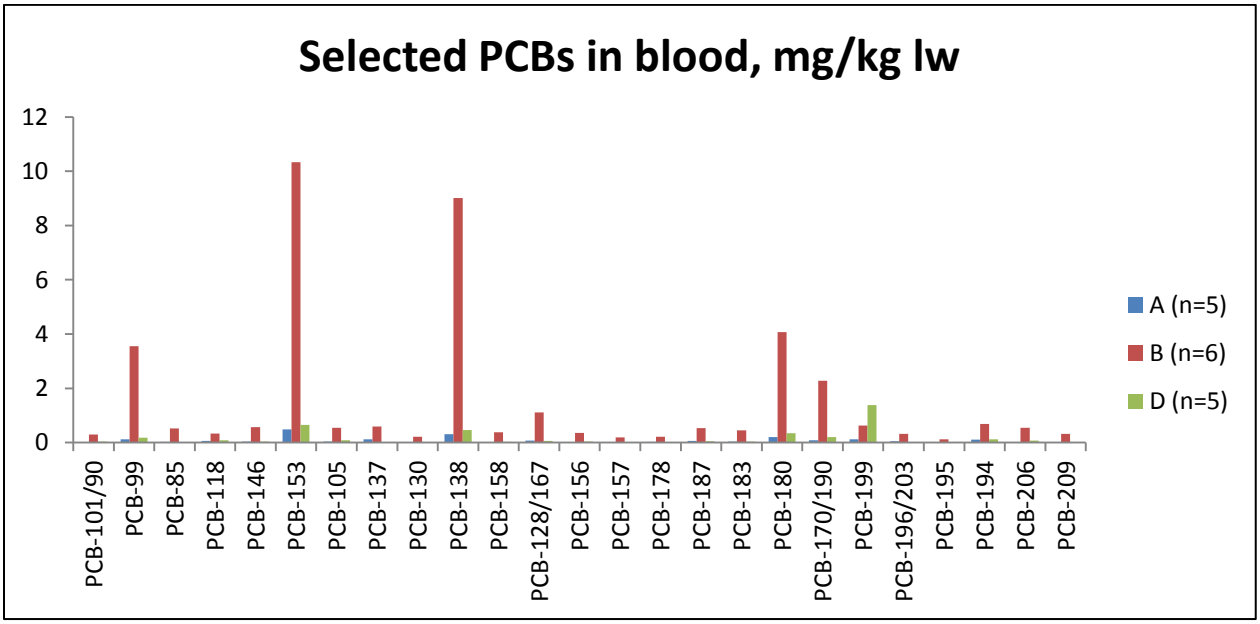


Figure 3.3b: Geometric mean for specific Polychlorinated Biphenyl (PCB) congeners (mg/kg lw) from river otter scat collected from active latrine sites on Southern Vancouver Island, BC, Canada between November 2009 and October 2010. Presented by zone; Oak Bay (A), Victoria Harbour (B), Esquimalt Harbour (C) and Colwood-Metchosin (D).

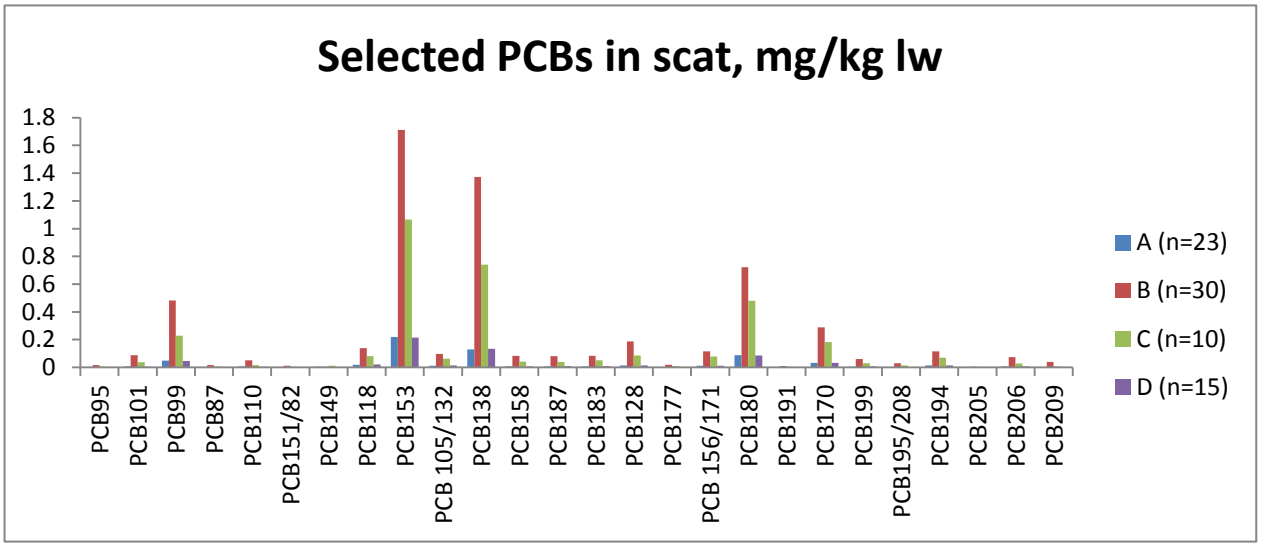


Figure 3.4: Geometric mean for sum Polychlorinated Biphenyl (PCB) from river otter samples (blood and scat) collected on Southern Vancouver Island, BC, Canada between November 2009 and October 2010. Presented by zone; Oak Bay (A), Victoria Harbour (B), Esquimalt Harbour (C) and Colwood-Metchosin (D). Includes standard deviation.

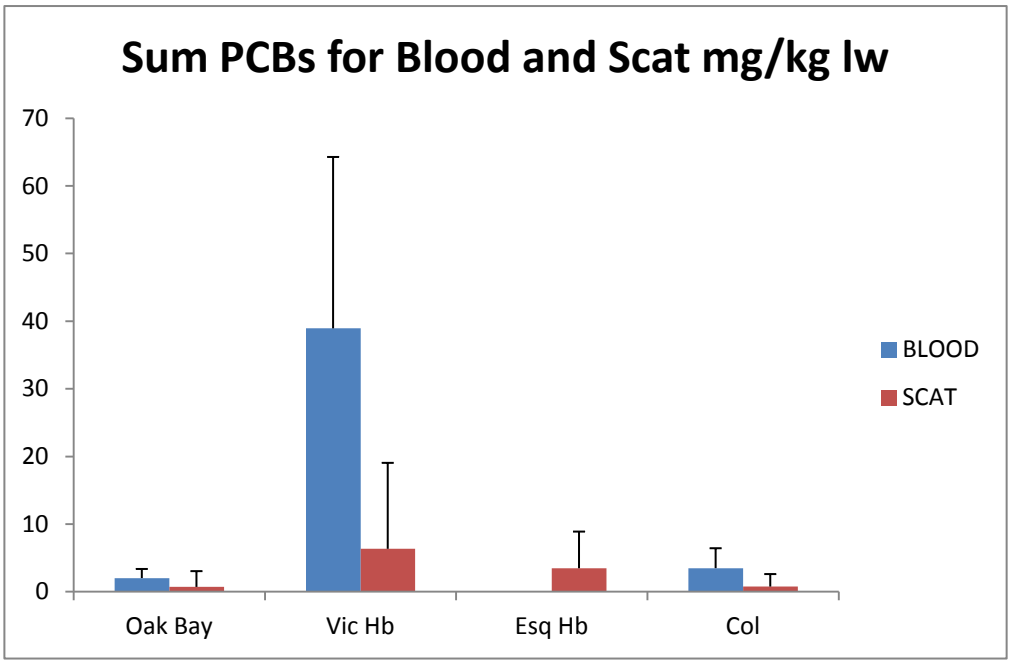


Figure 3.5: Polychlorinated Biphenyl (PCB) patterns (percentage of congener concentration to sum PCB concentration) in river otter otter samples (blood and scat) collected on Southern Vancouver Island, BC, Canada between November 2009 and October 2010.

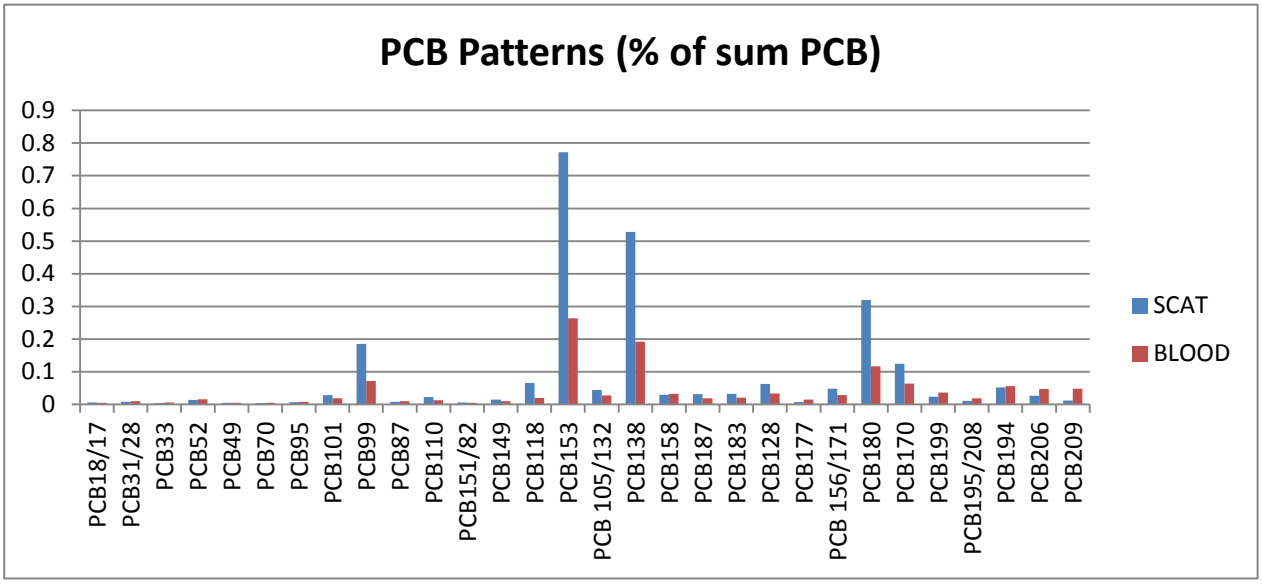


Figure 3.6: Geometric means of sum Polychlorinated Biphenyls (PCBs) in river otter scat collected on Southern Vancouver Island at multiple time points (1998, 2004, 2006, and 2010). Each year begins with Victoria Harbour (right to left). Sample size in brackets (x).

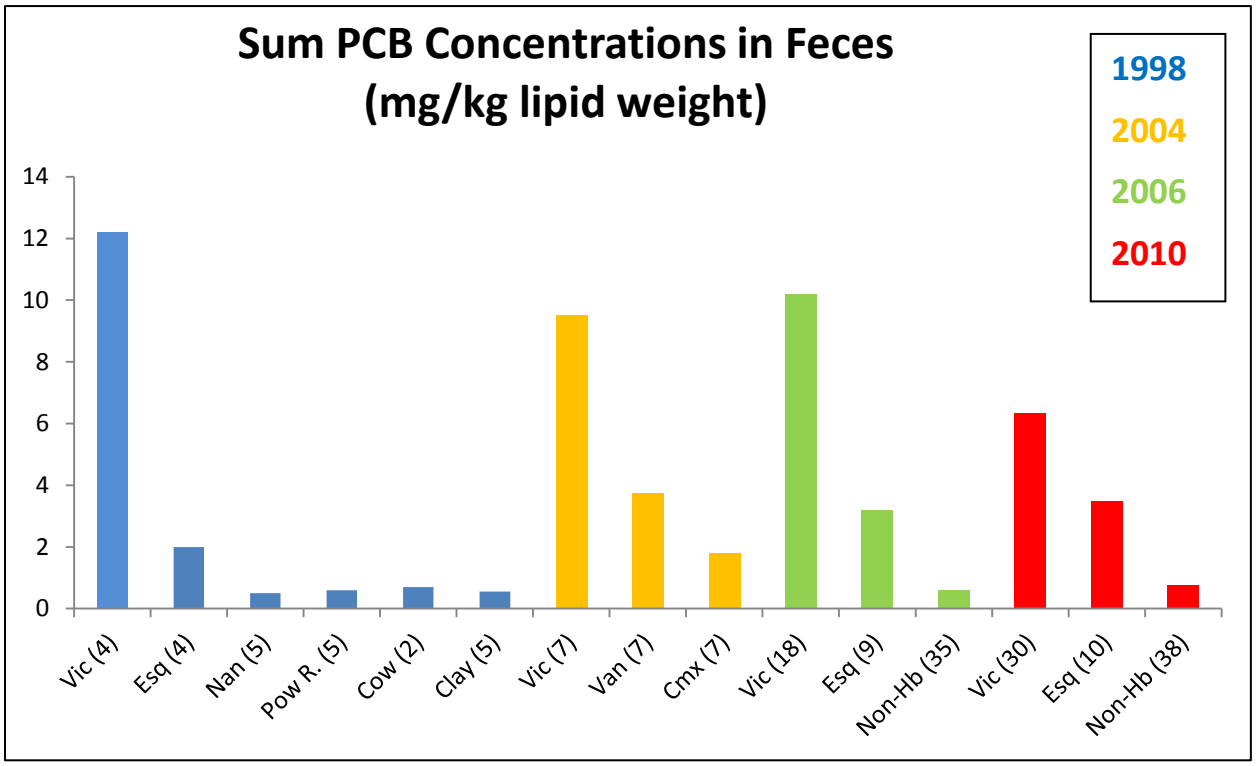
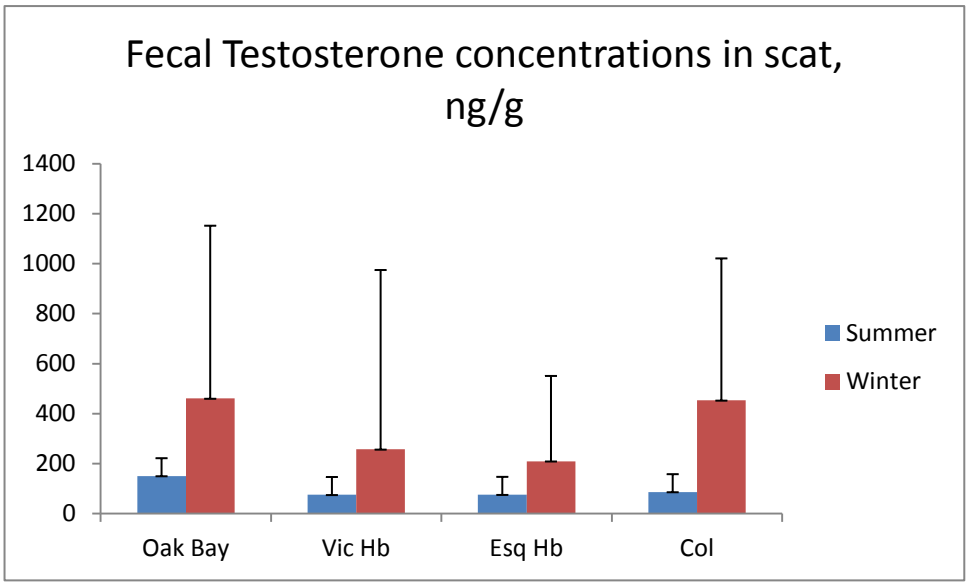


Figure 3.7: Geometric mean of testosterone concentration (ng/g) in river otter scat collected from active latrine sites on Southern Vancouver Island, BC, Canada between November 2009 and October 2010. Presented by zone; Oak Bay (A), Victoria Harbour (B), Esquimalt Harbour (C) and Colwood-Metchosin (D). Includes Standard deviation.



CHAPTER 4 – GENERAL DISCUSSION AND CONCLUSIONS

River otters (*Lontra canadensis*) are excellent sentinel species for investigating the impacts of environmental contamination on ecosystem health (Bowyer et al 2003). Environmental contamination has been, in part, responsible for significant population declines in European and North American river otters (Mason 1989[b], Lariviere and Walton 1998). River otter populations in the Pacific Northwest have not suffered the declines seen in other regions. There is not much known about coastal river otter ecology in BC because they have not been a conservation concern. However, as a top predator species, river otters have an important function in maintaining stability in their ecosystems, so it is valuable to understand their response to anthropogenic stressors such as contaminants.

Previous studies of this population of river otters presented population genetics and contaminant data derived from scat sampling. River otters within Victoria Harbour were being exposed to elevated levels of Polychlorinated Biphenyls (PCBs) (Elliott et al 2008, Guertin et al 2010[a]). The levels observed were above the levels of reproductive toxicity recorded for the European otter (Mason and MacDonald 1993). Comparable measures have not yet been established for the North American river otter, so the effects at these levels are unknown.

Fecal DNA analyses revealed two local subpopulations, one overlapping with the contaminated sites within the Harbour. The harbour subpopulation demonstrated high levels of relatedness, self-recruitment and emigration (Guertin et al, In Press). This result indicated that although there were elevated levels of exposure to PCBs, there appeared to be acceptable levels of survival and reproduction.

The purpose of my study was to confirm the above findings by testing aspects of the scat sampling techniques. I combined radio telemetry, live animal sampling and non-invasive scat sampling to investigate the impacts of contaminant exposure on marine foraging river otters and their ecosystem. The distribution and movement of river otters in relation to the contaminated sites was defined through radio telemetry and home range analyses. The hypothesis of population structuring was tested on the basis of limited mixing of individuals from the two proposed subpopulations. Limited movement would support this hypothesis and would indicate localized contaminant exposure. The relationship between fecal contaminant levels and actual body burden was examined by scat and live animal sampling. The hypothesis that fecal contaminants would reflect internal levels was tested by comparing blood (plasma) and field collected fecal material (scat and anal jelly). It was also expected that PCB levels would be elevated in Victoria and Esquimalt Harbours, as previously reported in scat data (Elliott et al 2008, Guertin et al 2010), and that this might correlate to a response in hormones levels, used as indicators for physiological effects.

The main finding of the telemetry study and home range analysis was that marine foraging river otters on southern Vancouver Island used relatively small spans of coastline (males = 16.84 km, females = 16.22 km). The home ranges of both males and females were seasonally constant, indicating that they were resident animals. As otters were confined to their small ranges, there was limited, if any, mixing of individuals between the subpopulations. River otter home ranges did overlap within a subpopulation but not between them. Certain otters remained within the Harbour over the course of the year and could have been exposed to contaminants full time, while other otters were exposed part time or not at all. In addition to supporting the hypothesis of small scale population structuring, these findings indicated localized PCB exposure.

Our increased knowledge of spatial ecology in coastal river otters was helpful in the analysis and interpretation of the contaminants study. The telemetry study revealed that an individual otter would use the space within the defined areas, e.g, Victoria Harbour (B). The spatial comparisons made here can therefore be considered biologically relevant for a river otter inhabiting a marine environment in this geographic region.

In future studies we plan to use the explicit data on habitat use by otters with a known genotype to compare movements and population structure with parallel information inferred from modeling of fecal DNA results throughout an annual cycle, building on previous work of Guertin et al (In press).

Contaminant levels in river otter plasma and scat were measured and compared between areas. The contaminant data revealed elevated levels of PCBs in river otter blood and scat from the Harbour sites. This was consistent with previously reported scat data (Elliott et al 2008, Guertin et al 2010[a]). Victoria Harbour had the highest sum PCB concentrations in blood (38.94 mg/kg lw) and scat (6.35 mg/kg lw), followed by Esquimalt harbour in scat (3.47 mk/kg lw), as there was no blood taken from Esquimalt Harbour. Blood and scat samples revealed similar patterns (ratio of sum) of PCB congeners. The most prevalent PCBs in both blood and scat were higher chlorinated congeners. As such, the hypothesis that scat contaminant levels would reflect internal levels was supported.

There did not appear to be a hormone response that could be attributed to elevated levels of PCB exposure. Testosterone measured in scat was lower in the Harbour sites, relative to non-harbour sites; however the only significant location effect was between Oak Bay and Esquimalt Harbour. Guertin et al (In press) proposed that the effects of chronic contaminant exposure at a population

level might be inadvertently mitigated by the avoidance of poor quality habitat in the harbours. Perhaps the avoidance of poor quality habitat is also influencing the demographics and number of reproducing individuals within the harbours. The trend in testosterone levels could be due to the avoidance of harbours by dominant males, rather than a direct indicator of adverse physiological effects.

The limited movement and small home ranges are probably an indication that these river otters have access to all of the required resources within a selected stretch of coastline. Due to the abundance of prey in productive marine environments river otters typically have smaller home ranges than those in fresh water systems (Blundell et al 1999).

The river otter population was partitioned into 2 genetics clusters; otters from the west side of the study area (including Colwood, Metchosin, Esquimalt Harbour and Victoria Harbour) and the east side (including Oak Bay and Cadboro Bay) (Guertin et al, In Press). Our data illustrates exposure and movement patterns on a slightly smaller scale. The overlapping home ranges from individuals in the west side, would suggest that they could be mating with one another and contributing to the genetic grouping. However, over the course of a year, their core areas of use were more localized. They might be considered part of the same subpopulation but their exposure to contaminants was occurring at a finer scale, only affecting individuals closest to the Harbour.

Based on otter latrine activity, it was previously known that otters were using the harbours. Through radio-telemetry, we now know that a number of those otters are resident to Victoria Harbour and are likely exposed to contaminants year round. Genetic data from the west subpopulation suggested high relatedness, high self-recruitment and high emigration (Guertin et

al, In press). This implied that the subpopulation was healthy and reproducing at acceptable rates. This is likely true for the subpopulation as a whole, however the health status of otters within the Harbour might not be the same for otters in Metchosin.

Fecal sampling can only provide a measure of contaminants or hormones at a particular moment in time and space. These snap-shots are a practical and common approach in contaminant studies (Elliott et al 2008, van den Brink and Jansmen 2006) but the temporal and spatial variability can present problems when interpreting the data. Fecal hormone levels, for example, will vary depending on a number of physiological and environmental factors (Bateman et al. 2009) that we were not able to measure. As such I acknowledge that these data are best applied when examining general population trends, unless genetic identification and re-sampling of individuals is possible.

Although the goal of this study was to advance the use of non-invasive techniques, the application of radio-telemetry and live animal sampling provided important information to the contaminants study. In an area of localized contamination an animal's history at that site is important in quantifying exposure (Eberhardt and Cadwell et al 1985). Most samples from a contaminated site will show elevated levels, regardless of the time the animal has spent there; however, the contaminant burden within the animal takes time to accumulate to toxicologically significant levels. Radio telemetry can provide an animal's movement patterns to and from the sites as well as their residence history. When interpreting contaminants data, this information was considered.

Our sampling design provided us with an opportunity to collect blood from known river otters. Mason and O'Sullivan (1992) used a bioaccumulation model that estimated body burden from

fecal levels. Blood and fat samples could be used to validate this model for the North American river otter. This sampling design could also be expanded to include prey samples from the areas of interest. Contaminant levels could then be compared between prey, scat, blood and fat to elucidate the transfer and biomagnification of contaminants through this marine based food chain.

This study demonstrated that data derived from scat, animal samples and radio telemetry produced the same general information about river otter toxicology and population metrics. We now know that fecal PCB levels reflect levels in circulation and that fecal genetics can be used to infer spatial organization and population structuring. This supports non-invasive scat sampling as an effective means of characterising the impacts of environmental contamination in an aquatic environment. Certain non-invasive techniques, such as measuring fecal hormones as indicators for stress and physiological effects, still need to be refined but will be important in determining population level impacts of contaminant exposure.

Non-invasive scat sampling can be a valuable management tool. These techniques provide a relatively efficient and logistically practical means of obtaining information pertaining to population ecology and wildlife's interactions with their environment. Scat sampling can be applied to river otter populations in other areas/regions where environmental contamination is a concern and to other wildlife species as a means of measuring ecosystem health and cumulative effects.

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APPENDICES

Appendix 3.1: Geometric means by zone (A=Oak Bay, B=Victoria Harbour, C=Esquimalt Harbour, D=Colwood/Metchosin) for specific congeners of all contaminants measured in river otter scat and blood.

BLOOD (mg/kg lw)	A	B	D	SCAT (mg/kg lw)	A	B	C	D
PCB-18/17	0.0294	NIL	NIL	PCB18/17	0.0046	0.0047	0.0047	0.0031
PCB-16/32	0.0437	NIL	NIL	PCB31/28	0.0045	0.0112	0.0047	0.0028
PCB-31/28	0.0571	NIL	NIL	PCB33	0.0055	0.0020	0.0021	0.0070
PCB-33/20	0.0369	NIL	NIL	PCB52	0.0051	0.0311	0.0176	0.0029
PCB-22	NIL	NIL	NIL	PCB49	0.0013	0.0073	0.0029	0.0007
PCB-52	0.0335	0.1144	NIL	PCB44	0.0018	0.0027	0.0028	0.0011
PCB-49	NIL	0.0323	NIL	PCB70	0.0035	0.0033	0.0036	0.0011
PCB-47/48	NIL	0.0880	NIL	PCB95	0.0033	0.0155	0.0052	0.0016
PCB-44	NIL	NIL	NIL	PCB101	0.0072	0.0870	0.0363	0.0056
PCB-42	NIL	NIL	NIL	PCB99	0.0483	0.4829	0.2284	0.0458
PCB-64/41	0.0392	NIL	NIL	PCB87	0.0021	0.0176	0.0071	0.0022
PCB-74	0.0453	0.0661	0.0402	PCB110	0.0082	0.0520	0.0164	0.0054
PCB-70/76	NIL	0.0303	NIL	PCB151/82	0.0022	0.0119	0.0056	0.0012
PCB-66	0.0606	0.0359	NIL	PCB149	0.0051	0.0380	0.0122	0.0033
PCB-56/60	NIL	NIL	NIL	PCB118	0.0192	0.1384	0.0815	0.0207
PCB-95	NIL	0.0550	0.0367	PCB153	0.2204	1.7119	1.0659	0.2159
PCB-92	NIL	NIL	NIL	PCB 105/132	0.0115	0.0966	0.0619	0.0146
PCB-101/90	NIL	0.2221	0.0344	PCB138	0.1293	1.3727	0.7402	0.1352
PCB-99	0.1306	2.6708	0.1660	PCB158	0.0071	0.0830	0.0428	0.0066
PCB-97	NIL	0.0247	NIL	PCB187	0.0070	0.0805	0.0402	0.0099
PCB-87	NIL	0.0586	NIL	PCB183	0.0078	0.0827	0.0501	0.0086
PCB-85	0.0248	0.3640	NIL	PCB128	0.0135	0.1879	0.0857	0.0144
PCB-110	0.0750	0.0928	0.0382	PCB177	0.0020	0.0182	0.0095	0.0017
PCB-151	NIL	NIL	NIL	PCB 156/171	0.0116	0.1150	0.0779	0.0127
PCB-149	0.0594	0.0558	NIL	PCB180	0.0879	0.7227	0.4803	0.0861
PCB-118	0.0625	0.2947	0.0752	PCB191	0.0014	0.0098	0.0056	0.0009
PCB-114	NIL	NIL	NIL	PCB170	0.0334	0.2897	0.1825	0.0338
PCB-146	0.0417	0.4511	0.0362	PCB199	0.0052	0.0601	0.0315	0.0073
PCB-153	0.5458	8.5747	0.6221	PCB195/208	0.0028	0.0295	0.0132	0.0029
PCB-105	0.0492	0.4639	0.0878	PCB194	0.0145	0.1166	0.0698	0.0143
PCB-141	0.0602	0.0310	NIL	PCB205	0.0011	0.0043	0.0029	0.0007
PCB-137	0.1060	0.4773	NIL	PCB206	0.0063	0.0734	0.0289	0.0070
PCB-130	NIL	0.1794	NIL	PCB209	0.0027	0.0407	0.0077	0.0032
PCB-138	0.3485	7.2763	0.4310	Sum PCB	0.7128	6.3458	3.4871	0.7657
PCB-158	NIL	0.2764	0.0237					
PCB-128/167	0.0709	0.8409	0.0553	1,2,4,5-TCB	0.0089	0.0080	0.0136	0.0030
PCB-156	NIL	0.2765	0.0435	1,2,3,4-TCB	0.0029	0.0021	0.0020	0.0029
PCB-157	NIL	0.1663	NIL	QCB	0.0033	0.0023	0.0027	0.0020
PCB-179	NIL	NIL	NIL	HCB	0.0482	0.0517	0.0490	0.0416
PCB-176	NIL	NIL	NIL	OCS	0.0008	0.0019	0.0011	0.0006
PCB-178	0.0329	0.1688	0.0124	Nonachlor	0.0116	0.0326	0.0097	0.0099
PCB-187	0.0489	0.4697	0.0450	pp'-DDE	0.0424	0.1643	0.0614	0.0401
PCB-183	0.0346	0.3768	0.0339	Mirex	0.0009	0.0032	0.0008	0.0006
PCB-174	NIL	NIL	NIL	a-BHC	0.0016	0.0017	0.0023	0.0010
PCB-177	0.0306	0.1056	NIL	b-BHC	0.0205	0.0175	0.0163	0.0144
PCB-171	0.0838	0.1044	NIL	g-BHC	0.0006	0.0007	0.0019	0.0004
PCB-172	NIL	0.1080	NIL	Chlordane	0.0308	0.0706	0.0319	0.0190

BLOOD (mg/kg lw)	A	B	D
PCB-180	0.2245	3.5145	0.3267
PCB-170/190	0.1063	2.0244	0.1958
PCB-189	NIL	0.0633	NIL
PCB-202	NIL	0.0883	NIL
PCB-200	NIL	NIL	NIL
PCB-199	0.1162	0.5973	0.1670
PCB-196/203	0.0500	0.2928	NIL
PCB-195	NIL	0.1108	NIL
PCB-194	0.1059	0.5898	0.1163
PCB-205	NIL	NIL	NIL
PCB-201	NIL	NIL	NIL
PCB-208	NIL	0.0464	NIL
PCB-207	NIL	NIL	NIL
PCB-206	NIL	0.5157	0.0631
PCB-209	NIL	0.2911	NIL
SUM PCBs	1.7703	32.5951	2.7604
1,2,4,5-Tetrachlorobenzene	NIL	NIL	NIL
1,2,3,4-Tetrachlorobenzene	NIL	NIL	NIL
Pentachlorobenzene	NIL	NIL	0.0861
α-Hexachlorocyclohexane	NIL	NIL	NIL
Hexachlorobenzene	0.1373	0.1593	0.1782
β-Hexachlorocyclohexane	NIL	NIL	NIL
γ-Hexachlorocyclohexane	NIL	NIL	NIL
Octachlorostyrene	NIL	NIL	NIL
Heptachlor epoxide	NIL	NIL	NIL
Oxychlordane	0.1277	0.3631	0.1580
t-Chlordane	NIL	NIL	NIL
c-Chlordane	NIL	NIL	NIL
t-Nonachlor	0.0535	0.0647	NIL
p,p'-DDE	0.2311	0.3314	0.1539
Dieldrin	NIL	NIL	0.5806
p,p'-DDD	NIL	0.0282	NIL
c-Nonachlor	NIL	NIL	NIL
p,p'-DDT	NIL	NIL	NIL
Photomirex	NIL	NIL	NIL
Mirex	NIL	NIL	NIL
SUM Organochlorines	0.5373	0.9311	0.6169
alpha-TBECH	NIL	NIL	NIL
beta-TBECH / BDE-15	NIL	NIL	NIL
BDE-17	0.0479	0.0355	0.0355
BDE-28	N	NIL	NIL
BDE-47	0.2008	0.8179	0.8179
BDE-99	0.0369	0.0511	0.0511
HBB	NIL	NIL	NIL
BDE-49	NIL	NIL	NIL
BDE-66	NIL	NIL	NIL
BB-101	NIL	NIL	NIL
BDE-100	0.0249	0.0749	0.0749
BDE-154 / BB-153	0.0576	0.0592	0.0592
BDE-190	NIL	NIL	NIL

SCAT (mg/kg lw)	A	B	C	D
Chlordane	0.0015	0.0026	0.0011	0.0006
Chlordane	0.0021	0.0035	0.0013	0.0008
pp'-DDD	0.0033	0.0116	0.0044	0.0021
Nonachlor	0.0013	0.0031	0.0009	0.0006
pp'-DDT	0.0070	0.0163	0.0051	0.0029
Sum OCPs	0.2143	0.4441	0.2168	0.1541
BDE-7	0.0231	0.0028	NIL	0.0148
BDE-15	NIL	0.0055	0.0204	0.0012
BDE-28	NIL	0.0013	NIL	0.0004
BDE-49	0.0005	0.0165	0.0155	0.0144
BDE-47	0.1250	0.2572	0.1052	0.1072
BDE-100	0.0038	0.0177	0.0059	0.0109
BDE-119	0.0082	0.0004	NIL	0.0029
BDE-99	0.0020	0.0087	0.0121	0.0051
BDE-85	0.0057	0.0010	0.0096	0.0574
BDE-126	0.0075	0.0040	0.0062	0.0020
BDE-154	0.0006	0.0013	0.0004	0.0002
BDE-153	NIL	0.0045	0.0016	0.0007
BDE-138	NIL	NIL	0.0017	NIL
BDE-183	NIL	0.0052	NIL	0.0007
BDE-191	0.0001	0.0007	NIL	0.0009
BDE-197	NIL	0.0136	0.0085	NIL
BDE-196	NIL	0.0240	0.0007	0.0004
BDE-207	0.0001	0.1000	NIL	NIL
BDE-206	NIL	0.1781	NIL	NIL
BDE-209	NIL	394.1433	NIL	NIL
SPBDEs	0.1374	0.2863	0.1424	0.1279

BLOOD (mg/kg lw)	A	B	D
BTBPE	NIL	NIL	NIL
HBCDD	NIL	NIL	NIL
BDE-183	NIL	NIL	NIL
BDE-138	NIL	NIL	NIL
BDE-153	0.0373	0.0796	0.0796
BDE-85	NIL	NIL	NIL
syn-DP	NIL	NIL	NIL
anti-DP	NIL	NIL	NIL
BDE-209	NIL	NIL	NIL
SUM BDE/BFRs	0.2638	1.1208	1.1208