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Exploring Linkages Between Landscape Patterns and Freshwater and Estuarine Bivalves in the Coast Range of Oregon

Kaegan Michael Scully-Engelmeyer
Portland State University

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by

Kaegan Michael Scully-Engelmeyer

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Dissertation Committee:
Elise F. Granek, Chair
Max Nielsen-Pincus
Steven Rumrill
Jennifer Allen
Andy Lanier (non-voting member)

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Abstract

Spatial configurations of landscape variables (biotic, abiotic, and socio-ecological) affect and are affected by ecological processes and species in watersheds. This dissertation explores relationships among landscape patterns, ecosystem processes and bivalve species dynamics in coastal watersheds in Oregon, USA. I approached this broad topic through two primary avenues of research: investigating cross-ecosystem threats from pesticide use in forestland management to downstream aquatic environments, and the landscape ecology of an at-risk freshwater mussel species.

Terrestrial land use activities present cross-ecosystem threats to riverine and marine species and processes. Specifically, pesticide runoff can disrupt hormonal, reproductive, and developmental processes in aquatic organisms, yet non-point source pollution is difficult to trace and quantify. In Oregon, state and federal forestry pesticide regulations, designed to meet regulatory water quality requirements, differ in buffer size and pesticides applied. To identify exposure and uptake of contaminants in coastal watersheds, I collected freshwater and estuarine bivalves *Margaritifera falcata*, *Mya arenaria*, and *Crassostrea gigas* from eight Oregon Coast watersheds to examine forestry-specific pesticide contamination. Additionally, during a 45 day period in the spring of 2019, I sampled sixteen coastal watersheds for current-use water-borne herbicides commonly used in forestland vegetation management. In 38% of bivalve samples, one or more of twelve unique pesticides were detected (two herbicides; three fungicides; and seven insecticides). Frequency and maximum concentrations varied by

season, species, and watershed, with indaziflam (herbicide) the only current-use forestry pesticide detected. At 80% of sampling locations integrative passive water samplers detected at least one of four commonly used herbicides, with hexazinone and atrazine most commonly detected. An additive effects model using slope, herbicide activity notified during the sampling window, and recent clearcut harvest notifications predicted variation in total herbicide accumulation (R^2=0.8914). The model was then applied to predict concentrations in un-sampled watersheds throughout Oregon's coastal region at three watershed scales using Hydrologic Unit Codes (HUCs) 8, 10, and 12. Details about types and levels of exposure provide insight into effectiveness of current forest management practices in controlling transport of forest-use pesticides at multiple scales.

Freshwater mussels have declined across the region following widespread degradation of freshwater habitat and other aquatic species, including parallel declines in salmonid species, which serve as host fish for larval western pearlshell mussels (*Margaritifera falcata*). *M. falcata* are native to Pacific coastal watersheds in Oregon and beyond, but their comparative distribution, habitat, host species interactions, and health have not been investigated in detail. To understand population dynamics of extant *M. falcata* in Oregon's small coastal watersheds, I analyzed a dataset of stream survey observations collected over a recent ten year period for presence/absence of mussels, explored reach-scale habitat characteristics in relation to persistence of populations, and summarized the current distribution of surveyed mussels and their co-occurrence with host fish species in coastal drainages. I also collected *M. falcata* at eight locations within Oregon's Coast Range and compared condition indices among sites. Overall naïve

occupancy in surveyed areas was 12.3%, close to half of predicted occupancy (ψ= 0.24, CI= 0.19-0.31) based on modeling repeated visits over a ten year assumed closure period. Mussel occupancy was positively correlated with habitat variables (% of pool, count of boulders and stream temperature), providing new information about reach-scale habitat associations in Oregon's coastal watersheds. Using a host fish co-occurrence analysis, I found that probability of mussel observations was positively correlated with presence of coho (*Oncorhynchus kisutch*) and chinook (*O. tshawytscha*) salmon, and negatively associated with steelhead (*O. kisutch*) based on logistic regression. Condition varied significantly among mussel collection locations (n=8), and healthiest animals were found in areas draining small catchments. Spatial relationships between existing distribution, host species and habitat variables outlined in this study answer questions about coastal freshwater mussel populations in Oregon and identify “priority areas" for further research, conservation, and population assessment within this region.

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Preface

Chapters 2, 3, and 4 will or have been submitted for peer-reviewed publication. Chapter 2 has been published, Chapter 3 is under peer review, and Chapter 4 is currently in preparation for submission. As a result there is some repetition of concepts in the introductions of those chapters. Additionally, I use “we” throughout those chapters to include co-author contributions.

Chapter 1: Introduction

Spatial patterns within landscapes influence ecological processes and species dynamics across multiple scales and timeframes (Turner, 1989). Investigations into the effects of patterns on processes at the landscape scale require a broad perspective, integrating larger socio-ecological, biological, and geographical considerations into research objectives (Turner, 1989). Understanding the spatial and temporal dynamics of cross-ecosystem impacts of terrestrial conditions on aquatic and marine species is a challenging but essential step in designing effective land-sea planning, management, and conservation (Álvarez-Romero et al., 2011). Coastal ecosystems, due to their transitional position bridging marine and terrestrial environments, force managers to think more broadly about threats and impacts of terrestrial environments on aquatic and marine systems (Ruttenberg and Granek, 2011). Region-specific considerations guide priorities in investigations into landscape processes affecting coastal species and ecosystems.

Oregon's coastal zone, a region encompassing biogeographically similar coastal watersheds from the mouth of the Columbia River to the California border, extends from the crest of the Coast Range Mountains (with exceptions in the southern coast) to three nautical miles offshore (Figure 1). The terrestrial area is predominately forested and characterized by cool dry summers and mild wet winters, making it one of the most productive forest ecosystems worldwide (Spies et al., 2002). Much of Oregon's coastal zone also closely overlaps the US Environmental Protection Agency's (EPA) Coast Range ecoregion designation (Level III) in Oregon (Figure 1) (Omernik, 1987).

Throughout this research, references are made to the Coast Range and the coastal zone; though not directly interchangeable they encompass the same coastal watersheds and biogeographical region. Temperate rainforests found in this region support a diversity of species, many of which are dependent on complex ecological processes associated with late seral and mature conifer forest habitats that once dominated the region (Molina et al., 2006). In addition to supporting biological diversity, the productive forestlands of the region have also been central in underpinning Oregon's natural resource economy since early in its statehood (LeMonds, 2001). Approaches to forestland management during the mid-late 1900s led to extensive declines in historical landscape patterns of large conifer dominated forests, and were replaced by small-medium conifer forests managed for timber production (Kennedy and Spies, 2004). This transformation of the forested landscape by the early timber industry has had dramatic repercussions on the landscape and forest dependent communities that are still evident today (Kelly and Bliss, 2012).

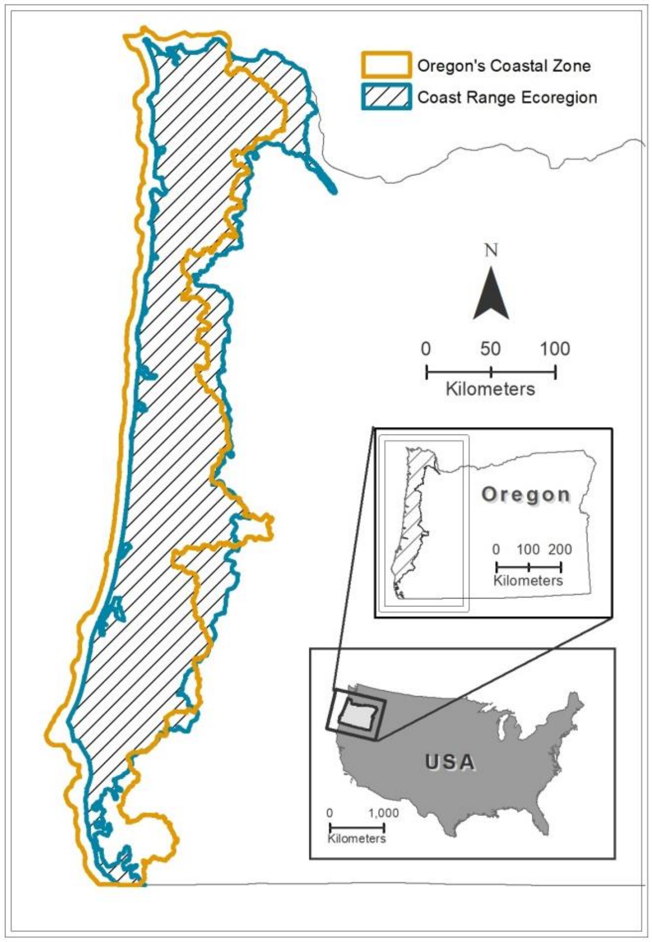

Figure 1: Small coastal drainages along the Pacific coast comprise Oregon's coastal zone, a watershed based zone designated by Oregon Legislature, which closely overlaps the EPA designated Coast Range ecoregion.

In Oregon, contemporary forestry management can be separated into two main regulatory regimes: management of federal lands (US Forest Service & Bureau of Land Management), guided by the Northwest Forest Plan (NWFP) and private, industrial, state, and tribal lands, regulated under Oregon's Forest Practices Act (FPA) (Hairston-Strang et al., 2008; Thomas et al., 2006). Both plans lay out Best Management Practices (BMPs) for timber harvest, pesticide application, road construction guidelines, and riparian buffer regulations for each land ownership type. Though management is further differentiated within each ownership class, regulatory direction is attributed to the overarching plan.

For example, federal, state, and tribal forests are managed under individual guiding documents, but the regulations guiding permitted activities and objectives fall under the NWFP and FPA. Each plan is designed to meet federal regulatory requirements ensuring adequate protections for listed species and water quality under the Endangered Species Act (ESA) and the Clean Water Act (CWA), though levels of responsibility and conservation to meet objectives are not shared evenly between ownership types. The NWFP relies heavily on the tenets of Ecosystem Based Management (EBM) to prioritize objectives, expanding management activity beyond timber harvest to promote biological diversity, ecosystem function, and endangered species conservation (FEMAT, 1993; Noon and Blakesley, 2006). The shift in priorities towards conservation, though included in some capacity by state forest management plans, was not echoed to the same extent on state "working forests", private or industrial lands. As a result, the majority of current forestlands managed primarily for timber production are concentrated on state, private, and industrial land (Andrews and Kutara, 2005). Within the context of this divergent management landscape, further research is needed on relationships among regulatory regimes, land ownership, and aquatic ecological systems across multiple scales to better understand cross-ecosystem threats and factors influencing regional aquatic species populations.

1. Cross-ecosystem threats and bivalve populations

Broad patterns of biotic, abiotic, and socio-ecological drivers influence species and ecological processes across multiple spatial and temporal scales (Figure 2). In my research, I focus on interactions among a set of landscape drivers (factors identified in

Figure 2) in Oregon's coastal watersheds, and investigate aspects of those interactions to answer questions about landscape patterns relevant to coastal bivalve species and ecosystem processes.

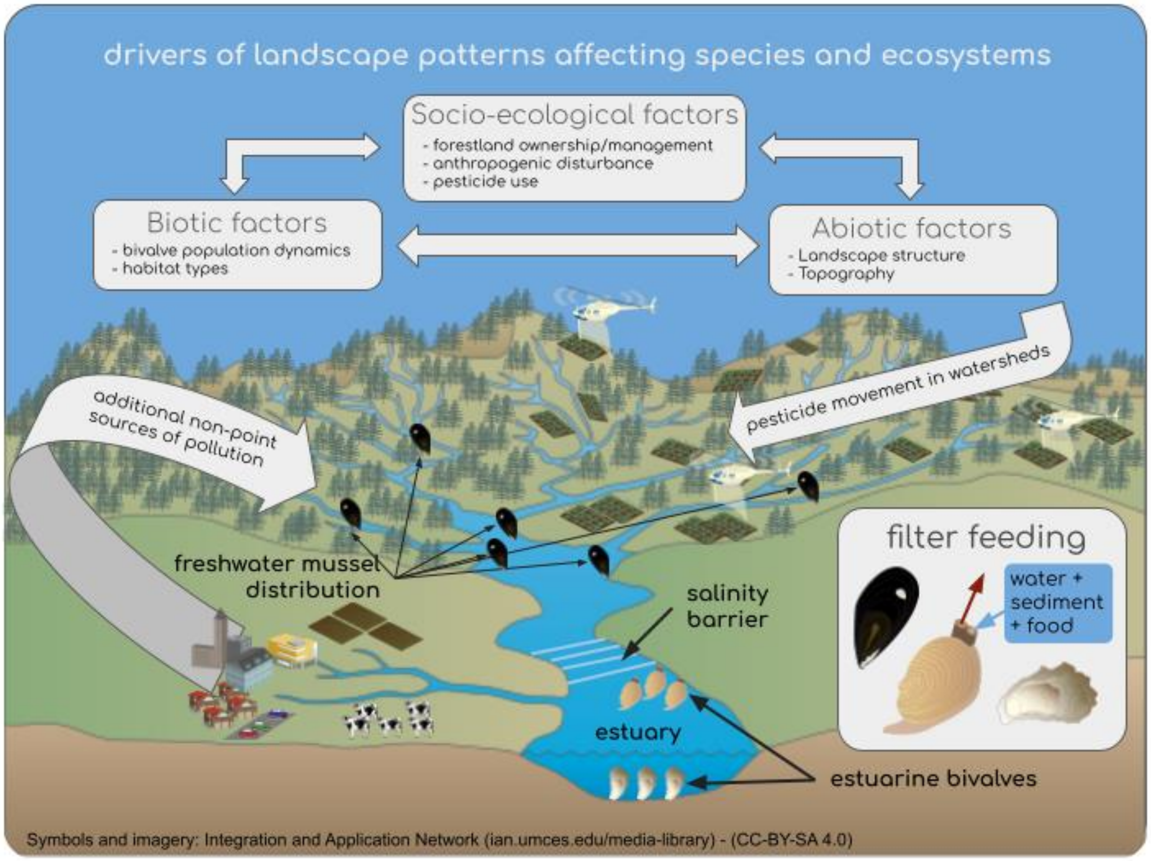

Figure 2. Drivers of landscape patterns that influence bivalve species and ecosystem processes across spatial and temporal scales. Biotic, abiotic, and socio-ecological factors affect pesticide movement (and additional non-point sources of pollution) in watersheds as well as distribution, abundance, and condition of bivalves living in freshwater and estuarine habitats.

Most present-day forestry practices that involve regeneration harvest, vegetation management, reforestation, and stand management (known collectively as Intensive Forest Management; IFM) rely on the use of chemicals to meet management objectives. Chemical treatments generally fall into the following categories: site preparation, conifer release, invasive species control, rodent control, disease control, or insect/pest control. To effectively accomplish these objectives, application methods vary based on factors such as parcel size, terrain, weather, ownership, and management plan. Previous research has shown that vegetated riparian management areas (RMAs) successfully mitigate impacts to water quality in terms of runoff and direct infiltration into stream networks, although there are ongoing debates about the minimum size for effective buffers (Mazza and Olson, 2015; Michael and Neary, 1993). Forestry investigations that document site-level impacts of pesticide application to downstream water quality demonstrate variability in episodic exposure scenarios, wherein low pulsed concentrations of applied chemicals are observed following application events (Caldwell and Courter, 2020), with most monitoring efforts generally at and below single treatment parcels (Dent and Robben, 2000; Louch et al., 2017). Once they are applied, a coalescence of environmental and chemical-specific variables influence pesticide transport pathways within watersheds (Lee, 2002; Müller et al., 2004).

Prolonged or pulsed exposure to low concentrations of pesticides has the potential to affect aquatic communities downstream and disrupt hormonal, reproductive, and developmental processes in organisms (Álvarez et al., 2015; Hayes et al., 2006; Munn et al., 2006). Furthermore, episodic exposure scenarios have the potential to affect aquatic plant communities, with repercussions throughout the aquatic food web (Vonk and Kraak, 2020). Non-point sources of pollution such as those associated with chemical runoff from forestlands are difficult to trace and quantify due to the transient nature of contamination, but may be investigated via biomonitoring (Hapke et al., 2016; Kennish, 1997). This research utilizes filter feeding bivalves as indicators of upstream pesticide transport across watershed catchments of variable sizes.

Filter feeding bivalves have long been recognized as sentinel species and good surrogates for monitoring water quality and watershed health (National Research Council, 1991). They have been frequently used in chemical biomonitoring research because: (1) they continually filter water and/or sediment, two major pathways of chemical exposure; (2) they are sedentary, making them good indicators of upstream conditions; (3) residues of chemical contamination in tissues respond to ambient environmental exposure; and (4) they are available commercially and recreationally for consumption, therefore contamination may have human-health implications (Farrington et al., 1983; Grabarkiewicz and Davis, 2008; Lehotay et al., 1998; National Research Council, 1991; Renault, 2011). In this research, Softshell clams (*Mya arenaria*), Pacific oysters (*Crassostrea gigas*), and Western pearlshell mussels (*Margaritifera falcata*) were chosen as suitable study organisms as they persist in various aquatic realms within Oregon's coastal ecosystems ranging from low in estuaries to high in freshwater streams. GIS and spatial modeling serve as effective aids to understand potential threats to aquatic systems and organisms by incorporating landscape characteristics and management/usage patterns into analysis on the watershed scale (Coulson et al., 1987, Basnyat et al., 2000).

Apart from being water quality indicators, bivalves provide valuable ecosystem services to aquatic environments by filtering bacteria and contaminants from the water column, storing and cycling nutrients, and creating biogenic habitat (Olivier et al., 2020; Vaughn, 2018; Vaughn and Hoellein, 2018). Populations, distribution, and ecological considerations of estuarine species in this research (*M. arenaria* and *C. gigas*) are well understood and documented across the region as both were introduced for commercial purposes and have been monitored over time (Dumbauld et al., 2009; Palacios et al., 2000). However, native freshwater mussel population dynamics (*M. falcata* and others) are less understood (Strayer, 2008). Research throughout the region has documented important habitat and distribution characteristics about the species (Blevins et al., 2017; Howard and Cuffey, 2003; Mock et al., 2013; Stone et al., 2004), but critical knowledge gaps remain within finer-scale regional contexts about habitat preferences and threats to species to guide conservation and management.

2. Research Objectives

The purpose of this research is to explore the landscape ecology of bivalve populations in Oregon's coastal watersheds by investigating cross-ecosystem threats to estuarine (*M. arenaria* and *C. gigas*) and freshwater species (*M. falcata*) and population dynamics of freshwater species (*M. falcata*). This investigation was carried out by means of studying the relationship between pesticide management practices in Oregon's coastal forestlands and contamination/exposure in downstream freshwater and estuarine bivalve mollusc populations (Chapter 2), identifying key variables influencing measured pesticide exposure and modeling predicted exposure in unmetered watersheds at multiple scales (Chapter 3), and exploring current distribution, condition, habitat requirements, and host species interactions of coastal freshwater mussel populations (*M. falcata*) (Chapter 4).

2.1. Exploring relationships between chemical use in forestlands and bivalve uptake and exposure

In Chapter 2, I investigate exposure and uptake of chemical contaminants related to forestland management by documenting pesticide body burdens of three bivalve species and measuring in-water exposure via integrative passive water sampling. I collected replicate composite bivalve samples (three composites of five individuals) over two seasons across eight watershed areas; composite samples were screened for a wide range of pesticide contamination. Additionally, I deployed passive water samples for a 45 day period coinciding with spring spray activities on forestlands. Results from this investigation offer insight into the effectiveness of current management practices in controlling the transport of pesticides in coastal watersheds, as well as provide new information about pesticide exposure and uptake in Oregon coastal bivalves.

2.2. Predicting springtime herbicide exposure across multiple scales in coastal watersheds

In Chapter 3, I further investigate landscape variables influencing the presence of herbicide concentrations measured in my second chapter, and develop a predictive model explaining the variation in detections using multiple regression. I then apply this model to un-sampled catchment areas in the Coast Range across three watershed scales (sub-basin, watershed, and sub-watershed) to explore the influence of scale and management intensity in coastal watersheds on predicted downstream concentrations. Results from this inquiry provide information about aquatic resource protection at multiple scales within coastal watersheds.

2.3 Assessing current populations of freshwater mussels in the context of legacy land-use impacts

Chapter 4 focuses on coastal populations of *M. falcata*, a freshwater mussel found throughout the western United States. In this portion of my research I utilize Western Oregon Rearing Project (WORP) survey data collected at random locations throughout Oregon's coastal drainages to summarize *M. falcata* occurrence, distribution, habitat needs, and host fish co-occurrence. Additionally, I compare condition indices of freshwater mussel samples collected at eight locations during sample collection for Chapter 2 to explore relative organism fitness within the region. This chapter adds to the limited population distribution and habitat information about freshwater mussels in the Coast Range, and provides new information about host fish co-occurrence in streams. Condition analysis provides a snapshot into *M. falcata* health in several Oregon coastal watersheds. Spatial analysis of existing distribution and important habitat variables at populated sites help assess "priority areas" for further research, conservation, and population assessment within this management unit.

Biogeographically similar watersheds found in Oregon's Coast Range provide an opportunity to investigate how landscape patterns of biotic, abiotic, and socio-ecological factors across watersheds affect bivalve species contaminant exposure/uptake, predicted pesticide movement in watersheds, and population dynamics of freshwater mussels (Figure 2). Together, these research chapters provide three avenues of investigation into how spatial patterns within coastal Oregon watersheds influence ecological processes and bivalve species across multiple scales and timeframes.

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Chapter 2: Exploring biophysical linkages between coastal forestry management practices and aquatic bivalve contaminant exposure

Special note: This article is an invited paper to a special issue in Toxics entitled: *Impacts of Agrochemicals on Aquatic Ecosystems: Assessing Responses across Biological Scales*. It was published online on March 2nd, 2021. The manuscript and supplementary material are available at: https://www.mdpi.com/2305-6304/9/3/46;

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1. Introduction

Coastal zone management has evolved into a complex and multidisciplinary framework incorporating management priorities and considerations beyond the shoreline to include processes and conditions in adjacent terrestrial and riverine environments (Granek et al., 2010; Lester et al., 2010). This approach relies on sufficient physical and socioecological knowledge of land–sea connections to understand cross-ecosystem threats to coastal and marine resources and guide management decisions that protect ecosystem functions (Álvarez-Romero et al., 2011; Stoms et al., 2005). Additional research and case-history investigations are needed to better understand how diverse land uses affect coastal species and ecosystems (Munns, 2006).

Oregon’s coastal zone, on the West Coast of the United States, encompasses the state’s coastal watersheds and extends approximately three miles seaward into nearshore marine waters (DLCD, 2020). Oregon coastal watersheds are largely forested and managed under several forestry management regimes (Spies et al., 2007), with the exception of Christmas tree farms, sporadic lowland agricultural lands, and rural towns and communities scattered throughout the region. The orientation of multiple small coastal watersheds along the linear Oregon coast, coupled with broad similarity in local climatic conditions, presents an opportunity to develop comparative case histories that explore effects of forestland management practices on coastal watersheds under contrasting management regimes.

Empirical investigations have found significant relationships between the scale of actively managed forestlands and cumulative effects on downstream water quality and quantity within watersheds (Johnson & Jones, 2011; T. D. Perry & Jones, 2017). Despite substantial research effort on cumulative effects of many forestry practices (such as road building, clearcutting, planting, and thinning), little is known about cumulative effects from multiple applications of chemical mixtures within watersheds and their transport away from the primary application site (Clark et al., 2009; Norris et al., 1991). Most research on chemical applications and development of germane best management practices (BMPs) is focused on site-level effectiveness; this study aims to address lingering data gaps on the effects of chemical applications across multiple catchments on the fate and transport of compounds and mixtures.

1.1. Forest Management in Oregon's Coastal Zone

Oregon's forests are managed under two regulatory regimes: 1) federal lands, regulated under the Northwest Forest Plan (NWFP); and 2) private, industrial, state, and tribal lands, regulated under Oregon's Forest Practices Act (OFPA), with Oregon's State Forestry Management Plan building upon OFPA to offer additional protections and management objectives within state forests. Each plan prescribes a set of BMPs to guide activities such as timber harvest, pesticide use and application, road construction, and riparian vegetated buffer retention for each land ownership type.

NWFP implementation in 1994 instituted a dramatic shift in forestry management on federal lands throughout the Pacific Northwest region as the ecosystem-based management (EBM) approach extended management considerations beyond timber production (Spies et al., 2018). Revised management objectives on federal lands resulted in significant portions of federal forestland being taken out of harvest rotation and allocated for other uses such as conserving biological diversity and endangered species (Spies et al., 2018; Thomas et al., 2006). Since NWFP implementation, state-regulated forests, including private and industrial forestlands, have comprised the majority of regeneration harvest, vegetation management, reforestation, and stand management (collectively known as intensive forest management; IFM) (FEMAT, 1993; Kaplan & White, 2002). IFM activities on private, industrial, and tribal land are subject to the Forest Practices Administrative Rules under the OFPA. Hardwood timber, orchard trees, and Christmas tree production are designated as agriculture rather than timber operations under state law and therefore not subject to prescriptive regulations under the OFPA (Boisjolie et al., 2017).

Though BMPs are designed to guide activities under both plans to meet federal regulatory requirements for the Endangered Species Act (ESA) listed species and Clean

Water Act (CWA) water quality guidelines, differences in stream protections between federal and state standards vary substantially, including clear differences in vegetated buffer protections and chemical application guidelines in coastal watersheds (Adams, 2007; Boisjolie et al., 2017). For example, riparian management area (RMA) designations, which are generally determined by stream size, flow duration, fish presence, and/or domestic water usage, vary widely among land ownership types, with the largest protections on federal lands (30~152 m), followed by state (7.6–52 m) and private/industrial lands (0–30.5 m) (Boisjolie et al., 2017). Furthermore, at the time of the study, foresters operating under the OFPA in the Coast Range are generally not required to establish chemical-free buffers for aerial or ground spray adjacent to headwater streams classified as small non-fish-bearing, intermittent, or ephemeral, though new regulations now require an 18.3 m buffer if the stream is flowing at the time of application (OAR × 629–640–0400) (*Oregon Secretary of State Administrative Rules*, n.d.; Senate Bill 1602, 80th Oregon Legislative Assembly, 2020 Special Session, 2020). In contrast, on federal lands in Oregon west of the Cascade Mountain Range, aerial application of herbicides for tree production is not permitted (US Bureau of Land Management, 2010).

1.2. Chemical Applications in Forestry Practices

Contemporary IFM relies on numerous chemical products to re-establish and maintain tree plantations by managing competitive native and non-native vegetation and controlling pests that interfere with seedling or plantation success (Peachy, 2020). Spring and late summer/fall are the most common seasons for herbicide treatments in Oregon

IFM, as application timing and effectiveness is prompted by phenological cues associated with conifer tolerances and target plant vulnerability (Peachy, 2020).

Increased complexity and specificity of forest management areas coupled with increased diversity and targeting of chemical applications has led to over 900 chemical products comprising over 200 active ingredients currently registered for use in Oregon's conifer forests (PICOL, 2020). Since the molecular formulations of these chemical compounds are targeted to control a specific type or suite of species, managers commonly use mixtures to maximize effectiveness of chemical application events (Clark et al., 2009).

The state of Oregon and federal agencies each have reporting systems to track pesticide applications on timberlands within their jurisdiction. Planned management actions on state, private, and tribal lands must be submitted to the Forestry Activity Electronic Reporting and Notification System (FERNS), which provides a record of approved activities and their locations. Management activities on federal lands are recorded by the U.S. Forest Service Activity Tracking System (FACTS) database, and for the U.S. Bureau of Land Management (BLM) by a separate online record system.

1.3. Management Practices and Ecotoxicology

Knowledge about the effectiveness of current forestry practices in protecting down-stream resources during chemical applications is limited for Oregon Coast Range watersheds. For example, little information exists to document the effects of no buffer protections under the OFPA for non-fish bearing streams (at the time of sampling), although they comprise up to 70% of the river miles in some watersheds (Dent &

Robben, 2000; Louch et al., 2017; Spies et al., 2018). Investigations in the neighboring Washington state have led to restrictions in chemical types and buffers on intermittent streams to improve protection of downstream resources (Rashin, E., & Graber, C., 1993).

The effect of chemical mixtures used in forestland management, particularly the potential for transport off-site and encounter by non-target species such as invertebrates, fish, and aquatic plants located downstream, is also poorly understood (Cox & Surgan, 2006; Laetz Cathy A. et al., 2009). Toxicity benchmarks used to assess risk are derived using LC50 measurements (lethality of compound to 50% of test organisms), yet in the environment, non-target organisms are likely exposed at lower doses and may experience sublethal effects such as disruptions in developmental, hormonal, and reproductive systems (Greco et al., 2011; Gunderson et al., 2011; Renault, 2011; Tanguy et al., 2005). Additionally, research in agricultural systems demonstrates that compound mixtures can exhibit a variety of effects that differ from toxicity of the individual compounds (Hayes et al., 2006; Kudsk & Mathiassen, 2004). Though the body of research demonstrating effects of chemical mixtures on non-target organisms grows annually, such findings are largely unaccounted for in forestry BMP protocols, creating a knowledge gap in forestry research and management decision making (Michael, 2004). Moreover, the considerable research focused on behavior of phenoxy herbicides (e.g., 2,4-D) in the forest environment may not adequately describe entry and movement of other commonly used classes of compounds such as triazines (e.g., atrazine) and prevailing mixtures (Norris et al., 1991).

1.4. Monitoring Considerations

Non-point sources of pollution, such as those associated with forest practices, are difficult to trace and hard to quantify due to the transient nature of aquatic contaminants. Cumulative effects and pulsed exposures, however, may be examined by tracking occurrence and bioaccumulation in filter feeding organisms (Jacomini et al., 2006; Kennish, 1997) and via passive water sampling (Metcalfe et al., 2019). Filter feeding bivalves are recognized as sentinel organisms for monitoring water quality, and are frequently used for biomonitoring of chemical exposure because they continually filter water and/or sediment (Council et al., 1991). Limited mobility of sedentary bivalves makes them good indicators of upstream conditions as residues of chemical contamination in their tissues respond to ambient environmental exposure (Council et al., 1991; Phillips & Rainbow, 1998). Changes in organismal lipid content throughout the year, which can fluctuate based on reproductive timing (Siah et al., 2002) and seasonal changes in temperature and food availability (Haider et al., 2020; Liu et al., 2013), can influence contaminant uptake and storage (LeBlanc, 1995).

Environmental behaviors and transport pathways of forestland chemicals are determined by a variety of chemical properties including octanol/water partition coefficient (K_{ow}), volatility, soil adsorption coefficient (K_{oc}), water solubility, and rates of hydrolysis and photolysis. These properties are influenced by environmental mechanisms and ambient conditions including the environmental matrix, temperature, and water chemistry (Lee, 2002). Many lipophilic compounds (log $K_{ow} > 3$ and often high K_{oc}), which can pass through and accumulate in lipid membranes in aquatic and terrestrial organisms (Tzilivakis, 2020), easily sorb to soil and organic matter (high K_{oc}) and are more likely to be transported away from primary site of application via particles (i.e., erosion, landslides, or other sediment movement within a watershed) (Lee, 2002). In contrast, most current-use herbicides are hydrophilic compounds (log $K_{ow} < 3$; dissolve easily into water), and are typically transported via surface water runoff, groundwater and/or macropore infiltration, and direct application to waterways (Michael, 2004).

1.5. Project Goals

Our research sought to elucidate the relationship between current pesticide use in forestland management and its effects on downstream coastal resources. We conducted an empirical study to examine linkages between coastal forest management and forestry-use chemical signatures in estuarine systems by tracking targeted chemical mixtures along a downstream flowpath within Oregon's coastal watersheds. We measured pesticide tissue concentrations in bivalves to document uptake of a variety of chemicals under a range of active management conditions and prescriptions. We then deployed a series of integrative passive water samplers to monitor organism exposure to hydrophilic com-pounds that typically go unmeasured in biomonitoring efforts. In addition, we measured in-tissue concentrations of pesticides used outside of IFM to document potential alternate land-uses contributing to bivalve contaminant loads in coastal drainages. Our primary research objectives were to: (1) describe and characterize seasonal differences in bivalve contaminant levels and classes; (2) quantify differences in chemical types, mixtures, and concentrations between bivalve tissue and water samples; and (3) examine variation in chemical exposure based on forestry practices permitted under different management regimes, while noting other sources of detected contamination.

2. Materials and Methods

2.1. Site Selection

Eight watersheds within Oregon's coastal zone were selected to encompass a range of forestland management activities across different ownership types (Figure 1). The coastal watersheds were characterized with ArcMap 10.7 to identify key attributes and spatial data regarding federal ownership and land-use zoning under the NWFP, and to characterize state, private, industrial, and tribal ownership areas associated with land-use zoning under the OFPA (Table 1, Figure 1). Sampling sites were selected within watersheds based on the presence, availability, and habitat for target species of bivalves (Table 2), land use (Table 1), and accessibility to stream reaches.

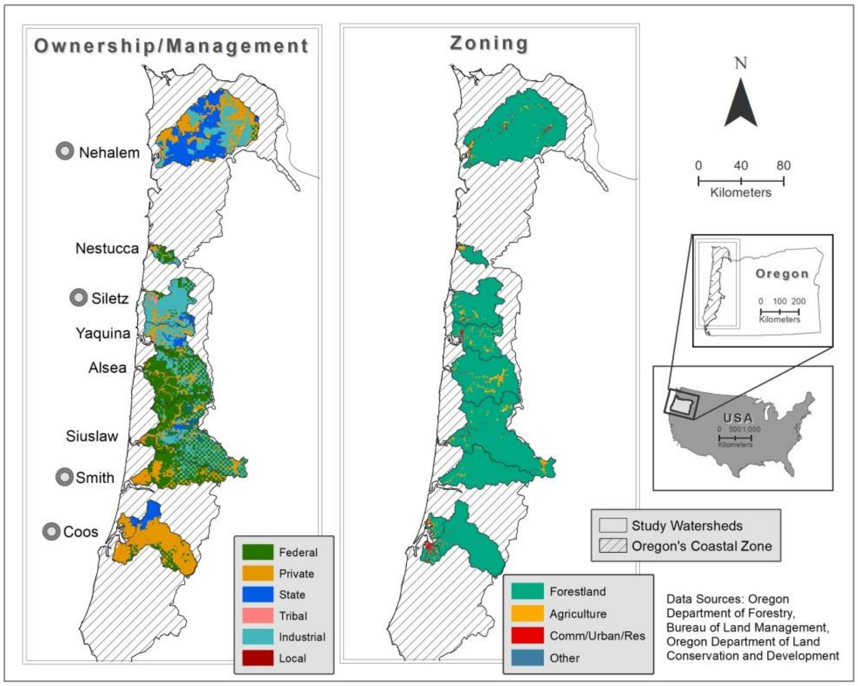

Figure 1. Location of eight watershed areas within the Oregon coastal zone where three species of bivalves were collected for biomonitoring. Colors indicate key land use (ownership and zoning attributes of study watersheds). Circles indicate a subset of watershed areas where passive water sampling was also conducted. Abbreviations: Res/Comm/Indust = zoned for residential, commercial, and industrial uses.

Table 1. Key attributes, zoning, and ownership/management characteristics of the forested watershed basins along the Oregon Coast Range. Abbreviations: Res/Comm/Indust = zoned for residential, commercial, and industrial uses.

Watershed	Watershed Area (sq. kilometers)	Mean Annual Precip (centimeters)	Mean Slope (degrees)	Zoning (%)				Ownership/Management (%)				
				Forestland	Agriculture	Res/Comm /Indust	Other	Federal	State	Industrial/ Private	Tribal	Local/ Water
Alsea	1168.1	218.7	18.9	93.1	6.3	0.4	0.2	65.2	0.2	34.3	0.1	0.2
Coos	1358.7	178.1	17	92.5	2.5	2.7	2	10.9	13.4	74.9	0	0.8
Nehalem	2150.7	313.2	14.2	96.6	1.5	1.3	0.4	0.8	40.4	58.6	0	0.1
Nestucca	152.8	256.5	13.4	89.9	7.6	2.2	0.4	51.6	3.1	45.3	0	0.0
Siletz	787.4	266.7	17.2	95.3	3.4	0.7	0.5	11.2	3.8	82.2	2.4	0.4
Siuslaw	1779.3	176.3	19.6	96.2	2.8	0.9	0.1	51.7	5.3	42.6	0	0.4
Smith	955.7	185.9	22.2	98.1	1.4	0.1	0.5	57.7	0	41.9	0	0.3
Yaquina	569.8	193.8	17.4	90	6.3	2.2	1.5	15.2	13.2	70.8	0	0.8

2.2. Field Sampling Methods

Given the differences in environmental fate and transport of pesticides both singularly and in mixtures in the forest environment, we designed our sampling methods to explore exposure of filter feeding bivalves to hydrophilic and lipophilic chemicals. We employed biomonitoring and passive water sampling to explore bivalve exposure to both classes of chemicals given their inherent behavioral differences in the environment.

2.3. Biomonitoring of Bivalves

We selected three bivalve mollusk species that inhabit different habitat types within Oregon coastal watersheds: Western pearlshell mussel (*Margaritifera falcata*), softshell clam (*Mya arenaria*), and Pacific oyster (*Crassostrea gigas*). Species attributes such as water salinity tolerances, habitat requirements, feeding type, life history characteristics, life span, and management status differ among these bivalves (Table 2).

Western pearlshell mussels (*M. falcata*), the target species for freshwater habitats, were historically abundant but are increasingly rare with patchy populations due to major population declines throughout their native range (Blevins et al., 2017; Nedeau, E. et al., 2009). Information about the current spatial distribution and abundance of freshwater mussels (including *M. falcata*) in Oregon aquatic systems is limited, and abundance thresholds at sample sites were required to limit potential impacts of this study to the at-risk populations. Several factors were considered in selecting collection sites of *M. falcata*, including: watershed spatial scale (preference toward smaller catchment basins), information about distribution and abundance of current populations, local forestland management practices (sampling areas span a diversity of management types), and access to stream reaches. Three composite samples (five individuals) of *M. falcata* were collected by hand or during snorkel dives from five sites located in four study watersheds during the summer of 2017 (July-August) and three sites in three study watersheds during the spring of 2018 (May-June) (see supplementary material (SM): Figure S1).

Softshell clams (*M. arenaria*), selected as an estuarine species with high tolerance for brackish water, typically inhabit the upper (riverine) region of the estuaries where freshwater drains down from forested watersheds. Exposure of the softshell clams to freshwater was a priority for sample sites, and we collected softshell clams from the uppermost (mesohaline) region of each estuary. Three composite samples (five individuals) of *M. arenaria* were collected from a single site in each of six watersheds during the summer of 2017 (July-August) and eight watersheds during the spring of 2018 (May-June) by digging in the soft mud or sand (SM: Figure S1).

Pacific oysters (*C. gigas*) are non-native bivalves cultured for commercial purposes in the middle (polyhaline) regions of several Oregon estuaries. Composite samples of *C. gigas* (five individuals) were obtained from commercial mariculture operators from two watersheds during summer (2017) and spring (2018) seasons (SM: Figure S1). All wild-stock bivalves (*M. arenaria* and *M. falcata*) were collected under the authority of Oregon Department Fish and Wildlife Scientific Taking Permits (#21207 and #22121).

Table 2. Bivalve species selected for the study exhibit a wide variety of life history characteristics, habitat requirements, salinity tolerances, and life span (Abraham & Dillon, 1986; Blevins et al., 2017; Haag, 2012; Kozloff, 2000; Nedeau, E. et al., 2009; Pauley et al., 1988). Abbreviations: psu= practical salinity units, IUCN= International Union for Conservation of Nature.

Species Attributes	***Margaritifera falcata***	***Mya arenaria***	***Crassostrea gigas***
Native Biogeographic Range	Western USA and Canada	East coast of USA, naturalized along west coast	Pacific coast of Asia
Habitat Type	Gravel and cobble substrates	Muddy substrate	Hard or rocky substrate
Water Salinity Preference (psu range)	Freshwater (0)	Upper estuarine; mesohaline, polyhaline (5–30)	Mid estuarine; polyhaline (20–25)
Management and conservation status	Designated as Near Threatened – (IUCN Red List)	Managed as a recreational fishery in Oregon	Commercial mariculture
Life-history Characteristics	Complex life-cycle with demersal glochidia larvae that attach to fish	Complex life-cycle with planktonic veliger larvae	Artificial propagation in hatcheries
Feeding Type	Suspension and deposit feeders	Suspension and deposit feeders	Suspension feeders
Life Span	>100 years	Up to 19 years, generally 10–12 years	Up to 40 years in northern latitudes

All sampled bivalves were held in ambient water collected on site (estuarine or freshwater) and transported in a cooler with wet ice to the Applied Coastal Ecology (ACE) Laboratory at Portland State University (Portland, OR; 280 samples) or the Hatfield Marine Science Center (Newport OR; 105 samples) for initial sample processing. Individual bivalves were weighed, shucked, drained, and final shell and tissue wet weights were recorded (SM: Table S5). Samples were composited (five individuals per sample) and frozen at -80 °C, and then homogenized using a CoorsTek mortar and pestle or Waring pulverizor (WSG30 Series), and lyophilized on a HarvestRight or VirTis BenchTop Pro Freeze Drier. Subsamples were sent to the USGS Organic Chemistry Research Laboratory in Sacramento, CA for analysis of pesticides in the bivalve tissues.

2.3.1. Laboratory Analytical Methods

Chromatographic and spectrometric analyses were conducted to determine bivalve tissue concentrations for a wide diversity of fungicides, insecticides, herbicides, and other compounds. Prior to extraction, freeze-dried tissue samples (0.2–0.3 g) were homogenized with sodium sulfate (Na_2SO_4) and spiked with $^{13}C_{12}$-*p,p'*-DDE, $^{13}C_4$-fipronil, d_4-imidacloprid, $^{13}C_6$-cis permethrin, and d_{10}-trifluralin (Cambridge Isotope, Cambridge MA) as recovery surrogates, followed by extraction with 50:50 acetone: dichloromethane (DCM) using a Dionex 200 accelerated solvent extractor (ASE) at 1500 psi and 100 °C. The extract was exchanged into 6 mL of acetonitrile, coextracted matrix interferences were removed with 0.5 g Z-sep+ (Sigma-Aldrich, St. Louis, MO), the eluent was reduced to 0.2 mL, and internal standards were added (d_{10}-acenaphthene and d_{10}-phenanthrene and d_3-clothianidin). The bivalve tissue samples were analyzed for a total of 146 pesticides and pesticide degradates (six of which are IFM current-use compounds; see SM: Table S1) using either gas chromatography—tandem mass spectrometry (GC–MS/MS; Agilent 7890 GC coupled to an Agilent 7000 MS/MS operating electron ionization (EI) mode), or liquid chromatography–tandem mass spectrometry (LC–MS/MS; Agilent 1260 bio-inert LC coupled to an Agilent 6430 MS/MS; see (Hladik et al., 2016) for further details). Data for all pesticides were collected in a multiple reaction monitoring (MRM) mode with each compound having one quantifier MRM and at least one qualifier MRM. Ten percent by volume of each raw extract was allowed to evaporate to a constant weight in a fume hood for gravimetric lipid determination to the nearest 0.001 g using a microbalance.

2.4. Passive Water Sampling

Integrative passive water sampling was used to characterize pulsed/episodic exposure of the aquatic habitats to contaminants over a longer timeframe (Alvarez, 2010) because short-term exposure events can easily be missed by grab or composite water sampling efforts. Polar organic chemical integrative samplers (POCISs; developed by the United States Geological Survey (USGS)) capture water soluble organic chemicals from the water column during deployment in a solid phase extraction resin (Oasis HLB sorbent) within two microporous (0.1 micron pore) membranes (Alvarez, 2010). Following USGS sampling protocols (Alvarez, 2010), we deployed the POCIS at sixteen locations during March 26–29, 2019 and retrieved them in identical order May 7–10, 2019 to capture episodic runoff events coinciding with the spring spray events. Exact dates/times and locations of spring spray events were not known, so the timing of deployment and retrieval was determined by the notification of spray events in the FERNS database and documented timing of spray events from previous research in the Coast Range (Oregon Health Authority, 2014). Documenting spring season exposure was of particular interest because of the reproductive timing of *M. falcata* and *M. arenaria* and their increased vulnerability during early life stages (Allard et al., 2017; Lindsay et al., 2010).

Following retrieval from the field, the POCIS disks were chilled on wet ice, transported to the PSU ACE laboratory, frozen, and shipped to Environmental Sampling Technologies (EST; Missouri) for processing and extraction. Each passive sampler was extracted individually using 25 mL methanol (MSl lot DU 136-US). Following extraction the samplers were blown down over ultra-high pure nitrogen (Air Gas), filtered through glass fiber filter paper (Whatman, GF/D), pooled, blown down again, and quantitatively transferred to 5 mL amber ampules using methanol as the transfer solvent. The ampules were chilled in dry ice and flame sealed. Sample extracts (composites of three POCIS discs) were sent to Anatek Labs (Idaho) for pesticide analysis. Samples were screened for 14 herbicides and one surfactant (all of which are IFM current-use compounds; see SM: Table S2) using either gas chromatography–tandem mass spectrometry (GC–MS/MS) or liquid chromatography–tandem mass spectrometry (LC–MS/MS) (EPA Methods 8151A, 8321A, and 625.1). Resultant concentrations are presented in ng/POCIS, as concentration of chemical per POCIS sample. Detection limits ranged from 3 to 500 ng/POCIS. Maximum and time weighted average concentrations in water could not be calculated due to the dynamics of uptake/degradation of compounds, unknown quantities of total water sampled over the deployment period, and the lack of performance reference compounds. Thus data are used to compare compounds and concentrations across sites (presence/absence and relative concentrations).

2.5. Spatial Analysis of Oregon Coast Watersheds

Watershed areas above sampling locations were delineated using StreamStats: Streamflow Statistics and Spatial Analysis Tools for Water-Resources Applications version 4 developed by USGS. Within each watershed boundary we explored planned management activities, physical watershed attributes, and policy/ownership characteristics as factors to explain variation in detections/concentrations among sampling locations. Using StreamStats watershed delineations, physical basin variables were calculated such as average slope, annual rainfall, and area using continuous parameter grids based on 30 m digital elevation models (DEMs). The FERNS database was used to summarize planned management activities within study watersheds. FERNS polygon, line, and point data associated with each activity are accessible through the Oregon Department of Forestry website (ODF, 2020), and the individual detailed notification information is available through a free subscription to the database (FERNS, 2020). Notifications in the FERNS database of aerial herbicide applications active during the sampling period were sorted, imported into ArcMap, and joined with notification polygons. Polygons were clipped to watershed boundaries above sample locations and used to calculate percentage of active notifications within each watershed. Federal forestry activities are available through the FACTS reporting system (USFS land) and the BLM Oregon data library, yet no wide scale reported activities occurred within our study watersheds during the study. Watershed policy/ownership characteristics, summarized as ratios of forestland ownership, were surrogates for land management guiding documents (NWFP, OFPA). Physical watershed characteristics included watershed area, average slope, average annual precipitation, and water temperature at collection (or averaged between deployment and retrieval for passive water samples) derived from StreamStats delineations and field measurements (water temperature).

2.6. Statistical Analyses

Organismal lipid content is known to influence bioaccumulation of hydrophobic contaminants in bivalve tissues (Bruner et al., 1994). Since lipid content can vary annually and among species, we analyzed whether lipid content differed among bivalve species. Differences in lipid content were examined using Kruskal–Wallis nonparametric tests, and pairwise differences were examined using Wilcoxon rank sum tests (R Studio; Version 1.2.5033). Seasonal differences in lipid content within species were explored using Wilcoxon rank sum tests. Lipid-normalized concentrations of chemicals (C_L) in tissue samples are defined using the following equation:

$$C_L = C_i \div F_L$$

where:

C_L= lipid-normalized concentration;

C_i = initial concentration of the chemical in the bivalve tissue (ng/g);

F_L = fraction of the tissue that is lipid.

Non-metric multidimensional scaling (NMDS) was used to explore patterns in herbicide detections across POCIS sampling their relationship to watershed variables. This non-parametric approach was used due to its ability to explore patterns independent of underlying distribution assumptions (e.g., non-detect values and skewness). We developed two dimensional ordinations of chemistry profiles detected with sufficient frequency to examine their overlays with land ownership/management and physical watershed variables. Chemistry concertation profiles in NMDS underwent log transformation and Wisconsin double standardization, and the distance matrix was calculated using the Bray–Curtis metric. Correlation matrices were used to visualize relationships between total accumulation in POCIS and watershed variables (see SM: Figure S2). Correlation matrices were used to explore the relationships between both upstream forest management activities and physical watershed characteristics and downstream concentrations of detected herbicides. Least squares linear regressions were used to compare highly correlated variables within categories. Variables were square root transformed to meet regression assumptions.

2.7. Quality Assurance/Quality Control

Quality assurance was assessed through the following considerations. During tissue pesticide analysis, the limits of detection (LOD) for tissue contaminants, defined as the value greater than three times the signal-to-noise ratio, were 5–10 ng/g for 0.2 g tissue samples. Additional samples included three laboratory blanks, which did not detect any tissue contaminants, and an acceptable surrogate and matrix spike recovery of 70–130% (all samples were in this range). For the second round of data there were two matrix spikes (acceptable recovery of 70–130%) and two replicates, the relative percent difference between detections was <25%.

Three POCIS discs were composited into one sample per sampling location. At three random sampling locations three replicates (9 POCIS discs) were deployed to assess total method variance. Three field blanks and three laboratory blanks were also used to ensure quality control (QC) throughout deployment, retrieval, and processing. At the three randomly selected replicate sites detections were averaged across the three canisters and the resultant standard deviation was used to assess total method variance.

3. Results

3.1. Biomonitoring of Bivalves

3.1.1. Bivalve Lipid Content

We collected a total of 385 individual bivalves from 18 watershed sites over two sampling periods (summer 2017 and spring 2018), and the specimens were combined into 77 composite samples of 5 individuals for analysis of pesticide residues (three composite samples per site). Due to low population density at one *M. falcata* collection site (Siletz River), only two replicate composite samples were collected. As expected, shell dimensions and tissue weight varied among species (see SM: Table S5). Bivalve lipid content averaged 6.1% (range 1.7–15.7%) and varied among species (Figure 2A, Kruskal–Wallis, p = 4e−08) with *C. gigas* having the highest average lipid content, followed by *M. falcata* and *M. arenaria*. In contrast, lipid content did not differ seasonally for any of the species (Figure 2B).

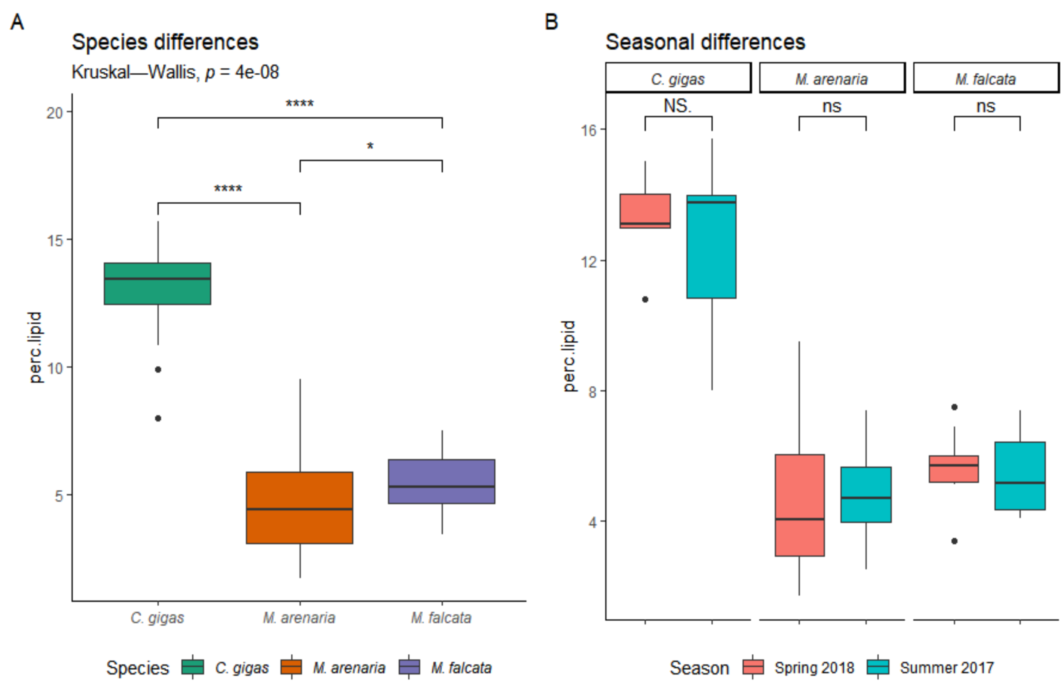

Figure 2. Lipid content of bivalve tissues varied between species (A) but not between seasons (B) for three species of bivalves that inhabit different areas of the coastal watersheds. *= $p \leq 0.05$, **** = $p \leq 0.0001$, ns = not significant.

3.1.2. Tissue Pesticide Analysis

Nine unique pesticides and three pesticide metabolites were detected in bivalve tissues collected during summer 2017 and five were detected in samples collected in spring 2018 across 38% (n = 77) of all samples. All study watersheds had at least one detection, though frequency and maximum concentrations varied by season, species, and watershed (Table 3 and Figure 3A). Detections included three fungicides, seven insecticides (including the metabolite), and two herbicides. The fungicide fluopicolide was most frequently detected chemical (23% of samples) and was identified in all three species, followed by the insecticide bifenthrin (8% of samples; Table 3). Bifenthrin, indaziflam (herbicide), metolachlor (herbicide), permethrin (insecticide), and pyraclostrobin (fungicide) were the only detected compounds currently registered for use in Oregon conifer plantations (PICOL, 2020), indaziflam (trade name Esplanade F) being the only one used in modern IFM within Oregon (FERNS, 2020). Fluopicolide is registered for use on conifers in neighboring Washington State, but in Oregon is used to control oomycetes in orchards, nursery, or agriculture settings (PICOL, 2020). Legacy insecticides (DDTs), once widely used in Oregon forestlands to control pests (Moore & Loper, 1980), were detected in one *M. falcata* and two *C. gigas* samples from summer 2017. Watershed sites exhibited a variety of chemical mixtures (summarized for each watershed in Figure 3A), with the greatest accumulation across all chemical classes in the Siuslaw and Smith, followed by the Coos watersheds. Accumulation of indaziflam, the only pesticide detected in tissue with widespread current use in forestland management, was inconsistent across watersheds, limiting further statistical analyses on watershed and management variables.

Table 3. Seasonal variability in the class of pesticides, detected compounds, frequency of detection, and maximum concentrations (ng/g dry weight) observed in *C. gigas*, *M. arenaria*, and *M. falcata* tissue during summer 2017 and spring 2018. Approximate method detection limits (MDLs) are 5–10 ng/g, ND indicates non-detect. * indicates a metabolite of a parent compound in this class.

Pesticide class	Detected Compounds	*C. gigas*		*M. arenaria*		*M. falcata*	
		Frequency	Max conc. (ng/g dry weight)	Frequency	Max conc. (ng/g dry weight)	Frequency	Max conc. (ng/g dry weight)
				Summer 2017			
Fungicides	Fenbuconazole	1/6	16.7	1/18	21.1	0/14	ND
	Fluopicolide	1/6	114.8	4/18	532.5	3/14	191.7
	Pyraclostrobin	0/6	ND	1/18	13.1	0/14	ND
Insecticides	Permethrin	0/6	ND	1/18	238.8	0/14	ND
	Bifenthrin	0/6	ND	2/18	12.7	0/14	ND
	*Clothianidin Desmethyl	1/6	52.2	1/18	24.6	0/14	ND
	p,p′-DDT	0/6	ND	0/18	ND	1/14	10.5
	**p,p′*-DDD	0/6	ND	0/18	ND	1/14	10.9
	**p,p′*-DDE	2/6	8.7	0/18	ND	1/14	9.8
Herbicides	Metolachlor	0/6	ND	0/18	ND	1/14	7.8
	Indaziflam	0/6	ND	1/18	235.8	1/14	26.6
				Spring 2018			
Fungicides	Fenbuconazole	1/6	11.8	2/24	215.7	0/9	ND
	Fluopicolide	1/6	264.6	9/24	2421.3	0/9	ND
Insecticides	Bifenthrin	0/6	ND	0/24	ND	4/9	11.6
	Indoxacarb	0/6	ND	2/24	374.6	0/9	ND
Herbicide	Indaziflam	1/6	107.4	2/24	1298.2	0/9	ND

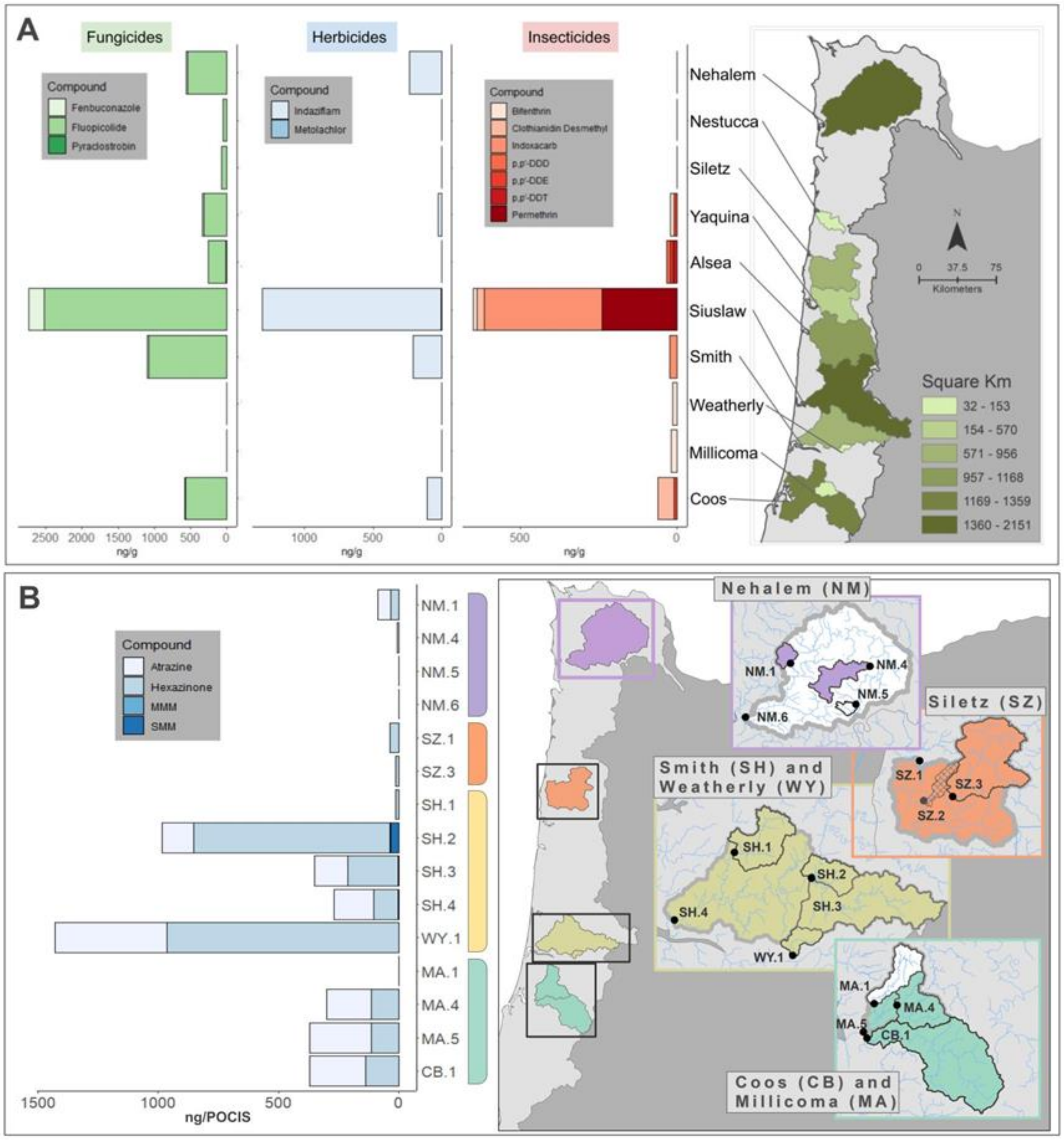

Figure 3. A. Total ng/g (dry weight) detected of insecticides, fungicides, and herbicides in tissues samples across each watershed. Detections varied across sites with Siuslaw watershed displaying consistently elevated levels compared to other watersheds. B. Herbicide detections in polar organic chemical integrative sampler (POCIS) passive water samplers (ng/POCIS). Site abbreviations in the bar chart are associated with mapped sample locations. Detections varied across sites with Weatherly and Smith watersheds displaying elevated levels compared to other watersheds. Hexazinone was the most frequently detected compound, followed by atrazine. Overlaid colors indicate watershed areas and presence of herbicides.

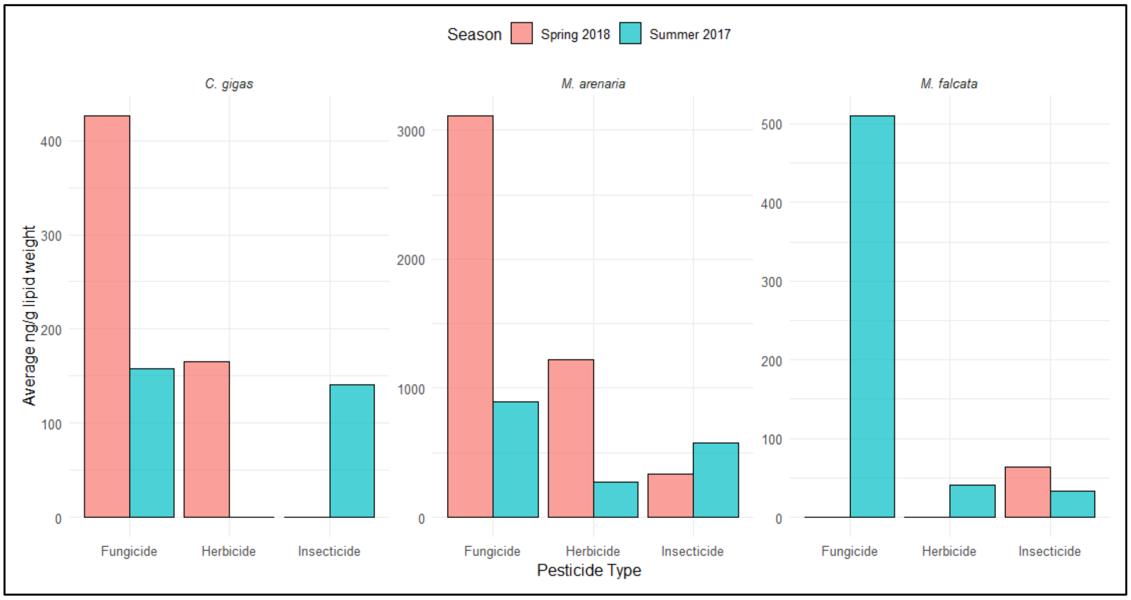

Figure 4. Seasonal changes in mean fungicide, herbicide, and insecticide lipid-normalized concentrations in *C. gigas*, *M. arenaria*, and *M. falcata* tissues. Due to low population sizes, collection sites of *M. falcata* differ by season. **Note the differences in the y-axis scales.**

Lipid-normalization allowed for further distinction of fungicide, herbicide, and insecticide concentrations among bivalve species. The greatest cumulative and average concentrations of all pesticide classes were observed in *M. arenaria*, and the average body burden observed in the species was further exaggerated after accounting for lipid content (SM: Figure S3A,B). Average concentrations of fungicides and herbicides were elevated in spring 2018 for the estuarine bivalves (*M. arenaria* and *C. gigas*). In contrast, average concentrations of fungicides were elevated in the tissues of freshwater bivalves (*M. falcata*) during the summer 2017 (Figure 4), but seasonal comparisons for this species are difficult because collection locations varied between seasons. Average insecticide concentrations were high in the estuarine bivalves during the summer 2017, and the highest insecticide concentrations were observed in freshwater mussels sampled during spring 2018 (Figure 4).

3.2. Analysis of Passive Water Samples

3.2.1. POCIS Deployment

Less than two weeks into the POCIS deployment period (2019), southern coast watersheds experienced abnormally severe spring storms from April 6 to 21st that toppled trees and substantially raised river levels, causing widespread flooding and landslides across the region. Damage incurred from flooding, severe weather, and landslides during the significant storm event resulted in a major disaster declaration (FEMA 4452-DR–OR) in July 2019[1]. Shortly after the rivers receded, POCIS canisters at two sites (west fork Millicoma River: MA.1; and north fork Smith River: SH.1) were partially stranded on the shore after being deposited there during high waters. The Oasis HLB media in those POCIS canisters was intact so they were processed and reported, yet the duration of time submerged in the river is unknown, so detected chemical concentrations at those locations may under-represent aquatic exposure. Additionally, the membranes in the POCIS canister at the Euchre Creek location (Siletz River: SZ.2) were destroyed at some point during its deployment, with insufficient HLB media remaining for analysis.

3.2.2. POCIS Detections

Four current-use herbicides commonly applied in spring forestland applications (pre-emergent and site preparation treatments) ranged from 1.16 to 936 ng/POCIS and

[1] https://www.fema.gov/disaster-federal-register-notice/oregon-severe-storms-flooding-landslides-and-mudslides-public

averaged 277 ng/POCIS. Standard deviations at the randomly selected replicate sites were averaged across the three sites for a method standard deviation of 8.06 ng/POCIS (range 0–12.2 ng/POCIS). Detections of the forestry application compounds varied across sampling locations, with the greatest accumulations observed at sites within the Weatherly (predominantly privately managed land) and Smith (mixed federal and private management) watersheds (Figure 3B). Hexazinone was the most commonly detected herbicide (73% of samples) followed by atrazine (60%), sulfometuron-methyl (SMM; 40%), and metsulfuron methyl (MSM; 7%). Field and laboratory blanks returned no detections.

3.2.3. Relationships between Compound Detections and Forestland Management

NMDS analysis elucidates associations between watershed variables and the compounds detected by POCIS monitoring, with biplots indicating relationships between compounds and forestland ownership based on shared vector direction. Federal ownership appears to be associated with SMM loading, tribal ownership is associated with hexazinone loading, and private and state ownership is associated with atrazine loadings (Figure 5A, stress = 9.62e-05). Physical characteristics of the coastal watersheds appeared to have only minor associations with the chemical compound variability (Figure 5B).

Least squares linear regressions of management and physical watershed variables (run separately) revealed that aerial herbicide application (% of watershed) and slope accounted for the greatest variation in total herbicide accumulation in POCIS samplers (Figure 6). Based on simple linear regression, the total herbicide load captured in the

POCIS was positively correlated with percentage of active aerial application notifications during the deployment window (R^2 = 0.694, p = 0.0005; Figure 6A), and average watershed slope in upstream catchments (R^2 = 0.487, p = 0.0007; Figure 6B). Negative y-intercept observed in the slope regression relates to high slope catchments (SZ.3 and SH.1) where low concentrations were detected.

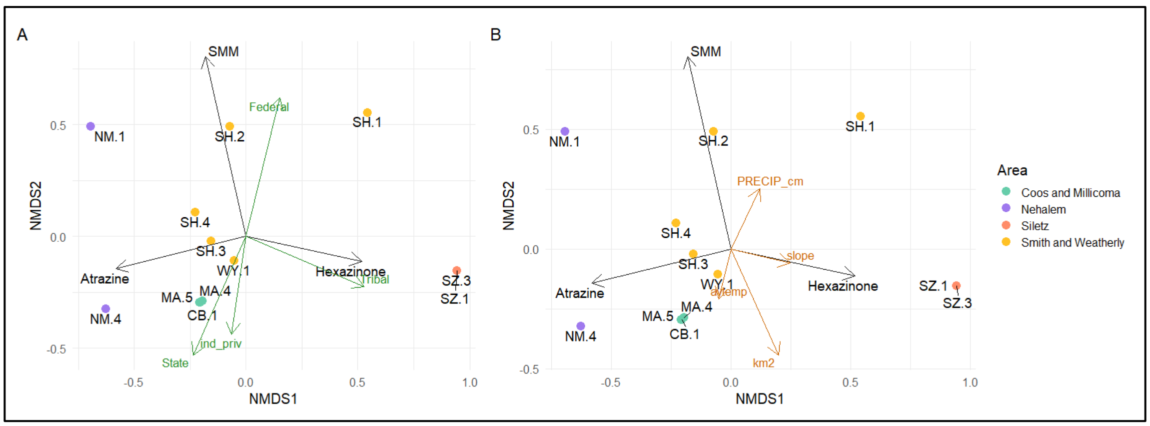

Figure 5. Non-metric multidimensional scaling (NMDS) biplots (stress = 0.017) indicate types of herbicide detections (black vector arrows) across (A) site types: ownership/management variables (green vector arrows) and (B) associated watershed characteristics: physical watershed variables (orange vector arrows). Similar vector directions of compounds and watershed characteristics indicate associations between the two. Watershed areas are indicated by point color. Detection concentrations were log transformed and the distance matrix was calculated using the Bray–Curtis metric. Abbreviations: ind_priv = industrial and private land ownership, avertemp = average temperature, km^2 = square kilometers of watershed, PRECIP_cm = annual precipitation in centimeters, SMM = sulfometuron methyl.

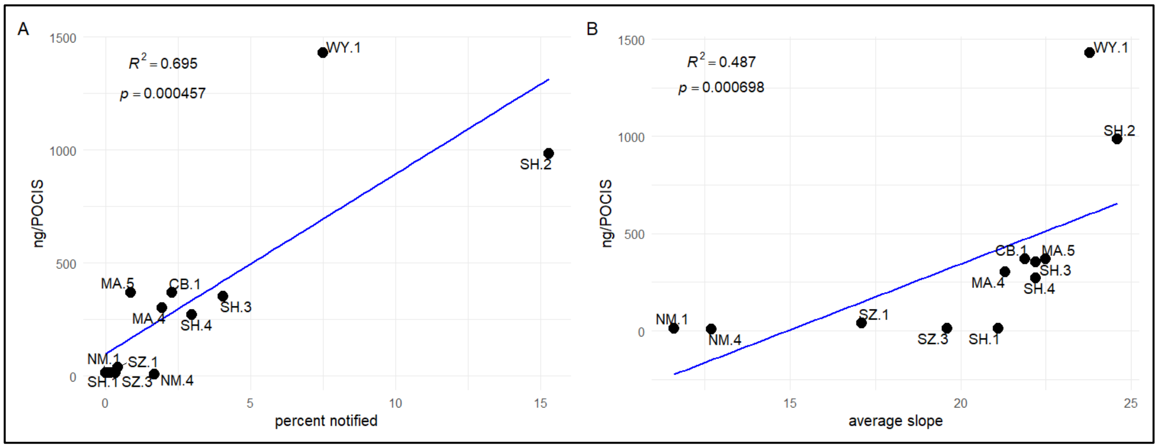

Figure 6. Linear regression indicated that total herbicide load captured in the POCIS was positively correlated with (A) active aerial herbicide notifications during deployment window ($R^2 = 0.695$, $p \leq 0.001$) and (B) average watershed slope ($R^2 = 0.487$, $p \leq 0.001$). Formulae and results in plots reflect calculations with both variables square root transformed to meet regression assumptions.

3.3. Combined Chemical Results and Considerations

This study detected three classes of pesticides (herbicide, insecticide, and fungicide) that exhibit a variety of chemical traits affecting their environmental fate and transport (Table 4). Detected compounds showed wide ranges of water solubility (0.001–33,000 mg/l), octanol/water partition coefficients (Log Kow: -1.87–6.91), soil adsorption coefficients (Koc; 54–236,610), and leaching potential (-3.89–4.43) (Table 4). Ranges and associated compound detection matrix (tissue or water) were closely aligned with expected behavior in the environment. Passive water samplers detected chemicals that display hydrophilic behavior such as high water solubility, potential for leaching, low bioconcentration factors, and low Log Kow values. With the exceptions of indaziflam and fluopicolide (which straddle the hydrophilic/lipophilic classification, as a consequence of their lower Log Kow values), compounds detected in bivalve tissues are predominantly classified as lipophilic (Table 4). Detected pesticides comprise a variety of registered uses (PICOL, 2020) and demonstrate a diversity of modes of action in their respective pesticide classes (Table 4) (Lewis et al., 2016). Five herbicides (atrazine, hexazinone, indaziflam, metsulfuron-methyl, and sulfometuron-methyl) were the only commonly used forestry-use compounds detected of the eighteen we tested for (SM; Tables S1 and S2). Of the forestry-use compounds we analyzed in both water and tissue samples (atrazine, hexazinone, and sulfometuron-methyl) none were detected in both matrices. Combined results of tissue and water sampling efforts document exposure and uptake of forestry-specific contaminants, and lipophilic compounds from other sources, contributing to pesticide bio-burdens in coastal bivalves.

Table 4. Detection frequency, current status, and matrix of compounds observed in tissue and water sampling; along with pesticide characteristics that explain environmental behavior (Lewis et al., 2016; PICOL, 2020). * indicates a metabolite of a parent compound in this class. BCF=bioconcentration factor

Compound	Sampling matrix	Detection matrix and frequency	Year intro-duced	Active registration (in OR forestry)	Pesticide class	Mode of action	Water solubility - at 20 °C (mg l^{-1})	Log Kow at pH 7, 20 °C	Koc	Groundwater Ubiquity Score (Leaching potential)	BCF (potential concern)
Atrazine	Tissue, water	Water, 60.0% (*n* = 15)	1957	Yes (yes)	Herbicide	Inhibits photosynthesis (photosystem II)	35	2.7	100	2.57 (Moderate)	4.3 (Low)
Bifenthrin	Tissue	Tissue, 7.8% (*n* = 77)	1984	Yes (yes)	Insecticide	Sodium channel modulator	0.001	6.6	236,610	-2.66 (Low)	1703 (Threshold for concern)
Clothianidin Desmethyl*	Tissue	Tissue, 2.6% (*n* = 77)		Yes (no)	Insecticide *	n/a	n/a	n/a	n/a	n/a	n/a
DDTs	Tissue	Tissue, 3.9% (*n* = 77)	1944	No (no)	Insecticide	Sodium channel modulator	0.006	6.91	151,000	-3.89 (Low)	3173 (Threshold for concern)
Fenbuconazole	Tissue	Tissue, 6.5% (*n* = 77)	1992	Yes (no)	Fungicide	Inhibits sterol biosynthesis in fungi	2.47	3.79		0.63 (Low)	160 (threshold for concern
Fluopicolide	Tissue	Tissue, 23.4% (*n* = 77)	2006	Yes (no)	Fungicide	Delocalizes spectrin-like proteins (novel)	2.8	2.9		3.2	121 (Threshold for concern)
Hexazinone	Tissue, Water	Water, 73.3% (*n* = 15)	1975	Yes (yes)	Herbicide	Inhibits photosynthesis (photosystem II)	33,000	1.17	54	4.43 (High)	7 (Low)
Indaziflam	Tissue	Tissue, 6.5% (*n* = 77)	2010	Yes (yes)	Herbicide	Inhibits cellulose biosynthesis (CB Inhibitor).	2.8	2.8	1000	2.18 (Moderate)	Low risk (based on Kow)
Indoxacarb	Tissue	Tissue, 2.6% (*n* = 77)	1996	Yes (no)	Insecticide	Voltage-dependent sodium channel blocker.	0.2	4.65	4483	0.27 (Low)	77.3 (Low)
Metolachlor	Tissue	Tissue, 1.3% (*n* = 77)	1976	Yes (yes)	Herbicide	Inhibition of VLCFA (inhibition of cell division)	530	3.4	120	2.36 (Moderate)	68.8 (Low)

Metsulfuron-methyl	Water	Water, 6.7% (n = 15)	1983	Yes (yes)	Herbicide	Inhibits plant amino acid synthesis -	2790	-1.87		3.28 (High)	1 (Low)
Permethrin	Tissue	Tissue, 1.3% (n = 77)	1973	Yes (yes)	Insecticide	Sodium channel modulator	0.2	6.1	100,000	-1.62 (Low)	300 (Threshold for concern)
Pyraclostrobin	Tissue	Tissue, 1.3% (n = 77)	2000	Yes (yes)	Fungicide	Respiration inhibitor (QoL fungicide)	1.9	3.99	9304	0.05 (Low)	706 (threshold for concern)
Sulfometuron-methyl	Tissue, Water	Water, 40.0% (n = 15)	1982	Yes (yes)	Herbicide	Inhibits plant amino acid synthesis -	244	-0.51	85	3.92 (High)	(Low)

4. Discussion

4.1. Interpreting Project Goals and Analyses

This study improves understanding about transport of pesticides applied within Oregon coastal watersheds and subsequent exposure and uptake by bivalves in down-stream freshwater and estuarine habitats. In 38% of the bivalve tissue samples, we detected at least one pesticide, with the frequency and maximum concentration of pesticides varying by season, species, and watershed. The greatest tissue accumulation across all chemical classes occurred in the Siuslaw watershed (1780 km2) and the Smith watershed (956 km2), a coastal sub-basin of the expansive Umpqua drainage system (12,000 km2). The Siuslaw and Smith watersheds both encompass a land-use matrix of federal (51.7%; 57.7% of the watershed area respectively) and private (41.9%; 42.6%) forestlands, some agricultural uses (1.4; 2.8%), and small enclaves of rural populations (0.9; 0.1%: Table 1). Our sampling detected a diversity of compounds in downstream waters and bivalve tissues, including three fungicides, seven insecticides, and two herbicides. The fungicide fluopicolide was the compound most frequently detected in bivalve tissues (23.4% of samples), followed by the insecticide bifenthrin (7.8% of samples) and herbicide indaziflam (6.5% of samples). The suite of compounds identified in tissue samples suggests a variety of potential sources may contribute to pesticide burdens, including but not limited to forestland applications, and provide new documentation about types of cur-rent-use pesticide contaminants found in Oregon's coastal bivalves.

Pesticide compounds commonly applied to commercial forestlands were detected by passive water samplers (atrazine, hexazinone, sulfometuron-methyl, and metsulfuron-methyl) and within the tissues of *Margaritifera falcata*, *Mya arenaria*, and *Crassostrea gigas* (indaziflam) in stream and estuarine habitats located considerable distances down-stream of the application areas. Water-borne herbicide exposure documented during the spring spray season displayed significant correlations with average watershed slope and planned herbicide activity during the sampling window. These finding suggest a fundamental connection between the spatial patterns of management activities, natural watershed features, and downstream multiscalar ecological processes within the study region (as outlined in Chapter 1; Figure 2).

4.1.1. Seasonal and Species Differences in Contaminant/Exposure Levels

Pesticide contaminants were more frequently detected in bivalve tissues during the summer of 2017 during low runoff conditions, and higher concentrations were detected in the spring of 2018 during high runoff conditions (Table 3). Elevated contaminant levels in spring are expected due to the timing of spring pesticide applications to commercial forestlands and resultant high flow downstream (Hapke et al., 2016; Oregon Health Authority, 2014). Bivalve tissues frequently exhibit seasonal variability in lipid content due to gametogenesis and reproduction, which can influence the composition and concentration of stored contaminants (Capuzzo et al., 1989). However, bivalve lipid content did not vary significantly between summer and spring sampling seasons, but varied significantly among the three bivalve species (Figure 2).

Interspecific comparison of lipophilic compound accumulation among bivalves is challenging due to differences in habitat, salinity, feeding mechanism, reproductive timing, life span, and other life-history characteristics. Lipid normalization allows for comparisons among diverse bivalve species to evaluate differences in tissue pesticide detections between the wet and dry seasons (Choi et al., 2016; Thompson et al., 2017). In our samples, lipid normalization inflated existing differences among species' contaminant burdens, further widening the gap between *M. arenaria* and the other species, while narrowing the range of concentrations between *C. gigas* and *M. falcata* (SM: Figure S3A,B). Elevated pesticide concentrations in *M. arenaria* are likely associated with the location of their preferred habitat at the interface between freshwater and estuarine regions of the watershed (salinity range >5 psu; Table 1) where they are presumably exposed to a diversity of waterborne pollutants carried downstream from multiple points of origin.

4.1.2. Contrast in Compounds Detected in Waters and Bivalve Tissues

Different chemicals detected in tissue versus water samples demonstrate two avenues of chemical fate and transport in the environment, critical in understanding environmental exposure and uptake. The suite of chemical compounds detected in passive water samplers did not overlap with the pesticides detected in tissue samples, with no common compounds detected in both sampling media. These differences are likely attributed to differing biochemical properties and transport pathways (Table 4), suggesting that although forest management activities expose bivalves to herbicide runoff, most current-use herbicides (with the exception of indaziflam) do not accumulate in their tis-sues. Low bioaccumulation in bivalve tissue is not surprising given the hydrophilic nature of most current-use forestry herbicides. In contrast, the current-use rainfall-activated herbicide indaziflam (Esplanade F (FERNS, 2020)), used to control vegetation by ground or aerial application and promoted for its persistence in soil (half-life >150 days) (Kaapro, J., & Hall, J., 2012; Peachy, 2020), was detected in bivalve tissue in five of eight coastal watershed areas. Widespread detection of indaziflam in bivalve tissue is especially notable as the compound (registered in 2010; Table 4) is classified as both "very toxic to aquatic life" and "very toxic to aquatic life with long lasting effects" by the Globally Harmonized System of Classification Labeling of Chemicals (GHS) (National Center for Biotechnology Information, 2021).

4.1.3. Forestland Management Regimes and Exposure of Bivalves to Pesticides

We documented accumulation of an array of insecticides, herbicides, and fungicides in bivalve tissue across multiple Oregon Coast Range watersheds. Detections were not consistent across sample locations, hindering statistical analysis relating tissue concentrations with watershed variables (SM; Table S3). Some tissue-detected pesticides are registered for use in plantation forestry management, but others are used in a variety of other crops including orchards, vineyards, and Christmas tree farms (PICOL, 2020). Water protection standards for Christmas tree farms and orchards are not prescriptive, and analysis of upstream rates of usage, prevalence, management activities, and linkages to tissue concentrations remains elusive. According to FERNS notification data, indaziflam is the only detected tissue-bound compound currently applied within the region during vegetation management activities on forestlands (FERNS, 2020).

Comparison of POCIS detections among sites indicate that compound accumulation was related to the amount of notified herbicide activity in upstream watersheds (Figure 6A), with types of compounds detected related to ownership/management (Figure 5A). These observations suggest that freshwater and estuarine bivalves in some watersheds may be at risk of pesticide exposure based on upstream forestland management regimes and the pervasiveness of activities. Our NMDS analysis suggests that forestland owner-ship (a surrogate for pesticide application policy) is related to the types of compounds in water samples (Figure 5A). For example, atrazine (the only herbicide of the four detected in POCIS sampling that is not permitted for use under the NWFP) exhibited a negative association with federal land ownership. In linear modeling, forestland ownership alone was not a strong predictor of chemical exposure, but management practices such as planned forestry herbicide applications influenced aquatic chemical concentrations. In particular, increases in notification of planned aerial herbicide application predicted in-creases in chemical loads of that pesticide class downstream (Figure 6A).

4.2. Additional Factors Affecting Pesticide Exposure and Transport in Coastal Watersheds

4.2.1. Spatial Scale and Complexity of Watershed Drainages

Exploration of downstream pesticide transport following multiple applications allowed us to examine the impact of forestland ownership and management on organismal exposure at the watershed scale. The percentage of coastal watersheds under notice for herbicide spray applications correlated with the concentration of herbicides detected in passive water samples. This relationship indicates a plausible connection between cumulative effects of herbicide applications within a catchment basin and the type and amount of chemical exposure to downstream organisms. However, previous BMP re-search has highlighted the role of variable abiotic factors, which were not controlled in our study, in understanding offsite movement of chemicals (Boyle et al., 1997; Caldwell & Courter, 2020). Caldwell and Courter (2020) found that proximity to herbicide application sites followed by rainfall had the greatest influence on herbicide concentration in downstream Oregon coastal waters (Caldwell & Courter, 2020). Our findings are consistent with these studies and indicate that a rainfall event may result in higher herbicide concentrations in areas with more herbicide applications upstream. Watershed slope was positively correlated with total POCIS accumulation and the best fit for our stepwise linear regression of physical watershed variables (Figure 6B). Watershed slope is consistently an important factor in offsite herbicide transport during site-scale investigations (Müller et al., 2004) as well as a critical input parameter for modeling pesticide runoff (Morselli et al., 2018; Zhang & Zhang, 2011). Given that surface runoff is a key process affecting pesticide presence in water (Schriever et al., 2007) the positive association between average watershed slope and the concentrations of herbicides detected by passive water samplers deployed downstream is not surprising.

4.2.2. Ecotoxicity of Pesticide Mixtures and Pulsed Exposures

The wide range of properties associated with detected compounds highlights the variability in chemical partitioning and movement in aquatic ecosystems, and the importance of documenting multiple routes of exposure across scales and timeframes within watersheds. The in-tissue and passive water pesticide mixtures observed in our study align poorly with USEPA toxicity information and established regulatory benchmarks that assume dose–response toxicity of single reference compounds on a small group of selected species (Touart & Maciorowski, 1997). Chemical interactions within complex mixtures (in tank mixes and observed in the field) may result in additive, synergistic, or antagonistic effects on organisms at or below established benchmarks (Lydy et al., 2004; Sobiech & Henry, 2002). Additional research is needed to better understand organisms' risks from sublethal exposures based on the documented chemical mixtures of lower doses of forestry (and other) pesticides (Michael, 2004; Norris et al., 1991). The discrepancy between pesticide registration requirements and our field observations of chemical mixtures highlights an important knowledge gap and topic for future research.

Organismal age has been identified as an important factor in understanding the impacts of episodic exposure (the commonly observed route of exposure in forestry runoff) to toxicity stressors (Gordon et al., 2012). Sublethal effects of episodic toxicant exposure can influence population dynamics, especially if exposure occurs to highly sensitive early life stages—juveniles, larva, or during reproduction (Boyle et al., 1997; K. Perry & Lynn, 2009; Schriever et al., 2007; Touart & Maciorowski, 1997). Low concentrations of atrazine may alter behavior at non-monotonic dose–responses as observed when short term exposure (72 h) to atrazine (1.5 and 150 ug/L) decreased spatial aggregation (associated with reproduction) by the freshwater mussel Ellipitio complanata (Flynn & Spellman, 2009). Freshwater mussels, which are particularly susceptible to contaminant exposure from surface water during their glochidial stage (Cope et al., 2008), are among the most sensitive aquatic organisms, and exposure to environmental concentrations of current use pesticides and surfactants have resulted in developmental and genotoxic responses below individual NOEC concentrations of test chemicals (Bringolf et al., 2007; Conners & Black, 2004). Reproductive timing of *M. falcata* is linked to springtime changes in water temperature in Oregon, and glochidia have been observed in the water column from April to mid-June (Allard et al., 2017). Our finding of forestry-specific herbicides in the water column during this timeframe suggests that larval mussels in coastal watersheds could be exposed to herbicide mixtures during this sensitive life stage.

4.2.3. Management Practices

Herbicides (such as atrazine) applied to ephemeral stream channels during dry conditions may become mobilized and transported during subsequent rainfall events (Norris et al., 1991). Additionally, climatic conditions influence dissipation of atrazine in plantation forestlands, and high rainfall events in temperate locations increase the likelihood of longer persistence in soils and higher offsite mobility (Kookana et al., 2010). Three detected current-use herbicides (atrazine, indaziflam, and hexazinone) are activated by rainfall for uptake and absorption into the roots of target plants (Peachy, 2020). Reliance on rainfall as the activation mechanism for popular herbicides, combined with a lack of buffer requirements on small type-N and intermittent streams, could explain why increasing compound detections were associated with increased herbicide applications upstream. Atrazine formulation labels typically list buffer restrictions, a 122 m minimum upwind buffer from sensitive vegetation and a 20 m buffer from points where surface water runoff enters perennial or intermittent streams (EPA Reg. No. 35915–4); these are more stringent than OFPA requirements. Indaziflam formulations require a 7.62 m spray buffer around water bodies such as streams or lakes during aerial application (EPA Reg. No. 432–1517). However, no information is available to characterize the level of applicator compliance with these label restrictions.

Vegetated riparian management areas (RMAs) can successfully mitigate contaminant impacts to water quality from runoff and direct infiltration into stream networks, though the minimum size for effective buffers is debated (Mazza & Olson, 2015; Michael & Neary, 1993). Studies of site-level effects of forestry pesticide application to downstream water quality indicate variability in episodic exposure scenarios, wherein low pulsed concentrations of applied chemicals are observed following application events (Caldwell & Courter, 2020), with most monitoring efforts generally at and below single treatment parcels (Dent & Robben, 2000; Louch et al., 2017; Tatum et al., 2017). However, earlier research has not specifically investigated movement of chemicals in areas without spray buffers, such as perennial and intermittent stream channels (Dent & Robben, 2000; Louch et al., 2017). As a result, test conditions and results from previous studies may not fully reflect permitted forestry management practices. Controversy exists between the timber industry and conservation communities around the issue of pesticide use in Oregon's forestland management, but recent developments indicate a collaborative and cooperative path forward may be on the horizon. A recently adopted Oregon Senate bill (S.B. 1602) provides support and structure for a mediated science-based approach to address shortcomings of OFPA aquatic resource protective measures, but specific approaches to achieve such outcomes have yet to be determined (Senate Bill 1602, 80th Oregon Legislative Assembly, 2020 Special Session, 2020).

4.3. Caveats and Lessons Learned

Understanding cross-ecosystem linkages, specifically effects of terrestrial activities on riverine and marine species, is a challenging but essential step in designing effective and comprehensive land-sea planning, management, and conservation (Álvarez-Romero et al., 2011). Unknown parameters and inherent variability at large spatial scales contribute uncertainty and important limitations or caveats when developing characterizations at the watershed scale (Milner-Gulland & Shea, 2017). Integrating ecological research such as ours directly into management decisions is complicated by the imperfect picture provided by watershed scale research, in contrast to that provided by controlled laboratory or small-scale field settings with lower inherent variability.

Our efforts to explain the biophysical linkages between coastal watershed forestry practices and bivalve exposure to waterborne toxicants in downstream systems were limited by potentially confounding factors. For example, bivalve sampling across two non-consecutive seasons confounds identification of seasonal differences in pesticide exposure as an underlying factor (SM: Figure S1). Inter-annual variation in pesticide application levels, timing, and concurrent rainfall are also controlling factors. Similarly, non-forestry sources of contamination can vary annually and spatially. Differences in the habitats, feeding mechanisms, and life-spans of the bivalves studied may contribute to variability in contaminant body burdens. Uncertainty about the specific timing and location of herbicide application activities during the spring spray season required us to extend the deployment of our passive water sampling, making it impossible to calculate realistic time-weighted average water concentrations for the detected herbicides. Consequently, our measurements of forestry herbicides in downstream waters and bivalve tissues are useful to understand compounds' presence/absence across watersheds and document complex exposure mixtures over time, but do not provide in-water pesticide concentrations to predict toxicity. Differences in the hydrology of the coastal watersheds, and variability in the chemistry of streams and soils, local climates, and the legacy impacts of forestry management practices are only a few of the many uncontrolled factors that may influence our findings.

5. Conclusions

Our study identified that bivalves (and likely other aquatic organisms) in Oregon's coastal watersheds are exposed to a suite of herbicides commonly used in forestland chemical applications during the spring spray season. Accumulation of measured herbicides in passive water samples was associated with land-use and physical watershed characteristics upstream (frequency of notified herbicide application and average watershed slope). Transient exposures captured in POCIS sampling coupled with varying levels of pesticide residues in bivalves identify specific pesticide compounds, pathways for pesticide transport, and levels of exposure. These findings highlight the need to ad-dress management practice effectiveness in controlling transport of potentially harmful compounds throughout the Oregon Coast Range. The precise timing of runoff events remains unknown, and the extent to which such runoff coincides with bivalve reproduction and resultant toxicity exposure in downstream habitats is still speculative. Our study highlights information gaps and research needs to: (1) quantify the extent to which variation in the widths of herbicide spray buffers across stream types function to protect downstream aquatic habitats; (2) explore precise fate and transport of the variety of chemicals used in coastal forest management; and (3) reconcile exposure concentration/duration with chronic or sublethal toxicity endpoints. As scientific understanding of ecotoxicology evolves and new monitoring techniques become available, efforts to understand cross-ecosystem stressors are critical, especially to incorporate ecosystem-based management into watershed-scale or regional land management objectives that go beyond managing for single land uses and individual classes of chemicals.

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Chapter 3: Predicting springtime herbicide exposure across multiple scales in Oregon's Coastal drainages

Under peer review at ***Ecological Indicators***

1. Introduction

Offsite movement of pesticides throughout watersheds is a universal concern for managers and scientists, especially in light of research on sublethal effects of low dose exposures to aquatic organisms. Approaches to understand risk in these contexts vary, but a central challenge is collecting sufficient data at appropriate scales and time intervals to make informed decisions about how pesticides affect aquatic ecosystems. Monitoring results from field collected data can be useful not only to inform managers about transport within the sampled locations but also to predict concentrations in un-sampled areas through modeling (Holvoet et al., 2007).

The foundational principles of landscape and riverscape ecology, emphasizing the relationships between spatial patterns and ecological processes in watersheds, have influenced the way contemporary ecologists conceptualize and study the environment (Turner and Gardner, 2015). Investigations into pesticide movement in watersheds require considerations of biotic, abiotic, and socio-ecological factors in understanding landscape processes and patterns of exposure (Chapter 1; Figure 2). Commonly in landscape scale research, multi-site comparisons and empirical modeling are implemented to record the influence of natural and anthropogenic variables - such as land-use, on in-stream conditions (Allan, 2004). Effects of land-based activities on river systems occur across many scales, highlighting the importance of river research to contextualize effects within ecosystems (Allan et al., 1997). Furthermore, by exploring research questions across a diversity of scales, cumulative effects of land-use practices can be better understood.

The Coast Range region of Oregon encompasses the majority of Oregon's coastal watersheds and is largely forested (Spies et al., 2002). The defining feature is the Coast Range Mountains, which separates the coastal watersheds from the inland portion of the state, both topographically and climatically (Franklin and Dyrness, 1973). Unlike other regions in Oregon, drainage basins in the Coast Range (aside from some sections of the Umpqua) are dominated by forestland from headwater to mouth (Spies et al., 2002). This unique geographic scenario provides a valuable and unique opportunity to explore how forestland management practices affect watershed health at multiple scales, without excessive confounding factors from widespread interspersed agricultural or urban land uses.

1.1 Forestland management in the Coast Range

Forestlands in the Coast Range region are managed under two governing documents: federal lands rely on the Northwest Forest Plan (NWFP) to guide management activities, whereas activities on state, tribal, and private lands rely on the Oregon Forest Practices Act (OFPA) for guidance (Spies et al., 2002). Management objectives outlined under each set of guidelines differ widely between the two documents, wherein OFPA provides management standards for commercial activities related to harvest, regeneration, and management of trees, but objectives of the NWFP extend beyond regenerative harvest to include significant reserve network and conservation strategies designed to protect and enhance habitat for threatened and endangered species (Thomas et al., 2006). As a result, the majority of intensive forest management (IFM) in Oregon's coastal forestlands is concentrated on lands governed by the OFPA (Kaplan and White, 2002).

Investigation into cumulative effects of intensive forestry on water quantity has found significant relationships between the scale of operations and their contribution to water quantity deficits in downstream waterways (Perry and Jones, 2017). Substantial research has focused on cumulative effects of many types of forestry practices (road construction, clearcutting, planting, etc..), but less is understood about the effects of multiple chemical applications within watersheds and the transport of chemical mixtures away from application sites (Clark et al., 2009; Norris et al., 1991). Pesticide application on forestlands is often downplayed in comparison to agricultural applications based on the frequency of occurrence (herbicide applications take place 1-5 years after clearcutting versus multi-annual applications on agricultural lands) until replacement conifers are established. Most research concerning chemical applications on forestlands is focused on site-level effectiveness, and data gaps remain on the effects of chemical applications across larger spatial scales or multiple watersheds within regions. Exploring the effectiveness of management practices at the site scale provides valuable and critical information, but looking at other larger scales may provide more accurate information on exposure by organisms within a watershed. Similarly, chemical applications in forestlands commonly take place in concert with other land use and forestry practices, and should be considered within these contexts (Norris et al., 1991). Additionally, valuable and protected resources exist across scales highlighting the importance of looking beyond site-scale impacts to understand catchment or watershed level effects. Chemical movement in watersheds related to specific land uses such as vegetation management in forestlands may be counterproductive to downstream designated aquatic uses.

During late winter and spring, pre-emergent and site preparation herbicide treatments are commonplace in Oregon’s coastal forestlands. Chemical site preparation treatments accompany mechanical, manual, and fire-based methodologies as vegetation control measures that take place within the first year of the original cutting before reforestation occurs (Rose and Haase, 2006). Once trees have been planted, pre-emergent or “dormant applications” are utilized to control competing vegetation before conifer bud break takes place in late spring (Peachy, 2020). Competing vegetation targeted in these applications range from herbaceous grasses and ferns to early successional woody species such as vine maple and alder. Dormant applications are commonly applied in mixtures to target a variety of early successional vegetation (Table 1). Rainfall during spring months in Oregon’s Coast Range is substantial, and many compounds used in vegetation management during this period are rainfall activated products. Resultant runoff events following forestland pesticide application are generally characterized as episodic exposures, wherein “pulses" of higher chemical concentrations move downstream followed by decreasing concentrations (Louch et al., 2017). Unfortunately, the majority of forestry specific monitoring in the region has occurred during foliage applications occurring in the summer and fall months (Dent and Robben, 2000), with monitoring during spring runoff understudied. Despite the low number of spring season studies in Oregon, the highest levels of pesticides are frequently observed during springtime runoff periods (Hapke et al., 2016; Kelly et al., 2012).

Table 1: Herbicides commonly applied during spring months in forestlands during vegetation management applications (site preparation and pre-emergent (Peachy, 2020)).

Herbicide Compound Name	Common Product Names	Target vegetation	Application rate (active ingredient per acre)
2-ethylhexyl ester of 2,4-Dichlorophenoxyacetic acid (2,4-D)	Weedone LV-4, Weedone LV-6	Broadleaf weeds and woody plants	1 to 2 lb.
Atrazine	Aatrex 4L, Atrazine 4L, Atrazine 90	Grasses and herbaceous plants	3 to 4 lb.
Clopyralid	Transline	Herbaceous plants	0.19 to 0.49 lb.
Glyphosate	Rodeo, Roundup	Grasses and broadleaf weeds	1.5 to 3 lb.
Hexazinone	Velpar L, Velpar DF	Herbaceous and woody plants	1 to 3 lb.
Indaziflam	Esplanade F	Broadleaf weeds and grasses	0.73 to 1.46 oz. (not to exceed 10 oz./a of product annually)
Sulfometuron-methyl	Oust, Oust XP	Grasses and broadleaf weeds	1.5 to 3 lb. 0.375 to 0.94 oz.
Triclopyr	Garlon 4 Ultra	Woody plants	< 6 lb. ae (triclopyr)= 6 quarts

During spring and early summer in Oregon, changes in water temperature cue reproduction in several freshwater and estuarine species (bivalves, pacific lamprey, etc...) that inhabit coastal watersheds (Allard et al., 2017; Meeuwig et al., 2005). Since reproduction and larval life stages of aquatic organisms are considered the most sensitive to chemical contaminants (Bringolf et al., 2007; Cope et al., 2008; Perry and Lynn, 2009), understanding in-water concentrations of current-use herbicides during time periods coinciding with spring spray is critical to assess relative threats to non-target aquatic species.

Integrative sampling is a valuable method to explore in-water pesticide presence from pulsed exposures during a fixed timeframe, to detect hydrophilic compounds easily missed in grab sampling, and to capture compound mixtures to identify diffuse contaminant sources (Alvarez, 2010; Metcalfe et al., 2019). Since seasonal and annual monitoring across the Coast Range is time consuming and limited by funding constraints, modeling existing monitoring data can extrapolate measured concentrations to unsampled areas. Modeling results, though simplified representations, can predict exposure at multiple scales and guide future monitoring efforts addressing exposure from cumulative or mixed effects.

A previous phase of this project explored herbicide runoff during the spring spray season (six week deployment) to understand differing exposure of bivalves to current-use forestry pesticides based on management regime (Scully-Engelmeyer et al., 2021). Using integrative passive water sampling, we detected four current-use herbicides downstream from actively managed catchments, which, along with bio-monitoring efforts, allowed us to examine bivalve exposure in Oregon coastal watersheds (Scully-Engelmeyer et al., 2021). We explored watershed variables related to management and physical characteristics to explain variation in herbicide detections in passive water samples and found that slope and active notifications for aerial herbicide application during the deployment window were the two best individual predictors of total herbicide accumulation in passive water samplers. Here we develop a multiple linear regression model to explain relative pesticide concentrations and: (1) identify the combination of watershed variables that best explain the variation in detected concentrations, (2) assess to what extent modeling can be used to predict the relative presence of herbicides in un-sampled coastal watersheds, and (3) identify the scale effects and regional patterns in measuring predicted concentrations. Additionally, we examine detected herbicides in the context of other protected and valuable aquatic resources in the Coast Range. We expect that variables related to herbicide use and watershed slope in upstream forestlands will best predict downstream concentrations detected in POCIS sampling, and that regional differences in measured pesticide concentrations will be reflected in predicted concentration values.

2. Methods

2.1 Passive water sampling

Sixteen catchments associated with four main watershed areas were selected for passive water sampling to encompass a range of active forestland management across multiple scales and different latitudes in the Coast Range (Figure 1). Integrative passive water sampling was utilized to capture episodic chemical exposure in selected catchment areas. Polar organic chemical integrative samplers (POCIS) were deployed (three replicate disks per sample) for six weeks beginning March 26-29, 2019; samplers were retrieved in identical deployment order. POCIS samplers use two microporous membranes (0.1 micron pore) to continually capture water soluble organic compounds from the water column in a solid phase extraction resin (Oasis HLB sorbent) during their deployment period. Upon retrieval, POCIS disks were sent to Environmental Sampling Technologies (EST; Missouri) for extraction. Composited ampules (three disks per ampule) were then sent to Anatek labs (Idaho) for pesticide analysis of commonly used forestry compounds (Supplementary Material (SM); Table S1). Field replicates were deployed at three randomly selected locations to assess method variance, and field and laboratory blanks were implemented to assess unintended contamination during field work and processing. Deployment, retrieval, and quality control measures were implemented in accordance with the guiding document on POCIS monitoring developed by the United States Geological Service (USGS) (Alvarez, 2010). Detailed processing and extraction information can be found in Scully-Engelmeyer et al. (2021).

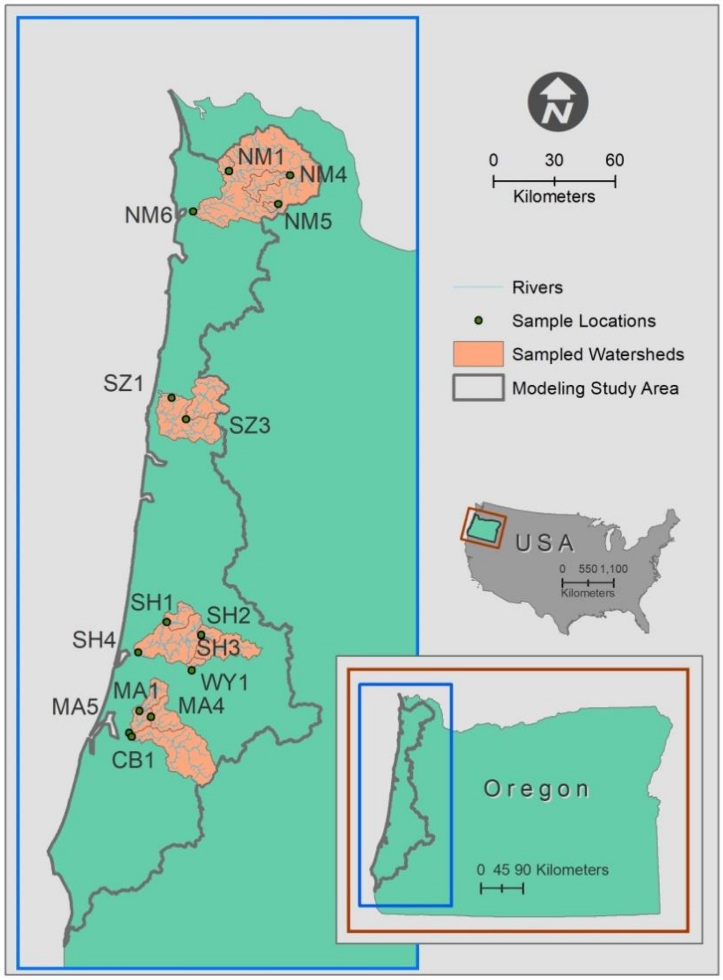

Figure 1. Watershed areas sampled using integrative passive water samplers. Outlined area shows modeling study area

2.2 Model Development

2.2.1 Catchment characterization

Catchment areas above sampling locations were delineated using USGS's online StreamStats application: Streamflow Statistics and Spatial Analysis Tools for Water-Resources Applications (Version 4). Delineations calculated basin characteristics within catchment areas using continuous parameter grids based on 30 meter Digital Elevation Models (DEM) (Cooper, 2005; Risley et al., 2008). Variables such as annual precipitation, slope, and elevation were calculated in this way (Table 2). Additionally, drainage density and length of roads were automatically calculated during delineation (Cooper, 2005; Risley et al., 2008). ArcMap version 10.7 was used to determine and export additional characteristics above sampling locations based on catchment delineations from StreamStats. Forest loss data ((Hansen et al., 2013) version 1.7) was imported to ArcMap and converted to polygons. Forest loss from 2016-2019 was selected, clipped within study watersheds, and exported. Oregon Department of Forestry hazard slope shapefiles indicating slope above 40% were used to develop a steep slope variable (Table 2).

Notifications regarding management activities taking place on state, private, and tribal lands are recorded and publicly available through the Forest Electronic Reporting and Notification System (FERNS), and activities on federal lands are accessible through the U.S. Forest Service Activity Tracking System (FACTS) database and a separate online record system for U.S. Bureau of Land Management (BLM) lands. Notification data available in the FERNS dataset outlines types and date ranges of planned management activities, implementation methods, and potential chemicals proposed for use (in the case of pesticide application notifications). Additionally, polygon and line shapefiles, available from the Oregon Department of Forestry's spatial data library, contain notification identification numbers matching pesticide application notifications available from FERNS. The exact date and precise chemical mixtures used in the final activity are not included in this notification data. FERNS notification data were sorted and filtered in excel to encompass the desired timeframes and activity types, then categorized into watershed variables for analysis. Sorted data were imported into ArcMap and joined with FERNS polygons based on identification number; only matching records were retained. Polygons were then re-selected based on desired activity type to exclude irrelevant activities that were inadvertently retained under the same NOAP id number during the first step. Remaining polygons were aggregated (using the Dissolve tool) and clipped to watershed boundaries; the Identity tool was used to compute the variables within study watersheds. Final polygons for each variable were catalogued, exported, and used in regression analysis to configure ideal model variables (Table 2).

Table 2. Watershed characteristics - including physical variables calculated above each sampling location and management variables at each location - used in regression analyses. dv= dimensionless variable, km2 = square kilometer.

Watershed Characteristics	Abbreviation	Unit
Physical Variables		
Area	area	Km^2
Steep slopes (slope above 40%)	slp_abv	%
Road density	rd_den	dv
Drainage density (Σ stream length / watershed area)	drn_den	dv
Forest loss	floss	%
Stream temperature change (between deployment and retrieval)	avtemp_c	Celsius
Average annual precipitation	precip_cm	centimeters
Management Variables		
Area notified for clearcut within 1 year of deployment	cc1yr	%
Area notified for clearcut within 3 years of deployment	cc3yr	%
Area notified for herbicide application during deployment	allherb_dep	%
Area notified for aerial herbicide application during deployment	aerial_dep	%
Area notified for herbicide application within 1 year of deployment	allherb_1yr	%
Area notified for aerial herbicide application within 1 year of deployment	Aerial_1yr	%

2.2.2 Best fit model development

Independent variables were scaled and square root transformed, and the dependent variable was square root transformed to meet regression assumptions.

Correlation matrices were used to investigate relative correlation between total accumulation in water samples and environmental variables as well as multicollinearity of environmental variables. Additive relationships were explored using manual forward selection stepwise multiple linear regression until coefficient of determination explained close to 90% of the variation. Since scale is one of the primary output explorations, it was critical to rule out watershed size as a predictor in developing the model. The final model assumptions of normality and multicollinearity were tested using a Shapiro test of residuals and variance inflation factors (VIFs). Remaining model assumptions of skewness, kurtosis, and heteroscedasticity were tested using the Global Validation of Linear Models Assumptions (GVLMA) package. Model validation was done using the leave-one-out cross validation method (LOOCV), which was chosen for its utility in working with small datasets.

2.3 Model application

Based on the best fit model, independent explanatory variables were calculated and projected across the entirety of the Coast Range province. Hydrologic Unit Code (HUC) catchments at 8, 10, and 12 digit scales from the Watershed Boundaries Dataset (WBD) were then overlaid above Coast Range watersheds, defining the study area. Within the 10 and 12 digit scales, HUC unit boundaries used in model analysis were restricted to catchments containing a complete drainage area to avoid misapplication of model output on HUC units representing partial watershed context (Omernik et al., 2017). This method was applied to avoid misrepresentation of downstream HUC segments as complete watersheds when they are more accurately defined as partial catchment units. HUCs modeled using this selection method represent complete catchments at small (HUC 12), medium (HUC 10), and large (HUC 8) scales within the Coast Range. Ratios of each predictor variable were calculated separately within each HUC across the three scales and exported to excel. Variable values for each catchment were then used to calculate the predicted concentration within each HUC unit based on the best fit model formula.

2.4 Model output analysis

2.4.1 Comparing model output across scales

Predicted values within each catchment across the three scales were displayed in choropleth format across the study area to visually explore patterns of predicted exposure at the three scales investigated. Boxplots and non-parametric Kruskal-Wallis one-way analysis of variance was used to compare the predicted values at the HUC 8, 10, and 12 digit scale.

2.4.2 Exploring regional differences in variables and model outputs

Ratio values of each predictor variable, calculated within each watershed scale, were displayed in a series of choropleth maps of the area to explore regional differences among predictor variables across scales. Boxplots were used to compare values of each predictor variable among scales. Predicted values projected within HUC boundaries across the coast range were displayed via choropleth mapping to visually explore regional differences in predicted exposure. HUC 12 catchments were then grouped into HUC 8 categories to explore how predicted values at the small catchment scale match up within larger drainages/subbasins across the study area. Kruskal-Wallis analysis of variance was used to compare predicted values in the smaller catchments (HUC 12 subwatersheds) across the HUC 8 subbasins (as the grouping variable).

3. Results

3.1 POCIS Deployment and Detections

During the POCIS deployment period, a severe spring storm blanketed south coast watersheds, raising river levels and causing flooding and landslides (FEMA 4452-DR-OR). Upon receding, POCIS canisters at two sites (west fork Millicoma River: MA.1, and north fork Smith River: SH.1) were partially stranded on the bank where they had been deposited while river levels were elevated. Oasis HLB media were still intact in those canisters, so they were processed and included in the results. The submerged sampling interval for those canisters cannot be determined, so concentrations may under-represent exposure over the 45 day sampling period. Additionally, the membranes and HLB media in the Euchre Creek canister (Siletz River: SZ.2) were destroyed during the deployment period, restricting analysis of sampling results at that site.

Of the fourteen herbicides and one surfactant included in POCIS canister analyses, four commonly applied herbicides were detected (hexazinone, atrazine, sulfometuron methyl, and metsulfuron methyl). Herbicides were detected at 80% of sample locations (Table 3). Detections ranged from 1.16-936 ng/POCIS, averaged 277 ng/POCIS, and varied across locations (Table 3). Concentrations were not detected in field or laboratory blanks.

Table 3. Herbicides detected in POCIS samples. Sample locations are organized from north to south along the coast. SMM=sulfometuron methyl, MSM= metsulfuron methyl, RL = reporting limit.

Sampling Location	ng/POCIS				
	Atrazine	Hexazinone	SMM	MSM	Total Accumulation
NM.1	11.93	<RL	1.8	<RL	13.7
NM.4	6.05	1.09	<RL	<RL	7.1
NM.5	<RL	<RL	<RL	<RL	<RL
NM.6	<RL	<RL	<RL	<RL	<RL
SZ.1	<RL	38	<RL	<RL	38
SZ.3	<RL	14	<RL	<RL	14
SH.1	<RL	11.6	1.55	<RL	13.2
SH.2	131	816	36.3	1.4	984.7
SH.3	139	212	1.92	<RL	352.9
SH.4	164	103	2.78	<RL	269.8
WY.1	466	963	1.16	<RL	1430.2
MA.1	<RL	<RL	<RL	<RL	<RL
MA.4	185	117	<RL	<RL	302
MA.5	253.3	117.3	<RL	<RL	370.6
CB.1	232	138	<RL	<RL	370

3.2 Model development

Correlation matrices and Pearson's correlation suggest strong relationships between total detected herbicide concentrations in POCIS samplers and upstream watershed variables, as well as collinearity among variables (Appendix A, Figures A1 & A2). Additionally, several notable variables did not correlate with POCIS accumulations, such as watershed size and drainage and road density (Figure A2). Manual additive multiple regression analysis determined a model with three independent variables best predicted total herbicide accumulation in passive water samplers without violating multicollinearity assumptions. A multiple linear regression was determined to predict total herbicide accumulation based on watershed characteristics including within the last year (cc1yr); (F (3, 8) = 31.1, $p < .000$), with an R^2 of .8914. POCIS predicted concentration = 15.016 + (3.854 *slp_abv) + (5.212* allherb_dep) + (4.855 *CC1yr), where all variables are measured as percentages of upstream catchment areas and were significant predictors of total concentration (Table 4). Variable inflation factors (VIF) for final variables were 1.460, 2.001, and 1.463 for slp_abv, allherb_dep, and CC1yr respectively (Table 4). Cross validation using LOOCV resulted in a model root mean squared error of 4.567 ng/POCIS, a mean absolute error of 3.783 ng/POCIS and an R^2 of 0.8358.

Table 4. Final multiple regression model summary statistics. CI= confidence interval, β = standardized beta coefficient, VIF = variable inflation factor.

	B	Std. Error	β	*t*	*p*-value	95% CI	VIF
Constant	*15.016*	*1.096*		*13.699*	*0.000*	*12.49,17.54*	
allherb_dep	*5.212*	*1.623*	*0.452*	*3.212*	*0.012*	*1.47,8.96*	*2.010*
cc1yr	*4.855*	*1.385*	*0.421*	*3.505*	*0.008*	*1.66,8.05*	*1.464*
slp_abv	*3.854*	*1.383*	*0.334*	*2.786*	*0.024*	*0.66,7.04*	*1.460*

Final model variables were calculated within each HUC scale across the study area (Figures 2 A, B & C), exported to excel, and imported to Rstudio (version 4.0.4) to calculate predicted values. Overall, variables within HUC 12 watersheds displayed the largest ranges across all categories, followed by HUC10 and HUC8 scales (Figure 2A, B & C, Table 5). Though ranges varied widely between scales, no significant differences were seen among HUC group means for any of the predictor variables based on Kruskal—Wallis tests (Table 5).

Table 5. Summary statistics for final predictor variables [steep slopes above 40 percent (slp_abv), area notified for herbicide application during deployment (allherb_dep) and area notified for clearcut within 1 year of deployment (cc1yr)] and predicted values in each HUC level.

Watershed size	**Predictor variables: $\bar{x}$ (range)**			**Model predicted values (ng/POCIS)**
	slp_abv (%)	allherb_dep (%)	cc1yr (%)	
HUC8	25.7 (15.5-33.0)	1.5 (0.4-3.2)	0.89 (0.5-1.3)	294.6 (99.5-516.8)
HUC10	29.6 (5.6-72.2)	1.5 (0-8.5)	0.8 (0.1-1.8)	289.0 (17.3-1301.8)
HUC12	27.8 (0.2-79.4)	1.7 (0-16.8)	0.96 (0-4.28)	303.5 (0.1-2445.1)
Overall	28.1 (0.2-79.4)	1.65 (0-16.8)	0.9 (0-4.28)	299.6 (0.1-2445.1)
Kruskal—Wallis	H(2)=0.704, *p*=0.7033	H(2)=0.315, *p*=0.8538	H(2)=0.316, *p*=0.8542	H(2)=2.1409, *p*=0.3428

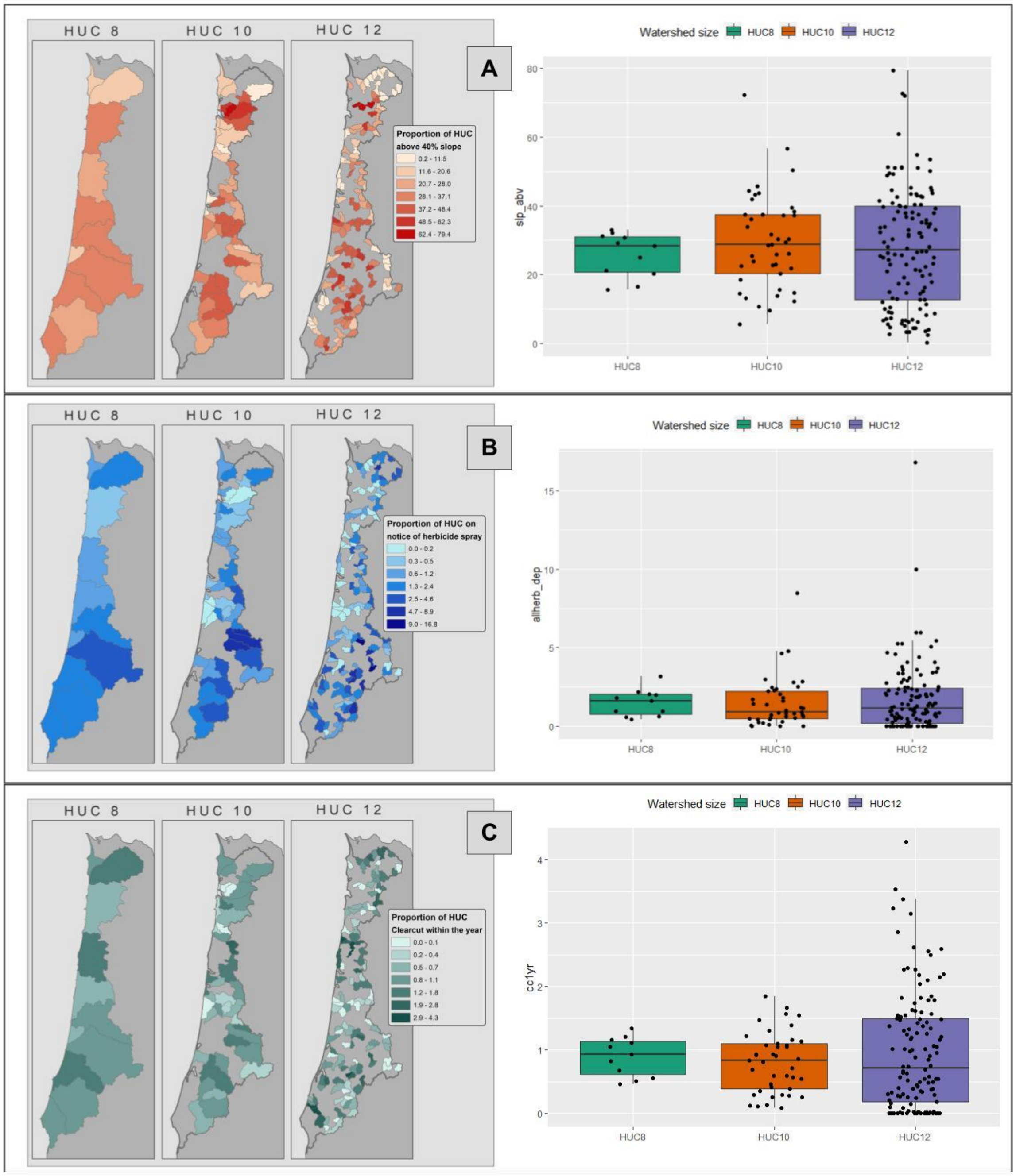

Figure 2. Percentage of each catchment with steep slopes (A), herbicide notifications during deployment window (B), and clearcuts within a year of deployment (C) were calculated across three HUC scales within the study area.

3.3 Model predicted concentration values

Predicted concentrations based on the best fit multiple regression model produced values ranging from 0.1 to 2445.1, and averaged 299.6 ng/POCIS across all categories (Table 5). Similar to predictor variables, the largest ranges were seen in HUC12 watersheds, followed by HUC10 and HUC8. No significant differences were observed between watershed scales (Table 5, Figure 3B). Predicted values varied geographically, with the highest values seen in the southern portion of the study area across all three scales (Figure 3A). Comparisons of HUC 12 predicted values grouped by HUC 8 catchment indicate regional differences in predicted concentrations, wherein predicted values in the Coos watershed were significantly higher than the group mean, and those within Wilson-Trask-Nestucca were significantly lower (Figure 4). The highest overall predicted values were seen within sub-watersheds of the Umpqua watershed.

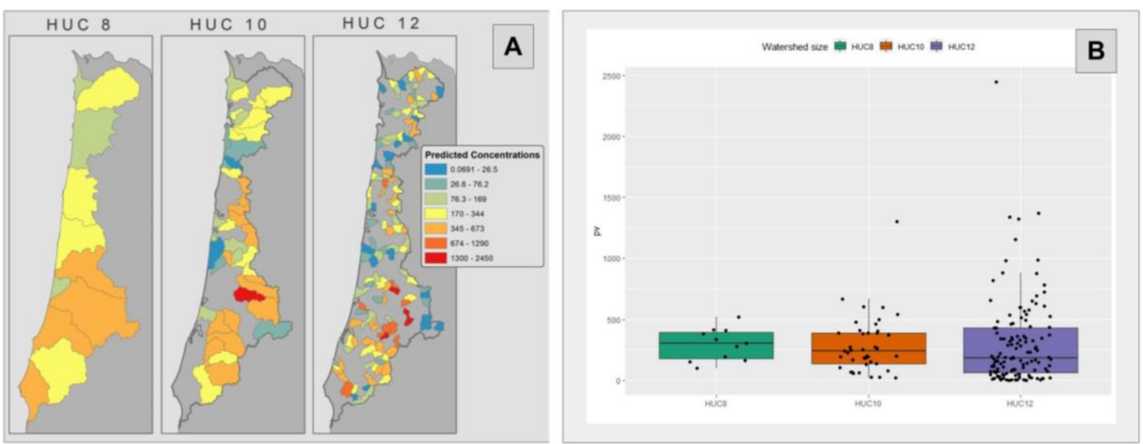

Figure 3. Model predicted concentrations across HUC 8, 10, and 12 scales in the Coast Range (A), and compared in boxplots (B).

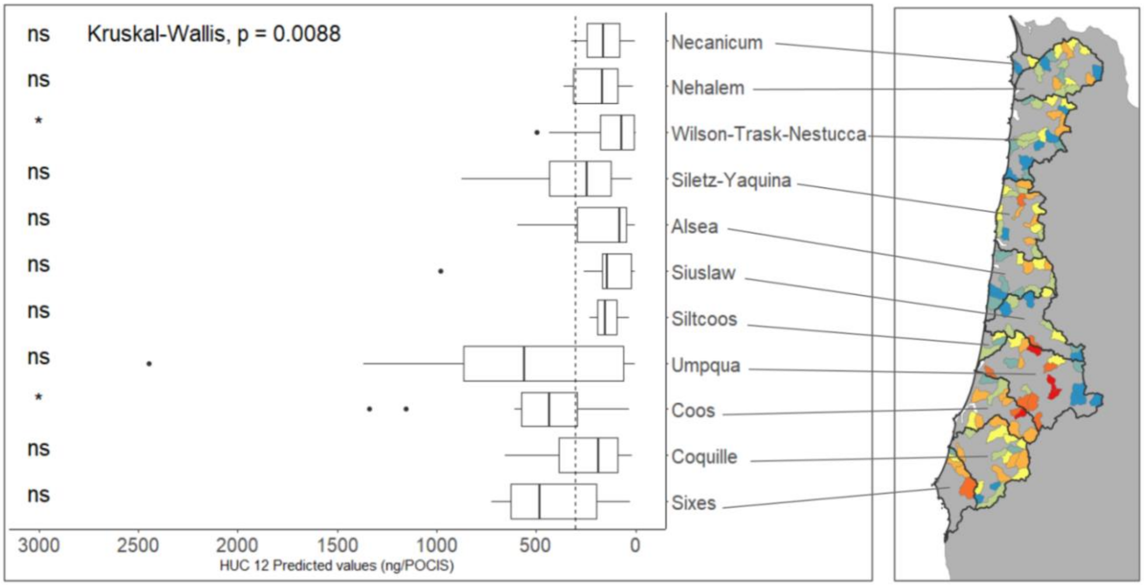

Figure 4. Predicted concentration values within HUC 12 catchments grouped by HUC 8 with multiple pairwise tests against the base mean. Abbreviations: ns= not significant, * = $p \leq 0.05$

4. Discussion

4.1 Passive water samples and independent variable correlation

Concentrations of four commonly applied current use forestry herbicides detected in passive water samples during the spring of 2019 ranged across watersheds and at least one compound detected above reporting limits in 80% of the samples (Table 3). Correlation matrices indicated many correlative relationships between total accumulation in samplers and independent watershed characteristics, as well as among watershed variables. In many instances catchment size is an important predictor in aqueous pesticide concentrations (Schulz, 2004), but in this case watershed size was not correlated with total accumulation in POCIS canisters, signifying that an exploration into factors across multiple scales would be appropriate for these data (Figure A1). Another explanatory variable that did not correlate with accumulation was road density, which is important to note as roadside spray activities are considered a potentially confounding source of herbicide runoff in watersheds (Huang et al., 2004; Massoudieh et al., 2005)(Figure A1).

4.2 Final explanatory variables

Multiple regression revealed that watershed variables: steep slopes and notified herbicide and clearcut activity best predicted herbicide accumulation in passive water samplers. Watershed slope is an important factor in determining runoff potential within watersheds (Dabrowski et al., 2002; Zhang and Zhang, 2011), so its significance in predicting pesticide exposure is logical. Additionally, small scale watershed research indicates that steep slopes significantly increase herbicide loss due to runoff (Müller et al., 2004). Herbicide concentration correlated with notified clearcut activity during the previous year, suggesting that site preparation treatments (which occur within the first year post-harvest, before reforestation (Rose and Haase, 2006)) may have contributed to herbicides detected in integrative samplers. Herbicide applications notified during the deployment period was the final predictor in our multiple regression model. Based on the time of year, active notifications during the sampling window (March-May) were likely comprised of pre-emergent (dormant) applications to help established plantations, as well as site preparation treatments.

Final model variables displayed spatial variability (observable in Figure 2) suggesting regional differences in management (recent clearcuts and herbicide usage) and physical watershed characteristics (slope) within the Coast Range. Steep slopes were most prominent in the north coast watersheds at the HUC 10 and 12 scales near the Kilchis and Wilson rivers (Figure 2A). Notified herbicide activity was highest in south coast watersheds, especially in tributaries of the Smith, Siuslaw, and Umpqua Rivers (Figure 2B). Clearcuts notified within the previous year were noticeable throughout the study area, with the highest percentages seen in the Nehalem watershed in the north coast, Siletz watershed in the mid coast, and near the Coquille and Sixes rivers in the south coast (Figure 2C). The combined additive effects of these variables across the landscape served as indicators of predicted herbicide concentration based on the measured sampling window. Across the three scales, the widest ranges of variables were observed within the HUC 12 watersheds followed by the 10 and 8 scales. This is not surprising since smaller catchments are more prone to dominance by single land use types/features, which can translate to higher and lower values of these variables. At larger scales, the complexity of the landscape has a dampening effect on the range of individual variables, as they are averaged across the entire watershed. Across scales, mean values for each variable were not significantly different (Table 5).

4.3 Model outputs/predicted concentration values

Similar to individual independent variables, predicted concentration values based on regression model output displayed regional differences in high values. Tributaries of the Umpqua, Coos, and Smith rivers displayed the highest values at the HUC 12 scale, followed by tributaries of the Alsea and Sixes rivers. At the HUC 10 scale, the upper Smith River had the highest predicted value followed by a number of other headwater catchments in the central and south coast. HUC 8 predicted herbicide concentrations were highest in south coast watersheds. Data structure of predicted concentrations was similar to predictor variables, wherein HUC 12 catchments displayed the largest ranges of values, followed by HUC 10 and 8 scales (Figure 3). Despite differences in range, differences among scales were not significant (Table 5), which is not surprising given the nested nature of the HUC watersheds in the study area. Predicted concentrations calculated across scales based on watershed slope, herbicide activity, and notified clearcuts highlights the importance of looking at potential impacts to aquatic ecosystems from a landscape pattern perspective, beyond the site level.

Subwatersheds (HUC 12) grouped by subbasin (HUC 8 scale) allowed for quantification of regional differences in predicted values (Figure 4). In our analysis, South coast watersheds had higher average predicted concentrations than mid or north coast watersheds, but Coos was the only HUC8 group significantly higher than the base mean, and the Wilson-Trask-Nestucca was the only watershed group with significantly lower predicted concentrations (Figure 4). Regional patterns from this analysis are similar to field-collected data, wherein south coast locations exhibited higher on average concentrations compared with mid and north-coast counterparts. These observations may represent the amount of active management taking place in southern watersheds or could be an artifact of spray timing/management differences between the areas.

4.4 Other aquatic resources across scales

Considerations of the spatial configuration of landscape variables (land use, management, environmental characteristics) are critical in understanding anthropogenic activities threatening watershed water quality, ecological processes, and aquatic resources (Lee et al., 2009). Within the context of the Oregon Coast Range, watershed scale aquatic resources exist at multiple points along stream networks, and are therefore influenced by upstream conditions at multiple scales. Interpreting potential impacts to these resources at the scales in which they are found is challenging, especially given the wide range of ownership, management, and physical watershed characteristics in upstream drainages. Study results suggest that the potential for both higher and lower herbicide exposure is greater at smaller watershed scales, but overall watershed size does not impact the average exposure among the three scales investigated. Our investigations provide predicted concentrations at established HUC scales, but on the, resources exist independent of established scale boundaries such as the HUC system. Figure 5 offers a subset of Oregon Coast Range aquatic resources, such as drinking water sources (surface and groundwater), salmonid runs, and aquaculture areas within watersheds, which are influenced by catchments of various sizes. Drinking water originating from surface water is a good example of a resource that, though permitted and collected at a specific point, is influenced (and potentially threatened) by upstream catchment characteristics such as land uses and practices (Lari et al., 2014). As indicated in figure 5, herbicide detections at sampling locations varied along the coast, with the highest values seen at the south coast sites. Furthermore, this figure illustrates the overlapping nature of detection sites and other aquatic resources within the Coast Range.

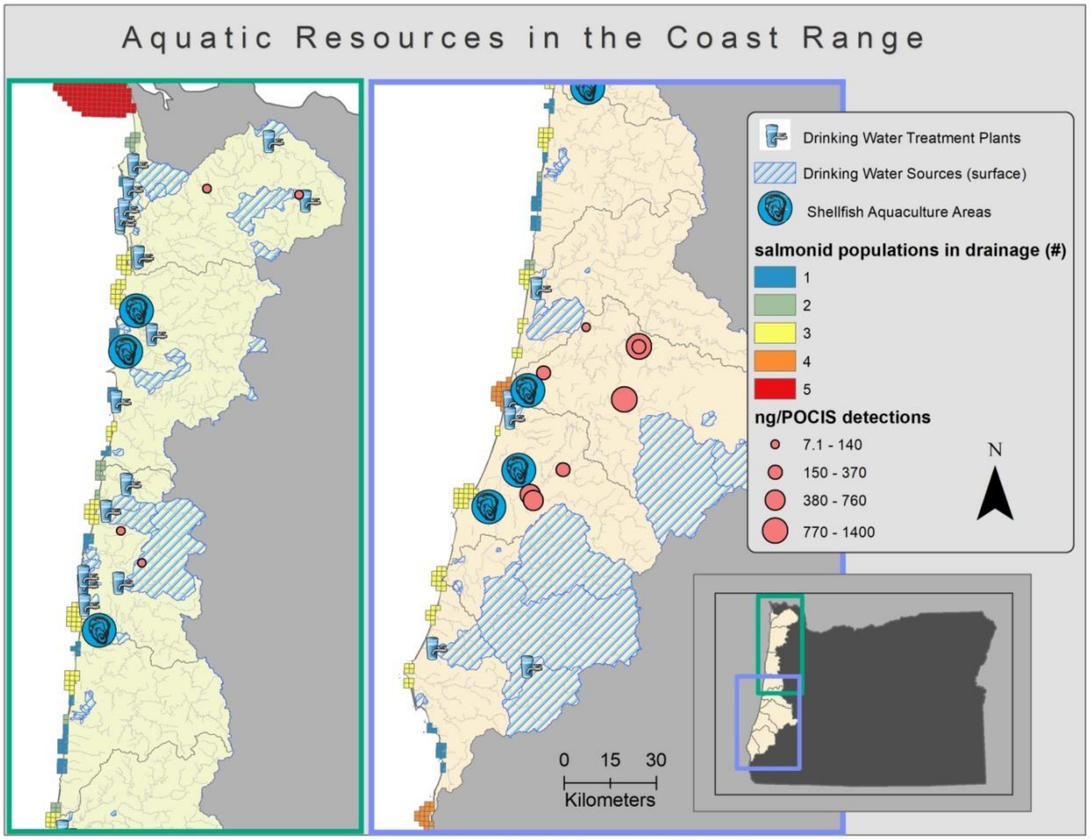

Figure 5. A subset of aquatic resources in the Coast Range, and the various scales they occupy. Total herbicide accumulation detected in POCIS samplers (ng/POCIS) is overlaid at sampling locations. Data sources: Oregon Department of Fish and Wildlife, Oregon Department of Environmental Quality, Oregon Department of Agriculture

4.5 Scale, complexity, and uncertainty

This investigation into springtime herbicide exposure across multiple scales in coastal watersheds is one of many potential avenues of inquiry into non-point source pesticide pollution, and like many monitoring and modeling efforts is limited by available data. Our sampling window characterizes one time period, and though results are useful in explaining relationships between upstream variables and observed concentrations, considerable inter-annual variation in management activities throughout the Coast Range introduces uncertainty about the suitability of our model to other timeframes or regions. Inconsistency in management regimes applied to Oregon forestlands based on developments in ownership, guiding regulations/practices, and technology throughout time present a complicated picture of the landscape ecology in coastal watersheds. Harvest rotations for contemporary intensive forest management are generally 30-50 years long, and over the timeframe of one harvest cycle, updates to methodology and regulations can evolve. Our results provide insight into herbicide movement through the water column during a 45 day deployment period, and associated catchment variables that can predict concentrations in this context, but herbicide movement during other times of year as well as during the same time frame across years may not be well characterized by these data.

Our results suggest fundamental connections between landscape patterns of watershed management/characteristics and downstream pesticide exposure can be predicted based on relatively simple indicators, but the applicability of these indicators (slope, herbicide use, and clearcuts) in different regions remains elusive. For example, our model may not be useful beyond the southern portion of the Coast Range region (past Cape Blanco to the south), where biogeographical, management and climatic differences in the landscape makeup likely impact the ability of this regional specific model in predicting movement of pesticides in watersheds. Similarly, in eastern portions of the state, federal and state forestry herbicide use regulations diverge from coastal provisions (US Bureau of Land Management, 2010)(OAR 629-642-0400), which coupled with differing in biogeographical features (Franklin and Dyrness, 1973) between regions further constrain model applicability. However, data collection in these areas and other seasons could be utilized to build similar predictive models.

The limited number of observations we relied on to build our statistical model (n=15) introduces additional uncertainty/limitation to our modeling results. Additional sampling locations were discussed during project design, but we opted for replication at three of the sampling sites in order to have more confidence in the results at each location. This data availability constraint limited our ability to account for nested watershed dynamics via hierarchical or two-stage modeling.

5. Conclusions

In this investigation we found that a physical watershed variable (steep slopes) coupled with notified forestland management activities (herbicide use and clearcut harvest) successfully predicted measured herbicide presence (R^2= 0.8914) during the spring spray period (March to May). These results highlight connections between spatial landscape patterns of environmental factors, anthropogenic land-uses, and offsite herbicide movement in coastal watersheds in Oregon. When applied to unsampled watersheds in the same region, predicted concentrations from our model exhibited similar spatial patterns as measured concentrations, wherein south coast watershed displayed higher on average concentrations compared to mid and north coast watersheds. Across three watershed sizes (scales) we found that the greatest ranges in predicted values were seen in smaller catchments (HUC 12), followed by medium and large catchments (HUCs 10 & 8), but the average concentrations did not differ among scales. The final model provides insight into patterns of herbicide use and movement in coastal watershed in Oregon, but its application is constrained by the sampling window from which the data were derived and the region-specific context. Furthermore, herbicide detections overlap with important aquatic resources, highlighting the need for further research to determine effects of transported herbicides on these resources. This research demonstrates the importance of approaching interpretation of non-point sources of pollution at appropriate landscape scales and contexts.

Chapter 3 References

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Appendix A: Supplementary material for Chapter 3

Figure A1. Correlation matrix of physical watershed variables and total herbicide accumulation (totalng). Variable abbreviations are provided in Table 2 (section 2.2.1) of the document.

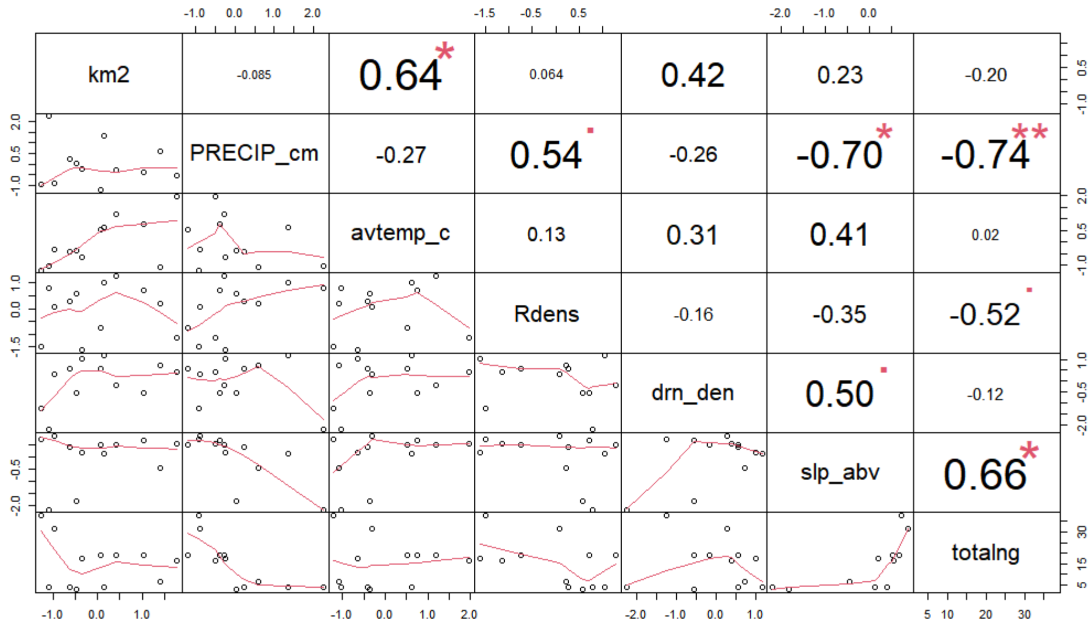
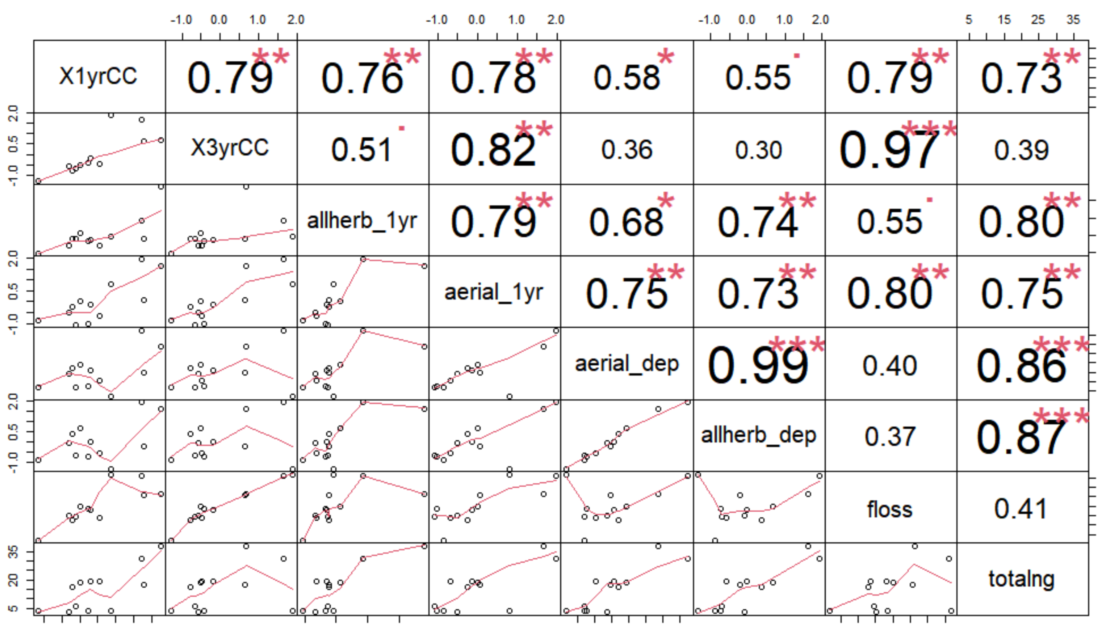


Figure A2. Correlation matrix of management watershed variables and total herbicide accumulation (totalng). Variable abbreviations are provided in Table 2 (section 2.2.1) of the document.



Chapter 4: Landscape ecology of freshwater mussel populations in Pacific Coast watersheds of Oregon: survey distribution, habitat, condition, and host species interactions

1. Introduction

1.1 Western pearlshell in Oregon

A relatively understudied class of organisms, freshwater mussels (Bivalvia:Unionida) are among the most imperiled freshwater species groups worldwide, with many species and populations lacking sufficient population/abundance data and without conservation status throughout their current ranges ("IUCN Red List of Threatened Species," 2021; Lydeard et al., 2004). North America is a biodiversity hotspot for freshwater mussels, with the highest species diversity in the Mississippi basin (Haag, 2010). Three extant taxonomic groups persist in Oregon, the western pearlshell (*Margaritifera falcata*), the western ridged mussel (*Gonidea angulata*), and species of floaters (genus *Anodonta*; currently undergoing taxonomic revision), each of which, except the Oregon floater (*Anodonta oregonensis*), are thought to be declining or in danger of extinction based on comparisons of historical and recent distributions (Blevins et al., 2017). Freshwater mussels have been of increasing interest for freshwater aquatic conservation and restoration groups across the region, but considerable population-specific information is still needed to facilitate comprehensive management and conservation.

Margaritifera falcata (western pearlshell), misidentified until the mid-1970s as its close relative *M. margaritifera* (freshwater pearl mussel, native to eastern North America, and temperate regions of western Russia and Europe), is found west of the Rocky Mountains to the Pacific Coast, from northern California to Alaska, with some small isolated populations persisting east of the continental divide in the headwaters of the Missouri River (Nedeau, E. et al., 2009). This species is documented from numerous ecoregions and watersheds in Oregon, including the Columbia River and its subbasins, the Klamath basin, Oregon's endorheic basins (having no outflow), and coastal watersheds. As with other freshwater mussels, *M. falcata* has evolved a set of unique life history traits that utilize a host fish species for metamorphosis. *M. falcata* is a functionally hermaphroditic species (giving them the ability to self-fertilize, although they also cross-fertilize) and is the only long-lived mussel species with this reproductive trait in North America (Haag, 2012). The species displays an obligate relationship with salmonids, releasing glochidia in conglutinates into the water column, where they can make contact with the host fish. Once glochidia attach themselves to the fish (usually the gills), they encyst, generally for several weeks before metamorphosing into the juvenile stage (Haag, 2012; Nedeau, E. et al., 2009). The length of the encysted stage is dependent on water temperature (Roscoe and Redelings, 1964). As juveniles, they inhabit the stream substrate, where they will grow for up to a decade before reaching reproductive maturity and spend most of their lives as filter feeders. Once they mature they can live for over a century, making them among the longest-lived animal species on the planet (Haag, 2012).

Margaritifera species have a low metabolic rate compared to many other freshwater mussel species, and have adapted to persist in rivers with low food availability

(Bauer et al., 1991). *M. falcata* and other filter feeding bivalves play an important role in nutrient movement, slowing the downstream transport of nutrients in watersheds by filtering water and depositing unused nutrients as feces and pseudofeces as well as sequestering nutrients through the formation of shell material (Nalepa et al., 1991; Vaughn, 2018). These alterations in nutrient flow increase growth in other suspension feeders such as Pacific lamprey (*Lampetra tridentata*), another understudied species experiencing regional decline (Limm and Power, 2011; Wicks-Arshack et al., 2018). Seasonal mussel biodeposition fluxes have also shown to increase abundance of other aquatic macroinvertebrates (Howard and Cuffey, 2006a). Additionally, freshwater mussel beds provide habitats within rivers, creating biogenic habitat, stabilizing substrate, and reducing shear stress (Hopper et al., 2019; Zimmerman and de Szalay, 2007).

1.2 Regional context and local threats to the species

Within the species' broader distribution (see above), *M. falcata* is known from coastal watersheds spanning the Pacific Coast of North America, including every coastal watershed in Oregon and Washington and nearly every coastal watershed from Monterey Bay northward in California (Xerces Society and CTUIR, 2021). In Oregon, the coastal watersheds considered in this study are within the Coast Range Ecoregion, defined by a series of biogeographically similar coastal watersheds draining from a low coastal mountain range, commonly referred to as the Pacific Coast Range, to the Ocean. The region is characterized by steep slopes, a wet and mild climate, and high forest productivity (Wimberly and Ohmann, 2004). Steep slopes and high rainfall in forested watersheds in the Coast Range affect debris flow and sediment transport in low order streams, resulting in highly dynamic channel morphology (May and Gresswell, 2003).

The majority of these watersheds drain comparatively small areas relative to large regional drainage systems like the Puget Sound, or Columbia, Willamette, Klamath, or Sacramento rivers (Figure 1). The dynamic instream conditions in this region also suggest that mussels in Coast Range watersheds may respond to a different set of habitat associations compared to other populations throughout the species' range. Additionally, the relative isolation of these smaller coastal watersheds suggest that mussel populations may be subject to a suite of factors influencing distribution, persistence, or condition that are affected by decreased connectivity among populations. In fact, regional population investigations comparing genetic variability between and among species (Mock et al., 2013) or distribution within larger watersheds (Brim Box et al., 2003; Davis et al., 2013) have highlighted the importance of life history traits and habitat characteristics in shaping distribution of *M. falcata* within other watersheds. Understanding the current status of isolated populations of freshwater mussels is particularly important as remnant populations may contain unique genetic diversity (Mock et al., 2013, 2010; Wacker et al., 2019; Walton et al., 2020).

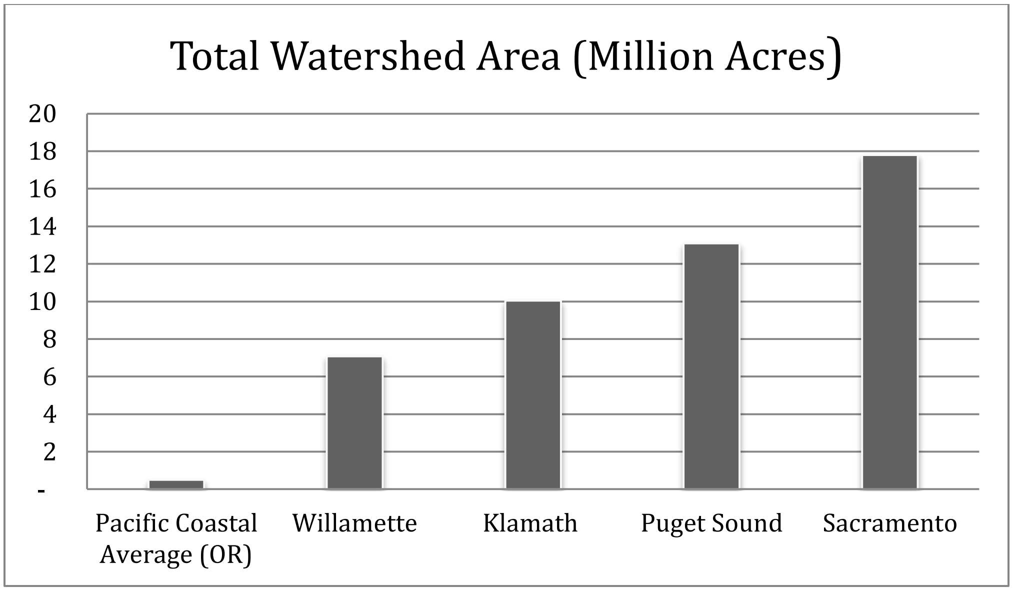


Figure 1. Pacific coastal watersheds in Oregon drain a smaller area on average than other river basins in the region with resident *M. falcata*. Not shown is the Columbia River system (of which the Willamette is a subbasin), which drains nearly 194 million acres before reaching the Pacific Ocean. Of the 26 coastal drainages in Oregon included in this study (excluding the lower Columbia), only 2 consist of more than one subbasin (HUC8), while the Willamette consists of 11, the Klamath of 12, Puget Sound of 21, and the Sacramento of 28.

Co-evolution and reliance of *M. falcata* on salmonid host species for reproduction, which are in decline across the region, further compounds the potential for reduced connectivity among biogeographically isolated populations, particularly at finer scales. In general, freshwater mussel populations inhabiting coastal drainages are thought to be functionally isolated from each other and from other larger watershed networks (Archambault et al., 2018; Karlsson et al., 2014; Sepkoski and Rex, 1974). Several theories propose movement pathways of freshwater mussels between unconnected drainages in the eastern US, varying from aerial bird transport to initial colonization being reliant on geomorphic stream capture processes (Ortmann, 1913; Sepkoski and Rex, 1974). Initial colonization of coastal drainages likely took place thousands of years ago, facilitated by altered entrapment and river connectivity between basins. There is some evidence that a subset of salmonid life histories involve movement between catchments before ocean migration, which is a potential route of dispersion for mussels during their parasitic stage, but there are no data to verify if mussels are able to move between drainages this way (Strayer, 1987). The isolation of individual coastal basins indicates that coastal mussel populations may function as non-equilibrium metapopulations, unlike inland metapopulations observed throughout the region, which are better classified by patchy or classical metapopulation structure. Subpopulations with classical and patchy metapopulation structures inhabit connected habitat patches and necessitate an adequate rate of migration among subpopulations, while non-equilibrium metapopulations are defined as completely independent populations without migration between habitat patches (Harrison, 1991). We propose that each coastal drainage comprises a distinct non-equilibrium metapopulation, wherein contiguous river segments and distribution of salmonids effectively define the extent of potential distribution.

M. falcata obligate host fish species have declined regionally, with multiple threatened or endangered salmonid species persisting at a fraction of historical numbers (Gavin et al., 2018; Naiman et al., 2002; Nehlsen et al., 1991). In Oregon's coastal watersheds there are four anadromous salmonid species with widespread occupancy throughout freshwater habitats: coastal coho (*Oncorhynchus kisutch),* chinook *(O. tshawytscha),* steelhead *(O. mykiss),* and coastal cutthroat (*O. clarki*). Each of these species is a potential host fish for *M. falcata*, though evidence suggests susceptibility to parasitism (host fish compatibility) may vary by species (Karna and Millemann, 1978; Meyers and Millemann, 1977). Chum salmon (*O. keta*) are also found in several coastal watersheds, but their limited dispersion in freshwater environments and immediate return to estuarine and ocean environments as juveniles limit their potential as host fish for *M. falcata*. Salmonids demonstrate a wide variety of life history characteristics related to reproductive timing/frequency, level of anadromy, and juvenile maturation and movement in watersheds that can vary both among and within species (Groot et al., 1991; Willson, 1997). The combined effect of differences in salmonid life histories with species-specific susceptibility to parasitism have the potential to influence successful reproduction and distribution of freshwater mussels within coastal watersheds, but these relationships have only recently begun to be investigated (Österling et al., 2020).

Additionally, *M. falcata* face a myriad of combined stressors that may further impede their success. Due to their long life spans and slow growth rates, isolated populations may be slow to adapt to changes in the environment, accruing extinction debt that may not be perceptible at shorter timeframes (Newton et al., 2008). Additionally, climate change is projected to alter flow regimes and increase instream temperatures, which may further disrupt extant populations via direct and indirect impacts to mussels and host fish species (Blevins, 2018; Terui et al., 2014). Research throughout the region has identified habitat factors and environmental variables that influence age structure and distribution, which provides critical first steps in assessing intrinsic habitat potential of streams and rivers within subregions (Anderson, 2002; Brim-Box et al., 2003; Davis et al., 2013; Howard and Cuffey, 2006b, 2003; Stone et al., 2004).

An important component in understanding the population dynamics of freshwater mussels is applying a landscape ecology perspective to guide conservation efforts, particularly to understand distribution and connectivity of populations as well as relationships between mussels and host fish (Newton et al., 2008). In this approach, spatial patterns of natural and anthropogenic variables are expected to influence population dynamics and distribution within and across watersheds (Chapter 1; Figure 2). Documenting the extent of current population distribution and occupancy of these organisms in region-specific contexts is critical, both to manage for ecological functions in fragile ecosystems as well as to ensure the continued existence of non-equilibrium metapopulations in isolated coastal drainages. Unique reproductive life history traits of *M. falcata* paint a complicated picture for managing current populations, further highlighting the need to investigation region-specific populations and their relationships with host fish species. Furthermore, heterogeneity in upstream conditions can influence habitat food availability, and extant isolated patchy populations may exhibit a range of physical fitness.

1.3 Project goals

We conducted a mixed-methods analysis of *M. falcata* to explore occupancy and distribution patterns, habitat requirements, and host fish associations within Pacific coastal watersheds in Oregon using a comprehensive dataset collected through the Western Oregon Rearing Project (Oregon Department of Fish and Wildlife, 2011). We also compared physical condition among mussels collected in eight Oregon coast watersheds. Our goals were to understand:

1) What is the current distribution and occupancy of *M. falcata* in Oregon's small coastal drainages?

2) Which reach-scale habitat variables best predict mussel occupancy?

3) Is there a relationship between host fish species abundance and mussel presence at sample locations?

4) In addition to distribution and occupancy, does mussel condition vary across the sampling range?

Catalogued mussel observations throughout the coastal region of Oregon suggest a wide distribution pattern and presence across the region, and we expect survey data will mirror this wide distribution pattern (Xerces Society and CTUIR, 2021). We expect that important habitat variables in this region will include those associated with low stream velocity, such as areas with lower gradient and sand/silt substrates (Hegeman et al., 2014; Nedeau, E. et al., 2009). Host fish infection research in the region suggests that *O. tshawytscha* are the most suitable hosts, followed by *O. clarki*, *O. mykiss,* and *O. kisutch* (Karna and Millemann, 1978), so we expect host fish associations to reflect this order in terms of co-occurrence. We expect mussel condition to vary between sites, but considering the myriad of factors contributing to mussel condition not accounted for in this analysis (food/nutrient availability, environmental stressors, disease, legacy impacts, etc.) we cannot offer predictions about patterns of condition. From these questions, we identify region-specific research priorities that outline future steps to better understand the landscape ecology, conservation, and management needs of this species.

2. Methods

2.1 Study area and geography

Oregon's coastal drainages south of the Columbia River estuary to Cape Blanco encompass a unique biogeographic and climatic area called the Coast Range region (Figure 2). The upper portion of the Umpqua river drainage, which is often not included in the Coast Range designation, was included in a portion of this analysis because of its continuity of dispersion of host salmonid species. Within this region, there are 26 distinct coastal drainages (Figure 2). Since colonization of freshwater mussels across open ocean sections has not been documented, populations within distinct watersheds are considered isolated. Coastal drainages were further divided into four regions based on salmonid "biogeographic strata" designations based on evolutionarily significant units (Wainwright et al., 2008) to provide a useful framework for evaluating regional variation in mussel observations and habitat characteristics. These include the North Coast (Nehalem, Nestucca, Tillamook area watersheds), Mid Coast (Siletz, Yaquina, Alsea Rivers), Umpqua (Smith and North and South Umpqua Rivers), and Mid-South Coast (Coos and Coquille Rivers) regions. See Appendix B1 for full list of rivers within each region.

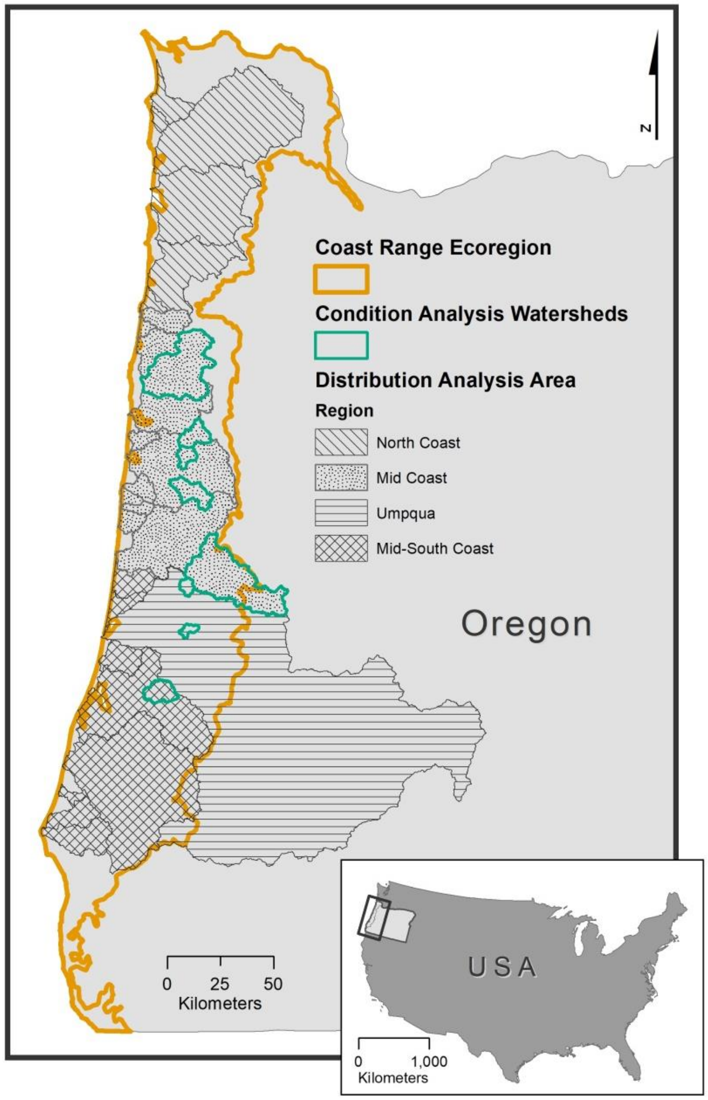


Figure 2 – A. The Coast Range ecoregion (orange) extends from the southern portion of Oregon north to the Columbia River, and comprises watersheds originating in the Coast Range Mountains (Omernik, 1987). Collection locations and upstream watersheds for condition analysis comparisons are outlined in green. The occupancy/distribution survey area (patterned) is comprised of the coastal watersheds in the Coast Range of Oregon, and divided into regions based on salmonid diversity strata designations.

2.2 Mussel and stream survey data

The Oregon Department of Fish and Wildlife (ODFW) conducts annual stream and aquatic species inventories through the Western Oregon Rearing Project (WORP), during which they also collect incidental observation data on the presence and abundance of freshwater mussels. Although WORP freshwater mussel observations are not identified by species, based on verified observations of mussel beds present in the Coast Range ecoregion, it is likely that all freshwater mussel observations included in this analysis are *M. falcata* (Xerces Society and CTUIR, 2021). As part of the WORP sampling design, sampling locations among first through third order stream segments were randomly selected with spatial balance throughout the study area using a Generalized Random Tessellation Stratification (GRTS) survey design (Stevens and Olsen, 2004). Selected stream segments (1km in length) were sampled using a rotating panel design, dividing locations equally between four survey rotations (annually, 3yr, 9yr, 27yr; (Stevens, 2002)). Each year between 2010 and 2020, an average of 154 locations (811 unique stream reaches and 1,693 surveys overall) were surveyed by ODFW staff per year during low flow periods (July through October) throughout the 27 drainages of the Oregon Coast Range. Each location was surveyed according to stream habitat and snorkel survey protocols developed by ODFW (Oregon Department of Fish and Wildlife, 2011). Along with in-stream habitat information (substrate type, water temperature at each visit, pool frequency), fish species presence/counts, mussel observations, and geomorphic characteristics were also recorded (Table 1).

Table 1. In-stream, geomorphic, and salmonid species information collected during stream habitat and snorkel surveys.

Survey variables	Description	Unit
In-stream variables		
Mussel presence	Observations of mussels on the reach scale	Categorical: 0 = no mussels observed 1 = few mussels observed (1-50) 2 = many mussels observed (51-200) 3 = dense mussels observed (>200)
Pools	Percentage of pools by surface area	%
Boulders	Count of large boulders	count
Sand or Organics	Percentage of substrate comprised of sand or organic material	%
Gravel	Percentage of substrate comprised of gravel	%
Bedrock	Percentage of substrate comprised of bedrock	%
Water Temperature	Stream temperature recorded at each reach	°C
Geomorphic Variables		
Gradient	Slope of the water surface across sampling unit	% change in elevation
Active Channel Width (ACW)	Distance across channel at "bankfull" flow	meters
Valley Width Index (VWI)	Estimate of how many ACW can fit within the valley between hillslope bases. Valley floor width/ ACW	dimensionless ratio
Valley Form	The morphology of the active channel	Categorical NVF = Narrow Valley Floor BVF = Broad Valley Floor
Salmonid Species		
Coho (Oncorhynchus kisutch)	Presence and abundance	count
Chinook (O. tshawytscha)	Presence and abundance	count
Steelhead (O. mykiss)	Presence and abundance	count
Cutthroat (O. clarki)	Presence and abundance	count

2.3 Mussel condition sampling

Physical measurements of *M. falcata*, collected during a previous survey of bivalve pesticide contaminants in the Coast Range (Scully-Engelmeyer et al., 2021), were used to compare the condition of mussels between eight watersheds in Oregon. Established spatial distribution and abundance information about *M. falcata* in Oregon's coastal drainages is limited, and abundance thresholds at collection sites were required to limit potential impacts to the at-risk populations. Oregon collection sites were selected based on: watershed spatial scale (preference toward smaller catchment basins), information about distribution and abundance of current populations, and access to stream reaches. Fifteen individuals were collected by hand, wading or during snorkel dives, from five sites located in four watersheds during the summer of 2017 (July-August) and three sites in three watersheds during the spring of 2018 (May-June). All samples were held in ambient water collected on site and transported in a cooler with wet ice to the Applied Coastal Ecology (ACE) Laboratory at Portland State University (Portland, OR; 100 samples) or the Hatfield Marine Science Center (Newport OR; 15 samples) for sample processing. Individual bivalves were weighed, shucked, drained, and final shell and tissue wet weights and shell lengths were recorded (Crosby and Gale, 1990).

2.4 Statistical analyses

2.4.1 Mussel observation and distribution analysis

Prior to modeling predicted occupancy, we analyzed proportional data about observation frequency at each WORP site (n=811) to understand relative distribution (naïve occupancy) of *M. falcata* among and within Oregon coastal drainages. Using ArcMap 10.7.1, we calculated the number of sites per catchment with detections (frequency) across the sampling period (10 years) and displayed the results in a choropleth map. We then compared the overall naïve occupancy proportion throughout the study area to each catchment and mapped the deviation from the mean to examine the relative spatial distribution of mussels within coastal watersheds.

2.4.2 Predicted mussel occupancy and habitat covariate analysis

To estimate true occupancy (i.e., accounting for imperfect detection), and to explore the relationship between presence/absence of mussels and reach-scale habitat variables, we applied a static occupancy model to surveyed locations, including all sites surveyed at least two times over the ten-year period (n=251). Due to the long lived and sessile nature of *M. falcata* mussel beds, the ten-year sampling period was considered closed to changes in mussel occupancy (closure period) and we modeled detection (p) as constant based on repeated annual visits. We modeled occupancy (ψ) probabilities using habitat variables (in-stream and adjacent geomorphic; Table 1) averaged across site visits to account for differences in surveyor estimations and uneven habitat data collection frequencies across sites. Temperature measurements taken during each site visit were averaged across all repeated visits for an average site temperature. We also considered watershed size as a site covariate to explore subpopulation isolation (smaller watersheds ~ more isolated). Habitat variables were compared via correlation matrices and one of each pair of highly correlated variables were excluded (Pearson's correlation coefficient >0.40). Final covariates were scaled. We estimated reach level detection (p) and occupancy (ψ) probabilities using Rstudio (version 1.2.5033; unmarked, AICmodavg, and MuMIn packages). Since all covariates could be influential in mussel occupancy we developed an "all subsets" candidate model set based from a global model and compared summed model weights to determine relative covariate importance (Arnold, 2010). We compared candidate models ranked based on Akaike Information Criterion (AIC), and the best models were selected for averaging based on AIC weights within 2 of the highest ranking model (Burnham and Anderson, 2002; MacKenzie et al., 2017). Goodness of fit was simulated using 500 bootstrapped samples (MacKenzie and Bailey, 2004)

The most important covariate identified via model averaging AIC weights was then compared among regions to explore whether regional habitat differences may explain mussel presence. The highest ranking variable was compared across the four regions of the study area (Figure 3A) using non-parametric analysis of variance (Kruskal-Wallis).

2.4.3 Mussel host fish analysis

Relationships between presence/absence of mussels and counts of salmon species (*O. tshawytscha*, *O. clarki*, *O. mykiss*, and *O. kisutch)* observed in snorkel surveys were investigated using binomial logistic regression analysis. Species counts at sites were averaged across sampling events. Backwards model selection was performed to determine the best fit model.

2.4.4 Mussel condition analysis

For the condition analysis, basic physiological health among organisms was summarized by calculating a live mussel body condition index (BCI) metric based on measurements of collected mussels (wet tissue weight, shell length) (Nobles & Zhang, 2015).

$$BCI = \text{Soft tissue wet weight (g)} \div \text{Shell length (mm)}$$

BCI was compared between sampling locations using boxplots and Kruskal-Wallis non-parametric analysis of variance to determine if measurable differences in health were detectable among sampled populations. Organism allometry, the scaled relationship between variation in organism morphology and organism size (Gayon, 2000), can be a useful metric in measuring how organisms function in environments (feeding/growth rates, water filtration, etc.) (Kreeger, 2011). Bivalve molluscs are known to have highly correlated relationships between shell height and tissue weight, and documenting these relationships in sacrificed organisms is helpful for future non-lethal biomass sampling. We performed least squares regression to explore the allometric length-weight relationship between shell height (mm) and wet tissue weight (g).

3. Results

3.1 Mussel distribution and abundance

WORP surveys were conducted at 811 1-km stream sites between 2010 and 2020, and sites were visited 2.1 times on average (minimum=1, maximum = 10) during that time period. Survey location selection was randomized across the study area and relative numbers of surveys within each region are presented in Figure 3A. Mussels were observed at least once at 100 of the sites, for a naïve occupancy proportion of 12.3%. Frequency of mussel observations varied across the study area, with the highest frequencies seen in the Umpqua watershed, and lowest frequencies observed on the North Coast (Figure 3B). When standardized by watershed size and compared with the average proportion of observation frequency, southern coast watersheds had the highest deviations above coast-wide averages, but smaller catchments such as Floras Creek and Tahkenitch Lake were significantly elevated compared with larger watersheds (Figure 3C).

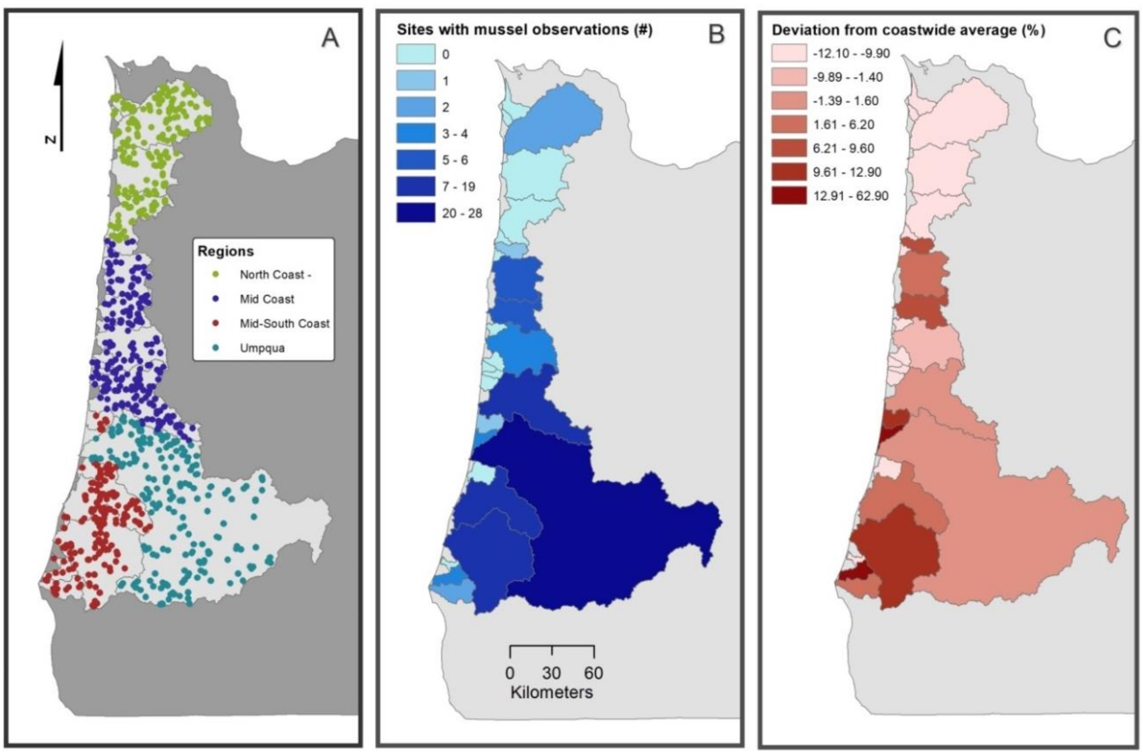


Figure 3. (A) Survey location pins divided into larger regional categories: North, Mid, Mid-South Coast, and Umpqua. (B) Frequency of sites with detections/observations summarized by watershed and displayed as a choropleth map divided and classified using natural breaks (Jenks and Caspall, 1971). (C) Proportions of detections from the total sites summarized within each watershed and presented as the deviation from the coastwide average (12.3%) as a choropleth map; classified using natural breaks.

Reach level habitat profiles were developed at 658 of the 811 total distribution survey sites by averaging repeated measurements over multiple visits. Means and ranges of continuous variables and proportions of categorical variables across sites are summarized in Table 2.

Table 2. Habitat characteristics across the distribution survey area summarized as mean values (min-max) for continuous variables and count (%) for categorical variables. Units of measurement are available in Table 1. Abbreviations: ACW= Active Channel Width; VWI = Valley Width Index

Characteristic	**North Coast**, N = 170[1]	**Mid Coast**, N = 176[1]	**Umpqua**, N = 166[1]	**Mid-South Coast**, N = 146[1]
Stream variables				
Boulders	250 (0-2,221)	209 (0-1,679)	194 (0-1,505)	356 (0-4,881)
SandOR	28 (6-96)	27 (2-94)	24 (0-72)	26 (2-100)
Gravel	31 (0-61)	36 (4-73)	33 (6-76)	29 (0-71)
Bedrock	8 (0-53)	12 (0-62)	18 (0-80)	14 (0-68)
Pools	34 (1-91)	46 (1-96)	44 (1-95)	49 (1-97)
Temperature	12.34 (8.00-17.00)	13.49 (8.00-20.00)	14.17 (8.00-23.00)	14.25 (8.50-20.00)
Geomorphic and Watershed variables				
Gradient	3.18 (0.20-13.68)	2.32 (0.19-10.35)	2.24 (0.24-12.03)	2.52 (0.11-18.92)
ACW	8.4 (2.3-28.2)	7.5 (1.6-31.0)	7.1 (1.6-26.8)	9.7 (1.4-36.8)
VWI	7.2 (1.1-20.0)	8.3 (1.2-20.0)	8.9 (1.0-26.3)	4.8 (1.0-20.0)
ValleyForm				
BVF	121 (71%)	146 (83%)	116 (70%)	63 (43%)
NVF	49 (29%)	30 (17%)	50 (30%)	83 (57%)
Watershed_Size	1,530 (56-2,212)	1,282 (43-2,006)	12,131 (12,131-12,131)	1,806 (55-2,737)

[1] Mean (Minimum-Maximum); n (%)

3.2 Occupancy and habitat analysis

Of the 811 total survey locations, 658 had complete habitat data accompanying mussel occurrence data (Table 2) and 251 of those locations were visited more than once during the survey period and could be modeled. The null model determined $p = 0.45$ (95% CI = 0.38-0.52) and was modeled as constant throughout occupancy modeling. Gradient, VWI and bedrock variables were removed based on multicollinearity with other covariates (pools, sand and organic matter, and bedrock respectively). Models incorporating covariates pools, boulders, and water temperature into ψ estimates frequently rated high, had the highest cumulative AIC weights based on all combinations of models (n= 256 candidate models) (Table 3), and were the three variables in the top model. The top model (p (.) ψ (*pools + boulders + temp*)) suggested positive relationships between predicted occupancy and percentage of pools (2.17, CI = 1.29-3.06), boulder counts (0.93, CI= 0.31-1.55) and temperature (0.77, CI= 0.01-1.52). We saw no indication of oversimplification in goodness of fit simulations.

Table 3. Cumulative AIC weights (w_i) of occupancy model covariates for *M. falcata* in 1st-3rd order streams in the western Oregon watersheds.

Model	w_i
ψ pools	1.00
ψ boulders	0.89
ψ temperature	0.75
ψ gravel	0.38
ψ valley form	0.35
ψ ACW	0.34
ψ sand and organics	0.27

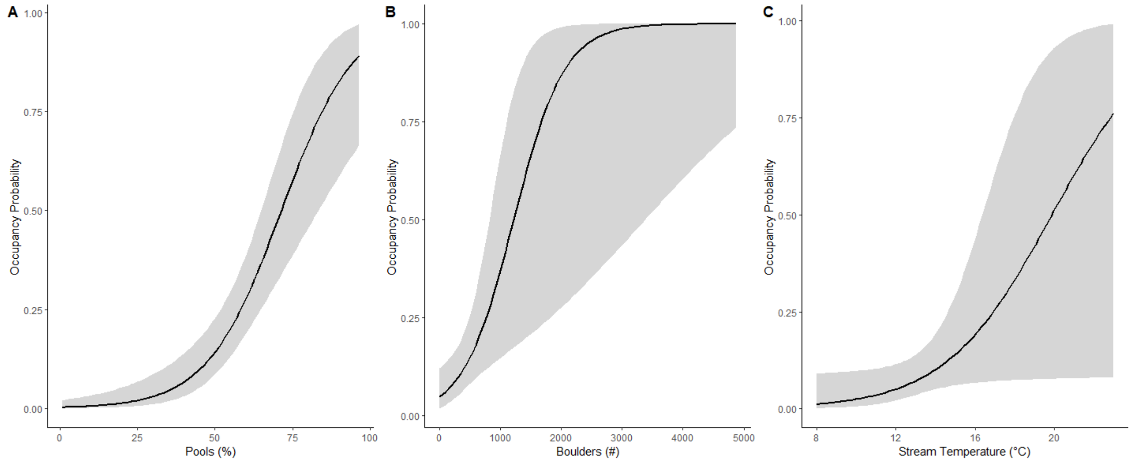


Figure 4. Percentage of pools as a proportion of surface area across the sampled reach (1km) was the strongest covariate in predicted mussel occupancy (A), followed by counts of boulders (B) and average stream temperature (C). The solid line represents changes in predicted mussel occupancy based on the amount of pools, grey area represents confidence intervals.

The highest ranking habitat covariates that best explained mussel presence (pools, boulders, and temperature) were compared across delineated regions to explore whether regional variation in habitat characteristics is responsible for spatial variability in mussel naïve occupancy (see Figure 3A & B). Kruskal-Wallis analysis of variance of the highest ranking variables (Figure 5) indicated a significant difference between regions for all variables. Pairwise Wilcox tests between regional groups indicated that percentage of pools at North Coast survey locations were significantly lower than the other regions (Figure 5A). Pairwise analysis of boulder counts between region found that Mid-South coast site had significantly higher counts than all other regions (Figure 5B). Pairwise tests of average site temperature between regions found that North Coast sites were significantly lower than sites in other regions, and Mid Coast sites were signifiantly lower than Umpqua and Mid-Coast sites (Figure 5C).

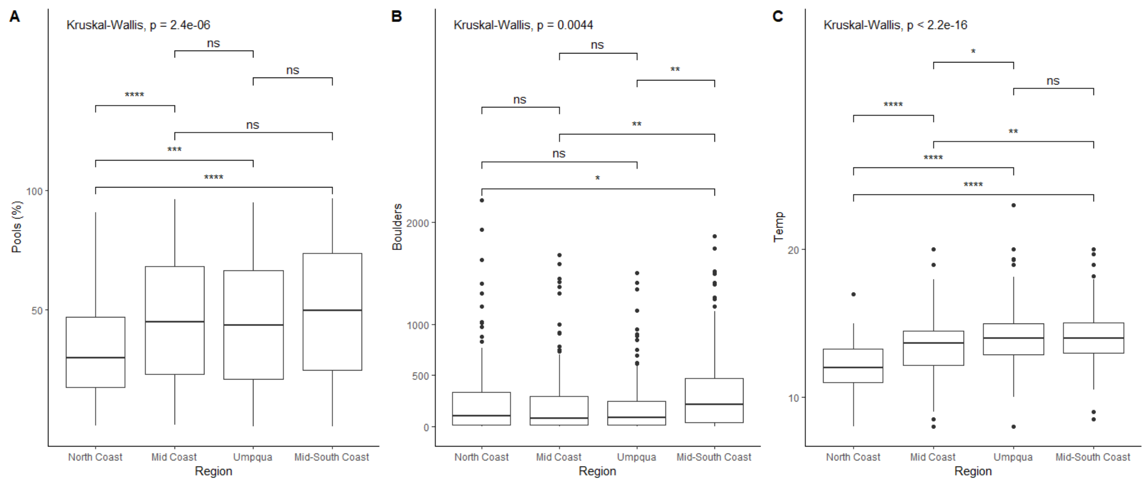


Figure 5. Regional comparison of the highest ranking covariates in predicting mussel presence, (A) percentage of pools, (B) count of boulders, and (C) averaged water temprature (celcius). Boxes indicate interquartile range, with the central line indicating sample median. Lines represnent the sample ranges without outliers, which are shown as dots. '****' = pval ≤ 0.0001, '***' = pval ≤ 0.001, 'ns' = pval > 0.05

3.3 Host Species

Fish counts and mussel presence or absence was recored at every survey locaton, and as a result, all 811 sites were used in the development of the binomial logistic regression model. Explanitory variables (fish species/counts) produced a model that predicted presence/absence of mussels significantly better than the null model (likelihood ratio chi squared test= 33.46, with 4 degrees of freedom; p <0.0001). The concordance index was 0.654, indicating an above average predictive model. Of the four fish species included in the model, counts of *O. kisutch* covaried the strongest, followed by *O. tshawytscha* and *O. mykiss. O. clarki* was not a significant covariate in the model.

Table 4. Binomial logistic regression results for predicting observations of mussels based on host fish counts

	Estimate (Standard Error)	**Standaridzed Coefficients**	**P value[a]**	**Odds Ratio (Confidence Interval)**
Intercept	-2.208 (0.16)		< 2e-16	0.108 (0.08-0.143)
O. kisutch	0.001 (0.00)	0.284	**3.26e-07**	1.001 (1.001-1.001)
O. tshawytscha	0.023 (0.01)	0.169	**0.000626**	1.024 (1.011-1.039)
O. mykiss	-0.011 (0.01)	-0.213	**0.023629**	0.988 (0.978-0.996)
O. clarki	-0.002 (0.01)	-0.027	0.736200	0.998 (0.985-1.01)

Observations: 811
AIC: 582.29
C: 0.654

[a] p values less than 0.05 are bolded

O. kisutch and *O. tshawytscha* species counts predicted mussel observations (binomial logistic regression; Figure 6 A & B), both exhibiting positive relationships with observation probability. *O. mykiss* displayed a weak negative relationship, appearing not to influence predicted probability of mussel observation above 15 % (Figure 6C).

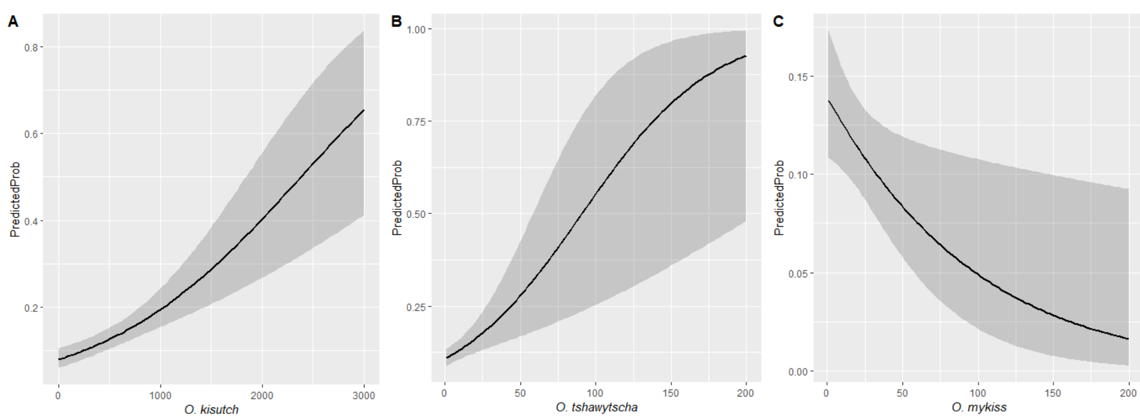


Figure 6. Predicted probability of mussel observation as counts increase for three covariate salmonid species *O. kisutch* (A), *O. tshawytscha* (B), *and O. mykiss* (C) based on logistic regression model. Shaded area denotes 95% confidence interval. Note the differences in x and y scales.

3.4 Condition Index Comparison

Fifteen mussels were collected at seven sites, and ten mussels were collected at one site (due to low abundance at that site; SZ) across three sub-regions within the Coast Range. Body condition indices (BCI) were significantly different among sites (Kruskal-Wallis, chi-squared= 44.482, df=7, *p-value* <0.001), with Siletz and Big Elk (Yaquina) sites significantly lower than the mean, and Fall Creek (Alsea), Smith, and Weatherly (Umpqua) sites significantly higher (Figure 7). BCI variables (shell length and body wet weight) displayed a strong positive relationship, and the largest/heaviest mussels were found at sites with the smallest upstream catchments (Figure 8). Allometric length-weight measurements were fit using least squares regression and log-log transformation, resulting in a significant regression equation (F (1,113) = 1,743, pval < 0.001), with an R^2 of 0.94. Predicted log mussel body weight (g) is equal to -9.44 + 2.79 (log shell length), where length is measured in mm.

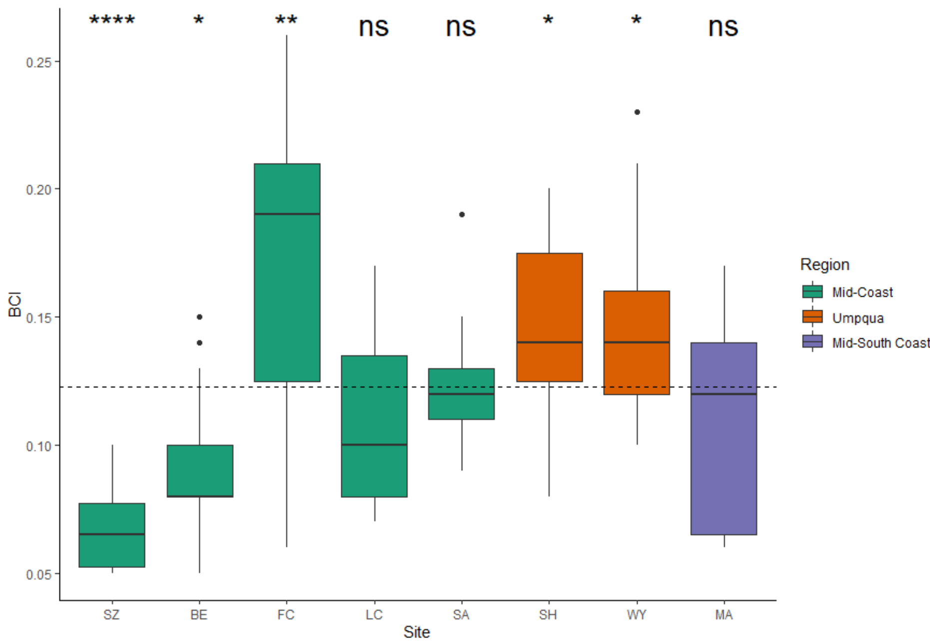


Figure 7. Mussel condition index (body weight ÷ shell length) was significantly different among sampling sites. Sites are ordered from north (left) to south (right) and color coded by region. Dotted line indicates mean BCI across all sites. ‘****’ = pval ≤ 0.0001, ‘**’ = pval ≤ 0.01, ‘*‘ = pval ≤ 0.05, ns’ = pval > 0.05

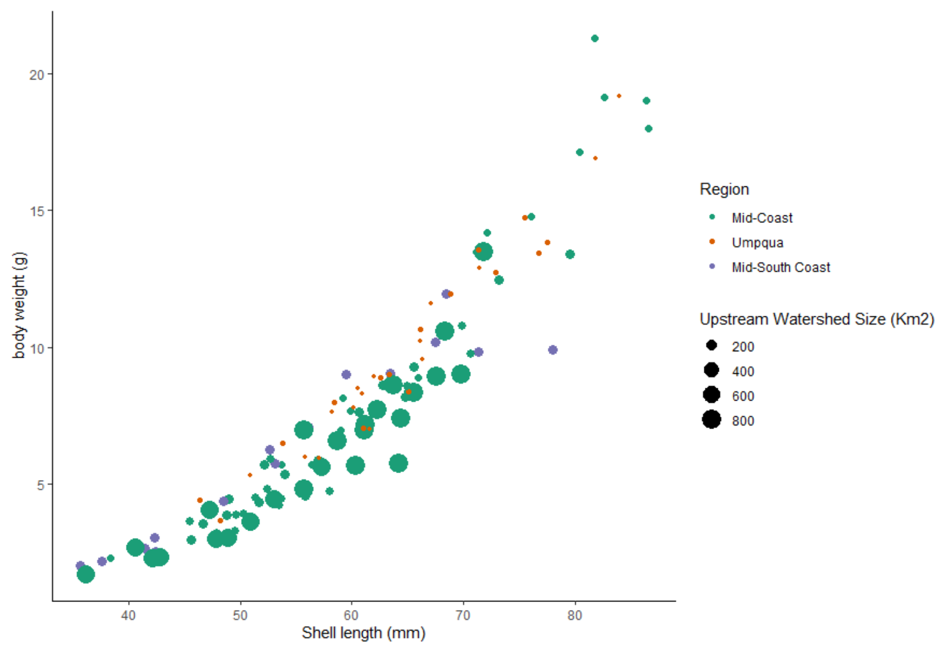


Figure 8 – Allometric relationship between body weight and shell length (variables used to calculate Body Condition Index) displayed a strong positive linear relationship (log-log transformed variables: $R^2 = 0.94$, pval < 0.001). Dot size corresponds with upstream watershed size and color signifies region.

4. Discussion

This first synthesis of Oregon Coast Range *M. falcata* observation frequency and modeled occupancy probability in a large sample of randomly surveyed headwater stream reaches highlights how mussel observation differs from predicted mussel occupancy. Overall, naïve mussel occupancy was low (12.3% of sites), and modeling indicated observed occupancy underrepresented predicted occupancy (ψ= 0.24, CI= 0.19-0.31) due to detection probability over a ten year assumed closure period (p=0.447, CI = 0.38-0.52). Researchers exploring freshwater mussel occupancy in North Carolina estimated a similar detection probability in their results (p=0.42, CI = 0.37-0.47) during a single season survey of 15 mussel species (Pandolfo et al., 2016). The similarity of these results suggest modeling mussel occupancy over a multiple year closure period is a practical means to account for imperfect detection of long lived freshwater mussels and address lingering questions about regionally important habitat characteristics.

4.1. Regional differences and habitat considerations of ***M. falcata*** *in coastal watersheds*

Proportion of pools (≥ 20 cm maximum depth; ≥ 6m^2 surface area), presence of boulders, and stream temperature within sampling reaches were strong predictors of mussel occupancy in Oregon coastal headwater streams, which aligns with previous research in Washington state indicating the importance of areas of lower shear stress (preference towards boulder-dominated substrate) in mussel habitat requirements (Stone et al., 2004). Boulder-stabilized substrate has also been linked to juvenile micro-habitat preferences in closely related *M. margaritifera* (Hastie et al., 2000). In our study, warmer averaged water temperature at each site was a positive predictor of mussel occupancy, though the confidence interval was exaggerated at higher temperatures, suggesting that effects of water temperature on predicted occupancy may only be relevant at lower temperatures (Figure 4C). Low temperature has been associated with poor recruitment success in freshwater mussels, likely due to temperature cues for reproduction (Howard and Cuffey, 2006b; Hruska, 1992). Though *M. falcata* are known to be more tolerant of lower temperatures than other freshwater mussels, their brooding and parasitic periods are extended by cold water conditions (Roscoe and Redelings, 1964), suggesting regional differences in reproductive timing based on varying water temperature.

Pool formation in watersheds has been shown to decrease in volume and prevalence with greater channel gradient, which is driven by tendency for debris flow scour in these systems (Buffington et al., 2002). Therefore, the volume and frequency of pools in higher gradient segments is largely associated with increased stream complexity and the presence and size of large wood debris (LWD), which trap and store sediment (Beechie and Sibley, 1997; Buffington et al., 2002; Rosenfeld and Huato, 2003). This dynamic mechanism of channel morphology in coastal watersheds relies on upstream sources of large wood, and is influenced by riparian area complexity (Collins et al., 2012). Further in-depth investigation into connections between mussels and underlying processes such as this, which generate and maintain habitat features utilized by *M. falcata*, is critical for future conservation of these species, especially in isolated catchments where productive downstream migration may be limited by watershed size.

North Coast watersheds exhibited low occupancy in randomly surveyed 1-3rd order streams, both in counts of sites with detections and as deviations from coast-wide average observation proportions (Figure 3). To investigate whether habitat characteristics may be contributing to regional differences in mussel observations (Figure 3), we compared the highest ranking reach-level habitat variables across the sampling regions. Headwater stream segments surveyed in North Coast watersheds have significantly fewer pools (Figure 5) and higher average gradient (Table 2) compared to other regions surveyed in this analysis. Difference in flow and shear stress are known to influence benthic habitat stability and thought to be linked with mussel mortality and/or downstream transport of mussels (Niraula et al., 2016; Strayer, 1999), which may explain why noticeably fewer mussels were observed in steeper North Coast headwater survey locations compared to other areas. Mussel aggregations respond to habitat needs and hydrological variables across micro and meso, scales within watersheds (Newton et al., 2008), but regional associations provide useful information for species conservation, especially in light of connectivity constraints among populations. Lack of mussel observations in North Coast headwater streams does not indicate that mussels are not present in the region, but that they may be confined to lower portions of some watersheds. Site-averaged temperature increased from north to south, with north coast being significantly lower than all other regions and mid coast being significantly lower than the southern regions (Umpqua and mid-south) (Figure 5C).

Though this study identified habitat characteristics associated with mussel presence in 1st -3rd order (headwater) streams and found regional differences in habitat availability, it is unclear how regional habitat differences affect mussel populations lower in watersheds, where channel morphology and gradient may provide more consistent habitat over time compared with dynamic headwaters. Habitat characteristics and substrate suitability are important considerations in understanding patch dynamics of freshwater mussel populations, but the complex (and lengthy) life history of *M. falcata* requires consideration of additional controlling factors in their persistence such as host fish and population condition (Strayer et al., 2004).

4.2 Host fish abundance and mussel presence

Coho (*O. kisutch*) presence were strongly positively associated with mussel observations (binomial logistic regression), followed by chinook (*O. tshawytscha*), and steelhead (*O. mykiss*) were negatively associated. Cutthroat (*O. clarki*) presence were not associated with mussel presence (Table 4). These findings differed from expectations based on host fish susceptibility to parasitism rankings by Karna and Millemann (1978), though their results were from a caged experiment in a single watershed, so may not reflect co-occurrence under natural conditions across coastal watersheds. The survey effort we analyzed was designed to coincide with juvenile coho presence in watersheds, which may have driven the higher mussel co-occurrence with coho and may not represent year-round co-occurrence. The timing and type of juvenile salmon present during the summer months raises an important point regarding co-occurrence with mussel populations during periods of glochidial release into the water column. Timing of *M. falcata* conglutinates can be variable, but have been detected in water samples between late March and June in a small tributary of the Columbia River (NE of our study area) (Allard et al., 2017). Allard et al. (2017) attributed the timing of glochidial release to seasonal changes in daily water temperature fluctuation. If coho are more abundant during the season of reproduction but not optimal hosts (per Mille mean 1978), coastal populations of *M. falcata* may be presented with barriers to successful reproduction based on host species co-occurrence. Freshwater pearl mussels (*M. margaritifera*) possess subpopulation-level adaptations to different host species based on coinciding historical presence and conditions (Salonen et al., 2017). *M. falcata* may exhibit similar subpopulation adaptations, but regional relationships of *M. falcata* and host-species adaptations have not been investigated. Considering the richness and diversity of life histories and species of potential salmonid hosts throughout the Pacific Northwest, significant data gaps remain in current understanding about *M. falcata* host species relationships and potential subpopulation adaptations.

Co-occurrence of *M. falcata* and salmonids may be more suggestive of habitat preference similarities among the species during the sampling season. LWD presence (and associated pools) supports higher densities of *O. mykiss*, *O. clarki*, and *O. kisutch* during winter months, and higher densities of *O. kisutch* and lower densities of *O. mykiss* during the summer (Roni and Quinn, 2011). These patterns of pool occupancy during the summer may, in part, explain the relative counts and associations we observed in our co-occurrence analysis, wherein *O. kisutch* were positively associated and *O. mykiss* were negatively associated with mussel presence.

4.3 Mussel condition and allometric comparisons

Understanding *M. falcata* presence/absence across the Coast Range is useful in determining distribution of extant populations, but is not an indication of whether mussel populations are thriving. Our condition analyses indicate that subpopulation fitness differs across coastal watersheds/scales, with some populations exhibiting significantly higher or lower BCI when compared to the sample mean (Figure 7). Upstream watershed size, indicated by varying dot size in the comparison of shell length and body weight (Figure 8), indicates the largest and heaviest mussels collected in this study originated from locations with smaller upstream catchment areas. As anticipated, shell length was strongly correlated with body weight (Figure 8), consistent with previous findings about freshwater Unionida allometry (Atkinson et al., 2020), though we explored relationships using wet instead of dry mass. Documentation of length-mass relationships provides useful information that could inform any future biomass assessment of *M. falcata*, as a non-lethal means to measure function and contribution of populations to ecosystems (Atkinson et al., 2020).

4.4 Additional considerations for M. falcata management and conservation in the Coast Range

4.4.1 Land use/land management practices

Long-lived sessile organisms such as *M. falcata* are subject to a wide range of influences and conditions over their life span, especially in terms of landscape patterns of anthropogenic disturbance. In the Coast Range, natural disturbance, approaches to forestland management, and the evolution of policy/regulation have influenced regional landscape dynamics and affected ecological processes across multiple scales and timeframes (Nonaka and Spies, 2005). Historical practices such as splash damming[2] and

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[2] Splash dam - a common practice from the 1880s-1950s, splash dams are temporary wooden dams built to raise water levels in streams, allowing for log transport downstream upon demolition (Miller, 2010)

log drives[3] were once commonplace within coastal rivers, and legacy impacts from those activities (depleted gravel, scoured substrates) may continue to affect freshwater environments and mussel habitat within these systems (Miller, 2010). Legacies of intensive plantation forest management (even age, single species, densely planted stands) are present across the Coast Range to varying degrees, and are largely influenced by ownership and changes to regulatory policy over time (Spies et al., 2007). Forest plantations have been shown to promote stream flow deficits during summer months, particularly as compared to older aged stands (Perry and Jones, 2017), which can be particularly taxing to sessile organisms such as *M. falcata*. Furthermore, because of their long life span, *M. falcata* populations may experience additional stress from contemporary and/or historical pesticide use/exposure (Scully-Engelmeyer et al., 2021). Forestland (the dominant land cover in the Coast Range) managers in Oregon's coastal watersheds have relied on numerous chemical products to establish and maintain forest plantations since the mid-1900s. Pesticide use and regulatory frameworks have evolved significantly to reduce the amount of chemical contamination permitted in aquatic environments, but contemporary pesticide use is still a source of contamination and is one of many stressors in aquatic ecosystems (Scully-Engelmeyer et al. 2021). In a recent investigation into bivalve contaminant uptake in the Coast Range, Scully-Engelmeyer et al. (2021) found *M. falcata* samples were contaminated with compounds originating from a variety of potential sources, including forestry. Additional pilot research into adjuvant

[3] Log drive – the method of moving logs from harvest location to downstream mills via river current (Miller, 2010)

contamination also indicates high retention of alkylphenol ethoxylates in *M. falcata* tissue (an order of magnitude higher) compared to estuarine bivalves; these compounds are known endocrine disruptors with effects on growth and reproduction that may affect long term population projections (Granek, unpublished data; see Table B1). Moreover, older *M. falcata* within coastal watersheds may have lived during the entire history of chemical pesticide use in Oregon, though neither this analysis nor others have examined age structures across *M. falcata* populations and regions.

4.4.2 Food/nutrient sources

Marine derived nutrients are thought to have been a significant source of nutrients in freshwater aquatic ecosystems, but they have declined in proportion to terrestrial nutrient influx in the Pacific Northwest, likely due to declines in salmon runs (Gende et al., 2002; Holtgrieve and Schindler, 2011). Beyond the effect of host fish declines on *M. falcata* reproductive success, this temporal shift in nutrient subsidies may also influence nutrient types and availability for mussels in these systems. Individual *M. falcata* have been shown to preserve spatially averaged measurements of instream base level nitrogen and carbon isotope ratios within watersheds (Howard et al., 2005), and could be a useful measure of relative variability in nutrient dynamics among coastal watersheds and provide insight into food abundance/sources.

4.4.3 Study Limitations/research directions

Mussel observations during WORP surveys were collected as incidental data to fish counts, which allowed for this preliminary analysis of distribution and occupancy of mussels in 1st-3rd order streams throughout coastal watersheds. Changes in mussel occupancy over time and assessment of colonization and extinction rates, which could vary according to land use/land management practices or other habitat variables expected to change over time (e.g., temperature, disturbance events), could not be investigated with these data, but are important variables worth investigating in future *M. falcata* monitoring in coastal watersheds. Asymmetrical dispersion patterns are of particular interest in small coastal watersheds with dynamic sediment movement regimes, as downstream migration of mussels over time may deplete reproductive subpopulations of mussels in headwaters, which can be important for metapopulation dynamics (Terui et al., 2014). Mussel occupancy was held at constant throughout the survey period in order to explore survey detection probabilities, but this may underappreciate mussel "migration" in morphologically dynamic headwaters.

This study identified a subset of coastal watersheds where mussels were not observed in 1st-3rd order streams during the ten year WORP survey period (Appendix B1). Environmental DNA (eDNA) monitoring technology has evolved as an effective monitoring tool to assess presence/absence of aquatic species in watersheds, and recent applications have evolved to incorporate freshwater mussels assays (Rodgers et al., 2020). Identified watersheds should be prioritized for future monitoring to determine the status of mussel presence and/or extirpation in coastal watersheds to help guide future efforts in population dynamics and extinction debt research in isolated subpopulations.

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Appendix B: Supplementary material for Chapter 4

Figure B1. Coastal drainages wherein surveys were conducted in 1st-3rd order streams. Watersheds with at least one mussel observation during the survey period are italicized and underlined.

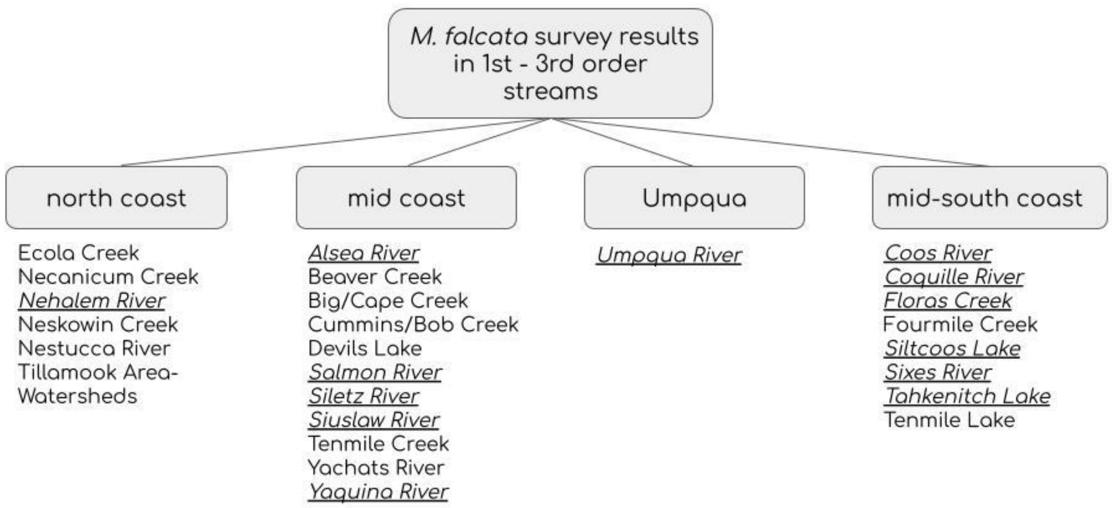

Table B1. Pilot adjuvant tissue retention analysis data (LC MS/MS, method MLA-080 Rev 2) quantifying alkylphenol ethoxylate contamination in freshwater (*M. falcata*) and estuarine (*M. arenaria*) bivalves in Oregon coastal watersheds. Analysis via SGS AXYS Analytical Services. RL=reporting limit

Species	Site	Compound (ng/g, wet weight basis)			
		4-Nonylphenol[1]	4-Octylphenol[2]	Nonylphenol monoethoxylate[3]	Nonylphenol diethoxylate[4]
Margaritifera falcata	SA-MS	**504**	**4.66**	**11.2**	**1.41**
	AA-FC	**256**	**7.61**	**<RL**	**0.646**
Mya arenaria	AA	**23.2**	**0.592**	**1.06**	**<RL**
	SH	**5.95**	**<RL**	**<RL**	**<RL**
	SA	**8.6**	**0.509**	**<RL**	**<RL**
	CB	**8.87**	**0.521**	**<RL**	**<RL**

Lab blanks (ng/g): [1] = 8.49, [2] = 0.777, [3] = 0.791, [4] = 0.5

Chapter 5: Conclusions

Spatial configurations of landscape variables (land use, ownership/management, watershed characteristics, etc.) aggregate to influence habitats, water quality, nutrient movement, and hydrological and morphological processes within watersheds across multiple scales (Canham et al., 2004; Lee et al., 2009; Stanfield et al., 2002). The effects of spatial landscape patterns on ecological processes and species dynamics, the underlying theme of this research (illustrated in Chapter 1; Figure 2), was explored through several avenues of inquiry within the Coast Range of Oregon. Chapter 2 and 3 focused on how patterns of forestland management and physical watershed characteristics influence herbicide movement in coastal watersheds, and Chapter 4 focused on how patterns of freshwater mussel occupancy in headwater streams were explained by reach-scale habitat features and host fish co-occurrence. Overall, this research highlights the utility of approaching questions about the effects of landscape patterns on coastal bivalve populations at appropriate scales and contexts.

1. Research summary/findings

Results from biomonitoring and passive water sampling in Chapter 2 provide insight into fate and transport of pesticides used in contemporary forestland management. Pesticide compounds commonly applied to commercial forestlands were detected by passive water samplers (atrazine, hexazinone, sulfometuron-methyl, and metsulfuron-methyl) and within the tissues (indaziflam) of *Margaritifera falcata*, *Mya arenaria*, and *Crassostrea gigas* in stream and estuarine habitats located considerable distances downstream of the application areas. Water-borne herbicide exposure documented during forestry's spring spray season displayed significant correlations with average watershed slope as well as planned herbicide activity during the sampling window. These finding suggest a fundamental connection between the spatial patterns of management activities, natural watershed features, and downstream multi-scalar ecological processes within the study region. Additionally, pesticides found in bivalve tissues originated from a variety of potential sources including household pest control, agriculture, nurseries, and Christmas tree farms. Documenting these contaminants offers valuable information about sources of contaminant burdens in bivalves in Oregon's coastal zone and a glimpse of the multiple stressors they endure.

In Chapter 3, multiple linear regression successfully predicted passive water sampler concentrations captured during deployment (R^2=0.89) based on three variables: percentage of steep slopes in upstream catchments (>40%), notified clearcuts within upstream watersheds over the last year, and notified herbicide activity during sampled timeframe. Model variables were calculated within three catchment sizes across the Coast Range (HUC 8, 10 and 12), and values were used to calculate predicted concentrations within those watersheds. Across HUC scales, larger ranges of values were seen at smaller watershed scales, but overall there were no significant differences between HUC group means based on Kruskal-Wallis analysis of variance. Regional variation in predicted values was observed, with catchments on the southern coast displaying higher predicted concentrations than mid and north coast catchments, which aligned with field-collected herbicide data. Model variables successfully predicted concentrations, but are confined by this context and timeframe, and likely do not properly predict exposure during other sampling windows or in other regions. Results from this chapter provide valuable information about the influence of scale and management intensity on predicted springtime herbicide exposure in Oregon's coastal drainages, and offer insight into pesticide exposure in unmeasured watersheds across multiple scales.

Investigation into the distribution, habitat variables, condition, and host species co-occurrence of *M. falcata* across Oregon's coastal drainages (Chapter 4) provides valuable and timely information to guide future management and conservation of coastal populations of the species. Mussel presence/absence and host fish counts were surveyed at 811 randomly selected 1-km segments of 1^{st}-3^{rd} order streams in coastal drainages; reach-scale habitat characteristics (geomorphic and in-stream) were collected at a subset of sampling locations (n=658). Mussels were observed at least once at 100 of the sites, for a naïve occupancy proportion of 12.3%. Frequency of mussel observations varied across the study area, with the highest frequencies seen in the Umpqua watershed, and lowest frequencies observed on the North Coast. Visual inspection of occupancy proportions within coastal watersheds revealed a clear distribution pattern throughout the study area, wherein occupied survey locations in north coast watersheds were below average compared to mid and southern coast locations. Modeled occupancy based on sites with 2 or more visits over the 10 year sampling period (n=251) estimated detection probability (p) to be 0.442 (95% CI = 0.37-0.51), and null occupancy probability (ψ) to be 0.24 (95% CI = 0.03-0.31). High ranking habitat covariates percentage of pools, count of boulders, and water temperature helped further explain mussel occupancy. Significant habitat covariates were summarized across regions to see whether differences in habitat might be driving occupancy. The north coast had significantly fewer pools compared with the other regions surveyed, suggesting that lack of some suitable habitat features (pools in this case) may contribute to low mussel observations in that region. Higher gradient streams, especially in the Coast Range, often have very dynamic stream morphologies (May, 2002), and these characteristics may contribute to downstream asymmetric dispersion of *M. falcata* over time via sediment and debris flow transport.

Host fish co-occurrence with *M. falcata* presence/absence observations was evaluated using binomial logistic regression analysis (n=811). Regression results indicated *O. kisutch* (coho) had the strongest correlation with mussel presence, followed by *O. tshawytscha* (chinook) and *O. mykiss* (steelhead). *O. clarki* (cutthroat) did not covary with mussel observations. Counts of coho and chinook showed positive relationships with mussel presence, and steelhead demonstrated a weak negative relationship. Survey timing was designed to monitor rearing juvenile coho, which may have influenced the relative presence of that species compared with other salmonids.

2. Directions for future research

Through the process of my dissertation research, I've identified several priority areas for future research expanding on the dissertation topics and concepts. Future directions within broader research topics of pesticide fate/transport and *M. falcata* landscape ecology in Oregon's coastal watersheds are displayed in Figure 1, along with connections to drivers of landscape change. The following paragraphs detail the priority research areas in Figure 1.

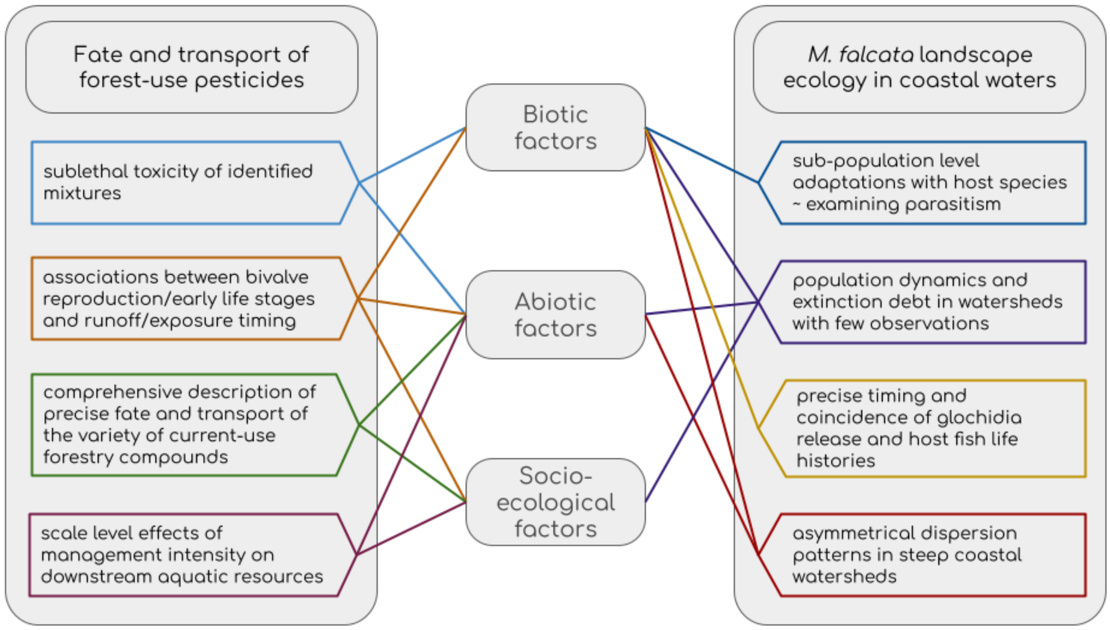

Figure 1. Conceptual diagram outlining priorities for future research directions identified throughout the course of this dissertation research.

These investigations into the fate and transport of forest-use pesticides provide new documentation about waterborne mixtures in coastal watersheds, but questions remain about potential sublethal effects of observed mixtures on bivalves at environmentally relevant concentrations. Exposure to pesticides during reproduction or early life stages are of particular interest, as those stages have been identified as the most sensitive to sublethal behavioral, developmental, or genotoxic endpoints (Conners and Black, 2004; Cope et al., 2008; Flynn and Spellman, 2009). Documenting exposure and uptake of compounds is helpful in identifying combinations of chemicals for future research into sublethal effects at environmentally relevant levels. Furthermore, the range of chemical properties associated with compounds detected in tissue and water samples indicate different routes of chemical movement in the environment, and highlight the importance of considering different routes of exposure when managing offsite chemical movement in watersheds. Further research is needed to better describe the precise fate and transport of the variety of current-use compounds commonly applied in forestland applications, especially regarding lingering questions about minimum effective spray and vegetative buffer widths across stream types. Beyond site level understanding of pesticide movement, future investigation into management practices at the landscape scale should incorporate scale level effects of management intensity and timing on downstream aquatic resources.

Of particular interest for future *M. falcata* population research are drainages that had few or no observations during headwater stream surveys (Chapter 4, Appendix B1). In these systems (which were primarily in the northern portion of the study area), populations may have been extirpated, or may be confined to lower in the watershed. Asymmetrical dispersion analysis of another freshwater mussel species (*M. laevis*) in the Shubuto River, Japan, highlighted the importance of reproductive upstream subpopulations as colonizers for downstream populations (Terui et al., 2014). Environmental DNA (eDNA) monitoring technology has developed as an effective monitoring tool to assess presence/absence of aquatic species in watersheds, and recent applications have evolved to incorporate freshwater mussel assays, which may be a useful and efficient means to identifying upstream source populations. Future research and monitoring of coastal mussel populations should also consider the effect of asymmetric “migration” over time in distribution analyses, especially in small/isolated watersheds where fruitful downstream movement may be limited.

Another avenue that merits further exploration is the precise timing and coincidence of *M. falcata* glochidial release with that of anadromous host fish run timing and juvenile life stage presence/dispersion in coastal watersheds, as these factors influence the genetic structure of mussel populations (Österling et al., 2020). These avenues of investigation are especially relevant to answer questions about mussel population dynamics in Oregon’s coastal watersheds in light of research suggesting host fish susceptibility to parasitism can vary based on fish species and life stage (Karna and Millemann, 1978). Freshwater pearl mussels (*M. margaritifera*) have been shown to possess subpopulation-level adaptations to different host species based on coinciding historical presence and conditions (Salonen et al., 2017). *M. falcata* may exhibit similar subpopulation adaptations, but regional relationships of *M. falcata* and host-species adaptations have not been fully investigated. Considering the richness and diversity of life histories and species of potential salmonid hosts throughout the Pacific Northwest, significant data gaps remain in current understanding about *M. falcata* host species relationships and potential subpopulation adaptations. Effective conservation and management of *M. falcata* throughout the Coast Range will require continued research and monitoring of population distribution, abundance, habitat range, and host species interactions/adaptations.

Chapter 5 References

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