Johnson's Hairstreak Surveys in Oregon and Washington

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Photo by Matt Schroeder – Wind River Canopy Crane Research Facility

Abstract

In 2008 the Interagency Special Status/Sensitive Species Program (ISSSSP) funded the development of a habitat suitability model and survey protocols for the Johnson's hairstreak butterfly (*Callophrys johnsoni*). The draft habitat suitability map (v1.0) was used to focus field surveys in Oregon and Washington. In 2009, ISSSSP funded field surveys to test the survey protocol and the usefulness of the habitat suitability map. Two different survey techniques were tested; adult surveys and larval surveys. Probability of detection was highest (50%) during the earlier portion of the flight season survey and quickly decreased as the season progressed, indicating that for 2009, the flight season may have occurred a week or two earlier than expected. The overall estimated probability of detection for adult surveys was 33% (95% confidence interval of 18 – 54%). The larval survey technique produced a 100% probability of detection at occupied sites. Occupancy modeling indicates habitat suitability maps can be used to increase the odds of finding occupied sites and can be used to focus surveys. Presence of the larval host plant dwarf mistletoe (*Arceuthobium* spp) is the most important site variable and in moderate to high abundance it can greatly increase the probability of occupancy for Johnson's hairstreak.

INTRODUCTION

The Johnson's Hairstreak (*Callophrys johnsoni*) is a rare butterfly that inhabits in the Pacific Northwest region of the United States of America. Prior to this survey, there were a total of 85 documented sites of adults or larvae, scattered throughout Oregon and Washington, dating back to 1891. All but 25 of these sightings predated the implementation of the Northwest Forest Plan in 1994 (USDA/USDI 1994), which provided increased protection of late-successional forests over the main portion of this species' range. Within this portion of its range, older coniferous forests, especially western hemlock (*Tsuga heterophyla*) forest types that are infected by *Arceuthobium tsugense*, a dwarf mistletoe, appear to be its key habitat. However, it is also found in other forest types, most notable, in Ponderosa pine forests infected with *A. camplypodum* in a disjunct population located in northeastern Oregon, near the Idaho border. It also has been documented to use *A. abietinum* in true firs such as white fir (*Abies concolor*).

A draft survey protocol was developed and tested in 2009. The primary objective of the survey was to field-test survey methods for detecting presence of this butterfly by means of repeated surveys at locations where species presence is likely based on habitat modeling and specific survey site selection criteria. Two types of field surveys were tested; 1) adult surveys during the estimated peak flight season and 2) larval surveys during the egg-laying and larval season. The results of these surveys were used to estimate site occupancy (site = transect) and detection probabilities for two survey methods. Secondary objectives included habitat map validation and improving our understanding of the ecology and biology of this little studied and poorly-understood butterfly.

METHODS

Field surveys

The Interagency Special Status/Sensitive Species Program (ISSSSP) provided funding to conduct surveys in the Oregon Cascades and field surveys were conducted using the draft survey protocol v1.0 (Davis et al. 2009). This draft protocol is attached to this report as Appendix A. The 2009 surveys were focused in areas that had a high potential for *C. johnsoni* presence based on habitat modeling and documented sightings. The framework for these surveys was the Environmental Protection Agency's (EPA 2002) Environmental Monitoring & Assessment Program (EMAP) hexagons (65,000 hectares each). Initially, four EMAP hexagons were selected for protocol testing in the draft protocol (Appendix 3 in draft protocol). Additional funding from ISSSSP allowed the addition of one more survey hexagon located in the Oregon Coast Range, and partnerships developed with the Mount Baker Snoqualmie National Forest in Washington and the Pacific Northwest Research Center, La Grande, Oregon added 2 additional EMAP hexagons. Two of these three 3 additional hexagons were located in areas of interest (see Appendix 3 in the draft protocol). The survey protocol called for 4 adult surveys along the 2-km transects during the estimated peak flight season for the butterfly. Transects were located in areas that habitat modeling indicated had conditions suitable for *C. johnsoni*, and that met certain site-specific ground conditions criteria. For every hexagon surveyed, one section (triangle) of the hexagon with the lowest and one section with the highest habitat suitability were surveyed to test whether or not the habitat suitability maps were useful for predicting occurrence of this species.

The survey methods described in the draft protocol (Appendix A) were followed with slight modifications or clarifications. The first clarification was that survey transects were placed at least 2km apart from each other. This was done to ensure site "closure" for occupancy modeling. This distance was believed to be outside of the maximum flight distance of *C. johnsoni* (McCorkle pers. comm.). Modifications were made to survey route selection criteria on page 4 of the draft protocol as follows:

1. Adjacency to older coniferous forest (>=80yr approx.)
2. Presence of dwarf mistletoe on western hemlock, true fir (*Abies* spp.) or Ponderosa pine on any portion of the transect.
3. If dwarf mistletoe was not detected along any transects in the low HS% hexagon sections (triangles), then presence of western hemlock (or other host trees) was required.
4. Drivable or walkable road (consider trails as last resort if no roads meet above criteria and if the trails have open gaps and edges with good nectar sources and sun exposure).

A third survey modification was made for the larval surveys. Instead of collecting dwarf mistletoe at eight "sample site centers" spaced every 250m, each transect was split into eight 250m segments, and a time-constrained search for dwarf mistletoe was conducted within each segment. Specifically, each segment was to be surveyed for 15 minutes, or until a maximum of 10 dwarf mistletoe clumps were collected, whichever happened first. Clumps were to be collected from within 50m of each side of the transect, instead of at sample centers. This allowed more flexibility in finding dwarf mistletoe clumps.

Additional "focused" surveys were conducted for both adults and larvae. Focused surveys are similar to "checklist" surveys (Royer et al. 1998), and focused only on likely habitats of *C. johnsoni*. Like checklist surveys, the surveyor is free to search out places where this specific butterfly typically would inhabit or lay its eggs and the technique is procedurally simpler than the transect surveys. Because it imposes few procedural constraints, checklist counting is also more flexible than transect sampling. However, the survey technique is inadequate to meet the rigors for statistical analysis. Our focused surveys entailed using the habitat suitability map (v1.0) to identify large (avg. 8,000-ha) concentrations of high habitat suitability. Five areas were selected for adult surveys and one area for larval surveys. An experienced lepidopterist searched these five areas for signs of dwarf mistletoe and adult butterflies during the flight season. A wildlife biologist, with no butterfly experience, was given basic instructions to search the sixth area during the larval season for dwarf mistletoe to collect clippings and inspect them for eggs or larvae.

Occupancy analysis

The probability of a site (transect in our analysis) being occupied (ψ) and the probability of detecting the species (p) at occupied sites was estimated using computer program PRESENCE (MacKenzie et al. 2002). This analysis assumes sites are closed to immigration or emigration and that detection at one site is independent of detections at other sites. Analysis of the larval survey data was limited to the genus *Callophrys*, specifically Johnson's Hairstreak (*C. johnsoni*) and Thicket Hairstreak (*C. spinetorum*) because identification between these closely related species from larvae is not possible until they pupate and eclose, which has not yet fully occurred at the time of this report.

RESULTS

Field survey

A total of 7 EMAP hexagons were surveyed, for a total of 28 transect surveys. A total of 19 adult *C. johnsoni* (Figure 1) were documented at 8 transects. Adult transect surveys began on May 22 in Washington and June 1 in Oregon and ended by July 9 in both states. Temperatures ranged between 14-30ºC (average = 21.4ºC) and wind speeds from 0-4 kph. Most surveys were conducted during sunny to partly cloudy skies. However, inclement weather resulted in non-completion of two surveys. Late snow melt postponed the start of a few surveys due to cool weather and poor road access. All adult *C. johnsoni* records from this survey and a few additional observations made during 2009 were fitted to a Poisson curve and compared to the historic data used to estimate the peak flight season for this survey in the draft protocol (Figure 1 in Appendix A). It appears that the peak of the flight season in 2009 likely happened at least a week earlier than anticipated (Figure 2).

Figure 1. Johnson hairstreak adult butterfly vouchered on the Mt. Hood National Forest (photo by Alan Dyck).

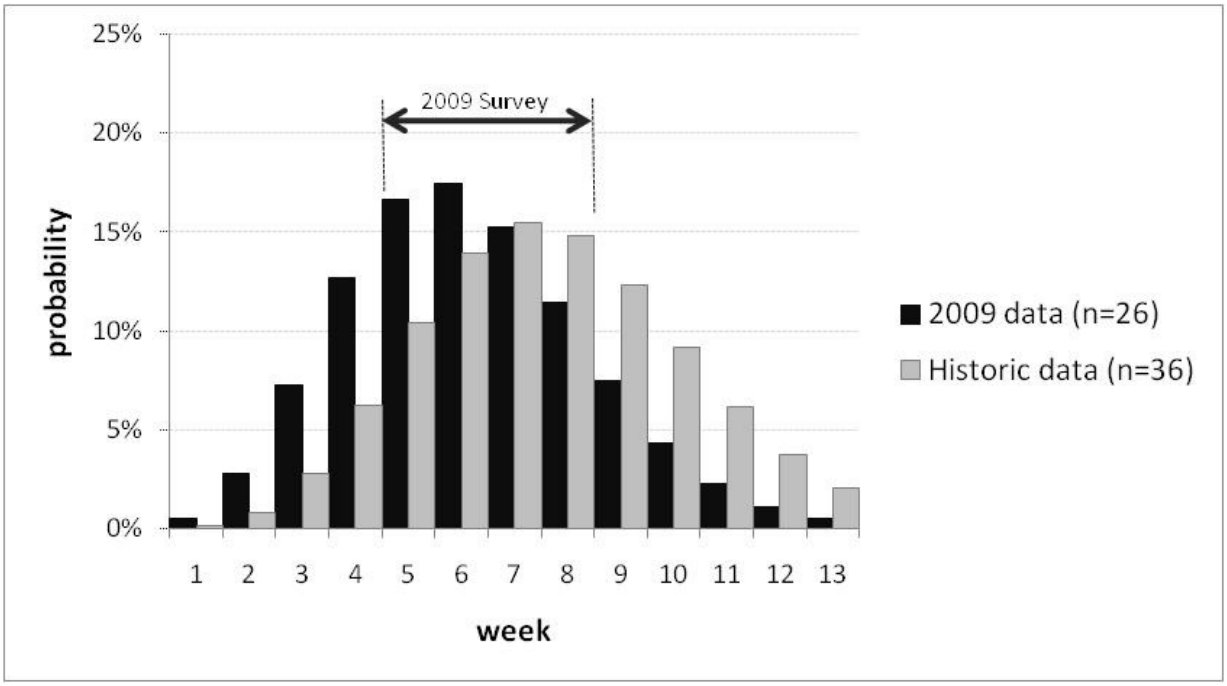

Figure 2. Johnson hairstreak adult observations from 2009 (black bars) and historic data (gray bars) from Figure 1 in the draft protocol (Appendix A) fit to a Poisson curve. Based on this data, it appears that the peak flight season for Johnson's hairstreak in 2009 may have occurred 1 to 2 weeks earlier than historical records indicated for Oregon. Week #1 = May 1 – 7.

Larval surveys were performed between July 13 through August 3. A total of 26 *Callophrys* spp. larvae were collected (and successfully reared to pupa stage) at 7 transects. At the time of the writing of this report, 14 *C. spinetorum* have eclosed, 4 pupa appear dead, and 1 was killed by a parasitoid wasp (Figure 2), which eclosed from the pupa. Final species identification of the remaining 7 pupae is pending upon their eclosure or breakthroughs in the genetic analysis currently being conducted.

Figure 3. Encytrid wasps (*Copidosoma* spp.), important pollinators of dwarf mistletoe, are also parasitoids. This specimen eclosed from a *Callophrys* spp. pupa collected on *Arceuthobium tsugense*.

Focused surveys for adults occurred between June 13 and July 2 and consisted of two visits during appropriate weather conditions (as defined in draft survey protocol). Only one *C. johnsoni* adult was vouchered in one of the five adult survey areas, which was the only one of the five areas that had an overall average habitat suitability (based on the maps) greater than 50. The focused larval surveys occurred on August 10-11, this site only being visited once. The average overall habitat suitability value for this survey area was 35. Hatched eggs and larvae were identified and 4 larvae were reared to eclosure. All eclosed larvae were *C. spinetorum*.

Occupancy analysis

A total of 19 transects met quality control standards for inclusion in an occupancy analysis using the computer program PRESENCE (MacKenzie et al. 2002). Each transect represented one site, and closure was assumed because transects were spaced no nearer than 2km to each other, as this is outside the estimated limit of flight distance of an adult *C. johnsoni* (McCorkle per comm.). Four transects had only 3 adult visits that met protocol, however occupancy models and program PRESENCE are designed to handle missing surveys allowing inclusion of these transects in the analysis (MacKenzie et al. 2002).

To estimate the detection probabilities for these surveys and assuming constant occupancy $\psi(.)$ between sites, three models with were compared; 1) detection is constant between survey days and methods (adult and larval surveys), represented by [p(.)], 2) detection varies by survey method [p(method)], and 3) detection varies by survey day [p(day)] (Table 1). Analysis results supported model #3, with detection rates that varied by survey day (over time) and also model #2, where detection rates varied by survey method. During the adult survey, detection probabilities were highest (0.5, SE = 0.204) during the first half of the survey and declined to zero by the end of the survey period, with no adults caught during the 4th visits. Overall, the adult survey method had a detection probability of 33% (95% confidence interval of 18 – 54%). The probability of detection using the larval survey method, which was also the last survey done at each site, was 100%. In other words, everywhere adults were observed (documented that the site was occupied) we also found larvae and at one site where adults were never observed, but larvae were collected. Since adults are "eruptive" and mobile, it makes sense that detecting adults would be harder than detecting stationary larvae. Constant detection between survey

visits and methods was not supported by this analysis and this variation in detection rates over the season is consistent with the Poisson distribution of adult observations (Figure 2).

Table 1. Model selection results for detection probability models including Akaike's information criteria (AIC), differences between the best model and all other models (ΔAIC), and AIC model weights (AIC wt). Model likelihood, number of parameters (k) and -2*loglikelihood are also included.

	Model	AIC	ΔAIC	AIC wgt	Model Likelihood	no.Par.	-2*LogLike
model 3	ψ(.),p(day)	59.97	0	0.5307	1	6	47.97
model 2	ψ(.),p(method)	60.25	0.28	0.4614	0.8694	3	54.25
model 1	ψ(.),p(.)	68.39	8.42	0.0079	0.0148	2	64.39

I used the best model structure on detection rates [p(survey)] to model occupancy and included three site covariates in four candidate models; 1) occupancy is constant between sites [ψ(.)], 2) occupancy varies with mean habitat suitability of EMAP hexagon [ψ(hexagon)], 3) occupancy varies with mean habitat suitability of the transect [ψ(transect)], 4) occupancy varies with amount of dwarf mistletoe along the transect [ψ(%dwarf mistletoe)] (Table 2).

Table 2. Results of the occupancy probability analysis.

	Model	AIC	ΔAIC	AIC wgt	Model Likelihood	no.Par.	-2*LogLike
model 4	ψ(%dwarf mistletoe),p(survey)	38.27	0	0.9914	1	7	24.27
model 3	ψ(transect),p(survey)	47.87	9.6	0.0082	0.0082	7	33.87
model 2	ψ(hexagon),p(survey)	53.71	15.44	0.0004	0.0004	7	39.71
model 1	ψ(.),p(survey)	59.97	21.7	0	0	6	47.97

Model results suggested that occupancy was not constant across sites, as all site covariate models performed better than the constant occupancy model (Table 2). The analysis showed that inclusion of site covariates fit the survey data better than the constant occupancy model. The evidence ratios between models showed that the hexagon model was about 20x more likely than constant occupancy, the transect model was about 20x more likely than the hexagon model and that the dwarf mistletoe model was by far (120x) the best model. The results of this analysis make biological sense, as dwarf mistletoe is the larval host plant for both *C. johnsoni* and *C. spinetorum*, and without it, these species could not survive.

In summary, the results of these analyses needs to be put into the context of the genus *Callophrys*, specifically for the closely related Johnson's and Thicket Hairstreak, as most of the larvae have eclosed as *C. spinetorum*, to date. Habitat use is similar between these species, and they overlap in many areas of the Johnson's Hairstreak's range. In addition, the limited number of sample sites limited the statistical power of this analysis. However, the relationships and patterns observed between these three site covariate and occupancy rates (Figure 4) are noteworthy for further study.

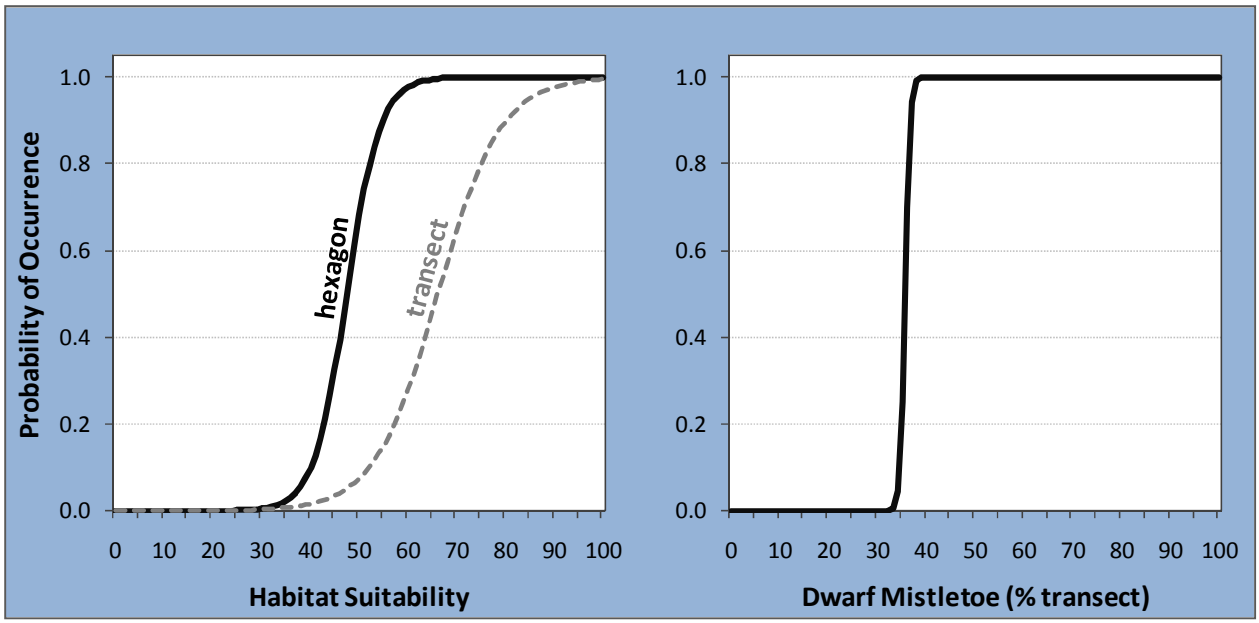

Figure 2. Relationship between habitat suitability map estimates at two different spatial scales; large-scale (EMAP hexagon) and small-scale (transect), and relationship between abundance of dwarf mistletoe and probability of occurrence.

DISCUSSION

Results of this pilot survey indicate clearly that the larval survey is the most effective survey technique for detecting presence of *C. johnsoni/spinetorum* and the results of this analysis were backed up by the results of the focused surveys, which documented adult *C. johnsoni* after 2 visits by an experienced lepidopterist, but only in a large (8,000-ha) area with an average HS>50. Whereas only one visit by an wildlife biologist with very little training was needed to produce documentation of *Callophrys* larvae (these larvae eclosed by November and were *C. spinetorum*). Unfortunately, our current inability to distinguish between larval *C. johnsoni* and *C. spinetorum* in areas where both species occur makes larval surveys problematic. Identification via color markings in later instar stages is uncertain, as wide variations of larvae coloration were observed, including darker 1st and 3rd dorsal ridges that turned out to eclose as *C. spinetorum*. This leaves rearing of larvae until they pupate and then waiting for eclosure as the only certain method for species identification. However, this requires the use of proper techniques and a major time commitment as larvae need to be fed, containers cleaned and maintained for about one month before they pupate. Eclosure could be accelerated by keeping pupae indoors at room temperature, and *C. spinetorum* began eclosing as early as August, and continued until December. As of the writing of this report, no *C. johnsoni* have eclosed (assuming that some or all of the remaining pupae are of that species). A more efficient approach would involve genetic analysis of larval vouchers collected during surveys. This is currently not possible as no genetic sequencing has been done on these species. However, adult specimens from this year's survey were sent for genetic analysis and progress is being made on developing genetic markers that may allow species identification from collected larval vouchers.

The probability of occupancy increased with increased mean habitat suitability at the hexagon scale, with an even better relationship observed between butterfly occupancy and habitat suitability at the transect scale. Thus, the large-scale hexagon map can be used to focus surveys, but the site specific map (1km resolution) is better for identifying potential habitat for this species. However, ultimately, site specific information on the presence of the larval host plant (i.e., dwarf mistletoe) is needed to determine exactly where to conduct surveys. Based on our experiences in 2009, presence of dwarf mistletoe was sometimes hard to find within the lowest habitat suitability sections of the EMAP hexagon. Since landscape density of dwarf mistletoe was an important variable in habitat suitability modeling, this was not surprising. In some cases signs of dwarf mistletoe infection were present, such as branch swelling and witches' brooms, however no live plants were found. In these situations, it is likely that fungi that infect *Arceuthobium* spp., such as *Colletotrichum gloesporioides* or *Neonectria neomacrospora*, may be the reason for its absence. Occasionally, active signs of the host plant could not be found. In these situations where active dwarf mistletoes were not readily apparent, survey transects were laid out in stands that had the proper host tree species such as western hemlock (*Tsuga heterophylla*).

The fact that most of the larvae collected during this survey were *C. spinetorum*, even at sites occupied by *C. johnsoni* is puzzling. Possible reasons for this may be because of the late timing of the larval survey, where *C. johnsoni* larvae were in more mobile, later instar stages and perhaps more dispersed throughout the trees infected by dwarf mistletoe or had already pupated. Or, perhaps *C. johnsoni* lay their eggs higher in the tree canopy, whereas *C. spinetorum* lay their eggs lower in the canopy, where we collected our clumps of dwarf mistletoe. However, *C. johnsoni* larvae have previously been collected on infected branches at ground level, and on younger trees (McCorkle 1973).

Finally, during our surveys we looked for presence of the invasive, non-native multicolored Asian lady beetles (*Harmonia axyridis*), which might pose problems to larval butterfly. No Multicolored Asian lady beetles were found during our surveys.

THE NEXT STEPS

A wider dispersion of surveys is planned for 2010. The basis for survey area selection will be the v1.1 habitat suitability map (map attachment 3). This map has also been summarized at the EMAP hexagon scale, showing average habitat suitability for each hexagon (map attachment 2). These maps can be used in conjunction with the graphs shown in Figure 4. In general, surveys should focus in areas with the highest habitat suitability scores. As an example, using the hexagon map (attachment 2) and the graph in Figure 4, hexagons with overall habitat suitability scores of 50 should have a probability of occupancy of around 80%. At the finer spatial scale, using map attachment 2 and the graph in Figure 4, pixels (1km resolution) with HS scores >75 would have a probability of occupancy of around 80%. Survey areas should focus on concentrations of high suitability. The HS gradients of the maps allow flexibility in choosing survey locations.

The use of the habitat suitability maps is just the first step. Once potential survey areas have been identified using these maps, ground visits prior to the egg laying season need to occur to confirm the presence of active dwarf mistletoes. As seen in the graph in Figure 4, areas with low concentrations of dwarf mistletoe <35% of the area searched, have a very low probability of occupancy. Survey areas should be selected in areas with both high habitat suitability and high amounts of dwarf mistletoe.

The next version of the survey protocol will be distributed prior to the 2010 survey season. This protocol will be a refined version of the draft protocol (Appendix A) based on the results of the 2009 surveys. It will include both the adult and larval survey techniques. However, given that the larval surveys have the highest probability of detecting this species, they will be the primary method used for surveys conducted in 2010.

The objective of the 2010 survey is to determine the extent of *C. johnsoni* distributions in Oregon and Washington. The focus will be on areas with high mapped habitat suitability and presence of active dwarf mistletoe where no previous documentation of *C. johnsoni* exists.

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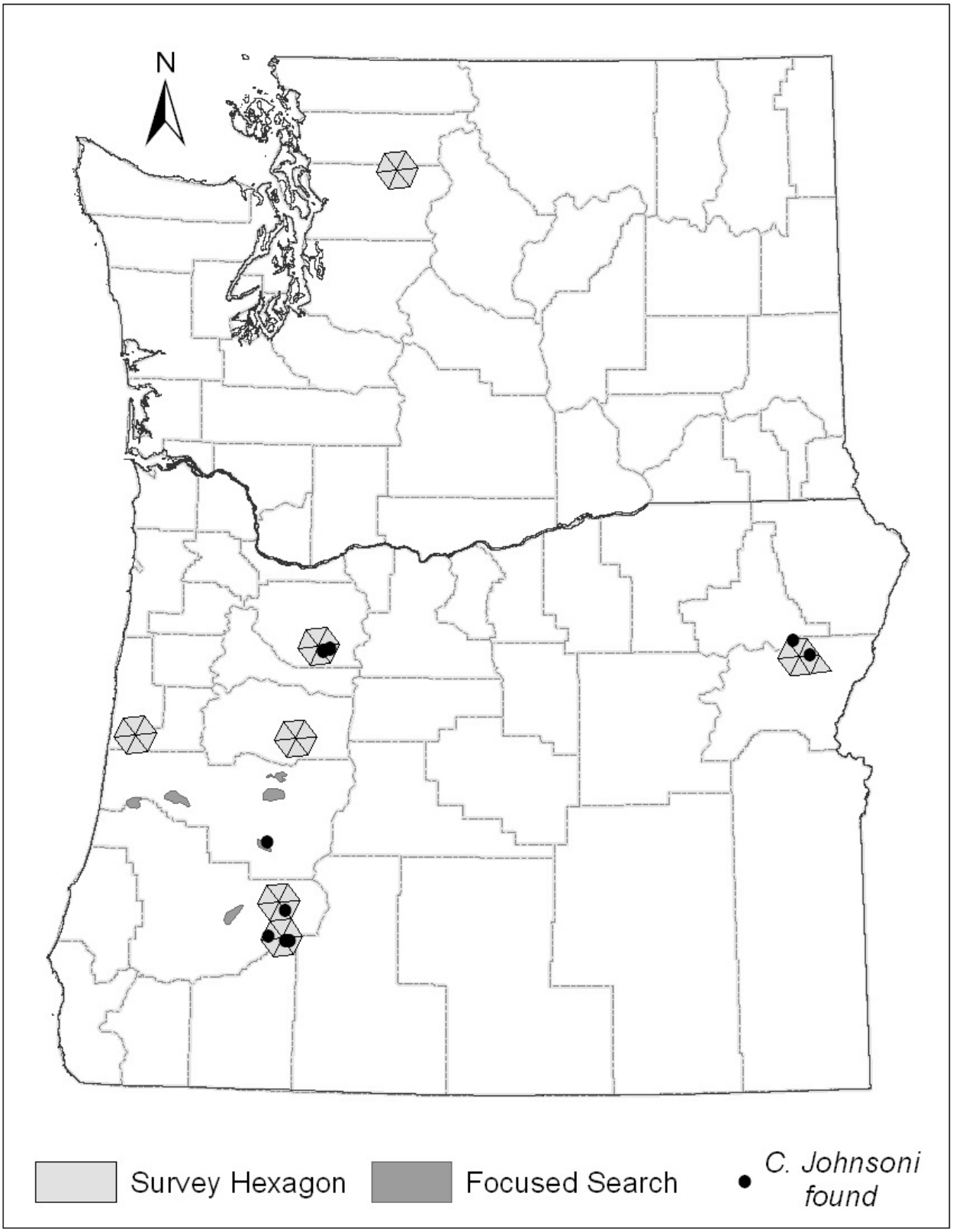
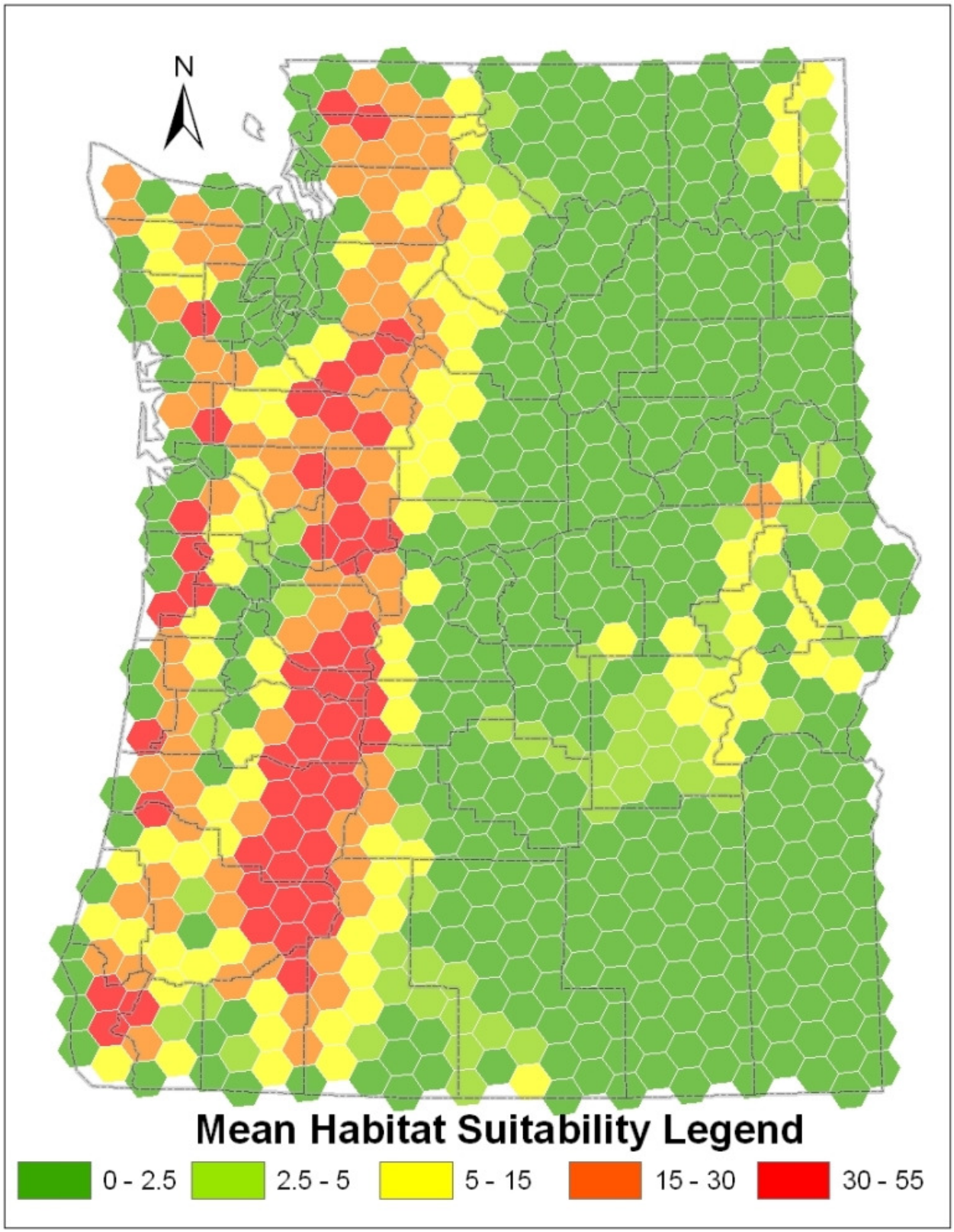
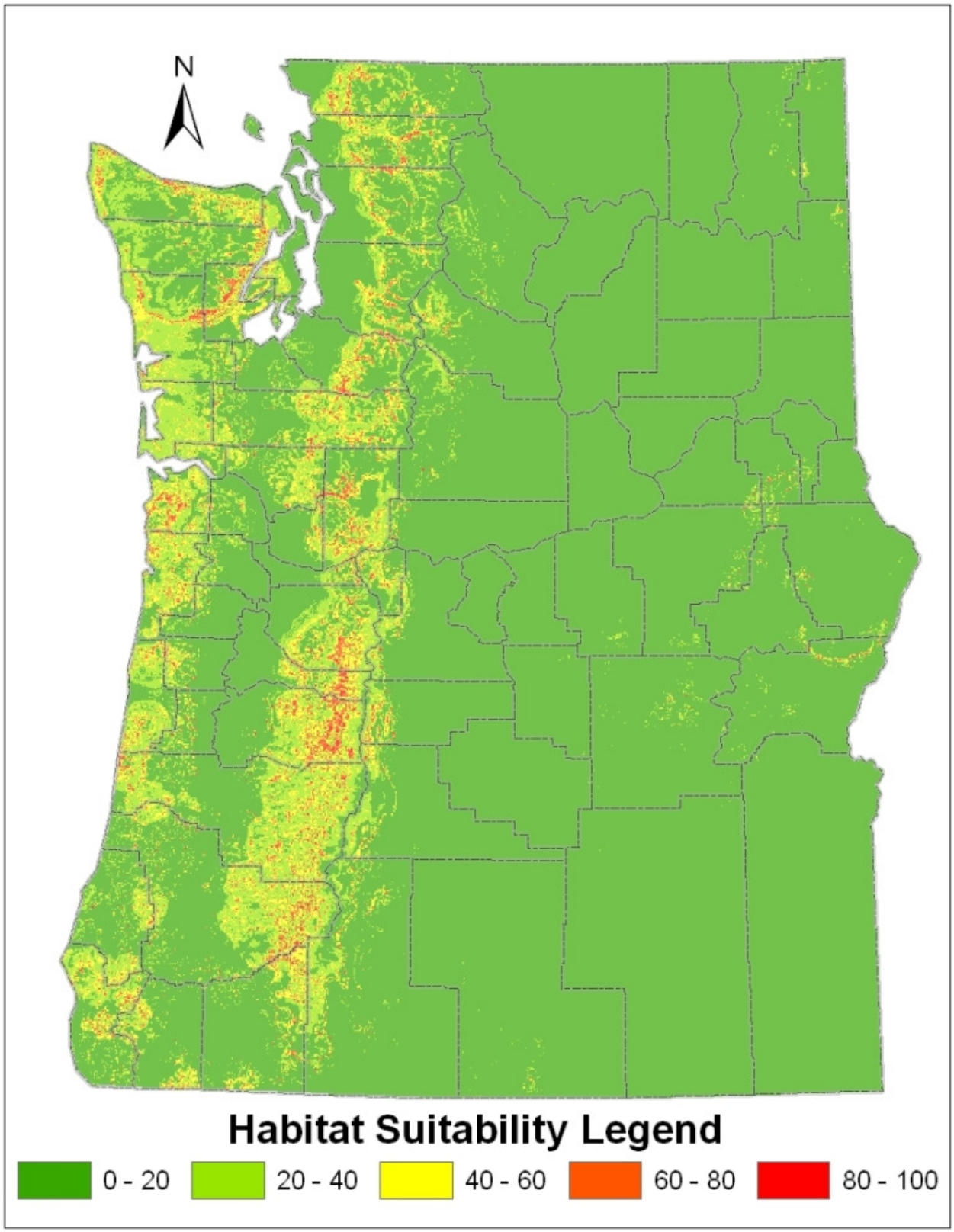
Map Attachment 1 – Areas surveyed in 2009



Map Attachment 2 – Hexagon map showing average habitat suitability scores.



Map Attachment 3 – Version 1.1 habitat suitability map (1km resolution).



APPENDIX A

Draft Survey Protocol (v1.0) for
Johnson's Hairstreak Butterfly (*Callophrys johnsoni*)
in Washington and Oregon
March 2, 2009

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Background

Inventories under the Interagency Special Status/Sensitive Species Program (ISSSSP) for Oregon/Washington Bureau of Land Management and Region 6 (R6) Forest Service are designed to increase our knowledge of the species listed under this program to inform land managers for species conservation. For the Johnson's hairstreak butterfly (*Callophrys johnsoni*), the first step in this process was to compile existing information on its presence for habitat modeling, based on our current understanding of its ecology. The habitat modeling procedures provided insight into this species ecological niche and use of available habitat components and produced a map to help focus these surveys. Information from these surveys and the habitat map may ultimately improve land management planning decisions in regards to this species conservation. As we learn more about this species through these efforts, we expect that the survey and monitoring techniques and habitat modeling will adapt to the new information.

Objective

The primary objective of the surveys for 2009 is to field-test survey methods for detecting presence of *C. johnsoni* by means of repeated surveys at locations where species presence is likely based on habitat modeling and specific survey site selection criteria. According to Pellet (2008), preliminary survey protocols should be designed to assess detection probabilities of the target species. Information from these surveys will be used to estimate site occupancy (site = transect) and to determine the detection probability of this survey method. If successful, this information will aid in the development of an effective survey protocol for this species within its range in Washington and Oregon. It is hoped that this protocol may be used at the project-level scale and that the data collected at this scale would fit into the larger regional-scale inventory scheme that might tell us something about population trends. For now, we will employ commonly used methods for conducting butterfly surveys, customized to fit the ecology of *C. johnsoni*, and logistically focused in areas that may have a higher chance of detection, based on documented presence ***and*** high amounts of [modeled] suitable habitat. Secondary objectives include increasing our understanding of the species' geographic distribution, habitat map validation and improving our understanding of its habitat use.

Species Information

Johnson's hairstreak is a member of the family Lycaenidae (gossamer-winged butterflies); subfamily Theclinae (hairstreaks). Taxonomic nomenclature remains unclear. Some place this species under the genus *Callophrys*, while others use *Mitoura* or *Loranthomitoura*. For consistency, we use *Callophrys* as it is the genus currently used in the Regional sensitive species list.

Johnson's hairstreak is currently known to occur from southwestern British Columbian and southward along the west coast and Cascade Mountains to central California. A disjunct population of *C. johnsoni* occurs at the Oregon/Idaho border in Baker and Union counties, Oregon and in Adams County, Idaho (see Appendix 1).

The flight period for Johnson's hairstreak begins in May and lasts until July, but varies with latitude, altitude and weather. In this region there are a few records from early August. The primary flight season probably lasts one month, and may vary from year to year with weather patterns. In Oregon and Washington it is believed to have only one brood per year. However, in California it has two, so it is possible that it may have two broods in certain parts of this region also.

The average lifespan of adults is estimated at 7-10 days, longer if there is extended inactivity due to inclement weather. Eggs are usually laid individually less often in small clusters on its larval host plant, dwarf mistletoe (*Arceuthobium spp.*). Females can lay as many as 160 fertile ova (McCorkle 1973). After hatching, the yellow-green larvae are small (1.2mm) and blend in well with their host plants (Appendix 7). They develop through 4-5 instars (James and Nunnalle in prep, Ballmer and Pratt 1992) and typically pupate within 5 weeks of hatching. The pupa is dark brown and is attached to the tree or mistletoe clump by a few fine silk threads. It spends the winter in diapause and the adult ecloses the following year.

Johnson's hairstreak adults are slightly larger than most other hairstreaks in this region with a wingspan of 25-30mm. The wing color is brownish and visible in flight. Its flight pattern is erratic, with rapid wing beats making it difficult for human eyes to follow. When perched, its wings are closed, showing only the undersides and their distinctive postmedian white line with three submarginal spots. Male adults have a dark oval stigma on the upperside of the forewing.

This species is believed to be closely associated with old-growth forests (Miller and Hammond 2007, Warren 2005, and LaBonte et al. 2001) that are infected with dwarf mistletoe (*Arceuthobium spp.*). However, eggs and larvae have been found from infected second-growth stands (McCorkle 1973). The primary host trees for dwarf mistletoes that are associated with *C. johnsoni* presence are western hemlock (*Tsuga heterophylla*), white fir (*Abies concolor*) and Ponderosa pine (*Pinus ponderosa*). Dwarf mistletoe can occur on all age classes of forest (Muir and Hennon 2007), but is most abundant in mature stands and old-growth.

Johnson's hairstreak is seldom seen, perhaps because it spends most of its adult life high in the forest canopy. Abundance of adults is thought to be highly variable from year to year, but occasionally large numbers have been reported (Warren 2005, Layberry 1998). Adults occasionally come down to ground level to nectar and to puddle at moist soil. Nectar plants include vine maple (*Acer circinatum*), dogbane (*Apocynum spp.*), manzanita (*Arctostaphylos spp.*), Oregon grape (*Berberis nervosa*), pussy paws (*Calyptridium umbellatum*), Whitethorn ceanothus (*Ceanothus cordulatus*), Mahala mat (*Ceanothus prostratus*), mountain balm (*Ceanothus velutinus*), bunchberry *(Cornus canadensis*), Pacific dogwood (*Cornus nuttallii*), wild strawberry (*Fragaria spp.*), yellow cress (*Rorippa spp.*), and dewberry (*Rubus ursinus*) . Most of these plants have whitish flowers, some with yellow and pinkish hues.

Habitat Modeling

To focus surveys for *C. johnsoni* in 2009, the existing knowledge of the species occurrence was compiled into a GIS database and a portion of these sites were used to "train" a habitat model. Species locations were consolidated from various sources of information such as Hinchliff's butterfly atlases (Hinchliff 1994, 1996), the butterfly database of the Evergreen Aurelians, and museum specimens as well as other sources. The recorded description of each location was cross-referenced with several different GIS layers, including counties, townships, rivers, creeks, cities and roads, to ensure it spatial accuracy as much as possible. In addition, locations were verified with the actual observer, when possible, using high resolution aerial imagery and topographic maps in ArcGIS and Google Earth. This work resulted in a compilation of 85 *C. johnsoni* sites within Oregon and Washington. Of these, 55 sites (most recent since ≥ 1970 and spatially accurate) were used to train the habitat model.

Habitat modeling was done with BioMapper v4.0 (Hirzel et al. 2007). The habitat variables used included a distribution map of dwarf mistletoe densities based on FIA/CVS plot data, climate data, forest cover data and elevation data. Habitat modeling indicated that this species' distribution is strongly associated with the distribution of dwarf mistletoe, primarily *Arceuthobium tsugense*, but it is also documented to use *A. abietinum* and *A. campylopodum*. Johnson's hairstreak occurs in areas that receive higher than average rainfall for the Oregon and Washington region, and areas that are more humid and cooler than average for the region. It appears to avoid areas that have large variations between maximum and minimum average annual temperatures. Elevations from sites used for habitat modeling ranged from 100 to 6,400ft (average = 3,100ft).

The habitat modeling produced a map (Appendix 2) with habitat suitability (HS) values from 0 to 100, where HS values closer to zero indicate habitat variable conditions at that site (map pixel) that are dissimilar to where *C. johnsoni* occurs and values closer to 100 are more similar. The resulting map was classified into 5-equal intervals (e.g., HS 0-20, 20-40, …80-100) and validated using a k-fold cross validation process where a different random portion of the training sites were set aside for 4 different replicates of the model, created with the remaining sites. The model was fairly robust (Spearman rank = 0.75) at predicting presence of *C. johnsoni* using these five map classifications. Based on the cross-validation curve, a cut-off of HS≥50 was chosen to represent potential *C. johnsoni* habitat for this map version. Of the additional 30 sites not used for modeling (some of these were compiled after model completion), about 78% were in or within 1-km of modeled habitat, well within the limit of spatial accuracy for these point locations. Most of the remaining 22% were old historic sites, which no longer have appropriate habitat (e.g., urbanization).

Survey Hexagon Selection

Once the habitat model was completed, broad survey areas were identified by overlaying the habitat model with EPA's Environmental Monitoring & Assessment Program[4] (EMAP) hexagons (65,000 hectares each). Hexagons with the highest amount of potential habitat (model HS ≥ 50)

[4] EMAP hexagons are used to develop species distribution maps by the Oregon Gap Analysis and Oregon Natural Heritage Information Center (ORNHIC).

and documented presence of *C. johnsoni* were selected for field surveys in 2009 (Appendix 3). There were 8 hexagons in the region that met these criteria. They occurred in the Western Cascades of Oregon, extending from the Mount Hood to the Rogue River National Forests. Given budget constraints, only one survey hexagon was selected from each National Forest within this area, for a total of 4 "survey hexagons".

Seven other hexagons were highlighted as "areas of interest" across the region. These are areas on the "edges" of the butterfly's know distribution, which the habitat model indicates similarity in habitat variables; however (in most cases) there is no documentation of *C. johnsoni*. These areas of interest may be surveyed using standard "checklist" survey techniques (Royer et al. 1998).

Survey Route Selection

Each survey hexagon was divided into 6 equal triangular sections that are 10,833ha each. Two of these sections were selected from each hexagon for surveys (Appendix 4). These were the sections with the most and the least amount (%) of potential modeled habitat[5] (model HS ≥ 50), regardless of presence of *C. johnsoni*. Two 2km survey routes (a.k.a. transects) were identified within each survey section. Survey routes are preliminary and need to be field verified to meet all of the following criteria:

1. Adjacent to older coniferous forest (≥80 years stand age)
2. Presence of dwarf mistletoe
3. Drivable or walkable road

Conducting the Survey during the Adult Flight Period

For Washington, past detection history (Figure 1) suggests that the peak flight period for adult *C. johnsoni* is from May 22nd thru June 18th (weeks 4-7 for a 13-week period begin May 1). In Oregon, the peak flight season appears to be from May 29th through July 2nd (weeks 5-9). Peak flight seasons may last from 1-2 weeks for small populations and 3-4 weeks for larger populations. The flight season in Oregon appears to be slightly longer than in Washington, and there are some indications of a second brood, especially east of the Cascade Crest. For instance, detections have been recorded as early as April 15th in Baker County (K. Smith) and May 4th in Jefferson County (H. Rice), with the latest recorded detections from July 9th and August 17th in these same areas, respectively.

[5] This was done for field validation purposes for the habitat map v1.0

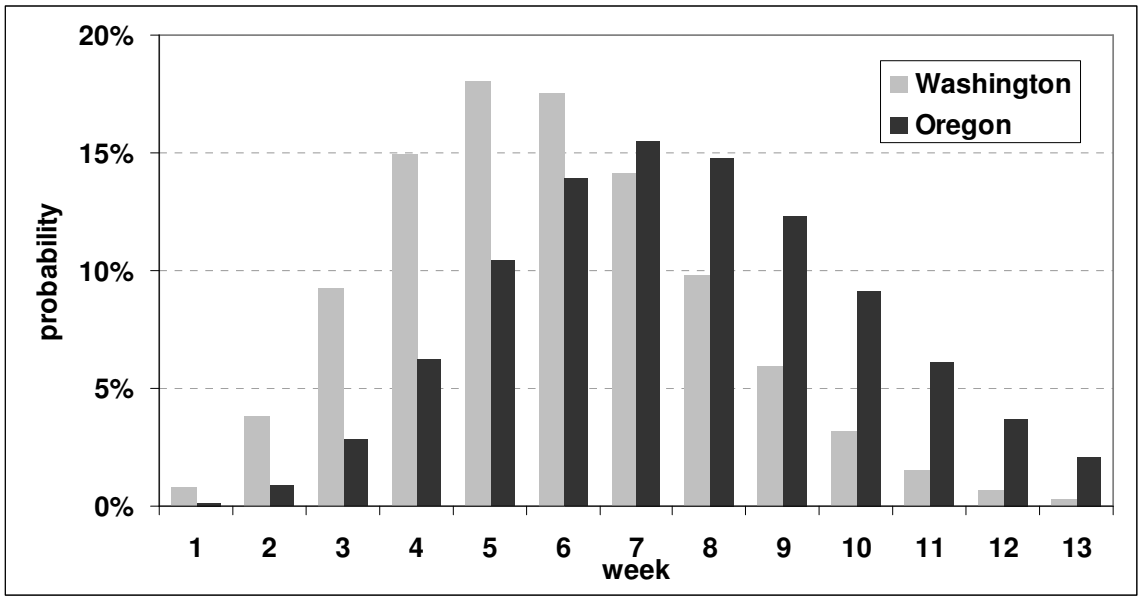

Figure 3. Weekly detection history for *C. johnsoni* sightings in Washington (n=40) and Oregon (n=36) fitted to a Poisson curve for the time period of May 1 thru July 31.

Emergence date of butterflies vary year to year with annual fluctuations in the weather. We estimate that peak emergence might vary from 1-2 weeks. Therefore, initiation of surveys should consider the seasonal weather, and phenology of the butterfly's nectar plants. Once initiated, each survey route will be surveyed 4 times, with at least a week between surveys. Surveys will be timed to increase odds of coinciding with flights of *C. johnsoni*. As such, they should only be conducted on sunny days with little to no wind, when ambient air temperature is >13ºC. Local winds can vary considerably depending on habitat and topography, but surveys should not be conducted when the average wind speed (along the transect) exceeds 16 kilometers per hour or Beaufort Scale 3 (gentle breeze). Windy and/or foggy, cloudy conditions may develop during a survey, so be prepared to wait out these conditions and resume surveys when they pass. Surveys need to occur between 1000 and 1600 hours.

Each 2km route will be walked from end-to-end at a slow pace, such that the opposite end is reached after 1.5 hours of searching. Focus on sunny edges and gaps. Walk cautiously along edges of mature conifer stands infected with dwarf mistletoe while inspecting nectar flowers and moist soil for hairstreaks before slowly sweeping the net over each area once observations have been made. This butterfly spends most of its time perched. It is not usually a patrolling insect, although males will cruise flowers for females. When disturbed it tends to flush upward, flying up high into the trees (James and Nunnallee in prep.). It may be useful to search tree canopies periodically with binoculars to spot flying or perching butterflies. Also, inspect mud puddles on the road, road ditches and culverts. Look for butterflies on animal scat on the road. Once the transect end is reached, the route can be walked back at a regular pace to where your vehicle is parked. Any *C. johnsoni* observed during this return trip would be recorded as incidental to the survey, but recorded in the field form. All hairstreaks should be netted and transferred into a viewing jar for closer observation and species confirmation. Collect one voucher per route whenever possible. Photo vouchers can be made in addition to collected specimens.

Conducting the Survey during the Egg and Larval Period

Adult hairstreak butterflies are often hard to detect during transect surveys and supplemental surveys for eggs and larvae may be needed (Warren 2005, Zonneveld et al. 2003). As stated above, certain species of dwarf mistletoe are the larval host plant for *C. johnsoni*. The Thicket hairstreak (*C. spinetorum*) is the only other hairstreak in this region that lays its eggs on dwarf mistletoe. These two species overlap in certain parts of this region and are very similar in appearance in both the adult and larval stages (Appendix 8). However, they can be distinguished by certain wing patterns and according to James and Nunnallee (in prep.) by coloration of the first and third dorsal segments (darker in *C. johnsoni*) and white edgings to dorsal segments (more prominent in *C. johnsoni*) in the later instar larval stages. Occasional hybrids are found.

This part of the survey will focus on collection of mistletoe clumps and searching them for eggs and larvae. Lower branches of infected trees along transects will be searched for dwarf mistletoe clumps. Clumps will be collected at 250m intervals so that a total of 8 samples will be collected along each transect (see Appendix 5). Sample site centers are spaced every 250m but if needed, clumps can be collected from within 50m of each side of the center. Young dwarf mistletoe clumps seem to have low stimulation value to ovipositing females (McCorkle 1973), therefore each sample should consist of 6-12 clumps per sample site larger than a golf ball in size, preferably from branches that have good exposure to sunlight. Place the clumps from each sample site in white plastic trash bags and mark the bags clearly with transect and sample site information. During transport, keep trash bags out of direct sunlight and do not keep in parked vehicle to prevent overheating and killing the eggs and larvae inside.

Clump sampling should occur after the peak flight period is over. For Washington this would begin on week 8 and week 10 in Oregon. Note that clump sampling can be done as long as there is daylight and in most weather conditions. However, carry along a net and be prepared for adults. The route can be walked or driven between dwarf mistletoe sample sites. Any adults observed during this 5th visit will be recorded as incidental observations.

Dwarf mistletoe occurs in all stand ages, but is most abundant in older stands (Muir and Hennon 2007). In younger stands it occurs on infected trees that survived the former stand disturbance or is present due to its adjacency to older infected stands. Single-storied, stem exclusion stands probably have the poorest conditions for dwarf mistletoe, as it needs sunlight and multiple tree layers for optimal growing conditions. It's occurrence and biomass increases with canopy height and exposure to sunlight (Muir and Hennon 2007, Shaw and Weiss 2000).

Upon return to the office, carefully arrange the clumps on a flat surface by spreading them out in their bags. Over the next few days, each bag will be inspected for signs of eggs and larva (Appendix 7). If egg and larvae are found, examine for details under a dissecting microscope (be careful not to overheat with the light source). Record your observations and take photographs using a digital camera with macro focus.

Equipment

The following field equipment is recommended for conducting Johnson's hairstreak butterfly field surveys:

- Binoculars
- Thermometer
- Hand held wind meter
- Digital camera with at least a 10x optical zoom and macro focus
- GPS unit to record survey observations
- Butterfly field guide(s)
- Insect net with lightweight aerial net bag
- Spatula bladed tweezers for handling live adults
- Clear, specimen-viewer "bug box"
- Specimen envelopes and hard, protective, envelope storage container
- Hand pruner for collecting dwarf mistletoe
- White plastic kitchen trash bags with twist ties
- Black sharpee pen to record information on trash bags and field maps
- Magnifying glass for examination of mistletoe clumps
- Dissecting microscope for examination of eggs and larvae

Verification Process

Identifications should be confirmed by an expert lepidopterist. Collecting voucher specimens is a standard entomological method for verifying species identification and documenting new populations of target taxa. At a minimum, a voucher specimen should be collected at any new site greater than 3 kilometers from the nearest previously documented site. Aim to collect a single voucher per route.

In addition to a specimen voucher, photographic vouchers should be collected. When doing so, at least one photo must include a clear side view showing the ventral hindwing pattern. To photograph the specimen, carefully place it in a container with a clear, flat surface to shoot through to avoid distortion. A clear acrylic "bug box" with a magnifier lid (Figure 2) works well for this purpose. The smaller the container, the easier it is to focus on the butterfly and the less likely it is that the butterfly will move in or out of focus.

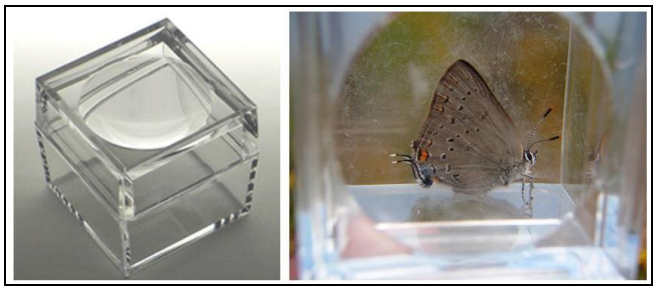

Figure 4. Clear acrylic boxes with magnifier lids are useful in taking photographic vouchers of hairstreak butterflies. This is an example of a Sylvan hairstreak (*Satyrium sylvinus*) taken in a 1½ inch x 1½ inch square "bug box" (butterfly photo by R. Davis).

Permits

Capturing or holding butterflies for identification, photography, or collection in the State of Washington requires a scientific collection permit from Washington Department of Fish & Wildlife (WDFW). Coordination with WDFW prior to submitting a permit application is recommended as several people can collect under the same Collection Permit: all names must be listed as sub-permittees. A $12.00 permit fee is charged. Up to 45 days may be required for a collection permit to be issued in Washington. Oregon does not currently require a collecting permit for butterflies, although a permit may be required for certain ecologically sensitive areas. If in doubt, it is best to check with the land owner or managing agency before collecting on any land.

Surveyor Qualifications and Training

Surveyors should have long attention spans with the ability to see minute details and should have a sufficient knowledge of the ecology and characteristics of hairstreak butterflies. Training will be required to conduct protocol surveys for Johnson's hairstreak butterfly. Training objectives will include:

1. Instructing surveyors to detect and identify hairstreaks and specifically Johnson's hairstreak.
2. Instructing surveyors to identify conifer tree species and dwarf mistletoe infections.
3. Providing surveyors with basic knowledge to recognize members of different butterfly families, and commonly occurring local species that are present during the Johnson's hairstreak flight period.
4. Review of the survey protocol and providing basic butterfly survey and collection techniques to conduct adequate field surveys.
5. Handling and preparation of voucher specimens.

Training should include a classroom day for instruction followed by at least one day of field training in survey methods.

Safety

Surveys will be conducted by individuals. Make sure you maintain a sign in/sign out method to ensure everyone returns from the field as well as to know exactly where the surveyor is working. When working alone, carry a handheld radio or some other communication device. Johnson's hairstreak butterfly surveys will sometimes be along roads with varying amounts of traffic. Surveys require focusing on butterflies, but the surveyor needs to always be aware of the traffic situation. It will be left up to the surveyor as to how to travel the survey route safely. Surveys should always be done on foot and not from the vehicle. Always park your vehicle in a safe location and use a chock-block and parking brake on slopes. These safety considerations are not meant to be an inclusive list of hazards expected to be encountered while performing this protocol survey method. They are simply some important ideas to keep in mind. Each surveyor must be mindful of safety and follow their agency's safety policies and procedures.

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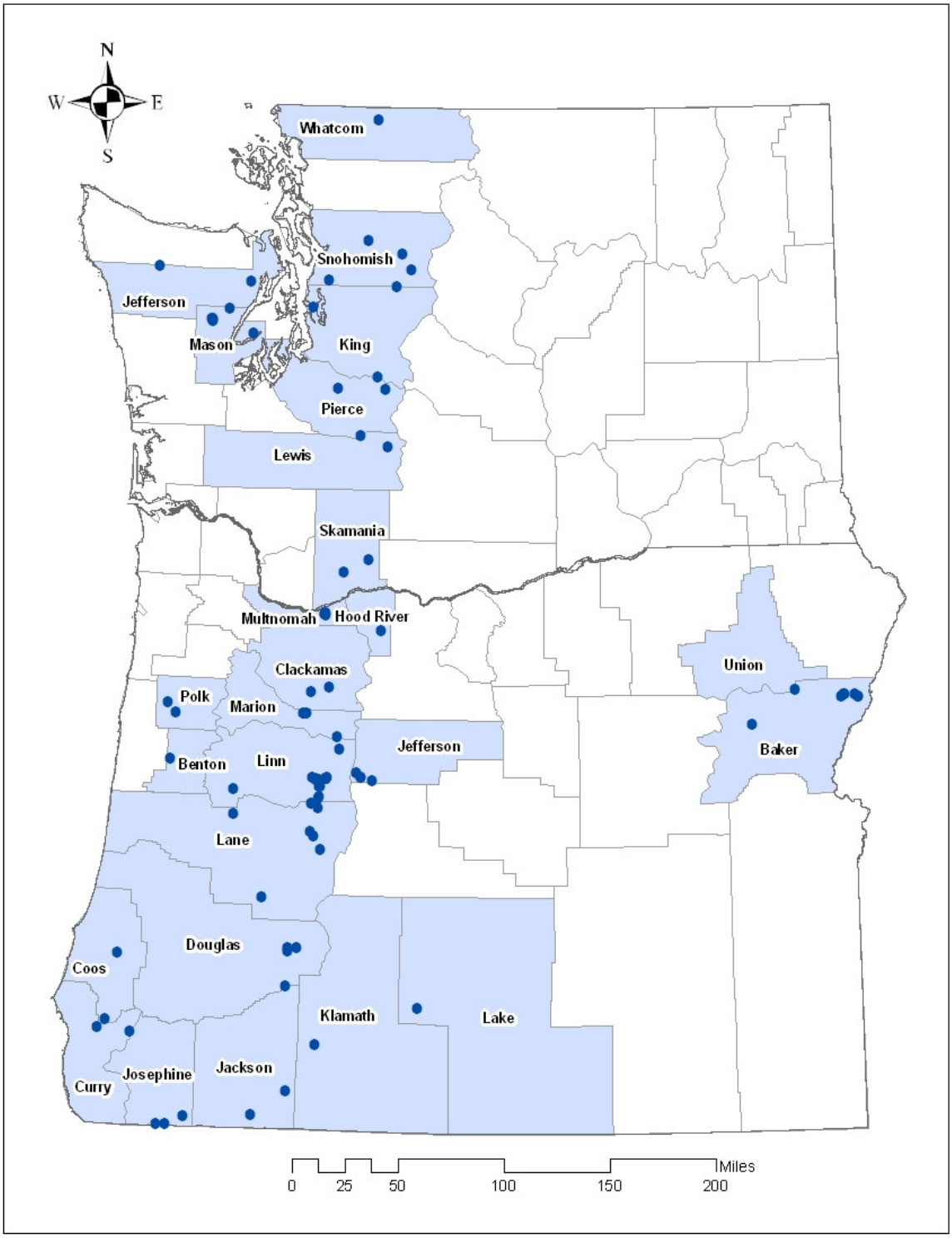
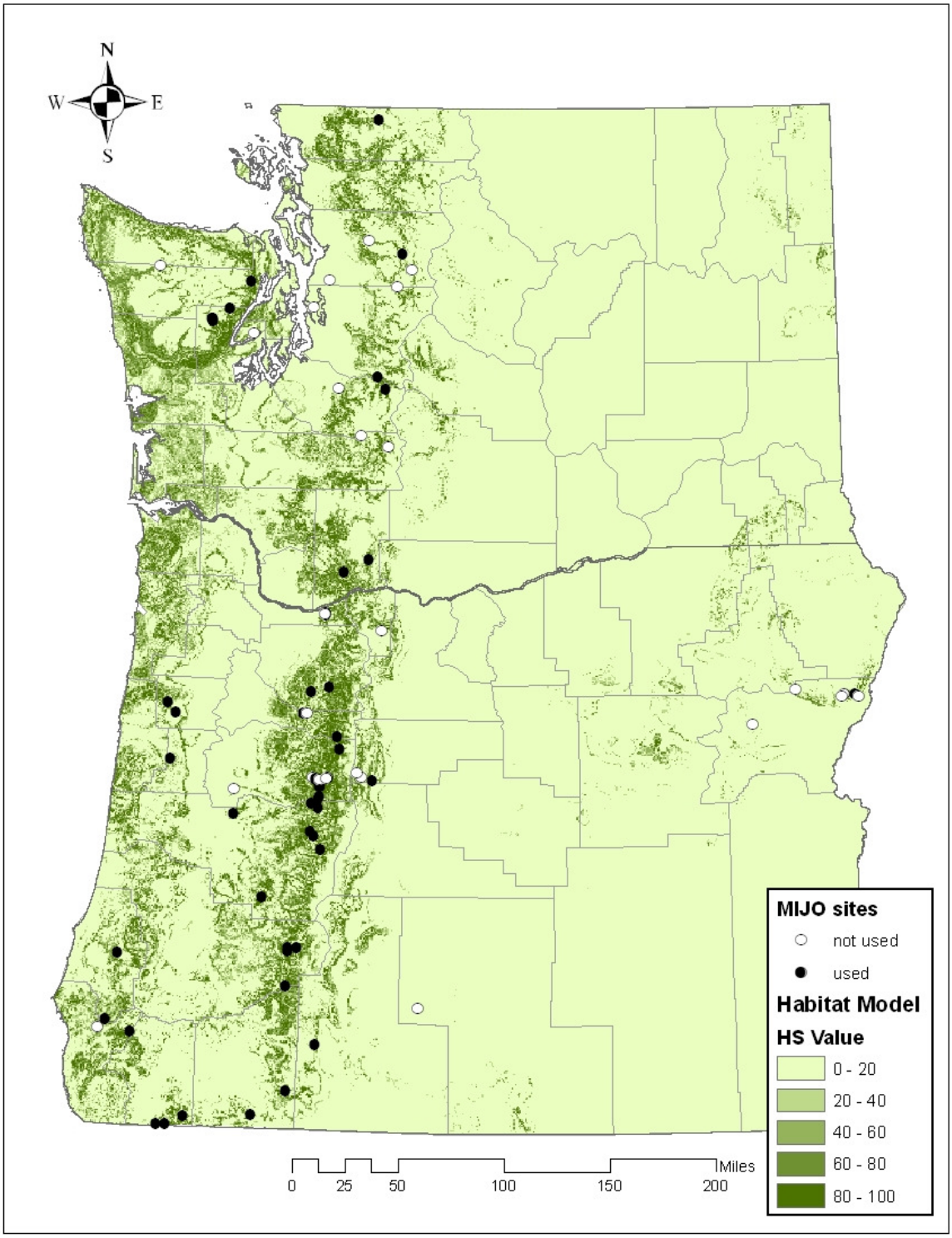
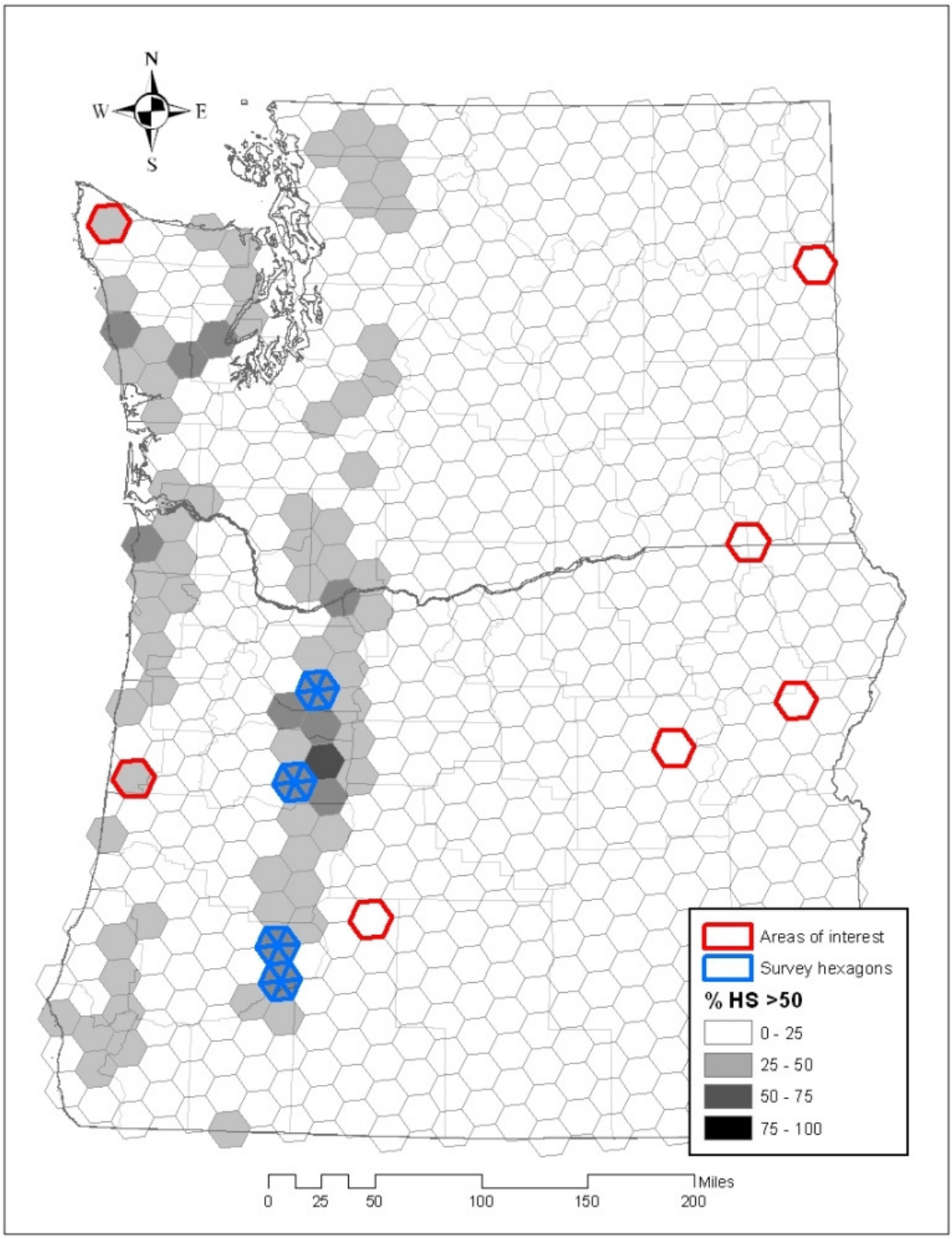
APPENDIX 1 – Current known distribution of Johnson's hairstreak (*Callophrys johnsoni*) in Oregon and Washington



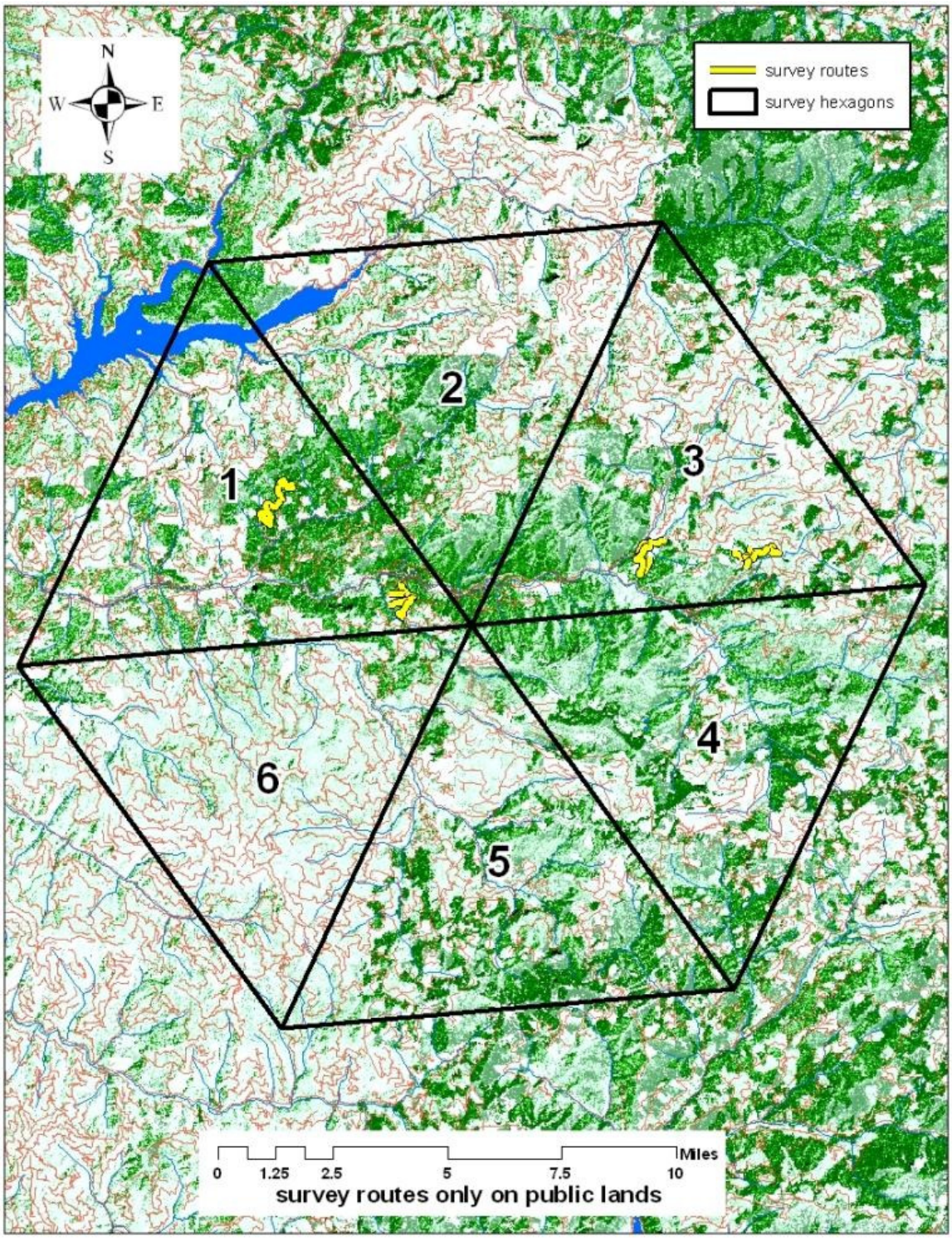
APPENDIX 2 – Habitat Model (v1.0)



APPENDIX 3 – Survey hexagons for 2009



APPENDIX 4 – Example of survey route (a.k.a. transect) layout



Darker shades of green represent older coniferous forest.

APPENDIX 5 – Example field form and survey maps

Johnson Hairstreak Butterfly Field Survey Form (2009)

Survey route_____ Visit#[1]_____ Date_____

Start: Time_____ Cloud cover%____ Overcast type____ Wind speed____ Temp (C)____

Stop: Time_____ Cloud cover%____ Overcast type____ Wind speed____ Temp (C)____

Comments_____

_____ Surveyed by_____

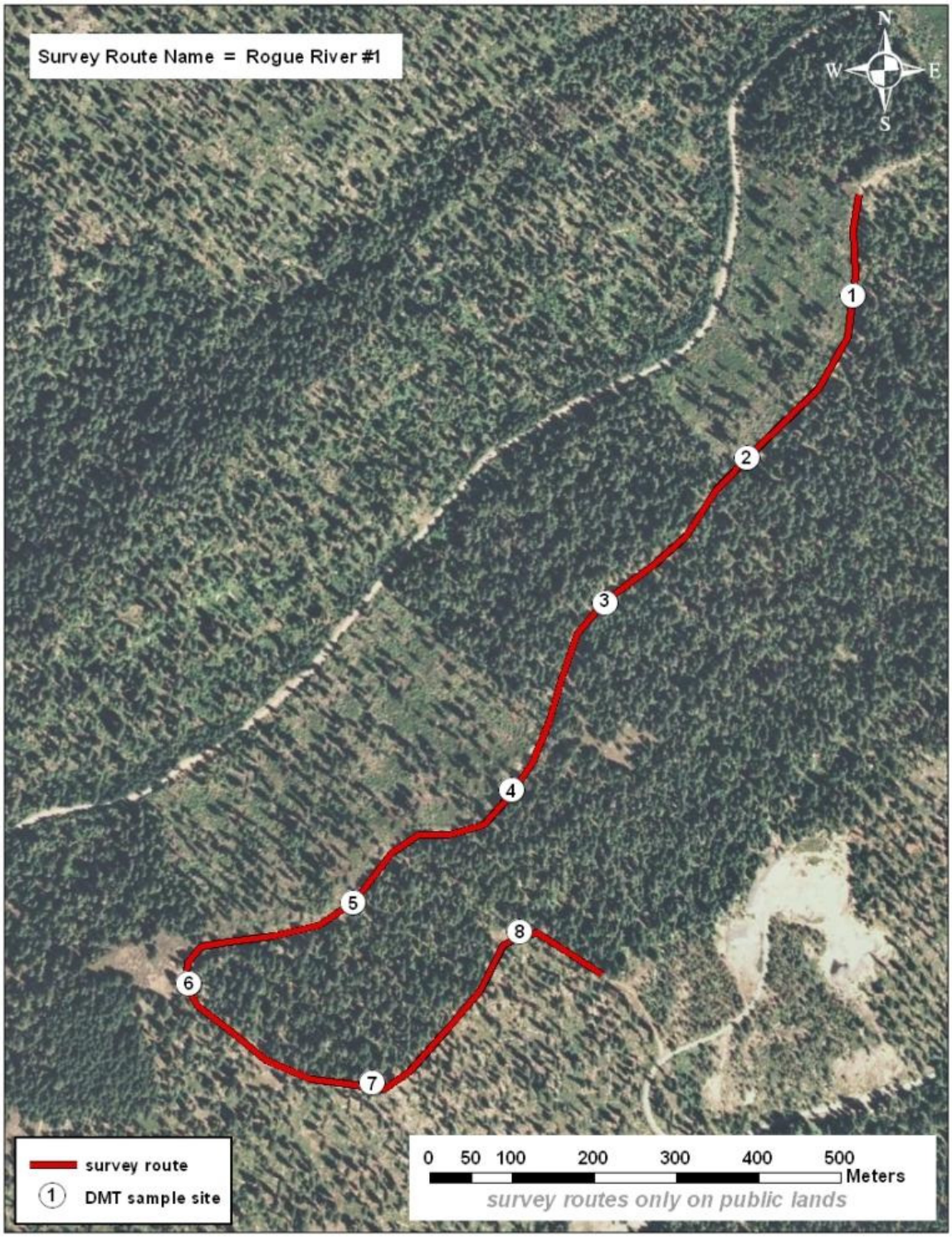
Obs#	Qty	Time	Incidental (Y/N)	Activity	Notes
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					

other butterfly species detected and other comments:

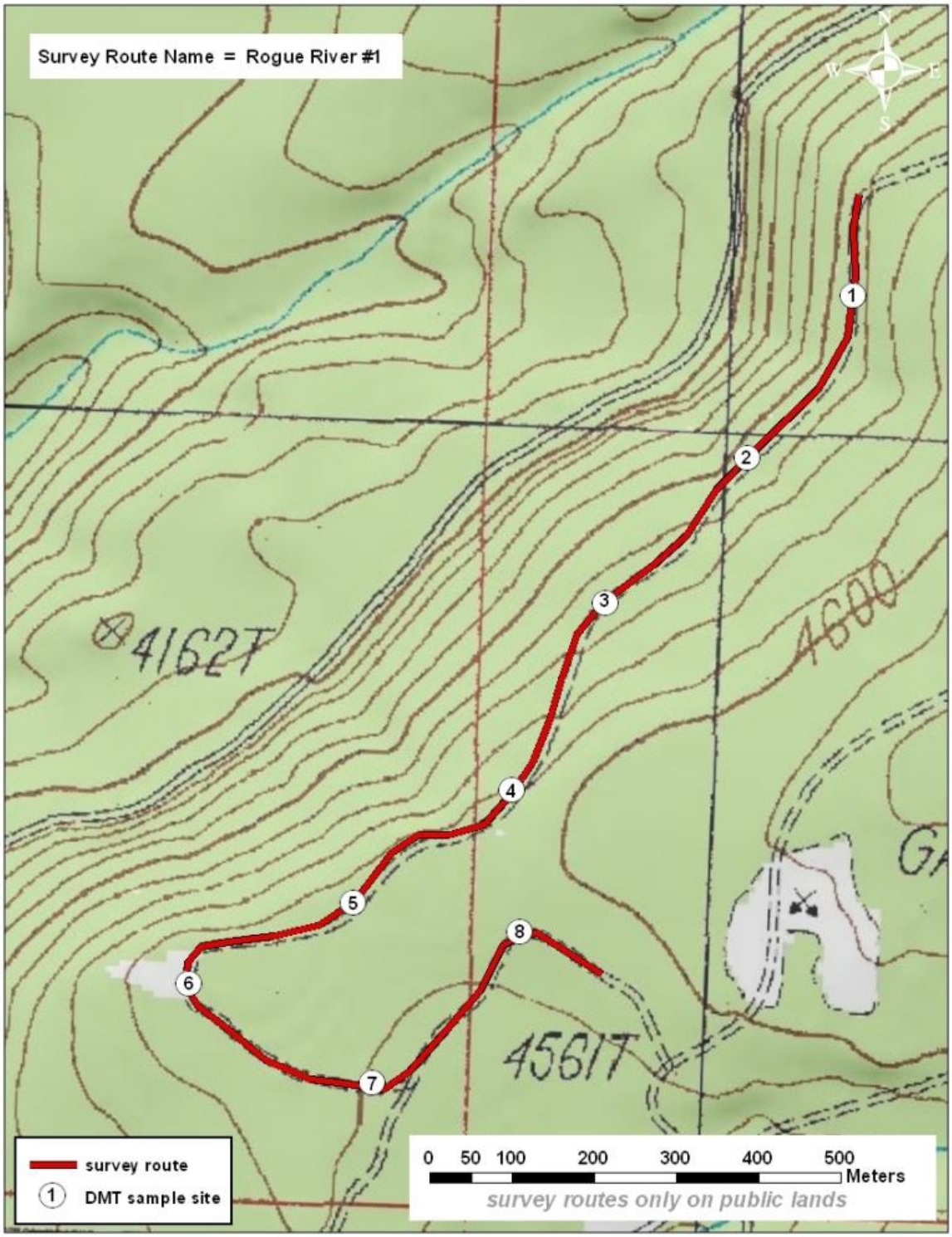
Cloud cover% – percent of sky covered by clouds, fog, smoke…etc.
Overcast type – CLD = clouds, FOG = fog, SMK = smoke/haze
Wind Speed – record average wind speed using wind gauge in kilometers per hour (kph) or Beaufort Scale:
0 = calm (<1.8 kph)
1 = light air (1.8 – 6.4 kph)
2 = light breeze (6.5 – 12 kph)
3 = gentle breeze (12 – 16 kph)
Temp – record air temp in degrees ºC
Obs# – record Johnson Hairstreak observation # on survey map using a sharpie and take GPS waypoint
Qty – record number of CAJO adults observed
Time – record time of observation
Activity Codes – FLY = flying, PER = perching, NEC = nectaring (record plant spp in notes), PUD = puddling, OTH = other (record in notes)

[1] Visit #5 is to collect dwarf mistletoe clumps. Use this field form for this survey and Obs# will represent Sample Site#. QTY = number of clumps (between 6-12). Use notes to describe collection distance from sample site center.

APPENDIX 5 (cont.) – Example field form and survey maps



APPENDIX 5 (cont.) – Example field form and survey maps



APPENDIX 6 – Similar looking hairstreaks

Johnson's Hairstreak - Male
(*Callophrys johnsoni*)

Johnson's Hairstreak - Female
(*Callophrys johnsoni*)

Similar "look-alike" hairstreak butterflies
(photographs below are property of Nearctica.com)

Thicket Hairstreak
(*C. spinetorum*)

Cedar Hairstreak
(*C. gryneus nelsoni*)

Juniper Hairstreak
(*C. gryneus*)

Hedgerow Hairstreak
(*Satyrium saepium*)

APPENDIX 7 – Johnson's hairstreak eggs, larvae and pupa

Eggs (0.7mm)

3rd Instar (3-5mm)

4th Instar (12-19mm)

Pupa (11-12mm)

Things to look for during inspection of mistletoe clippings in the lab

Signs of larvae include holes in dwarf mistletoe, tiny caterpillar droppings found underneath mistletoe clippings, egg shells and of course, larvae themselves (2nd – 3rd instar)

APPENDIX 8 – Distinguishing between *C. johnsoni* and *C. spinetorum*

Adult Johnson's Hairstreak
(photo courtesy of James and Nunnallee)

Adult Thicket Hairstreak
(photo courtesy of James and Nunnallee)

4th-instar of Johnson's Hairstreak (photo courtesy of James and Nunnallee)

4th-instar of Thicket Hairstreak (photo courtesy of James and Nunnallee)

APPENDIX 9 – Multicolored Asian lady beetle (*Harmonia axyridis*)

Non-native, invasive species are of major concern because of their potential impacts to the ecosystems they invade (both economical and ecological). One of the most effective methods for controlling spread of invasive species is early detection.

During the late 1970s and early 1980s USDA Agricultural Research scientists released the multicolored Asian lady beetle (*Harmonia axyridis*) as a biological control agent for pear psylla and other soft bodied insect pests. Others may have arrived here as unintentional passengers aboard cargo ships from the Orient (Suomi 2008).

Numerous releases occurred in the US; the closest to western Washington were in Chelan, Klickitat, and Yakima Counties. The beetle was extremely abundant in western Washington and Oregon throughout 1993–1994 (LaMana and Miller 1995), extending west from the coast to an elevation of 1371m in the Cascades. The range of this species is believed to still be increasing to the south in the Pacific Northwest.

This species was recently identified as a potential hazard to immature monarch butterflies (Koch et al. 2005) and its larvae have been seen on dwarf mistletoe during the egg-laying period for Johnson's hairstreak (McCorkle pers. comm.). There is reason to suspect it may be impacting this butterfly species too. Document its presence in this butterfly's habitat, by collecting vouchers along transects. Note any observations of these or other predators attacking *C. johnsoni* ova or larvae.

The name "multicolored" refers to color variations in adults, ranging from solid red or black with two spots, to red with up to 19 black spots. It can be distinguished from other lady beetle species by the black "M" on the white pronotum formed by black dots.

Koch R.L., R.C. Venette, and W.D. Hutchison. 2005. Influence of alternate prey on predation of monarch butterfly (Lepidoptera: Nymphalidae) larvae by the multicolored Asian lady beetle (Coleoptera: Coccinellidae). Environ Entomol 34:410–416

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