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

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Background

Inventories under the Interagency Special Status/Sensitive Species Program (ISSSSP) for Oregon/Washington Bureau of Land Management and the Pacific Northwest Region (Region 6) of the Forest Service are designed to increase our knowledge of the species listed by this program. This information can be used for environmental analysis of proposed projects, to aid in conservation, and ultimately avoid Federal listing of the species. For the Johnson's Hairstreak butterfly (*Callophrys johnsoni*), the first step in this process was to compile existing information on its presence, which was used for habitat modeling based on the current understanding of its ecology. The habitat modeling procedures provided insight into the species ecological niche and produced a regional map to help focus field surveys. These field surveys were then tested for their effectiveness in detecting the species, as well as their logistic feasibility and costs. Information collected from these surveys was also used to update the habitat models, improving our understanding of this species geographic distribution. This work culminated in the survey protocols described herein. As we learn more about this species through continued survey efforts, we expect the additional information gathered will be used to periodically update the habitat models and advance our knowledge of the species.

Species Information

Johnson's Hairstreak is a member of the family Lycaenidae (gossamer-winged butterflies); subfamily Theclinae (hairstreaks). Taxonomic nomenclature remains unclear. Some put this species under the genus *Callophrys*, while others use *Mitoura* or *Loranthomitoura*. We use *Callophrys* as it is currently used by the Integrated Taxonomic Information System, the national naming convention used by ISSSSP.

This butterfly is known to occur from southwestern British Columbia and Vancouver Island in Canada, southward along the west coast and Cascade Mountains to the Sierra Mountains in central California. Elevations range from 90 to 930m (median = 390m) in Washington, and 30 to 1,950m (median = 1,070m) in Oregon. In Washington, it is only know to occur west of the Cascade crest. A disjunct population occurs at the Oregon/Idaho border in Baker and Union counties, Oregon and Adams County, Idaho (appendices 1 and 2). This disjunct population may be a relict population isolated by climate changes.

Figure 1. Adult Johnson's Hairstreak (photo by Alan Dyck).

Adults (fig. 1) are slightly larger than other hairstreaks in this region with a wingspan of 25 to 35mm. The uppersides of the wings are brownish with orange-brown near the tail. The undersides are also brown with a white postmedian line bordered with black or darker brown on the inner edge. The hindwing has three black submarginal spots, the center one with orange-brown scales separating it. Some silver-blue and orange scales occur near the tail. Its flight pattern is erratic, with rapid wing beats making it hard for human eyes to follow. However, the brown wing color is visible in flight. It perches with its wings closed, showing only the undersides with the distinctive white line and hindwing spots. Males have an oval-shaped stigma on the upperside of the forewings (fig. 2).

Figure 2. Upperside (dorsal) wings of a female (left) and male (right) Johnson's Hairstreak. The stigma is a section of scent scales located on the forewings of male hairstreaks that produces pheromones for attracting females.

The average lifespan of adults is estimated to be 7 to10 days; longer if there is extended inactivity due to inclement weather. Adult coloration can fade with age as it loses its wing scales, and the wing conditions can become tattered or frayed about the edges (fig. 3B).

Figure 3. Underside wings in fresh adult (**A**) and weathered adult (**B**).

Based on historical records from Oregon and Washington, the flight period for Johnson's Hairstreak begins in late April and lasts until early August (appendix 4), but varies with latitude, altitude and weather. There are very few records from April and August, and the primary flight season runs from late May to early July.

Egg laying begins as soon as the adults eclose (hatch) from their pupae and begin flying. Eggs are usually laid individually (fig. 4), less often in small clusters on or very near dwarf mistletoe (*Arceuthobium spp.*). Surveys have documented egg densities ranging from 0.1 to 0.7 eggs per dwarf mistletoe clump collected (McCorkle 1973). Females can lay as many as 160 fertile ova (McCorkle 1973). It takes about one week for eggs to hatch. After hatching, the yellow-green first instar larvae are small (1mm) and blend in very well with their host plant (fig. 5A). Larvae

Figure 4. Eggs on *Arceuthobium tsugense***.**

have a fleshy hood that covers the head as it feeds. They develop through four instars, forming dorsal ridges in the later stages (James and Nunnallee in press, Ballmer and Pratt 1992). They 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 (fig. 5F). In Oregon and Washington it is believed to have only one brood per year. However, in California it has two broods, so it is possible that it may also have two broods in certain parts of this region. It spends the winter in diapause and the adult ecloses from the pupa in the following year.

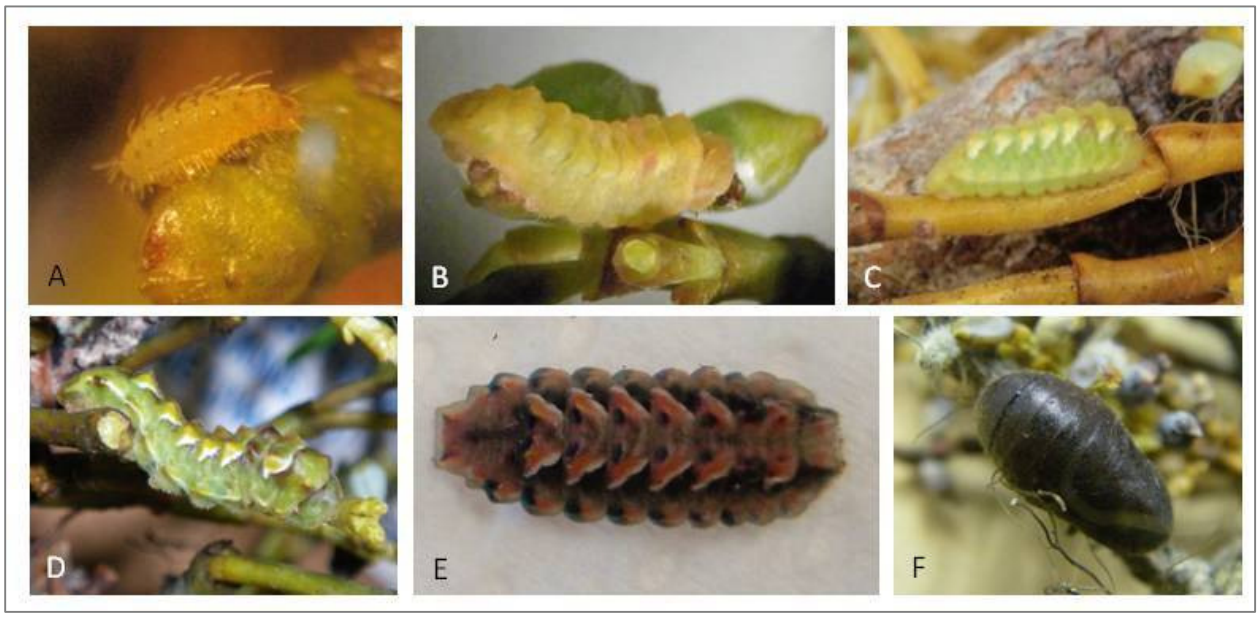

Figure 5. Larval stages are; [**A**] first instar (1-2mm), [**B**] second to third instar (2-6mm), [**C**] early fourth instar (6-12mm), [**D**] late fourth instar (12-19mm), [**E**] prepupa (8-15mm), and [**F**] pupa (10-12mm).

Adult Johnson's Hairstreaks are seldom seen, perhaps because they spend most of their adult life high in the forest canopy. Abundance of adults is thought to be highly variable from year to year, and occasionally large numbers have been reported (Warren 2005, Layberry 1998). Adults do occasionally come down to ground level to nectar and to puddle at moist soil.

In Oregon and Washington, there is only one other hairstreak butterfly that has a similar life history as the Johnson's Hairstreak and similar larval host plant. It is known as the Thicket Hairstreak (*C. spinetorum*). This species is similar in appearance, but can be distinguished from *C. johnsoni* in its adult stage as the ventral side of the hindwings have three additional submarginal spots (Fig. 6A) and the dorsal side of both wings are gunmetal blue in color (fig. 6B). One can often see glints of this color as it flies. However, there is no current physical or coloration description that allows for distinguishing the species in their larval stages. There is also evidence of hybridization occurring between these two species in portions of their range (McCorkle 1973, Davis and Weaver 2010). Another similar looking species is the smaller Cedar Hairstreak (*C. gryneus*), see appendix 5 for distinguishing features.

Figure 6. An adult female Thicket Hairstreak (*C. spinetorum*). Note the additional submarginal spots (see arrow) on the underside of the hind wings (**A**) and the gunmetal blue of the uppersides (**B**).

Habitat Information

This species is believed to be closely associated with late-successional and old-growth coniferous forests (Miller and Hammond 2007, Warren 2005, and LaBonte et al. 2001) that are infected with dwarf mistletoe (*Arceuthobium spp*.) (fig. 7). However, eggs and larvae have been found from infected younger stands (McCorkle 1973). The amount of late-successional forest within a 500-m radius of *C. johnsoni* sites from regionwide 2009 survey ranged from 30-80%, with an average of 50%. All *C. johnsoni* detections during that survey were directly adjacent to older stands, but not always within the stand and sometimes along the edges of these stands.

Figure 7. Typical habitat consists of older conifer forests that are actively infected with dwarf mistletoe. These pictures show stands with western hemlock (*Tsuga heterophylla*) infected with *Arceuthobium tsugense*.

While dwarf mistletoe can occur on all age classes of forest (Muir and Hennon 2007), it is most abundant in mature stands and old-growth. 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). However, clumps can readily be found on lower branches and younger understory trees, especially when trees and branches have good exposure to sunlight (fig. 8).

Figure 8. In heavily infected stands, dwarf mistletoe clumps can be collected from smaller understory trees, or from the lower branches of larger trees. The white arrows in these photos point to clumps.

Light can be a limiting factor in dense coastal forests (Muir and Hennon 2007). It takes 2 to 5 years for seeds to produce aerial shoots after landing on a branch (Hawksworth and Wiens 1996). The plants are dioecious (fig. 9) and while perennial, live for only 4 to 8 years, although the infection can last many more years. Thinning that increases light has been documented to encourage reproduction in dormant infections of hemlock dwarf mistletoe (Baranyay 1962).

Figure 9. Female (pistillate) shoots of *A. tsugense* are yellow-green to reddish-purple in color, whereas male (staminate) shoots are usually olive colored to yellow-green.

Most shoots produce at least two successive crops of seeds (Hawksworth and Wiens 1996). Shoot growth mainly occurs between May and August, with seeds being dispersed in September (appendix 9). Signs of dwarf mistletoe infections include branch swelling, witches' brooms, stunted tree growth, defects, and dead branches and tops in older trees (fig. 10).

Figure 10. Signs and symptoms of dwarf mistletoe infections include branch swelling (**A**), witches' brooms (**B**), dead branches (**C**), and dead tree tops (**D**).

Sometimes the signs of infection are apparent, but aerial shoots are absent or sparse. In these situations it is likely that "dead" infections may be caused by lack of sunlight (Bloomberg and Smith 1982), or from fungi such as *Colletotrichum gloeosporioides* and *Neonectria neomacrospora* (Hawksworth and Wiens 1996, Muir and Hennon 2007).

Wildfires have been the most important single factor governing the distribution and abundance of dwarf mistletoes (Hawksworth and Wiens 1996). The effects of fire on presence of dwarf mistletoe are mostly negative, as fires have been documented to reduce the abundance of dwarf mistletoes (Koonce and Roth 1980, Zimmerman and Laven 1987). Therefore, recent fire history should be considered in selection of potential survey areas.

To date, 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*). In southwestern Oregon, Brewer's spruce (*Picea breweriana*) and Jeffrey pine (*Pinus jeffreyi*) are also host trees.

There is a strong relationship between abundance of dwarf mistletoe and presence of *C. johnsoni.* An occupancy analysis conducted on survey data from 2009 (Davis 2010) showed that a transect with dwarf mistletoe along at least 35% of its length had a high probability of occurrence and transects with less than this had very low probability of occurrence.

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

Two different species distribution modeling (SDM) software packages were used to create region-wide habitat suitability models for Oregon and Washington. The SDMs used were BioMapper v4.0 (Hirzel et al. 2007) and MaxEnt v3.3.2 (Phillips et al. 2010). Both SDMs use species presence locations to train the model based on the relationship between species locations within a set of environmental predictor variables that is then extrapolated to the larger modeling region. In general, the more similar the environmental conditions are to those of known species occurrence, the higher the habitat suitability value is for that particular location. Both models were trained using 64 current *C. johnsoni* locations dating back to 1970, included 15 new sites found during the 2009 season.

The environmental predictor variables used in these models included a distribution map of dwarf mistletoe densities based on FIA/CVS plot data, climate data, forest cover data, and elevation data (Davis 2010). Not surprisingly, the habitat modeling indicated that this species' distribution is strongly associated with the distribution of coniferous forests infected by dwarf mistletoe, primarily *Arceuthobium tsugense*, but also *A. abietinum* and *A. campylopodum*. The models also indicated that species occurrence is more likely in areas that receive higher than average rainfall for the region, and have more humid and cooler climates. It appears to avoid areas that have large annual seasonal temperature variations between summer and winter.

A portion of the species presence data is held out from model training, and used to test the model predictions. The BioMapper habitat suitability model was fairly robust (Spearman rank = 0.98, $p<0.001$) at predicting these held out locations of *C. johnsoni*. The MaxEnt habitat suitability model also validated well, based on 100 bootstrapped replicates, where each replicate was trained with 75% of the data (n=48) and tested with a randomly held-out sample of 25% (n=16) (Davis 2010).

Binary maps (not suitable/suitable) from these two models were combined to produce a "combination" map (appendix 3) where the probability of occurrence is "low" where neither model predicted suitable habitat conditions, "moderate" where one of the models predicted suitable habitat, and "high" where both models predicted suitable habitat conditions. This map was developed to focus surveys in a broad geographic sense across the region. However, it can also be used for pre-field assessments to determine the likelihood of species occurrence in your area of interest. This can be used to determine the need for conducting surveys. Surveys should try to focus on areas with the highest probability of species occurrence, but it is possible that areas of low probability may contain habitat, and the use of the model should always be balanced with local, site specific information. Likewise, not all areas that were modeled as high probability will contain habitat, although the probability that they will is higher. The use of the habitat model is only the first step, and it is important to verify on the ground conditions, and specifically, the presence of active dwarf mistletoe infections prior to conducting a survey.

Survey Methods

The protocols contained herein are intended to provide standardized methods, which can be used by field personnel to survey for this species, and to document species locations and habitats in a consistent format. These methodologies can be used during project planning and also for general inventories or other efforts to determine species geographic distribution or habitat associations.

Before planning or implementing any survey, it is important to determine what the objectives of your survey are and what type of information is needed to provide the best results. We provide protocols for two levels of survey intensity; (1) a transect design that can be used for assessing relative abundance or occupancy analyses, and (2) an informal "checklist" or "focused" survey design that is streamlined to provide a rapid assessment on species or habitat presence. It cannot be used for determining species absence.

Transect Surveys

Transect surveys were originally designed for conducting adult surveys followed by a larval survey. However, the option for conducting one or the other (or both) is left to the discretion of the surveyor. Transect surveys are time consuming, as they require some pre-field assessment time to determine potential survey areas, followed by field verification of ground conditions, and then transect layout. Once laid out, an adult transect survey requires four days to meet protocol, whereas a larval transect survey can be conducted in a single day to meet protocol.

A transect is 2km in total length and, given the estimated distance an adult butterfly can fly, covers an area that extends out to about 1km from the transect, or roughly 600 to 700 ha depending on the shape of the transect. Larger areas of habitat will require more than one transect to be adequately covered. Transects are laid out along either roads or trails. The following criteria must be met before establishing a survey transect:

1. A transect must be within or along the edge of potentially suitable habitat with at least one side of the transect directly bordering suitable habitat. Small skips or gaps are allowable, as long as at least 80 percent of the transect length is adjacent to potentially suitable habitat.
2. At least one transect segment must have documented presence of active (living) dwarf mistletoe clumps that are accessible from the ground. The more segments that have live dwarf mistletoe, the higher the probability of detecting the species.

The best way to determine areas for potential transect surveys is to use GIS to do a pre-field review of potential survey areas using the habitat model provided in appendix 3, forest vegetation maps, and road or trail data. Roads are preferable to trails, unless the trails are along areas that have large canopy gaps, or are along edges of large natural openings. High resolution color aerial photographs (e.g., NAIP) are very useful in this process. Consult with your local silviculturist for information on the locations of active dwarf mistletoe infections.

An example of a 2km transect is shown in figure 11. Each transect is divided into eight 250m segments. They don't necessarily need to be along the same road, but can branch out at intersections. When designing a transect, one should give consideration to the transect's exposure to sunlight as it is important for the presence and size of dwarf mistletoe clumps as well as nectar plants. A transect located on a southerly aspect will usually get more sun exposure than one on a northerly aspect, especially if it is not located along the bottom of a deep canyon. Orientation of transects should also be factored into the timing of the survey. For instance, a north-south oriented transect with tall trees along its eastern margin will generally experience more sustained sunlight later in the day when the sun is higher in the sky, so beginning the survey around noon might provide better results than starting it earlier in the morning, when shadows prevail.

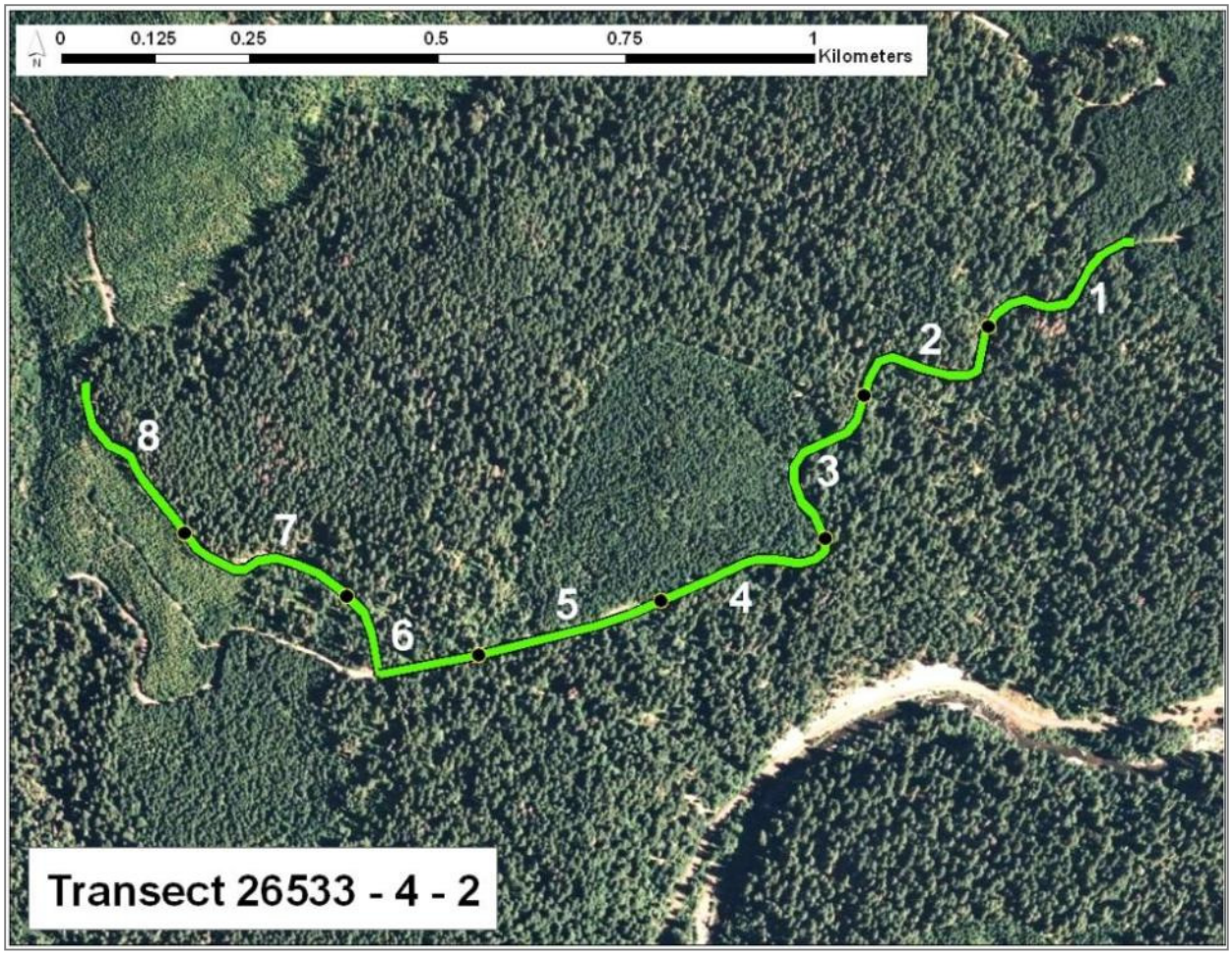

Figure 11. Example of a 2km survey transect, divided into eight 250m segments.

Once a potential survey area and transect route is determined, it should be field reviewed to verify the presence of active dwarf mistletoe. Once both criteria outlined above are met, the transect can be laid out on the ground. The start and stop location should be flagged, labeled, and GPSed. Each segment break should be flagged and labeled, ensuring that each is 250m in length. Once field layout is completed, field survey maps (appendix 6) should be prepared in the office. Laminated copies of these field survey maps are useful for recording information in the field while conducting the actual surveys.

Adult Surveys

Adult hairstreak butterflies are often hard to detect during transect surveys, with an estimated detection rate of 33 percent following this protocol (Davis 2010). However, adult butterflies are more easily identified and this type of survey does not require the additional time and costs of collecting dwarf mistletoe clumps and species identification via DNA analysis. The survey effort for adult surveys requires the most time, as it takes 4 day-visits, which are constrained by weather conditions, to meet protocol.

Timing adult surveys to coincide with the peak flight period and suitable flying weather is critical for improving your odds of detection. The earliest recorded detection of adults in Washington is May 13, and based on the latest analysis of historical detection data, the peak flight season

seems to occur during the last week of May and first week of June (appendix 4). In Oregon, the earliest adult detection is April 15 (from eastern Oregon) and the peak flight season appears to occur during mid-June (appendix 4). Peak flight seasons can last from 1 to 2 weeks for small populations and 3 to 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 recorded detections from July 9th and August 17th in these same areas, respectively.

Emergence dates will vary from year to year with annual fluctuations in the weather and climatic events such as El Niño. Therefore, initiation of adult surveys should consider the seasonal weather patterns and phenology of the butterfly's nectar plants. Once initiated, each survey transect will be surveyed 4 times, with no less than 5 days and no more than 10 days since the previous visit. Surveys should only be conducted on sunny or partly sunny days with little to no wind, when the ambient air temperature is >13°C. Local winds can vary considerably depending on habitat and topography, and surveys should not be conducted when the average wind speed 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 the survey when they pass. If the weather does not improve, the visit should be repeated on a day that meets the weather and timing criteria above. Surveys need to occur between 1000 and 1600 hours.

Figure 12. Conducting an adult butterfly survey along a forest road transect (photo by C. Fallon).

Each transect must 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 (fig. 12). 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 and while perched, its wings are closed. 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 branches, canopies, and the road ahead periodically with binoculars to spot flying or perching butterflies. Also, look for butterflies on animal scat on the road.

Once the transect end is reached, the surveyor should walk back at a regular pace to the starting point, where your vehicle is parked. Any *C. johnsoni* observed during this return trip would be recorded as incidental to the survey, and recorded as such in the field form (appendix 6). All hairstreaks should be netted and transferred into a viewing jar or "bug box" for closer observation and species identification. Collect one voucher per transect whenever possible, using a specimen envelope labeled with the appropriate information. Additional photo vouchers can be made once the initial voucher specimen has been collected. Effort should be taken to ignore other butterflies along the transect that are clearly not hairstreaks. Other look-alike species are expected to be netted and released after species ID. If you are uncertain of species ID, a voucher can be collected for confirmation by an experienced lepidopterist.

Larval Surveys

Surveys for eggs and larvae can improve the odds of detecting this species in occupied habitats (Warren 2005, Zonneveld et al. 2003). Larval transect surveys can provide the same information as the adult surveys, but have a much higher probability of detection in occupied habitat (Davis 2010). They are logistically less complicated, requiring only one day to complete, are not dependent on weather conditions, and also provide information of larval host plant abundance. However, they require additional time and expense for storing dwarf mistletoe clumps, sorting through the clumps to find eggs and larvae, and vouchering of larvae for subsequent DNA analysis.

Figure 13. Clipping dwarf mistletoe clumps from lower tree branches (photo by A. Dyck).

The larval survey technique involves collection of dwarf mistletoe (fig. 13) and carefully searching them later for eggs and larvae. This survey occurs within each 250m segment of the transect. A time-constrained search is used to collect a maximum of 10 dwarf mistletoe clumps, or for 15 minutes (total search time) per segment, whichever occurs first. Clumps of dwarf mistletoe can be found on the lower branches of infected trees, or on small understory trees beneath infected overstory trees. The search area is confined to within 50m of either side of the transect (road or trail). Within this search area, the surveyor should collect clumps that are larger than a golf ball in size, if possible. Smaller clumps have low stimulation value to ovipositing females (McCorkle 1973), therefore, the larger the clump, the more likely it may contain eggs and/or larvae. However, if limited, clumps of any size can be collected.

Figure 14. Branch clipping with dwarf mistletoe clump (photo by A. Dyck).

To collect a dwarf mistletoe clump, hand pruners (fig. 14) are used to clip branches about 3 to 4cm on either side of the swelling and trim off twigs so that the clump fits into a 1-gallon Ziploc® bag. Note tree species. Keep clippings separated between segments. Place clippings from each segment into a 13-gallon white plastic trash bag and label the trash bag with transect and sample segment information. White trash bags are necessary as they reflect sunlight and help keep clippings cooler than a black trash bag. Keeping your clippings cool is critical, as excessive heat will kill eggs and larvae, rendering your sample useless. During transport, place filled trash bags in a large camping cooler out of direct sunlight and do not keep them in a parked vehicle for a prolonged period of time in sun.

Larval surveys should occur at least one week after the peak flight period is over. This will improve your chances of finding larvae. Note that larval sampling can be done as long as there is daylight and in most weather conditions. The route can be walked or you can drive between sample segments. Any adults observed during larval surveys will be recorded as incidental observations.

Upon returning to the office, carefully remove each clump from their collection bags, place it on a clean sheet of white paper, and inspect it closely for eggs and larvae. A large magnifying glass and good lighting is essential for this process. While in the process of inspecting each clump, clip off excess twigs and all green conifer needles to slow down the desiccation of the

clump. Record the number of eggs and/or larvae found on the larval survey field form (appendix 6). Voucher all larvae found that are at least 2mm in length. If eggs or larvae that are smaller than 2mm in length are found, transfer the clump to its individual container and record the appropriate information on the container. If no eggs or larvae were detected, return the clump to the collection bag (trash bag) and arrange them by spreading/spacing them out in the bag. Once completed, lightly seal the opening of the bag by folding it over to maintain some humidity inside it to slow down desiccation of the clumps, but prevent larvae from crawling out of the bag.

Figure 15. Larval frass.

Over the next few days, carefully conduct daily inspections of each clump, starting with a search for signs of larval frass under each clump (fig. 15 and appendix 10). The presence of frass (fig. 15) indicates the presence of larvae, if it is observed, carefully remove the clump under which the frass has accumulated and inspect it carefully for larvae. Occasionally, the larvae of other species, such as the hemlock looper (*Lambdina fiscellaria*) can be found, and should be removed (fig. 16). Once *Callophrys* spp. larvae are found, they should be vouchered if at least 2mm in length, or if smaller, the clump should be placed in its own container, until the larvae has reached the appropriate voucher length. If after one week, no frass is detected, then discard the clumps and collection bag, and record the information in the larval survey form.

Small plastic snap-top containers, such as Ziplocs®, make good storage containers for rearing of larvae. The bottom of the container should be lined with a piece of white paper towel or tissue. Containers should be kept sealed to keep moisture in and clumps from drying out too quickly. The containers should be inspected and cleaned daily, otherwise fungi will grow and larvae may die. Cleaning involves removing frass and replacing the paper towel or tissue liner on the bottom of container.

Figure 16. Hemlock looper larva.

If the decision is made to rear larvae until the pupa stage, for species identification at eclosure, then additional time will be required and it will be necessary to replace desiccated dwarf mistletoe with fresh clippings until the larva enters the prepupal stage and stops eating. Indications of of this include a darkening of the larvae (fig. 17) and lessening in movement or sometimes leaving the clump and crawling under the paper towel at the bottom of the container.

Figure 17. After about one month, the larva begins to darken (**1**) and stops eating. Within a few days, it will begin to shed its skin (**2 thru 5**) then harden as it enters pupa stage (**6**).

Focused (Checklist) Survey

The simplest form of survey is a focused survey. Focused surveys are similar to "checklist" surveys (Royer et al. 1998), and focus on the likeliest habitats of *C. johnsoni*. Like checklist surveys, the surveyor is free to search out places where this butterfly would typically inhabit or lay its eggs and the technique is procedurally simpler than the transect surveys. However, the survey technique is inadequate to meet the rigors for many types of statistical analysis, and is only an indicator for species presence, but not absence.

Either adult or larval survey techniques (or both) can be applied during focused surveys; however, larval survey techniques produce better results in detection. If adult surveys are used, then repeat visits are highly recommended. Only one visit is needed for the larval surveys.

In general, areas with the highest likelihood *of C. johnsoni* occurrence should be selected, and searched during optimal weather conditions and seasonal timing. The surveyor should cover as much of the area as possible, and document the survey effort accordingly. This type of survey can be used in areas with highly fragmented habitat, where the more formal transect surveys cannot be laid out to protocol. Each site surveyed should be GPSed and information recorded in the focused survey field form in appendix 7.

Dwarf mistletoe collection is not time constrained, but otherwise, the procedures described for the transect larval survey should be used to collect, store, and inspect dwarf mistletoe clumps for eggs and larvae. A minimum of 15 minutes should be spent at a site, searching for clumps.

Survey 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
- Plastic Ziploc® bags (1 gallon)
- White plastic kitchen trash bags (13 gallon)
- Black sharpie pen to record information on Ziploc® and trash bags and field maps
- Field forms (see appendices)

Verification Process

Adult butterfly

Adult butterfly identifications should be confirmed by an expert lepidopterist. Collecting adult vouchers 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 2 to 3 kilometers from the nearest previously documented site. Aim to collect a single voucher per route. Specimen envelopes are used for this process.

Figure 18. Johnson's Hairstreak in a 1½ inch x 1½ inch square "bug box" (photo by G. Pearson).

In addition to a specimen voucher, photographic vouchers can and 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 (fig. 18) 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. More than one picture per specimen is advised, to make identification easier.

Larval butterfly

Currently, a genetic analysis is needed to distinguish between *C. johnsoni* and *C. spinetorum* larvae. Vouchering of larval specimens is conducted in the office or lab from dwarf mistletoe clippings that are collected in the field. Based on current genetic analyses, the optimal voucher size is between 2 to 4mm in length, or during the second or third instar stages (Davis and Weaver 2010). Once larvae reach this size range they should be vouchered as soon as possible using the following procedure. Larger specimens can also be vouchered, but have resulted in poorer success in DNA sequencing, thought to be related to the increased amount of chitin that develops in larger caterpillars.

With fine-point tweezers or forceps, carefully place individual caterpillars into a small laboratory grade sample vial with 75% ethanol (ethyl alcohol). Do not use isopropyl alcohol (a.k.a. rubbing alcohol) as it will break down genetic material rendering the voucher useless. Small glass vials (1 dram or 3.7 ml) with "polyseal" caps work well as they are large enough to contain all larval stages. The polyethylene liner in the cap provides a double seal when screwed on tightly, and prevents leakage or evaporation of alcohol in the vial. Keep each caterpillar voucher separated (one per vial) as you may find both *C. johnsoni* and *C. spinetorum* at the same site, and on the same dwarf mistletoe clump. Clean tweezers between handling each larvae, to prevent chances of cross contamination. Wear safety goggles and work in a well ventilated area when working with ethyl alcohol.

Label each vial with a voucher number that corresponds to the information in appendix 8. Larva length is easier to measure after larvae are placed in the glass vial. Make sure to specify the UTM projection and datum used. Package the vials in a well-padded cardboard box for mailing to the genetic lab. Ensure polyseal caps are on tight, to prevent leakage during shipping. Include a paper copy of the larvae DNA voucher form (appendix 8) inside the package along with the vouchers. Vouchers should be consolidated and sent in bulk to a laboratory for DNA analysis. The information in the DNA voucher forms should correspond exactly to the information from your survey field forms (appendix 6 and 7). UTM coordinates from transect segments can be determined in the office using GIS, where all transects should be entered into.

The following equipment is recommended for vouchering larval specimens:

- Lighted magnifying glass for examination of mistletoe clumps
- Dissecting microscope for examination of eggs and larvae
- Tweezers or forceps (fine point)
- Ethanol (ethyl alcohol) diluted to a 75% solution (using distilled water)
- Glass sample vials (1dram or 3.7ml) with polyseal caps
- Safety goggles for handling ethanol

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.

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 will be working at. 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. Do not climb trees to collect dwarf mistletoe clumps. They can be obtained from the ground. 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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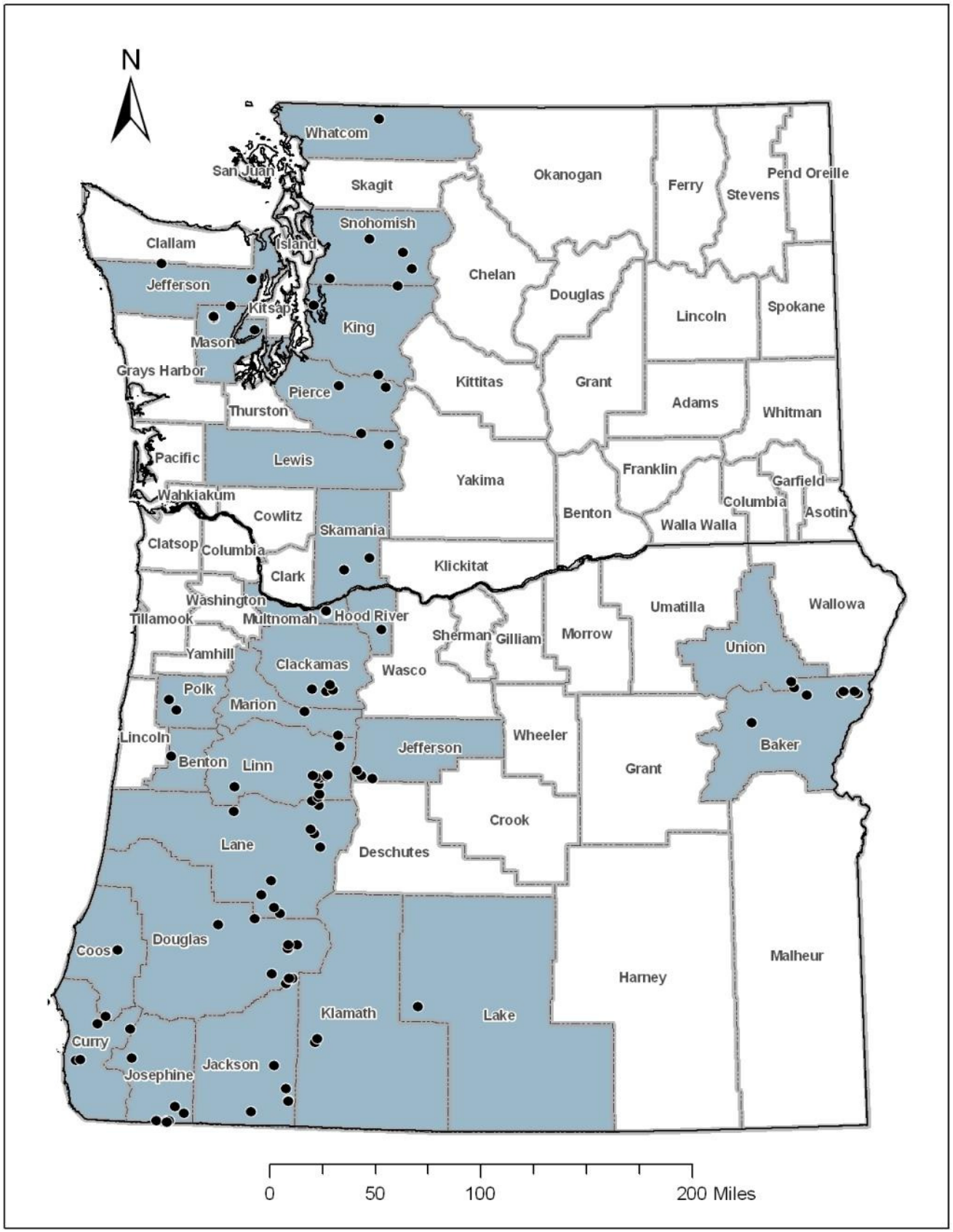
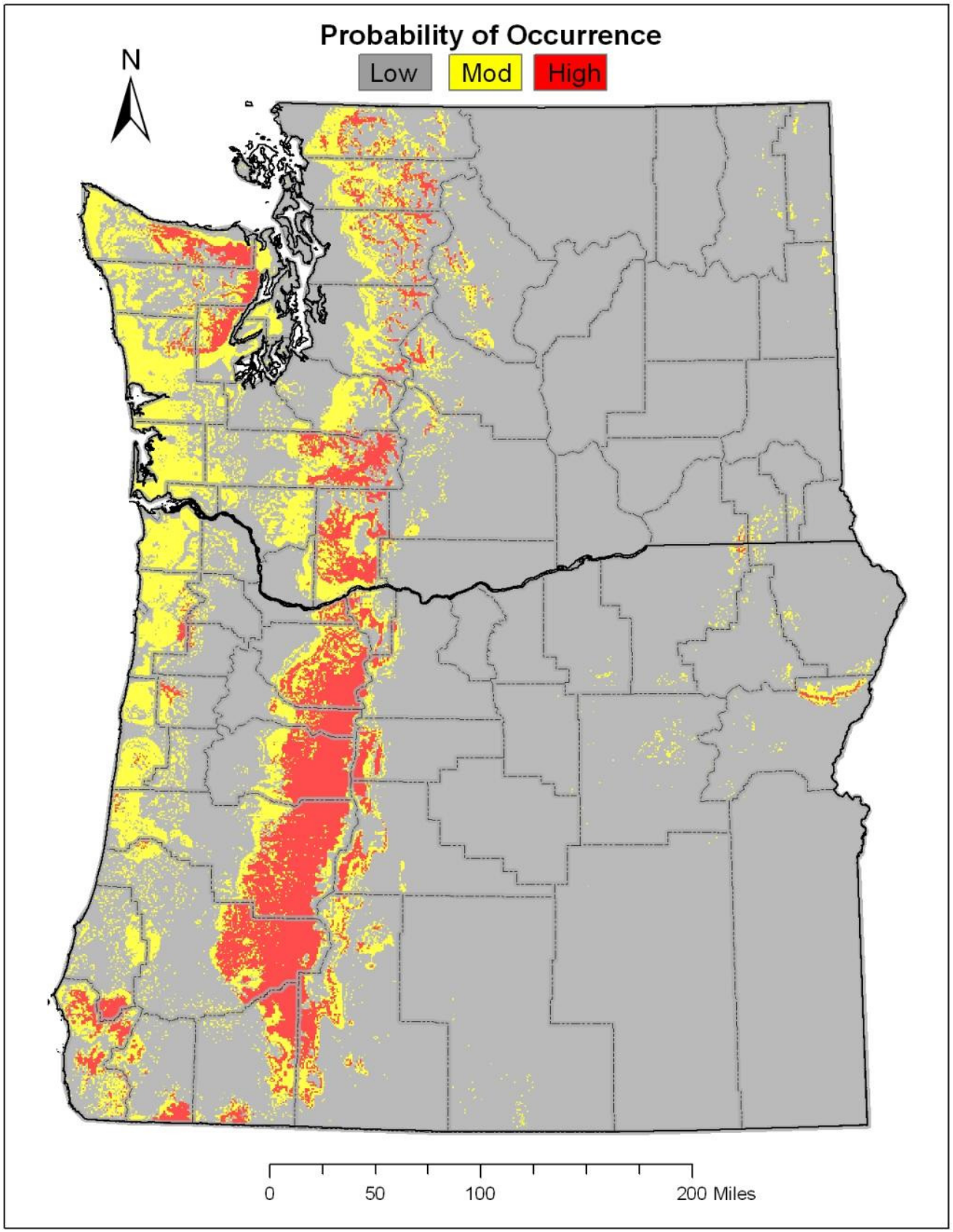
APPENDIX 1 –Distribution of Johnson's Hairstreak (*Callophrys johnsoni*) Locations (Historic and Current) in North America

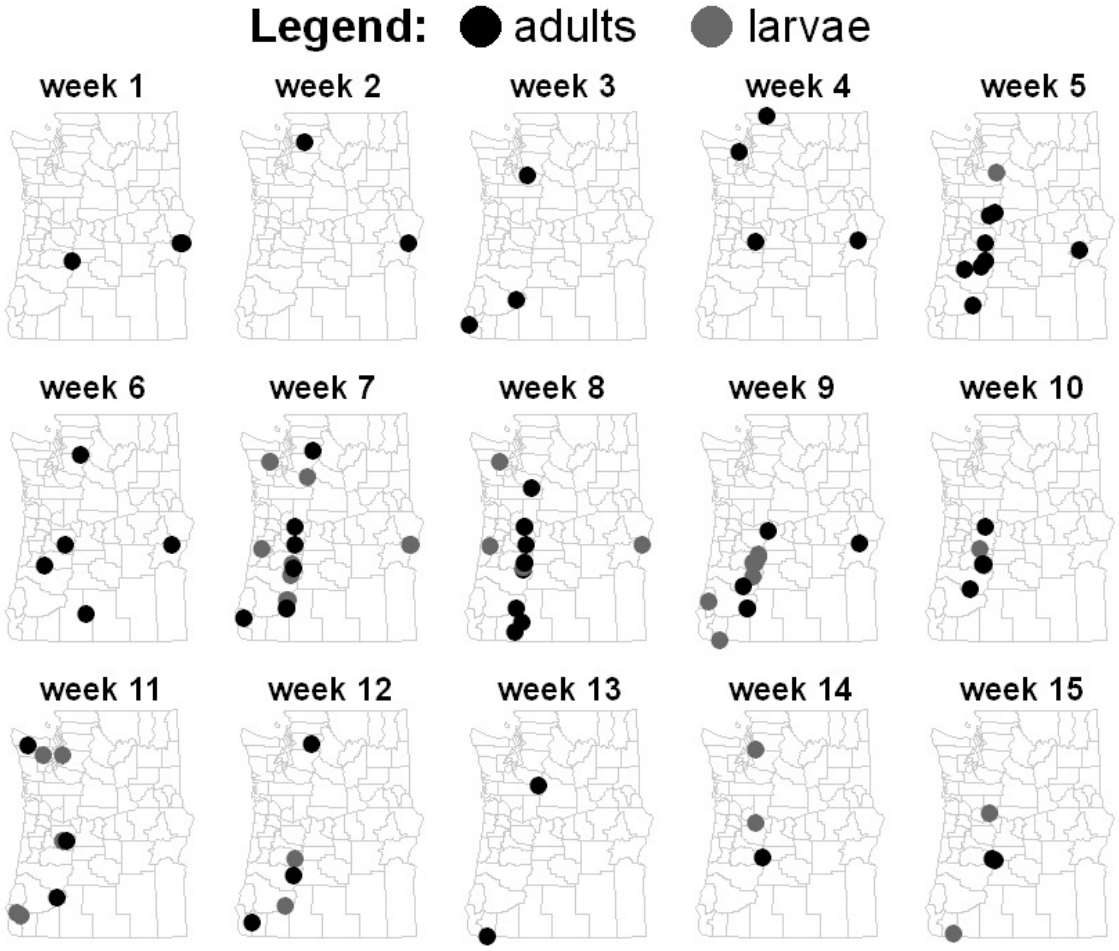
APPENDIX 2 –Oregon and Washington Records of Johnson's Hairstreak

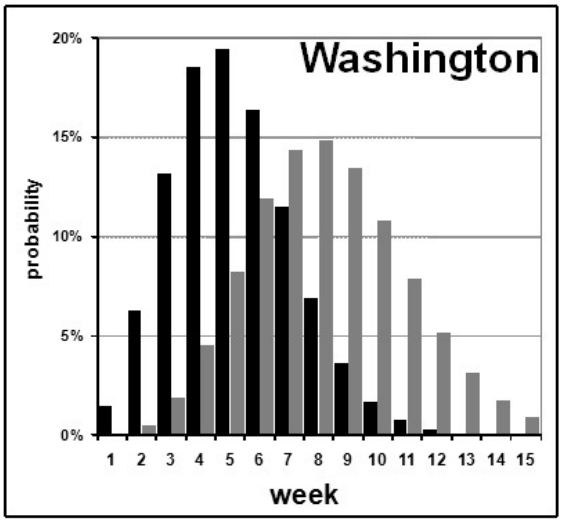


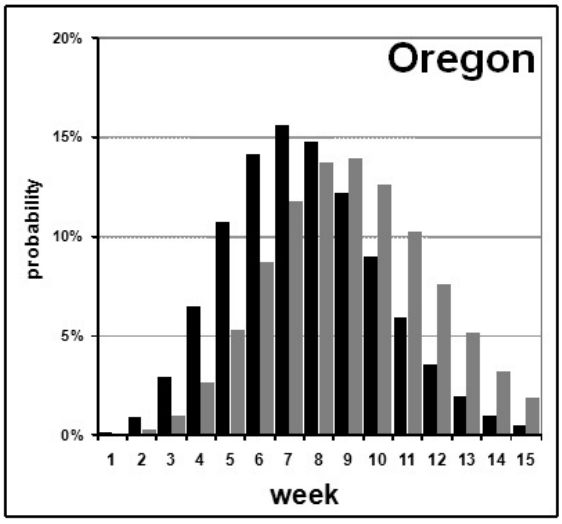
APPENDIX 3 –Habitat Model (v1.1)



APPENDIX 4 –Adult and Larval Seasonal Trends

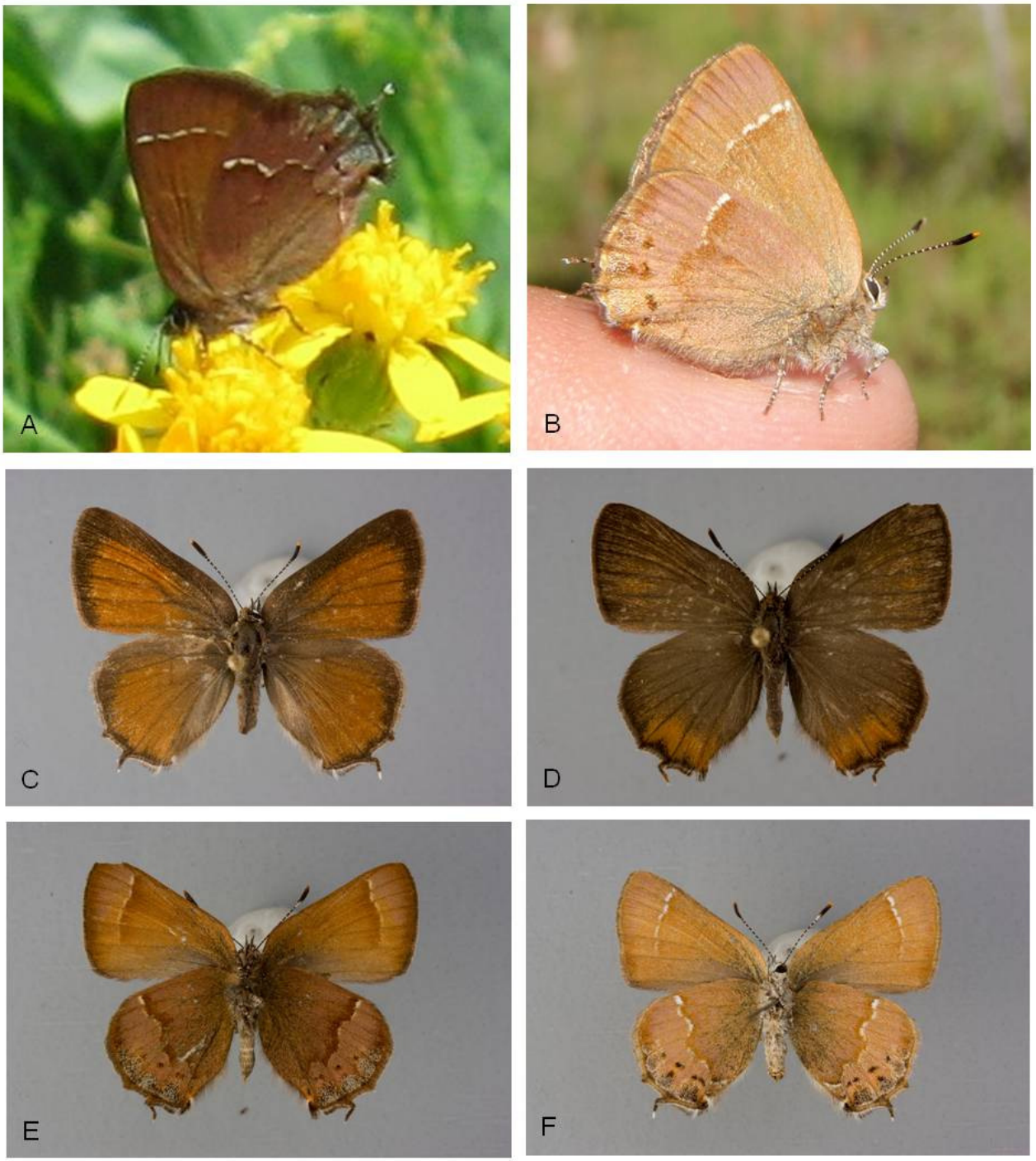






1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
MAY					JUN				JUL				AUG	
1-7	8-14	15-21	22-28	28-31	1-4	5-11	12-18	19-25	26-30	1-2	3-9	10-16	17-23	24-31	1-7	8-14

APPENDIX 5 –Cedar Hairstreak (*Callophrys gryneus*)



Cedar hairstreaks are smaller than *C. johnsoni* with a wingspan of 21-27mm. The main distinguishing features between the species include dark brown, almost black thick marginal edges on the upperside of the wings, especially the forewings (frames C and D). The postmedian line is white, but never complete and sometimes absent (E and F). The coloration of the uppersides can be very similar to that of *C. johnsoni*, but has much variation, as shown in frames A, B, E, and F. In sunlight, there is often a lilac or violet tinge to the underside color.

APPENDIX 6 –Transect Survey Field Forms

Transect_____ Visit #____ Date_____ Surveyed by_____

Start time_____ Cloud cover %_____ Overcast type_____ Wind (kph) _____ Temp (°C)_____

Stop time_____ Cloud cover %_____ Overcast type_____ Wind (kph) _____ Temp (°C)_____

Site notes_____

SEG #	QTY	TIME	TALLY (Y/N)	SEX	AGE	ACT	SEGMENT NOTES
1							
2							
3							
4							
5							
6							
7							
8							

Additional notes:

Codes:
Cloud cover %: Percent of sky covered by clouds, fog, smoke…etc.
Overcast type: C = clouds, F = fog, S = smoke, H = haze, N = none
Wind speed: Record average wind speed (1 minute average) using wind gauge in kilometers per hour (kph)
Temp: Record air temp in degrees ºC, keep thermometer out of direct sunlight
SEG #: Each row represents a transect segment #. Indicate on survey map using a sharpie and take GPS waypoint,
QTY (quantity): Record number of adults observed during adult survey – OR – Record number of clumps collected (up to 10 max.) during larval survey.
TIME: Record time of adult CAJO observation (use military time) – OR – Record stop time of dwarf mistletoe collection for each segment.
TALLY: Y = adults captured, identified, or vouchered during official adult survey, N = adults cap/id/vouch on return walk back to vehicle.
SEX: F = female, M = male, U = unknown
AGE: A = adult, E = egg, L= larva, O = other, U = unknown
ACT (activity): F = flying, PE = perching, N = nectaring (record plant spp in notes), PU = puddling, O = other (record in notes)

APPENDIX 6 (cont.)–Example Adult Survey Field Form

APPENDIX 6 –Transect Survey Field Forms

Transect 26759-4-1 Visit # 1 Date 6/18/09 Surveyed by R. DAVIS

Start time 1032 Cloud cover % 0 Overcast type N Wind (kph) 3.1 Temp (°C) 17.0

Stop time 1217 Cloud cover % 0 Overcast type N Wind (kph) 1.0 Temp (°C) 20.7

Site notes WILD STRAWBERRY, PINEMAT MANZANITA, & TRAILING BLACKBERRY IN BLOOM

SEG #	QTY	TIME	TALLY (Y/N)	SEX	AGE	ACT	SEGMENT NOTES
1							
2							
3	1	1250	N	F	A	PE	- PERCHED ON SAPLING WESTERN HEMLOCK BRANCH. - VOUCHERED
4							
5							
6	1	1156	Y	F	A	PU	- PUDDLING ON DAMP GROUND ON ROAD SURFACE - VOUCHERED
7							
8							

Additional notes:

- MANY TORTOISE SHELL BUTTERFLIES, ONE SWALLOWTAIL, ONE FRITILLARY, FEW SULPHURS. NETTED CEDAR HAIRSTREAK, BROWN ELFIN, AND GREEN HAIRSTREAK - RELEASED.
- NETTING SUCCESS WAS 6:10 (# SUCCESSFUL NETS : ATTEMPTS)

Codes:

Cloud cover %: Percent of sky covered by clouds, fog, smoke...etc.
Overcast type: C = clouds, F = fog, S = smoke, H = haze, N = none
Wind speed: Record average wind speed (1 minute average) using wind gauge in kilometers per hour (kph)
Temp: Record air temp in degrees °C, keep thermometer out of direct sunlight
SEG #: Each row represents a transect segment #. Indicate on survey map using a sharpie and take GPS waypoint.
QTY (quantity): Record number of adults observed during adult survey – OR – Record number of clumps collected (up to 10 max.) during larval survey.
TIME: Record time of adult CAJO observation (use military time) – OR – Record stop time of dwarf mistletoe collection for each segment.
TALLY: Y = adults captured, identified, or vouchered during official adult survey, N = adults cap/id/vouch on return walk back to vehicle.
SEX: F = female, M = male, U = unknown
AGE: A = adult, E = egg, L= larva, O = other, U = unknown
ACT (activity): F = flying, PE = perching, N = nectaring (record plant spp in notes), PU = puddling, O = other (record in notes)

APPENDIX 6 (cont.)–Example Larval Survey Field Form

APPENDIX 6 –Transect Survey Field Forms

Transect 26759-4-2 Visit # 5 Date 7/29/09 Surveyed by R. DAVIS

Start time 1138 Cloud cover % 5 Overcast type C Wind (kph) 0 Temp (°C) 32.8

Stop time 1411 Cloud cover % 80 Overcast type C Wind (kph) 0 Temp (°C) 27.1

Site notes HOT & MUGGY, SOME PRECIP. @ END OF SURVEY

SEG #	QTY	TIME	TALLY (Y/N)	SEX	AGE	ACT	SEGMENT NOTES
1	0	1155	—	—	—	—	COULD NOT FIND CLUMPS AT GROUND LEVEL. LOTS OF LARGE WESTERN HEMLOCK BUT NOT MUCH SIGN OF D.M.
2	10	1203	—	—	1E 2L	—	COLLECTED CLUMPS FROM WESTERN HEMLOCK. FOUND 1 HATCHED EGG & 2 LARVAE IN 2ND INSTAR STAGE
3	10	1212	—	—	2L	—	COLLECTED FROM BOTH WESTERN HEMLOCK AND WHITE FIR. FOUND LARVAE ON WHITE FIR CLUMPS, 1ST & 2ND INSTARS
4	10	1225	—	—	—	—	COLLECTED FROM WHITE FIR. SMALL CLUMPS. NO LARVAE FOUND.
5	2	1245	—	—	1L	—	COLLECTED FROM W. HEMLOCK. SMALL CLUMPS, 1 LARVA (2ND INSTAR) FOUND.
6	5	1330	—	—	—	—	COLLECTED FROM W. HEMLOCK SMALL CLUMPS, NO LARVAE FOUND
7	0	1350	—	—	—	—	NO SIGNS OF D.M.
8	0	1411	—	—	—	—	NO SIGNS OF D.M.

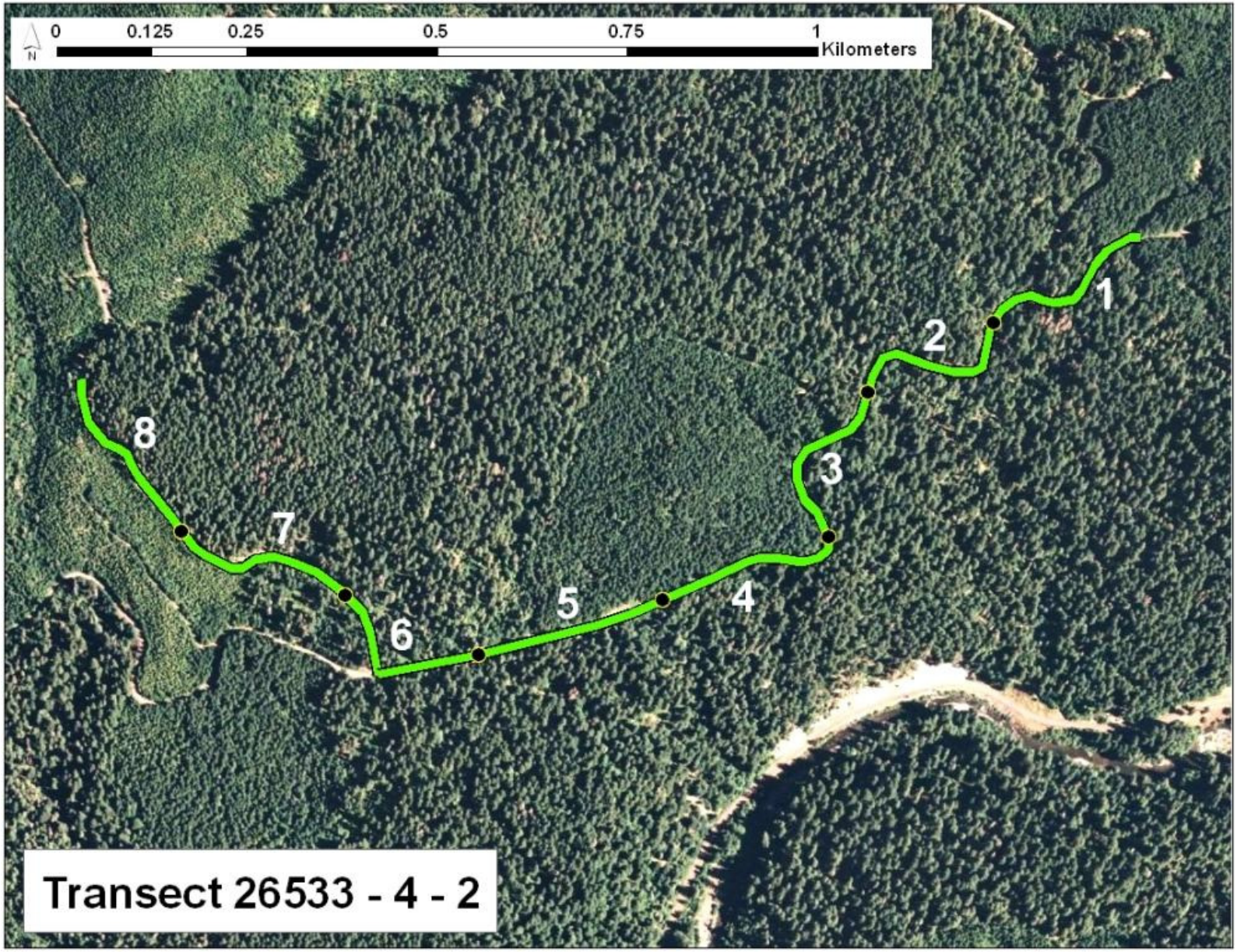
Additional notes:

A TOTAL OF 5 LARVAE VOUCHERED W/IN ONE WEEK OF FIELD COLLECTION DATE. VOUCHER DATES ARE RECORDED ON DNA VOUCHER FORM.

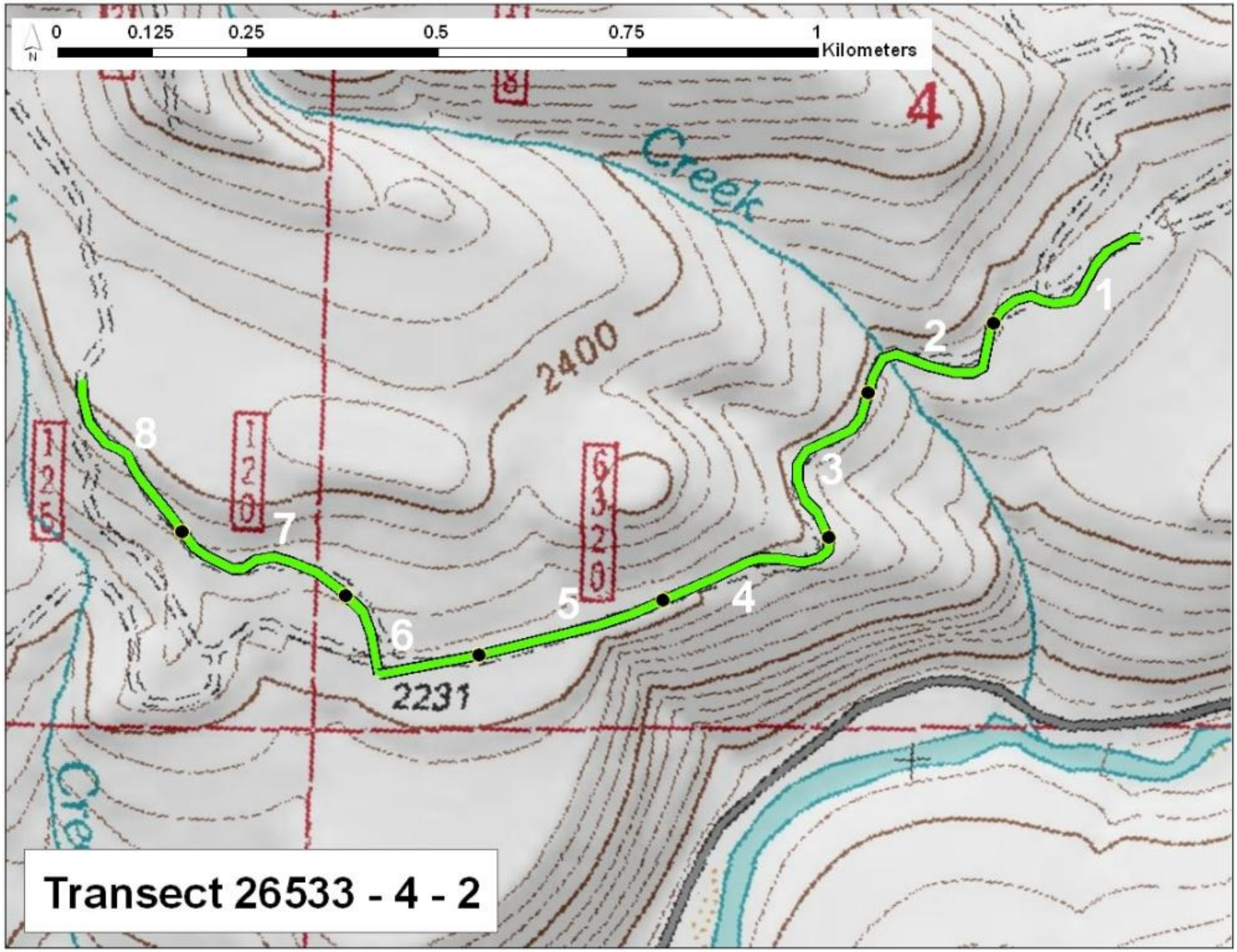
Codes:

Cloud cover %: Percent of sky covered by clouds, fog, smoke...etc.
Overcast type: C = clouds, F = fog, S = smoke, H = haze, N = none
Wind speed: Record average wind speed (1 minute average) using wind gauge in kilometers per hour (kph)
Temp. Record air temp in degrees °C, keep thermometer out of direct sunlight
SEG #: Each row represents a transect segment #. Indicate on survey map using a sharpie and take GPS waypoint.
QTY (quantity): Record number of adults observed during adult survey – OR – Record number of clumps collected (up to 10 max.) during larval survey.
TIME: Record time of adult CAJO observation (use military time) – OR – Record stop time of dwarf mistletoe collection for each segment.
TALLY: Y = adults captured, identified, or vouchered during official adult survey, N = adults cap/id/vouch on return walk back to vehicle.
SEX: F = female, M = male, U = unknown
AGE: A = adult, E = egg, L= larva, O = other, U = unknown
ACT (activity): F = flying, PE = perching, N = nectaring (record plant spp in notes), PU = puddling, O = other (record in notes)

APPENDIX 6 (cont.)–Example Transect Survey Field Maps



APPENDIX 6 (cont.)–Example Transect Survey Field Maps



APPENDIX 7–Focused Survey Field Form

Surveyed by_____ Date_____ Weather Conditions_____

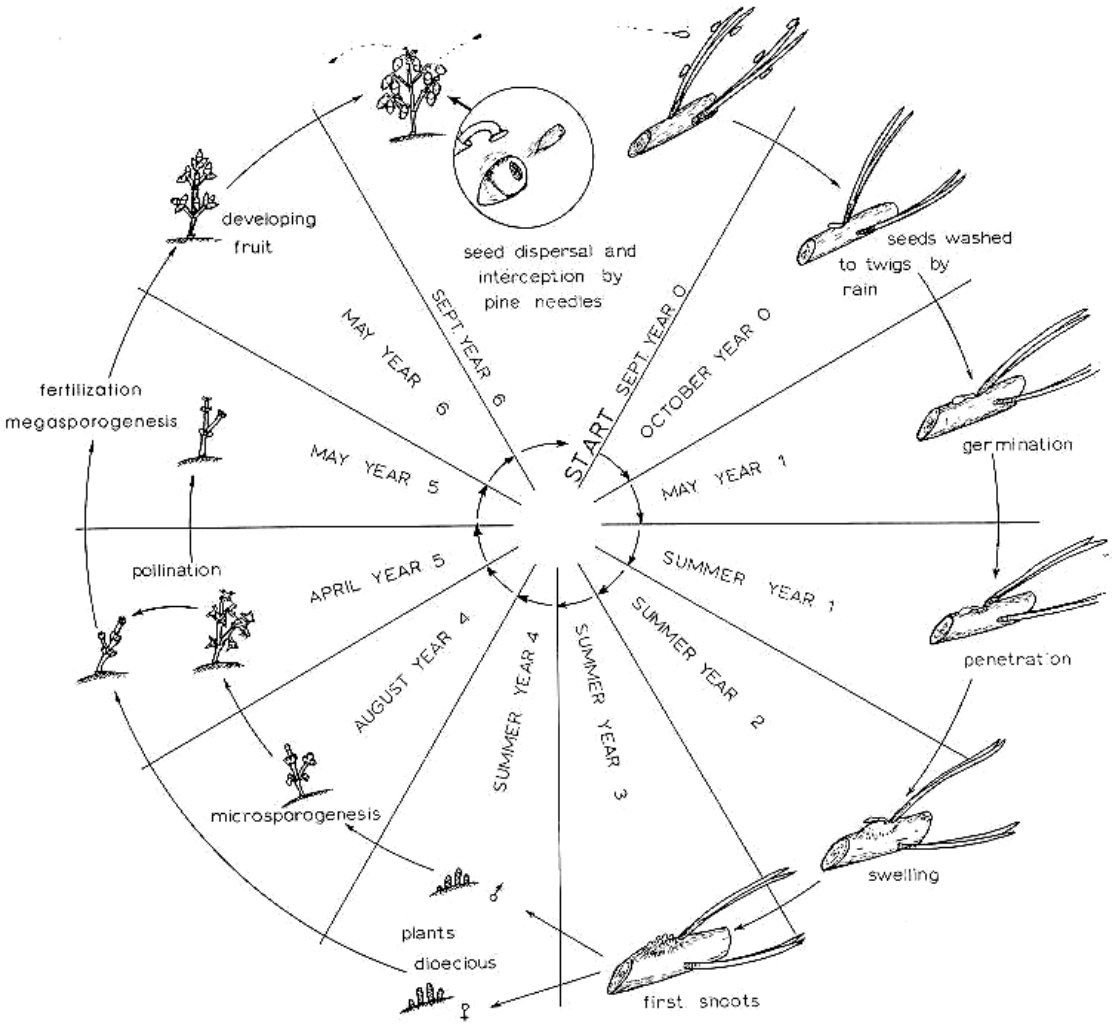
UTM X	UTM Y	Datum/ Projection	Site Name	Collected/Vouchered			Notes
				Adults	Clumps	Larvae	

Additional notes:

APPENDIX 8–Larvae DNA Voucher Form

Voucher ID	Site Name	UTM X	UTM Y	Collected By	Date Collected	Vouchered By	Date Vouchered	Length (mm)	Life Stage	Host Tree
		Datum/Projection								

APPENDIX 9– Generalized Life Cycle of Dwarf Mistletoe as Exemplified by *Arceuthobium americanum* on *Pinus contorta* (from Hawksworth and Wiens 1996)



Hawksworth, F.G. and D. Wiens.1996. Dwarf mistletoes: biology, pathology and systematics. Agric. Handbook 709. Washington, DC: U.S. Department of Agriculture, Forest Service, Government Printing Office. 410 p.

APPENDIX 10– Example of Clipped Dwarf Mistletoe Clump

There are two 2nd-3rd instar larvae on this clipping of dwarf mistletoe. Can you find them?

APPENDIX 11– Multicolored Asian Lady Beetle

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

LaMana M.L. and J.C. Miller.1996. Field observations on *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) in Oregon. Biol Control 6:232–237

Suomi, D.A. 2008. Good bug gone bad or biocontrol gets a black eye. Information found on internet at http://whatcom.wsu.edu/ag/homehort/pest/ladybug.htm