CHEHALIS BASIN STRATEGY

STREAM-ASSOCIATED AMPHIBIANS

Study Goals and Objectives

Stream-Associated Amphibians (SAAs) include Coastal Tailed Frog (*Ascaphus truei*), two species of Torrent Salamander (*Rhyacotriton* spp.), and Giant Salamander (*Dicamptodon* spp.). Tailed Frogs are an ASRP indicator species and the Torrent and Giant Salamanders include two Washington Species of Greatest Conservation Need. Because SAAs inhabit headwater streams (~1st to 3rd orders), they are particularly sensitive indicators of habitat impacts associated with climate change and land use. Our SAA surveys throughout the Chehalis Basin support ASRP goals by identifying the distribution, habitat associations, and long-term trends of these sensitive indicator species.

From 2018-2021 we sampled over 1,110 stream reaches throughout the Chehalis Basin (Humptulips, Satsop, Wynoochee, Upper Chehalis, and Stillman) as a preliminary assessment of the distribution of these species. In 2022, we pivoted from this broad status assessment and selected a series of 117 reaches across these five watersheds for long-term status and trends monitoring. These long-term sites will be used to assess changes in the occupancy and abundance of SAAs as bellwethers of climate change and altered habitat conditions.

Our project goals are to describe the relationship between SAA occupancy and stream characteristics including stream order, land use, canopy cover, substrate, functional wood, and stream temperature. What interventions are needed to protect and restore headwater habitats remains unclear and this work will provide critical information need to prioritize areas of the Basin that could benefit from acquisition and other interventions. The results from our analyses will ideally provide insights into which stream, riparian, or landscape interventions may improve conditions for these species as well. Additionally, we have partnered with Dr. Lauren Chan at Pacific University to conduct genetic analyses on over 250 samples from our surveys. The goal of these genetic results is to identify the degree of connectivity among watersheds within the Chehalis Basin to identity functional units for prioritizing protection and restoration efforts.

Methods / Study Design

Based on our survey of over 1,100 stream reaches from 2018-2021, we established a subset of long-term monitoring sites that are representative of the distribution of these species across 1st-3rd order streams, throughout each of the five watersheds, and spatially distributed (**Table 1, Figure 1**). At these 117 sites in 2022 we marked permanent 30-m transects where we conduct SAA and habitat surveys. Each year we collect triple-pass light touch surveys (habitat-sensitive surveys in three sequential days). First order streams are sampled in April/May, second order streams in July/August, and 3rd order streams in August/September; We randomly select which sites to survey during each sample season and stratify stream order across the season to follow the water year given first order streams are more likely to dry than second and third order streams. Doing so minimizes the effect of interannual variation across stream orders.

Watershed	1st Order	2nd Order	3rd Order	Total
Humptulips	9	8	7	**24**
Wynoochee	9	9	9	**27**
Satsop	9	9	8	**26**
Stillman	6	5	4	**15**
Upper Chehalis	9	8	8	**25**
Total	**42**	**39**	**36**	**117**

Table 1. Distribution of long-term SAA monitoring sites across watersheds and stream orders.

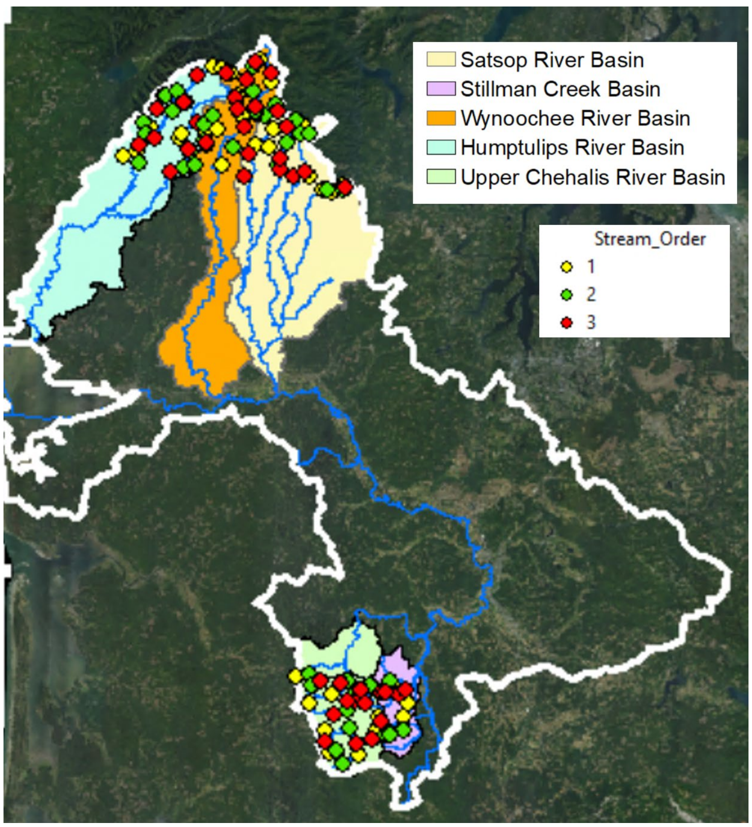

Figure 1. Long-term SAA monitoring sites throughout the Chehalis Basin that were established in 2022.

Each year at the beginning of each triple pass survey, we record several physical parameters including land use, conductivity, pH, gradient, wetted width, canopy cover/species, substrate and functional wood. All vertebrate species are identified and recorded with special emphasis on the direct cover habitat associated with the target amphibians. In 2022, we collected representative tissue samples from each amphibian species to maintain a tissue library reflective of the distribution of the SAAs in the basin. Triple pass surveys are necessary for SAAs because these species have a notoriously low detection probability and so three surveys in sequence increase the probability of detecting a species at a given location if it is present.

For 2022 SAA data, we used principal components analysis (PCA) to collapse habitat variables into multiple principal component (PC) axes for statistical analysis. We chose the top six PC axes which in aggregate accounted for over 70% of the variation in habitat data. We then used binomial generalized linear models to analyze the probability of each species' occupancy at a given site as a function of Watershed, Stream Order, Land Use, and the six PC axes. We then used an information theoretic approach in R to identify which suite of habitat variables were most associated with SAA occupancy.

For genetic analyses, we used existing microsatellites for Tailed Frogs and Giant Salamanders and a single mitochondrial locus for Torrent Salamanders. Tailed Frog and Giant Salamander analyses uses landscape genetics methods to determine gene flow and population differentiation among watersheds as well as which – if any – landscape features influenced connectivity within and among watersheds. The Torrent Salamander analysis was centered on confirming the validity of the two Torrent Salamanders as separate species (which has not been done yet) and using environmental niche models to assess differences in Chehalis Basin niches.

Summary of Results

In 2022, we detected Tailed Frogs at 69 sites (59%), Giant Salamanders at 78 sites (67%), and Torrent Salamanders at 51 sites (44%). 14 sites (12%) had no SAAs detected. At 71 sites (61%), at least two SAA species were detected and all three taxa were detected at 24 sites (21%).

Our habitat PCA produced 23 PC axes, the first six of which represented over 70% of the variation in the habitat data. For Habitat PC1, canopy cover, wetted width, and the percentage of boulder in the stream load positively whereas the percentage gravel and fines load negatively. For Habitat PC2, the percentage of boulders and pH load strongly positively whereas canopy cover loads strongly negatively. For PC3, the percentage of bedrock loads strongly positively, and the percentage of gravel loads strongly negatively. For PC4, the percentage of fines loads strongly positively, and the percentage of bedrock loads strongly negatively. For PC5, wetted width, gradient, and the percentage of gravel load strongly positively whereas conductivity loads negatively. For PC6 canopy cover and the percentage of boulders loads positively whereas wetted width, conductivity, and the percentage of cobbles load negatively.

For Tailed Frogs, model selection identified three models with similar rankings; the first carried 16% of model weight, the second carried 8%, and the third carried 7%. All three models included among-watershed differences and positive associations along Habitat PC1 and PC2 (**Figure 2**). Tailed Frog occupancy was lowest in the Upper Chehalis, highest in Satsop, and intermediate in the Stillman, Wynoochee, and Humptulips watersheds. Interestingly, Tailed Frogs in the Upper Chehalis display an opposite associated with Habitat PC1 than Tailed Frogs in all other watersheds, however this was likely due to the low occupancy in this watershed. In general, Tailed Frogs are associated with a higher percentage of boulders, higher pH, a greater wetted width, and a higher canopy.

For Giant Salamanders, there were seven models with similar rankings, but each only had 2-5% of the model weight. Positive associations with Habitat PC1 and PC2 were in all models and Land Use was a factor in 6 of the 7 models. For simplicity, we assessed the top-ranked model which contained Land Use, PC1, and PC2 only (**Figure 3**). Although Land Use was included in the best models, Tukey's post hoc tests could not identify differences in occupancy among Land Use categories, likely due to relatively small sample sizes in some Land Use categories (e.g., only eight recent clear cuts were assessed, two of which had Giant Salamanders). Even so, visual assessments of the data suggest that Giant Salamander occupancy is substantially reduced in recent clearcuts, and similarly high in mid-age and old forest stands with a tendency towards higher occupancy in older stands (**Figure 3**).

For Torrent Salamanders, there were 14 models with similar rankings, but each only had 2-4% of the model weight. 13 of these included among-watershed differences, all included a negative associated with Habitat PC4, 9 included an effect of Stream Order, and 8 included a positive association with PC5. Here, we assess a model with each of these four variables (the 4th ranked model; **Figure 4**). Although among-basin differences were included all but one of the top models, Tukey's post hoc tests could not identity differences in occupancy between basin; even so, there was a nearly-significant trend with lower Torrent Salamander occupancy in the Satsop drainage compared with both the Upper Chehalis and Stillman basins.

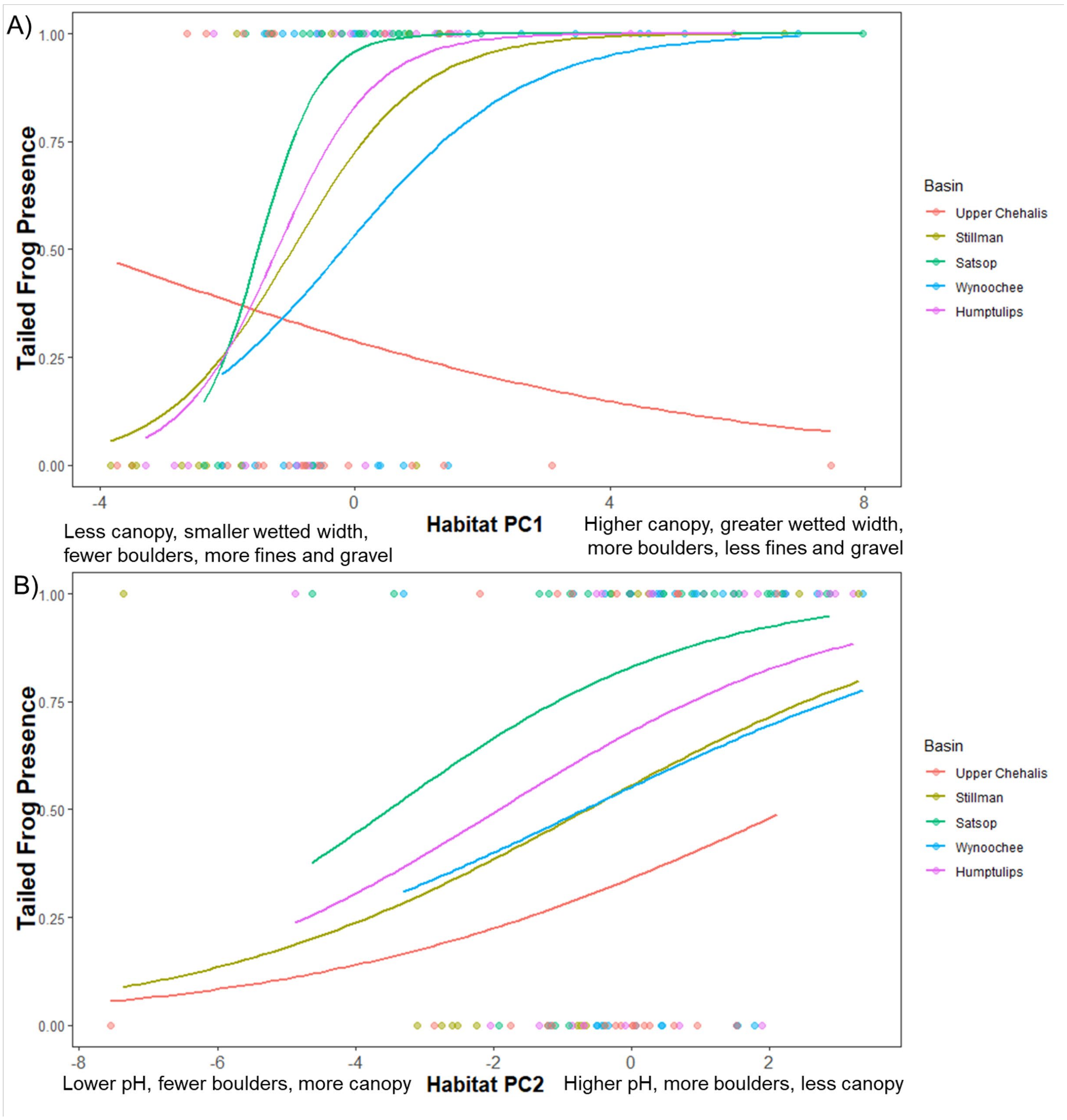

Figure 2. Habitat associations for Chehalis Basin Tailed Frogs based on 2022 triple pass surveys at 117 sites.

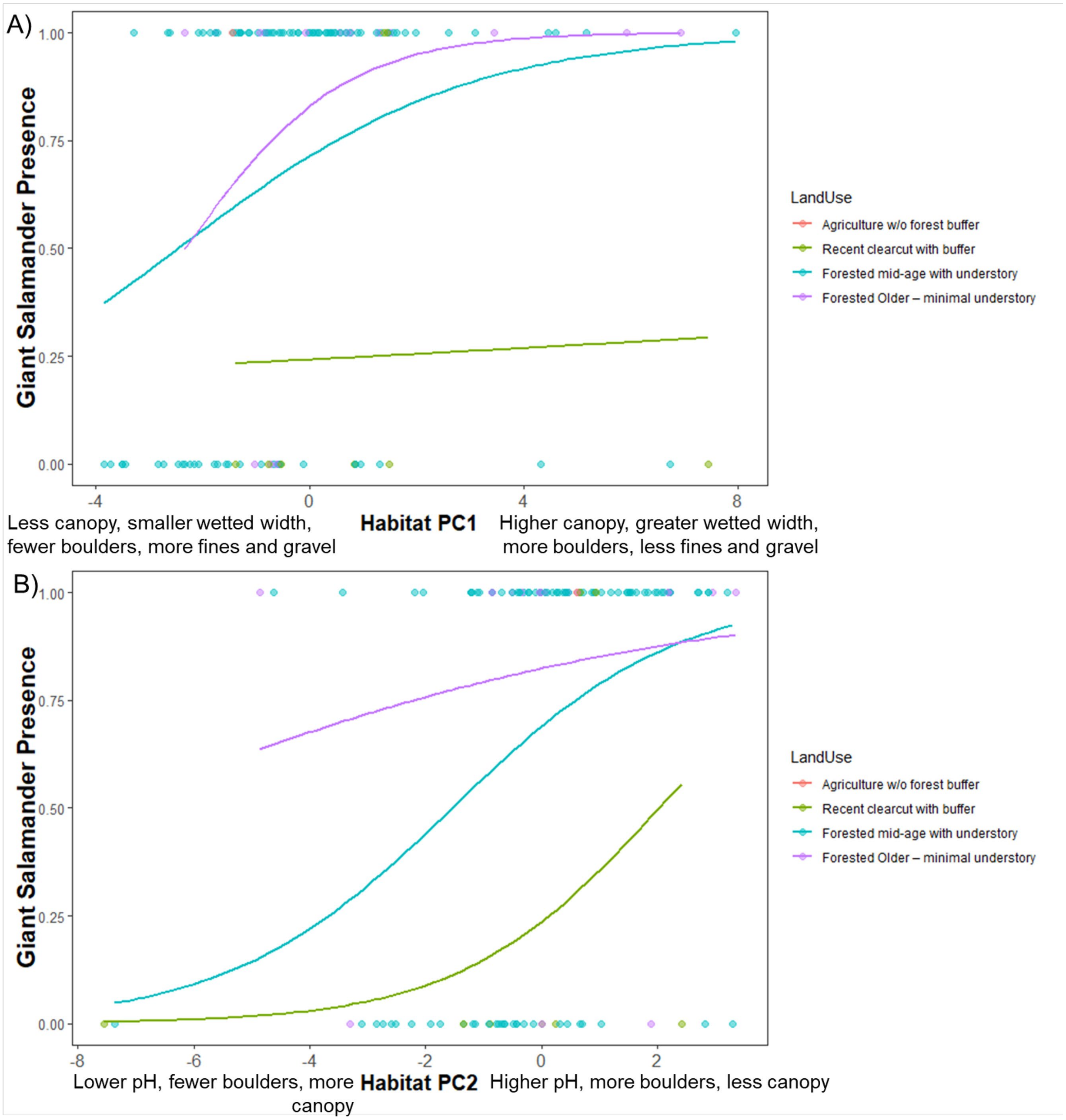

Figure 3. Habitat associations for Chehalis Basin Giant Salamanders based on 2022 triple pass surveys at 117 sites.

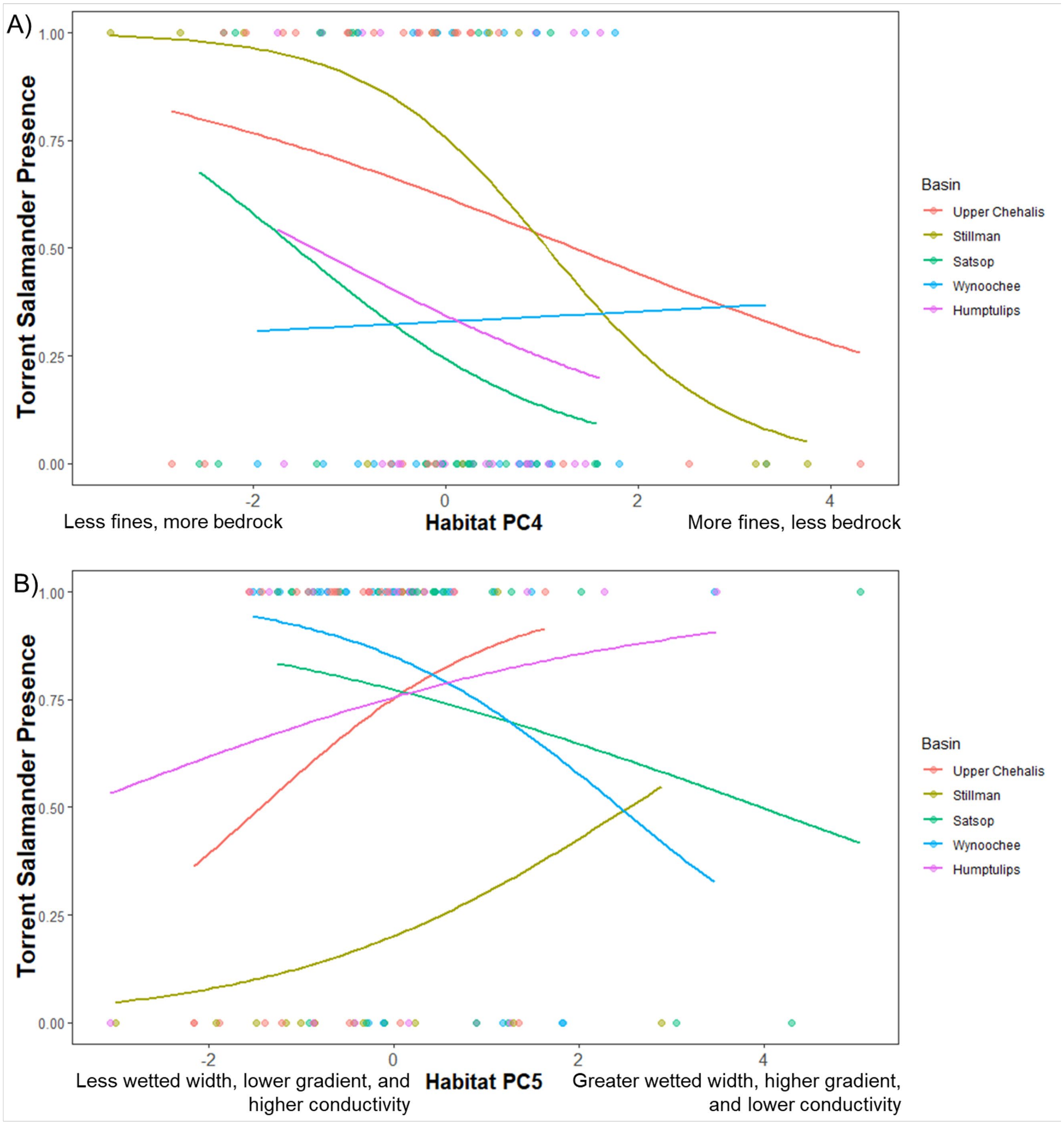

Figure 4. Habitat associations for Chehalis Basin Torrent Salamanders based on 2022 triple pass surveys at 117 sites.

Genetic Results: Landscape genetic analyses found that Tailed Frogs and Giant Salamanders differed in their response to landscape features such as stream order, geographic distance, slope, canopy cover, or land use. Specifically, Tailed Frogs showed substantial admixture throughout the Olympics with little effect of landscape features. Tailed Frogs in the Upper Chehalis and Stillman watersheds are genetically distinct from each other and from the Olympics, but all Olympic watersheds are admixed (**Figure 5**). Giant Salamanders show a similar separation between the upper Chehalis and the southern Olympics but also population differentiation within the

Olympics (**Figure 6**). Further, Giant Salamander gene flow and connectivity is influenced by canopy cover, land use, and other landscape features.

Mitochondrial analyses confirmed decades-old allozyme results and establish that the Olympic Torrent Salamander (*R. olympicus*) and Columbia Torrent Salamander (*R. kezeri*; coastal southwest Washington and northwest Oregon) are distinct species such that the Chehalis River represents a historical and ongoing barrier for the species. Environmental niche models emphasize that these two Torrent Salamander species also have distinctly different climatic niches (**Figure 7**) which has potential implications for managing these two species under climate and land use change.

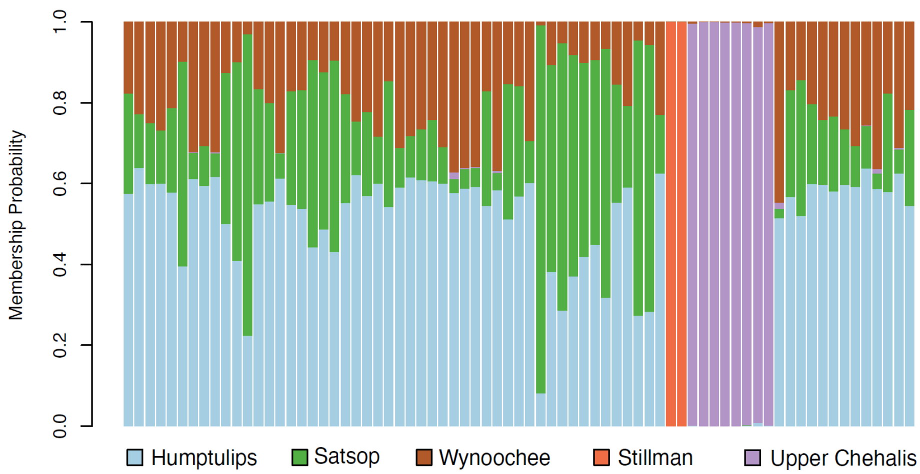

Figure 5. Population genetic structure for Tailed Frogs in the Chehalis Basin. Upper Chehalis and Stillman watershed Tailed Frogs are genetically distinct from each other and from the Olympics. However, Olympic watersheds (Wynoochee, Humptulips, and Satsop) shows widespread admixture and no effect of landscape features on genetic connectivity.

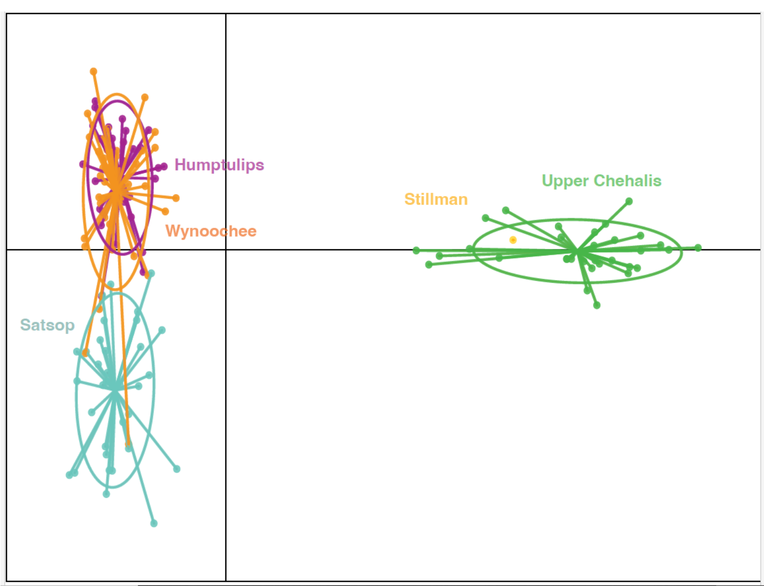

Figure 6. Population genetic structure for Giant Salamanders in the Chehalis Basin. Substantial genetic structure occurs separating the Olympics from the Upper Chehalis as well as the Satsop watershed from the Wynoochee and Humptulips. Within the Olympics, Giant Salamander genetic connectivity is influenced by stream order, canopy cover, slope, and land use.

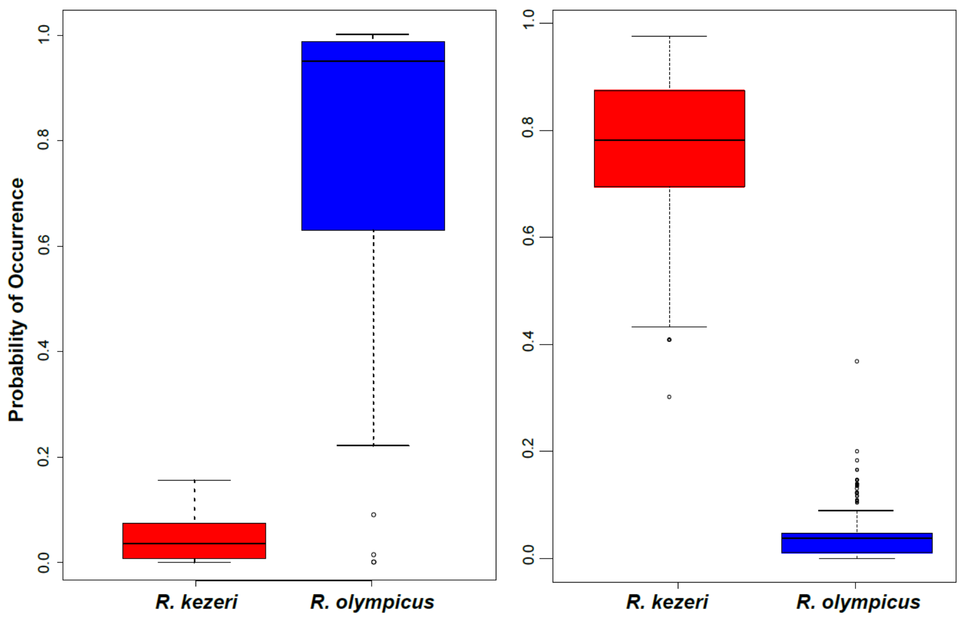

Figure 7. Environmental niche models for the two Torrent Salamander species illustrating that each has a high probability of occurrence in their own climate niche but a low probability in the others' climate niche.

Discussion

The results presented here are the first year of intensive long-term monitoring for SAAs. As such, additional years of data are necessary to determine whether habitat associations are consistent for these species or fluctuate with changing climatic and land use conditions. Regardless, our analyses provide robust habitat association results that are distinct for each species. Such findings are essential should the ASRP choose to prioritize certain habitats for additional protections or habitat enhancement actions.

Tailed Frogs show an association with canopy cover as well as in-water conditions. Interestingly, genetic analyses show substantial gene flow in the southern Olympics but relatively high structuring between the Stillman and Upper Chehalis watersheds which may indicate that the more intensive forestry operations in the southern half of the Chehalis Basin may be constraining habitat availability and connectivity for Tailed Frogs. Indeed, we detected Tailed Frogs at fewer than 1/3 of samples sites in the Upper Chehalis Watershed and ~50% of Stillman reaches compared to 60-80% of reaches in each of the Olympic watersheds. In contrast, Torrent Salamanders had the highest occupancy in the Upper Chehalis and Stillman watersheds, occurring at ~ 2/3 of reaches surveyed, but occurred at only ~1/3 of reaches surveyed in each of the Olympic watersheds. Habitat association models for Torrent Salamanders showed little influence of canopy cover but in-stream conditions like substrate, wetted width, gradient, and conductivity. Further, the two Torrent Salamanders show distinct climatic niches between the northern and southern halves of the Chehalis Basin. Increased forest protections and connectivity may therefore be best suited for enhancing habitat and resiliency for Tailed Frogs but Torrent Salamanders likely require protections of streams with particular hydrologic and geomorphic conditions that may not be readily manipulatable.

Findings for Giant Salamanders are particularly interesting as our habitat association modeling suggests a relatively strong association with canopy cover and stand age on this taxon's occupancy. Genetic results further underscore that landscape conditions including canopy cover influence the genetic connectivity of this species. As such, forest protections targeted at Tailed Frogs will likely have co-benefits for Giant Salamander in addition to prioritizing streams with substrates comprised of larger rocks which both species are associated with.

In 2023 we initiated the second full year of triple-pass monitoring at these newly established long-term monitoring sites and are eager to assess whether the patterns identified here are consistent over time. Interannual variation in weather conditions, longer term changes in climate (temperature and precipitation), as well as changes in land use (timber operations) within and across watersheds will allow us to detect how habitat alterations influence the occupancy of these species. Even without multiple years of data available, the habitat association data here can begin to provide guidance for the types of habitat conditions that are best suited for protection under the ASRP for SAAs. Further, our analyses underscore the utility of SAAs as sensitive indicators of habitat conditions and climate change for the Chehalis Basin.

Adaptive Management

This work provides adaptive management information for the ASRP Steering Committee based on both science and policy loops. In particular, SAAs are considered sensitive indicators of environmental conditions. The analyses presented here illustrate the tight association of each SAA to particular in-stream, landscape, and/or climatic variables. Over time, these long-term monitoring sites will provide critical information as to the impacts of climate change and land use on these species and underscore regions of the Basin that may require additional protections or restoration actions. Further, by illuminating the specific habitat conditions associated with each species, the Steering Committee can better provide guidance to project sponsors as to which lands may be best suited for acquisitions and protections as well as which types of stream reaches are most likely to serve as quality SAA habitat. Currently, beyond protecting existing forest and enhancing forested upland conditions, few habitat interventions are available for SAAs. Our long-term monitoring may eventually point to habitat features that could be manipulated to benefit these and other species. However, this work in the context of the broader body of science underscores a need for additional research targeting what habitat interventions would bolster these species, particularly under climate change. Our study sites provide a useful baseline for which to compare any future experimental restoration work aimed at SAAs. Further, such experimental restoration efforts may benefit from occurring at a subset of our long-term monitoring sites to leverage our existing data to best clarify restoration effectiveness. Given Tailed Frogs, specifically, are an indicator species for the ASRP and they are notably depauperate in the Upper Chehalis and Stillman watersheds, our data point to the potential value for additional protections in these regions. Further, the Columbia Torrent Salamander (in the Upper Chehalis and Stillman watersheds) and Cope's Giant Salamander (found throughout the Chehalis Basin) are WA Species of Greatest Conservation Need and are undergoing Federal Status Review. Proactive measures for these species could leverage habitat conclusions from our work to guide project applicants' decision making.