Trophic Magnification of Legacy Persistent Organic Pollutants and Emergent Contaminants within a Terrestrial Food-Web of an Avian Apex Predator, the Cooper's Hawk (*Accipiter cooperii*)

by

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Abstract

Several types of legacy and lipophilic persistent organic pollutants (POPs), such as organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs), and emergent proteinophilic POPs like perfluorinated compounds (PFCs) are released from multiple sources into the environment and negatively impact endocrine functions within exposed wildlife. Protocols to assess bioaccumulation of these persistent chemicals within terrestrial systems are far less developed compared to aquatic systems. Consequently, regulatory agencies in Canada, the United States, and the European Union use only aquatic information to assess bioaccumulation potential of chemicals. However, recent studies have shown that some chemicals that are not bioaccumulative in aquatic food-webs do biomagnify in terrestrial food-webs. To better understand the bioaccumulation behaviour of chemicals in terrestrial systems, we assessed the biomagnification of lipophilic and proteinophilic POPs in a terrestrial food-web that included an avian apex predator, the Cooper's Hawk (*Accipiter cooperii*). Over 100 samples were collected from various trophic levels of the food-web including hawk eggs, songbirds, invertebrates, and berries. We estimated the trophic position of each organism using stable isotope analysis of $\delta^{13}C$ and $\delta^{15}N$ signatures of the hawks, songbirds, invertebrates, and berries. We analyzed the biota samples for concentrations of 38 PCB congeners, 20 OCPs, 20 PBDE congeners, 7 other brominated flame retardants (BFRs), and 18 PFCs listed on the Government of Canada's Chemicals Management Plan. We used censored regression by maximum likelihood estimation to assess the relationship between the natural logarithm of each contaminant concentration and trophic position. Trophic magnification factors (TMFs) were determined as the antilog of the regression slope. We determined TMFs for contaminants that were detected at appreciable levels in all of the biota samples (i.e. had 50% or greater detection frequency) and compared these terrestrial TMFs to those observed in aquatic systems. TMFs of legacy and lipophilic POPs ranged from 0.77 to 15.66, indicating that the majority of those POPs are biomagnifying. TMFs of PFCs ranged from 13.02 – 86.19, indicating PFCs are also readily biomagnifying and perhaps at a greater extent than lipophilic POPs. Terrestrial TMFs for legacy POPs were comparable or higher than aquatic TMFs; whereas, terrestrial TMFs for PFCs were considerably higher than aquatic TMFs.

Keywords: avian apex predator, legacy POPs, emergent POPs, lipophilic, proteinophilic, trophic magnification

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Preface

Protocols to assess bioaccumulation of persistent organic pollutants (POPs) are far less developed for terrestrial systems than compared to aquatic systems (*1*). At present, regulatory agencies in Canada, the United States, and the European Union commonly use two criteria developed from aquatic studies to evaluate the bioaccumulation potential of chemicals. The first being the octanol-water partition coefficient (K_{OW}), which estimates the level of lipophilicity/hydrophobicity of a chemical (*2-4*). The second criterion is based on empirical bioaccumulation data from fish and aquatic studies, which express the chemical concentration within an organism compared to its aquatic, environmental medium as a bioconcentration factor (BCF) or bioaccumulation factor (BAF; *2, 4*). However, these metrics are not appropriate for estimating bioaccumulation of chemicals within terrestrial environments as BCFs and BAFs apply only to aquatic or water-respiring organisms and generally do not consider dietary exposure. Hence, regulators need to establish separate standards and bioaccumulation criteria for terrestrial systems.

Bioaccumulation of POPs in terrestrial environments may be more accurately represented by the octanol-air partition coefficient (K_{OA}) since terrestrial organisms breathe air rather than water. In addition, many contaminants with a low K_{OW} and high K_{OA} will biomagnify in terrestrial organisms because of a low rate of respiratory elimination but generally will not biomagnify in water-respiring organisms (*3*). Alternative biomagnification criteria more appropriate to use for terrestrial systems with air-respiring organisms include biomagnification or trophic magnification factors (BMF or TMF, respectively), as they account for dietary exposure and can be applied to both water- and air-respiring organisms (*5, 6*). To date, only a handful of studies have examined trophic magnification of POPs in terrestrial environments (*7-11*). Therefore, there is a critical need for more empirical data and bioaccumulation studies from terrestrial systems to improve the regulatory bioaccumulation assessment of chemicals and to help set separate standards for terrestrial environments. To address this information gap, we designed a terrestrial field study to assess the extent of biomagnification of POPs in a food-web that included a primary producer, detritivores, primary and secondary consumers, and an avian apex predator, the Cooper's Hawk (*Accipiter cooperii*).

In Chapter 1, we focused our attention on legacy and emergent lipophilic POPs listed under the Stockholm Convention on POPs, a United Nations treaty signed in 2001 (www.pops.int). Legacy POPs, such as organochlorine pesticides (OCPs), polychlorinated

biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs), are lipophilic substances banned or restricted in usage since the early 1970s. Whereas, emerging, lipophilic POPs, such as polybrominated diphenyl ethers (PBDEs), effectively began commercial production in 1965 and have only been discontinued in the United States and Europe since 2004 (*12-14*). For simplicity we refer to these contaminants as legacy and lipophilic POPs in Chapter 1.

Our primary objectives in Chapter 1 included estimating the trophic positions of biotic samples of berries, invertebrates, songbirds, and Cooper's Hawks using a literature-based trophic position model and stable nitrogen isotope comparisons. We then analysed over 100 biota samples for concentrations of 38 PCB congeners, 20 OCPs, 20 PBDE congeners, and 7 other brominated flame retardants (BFRs) listed on the Government of Canada's current Chemicals Management Plan (CMP). Finally, we determined TMFs for legacy and lipophilic POPs that were detected at appreciable levels in all of the biota samples (i.e. have a detection frequency > 50% across all samples) and compared these terrestrial TMFs to those reported for aquatic systems.

In Chapter 2, we focused our attention on perfluorinated chemicals (PFCs), which are considered proteinophilic contaminants rather than lipophilic. PFCs are considered emerging POPs as they have been manufactured worldwide since the 1940s but did not become widely detected in environmental samples until the early 2000s (*15*). Consequently, a slow phase out in the production and use of a small number of PFCs began in the early 2000s in the United States, Canada, and the European Union (*15, 16*). At present, perfluorooctane sulfonic acid (PFOS) is the only PFC listed under the Stockholm Convention (www.pops.int). However, perfluorooctanoic acid (PFOA) and perfluorohexane sulfonic acid (PFHxS) are candidate POPs proposed for listing under the Stockholm Convention (www.pops.int).

Our primary objectives in Chapter 2 included analysing roughly 50 biota samples for concentrations of 18 PFCs listed on the Government of Canada's CMP. Then we determined TMFs for PFCs that were detected at appreciable levels in all the biota samples and compared these terrestrial TMFs to those reported for aquatic systems. As we already estimated the trophic positions of biotic samples of berries, invertebrates, songbirds, and Cooper's Hawks in Chapter 1, we simply used the same trophic relationships and positions in Chapter 2 to estimate TMFs for PFCs.

1. Trophic Magnification of Legacy and Lipophilic Persistent Organic Pollutants within a Terrestrial Food-Web of an Avian Apex Predator

1.1. Introduction

Protocols to assess bioaccumulation of persistent organic pollutants (POPs) are far less developed for terrestrial systems than compared to aquatic systems (*1*). At present, regulatory agencies in Canada, the USA, and the EU primarily use bioaccumulation data from fish studies to assess the bioaccumulation potential of chemicals expressed as either a bioconcentration factor (BCF) or bioaccumulation factor (BAF; *1, 5, 6*). However, BCF and BAF metrics apply only to aquatic or water-respiring organisms and generally do not consider chemical exposure from the diet of an organism. Moreover, many chemicals are known to behave differently in terrestrial ecosystems due to their physicochemical properties, such as the octanol-water partition coefficient (K_{OW}) and the octanol-air partition coefficient (K_{OA}) (*3*). For example, contaminants with a low K_{OW} (log K_{OW} ~2 to 5) and high K_{OA} (log K_{OA} ~6 to 12) usually biomagnify in air-respiring organisms because of a low rate of respiratory elimination but generally do not biomagnify in water-respiring organisms (*3*). Thus, separate models and metrics for terrestrial systems need to be considered when evaluating the total bioaccumulation potential of a chemical.

Alternative bioaccumulation metrics that could be used for terrestrial food-webs include biomagnification or trophic magnification factors (BMF or TMF, respectively), as they account for dietary exposure and can be applied to both air-respiring and water-respiring organisms (*5, 6*). However, TMFs are often difficult to determine if organisms in the food-chain have overlapping trophic positions or if chemicals are not present at detectable concentrations in the environment, which is common at the lower levels of the food-chain (*6*). For instance, a recent study in China evaluated biomagnification of POPs in an urban, terrestrial food-web (*17*), but was unable to determine TMFs because the trophic positions of the apex predator overlapped with other consumers. Additionally, the few studies that have evaluated biomagnification of POPs in terrestrial environments have estimated TMFs using simple models (*7, 8*) or used data collected from a limited number of trophic levels or species groups (*9, 10, 18*). Consequently, many scientists (*1, 19*) have stressed that there is a critical need for more terrestrial field studies that assess biomagnification and provide essential empirical field data on ionic and ionogenic chemicals like POPs.

Legacy POPs, such as organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs), are lipophilic substances banned or restricted in usage since the early 1970s. Hence, these were the initial POPs listed under the Stockholm Convention on POPs, a United Nations treaty signed in 2001 (www.pops.int). Emergent, lipophilic POPs, such as polybrominated diphenyl ethers (PBDEs), were added later to the Stockholm Convention as their production and use were only discontinued in the United States and Europe since 2004 (*12, 13*). However, even after decades of restrictions, legacy POPs combined with emergent POPs continue to be detected at elevated levels in apex predators posing a significant risk to them (*20-25*). Avian apex predators are particularly at risk because legacy and emergent, lipophilic POPs are often detected at much higher concentrations in raptors than compared to many mammalian apex predators (*12, 17, 26, 27*). For instance, a Cooper's Hawk (*Accipiter cooperii*) from Metro Vancouver, British Columbia had the highest ΣPBDE concentration recorded to date for a wild bird at 194 μg/g lipid (*24*), which is also considerably higher than most ΣPBDE concentrations reported in large mammalian predators (*18, 27*). This obvious bioaccumulation is a concern since most POPs are known to behave as endocrine disrupting chemicals that can adversely impact reproduction, metabolism, growth, and behaviour of exposed wildlife. For example, a recent study found evidence that as ΣPCBs, ΣPBDEs, and dieldrin concentrations increased in blood plasma of adult Cooper's Hawks from Metro Vancouver, triiodothyronine (T_3) levels in adults decreased and nesting success declined (*28*). As terrestrial raptors like the Cooper's Hawk often have higher concentrations of lipophilic POPs than compared to many aquatic species (*29, 30*), we suspect that their terrestrial food-webs may also exhibit higher biomagnification of these contaminants as well (*24, 28, 31*).

To answer this question, we designed a terrestrial field study to assess the extent of biomagnification of legacy and emergent, lipophilic POPs in a food-web that included a primary producer, detritivores, primary and secondary consumers, and an avian apex predator, the Cooper's Hawk. We estimated the trophic positions of biotic samples of berries, invertebrates, songbirds, and Cooper's Hawks using a literature-based trophic position model and stable nitrogen isotope comparisons. Then we analysed over 100 biota samples for concentrations of 38 PCB congeners, 20 OCPs, 20 PBDE congeners, and 7 other brominated flame retardants (BFRs) listed on the Government of Canada's current Chemicals Management Plan (CMP; Appendix 1). For simplicity we refer to these contaminants as legacy and lipophilic POPs. Lastly, we determined the TMFs for legacy POPs that were detected at appreciable levels in all of the

biota samples (i.e. had a detection frequency > 50% across all samples) and compared these terrestrial TMFs to those reported for aquatic systems.

1.2. Methods

1.2.1. Field Sampling Methods

Study Area

We assessed the trophic transfer dynamics and biomagnification of legacy POPs within a terrestrial food web in urbanized regions of Metro Vancouver, British Columbia, Canada. We chose an urban area primarily because previous studies in Metro Vancouver showed relatively high concentrations of POPs in Cooper's Hawks (*24, 28*) increasing the likelihood of observing detectable concentrations in the lower trophic levels of the food-web. We also believe that an urban food-web might be less complex resulting in a more linear or direct transfer of contaminants up the food-chain, especially as Cooper's Hawks in rural areas are known to have more diverse diets compared to urban hawks (*32-34*). We focused sample collection efforts in urban parks and residential areas of Metro Vancouver, which is comprised of 21 municipalities. Our sampling of food-web biota was limited to municipalities with known active Cooper's Hawk nests. Our study area included 6 sampling regions within 5 municipalities: North Vancouver (the District of North Vancouver), Vancouver-West (City of Vancouver), Vancouver-South (City of Vancouver and City of Burnaby), North Burnaby-East Vancouver (City of Burnaby and City of Vancouver), Richmond (City of Richmond), and Ladner (City of Delta) (Figure 1.1).

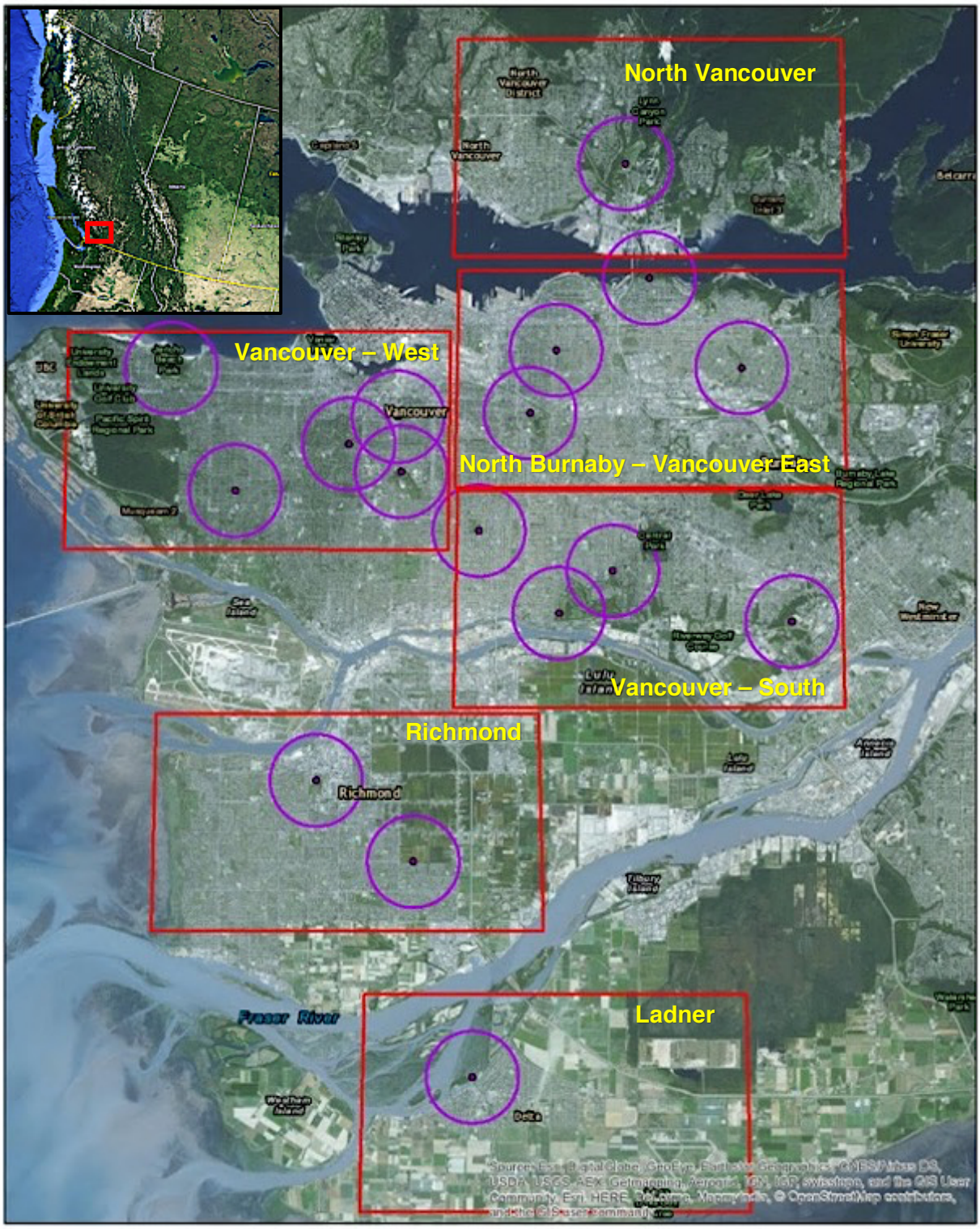

Figure 1.1. Study area separated into six sampling regions (red squares) with Cooper's Hawk nests (purple dots) and nesting territories (purple circles) in Metro Vancouver, British Columbia, 2016.

Food-Web

The Cooper's Hawk is considered a generalist apex predator but predominantly eats avian prey (*35*). Although typically considered a migratory or partially migratory species, the Cooper's Hawk population of Metro Vancouver resides year-round near their breeding sites (*28, 36, 37*). In southwestern BC, urban Cooper's Hawk typically prey upon American Robins (*Turdus migratorius*), European Starlings (*Sturnus vulgaris*), and House Sparrows (*Passer domesticus*; *38*), which are also year-round residents in Metro Vancouver. American Robins and European Starlings predominately eat terrestrial invertebrates (such as beetles, butterfly larvae, crane fly larvae, and earthworms) and seasonal wild fruit or berries (*39-44*). Whereas, House Sparrows mostly eat grains and seeds, some invertebrates, and small quantities of fruit (*45, 46*). Ground beetles generally eat earthworms, snails and slugs, other insects, and limited quantities of seeds and fruit (*47-50*). Songbirds and ground beetles comprised the secondary and primary consumers in this food-web. The detritivores included earthworms and isopods, such as pillbugs and sowbugs, which primarily eat organic matter in soil and decomposing vegetation, respectively (*51-53*). Himalayan blackberry (*Rubus armeniacus*), the representative primary producer in this food-web, is an invasive shrub species that is widely distributed across Metro Vancouver with abundant fruit consumed by numerous mammal and bird species, such as American Robin, European Starling, and Spotted Towhee (*Pipilo maculatus*; *54, 55*). We chose Himalayan blackberry because it typically ripens in the late summer (late July to early August), and songbirds like the American Robin generally forage more heavily on berries and fruit in the late summer and early fall than compared to the spring and early breeding season (*56*). We collected all samples in this food-web from May to September in 2016.

Biotic Sample Collection

Cooper's Hawk

We obtained tissue samples of Cooper's Hawk by collecting eggs from active nests (Table 1.1). Eggs were chosen to represent the apex predator trophic level because eggs represent a maternal transfer of contaminants from the female hawk to the eggs and are frequently used as a matrix for environmental contamination monitoring (*57-59*). One egg can be removed from each active nest with minimal impact to reproductive success since Cooper's Hawks typically lay a clutch of three to five eggs and fledge an average of 2 to 4 juveniles each breeding season (*37*). Eggs we collected ranged in mass from 30.4 to 40.6 g.

Cooper's Hawks are tolerant to nest site disturbance and are well-adapted to urban environments (*36, 38, 60-62*). They have high nest site fidelity and will generally nest in the same area or nest each year (*60, 63*). To locate active nest sites, we referred to historical nest records from Bird Studies Canada, eBird Canada, and previous research studies (*28, 36*). During the pre-incubation period between mid-February and early May 2016, we visited all potential nest locations and used call play-back methods to determine nest occupancy and breeding activity (*63, 64*). Once a nest was identified as active (i.e. one adult or a pair were observed near or within the nest), we regularly monitored it to determine when eggs were laid or present. During the 30 – 36 day incubation period, we accessed each active nest using the services of a professional tree climber or bucket truck to determine clutch size and collect one fresh egg. We continued to monitor nests after eggs were harvested to assess nest productivity and survival of chicks/fledglings. No nests were deserted as a result of our egg collection activities.

We processed eggs by recording each eggs' size and weight, scoring the shell circumference with a chemically rinsed scalpel to open the egg, depositing egg contents into chemically rinsed glassware, extracting separate subsamples of yolk and albumen, and storing at – 20°C. Egg collection was approved by the University Animal Care Committee of Simon Fraser University and authorized by the Ministry of Forests, Lands and Natural Resource Operations (Surrey, BC) under permit SU16-225842.

Table 1.1. Sample sizes and total biomass of species collected from terrestrial, urban food web across Metro Vancouver, BC in 2016.

Trophic Guild	Species	Scientific Name	*n* [1]	No. Individuals or Mass[2]
Apex Predator	Cooper's Hawk	*Accipiter cooperii*	17	17
Secondary Consumers	American Robin	*Turdus migratorius*	9	22
	European Starling	*Sturnus vulgaris*	5	19
	Northern Flicker	*Colaptes auratus*	6	20
	Pigeon/Dove: Rock Pigeon, Eurasian Collared Dove	*Columba livia, Streptopelia decaocto*	6	27
	Sparrow spp.: House Sparrow, Dark-eyed Junco, White-crowned Sparrow, Fox Sparrow, Song Sparrow, Golden-crowned Sparrow, Spotted Towhee	*Passer domesticus, Junco hyemalis, Zonotrichia leucophrys, Passerella iliaca, Melospiza melodia, Zonotrichia atricapilla, Pipilo maculatus*	6	28

Trophic Guild	Species	Scientific Name	*n* [1]	No. Individuals or Mass[2]
	Thrush spp.: Varied Thrush, Swainson's Thrush, Hermit Thrush	*Ixoreus naevius, Catharus ustulatus, Catharus guttatus*	6	13
Primary Consumers	Large Beetles	*Pterostichus melanrius, Carabus nemoralis, Carabus granulatus, Pterostichus sp.*	6	77.9
	Small Beetles	*Harpalus affinis, Calathus fuscipes, Anisodactylus binotatus, Agonum mülleri, Philonthus politus, Anatrichis minuta, Amara sp., Staphynlidae, Harpalitae*	6	33.9
	Insecta: Milipedes, Centipedes, Spiders, Ants	*Julida, Chilopoda, Arachnida, Formicidae*	5	1.2
Detritivores	Earthworms	*Lumbricidae*	25	496 g
	Oniscidea: Sowbugs and Pillbugs	*Oniscus asellus, Porcellio scaber, Armadillidium vulgare*	6	145 g
Primary Producer	Himalayan blackberry	*Rubus armeniacus*	6	662 g

[1]Refers to number of samples analyzed per species group;

[2]Refers to total number of eggs and/or songbird carcasses collected per species or group and total weight (g) of invertebrates and berries collected.

Avian Prey

We obtained samples of American robins, European starlings, and house sparrows by collecting an egg or nestling from active nests located within a 2 km radius of each active hawk nest (Figure 1.1). The nesting territory of resident Cooper's Hawks in Metro Vancouver was estimated to be approximately 4.75 km^2 (*28, 36*).

To locate active nests of American robins, European starlings, and house sparrows, we systematically surveyed vegetated areas within the nesting territory of each nest site. One or two surveyors walked transects 10 m apart and searched for nest sites and signs of active breeding (i.e. nest building, food carrying, or fecal sac carrying). If a songbird nest was found, we collected 1 fresh egg or nestling from the nest. We processed songbird eggs by recording size and weight of each egg, depositing egg contents into chemically rinsed glassware, and storing at –20°C until

sample homogenizing and chemical analysis. We also captured 5 adult European starlings and 5 adult house sparrows in mist nets within the Ladner sampling region at Tecarte Farm. We anaesthetized nestlings and birds captured in mist nests with isoflurane, euthanized them by cervical dislocation, then stored them at –20°C until sample processing and chemical analysis.

We supplemented our targeted prey species collection efforts with samples of 12 other known Cooper's Hawk prey species, including Varied Thrush (*Ixoreus naevius*), Hermit Thrush (*Catharus guttatus*), Swainson's Thrush (*Catharus ustulatus*), Spotted Towhee (*Pipilo maculatus*), Song Sparrow (*Melospiza melodia*), White-crowned Sparrow (*Zonotrichia leucophrys*), Golden-crowned Sparrow (*Zonotrichia atricapilla*), Dark-eyed Junco (*Junco hyemalis*), Fox Sparrow (*Passerella iliaca*), Rock Pigeon (*Columba livia*), Eurasian-collared Dove (*Streptopelia decaocto*), and Northern Flicker (*Colaptes auratus*) that had been euthanized by a wildlife rehabilitation facility, Wildlife Rescue Association (Table 1.1). We only collected and processed bird samples from Wildlife Rescue Association if each specimen's point of origin was associated with a sampling region near a Cooper's Hawk nest (Figure 2.1). When we had a limited number of samples for individual species we grouped individual birds into representative family groups, including Emberizidae or Sparrow spp., Turdidae or Thrush spp., and Columbidae or Pigeons/Doves, as the species in these family groups are known to have similar behaviours, habitat preferences, and diets (*45, 65-69*). In order to manage the cost of the analytical chemistry and to have enough biomass for each sample, we pooled bird samples into six avian groups per sampling region as follows: American Robin, European Starling, Sparrow spp., Thrush spp., Pigeon/Dove, and Northern Flicker (Table 1.1).

Egg collection and animal capturing, handling, and euthanasia were approved by the Animal Care Committee of Simon Fraser University and authorized by the Canadian Wildlife Service – Environment Canada under permit BC-16-0010.

Invertebrates

We obtained invertebrate samples from five subsampling stations within each sample region, collecting invertebrates from a total of 30 stations (Figure 1.1). We randomly selected subsampling stations by overlaying a 1 x 1 km grid above the study area and pinpointing crosshairs. All subsampling stations were required to: 1) be located within a 2 km radius of an active Cooper's Hawk nest; 2) occur within a public park; and 3) contain both forested vegetation

and open fields. At each subsampling station, we used a random number generator to obtain a random bearing to select the nearest forested edge habitat.

We collected terrestrial invertebrates using a plastic pitfall trap (Dr. R. Vernon, Agriculture and Agri-Food Canada, Agassiz, BC, CAN; Plate 1.1). We placed 30 traps in edge habitat either near or under vegetation to help conceal the trap from public view (Plate 1.2). We monitored the traps weekly from August to September 2016, placed captured invertebrates into chemically rinsed clear jars, and froze them at –20°C. If no invertebrates were captured within one week, we moved the trap to a new location within a 10 m radius of the previous location. We identified all specimens collected at each station to species or genus, sorted them into pooled regional groups for analysis, placed them into new chemically rinsed amber jars, and stored them at –20°C. We pooled invertebrate samples into groups which included large beetle species, small beetle species, isopods, and Insecta species (e.g. centipedes, millipedes, ants, and spiders; Table 1.1) in order to manage the cost of the analytical chemistry and to have enough biomass per sample.

Plate 1.1. The trap designed by R. Vernon has small pins extending down from the lid to allow invertebrates in but to exclude small mammals or rodents

Photo: Wim van Herk, Agriculture and Agri-Food Canada, 2016.

Plate 1.2. Pitfall trap placed in edge habitat under concealing vegetation

Photo: K. Fremlin, 2016.

Near the 30 pitfall stations, we also collected earthworms in areas where American Robins were observed foraging and with moist lawn vegetation (e.g. lawn moss [*Byrum* spp.] or creeping buttercup [*Ranunculus repens*]). We used allyl isothiocyante (AITC; Fisher Scientific, Ottawa, ON, CAN; 94%; density 1.0175) as a chemical expellant to bring the earthworms to the surface (*70-72*). We cleared the area within a 60 cm^2 wooden quadrat of surface debris with a rake and clipped

the grass and other vegetation to its base or pulled it out (*73*). We diluted AITC with isopropanol (Fisher Scientific, Ottawa, ON, CAN; 100%; density 0.785) to make a stock solution of 5 g/L and used a plastic watering can to sprinkle 10 L of water mixed with 100 mg/L of the stock solution over the quadrat to force earthworms to the surface (*70*). This concentration and expulsion technique has been shown to be more effective at sampling earthworms than compared to hand digging, and is considered comparable to, yet much safer than formalin, which is a known carcinogen (*70-72*). Earthworms that came to the surface were immersed in clean water to remove surficial AITC and to keep the earthworms alive until their guts could be cleared of soil. To clear gut contents, we placed worms on moist paper towel in aluminum trays for at least 24 hours. Purged earthworms were placed into chemically rinsed jars and frozen at –20°C. We were unable to identify earthworms to genus or species due to the lack of visible setae post-mortem, so all earthworms were simply grouped under the family *Lumbricidae* (Table 1.1).

Berries

We collected samples of Himalayan blackberry from each of the 30 subsampling stations used to sample invertebrates. We collected approximately 150 to 250 mL of berries from the nearest shrubs at each subsampling station (Table 1.1). We used needle-nose pliers to pluck the berries and place them into chemically rinsed jars. The needle-nose pliers were cleaned with a 10% ethanol solution prior to berry picking. The berries were then frozen at –20°C prior to sample preparation and chemical analysis.

1.2.2. Analytical Methods for Determining Contaminant Concentration

Biotic Sample Preparation and Analysis

We shipped all of the frozen samples on dry ice to the National Wildlife Research Centre (NWRC) in Ottawa, ON where they were stored at –40°C. We processed and homogenized all biotic samples in the Tissue Preparation Lab at the NWRC. We homogenized eggs by whisking the yolk and albumen together. Prior to processing the songbirds, we plucked them of feathers and clipped off large keratinized or boney tissues (e.g. beaks, wings, legs, and feet) as Cooper's Hawks typically pluck their prey then eat the head, viscera, and muscle tissues in sequence (*60*). It is preferable to use the whole body of the prey, rather than just its liver or blood, to reflect the total pollutant concentration ingested by the Cooper's Hawk (*17, 74*). We then processed all the animal samples by cutting tissues into small pieces and homogenizing them with a ball-mill

(Retsch^{TM} MM400 Mixer Mill, Fisher Scientific). One to seven individual birds from each species group within each sampling region were pooled and homogenized for a total of 35 pools (Appendix 2). We stored all the homogenized samples at –40°C prior to analysis. The avian prey, invertebrates, and berry samples were transferred to the Great Lakes Institute for Environmental Research (GLIER) in Windsor, ON for chemical analysis and the Cooper's Hawk eggs were analysed for contaminants at NWRC.

National Wildlife Research Centre

Sample extraction methods used at NWRC have been described in detail in comparative studies on FRs within eggs of various species (*75-77*). Approximately 0.25 – 3.0 g of biota sample homogenate was ground with diatomaceous earth (J.T. Baker, NJ, U.S.A.), spiked with 25 µL of a standard solution, and then extracted with a 50:50 dichloromethane:hexane (DCM:HEX) solvent mixture using an accelerated solvent extraction system (ASE, Dionex ASE 350, CA, USA). After gravimetric determination of lipid content using 10% of the extract, the remaining extract was subjected to gel-permeation chromatography (GPC; GX-271 Liquid Handler, Gilson, Inc., WI, USA), followed by cleanup with solid phase extraction (SPE) to remove any remaining small lipids that were not removed using GPC. The cleaned-up sample was concentrated to 100 µL using nitrogen evaporation, then 400 µL of iso-octane was added to the sample prior to instrumental analysis.

The PCBs/OCPs in the hawk eggs were analyzed using an Agilent 7890 gas chromatograph (Agilent Technologies, CA, USA) coupled to a single quadruple mass analyzer (Agilent 7000 MS) in electron impact ionization (MS-EI) mode. We used a 15 m DB-5MS column (0.25 mm ID, 0.25 µm film thickness; J&W, Agilent Technologies) and the injector was operated in splitless mode, held at 280°C. Initial oven temperature was held at 60°C for 1 min, increased to 120°C at 40°C/min, and finally to 310°C at 5°C/min. OCP/PCB quantification was determined via selected ion monitoring (MRM). The internal standards for quantification were carbon labelled and were selected to cover the range of tri- to octa-PCBs: ^{13}C-PCB28, ^{13}C-PCB52,^{13}C-PCB118, ^{13}C-PCB153, ^{13}C-PCB180 and ^{13}C-PCB194. Congeners that co-elute are reported as a sum and are listed in the form PCB xx/xx (e.g. PCB 28/31 is the sum of the co-eluting congeners PCB-28 and PCB-31).

The PBDEs were analyzed using an Agilent 7890 gas chromatography (Agilent Technologies, CA, USA) coupled to a single quadruple mass analyzer (Agilent 5977 MS) in

electron capture negative chemical ionization (MS-NCI) mode (similar to methods used in *75, 76, 77, 78*). The column used was a 15 m DB-5ht fused silica column (0.25 mm ID, 0.10 µm film thickness; J&W, Agilent Technologies) and the injector was operated in pulsed splitless mode, held at 280°C. Initial oven temperature was held at 100°C for 2 min, increased to 250°C at 25°C/min, then to 260°C at 1.5°C/min, and finally to 325°C at 25°C/min and held for 7 min. PBDE quantification was determined via selected ion monitoring (SIM) for $^{79}Br^-$ and $^{81}Br^-$, except for BDE-209 (*m/z* 487) and $^{13}C_{12}$-BDE-209 (*m/z* 495). The molecular ion (*m/z* 652) was used for quantifying *syn*– and *anti* -Dechlorane Plus (DP) isomers.

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Homogenized tissue samples sent to GLIER underwent similar extraction methods with some exceptions. Approximately 2.0 – 5.0 g of homogenate was ground with anhydrous sodium sulphate, spiked with a recovery internal standard of either 50 µL of PCB-34 or 100 µL of BDE-71, extracted with a 50% DCM:HEX solvent mixture, rotary-evaporated (Bühi Rotavapor RE 111) to approximately 5 mL, and then mixed with 25 mL of hexane. Approximately 2 mL of this sample extract was removed for lipid content determination; the remaining 23 mL of extract was rotary-evaporated to 2 mL and subjected to GPC and/or Florisil® cleanup and separation. During Florisil® cleanup, the remaining sample extract was transferred to a glass column, plugged with glass wool, filled with hexane and 6 g of activated Florisil® absorbent (60-100 mesh), and then topped with 50 g of sodium sulphate as a moisture trap. As the sample extract passed through the column to the top, the column was rinsed with 50 mL of Hexane, followed by 50 mL of 15% DCM:HEX, and eluted to a flat bottom flask. Afterwards, 150 mL of 60% DCM:HEX was added to the column and the remaining extract was eluted to a separate flask. Finally, approximately 5 mL of iso-octane was added to each flask and concentrated to roughly 1 mL by rotary evaporation.

The OCPs/PCBs in the extracted samples were analyzed using an Agilent 6890 gas chromatograph (Agilent Technologies, CA, USA) coupled to a single quadruple mass analyzer (Agilent 5973 MS) in electron impact ionization (MS-EI) mode. For the analysis, a 60 m DB-5MS column (0.25 mm ID, 0.10 µm film thickness; J&W, Agilent Technologies) was injected using splitless injection mode and held at 280°C. Initial oven temperature was held at 90°C for 1 min, increased to 200°C at 20°C/min and held for 2 min, then to 280°C at 3°C/min and held for 5 min, and finally to 300°C at 20°C/min and held for 2 min.

The low molecular weight PBDEs were also analyzed using an Agilent 6890 gas chromatograph coupled to a single quadruple mass analyzer (Agilent 5973 MS) in MS-EI mode. For the analysis, a 30 m Rtx-1614 fused silica column (0.25 mm ID, 0.10 µm film thickness; Restek Corporation) and 15 m Rtx-1614 column (0.25 mm ID, 0.10 µm film thickness; Restek Corporation) were each injected with 2 µL of sample using splitless injection mode and held at 250°C and 260°C, respectively. In the 30 m Rtx-1614 column, initial oven temperature was held at 100°C for 1 min, increased to 265°C at 15°C/min, then to 280°C at 8.0°C/min, and finally to 310°C at 25°C/min and held for 12 min. In the 15 m Rtx-1614 column, initial oven temperature was held at 100°C for 1 min then increased to 320°C at 13°C/min and held for 3 min. The interface temperature was set to 280°C.

The high molecular weight PBDEs (e.g. BDE-197, -196, -206, -207, -209) were analysed using an Agilent 6890 gas chromatograph coupled to a GCT high resolution time-of-flight (TOF) mass spectrometer (Micromass, Manchester, UK) in electron impact ionization mode. For the TOF analysis, a 30 m Rtx-1614 fused silica column (0.25 mm ID, 0.10 µm film thickness; Restek Corporation) and 15 m Rtx-1614 (0.25 mm ID, 0.10 µm film thickness; Restek Corporation) were each injected with 1 µL of sample using splitless injection mode and held at 260°C. In the 30 m Rtx-1614 column, initial oven temperature was held at 100°C for 1 min, then increased to 180°C at 20°C/min, and finally to 325°C at 5.0°C/min and held for 10 min. In the 15 m Rtx-1614 column, initial oven temperature was held at 100°C for 1 min then increased to 320°C at 13°C/min and held for 3 min. The shorter Rtx-1614 column is used for analyzing highly brominated PBDEs.

Standards and Chemicals

All PCB, OCP, and PBDE standards were purchased from Wellington Laboratories (Guelph, ON, Canada) or from the National Institute of Standards Technology (Gaithersburg, MD). All solvents used were HPLC or Optima grade and purchased from Fisher Scientific (Ottawa, ON).

Quality Control and Assurance

In both labs, method procedural blanks were processed to monitor interferences and contamination. The analytical accuracy and precision were evaluated by running an aliquot of a Certified Standard Reference Material (SRM; NIST 1947 Lake Michigan Fish Tissue), as well as duplicates of randomly selected egg or animal samples. For each contaminant, the method limit of quantification (MQL), defined as a minimum amount of analyte producing a peak with a signal to noise ratio (S/N) of 10, and the method detection limit (MDL), defined as S/N = 3, are listed in

Appendix 1. The results for method validation are accepted based on 20% accuracy with respect to the known value.

We also collected sample field blanks during invertebrate identification and sorting to monitor possible contamination from handling. Sample field blank collection consisted of opening an empty chemically rinsed jar within the fume hood during invertebrate sorting activities then closing it upon completion of sorting for each sampling region. One blank was collected for each sampling region for a total of six blanks. No contaminants were detected in these sample blanks, with the exception of γ-Hexachlorocyclohexane (γ-HCH), which was detected in three sample blanks. Concentrations of γ-HCH within invertebrates were blank corrected with the average concentration of γ-HCH from the sample blanks.

Lipid Equivalent Concentrations

Lipid contents were measured in all biota samples using a gravimetric method (Appendix 3). Approximately 1 mL (or 10%) of the extracted sample was transferred into a pre-weighed aluminum dish, allowed to air dry in a fume hood for 30 min, and then re-weighed to calculate the lipid content on a wet weight basis.

We expressed the observed wet weight concentrations in terms of lipid equivalent concentrations ($POP_{lipid\ eq}$; ng/g of lipid equivalent) to remove the effect of differences in lipid contents or other sorbing matrices between organisms. However, as some organisms, such as berries, earthworms, and isopods, had very low lipid contents and high organic carbon contents, we also included non-lipid organic matter as an important matrix for chemical accumulation (*3, 79*). We incorporated lipid, protein, and non-lipid organic carbon contents into the lipid equivalent normalization for all biota on a sample specific basis similar to (*3, 27*) and according to

$$C_{lipid\ eq.} = \frac{C_{wet}}{[L_{wet} + P_{wet}(0.05) + OC_{wet}(0.1)]} \quad (1)$$

in which *L* is the lipid fraction of the sampled tissue (g of lipid/g of wet tissue); *P* is the protein fraction estimated as the product of the percent of nitrogen measured during elemental analysis (g of N/g of wet tissue; refer to section 2.3 Stable Isotope Analysis) and a nitrogen:protein conversion factor (Appendix 3; g of protein/g of wet tissue (*80-82*)); and *OC* is the fraction of non-lipid organic carbon (i.e. lipid content subtracted from total organic carbon content) estimated as the percent of organic carbon measured during elemental analysis (i.e. g of C/g of wet tissue).

The constant 0.05 represents that proteins exhibit 5% the sorptive capacity of lipids (*3, 83*), and the constant 0.1 assumes that non-lipid organic carbon behaves similar to carbohydrates and exhibits 10% the sorptive capacity of lipid. Even though there is considerable uncertainty with this proportionality constant for non-lipid organic matter, any errors in the normalization should affect all the legacy POPs similarly assuming that lipid normalization applies to all these contaminants in a similar manner.

1.2.3. Stable Isotope Analysis

Stable isotope ($\delta^{15}N$ and $\delta^{13}C$) analyses were performed at the G. G. Hatch Stable Isotope Laboratory (G. G. Hatch) at the University of Ottawa (Ottawa, ON, Canada). Briefly, homogenized subsamples of the biotic samples (n = 98) were freeze-dried and then approximately 1.0 mg of each subsample was weighed into tin capsules (~6 mm). Samples were combusted at 1800°C in a Vario EL Cube elemental analyzer (Elementar, Germany) interfaced to a Delta Advantage isotope ratio mass spectrometer (IRMS; Conflo III, Thermo Scientific, Germany). The resulting gas products are carried by helium through columns of oxidizing/reducing chemicals optimized for CO_2 and N_2, then the gases are separated by a "purge and trap" absorption column and eventually sent to the IRMS.

The internal standards used were ($\delta^{15}N$, $\delta^{13}C$ in ‰): C-51 Nicotiamide (0.07, -22.95), C-52 mix of ammonium sulphate and sucrose (16.58, -11.94), C-54 caffeine (-16.6, -34.46), and blind standard C-55 glutamic acid (-3.98, -28.53). These standards cover the natural range and the data is reported in Delta notation δ with units in per mil (‰) and defined as δ = ((Rx-Rstd))/Rstd)*1000 where R is the ratio of the abundance of the heavy to the light isotope, x denotes sample and std is an abbreviation for standard. All $\delta^{15}N$ is reported as ‰ vs. AIR and normalized to internal standards calibrated to International standards IAEA-N1 (+0.4‰), IAEA-N2 (+20.3‰), USGS-40 (-4.52‰) and USGS-41 (47.57‰). All $\delta^{13}C$ is reported as ‰ vs. V-PDB and normalized to internal standards calibrated to International standards IAEA-CH-6 (-10.4‰), NBS-22 (-29.91‰), USGS-40 (-26.24‰) and USGS-41 (37.76‰). At the G. G. Hatch Lab, analytical precision is based on their internal standard (C-55) which is not used for calibration and is usually better than 0.2 ‰.

1.2.4. Trophic Position of Organisms within the Food-web

We used two methods to measure the trophic position of organisms within the food-web: 1) an estimate based on dietary preferences of each species obtained from the literature (*32-35, 37-39, 41-53, 68, 69, 84-90*) and 2) an estimate inferred from stable nitrogen isotope comparisons (*79, 91*). Based on the dietary preferences, the trophic position (TP) of each species was calculated according to equation 2 (*91*)

$$\mathrm{TP}_{predator} = \left(\sum_{i=1}^{n} \mathrm{TP}_{prey\ i} \times\ p_{prey\ i}\right) +\ 1 \qquad (2)$$

in which $p_{\text{prey i}}$ is the proportion of prey item *i* in the diet of the predator.

For the stable nitrogen isotope comparison, the δ^{15}N of each consumer was compared to an average δ^{15}N of the detritivores and/or primary producer and calculated as per equations 3 and 4, respectively (*5*):

$$\mathrm{TP}_{consumer} = \left(\frac{\delta^{15}\mathrm{N}_{consumer} - \delta^{15}\mathrm{N}_{detritivore}}{\Delta^{15}\mathrm{N}}\right) + 2 \qquad (3)$$

or

$$\mathrm{TP}_{detritivore} = \left(\frac{\delta^{15}\mathrm{N}_{detritivore} - \delta^{15}\mathrm{N}_{berry}}{\Delta^{15}\mathrm{N}}\right) + 1 \qquad (4)$$

in which 2 or 1 are the assumed trophic positions of the detritivores or berries and Δ^{15}N is the isotopic enrichment factor constant. Very few bioaccumulation studies have estimated a study-specific isotopic enrichment factor from organisms within food-webs since a factor of 3.4% is often used for biomagnification assessments (*5, 11*). However, we used 2.88% because it represented the δ^{15}N enrichment that was measured within our isotope data set and was comparable to 2.4% estimated for muscle tissue δ^{15}N enrichment of captive, adult Common Cormorants (*Phalacrocorax carbo*; *92*)

1.2.5. Statistical Analysis

Stable Isotopes and Isotopic Enrichment

To compare the average stable nitrogen and carbon isotopes between species groups and identify differences between the species groups, we used a one-way ANOVA (Type III for unbalanced data) with a Tukey's multiple comparison's test (MCT) in the R program (*93*). Statistical significance of *p*-values for mean estimates were assessed at $\alpha = 0.05$. We also assessed the linear relationship between $\delta^{15}N$ and $\delta^{13}C$ with a simple linear model.

To determine our study-specific isotopic enrichment factor constant, we compared the average $\delta^{15}N$ of each predator to an overall average $\delta^{15}N$ of available prey with a linear mixed effects model that included study region or city as a random effect (Appendix 4). We did not use a predator-specific proportional sum of the $\delta^{15}N$ of each prey species since we had a limited representation of the prey species that each bird or insect is known to eat.

Contaminant Concentrations with Non-Detect Data

Environmental samples often contain concentrations of contaminants that are below the level of detection. We calculated average concentrations of legacy POPs detected within each species, using the Nondetects and Data Analysis for Environmental Data (NADA) package (*94*) in the R program (*93*) as recommended for left censored data (*95*). This package has several functions that appropriately handle concentration data with values below MDLs. If a concentration was censored (i.e. below the detection limit) within a sample, we used the chemical's MDL in equation 1 to determine a sample specific, lipid equivalent detection limit (DL). We calculated the mean concentration, standard deviation, and standard error of each legacy POP detected within each trophic level using a Kaplan-Meier (KM) statistical model (cenfit) in the NADA package. Congener means were then used in another KM model to compute an overall mean for PCBs, chlorobenzenes (CBZ), chlordanes (CHL), hexachlorocyclohexanes (HCH), and PBDEs within each trophic level. These overall means were multiplied by the number of respective congeners to compute ∑PCB, ∑CBZ, ∑CHL, ∑HCH, and ∑PBDE as recommended in (*96*). If trophic levels had some or all congeners with sample concentrations all below detection limits, we averaged the sample-specific DLs for the congener then multiplied the overall mean DL by the number of congeners to compute a censored ∑. ∑PCB and ∑PBDE values for each species group only included congeners that were assessed for in that trophic level. For example, congeners, such as

PCB 82 and 191, that were not analysed in the Cooper's Hawk samples were not included in the ∑PCB average for the apex predator but were included in the ∑PCB averages for the other trophic levels. However, only BDE congeners were included in the ∑PBDE averages for each trophic level whereas the other BFRs analysed exclusively in the Cooper's Hawk samples were excluded from the ∑PBDE average for the apex predator.

Trophic Magnification Factor

We determined trophic magnification factors (TMFs) for contaminants that were detected in more than 50% of all samples by using a log-linear regression between the natural logarithm of a contaminant lipid equivalent concentration and the trophic position of each sample. We only determined TMFs for POPs that had a detection frequency greater than 50% because the error in the estimated TMF will increase as the detection frequency decreases. We used a censored regression function (cenreg) in the NADA package, which uses maximum likelihood estimation and an assumed log-normal distribution, to estimate the slope coefficient that had the highest likelihood of producing the observed values for the detected observations and the observed proportion of data that was below each detection limit (*95*). The TMF is then computed based on the antilog of the slope *m* (i.e. TMF = e^m). A TMF greater than 1 indicates that the contaminant is biomagnifying in the food-web; whereas, less than 1 indicates trophic dilution. We determined TMF variability as the antilog of the lower and upper 95% confidence limits for the slope and standard error associated with the TMF was equal to the variability associated with the slope multiplied by the TMF (*5*). Statistical significance of *p*-values for slope estimates were assessed at $\alpha = 0.05$. We report the likelihood-*r* correlation coefficient (Loglik-*r* = square root of likelihood r^2), which expresses the strength of the relationship between lipid equivalent concentrations and isotopic trophic positions as measured by maximum likelihood estimation (*95*).

The variation in trophic magnification observed between many POPs can often be explained by differences in their chemical properties, such as K_{OW} and K_{OA}. To better understand the variation in trophic magnification observed, we examined the relationship between our estimated TMF and log K_{OA} of each legacy POP using a linear-mixed effects model with type of POP (e.g. PCB, OCP, or PBDE) as a random effect. K_{OA} and K_{OW} values of each legacy POP were compiled from various sources (*4, 27, 79, 97-101*). As we are working with air-breathing organisms, we simply looked at the relationship between TMF and K_{OA}.

1.3. Results

1.3.1. Stable Isotopes and Trophic Position

Stable Isotopes and Isotopic Enrichment

We conducted stable nitrogen and carbon isotope analysis on a total of 98 samples from across the food-web (Table 1.1; Appendix 3). $\delta^{15}N$ varied with species group ($F_{11,86} = 54.64$, $p < 0.001$; Table 1.1) with average $\delta^{15}N$ values ranging from 0.48 ‰ in Himalayan blackberry to 9.23 ‰ in Cooper's Hawks. $\delta^{13}C$ also varied with species group ($F_{11,86} = 20.29$, $p < 0.001$; Table 1.1) but there was considerably more overlap in the $\delta^{13}C$ values of species groups in the food-web (Table 1.1; Figure 1.2). Nevertheless, we observed a positive relationship between the $\delta^{15}N$ and $\delta^{13}C$ values from the samples in the food-web ($\delta^{15}N = 1.05(\delta^{13}C) + 32.55$; $F_{1,96} = 102.7$, $p < 0.001$, $r^2 = 0.51$). From our isotope data, we determined the isotopic enrichment factor to be 2.88% (Figure 1.3; Appendix 4).

Table 1.1. Average stable nitrogen and carbon isotopes measured in each species group. No isotope data available for Insecta species as there was not enough biomass available. SE = standard error; LCL = lower 95% confidence limit; UCL = upper 95% confidence limit.

Species	*n*	$\delta^{15}N$ (‰)				Compact Letter Display	$\delta^{13}C$ (‰)				Compact Letter Display
		Average	SE	LCL	UCL		Average	SE	LCL	UCL	
Himalayan Blackberry	6	0.48	0.30	-0.29	1.25	A	-28.72	0.24	-29.33	-28.11	A
Lumbricidae	25	2.59	0.30	1.97	3.21	B	-27.46	0.24	-27.96	-26.97	AB
Oniscidea	6	3.35	0.43	2.24	4.46	BC	-25.75	0.15	-26.13	-25.36	CDE
Thrush species	6	5.01	0.38	4.03	5.99	CD	-26.30	0.38	-27.27	-25.32	BCD
American Robin	6	5.30	0.39	4.28	6.31	CD	-25.85	0.28	-26.57	-25.13	BCDE
Small Beetles	3	5.04	0.38	3.40	6.68	CDE	-27.05	0.24	-28.10	-26.01	ABCD
Pigeon/Dove	6	6.08	0.24	5.46	6.69	DEF	-23.98	0.64	-25.63	-22.32	EF
Large Beetles	6	6.39	0.31	5.59	7.18	DEF	-27.38	0.31	-28.18	-26.58	ABC
Sparrow species	6	6.65	0.58	5.16	8.14	DEF	-24.10	0.93	-26.49	-21.70	EF
European Starling	5	7.63	0.30	6.80	8.45	EFG	-25.47	0.43	-26.66	-24.28	CDE
Northern Flicker	6	8.06	0.49	6.80	9.31	FG	-24.85	0.24	-25.46	-24.25	DEF
Cooper's Hawk	17	9.23	0.13	8.95	9.51	G	-23.54	0.23	-24.02	-23.06	F

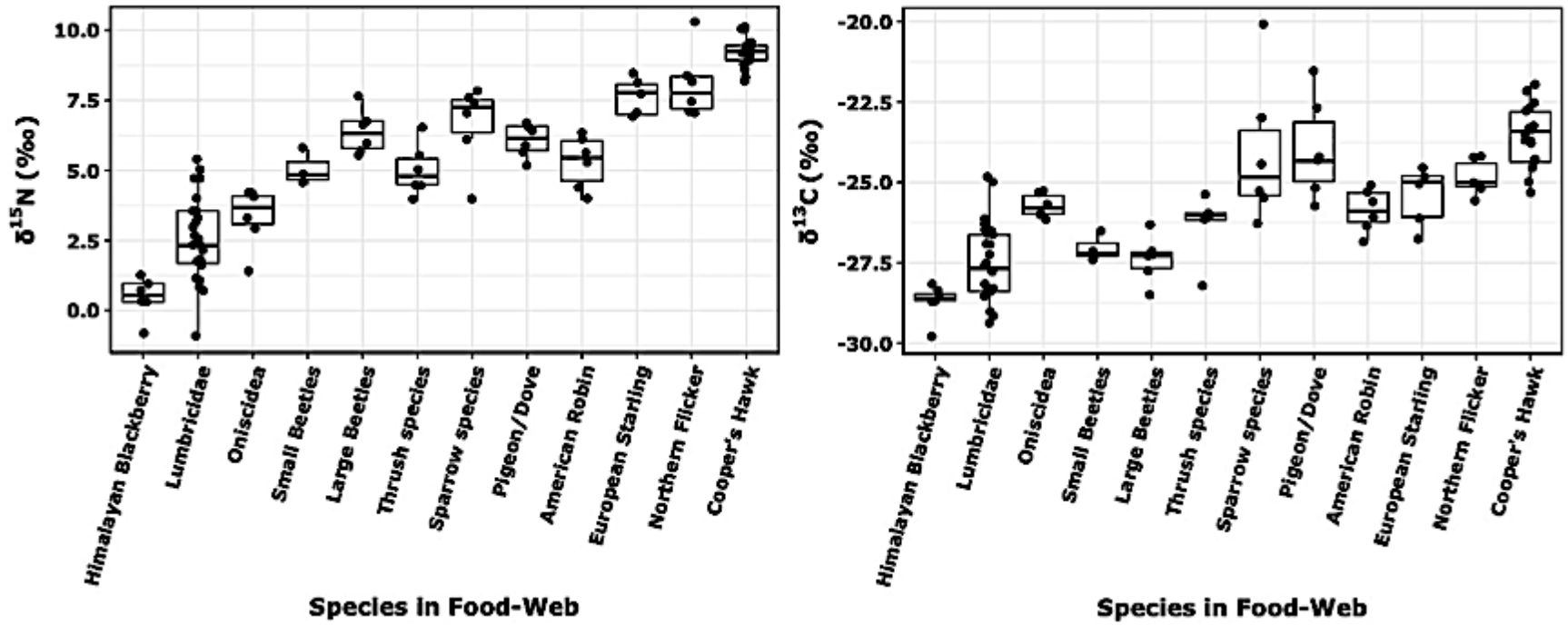

Figure 1.2. Stable δ15N and δ13C isotope signatures in the biotic levels of the food-web. Several distinct trophic levels can be seen in the stable nitrogen isotope data (left); whereas, there is considerable overlap in the stable δ13C isotope data across the food web (right).

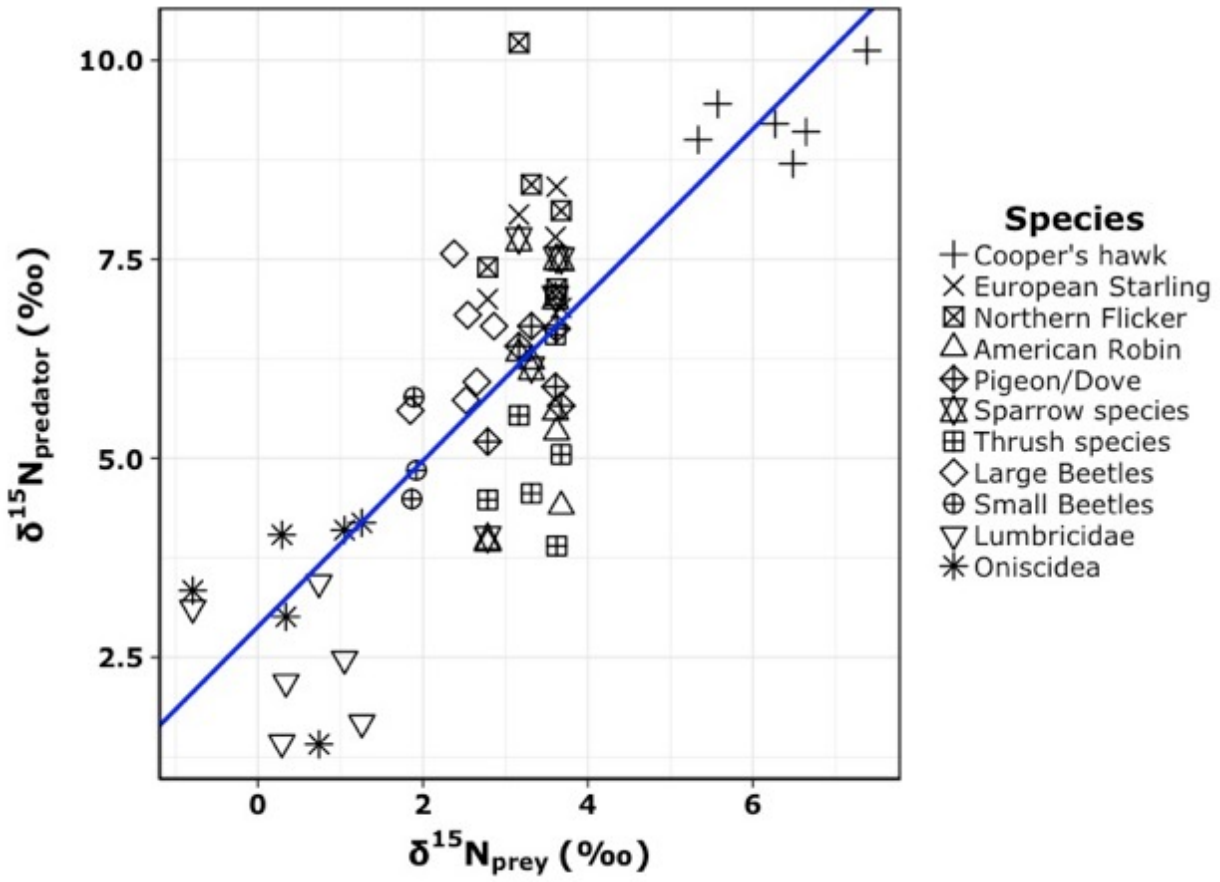

Figure 1.3. Isotopic enrichment factor constant represented by relationship between average δ15N of predator species and overall average δ15N of prey species within six sampling regions (δ15Npredator = 1.04(δ15Nprey) + 2.88; F1,55 = 101.3; p <0.0001).

Trophic Position and Food-web Characterization

We assumed detritivores, i.e. earthworms and sowbugs/pillbugs, had a dietary trophic position of 2.00 and the primary producer, Himalayan blackberries, to have a dietary trophic position of 1.00. The estimated dietary trophic positions of the other species groups ranged from 3.00 in Insecta species up to 4.03 in Cooper's Hawk (Table 1.2). We used the average $\delta^{15}N$ values of the detritivores and primary producer in Table 1.1 to determine the isotopic trophic position of each consumer or detritivore in equation 3 (Appendix 5). Average isotopic trophic positions ranged from 1.96 (0.10 SE) in the earthworms to 4.27 (0.05 SE) in the Cooper's Hawk (Table 1.3, Appendix 5). The isotopic trophic positions were generally comparable to, or slightly higher, than the dietary trophic positions, indicating that $\delta^{15}N$ provides a reasonable estimate of trophic position in this food-web (Table 1.3; Appendix 5). We were unable to estimate the isotopic trophic

position for Insecta species because there was not enough biomass available, so we used the dietary trophic position of 3.00 for estimating trophic magnification.

Table 1.2. Trophic position model, feeding preferences, and food-web relationships determined from literature resources.

Species	Trophic Position	Prey Species	Prey Trophic Position[1]	Dietary Proportion (%)[2]	References
Cooper's Hawk	4.03	Northern Flicker	3.28	3.00	(*34, 38*)
		Pigeon/Dove	2.46	10.00	(*32, 34, 38*)
		American Robin	3.12	30.00	(*32, 38*)
		Sparrow	2.71	22.00	(*32, 38*)
		European Starling	3.22	35.00	(*32, 38*)
		Thrush	3.02	1.00	(*38*)
Northern Flicker	3.28	Fruit	1	32.30	(*86, 87*)
		Coleoptera	3	3.30	(*86, 87*)
		Carabidae	3	3.30	(*86, 87*)
		Orthoptera	2	1.50	(*86, 87*)
		Hemiptera	2	1.80	(*86, 87*)
		Lepidoptera	2	2.10	(*86, 87*)
		Invertebrates	2	1.90	(*86, 87*)
		Formicidae	3	53.80	(*86, 87*)
European Starling	3.22	Fruit	1	25.00	(*39, 40*)
		Carabidae	3	5.00	(*40*)
		Coleoptera	3	25.00	(*40, 41*)
		Orthoptera	2	1.00	(*40*)
		Formicidae	3	1.00	(*40*)
		Tipulid	2	15.00	(*40, 41*)
		Lepidoptera	2	7.00	(*40, 41*)
		Lumbricidae	2	7.00	(*40, 41*)
		Gastropoda (Snails and Slugs)	2	2.00	(*40, 41*)
		Arachnidae	3	2.00	(*40, 41*)
		Oniscidea	2	2.00	(*40, 41*)
		Human food waste	3	10.00	(*40, 41*)
American Robin	3.12	Fruit	1	35.00	(*42, 43*)
		Lepidoptera	2	7.00	(*42*)
		Carabidae	3	10.00	(*42*)
		Coleoptera	3	30.00	(*42*)
		Formicidae	3	5.00	(*42, 43*)
		Arachnidae	3	2.00	(*42*)
		Lumbricidae	2	10.00	(*44*)
		Oniscidea	2	1.00	(*42*)
Pigeon/Dove	2.46	Grains and Seeds	1	75.00	(*68, 69*)
		Fruit	1	1.00	(*68, 69*)

Species	Trophic Position	Prey Species	Prey Trophic Position[1]	Dietary Proportion (%)[2]	References
		Insects and Invertebrates	2	5.00	(*68, 69*)
		Human food waste	3	20.00	(*68, 69*)
Sparrow	2.71	Fruit	1	5.00	(*45, 84*)
		Grains and Seeds	1	50.00	*(45, 46, 84)*
		Lepidoptera	2	5.00	(*45*)
		Orthoptera	2	4.00	(*45*)
		Tipulid	2	2.00	(*45*)
		Hymenoptera	3	5.00	(*45*)
		Coleoptera	3	10.00	(*45, 84*)
		Formicidae	3	4.00	(*45, 84*)
		Julida	3	2.00	(*45*)
		Arachnidae	3	5.00	(*84*)
		Gastropoda (Snails and Slugs)	2	2.00	(*45*)
		Lumbricidae	2	2.00	(*84*)
		Oniscidea	2	4.00	(*46*)
Thrush	3.02	Fruit	1	30.00	(*65-67, 88, 89*)
		Seeds	1	5.00	(*65-67, 88, 89*)
		Carabidae	3	5.00	(*65-67, 88, 89*)
		Coleoptera	3	10.00	(*65-67, 88, 89*)
		Lepidoptera	2	10.00	(*65-67, 88, 89*)
		Hemiptera	2	8.00	(*65-67, 88, 89*)
		Diptera	2	6.00	(*65-67, 88, 89*)
		Formicidae	3	15.00	(*65-67, 88, 89*)
		Hymenoptera	3	6.00	(*65-67, 88, 89*)
		Arachnidae	3	1.00	(*65-67, 88, 89*)
		Lumbricidae	2	2.00	(*65-67, 88, 89*)
		Oniscidea	2	2.00	(*65-67, 88, 89*)
Large Beetles	3.28	Lumbricidae	2	35.00	(*50*)
		Insects	3	25.00	(*47, 50*)
		Gastropoda (Snails and Slugs)	2	40.00	(*49*)
		Oniscidea	2	1.00	(*48*)
		Seeds and Plants	1	1.00	(*47, 50*)
Small Beetles	3.00	Seeds	1	35.00	(*49*)
		Insects	3	35.00	(*49*)
		Gastropoda (Snails and Slugs)	2	30.00	(*49*)
Insecta	3.00	Insects and Invertebrates	3	35.00	(*51*)
		Plants	1	35.00	(*51*)

Species	Trophic Position	Prey Species	Prey Trophic Position[1]	Dietary Proportion (%)[2]	References
		Detritus - Plant and Animal	2	30.00	(*51*)
Earthworms	2.00	Soil	1	50.00	(*52*)
		Detritus - Plant	1	50.00	(*52*)
Oniscidea	2.00	Detritus - Plant	1	100.00	(*51, 53*)
Himalayan Blackberry	1.00	-	-	-	

[1]Trophic positions of invertebrate prey species are assumed from dietary preferences (i.e. herbivores = 2, omnivores = 3, carnivores = 3) and trophic positions of plants and soil are assumed to be 1;

[2]Dietary proportion is an average roughly estimated or assumed from available literature references.

Table 1.3. Summary of trophic positions determined by dietary references and stable isotope analysis

Species	Dietary Trophic Position	Isotopic Trophic Position		
		Mean	SD	SE
Cooper's Hawk	4.03	4.27	0.19	0.05
Northern Flicker	3.28	3.86	0.42	0.17
European Starling	3.22	3.76	0.24	0.11
American Robin	3.12	2.91	0.33	0.14
Thrush	3.02	2.81	0.32	0.13
Sparrow	2.71	3.38	0.49	0.20
Pigeon/Dove	2.46	3.18	0.20	0.08
Large Beetles	3.28	3.28	0.26	0.11
Small Beetles	3.00	2.81	0.23	0.13
Insecta[1]	3.00	-	-	-
Oniscidea	2.00	2.23	0.37	0.15
Earthworms	2.00	1.96	0.52	0.10
Himalayan Blackberry[2]	1.00	-	-	-

[1]Stable isotope analysis was not completed for Insecta as there was not enough biomass available, so dietary trophic position was used in TMF regressions;

[2]A trophic position of 1 was assumed for Himalayan blackberry and used in TMF regressions.

1.3.2. Legacy POP Concentrations

Polychlorinated Biphenyls (PCBs)

We detected all 38 targeted PCB congeners within samples of at least one species group (Appendix 6). Fifteen (40%) of the 38 congeners were detected in > 50% of all the samples (Table 1.4). Generally, total detection rates increased with chlorination. Hexachlorobiphenyls, such as PCB 138 and 153, typically had the highest detection rates in all of the species groups, excluding Himalayan blackberry, with detection rates > 70% (Appendix 6). More highly chlorinated congeners, such as octachlorobiphenyls PCBs 194 – 209, were often detected at rates > 50% but only in 6 species groups (Appendix 6). PCB 180, 158, 105, 138, and 153 had the highest total detection frequencies ranging from 69 – 89% (Table 1.4).

Table 1.4. Frequency of detection for PCBs across all samples.

PCB	*n*	*n* Censored	*n* Detected	Detection Frequency (%)
PCB-17	109	108	1	1
PCB-18	109	107	2	2
PCB-28/31	109	51	58	53
PCB-33	109	98	11	10
PCB-44	109	85	24	22
PCB-49	109	86	23	21

PCB	*n*	*n* Censored	*n* Detected	Detection Frequency (%)
PCB-52	109	66	43	39
PCB-70	109	73	36	33
PCB-74	109	58	51	47
PCB-82*	92	90	2	2
PCB-87	109	61	48	44
PCB-95	109	65	44	40
PCB-99	109	37	72	66
PCB-101	109	35	74	68
PCB-105	109	20	89	82
PCB-110	109	46	63	58
PCB-118	109	36	73	67
PCB-128	109	50	59	54
PCB-138	109	13	96	88
PCB-149	109	35	74	68
PCB-151	109	72	37	34
PCB-153	109	12	97	89
PCB-156	109	54	55	50
PCB-158	109	25	84	77
PCB-170	109	57	52	48
PCB-171	109	65	44	40
PCB-177	109	63	46	42
PCB-180	109	34	75	69
PCB-183	109	60	49	45
PCB-187	109	28	81	74
PCB-191*	92	83	9	10
PCB-194	109	61	48	44
PCB-195	109	72	37	34
PCB-199	109	58	51	47
PCB-205	109	86	23	21
PCB-206	109	57	52	48
PCB-208	109	76	33	30
PCB-209	109	69	40	37

**Not analysed in Cooper's Hawk samples.*

Average concentrations for the 15 congeners detected in > 50% of the samples varied considerably within each species group (Appendix 7). PCB 153 had the highest average concentrations across the food-web ranging from 4.33 (0.45 SE) ng/g lipid eq. in earthworms to 3,242.86 (455.69 SE) ng/g lipid eq. in Cooper's Hawks (Appendix 7). Other congeners detected in high concentrations across the food-web included PCB 180, 138, and 187 with averages ranging from 2.23 – 2,088.14 ng/g lipid eq., 4.52 – 2,048.41 ng/g lipid eq., and 0.96 – 1,438.13 ng/g lipid eq., respectively (Appendix 7). PCB 153, 180, and 138 are typically known to dominate in avian tissues collected from many locations and diverse taxonomic groupings (*102*). Generally, PCBs with greater chlorination had higher concentrations detected in each species group. We report the estimated lipid equivalent concentrations for each sample in Appendix 8.

∑PCB average concentrations in the Cooper's Hawk were roughly 10x greater than in the species groups with lower estimated trophic positions (Figure 1.4; Appendix 7). ∑PCB concentrations increased from non-detectable levels in the primary producer to 13704.15 ng/g lipid eq. in the apex predator (Figure 1.4; Appendix 7). Among the avian prey species, ∑PCB average concentrations were highest in Northern Flickers at 1465.09 ng/g lipid eq., European Starlings at 1016.62 ng/g lipid eq., and American Robins at 599.02 ng/g lipid eq. and lowest in Sparrows at 314.26 ng/g lipid eq., Thrushes at 174.44 ng/g lipid eq., and Pigeons/Doves at 62.29 ng/g lipid eq. (Figure 1.4; Appendix 7).

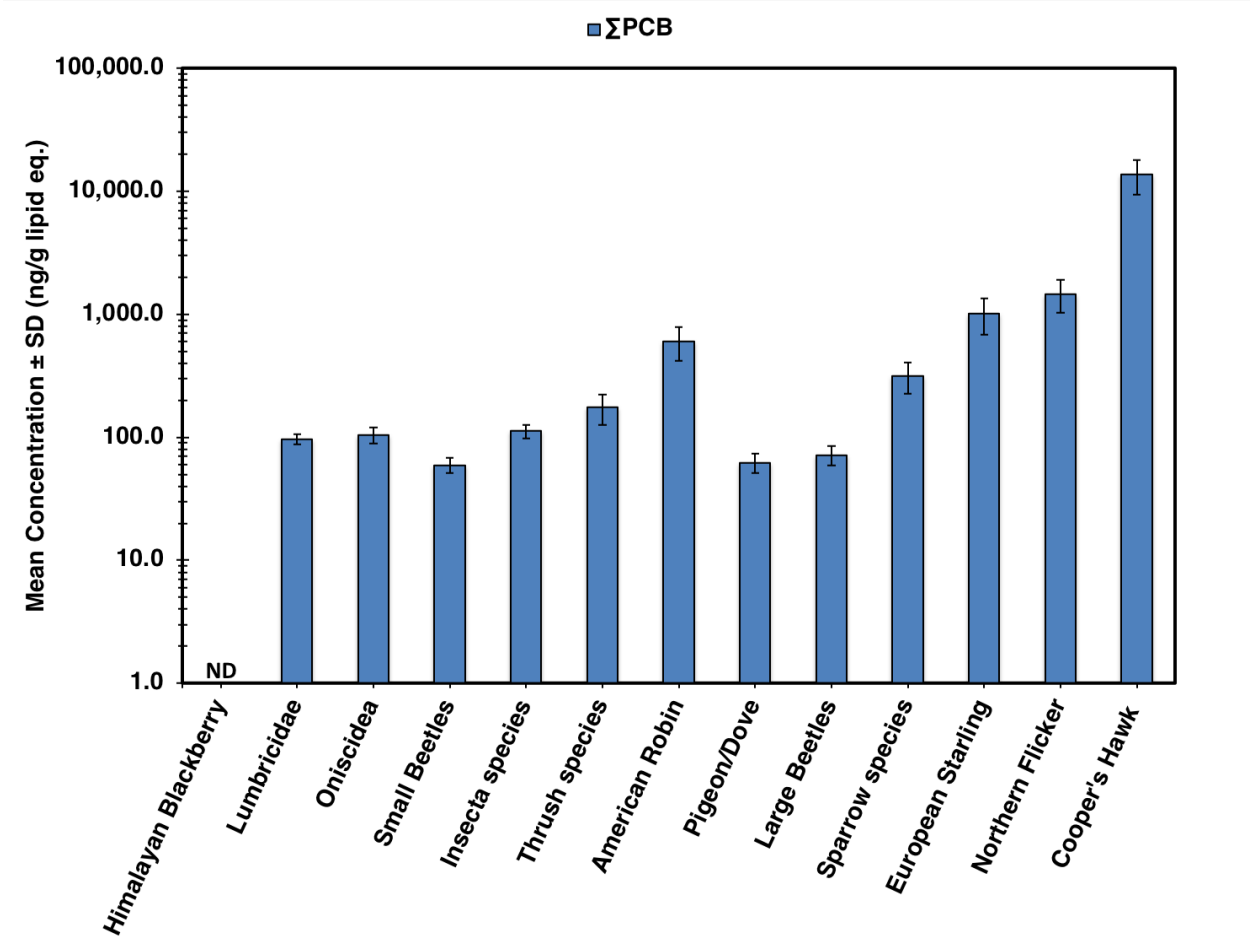

Figure 1.4. Total PCB average concentrations observed in each species group. ND = Non-detection with sample concentrations below MDL.

Organochlorine Pesticides (OCPs)

We detected all 20 OCPs within at least one trophic level. OCPs detected at rates higher than 80% in every species group (excluding Himalayan blackberry) included oxychlordane (OXY), dieldrin (DIEL), and *p,p*-dichlorodiphenyldichloroethylene (DDE; Appendix 6). However, only 9 (45%) of the 20 OCPs were detected in > 50% of all the samples across the food-web (Table 1.5). OXY, DDE, DIEL, hexachlorobenzene (HCB), and heptachlor epoxide (HEP) had the highest total detection frequencies ranging from 75 – 96%.

Table 1.5. Frequency of detection for OCPs across all samples.

OCP	*n*	*n* Censored	*n* Detected	Detection Frequency (%)
1,2,4,5-TCB	109	104	5	5
1,2,3,4-TCB	109	102	7	6
QCB	109	35	74	68
HCB	109	16	93	85
α-HCH	109	105	4	4
β-HCH	109	106	3	3
γ-HCH	109	75	34	31
OCS	109	83	26	24
HEP	109	27	82	75
OXY	109	4	105	96
trans-CHL	109	101	8	7
cis-CHL	109	95	14	13
trans-NON	109	29	80	73
cis-NON	109	62	47	43
DIEL	109	11	98	90
DDE	109	6	103	94
DDT	109	34	75	69
DDD	109	41	68	62
MIR	109	60	49	45
PMIR*	17	0	17	100

**Analysed only in Cooper's Hawk samples.*

Average concentrations for the 9 OCPs detected in > 50% of the samples varied considerably in each species group (Appendix 7). DDE had the highest average concentrations detected across the food-web ranging from < 5.39 ng/g lipid eq. in the primary producer up to 23,927.59 (2,667.96 SE) ng/g lipid eq. in the apex predator (Figure 1.5; Appendix 7). Other OCPs detected in > 50% of the samples with high concentrations included *trans*-nonachlor (*trans*-NON), DIEL, and OXY with averages ranging from 1.20 – 2,489.74 ng/g lipid eq., 5.15 – 1,716.45 ng/g lipid eq., and 4.13 – 1,332.41 ng/g lipid eq., respectively (Appendix 7). We report the estimated lipid equivalent concentrations for each sample in Appendix 9.

∑CBZ average concentrations generally increased with the estimated trophic position of the species groups. ∑CBZ concentrations ranged from 9.04 ng/g lipid eq. in large beetles to 163.17 ng/g lipid eq. in Cooper's Hawks (Figure 1.5; Appendix 7). HCB was the predominant CBZ with average concentrations ranging from 1.11 (0.21 SE) ng/g lipid eq. in Pigeons/Doves to 82.53 (17.39 SE) ng/g lipid eq. in Cooper's Hawks (Appendix 7). γ-Hexachlorocyclohexane (γ-HCH) was the dominant HCH observed within the food-web with average concentrations ranging from 0.79 (0.15 SE) ng/g lipid eq. in Pigeons/Doves up to 254.33 (113.97 SE) ng/g lipid eq. in Insecta species. ∑HCH average concentrations were generally very low or decreasing with the average trophic positions of the species groups (Figure 1.5; Appendix 7). Octachlorostyrene (OCS) average concentrations did not vary greatly but somewhat decreased with the estimated trophic position of the species groups ranging from 0.80 (0.47 SE) ng/g lipid eq. in Pigeons/Doves to 20.83 ng/g lipid eq. in Insecta spp. (Figure 1.5; Appendix 7). ∑CHL average concentrations increased with the estimated trophic position of the species groups and ranged from 10.61 ng/g lipid eq. in Pigeons/Doves to 4,690.42 ng/g lipid eq. in Cooper's Hawks (Figure 1.5; Appendix 7). The predominant CHLs were *trans*-NON and OXY with average concentrations ranging from 1.20 ng/g lipid eq. in Pigeons/Doves to 2,489.74 (475.13 SE) ng/g lipid eq. in Cooper's Hawks for *trans*-NON and 4.13 (0.70 SE) ng/g lipid eq. in Pigeons/Doves to 1,322.41 (150.60 SE) ng/g lipid eq. in Cooper's Hawks for OXY (Appendix 7). Average concentrations of *p,p*-dichlorodiphenyltrichloroethane (DDT) and *p,p*-dichlorodiphenyltrichloroethane (DDD) within each species group were similar ranging from 1.97 (0.67 SE) ng/g lipid eq. in small beetles to 358.26 (10.2.21 SE) ng/g lipid eq. in Cooper's Hawks for DDT, and 0.93 (0.18 SE) ng/g lipid eq. in small beetles to 353.98 (48.35 SE) ng/g lipid eq. in Cooper's Hawks for DDD (Figure 1.5; Appendix 7). Finally, Mirex (MIR) had high concentrations comparable to DIEL in the species groups with higher estimated trophic positions ranging from 0.88 (0.23 SE) ng/g lipid eq. in Pigeons/Doves to 1,562.34 (1,200.44 SE) ng/g lipid eq. in Cooper's Hawks (Figure 1.5; Appendix 7).

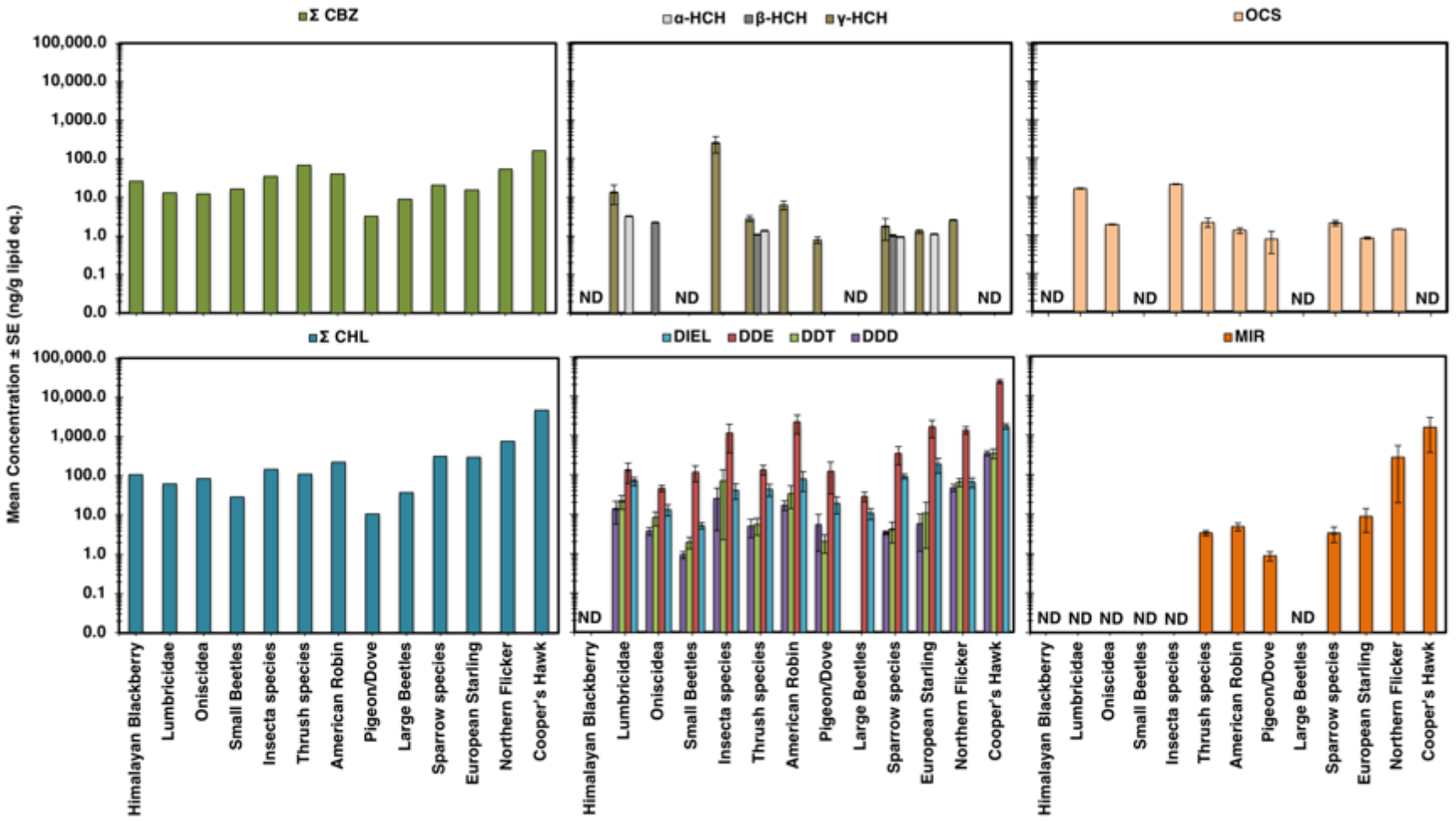

Figure 1.5. OCP average concentrations observed in each species group. ∑CBZH include 1,2,4,5-TCB. 1,2,3,4-TCB, QCB, and HCB. ∑CHL include HEP, OXY, cis- and trans-CHL, and cis- and trans-NON. ND = Non-detection with sample concentrations below MDL.

Polybrominated Diphenyl Ethers (PBDEs)

We detected 17 (85%) of the 20 PBDE congeners within at least one trophic level. Highly brominated congeners, such as BDE-183 and -209, were infrequently detected (< 20%) in samples from species groups with lower estimated trophic positions but were detected in 100% of the Cooper's Hawk samples (Appendix 6). Two (10%) of the 20 PBDE congeners, BDE-47 and -99, were detected in > 50% of all the samples (Table 1.6; Appendix 6). Congeners BDE-119, -196, -197, -206, and -207, which were not analysed in the Cooper's Hawk samples, had very low detection rates of < 20% in the species groups with lower estimated trophic positions (Table 1.6; Appendix 6). The majority (86%) of the 7 BFRs analysed exclusively within Cooper's Hawk eggs had total detection frequencies > 50%, with hexabromocyclododecane (HBCDD) and *syn*-Dechlorane Plus (syn-DP) both having the highest detection frequency at 100% (Table 1.6; Appendix 6).

Table 1.6. Frequency of detection for PBDEs and other BFRs across all samples.

PBDE	*n*	n Censored	n Detected	Detection Frequency (%)
BDE-15	109	109	0	0
BDE-17	109	92	17	16
BDE-28	109	94	15	14
BDE-47	109	24	85	78
BDE-49	109	91	18	17
BDE-66	109	93	16	15
BDE-85	109	87	22	20
BDE-99	109	35	74	68
BDE-100	109	64	45	41
BDE-119[1]	92	90	2	2
BDE-138	109	90	19	17
BDE-153	109	62	47	43
BDE-154	109	66	43	39
BDE-183	109	90	19	17
BDE-190[2]	17	17	0	0
BDE-196[1]	92	91	1	1
BDE-197[1]	92	90	2	2
BDE-206[1]	92	92	0	0
BDE-207[1]	92	91	1	1
BDE-209	109	91	18	17
α-TBECH[2]	17	17	0	0
HBB[2]	17	8	9	53
BB-101[2]	17	1	16	94
HBCDD[2]	17	0	17	100
BTBPE[2]	17	5	12	71
syn-DP[2]	17	0	17	100

PBDE	*n*	n Censored	n Detected	Detection Frequency (%)
anti-DP[2]	17	3	14	82

[1]Not analysed in Cooper's Hawk samples;
[2]Analysed only in Cooper's Hawk samples

Average concentrations for most of the PBDE congeners generally increased with the estimated trophic position of the species groups (Appendix 7). BDE-47 and -99, which were detected in > 50% of all the samples, had the highest average concentrations observed across the food-web. Concentrations of BDE-47 ranged from < 1.45 ng/g lipid eq. in Insecta species to 633.23 (75.54 SE) ng/g lipid eq. in the Cooper's Hawks and BDE-99 concentrations ranged from < 1.29 ng/g lipid eq. in Insecta species to 1,803.84 (258.21 SE) ng/g lipid eq. in the Cooper's Hawks (Appendix 7). Congeners BDE-100 and -153 also had particularly high average concentrations observed across the food-web. Concentrations of BDE-100 ranged from 4.62 ng/g lipid eq. in the small beetles up to 535.14 (64.07 SE) ng/g lipid eq. in the Cooper's Hawks and BDE-153 concentrations ranged from < 0.71 ng/g lipid eq. in small beetles up to 795.51 (162.46 SE) ng/g lipid eq. in the Cooper's Hawks (Appendix 7). Congeners BDE-47, -99, and -100 are commonly known to dominate in the eggs of water-birds and raptors (*102*). We report the estimated lipid equivalent concentrations for each sample in Appendix 10.

Average concentrations for the six BFRs detected within the Cooper's Hawks' eggs were generally low ranging from 1.48 (0.16 SE) ng/g lipid eq. for BB-101 to 13.71 (2.11 SE) ng/g lipid eq. for *anti*-Dechlorane Plus (anti-DP). However, HBCDD had a very high average concentration at 389.67 (103.95 SE) ng/g lipid eq. (Appendix 7).

∑PBDE average concentrations were quite high across the food-web in comparison to ∑PCB average concentrations but generally increased with the estimated trophic position of the species groups (Figure 1.6). ∑PBDE average concentrations were typically 10X higher in the apex predator compared to the species groups with lower estimated trophic positions, which was similar to ∑PCB average concentrations (Figure 1.6). ∑PBDE average concentrations ranged from 64.21 ng/g lipid eq. in the Pigeons/Doves to 5,745.62 ng/g lipid eq. in the Cooper's Hawks (Figure 1.6; Appendix 7).

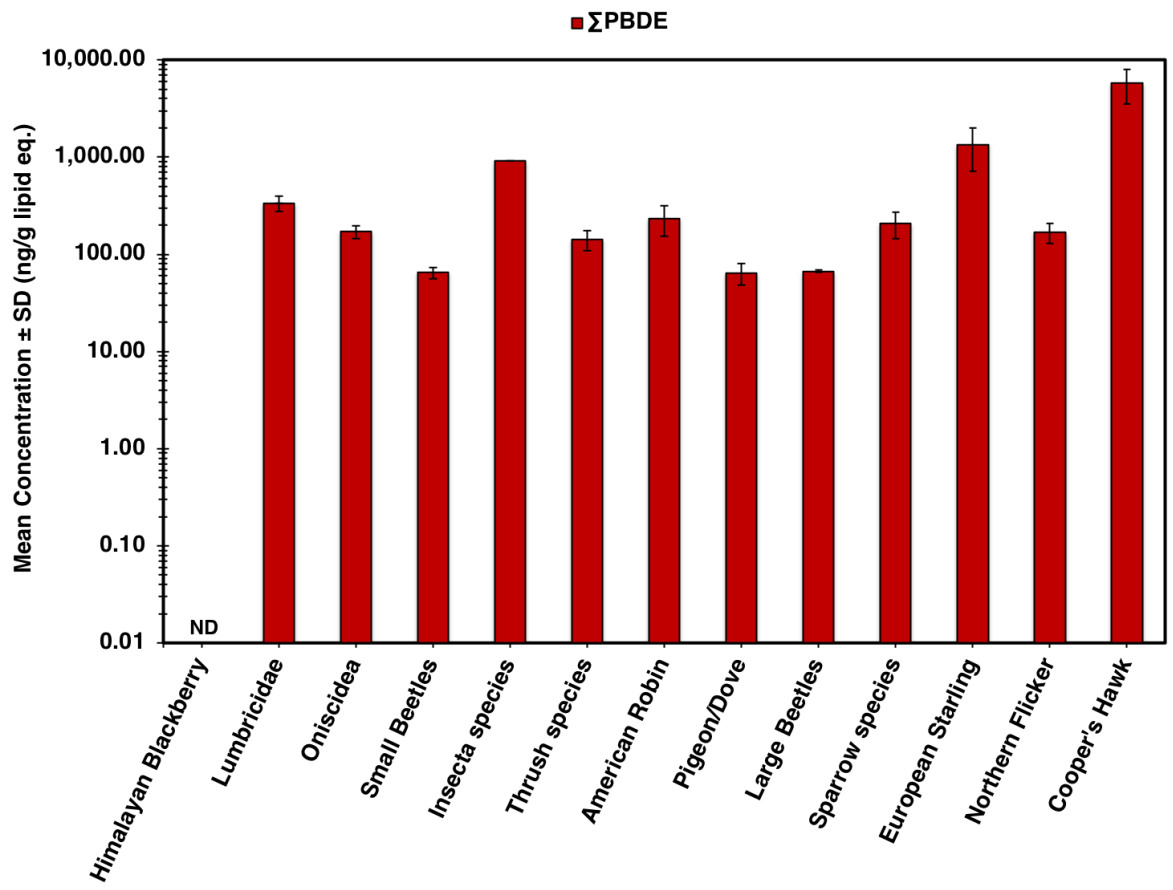

Figure 1.6. Total PBDE average concentrations observed in each species group, excluding BFRs. ND = Non-detection with sample concentrations below MDL.

1.3.3. Trophic Magnification of Legacy POPs

We found that most of the PCBs had TMFs greater than 1 indicating biomagnification in the food-web (Table 1.7; Appendix 11). However, PCB-28/31 had a TMF roughly equal to 1 (p = 0.311; Z = 1.01) with a 95% confidence interval that bounded 1 indicating that on average it was not biomagnifying in the food-web (Figure 1.7). Of the three PCBs known to dominate in avian tissues, PCB-180 had the highest TMF at 15.66 (4.05 SE; Figure 1.7) as well as the second highest average concentrations detected across the food-web. Conversely, PCB-153, which had the highest average concentrations detected across the food-web, had a much lower TMF at 7.40 (1.24 SE; Figure 1.7).

Table 1.7. Statistical results from censored regressions to determine TMFs of legacy POPs with detection frequencies greater than 50%. SE = standard error; LCL = lower 95% confidence limit; UCL = upper 95% confidence limit.

Analyte	Slope	SE	LCL	UCL	χ^2	Loglik-*r*	*p*-value	TMF	SE	LCL	UCL
PCB-28/31	0.18	0.18	-0.17	0.53	1.03	0.097	0.311	1.20	0.21	0.85	1.69
PCB-99	2.12	0.22	1.68	2.56	91.40	0.753	< 0.001	8.30	1.87	5.35	12.90
PCB-101	1.36	0.17	1.03	1.68	65.61	0.672	< 0.001	3.88	0.65	2.80	5.39
PCB-105	1.51	0.16	1.20	1.82	76.20	0.709	< 0.001	4.53	0.71	3.33	6.15
PCB-110	0.57	0.15	0.29	0.86	15.90	0.368	< 0.001	1.78	0.26	1.33	2.37
PCB-118	2.16	0.22	1.73	2.58	93.39	0.759	< 0.001	8.63	1.87	5.64	13.20
PCB-128	2.19	0.27	1.67	2.72	70.41	0.690	< 0.001	8.97	2.39	5.32	15.13
PCB-138	1.85	0.17	1.52	2.18	89.93	0.750	< 0.001	6.35	1.08	4.55	8.86
PCB-149	1.23	0.18	0.88	1.59	45.14	0.582	< 0.001	3.43	0.62	2.41	4.89
PCB-153	2.00	0.17	1.67	2.33	100.52	0.776	< 0.001	7.40	1.24	5.33	10.28
PCB-156	2.43	0.29	1.86	3.00	74.02	0.702	< 0.001	11.34	3.30	6.41	20.05
PCB-158	1.63	0.18	1.27	1.98	72.89	0.698	< 0.001	5.08	0.91	3.58	7.22
PCB-180	2.75	0.26	2.24	3.26	106.52	0.790	< 0.001	15.66	4.05	9.44	25.99
PCB-187	2.10	0.23	1.65	2.55	74.26	0.703	< 0.001	8.17	1.87	5.21	12.79
QCB	0.84	0.14	0.56	1.12	36.07	0.531	< 0.001	2.31	0.33	1.74	3.06
HCB	0.93	0.11	0.72	1.15	60.47	0.653	< 0.001	2.54	0.28	2.05	3.16
HEP	1.68	0.20	1.29	2.07	61.53	0.657	< 0.001	5.37	1.08	3.62	7.95
OXY	1.08	0.15	0.79	1.38	42.79	0.570	< 0.001	2.95	0.45	2.20	3.97
trans-NON	1.88	0.26	1.37	2.39	44.94	0.581	< 0.001	6.55	1.70	3.93	10.91
DIEL	1.09	0.17	0.75	1.43	34.41	0.520	< 0.001	2.97	0.52	2.11	4.19
DDE	2.05	0.18	1.70	2.40	91.18	0.753	< 0.001	7.79	1.38	5.50	11.03
DDT	0.98	0.23	0.53	1.42	17.72	0.387	< 0.001	2.66	0.60	1.70	4.15
DDD	1.48	0.26	0.98	1.99	31.96	0.504	< 0.001	4.41	1.14	2.66	7.32
BDE-47	1.14	0.18	0.78	1.49	36.20	0.532	< 0.001	3.11	0.56	2.19	4.43
BDE-99	1.99	0.24	1.52	2.46	61.89	0.658	< 0.001	7.32	1.77	4.55	11.76

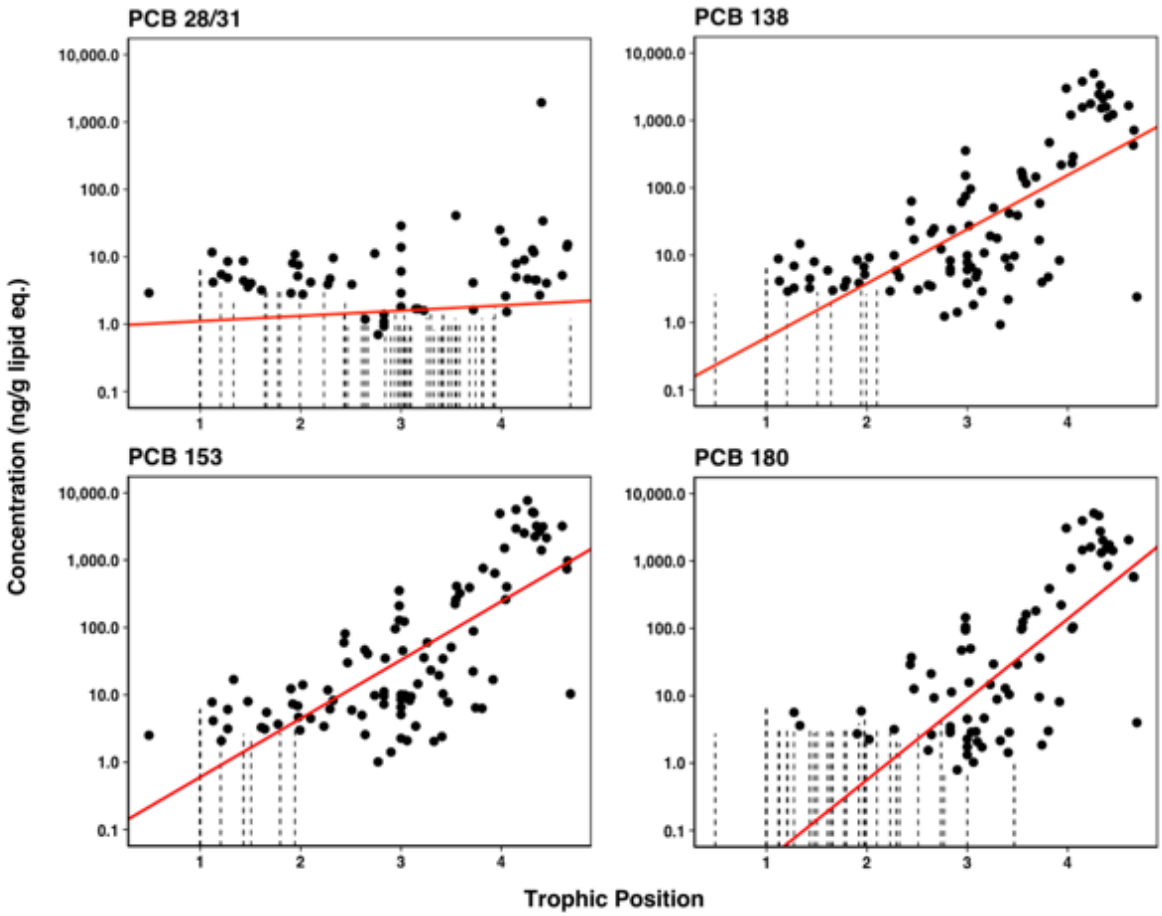

Figure 1.7. PCB concentrations in organisms within an urban, terrestrial food-web (ng/g lipid eq.) versus trophic position (TP) for PCB 28/31 and PCBs 138, 153, and 180, which are known to dominate in avian tissues. Black circles represent detected observations and dashed lines represent non-detected observations below a sample specific, lipid normalized MDL. Red lines represent the log-linear regression of lipid equivalent concentration to TP across the food-web.

We found that all of the OCPs we evaluated had TMFs greater than 1 indicating biomagnification in the food-web (Table 1.7; Appendix 11). OCPs *p,p*-DDE and *trans*-NON had the highest TMFs at 7.79 (1.38 SE) and 6.55 (1.70 SE) and also had the highest average concentrations detected across the food-web (Table 1.7; Figure 1.8).

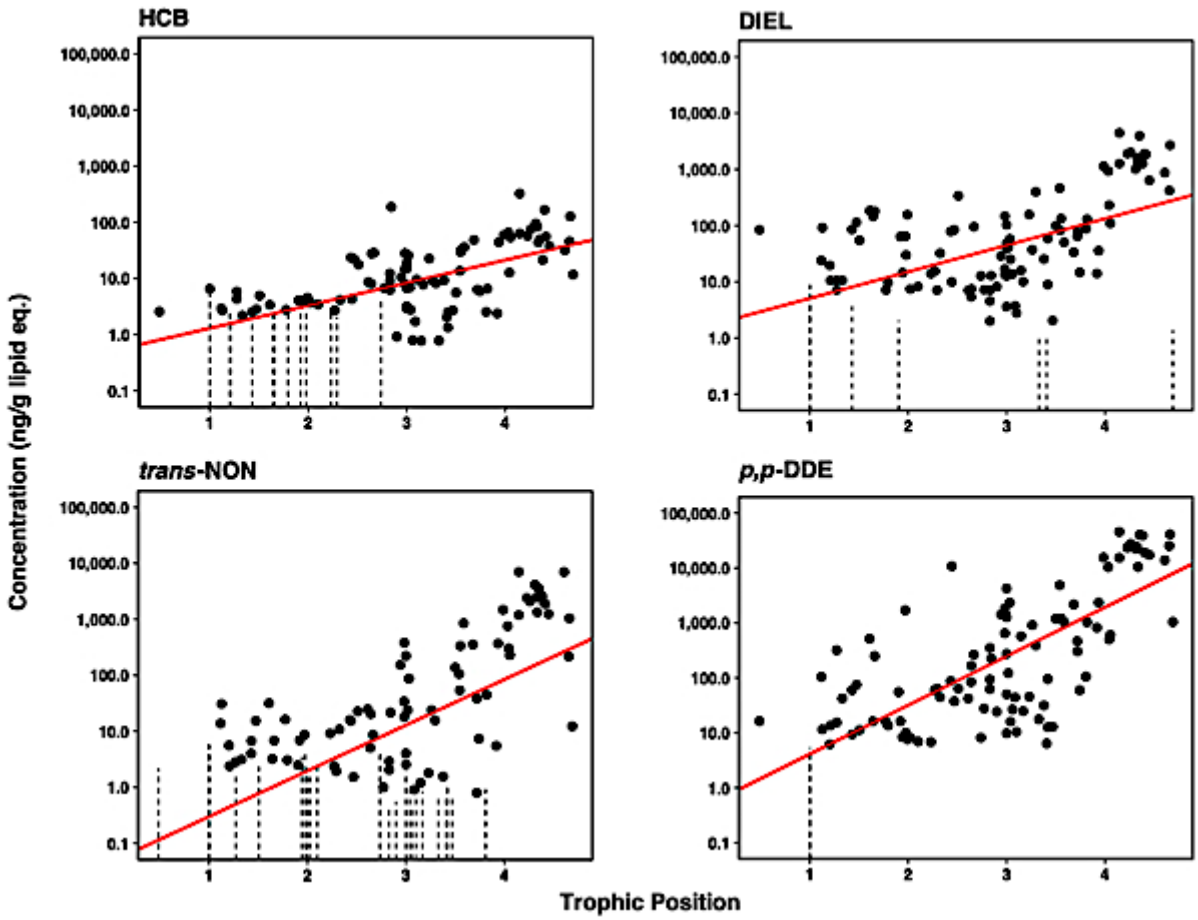

Figure 1.8. OCP concentrations in organisms within an urban, terrestrial food-web (ng/g lipid eq.) versus trophic position (TP) for HCB, trans-NON, DIEL and p,p-DDE, which were dominant types of OCPs in the food-web. Black circles represent detected observations and dashed lines represent non-detected observations below a sample specific, lipid normalized MDL. Red lines represent the log-linear regression of lipid equivalent concentration to TP over the entire food-web.

BDE-47 and -99 both had TMFs greater than 1 indicating biomagnification in the food-web (Table 1.7; Appendix 11). However, BDE-99 had a higher TMF at 7.32 (1.77 SE), which was comparable to many of the high TMFs for PCBs as well as for *p,p*-DDE and *trans*-NON (Table 1.7; Figure 1.9).

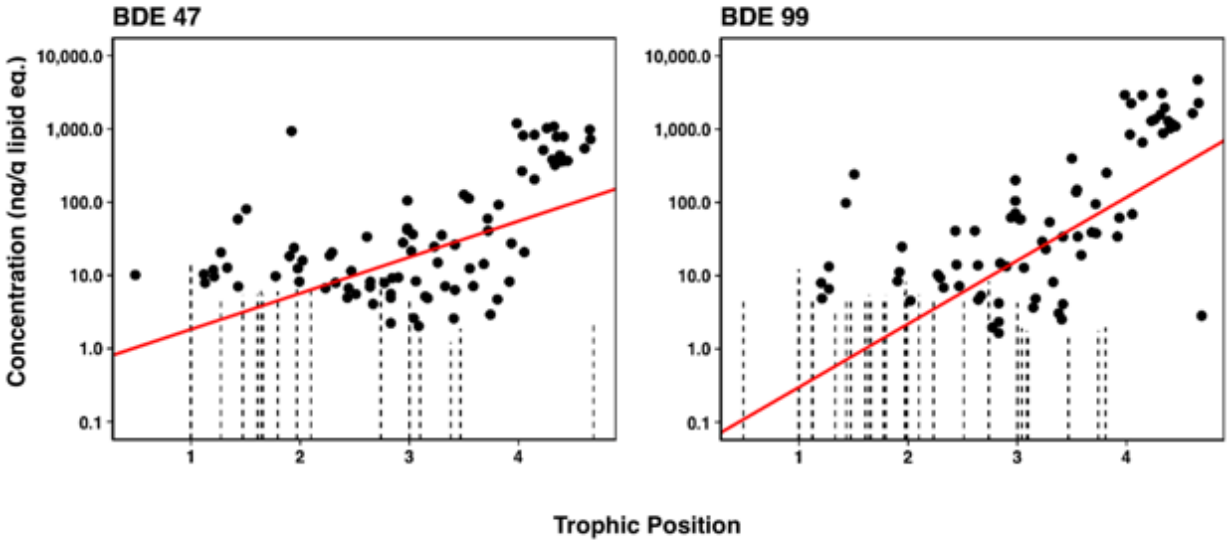

Figure 1.9. PBDE concentrations in organisms within an urban, terrestrial food-web (ng/g lipid eq.) versus trophic position (TP) for BDE 47 and 99, which were the dominant congeners in the food-web. Black circles represent detected observations and dashed lines represent non-detected observations below a sample specific, lipid normalized MDL. Red lines represent the log-linear regression of lipid equivalent concentration to TP over the entire food-web.

Legacy POPs typically have high log K_{OA} and log K_{OW} values (i.e. > 5) indicating that they will biomagnify in both terrestrial and aquatic environments (Table 1.9; *3*). Log K_{OA} values reported in the literature for PCBs ranged from 8.45 for PCB 28/31 to 10.95 for PCB 187 (Table 1.8). Log K_{OA} values reported in the literature for OCPs ranged from 7.11 for HCB to 10.75 for DDT (Table 1.8). Log K_{OA} values reported in the literature for BDE-47 and -99 were 9.90 and 10.70, respectively (Table 1.8). We saw a strong positive relationship between log K_{OA} and the TMF of each legacy POP ($F_{1,22.15}$ = 6.93; p = 0.0152) indicating that the TMF increased by 1.55 (0.59 SE) units for every unit increase in log K_{OA} (Figure 1.10). PCBs had the highest TMFs and log K_{OA} values and both variables generally increased with chlorination (Table 1.8; Figure 1.10).

Table 1.8. Estimated terrestrial and aquatic TMFs and Log K_{OA} and K_{OW} for each legacy POP. NA = Value was not available/provided in reference.

Analyte	TMF	LCL	UCL	Log K_{OA}	Log K_{OW}	Aquatic TMF	SE	LCL	UCL	Reference
PCB-28/31	1.20	0.85	1.69	8.45	5.67	2.90	NA	2.40	3.40	(27)
PCB-99	8.30	5.35	12.90	9.71	6.39	5.94	NA	2.68	13.14	(79)
PCB-101	3.88	2.80	5.39	8.60	6.40	9.80	NA	6.80	14.00	(27)

Analyte	TMF	LCL	UCL	Log K_{OA}	Log K_{OW}	Aquatic TMF	SE	LCL	UCL	Reference
PCB-105	4.53	3.33	6.15	10.00	6.65	4.01	NA	3.23	4.79	(*101*)
PCB-110	1.78	1.33	2.37	8.64	6.48	3.26	NA	2.82	3.70	(*101*)
PCB-118	8.63	5.64	13.20	8.50	6.74	4.10	NA	3.38	4.82	(*101*)
PCB-128	8.97	5.32	15.13	10.59	6.74	6.00	NA	4.23	7.77	(*101*)
PCB-138	6.35	4.55	8.86	9.20	6.80	10.00	NA	7.60	13.00	(*27*)
PCB-149	3.43	2.41	4.89	8.53	6.67	4.18	NA	3.44	4.92	(*101*)
PCB-153	7.40	5.33	10.28	9.80	6.90	11.10	NA	8.60	14.00	(*4, 27*)
PCB-156	11.34	6.41	20.05	9.83	7.18	6.24	NA	4.38	8.10	(*101*)
PCB-158	5.08	3.58	7.22	10.17	7.62	–	–	–	–	(*99*)
PCB-180	15.66	9.44	25.99	10.70	7.50	10.00	NA	7.20	14.00	(*4, 27*)
PCB-187	8.17	5.21	12.79	10.95	7.17	5.60	NA	4.28	6.92	(*101*)
QCB	2.31	1.74	3.06	8.17	5.03	–	–	–	–	(*99, 103*)
HCB	2.54	2.05	3.16	7.11	5.50	2.90	1.70	NA	NA	(*97*)
HEP	5.37	3.62	7.95	10.53	5.40	–	–	–	–	(*99, 103*)
OXY	2.95	2.20	3.97	10.53	6.02	9.63	2.76	NA	NA	(*98*)
trans-NON	6.55	3.93	10.91	10.00	6.35	3.60	1.50	NA	NA	(*97*)
DIEL	2.97	2.11	4.19	8.73	5.40	1.50	0.50	NA	NA	(*97*)
DDE	7.79	5.50	11.03	9.44	6.96	6.28	NA	3.07	12.80	(*4*)
DDT	2.66	1.70	4.15	10.75	6.91	4.90	NA	NA	NA	(*100*)
DDD	4.41	2.66	7.32	10.34	6.50	7.10	NA	NA	NA	(*100*)
BDE-47	3.11	2.19	4.43	9.90	7.30	1.60	NA	1.20	2.00	(*4, 27*)
BDE-99	7.32	4.55	11.76	10.70	7.60	0.76	NA	0.57	1.00	(*4, 27*)

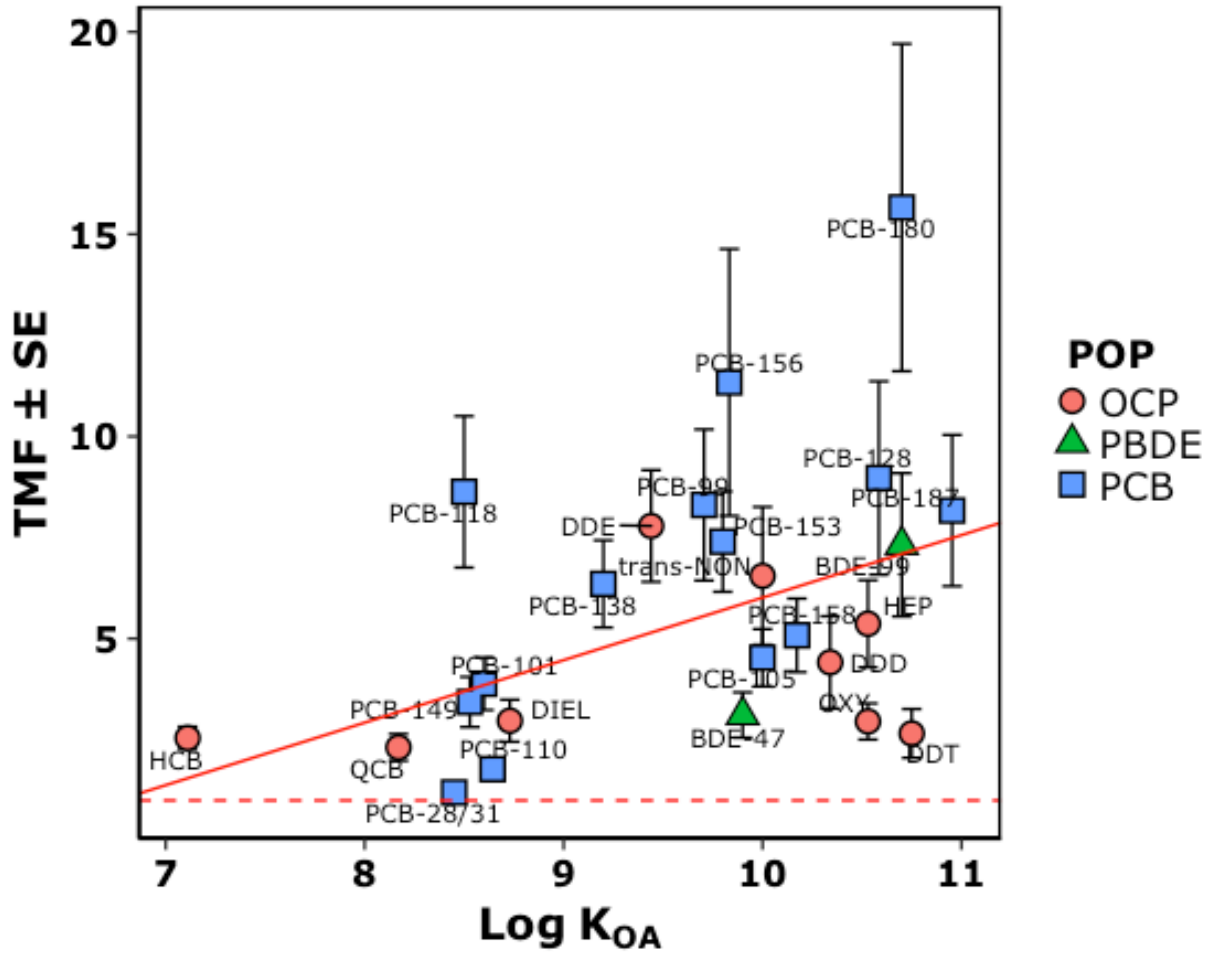

Figure 1.10. Relationship between estimated TMF and log K_{OA} of each legacy POP (TMF = 1.55(Log K_{OA}) – 9.44; $F_{1,22.15}$ = 6.93; p = 0.0152). Solid red line represents the linear relationship between TMF and log K_{OA} and the dashed red line represents a TMF of 1, indicating no biomagnification.

1.4. Discussion

1.4.1. Stable Isotopes and Trophic Position

The use of stable isotope analysis has significantly advanced the fields of ecotoxicology since diet has long been known to be a primary route of exposure to persistent contaminants and stable isotopes can be used to infer the diet and trophic position of organisms within a food-web (*5, 104, 105*). Consequently, many authors argue that every biomagnification study should include stable isotope analysis to assess TP of organisms in the food-web of interest (*5, 6, 106*). We estimated the TPs of biotic samples of berries, invertebrates, songbirds, and Cooper's Hawk eggs using a literature-based trophic position model and stable nitrogen isotope comparisons. The

literature-based and average $\delta^{15}N$-determined TPs were comparable indicating that both methods are reasonable indicators of trophic position. However, there were some notable discrepancies between the estimated TPs of some species groups, such as Sparrow spp. and Piegons/Doves, likely due to assumptions regarding local diet and the trophic positions of various invertebrate prey items (Table 1.2). Therefore, we encourage using a $\delta^{15}N$-determined TP because it corrects for the baseline variation in $\delta^{15}N$ that occurs between or within systems as a result of natural or anthropogenic inputs of N (*5*). The $\delta^{15}N$-determined TPs also have the additional advantage of incorporating enrichment factors unique to ecosystems, species, or animal groups to improve our estimates of TP and consequently TMFs (*5, 6*). We believe our approach improves on previous bioaccumulation studies that have simply used $\delta^{15}N$ rather than integer-based TPs to assess biomagnification (*5, 24, 28, 79*). However, knowledge of the TP of a given population is not necessarily representative of other populations for that species (*91, 107*). For example, Herring gulls in the Great Lakes have been shown to have differing trophic positions among colonies (*108*); emphasizing that it is imperative to include stable isotope analyses for every biomagnification study that considers TP.

The accuracy of $\delta^{15}N$-determined TPs will depend on the choice of baseline organisms and $\Delta^{15}N$ used in equations 3 and 4. There is considerable debate over the most appropriate organism to use as the baseline consumer (*104, 107*). Studies have typically used bivalves but other organisms such as gastropods, copepods, and other invertebrates (*104, 107*) have also been used. Nevertheless, Vander Zanden and Rasmussen (*107*) advocate the use of a long-lived baseline consumer rather than a primary producer because their longevity results in less seasonality in $\delta^{15}N$ signatures. We chose to sample earthworms and woodlice as our baseline consumers, which is likely appropriate since the most widespread earthworm species, *Lumbricus terrestris*, has an average lifespan of 6 years in the wild (*109*) and their low $\delta^{15}N$ signatures confirm that earthworms and woodlice are at the base of the food-web. Pooled estimates of $\Delta^{15}N$ commonly used in bioaccumulation studies range from 2.0 – 3.4% (*104*). We estimated $\Delta^{15}N$ from the average predator and prey $\delta^{15}N$ signatures to more accurately reflect $\Delta^{15}N$ across this specific food-web. Ideally, studies should rear each study species on a known diet then calculate $\Delta^{15}N$ directly for each species, but this is costly and labour-intensive (*104*).

1.4.2. Contaminant Concentrations

We detected 98% (83) of the 85 legacy POPs within at least one trophic level and 35% (30) were detected within most (>60%) of the species groups in the food-web; demonstrating that although they have been restricted in use for more than 40 years, these contaminants are still ubiquitous and persistent within the environment. Many of these contaminants were frequently detected at high concentrations within samples across the food-web, but particularly within our apex predator. Previous research has shown that the high levels of legacy POPs present in this urban population of Cooper's Hawks is potentially causing reproductive or other sub-lethal effects as nesting success appeared to decline as dieldrin concentrations increased in blood plasma of adults (*28*).

Exposure to many legacy POPs, particularly PCBs, PBDEs, and DDE, has been linked to reduced hatching and fledging success, delayed clutch initiation, reduced incubation consistency, and reduced eggshell thickness and strength in many raptor species (*59, 102, 110-114*). Minimum reproductive effects thresholds for ΣPCB, ΣPBDE, and DDE were estimated at 29 – 34 μg/g ww, 1 – 2 μg/g ww, and 7 – 10 μg/g ww, respectively, within the eggs of American Kestrels (*Falco sparverius*) and Eurasian Sparrowhawks (*Accipiter nisus*) (*102, 115*). However, lower threshold concentrations of ΣPCB were estimated at 2 – 4 μg/g ww within plasma of adult American Kestrels (*102*). In addition, the lowest dietary concentration of DDE that resulted in critical eggshell thinning and reduced egg production was estimated at 1 μg/g ww within the eggs of Peregrine Falcons (*Falco peregrinus*) (*115*). In secondary consumers, such as European Starlings, critical effects thresholds for ΣPCBs were estimated at 6 – 9 μg/g ww (*102*). However, ΣPCB, ΣPBDE, and DDE egg concentrations as low 92 ng/g ww, 218 ng/g ww, and 27 ng/g ww, respectively, have been associated with reduced fledging success, lower provisioning rates, and smaller eggs and chicks in European Starlings from Delta, BC (*41*).

Up till the late 1990s, lethal concentrations of chlordane compounds, such as oxychlordane and *trans*-nonachlor, and dieldrin were identified as the cause of death for numerous songbird species and the subsequent secondary poisoning of Cooper's Hawks (*112*). Lethal concentrations of oxychlordane for Cooper's Hawks, American Robins, European Starlings, and House Sparrows were estimated as low as 1.5 μg/g ww, 0.39 μg/g ww, 0.11 μg/g ww, and 0.58 μg/g ww, respectively (*112*). Whereas, lethal concentrations of *trans*-nonachlor for Cooper's Hawks, American Robins, European Starlings, and House Sparrows were comparable

or considerably higher than oxychlordane at 1.4 μg/g ww, 2.8 μg/g ww, 2.2 μg/g ww, and 2.4 μg/g ww, respectively (*116*). Critical threshold concentrations of dieldrin associated with population decline due to impacted hunting behaviour were estimated at 0.7 μg/g ww within Sparrowhawk eggs (*112*). However, one study suggested that plasma and brain concentrations as low as 0.1 μg/g ww were associated with reduced survival of juvenile Great Horned owls (Bubo virginianus; *117*).

These critical thresholds and lethal concentrations are not directly comparable to our sample concentrations; nevertheless, we can convert these concentrations to lipid equivalent concentrations based on average lipid equivalent values from our trophic groups (Table 1.9). In our apex predator, sampled concentrations for the major legacy POPs were all below the lipid adjusted thresholds; however, sampled concentrations of DDE were actually higher than the lowest lipid adjusted critical threshold (Table 1.9). In the dominant prey species of the Cooper's Hawk, sampled concentrations were also generally below the lipid adjusted thresholds. Conversely, sampled concentrations of DDE and ΣPCB within European Starlings were roughly 3x higher or comparable to the lowest lipid adjusted thresholds (Table 1.9), indicating that prey species of the Cooper's Hawk may be experiencing sub-lethal effects that contribute to potential effects observed in the predator.

Table 1.9. Threshold concentrations (μg/g ww) of legacy POPs lipid adjusted and compared to sampled concentrations (μg/g lipid eq.) within Cooper's Hawks and their dominant prey species.

Species	% Lipid Eq.	Concentration	ΣPCB	ΣPBDE	DDE	OXY	*trans*-NON	DIEL
Cooper's Hawk	6.49%	Threshold	29 – 34	1.0 – 2.0	7.0 – 10.0 (lowest = 1.0)	< 1.5	< 1.40	0.70
		Lipid Adjusted	447 – 524	15.4 – 30.8	107.9 – 154 (15.4)	23.0	21.6	10.8
		Sampled	13.7	5.75	23.9	1.30	2.50	1.70
European Starling	5.76%	Threshold	6.0 – 9.0 (lowest = 0.091)	0.218	0.0274	< 1.80	< 2.20	< 0.20
		Lipid Adjusted	104 – 156 (1.58)	3.78	0.476	31.3	38.2	3.5
		Sampled	1.20	1.35	1.67	0.087	0.120	0.190
American Robin	4.32%	Threshold	6.0 – 9.0	-	15.0 – 151	< 4.00	< 2.80	< 0.15
		Lipid Adjusted	139 – 208	-	347 – 3,495	92.6	64.8	3.50
		Sampled	0.60	0.23	2.21	0.082	0.084	0.08
Sparrows	6.33%	Threshold	6.0 – 9.0	-	-	< 2.80	< 2.40	< 0.19
		Lipid Adjusted	94.8 – 142	-	-	44.2	37.9	3.00
		Sampled	0.31	0.21	0.36	0.19	0.015	0.092

In comparison to our Cooper's Hawk egg samples, ΣPCB, ΣPBDE, and DDE concentrations reported within blood of adult Cooper's Hawks from Metro Vancouver were approximately 16 – 30% higher (Table 1.10). One caveat with this comparison is that total lipid content in blood plasma is not always determined by the traditional gravimetric method used for eggs and other tissues but instead by an alternative method using enzymatic assays (*118, 119*). However, studies have found that total lipid contents estimated by both methods were generally comparable (*118, 119*). Concentrations of legacy POPs in eggs are often directly indicative of female body burden as female herring gulls were reported with ratios of egg/liver concentrations equal to one (*112*), yet this 1:1 ratio appears to apply only to ΣPCB in this population of Cooper's Hawks (Table 1.10). Conversely, concentrations reported within blood of chicks for most of these contaminants were roughly 50% lower demonstrating growth dilution in the chicks (Table 1.10). These concentration differences suggest that we may want to consider incorporating adult blood or liver samples into our TMF evaluations in the future to better understand the bioaccumulative behaviour of legacy POPs during the life stages of the hawk.

Table 1.10. Concentrations of legacy POPs (μg/g lipid eq.) detected in Cooper's Hawks across Metro Vancouver. Blood plasma concentrations for chicks and adults obtained from (28) and lipid adjusted with an average lipid equivalent value of 0.58%. Liver concentrations (μg/g lipid) for adults obtained from (24).

Analyte	Eggs	Chicks	Adults	
			Blood	Liver
∑PCB	13.7	7.00	67.2	16.1
∑PBDE	5.80	1.70	16.0	18.7
DDE	23.9	8.90	142	47.2
***trans*-NON**	2.50	1.20	8.90	-
DIEL	1.70	0.40	4.60	3.96

1.4.3. Trophic Magnification

Biomagnification experts recommend using TMF estimation of PCB-153 as a positive control to evaluate the efficiency of the study design since TMFs for PCB-153 are consistently > 1 in almost all food-webs that have been studied (*5*). Therefore, if PCB-153 is present at detectable levels across the food-web but does not result in a statistically significant TMF, it indicates that there may be a problem with the study design or statistical method used. As most legacy POPs have high log K_{OA} and log K_{OW} values, we expect them to biomagnify in both aquatic

and terrestrial systems (*1, 3, 7*). In our terrestrial bioaccumulation study, we confirmed that most of the legacy POPs biomagnified up the food-web as we expected, including PCB-153 indicating that our study design and statistical method were effective for evaluating the biomagnification potential of other chemicals. However, one legacy POP, PCB-28/31, did not biomagnify in this terrestrial food-web even though it has high log K_{OA} and K_{OW} values. This was a bit surprising since PCB-28/31 concentrations within the Cooper's Hawk were relatively high at 125.00 (113.95 SE) ng/g lipid. Yet, one reason that we did not observe biomagnification of PCB-28/31 could be due to the fact that it co-elutes between two congeners and thus masks individual congener bioaccumulation behaviour. Also, as PCB-28/31 is a lower chlorinated compound, it was likely readily metabolized and biotransformed by the avian prey species.

We were unable to examine trophic magnification of several legacy POPs; such as β- and γ-HCH, *cis*-and *trans*-chlordane, or Mirex, due to low detection rates. However, some aquatic and terrestrial biomagnification studies have shown that β-HCH and Mirex biomagnify in both aquatic and terrestrial systems (*3, 9, 103*). Our sample data for Mirex also confirms this bioaccumulative behaviour since average concentrations generally increased with the estimated trophic position of the species groups (Figure 1.8). Yet, our sample data for β-HCH indicates that it was readily eliminated in our terrestrial food-web since there was an overall lack of detected concentrations across the food-web (Figure 1.8). This is surprising since β-HCH has high log K_{OA} (8.9) and low log K_{OW} (3.8) values so is expected to biomagnify in terrestrial air-breathing organisms (*3, 9*). However, it likely does not biomagnify in our terrestrial organisms because birds typically have faster respiratory elimination and metabolic rates than mammals (*120*).

TMFs reported in aquatic food-webs for most of the legacy POPs were generally greater than 1 demonstrating biomagnification and were comparable to the TMFs we estimated in our terrestrial food-web (Table 1.8). Surprisingly, the aquatic TMF reported for BDE-99 by Kelly, Ikonomou, Blair and Gobas (*27*) was considerably lower at 0.76 (0.10 SE) in contrast to our terrestrial TMF at 7.32 (1.77 SE) revealing significant biomagnification differences between aquatic and terrestrial environments (Table 1.8). This discrepancy is likely due to the mixed composition of both air and water respiring organisms in the aquatic food-web versus only air respiring in the terrestrial, as air respiring organisms often exhibit higher biomagnification levels than water respiring because of their greater ability to absorb and digest their diet (*3*). It may also be due to increased biotransformation and debromination of higher brominated congeners like BDE 99 resulting in elevated concentrations of lower molecular weight PBDEs in the marine

mammal organisms (*27*). Additionally, our food-web is dominated by bird species and many groups of birds are often known to have higher biomagnification than mammals (*121*) due to lower mono-oxygenase enzyme activity, which limits their elimination of lipophilic contaminants (*26*). For example, TMFs reported for BDE-47 and -99 in an arctic, terrestrial food-web indicated that BDE-47 diluted and BDE-99 did not biomagnify in a food-web comprised of large mammals (*18*). Furthermore, these contaminants do not occur in isolation within organisms but could, in fact, interact by inhibiting the enzymatic biotransformation of other chemicals (*122*) and consequently increase their bioaccumulative behaviour. Another reason could simply be the close proximity of our food-web to an urban environment with a large human population since bird populations living in close proximity to urban environments typically have higher concentrations of contaminants than birds living in remote or rural places (*20, 21*). For instance, European starling eggs collected from nest boxes adjacent to Canadian landfill sites had concentrations of flame retardants close to 12 times higher than compared to eggs collected from nest boxes in rural areas (*77*). In addition, the arctic study by Kelly, Ikonomou, Blair and Gobas (*27*) had a much larger geographical range compared to our urban one, which likely increased the spatial concentration gradient of BDE-99 and thus may have systematically biased the TMF to be < 1 (*5, 123*).

1.5. Conclusions

Despite cessation of usage for over 40 years for many of these legacy POPs, such as OCPs and PCBs, they still remain prevalent and continue to biomagnify in aquatic and terrestrial systems. In comparison, PBDEs are considered an emergent POP as they were not recognized as global contaminants until 1987 and have just recently been discontinued in the last decade (*14*). Nonetheless, PBDEs have also become widespread causing a general repeat of the global contamination crisis created by the legacy POPs. This biomagnification of legacy and emergent, lipophilic POPs highlights that these contaminants could continue to have biological or sub-lethal effects on apex predators if bioaccumulation levels exceed toxicity thresholds. It also appears that avian species in our urban, terrestrial food-web had higher biomagnification of PBDEs than mammalian species in an arctic, terrestrial environment (*18*) emphasizing a need for more biomagnification studies with a diversity of terrestrial species. However, it would appear that biomagnification in this urban population of Cooper's Hawk may be sufficiently low enough to not cause many adverse reproductive effects. Yet, as more commercial chemicals are introduced into the global market, there is a substantial need to understand how they will behave in both aquatic and terrestrial environments. Moving forward we plan to use our empirical data to develop a

terrestrial bioaccumulation model that can be used by regulatory agencies to assess the bioaccumulative behaviour of new commercial chemicals.

2. Trophic Magnification of Proteinophilic Emergent Contaminants within a Terrestrial Food-Web of an Avian Apex Predator

2.1. Introduction

Regulatory agencies in Canada, the United States, and Europe commonly use two criteria to evaluate the biomagnification potential of chemicals. The first being the octanol-water partition coefficient (K_{OW}), which estimates the level of lipophilicity/hydrophobicity of a chemical (*2-4*). So, the more lipophilic the compound, the higher the likelihood it will bioaccumulate in organisms. The second criterion is based on empirical bioaccumulation data from fish and aquatic studies, specifically the bioconcentration factor (BCF) or bioaccumulation factor (BAF), which express the chemical concentration in an organism compared to its aquatic, environmental medium (*2, 4*). However, neither of these criteria are appropriate for understanding the bioaccumulation potential of perfluorinated compounds (PFCs) within terrestrial systems because K_{OW} cannot be reliably measured for ionic surfactants like PFCs (*2, 4*), and BCF or BAF metrics apply only to aquatic or water-respiring organisms. Many PFCs, such as perfluorinated carboxylic acids (PFCAs) and perfluorinated sulfonic acids (PFSAs), are frequently detected around the world, including remote oceanic areas, in an array of wildlife species much like legacy and other emergent persistent organic pollutants (POPs; *2, 4, 124*). We know that many chemicals, such as legacy POPs, exhibit different biomagnification behaviour in aquatic systems compared to terrestrial ones due to differences in hydrophobicity and other physicochemical properties (*3*). Therefore, regulatory agencies need alternative criteria and additional bioaccumulation data for terrestrial systems in order to better understand the bioaccumulative behaviour of PFCs.

PFCs are man-made chemicals commonly used in the production of commercial stain repellents, surface coatings, firefighting foams, insecticides, and cleaners (*2, 4*). PFCs are considered emergent POPs as they were not widely documented in environmental samples until the early 2000s even though some PFCs have been manufactured worldwide since the 1940s (*15*). At present, perfluorooctane sulfonic acid (PFOS) is the only PFC listed under the United Nations Stockholm Convention on POPs (www.pops.int). However, perfluorooctanoic acid (PFOA) and perfluorohexane sulfonic acid (PFHxS) are candidate POPs proposed for listing under the Stockholm Convention (www.pops.int). The production and use of PFOS and PFOA

has been slowly phased out in the United States since 2000, in Canada since 2006, and in the European Union since 2008 (*15, 16*).

Bioaccumulation of PFCs may be more accurately represented by protein-water (K_{PW}) and protein-air (K_{PA}) partition coefficients, as several studies have shown PFCs to be proteinophilic (*2, 4*) rather than lipophilic like legacy POPs. Alternative biomagnification criteria appropriate to use for terrestrial systems with air-respiring organisms include biomagnification or trophic magnification factors (BMF or TMF, respectively), as they account for dietary exposure and can be applied to both water- and air-respiring organisms unlike BCFs or BAFs (*5, 6*). However, TMFs should be protein normalized rather than lipid normalized to account for differences in protein content between species and organisms since lipids are not the main sorbing matrix of PFCs within biota (*2, 4*). A few studies have indicated that some PFCs have low bioaccumulation potential, yet several other studies have shown that many PFCs continue to be detected in apex predators, such as polar bears, dolphins, wolves, and birds of prey (*4, 11, 125-130*). Consequently, the bioaccumulative behaviour of PFCs has been well documented in many marine and freshwater aquatic food-webs with apex predators (*2, 4, 125, 131*). However, very few studies have examined the bioaccumulative potential of PFCs within terrestrial food-webs (*132*) and those that have been completed were located in remote arctic regions and only assessed terrestrial mammal species (*11*). The scarcity of terrestrial studies highlights a need to examine PFC bioaccumulation within more species from terrestrial systems, particularly avian species as they often exhibit higher biomagnification of chemicals than mammals (*121*).

In this study, we designed a terrestrial field study to assess the extent of biomagnification of PFCs within a terrestrial food web that included a primary producer, detritivores, primary and secondary consumers, and an apex predator, the Cooper's Hawk (*Accipiter cooperii*). We chose an avian apex predator because terrestrial raptors may experience greater biomagnification of PFCs than mammalian predators due to their greater energy requirements for flying (*121*); faster, more efficient digestive systems; and lower biotransformation capacity to metabolize POPs. Moreover, marine studies have shown that many seabird species had higher BMF values reported for legacy POPs than compared to ringed seals (*Pusa hispida*; *121*). The trophic positions of the organisms in this food-web were previously estimated in Chapter 1 using a literature-based trophic position model and stable nitrogen isotope comparisons. Here, we analysed approximately 50 biota samples for concentrations of 18 PFCs that are listed on the Government of Canada's current Chemicals Management Plan (CMP; Table 2.1; Appendix 12). We then estimated the TMFs for PFCs that were detected at appreciable levels in all the biota samples (i.e. had a

detection frequency > 50% across all samples) and compared these terrestrial TMFs to those observed in aquatic systems.

Table 2.1. List of PFCs on the CMP analysed in samples from an urban terrestrial food-web in Metro Vancouver.

Type of PFC	Analyte	Acronym	No. Fluorinated Carbons
Perfluorinated Carboxylic Acids (PFCAs)	Perfluoro-n-butanoic acid	PFBA	4
	Perfluoro-n-pentanoic acid	PFPeA	4
	Perfluoro-n-hexanoic acid	PFHxA	5
	Perfluoro-n-heptanoic acid	PFHpA	6
	Perfluoro-n-octanoic acid	PFOA	7
	Perfluoro-n-nonanoic acid	PFNA	8
	Perfluoro-n-decanoic acid	PFDA	9
	Perfluoro-n-undecanoic acid	PFUdA	10
	Perfluoro-n-dodecanoic acid	PFDoA	11
	Perfluoro-n-tridecanoic acid	PFTrDA	12
	Perfluoro-n-tetradecanoic acid	PFTeDA	13
	Perfluoro-n-hexadecanoic acid	PFHxDA	15
	Perfluoro-n-octadecanoic acid	PFODA	17
Perfluorinated Sulfonic Acids (PFSAs)	Perfluoro-1-butanesulfonic acid	PFBS	4
	Perfluoro-1-hexanesulfonic acid	PFHxS	6
	Perfluoro-4-ethylcyclohexane sulfonic acid	PFEtCHxS	8
	Perfluoro-1-octanesulfonic acid	PFOS	8
	Perfluoro-1-decanesulfonic acid	PFDS	10

2.2. Methods

2.2.1. Field Sampling Methods

Study Area

We assessed the trophic dynamics and biomagnification of PFCs within a terrestrial food web in urbanized regions of Metro Vancouver, BC. Our study area included 6 sampling regions within 5 municipalities: North Vancouver (the District of North Vancouver), Vancouver-West (City of Vancouver), Vancouver-South (City of Vancouver and City of Burnaby), North Burnaby-East Vancouver (City of Burnaby and City of Vancouver), Richmond (City of Richmond), and Ladner (City of Delta) (Figure 2.1).

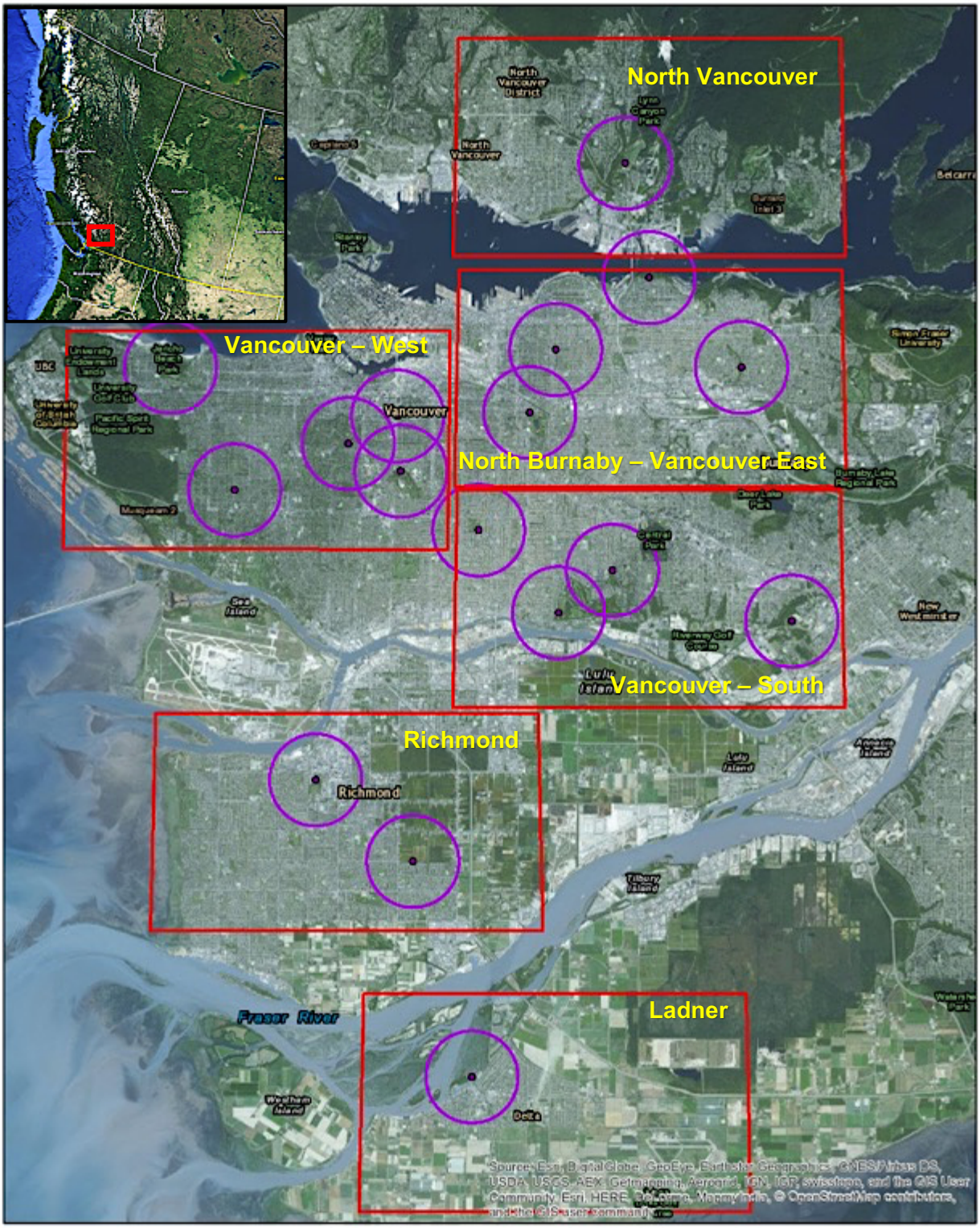

Figure 2.1. Study area separated into six sampling regions (red squares) with Cooper's Hawk nests (purple dots) and nesting territories (purple circles) in Metro Vancouver, British Columbia, 2016

Food-Web Characterization

We designed a terrestrial food-web that included a primary producer, detritivores, primary and secondary consumers, and an apex predator. Our representative species included the Cooper's Hawk, a generalist apex predator; the hawks' main local prey species American Robins (*Turdus migratorius*), European Starlings (*Sturnus vulgaris*), and House Sparrows (*Passer domesticus*; *38*) plus some other occasional avian prey species. The lower levels of the food-web were represented by terrestrial invertebrates (such as beetles, earthworms, and sowbugs) commonly eaten by birds and seasonal wild Himalayan blackberries (*Rubus armeniacus*) also frequently eaten by local birds (Table 2.2; *39, 40-44*). We characterized the trophic positions for 11 species groups within this food-web using a literature-based trophic position model and stable nitrogen isotope comparisons. Details of these two methods are provided in Chapter 1. The two methods provided comparable trophic position estimates, but we used the trophic positions based on the isotope comparisons for all subsequent TMF analyses.

Table 2.2 .Species included in terrestrial food-wed within Metro Vancouver, BC. Sampling regions and biota samples analysed for PFC analyses were randomly chosen.

Species	Scientific Name	Region/City	*N*
Cooper's Hawk	*Accipiter cooperii*	All	17
American Robin	*Turdus migratorius*	Ladner North Vancouver Vancouver - West	3
European Starling	*Sturnus vulgaris*	Ladner North Vancouver Vancouver - South	3
Northern Flicker	*Colaptes auratus*	Ladner North Burnaby - Vancouver East	2
Pigeon/Dove: Rock Pigeon, Eurasian Collared Dove	*Columba livia, Streptopelia decaocto*	Richmond Vancouver - West	2
Sparrow: House Sparrow, Dark-eyed Junco, White-crowned Sparrow, Fox Sparrow, Song Sparrow, Golden-crowned Sparrow, Spotted Towhee	*Passer domesticus, Junco hyemalis, Zonotrichia leucophrys, Passerella iliaca, Melospiza melodia, Zonotrichia atricapilla, Pipilo maculatus*	Ladner Richmond Vancouver - South	3
Thrush: Varied Thrush, Swainson's Thrush, Hermit Thrush	*Ixoreus naevius, Catharus ustulatus, Catharus guttatus*	North Burnaby - Vancouver East Ladner Richmond	3
		Ladner	3

Species	Scientific Name	Region/City	*N*
Carabidae: Large Beetles and Small Beetles	*Pterostichus melanrius, Carabus nemoralis, Carabus granulatus, Pterostichus sp., Harpalus affinis, Calathus fuscipes, Anisodactylus binotatus, Agonum mülleri, Philonthus politus, Anatrichis minuta, Amara sp., Staphynlidae, Harpalitae*	North Vancouver Vancouver - South	
Earthworms	*Lumbricidae*	All	6
Oniscidea: Sowbugs and Pillbugs	*Oniscus asellus, Porcellio scaber, Armadillidium vulgare*	Ladner North Vancouver Vancouver - West	3
Himalayan blackberry	*Rubus armeniacus*	North Vancouver Vancouver - West Vancouver - South	3

Biotic Sample Collection

Cooper's Hawk

We obtained tissue samples of Cooper's Hawk by collecting eggs from active nests (Table 2.2). During the pre-incubation period, we visited potential nest locations and used call play-back methods to determine nest occupancy and breeding activity (*63, 64*). We referred to historical nest records from Bird Studies Canada, eBird Canada, and previous research studies to locate active nest sites (*28, 36*). We regularly monitored active nests to determine when eggs were present then accessed each nest using the services of a professional tree climber or bucket truck to collect one egg.

We processed each egg by recording its size and weight, scoring the circumference to open the shell, depositing egg contents into chemically rinsed glassware, extracting separate subsamples of yolk and albumen, and storing at – 20°C. Egg collection was approved by the University Animal Care Committee of Simon Fraser University and authorized by the Ministry of Forests, Lands and Natural Resource Operations (Surrey, BC) under permit SU16-225842.

Avian Prey

We obtained samples of American Robins, European Starlings, and House Sparrows by collecting an egg or nestling from active nests located within a 2 km radius of each active hawk nest (Figure 2.1). We supplemented our targeted prey species collection efforts with samples of 12 other known Cooper's Hawk prey species, including Varied Thrush (*Ixoreus naevius*), Hermit Thrush (*Catharus guttatus*), Swainson's Thrush (*Catharus ustulatus*), Spotted Towhee (*Pipilo maculatus*), Song Sparrow (*Melospiza melodia*), White-crowned Sparrow (*Zonotrichia leucophrys*), Golden-crowned Sparrow (*Zonotrichia atricapilla*), Dark-eyed Junco (*Junco hyemalis*), Fox Sparrow (*Passerella iliaca*), Rock Pigeon (*Columba livia*), Eurasian-collared Dove (*Streptopelia decaocto*), and Northern Flicker (*Colaptes auratus*) that had been euthanized by a wildlife rehabilitation facility, Wildlife Rescue Association (Table 2.2). We pooled individual birds into representative family groups (i.e. Emberizidae or Sparrow spp., Turdidae or Thrush spp., and Columbidae or Pigeons/Doves) when we had a limited number of samples for individual species.

Egg collection and animal capturing, handling, and euthanasia were approved by the Animal Care Committee of Simon Fraser University and authorized by the Canadian Wildlife Service – Environment Canada under permit BC-16-0010.

Invertebrates

All the collection methods for invertebrates mentioned in Chapter 1 apply to the invertebrate samples we analysed for PFCs. Briefly, we installed 30 plastic pitfall traps within edge habitat across our study area. We monitored traps weekly from August to September 2016, placed captured invertebrates into chemically rinsed clear jars, and froze them at –20°C. We sorted and pooled invertebrate samples into groups which included large beetle species, small beetle species, isopods (Oniscidea), and Insecta species (e.g. Julida, Chilopoda, Formicidae, and Arachnida; Table 2.2). We did not include the Insecta species for PFC analysis because there was not enough biomass available. We pooled the large and small beetle species together in order to have enough biomass to analyze for PFCs (Table 2.2).

Near the 30 pitfall stations, we also collected earthworms. Again, we used the same collection methods described in Chapter 1 for the earthworm sampling. We focused our sampling efforts in areas with moist lawn vegetation. We cleared a 60 cm^2 area of surface debris and vegetation then applied allyl isothiocyante (AITC; Fisher Scientific, Ottawa, ON, CAN; 94%; density 1.0175) as a chemical expellant to bring the earthworms to the surface. We made a 5 g/L

stock solution of AITC with isopropanol (Fisher Scientific, Ottawa, ON, CAN; 100%; density 0.785) then mixed 100 mg/L of the stock solution with 10 L of water to sprinkle over the area. We immersed earthworms that came to the surface in clean water then placed them on moist paper towel in aluminum trays for at least 24 hours to clear their gut contents. Purged earthworms were placed into chemically rinsed jars and frozen at –20°C. We were unable to identify earthworms to genus or species due to the lack of visible setae post-mortem, so we grouped all earthworms under the family *Lumbricidae*. In order to have enough biomass to analyze for PFCs, we pooled the earthworm samples collected within each sampling region together for a total of 6 pools (Table 2.2).

Berries

We collected samples of Himalayan blackberry (*Rubus armeniacus*) from each of the 30 subsampling stations used to sample invertebrates. We collected approximately 150 to 250 mL of berries from the nearest shrubs at each subsampling station and created six sampling pools.

2.2.2. Analytical Methods for Determining Contaminant Concentration

Biotic Sample Preparation and Analysis

We collected all samples within the food-web from May to September in 2016 and shipped all of the frozen samples on dry ice to the National Wildlife Research Centre (NWRC) in Ottawa, ON. We processed and homogenized all biotic samples in the Tissue Preparation Lab at the NWRC by cutting tissues into small pieces and homogenizing them with a ball-mill (Retsch™ MM400 Mixer Mill, Fisher Scientific). In order to mitigate the costs associated with PFC analysis, we randomly selected pooled samples from two or three sampling regions within nine of the species groups (Table 2.2). The animal samples remained at NWRC for chemical analysis and the berry samples were transferred to AGAT Laboratories in Montreal, QC for analysis.

National Wildlife Research Centre

Sample extraction methods used at NWRC have been described in detail in comparative studies on PFCs within eggs of seabirds (*133*). Briefly, approximately 0.2 – 1.0 g of sample homogenate was spiked with 100 µL of mixed internal standard solutions (100 ng/mL) for Perfluoroalkyl Substances (PFASs), Perfluorinated Carboxylic Acids (PFCAs), and Perfluorinated Sulfonic Acids (PFSAs) analysis, and extracted with 3 mL of formic acid acetonitrile/water (0.2%)

solution. After extraction, the extract was diluted with water and the target compounds were enriched and fractioned on a Waters Oasis weak anion exchange (WAX) solid phase extraction (SPE) cartridge. The first fraction contained fluorotelomer alcohols (FTOHs) and perfluorosulfonamides (FOSAs); the second fraction contained PFSAs, PFCAs, and fluorotelomer unsaturated acids (FTUCAs). The separation of the target compounds in each fraction was determined on a Waters Acquity Ultra Performance Liquid Chromatography (UPLC) system paired with a Xevo TQ-S mass spectrometer (MS/MS) system (Waters, ON, Canada). The UPLC-MS/MS was equipped with a Cortecs^{TM} UPLC® C_{18} analytical column (1.6 µm particle size, 2.1 × 50 mm; Waters, ON, Canada) and an ACQUITY UPLC™ guard column (0.2 µm particle size, 2.1 mm x 50 mm; Waters, ON, Canada) and injected with 5 µL of extracted sample. The column temperature was held at 50°C while the sample was maintained at 20 °C. The PFCs were detected by negative electrospray ionization (ESI^-) in multiple reaction monitoring scanning mode (MRM). The relative response of a given analyte to its mass-labelled internal standard is used to calculate the concentration of the analyte, which corrects for any experimental losses of both the analyte and its internal standard.

AGAT Laboratories

Sample extraction methods used at AGAT Laboratories followed a modified EPA 537, ISO 25101 standard but were similar to methods used at NWRC. Approximately 0.5 g to 5.0 g of sample homogenate was mixed with 0.1% ammonium hydroxide in methanol, spiked with internal standard solutions, concentrated to dryness with nitrogen in a heated water bath, then extracted with formic acid. The extract was then diluted with water and the target compounds were cleaned-up and fractioned on a Waters Oasis WAX SPE cartridge. The target compounds were separated and determined on a Waters Acquity UPLC system paired with Xevo a TQ-S MS/MS system (Milfor, Massachusetts, USA) equipped with an Agilent Poroshell 120 EC-C_{18} analytical column (2.7 µm particle size, 2.1 × 100 mm; Agilent Technologies, CA, USA) and an Agilent Eclipse Plus C_8 guard column (3.5 µm particle size, 4.6 × 50 mm; Agilent Technologies, CA, USA). The PFC analytes were identified by negative ESI^- and by comparing the acquired mass spectra and retention times to reference spectra and retention times for calibration standards acquired under identical UPLC/MS/MS conditions.

Standards and Chemicals

Non-labelled standards for the PFSAs [C_4 (PFBS), C_6 (PFHxS), C_8 (PFOS), C_{10} (PFDS)], PFCAs (C_6 − C_{15} chain lengths: PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA, PFTrA, PFTeA, and PFPA, respectively), 6:2, 8:2, and 10:2 FTUCAs, 6:2, 8:2, and 10:2 FTOHs, and two FOSAs [perfluorooctanesulfonamide (PFOSA), methylated perfluorooctanesulfonamide (N-Me-FOSA)] as well as all internal ^{13}C or ^{18}O-enriched standards were obtained from Wellington Laboratories (Guelph, ON, Canada). Recoveries of the PFCAs and PFSAs internal standards averaged from 59 – 92%. All solvents used were HPLC or Optima grade and purchased from Fisher Scientific (Ottawa, ON).

Quality Control and Assurance

At NWRC, a pool of Double-Crested Cormorant (*Phalacrocorax auritus*) egg homogenate (DCCO 03L86S01, Environment Canada, National Wildlife Specimen Bank) was used as a Certified Standard Reference Material when analyzing each batch of 9 or 10 samples to evaluate the analytical accuracy and precision. At both labs, a sample blank (0.5 mL deionized water) containing all the components except the tissue (animal or plant) is run with the samples to assess contamination by target compounds in all the processes (i.e. extraction, clean-up, concentration, and determination by UPLC/MS/MS). Duplicate extraction of three samples was done to assess the precision of the extraction protocol. A solvent blank (methanol) is run by UPLC/MS/MS before and after each batch of 12 samples to monitor carry-over and contamination from the UPLC system.

The Method Detection Limits (MDLs) and Method Quantification Limits (MQLs) of each PFC congener were pre-determined by repetitive sample analysis during the initial method validation procedure and are reported in Appendix 12. PFC concentrations reported are corrected for background contamination by subtracting their respective method blank concentration values.

Protein Equivalent Concentrations

We estimated the fraction of protein (*P*) in all biota samples by multiplying the percent of nitrogen measured during elemental analysis (g of N/g of wet tissue; refer to section 2.3 Stable Isotope Analysis) with a nitrogen:protein conversion factor (Appendix 3; g of protein/g of wet tissue (*80-82*)). Lipid contents were measured in all biota samples using a gravimetric method and are reported in Appendix 14. Approximately 1 mL (or 10%) of the extracted sample was transferred

into a pre-weighed aluminum dish, allowed to air dry in a fume hood for 30 min, and then re-weighed to calculate the lipid content on a wet weight basis. We estimated the fraction of non-lipid organic carbon (*OC*) by subtracting the fraction of lipid from the percent of organic carbon measured during elemental analysis (i.e. g of C/g of wet tissue).

We expressed the observed wet weight concentrations in terms of protein equivalent concentrations ($C_{protein\ eq}$; ng/g of protein equivalent) to remove the effect of differences in protein contents or other sorbing matrices between organisms. However, as some samples, such as berries, had very low protein contents and high organic carbon contents, we also included non-lipid organic matter as an important matrix for chemical accumulation in all samples (*3, 4*). We incorporated protein and non-lipid organic carbon contents into the lipid equivalent normalization for all biota on a sample specific basis similar to (*4*) and according to

$$C_{protein\ eq.} = \frac{C_{wet}}{[P_{wet} + OC_{wet}]} \quad (1)$$

in which *P* is the sample-specific protein fraction and *OC* is the sample-specific fraction of non-lipid organic carbon. We assume that non-lipid organic carbon behaves similar to protein and exhibits a similar sorptive capacity in perfluorinated compounds. Even though there is considerable uncertainty with this protein normalization method, any errors in the normalization should affect all the PFCs similarly if we assume that protein normalization applies to all these contaminants in a similar manner.

2.2.3. Statistical Analysis

Contaminant Concentrations with Censored Data

We calculated average concentrations of PFCs detected within each species, using the Nondetects and Data Analysis for Environmental Data (NADA) package (*94*) in the R program (*93*) as recommended for left censored data (*95*). If a concentration was censored (i.e. below the method detection limit) within a sample, we used the chemical's MDL in equation 1 to determine a sample specific, protein equivalent detection limit (DL). We calculated the mean concentration, standard deviation, and standard error of each PFC detected within each trophic level using a Kaplan-Meier (KM) statistical model (cenfit) in the NADA package. The PFC means were then used in another KM model to compute an overall mean for PFCAs and PFSAs within each trophic

level. The overall mean PFCAs and PFSAs values were then multiplied by the number of respective PFCs to compute a ∑PFCA and ∑PFSA as recommended in (*96*). If trophic levels had some or all PFCs with sample concentrations below detection limits, we averaged all the sample-specific DLs for each PFC; then we averaged all the mean DLs and multiplied by the number of PFCs to compute the censored ∑.

Trophic Magnification Factor

We previously described the trophic positions of the organisms in the food-web in Chapter 1 and used the same isotopic trophic positions to estimate trophic magnification of PFCs. However, there were some exceptions since we pooled the large beetle and small beetle species from the same sampling regions and pooled the earthworms into 6 pools. Therefore, we averaged the isotopic trophic positions determined for each sample to estimate a pool-specific trophic position. These averaged isotopic trophic positions were then used in the regressions to estimate trophic magnification.

We determined trophic magnification factors (TMFs) for contaminants that were detected in more than 50% of all samples by using a logistic regression between the natural logarithm of a contaminant protein equivalent concentration and the trophic position of each sample. We used a censored regression function (cenreg) in the NADA package, which uses maximum likelihood estimation and an assumed log-normal distribution, to estimate the slope coefficient that had the highest likelihood of producing the observed values for the detected observations and the observed proportion of data that was below each detection limit (*95*). The TMF is then computed based on the antilog of the slope *m* (i.e. TMF = e^m). A TMF greater than 1 indicates that the contaminant is biomagnifying in the food-web; whereas, less than 1 indicates trophic dilution. The likelihood-*r* correlation coefficient (Loglik-r = square root of likelihood r^2) expresses the strength of the relationship between PFC protein equivalent concentrations and isotopic trophic positions as measured by maximum likelihood estimation (*95*). We determined TMF variability as the antilog of the lower and upper 95% confidence limits for the slope and standard error associated with the TMF was equal to the variability associated with the slope multiplied by the TMF (*5*). Statistical significance of *p*-values for slope estimates were assessed at $\alpha = 0.05$.

The variation in trophic magnification observed between many chemicals can often be explained by differences in their chemical properties, such as octanol-water partition coefficient (K_{OW}), octanol-air partition coefficient (K_{OA}), protein-water partition coefficient (K_{PW}), and protein-

air partition coefficient (K_{PA}). To better understand the variation in PFC trophic magnification observed, we examined the relationships between our estimated TMFs and log K_{OA} and log K_{PA} values using quadratic regression models. We chose quadratic models as they provided better fits than linear models (as per Adjusted R^2 values). We compiled partition coefficient values of each PFC from (*4*). We focused our quadratic regression models on log K_{OA} and log K_{PA} values as they are more applicable to air-respiring organisms.

2.3. Results

2.3.1. Isotopic Trophic Position

We presented the results for dietary and isotopic trophic positions in Chapter 1. We used all the previously determined isotopic trophic position results from Chapter 1 to estimate our PFC TMFs. However, average isotopic trophic positions for the pooled Carabidae species (i.e. Large and Small Beetles) and pooled earthworms were used to estimate TMFs. Average isotopic trophic positions ranged from 1.85 (0.13 SE) for the earthworms to 4.27 (0.05 SE) for the Cooper's Hawks (Table 2.3). The new isotopic trophic positions were generally comparable to, or slightly higher than, the dietary trophic positions and previously reported isotopic trophic positions.

Table 2.3. Average isotopic trophic positions determined by stable isotope analysis and compared to dietary trophic positions

Species	Dietary Trophic Position	*n*	Mean	SD	SE
Cooper's Hawk	4.03	17	4.27	0.19	0.05
Northern Flicker	3.28	2	4.06	0.78	0.55
European Starling	3.22	3	3.78	0.25	0.15
Sparrow species	2.71	3	3.29	0.73	0.42
Pigeon/Dove	2.46	2	3.20	0.25	0.17
American Robin	3.12	3	3.13	0.19	0.11
Thrush species	3.02	3	3.05	0.27	0.15
Carabidae[1]	3.14	3	3.02	0.19	0.11
Oniscidea	2.00	3	2.08	0.47	0.27
Lumbricidae	2.00	6	1.85	0.31	0.13
Himalayan Blackberry[2]	1.00	3	–	–	–

[1]Dietary trophic positions for Large and Small Beetles were averaged since they were pooled;

[2]A trophic position of 1 was assumed for Himalayan blackberry and used in TMF regressions.

2.3.2. Detection Frequency and Contaminant Concentrations

We detected 15 (83%) of the 18 PFCs in at least one species group within the food-web (Table 2.4). Twelve PFCs were detected in most (> 88%) of the apex predator samples. Eleven PFCs were detected within one or more of the avian prey samples (> 6%; Table 2.4). However, only six PFCs were detected in the invertebrate groups and only one PFC was detected in Himalayan blackberry (Table 2.4). Nonetheless, we detected seven (39%) of the 18 PFCs in > 50% of all the samples across the food-web. PFOS had the highest total detections at 75% followed by PFTeDA, PFDoA, and PFOA at 63%, 58%, and 54%, respectively. Generally, frequency of detections across species groups increased as carbon fluorination increased.

Average concentrations for the seven PFCs detected in > 50% of all the samples varied considerably across the species groups (Table 2.5). However, the average concentrations for each PFC generally increased with the average trophic position of the species groups. PFOS had the highest average concentrations across the food-web ranging from non-detectable levels in the berries, earthworms, and sowbugs/pillbugs to 596.41 (46.51 SE) ng/g protein eq. in the Cooper's Hawks (Table 2.5). PFTeDA and PFDoA also had relatively high average concentrations in the Cooper's Hawks at 94.93 (10.27 SE) ng/g protein eq. and 80.51 (9.38 SE) ng/g protein eq., respectively. But average concentrations in the species groups with lower average trophic positions were generally low ranging from 0.22 – 16.94 ng/g protein eq. for PFTeDA and 0.54 – 20.70 ng/g protein eq. for PFDoA. We report the estimated protein equivalent concentrations for each sample in Appendix 13.

∑PFSA average concentrations generally increased with the average trophic position of the species groups but had particularly high concentrations in Pigeons/Doves at 975.72 ng/g protein eq. and in Sparrows at 865.67 ng/g protein eq. (Table 2.5; Figure 2.2). Whereas, ∑PFCA average concentrations appeared to fluctuate across the food-web with rather high concentrations in Himalayan blackberries at 165.51 ng/g protein eq., moderately high concentrations in the predominately fruit-eating Thrushes and American Robins at 68.61 ng/g protein eq. and 27.72 ng/g protein eq., and then peaking at 291.0 ng/g protein eq. in the Cooper's Hawks (Table 2.5; Figure 2.2). ∑PFCA average concentrations were surprisingly high for Himalayan blackberries, but this was simply due to integrating the large number of censored concentrations with the high detected concentration of PFNA into the Kaplan-Meier estimation of the sum. ∑PFCA and ∑PFSA average concentrations were also high for Himalayan blackberries due to berries having substantially higher MDLs compared to the other species groups (Appendix 12).

Table 2.4. Frequency of detection (% of samples with detections) of each PFC within species groups across the food-web. ITP = Mean Isotopic Trophic Position.

	HBB	LMB	ONI	CAR	THRU	AMRO	ROPI	SPAR	EUST	NOFL	COHA	Total
ITP	1.00	1.85	2.08	3.02	3.05	3.13	3.20	3.29	3.78	4.06	4.27	-
n	3	6	3	3	3	3	2	3	3	2	17	**48**
PFC	**Detection Frequency (%)**											
PFBA	0	0	0	0	0	0	0	0	0	0	0	0
PFPeA	0	0	0	0	0	0	0	0	0	0	53	19
PFHxA	0	0	0	0	0	0	0	0	0	0	18	6
PFHpA	0	0	0	0	0	0	0	0	0	0	18	6
PFOA	0	17	0	67	67	67	0	0	33	50	100	54
PFNA	33	0	0	0	33	0	0	0	0	50	100	42
PFDA	0	0	0	0	67	100	0	67	33	50	100	54
PFUdA	0	0	0	0	67	100	0	33	0	50	100	50
PFDoA	0	17	33	0	67	100	0	67	33	50	100	58
PFTrDA	0	17	0	0	67	100	0	33	0	50	100	52
PFTeDA	0	67	0	0	67	100	0	33	67	50	100	63
PFHxDA	-	0	0	0	33	0	0	0	0	0	94	38
PFODA	-	0	0	0	0	0	0	0	0	0	0	0
PFBS	0	0	0	0	0	0	0	0	0	0	0	0
PFHxS	0	0	0	100	67	33	0	0	0	0	88	44
PFEtCHxS	-	0	0	0	0	0	0	0	0	0	100	38
PFOS	0	0	0	100	100	100	100	100	100	100	100	75
PFDS	0	0	0	0	33	67	0	0	0	50	100	44

HBB = Himalayan Blackberry, LMB = Lumbricidae, ONI = Oniscidea, CAR = Carabidae, THRU = Thrush spp., AMRO = American Robin, ROPI = Pigeons/Doves, SPAR = Sparrow spp., EUST = European Starling, NOFL = Northern Flicker, COHA = Cooper's Hawk

Table 2.5. Concentrations of PFCs (ng/g protein eq.) within each species group collected across Metro Vancouver in 2016. Concentrations are means with standard error determined by Kaplan-Meier method. Total PFCA and PFSA are overall sums determined by K-M method (i.e. an overall mean multiplied by the number of respective PFCAs or PFSAs as recommended in (96)). ND = Non-detection. – = Not analysed in samples.

	HBB	LMB	ONI	CAR	THRU	AMRO	ROPI	SPAR	EUST	NOFL	COHA
ITP	1	1.85	2.08	3.02	3.05	3.13	3.20	3.29	3.78	4.06	4.27
PFBA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
PFPeA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.34 ± 0.74
PFHxA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.48 ± 0.30
PFHpA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.36 ± 0.36
PFOA	ND	0.79	ND	0.29 ± 0.08	2.46 ± 0.85	0.29 ± 0.09	ND	ND	0.09	0.33	4.53 ± 0.64
PFNA	14.96	ND	ND	ND	0.86	ND	ND	ND	ND	1.95	9.13 ± 1.01
PFDA	ND	ND	ND	ND	5.71 ± 2.59	1.25 ± 0.65	ND	0.37 ± 0.01	0.25	5.03	27.51 ± 3.52
PFUdA	ND	ND	ND	ND	4.45 ± 2.88	2.07 ± 1.00	ND	0.72	ND	1.77	17.30 ± 1.91
PFDoA	ND	1.79	0.54	ND	20.70 ± 12.16	9.64 ± 2.61	ND	1.69 ± 1.40	1.32	7.33	80.51 ± 9.38
PFTrDA	ND	0.99	ND	ND	11.60 ± 9.92	4.44 ± 1.53	ND	1.71	ND	2.87	51.92 ± 5.74
PFTeDA	ND	3.03 ± 0.93	ND	ND	16.94 ± 10.08	7.98 ± 1.56	ND	1.80	0.22 ± 0.11	5.07	94.93 ± 10.27
PFHxDA	–	ND	ND	ND	1.58	ND	ND	ND	ND	ND	2.27 ± 0.40
PFODA	–	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
∑PFCA	164.51	13.76	6.98	3.76	68.61	27.72	< 0.41	9.23	2.68	26.34	291.00

	HBB	LMB	ONI	CAR	THRU	AMRO	ROPI	SPAR	EUST	NOFL	COHA
ITP	1	1.85	2.08	3.02	3.05	3.13	3.20	3.29	3.78	4.06	4.27
PFBS	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
PFHxS	ND	ND	ND	2.25 ± 1.22	2.63 ± 1.25	0.30	ND	ND	ND	ND	2.54 ± 0.38
PFEtCHxS	–	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.49 ± 0.12
PFOS	ND	ND	ND	49.64 ± 12.73	175.94 ± 60.71	88.06 ± 23.89	195.14 ± 101.61	173.13 ± 89.02	111.33 ± 46.93	188.30 ± 11.06	596.41 ± 46.51
PFDS	ND	ND	ND	ND	4.98	2.93 ± 1.28	ND	ND	ND	2.99	39.39 ± 4.48
∑PFSA	< 97.77	< 0.23	< 0.14	58.66	188.80	91.88	975.72	865.67	556.67	200.26	639.32

HBB = Himalayan Blackberry, LMB = Lumbricidae, ONI = Oniscidea,, CAR = Carabidae, THRU = Thrush spp, AMRO = American Robin, ROPI = Pigeons/Doves, SPAR = Sparrow spp., EUST = European Starling, NOFL = Northern Flicker, COHA = Cooper's Hawk

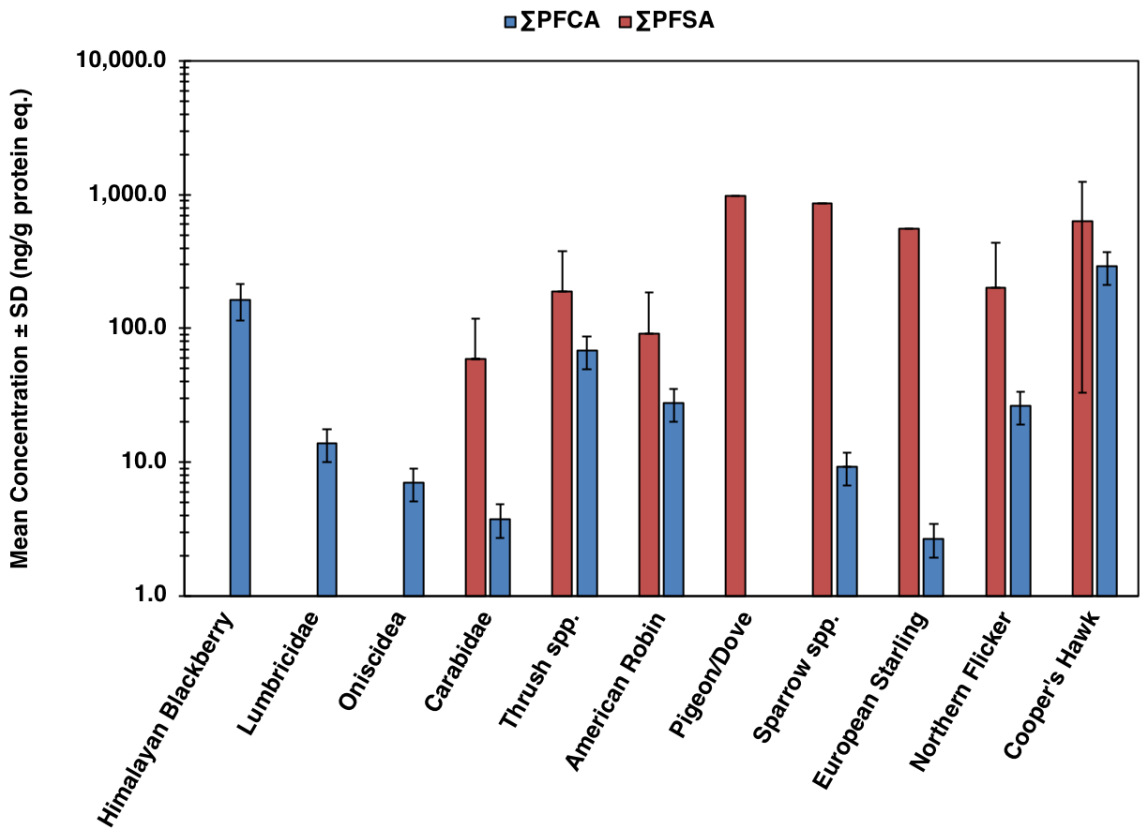

Figure 2.2. Total PFCA and PFSA average concentrations (ng/g protein eq.) observed in each trophic group across the food-web.

2.3.3. Trophic Magnification of PFCs

We were able to estimate TMFs for six PFCAs and one PFSA that were detected in > 50% of all food-web samples (Table 2.6). All seven PFCs had TMFs greater than 1, indicating biomagnification within this terrestrial food-web (Table 2.6; Appendix 14). Our estimated TMFs ranged from 13.02 (10.10 SE) for PFTeDA to 86.19 (85.70 SE) for PFUdA demonstrating large variation in trophic magnification (Table 2.6). Four PFCAs, including PFDA, PFUdA, PFDoA, and PFTrDA had the highest TMFs but also had the largest estimates of uncertainty (Table 2.6; Figure 2.3). The remaining two PFCAs, PFOA and PFTeDA, had the lowest TMFs but also had smaller estimates of uncertainty compared to the other PFCAs (Figure 2.3). The estimated TMF for PFOS, the PFC with the highest average concentrations detected across the food-web, was quite high at 42.14 (16.72 SE), but also had a smaller estimate of uncertainty compared to the four PFCAs with high TMFs (Figure 2.4).

Table 2.6. Statistical results from censored regressions to determine TMFs of PFCs with detection frequencies greater than 50%. SE = standard error; LCL = lower 95% confidence limit; UCL = upper 95% confidence limit.

Analyte	Slope	SE	LCL	UCL	Loglik-*r*	χ^2	*p*-value	TMF	SE	LCL	UCL
PFOA	2.64	0.60	1.45	3.82	0.588	20.35	<0.001	13.98	8.45	4.27	45.73
PFDA	4.40	0.85	2.74	6.06	0.697	31.88	<0.001	81.44	69.15	15.42	430.06
PFUdA	4.46	0.99	2.51	6.41	0.635	24.77	<0.001	86.19	85.70	12.28	605.14
PFDoA	3.80	0.87	2.09	5.50	0.586	20.17	<0.001	44.48	38.62	8.12	243.85
PFTrDA	4.04	0.96	2.15	5.93	0.577	19.46	<0.001	56.82	54.68	8.62	374.62
PFTeDA	2.57	0.78	1.04	4.09	0.454	11.07	<0.001	13.02	10.10	2.84	59.60
PFOS	3.74	0.40	2.96	4.52	0.849	61.10	<0.001	42.14	16.72	19.37	91.71

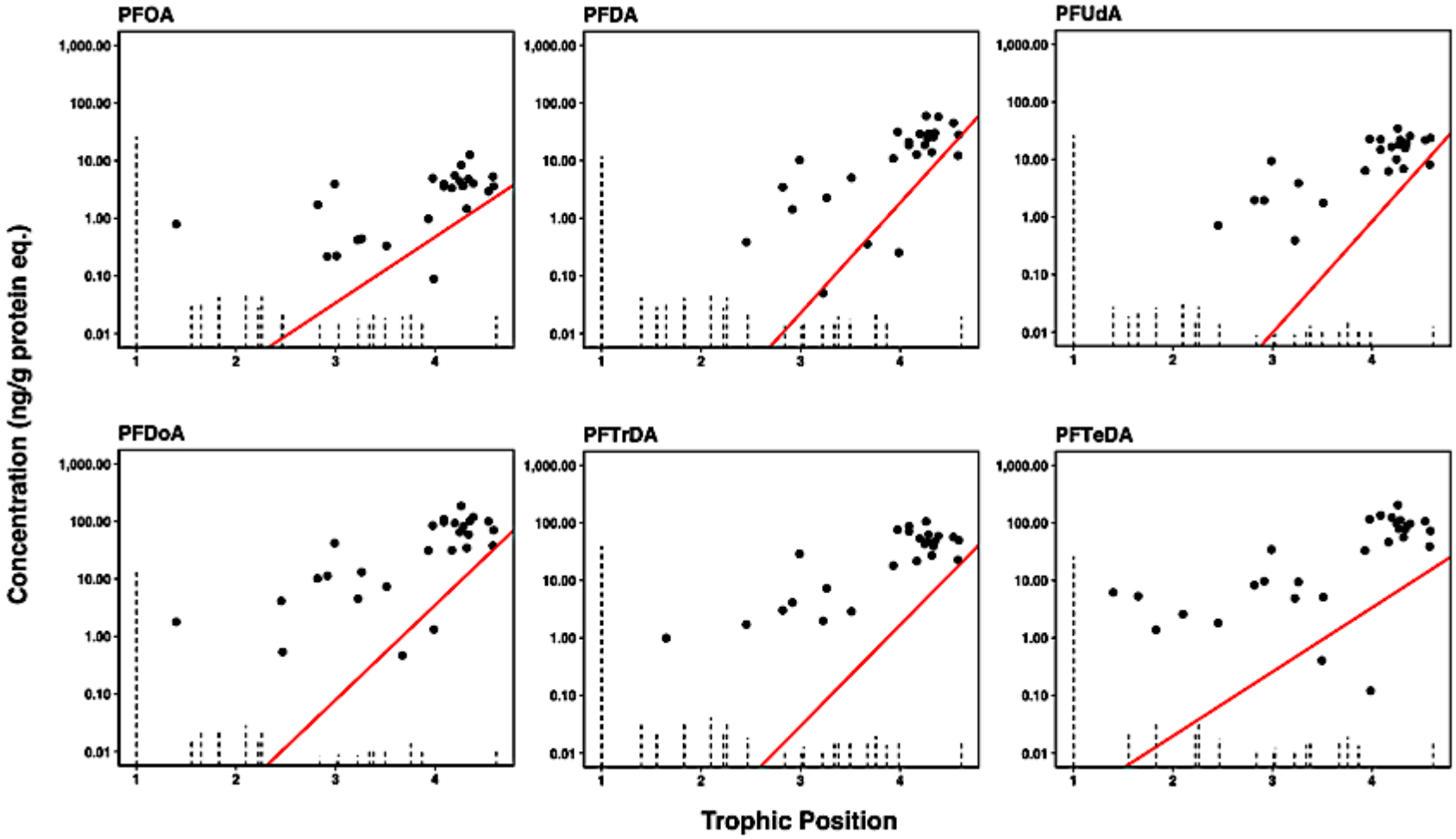

Figure 2.3. PFCA concentrations in organisms within an urban, terrestrial food-web (ng/g protein eq.) versus trophic position (TP) for PFDA, PFDoA, PFOA, PFTeDA, PFTrDA, and PFUdA. Black circles represent detected observations and dashed lines represent non-detected observations below a sample specific, protein normalized MDL. Red lines represent the log-linear regression of protein equivalent concentration to TP across the food-web.

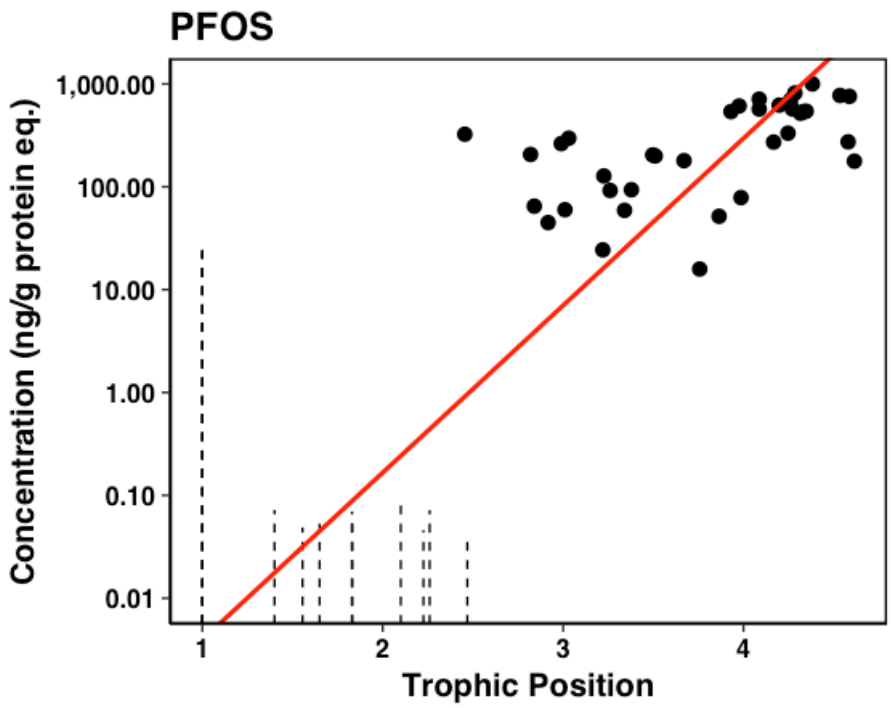

Figure 2.4. PFSA concentrations in organisms within an urban, terrestrial food-web (ng/g protein eq.) versus trophic position (TP) for PFOS. Black circles represent detected observations and dashed lines represent non-detected observations below a sample specific, protein normalized MDL. Red lines represent the log-linear regression of protein equivalent concentration to TP across the food-web.

Log K_{OA} values that we compiled for the seven PFCs ranged from 6.3 – 8.0 and log K_{OW} values ranged from 3.6 – 8.8 (Table 2.7). The log K_{OA} values for PFOA and PFOS were high (i.e. > 5), whereas, the log K_{OW} values were low indicating that these two PFCs would likely biomagnify in air-breathing organisms but not water-breathing (Table 2.7)(*4*). In comparison, the log K_{OA} and K_{OW} values we compiled for PFDA, PFDoA, PFTeDA, and PFUdA were both high, indicating that these four PFCs would likely biomagnify in both air-breathing and water-breathing organisms. The log K_{PA} and log K_{PW} values for the seven PFCs were both generally low ranging from 4.4 – 5.5 and 2.5 – 5.6, respectively (Table 2.7). There appeared to be curvilinear relationships between TMF and log K_{OA} values (R^2 = 0.673, p = 0.0869, $F_{2,3}$ = 6.14; Figure 2.5) and log K_{PA} values (R^2 = 0.413, p = 0.209, $F_{2,3}$ = 2.76; Figure 2.5). TMFs peaked when log K_{OA} values reached 7 and log K_{PA} values reached 5 (Figure 2.5). In addition, TMFs of PFCAs generally peaked when fluorinated carbon chain length reached 10.

Table 2.7. Estimated terrestrial and aquatic TMFs based on protein normalized concentrations with partition coefficients for each PFC. No aquatic TMFs and reliable partition coefficients were available for PFTrDA. Aquatic TMFs are either from food-webs with both air- and water-breathing organisms or just water-breathing.

PFC	TMF ± SE	Log K_{OA}	Log K_{OW}	Log K_{PA}	Log K_{PW}	Air + Water Aquatic TMF (95% CI)	Water Aquatic TMF (95% CI)	Reference
PFOA	13.98 ± 8.45	6.3	3.6	4.4	2.5	1.93 (1.40 – 2.64)	0.40 (0.30 – 0.53)	(*4*)
PFDA	81.44 ± 69.15	6.8	5.4	4.8	3.3	4.81 (3.31– 6.99)	0.60 (0.39 – 0.99)	(*4*)
PFUdA	86.19 ± 85.70	7.1	6.4	4.9	4.5	4.79 (3.63 – 6.32)	1.09 (0.75 – 1.58)	(*4*)
PFDoA	44.48 ± 38.62	7.4	7.1	5.2	5.0	2.96 (2.34 – 3.76)	1.01 (0.71 – 1.44)	(*4*)
PFTrDA	56.82 ± 54.68	–	–	–	–	–	–	–
PFTeDA	13.02 ± 10.10	8.0	8.8	5.4	5.6	1.97 (1.50 – 2.60)	0.34 (0.23 – 0.48)	(*4*)
PFOS	42.14 ± 16.72	7.8	4.3	5.5	3.0	11.00 (6.90 – 17.40)	0.47 (0.27 – 0.85)	(*4*)

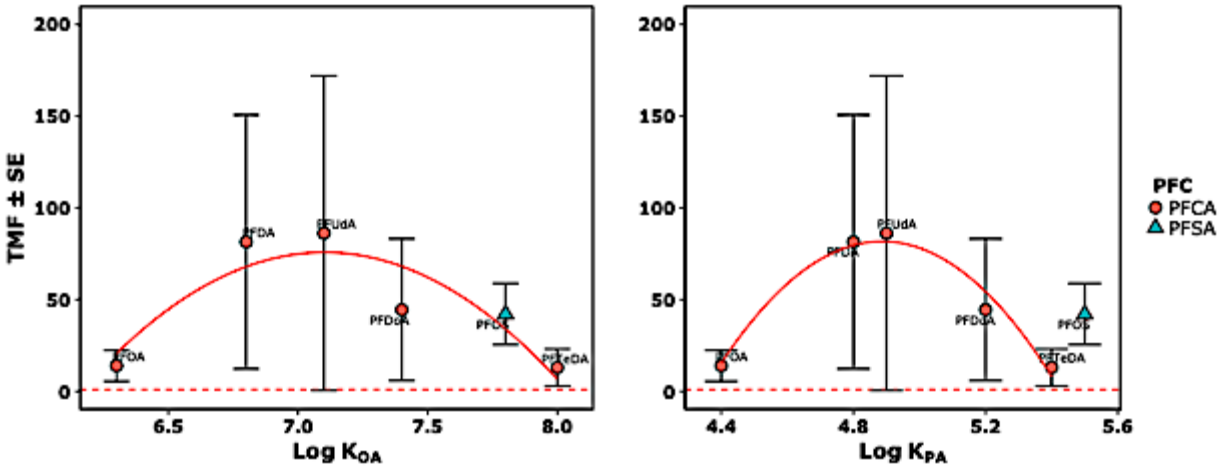

Figure 2.5. Curvilinear relationship between estimated TMF and log K_{OA} values (TMF = 46.9 – [13.1 x log K_{OA}] – [62.0 x log K_{OA}]^2) and log KPA values (TMF = 46.9 – [8.11 x log K_{PA}] – [56.3 x log K_{PA}]^2) of each PFC (excluding PFTrDA). Curved solid red line represents the quadratic regression and the dashed red line represents a TMF of 1, indicating no biomagnification.

2.4. Discussion

Here we show that several PFCs are present throughout an urban, terrestrial food-web and are prevalent in higher trophic levels, particularly within our apex predator. We also provide evidence that PFCs biomagnify in terrestrial systems and that our estimated terrestrial TMFs for PFCs far exceed the estimated TMFs obtained for aquatic environments. Our results confirm that regulatory agencies need to consider bioaccumulative behaviour within both terrestrial and

aquatic environments. Ideally, regulators should request that bioaccumulation data and models be provided for both terrestrial and aquatic environments during the assessment process of new chemicals.

2.4.1. Contaminant Concentrations and Bioaccumulation

We detected 83% of the 18 PFCs in at least one trophic group within our urban terrestrial food-web. We detected six PFCAs and PFOS at high concentrations within our apex predator and some of the avian prey species, but there was substantial variation in prevalence across the food-web. In general, PFOS dominated the PFSA and overall PFC profile in all of our terrestrial species groups, which is commonly observed in most aquatic wildlife as well (*125, 133*). PFOS was present in roughly 75% of all the biota samples with concentrations ranging from non-detectable levels in the berries and earthworms up to 999.85 ng/g protein eq. in the Cooper's Hawk. This evident biomagnification indicates that Cooper's Hawks and secondary avian consumers may experience significant adverse effects if concentration levels exceed toxicity thresholds. However, avian toxicological studies on PFCs are rather limited and have mostly focused on PFOS and PFOA, which have both been linked to reduced hatching or pipping success in several species at relatively high concentrations (*134, 135*). Benchmark doses based on the dose-response curve where a 10% effect (BMD_{10}) is observed for embryo mortality in eggs of the domestic White Leghorn chicken (*Gallus gallus domesticus*) with a 95% lower confidence limit (BMDL) are reported for PFOS at 1.26 (0.42 LCL) μg/g ww and PFOA at 1.01 (0.60 LCL) μg/g ww (*135*). However, smaller wild avian species, such as Tree Swallows (*Tachycineta bicolor*), had reduced hatching success with PFOS levels estimated as low as 150 – 200 ng/g ww (*136*). In contrast, larger wild avian species, such as the Herring Gull (*Larus argentatus*) and Great Cormorant (*Phalacrocorax carbo sinensis*), appear to be much less sensitive than chickens with dose-response curves roughly 1.6 – 2.6 times higher for PFOS and 3.5 – 8.2 times higher for PFOA (*135*). These dose-response curves and negative effects concentrations are not directly comparable to our sample concentrations, but we can convert them to protein equivalent concentrations based on average protein equivalent values from our species groups (Table 2.8). In our apex predator, sampled concentrations of PFOS and PFOA were well below the estimated BMD_{10}/BMDL for chicken eggs (Table 2.8). In the dominant prey species, sampled concentrations of PFOS were also well below protein adjusted negative effects concentrations for Tree Swallows (Table 2.8). Thus, it would appear that Cooper's Hawks and their main avian prey species are not

experiencing reduced hatching success greater than 10% due to PFOS bioaccumulation at this time.

Nonetheless, very little information is known of how PFOA and other long-chained PFCAs affect many wild avian species either solely or in combination with each other (*135*). This is likely due to the fact that PFOA, the most widely studied PFCA, and other PFCAs are typically not detected or are found only at low concentrations in most aquatic biota (*125, 133, 137*). However, we detected six PFCAs, including PFOA, PFDoA, PFDA, PFTeDA, PFTrDA, PFUdA, in > 50% of all the biota samples from our terrestrial food-web and observed fairly high concentrations of these PFCAs in many of the avian prey species and our apex predator. These high concentrations indicate that PFCAs also bioaccumulate in terrestrial organisms and appear to biomagnify.

Table 2.8. Benchmark doses where a 10% effect is observed with a 95% lower confidence limit (BMD_{10}/BMDL) for PFOS and PFOA (μg/g ww) within chicken eggs were protein adjusted and compared to sampled concentrations (μg/g protein eq.) within Cooper's Hawks. Minimum range of PFOS concentrations that caused negative effects (μg/g ww) within Tree Swallow eggs was protein adjusted and compared to sampled concentrations (μg/g protein eq.) within dominant prey species.

Species	% Protein Eq.	Measurement	PFOS (μg/g)	PFOA (μg/g)
Cooper's Hawk	20.58%	BMD_{10}/BMDL	1.26 (0.42)	1.01 (0.60)
		Protein Eq.	6.12 (2.04)	4.91 (2.92)
		Sampled Concentrations	0.600	0.00450
European Starling	35.01%	Negative Effects	0.15 – 0.20	-
		Protein Eq.	0.43 – 0.57	-
		Sampled Concentrations	0.11	-
American Robin	31.96%	Negative Effects	0.15 – 0.20	-
		Protein Eq.	0.47 – 0.63	-
		Sampled Concentrations	0.088	-
Sparrows	30.81%	Negative Effects	0.15 – 0.20	-
		Protein Eq.	0.49 – 0.65	-
		Sampled Concentrations	0.17	-

2.4.1. Trophic Magnification

We assessed six PFCAs and PFOS for trophic magnification in our urban terrestrial food-web and all of them showed evidence of biomagnification. However, TMFs estimated for several of the PFCAs had large estimates of error, indicating that there was some uncertainty in our TMFs.

PFOS, on the other hand, had a much more reliable TMF estimate with a smaller measure of error (Table 2.6). This greater confidence was likely due to the very high PFOS concentrations we detected across the food-web. Moreover, PFSAs are usually more bioaccumulative than PFCAs with the same fluorinated carbon chain length (*2*). In fact, many laboratory studies have shown that bioaccumulation has a positive relationship with fluorinated carbon chain length likely because PFSAs and longer chained PFCAs bind more tightly to proteins (*2, 133*). Yet, it has also been suggested that the odd-chain length PFCAs are more bioaccumulative than the even-chain length PFCAs in wildlife (*2, 133*). Our concentration data appears to support these findings as the PFCAs with the highest concentrations across our food-web either had odd-chain lengths (i.e. PFTeDA, PFDoA, PFDA, and PFOA) or had even-carbon chain lengths greater than 10 (i.e. PFTrDA and PFUdA; Table 2.6).

In comparison to TMFs estimated in aquatic systems, the terrestrial TMFs we estimated were 4 – 20 times higher. This large discrepancy is likely due to the fact that our food-web is comprised of air-breathing organisms. For example, if we compare TMFs estimated in aquatic food-webs comprised solely of water-breathing organisms with aquatic food-webs with both water- and air-breathing organism, we can see that the TMFs increased by 1 – 2 orders of magnitude in the mixed organism food-web. Whereas, within the water-breathing aquatic food-web, all the PFCs had TMFs roughly less than 1 indicating that they either were not biomagnifying or were diluting. We also may have obtained larger TMFs because our food-web was in an urban environment rather than a remote, arctic one since wildlife closer to urban or industrialized areas typically have higher concentrations of PFCAs or PFOS than wildlife in non-urban or remote locations (*133, 137*). In addition, our food-web is dominated by bird species and many groups of birds are often known to have higher biomagnification than mammals (*121*) due to lower mono-oxygenase enzyme activity which limits their biotransformation of contaminants (*26*). Furthermore, these PFCs do not occur in isolation within organisms but rather may interact by inhibiting the enzymatic biotransformation of other chemicals (*122*) and consequently increase their observed bioaccumulative behaviour.

Generally, compounds with log K_{OW} values > 5 are bioaccumulative in water-breathing organisms. However, K_{OW} based criteria are not considered appropriate for predicting the bioaccumulative potential of PFCs because lipids are not the main depository of PFCs in organisms (*2*). Thus, trophic magnification differences between PFCs may be better understood by K_{PW} or K_{PA} (*4*). Log K_{PW} and log K_{PA} values for the PFCs we examined ranged from 2.5 – 5.6 and 4.4 – 5.5, respectively. Previous aquatic studies demonstrated that PFC TMFs generally

increased between log K_{PA} values from 4 to 5 and then declined when log K_{PA} values were > 5 (*4*). Our results supported a similar curvilinear relationship with PFC TMFs declining when log K_{PA} values were > 5, confirming that the overall pattern of trophic magnification of PFCs we observed in our terrestrial food-web is reasonably accurate even though the actual magnitude of trophic magnification may be somewhat unclear. We also observed a similar trend between PFC TMFs and log K_{OA} values demonstrating that lipophilicity and decreasing volatility appear to play a role in biomagnification of PFCs in terrestrial organisms. Overall, our results confirm that regulatory agencies need to consider separate bioaccumulation criteria for terrestrial systems particularly when contaminants do not bioaccumulate in aquatic systems comprised solely of water respiring organisms.

2.4.2. Limitations

Only one study to date has estimated trophic magnification of PFCs in a terrestrial food-chain (*11*). Ours is the second terrestrial study to confirm that PFCs do in fact biomagnify in terrestrial food-webs, but we acknowledge that our study has several limitations. Primarily, we were only able to estimate TMFs for seven of the 18 PFCs, and even for these chemicals with total sample detections > 50% there was considerable uncertainty around our estimated TMFs. The large estimates of error for many of our TMFs were likely due to the large variability among sample concentrations within some of the species groups as well as the limited sample size of organisms in our food-web. For instance, a power analysis of results from several studies indicated that biomagnification studies require a minimum of 30 – 40 samples in order to detect statistically significant regression slopes (*5, 138*). However, large variability in concentrations versus trophic position combined with low detection frequencies will often drive down the statistical sensitivity to the point that approximately 100 – 150 samples would be required to obtain a statistically significant regression slope (*5, 138*). In our previous study that examined biomagnification of lipophilic legacy POPs, we used a sample size of 109 and obtained reasonably small estimates of error for our TMFs. Furthermore, Kelly, Ikonomou, Blair, Surridge, Hoover, Grace and Gobas (*4*) used a sample size of approximately 110 and consequently reported small estimates of uncertainty for TMFs of PFCs. Yet, their study also had higher detection frequencies of PFCs within samples from lower trophic levels. Whereas, many of our species groups with lower trophic positions, such as Himalayan blackberry and sowbugs/pillbugs, primarily had censored concentration data. Consequently, we may want to increase the number of samples analysed for PFCs, particularly within our species groups with low detection frequencies. Or, we

may want to consider incorporating potential direct sources of PFCs, such as soil or air samples, as many PFCs experience long-range transport similar to legacy POPs (*2, 126, 132*). For instance, PFCAs and PFSAs have been detected on atmospheric particles (*132*) and many PFCs have been shown to correlate with measures of air movement, such as the North Atlantic Oscillation index (*126*). Thus, many PFCs in the Pacific Northwest may correlate with the Pacific Decadal Oscillation or El Niño Southern Oscillation indices by altering the availability of contaminated prey species or by directly changing the levels of PFCs in the environment.

Secondly, we assumed that protein normalization applied to all the PFCs we analysed in a similar manner since several studies have suggested that most perfluorinated acids are proteinophilic (*2, 4*) and generally exhibit a positive correlation with protein content (Hoff et al. 2003 in *4*). However, this assumption is another likely source of uncertainty, particularly since shorter PFCs (with three or four fluorinated carbons) have been found to be 1 – 2 orders of magnitude less proteinophilic than PFCs with longer carbon chain lengths (Jones et al. 2003 in *2*). In fact, even though the carboxylate or sulfonate functional groups in the "tail" of PFCs are highly hydrophilic and lipophobic, the fluorocarbon "head" is hydrophobic and lipophilic. So, we may want to consider incorporating a small fraction of lipid into the protein equivalent concentration for each sample, especially for shorter carbon chain length PFCs. This lipid incorporation may also be helpful for organisms like Himalayan blackberry that have very low protein content. Indeed, we may want to consider developing a different protein normalization for plant material as the censored concentration data from the berries appeared to strongly bias the MLE regression.

In addition, studies typically estimate PFC biomagnification based on whole body concentrations (*6*), but these types of concentrations are not always easily obtained or measured so concentrations in blood, serum, or plasma are often used as surrogates (*2*). However, PFCs are known to accumulate in protein-rich fluids and tissues, such as the blood, liver, or kidney (*6, 139*), and thus, often have concentrations several orders of magnitude higher than in other tissues or organs (*2*). Consequently, these tissue and fluid concentrations are not appropriate for exclusively assessing bioaccumulation potential as they tend to overestimate biomagnification (*2*). For example, in the terrestrial study by Müller, De Silva, Small, Williamson, Wang, Morris, Katz, Gamberg and Muir (*11*), the TMFs based on liver PFC concentrations were 2 – 3 times higher than the TMFs based on whole body concentrations. Comparatively, in our study we likely achieved a reasonable estimate of PFC biomagnification since we sampled the entire body of each organism and homogenized all the body tissues together. Ideally, total protein content

should be measured within each sample to determine protein equivalent concentrations; however, as an alternative, some studies have simply used total protein content values reported in the literature (*4*). But often these values are not available in the literature for many species, so we estimated the fraction of protein in each of our samples using protein:nitrogen conversion factors (*80-82, 140*). These conversion factors resulted in protein estimates comparative to total protein values reported in the literature. For instance, our protein fraction estimates for Cooper's Hawk eggs ranged from 11.1 – 16.0% which were similar to chicken eggs with ~12.6% total protein content (Appendix 13).

2.5. Conclusions

This study highlights the value of obtaining empirical estimates of trophic magnification of PFCs in terrestrial systems. Particularly, since some PFCs appear to have greater biomagnification in terrestrial food-webs with air-breathing organisms than in aquatic food-webs with water- and air-breathing organisms. Additionally, our results emphasize the need to examine both urban and remote ecosystems or landscapes, as it appears that avian species in our terrestrial urban food-web had higher bioaccumulation and biomagnification of PFCs than compared to mammalian species in a terrestrial arctic environment (*11*). Nonetheless, moving forward we plan to incorporate soil and air samples into our estimated TMFs for PFCs, which may help to reduce the level of uncertainty in our estimates. Eventually, we will also use our empirical data to develop a terrestrial bioaccumulation model for emergent POPs in order to assist regulatory agencies during the environmental review of new commercial chemicals.

Final Conclusions

In this thesis, we demonstrate that many legacy and emergent POPs are biomagnifying in a terrestrial food-web. We were able to estimate TMFs for 15 PCBs, 9 OCPs, 2 PBDEs, and 7 PFCs and found that all of them showed evidence of trophic magnification, except for PCB-28/31. Our concentration data also indicated that most of these contaminants are bioaccumulating in our various species groups, particularly the avian prey species and our apex predator. So, despite cessation of usage for over 40 years for many of these POPs, they are still prevalent and continue to biomagnify in wildlife. This evident biomagnification highlights that these contaminants could continue to have biological or sub-lethal effects on apex predators if bioaccumulation levels exceed toxicity thresholds. However, it appears that concentration levels of most of the POPs we examined in the avian species from our terrestrial food-web were below minimum reproductive effects thresholds, so it is unclear if these concentration levels are causing sub-lethal effects on these avian species in Metro Vancouver.

Biomagnification experts recommend using TMF estimation of PCB-153 as a positive control to evaluate the efficiency of the study design since TMFs for PCB-153 are consistently > 1 in almost all food-webs that have been studied (*5*). Therefore, if PCB-153 is present at detectable levels across the food-web but does not result in a statistically significant TMF, it indicates that there may be a problem with the study design or statistical method used. In our terrestrial bioaccumulation study, we confirmed that PCB-153 biomagnified indicating that our study design and statistical method were effective for evaluating the biomagnification potential of other chemicals. In addition, we also observed a positive trend in the relationship between the TMFs of lipophilic contaminants and their estimated log K_{OA} confirming that as log K_{OA} increased, the estimated TMF generally increased as well. We expected this result as many lipophilic POPs with high log K_{OA} values are known to biomagnify in terrestrial systems (*1, 3, 7*). We also found that K_{OA} and protein-air (K_{PA}) partition coefficients exhibited quadratic relationships with proteinophilic PFCs. We also expected this result as this relationship has been previously shown in aquatic systems with air-breathing organisms (*4*).

TMFs reported in aquatic food-webs for most of the legacy and lipophilic POPs were generally comparable to the TMFs we estimated in our terrestrial food-web (Table 1.8). However, the aquatic TMF reported for BDE-99 by Kelly, Ikonomou, Blair and Gobas (*27*) was considerably lower at 0.76 (0.10 SE) in contrast to our terrestrial TMF at 7.32 (1.77 SE) suggesting that there

are significant biomagnification differences between aquatic and terrestrial environments for brominated chemicals. Similarly, the TMFs reported in aquatic food-webs for the PFCs were 4 – 20 times lower than the TMFs we estimated in our terrestrial food-web. These biomagnification discrepancies are likely due to the mixed composition of both air and water respiring organisms in the aquatic food-webs versus only air respiring in our terrestrial food-web; as air respiring organisms often exhibit higher biomagnification levels than water respiring because of their greater ability to absorb and digest their diet (*3*). For instance, if we compare TMFs of PFCs estimated in aquatic food-webs comprised solely of water-breathing organisms with aquatic food-webs with both water- and air-breathing organism, we can see that the TMFs increased by 1 – 2 orders of magnitude. Whereas, within the water-breathing aquatic food-web, all the PFCs had TMFs roughly less than 1 indicating that they either were not biomagnifying or were diluting. In addition, our food-web is dominated by bird species and many groups of birds are often known to have higher biomagnification than mammals (*121*) due to lower mono-oxygenase enzyme activity which limits their biotransformation of contaminants (*26*). We also may have obtained larger TMFs for BDE-99 and PFCs because our food-web was in an urban environment rather than a remote, arctic one since wildlife closer to urban or industrialized areas typically have higher concentrations of flame retardants, PFCAs, or PFOS than wildlife in non-urban or remote locations (*20, 21, 133, 137*). Furthermore, all these POPs do not occur in isolation within organisms but instead may interact by inhibiting the enzymatic biotransformation of other chemicals (*122*) and consequently increase their observed bioaccumulative behaviour.

Moving forward we plan to incorporate soil and air samples into our estimated TMFs for PFCs as well as lipophilic POPs, which may help to reduce the level of uncertainty in our estimates. These direct sources of contamination will improve our sample size but also help us to better understand the trophic dynamics occurring in this terrestrial system. We collected soil and air samples from the food-web during our biota sampling efforts, so we plan to convert the observed air and soil concentrations with respect to concentrations observed in the other organism to fugacity ratios. We may also consider using other regression techniques, such as ordinary least squares, logistic, or robust regression on order statistics, to see if they help to reduce the level of uncertainty in our TMF estimates. In addition, we may also consider performing a post-hoc power analysis of the PFC data or an uncertainty analysis to explore the spread or deviations from the average concentrations in the food-web biota.

Overall, our terrestrial study has provided critical empirical data to the field of bioaccumulation. It also highlights the value of obtaining empirical estimates of trophic

magnification of emergent POPs like PFCs and PBDEs in terrestrial systems. Particularly, since PFCs and PBDEs appear to have greater biomagnification in food-webs with terrestrial air-breathing organisms than compared to aquatic water- or air-breathing organisms. Our study also demonstrates that terrestrial food-webs with avian predators likely biomagnify PFCs and PBDEs to a greater extent than terrestrial food-webs with mammalian predators as TMFs reported for BDE-47 and -99 in an arctic food-web with wolves indicated that BDE-47 diluted and BDE-99 did not biomagnify (*18*). Additionally, our results emphasize the need to examine both urban and remote ecosystems or landscapes, as it appears that avian species in our terrestrial urban food-web had higher bioaccumulation and biomagnification of PFCs than compared to mammalian species in a terrestrial, arctic environment (*11*). Our results also stress the importance of establishing separate standards and bioaccumulation criteria for terrestrial systems. Eventually, we hope to use our empirical data to develop a field-derived bioaccumulation model that would ideally be used as a regulatory standard to predict bioaccumulation potential of new chemicals in terrestrial environments.

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Appendices

Supplementary Data File

Description:

The accompanying Excel workbook contains Appendices 1 – 14, which include the following information:

- Appendix 1 – List of POPs analysed in biotic samples with method detection limits (MDL) and method quantification limits (MQL) from both labs.
- Appendix 2 – Number of individual birds or eggs within each pooled species group.
- Appendix 3 – Measured (moisture, lipids, δ15N, δ13C, % carbon, and % nitrogen) and calculated (proteins, DTP, ITP, and lipid eq.) properties of each sample.
- Appendix 4 – Stable isotope data used to determine proportion of prey δ15N and prey δ13C and average prey δ15N and prey δ13C for each predator, which were subsequently used in linear mixed effects model to determine trophic enrichment factor.
- Appendix 5 – Stable isotope data used to calculate isotopic trophic position based on isotopic enrichment factor from Mizutani et al. 1991, from averaged prey, and from proportional prey.
- Appendix 6 – Frequency of detection of each legacy POP within samples across the food-web.
- Appendix 7 – Concentrations of POPs within each trophic level collected across Metro Vancouver in 2016. Chemical concentration data (ng/g lipid eq.) are means with standard error determined by Kaplan-Meier method. ND = Non-detection so sample is below detection limit. NA = no standard error. Total PCBs, OCPs, and PBDEs are overall sums determined by KM method.
- Appendix 8 – Lipid equivalent concentrations (ng/g lipid eq.) of PCBs within each sample collected across Metro Vancouver in 2016. Each PCB has a corresponding

column that indicates if the concentration was censored (i.e. below the method detection limit) in the sample.

- Appendix 9 – Lipid equivalent concentrations (ng/g lipid eq.) of OCPs within each sample collected across Metro Vancouver in 2016. Each OCP has a corresponding column that indicates if the concentration was censored (i.e. below the method detection limit) in the sample.

- Appendix 10 – Lipid equivalent concentrations (ng/g lipid eq.) of PBDEs and BFRs within each sample collected across Metro Vancouver in 2016. Each PBDE and BFR has a corresponding column that indicates if the concentration was censored (i.e. below the method detection limit) in the sample.

- Appendix 11 – Statistical results from censored regressions to determine TMFs of legacy POPs with detection frequencies greater than 50%.

- Appendix 12 – List of PFCs analysed in biotic samples with method detection limits (MDL) and method quantification limits (MQL).

- Appendix 13 – Lipid equivalent concentrations (ng/g lipid eq.) of PFCs within each sample collected across Metro Vancouver in 2016. Each PFC has a corresponding column that indicates if the concentration was censored (i.e. below the method detection limit) in the sample.

- Appendix 14 – Statistical results from censored regressions to determine TMFs of PFCs with detection frequencies greater than 50%.

Filename:

KFremlin_Thesis_Supplementary Data.exls