NATIVE FISH OCCUPANCY AND DENSITY

Study Goals and Objectives

The Aquatic Species Restoration Plan (ASRP, Aquatic Species Restoration Plan Steering Committee 2019) and the associated Monitoring and Adaptive Management Plan (M&AM) focus on protecting and preserving aquatic species and habitats, restoring degraded ecosystems, and reestablishing the natural watershed processes that are important in the formation, condition, and function of aquatic habitats. To identify restoration actions, modeling efforts (e.g., Ecosystem Diagnosis and Treatment (EDT)) largely focus on benefits to salmon and steelhead in part due to data availability. Our study informs the ASRP and M&AM by helping to answer the question, "Will restoration actions targeting salmon and steelhead also benefit other native fish species in the Chehalis River?"

Several of the native freshwater fishes and shellfish in the Chehalis Basin are listed as Species of Greatest Conservation Need in the Washington State Wildlife Action Plan, including Pacific lamprey, river lamprey, and Olympic mudminnow (Washington Department of Fish and Wildlife 2015). Furthermore, the Aquatic Species Enhancement Plan Data Gaps Report (Aquatic Species Enhancement Plan Technical Committee 2014), section 4.1.1 Species-Specific Life History and Population Data Gaps for In-Channel Species identified detailed life history, distribution, and abundance information for native freshwater fishes as an important data gap. While there has been an effort in recent years to increase our understanding of native freshwater fishes in the Chehalis Basin (e.g., Winkowski et al. 2016, Winkowski et al. 2017, Winkowski and Kendall 2018), distribution information across the Chehalis Basin still represents a large data gap.

The Chehalis River Occupancy and Density Study (ODS) is a multi-year study that includes all native fish and shellfish across a large portion of the Chehalis Basin. For this study, we investigated the occupancy patterns of native freshwater fishes and native freshwater shellfish associated with physical (e.g., landscape and thermal) and biological features. We used this information to better understand the relationships between occupancy and the physical features (e.g., how does the probability of occupancy change with varying large woody debris counts). We also used this information to develop a multispecies occupancy model (MSOM) with which we can predict occupancy patterns outside of surveyed locations. The results of our study provide easily accessible occupancy information for native fish and shellfish throughout much of the watershed, are critical for evaluating effectiveness of restoration efforts, and inform the spatial and temporal status and trends of freshwater fishes and shellfish in the Chehalis Basin.

Objectives:

1. Collect native fish occupancy information that will inform a multispecies occupancy model (MSOM) and predict occupancy patterns across the study area.

2. Describe relationships between habitat and native freshwater fishes.

Methods / Study Design

<u>Sampling frame and site selection</u>

To define our sampling frame, we focused on 3rd order and greater streams to represent fish-bearing streams. We removed the mainstem portion of the Chehalis River downstream of the South Fork Chehalis because of sampling restraints (e.g., water depth and visibility) as well as estuary tributaries due to survey feasibility (e.g., travel time to sites). To stratify our samples, we selected ecologically diverse regions (EDRs) based on the Aquatic Species Restoration Plan Steering Committee (2019) and combined the Black Hills, Black River, and Lowlands into one Central Lowlands macro ecologically diverse region (MEDR) resulting in four regions. They included: 1) Olympic Mountains, 2) Central Lowlands, 3) Cascade Mountains, and 4) Willapa Hills (Figure 1). Sites sampled were drawn from points along the stream network, stratified within ecologically diverse regions, and spaced every kilometer using a spatially balanced, randomized approach (generalized random tessellation stratified: GRTS)). Points along the stream network were established with the Chehalis Thermalscape (Winkowski and Zimmerman 2019), a Spatial Stream Network (SSN) model (e.g., Peterson et al. 2013, Isaak et al. 2016, Isaak et al. 2017) that used the National Stream Internet (NSI). GRTS sample draws were completed using spsurvey package (Kincaid et al. 2016) in R v. 4.0.2 (R Core Team 2020). We sampled additional sites that added spatial coverage and increased the explained habitat variation. We also repeated sites each year from the previous year to monitor interannual variation in occupancy.

Each selected site was assessed for sampling feasibility (i.e., landowner permissions, wetted, perennial freshwater, flowing, and naturally occurring). In 2019, we completed 8 sites per MEDR for a total of 32 sites. In 2020, we completed 17 sites per MEDR except in the Cascade Mountains MEDR where we completed 16 sites for a total of 67 sites. In 2021, we completed 24 sites per MEDR except in the Central MEDR where we completed 25 sites for a total of 97 sites.

<u>Data collection</u>

We established a sampling reach 20 times the average bankfull width, which was adapted from Washington State Department of Ecology (2018). We determined bankfull width at three locations, five meters apart, immediately downstream of each sampling site. The minimum and maximum reach lengths were 100 m and 400 m, respectively.

We collected fish and shellfish occupancy data using environmental DNA (eDNA) as well as traditional sampling techniques (snorkeling and electrofishing) on a wide range of freshwater fishes and shellfish present within the Chehalis Basin (Table 1). We used eDNA collection because it can simultaneously target many species, be completed more quickly allowing us to increase our sample size and detect cryptic and rare species without the risks of handling them physically. For traditional sampling, we used snorkeling and electrofishing to target both pelagically- and benthically oriented fish species.

We collected eDNA samples moving downstream to upstream, in duplicate at three locations along the reach – the downstream most point, the approximate midpoint, and the upstream most point of each reach. We filtered water samples using the Smith-Root eDNA water sampling backpack (https://www.smith-root.com/edna/ande) and followed protocols outlined by the Washington Department of Fish and Wildlife Genetics Lab (Brown 2019), which uses a metabarcoding approach adapted from Jeanniard-du-Dot et al. (2017). At each of the three sampling locations within a site, we filtered 1 Liter of water, through a 1.0 μM pore size filter in duplicate equating to 6 samples per site. To detect potential contamination, we filtered 500 ml of sterile water after each sampling day, as an equipment control. Filters were stored in 100% ethanol in 2 ml tubes or 15 mL of desiccant beads at room temperature until DNA extraction.

At sites where traditional sampling was completed, we conducted snorkeling surveys using an approach adapted from Winkowski and Zimmerman (2017). For each snorkel survey, one or two snorkeler(s) surveyed from downstream to upstream in the river while identifying and counting fish. For streams with wetted width > 15 m, we used two snorkelers. Snorkelers made data recording stops when they observed fish in large aggregations to avoid duplicate counts. Either by switching banks (e.g., snorkeler 1 surveys river left on pass 1 then river right on pass 2) or switching snorkelers, two passes were completed (waiting a minimum of 10 minutes between passes) to determine detection efficiency. Each snorkeler estimated the amount of stream they were not able to snorkel. Reasons for not snorkeling a section of river included depth, water velocity, or structure (e.g., brush, logjam).

At sites where traditional sampling was completed, we also completed a single pass electrofishing effort, attempting to move upstream in a zigzag pattern. In larger streams, we were limited by water depth (<1.5 m) and sampling time, so we did not sample the entire reach and instead focused on sampling each type of habitat and substrate (e.g., pool, riffle, run, silt, sand, gravel, cobble, boulder). We estimated the area that was not electrofished. Initially, we set the electrofisher to 25 Hz, 15% duty cycle, and 350 V. We raised the voltage in 20 V increments (max 450 V) until adequate galvanotaxis occurred within the zone of influence. In sections of fine substrate (e.g., silt, sand, and organic detritus) with a higher likelihood of larval lamprey occupancy (Clemens et al. 2022), we switched the electro fisher to dual channel setting (Moser et al. 2007, Dunham et al. 2013). The primary channel was set to a burst pulse at 125-150 V (depending on fish response), 1 Hz (cycle frequency), 4 Hz (burst frequency), and 33% duty cycle. The secondary channel was set to a standard pulse, 125 V, 30 Hz, and 12% duty cycle. We placed collected fish in buckets with fresh water and aerators and we monitored the bucket water temperature and captured fish for signs of stress. We identified collected fish to species and life stage, except for *Cottus* which were identified to genus. We identified larval lamprey > 70 mm length as *Lampetra* or *Entosphenus* using Goodman et al. (2009). In 2020, fin clips from 46 sculpin were collected within a subset of reaches, successfully amplified for genetic analysis, and identified to species. These data were added to the regional database for metabarcoding eDNA, subsequently improving eDNA sculpin identification.

We collected habitat data for each site following a modified approach from Dunham et al. (2013) and Zimmerman and Winkowski (2022). We established 11 evenly spaced transects perpendicular to the flow along the sampling reach, starting at the most downstream point and moving upstream. At each transect, we measured bankfull width and wetted width. In addition, we collected water depth and substrate size at five equally spaced stations along each transect. We counted water inputs (e.g., tributaries and seeps), pools, side channels, and large wood (diameter ≥ 30 cm and a length ≥ 2 m) and measured the maximum depth within the reach and the temperature at the beginning and end of the sampling period. We categorized the channel type following Montgomery and Buffington (1997).

<u>DNA Metabarcoding Analysis</u>

We used a metabarcoding approach, which utilizes universal primers to amplify conserved regions in the mitochondrial genome. We utilized a mitochondrial metabarcoding marker, Cytochrome Oxidase I (COI), to identify and quantify species from eDNA filters. The COI marker (~500 bp) was used to quantify and identify metazoan species (Geller et al. 2013, Leray et al. 2013). In 2019, while we utilized two universal primers to amplify conserved regions in the mitochondrial genome for the metabarcoding approach, COI and 16S rRNA (16S), to identify and quantify species from eDNA filters. No significant difference was found between the species detected by each marker (V=134, p=0.6559) so we used COI to identify and quantify species from eDNA filters.

<u>Occupancy model</u>

To evaluate the distribution of species and relationships with habitat covariates, we used a multi-species occupancy modeling framework similar to Broms et al. (2016). To account for the non-independence of occupancy within sites across years, we included an auto-logistic effect. We modeled the probability, $\psi_{y,i,s}$, that species i was present at site j during surveys in the first year y as,

$$\psi_{ij,y=1} = \frac{\text{logit}^{-1}\left(\alpha_i + \sum_{n=1}^{N} x_{j,n}\beta_{i,n}\right)}{\text{logit}^{-1}\left(\alpha_i + \sum_{n=1}^{N} x_{j,n}\beta_{i,n}\right) + \left(1 - \text{logit}^{-1}\left(\alpha_i + \left(\sum_{n=1}^{N} x_{j,n}\beta_{i,n}\right) + \rho\right)\right)} \qquad (1)$$

where α_i is a species-specific intercept, $x_{j,n}$ is the n^{th} site-specific habitat covariate or categorical effect of a total of N such variables, $\beta_{i,n}$ is a species-specific coefficient, ρ is an auto-logistic effect, and logit^{-1} is the inverse logit function. This equation represents the expected occupancy probability in a randomly selected year, in the presence of an auto-logistic effect, reflecting the fact that we have no information on occupancy prior to the first year. The probability of occupancy in subsequent years was,

$$\psi_{ij,y} = \psi_{i,j,y-1}^{\text{cond}} \left[\text{logit}^{-1} \left(\alpha_i + \sum_{n=1}^{N} x_{j,n} \beta_{i,n} + \rho \right) \right] + \left(1 - \psi_{i,j,y-1}^{\text{cond}}\right) \left[\text{logit}^{-1} \left(\alpha_i + \sum_{n=1}^{N} x_{j,n} \beta_{i,n} \right) \right] \quad (2)$$

where $\psi_{i,j,y-1}^{\text{cond}}$ is the conditional probability of occupancy given the observed data through year $y-1$. We assumed that the species-specific intercepts were drawn from a hyperdistribution $\alpha_i \sim N(\mu^{\alpha}, \sigma^{\alpha})$, with mean μ^{α} and standard deviation σ^{α}.

We chose to model covariate effects independently for each species rather than hierarchically for two reasons. First, the species included in the model represent a wide range of taxa that could have different habitat preferences. Second, it facilitated the use of regularizing priors on coefficients $\beta_{i,n}$, to reduce the variance of estimates given that we included several colinear covariates. The prior was equivalent to the penalty used in ridge regression, $\beta_{i,n} \sim N\left(0, \sigma_{i,n}^{\beta}\right)$, $\sigma_{i,n}^{\beta} \sim exp(\lambda_i)$, where $\sigma_{i,n}^{\beta}$ is a species-specific standard deviation for a zero-centered normal distributions and λ_i is a species-specific parameter that control the magnitude of penalization across coefficients. We applied an informative half-normal penalty with standard deviation of 2.0 on each λ_i to help with convergence of the weakly informed λ_i parameters.

We evaluated the effects of the following covariates on occupancy: regional location within the basin (MEDRs), geologic types (sandstone, glacial, basalt, and alluvium), temperature, precipitation, slope, elevation, cumulative drainage area, canopy, bankfull index. All continuous covariates were Z-scored prior to inclusion in the model. Geologic types came from USGS geologic map databases for the United States (Ludington et al. 2005). Covariate values for precipitation, slope, elevation, cumulative drainage area, canopy, bankfull index came from the National Hydrography Dataset Plus (NHDPlus Version 2; Moore et al. 2019). Out temperature covariate came from the Chehalis Thermalscape model (Winkowski et al in review). We estimated the future occupancy using our MSOM with temperatures adjusted for 2040 and 2080 climate scenarios. Climate scenarios were based on Chehalis Thermalscape predictions for 2040 and 2080.

Summary of Results

We sampled 166 unique sites and 36 repeat sites from 2019 to 2021 (Figure 1) and developed a MSOM predicating occupancy for 19 native fishes and two freshwater shellfish (Figure 2), summarized native fish occupancy for current (2019) and future (2080) temperature scenarios (Figure 3), and described individual species' relationships to landscape variables in terms of MSOM covariates (Figure 4).

Detections varied among species with prickly sculpin and cutthroat being detected most often; they were found at 85% and 84% of our sites, respectively. Conversely, Olympic mudminnow and coastrange sculpin were detected at only 2% of our sites.

Occupancy patterns also varied among species, with increased occupancy in downstream portions of a given river system (Figures 2 and 3). Predicted occupancy for Pacific lamprey, for example, was more expansive than longnose dace and both species' occupancy decreased in the headwater habitats (Figure 2 Pacific lamprey). Conversely, *Lampetra* spp. were found at many of our sampled sites and were predicted to occupy more of the basin, and, like other species, occupancy decreased in headwater habitats (Figure 2 *Lampetra* spp.). Species predicted to have widespread occupancy included rainbow trout, coho salmon, torrent and prickly sculpin, signal crayfish, and cutthroat trout. For other species, including riffle sculpin, coastrange sculpin, and Olympic mudminnow, predicted occupancy was relatively limited.

For some species, occupancy was focused regionally within the Chehalis. Chinook salmon had the highest probability of occurrence in the Skookumchuck and Newaukum Rivers, whereas shorthead sculpin's predicted occupancy was highest in both the headwaters of the Cascade and Olympic Mountain ecological diversity regions. Mountain whitefish and redside shiner had relatively higher predicted occupancy in mainstem tributary or larger stream habitats (Figure 2).

Overall, the native fish species richness ranged from 0.65 to 14.23 species within a modeled reach of the NSI (i.e., spaced every kilometer) with an average of 7.07. The highest richness was in portions of the mainstem tributary or larger stream habitats and decreased upstream (Figure 3).

In 2080 the native fish richness was predicted to range from 0.64 to 14.40 with an average of 7.50--an increase of 6% from current. Changes to individual species' overall predicted occupancy from current to future predictions varied from -13.7% in 2040 and -31.1% in 2080 for shorthead sculpin to 43.9% in 2040 and 148.4% in 2080 for three-spined stickleback (Figure 5). All species were predicted to increase occupancy except for coastrange sculpin (-6.1%), Olympic mudminnow (-13.3%), and shorthead sculpin (-31.1%).

We present and describe the parameters associated with these predicted occupancy patterns (Figure 4). Relationships varied by species with cumulative drainage being the largest positive covariate (redside shiner, 2.22) and elevation being the largest negative covariate (Pacific lamprey, -1.12). Across all species, cumulative drainage and elevation were also the most frequent largest positive and negative covariates, respectively.

Discussion

We described occupancy patterns for 19 native freshwater fish and two native freshwater shellfish in the Chehalis River basin, providing insight into the native fishes' basin-wide distributions. These inform critical data gaps and represent the first-time predictions of occupancy are available for the entire Chehalis Basin for many of these species, including the cyprinids, cottids, and unionids, which have not previously been the focus of research. This information enables managers to focus and prioritize conservation efforts at locations and scales across management areas that will affect the most change for an organism or population. It also provides an easily accessible tool to visualize and integrate occupancy information into watershed management. These data help inform and plan restoration projects by describing the likelihood that these fishes occupy a location. For example, if you were targeting culvert replacement, this tool could help you prioritize based on the number of native species that would be impacted by a given project or where a target species is predicted to occur. Additionally, our MSOM showed minimal predicted occupancy for Spring Chinook in tributaries within the Chehalis other than the Skookumchuck River and Newaukum River drainages. This reinforces that restoration efforts for Spring Chinook should be focused in these rivers.

We evaluated the relationships between landscape variables (parameters for covariates in our MSOM) and species occupancy. These data can inform the likelihood of occupancy in a stream or reach based on current or proposed changes to temperature or canopy. For example, our model indicates the temperature covariates for northern pikeminnow and three-spined stickleback are some of the largest positive values in our model, so increased temperature would increase the likelihood of their occupancy.

In addition, we estimated changes to individual species occupancy in 2040 and 2080, highlighting the groups of native fishes and shellfish that will increase and decrease their predicted occupancy and to what degree based on future climate change conditions. We also provided predicted occupancy patterns for native fishes under a 2080 climate scenario. This enables mangers to identify areas that have the most native fish for targeting forward-looking restoration and identify which areas are more sensitive to change.

Adaptive Management

This work can directly inform the Steering Committee decisions and provide guidance to sponsors developing habitat project by informing occupancy patterns for aquatic species for which little to no information was previously available. The Steering Committee and sponsors can compare restoration actions to ensure that target species are included. It also highlights where we can expect high and low native fish occupancy throughout the basin, which is useful for project scoping and locating projects. For example, looking at species with regional occupancy patterns, this study could inform targeted areas for conservation. Chinook salmon had the highest probability of occurrence in the Skookumchuck and Newaukum Rivers whereas shorthead sculpin’s predicted occupancy was highest in both the headwaters of the Cascade and Olympic Mountain ecological diversity regions. Mountain whitefish and redside shiner had relatively higher predicted occupancy in mainstem tributary or larger stream habitats.

Figures and Tables

Figure 1: Sites sampled in the Chehalis River Basin from 2019, 2020, and 2021 field seasons as well as the ecological diversity regions (EDRs). Inset shows the location of the Chehalis Basin within Washington State, USA.

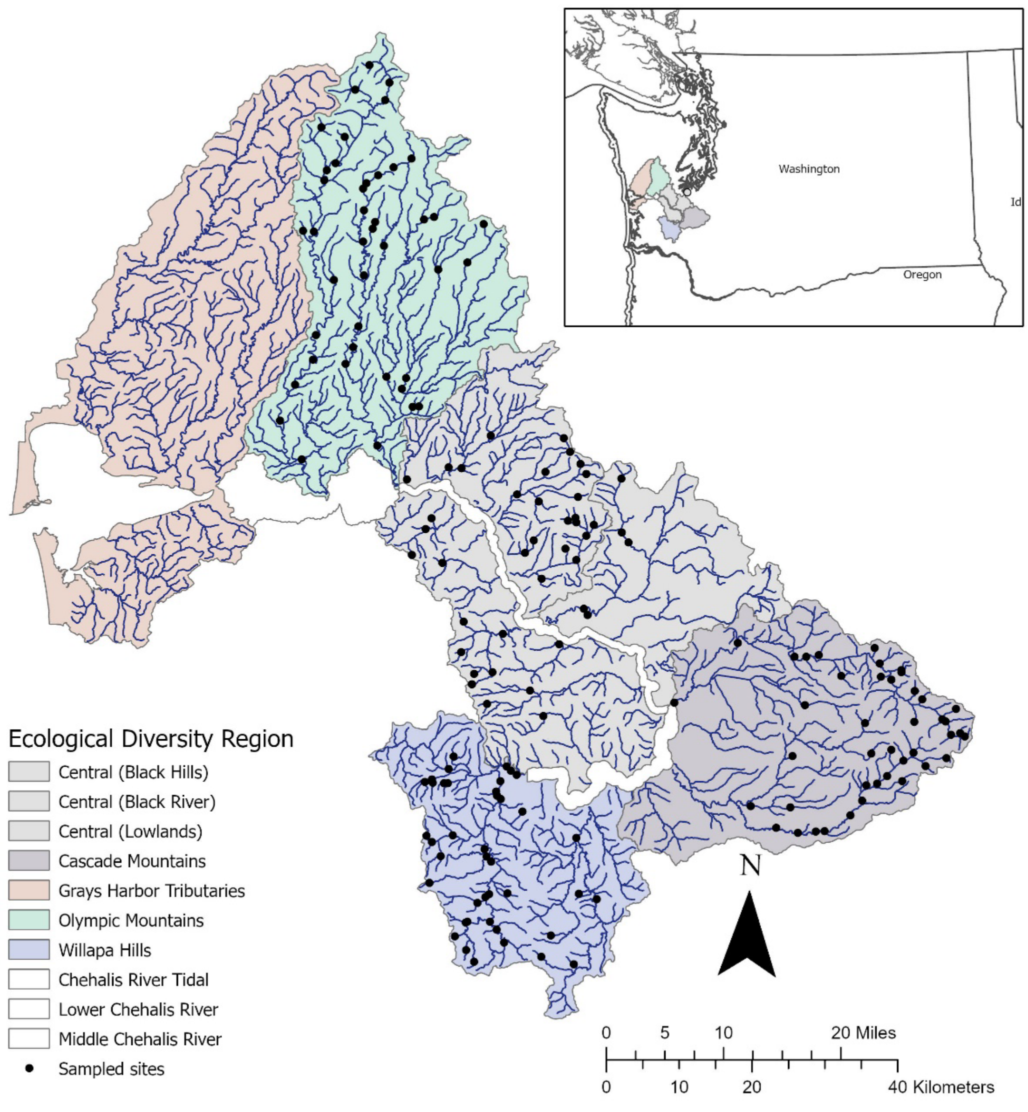
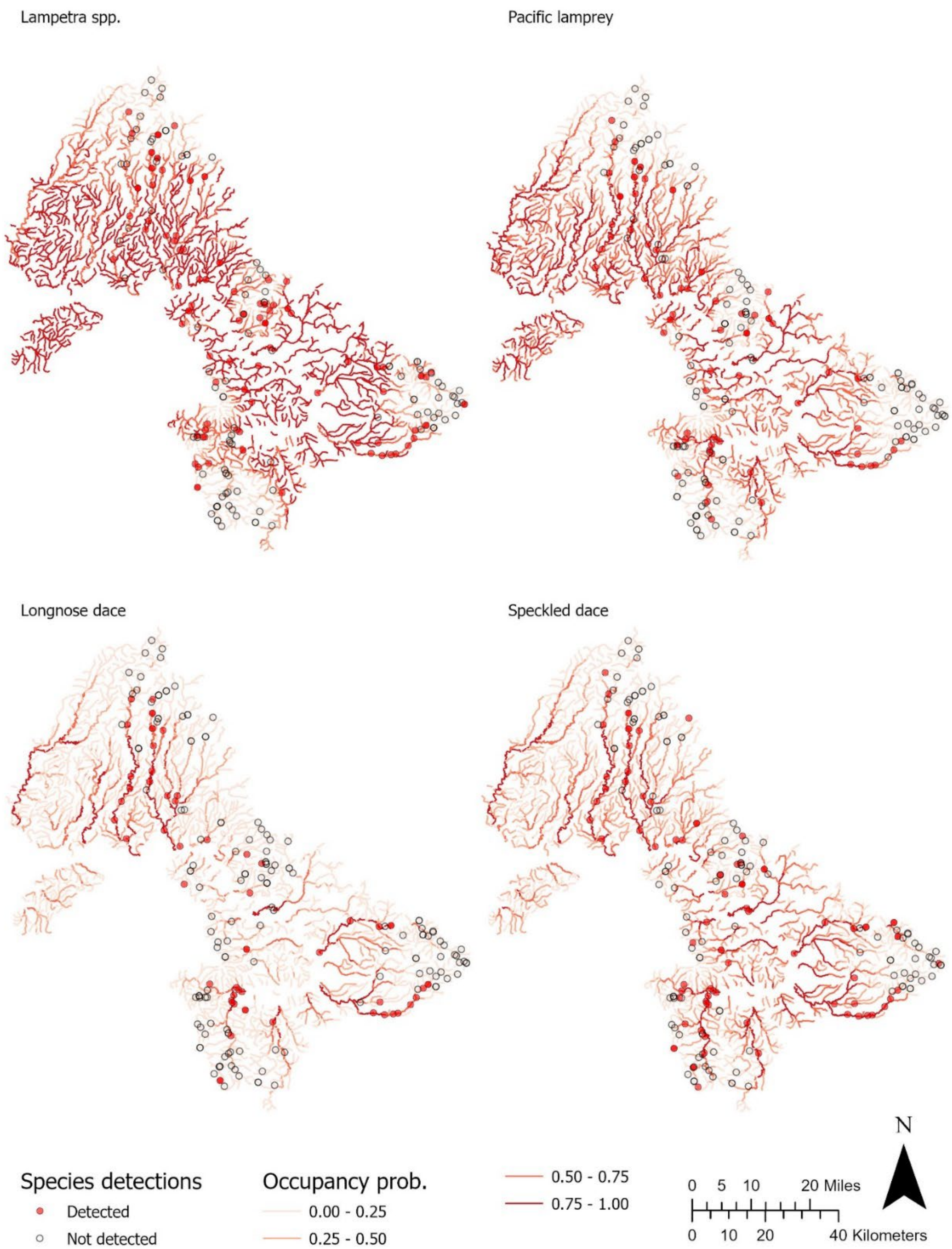


Figure 2: Native fish and shellfish detections at sampled sites (from on eDNA, electrofishing, and snorkeling) and probability of occupancy predicted by our multispecies species occupancy model (MSOM) for the Chehalis Basin.




Mountain whitefish
Rainbow trout
Chinook salmon
Coho salmon
Species detected
Not detected
Detected
Occupancy prob.
0.0 - 0.25
0.25 - 0.50
0.50 - 0.75
0.75 - 1.00
0 5 10 20 Miles
0 10 20 40 Kilometers
N


Northern pikeminnow
Largescale sucker
Redside shiner
Threespine stickleback
Species detected
Detected
Not detected
Occupancy prob.
0.00 - 0.25
0.25 - 0.50
0.50 - 0.75
0.75 - 1.00
0 5 10 20 Miles
0 10 20 40 Kilometers
N


Torrent sculpin
Riffle sculpin
Shorthead sculpin
Prickly sculpin
Species detected
Detected
Not detected
Occupancy prob.
0.00 - 0.25
0.25 - 0.50
0.50 - 0.75
0.75 - 1.00
0 5 10 20 Miles
0 10 20 40 Kilometers
N


Coastrange sculpin
Olympic mudminnow
Signal crayfish
Western pearlshell mussel
Species detected
Detected
Not detected
Occupancy prob.
0.00 - 0.25
0.25 - 0.50
0.50 - 0.75
0.75 - 1.00
0 5 10 20 Miles
0 10 20 40 Kilometers
N

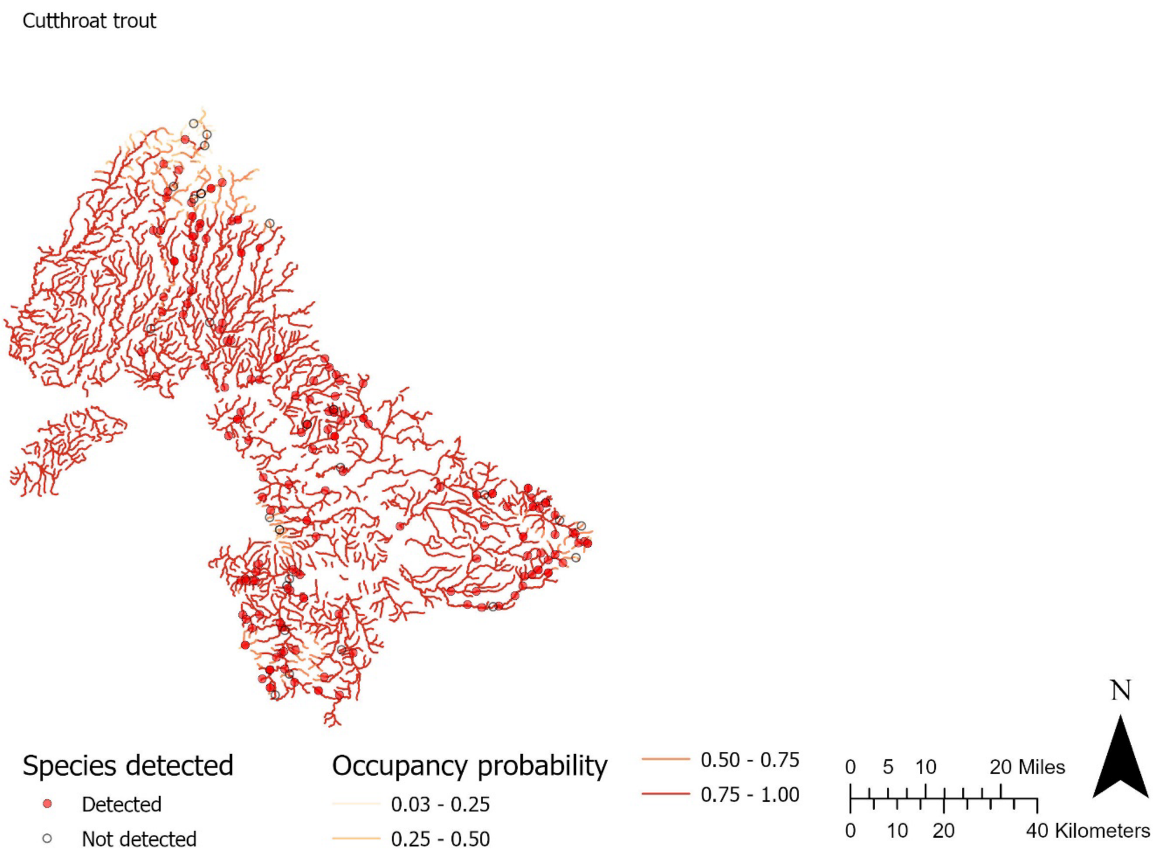

Cutthroat trout
Species detected
Detected
Not detected
Occupancy probability
0.03 - 0.25
0.25 - 0.50
0.50 - 0.75
0.75 - 1.00
0 5 10 20 Miles
0 10 20 40 Kilometers
N

Figure 3: Native fish species richness predicted by our multispecies species occupancy model (MSOM) for the Chehalis Basin for 2019 and predicted change in occupancy for native fishes in 2080.

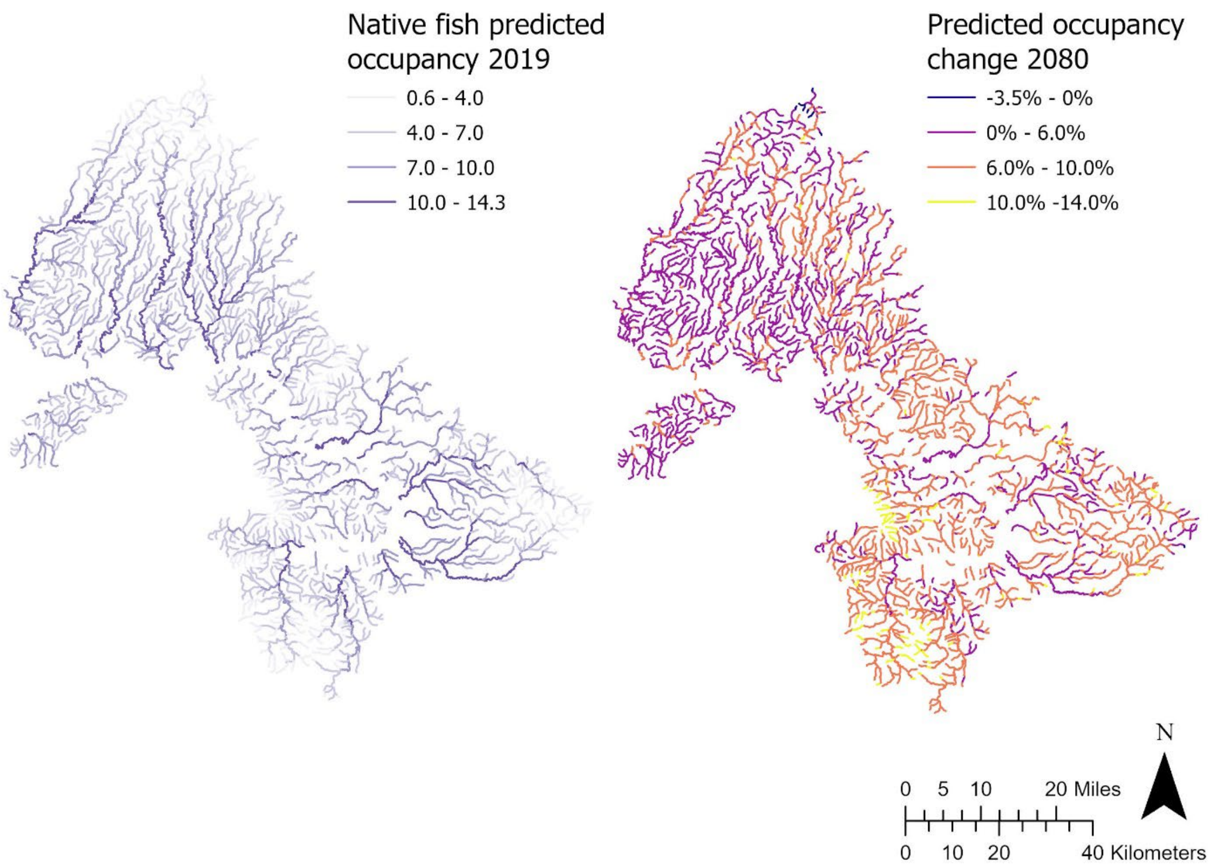
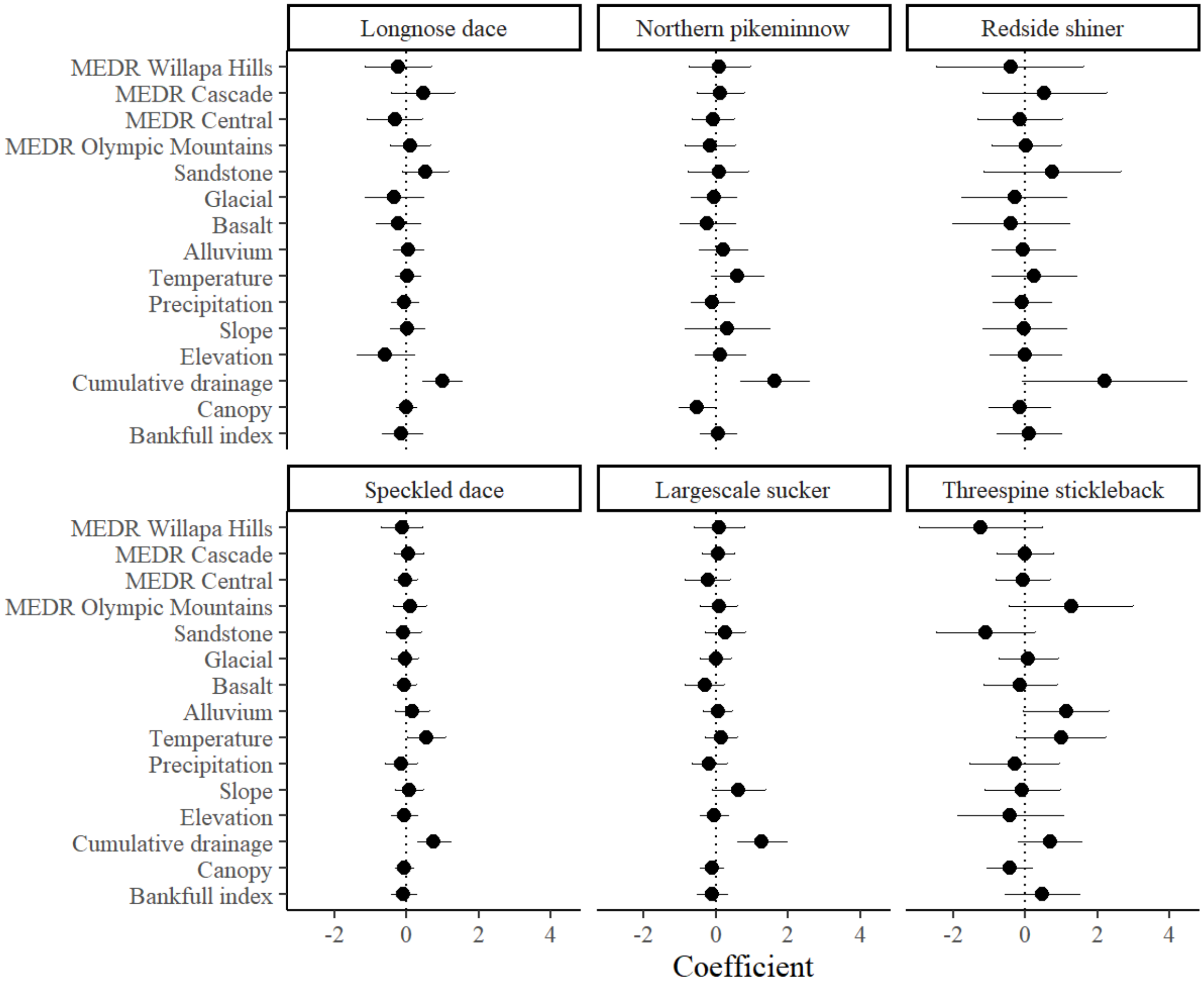
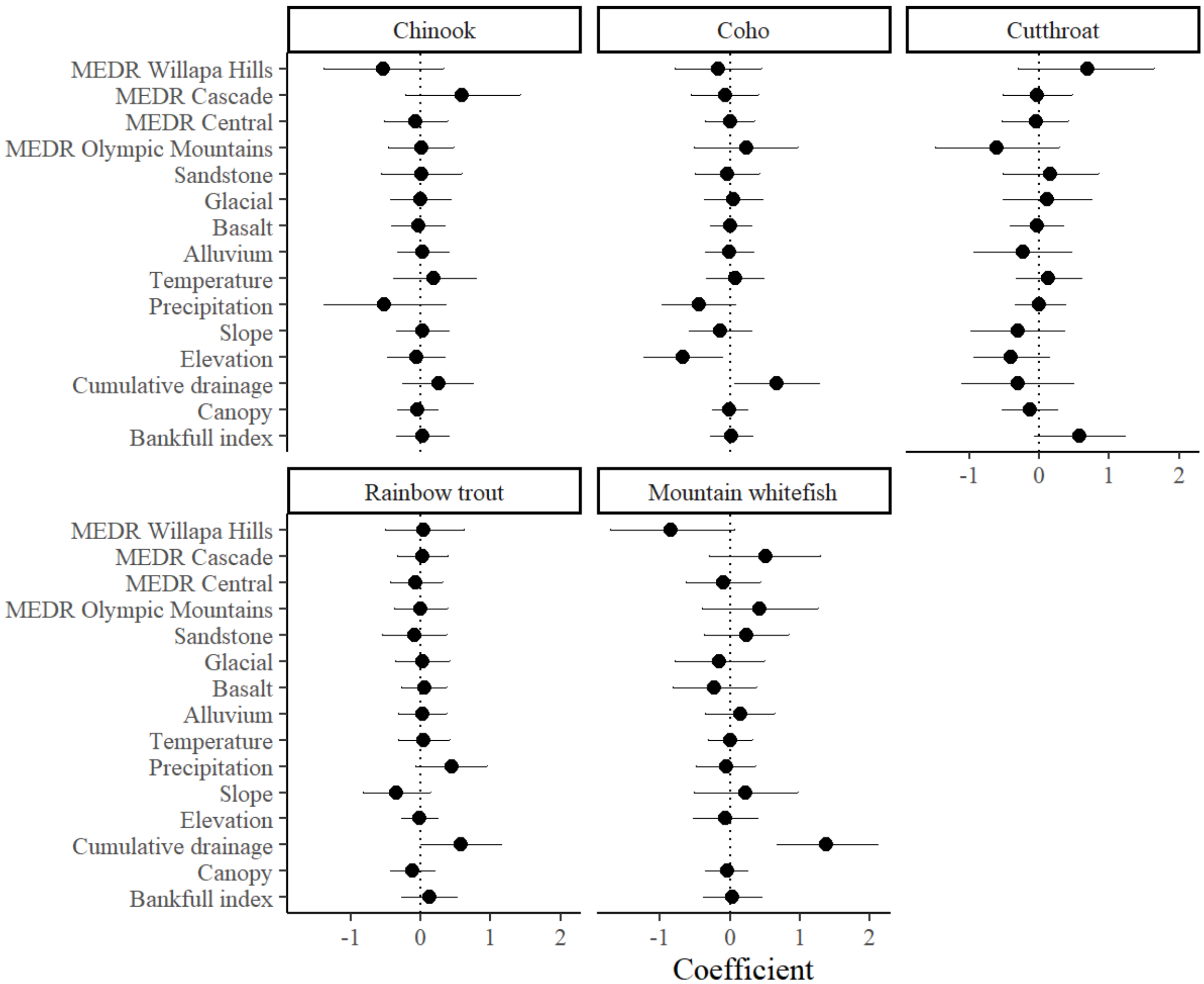
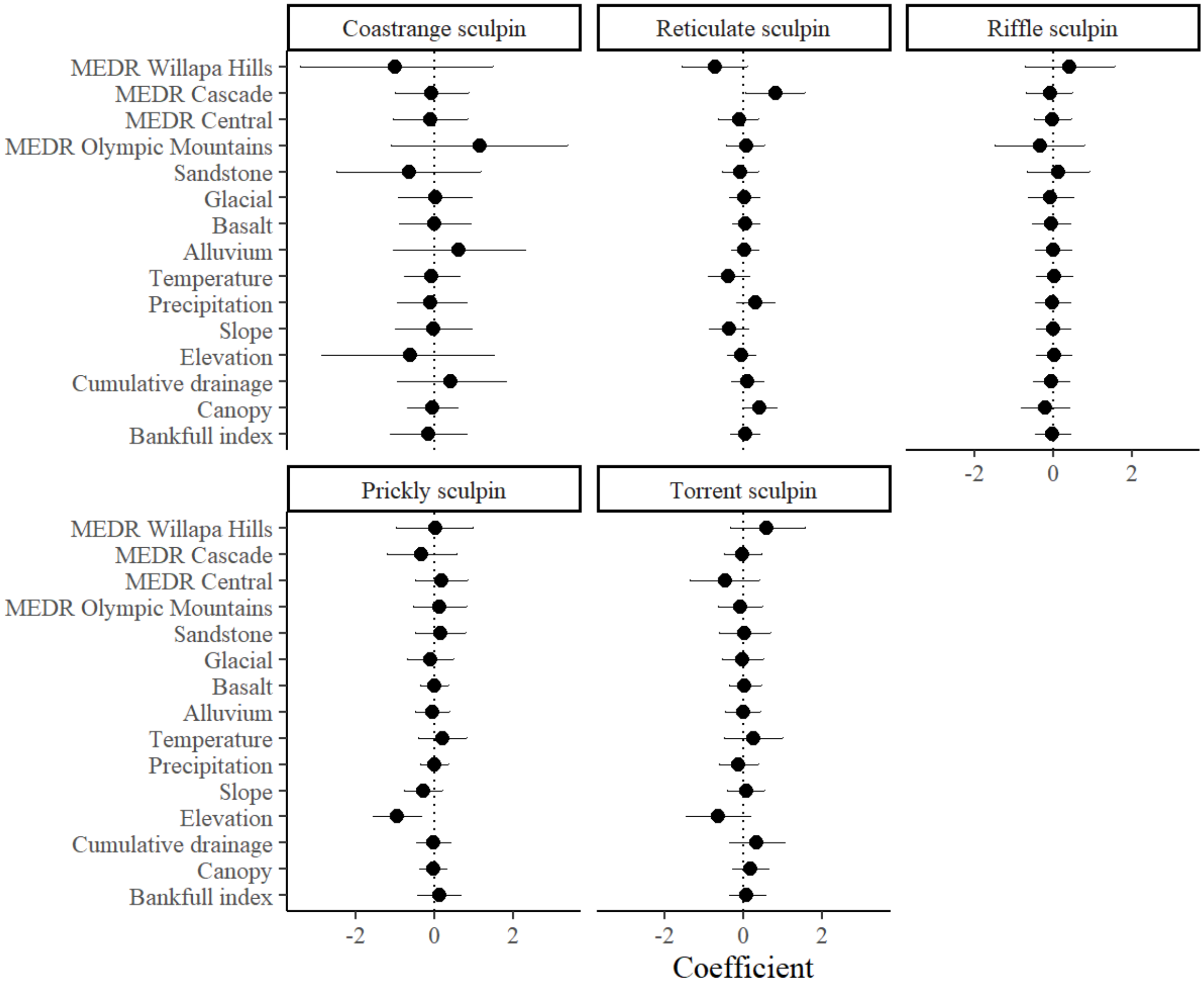


Figure 4: Parameters for covariates from Multispecies Occupancy Model (MSOM) for individual species. Points are median values and lines are upper and lower 95% confidence intervals




Chinook
Coho
Cutthroat
Rainbow trout
Mountain whitefish
MEDR Willapa Hills
MEDR Cascade
MEDR Central
MEDR Olympic Mountains
Sandstone
Glacial
Basalt
Alluvium
Temperature
Precipitation
Slope
Elevation
Cumulative drainage
Canopy
Bankfull index
-1
0
1
2
Coefficient


Coastrange sculpin
Reticulate sculpin
Riffle sculpin
Prickly sculpin
Torrent sculpin
MEDR Willapa Hills
MEDR Cascade
MEDR Central
MEDR Olympic Mountains
Sandstone
Glacial
Basalt
Alluvium
Temperature
Precipitation
Slope
Elevation
Cumulative drainage
Canopy
Bankfull index
-2
0
2
Coefficient

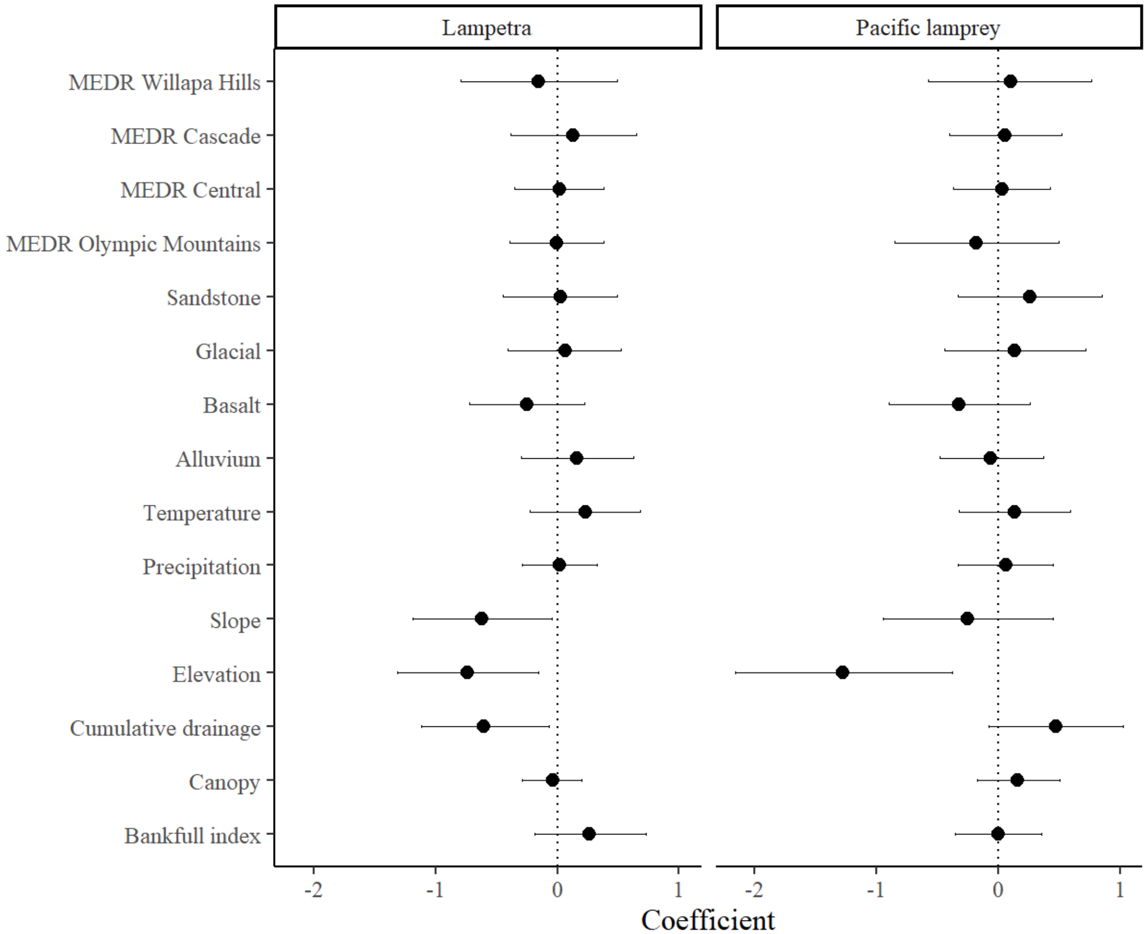
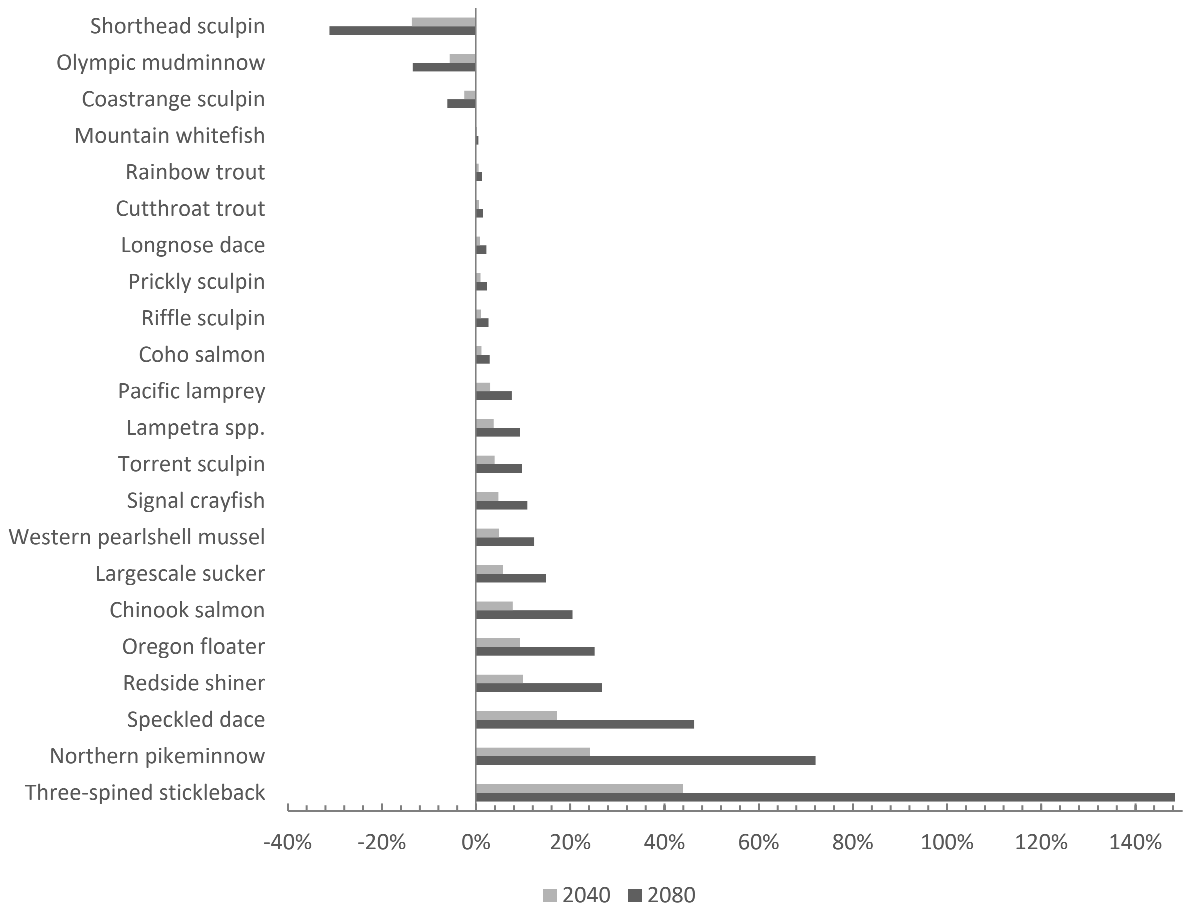

Lampetra
Pacific lamprey
MEDR Willapa Hills
MEDR Cascade
MEDR Central
MEDR Olympic Mountains
Sandstone
Glacial
Basalt
Alluvium
Temperature
Precipitation
Slope
Elevation
Cumulative drainage
Canopy
Bankfull index
-2
-1
0
1
Coefficient

Figure 5: Predicted occupancy changes (%) from current (2019) to 2040 and 2080 Chehalis Thermalscape temperature scenarios.



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