

BUMBLE BEE COMMUNITY COMPOSITION AND POPULATION GENETIC DIVERSITY IN THE NORTH CASCADES AND COAST NETWORK

2013 PROGRESS REPORT

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ABSTRACT

Several bumble bee species are experiencing dramatic declines in abundance and range throughout the United States. In the present study we conducted standardized surveys at 21 different locations across seven Pacific Northwest National Parks. The most abundant species detected in our survey was *B. flavifrons*, found in five of the parks, while the least abundant species were *B. griseocollis* and *B. nevadensis* both found only at one park. The average species diversity across the survey sites is 4.8 ± 1.4 species. Our results show that species diversity is positively correlated with elevation ($R = 0.49$, $P < 0.05$) and not latitude ($R = 0.04$, $P = 0.84$) across the parks. The highest species diversity was found in Olympic National Park (OLYM), where nine species were identified. In OLYM we detected the imperiled *B. occidentalis*, and expanded the known distribution of *B. sylvicola* onto the Olympic peninsula. Regardless of the park, we found that high elevation sites were more similar in species diversity, and harbored elevation-restricted species. Habitat suitability models of each species suggests that high elevation communities will likely be at greater risk to temperature and precipitation changes in the next 80 years as predicted by Intergovernmental Panel on Climate Change. Our population genetic study is ongoing, and we will submit the results of the study when completed. We also conducted workshops on bumble bee natural history and conservation for park personnel and the general public, presented two evening lectures for park visitors, and distributed a handout on topics covered during the identification and conservation workshops. Finally, in an effort to make our research accessible to park visitors, we have summarized our research on bumble bee diversity in the park in the form of poster.

INTRODUCTION

Pollinator communities worldwide are undergoing dramatic changes in both abundance and composition that may put pollination service at risk in many terrestrial ecosystems (Winfrey et al. 2009). These changes may not be unidirectional declines in species abundance, but may manifest as shifts in geographic range or increases in abundance where new habitat is formed. To

date, documented changes in pollinator communities have been attributed to many factors, including pathogen outbreaks, pesticides, and land-use change (Goulson et al. 2008). However, the underlying mechanisms of these changes are not fully understood (Cameron et al. 2011, Bartomeus et al. 2011). Identifying the factors affecting pollinator communities can be difficult as most strategies investigate distinct taxonomic groups (guilds) (Cameron et al. 2011) or attempt to isolate specific threats, measuring a single species' responses to threat in question (Whitehorn et al. 2012).

Climate change is an emerging threat to pollinators, yet it remains poorly studied because isolating the effects of climate from other potential factors is difficult (Bartomeus et al. 2011, Williams et al. 2008). To date, the effects of climate warming on bees have only been studied from museum records of bee phenology (Bartomeus et al. 2011) which showed earlier springtime activity of bees in the northeastern United States as climate warmed. In montane regions, animals are predicted to follow plant distributional shifts up slope as climates warm (citation), but where species are currently adapted to high elevation habitat it is unclear if they can adapt *in situ*. Under currently projected climate models bumble bee distributions are predicted to shift to higher latitudes in the cases where elevation is limited.

Bumble bees (*Bombus*) are important pollinators of wild flowering plants (Bauer 1983) especially in montane and alpine environments. The Pacific Northwest is rich in both wildflower and bumble bee diversity, largely in part to the environmental heterogeneity resulting from the region's complex topography (Koch et al. 2012). In the Pacific Northwest, one bumble bee, *Bombus occidentalis*, is known to be at risk, while another, *Bombus vosnesenskii*, may be expanding in range (Cameron et al. 2011, Fraser et al. 2013). Several other species are also suspected of undergoing changes in range or abundance in the region, yet empirical data is currently lacking (Cameron et al. 2010). The North Cascade and Coast Network (NCCN) National Parks and Monuments are situated across an elevation and latitude gradient that allows direct study of the community composition and genetic diversity of bumble bee pollinators and further understanding of anthropogenic community changes. A recent, comprehensive study has documented changes in bumble bee abundance within National Parks, including the disappearance of *Bombus occidentalis* from most of the Pacific Northwest (Cameron et al. 2011). Investigating bumble bee community composition and genetic diversity in the North Coast and Cascades Network (NCCN) will provide park management baseline data on bumble bee pollinator health, which may be used as a benchmark to future monitoring efforts (Cameron et al. 2010).

The aims of our study are as follows:

Aim 1: Survey and identify the composition of bumble bee communities and genetic diversity across an elevation transect in Mt. Rainier (MORA), North Cascades (NOCA), Olympic (OLYM) National Parks, and San Juan Island (SAJH), Ebey's Landing (EBLA), Fort Vancouver (FOVA), and Lewis and Clark National Historic Park (LEWI). We hypothesize, based on our preliminary data, that bumble bee species diversity will be highest at mid-elevations with low and high elevations exhibiting lowest diversity.

Aim 2: *Train NCCN park staff and collaborating researchers to survey sites in National Park to monitor bumble bee community composition and genetic diversity.* As part of the ongoing initiative by the NCCN Science Learning Network (SLN), our secondary goal in our study is to train NCCN park staff on survey and identification techniques of wild bumble bee pollinators.

Aim 3: *Provide the SLN with a narrative that identifies the importance of research and monitoring of bumble bee pollinators in the NCCN national parks.* We will include the results of our survey efforts and historic specimen data from natural history collections that identify the bumble bee pollinators found in NCCN national parks. The narrative will be in the form of a brochure or poster that can be integrated into visitor information and education.

MATERIALS AND METHODS

Aim 1: Study of Bumble Bee Community Composition and Genetic Diversity

To assess the bumble bee community composition within parks and at different elevations within parks, in July 2013 we visited 21 sites in the seven NCCN parks (Figure 1, Supplementary Table 1). In OLYM and MORA, two elevation transects were surveyed, while NOCA had one transect surveyed. In EBLA, LEWI, and FOVA, one site at each park was surveyed. In SAJH, two sites were surveyed. In NOCA and OLYM, we revisited some locations surveyed in a previous study (Cameron et al. 2011; Supplementary Table 1).

Sites were surveyed by teams of individuals using random and haphazard net collections of bumble bees at plots of approximately 0.5 ha however sites varied based on floral density and accessibility for off trail movement. To standardize collection effort, surveys were timed and collections were numerically standardized to 1.5 collector hours per site when feasible. Collectors surveyed with aerial insect nets (30 cm diameter) and collected bumble bees foraging on flowers directly into 20 mL plastic vials. The vials were placed on ice for 10-15 minutes until surveys were complete and the bees were immobilized by the cold. Upon completion of the survey period, the bumble bees were sexed and preliminarily identified to species following Thorp et al. (1983) and Koch et al. (2012). While the specimens were immobilized, we non-lethally sampled DNA from the bumble bees by removing a mid-leg from each individual (Holehouse et al. 2002). The mid-legs were individually stored in 95% ethanol for down-stream DNA analysis to verify the species identity. At each site, a worker and male of each captured species were sacrificed and retained as voucher specimens. All queens were released after legs were sampled.

During the survey period we associated and recorded floral hosts to each bee specimen and collected pertinent environmental site data. Voucher specimens of the flowering plants in the immediate area were collected and pressed for identification in the lab if the species could not be identified in the field. Each survey event was associated with a unique locality description and georeferenced with a Garmin GPSmap 60CS. We recorded temperature (°C), relative humidity (%), and wind speed (kph) data with a Kestrel 4000 Pocket Weather Tracker.

Voucher specimens were pinned and associated with a unique barcode ID and curated into the USDA-ARS National Pollinating Insect Collection (NPIC) in Logan, UT (Supplementary Table

2). Genotyped individuals will also be given a unique ID, an NPS accession number, and included in the NPIC database. The data will be stored digitally in a relational database at the NPIC and made readily available to the NCCN and broadly through Global Biodiversity Information Facility following NPS guidelines.

On return to Logan, UT, total DNA was extracted from the mid-leg and genotyped using 10-12 highly variable DNA microsatellite markers. These markers are selected not only for their utility in verifying the species of the field identification, but also for utility in studying population divergence and genetic diversity within populations (Cameron et al. 2011, Lozier et al. 2011). Population genetic studies are currently underway, and results will be provided to NCCN SLN upon completion.

We assessed species richness across our sites and further compared sites both among and within parks. We estimated species richness across sites with the inverse Simpson's D index (1/D) (Magurran 2004). Pairwise community dissimilarity was examined using the Bray-Curtis index in a non-metric dimensional scaling (NMDS) analysis and unpaired Wilcoxon Rank Sum Tests. The effect of elevation and latitude on species richness was examined with a simple linear regression. Indices and statistical analyses were conducted in R v2.15.1 (R Development Core Team, 2012). Contemporary (1950 – 1990) and future (2070) habitat suitability models (HSM) were constructed for by aggregating a suite of informative bioclimatic variables with contemporary distribution records of the target bumble bee species, accessed from <http://gbif.org> (accessed 17 November 2013). HSM procedures and validation follow Cameron et al. (2011) and Strange et al. (2009). HSM and distribution records from the contemporary surveys were visualized with ArcGIS v 10.0 (ESRI, 2013). Climate data used to estimate future HS for each species were taken from the Community Climate System Model 4 RCP60 greenhouse trajectory. The RCP 60 greenhouse trajectory represents a stabilizing scenario, where immediate action by world governments is taken in response to current upwards trends of greenhouse emissions (IPCC 2013).

Aim 2: Bumble Bee Workshops

Prior to the commencement of surveys, all participants met at OLYM Headquarters for a half-day workshop on survey techniques and methodology. Standard bumble bee sampling protocols including non-lethal sampling of DNA (Holehouse et al. 2003) were taught to all research participants and interested NPS staff. We included a discussion survey site selection, collection techniques, safe bumble bees handling, and species identification. We also addressed the topic of data collection etiquette when surveying bumble bees in the presence of the general public, especially near high traffic areas. Proper etiquette is imperative as pollinator decline can be a sensitive subject for the general public. By equipping park staff with knowledge of how to conduct standardized surveys, we may ensure the potential for effective long-term monitoring bumble bee pollinator health in NCCN. Additional bumble bee identification and conservation workshops were conducted throughout the survey period at NOCA, MORA, LEWI and FOVA. The handout from our workshop presentations is attached as a supplemental file (WorkshopHandout.pdf)

Aim 3: Education and Outreach

Our final goal is to effectively communicate the importance of pollinator research in NCCN National parks to both the general public and the citizen scientist. Considering the global concerns of managed pollinator decline, we are determined to elucidate the value of wild bumble bee pollinators in NCCN. Providing a narrative on both the natural history and rigorous science behind pollinator conservation will ensure that NCCN National Park visitors are aware of the importance of maintaining federally protected wild lands for wild bees and other important pollinators. Material for interpretation of our research results to the public is provided as a supplemental file (EducationOutreachText.pdf).

RESULTS

Aim 1: Study of Bumble Bee Community Composition and Genetic Diversity

In total, fifteen different bumble bee species were detected with the NCCN. We captured 766 bumble bees across 28 standardized field surveys at 21 unique field sites from 15 - 25 of July and 2 August (Table 1). Of the 766, we have a voucher of XX specimens which have been curated and are currently housed at the NPIC in Logan, Utah. Specimens not retained for the voucher were released at the collection site after field identification. Average temperatures during the field survey were 22.3 ± 0.69 °C, average relative humidity of $50.6 \pm 2.21\%$ and average wind speed of 1.9 ± 0.41 kph. The total specimens surveyed from each park are EBLA = 35, FOVA = 55, LEWI = 33, MORA = 231, NOCA = 118, OLYM = 173, SAJH = 121 (Figure 1). *Bombus flavifrons* was the most abundant species detected in our survey at 18.8% relative abundance, whereas *B. griseocollis* and *B. nevadensis* each constituted <0.01% relative abundance. *Bombus occidentalis* was detected at two sites in OLYM in the Royal Basin Area (Table 1). This is the first time since 1955 that *B. occidentalis* has been detected within the boundaries of OLYM (Figure 2a). However it should be noted that single *B. occidentalis* has been detected on Mt. Townsend in Olympic National Forest by a citizen scientist in 2011 (Don Rolfs pers. com.), and more recently in Seattle, Washington in 2013 (The Seattle Times, July 14, 2013).

In OLYM we captured 33 *B. sylvicola*, expanding the known distribution of the species onto the Olympic peninsula. Expanding the distribution of *B. sylvicola* is a significant scientific contribution as bumble bees are well studied insects in North America and have been under heavy investigation for over half a century (Thorp et al. 1983, Stephen 1957). In the Pacific Northwest *B. sylvicola* can be confused for *B. melanopygus*; however, *B. sylvicola* differs from *B. melanopygus* with having tergite 4 with yellow setae instead of black setae (Koch et al. 2013, Thorp et al. 1983, Stephen 1957). Furthermore, the anterior scutum of *B. sylvicola* is predominantly yellow, whereas *B. melanopygus* typically has an admixture of black and yellow, giving a cloudy appearance (Figure 2b, c). We will confirm the taxonomic diagnosis with molecular techniques, and present them in the IAR for this study. At present we have about 11% of the bumble bee specimens without identification. The remaining 89% are tentatively identified. As we have extracted whole DNA from all bumble bee specimens in this study, we will apply molecular approaches to confirm the species identify of all bumble bees detected in the NCCN survey.

Despite standardized survey efforts at each park, local weather patterns, and phenological variation did not provide optimal collections at every site. Thus, we elected to rarefy our surveys abundances to estimate species richness at each site of park. Our results suggests that species diversity is positively correlated with elevation ($R = 0.49$, $P < 0.05$) and not latitude ($R = 0.04$, $P = 0.84$) across the parks (Figure 3). Furthermore, as we hypothesized, we found that high elevation communities across all the parks were more similar to each other in species composition, evidenced by the degree of clustering in a NMDS analysis (Figure 4), than they were to low elevation sites within parks. In the context of community similarity, middle elevation sites were more similar to high elevation sites, whereas low elevation sites were highly variable among the parks. We found a significant difference in community composition (measured as similarity) between high and low elevation sites (two-sample Wilcox Test, $P < 0.0001$) but not between high and middle elevation sites (two-sample Wilcox Test, $P = 0.13$) or between middle and low elevation sites (two-sample Wilcox, $P = 0.19$).

Habitat suitability models for each of the 15 bumble bee species predicts that some species will expand their distribution, whereas others species will contract in distribution in the Pacific Northwest (Supplementary Figure 1). Expansion is observed primarily with low elevation species, whereas contraction is observed specifically with high elevation restricted species. An examination of habitat suitability (HS) differences between contemporary and future climate scenarios for each species reveals that high and middle elevation species will be subjected to decreases in HS (Figure 5). *Bombus sylvicola*, a rare, and newly discovered high elevation species in the Olympic Mountains may experience significant declines of suitable habitat over the next 80 years (paired Wilcox Text, $P < 0.001$). Broadly distributed species, those found across low, middle, and high elevations, will experience slight increases in habitat suitability, whereas low elevation species may likely experiences expansions in habitat suitability. The increases in HS in low elevation species is likely a reflection of northward latitudinal shifts in habitat suitability. However *B. rufocinctus* may experience decline of suitable habitat in lowland regions, forcing either adaptation or migration of the species to higher elevations (Figure 5).

Aim 2: Bumble Bee Workshops

During our field survey effort we also conducted five one-hour workshops on bumble bee natural history, conservation, and monitoring techniques. James Strange and Jonathan Koch facilitated the workshops at each of the parks. The workshops were designed to educate both national park staff and the general public on the value of pollinator research in the national parks. The average attendance at each workshop was about 15 people. Those in attendance included professional scientists, technical staff, park administrators, and education specialist. In FOVA, James Strange gave a public lecture on the conservation and decline of bumble bee pollinators. During each workshop we distributed at least one copy of *Bumble Bees of the Western United States* coauthored by Jonathan Koch, James Strange, and Paul Williams (Koch et al. 2012). Education on the value of long-term bumble bee monitoring is a goal of the International Union of the Conservation of Nature- Species Survival Commission (Cameron et al. 2010). It is our hope that the information provided to stakeholders of the NCCN-SLN will use the data gathered during our survey to educate the public on the importance of bumble bee pollinators in the park. In addition to a lesson on species identification, we also provided handouts to all persons in attendance

summarizing the information covered during the workshop. The handout is included in this progress report as part of the final product to the NCCN-SLN.

Aim 3: Education and Outreach

We anticipate that the pollinator research narrative provided to the SLN will be an important tool for educating the public on the importance of wild bumble bee pollinators in the park. We are certain that defining the importance of pollinator research in NCCN-SLN national parks will galvanize park visitors to be citizen scientist and participate in pollinator conservation and monitoring in their own communities. Online and open-source communities to engage in the monitoring wild bumble bee species include Bumblebee Watch (<http://bumblebeewatch.org>), BeeSpotter (<http://beespotter.mste.illinois.edu>), and BugGuide (<http://bugguide.net>). A pollinator education narrative is included in this progress report as part of the final product of the NCCN-SLN. We hope to collaborate with national park staff in getting information and design of the poster in a format that will be accessible to park visitors.

DISCUSSION

Our study of bumble bee communities in NCCN national parks underscores the importance of conducting standardized surveys across a variety of habitats. Despite the history of bumble bee surveys in the Pacific Northwest, we have likely expanded the known distribution of an alpine and temperate distributed bumble bee species, *B. sylvicola*, on the Olympic peninsula. Furthermore, we have detected *B. occidentalis* within the park boundaries, the first known records in over half a century. *Bombus occidentalis* is an important find as the species has undergone extensive range and population declines (Cameron et al. 2011). The detection of *B. sylvicola* is of great significance and conservation concern as it is a high elevation and high latitude species, typically associated with temperate environments.

In our survey we found that high elevation sites housed the most diverse bumble bee community assemblages relative to low elevation communities. In the context of global climate change, our findings suggest that high elevation communities will be at great risk to temperature and precipitation change in the next 80 years (IPCC 2013). We developed this hypothesis by modeling the potential distribution of suitable habitat for each bumble bee species detected in our field survey within the national park boundaries. We found that HS based on the climate variables will decline for high and middle elevation species. However, other species may experience small expansions in HS across the national parks (Figure 5). Considering the sensitivity bumble bees exhibit to temperature increases and precipitation variability (Bartomeus et al. 2011), it is likely that changes to community composition will be observed in the Pacific Northwest.

The pressing issue of bumble bee conservation has been presented to both national park staff through intensive workshops, as well as to the general public through media. In this progress report we present two products that have the potential to be distributed to national parks throughout the NCCN-SLN. We feel that our survey, as well as our effort to translate our

research is a model that other applicants can follow to educate the public on the importance of preserving the wildlife found in America's great outdoors.

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FIGURE AND TABLE LEGENDS

Figure 1. Map of the 21 bumble bee collections surveys across the national parks in the North Coast and Cascades Network.

Figure 2. Dorsal and lateral photographs of the (a) *B. occidentalis*, (b) *B. sylvicola*, and (c) *B. melanopygus* specimens detected within the Olympic National Park in July 2013.

Figure 3. Elevation distribution of rarified bumble bee community species richness across the national parks of the North Coast and Cascades Network ($R = 0.49$, $P < 0.05$). Species richness is calculated as the Inverseness of Simpson's D ($1/D$).

Figure 4. Nonmetric dimensional scaling analysis of bumble bee community dissimilarity across the national parks of the North Coast and Cascades Network. Locations clustered closer together suggest that bumble bee communities are more similar in composition. High elevation communities are more similar in composition than low and middle elevation communities.

Figure 5. Habitat Suitability (HS) comparisons across bumble bees surveyed in the national parks of the North Coast and Cascades Network. Comparisons for each species are made between modeled HS of contemporary and future distributions under a "middle of the road" climate change scenario (IPPC 2013). The X-axis represents the difference between contemporary and future HS. Values to the left of the dashed red line indicate a decrease in HS, whereas values to right of the dashed red line indicate an increase in HS.

Supplementary Table 1. Collection locations and associated environmental data of the bumble bee survey events across the national parks of the North Coast and Cascades Network.

Supplementary Figure 1. Contemporary and Predicted MaxEnt Habitat Suitability Models of the 15 bumble bee species found throughout the national parks in the North Coast and Cascades Network: (a) *B. appositus*, (b) *B. bifarius*, (c) *B. californicus*, (d) *B. caliginosus*, (e) *B. flavifrons*, (f) *B. griseocollis*, (g) *B. melanopygus*, (h) *B. mixtus*, (i) *B. nevadensis*, (j) *B. occidentalis*, (k) *B. rufocinctus*, (l) *B. sitkensis*, (m) *B. sylvicola*, (n) *B. vandykei*, (o) *B. vosnesenskii*.

Figure 1.

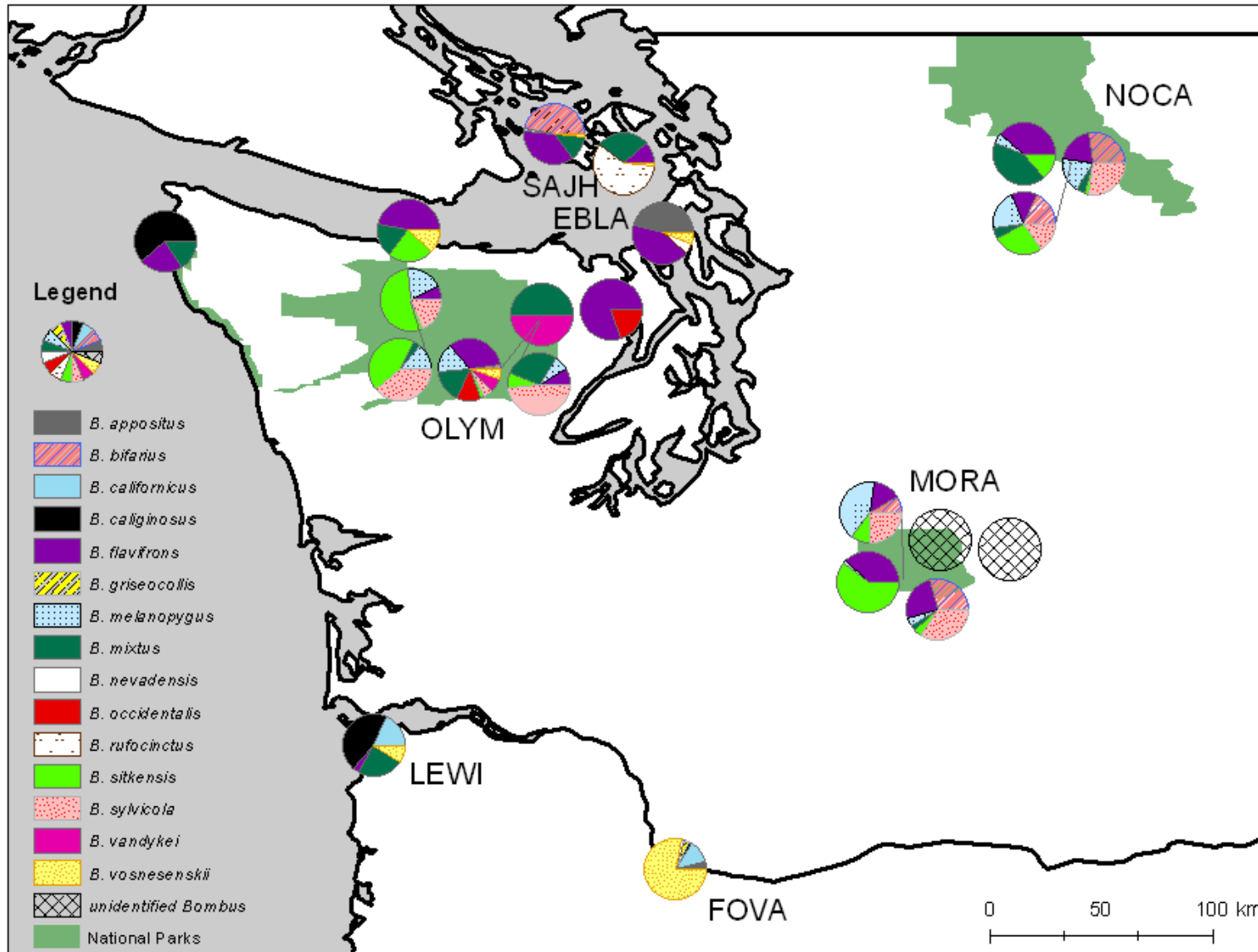


Figure 2.



Figure 3.

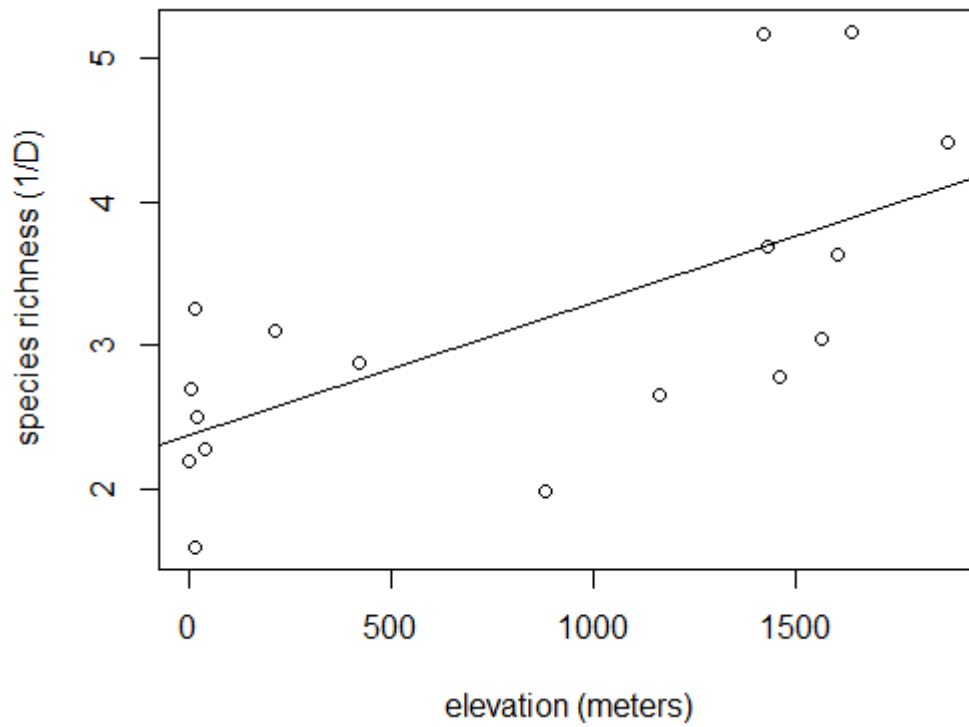


Figure 4.

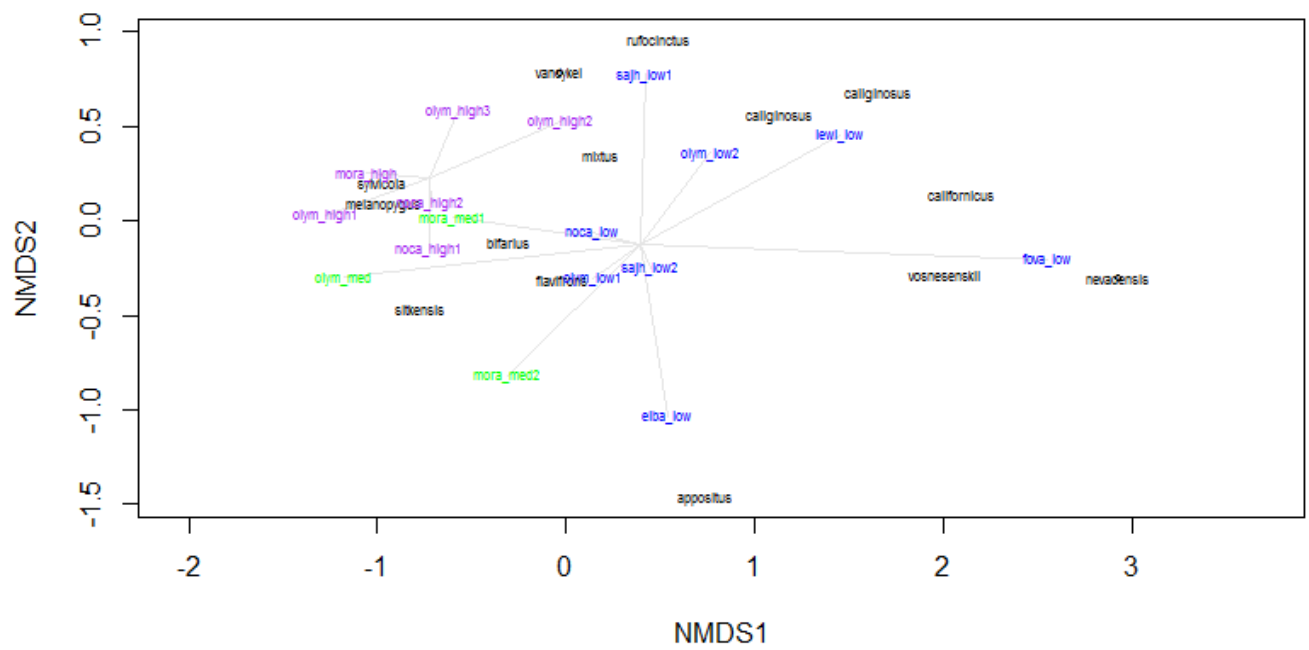
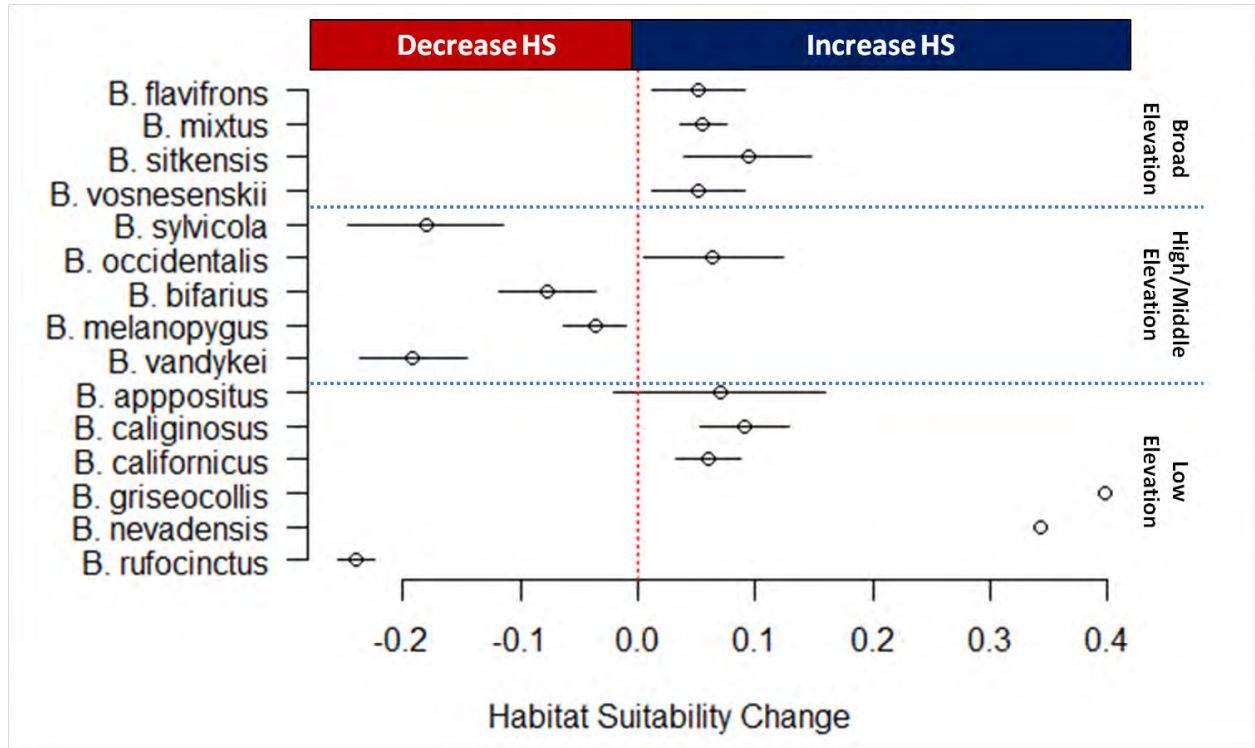


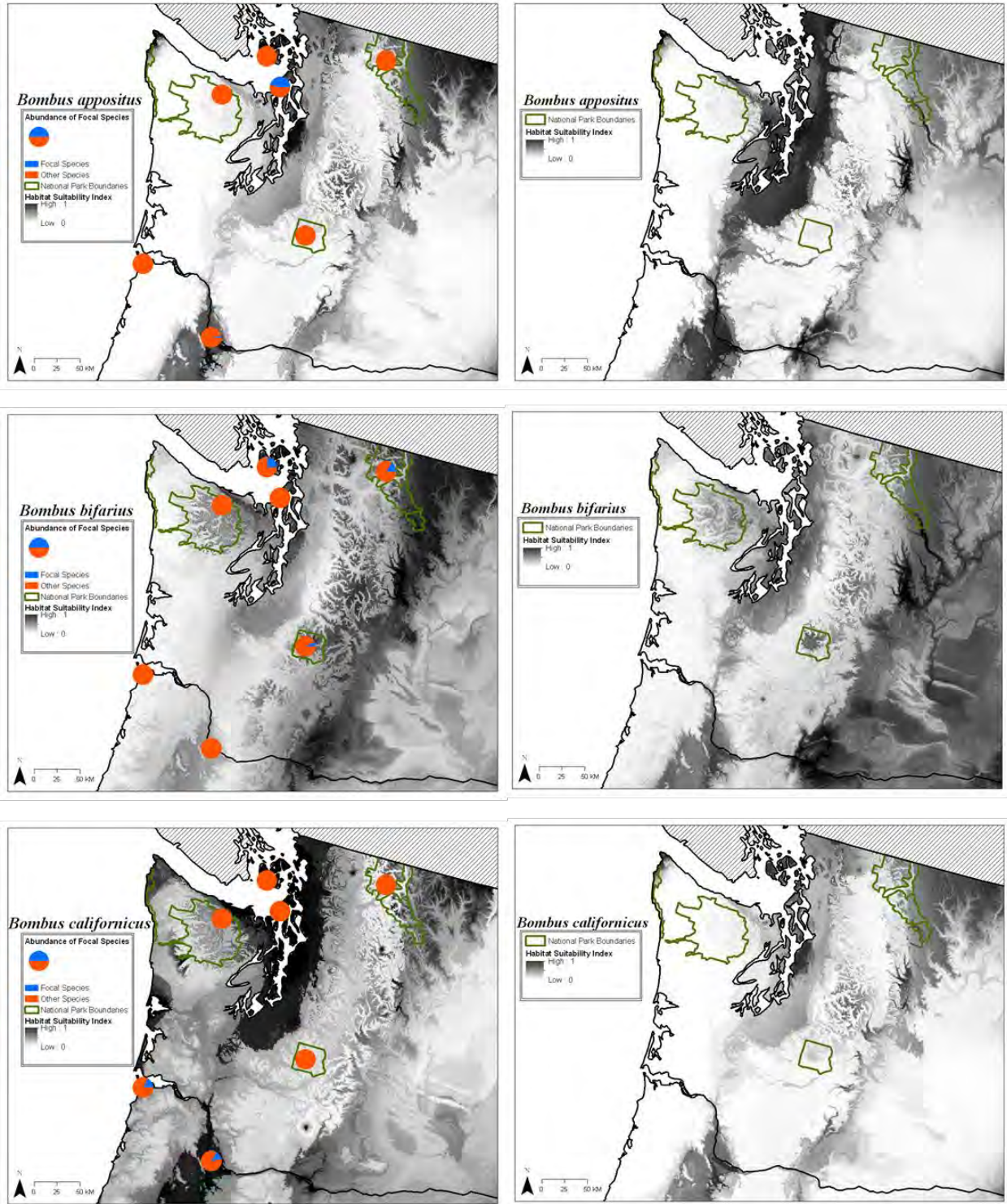
Figure 5.



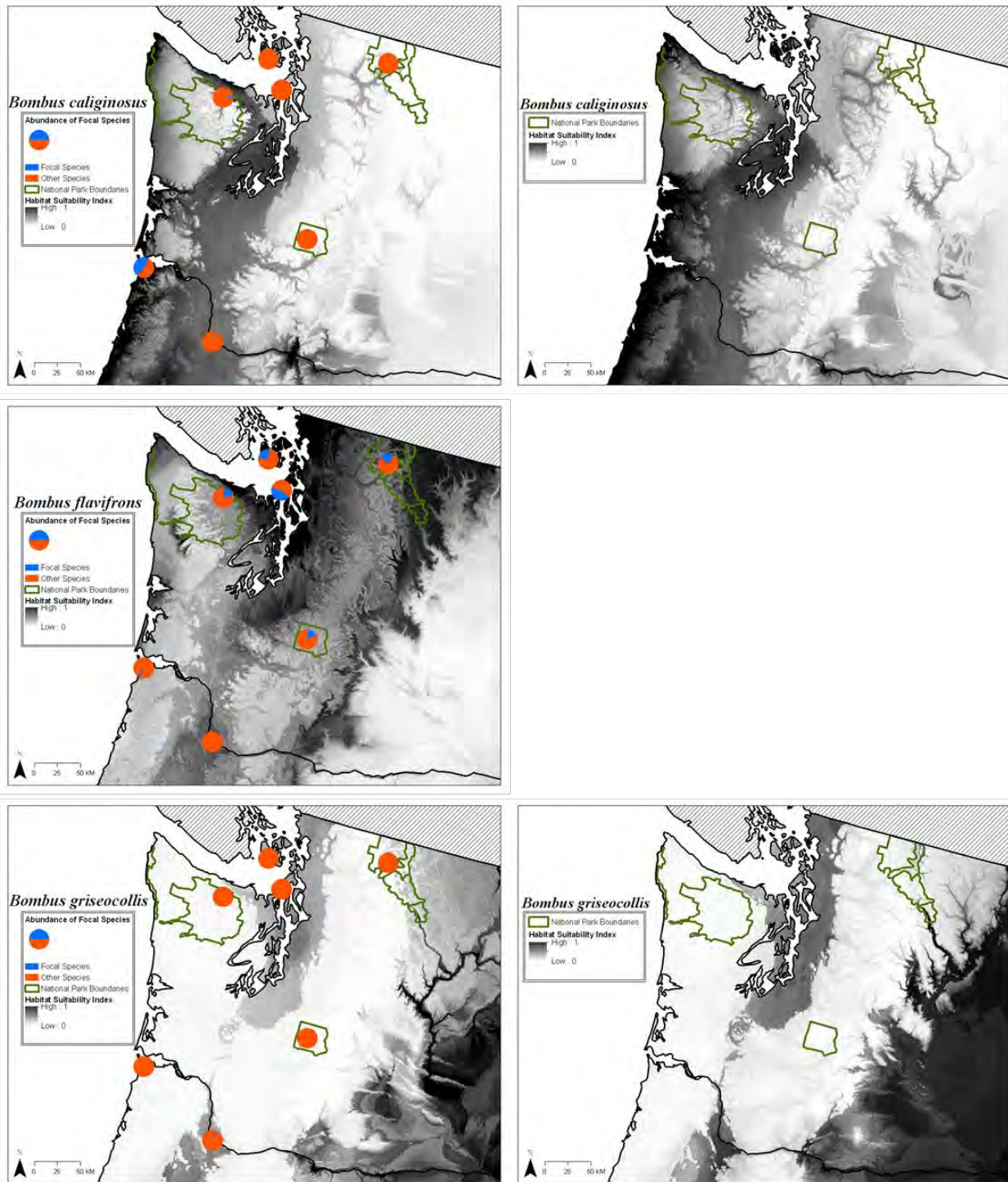
Supplementary Table 1.

Site Name	National Park	Lat	Long	Elev (m)	Day	Mon	Year	Time Start	Time End	Total Hours
Royal Basin R.S.	OLYM	47.83	-123.21	1564	16	7	2013	16:37		0.00
Lower Royal Basin	OLYM	47.84	-123.21	1421	16	7	2013	9:51	10:21	1.50
Lower Bridge Creek	OLYM	47.92	-123.73	1163	16	7	2013	17:00	17:30	2.00
Heart Lake	OLYM	47.91	-123.73	1460	16	7	2013	13:15	13:30	1.00
Heart Lake	OLYM	47.91	-123.73	1460	16	7	2013	15:00	15:15	0.50
Royal Basin Trail	OLYM	47.86	-123.20	1177	17	7	2013	10:21		0.00
Royal Basin Parking Lot	OLYM	47.88	-123.00	1170	17	7	2013	13:22		0.00
Crescent Lake	OLYM	48.09	-123.74	209	17	7	2013	12:30	12:45	0.75
Sand Point Loop	OLYM				1	8	2013			0.50
Ebey's Landing	EBLA	48.19	-122.71	20	18	7	2013	10:50	11:50	3.00
Sahale Arm Trail	NOCA	48.47	-121.52	1875	19	7	2013	16:08	16:38	1.50
Cascade Pass	NOCA	48.47	-121.06	1638	19	7	2013	13:30	14:00	2.00
Sibley Creek	NOCA	48.51	-121.25	419	20	7	2013	10:10	10:40	1.00
English Camp	SAJH	48.59	-123.15	1	19	7	2013	14:15	15:15	3.00
American Camp	SAJH	48.46	-123.02	38	19	7	2013	11:00	12:00	3.00
Fort Vancouver	FOVA	45.62	-122.66	12	23	7	2013			1.00
Lewis & Clark	LEWI			0	22	7	2013	16:00	16:30	0.50
West Side Road	MORA	46.78	-121.88	878	22	7	2013	18:00	18:30	1.50
Upper Crystal Lake 1	MORA	46.91	-121.51	1784	22	7	2013	16:00	16:45	1.50
Upper Crystal Lake 2	MORA	46.91	-121.51	1784	22	7	2013	18:15	18:30	0.50
Paradise Meadows	MORA	46.79	-121.74	1603	23	7	2013	10:00	12:00	6.00
Snow Lake	MORA	46.77	-121.71	1428	23	7	2013	14:50	15:35	2.25
Upper Crystal Lake 3	MORA	46.91	-121.51	1784	23	7	2013	8:30	8:45	0.50
Upper Palisades Lake 1	MORA	46.95	-121.59	1804	23	7	2013	14:30	15:00	1.00
Upper Palisades Lake 2	MORA	46.95	-121.59	1804	23	7	2013	17:50	18:05	0.50
Upper Palisades Lake 3	MORA	46.95	-121.59	1804	23	7	2013	20:00	20:10	0.33
Lower Palisades Lake 1	MORA	46.95	-121.59	1668	23	7	2013	16:10	16:50	1.33
Sunrise Meadows 1	MORA	46.91	-121.62	1907	24	7	2013	9:10	9:55	1.50

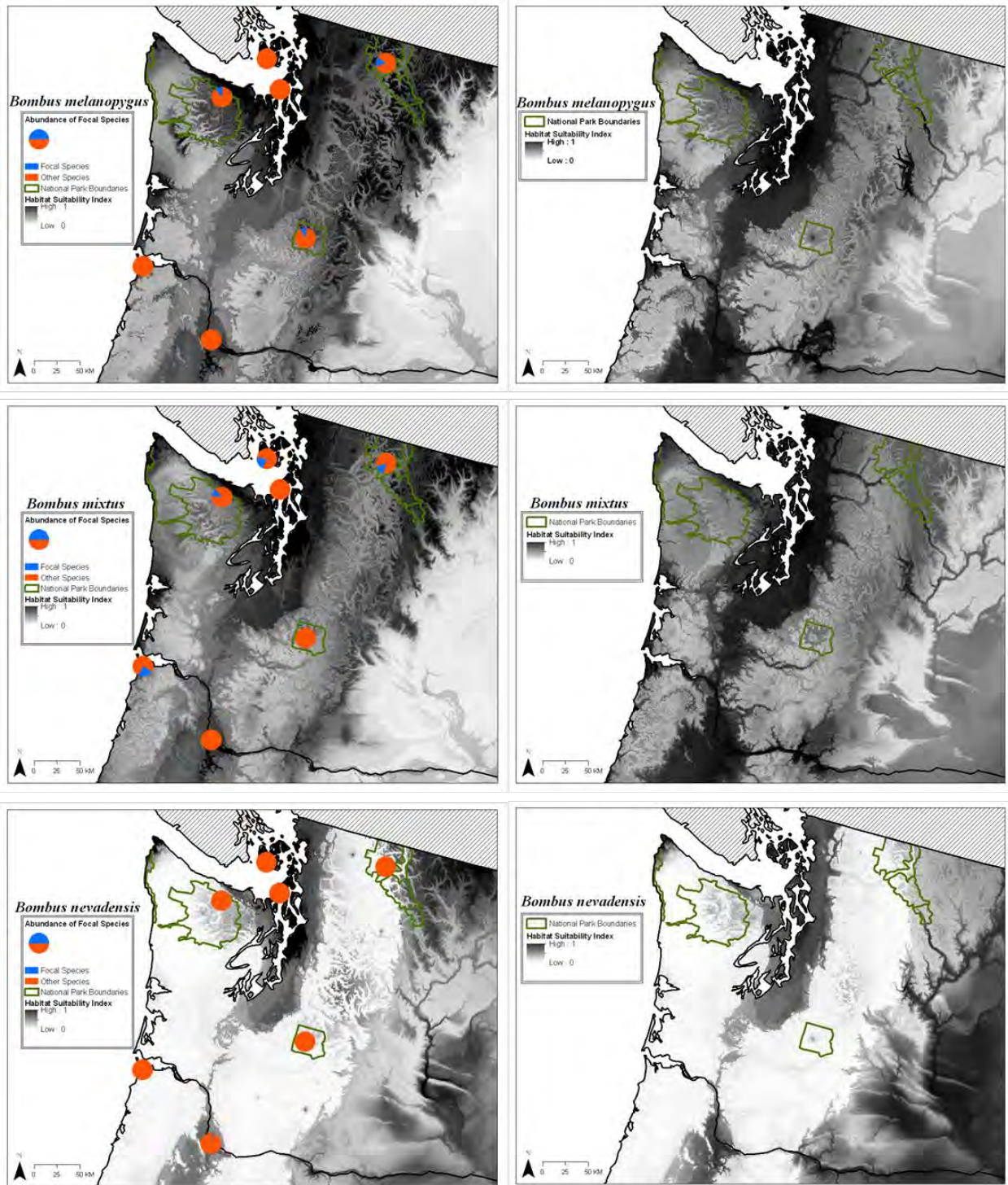
Supplementary Figure 1.



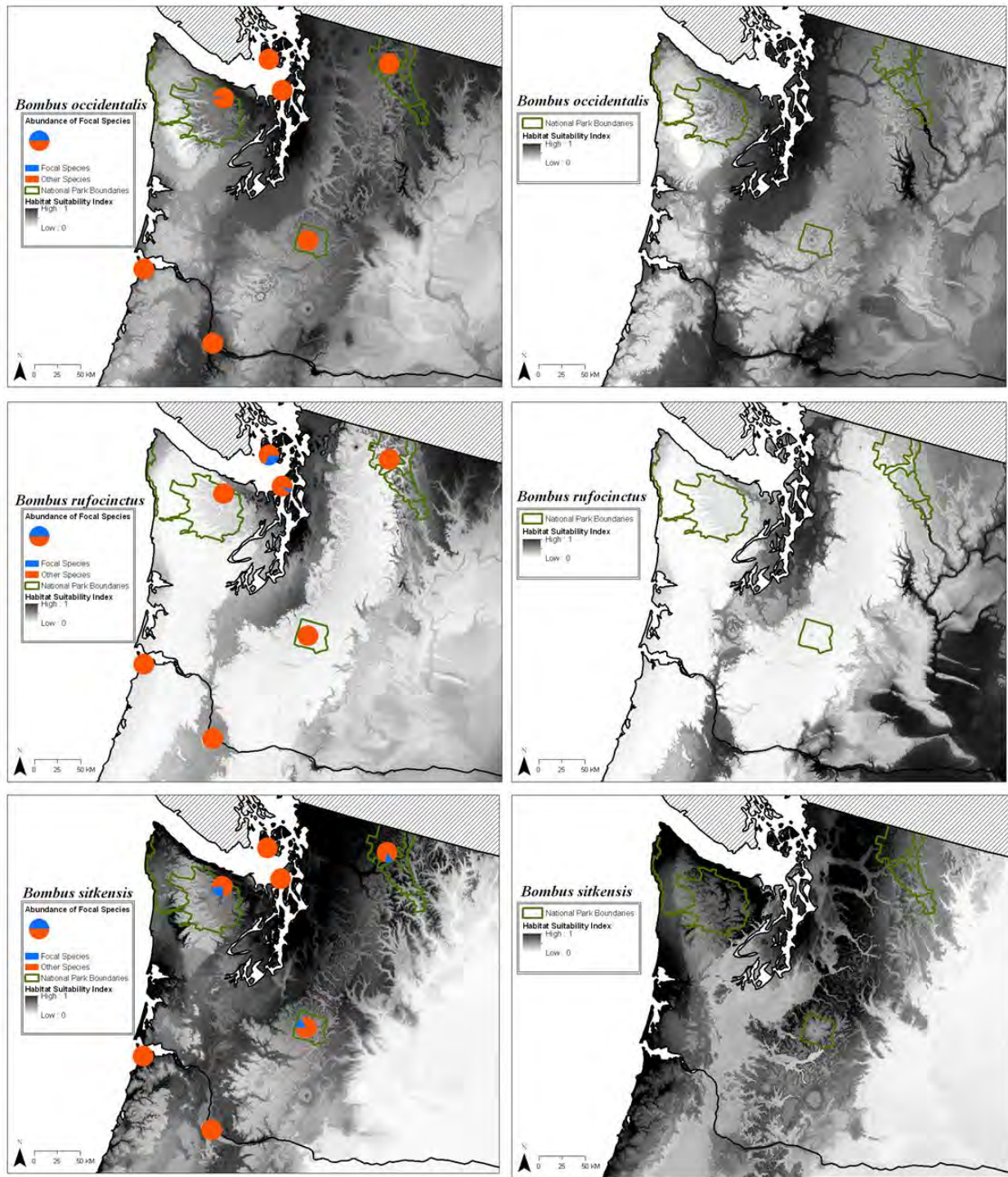
Supplementary Figure 1b.



Supplementary Figure 1c.



Supplementary Figure 1d.



Supplementary Figure 1e.

