AN ABSTRACT OF THE DISSERTATION OF

Allyson Kathleen Jackson for the degree of Doctor of Philosophy in Wildlife Science presented on May 16, 2017

Title: You are What, When, Where, and How you Eat: Mercury in Avian Food Webs across Multiple Spatial Scales.

Abstract approved:

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Collin Eagles-Smith

Human alterations of landscapes take many forms, one of which is anthropogenic pollution. Mercury (Hg) is a complex contaminant because its uptake into the food web is not driven entirely by loading to the system; methylation is necessary to make Hg bioavailable and toxic to fish and wildlife. Because methylation takes place primarily in aquatic systems, research has historically focused on local Hg exposure in fish and fish-eating taxa (including the human health implications of eating exposed fish). As our understanding of Hg movement out of the aquatic and piscivorous food web advances, we must shift focus to other potential recipients and spatial scales. This dissertation seeks to understand how Hg moves through food webs with avian endmembers, both with traditionally-studied piscivores at novel spatial scales and novel endmembers (riparian songbirds in western North America) at more traditional spatial scales. Moving from broad to narrow, I consider Hg exposure across continental (Chapter 2), regional (Chapter 3), and local scales (Chapter 4).

At the largest scale, in Chapter 2, I used fish Hg concentrations compiled across western North America at a 1 degree-by-1 degree grid cell resolution. Fish Hg concentrations were size corrected to reflect the primary prey base for a suite of five avian piscivores commonly used in Hg ecotoxicological studies: Bald Eagle, Osprey, Loons (Common and Yellow-billed), Grebes (Clark's and Western) and Belted Kingfisher. At a continental scale, I identified taxa and regions of increased potential Hg risk to avian endmembers. Avian piscivores foraging on larger-sized fish generally were at higher relative risk to Hg. Habitats with relatively high risk included wetland complexes (e.g., prairie potholes in Saskatchewan),

river deltas (e.g., San Francisco Bay, Puget Sound, Columbia River), and arid lands (Great Basin and central Arizona).

Chapter 3 focuses on one river basin in particular – the Willamette Valley in western Oregon. Mercury in fish and Osprey have been previously studied in this region; this study characterized differences in aquatic invertebrate and riparian songbird Hg exposure, starting at the headwater area which contains a point source of Hg from a historic Hg mine, moving downstream to a reservoir known to methylate Hg, and including all subsequent downstream reaches. While Hg exposure was highest in songbirds near the Black Butte Hg Mine, Hg concentrations were also elevated at the Cottage Grove Reservoir and several wetland complexes within the valley, reinforcing the importance of habitat on Hg methylation rates. Within the main stem Willamette River, birds in backwater areas had higher Hg concentrations than birds in main channel areas.

Chapter 4 examines why individual songbirds and different species of songbirds can vary dramatically in their Hg exposure. I sampled 11 sites within the main stem Willamette River and found that both aquatic invertebrates and riparian songbird Hg concentrations were higher in backwater habitats than main channel habitats, even over relatively small distances. After sampling both aquatic and terrestrial invertebrates, I used a two end member mixing model for $\delta^{13}C$ to determine the proportion of aquatic prey in the diet of composited riparian spiders and individual riparian songbirds. Birds sampled early in the season exhibited higher reliance on aquatic prey than those sampled later in the season and also had correspondingly higher blood Hg concentrations.

Taken together, the findings of this dissertation show that, for many avian species, Hg exposure is mitigated by what, when, where, and how they eat. What birds eat is driven both by taxonomic and behavioral differences (piscivores versus insectivores vs omnivores) and source of diet (aquatic versus terrestrial prey). For riparian songbirds, those individuals foraging early in the season rely more heavily on the pulsed aquatic carbon subsidy of emergent aquatic insects. Birds foraging near habitats that efficiently methylate Hg, such as backwaters or wetlands, exhibit higher Hg levels.

You are What, When, Where and How you Eat:
Mercury in Avian Food Webs across Multiple Spatial Scales

by
Allyson Kathleen Jackson

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

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Allyson Kathleen Jackson, Author

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CONTRIBUTION OF AUTHORS

Dr. Collin Eagles-Smith contributed to all aspects of this dissertation. Dr. David C. Evers, Dr. Joshua T. Ackerman, Dr. James J. Willacker, Dr. John E. Elliott, Jesse M. Lepak, Stacy S. Vander Pol, and Colleen E. Bryan were members of the Western North America Mercury Synthesis project and contributed data, along with help in study design, analysis and writing for Chapter 2. Dr. W. Douglas Robinson contributed to study design, analysis, and writing for Chapter 3. Colleen Emery contributed independent field and lab support as well as writing to Chapter 4.

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CHAPTER 1. GENERAL INTRODUCTION:

Mercury in avian food webs across multiple spatial scales

Food webs are driven by flow of nutrients (Lindeman 1942). These nutrients are not confined to one food web in one ecosystem. Instead, they can move between ecosystems in a variety of ways, described as cross-ecosystem subsidies (Polis et al. 1997, Nakano and Murakami 2001). In recent years, the importance of subsidies has been highlighted as they can move considerable nutrients between ecosystems and facilitate a wide range of physical, chemical, and biological processes. With the increasing urbanization of the planet, we are finding that these nutrients also move contaminants both within food webs of ecosystems and across ecosystem boundaries (Walters et al. 2008, Jones et al. 2013, Kraus et al. 2013).

Mercury (Hg) is a globally distributed, persistent environmental pollutant that has widespread impacts on human and ecosystem health (Wiener 2013, Sunderland and Selin 2013). Global sources of Hg include atmospheric release by fossil fuel combustion, waste incineration and gold and metal processing, whereas more localized sources include accidental or legacy point-source contamination from manufacturing plants and mining activity (Custer et al. 2007, Brasso and Cristol 2008, Hothem et al. 2008). Mercury is a concern whenever it is taken up by wildlife because, as a neurotoxin, Hg causes a variety of lethal and sublethal effects, ranging from abnormal behavior to direct mortality (Scheuhammer et al. 2007, Seewagen 2009). Mercury is unique among contaminants in the sense that its toxicity and bioavailability are directly tied to its propensity to be converted into a highly toxic organic form, methylmercury (MeHg). Mercury methylation occurs by iron- and sulfur-reducing bacteria commonly associated with anoxic aquatic environments. Thus, inorganic Hg loading in an area is only one of several components responsible for MeHg exposure (Wolfe et al. 1998). Instead, complex interactions among biogeochemical, hydrological, and ecological factors control the production and subsequent bioaccumulation of MeHg in a given locale (Evers et al. 2007).

Western North America is home to a wide diversity of habitat types, ranging from arid lands to temperate rainforest to high elevation mountains, which could each influence Hg cycling in different and distinct ways (Eagles-Smith et al. 2016). A defining feature throughout the region is the role of water in driving habitat and wildlife associations.

Additionally, there is both Hg and gold mining legacy pollution (Wiener and Suchanek 2008) and atmospheric deposition throughout the region (Jaffe et al. 2005).

The propensity of Hg to biomagnify as it moves up the food chain makes selection of indicator species crucial when trying to understand exposure and risk. Human risk of impairment from consuming contaminated prey is usually assessed through fish Hg concentrations (Lepak et al. 2016). Piscivorous wildlife species, such as birds, are good indicators of Hg at a water body or regional scale. Songbirds, on the other hand, are excellent indicators at a small scale because of their relatively small territory sizes and site fidelity during the breeding season, whereas their occurrence across a wide variety of habitats facilitates comparison of Hg risk across broad geographic areas (Jackson et al. 2015).

Because Hg methylation typically occurs in aquatic systems, research and monitoring have historically focused on fish and fish-eating wildlife as Hg indicator species (Evers et al. 2007). However, scientists have recently established that aquatic energetic subsidies facilitate the transfer of Hg through terrestrial food webs as well (Cristol et al. 2008). Many songbird species eat primarily invertebrate prey during the breeding season, and often concentrate near aquatic habitats ostensibly to exploit the emergent insect subsidy (Nakano and Murakami 2001, Hagar et al. 2012). Contaminants, such as Hg, heavy metals, and PCBs are all exported via emergent aquatic insects (Walters et al. 2008, Jones et al. 2013, Kraus et al. 2013) but no study has directly linked the magnitude of this subsidy with Hg exposure in insect-eating riparian songbirds.

Aquatic insect emergence from rivers can have far-reaching implications for terrestrial predators, concentrating both invertebrate and vertebrate predators near aquatic sources during peak emergences (Whitaker et al. 2000, Iwata et al. 2003, Baxter et al. 2005). This aquatic energy subsidy is especially important for songbirds before the bud break allows terrestrial invertebrates to become abundant, when terrestrial insect densities are low and insectivorous songbirds concentrate in areas around riparian zones (Gray 1993, Nakano and Murakami 2001). Birds that maximize this energetic subsidy may coincidentally put themselves at high risk to Hg exposure, as the emergent aquatic insects move Hg from the aquatic to riparian system (Walters et al. 2008, Sullivan and Rodewald 2012). Despite the fact that terrestrial birds are known to concentrate in riparian areas, few studies exist to show that they actually eat an aquatic-based diet (Gray 1993, Hagar et al. 2012).

Songbird species such as Tree Swallows (*Tachycineta bicolor*), which rely heavily on the aquatic prey base, are common bioindicators of Hg contamination because their foraging habits tie them directly to the flux of Hg out of the aquatic system (Custer et al. 2007, Brasso and Cristol 2008). Focusing Hg investigations on aerial insectivores ignores many species that are likely also exploiting the aquatic subsidy, either by directly consuming aquatic prey or by consuming invertebrate predators that rely on aquatic-based prey. All insectivorous songbirds in an area may respond to pulses of emergent prey, and even those not specializing on flying insects likely consume a large amount of aquatic prey when it is abundant (Uesugi and Murakami 2006). Additionally, because Hg biomagnifies as it moves up trophic levels, songbirds that eat a large amount of predatory invertebrates (i.e., spiders) may actually be heavily exposed to Hg (Cristol et al. 2008). Moreover, songbirds are sensitive to the effects of MeHg exposure, making them excellent sentinels of ecological impairment (Brasso and Cristol 2008, Hawley et al. 2009, Wada et al. 2009, Hallinger et al. 2010, Jackson et al. 2011).

Objectives. The data chapters of my dissertation examine the ecological pathways of Hg exposure in avian species across a range of spatial scales. The second chapter works within a traditional piscivorous bird context to identify biological Hg hotspots across western North America. At this broad spatial scale, I use primarily fish tissue Hg concentrations (the most commonly sampled matrix) to understand risk to a suite of fish-eating bird species that are less commonly sampled. My third chapter looks at the Willamette Valley region in Oregon to better understand how MeHg availability in aquatic invertebrates varies within the different habitat types of the region and whether riparian songbirds exhibit similar patterns. Considering prey availability through a different lens, my fourth chapter focuses on a small region of the Willamette River to understand riparian songbird diet traced through stable isotopes. I consider whether the factors governing Hg exposure in diverse songbird species are controlled largely by the amount of reliance on aquatic prey. My final chapter summarizes my major findings and why I believe "you are what, when, where and how you eat."

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CHAPTER 2. CITATION INFORMATION

MERCURY RISK TO AVIAN PISCIVORES ACROSS WESTERN UNITED STATES
AND CANADA

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CHAPTER 2. MERCURY RISK TO AVIAN PISCIVORES ACROSS WESTERN UNITED STATES AND CANADA

Allyson Jackson, David C. Evers, Collin A. Eagles-Smith, Joshua T. Ackerman, James J. Willacker, John E. Elliott, Jesse M. Lepak, Stacy S. Vander Pol, Colleen E. Bryan

Abstract

The widespread distribution of mercury (Hg) threatens wildlife health, particularly piscivorous birds. Western North America is a diverse region that provides critical habitat to many piscivorous bird species, and also has a well-documented history of Hg contamination from legacy mining and atmospheric deposition. The diversity of landscapes in the west limit the distribution of avian piscivore species, complicating broad comparisons across the region. Hg risk to avian piscivores was evaluated across the Western United States and Canada using a suite of avian piscivore species representing a variety of foraging strategies that together occur broadly across the region. Prey fish Hg concentrations were size-adjusted to the preferred size class of the diet for each avian piscivore (Bald Eagle = 36 cm, Osprey = 30 cm, Common and Yellow-billed Loon = 15 cm, Western and Clark's Grebe = 6 cm, and Belted Kingfisher = 5 cm) across each species' breeding range. Using a combination of field and lab-based studies on Hg effects in a variety of species, wet weight blood estimates were grouped into five relative risk categories including: background (<0.5 µg/g), low ($0.5 - 1$ µg/g), moderate ($1 - 2$ µg/g), high ($2 - 3$ µg/g), and extra high (>3 µg/g). These risk categories were used to estimate potential Hg risk to avian piscivores across the west at a 1 degree-by-1 degree grid cell resolution. Avian piscivores foraging on larger-sized fish generally were at higher relative risk to Hg. Habitats with relatively high risk included wetland complexes (e.g., prairie potholes in Saskatchewan), river deltas (e.g., San Francisco Bay, Puget Sound, Columbia River), and arid lands (Great Basin and central Arizona). These results indicate that more intensive avian piscivore sampling is needed across western North America to generate a more robust assessment of exposure risk.

Introduction

Avian piscivores (fish-eating birds) are valuable bioindicators of environmental health and exposure to aquatic contaminants, such as mercury (Hg) (Becker 2003, Scheuhammer et al. 2007). Their aquatic foraging habits and high trophic level place them among the most at-

risk wildlife groups with regard to Hg exposure (Scheuhammer et al. 2007). Published literature has demonstrated field-based Hg exposure and related effects in many species of avian piscivores (Evers et al. 2008a, Schoch et al. 2014, Ackerman et al. 2016b), and avian piscivores are key bioindicators for many long-term monitoring programs (Monteiro and Furness 1995, Weech et al. 2006, Burgess and Meyer 2008, Guigueno et al. 2012) and site-specific assessments (Weech et al. 2006, Guigueno et al. 2012). As part of a cross-national assessment of Hg cycling, distribution, and bioaccumulation (Western North America Mercury Synthesis), Hg risk to avian piscivores was assessed for Western North America – a region spanning north to Alaska, south to Arizona, west to the Pacific, and east to the Dakotas (see overview in Eagles-Smith et al. 2016a). Understanding Hg risk to avian piscivores across this large region is important for conservation of these sensitive species.

Efforts elsewhere to evaluate avian risk of Hg exposure across similarly broad geographic areas have relied on assessments of key prey fish species and often a wide distribution of only one target avian piscivore species (Depew et al. 2013). For example, the Common Loon (*Gavia immer*) and Yellow Perch (*Perca flavescens*) have been successfully used as effective bioindicators of risk in previous Hg syntheses in the Northeastern United States (Evers et al. 2008b), Great Lakes region (Evers et al. 2011) and across central and eastern Canada (Evers et al. 2005, 2011). In these studies, investigators mathematically transformed Hg concentrations of numerous fish species into single prey species equivalents to facilitate assessing risk to Common Loons across broad spatial extents. For those geographic regions, using a single widely distributed and well-studied sentinel species, (i.e., Common Loons) allowed for the identification of areas with greatest biological risk to Hg exposure (Evers et al. 2007).

Understanding risk to avian piscivores across Western North America is complicated by two important factors. First, Western North America covers a large diversity of freshwater and marine ecosystems, incorporating at least 19 bird conservation regions that ultimately support a diverse assemblage of avian piscivore species. Second, Hg distribution across Western North America is highly variable because of diverse landscape factors that facilitate methylation, an extensive mining history, and climatological variation that influences Hg transport and fate (Pinkney et al. 2015, Eagles-Smith et al. 2016a). The diverse landscape and climatological gradients of the West limit the geographical utility of single species assessments, because prey fish and avian piscivore assemblages vary greatly across the

region, which presents unrealistic assessments of risk to avian taxa that do not exist in certain regions. Limiting the analysis to only one species would not be representative of piscivores in this region, thereby necessitating the current approach.

The goal of this paper is to illustrate relative risk of Hg to freshwater avian piscivores across their Western North America breeding ranges. Avian piscivores were selected from freshwater systems that encompassed a range of foraging strategies, body sizes, and distributions; they include the Bald Eagle (*Haliaeetus leucocephalus*), Osprey (*Pandion haliaetus*), Belted Kingfisher *(Megaceryle alcyon)*, and two closely-related species groups: loons (Common, *Gavia immer* and Yellow-billed, *Gavia adamsii*) and grebes (Western, *Aechmophorus occidentalis* and Clark's, *Aechmophorus clarkii*). First, bird Hg concentrations in 20 common marine and freshwater avian piscivores were compared to better understand the Hg concentration ranges of the target freshwater piscivores across the study region. Because the geographic scope of the bird data was limited, the large fish monitoring and synthesis effort across the West (Eagles-Smith et al. 2016b) was leveraged to use fish Hg concentrations for modelling risk to the target avian piscivores in Western North America. This approach allows us to identify areas of concern for future research, and sets the context for understanding future changes to Hg bioaccumulation across this region. This approach can be used in the future across the entirety of North America to understand the location and patterns of biological Hg hotspots on a continental scale.

Materials and Methods

Fish and avian piscivore database compilation. Fish total Hg (THg) concentrations were compiled from numerous sources, summarized in more detail by Eagles-Smith et al. (2016b). Briefly, fish samples were compiled from federal, state, and provincial databases, constraining data to Alaska, Yukon Territories, Northwest Territories, British Columbia, Washington, Oregon, Idaho, California, Nevada, Arizona, New Mexico, Colorado, Utah, Wyoming, Montana, Alberta, Saskatchewan, North Dakota, and South Dakota. While the larger fish dataset included fish from Hawaii, for this analysis, Hawaiian and any marine fish were excluded. All fish THg concentrations were standardized to skinless, boneless fillet in wet weight. More information on these specific conversions can be found in Eagles-Smith et al. (2016b).

Contaminant databases that contained avian piscivore THg concentrations were also compiled, including data from the U.S. Fish and Wildlife Service Environmental Contaminants Data Management System (ECDMS), the Biodiversity Research Institute's Center for Mercury Studies, U.S. Geological Survey, Environment Canada, and the multi-partner Seabird Tissue Archival and Monitoring Project (STAMP). More extensive summaries of the bird datasets can be found in (Ackerman et al. 2016b). Hg concentrations were determined in established laboratories using a variety of instruments (e.g., ICP-MS, Direct Hg Analyzers) based on established standard operating procedures.

Avian Hg sample risk assessment. Risk assessment was focused on modeling five target piscivore species or closely related species groups: Bald Eagle, Osprey, Loons (Common and Yellow-billed) and Aechmophorus Grebes (Clark's and Western), and Belted Kingfisher (scientific names in Table 2.2). These species were chosen based upon their use in a variety of Hg monitoring projects and to represent a variety of foraging strategies and geographic areas (Table 2.1). To understand how these target piscivores compared to other bird species across western U.S. and Canada, avian piscivore tissue (whole blood, egg, and liver) THg concentrations for 20 commonly sampled species were compared across the west (Table 2.2). All tissue concentrations were converted to a whole blood equivalent THg concentration in order to standardize THg concentrations, and facilitate comparisons and risk categorization. For blood and liver samples, only adult birds were selected. To convert eggs to whole blood, the equation developed in Ackerman et al. (2016a) was applied to the egg data compiled for this paper (Eq. 1). Liver samples compiled for this paper were converted to whole blood using an equation developed by Eagles-Smith et al. (2008), which was derived from data for 4 species of waterbirds (Eq. 2).

Equation 1. (Ackerman et al. 2016a)
$$\ln(\text{Bird Blood THg}_{ww}) = (\ln(\text{Egg THg}_{fww}) + 0.75914)/0.9316$$
Equation 2. (Eagles-Smith et al. 2008)
$$\ln(\text{Bird Blood THg}_{ww}) = 0.970 * \ln(\text{liver THg}_{dw}) - 1.929$$

After converting THg in all tissues to the common unit of THg concentrations in whole blood, concentrations were compared across all 20 species (Figure 2.1). Although the sample size for each target species was relatively large (Table 2.2), they had relatively

constrained geographic distributions, making it difficult to estimate risk across their ranges (Figure 2.2). As a result, risk maps were developed based on models derived from fish THg concentrations.

Freshwater fish Hg sample risk assessment. The preferred prey fish size for each target piscivore species was determined using published literature (Table 2.1). For each avian piscivore species, the fish length that best fit their foraging preferences was used in the calculation of risk based upon prey fish THg concentration. Size adjustment of fish THg concentrations was conducted in R (version 3.1.0; www.r-project.org) and JMP (Version 12, SAS Institute Inc., Cary, NC) was used for all subsequent statistical analyses. Because THg concentrations often vary with fish length, the raw fish THg concentrations were size-adjusted to the preferred prey size of each piscivore (Table 2.1). For each target piscivore, the available fish data was constrained to only those species with individuals that fell within the birds prey size range, and where a likelihood ratio test suggested that the inclusion of length improved the fit of the data. Fish species with similar ecologies and physiologies were grouped into aggregate species for size-adjustment, because some species were poorly represented in the dataset (Eagles-Smith et al. 2016a). Species groups included in the dataset are shown in Table 2.2.

Sites were defined separately for lentic and lotic systems; fish locations in lentic systems were aggregated to the centroid of the water body, whereas fish locations in lotic systems were aggregated such that samples within 10 km of each other were categorized as originating from the same location. For each of these fish species groups, a linear mixed-effects model that contained fish total length as a fixed continuous factor; and site, fish species, and a species × total length interaction as random effects was used to predict the whole body total THg concentration of each fish at the preferred prey size of each piscivore (Bald Eagle = 36 cm, Osprey = 30 cm, Loons = 15 cm, Grebes = 6 cm, Belted Kingfisher = 5 cm; Table 2.1). For each predicted THg concentration, each individual fish's residual was added from the size-adjusted model to reincorporate variation not related to length into the size-adjusted THg concentrations for each individual fish. In some species, a likelihood ratio test indicated that the model including length did not fit the fish THg concentrations better than a null model; for these species, non size-adjusted, whole body THg concentrations were used.

The geospatial selection of fish samples for statistical analysis (using ArcGIS v 10.2.2) was conducted by constraining the dataset to only those locations that fell within each target piscivore's breeding range using species-specific range maps (Buehler 2000, Evers et al. 2010, Kelly et al. 2009, LaPorte et al. 2013, North 1994, Poole et al. 2002, Storer and Nuechterlein 1992). The study region— including the states and provinces listed previously—does not cover the entire North American range of any of these target avian piscivore species. Western and Clark's Grebes are mostly western species, and the study area covered 89% of their entire range. Other species had breeding distributions that expanded far beyond Western North America. For the Bald Eagle, the study area encompasses 78% of its breeding range. For the Osprey (found on all continents except Antarctica), the study area encompassed 72% of its North American breeding range. For the Belted Kingfisher, the study area covered only 69% of its breeding range. Common and Yellow-billed Loons had 78% of their North American breeding range within the western study area; Yellow-billed Loons also breed in Russia.

Grid cells (1-degree-by-1-degree) were created to cover the extent of the geographic range of each species to aid in creating species-specific risk maps. Grid cells have been used in previous synthesis efforts to illustrate risk across large geographic area with a wide range of representation of fish and bird THg concentrations (Evers et al. 2007, 2011). Grid cells covered multiple water bodies within a region and were drawn not based on state or provincial boundaries. Any grid cells with a low number of fish samples (n < 3) were excluded and results were classified based on the confidence in the estimate. Because each grid contains a different data density, confidence in the least squares mean was shown based on whether model-calculated standard error divided by the least squares mean was less than 0.25 (indicating low confidence in the least squares mean estimate). Because datasets for each avian piscivore were corrected for both fish that fell within their preferred size and within their breeding range, sample sizes of individual fish THg concentrations were different for each avian piscivore (Bald Eagle n = 56,096; Osprey n = 58,890; Loon n = 46,840; Grebe n = 46,203; Belted Kingfisher n = 54,673). By using this modeling approach, the fish dataset varies in species composition and sample size (Table 2.2).

All THg concentrations in fish samples were natural log (ln) transformed to improve normality of residuals. From this subset of data, the least squares mean prey fish THg concentration was modeled for each 1-degree-by-1-degree grid cell, including site and

species group as random effects. Back-transformed standard errors were estimated using the delta method (Seber 1982). In order to standardize the relative risk estimates, the least squares mean prey fish THg concentration in each grid was converted to the equivalent avian blood THg concentration using the global equation from Ackerman et al. 2015, which synthesized seven different study's equations (Eq. 3).

Equation 3. Ackerman et al. 2015

$$\ln(\text{Bird Blood THg}_{ww}) = 1.788 + 0.6182 * \ln(\text{Prey Fish THg}_{ww})$$

Important assumptions. The use of prey fish data THg concentrations to estimate avian THg exposure comes with four important assumptions. First, preferred prey size was used to model fish THg concentrations. Although using the preferred prey size likely provides an accurate estimate of typical risk to each piscivore, each species actually consumes variable sized fish depending on availability. Further, the size range of potential prey varies tremendously among avian piscivores (Table 2.1) suggesting that the variability in the predictions of risk may be underestimated more in some species in comparison to others. Bald Eagles, in particular, will eat much larger fish if scavenging dead prey, whereas Osprey are limited by the size of fish that they can carry (Buehler 2000, Poole et al. 2002). Second, the model also assumed that the birds were eating primarily fish. Loons and Osprey are known to focus on fish prey, but Bald Eagles often consume carrion or terrestrial prey not related to fish. Grebes and kingfishers will eat a variety of invertebrates in addition to small fish (Kelly et al. 2009; Storer and Nuechterlien, 1992; LaPorte et al. 2013), but prey fish THg concentrations are very highly correlated to grebe blood THg concentrations (Ackerman et al. 2015). Third, it was assumed that birds were eating the same fish species that were included within the fish Hg database. Although the size correction process likely accounted for much of this issue, it is still possible that avian piscivores select different fish species or sizes than those that are monitored by Hg monitoring programs. Foraging habitat also has a large impact on Hg exposure in fish and avian piscivores; this is partially taken into account by including site in the model for fish. Fourth, it is assumed that fish THg is correlated with bird blood THg and that this correlation is similar across species. Ackerman et al. (2015) synthesized 7 study's equations into one global equation which was used to correlate prey fish to bird Hg concentrations. Because published correlations between fish prey and bird Hg do not exist for all of the individual target piscivores, it is assumed that this global correlation is applicable

across species. While there may be relatively few studies that correlate prey fish to bird blood, the correlations that do exist are remarkably consistent across taxa and geographic areas, supporting the use of the global equation (Ackerman et al. 2015, Evers et al. 2011, Scheuhammer et al. 1998, Champoux et al. 2006, Burgess and Meyer 2008, Yu et al. 2011, Scheuhammer et al. 2016).

Hg Risk Categories for Avian Piscivores. Selection of risk benchmarks for avian piscivores is greatly complicated by substantial variation in sensitivity among taxa (Heinz et al. 2009), and limited data available on effects. A comprehensive assessment of risk benchmarks for birds based upon their THg concentrations is compiled elsewhere (Ackerman et al. 2016b) and their categorical approach was applied to standardize interpretation among papers. Blood THg concentrations (μg/g, ww) were classified into five categories that included: background (< 0.5 μg/g), generally below effects thresholds, low ($0.5 - 1$ μg/g) or elevated above background but below most effects thresholds, moderate ($1 - 2$ μg/g) or where adult physiological and behavioral abnormalities likely occur, high ($2 - 3$ μg/g) or likely reproductive impairment, to extra high (> 3 μg/g) or a high likelihood of significant reproductive failure.

Given the scale of this synthesis, it was most appropriate to use these broad categories, because more precise estimates may have limited basis for use across taxa. Although there are some studies of relative risk across different bird species (Heinz et al. 2009), there are few comprehensive benchmarks for the five target piscivores highlighted here. Although these risk categories approximately align with Hg risk thresholds developed for Common Loons (Depew et al. 2012), interpreting their meaning for the different species outlined in this study is complicated by uncertainty in interspecies differences. Thus, qualitative risk categories were presented to be used as a meaningful guide to evaluate concern at a population level.

Results

Avian piscivore Hg samples. THg concentrations in 4407 samples were compiled from avian piscivore tissues across western North America, and converted into blood Hg equivalent (μg/g, wet weight throughout) for comparison (Figure 2.1, Table 2.2). More than half of the bird samples (55%) fell into the background Hg risk category (0 μg/g – 0.5 μg/g), whereas 24% were in the low category (0.5 μg/g – 1 μg/g), 14% were in the moderate category (1 μg/g – 2 μg/g), 5% were in the high category (2 μg/g – 3 μg/g), and 2% were in

the extra high category (> 3 µg/g). While the majority of samples were less than 1 µg/g, individual species exhibited variation in the proportion of samples in each Hg category (Table 2.2). The species with the highest proportion of samples above 2 µg/g (high or extra high category) were Black-footed Albatross (75% above 2 µg/g), American White Pelican (25% above 2 µg/g), Common Loon (17% above 2 µg/g), Clark's Grebe (15% above 2 µg/g), Western Grebe (8% above 2 µg/g), Yellow-billed Loon (8% above 2 µg/g), and Least Tern (7% above 2 µg/g).

The geometric mean for all samples was 0.41 µg/g ww (SE = 0.01). On an individual species basis, geometric mean Hg concentrations in 11 of the 20 species were within the background Hg category (0 µg/g – 0.5 µg/g; Table 2.2). Black-footed Albatross had the highest geometric mean (± SE) concentration (2.72 µg/g ± 0.31) but also the lowest sample size (N = 16). American White Pelican samples had a geometric mean concentration of 1.02 µg/g ± 0.09 (moderate risk category), while seven other species exhibited geometric mean THg concentrations in the low Hg category (0.5 – 1 µg/g), including: Common Loon (0.91 µg/g ± 0.03), Clark's Grebe (0.90 µg/g ± 0.09), Least Tern (0.76 µg/g ± 0.4), Bald Eagle (0.69 µg/g ± 0.02), Laysan Albatross (0.65 µg/g ± 0.02), Yellow-billed Loon (0.64 µg/g ± 0.04), and Western Grebe (0.53 µg/g ± 0.03). Although the sample size for many of these species was relatively large (Table 2.2), the geographic distribution was quite constrained (Fig. 2.2). Therefore, the focused analysis is based upon predicted THg concentrations from the fish database, which provides a more robust spatial representation.

Fish Hg models. Although coverage was better than the bird dataset, the fish data did not cover the entire western breeding range of any of the avian piscivores. The amount of each species' western breeding range that was covered by the fish model was 44% for Bald Eagle, 51% for Osprey, 25% for loons, 79% for grebes, and 41% for Belted Kingfisher (Fig. 2.3). All THg concentrations are reported as µg/g, wet weight (ww) ± standard error. Fish size distributions are reported in cm ± standard error.

Bald Eagle: Fish data used to estimate Bald Eagle exposure (N = 56,096) was size corrected to 36 cm before the THg concentration of each fish was converted to its avian blood equivalent using Equation 1. Original fish total lengths ranged from 1.5 cm to 138 cm, with a mean of 45 cm ± 0.08. Blood THg concentrations derived from the Bald Eagle fish dataset ranged from 0.02 µg/g to 20.4 µg/g (geometric mean = 1.47 µg/g ± 0.004). The mixed effects model used to compute the least squares mean THg concentration of each grid cell had an

adjusted R^2 of 0.72, with a significant effect of grid cell ($F_{443,1382} = 3.7$, $P < 0.0001$). Least squares mean THg concentrations were estimated for 444 grid cells, ranging from 0.37 µg/g – 8.03 µg/g, with a mean THg concentration of 1.5 µg/g ± 0.03. The majority (71%) of grid cells with estimates that had high confidence (SE/mean < 0.25) fell into the moderate risk (1 – 2 µg/g) category, whereas 17% and 11% of grid cells fell into the low (0.5 – 1 µg/g) and high (2 – 3 µg/g) categories, respectively (Table 2.4). Relatively few grid cells were in the extra high (> 3 µg/g) range (1%). Based upon the grid maps, several geographic areas had relatively higher risk in comparison with the rest of the Bald Eagle range fish data occurred. High confidence grid cells greater than 2 µg/g occurred in the Great Basin (southeastern Oregon and southern Idaho) and the Prairie Pothole region (Saskatchewan). Other high confidence, greater than 2 µg/g grid cells occurred near Puget Sound, the Columbia River, Pinchi Lake region (British Columbia), central Arizona, and San Francisco Bay (Fig. 2.3a).

Osprey: Fish data used to estimate Osprey exposure (N = 58,890) was size corrected to 30 cm before each the THg concentration of each fish was converted to its avian blood equivalent using Equation 1. Original fish total lengths ranged from 1.5 cm to 138 cm, with a mean of 43.8 cm ± 0.08. Blood THg concentrations derived from the Osprey fish dataset ranged from 0.02 µg/g to 21.7 µg/g (geometric mean = 1.29 µg/g ± 0.003). The mixed effects model to compute the least squares mean of each grid cell for osprey prey fish had an adjusted R^2 of 0.70, with a significant effect of grid cell ($F_{489,1840} = 3.32$, $P < 0.0001$). Least squares mean THg concentrations were estimated for 490 grid cells, ranging from 0.33 µg/g to 4.57 µg/g, with a mean THg concentration of 1.31 µg/g ± 0.02 across all grid cells. A high proportion of grid cells fell into the 0.5 – 1 µg/g category (low risk, 21%) and 1 - 2 µg/g category (moderate risk, 71%) (Table 2.4). Parts of the Great Basin in Oregon, Idaho, and Nevada, along with the Prairie Pothole region of Saskatchewan contained areas of relatively higher risk in comparison to other geographic areas within their range (Fig. 2.3b).

Loon: Fish data used to estimate Loon exposure (N = 42,840) was size corrected to 15 cm before the THg concentration of each fish was converted to its avian blood equivalent using Equation 1. Original fish total lengths ranged from 1.5 cm to 138 cm, with a mean of 48.8 cm ± 0.08. Converted blood THg concentrations from the loon dataset ranged from 0.01 µg/g to 13.84 µg/g (geometric mean = 0.95 µg/g ± 0.003). The mixed effects model to compute the least squares mean per grid cell for loons had an adjusted R^2 of 0.56, with a significant effect of grid cell ($F_{340,941} = 2.75$, $P < 0.0001$). Least squares mean THg

concentrations were estimated for 341 grid cells, ranging from 0.22 µg/g to 3.05 µg/g, with a mean THg concentration of 0.90 µg/g ± 0.02. Most of the grid cells fell into the low (0.5 – 1 µg/g) and moderate risk (1 – 2 µg/g) categories (69% and 25%, respectively) with no grid exceeding the extra high risk threshold (> 3 µg/g; Table 2.4). Compared to Bald Eagle and Osprey, the loon map showed relatively fewer areas of high or extra high concern (> 2 µg/g). Only three grid cells along the Pacific Ocean in British Columbia were modeled to high risk categories (Fig. 2.3c).

Grebe: Fish data used to estimate Grebe exposure (N = 46,203) was size corrected to 6 cm before the THg concentration of each fish was converted to its avian blood equivalent using Equation 1. Original fish total lengths ranged from 1.7 cm to 104.6 cm, with a mean of 29.4 cm ± 0.08. Converted blood THg concentrations from the grebe dataset ranged from 0.01 µg/g to 11.72 µg/g (geometric mean = 0.92 µg/g ± 0.002). The mixed effects model to compute the least squares mean of each grid cell for grebes had an adjusted R^2 of 0.64, with a significant effect of grid cell ($F_{396,1957}$ = 3.06, P < 0.0001). Least squares mean THg concentrations were estimated for 397 grid cells, ranging from 0.21 µg/g to 4.1 µg/g with a mean THg concentration of 0.91 µg/g ± 0.02. Over 90% of the grids fell within the low and moderate risk category (65% within 0.5 – 1 µg/g, 30% within 1 - 2 µg/g, Table 2.4). This fish prey model indicated areas of concern in the Great Basin of Oregon and Nevada (Fig. 2.3d).

Belted Kingfisher: Fish data used to estimate Belted Kingfisher exposure (N = 54,673) was size corrected to 6 cm before the THg concentration of each fish was converted to its avian blood equivalent using Equation 1. Original fish total lengths ranged from 1.1 cm to 121.7 cm, with a mean of 32.7 cm ± 0.08. Converted blood THg concentrations from the Belted Kingfisher dataset ranged from 0.01 µg/g to 11.5 µg/g (geometric mean = 0.88 µg/g ± 0.002). The mixed effects model to compute the least squares mean of each grid cell for Belted Kingfisher had an adjusted R^2 of 0.64, with a significant effect of grid cell ($F_{519,2178}$ = 2.92, P < 0.0001). Least squares mean THg concentrations were estimated for 520 grid cells, ranging from 0.21 µg/g to 4.19 µg/g, with a mean THg concentration of 0.89 µg/g ± 0.02. Similar to grebes, over 90% of the grids fell within the low (0.5 – 1 µg/g) and moderate (1 – 2 µg/g) risk category (69% and 26%, respectively, Table 2.4). The risk map for kingfisher prey fish was similar to that for grebes, with increased risk in the Great Basin (Fig. 2.3e) and additional areas with low confidence in British Columbia.

Discussion

      Comparison to previous Hg synthesis work. In this study, Hg risk to multiple avian piscivores was assessed across a broad geographic area – the western United States and Canada. Unlike previous synthesis efforts that focused on a single avian piscivore and prey species for assessing risk from environmental Hg loads (Evers et al. 2007, 2011), there was not a single bird species that could be effectively used across the region. Instead, the approach targeted five breeding avian piscivores that collectively span the majority of the freshwater habitats occurring within western North America. The use of fish Hg concentrations to predict risk to avian piscivores had several advantages. First, fish are the most widely measured taxonomic group for Hg exposure. Second, there are numerous national-, state-, and provincial-scale fish Hg monitoring programs used as a basis for fish consumption guidelines for human health. Third, fish Hg concentrations can be converted to avian piscivore risk. Other papers within this special issue used either fish (Eagles-Smith et al. 2016b, Lepak et al. 2016) or bird (Ackerman et al. 2016b) Hg concentrations to characterize risk across the study region. By leveraging the established strong linkages between prey fish and avian piscivore Hg concentrations (Ackerman et al. 2015), risk for 5 target avian piscivores was characterized at a finer scale than piscivorous bird data alone would allow. Many areas in Western North America remain uncharacterized and using fish Hg concentrations as a proxy for bird Hg exposure allows for an examination of risk at a landscape level to better understand the spatial patterns of biological Hg hotspots.

      In freshwater ecosystems, the positive modeled relationship between fish length and Hg concentration demonstrated that avian piscivores that consume larger fish were classified into higher Hg categories across their breeding range (Table 2.4). Proportion of grid cells above 1.0 µg/g declined from 83% for Bald Eagle and 77% for Osprey to 26% for loons, 31% for grebes, and 27% for kingfishers. While these Hg categories are helpful for comparing risk on a standardized scale, this does not necessarily mean that smaller avian piscivores are not at risk. For example, in San Francisco Bay, Forster's Terns and invertivorous Black-necked Stilts had similar or higher THg concentrations than Caspian Terns despite the fact that Caspian Terns feed on larger fish at a higher trophic position (Eagles-Smith et al. 2009). Space use data showed that Forster's Terns and stilts selected foraging habitats with much higher baseline Hg concentrations than did Caspian Terns (Ackerman et al. 2007, 2008).

In addition to variation in habitat drivers of differential Hg bioaccumulation that can put birds that eat smaller fish at risk, there is also tremendous variation in the sensitivity of different avian species that can complicate toxicological risk (Heinz et al. 2009). Aside from the thresholds developed for Common Loons, there are few field-based effects thresholds that are well-developed for other bird species. Artificially incubated eggs dosed with Hg have shown that species have wide variation in their sensitivities to Hg, based on hatching success (Heinz et al. 2009, Braune et al. 2012). In a comparative egg dosing study of 21 bird species by Heinz et al. (2009), Osprey were among the most sensitive species to Hg in egg injections, which might translate to lower effects thresholds than presented here. Comparatively, loons are moderately sensitive (Kenow et al. 2011, based on similar protocols as Heinz et al. 2009). However, it is widely acknowledged that the MeHg injected into eggs is likely more toxic than maternally derived Hg, complicating the application of these effects benchmarks to successful reproduction in the wild, where parental incubation behavior and ability to catch prey (and subsequent ability to raise young) may be impaired by Hg contamination.

Ecosystems and regions of Hg concern. Although the focus of the analysis did not identify conclusive "biological Hg hotspots" as were developed in previous synthesis efforts in the Northeast (Evers et al. 2007) and Great Lakes (Evers et al. 2011), the results do show certain areas of concern for multiple avian piscivores. To better visualize the results, grid cells across all target species that were in the moderate category or above (> 1 µg/g) were compiled (Fig. 2.4). Some regions highlighted in the risk map have known sources of elevated Hg. For example, the Pinchi Lake region has been well characterized as an area with elevated environmental Hg loads due to cinnabar deposits and mining (Weech et al. 2004). Similarly, fish and birds in the Carson River complex in Nevada have well-documented elevated Hg concentrations as a result of Hg from legacy mining transported to wetland areas with high methylmercury production capacity (Henny et al. 2002, Hill et al. 2008, Seiler et al. 2004).

Several major river-fed systems across western North America were isolated as areas of moderate or high relative risk for multiple piscivores (e.g., Columbia River, Puget Sound, San Francisco Bay). San Francisco Bay in particular appears to be an area with highly elevated biotic Hg concentrations that are related to legacy mining activities and methylating habitats (Ackerman et al. 2008, Eagles-Smith 2008, 2009). Relatively elevated Hg concentrations were observed in areas of the Puget Sound and Columbia River; however, no

direct estimates exist regarding specific source in these systems. Models have suggested that delta regions of rivers associated with marine ecosystems can be affected by deposition of watershed transport of Hg, with climate change projections suggesting increased methylation rates and Hg releases (Amos et al. 2014, Fisher et al. 2012).

The Prairie Pothole region in Canada (particularly in southern Saskatchewan) was shown to have moderate or high relative risk for multiple piscivores. The Prairie Pothole region is a unique system with relatively high methylation potential due to high density of wetlands (Hall et al. 2009, Bates and Hall 2012). Although relatively few contaminant studies with avian bioindicators have occurred there (Allen et al. 1998, Winder et al. 2011, Fox et al. 2005), the Prairie Potholes is one of the largest waterfowl producing areas in North America making further study of Hg in this area critically important (Greenwood et al. 1995). This study also highlighted two arid land ecosystems: central Arizona and the Great Basin in Idaho, Oregon and Nevada. Parts of the Great Basin also are identified using only bird Hg concentrations (Ackerman et al. 2016b). Different areas of this large region have been studied previously because of concerns from legacy mining Hg use (Henny et al. 2002) or risk to waterfowl (i.e., the Great Salt Lake; Vest et al. 2009), but the area also supports a vast network of seasonal and permanent wetlands (Sada and Vinyard 2002) that may enhance methylmercury potential. A more integrated monitoring is needed across the region to better understand causality. New predictions of increased Hg deposition in the western United States that likely originate from increased global Hg emissions (Weiss-Penzias et al. 2016) also indicates that ecosystems sensitive to Hg input need to be monitored to track potential increases of biotic Hg body burdens.

Conclusions. Results highlight the need for future monitoring of Hg exposure in both birds and fish across North America. Compared to the risk maps created using bird Hg concentrations (Ackerman et al. 2016b), our results using prey fish identified additional areas of potential concern where bird sampling has not occurred. Although using fish Hg concentrations allows more extensive spatial coverage than would the use of only bird tissue sampling, the ability to draw conclusions about the overall Hg risk to each bird species was limited because of uncertainties related to the sensitivities of birds to Hg (Heinz et al. 2009). Although fish Hg concentrations covered from 25% to 79% of the western breeding ranges of the target piscivores, better geographic coverage and a defined sampling design is needed to fully evaluate the proportion of the breeding population impacted. Using the approach within

this paper, it is important to next examine Hg concentrations in prey fish and associated target avian piscivores across their entire North American breeding ranges. Standardized continental comparisons by Evers et al. (1998) for Common Loons found a significant declining west to east gradient of loon Hg concentrations, but this has not been established for other avian piscivores. Due to the climatological gradients of western United States and Canada, many aquatic-specialist bird species concentrate in relatively small areas to breed and forage, making those areas potentially of higher conservation concern. The next step for assessing risk of environmental Hg loads to avian piscivores is to understand their breeding density areas across the broad and varied ecosystems that they occupy.

An approach that can scale the risk of Hg to breeding populations of avian piscivores across a mosaic of ecosystems at continental levels is important for assessing potential impacts at global levels. In October 2013, 128 countries signed a new global treaty called the Minamata Convention (UNEP 2013) with the overall objective to "protect the human health and the environment from anthropogenic emissions and releases of mercury and mercury compounds." This Convention will be instrumental for reducing the use and release of Hg into the environment and metrics are being developed that could be used for evaluating its effectiveness (Evers et al. 2016). As such, one metric will likely be based on biotic Hg concentrations from key bioindicators. In the Convention (Article 19), "geographically representative monitoring of Hg and Hg compounds in vulnerable populations and in environmental media, including biotic media such as fish, marine mammals, sea turtles, and birds" is needed by the Parties to develop a plan for assessing improvements in ecological and human health. Monitoring strategies for Hg in fish, birds, marine mammals and other biota have been developed (Mason et al. 2005; Evers et al. 2008b) and inclusion of avian piscivores as sensitive and high-profile bioindicators is important for identifying biological Hg hotspots and tracking trends of environmental Hg loads over time. The results from the assessment of potential impacts from Hg on avian piscivores in Western North America will help meet the demands for evaluating the effectiveness of the Minamata Convention.

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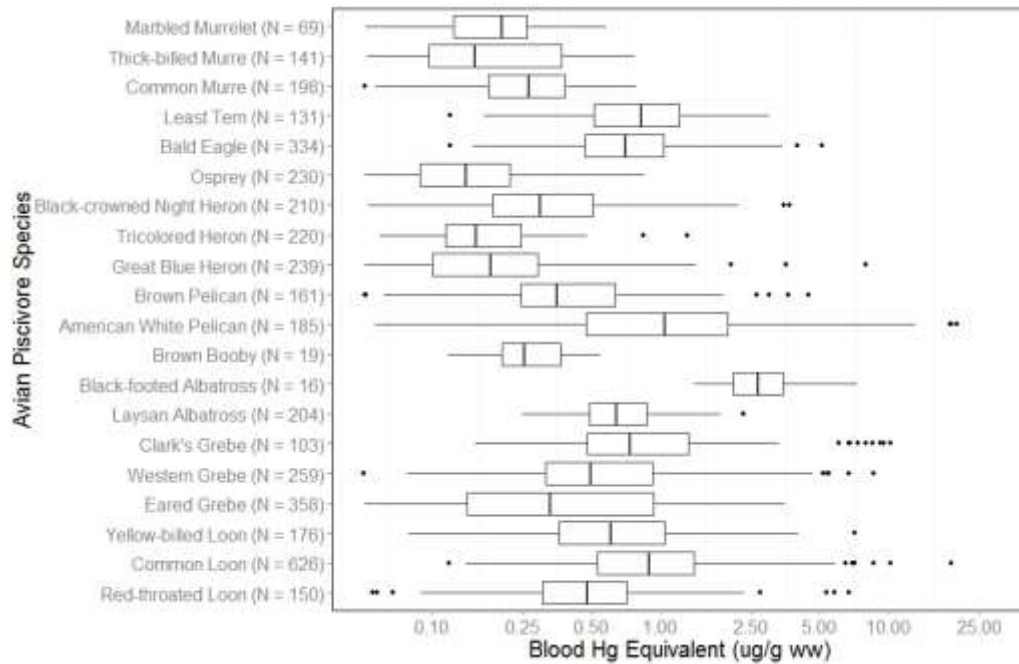
Figure 2.1. Whole blood Hg (µg/g wet weight) comparison across 20 avian piscivores in western United States and Canada. Birds (including sample size) are shown in taxonomic order. Egg and liver samples were converted into whole blood equivalent to aid in comparison using Equation 1 and 2 (see text).
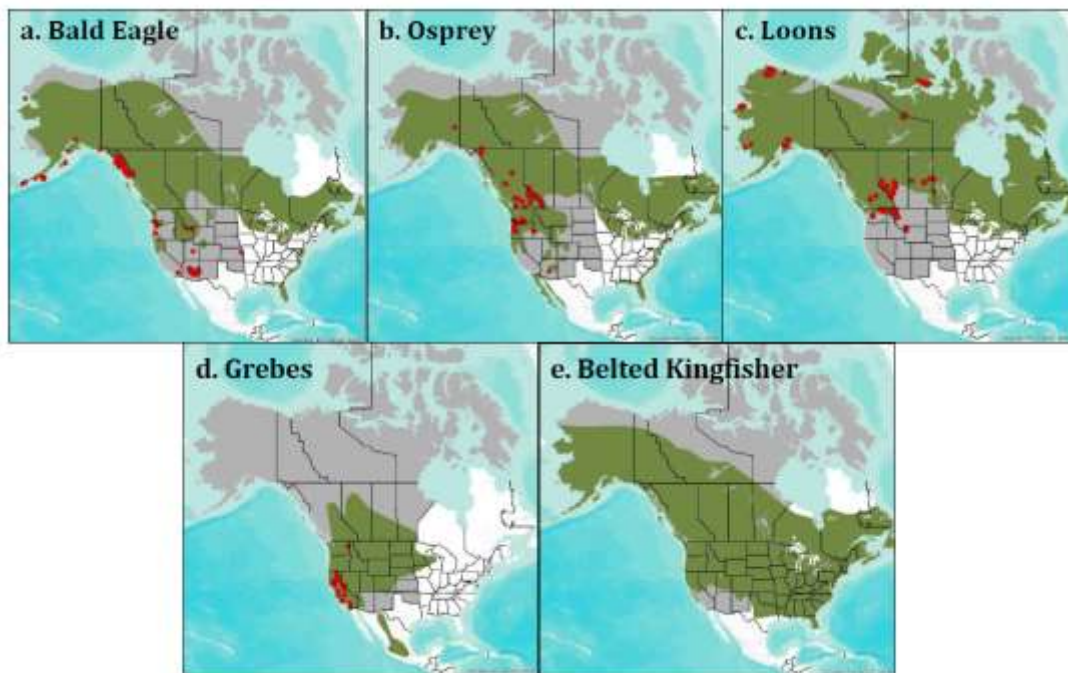
Figure 2.2. Distribution of avian samples (blood, egg, and liver combined) for target piscivores across their breeding ranges in western United States and Canada. The study area in western United States and Canada is shown in gray. Each species breeding range is show in green. Loons include Common Loons and Yellow-billed Loons (c). Grebes include Western Grebes and Clark's Grebes (d). There were no bird data for Belted Kingfishers (e).
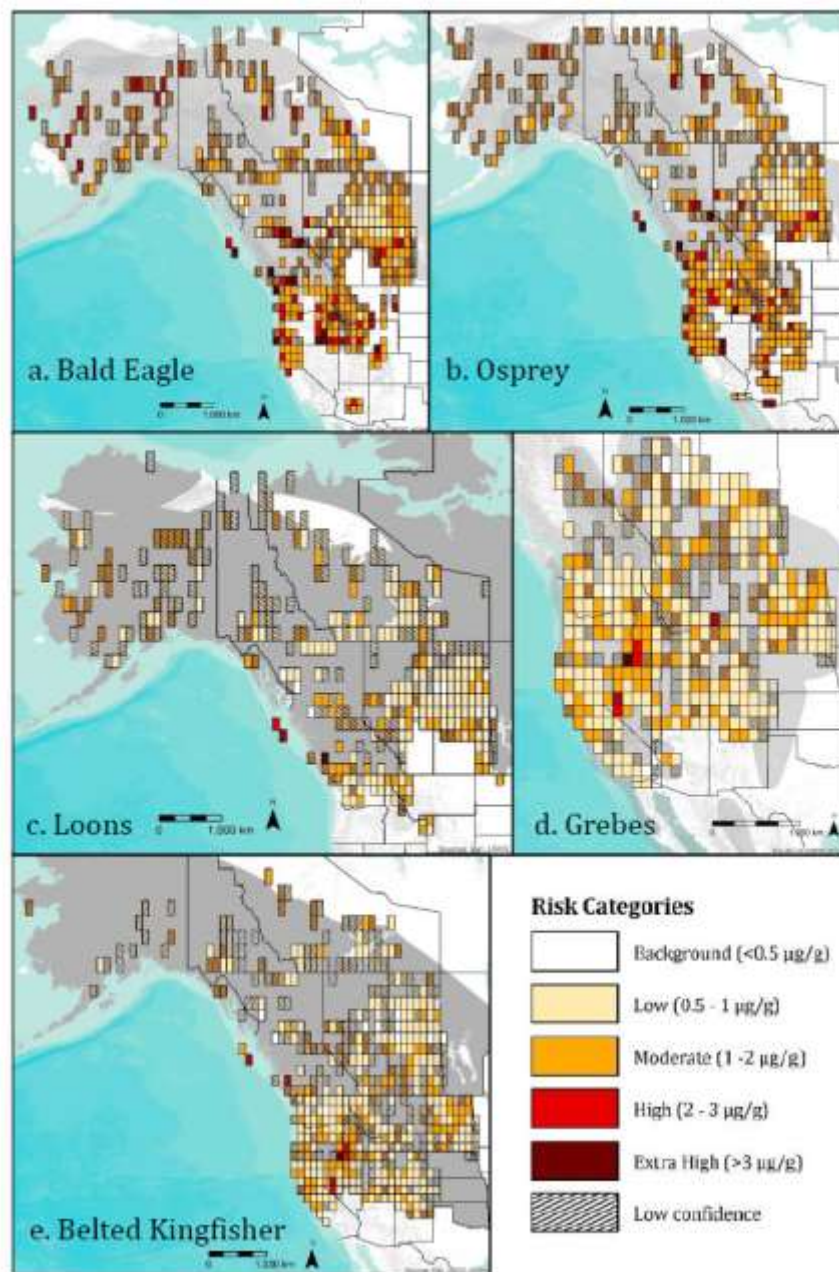
Figure 2.3. Relative Hg risk per 1 degree grid cell across the North American breeding range of five target freshwater piscivore species: a) Bald Eagle, b) Osprey, c) Loons (Common and Yellow-billed), d) Grebes (Clark's and Western), and e) Belted Kingfisher. The prey fish were size-corrected according to the preference of each species: Bald Eagle = 36 cm, Osprey = 30 cm, Loons = 15 cm, Grebes = 6 cm, Belted Kingfisher = 5 cm. All Hg concentrations have been converted to wet weight whole bird blood for comparison. Low confidence is estimated as any grid cell where the standard error is more than one quarter of the mean.
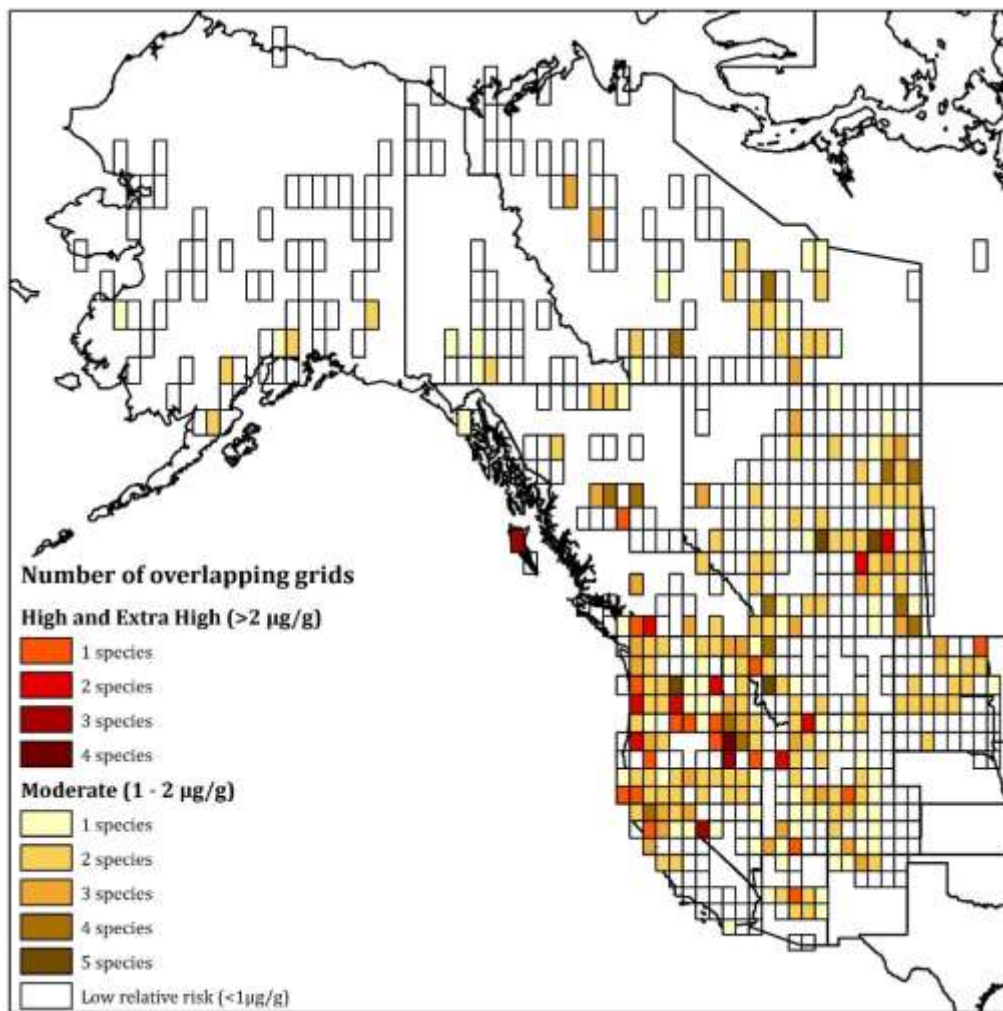
Figure 2.4. Areas of western United States and Canada where fish modeling indicates high confidence in moderate or higher relative Hg risk. Light colors indicate fewer species with overlapping risk. Empty grid cells indicate areas where fish data was modeled but risk was estimated to be low. Areas not covered by grid cells either have least square mean estimates with low confidence or no fish data to use in the model.

Table 2.1. Characteristics of target avian piscivore species or species groups. For consistency, piscivores from each ecosystem are shown in order from large to small prey.

Species or group	Foraging Habits during Breeding Season	Fish Size Range	Fish Size Preference
Bald Eagle[a]	Opportunistic forager (active fishing and scavenger)	20-75 cm	36 cm
Osprey[b]	Obligate piscivore, limited by weight of fish to carry	10-45 cm	30 cm
Loons[c]	Obligate piscivore, limited by size able to swallow	10-25 cm	15 cm
Grebes[d]	Small fish specialist (also some invertebrates)	2-15 cm	6 cm
Belted Kingfisher[e]	Small fish specialist (also some invertebrates)	1-10 cm	5 cm

[a] (Buehler 2000)

[b] (Poole et al. 2002)

[c] Common Loon (Evers et al. 2010); Yellow-billed Loon (North 1994)

[d] Clark's Grebe, Western Grebe, (Ackerman et al. 2015)

[e] (Kelly et al. 2009)

Table 2.2. Geometric mean blood Hg (equivalent) concentrations for 20 avian piscivore species in taxonomic order. Liver and egg tissues were standardized to whole blood equivalent (µg/g, ww).

Avian Piscivore Species	N	Mean Hg (µg/g)	Standard Error	Percent of samples in each Hg category				
				Background 0 - 0.5 (µg/g)	Low 0.5 - 1 (µg/g)	Moderate 1 -2 (µg/g)	High 2 - 3 (µg/g)	Extra High > 3 (µg/g)
Marbled Murrelet *Brachyramphus marmoratus*	69	0.19	0.01	97%	3%	0%	0%	0%
Thick-billed Murre *Uria lomvia*	141	0.12	0.01	94%	6%	0%	0%	0%
Common Murre *Uria aalge*	198	0.18	0.01	92%	8%	0%	0%	0%
Least Tern *Sternula antillarum*	131	0.76	0.04	24%	43%	27%	7%	0%
Bald Eagle *Haliaeetus leucocephalus*	334	0.69	0.02	30%	44%	22%	4%	0%
Osprey *Pandion haliaetus*	229	0.12	0.01	98%	2%	0%	0%	0%
Black-crowned Night Heron *Nycticorax nycticorax*	210	0.29	0.02	75%	16%	7%	1%	0%
Tricolored Heron *Egretta tricolor*	220	0.15	0.01	99%	0%	0%	0%	0%
Great Blue Heron *Ardea herodias*	238	0.19	0.01	90%	7%	2%	1%	0%
Brown Pelican *Pelecanus occidentalis*	161	0.35	0.02	68%	23%	6%	2%	1%
American White Pelican *Pelecanus erythrorhynchos*	185	1.02	0.09	28%	21%	26%	14%	11%
Brown Booby *Sula leucogaster*	19	0.27	0.03	84%	16%	0%	0%	0%

Table 2.2 (continued)

Avian Piscivore Species	N	Mean Hg (µg/g)	Standard Error	Percent of samples in each Hg category				
				Background 0 - 0.5 (µg/g)	Low 0.5 - 1 (µg/g)	Moderate 1 -2 (µg/g)	High 2 - 3 (µg/g)	Extra High > 3 (µg/g)
Black-footed Albatross *Phoebastria nigripes*	16	2.72	0.31	0%	0%	25%	56%	19%
Laysan Albatross *Phoebastria immutabilis*	204	0.65	0.02	26%	56%	17%	0%	0%

Table 2.3. Sample size for fish species groups used in each avian piscivore Hg risk model. Sample sizes vary for each piscivore because our modeling process only included fish species groups if they fell within the range preferred by avian piscivores. All fish Hg concentrations used in models were size adjusted to the preferred size of each piscivore (size shown in parenthesis in heading).

Fish Species Group	Bald Eagle (36cm)	Osprey (30cm)	Loons (15cm)	Grebes (6cm)	Belted Kingfisher (5cm)	Fish Species Total
Anadromous salmonid	288	335				623
Arctic grayling	263	274	284			821
Black basses	2744	3817	770	6015	4953	18299
Burbot	821	821	636			2278
Carp	355	533	85	1054	909	2936
catfishes	540	828	185	1845	1780	5178
Char	5001	5811	4479	2057	5473	22821
Chub	224	247	39	327	342	1179
Cichlids		8		7		15
Cisco	245	232	265			742
Clupeiformes	3	4		8	4	19
Crappie	251	325	24	625	605	1830
Freshwater drum	24		24			48
Goldeye	1154	1236	1053			3443
Killifish				3948	4169	8117
Minnows				1952	1932	3884
Morone bass	29	193				222
Pike	13995	13303	12853			40151
Pikeminnow	1844	1899	226	1905	1913	7787

Table 2.3 (continued)

Fish Species Group	Bald Eagle (36cm)	Osprey (30cm)	Loons (15cm)	Grebes (6cm)	Belted Kingfisher (5cm)	Fish Species Total
Sculpin				184	243	427
Stickleback				412	415	827
Suckers	3482	3585	1913	3554	4221	16755
Sunfishes	318	545	74	847	797	2581
Trout	3798	5681	1777	6189	6506	23951
Walleye/Sauger	13894	12437	12016	11337	17829	67513
Whitefish	5065	5334	4889	1561		16849
Yellow perch	1758	1442	1248	2376	2582	9406
Total	56096	58890	42840	46203	54673	258702

Table 2.4. Percentage of grid cells assigned to each risk category for each freshwater avian piscivore. High confidence grids had standard error that was within one quarter of the mean. All grids includes both those grids with high and low confidence (total dataset).

Species	Subset of Data	Background (< 0.5 µg/g)	Low (0.5 - 1 µg/g)	Moderate (1 - 2 µg/g)	High (2 - 3 µg/g)	Extra High (> 3 µg/g)	Total
Bald Eagle	High Confidence	1 (0%)	35 (17%)	148 (71%)	22 (11%)	3 (1%)	209
	All grid cells	3 (1%)	85 (19%)	294 (66%)	50 (11%)	12 (3%)	444
Osprey	High Confidence	3 (1%)	54 (21%)	182 (71%)	14 (5%)	2 (1%)	255
	All grid cells	7 (1%)	121 (25%)	323 (66%)	32 (7%)	7 (1%)	490
Loons	High Confidence	9 (5%)	125 (69%)	46 (25%)	1 (1%)	0 (0%)	181
	All grid cells	23 (7%)	218 (64%)	97 (28%)	2 (1%)	1 (0%)	341
Grebes	High Confidence	8 (3%)	176 (65%)	82 (30%)	3 (1%)	0 (0%)	269
	All grid cells	22 (6%)	252 (63%)	117 (29%)	5 (1%)	1 (0%)	397
Belted Kingfisher	High Confidence	11 (4%)	211 (69%)	81 (26%)	3 (1%)	0 (0%)	306
	All grid cells	36 (7%)	339 (65%)	138 (27%)	6 (1%)	1 (0%)	520

CHAPTER 3. REGIONAL DIFFERENCES IN MERCURY EXPOSURE IN AQUATIC INVERTEBRATES AND RIPARIAN SONGBIRDS ACROSS A RIVER-RESERVOIR SYSTEM WITH A LEGACY OF MERCURY CONTAMINATION

Allyson K. Jackson, Collin Eagles-Smith, and Colleen Emery

Abstract

Mercury (Hg) loading and methylation in aquatic systems causes a variety of deleterious effects for fish and wildlife populations. Relatively little research has focused on Hg movement into the riparian food web and how this is mitigated by habitat characteristics. This study characterized differences in Hg exposure in aquatic invertebrates and riparian songbirds across a large portion of the Willamette River system in western Oregon, starting at a Hg contaminated site in the headwaters (Black Butte Hg Mine) and including a reservoir known to methylate Hg (Cottage Grove Reservoir), all downstream reaches (Coast Fork and Willamette River) and off-channel wetland complexes (Willamette Valley National Wildlife Refuge Complex). After accounting for year, date, and site differences in a mixed effects model, MeHg in aquatic invertebrates varied spatially by Willamette region and taxonomically by invertebrate order. Similarly, THg in songbird blood varied by Willamette region, species, and age (adult or juvenile). The highest Hg concentrations occurred near the Hg mine, but Hg did not decline linearly with distance from the source of contamination. Birds were consistently elevated in Hg in habitats known to methylate Hg, such as backwater or wetlands. We found a positive but weak correlation between invertebrate MeHg and songbird THg on a site specific basis. We found stronger correlations between individual invertebrate orders (plecoptera and gastropoda) and Song Sparrow (*Melospiza melodia*) blood THg concentrations on a site-specific basis. Our findings indicate that areas of concern for songbirds in the Willamette Valley are not confined to the area of contamination near the Black Butte Hg mine or Cottage Grove reservoir but instead extends into the main stem Willamette River and Willamette Valley National Wildlife Refuges complexes.

Introduction

Human development has caused a variety of anthropogenic changes in land use, biogeochemical cycling, and biota (Vitousek 1997). Almost all freshwater ecosystems have

been structurally modified by human development in some way, either becoming channelized to make them more navigable and stable or dammed for hydroelectric power, recreational reservoir creation and flood or drought control (Payne and Baker 2002, Ormerod et al. 2010). Chemically, anthropogenic inputs of waste water, fertilizer and run-off have altered nutrient cycling and subsequent food web structure. Similarly, human development has caused release of heavy metal contaminants, such as mercury (Hg) into aquatic systems. These separate anthropogenic stressors can combine to produce unexpected and sometimes large effects (Ormerod et al. 2010).

Mercury is a persistent environmental contaminant that efficiently bioaccumulates through food webs in its organic form, methylmercury (MeHg), and can cause deleterious effects to fish and wildlife populations at elevated concentrations (Scheuhammer et al. 2007). Research has generally focused on fish and fish-eating species, because Hg methylation (and subsequent uptake by organisms) occurs primarily in aquatic systems. However, loading of inorganic Hg into a system is not usually a strong predictor of Hg exposure in fish and wildlife, instead its movement into the food web is linked to Hg methylation rates. Methylation rates are driven by habitat characteristics, which modulate the subsequent exposure in animals (Chasar et al. 2009). Wetlands, with their wetting-drying cycles and high dissolved organic carbon, are known to drive high methylation rates (Ravichandran 2004). Reservoir creation often increases Hg methylation because of the water-level fluctuations, drying cycles, and likely the high carbon content of the flooded vegetation (Eckley et al. 2015, Willacker et al. 2016).

Dam and reservoir creation not only impact aquatic systems, but have dramatically altered riparian ecosystems as well (Nilsson and Berggren 2000). Similarly, despite methylation occurring primarily in aquatic habitats, Hg can move into surrounding riparian areas as well (Cristol et al. 2008, Jackson et al. 2011b). Riparian songbirds living in contaminated areas may be exposed to high levels of Hg (Cristol et al. 2008). While some Hg methylation has been documented in terrestrial systems (Townsend et al. 2014), it is believed that the primary mechanism for moving aquatic contaminants from the aquatic to the terrestrial ecosystem is the emergence of aquatic invertebrates (Walters et al. 2008, 2010). These emergent aquatic insects become the prey base for riparian invertebrates and vertebrates, and thus move aquatic-based Hg into the terrestrial food web.

Despite the wealth of information about Hg methylation and bioaccumulation in aquatic systems, relatively little is still known about riparian songbirds at the landscape scale and how their Hg concentrations correlate with aquatic invertebrate MeHg concentrations. Songbirds may be uniquely sensitive to the effects of Hg on reproduction (Jackson et al. 2011a). In this study, we examined Hg exposure in riparian songbirds throughout a river-reservoir system with legacy Hg contamination to better understand the spatial variability associated with gradients in habitat type. Our objectives were four-fold. First, we compared Hg concentrations in aquatic invertebrates and songbirds among six regions of the larger Willamette basin: (1) headwater streams with a history of Hg mining and contamination, (2) a man-made reservoir downstream of the Hg mine, (3) Coast Fork Willamette River and (4) main channel and (5) backwater habitats of the Willamette River, and (6) wetlands in the Willamette Valley National Wildlife Refuge complex. Second, in the Willamette River proper, we examined whether Hg concentrations in aquatic invertebrates and riparian songbirds varied longitudinally or among distinct river segments (upper, middle, and lower). Third, we examined the relationship between MeHg concentrations in aquatic invertebrates with THg concentrations for riparian songbirds at a site-specific scale. Finally, we correlated Hg concentrations in the most well-sampled songbird species (Song Sparrow, *Melospiza melodia*) with MeHg concentrations in individual orders of aquatic invertebrates, to quantify which aquatic invertebrates best predict Song Sparrow Hg exposure.

Methods

Study site. The Willamette River (Oregon, USA) is a major tributary to the Columbia River. It is a relatively small river (~300 km total length) but ranks 19th in the US in terms of discharge volume. The river flows south to north through the Willamette Valley in western Oregon. Three of the largest cities in Oregon occur along the river: Eugene, Salem and Portland. The basin is in the Pacific Coastal Rainforest, which is characterized by wet winter and dry summers. In fact, summer precipitation accounts for only about 5% of the annual average total (Payne and Baker 2002). Historically, the river channel consisted of a series of braided channels with variable water flow depending on snowmelt and rainfall. From 1941 – 1969, the Army Corps of Engineers built 11 major water storage reservoirs in the Willamette Basin, to aid in irrigation, generate electricity and control floods (Payne and Baker 2002). The headwaters of the Willamette River lie in the Cascades Range, which has naturally

occurring Hg and gold deposits, both of which have been mined over the past 150 years. Despite the relatively short extent of the river, it is comprised of a variety of habitat types and extends through several ecoregions (Fig. 3.1).

In order to evaluate spatial and habitat variation in invertebrate and riparian songbird Hg concentrations we partitioned our sampling locations into six distinct categories (Fig. 3.1). The most upstream site was the Black Butte mine area (headwater streams in Western Cascades Lowlands and Valleys ecoregion, hereafter Black Butte), which was listed as an EPA Superfund site in 2010 (Eckley et al. 2015). Approximately 24 km downstream of Black Butte is the Cottage Grove reservoir (reservoir in Valley Foothills ecoregion, hereafter C.G. reservoir). The Coast Fork Willamette River (tributary of Willamette River in Valley foothills or Willamette River and Tributaries gallery forest, hereafter Coast Fork) flows out from the below the Cottage Grove dam to its confluence with the Middle River near Eugene to form the main stem Willamette River. We divided the Willamette River into backwater sites (hereafter W.R. backwater) and main channel Willamette River sites (hereafter W.R. main), (both Willamette River and Tributaries gallery forest). We also surveyed wetland complexes in the Willamette Valley National Wildlife Refuge system (in the Prairie terraces ecoregion, hereafter NWR wetland). Site characteristics are listed in Table 3.1.

Field sampling. We sampled aquatic invertebrates and riparian songbirds in 2013 and 2014. Aquatic invertebrates were collected via dip net in sections of the water body that could be waded. Invertebrates were placed in glass vials on ice in the field and frozen within 6 hours. Riparian songbirds were caught via mist net using playback recording of conspecific songs. Birds were banded with a USGS band and blood samples were taken from the brachial vein. Morphometric measurements of unflattened wing chord, tail length, tarsus, bill (nares to tip), and mass were taken before the bird was released at the same site where it was captured. All samples were collected under authority of appropriate scientific collection permits, including both State (invertebrates: Oregon DFW# 17648; birds: Oregon DFW# 062-13) and Federal (USFWS MBTA# MB28361A; USGS Banding # 20786) agencies. All birds were handled under approved animal care and use protocols (Oregon State University ACUP # 4408).

Lab analyses. In the lab, invertebrates from each site were sorted into families, rinsed three times with deionized water and composited into separate glass vials for each family at each site. The invertebrate samples were dried for a minimum of 48 hours at 50°C. They were

then ground with either a glass rod (primarily) or mortar and pestle (for large or especially hard samples) to homogenize all samples.

We analyzed MeHg concentrations in all invertebrate samples following EPA method 1630 (U.S. Environmental Protection Agency, 2001) at the USGS Forest and Rangeland Ecosystem Science Center. Briefly, 2-10 mg of dried homogenate was digested in 3-4ml 30% nitric acid at 60°C overnight (~15 hours), ethylated with 1% sodium tetraethylborate, then analyzed via cold-vapor atomic fluorescence spectrometry on a MERX-M (Brooks Rand Instruments, Seattle, Washington, USA) automated methylmercury analyzer. Quality assurance measures included analysis of two independently derived liquid calibration standards, two certified reference materials (scallop tissue [IAEA-452; International Atomic Energy Agency, Vienna, Austria], and lobster hepatopancreas [TORT-3; National Research Council of Canada, Ottawa, Canada]). Percent recoveries averaged 99% (SD = 8%) for 10pg MeHg standard, 96% (SD = 13%) for 100pg MeHg standard, 99% (SD = 21%) for IAEA-452, and 86% (SD = 5%) for TORT-3.

Songbird whole blood samples were not composited, but instead run on an individual basis as dried blood samples. Each sample was weighed onto a quartz filter and the wet weight was recorded before the sample was dried for a minimum of 48 hours at 50°C. Samples were returned to room temperature in a desiccator before dry weight was recorded. Blood samples were analyzed for total Hg following US EPA method 7473 using either a Milestone tri-cell DMA-80 Direct Hg Analyzer (Milestone, Shelton, Connecticut USA) or a Nippon MA-3000 Hg Analyzer (Nippon Instruments, College Station, Texas, USA) at the USGS Contaminant Ecology Research Lab in Corvallis OR. Certified reference material (dogfish muscle tissue [DORM-4; National Research Council of Canada, Ottawa Canada] and lobster hepatopancreas tissue [TORT-2; TORT-3; National Research Council of Canada, Ottawa Canada]), calibration verification (liquid standards), CRM duplicates, air blanks and boat blanks were included with each run. Milestone QA/QC included recoveries of 99.8% (SD=8.4%, N = 67) for calibration verification, recovery of 98% (SE = 11.5%, N = 100) for certified reference material and absolute percent difference of 2% (SD = 3%, N = 43) for duplicates. Nippon recoveries averaged 100% (SD = 5%, N = 15) for calibration verification and 96% (SD = 3%, N = 34) for certified reference materials.

Statistical analyses. We used mixed effects general linear models in a tiered fashion to evaluate the spatial patterns of Hg concentrations at different scales through the study area.

At the broadest scale, we examined variation in Hg concentrations among coarse habitat types across broad regions of the Willamette Valley. We then examined linear patterns in Hg concentrations along the main stem Willamette River through the valley floor. Finally, we assessed the relationship between site-specific MeHg concentrations in invertebrates and THg concentrations in songbirds across all sampling locations. All statistical modeling was conducted with Program R (Version 3.3.2, R Foundation for Statistical Computing). We developed mixed effects models using packages lme4 (Bates et al. 2015) and lmerTest (Kuznetsova et al. 2016).

We first evaluated MeHg concentrations in aquatic invertebrates and THg concentrations in songbirds among regions across the Willamette basin. To do so, we developed a series of general linear models for each taxonomic group (invertebrates and songbirds). For aquatic invertebrates, we developed a mixed effects model for MeHg concentrations with region (Black Butte, C.G. reservoir, Coast Fork R., NWR wetland, W.R. backwater, W.R. main) and invertebrate order (Coleoptera, Crustacean, Ephemeroptera, Gastropod, Plecoptera, Odonata, Hemiptera, Trichoptera) as main effects, while accounting for site, year, and Julian date as random effects. For riparian songbirds, our model for THg concentration in blood included region, songbird species, and bird age (adult or juvenile) as main effects, and site, year, and Julian date as random effects. We report back-transformed least squares mean Hg concentrations (with standard error) for all significant main effects from both models.

We also conducted a higher resolution assessment of Hg variability along the main stem Willamette River to better understand how Hg varied longitudinally and among previously characterized river segments. For aquatic invertebrates, our mixed effects model for MeHg concentration included river kilometer, Willamette river region (middle or upper), habitat type (main channel or backwater), and invertebrate order as main effects and site, year, and Julian date (as random effects). We did not collect any invertebrate samples from lower Willamette reaches. Similarly, for riparian songbirds, we developed a mixed effects model for THg concentration in blood that included river kilometer, Willamette river section (lower, middle or upper), habitat type (main channel or backwater), songbird species, and bird age (adult or juvenile) while accounting for site, year, and Julian date as random effects.

Finally, we examined the relationship in Hg concentrations between invertebrates and songbirds using estimates of site mean Hg concentrations to evaluate how predictive

invertebrate MeHg concentrations were of breeding songbird THg concentrations. To do so, we calculated least squares mean Hg concentrations for each individual site. Each model included site as a main effects, but our invertebrate models accounted for date, order, and year as random effects whereas our songbird model included date, species, age, and year as random effects. We examined the relationship between least squares mean invertebrate MeHg concentration at each site with the corresponding least squares mean songbird THg concentrations using a simple linear regression. We also examined the relationship between the most commonly sampled single songbird species and various invertebrate taxa across sites. Our target songbird species that occurred at every site sampled was the Song Sparrow (*Melospiza melodia*). We calculated geometric mean MeHg concentrations for each invertebrate order at each site and correlated it to geometric mean THg concentrations for adult Song Sparrows at each site using a simple linear model.

Results

We sampled invertebrates and songbirds from a total of 25 and 17 sites between 2013 and 2014, respectively (Table 3.1). Both MeHg and THg concentrations are reported in dry weight (dw). MeHg concentrations in aquatic invertebrates ranged from 5 ng/g to 922 ng/g, with the highest geometric mean in Hemiptera (154.7 ng/g, median = 137.5 ng/g) and the lowest geometric mean in Trichoptera (27.8 ng/g, median = 24.6 ng/g). For riparian songbirds, THg concentrations in blood ranged from 12 ng/g to 4758 ng/g. The highest geometric mean THg concentrations found in Willow Flycatchers (geometric mean = 1388 ng/g, median = 1054 ng/g) and the lowest THg concentrations were found in Black-headed Grosbeaks (geometric mean = 76.2 ng/g, median = 52.7 ng/g). Descriptive statistics for each invertebrate family and songbird species included in this analysis are compiled in Table 3.2.

Entire basin analysis. For the portion of the Willamette basin that we sampled (Fig. 3.1), our mixed effect model for aquatic invertebrates indicated significant differences among regions ($F_{5,23} = 2.9$, P = 0.04, Fig. 3.2a) and invertebrate orders ($F_{7, 342} = 30.5$, P < 0.001, Fig. 3.3a). The highest least squares mean MeHg concentration was in the Coast Fork region (146 ng/g, SE = 23.4), followed by the Black Butte region (136 ng/g, SE = 32.3), Cottage Grove reservoir (117 ng/g, SE = 18.5), NWR wetland region (86 ng/g, SE = 12.0), Willamette backwater (76 ng/g, SE = 7.6), and Willamette main channel (45 ng/g, SE = 3.6). There was a four-fold difference in MeHg concentrations between the order with the lowest MeHg

concentration (Trichoptera; 42 ng/g, SE = 3.2) and the order with the highest MeHg concentration (Hemiptera; 179 ng/g, SE = 13.0). Hemiptera and Odonata (163 ng/g, SE = 11.3) orders exhibited the highest least squares mean MeHg concentrations, followed by Coleoptera 114 ng/g, SE = 8.2), Gastropod (111 ng/g, SE = 7.9), and Crustacean (104 ng/g, SE = 7.7) orders. The lowest MeHg concentrations were found in Plecoptera (68 ng/g, SE = 5.9), Ephemeroptera (54 ng/g, SE = 3.9) and Trichoptera.

Blood THg concentrations in riparian songbirds differed among regions ($F_{5,208.}= 9.5$, P < 0.001, Fig 3.2b), bird age ($F_{1, 674} = 17.2$, P < 0.001), and species ($F_{9, 658} = 46.4$, P < 0.001, Fig 3b). THg concentrations in the Black Butte region (lsmean = 1153 ng/g, SE = 140.0) were significantly higher than all other regions, with the exception of the Coast Fork region (lsmean = 282 ng/g, SE = 51.0). There was no statistical difference among Coast Fork, Cottage Grove reservoir (lsmean = 344 ng/g, SE = 30.4), NWR wetlands (lsmean = 315 ng/g, SE = 28.6), nor Willamette backwater (lsmean = 270 ng/g, SE = 15.8). The Willamette main channel region showed the lowest THg concentration (lsmean = 169, SE = 8.9). Adult songbirds (lsmean = 410 ng/g, SE = 19.7) had significantly higher THg concentrations than juveniles (lsmean = 285 ng/g, SE = 16.6). After taking into account region, age, site, year, and Julian date, songbird species exhibited different lsmean THg blood concentrations (Table 3.3). Black-headed Grosbeak were significantly lower in THg concentrations than the rest of the species. The highest species included Bewick's Wren, Willow Flycatcher, and Common Yellowthroat (Table 3.3).

Willamette River analysis. MeHg concentrations of invertebrates of the Willamette River did not change with river kilometer ($F_{1,12.1} = 0.4$, P = 0.5) nor section ($F_{1,10.9} = 0.08$, P = 0.80). However, MeHg concentrations differed between habitat types ($F_{1,10.2} = 5.2$, P = 0.04) and among invertebrate orders ($F_{7,237.6} = 26.7$, P < 0.001). Least squares mean MeHg concentrations were almost twice as high in backwater habitats (lsmean = 78.2 ng/g, SE = 5.8) than they were in main channel habitats (lsmean = 44.7 ng/g, SE = 4.1). Invertebrate order had exposures similar to the region-wide analysis. MeHg concentrations decreased in each order as follows: Hemiptera (lsmean = 106.7 ng/g, SE = 8.1), Odonata (lsmean = 103.2 ng/g, SE = 7.2), Coleoptera (lsmean = 76.8 ng/g, SE = 5.4), Gastropod (lsmean = 69.6 ng/g, SE = 4.9), Crustacean (lsmean = 65.1 ng/g, SE = 4.7), Plecoptera (lsmean = 46.1 ng/g, SE = 4.14), Ephemeroptera (lsmean = 33.4 ng/g, SE = 2.3), and Trichoptera (lsmean = 25.4 ng/g, SE = 2.0) (Fig 3.4a)

Total Hg concentrations of songbirds in the Willamette River also did not change with river kilometer ($F_{1,2} = 0.59$, $P = 0.53$) nor region ($F_{2,2.1} = 0.23$, $P = 0.81$). Songbird THg concentrations differed between habitat types ($F_{1,3} = 12.7$, $P = 0.04$), species ($F_{7,502} = 48.3$, $P < 0.001$), and with age ($F_{1,527} = 8.8$, $P = 0.003$). Least squares mean THg concentrations were 50% higher in backwater habitats (lsmean = 251 ng/g, SE = 15.1) than main channel habitats (lsmean = 169 ng/g, SE = 8.1). Adult songbirds (lsmean = 237.3 ng/g, SE = 10.9) had significantly higher THg concentrations than juveniles (lsmean = 179.0 ng/g, SE = 10.6). Similar to the region wide analysis, songbird species decreased in order as follows (Fig 4b): Bewick's Wren (lsmean = 949.5 ng/g, SE = 72.8), Common Yellowthroat (lsmean = 303.0 ng/g, SE = 18.5), Black-capped Chickadee (lsmean = 250.3 ng/g, SE = 16.5), Song Sparrow (lsmean = 228.6 ng/g, SE = 11.0), Yellow Warbler (lsmean = 205.3 ng/g, SE = 113.9), Spotted Towhee (lsmean = 193.5 ng/g, SE = 11.1), Swainson's Thrush (lsmean = 113.9 ng/g, SE = 5.9), and Black-headed Grosbeak (lsmean = 43.6 ng/g, SE = 3.9).

Site comparison of invertebrates and birds. Although there were no linear trends in either invertebrate or songbird Hg concentrations within the main stem Willamette, there was substantial variation among sites (Fig 3.5). Some NWR wetlands exhibited Hg concentrations similar to those of Cottage Grove reservoir. Where overlap between invertebrate and songbird collection occurred, we tested the relationship between site-specific least squares mean MeHg concentrations of invertebrates (mixed effects model accounting for invertebrate order, year, and date as random effects) and site-specific least squares mean THg concentrations of songbirds (mixed effects model accounting for songbird species, age, year and date as random effects). We did not find a significant correlation between site-specific least squares mean Hg concentrations ($F_{1,19} = 2.8$, $P = 0.11$, $R^2 = 0.13$; Fig. 3.6). However, on an individual taxa basis, geometric mean THg concentrations in adult Song Sparrows at each site were correlated ($P < 0.05$) with MeHg concentrations in both plecoptera and gastropod orders, but not other invertebrate taxa.

Discussion

Hg concentrations in aquatic invertebrates and songbirds differed substantially among regions of the Willamette River basin. Although the headwaters of the basin contain an abandoned Hg mine and reservoir listed as EPA Superfund sites, there was no linear decline in invertebrate or songbird Hg concentrations with river mile. Instead, there was substantial

variability in site-specific Hg concentrations in riverine and wetland sites through the valley floor. Moreover, songbird THg concentrations were only weakly correlated with MeHg concentrations in invertebrates, suggesting a complex mechanism between emerging insects and uptake by riparian songbirds.

Ecological risk of Hg contamination is complicated by the fact that it is influenced by the availability of inorganic Hg, the processes that influence MeHg production, as well as the ecological factors that influence trophic transfer and bioaccumulation (Ullrich et al. 2001). Because of this, there can be a substantial disconnect between inorganic Hg concentrations in the environment, and MeHg concentrations in ecological receptors (Eagles-Smith et al. 2016). For Hg, methylation rates – and subsequent entrance of MeHg into the base of the food chain – are often higher in wetland habitats than fully lotic systems (Ullrich et al. 2001). Consistent with these processes, we found that small scale habitat differences are an important driver of Hg exposure in riparian songbirds, as wetland habitats far downstream from the source of contamination exhibited similar Hg concentrations to the Cottage Grove Reservoir (USEPA Superfund site). In the Willamette River main stem section, we observed higher Hg concentrations in both invertebrates and songbirds in backwater sites compared to main channel sites, even within close proximity to each other. The Willamette Valley National Wildlife Refuges are managed to maintain wetland habitat for conservation purposes, but this could also provide wetland habitat conditions that facilitate Hg methylation (U.S. Fish and Wildlife Service 2011).

Both invertebrate and songbird sampling present their own unique set of challenges for use as effective biomonitors; MeHg analysis of invertebrates is expensive and blood sampling of songbirds requires training and experience. We observed a weak correlation between site-specific invertebrate and songbird Hg levels on a site-specific basis, but we lack confidence from this analysis that we could use invertebrate samples to estimate songbird Hg or vice versa. There was a slightly stronger correlation when considering only our most cosmopolitan species, Song Sparrows, and some invertebrate taxa. There are many reasons why MeHg in aquatic invertebrates and THg in songbirds may not correlate on a site-specific basis, including both environmental and behavioral factors. Aquatic habitat factors that mediate aquatic productivity also govern the terrestrially-bound flux of emergent aquatic prey (Gratton and Vander Zanden 2009, Chumchal and Drenner 2015). Additionally, aquatic predators can mediate the biomass of emergent aquatic insects that survive to emerge

(Tweedy et al. 2013). From a bird foraging behavior standpoint, individuals and species vary in their dependence on emergent aquatic prey (Chapter 4, this dissertation) which can be further complicated by aquatic habitat type (Iwata et al. 2003). Although we accounted for variables in our model that we know influence Hg concentrations in songbirds, there are complex processes mitigating exposure in songbirds, making a simple correlation between aquatic invertebrates and songbirds difficult.

Another confounding factor is that we are limited in our ability to explicitly account for trophic position within our models. While we did not study species foraging habits for this analysis, our results generally align with expectations based on the preferences of the species as a whole. For example, Bewick's Wren show some of the highest Hg levels and those in Black-headed Grosbeak were commonly low, which is consistent with their foraging ecology. Wrens are gleaners that eat primarily invertebrate material (less than 3% vegetable material; (Kennedy and White 2013) whereas grosbeaks have powerful beaks that help them eat seeds and fruits and large bodied insects (eating 43% vegetable matter during the breeding season (Ortega and Hill 2010). While eating invertebrate prey over vegetable matter will place a bird at a higher trophic position, focusing on predatory invertebrates will also increase trophic position. Songbird species that eat more spiders generally have higher Hg exposure (Cristol et al. 2008).

In our analysis, adult songbirds show consistently higher blood Hg concentrations than conspecific juveniles (hatch-year). This is not surprising as hatch-year birds caught during the breeding season had recently (within the past few months) grown all of their feathers and so would have depurated a large amount of Hg (Condon and Cristol 2009). This, assessments of Hg risk to songbird populations using juvenile birds can be confounded because time since molt can complicate blood Hg levels.

Although songbirds are generally thought to be more sensitive to Hg exposure than other bird taxa (Heinz et al. 2009), there are few species for which direct toxicity has been measured. Additionally, most other studies report results in µg/g, wet weight. Assuming 80% moisture in blood samples we can convert to dry weight ($[dw] = 5*[ww]$). This study reported results in ng/g instead of µg/g. For example, Carolina Wrens on the contaminated South River in Virginia averaged Hg concentrations of 4.49 µg/g ww (22,450 ng/g dw), Tree Swallows averaged 3.66 µg/g ww (18,300 ng/g dw) and Song Sparrows averaged 2.69 µg/g ww (13,450 ng/g dw) (Cristol et al. 2008). The maximum concentration observed in this

study was 4758 ng/g dw, which is more comparable to birds at the reference
(uncontaminated) sites in Virginia (Carolina Wren 2050 ng/g dw, Song Sparrow 550 ng/g dw,
Tree Swallow 850 ng/g dw; Cristol et al. 2008). At the lowest end, however, Jackson et al.
(2011) modelled that a 10% reduction in the likelihood of a nest being successful could occur
at concentrations as low as 3500 ng/g dw (0.7 ug/g ww) which is well within the range of
values we report here.

It is important to note that no effects studies have been completed for any of the
actual species sampled here, resulting in considerable uncertainty with regard to potential
population-level harm to species in the Willamette Valley. Species and individuals vary in
their sensitivity to Hg exposure (Heinz et al. 2009, Varian-Ramos et al. 2013). It is most
likely that Hg could act as another stressor on already stressed populations. For example, Hg
can be mobilized during migration-induced fasting (Seewagen et al. 2016), can inhibit
immune responses (Hawley et al. 2009, Lewis et al. 2013), and change endocrine function
(Wada et al. 2009). Both Bewick's Wren and Willow Flycatcher populations are declining
and these two species exhibited some of the highest Hg concentrations in this study. While
speculative, Hg can be considered as another stressor on these populations that can inhibit
population growth rates, which has been observed in piscivorous birds (Schoch et al. 2014).
At a landscape scale, species that favor wetland habitats are more likely to come into contact
with higher Hg levels.

Conclusions. This is the first study to document riparian songbird Hg in the
Willamette River basin, which has a legacy of Hg and gold mining in its headwaters. The
majority of Hg research in the watershed has focused on fish and fish-eating birds closer to
the impacted Cottage Grove reservoir. Hg exposure is not confined to songbirds in the
headwater area where the EPA has designated a superfund site. In fact, Hg levels in songbirds
did not differ between reservoir locations and other wetlands in the Willamette Valley such as
the Willamette Valley National Wildlife Refuge complex. This has important conservation
implications for assessing ecosystem-wide risk to Hg exposure; monitoring of all habitats in
the watershed are important to fully understand the Hg footprint. Conservation goals for the
Willamette River include increasing channel complexity, especially in the less populated
upper reaches (Gregory et al. 2002). This would translate into more wetland habitats across
the landscape, and more potential for Hg methylation. As the human population of the

Willamette Valley is predicted to grow, demands for habitat and water resources will continue to change and research must keep pace.

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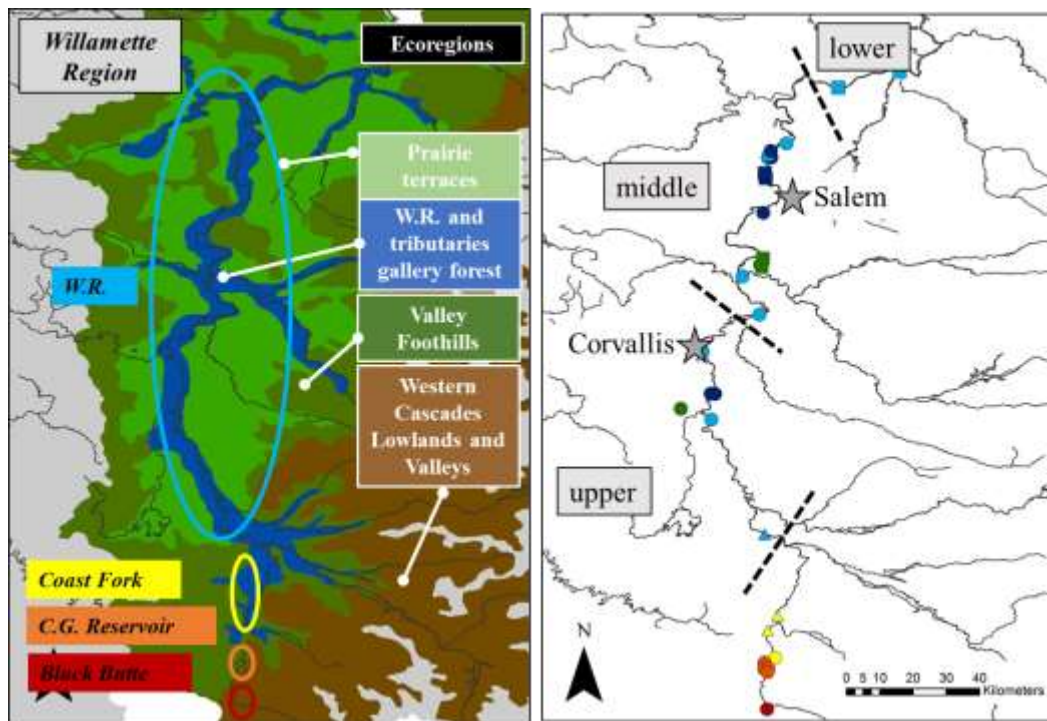
Figure 3.1. Study area in the Willamette Basin of western Oregon. Ecoregions are EPA Level-IV. Six Willamette basin region sites surveyed in this study were Black Butte (EPA superfund site), Cottage Grove Reservoir, Coast Fork Willamette River, main stem Willamette River, backwater Willamette River and wetlands in the Willamette Valley National Wildlife Refuge Complex.
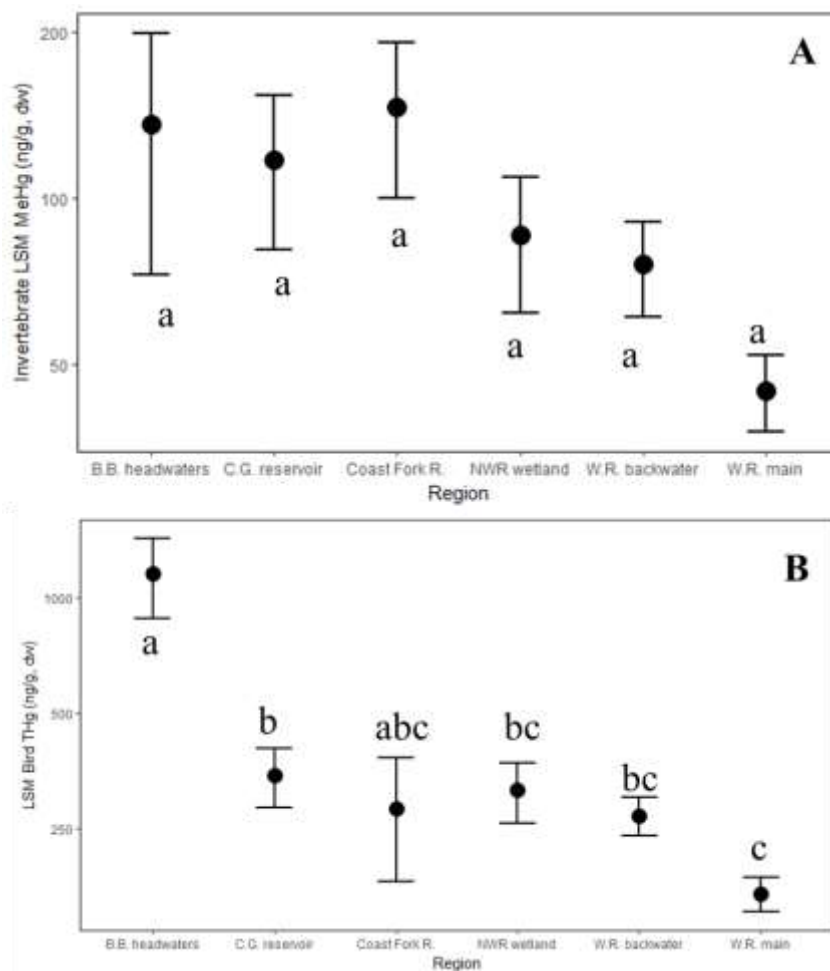
Figure 3.2. Least squares mean Hg concentrations (back-transformed with 95% confidence interval) among Willamette basin regions for A) invertebrates (mixed effects model accounting for invertebrate order, year, date and site) and B) riparian songbirds (mixed effects model accounting for species, age, year, date, and site. Different letters indicate significant differences in post hoc Tukey comparisons; regions with the same letters have no significant difference in Hg concentration.
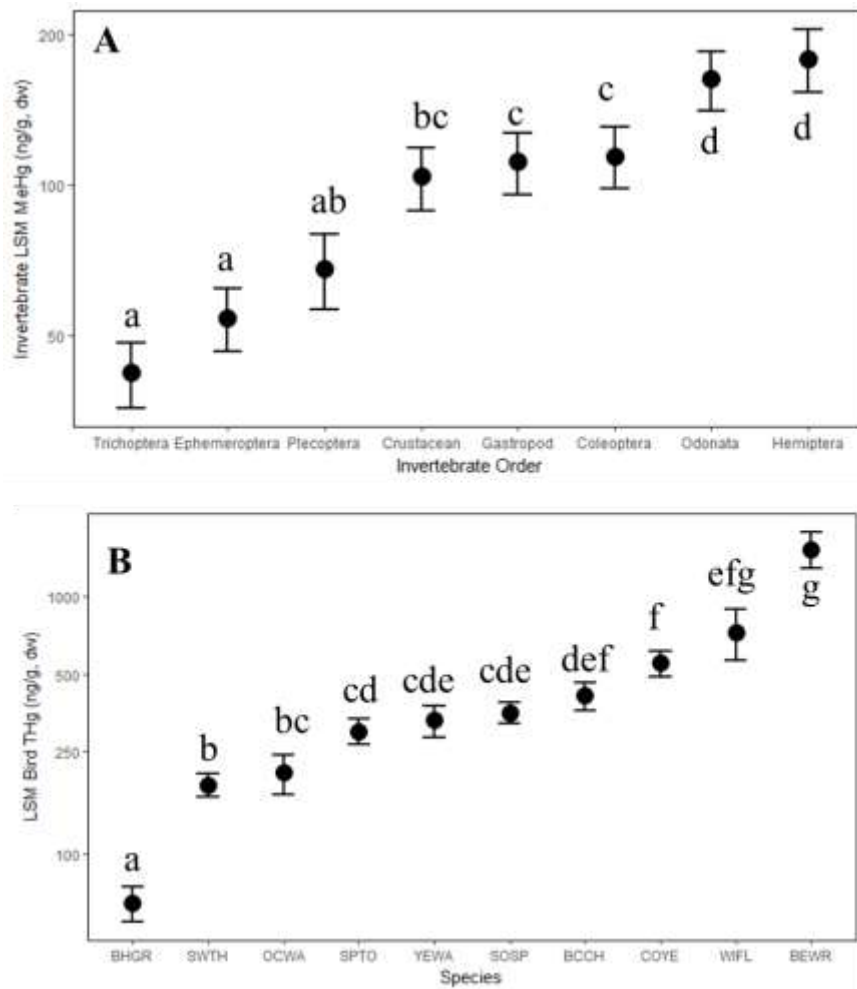
Figure 3.3. Least squares mean Hg concentrations (back-transformed with 95% confidence interval) for differences between A) invertebrates orders (mixed effects model accounting for region, year, date and site) and B) songbird species (mixed effects model accounting for region, age, year, date, and site. Different letters indicate significant differences in post hoc Tukey comparisons; regions with the same letters have no significant difference in Hg concentration.
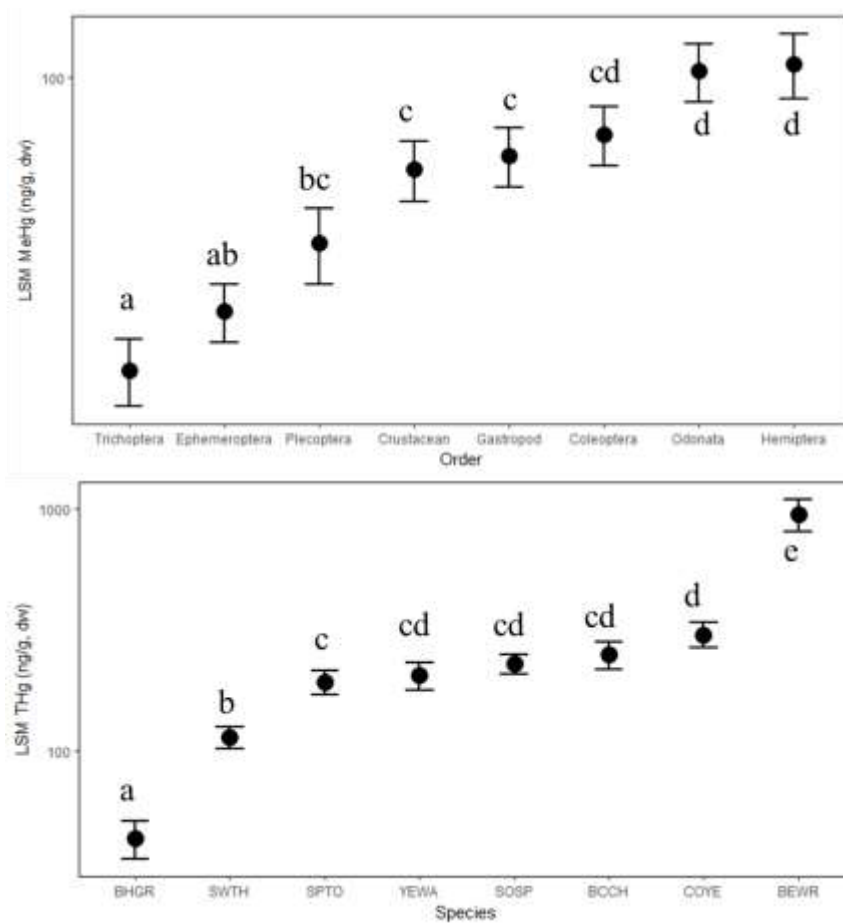
Figure 3.4. Least squares mean Hg concentrations (back-transformed with 95% confidence interval) results from Willamette River specific analysis (excluding Black Butte, C.G. reservoir, NWR wetlands, and Coast Fork region for A) invertebrates order (mixed effects model accounting for river kilometer, Willamette section, habitat type, year, date and site) and B) songbird species (mixed effects model accounting for river kilometer, Willamette section, habitat type, age, year, date, and site. Different letters indicate significant differences in post hoc Tukey comparisons; regions with the same letters have no significant difference in Hg concentration.
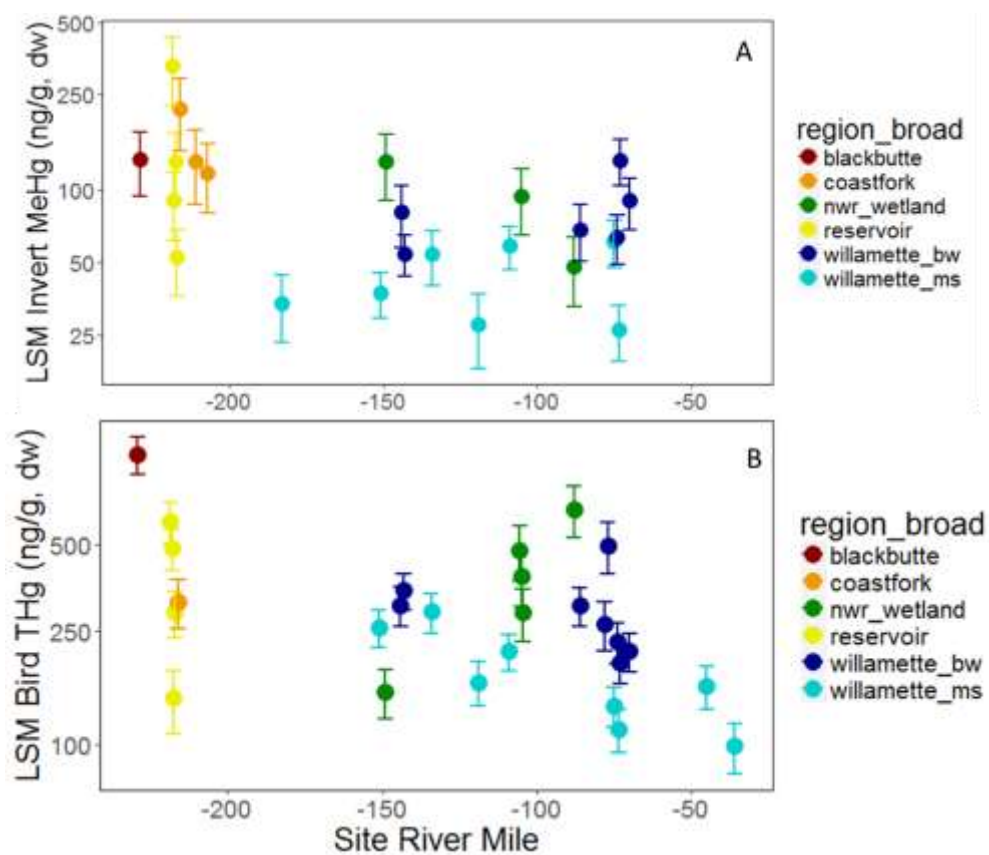
Figure 3.5. Modelled least square mean Hg concentrations at each site, in order from upstream to downstream sites for A) aquatic invertebrates and B) riparian songbirds.
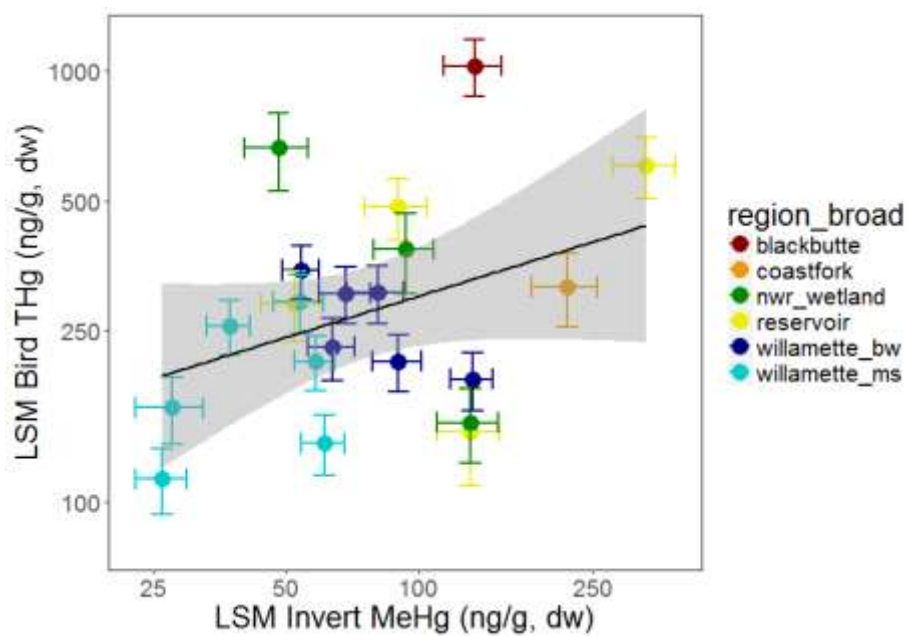
Figure 3.6. Model-calculated least square mean MeHg concentration for generic invertebrate correlated to model-calculated least square mean THg concentration for generic songbird.
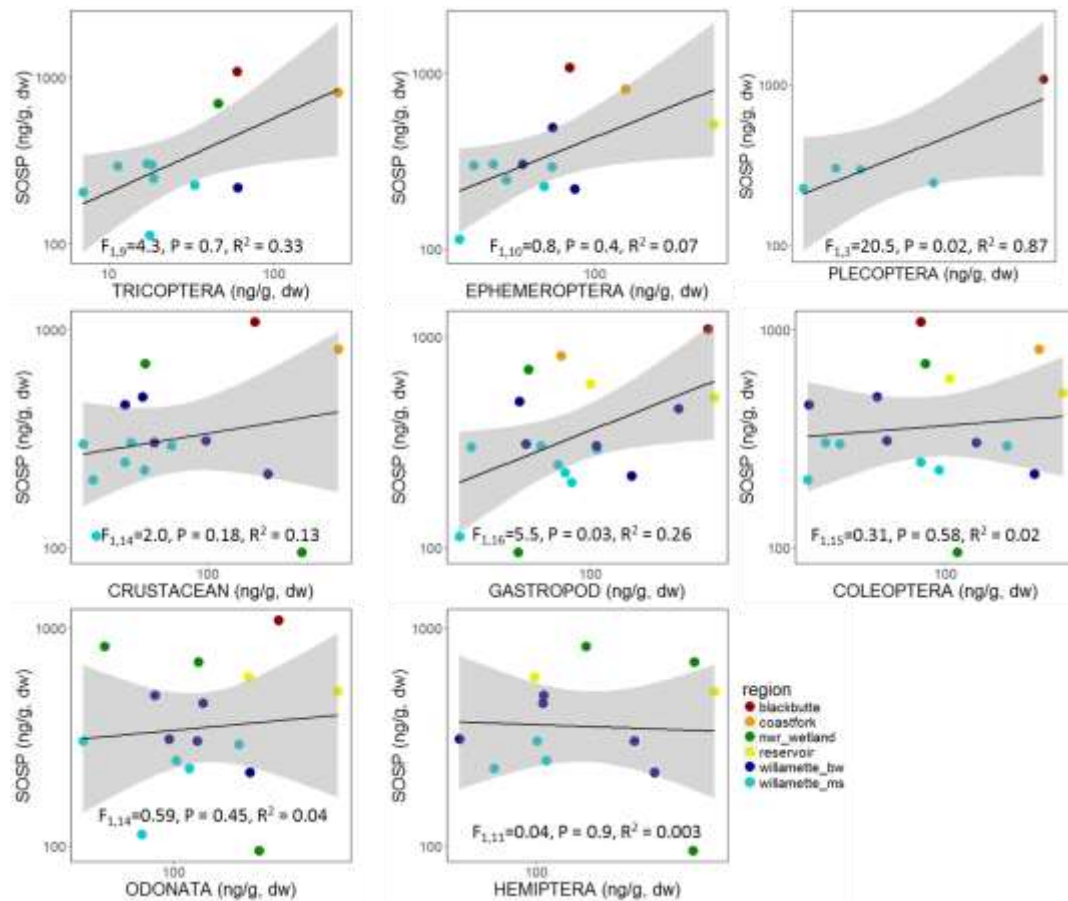
Figure 3.7. Simple linear correlation between geometric mean THg concentrations in Song Sparrows at each site and geometric mean MeHg concentrations of sampled invertebrate orders.

Table 3.1. Site characteristics of Willamette Basin sites sampled in 2013 and 2014.

				2013		2014	
River km	**Management Unit**	**Site Name**	**Region**	**N inverts**	**N birds**	**N inverts**	**N birds**
229	Black Butte - USEPA Superfund	Black Butte Mercury Mine Site	Black Butte		18	23	24
218	Cottage Grove Reservoir	east C.G. reservoir	C.G. Reservoir		15	9	5
218	Cottage Grove Reservoir	northwest C.G. reservoir	C.G. Reservoir			12	6
218	Cottage Grove Reservoir	southeast C.G. reservoir marsh	C.G. Reservoir		12	9	10
218	Cottage Grove Reservoir	west C.G. reservoir marsh	C.G. Reservoir			10	2
216	Cottage Grove Reservoir	Coast Fork downstream of dam	Coast Fork		3	6	2
211	Cottage Grove City	swinging bridge, downtown Cottage Grove	Coast Fork			5	
207	City of Saginaw	Saginaw Road overpass	Coast Fork			8	
183	City of Eugene	Alton Baker Park, Boat launch area	W.R. main			11	
151	Benton County	Irish Bend County Park	W.R. main	17	39	8	9
149	USFWS	Finley - Cheadle Marsh	NWR wetland		24	12	
144	USFWS	Snagboat Bend - lake	W.R. backwater	10	37		
143	USFWS	Snagboat Bend - alcove	W.R. backwater	18	46	12	35
134	city of Corvallis	Willamette Park	W.R. main	9	28		
119	city of Albany	Takena Landing City Park	W.R. main	5	13		
109	State of Oregon	Luckiamute North State Natural Area	W.R. main	25	52	17	21
105	USFWS	Ankeny - Eagle Marsh	NWR wetland		6		
105	USFWS	Ankeny - Wood Duck Pond	NWR wetland		9		
105	USFWS	Ankeny - Pintail Marsh	NWR wetland		8	12	

Table 3.1 (continued)

River km	Management Unit	Site Name	Region	2013		2014	
				N inverts	N birds	N inverts	N birds
88	USFWS	Baskett Slough, Traverner's Marsh	NWR wetland		10	8	
86	City of Salem	Minto-Brown Island City Park	W.R. backwater	10	45		
78	State of Oregon	Darrow Bar State Natural Area	W.R. backwater		7		
77	State of Oregon	Lincoln Access State Natural Area	W.R. backwater		13		
75	State of Oregon	Spring Valley State Natural Area	W.R. main	14	44	11	
74	State of Oregon	Willamette Mission State Park, alcove	W.R. backwater	15	17		
74	State of Oregon	Willamette Mission State Park, main stem	W.R. main	10	12		
73	State of Oregon	Willamette Mission State Park, pool	W.R. backwater	38	38	14	
70	State of Oregon	Grand Island State Natural Area	W.R. main	21	49		
45	State of Oregon	Champoeg State Park	W.R. main		29		
36	State of Oregon	Molalla River State Natural Area	W.R. main		19		

Table 3.2. Statistical summary of invertebrate and songbird species sampled in 2013 and 2014 in the Willamette basin. All mercury measurements are displayed as ng/g (dw).

Type	Order/Species	N	Maximum	Minimum	Median	Geometric Mean	Standard Error
Invertebrate	Coleoptera	54	321	8	89.2	90.4	4.1
	Crustacean	40	332	31	70.15	77.7	3.4
	Ephemeroptera	52	586	10	41.7	41.7	2.1
	Gastropod	56	685	12	104	94.8	4.7
	Hemiptera	50	922	31	137.5	154.7	6.7
	Odonata	79	579	34	141	140.7	4.3
	Plecoptera	17	99	11	39	41.8	2.8
	Trichoptera	32	246	5	24.6	27.8	2.3
Bird	Black-capped Chickadee	35	3321	46	311.3	346.6	21.7
	Bewick's Wren	16	2224	292	1079.3	936.4	59.3
	Black-headed Grosbeak	19	631	12	52.7	76.2	8.3
	Common Yellowthroat	72	3157	156	480.5	536.2	19.5
	Orange-crowned Warbler	13	1081	98	240.9	269.2	24.8
	Song Sparrow	291	2666	22	282.7	284.5	6.1
	Spotted Towhee	71	2584	43	223.1	241.3	8.9
	Swainson's Thrush	155	3124	28	144.6	163.5	5.7
	Willow Flycatcher	7	4758	750	1054.0	1388.4	157.9
	Yellow Warbler	31	697	108	286.9	292.4	8.6

Table 3.3. Model results for differences in riparian songbirds for entire Willamette region. Least squares mean THg in blood reported as ng/g dw (LS Mean THg). Standard error (SE) is back-transformed from model output.

Species Code	Species Common Name	Scientific Name	LS Mean THg	SE
BHGR	Black-headed Grosbeak	*Pheucticus melanocephalus*	65	5.1
SWTH	Swainson's Thrush	*Catharus ustulatus*	186	9.9
OCWA	Orange-crowned Warbler	*Oreothlypis celata*	207	18.2
SPTO	Spotted Towhee	*Pipilo maculatus*	301	17.3
YEWA	Yellow Warbler	*Steophaga petechia*	330	23.4
SOSP	Song Sparrow	*Melospiza melodia*	354	17.3
BCCH	Black-capped Chickadee	*Poecile atricapillus*	411	26.7
COYE	Common Yellowthroat	*Geothlypis trichas*	551	32.1
WIFL	Willow Flycatcher	*Empidonax traillii*	726	83.0
BEWR	Bewick's Wren	*Thryomanes bewickii*	1523	123.6

CHAPTER 4. PULSED RELIANCE ON AQUATIC CARBON SUBSIDIES INFLUENCES MERCURY EXPOSURE IN RIPARIAN SONGBIRDS

Allyson Jackson, Collin Eagles-Smith, and W. Douglas Robinson

Abstract

Cross ecosystem subsidies move substantial amounts of nutrients between ecosystems. Emergent aquatic insects are a particularly important prey source for riparian songbirds but may also move aquatic contaminants, such as mercury (Hg), to the riparian food web. This subsidy of nutrients and contaminants is complex for a variety of reasons. Because of seasonal weather changes and insect phenology, these subsidies are not constant but pulsed at specific times throughout the year. Mercury loading to the ecosystem is only one factor that influences Hg exposure, as Hg must be methylated before it can be bioavailable for organisms. Because methylation occurs through anaerobic bacterial processes, wetland habitats are more likely to foster a strong methylation environment. In the Willamette River system in western Oregon, riverine habitat varies considerably between main stem flowing reaches and backwater off channel habitats. We investigated the effect of habitat, date, and species differences on Hg exposure in aquatic invertebrates, terrestrial invertebrates and riparian songbirds. We found that riparian songbird blood total Hg (THg) concentrations are significantly higher in backwater sites than main channel sites. We used stable isotopes of $\delta^{13}C$ to determine the reliance of riparian predators on emergent aquatic carbon sources and correlated this with Hg exposure in avian species. We found that aquatic invertebrate methylmercury (MeHg) concentrations were higher at later sampling dates, but MeHg in most terrestrial invertebrate predators and THg concentrations in the blood of songbirds were lower at later sampling dates. We used a two end member mixing model for reliance on aquatic carbon and determined that both invertebrates and songbirds sampled later in the season exhibited a more terrestrial-foraging carbon signature than those sampled early in the season, who showed a more aquatic carbon signature. Most terrestrial invertebrate families are flexible in their prey preference, except for tetragnathid spiders that do not vary throughout the season. Songbird species vary in their foraging preferences from early to late time periods, with some species (i.e., Swainson's Thrush, *Catharus ustulatus*) switching more dramatically to terrestrial prey by the end of the season, some species (i.e., Yellow Warbler,

Steophaga petechia) showing little seasonal change in diet, and others shifting in less dramatic ways (i.e., Song Sparrow, *Melospiza melodia*). Our results offer the first demonstration of the relationship between pulsed reliance on aquatic carbon and Hg exposure in riparian predators.

Introduction

Cross-ecosystem nutrient subsidies are important components of ecosystem function (Polis et al. 1997). Freshwater ecosystems in particular, receive substantial allochthonous nutrient input from riparian leaf litter and detritus, and in return contribute nutrients and energy back to the surrounding riparian areas via aquatic insect emergence (Baxter et al. 2005, Ballinger and Lake 2006). These subsidies have been shown to increase the density and diversity of riparian predators, including lizards, birds and bats (Nakano and Murakami 2001, Sabo and Power 2002, Fukui et al. 2006).

Despite the benefits of aquatic insect emergence as energetic subsidies to riparian communities, they can also degrade riparian habitats through the import of aquatically-derived environmental contaminants. Many aquatic ecosystems are accumulation zones for environmental contaminants, such as mercury (Hg), other heavy metals, and polychlorinated biphenyls (PCBs). When assimilated by aquatic invertebrates, these contaminants can accompany their movement into surrounding terrestrial food webs (Walters et al. 2008, Jones et al. 2013, Kraus et al. 2013, Latta et al. 2015). As a result, many riparian taxa, ranging from invertebrates to invertebrate-eating birds and bats, have been shown to accumulate aquatic-sourced contaminants (Jackson et al. 2011b, Yates et al. 2014, Moy et al. 2016).

Although contaminant exposure in riparian taxa is well-documented, the pathways and magnitude of transfer are complex and not well elucidated. This may be because exposure is mitigated by the magnitude or biomass of insects that survive to emerge from the aquatic system, which can be mediated by habitat, water quality and fish abundance (Paetzold et al. 2011, Jones et al. 2013). For example, contaminants can reduce invertebrate fecundity at environmentally-relevant concentrations, ultimately constraining insect flux and contaminant transfer to riparian food webs (Paetzold et al. 2011, Kraus et al. 2013). In contrast, some contaminants, such as Hg, are not known to affect aquatic insect survival and emergence. Thus, in Hg contaminated areas, high insect emergence rates can move large amounts of Hg into the riparian zone (Tweedy et al. 2013).

Although Hg does not impact insect emergence, Hg methylation and subsequent exposure of wildlife is influenced by habitat type (Evers et al. 2007, Jackson et al. 2015). Hg must be methylated to be taken up by organisms and become toxic, a process that occurs mostly in anaerobic aquatic environments (Ullrich et al. 2001). Methylation rates vary based on physicochemical conditions, such as water flow, dissolved organic carbon, and temperature (Ullrich et al. 2001, Chaves-Ulloa et al. 2016).

Fluxes of Hg to riparian zones can also be influenced by intra-annual phenological shifts in the invertebrate food web. In temperate climates, aquatic and terrestrial ecosystems have major seasonal shifts between spring and summer months (Richardson 2001). Both terrestrial predatory invertebrates (e.g., spiders) and songbirds often concentrate near aquatic habitats ostensibly to exploit the emergent insect subsidy during peak emergence times (Hagar et al. 2012). After leaf out in the terrestrial ecosystem, riparian predators can exploit a terrestrially-based invertebrate food web (Nakano and Murakami 2001).

Hg exposure in riparian songbirds is likely driven by a variety of factors including: Hg methylation rates in the aquatic system, seasonal pulses of aquatic insect prey versus terrestrial insect prey, and individual and species-based foraging preferences. We use a combination of stable isotopes in both invertebrate prey and riparian songbird blood to determine differences in aquatic subsidies between main channel and backwater habitats of the Willamette River, Oregon, USA. We assess whether the proportion of aquatic carbon in the diet of riparian predators (songbirds and invertebrates) is associated with Hg exposure. Our objectives were threefold: 1) Determine differences in invertebrate food web in main channel and backwater habitats using stable isotopes of δ^{13}C and δ^{15}N. 2) Consider a wide suite of factors (including season, habitat, and species) that could influence Hg exposure in terrestrial invertebrates, aquatic invertebrates and riparian songbirds and 3) use terrestrial and aquatic endmembers to determine the proportion of aquatic carbon in the diet of terrestrial predators (invertebrates and songbirds) and correlate this to their Hg exposure.

Methods

Field methods. The Willamette River, Oregon, USA is a major tributary to the Columbia River and drains the eastern Coast Range and western Cascades. It also has a history of anthropogenic influences, including Hg contamination (Hope and Rubin 2005, Henny et al. 2005, Hope 2006). Prior to human development in the region, the Willamette

River was a braided river channel with extensive wetlands, backwater, and side channels. The river is now largely channelized, with only remnants of the original braided channels remaining in the form of backwater areas with varying connections to the main stem (alcoves, oxbow lakes). Many of the riparian areas have been converted to agriculture or urban centers (Baker et al. 2004). Field site placement was opportunistic, based on access and size of forest patch being large enough to allow for bird capture. Field sites were chosen in gallery forest sites along the Willamette River in western Oregon (Fig. 4.1). We selected a total of 12 sites that contained contiguous forest adjacent to the river. Sites were classified as either main channel (n = 7) or backwater (n = 5) based on broad aquatic habitat classifications (Fig. 4.1).

We sampled invertebrates and songbirds from May to August, 2013. We collected aquatic and terrestrial invertebrates from locations paired with songbird sampling locations. Our goal was to collect at least 30 individuals of the dominant invertebrate families at each site. Aquatic invertebrates were collected via kick net and dip net in the aquatic habitat near where mist nets had been set. Terrestrial invertebrates were collected by beat sheet and sweep net in forest or shrub habitat near mist nets. All invertebrates were composited by site, transferred to glass scintillation vials and kept on ice in the field until they could be transferred to a freezer (within 6 hours of sampling). We excluded any non-feeding emergent aquatic insects captured in our terrestrial sampling that were in their adult forms (i.e., adult mayfly, stonefly) as these would likely reflect aquatic and not terrestrial stable isotope signatures.

Playback recordings of conspecific songs were used to capture riparian songbirds in a mist net. Although numerous bird species were captured and sampled, the majority of samples were of five species present at all sites: Common Yellowthroat (*Geothlypis trichas*), Spotted Towhee (*Pipilo maculatus*), Swainson's Thrush (*Catharus ustulatus*), Song Sparrow (*Melospiza melodia*) and Yellow Warbler (*Setophaga petechia*). All birds were banded with an aluminum USGS band. Blood samples of each bird were taken from the brachial ulnar vein, using 27 gauge needles (BD PrecisionGlide, Fisher Scientific) and heparinized microhematocrit capillary tubes (Fisherbrand, Fisher Scientific). Samples were capped with Critocaps™ (Leica Microsystems) and stored on ice in the field until they could be transferred to a freezer (within 6 hours of sampling). No more than 1% of bird's body weight of blood was collected from each individual, usually between 20 µl and 100µl.

We also recorded morphometric measurements of unflattened wing length, tail length, tarsus length, and bill (nares to tip). We recorded presence of brood patch (for females) or cloacal protuberance (for males) as an indication of breeding condition and checked all feathers for molt (as an indication of post-breeding condition). We limited bird samples to only those individuals that were in breeding condition, not molting, and not young of the year. All samples were collected under authority of appropriate scientific collection permits, including both State (invertebrates: Oregon DFW# 17648; birds: Oregon DFW# 062-13) and Federal (USFWS MBTA# MB28361A; USGS Banding # 20786) agencies. All birds were handled under approved animal care and use protocols (Oregon State University ACUP # 4408).

Lab methods.

Invertebrates. Aquatic invertebrates were identified to family (Merritt et al. 2008) and terrestrial invertebrates were identified to either order (i.e., Hemiptera, Coleoptera, etc) or family for Aranadae. All invertebrates were composited based on the lowest taxon identified per site and sampling date, washed with deionized water, placed in glass vials and dried in an oven at 50°C for a minimum of 48 hours hrs. Once dried, they were homogenized into a fine powder with a clean glass rod.

All invertebrates were classified to coarse functional feeding groups (predators or non-predators; Table 4.1) so that we could separately examine bioaccumulation patterns between predators and non-predators in aquatic and terrestrial environments. Therefore, we classified invertebrates in four categories (hereafter invertebrate category): aquatic non-predators, aquatic predators, terrestrial non-predators, and terrestrial predators.

We analyzed MeHg concentrations in all invertebrate samples following EPA method 1630 (U.S. Environmental Protection Agency, 2001) at the USGS Forest and Rangeland Ecosystem Science Center. Briefly, 2-10 mg of dried homogenate was digested in 3-4ml 30% nitric acid at 60°C overnight (~15 hours), ethylated with 1% sodium tetraethylborate, then analyzed via cold-vapor atomic fluorescence spectrometry on a MERX-M (Brooks Rand Instruments, Seattle, Washington, USA) automated methylmercury analyzer. Quality assurance measures included analysis of two independently derived liquid calibration standards, two certified reference materials (scallop tissue [IAEA-452; International Atomic Energy Agency, Vienna, Austria] or lobster hepatopancreas [TORT-3; National Research Council of Canada, Ottawa, Canada]). Percent recoveries averaged 99%

(SD = 8%) for 10pg MeHg standard, 96% (SD = 13%) for 100pg MeHg standard, 99% (SD = 21%) for IAEA-452 averaged and 86% (SD = 5%) for TORT-3.

Songbird blood. Songbird whole blood samples were not composited, but instead run on an individual basis as dry weight. All samples were analyzed for total Hg using a Milestone tri-cell DMA-80 Direct Hg Analyzer (Milestone, Shelton, Connecticut USA) at the USGS Forest and Rangeland Ecosystem Science Center in Corvallis OR. This method uses combustion and gold amalgamation coupled with cold vapor atomic absorption spectrometry following US. Environmental Protection Agency method 7473. Certified reference material (dogfish muscle tissue [DORM-4; National Research Council of Canada, Ottawa Canada] and lobster hepatopancreas tissue [TORT-2; TORT-3; National Research Council of Canada, Ottawa Canada]), calibration verification (liquid standards), CRM duplicates, air blanks and boat blanks were included with each run. Milestone QA/QC included recoveries of 99.8% (SD=8.4%, N = 67) for calibration verification, recovery of 98% (SE = 11.5%, N = 100) for certified reference material and absolute percent difference of 2% (SD = 3%, N = 43) for duplicates.

Stable isotope analysis. Composited, dried and homogenized invertebrates and dried whole blood from birds were packed in tin capsules for stable isotope analysis at the University of California, Davis Stable Isotope Facility. All samples were analyzed using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDX Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Stable isotope values are reported as delta (δ) values using the equation ‰ = [(R_{sample} / $R_{standard}$) -1] *1000 where R = the ratio of the heavy isotope to the light isotope. Nitrogen samples were standardized against N_2 in air and carbon isotopes were standardized against Vienna PeeDee Belemnite. Based on replicate analysis of standard reference materials, we calculated the instrument standard for invertebrates and bird blood separately. For invertebrates, reference materials included bovine liver ($\delta^{13}C$ SD = 0.08, $\delta^{15}N$ SD = 0.29), USGS-41 glutamic acid ($\delta^{13}C$ SD = 0.14, $\delta^{15}N$ SD = 0.15), nylon-5 ($\delta^{13}C$ SD = 0.05, $\delta^{15}N$ SD = 0.17, and glutamic acid ($\delta^{13}C$ SD = 0.1, $\delta^{15}N$ SD = 0.2). For bird blood, reference materials included bovine liver ($\delta^{13}C$ SD = 0.85, $\delta^{15}N$ SD = 0.24), USGS-41 glutamic acid ($\delta^{13}C$ SD = 0.29, $\delta^{15}N$ SD = 0.31), nylon-5 ($\delta^{13}C$ SD = 0.34, $\delta^{15}N$ SD = 0.26, and glutamic acid ($\delta^{13}C$ SD = 0.3, $\delta^{15}N$ SD = 0.64).

Statistical Analysis. We used stable isotope analysis paired with Hg exposure in invertebrate and songbird communities to test for differences in aquatic-terrestrial energy

flow between main channel and backwater habitats and how those processes influenced Hg exposure. All statistical modeling was conducted with Program R (Version 3.3.2, R Foundation for Statistical Computing). We developed mixed effects models using packages lme4 (Bates et al. 2015) and lmerTest (Kuznetsova et al. 2016). For each model, we first developed a global model that included all main effects and two-way interactions of main effects. If an interaction had a p-value > 0.1, we excluded it and reran the model without it. We created stable isotope plots of carbon and nitrogen between non-predator invertebrates (i.e., baseline consumers), predatory invertebrates, and songbirds at main channel and backwater sites. We then tested for differences between carbon isotope signatures of non-predator aquatic and terrestrial prey in both main channel and backwater habitats, using a mixed effects general linear model that included invertebrate category and habitat type as main effects and site and invertebrate order as random effects.

We first evaluated the influence of variables influencing Hg exposure in invertebrates. We built a mixed effects model to test the effects of invertebrate category, Julian date, and habitat type on invertebrate MeHg concentrations. We included all two-way interactions of these main effects, while accounting for site (nested in habitat as a random effect) and invertebrate order (as a random effect). As in all subsequent models, if an interaction had a p-value > 0.1, we excluded it and reran the model without it.

To examine the effect of habitat and date on songbird blood THg concentrations, we built a model that included Julian date and habitat as main effects, and a Julian date x habitat interaction. We accounted for taxonomic and spatial variability by including species and site (nested in habitat type) as random effects. Next, we evaluated how our main effects might have differentially influenced Hg exposure among species. To do so, we used a reduced dataset that included only our five most well-represented species (Common Yellowthroat, Spotted Towhee, Swainson's Thrush, Song Sparrow and Yellow Warbler) and replicated the model structure associated with MeHg, including species, Julian date, and habitat type as main effects with all two-way interactions, and site nested in habitat type as a random effect. We reduced this model by removing any interactions with P > 0.1.

To illustrate changes in Hg concentrations through the season for any models with significant interactions that included Julian date, we compared model-estimated Hg concentrations at three time points in the season: early (i.e., our earliest sampling date, Julian

date =120 = April 30), mid (middle of the field season, Julian date = 160 = June 9) and late (our latest sampling date, Julian date = 200 = July 19).

We used a linear two-end-member mixing model to determine the proportion of aquatic carbon in the diet of riparian invertebrate predators and songbirds (Post 2002). Our stable isotope analysis indicated that there was distinct differentiation between aquatic and terrestrial carbon isotope ratios in main channel habitats but not in backwater habitats (see Results). Because of this, we focus only on main channel habitats for all mixing models. For main channel habitats, we used a two-end member mixing model for aquatic (mean $\delta^{13}C$ = -22.08) and terrestrial (mean $\delta^{13}C$ = -28.01) endmembers to calculate proportion of aquatic carbon in whole tissue samples of arachnids (tetragnathidae, salticidae and composite families), opiliones, and songbirds. We used a mixed effects model that accounted for site as a random effect to determine if Julian date or taxa (invertebrate family or songbird species) influenced proportion of aquatic carbon in both invertebrates and songbirds. We then ran models to determine if proportion of aquatic carbon and taxa influence Hg exposure in both predatory invertebrates and songbirds (while accounting for site as a random effect).

Results

Between 29 April 2013 and 24 July 2013, we sampled 314 composited invertebrates. Invertebrate MeHg concentrations ranged from 0.47 – 471 ng/g (dw). The geometric mean MeHg concentration across all invertebrates was 27.0 ng/g (SE = 3.5). Invertebrate families sampled in each habitat type are summarized in Table 4.1. Between 1 May and 24 July 2013, we sampled 287 adult breeding birds from 12 sites representing two distinct riparian habitats (main channel and backwater). All birds were captured within 150 m of the river's edge. Bird blood THg concentrations ranged from 10.0 – 1170 ng/g (dw) and the geometric mean concentration was 240 ng/g (SE = 14).

Stable isotope analysis. Carbon and nitrogen stable isotope ratios showed differing trends between the two habitat types for aquatic invertebrates only; there was no difference in terrestrial signatures (Fig. 4.2). At main channel sites, aquatic invertebrates were more enriched in both $\delta^{13}C$ and $\delta^{15}N$ isotopes than terrestrial invertebrates. At backwater sites, the main aquatic signature that we observed was more depleted in $\delta^{13}C$ isotope than the terrestrial signal. Aquatic invertebrates were enriched in $\delta^{15}N$ in both main channel and backwater habitats (Fig. 4.2).

Isotope ratios of $\delta^{13}C$ differed among habitat type ($F_{1,11} = 80.9$, P < 0.001) and invertebrate category ($F_{1,13} = 19.0$, P = 0.0007), but the interaction between these factors ($F_{1,191} = 107.7$, P < 0.0001) indicates the effect of habitat type differed among invertebrate category. There was no difference between terrestrial invertebrates from main channel and backwater habitats (T = 0.15, P = 0.9988). In contrast, the aquatic carbon signal from backwater habitats (least squares mean = -29.97‰, CI = -30.9‰ to -29.0‰, SE = 0.45) was significantly more depleted than the terrestrial signal (least squares mean = -27.82‰, CI = -28.8‰ to -26.9‰, SE = 0.47). Conversely, the aquatic carbon signal from main channel habitats (least squares mean = -22.12‰, CI = -22.9‰ to -21.3‰, SE = 0.36) was significantly more enriched than the terrestrial carbon signal (least squares mean = -27.91‰, CI = -28.8‰ to -27.0‰, SE = 0.43).

For predators from main channel habitats, we found that the carbon signal of both invertebrate (Fig. 4.3a) and songbird predators (Fig. 4.3c) fell between the terrestrial and aquatic non-predators. Conversely, in backwater habitats some invertebrate (Fig. 4.3b) and songbird predators (Fig. 4.3d) were more enriched than the terrestrial non-predator endmember. MeHg concentrations in aquatic invertebrates were almost an order of magnitude higher than those in terrestrial invertebrates in both main channel (Fig. 4.3e) and backwater habitats (Fig. 4.3f). The only exception was terrestrial spider groups, which tracked the carbon signal of aquatic invertebrates and also had MeHg concentrations that were elevated.

Spatial and temporal patterns in invertebrate MeHg. MeHg concentrations in invertebrates did not differ among invertebrate categories ($F_{3,207} = 2.4$, P = 0.07), nor habitat types ($F_{1,13} = 2.5$, P = 0.13), and did not vary with Julian date ($F_{1,298} = 0.0001$, P = 0.99). However, the significant interaction between invertebrate category and Julian date ($F_{3,294} = 4.0$, P = 0.008) indicated that there were taxonomic differences that varied over time. Our model indicated that terrestrial non-predators were significantly lower in MeHg than all other categories at early, mid and late points in the season (P < 0.05).

To simplify this interaction, we reran the model separately for aquatic and terrestrial invertebrates. The reduced aquatic model included a significant interaction of habitat type by Julian date ($F_{1,169} = 5.0$, P = 0.03) and a marginal interaction of invertebrate group by Julian data ($F_{1,165} = 3.5$, P = 0.06) and main effects of invertebrate category ($F_{1,169} = 2.0$, P = 0.15), Julian date ($F_{1,169} = 7.5$, P = 0.007), and habitat ($F_{1,133} = 7.4$, P = 0.007). At the early point in

the season (Julian = 120), there was a significant difference in MeHg concentrations between main channel and backwater habitats, with backwater least squares mean MeHg concentrations (67.5 ng/g, SE = 7.9) being 2 times higher than those in the main channel (33.1 ng/g, SE = 3.6). MeHg concentrations in invertebrates from backwater habitats varied by only 7% throughout the season, whereas and MeHg in invertebrates from main channel habitats increased by 83% throughout the season (Fig 4.4a). At the end of the season, there was no difference in least squares mean MeHg concentration between main channel (60.7 ng/g, SE = 6.3) and backwater (72.2 ng/g, SE = 8.3) aquatic invertebrates. There was also a marginal interaction of invertebrate category and Julian date ($F_{1, 165}$ = 3.5, P = 0.06). Early in the season, aquatic predators and non-predators did not differ in MeHg concentrations (P > 0.05). Over the season, both predators and non-predators increased in MeHg concentrations, but concentrations in aquatic predators were 13-fold higher at the end of the season while non-predators were only 5-fold higher (Fig. 4.4b). At the end of the season, there was a marginal difference, with predators showing higher MeHg concentrations than non-predators.

In terrestrial invertebrates, MeHg concentrations differed among invertebrate categories ($F_{1,5}$ = 27.2, P = 0.004) but not with Julian date ($F_{1, 111}$ = 2.8, P = 0.1) nor habitat type ($F_{1, 6}$ = 0.4, P = 0.55), and there were no significant 2-way interactions. MeHg concentrations in predatory invertebrates (least squares mean = 38.95 ng/g, SE = 7.36) were over 12 times higher than in non-predators (least squares mean = 2.81 ng/g, SE = 0.31).

Spatial and temporal patterns in songbird Hg exposure. Across all samples of breeding riparian songbirds (accounting for species and site as random effects) we found that blood THg concentrations differed with Julian date ($F_{1,268}$ = 24.8, P < 0.001) and by habitat ($F_{1, 9}$ = 5.0, P = 0.05). Across all species, sites and habitats, songbird THg least square means declined by over half between early and late points in the season (early = 266.4 ng/g, SE = 32.4, late = 123.2 ng/g, SE =15.0). Backwater sites (209.2 ng/g, SE = 25.6) were 1.4x higher in THg concentrations than main channel (150.5 ng/g, SE = 18.1).

For the five bird species where we had substantial replication across habitats, sites and dates (Common Yellowthroat, Spotted Towhee, Song Sparrow, Swainson's Thrush, and Yellow Warbler) we found that Hg concentrations differed among species ($F_{4,258}$ = 4.8, P = 0.001) and habitats ($F_{1,9}$ = 6.0, P = 0.04), as well as with Julian date ($F_{1, 266}$ = 15.3, P = 0.0001). However, the significant species x Julian date interaction ($F_{4,258}$ = 7.1, P < 0.001) suggests that the temporal patterns were not consistent across species. After accounting for

the effects of species, date, and site, we found that birds sampled from backwater habitats (305 ng/g, SE = 13.1) had elevated THg concentrations in comparison to birds sampled from main channel (223.5 ng/g, SE = 8.5) habitats (Fig. 4.5a). Additionally, there were no differences in THg concentrations among species early in the season, but species varied in the magnitude of change throughout the season. At the late sampling period, Swainson's Thrush exhibited lower Hg concentrations than all other species (Fig. 4.5b).

Aquatic subsidy effect on Hg exposure in songbirds and riparian predatory invertebrates. In backwater habitats, there was poor differentiation in $\delta^{13}C$ ratios between aquatic and terrestrial food webs (Fig. 4.3), thus we focus solely on main channel habitats. We ran a two-end member mixing model to model the magnitude of aquatic carbon reliance in both terrestrial invertebrate predators (groups including invertebrate families: Opiliones, Salticidae, and Tetragnathidae as well as composite arachnid samples) and riparian songbirds (Common Yellowthroat, Song Sparrow, Spotted Towhee, Swainson's Thrush, and Yellow Warbler). We used the average $\delta^{13}C$ for terrestrial non-predator invertebrates (-28.02‰) and the average $\delta^{13}C$ for aquatic non-predator invertebrates (-22.08‰) as endmembers in our two-end member mixing model. We ran a mixed effects general linear model to predict the proportion of aquatic carbon in predatory invertebrates based on Julian date ($F_{1,19}$ = 1.6, P = 0.22), invertebrate category ($F_{3,18}$ = 2.4, P = 0.1) and an interaction between Julian date and invertebrate category ($F_{3,18}$ = 3.8, P = 0.03). Early in the season, there was no significant differences among invertebrate categories but opiliones, salticidae and spider composite groups all declined throughout the season, whereas tetragnathids did not; at the late season time point, tetragnathids (least squares mean 84% aquatic carbon, SE = 7%) were higher in proportion aquatic carbon than opiliones (40% aquatic carbon, SE = 8%), salticidae (21% aquatic carbon, SE = 13%), and spider composites (27% aquatic carbon, SE = 7%) (Fig. 4.6a).

We then examined the relationship between aquatic carbon reliance and MeHg concentrations in terrestrial invertebrate predators using analysis of covariance (ANCOVA). MeHg concentrations differed among group (Opiliones, Salticide, Tetragnathidae, Spider composite, $F_{3,20}$=7.2, P = 0.002), and with proportion aquatic carbon ($F_{1,22}$ = 25.1, P < 0.0001). However, the interaction between proportion of aquatic carbon and group ($F_{3,19}$ = 4.4, P = 0.02) indicated that the slopes for the relationship between proportion of aquatic carbon and MeHg concentrations varied among taxa (Fig. 4.6b).

We ran similar models for riparian songbirds. First, we looked at factors that predicted proportion aquatic carbon in the diet. We found a simplified model that accounted for main effects of Julian date ($F_{1, 130} = 29.6$, $P < 0.0001$) and species ($F_{4,127} = 10.2$, $P < 0.0001$). Throughout the season, proportion of aquatic carbon in the diet of riparian songbirds declines, from 78% (SE = 4%) at the early sampling period to 50% (SE = 4%) at the late sampling point (Fig. 4.7a). Some species showed baseline higher reliance on aquatic carbon but overall there was high individual variation. Song sparrows (least squares mean = 72%, SE = 3%) and Yellow Warblers (least squares mean = 68%, SE = 5%) generally showed higher baseline reliance on aquatic prey than Common Yellowthroat (least squares mean = 55%, SE = 5%), Swainson's Thrush (least squares mean = 56%, SE = 4%) or Spotted Towhee (least squares mean = 59%, SE = 4%) (Fig. 4.7b).

We found that the THg concentrations in riparian songbirds were predicted by proportion aquatic carbon in diet ($F_{1, 131} = 30.5$, $P < 0.0001$) and species ($F_{4,128} = 14.8$, $P < 0.0001$) but there was no significant interaction between these variables. Species have different baseline THg concentrations. Baseline THg varied more than two-fold among species, with Swainson's Thrush (135 ng/g, SE = 7.2) showing the lowest baseline least squares mean THg concentration, followed by Spotted Towhee (197 ng/g, SE = 13.0), Yellow Warbler (220 ng/g, SE = 7.2), Song Sparrow (228ng/g, SE = 10.1) and Common Yellowthroat (441 ng/g, SE = 31.0) (Fig. 4.7c). Across species, as proportion aquatic carbon increases, THg concentration in the blood increases (Fig. 4.7d).

Discussion

We found strong influences of season and habitat on Hg exposure in both invertebrates and riparian songbirds. Aquatic invertebrates in backwater habitats were significantly higher in MeHg than those from main channel habitats early in the season, but this difference diminished across the season. Songbird blood THg concentrations in backwater habits were higher than in main stem habitats throughout the year. We believe that the seasonal drop in songbird MeHg concentrations was due to a shift from aquatic to terrestrial invertebrates as the breeding season progressed. Terrestrial invertebrates had lower MeHg than aquatic invertebrates, and their MeHg decreased across the season, whereas MeHg concentrations in aquatic invertebrates were higher later in the season. Consistent with this interpretation is

isotopic data showing a correlation between proportion of aquatic carbon in songbird diet and THg exposure in all songbird species sampled.

Previous studies have shown that habitat can influence Hg exposure in songbirds at a large scale (Jackson et al. 2015). Our finding, that riparian songbirds living near backwater habitats had higher Hg exposure than conspecifics living near main stem habitats, supports the notion that aquatic habitat differences, even within a small geographic region of one river system, can influence Hg exposure throughout the food web. Fish in alcove versus main stem habitats of the Willamette River show similar trends, with higher THg exposure in backwater habitats (Eagles-Smith, unpublished data). Backwater habitats provide important conservation areas for flood management and riparian habitat in this human dominated system (Baker et al. 2004), but may also increase Hg bioaccumulation through aquatic and terrestrial food webs. Similar to emergent or forested wetlands, these backwater habitats likely provide conditions where Hg methylation can take place at higher rates. Backwaters may increase risk associated with Hg exposure because of their potential for Hg methylation but we speculate that their documented conservation benefits likely outweigh the negative effects of Hg methylation in the Willamette River system (Gregory et al. 2002). Because the mercury concentrations in songbirds were, on average, well below concentrations associated with health effects, there may be a net gain in productivity due to the conservation of these backwater habitats. Further research is needed within the Willamette River system to fully understand the cost and benefit of backwater habitats. However, in riverine systems with higher Hg loading than the Willamette, backwater habitats such as these may significantly influence MeHg exposure to riparian food webs, potentially increasing the risk of deleterious toxic effects (Cristol et al. 2008, Jackson et al. 2011a).

Although there were clear differences in aquatic invertebrate MeHg concentrations among habitats, dietary shifts in riparian songbirds likely influenced their exposure to MeHg through the breeding season. Seasonal weather shifts from wet spring through dry summer months, which span the songbird breeding season, can influence terrestrial invertebrate abundance as well as emergence pulses of aquatic invertebrates (Nakano and Murakami 2001). Using isotopic signatures, we found that songbird diets from main channel habitats shifted from one largely reliant on aquatic-sourced carbon to a greater reliance on terrestrially sourced carbon. Songbird shifts in reliance on emergent aquatic insects have been documented using other techniques (Murakami and Nakano 2001) but we are not aware of

any that have used stable isotopes or linked this to Hg exposure. Our results show that although aquatic invertebrate MeHg concentrations are higher at the end of the breeding season, riparian bird Hg concentrations are lowest during that time. These contrasting patterns of bioaccumulation indicate that 1) birds caught later in the season are largely feeding on terrestrial insects instead of the high Hg emergent aquatic invertebrates and/or 2) the individuals sampled early in the season leave after fledging and their territories become occupied by other birds that had been previously foraging on terrestrial invertebrates. Although we gathered no data on timing of aquatic invertebrate emergences, it is widely accepted that the majority of emergences happen early in the season before terrestrial prey is readily available (Meck and Hoover 1992).

Hg concentrations were correlated with aquatic carbon reliance in both predatory terrestrial invertebrates and riparian songbirds. For invertebrates, we found that the relationship between reliance on aquatic carbon and MeHg exposure varied among families. For example, tetragnathid spiders, often used in contaminant studies (Walters et al. 2008, 2010, Otter et al. 2013), all had high reliance on aquatic carbon and similar MeHg concentrations, indicating they are fairly constrained in their prey preferences. Other spider groups are much more mobile with various hunting strategies and varied in both their reliance on aquatic carbon and their MeHg exposure.

Birds that rely more on aquatic carbon show higher Hg exposure, which follows findings of others who have shown that emergent aquatic insects are an important source of aquatic contaminants to terrestrial ecosystems (Walters et al. 2008, 2010). Very few studies have quantified individual or species-specific reliance on aquatic prey outside of aerial insectivores, which are known to focus on emergent insects (Brasso and Cristol 2008, Custer et al. 2008, Alberts et al. 2013). By focusing on other forest riparian songbirds, we elucidate the changes that occurred within the season in species that have more flexible foraging strategies. Within our main channel systems, it is clear that songbird reliance on aquatic sourced carbon declines throughout the season, presumably due to the later emergence of terrestrial invertebrates following the leafing out of deciduous trees and plants. Previous studies have used observations to determine aquatic reliance on terrestrial versus aquatic prey in riparian songbirds (Murakami and Nakano 2001, Uesugi and Murakami 2007) but few have used $\delta^{13}C$ isotope to determine this reliance at a coarse scale.

Overall, these Hg levels in songbirds are below general thresholds thought to cause reproductive harm (Jackson et al. 2011a, Varian-Ramos et al. 2014). It is important to understand, however, that these thresholds are developed for a limited numbers of species, none of which were sampled in this project. It is likely that species and individuals vary in their sensitivity to Hg and so taxa-wide threshold levels should be used with caution (Varian-Ramos et al. 2013). While the Hg levels are relatively low, we believe that our findings on habitat, species, and season can apply to other study areas with higher Hg loading and so reinforce the importance of studying interactions between behavior, season and habitat.

Conclusions. This study documents significant differences in Hg exposure in riparian songbirds between backwater and main channel habitats within a subsection of the Willamette River watershed. While maintaining and enhancing backwater habitats are important conservation goals to restore the historic ecosystem, we must take into account the added Hg methylation that occurs in them. This is also the first study to use stable isotopes of carbon to determine reliance on aquatic carbon in riparian songbirds other than for aerial insectivores traditionally used in contaminant studies. We were not only able to correlate Hg concentrations in songbirds to their reliance on aquatic-based food, but also showed that this subsidy is pulsed early in the season. There is still a lot to understand about how the actual flux of emergent aquatic insects varies across the season and future research should quantify the biomass and Hg flux of emergent aquatic insects seasonally.

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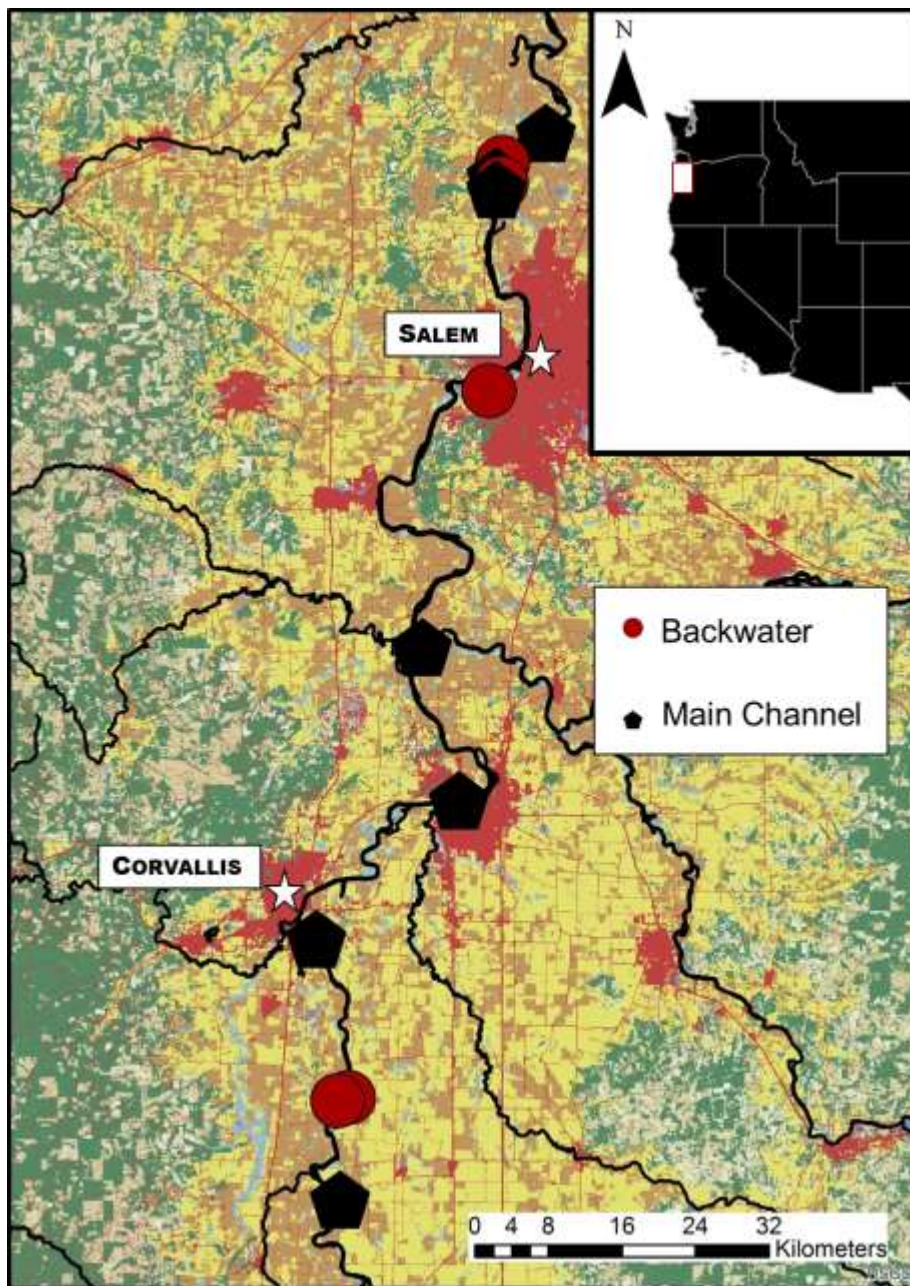
Figure 4.1. Study sites along the Willamette River in western Oregon, sampled in 2013. Backwater sites include pools, alcoves and lakes while main channel sites are riffle and run habitat on the main stem of the river.
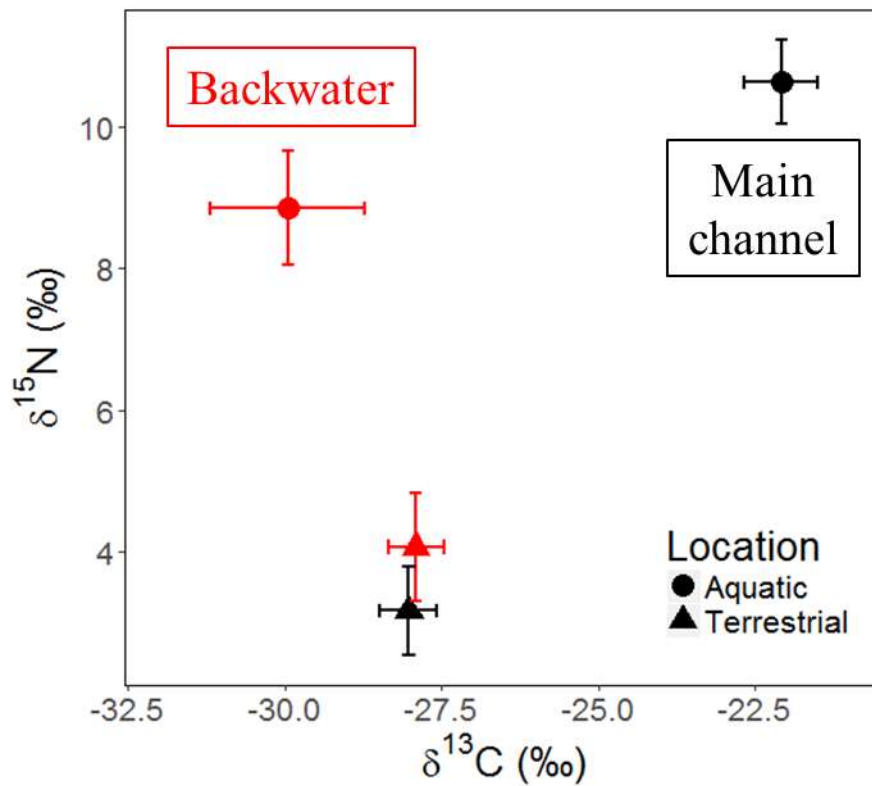
Figure 4.2. Stable isotopes (δ^{13}C and δ^{15}N) of non-predatory invertebrates as arithmetic means in each habitat type (main channel shown in black and backwater shown in red) and sampling location (aquatic shown as circles and terrestrial shown as triangles). Error bars indicate 95% confidence interval for the mean.
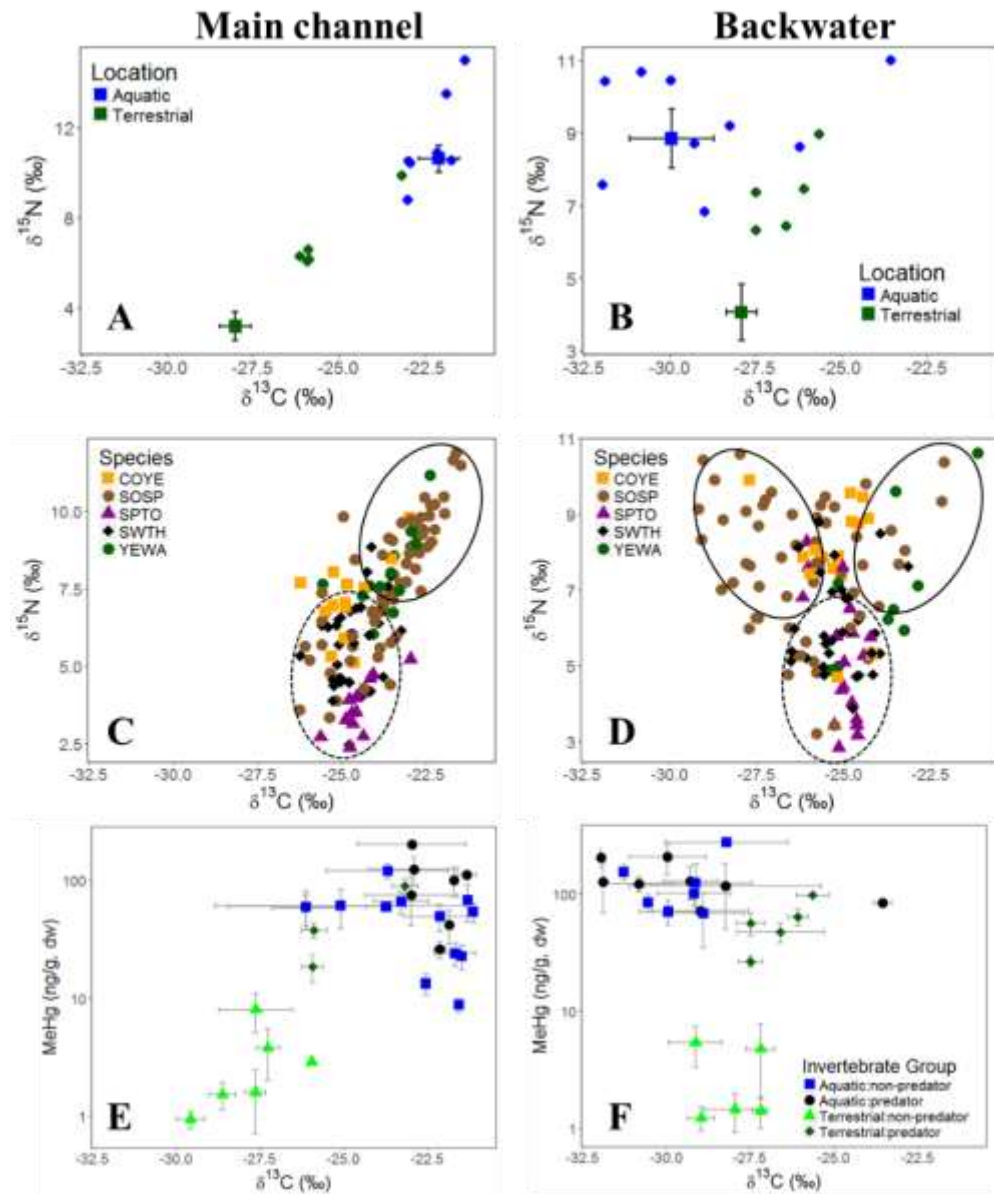
Figure 4.3. Comparison of main channel (left) and backwater (right) isotope plots. Relationship between $\delta^{13}C$ isotope and $\delta^{15}N$ isotope for invertebrates in a) main channel and b) backwater habitats. Error bars indicate mean for non-predatory invertebrate endmembers while points indicate mean values of predatory taxa from both aquatic (blue) and terrestrial (green) sampling locations. Relationship between $\delta^{13}C$ isotope and $\delta^{15}N$ isotope in riparian songbirds in c) main channel and d) backwater habitats. COYE = Common Yellowthroat, SOSP = Song Sparrow, SPTO = Spotted Towhee, SWTH = Swainson's Thrush, YEWA = Yellow Warbler. Dashed ellipses indicate isotope space for terrestrial carbon, solid ellipses indicate isotope for aquatic carbon. Relationship between $\delta^{13}C$ isotope and MeHg concentrations in invertebrates sampled in either terrestrial or aquatic locations of e) main channel or f) backwater habitats.
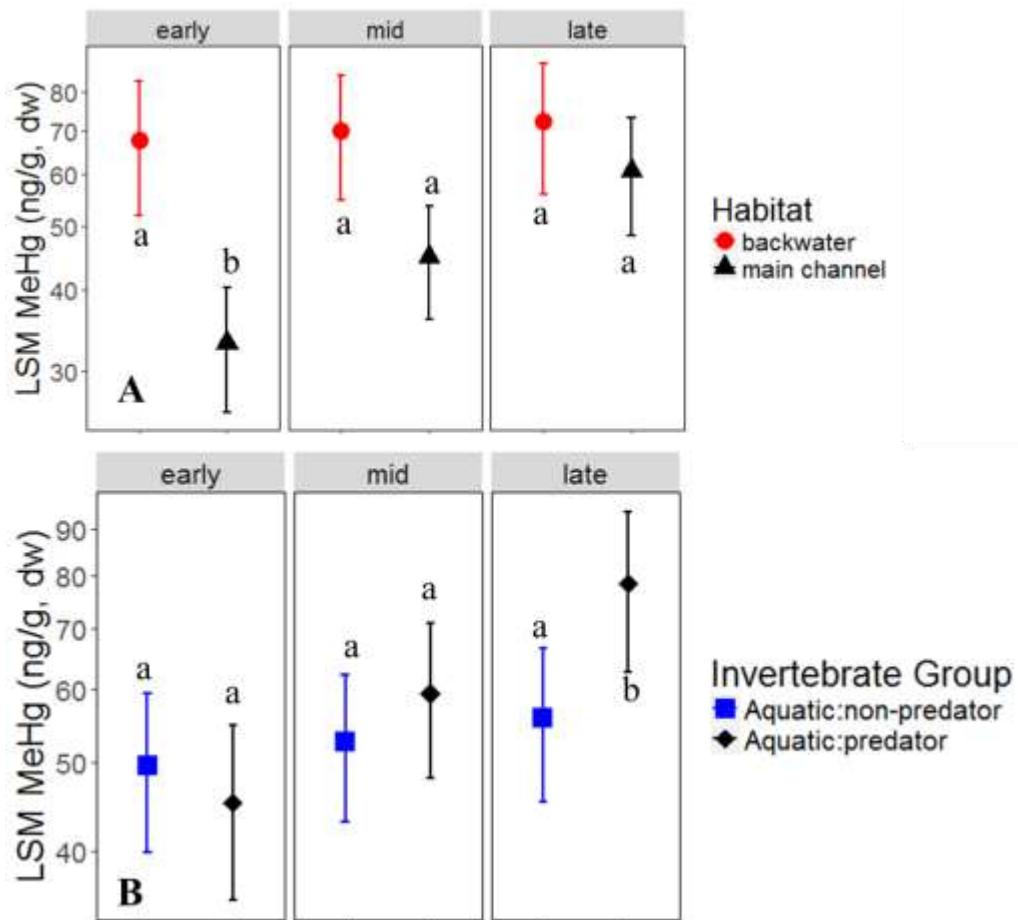
Figure 4.4. Least squares mean invertebrate MeHg concentrations for a) significant interaction of habitat by Julian date and b) marginal interaction of invertebrate group by Julian date. Mixed effects model accounts for site and invertebrate order as random effects.
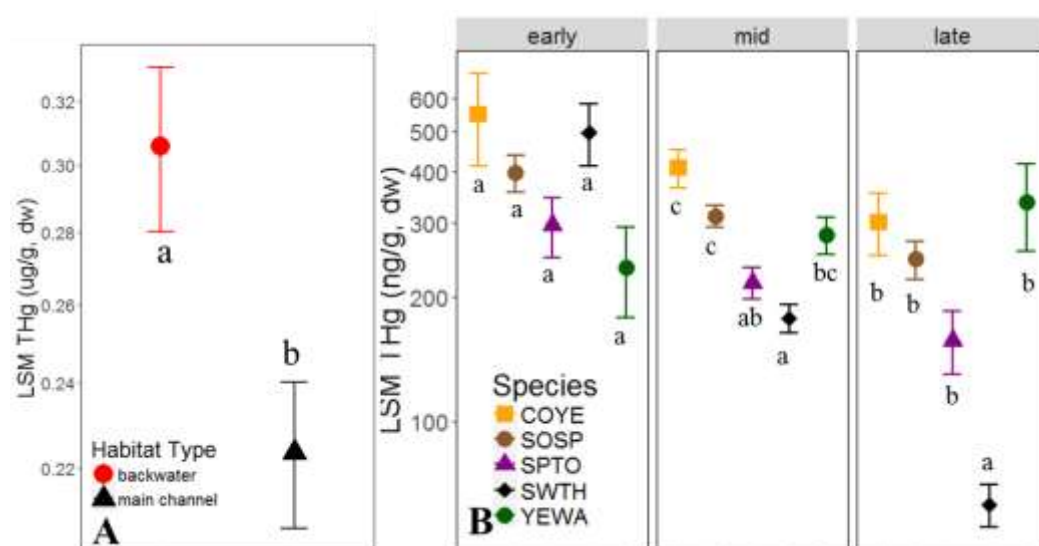
Figure 4.5. Least squares mean songbird blood THg concentrations for a) significant main effect of habitat and b) significant interaction of species by Julian date. Mixed effects model accounts for site and as a random effect.
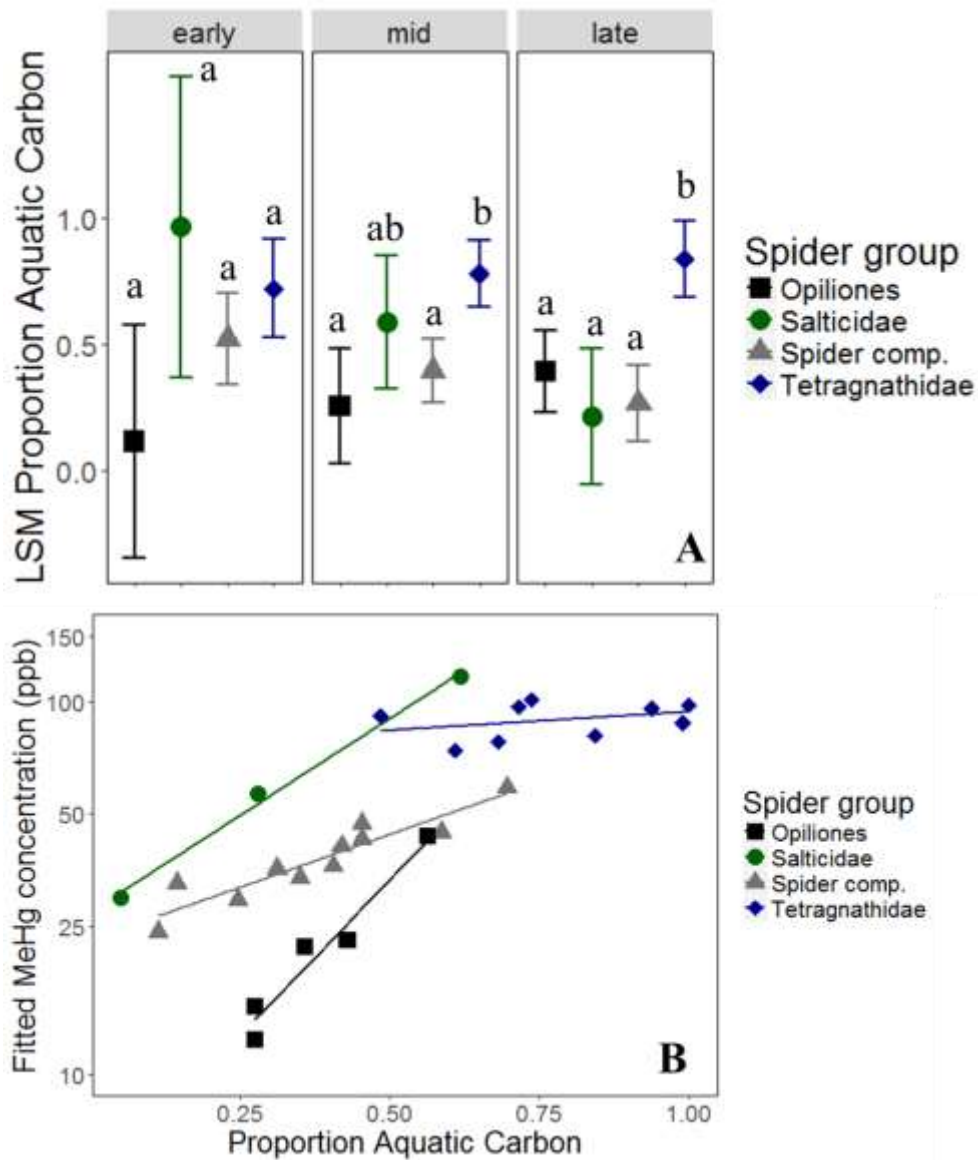
Figure 4.6. A) Significant interaction of spider group by julian date in a model to predict proportion of aquatic carbon in the diet of terrestrial predator invertebrates. B) Relationship between proportion aquatic carbon and MeHg concentration in terrestrial predatory invertebrates, after taking into account site and species differences.
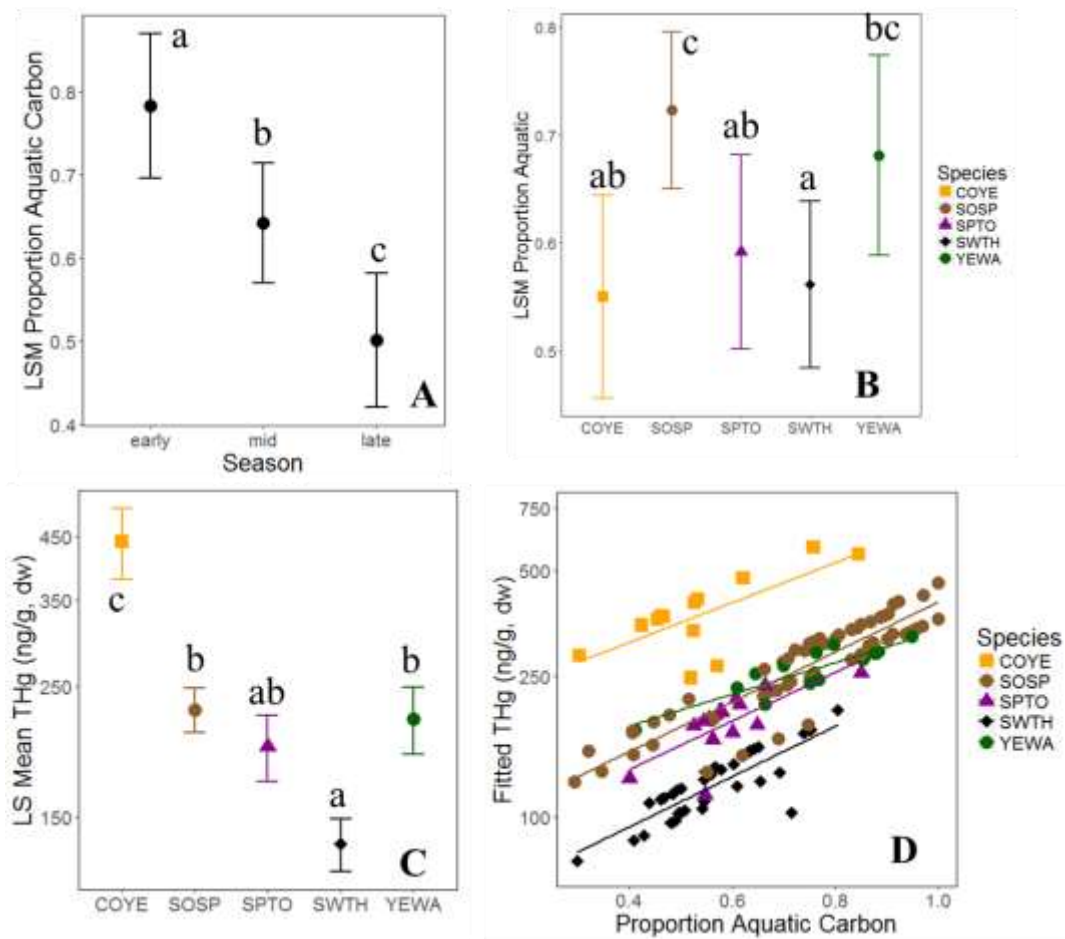
Figure 4.7. Significant effects of model to predict riparian songbird proportion of aquatic prey in the blood: a) main effect of julian date and b) main effect of species. Significant effects of model to predict songbird THg in blood based on c) species and d) proportion aquatic carbon in diet.

Table 4.1. Sample size for invertebrate composite samples in each invertebrate category.

Invertebrate Category	Order	Lowest taxon	backwater	Main channel	Total
Aquatic, non-predator	Coleoptera	Hydrophilidae	4	3	7
	Crustacean	amphipod	4	11	15
		Isopod	5		5
		Isopod &Amphipod composite	2		2
	Ephemeroptera	Caenidae	1		1
		Ephemerellidae	3	6	9
		Ephemeroptera comp.		1	1
		Heptagenidae	1	9	10
		Leptohyphidae	1	4	5
		Siphlonuridae	2	3	5
	Gastropod	Asian freshwater clam		1	1
		Hydrobiidae		3	3
		Lymnaeidae	1	1	2
		Physidae	2	2	4
		Planorbidae	4		4
		Pleuroceridae	2	10	12
		Snail comp.	3		3
	Hemiptera	Corixidae	7	4	11
	Trichoptera	Glossomatidae		3	3
		Hydropsychidae		5	5
		Limnephilidae	1	4	5
		Trichoptera comp.		1	1

Table 4.1 (continued)

Invertebrate Category	Order	Lowest taxon	Backwater	Main channel	Total
Aquatic, predator	Coleoptera	Dytiscidae	10	10	20
		Gyrinidae	1	2	3
	Crustacean	Crayfish		1	1
	Hemiptera	Belostomatidae	3		3
		Gerridae	3		3
		Notonectidae	3		3
	Odonata	Aeshnidae	6	2	8
		Coenagrionidae	9	3	12
		Corduliidae and Libellulidae	3		3
		Gomphidae	2	3	5
	Plecoptera	Chloroperlidae		2	2
		Perlidae		2	2
		Perlodidae		2	2
Terrestrial, non-predator	Arthropod Composite	Arthropod composite	1	1	2
	Coleoptera	Coleoptera	10	11	21
	Dermoptera	Dermoptera	1	1	2
	Hemiptera	Hemiptera	9	11	20
	Hymenoptera	Hymenoptera	1	2	3
	Lepidoptera	Lepidoptera adult	3	4	7
		Lepidoptera larvae	9	11	20
	Orthoptera	Orthoptera	4	4	8

Table 4.1 (continued)

Invertebrate Category	Order	Lowest taxon	Backwater	Main channel	Total
Terrestrial, predator	Araneae	Araneidae	2	1	3
		Salticidae	2	3	5
		Spider composite	8	11	19
		Tetragnathidae	5	10	15
	Opiliones	Opiliones	2	6	8

CHAPTER 5. GENERAL CONCLUSIONS: YOU ARE WHAT, WHEN, WHERE AND HOW YOU EAT.

In terms of Hg exposure, birds are what, when, where and how they eat. Here I summarize the findings across the multiple spatial scales—continental (Chapter 2), regional (Chapter 3), and local (Chapter 4) — studied in this dissertation.

You are what you eat. Broadly, "what" birds eat is one of the major differences in the avian taxa I studied. Chapter 2 details risk in traditional subjects of Hg exposure studies – fish and fish-eating birds (Scheuhammer et al. 2007). Chapters 3 and 4 focus on riparian songbirds, which have been more recently developed as bioindicators (Cristol et al. 2008, Jackson et al. 2015). Aside from broad-scale differences in taxa feeding requirements (fish versus invertebrates), my dissertation highlights how what each individual bird is eating impacts its Hg exposure. For piscivores, we modelled risk based on the fish prey they ate; birds that could eat larger (higher trophic position and older) fish were at more risk to Hg exposure. In riparian songbirds, individuals that consume more aquatic-based prey have higher Hg exposure than those that eat more terrestrial-based prey.

Besides feeding in different food webs, these two groups of birds also come from different evolutionary lineages, which could have implications for their ability to deal with Hg exposure. Mercury is a naturally occurring heavy metal found in the earth's crust. Mercury would have historically occurred in aquatic environments, but humans have mined, concentrated and rereleased Hg in high quantities (Pirrone et al. 2010). Because it is a naturally occurring element, some researchers believe that aquatic or marine species may have developed evolutionary mechanisms to demethylate MeHg in their liver, reverting it to inorganic Hg and decreasing its negative effects. This potential demethylation pathway has only been documented in aquatic-based species, such as fish-eating birds and mammals, where there is a logical pathway of Hg exposure throughout evolutionary history (Wagemann et al. 1998, Eagles-Smith et al. 2009). In my second chapter, I focused on fish and fish-eating bird species that likely have come in contact with Hg throughout their evolutionary past. In the other two chapters, I focused on songbird species that likely came in contact with relatively little Hg during their evolutionary history. I think that they likely lack demethylation strategies because their populations would not have had to interact with Hg until humans concentrated Hg through mining and industry. Preliminary studies have shown

that songbird taxa may be particularly sensitive to the effects of Hg on reproduction (Brasso and Cristol 2008, Jackson et al. 2011)

You are when you eat. My fourth chapter offers a first look at how temporal changes in insect phenology and bird foraging can influence Hg exposure. We showed that early in the season, sampled birds ate more aquatic carbon and had subsequently higher Hg exposure. Later in the season, sampled birds showed a much more terrestrial signal, coinciding with warmer temperatures, increased solar radiation, and increased terrestrial productivity. There are still many gaps in this research; future research should focus on measuring actual Hg flux (via emergent insect biomass) within the season. Relatively few studies have quantified this temporally, but researchers are finding correlations between emergent insect flux and Hg exported into the riparian zone (Tweedy et al. 2013, Chumchal and Drenner 2015). These temporal shifts have important implications for biomonitoring of bird populations because the sampling time period can influence which part of the population is sampled – and subsequently Hg concentrations exhibited.

You are where you eat. Throughout this dissertation, a uniting theme is that habitat matters. Although I did not test this at a large spatial scale, the hotspots of biological Hg contamination from Chapter 2 share similar habitat characteristics. Hg hotspots occur in cells with heavy mining influence, cells that encompass river estuaries or cells with high density of wetlands, all habitats of high Hg methylation. At the regional scale of the Willamette basin, distance to the source of contamination did not matter as much as habitat-specific characteristics. Wetland complexes had similar Hg concentrations to the superfund sites because methylation rate is probably a more important driver than input. Within our smallest spatial scale – the Willamette River – I showed that backwater habitats within a single river system showed higher Hg concentrations than main channel areas, likely driven by methylation rates. Although we know that Hg is driven by methylation, this is the first time these small scale differences have been identified in riparian songbirds with varying connections to aquatic prey.

You are how you eat. Although not explicitly tested in any chapter, there could be differences in songbird foraging ecology that would help explain differences in Hg exposure within sites. Foundational research in niche theory shows that songbirds often partition niche by foraging location and strategy within the canopy. This partitioning could also help explain our differences in Hg exposure because foraging strategy influences what type of prey the

birds consume. While ultimately related to what the birds are eating ("you are what you eat"), foraging strategies ("how you eat") can help explain why birds may be feeding on such different prey sources within the same area. For example, Spotted Towhee and Swainson's Thrush show some of the lowest reliance on aquatic carbon and lowest Hg and both of these species are classified as omnivorous ground foragers (De Graaf et al. 1985). Bewick's Wren, which showed the highest Hg concentrations in this region, are also ground foragers but focus on invertebrates and likely eat many spiders (Kennedy and White 2013). Although Common Yellowthroats exhibit relatively low reliance on aquatic carbon, they have some of the highest Hg levels shown here. Although classified similarly to Yellow Warblers (insectivore, lower canopy gleaner; De Graaf et al. 1985), riparian Common Yellowthroats likely feed at higher trophic levels due to their gleaning habits. Future research should focus on parsing these differences and understanding their implications for food web structure and contaminant exposure.

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