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Coloration, Camouflage, and Sexual Dichromatism in the Northern Pacific Rattlesnake (*Crotalus oreganus*)

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COLORATION, CAMOUFLAGE, AND SEXUAL DICHROMATISM

IN THE NORTHERN PACIFIC RATTLESNAKE

(Crotalus oreganus)

A Thesis

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by

Jefferson Davis Brooks

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CENTRAL WASHINGTON UNIVERSITY

Graduate Studies

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ABSTRACT

COLORATION, CAMOUFLAGE, AND SEXUAL DICHROMATISM IN THE NORTHERN PACIFIC RATTLESNAKE

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In some viperid snakes, natural selection has shaped coloration and patterning to match local habitats, and for males to show greater warning coloration than females due to a less sedentary lifestyle. The Northern Pacific Rattlesnake (*Crotalus oreganus*) is likely the most abundant ambush predator in eastern Washington, yet little is known about its color variation, how color patterns interact with their habitats, and if male and female snakes show differences in patterning or color. To explore how snake color might interact with the colors of their environment, I used standardized digital photography to record both snake and substrate coloration. I sampled 127 rattlesnakes from overwintering hibernacula and compared their coloration to that of the surrounding habitat. I also used satellite imagery and land cover classification to investigate possible relationships between snake coloration and habitat at a broader landscape level. The hypothesis that *C. oreganus* shows sexual dichromatism (inter-sexual differences in color

and/or pattern) was also tested by comparing both overall snake color, and the strength of contrast in tail bands between the sexes.

I found that *C. oreganus* varies in color significantly, both locally between hibernacula, and regionally. Snake coloration was not associated with the degree of forested area within a 0.5 km radius of the hibernacula, and no significant differences in color were observed between the sexes. Despite this, the color of male and female snakes interacted differently with the habitat surrounding the hibernacula, where male snakes showed increased Red/Blue color ratios in high forested areas, while females showed lower ratios. Importantly, males showed significantly greater contrast in their tail bands than did females, suggesting increased selection for warning patterns and coloration in male snakes.

Sexual dimorphism is usually attributed to sexual selection. In contrast, the sexually dichromatic warning coloration observed in this study is an example of “Ecologically Caused Sexual Dimorphism”, which has now been observed in at least two species of viper. My results suggest that other viper species with similar natural history traits to *C. oreganus* may also exhibit sexual dichromatism, where males have exaggerated warning colors in comparison to females.

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Chapter I

GENERAL INTRODUCTION

Ambush predators, like the Northern Pacific Rattlesnake (*Crotalus oreganus*), rely on cryptic coloration and patterning both to forage and avoid predators (Shine and Harlow 1998) (Fig. 1). Predator pressure can be intense on rattlesnake populations (Gomez 2007; Macartney 1979), and snakes are the primary food source for several raptor species in Washington State (Fitzner et al. 1981; Fitch et al. 1946). Natural selection, therefore, has likely acted upon snake color and patterns to match local habitats and substrates (Farallo and Forstner 2012). While this pressure has led to polymorphisms in other ambushing snake species (Farallo and Forstner 2012; Shine and Harlow 1998), distinct color polymorphisms have not been demonstrated in *C. oreganus* in Washington, although most naturalists familiar with the species notice considerable snake color variation (St. John 2002).

While the ability to blend in with their surroundings is likely important for both sexes of *C. oreganus*, natural selection may act upon males to match a broader array of habitats and substrates than females. During the active season, males often travel farther than females from the overwintering den (Macartney 1979), especially when females are gravid (Gomez 2007; Bertram et al. 2001; Macartney 1979). Consequently, males and females may be adapted to different habitats and substrate types. In viperid species of Europe, such inter-sexual differences in activity patterns have been implicated in the evolution of sexual dichromatism, where males of *Vipera berus* express aposematic

coloration, and females predominantly express more cryptic coloration and patterns (Shine and Madsen 1994).

My study seeks to investigate these ecological and evolutionary aspects of coloration in the Northern Pacific Rattlesnake, a species for which no study on variation in coloration and patterning has been published. The goals of my study were threefold: 1) to determine the extent to which color in *C. oreganus* varies across the landscape, 2) to investigate to what degree snake coloration is associated with their surrounding habitat, and 3) to test the hypothesis that Northern Pacific Rattlesnakes are sexually dichromatic (inter-sexual differences in coloration and pattern) by comparing overall coloration and tail banding in males and females.



Figure 1: A large male Northern Pacific Rattlesnake (*C. oreganus*) found in the Methow Valley, WA.

Chapter II

LITERATURE REVIEW

Color and Crypsis

Color's purpose in the animal kingdom has historically been broken into two general groups: advertisement and concealment (Cott 1940). While this distinction certainly does not capture all the roles of animal coloration (color has been implicated in the regulation of certain physiological processes (Bittner et al. 2002; May 1976; Walton and Bennet, 1993)), it does describe the major purposes of animal coloration in the wild. This classification method can be further subdivided to describe the myriad of functions for advertising and concealing coloration. For instance, animals may express concealing colorations/patterns to avoid predators (Cooper and Allen 1994; Kettlewell 1956), or to ambush prey themselves (Ayers and Shine 1997; Jones and Whitford 1989; Reinert et al. 1984). Conversely, the same diversity found in the application of concealing (cryptic) colors can be found in advertising colors as well. Many birds, for example, use bright advertising colors in conspecific communication (Dale and Slagsvold 1996), while other animals use bright advertising colors to communicate their toxicity to potential predators (Kuchta 2005).

Of particular importance is the role of color in avoiding predators. While advertising colors for breeding are certainly important for many animals, such colors and patterns may be selected against when there is significant predator pressure (Endler 1980). Additionally, some of the bright coloration typically associated with conspecific communication in birds (e.g. ultra-violet coloration in passerines) may have evolved

precisely because their predators were unable to see those colors (Lind et al. 2013; Håstad et al. 2005). Therefore, studies on the role of color in predator avoidance (be it through crypsis or aposematic coloration) are crucial to better understand how predators impact the evolution of a given organism.

Color polymorphisms have evolved in a variety of animals, where easily identifiable and distinct color morphs can be observed in the same species (Huxley 1955), likely enabling it to adapt to a variety of specific habitats and environmental conditions (Ruxton et al. 2004; Shine and Harlow 1998; Huxley 1955; Cott 1940). This is particularly true for cryptic species. Mottled Rock Rattlesnakes (*Crotalus lepidus*) exhibit distinct color morphs that are maintained by predator pressure, allowing snakes to more cryptically match their different habitats (Farallo and Forstner 2012), and both light and dark color morphs of Western Terrestrial Garter Snakes (*Thamnophis elegans*) select basking sites that optimize crypsis (Isaac and Gregory 2013). As ambush predators rely on crypsis to hunt, color polymorphisms have arisen in a wide variety of ambushing snake taxa (Shine and Harlow 1998) and are particularly prevalent in viperid snakes. Polymorphic viper species include Timber Rattlesnakes (*Crotalus horridus*; Brown 2007), Rock Rattlesnakes (*Crotalus lepidus*; Jacob and Altenbach 1977), the Asp Viper (*Vipera aspis*; Dubey et al. 2015), Russel's Viper (*Deboia russelii*; Thorpe 2007), and the Thai Palm Viper (*Trimeresurus wiroti*; Chanhom et al. 2011), to name a few.

While cryptic coloration is important for ambush hunting species for foraging (Ortolani 1999; Poulton 1890), it also serves to help avoid encounters with their own

predators. The Northern Pacific Rattlesnake (*Crotalus oreganus*) is a prey species to a host of organisms, including coyotes, raptors, badgers, and skunks (Gomez 2007; Macartney 1979). At the Hanford Site in Washington State, snakes compose over 61% of the diet of Swainson's Hawk nestlings (*Buteo swainsoni*) and over 35% of the diet of nesting Red-Tail Hawks (*Buteo jamaicensis*) (Fitzner et al. 1981), indicating that in some snake populations predator pressure can be intense. Additionally, in the San Joaquin Experimental Range in California, Red-Tailed Hawk pellets collected over two seasons in a relatively small area contained the remains of at least 70 rattlesnakes (Fitch et al. 1946). These observations indicate that *C. oreganus* may be subject to strong selective pressures favoring cryptic colorations.

Since it is advantageous for ambush predators to be polymorphic in color, and color polymorphisms have been observed in other *Crotalus* species (Sweet 1985; Jacob and Altenback 1977; Klauber 1956), *C. oreganus* may have similarly evolved distinct color polymorphisms to adapt to different environments. Despite this, no study to date has investigated the relationship between the color of *C. oreganus* and its habitat. This investigation seeks to do that by 1) determining how snakes vary in color in Washington State and 2) by examining the relationship between color and local habitat in the Northern Pacific Rattlesnake.

Sexual Dimorphism

Sexual dimorphism, a difference between the sexes in some trait (morphological or otherwise), is found in many animal taxa (Berns 2013). Fish (Endler 1980), insect (Emlen et al. 2005), bird (Selander 1966), mammal (Festa-Bianchet 1996), reptile (Weatherhead and Barry 1995), and amphibian species (Shine 1979) all exhibit sexual dimorphism in some way. Despite the seemingly ubiquitous nature of sexual dimorphism, there is still disagreement about the primary mechanisms driving the evolution of this phenomenon (Shine 1989). Darwin (1871) was the first to explain the evolution of sexual dimorphism by relating it to sexual selection. Sexual selection can drive sexual dimorphisms through either intra-sexual competition (competition within a sex for mates, resources, etc.; Mysterud 2003) or inter-sexual selection (where mate choice governs selective pressures; Godin and Dugatkin 1996). The theory of sexual selection has dominated biological conversations on sexual dimorphism, where differences in the sexes are typically attributed to sexual selection (Punzalan and Hosken 2010; Shine 1989). This is not without reason; the elaborate plumage of the Birds of Paradise (Family: *Paradisaeidae*) is a classic example of the incredible effects sexual selection can have on the evolution of sexual dimorphism (Irestedt et al. 2009).

There are alternative explanations to sexual selection as the primary driver of sexual dimorphism, and among the most compelling are ecological differences between the sexes (Shine 1989; Slatkin 1984). Alfred Russell Wallace first championed this view (1889) by hypothesizing that some sexually dimorphic characteristics can be explained by natural selection alone. For example, in Mexican Lance-Headed Rattlesnakes

(*Crotalus polystictus*), male and female head morphology was found to be sexually dimorphic (Meik et al. 2012). A similar dimorphism was described in cottonmouth snakes (*Agkistrodon piscivorous*), where females were more gape limited than males (Vincent 2004). In both cases, the dimorphisms were not caused by sexual selection, but rather ecological differences between the sexes.

The Natural Histories of Viperid Snakes

In many species of the genus *Vipera* (particularly *V. berus*), males typically exhibit bright dorsal “zig-zag” patterning. Conversely, females tend to exhibit more drab colors, occasionally completely without the dorsal “zig-zag” pattern (Shine and Madsen 1994) (Fig. 2). This sexual dichromatism (where a species is sexually dimorphic in color or pattern) in *V. berus* may be due to ecological pressures, such as differences in seasonal movement patterns between the sexes (Shine and Madsen 1994). Males travel extensively in the spring in search of mates whereas females do not (Madsen et al. 1993; Shine and Madsen 1994). The increased spring activity of males also increases their exposure to predators, implying different selective pressures between the sexes (Shine and Madsen 1994). Consequently, males have exaggerated aposematic and disruptive coloration/patterning in comparison to females (Niskanen and Mappes 2005; Wüster et al. 2004) which decreases their likelihood of being preyed upon (Lindell and Forsman 1996; Shine and Madsen 1994). Conversely, as female adders are typically much less active, they may benefit from the thermoregulatory (Forsman 1995) and cryptic (Lindell

and Forsman 1996) benefits of melanism more than they would from bright aposematic coloration. Therefore, the sexual dichromatism observed in *V. berus* has likely evolved in response to ecological pressures, and not sexual selection.



Figure 2: Color patterns typical of male (lighter snake) and female (darker snake) *V. berus* snakes.

Photo Credit: Malene Thyssen, 2005; “Two Color Variations of *Vipera berus* in Copenhagen Zoo”
Wikimedia Commons; GNU Free Documentation License.

Several *Crotalus* species also exhibit sex dependent dispersal patterns similar to the *Vipera* snakes of Europe. Male Prairie Rattlesnakes (*C. viridis*) in Wyoming show increased reproductive success when dispersing in straight-line movements away from the den, maximizing their probability of encountering a female (Duvall and Schuett 1997). In New Jersey, male *C. horridus* moved on average ~2 times more (6335 m) than non-gravid females (2779 m), ~3.5 times more than gravid females (1783 m) and had significantly larger home-ranges than did females (Reinert and Zappalorti 1988). In Nevada, male Speckled Rattlesnakes (*C. mitchelli*) moved twice as much per day than non-gravid females (15 times more than gravid females), and had home-range estimates ~3 times larger than non-gravid females (Glaudas and Rodriguez-Robles 2011).

Our local viper species, *C. oreganus*, also exhibits seasonal movement differences between the sexes. In a British Columbia population, home range estimates for males (39.2 ha) were ~3 times larger than home range estimates for females (14.1 ha) and over 300 times larger than the same estimate for gravid females (0.12 ha) (Bertram et al. 2001). Similarly, gravid females had activity ranges of 0.16-0.22 ha, while an adult male had an activity range of ~8 ha (Macartney 1979). This trend was also observed in a population of *C. oreganus* in California, where males expressed mean daily movements that were ~4 times larger than non-gravid females, and ~5 times larger than gravid females (Putman et al. 2013).

Differences between the sexes in seasonal movement patterns are compounded when the reproductive biology of *C. oreganus* is also considered. Female *C. oreganus* are

likely biennial breeders (Diller and Wallace 2002; Aldridge and Duvall 2002; Diller and Wallace 1984), but may reproduce multiple years in a row (Aldridge and Duvall 2002). Since female *C. oregonus* can be gravid so frequently, and gravid females rarely travel far from the den (SIRAWG 2016; Gomez 2007; Didiuk et al. 2004; Bertram et al. 2001; Macartney 1979), much more of the life of a female snake is spent near the den (< 100 m; Macartney 1979). This is contrasted by adult male snakes, who may regularly venture farther from the den (Gomez 2007; Bertram et al. 2001; Macartney 1979).

This sex specific dispersal pattern in rattlesnakes may be an adaptation to avoid inbreeding. In *C. horridus* of New York, most snakes (~99%) return to their natal den year after year to overwinter, leading to high levels of relatedness within a den (Clark et al. 2008). Simultaneously, relatedness between dens was low, and over 33% of snakes had male parents from a different den (Clark et al. 2008). This implies that many males travel to other dens to mate, rather than mating with females at their own den. This same trend is exhibited in *C. horridus* of Missouri, where males mate nearly twice as far from the den as females do (1001 m – 511 m respectively), while ~96% of snakes monitored returned to their original natal den after the active season (Anderson 2010). Populations of *C. oregonus* also appear to exhibit high den fidelity, likely leading to high levels of relatedness within an individual den as well. *C. oregonus* snakes of British Columbia in six different dens exhibited ~93% den fidelity over two seasons (Macartney 1979). Since populations need sufficient gene flow to avoid inbreeding, it is likely that male dispersal in communally denning rattlesnakes evolved, in part, to circumvent inbreeding and loss of genetic variation in snake populations with high natal den fidelity (Clark et al. 2008).

As *C. oregonus* exhibits natural history traits (e.g. male dispersal, and more sedentary females) that have been hypothesized to select for sexual dichromatism in other ambushing snake genera (Shine and Madsen 1994), it is possible that *C. oregonus* is also experiencing similar selective pressures. Females may express colorations more similar to the substrates immediately surrounding the den than males, as they likely spend more time there throughout their lives than males (SIRAWG 2016; Gomez 2007; Didiuk et al. 2004; Bertram et al. 2001; Macartney 1979).

Inter-sexual differences in seasonal movement patterns in *C. oregonus* may also select for different levels of warning coloration between the sexes. Northern Pacific Rattlesnakes have distinctive black and white banding patterns on the tail proximal to the rattle, an obvious warning signal (Klauber 1956). Strongly contrasting white and black color patterns in many animals are commonly regarded as having anti-predator functions (Caro 2009). A classic example is the skunk (Caro 2009, 2005; Cantú-Salazar and Hidalgo-Mihart 2004), where the contrasting black and white coloration warns potential predators of their noxious nature. Additionally, banding patterns in coral snakes and their mimics are classic examples of aposematic coloration (Brodie 1993, 1995; Greene and McDiarmid 1981), where bright banded patterns (including black and white banding) serve to reduce encounters with predators. In insects, especially bees and wasps, banded color patterns (including black and white) also play significant roles in predator avoidance (Schmidt 2016). Toads will refuse honeybees and their mimics after associating their banded colors with a sting (Brower and Brower 1962), and the coloration of bees has been linked to aposematism (Williams 2007; Plowright and Owen

1980). Using banded patterns in life preservers has even been proposed to reduce the prevalence of shark attacks (Burkhead et al. 1985). The ubiquity of black and white bands as an aposematic warning in many other species suggests that strongly contrasting tail banding serves a similar purpose in rattlesnakes. The exaggerated warning color observed in male European vipers (*Vipera* spp.) begs the question: is warning coloration exaggerated in male *C. oreganus* as well?

Questions of this Investigation

I. Do Rattlesnakes Vary in Color Across Washington State?

Color polymorphisms are common in ambushing snake taxa, as they enable the species to adapt to a variety of habitats and substrates. Since the Northern Pacific Rattlesnake is an ambush predator, it may also be color polymorphic, and express significantly different colorations across the state.

II. How Does Rattlesnake Color Relate to the Habitat Surrounding the Den?

As ambush predators, rattlesnakes must adapt to their local habitats and surroundings. Not only does this necessitate color and pattern variation within the species, but it also implies that it will be associated with some aspect of the local habitat.

III. Do Male and Female Rattlesnakes Differ in Color?

Sexual dichromatism in *V. berus* has been linked to the different dispersal patterns of male and female snakes. As *C. oregonus* shares these sex specific dispersal patterns, there is potential that our local viper species is also sexually dichromatic. This may be exhibited by the sexes differing in overall coloration, and/or in the sexes expressing different levels of warning coloration.

Chapter III

METHODS

Study Area

Snakes were sampled from several hibernacula (dens) east of the Cascades in Washington State (Fig. 3). During the active season, *C. oregonus* individuals may disperse over several kilometers from the den (Putman et al. 2013; Gomez 2007; Bertram et al. 2001; Macartney 1979). Therefore, dens that were sampled within 2 km of one another were assumed to be representative of the same population and were pooled together in analysis. One population (2 dens) in Frenchman Coulee and one population (1 den) in Umptanum Canyon were sampled in central Washington to represent snakes adapted to the arid shrub-steppe east of the Cascade Mountains. Five populations (6 dens) were sampled in the Methow Valley of north central Washington to represent snakes from more forested environments.

The dens at Frenchman Coulee (~330 m elevation) are surrounded by semi-disturbed shrub-steppe habitat where precipitation averages ~20 cm/yr (NOAA 2018). Vegetation consists of Big Sagebrush (*Artemisia tridentata*), Stiff Sagebrush (*Artemisia rigida*), buckwheat (*Eriogonum* sp.), and perennial grasses such as Bluebunch wheatgrass (*Pseudoroegneria spicata*) and Sandberg bluegrass (*Poa secunda*) (Barbour and Billings 1988). However, fires in 2015 significantly changed the landscape, allowing invasive cheatgrass (*Bromus tectorum*) to replace native perennial grass species (Chase 2017). Frenchman Coulee is largely devoid of trees except for small wetland areas created by agricultural runoff where Russian Olive (*Elaeagnus angustifolia*) is sporadic.

The den at Umptanum Canyon (624m elevation) is located high on a scree slope surrounded by shrub-steppe, and a small creek runs along the bottom of the canyon (Fig. 4). Cottonwood (*Populus trichocarpa*) and Ponderosa Pine (*Pinus ponderosa*) trees are present along the creek, and the area receives ~23 cm average annual precipitation (NOAA 2018). Although the Pacific Northwest has drier summer months in general (Franklin and Dyrness 1973), this trend can constrain plant growth more in the warmer and drier shrub-steppe, as surface soils dry out as early as June (Barbour and Billings 1988).

In contrast to the shrub-steppe habitats surrounding the Frenchman Coulee and Upper Umptanum dens, the habitat surrounding dens in the Methow Valley is more variable. The Bear Creek (894m elevation) and Uphill (712m elevation) dens are in rock outcroppings largely surrounded by shrub-steppe habitats associated with Ponderosa Pine (*Pinus ponderosa*) forests. This is typical of shrub-steppe/forest transition zones east of the Cascades in Washington State (Franklin and Dyrness 1973). The Falls Creek (2 dens; ~809m elevation), Lost River (1190m elevation), and Matrix (849m elevation) sites are located on ridges, either in scree slopes below cliff faces or rock outcroppings (Fig. 5). These dens are located in or near Ponderosa Pine/Douglas Fir (*Pseudotsuga menziesii*) mixed forests, representing considerably more forested habitats than those dens sampled in the shrub-steppe. Precipitation in the Methow Valley is variable; Winthrop, WA receives ~37 cm, and Mazama, WA receives ~57 cm of average annual precipitation (NOAA 2018).

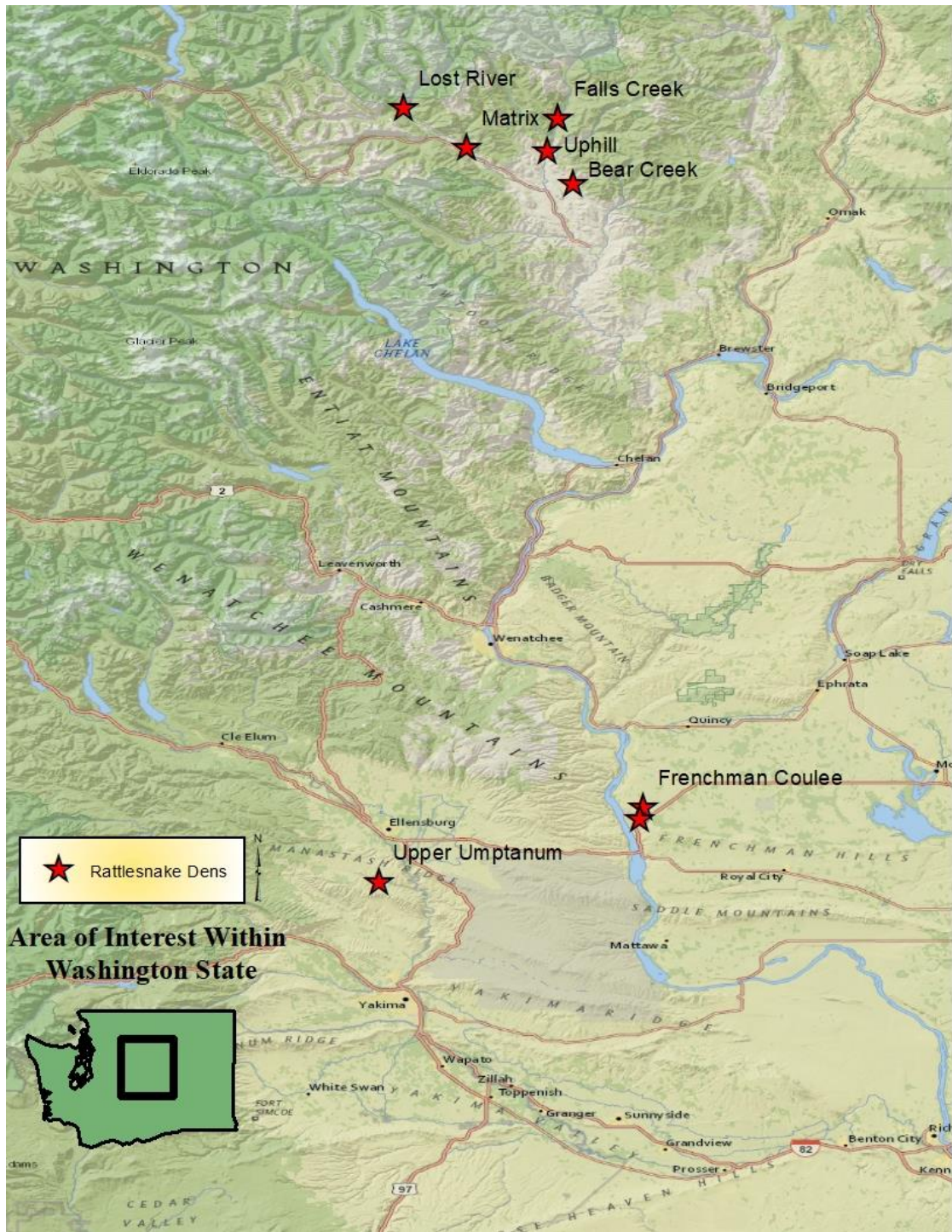


Figure 3: Map showing the locations of sampled rattlesnake dens throughout Washington State. Frenchman Coulee and Falls Creek populations are representative of two dens located within 2km of one another.



Figure 4: Representative photos of the Upper Umptanum den (Left; Photo Credit: Annika Hearn, 2017) and the Frenchman Coulee dens (Right; Photo Credit: Casey Croshaw, 2017). The photo at Upper Umptanum was taken during spring emergence, while the photo at Frenchman Coulee was taken during fall substrate sampling.



Figure 5: The view of the Methow Valley in spring from above Pearrygin Lake near the Bear Creek den (top), and a representative photo of the Falls Creek dens during spring emergence (bottom; Photo Credit: Casey Croshaw, 2017).

Study Species

Snakes were sampled at dens during spring emergence in April and May, 2017 which enabled relatively large samples to be gathered quickly (often 10+ snakes would be basking near the den). Snout-vent length (SVL) and tail length (measured from the vent to the beginning of the basal rattle segment) were measured to the nearest 0.5 cm. Sex was determined via recognizing the proportionately longer tail length of males and with caudal probing for hemipenes (Fitch 1960). Each snake was marked by clipping a unique number of ventral scales (Blanchard and Finster 1933), and a rattle segment was colored with permanent marker to aid in short term recognition of previously sampled individuals. After each snake was processed, it was placed in the “Photobucket” (see *Digital Photography* section) to acquire standardized photographs of the snake. Once all measurements and photographs were taken, snakes were released at the den entrance. All procedures followed approved Central Washington University reptile SOP (Standard Operating Procedures) and Institutional Animal Care and Use (IACUC) protocol no. A011405.

As there is some ontogenetic color change in rattlesnakes (Klauber 1956), only mature rattlesnakes were used in color analysis. However, *C. oreganus* may reach sexual maturity at a variety of ages (Diller and Wallace 1984), making age estimates via rattle length an unreliable method of determining sexual maturity. In northern Idaho, the age at first reproduction can range from 3-6 years for female snakes (Diller and Wallace 2002), and females in British Columbia reproduce later at 6-9 years (Macartney and Gregory 1988). While SVL at sexual maturity also varies considerably between populations, it has

been used to determine sexual maturity in past studies (Macartney and Gregory 1988). In a review of *C. oreganus* throughout the distribution of the species, mature adults were not observed below ~50cm SVL for both sexes (Ashton 2001). Consequently, any snake with an SVL above 50cm was considered sexually mature and included in this study.

Digital Photography

Digital photography is an effective method for analyzing color in field studies and circumvents the subjectivity inherent in human perception (Stevens et al. 2007). Color in digital photographs is a direct result of photon catches by the camera's sensor in three wavelengths of light in the visual spectrum (Red, Green, and Blue) (Stevens et al. 2007). This provides an objective measure of color, describing the actual spectral reflectance of an organism rather than how it is perceived. Spectrometry is a commonly used method of color analysis with equal or superior accuracy to digital photography, but conventional spectrometers only yield information for small points of interest, precluding their use (without complex sampling schemes) in studies on overall animal coloration (Stevens et al. 2007). Additionally, the portability and affordability of modern digital cameras makes photography an ideal method for field measurements in remote locations.

All photographs were taken in a standardized "Photobucket" (Fig. 6), consisting of a 5-gallon bucket with a frame attached to the top of the bucket to support the camera. This setup ensured that all photos were taken from the same height, providing identical focal distance for each photo. Using a standardized apparatus also facilitated maintaining standardized lighting conditions for photography (Krohn and Rosenblum 2016). A Nikon

D3200 DSLR camera, along with a Nikkor 18-55mm lens set at 35mm was used for all photography. The camera was set at manual, with ISO at 400, F-stop at 6.3, and shutter speed adjusted to reflect ambient light conditions. All photos were saved in RAW format to prevent the “lossy” compression (pixel averaging that degrades photo quality) caused by other common file formats (Stevens et al. 2007). To correct for white balance during post processing a standard 15% gray card was placed inside the bucket for every photo, as well as white and black standard cards to correct for differences in exposure (Krohn and Rosenblum 2016; Stevens et al. 2007; Gerald et al. 2001). Additionally, as many camera responses are not linear (Stevens et al. 2007), camera and workflow linearity was verified by plotting camera response to varying exposures for each color channel (Red, Green, Blue) in Microsoft Excel© before analysis ($R^2 > 0.97$ for all channels) to ensure that the camera and workflow were not distorting color values.

All photos were analyzed in Adobe Photoshop CC 2015.5©. For each photo, the “Lasso” tool was used to select an area of interest (AOI, Fig. 9-11), and the “Histogram” function was used to determine an average pixel value for the AOI for each color channel (Red, Green, Blue). Average pixel values for all AOIs were converted into ratios of the sum of all channels using Equation 1:

$$\text{Equation 1: } rX = X/(R + G + B)$$

Equation 1: Where rX is the ratio of the color channel of interest in the photograph (Red, Green, or Blue), and $R/G/B$ are the average pixel values for each color channel (Red, Green, and Blue) of a given AOI. This was repeated for all 3 color channels.

Ratios were used in preference to raw pixel values, as pixel values within a given channel are only meaningful relative to the values of another channel, and ratios are more resistant to distortion due to changes in lighting (Paterson and Blouin-Demers 2017; Teasdale et al. 2013; Bergman et al. 2009).



Fig. 6: A standardized “Photobucket” used for sampling rattlesnakes in the field.

Snake Photos

To measure overall body color (Fig. 7), the entire animal was selected as the AOI, enabling the calculation of color ratios for the entire body of the animal. Only photos that showed the entirety of a snake were used for analysis. The same snake photos used to analyze overall snake color were used to evaluate the contrast between adjacent dark and light tail bands on the snakes. Five light band and adjacent dark band AOIs were selected, starting at the rattle and extending proximally (Fig. 8). Average pixel value in the luminosity channel (a composite channel describing the perceived brightness of an image) was calculated for each set of five tail bands using the “Histogram” function. The absolute difference was then calculated between these sets of bands to yield a metric of contrast between the light and dark tail bands for each snake.

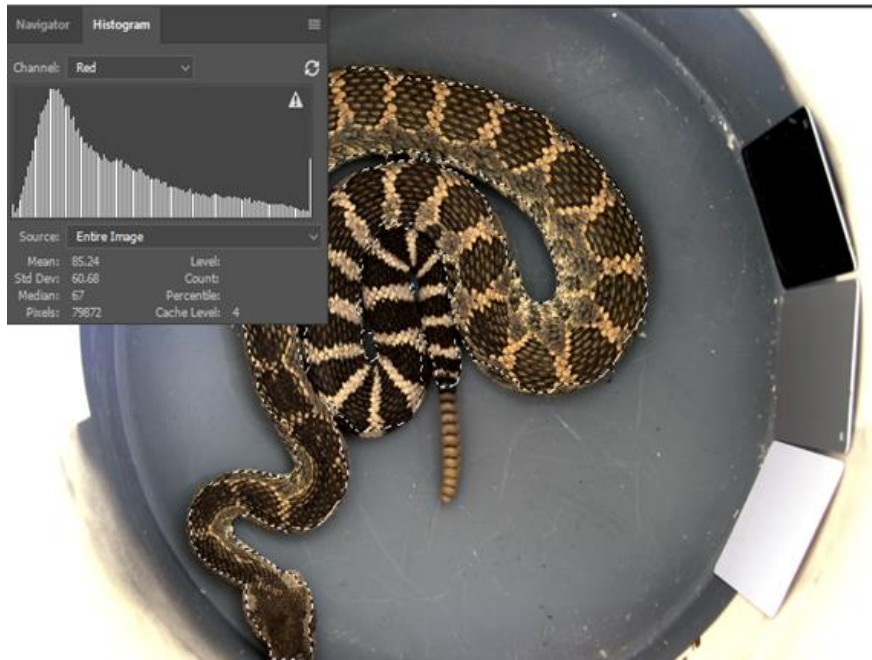


Figure 7: The reflectance histogram in the red channel of a wild caught male Northern Pacific Rattlesnake.

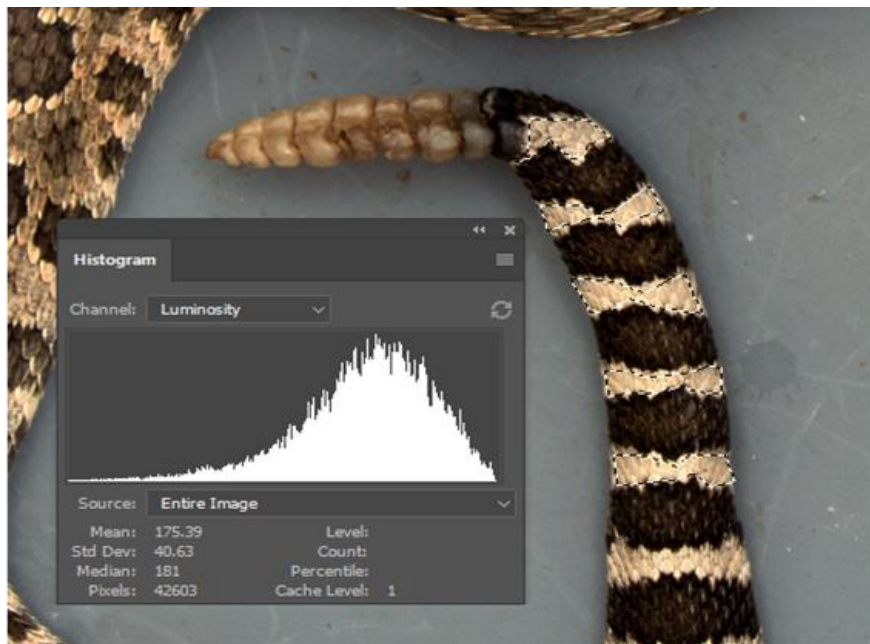


Figure 8: The reflectance histogram in the luminosity channel of the last five light bands on the tail of a wild caught Northern Pacific Rattlesnake in the “Photobucket”.

Substrate Photos

The same “Photobucket” used to photograph the snakes was used to photograph the substrates around the den. To do this, the bucket was placed on the ground with the bottom cut out and a photo of the substrate was taken. The entire exposed area of substrate visible in the bottom of the bucket was used as the AOI (Fig. 9), enabling a comparison of snake coloration to that of the microhabitats they might use in their environment. This provides a spatial resolution that might not be captured with aerial or satellite imagery, as aspects of the environment that snakes might use could be lost when capturing images from far above. This also ensures that both snake and substrate photos were subject to the same photographic procedures and standardizations with the same apparatus.

Substrate samples were taken twice during the field season for each den, once in late June and again in early September, to capture the effect of seasonal change in vegetation on color measurements (Wang et al. 2016). Five, 50m-long transects spaced 72 degrees centered at each den were used to sample substrates with a photo of the substrate taken every 10 meters along each transect. This resulted in 50 equally spaced photos describing a 50m radius around the den (Fig. 10). Some dens were in a location that prevented 5 equally distributed 50m transects, as often dens were above, or at the base of cliff faces. In these cases the angle between transects was adjusted to accommodate the local topography and maintain an even distribution of samples.

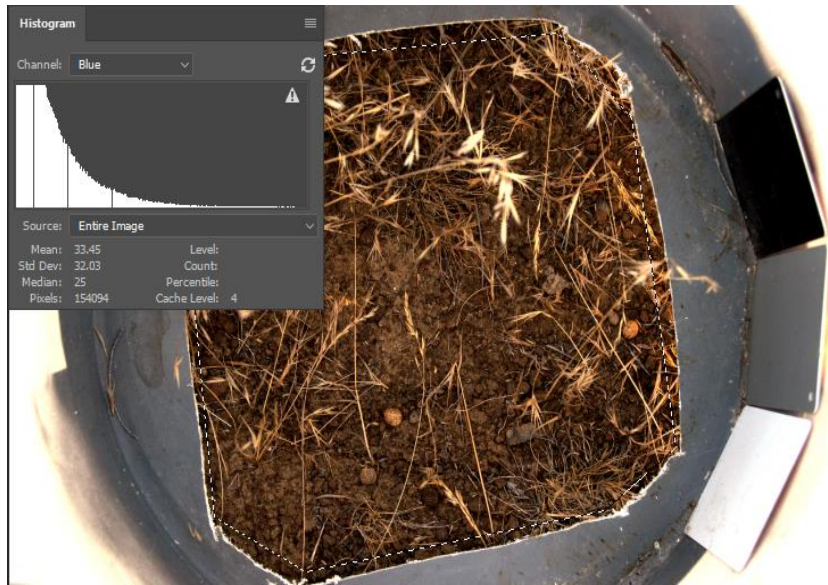


Figure 9: The reflectance histogram of a substrate sample in the “Photobucket” at Frenchman Coulee, WA.

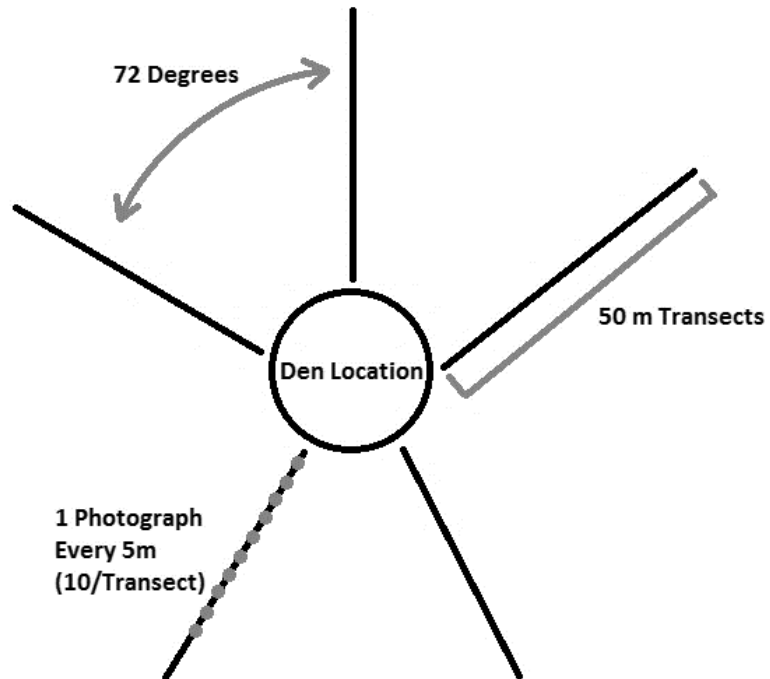


Figure 10: Sampling schematic for substrate photographs, yielding 50 equally spaced photographs of the substrate surrounding a snake den.

Accuracy of Photographic Measurements

The accuracy of color assessment from photos was verified in a trial where 10 photographs of an object in the “Photobucket” were taken under three different light conditions (where shutter speed was adjusted to simulate different lighting conditions – total of 30 photos) (Fig. 11). After standardization and color analysis procedures, the sum of all standard deviations for all three color channels over all light conditions was remarkably small (<1% variation in color assessment), indicating accurate color measurement over a variety of light conditions and high repeatability of measurements.

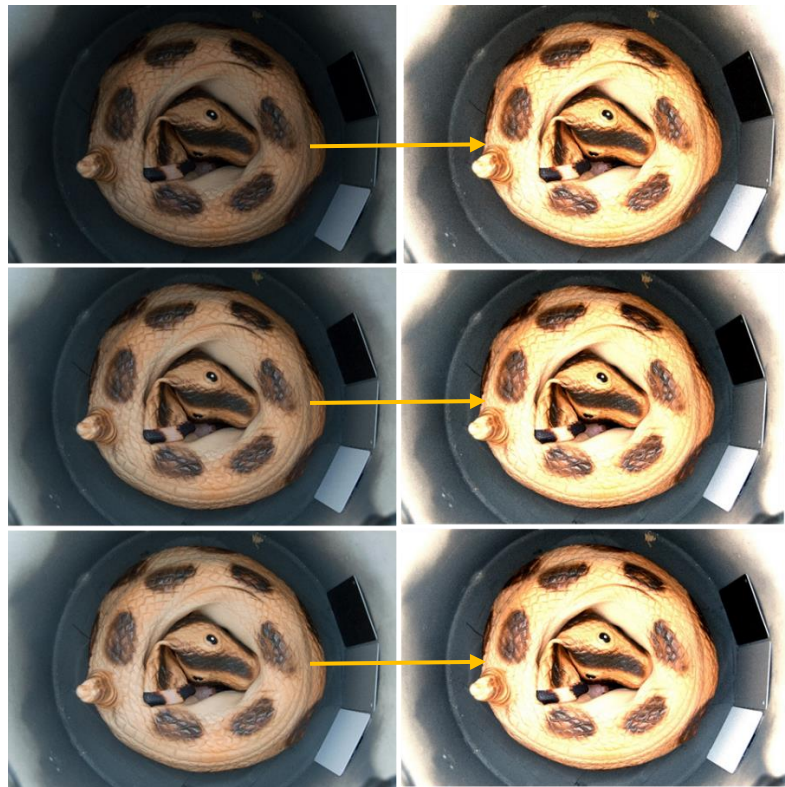


Figure 11: The results of a repeatability experiment where shutter speed was altered to simulate different light conditions/intensities. After standardization and photo analysis procedures, the sum of standard deviations for all color channel ratios (standard deviation of ratios for R = 0.003, G = 0.001, and B = 0.003) over all treatments (10 photos/light treatment, 3 light treatments) is 0.007, or 0.7% deviation in color values.

Remote Sensing and the Effect of Habitat on Snake Coloration

Remote Sensing methods were used to explore whether snake coloration is associated with the habitat surrounding the den at the landscape level. Maximum resolution air photos showing a half kilometer radius around the den was acquired from Google Earth[®]. All images used were taken between May and August of 2017, the same year as snake sampling occurred. Images were then georeferenced in ArcMap 10[®] using known point locations. A buffer of 0.5 km was created around the den location to represent the likely maximum distance a gravid female might travel from the den, and which males are likely to exceed during the active season (SIRAWG 2016; Didiuk et al. 2004; Bertram et al. 2001; Macartney 1979). Images were then classified using a Supervised Maximum Likelihood Classification (SMLC), where polygons were used to obtain training pixels for the Maximum Likelihood Classification (Table 1). A minimum of 3 land cover classes were used for each den with roughly 50 training polygons for each class (Fig. 12). Training polygons were only created within a 0.5 km buffer of the den location, as this was the area of interest. Classified images were then clipped to the 0.5 km buffer around the den.

Confusion matrices were used to assess the accuracy of classifications, using roughly 100 accuracy assessment points per class (a minimum of 300 points per den) created within the 0.5 km buffer of the den (Table 2). Accuracy assessment points were classified by hand to determine the most likely class for a given pixel by visually referencing the original image (Fig. 13). These user-defined classifications of assessment points were then compared to how the SMLC classified those same assessment points to

yield accuracy estimates for each SMLC.

After land cover classification, dens were described by the percent of forest classified pixels via the SMLC. Percent forested was used to determine associations between snake coloration and habitat, as the amount of forested land in an area is associated with a variety of other environmental characteristics such as soil characteristics, climate and temperature, vegetation, water balance, and fire regime (Chandra et al. 2016; Arno 1979; Grier and Running 1977; Bragg and Hulbert 1976). Consequently, differences in the percent forested habitat between dens likely represent differences in snake habitat as well.

Table 1: The number and type of training cells obtained from the training polygons used to classify the different land cover types at each den.

*The Frenchman Coulee den had an additional vegetation class to aid in discriminating between dense vegetation around small ponds and forested areas.

Den Name	Den	Classification Type						Sum
		Forest	Soil	Shadow	Road	Water	Riparian	
Bear Creek	BC	14894	73788	4986	0	0	0	93668
Falls Creek	FAL	23393	38189	3616	0	13738	0	78936
F. Coulee	FC	3067	76116	7804	12988	8011	9404	117390
Lost River	LR	25838	35782	8746	0	0	0	70366
Matrix	MAT	29968	40538	3272	15831	0	0	89609
Uphill	UP	48225	108317	4888	9082	22374	0	192886
Upper Ump.	UU	47576	242354	9488	0	0	0	299418

Table 2: The number and type of accuracy assessment points used to create confusion matrices, for each class at each den.

*Frenchman Coulee had an additional vegetation class to aid in discriminating between dense vegetation around small ponds areas and forested areas.

Den	Classification Type						Sum
	Forest	Soil	Shadow	Road	Water	Vegetation	
BC	117	100	97	0	0	0	314
FAL	114	115	99	0	61	0	389
FC	19	232	66	91	28	89	525
LR	121	168	108	0	0	0	397
MAT	138	125	142	47	0	0	452
UP	209	150	192	56	45	0	652
UU	93	218	70	0	0	0	381

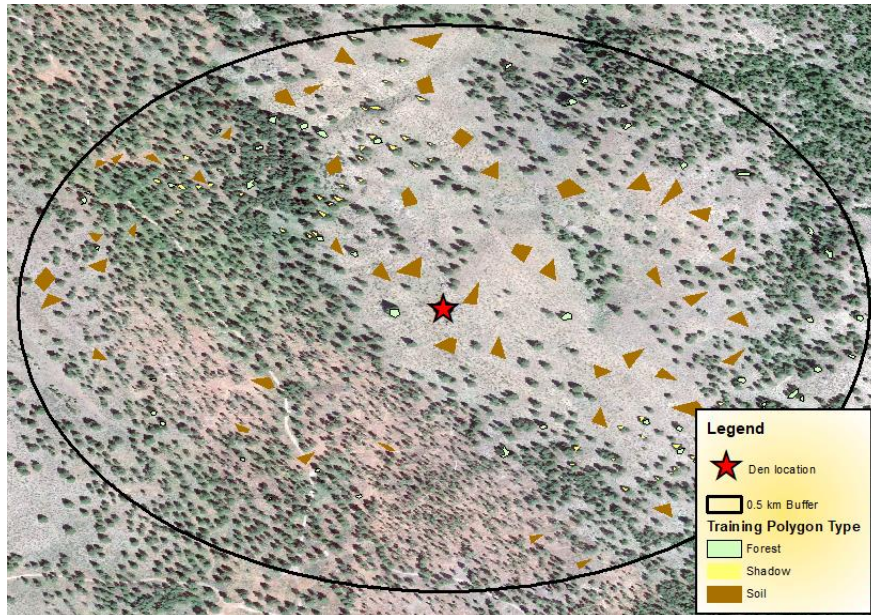


Figure 12: An example Supervised Maximum Likelihood Classification scheme (SMLCs), showing the training polygons used for the Bear Creek (BC) den in the Methow Valley, WA.

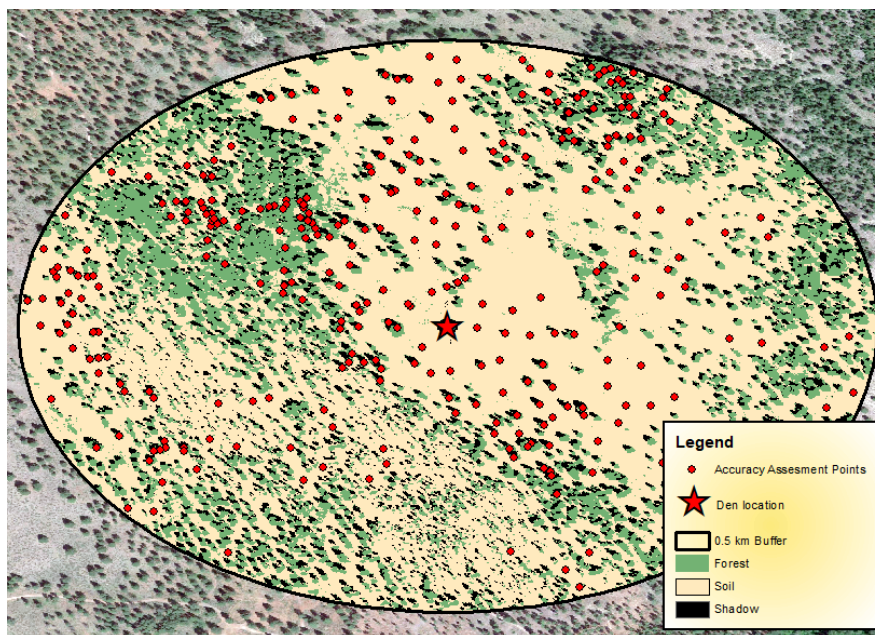


Figure 13: An example land cover classification resulting from the SMLC, showing the different classes and accuracy assessment points used for the Bear Creek (BC) den in the Methow Valley, WA.

Statistical Analyses

A General Linear Mixed Effects Model was used to determine if color is related to the habitat surrounding the hibernaculum (percent forested). Den was nested inside region (Ellensburg/Methow) and classified as a random effect, and an interaction between sex and percent forested was used as a fixed effect. To simplify statistical analysis only the R/B color ratio of snake photos was used, as snake color varied little in the Green color channel (Fig. 20 - Appendixes) and previous studies have simplified analysis in similar ways (Bergman et al. 2009). A natural-log transformation of the R/B color ratio was used to ensure normality of the response variable. To determine if the nesting structure (dens nested within region) had a significant effect on the model, two models were created: one with the nesting structure and one without. A Likelihood Ratio test was then conducted on the two models to determine if color varied between regions.

A General Linear Model was used to determine if individual dens were significantly different from one another in snake coloration, where den was classified as a fixed effect. Snake R/B color ratios were natural-log transformed to ensure a normal distribution of the response variable. Using the EMMEANS package in “R”, post-hoc Tukey adjusted pairwise comparisons were used to determine p-values for individual den comparisons.

To determine if female snakes were more cryptic in the immediate vicinity of the den than male snakes, the total absolute difference between a snake and a substrate photograph for all color channels was determined using Equation 2. All three color-

channels were used for this analysis, as the Substrate Color Contrast Level (SCCL) should reflect how well a snake matches its background overall.

$$\text{Equation 2: Substrate Color Contrast Level (SCCL)} = |R_k - R_s| + |G_k - G_s| + |B_k - B_s|$$

Equation 2: Where R/G/B are the ratios of each color channel (Red, Green, or Blue) for a given AOI (determined via Equation 1), and “k” is the snake body AOI, and “s” is the substrate AOI.

These values for an individual snake in comparison to its den’s 50 substrate photographs were then averaged. This yielded a metric of how closely on average that particular snake’s color matched the substrate color (SCCL) within 50 m of its den, a metric of how cryptic that snake is. Repeating this for all snakes for both season’s substrate photos yielded SCCLs for each snake relative to its own den’s surroundings, in both summer and fall.

A Linear Mixed Effects Model was used to detect any association between sex and the SCCL of rattlesnakes. A natural-log transformation was used on the SCCLs of snakes to ensure a normally distributed response variable. Den was nested inside region and classified as a random effect, with individual snakes nested within dens, as each snake had multiple SCCL measures (one for each season). Season and sex were classified as fixed effects to determine if either had a significant effect on SCCLs.

A Linear Mixed Effects Model was also used to model the effect of sex on the strength of contrast in tail bands. The contrast in tail bands of snakes was transformed by raising it to the power of 1.5 to ensure a normally distributed response variable. Den was

nested inside region and classified as a random effect, and sex was classified as a fixed effect in the model. Region was included to determine if any significant differences in tail banding might be due to differences in the region where the snakes were captured.

All linear models were created using the NLME package in the Rstudio 3.4.3 statistical software (Zuur et al. 2009), and, other than post-hoc analyses, the ANOVA output function was used to obtain all p-values via *F*-Tests (Bolker et al. 2008). Models were weighted by the variation within dens to accommodate heteroscedasticity between dens using General Least Squares regression (Zuur et al. 2009). Additionally, all models met the assumptions of linear models, as standardized residuals plotted against fitted values showed a homoscedastic distribution of residuals, while a QQ-Plot and histogram of the residuals both showed normal distributions (Zuur et al. 2009).

Chapter IV

RESULTS

A sample of 127 snakes, captured from 9 different dens (7 populations) during the spring of 2017, was used in this study. Two samples each from dens at Frenchman Coulee and Falls Creek were pooled in this analysis due to their proximity. Snakes were generally larger in the Methow, particularly in the Falls Creek den, where 8 of 32 snakes captured were over 90 cm SVL. In nearly all dens with more than 10 snakes, the samples were skewed towards males, the exception being Upper Umptanum (Table 3).

The overall accuracy of land cover classification was >86% for all den classifications, with the majority of classifications being >90%. Commission (non-forest pixels incorrectly classified as forest) and omission (forest pixels not correctly identified as forest) accuracy were both high as well, with all dens being >93% accurate. The exception was Frenchman Coulee, which had very low omission accuracy. The classification scheme for Frenchman Coulee underestimated percent forested by roughly half. However, the percent forested predicted by the classification scheme for this den is so low (0.98%) that this underestimation likely does not have a significant impact on any analyses. The percent forested predicted by the classification schemes for all dens varied significantly (~1 - ~51% forested), indicating that snakes were sampled from dens in a variety of habitats (Table 4).

Table 3: Names, abbreviations, study areas, and sample sizes of all dens sampled. Dens that were less than 2 km from one another were pooled in analysis. Dens that were located amongst topography that prohibited equal spread of transects had transect locations altered to maximize spread of transects.

* Indicates that two dens were in close proximity (< 2 km) and were pooled together in the analysis, and that some substrate transects were altered due to local topography.

Den Name	Den Abbreviation	Region of Study	Sample Size (M/F)	Average SVL (cm) (M/F)
Bear Creek	BC	Methow	3 / 6	76 / 63.42
Falls Creek	FAL*	Methow	24 / 8	82.75 / 63.75
F. Coulee	FC*	Ellensburg	29 / 10	65.59 / 56.8
Lost River	LR	Methow	6 / 1	76.92 / 52.5
Matrix	MAT	Methow	1 / 5	81.5 / 58.3
Uphill	UP	Methow	14 / 3	67.25 / 65
Upper Ump.	UU	Ellensburg	8 / 9	71 / 60.56
<i>All Dens Combined</i>			<i>85 / 42</i>	<i>72.46 / 60.06</i>

Table 4: Accuracy of Supervised Maximum Likelihood classification schemes for each den, and the percent of the 0.5 km buffer that was classified as forested. Shows omission and commission accuracy, as well as overall accuracy of the classification scheme for all assessment points at that den.

Den	Overall Accuracy (%)	Forest Commission Accuracy (%)	Forest Omission Accuracy (%)	Pixels Classified as Forested (%)
BC	100	100	100	22.55
FAL	98.16	100	98.26	47.08
FC	86.24	94.74	58.06	0.98
LR	98.49	98.33	96.72	50.59
MAT	97.78	97.82	95.07	41.76
UP	93.98	93.78	97.51	27.95
UU	97.61	98.91	91.92	15.27

Post-hoc Tukey analysis of snake color revealed that snakes varied in color considerably across the landscape. Several dens were significantly different from one another in the R/B color ratio, including two dens that were within close proximity (BC-UP, ~10km apart; Table 5). Many of the significant differences between dens were comparisons between dens in different regions, as in the case of BC-FC, BC-UU, FC-MAT, and MAT-UU. This indicates that while snakes do vary in color within a region, most snakes within a region are likely to share similar colorations (Table 5).

Male and female color (R/B ratio) interacted with the environment differently, indicating that they had significantly different associations between color and percent forested ($p = 0.018$; Table 6). Male color (R/B ratio) had a positive slope in response to percent forested, while female color was negative in response (Fig. 14). However, while the sexes did have opposing slopes in response to percent forested, the slopes for each sex were not significantly different from zero ($p = 0.329$; Table 6), indicating that percent forested does not explain snake color in any significant way. Additionally, the sexes did not express significantly different R/B color ratios across all dens, as indicated by the lack of a significant difference between the intercepts, or the averages for the sexes ($p = 0.664$; Table 6).

Table 5: Results of Tukey post-hoc pairwise comparisons of dens for a General Least Squares model of the natural-log transformed R/B color ratios of rattlesnakes sampled across Washington State.

Significant differences (p-value < 0.05, DF: 119) between dens are indicated by bolding.

Den Comparison	p-Value
BC – FAL	0.0749
BC – FC	< 0.0001
BC – LR	0.5936
BC – MAT	0.9995
BC – UP	0.0417
BC – UU	< 0.0001
FAL – FC	0.8739
FAL – LR	0.9985
FAL – MAT	0.4094
FAL – UP	0.9422
FAL – UU	0.0783
FC – LR	~1
FC – MAT	0.0066
FC – UP	~1
FC – UU	0.4598
LR – MAT	0.7395
LR – UP	~1
LR – UU	0.9841
MAT – UP	0.1518
MAT – UU	< 0.0001
UP – UU	0.9661

Table 6: Summary of a general linear mixed model on natural-log transformed R/B ratios of snakes, with regression weighted for each den (General Least Squares).

Significant differences (p-value < 0.05) are indicated by bolding.

To test if the nesting structure of the sampling design was significant, a likelihood ratio test was used, yielding a Likelihood Ratio test statistic (LR). For all other comparisons, the ANOVA output and F distribution were used to determine significance.

Statistical Comparison	D.F.	Test Statistic	p-Value
Difference in intercept (average R/B) between sexes	118	F = 0.189	0.664
Environment (% Forested) Associated with Snake Color	4	F = 1.233	0.329
Interaction Between Sex and Environment (% Forested)	118	F = 5.759	0.018
Region Associated with Snake Color	-	LR = 15.620	< 0.0001

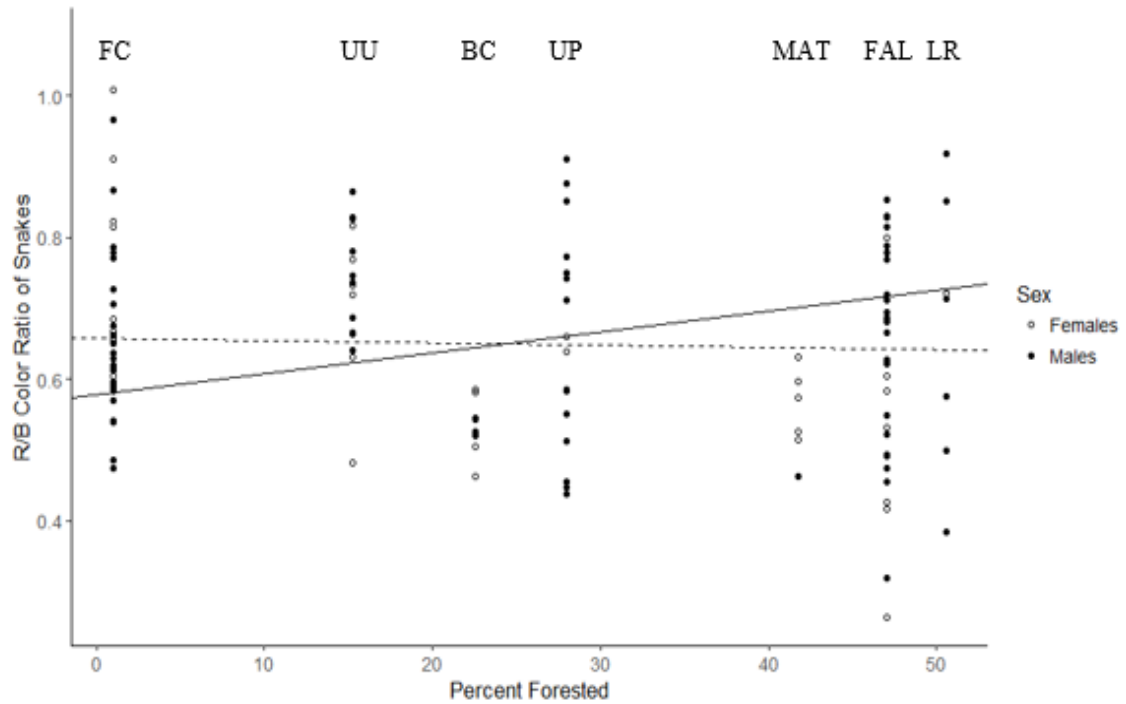


Figure 14: How snake coloration varies across the landscape. Summary of a general linear mixed model on natural-log transformed R/B ratios of snakes, with regression weighted for each den (General Least Squares). The hashed line is the model regression for females, and the solid line is the model regression for males. An interaction term between Sex and Percent Forested was significant (DF: 118, F-Statistic: 5.76, p-value: 0.018).

The effect of sex on the color of snakes compared to the color of their surrounding substrates (SCCL) was not significant. However, significant differences were found between the two seasons substrates that were sampled. Snakes contrasted the color of the surrounding substrates significantly less in the fall than in the spring ($p = 0.001$; Fig. 15), although the difference was slight.

While male and female snakes did not differ in overall color (Table 6), they did differ significantly in the amount of contrast (warning coloration) of their tails. Males on average exhibited stronger warning coloration than did females ($p = 0.0045$; Fig. 16), indicating that, while not sexually dichromatic in overall coloration, *Crotalus oreganus* is dichromatic in the degree of aposematic coloration exhibited on the tail.

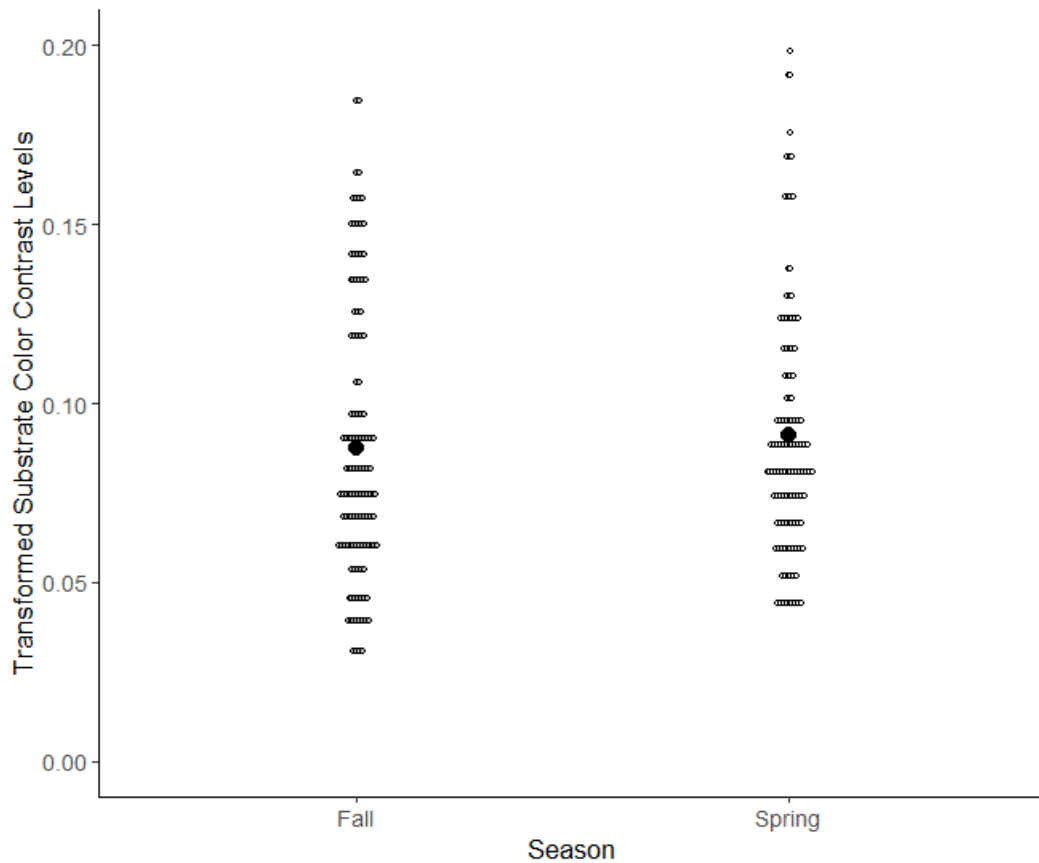


Figure 15: Summary of a general linear mixed model on the log transformed Substrate Color Contrast Level (SCCL) of snakes in comparison to their spring and fall substrate photographs, with regression weighted for each den (General Least Squares). Season was highly significant (DF: 119, F-Statistic: 238.0561, $p < 0.0001$). Black dots are averages for each season. Despite the significant p-value, the difference was marginal between seasons. The sexes did not differ in how cryptically colored they were relative to the substrates they inhabit (DF: 115, F-Statistic: 0.2068, $p = 0.6782$).

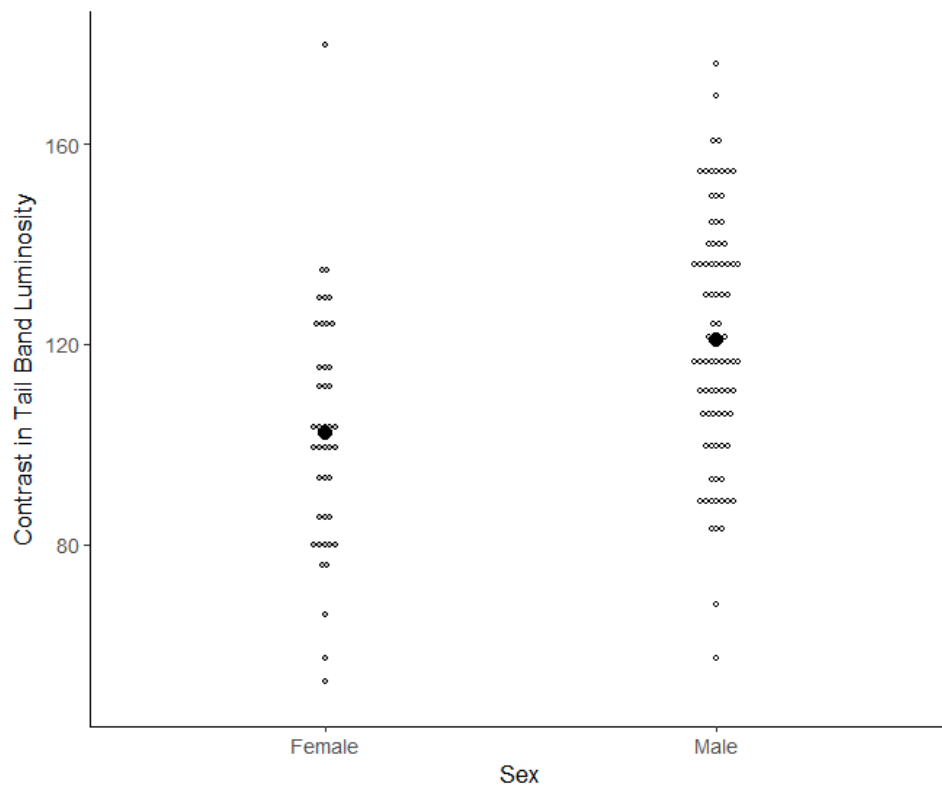


Figure 16: Summary of a general linear mixed model on the absolute difference in luminosity between light and dark snake tail bands (amount of warning coloration), transformed by raising to the 1.5 power, with regression weighted for each den (General Least Squares). Sex was highly significant (DF: 119, F-Statistic: 8.38, $p = 0.0045$). Black dots are averages for each sex (female: $102.13 \pm 3.72SE$, male: $120.33 \pm 3.04SE$). Photos are representative of typical female (above left) and male (above right) rattlesnake tails, illustrating the contrasting bands near the base of the rattle, as well as the difference in band contrast between the sexes.

Chapter V

CONCLUSION

I. How do Rattlesnakes Vary in Color across Washington State?

Many rattlesnake species have developed different colorations and patterns, presumably to adapt to local habitats and substrates (Farallo and Forstner 2012; Shine and Harlow 1998; Greene 1997), likely including the Northern Pacific Rattlesnake (*Crotalus oreganus*). *C. oreganus* does appear to vary in color across Washington State, as snakes in different regions and different dens were statistically different from one another in snake R/B color ratios (Tables 5 and 6). Distances between dens that were significantly different were not always large, indicating that snakes can vary locally in color as well (BC – UP; ~10km apart).

However, while statistically significant differences in snake color were detected between dens, these differences were not nearly as striking as the color differences that have been reported for polymorphic species like *C. l. lepidus* (Farallo and Forstner 2012). Snakes from the Methow region did appear qualitatively to be slightly more greenish-blue than snakes from the Ellensburg region, but snake color seemed to vary nearly as much within a den as it did between dens (Fig. 18 – Appendixes). Large within-den variation in snake color could explain why few rattlesnake dens were statistically different from one another, even when dens represented substantially different snake habitats (Table 5). Substrate Color Contrast Levels (SCCL) for snakes varied

significantly in response to the variability of substrate colors surrounding a den (Fig. 21 – Appendixes), indicating that there is no single advantageous coloration for rattlesnakes in such heterogeneous habitats. This is supported by previous research, as Eastern Collared Lizards (*Crotaphytus collaris*) found in desert environments were less cryptically colored than those found on a nearby lava flow where the habitat is more homogenous (Krohn and Rosenblum 2016).

In addition to the heterogeneous environments the Northern Pacific Rattlesnake inhabits, the lack of distinct color morphs in *C. oreganus* may also be attributable to the generalist nature of the species. The color polymorphic Banded Rock Rattlesnake (*C. l. lepidus*) shares portions of its range with other rattlesnake species, including the Western Diamondback Rattlesnake (*C. atrox*) (Farallo and Forstner 2012; Klauber 1956). However, one species is a habitat specialist, while the other is a generalist: *C. l. lepidus* is almost exclusively found in rocky outcroppings, while *C. atrox* can be found in a wide variety of habitats (Farallo and Forstner 2012; Beck 1995; Klauber 1956). Additionally, *C. lepidus* populations in Arizona typically have summer ranges of ~0.4km, compared to the much larger summer ranges of *C. atrox*, which may disperse as far as ~1.6km from the den (Schuett et al. 2016). Consequently, *C. atrox* does not exhibit the distinct color polymorphisms within a population that *C. l. lepidus* does (Schuett et al. 2016; Farallo and Forstner 2012), likely because it does not specialize in any one habitat.

As distinct color polymorphisms are prevalent when suitable habitats are patchy and genetic flow is limited (Gray and McKinnon 2006; Rosenblum 2006), it seems appropriate that a specialist species inhabiting dispersed rock outcroppings with limited

dispersal would be polymorphic, while a habitat generalist would not. Like *C. atrox*, *C. oregonus* is also a habitat generalist, having been described as “one of the most broadly tolerant of all rattlers in its choice of habitats” (Klauber 1956). With dispersal ranges of ~3.5km or more (Gomez 2007), *C. oregonus* may experience even more variation in habitat and substrates than many *C. atrox* populations. This is supported by this study, as snakes were sampled in habitats ranging from the shrub-steppe of eastern Washington to the mixed conifer forests of the eastern Cascade slopes (Table 4). This lack of habitat specialization in the Northern Pacific Rattlesnake may partially explain why rattlesnakes in Washington State do not exhibit the distinct color polymorphisms that have been observed in more specialized rattlesnake species.

II. How Does Rattlesnake Color Relate to the Habitat Surrounding the Den?

The amount of forested habitat surrounding the den was not significantly associated with snake coloration (Table 6). However, an interaction term between sex and percent forested was statistically significant (Table 6), indicating that male and female snakes interact differently with their habitat. Despite this, the slopes describing the relationship between color and percent forested for each sex were not significant, making the nature of this sex specific interaction with the environment unclear. Part of this may be due to unintentional sampling bias. All sparsely forested dens were sampled from the Ellensburg region, and all heavily forested dens were sampled in the Methow region. This makes it difficult to determine if snake color is associated with the degree of

forested habitat surrounding the den rather than some other variation between regions (other types of vegetation, dominant ground cover, etc.) (Fig. 19 - Appendixes). Future studies should seek to avoid this sampling problem by ensuring that both forested and non-forested den sites are sampled in each region. This would establish a more robust statistical approach to these data, and provide a more complete answer to the question of habitat's effect on snake coloration.

While sampling bias may be partially responsible, there are also a variety of ecological reasons for why the percent forested of the habitat surrounding the den did not significantly explain variation in snake color. While changes in the percent forested of an environment do correlate with changes in a host of environmental variables (precipitation, fire regime, etc.; Chandra et al. 2016; Arno 1979; Grier and Running 1977; Bragg and Hulbert 1976), changes in percent forest may not be correlated with environmental variables that are relevant for rattlesnakes. In the Eastern Massasauga Rattlesnake (*Sistrurus c. catenatus*), snakes preferentially spend time in specific microhabitats (shrub, rock, log etc.), with little preference for the landscape-scale habitats, like percent forested, that were measured in this investigation (Harvey and Weatherhead 2006). Consequently, using a coarse habitat metric like percent forested might not accurately capture differences between dens in a meaningful way for rattlesnakes.

Additionally, the amount of forested habitat currently surrounding a snake den may not be indicative of the selective pressures that shaped the evolution of snake

coloration. Habitats surrounding snake populations (and the selective pressures on those populations) have been changing for millennia (Wright et al. 1993), but the amount of forested landscape has changed even more drastically in recent history. Forests have been altered by human activity in many ways: logging has played a significant role in the reduction of forested areas (Swanson and Franklin, 1992), and fire suppression has been implicated in increasing the amount of forested areas (Bragg and Hulbert 1976), to name a few. Consequently, the habitat surrounding a snake population today may not be representative of the habitat used by that population during much of its evolutionary history when natural selection was shaping the genetics of coloration. Extensive forest management has occurred near many of the dens sampled in this investigation (Romain-Bondi 2006), potentially precluding the use of percent forested as an accurate metric for the selective pressures to which these snakes have adapted.

Snakes did vary in how well they matched substrate photographs surrounding the den seasonally. Snakes matched their substrates significantly more when compared to fall substrate photos than early summer substrate photos (Fig. 15). However, as there was considerable variation in the color of the substrates surrounding a den and how cryptic snakes were in comparison to them (Fig. 21 – Appendixes), it is unlikely that the seasonal changes captured in substrate photograph colors are biologically relevant for these snakes. A more thorough analysis of how snake color compares to seasonal changes in substrates would be required to assess how snake crypsis varies seasonally, and if that is even important for a generalist snake species.

III. Do Male and Female Rattlesnakes Differ in Color?

To circumvent the challenges associated with characterizing habitat at the landscape level, snake coloration was compared directly to the color of substrate photographs from the immediate area of the den. As females are likely biennial breeders (Diller and Wallace 2002, 1984), and gravid females likely spend more time in the immediate vicinity of the den (Gomez 2007; Macartney 1979), females may match the color of the substrates immediately surrounding the den more than males. However, statistical comparisons between the color of the sexes did not indicate that the sexes were significantly different in overall color (Table 6), and similar comparisons of snakes to their substrates did not yield any significant differences in SCCLs between the sexes (Fig. 15). This indicates that any differences in seasonal dispersal patterns between the sexes may not have an effect on overall snake coloration. Again, this may be due to both the generalist nature of this snake species and the heterogeneity of their habitats and environments. Even if male and female snakes are dispersing different distances from the den (and consequently occupying different habitats), a widely distributed generalist species like the Northern Pacific Rattlesnake may not exhibit significant habitat specific coloration (Gray and McKinnon 2006; Rosenblum 2006), particularly if the habitats they encounter are variable in color (Krohn and Rosenblum 2016; Stevens 2007).

Despite the lack of significant differences between the sexes in overall color, males showed significantly stronger warning patterns and coloration than females. This may be a response to increased predator pressure on males. Because female rattlesnakes

tend to move less than males, especially when gravid (Macartney 1989, 1979), males likely experience more predator pressure. In California, male *C. oregonus* moved significantly more per day than females did during the active season (Putman, Lind and Taylor 2013) and males in Idaho disproportionately represented snakes found moving across the landscape (Jochimsen 2005). As predator pressure plays a significant role in driving snake coloration (Farallo and Forstner 2012; Stevens 2007), particularly in the case of aposematic coloration (Niskanen and Mappes 2005; Wüster et al. 2004; Shine and Madsen 1994; Brodie 1993), this potential difference in predator pressure between the sexes in *C. oregonus* may explain the stronger aposematic coloration/patterning exhibited by male snakes.

Sexual dichromatism in warning coloration is a novel finding for *C. oregonus*. While sexual selection can drive sexual dichromatism in other species (Myserud 2003; Godin and Dugatkin 1996), the nature of this dichromatism in *C. oregonus* likely lends itself to ecological differences between the sexes, as black and white banding is typically regarded as having a role in predator avoidance (Caro 2009, 2005; Williams 2007; Cantú-Salazar and Hidalgo-Mihart 2004; Brodie 1993; Greene and McDiarmid 1981; Plowright and Owen 1980; Brower and Brower 1962). My findings support the ecological hypothesis for sexually dichromatic snake color posited by Shine and Madsen (1994), representing an example of ecological, and not sexually selected, sexual dichromatism.

Since *C. oregonus* (this study) and *Vipera* spp. (Shine and Madsen 1994) both exhibit increased male warning coloration and pattern, other vipers with similar

ecological traits (e.g. differences in activity level between males and females, communal denning, ambush hunting) might also exhibit increased male warning coloration. In *C. atrox*, males typically have more black and white tail bands than do females, which has been attributed to male snakes having longer tails (Klauber 1956). However, the dispersal patterns of male and female snakes may also play a role in shaping the observed warning colors and patterns in *C. atrox*. Future investigations on warning coloration in snakes should focus on other *Crotalus* species, to determine if increased male warning coloration is a trend for communally denning vipers.

Summary

Northern Pacific Rattlesnakes vary significantly in color across Washington State, and can vary in color both regionally and locally. However, these differences in color were not so substantial that they could be consistently observed with the naked eye; snakes varied in color nearly as much within a den as they did between dens.

Additionally, the habitat measurements employed in this study did not have any significant association with snake coloration. The percent forested area surrounding the den does not appear to correlate with any significant change in either male or female coloration, though this may be partially due to sampling bias.

C. oreganus was not sexually dichromatic in overall body color, nor did male and female rattlesnakes differ in how well they matched the substrates immediately surrounding their den. However, the sexes did differ substantially in the degree of warning color present on their tails. Males had significantly more warning coloration than did females, and this may be due to the increased predator pressure male snakes may experience. This sexually dichromatic tail coloration is a novel finding for *C. oreganus*, and is consistent with trends in other viper species. It also represents one of a few known instances of sexual dimorphism caused by differing ecology of the sexes, and not sexual selection.

Future studies should seek to establish a more balanced sampling design in capturing how forested dens are, and attempt to measure how snake color might be related to microhabitat selection as well. Capturing how much predator pressure is present, as well as tracking the movement patterns of sampled snake populations, might

elucidate the differential selective pressures on male and female snakes. Future studies should investigate the potential for sexual dichromatism in other *Crotalus* species to establish how these trends in seasonal dispersal might impact snake color and if they are consistent in selecting for sexually dichromatic warning colorations.

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APPENDIXES

Appendix A – Ternary Plots and Snake Color

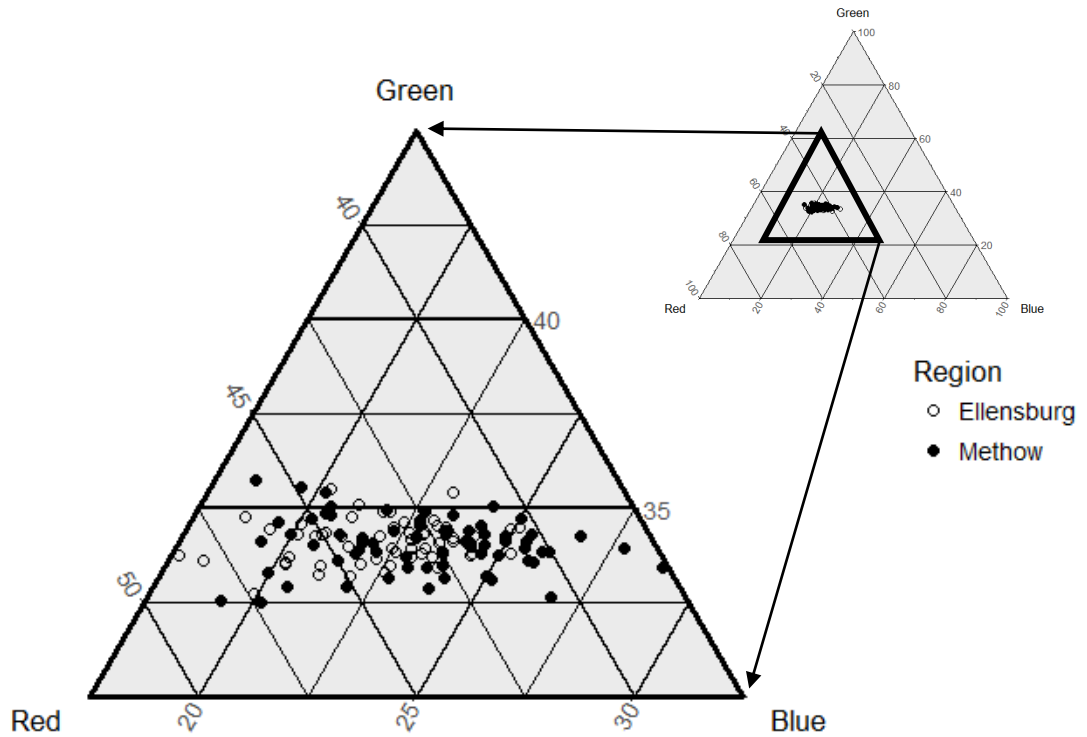


Figure 17: Ternary plot showing the distribution of snake coloration between the sampled regions. Values are each snake's color ratios for the Red (left axis), Green (right axis), and Blue (bottom axis) color channels in snake photographs, and sum to 100%. The entire scale of the possible color values was not used to display data as it becomes unintelligible. This is also inappropriate, as no snake will ever be 100% red, blue or green. To remedy this, the axes were adjusted to accommodate how snake color was grouped, and increase interpretability of the figure.

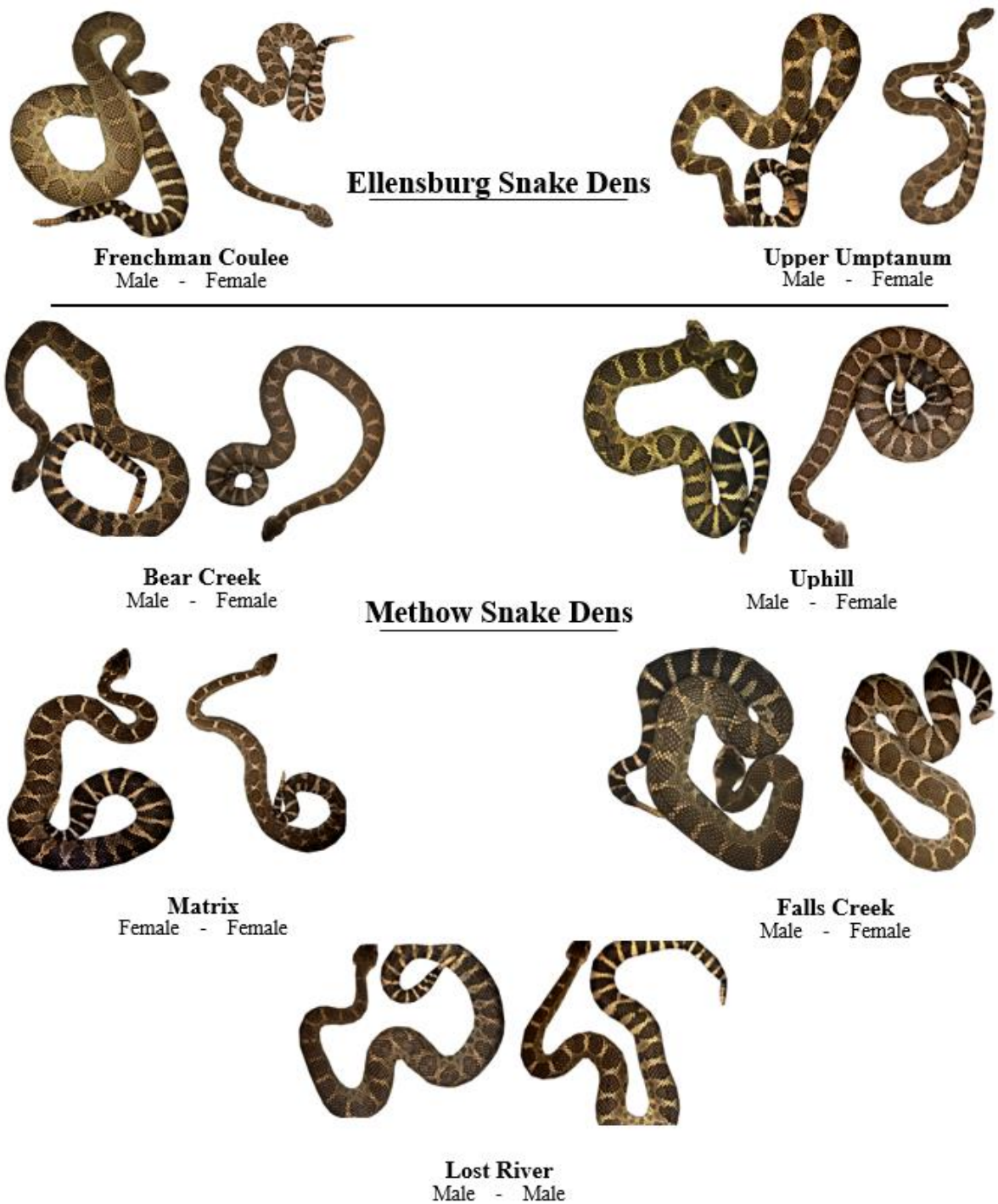


Figure 18: Representative snakes from each den sampled in this study, showing how snake color varies across the landscape.

Appendix B – Additional Statistical Information

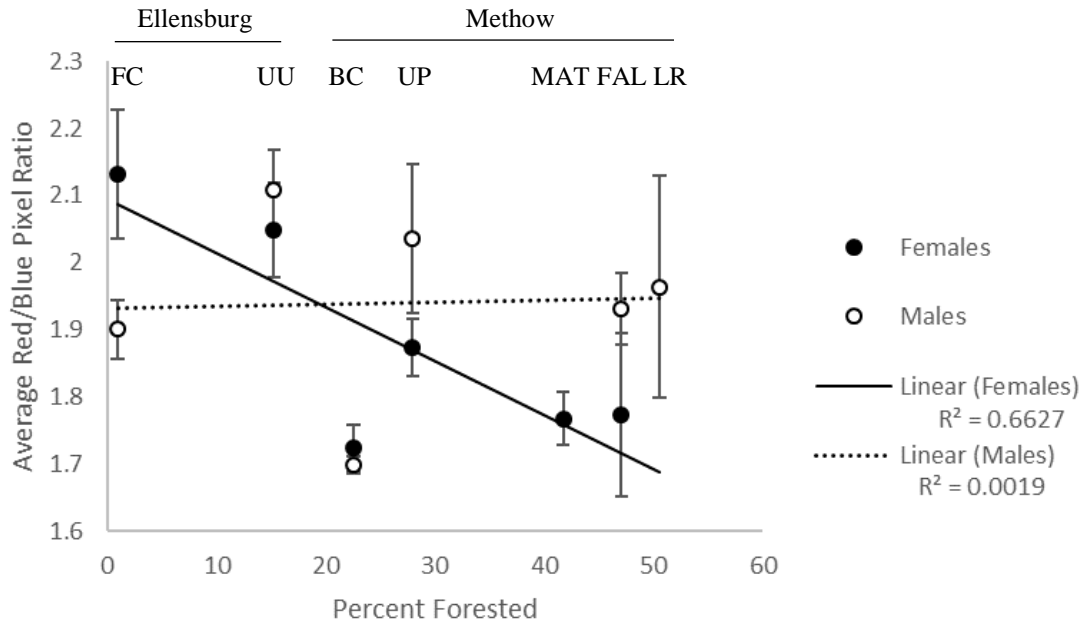


Figure 19: Depiction of how female coloration is strongly correlated with the percent forested area around a den ($R^2 = 0.6627$) whereas male coloration is not ($R^2 = 0.0019$), if the nested sampling design is ignored. While not statistically appropriate, this suggests that future research should incorporate forested and un-forested dens in both study areas to disentangle region and percent forested in analysis.

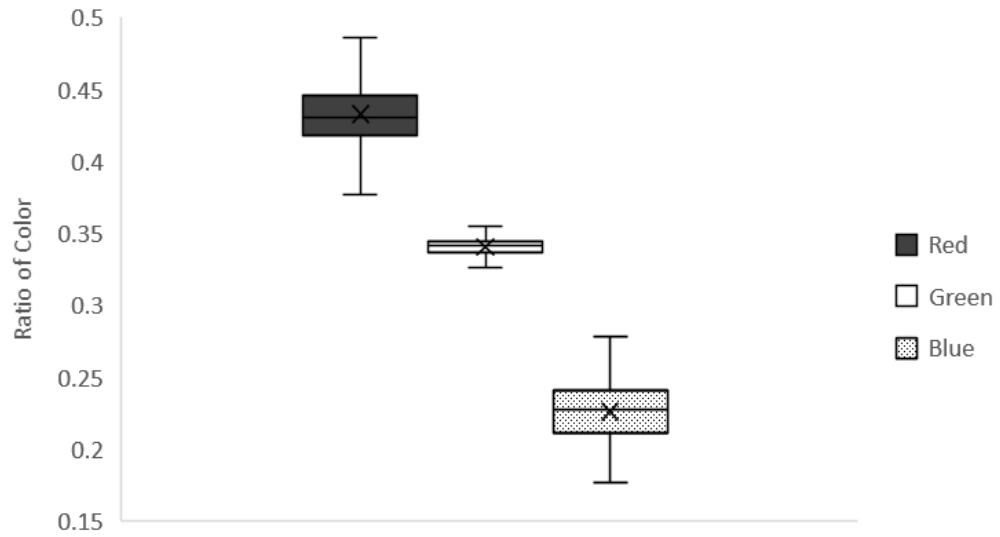


Figure 20: Box plots depicting the distribution of each color ratio for all snake photos. There was much less variation in snake color in the Green channel than the Red or Blue color channels (Standard Deviations: Red = 0.021, Green = 0.0064, Blue = 0.021).

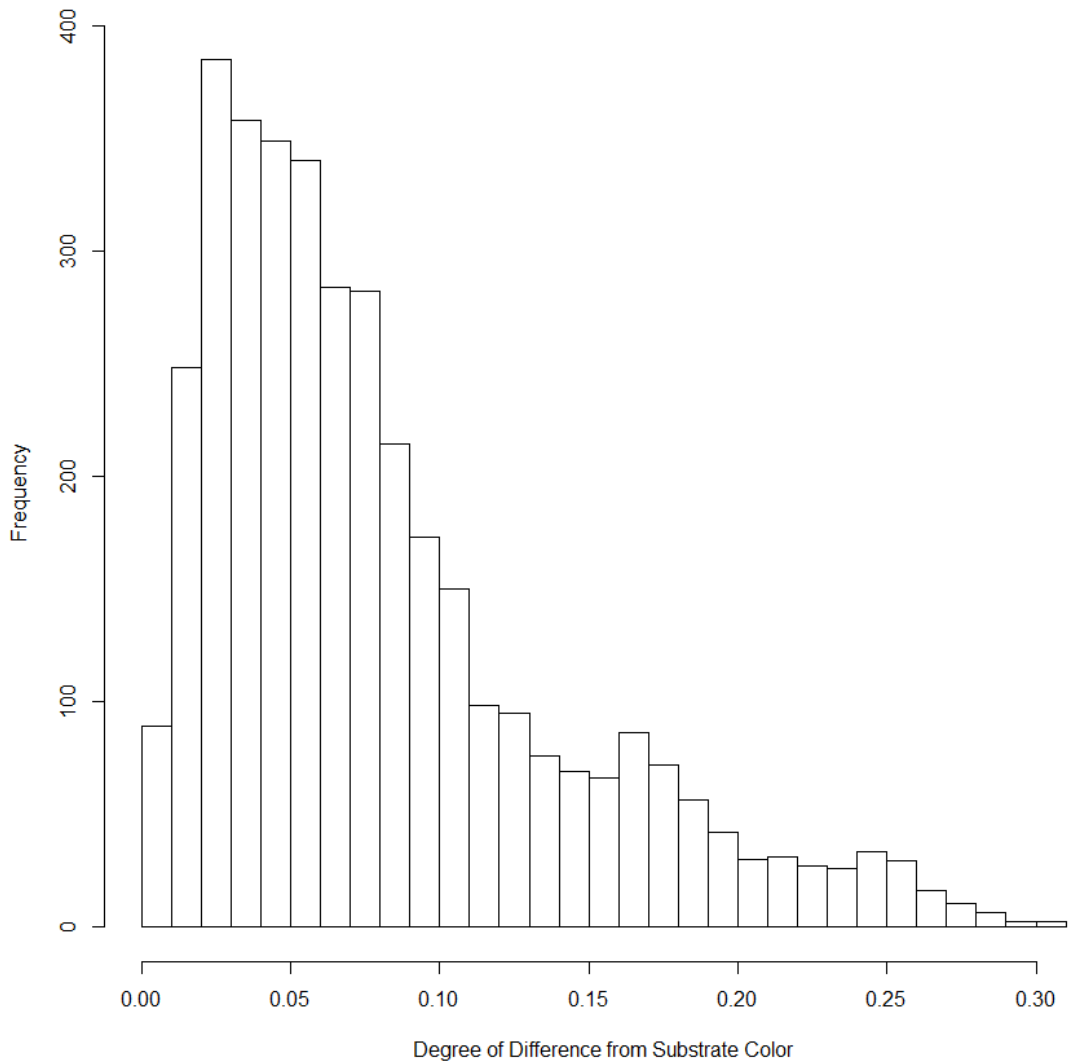


Figure 21: Substrate Color Contrast Levels (SCCLs) for snakes captured in the Frenchman Coulee (FC) den. Variation in how cryptic snakes were in comparison to substrates was high (standard deviation is ~74% of the mean: mean = 0.080 ± 0.059), where some substrate photographs were as much as 30% different in color from snakes. This implies that there is considerable heterogeneity in substrate colors, and likely no single adaptive coloration for snakes in these populations.

Appendix C – Additional Remote Sensing Information

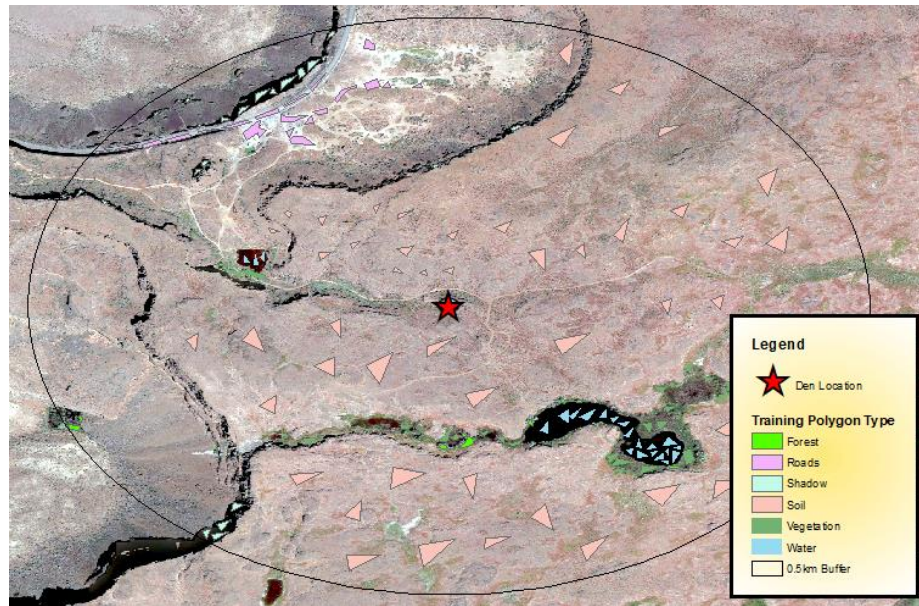


Figure 22: A Supervised Maximum Likelihood Classification scheme (SMLC), showing the training polygons used for the Frenchman Coulee (FC) den in the Ellensburg region, WA.

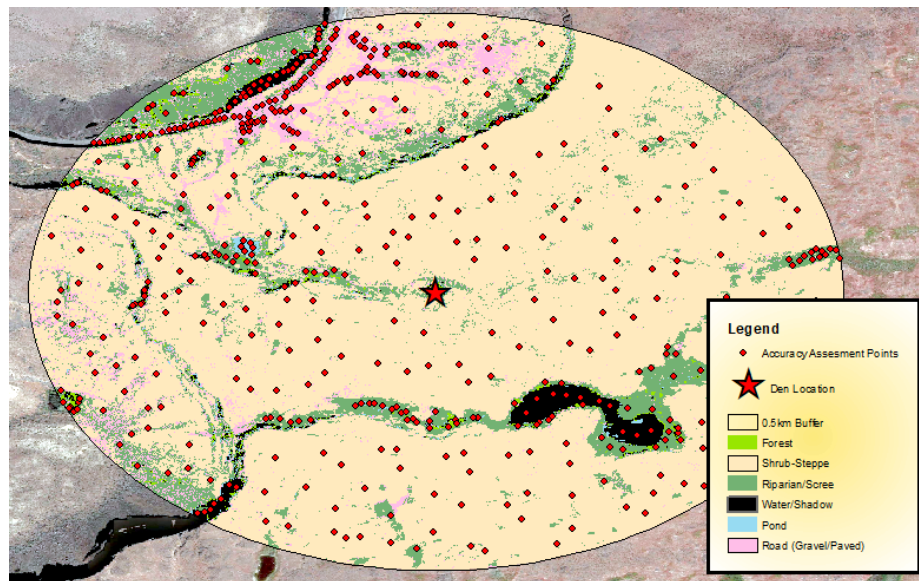


Figure 23: A land cover classification resulting from the SMLC, showing the different classes and accuracy assessment points used for the Frenchman Coulee (FC) den in the Ellensburg region, WA.

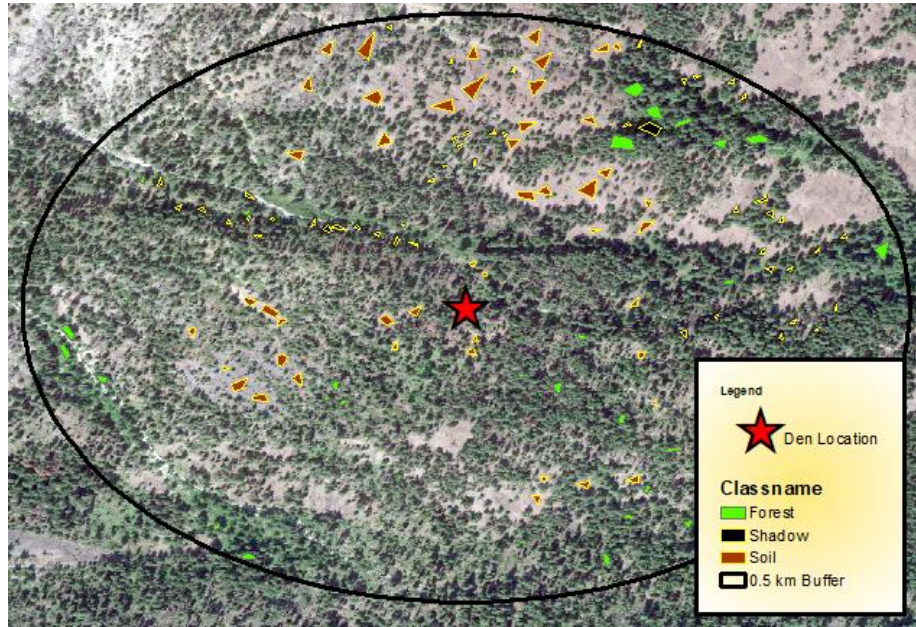


Figure 24: A Supervised Maximum Likelihood Classification scheme (SMLC), showing the training polygons used for the Lost River (LR) den in the Methow region, WA.

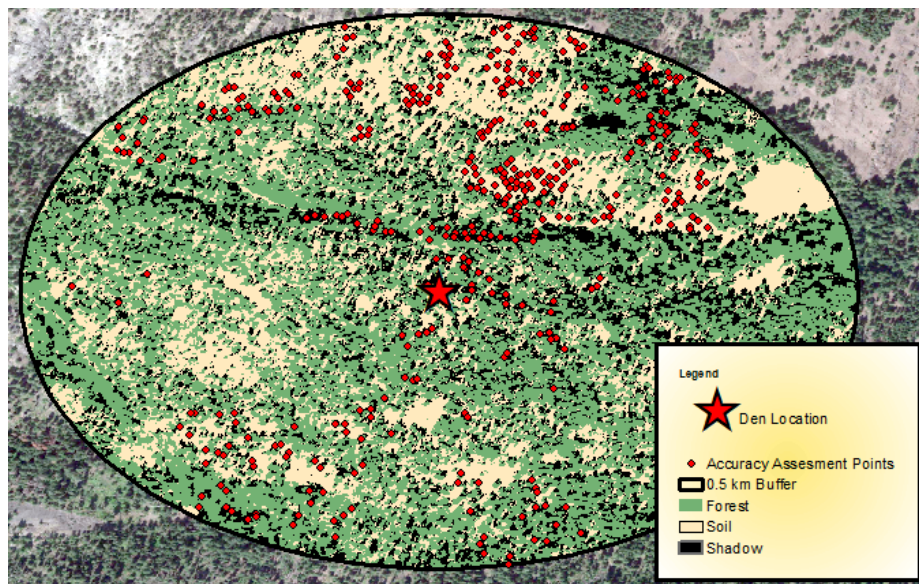


Figure 25: A land cover classification resulting from the SMLC, showing the different classes and accuracy assessment points used for the Lost River (LR) den in the Methow region, WA.

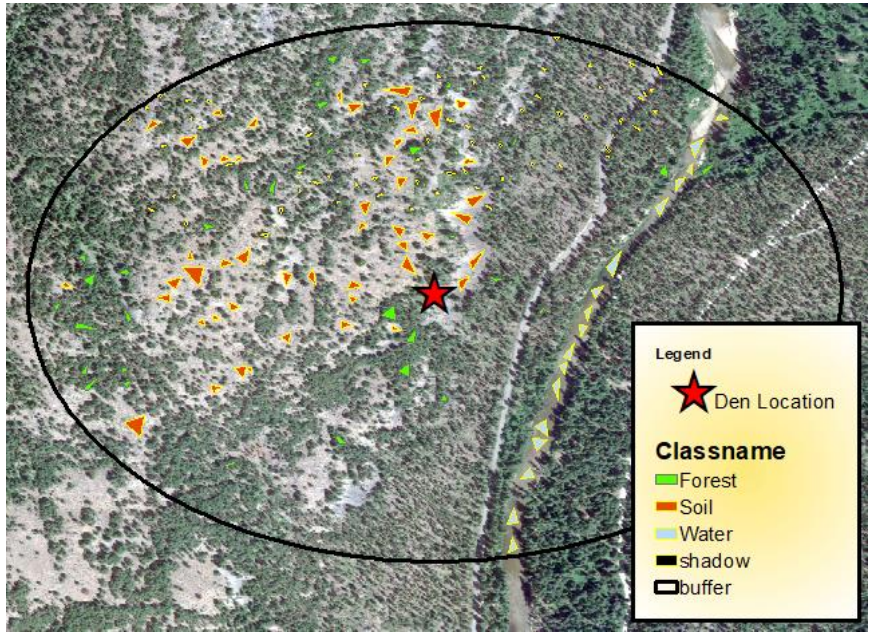


Figure 26: A Supervised Maximum Likelihood Classification scheme (SMLC), showing the training polygons used for the Falls Creek (FAL) den in the Methow region, WA.

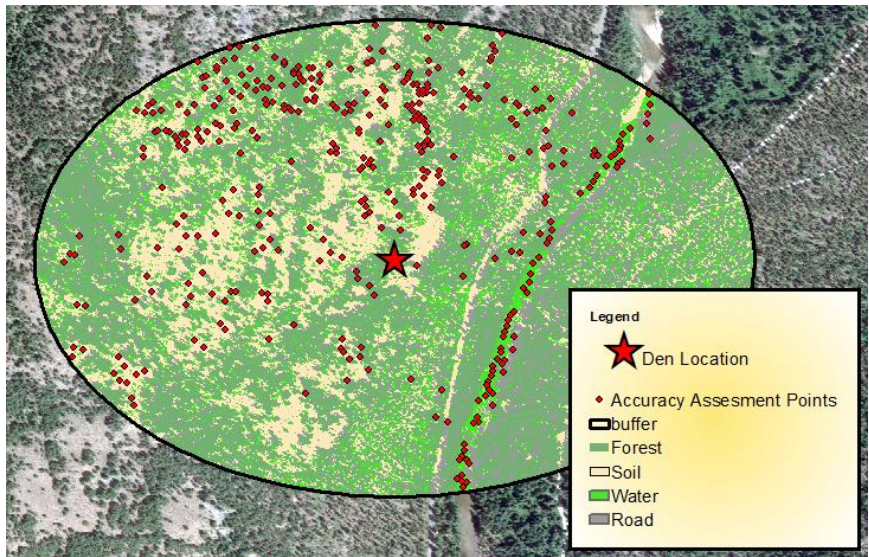


Figure 27: A land cover classification resulting from the SMLC, showing the different classes and accuracy assessment points used for the Falls Creek (FAL) den in the Methow region, WA.

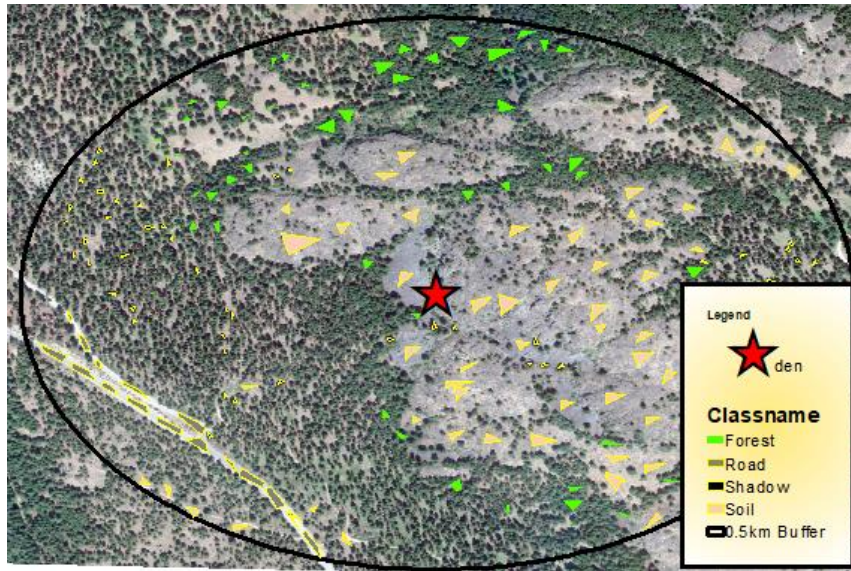


Figure 28: A Supervised Maximum Likelihood Classification scheme (SMLC), showing the training polygons used for the Matrix (MAT) den in the Methow region, WA.

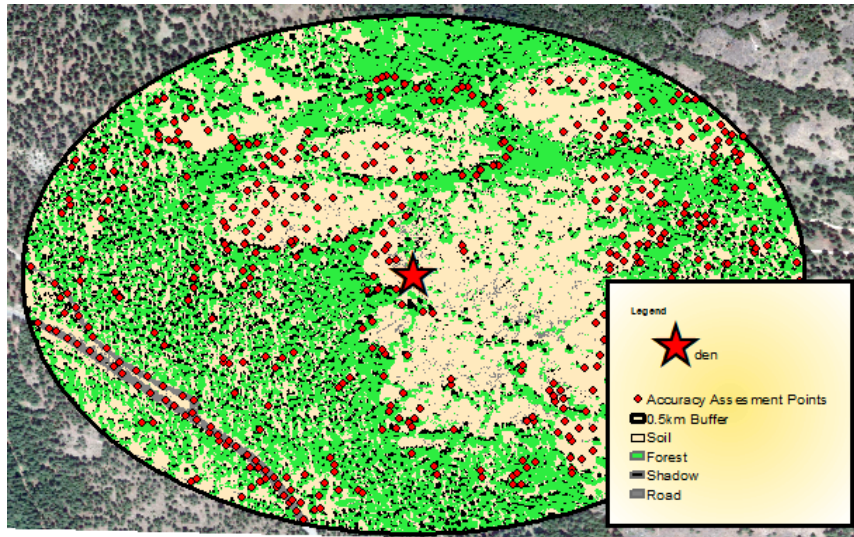


Figure 29: A land cover classification resulting from the SMLC, showing the different classes and accuracy assessment points used for the Matrix (MAT) den in the Methow region, WA.

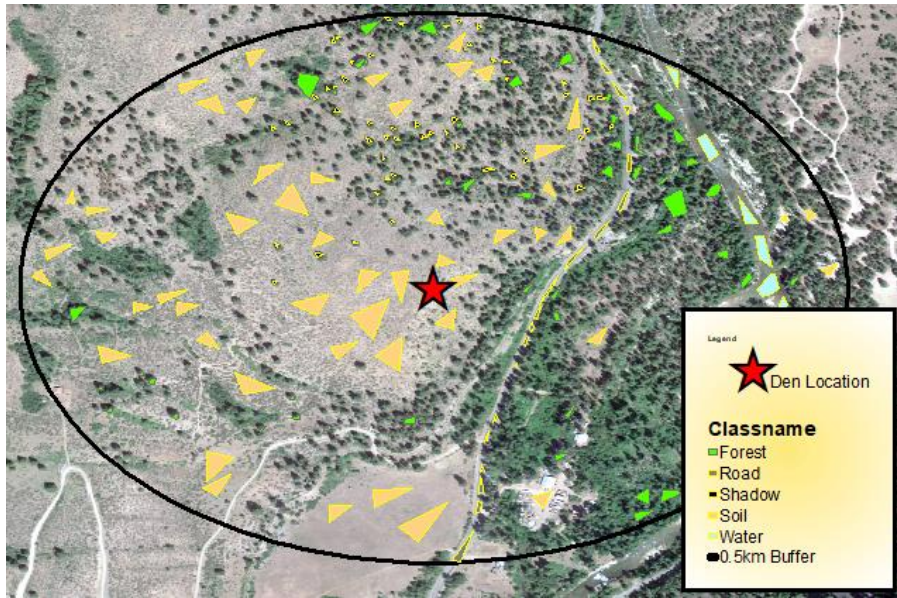


Figure 30: A Supervised Maximum Likelihood Classification scheme (SMLC), showing the training polygons used for the Uphill (UP) den in the Methow region, WA.

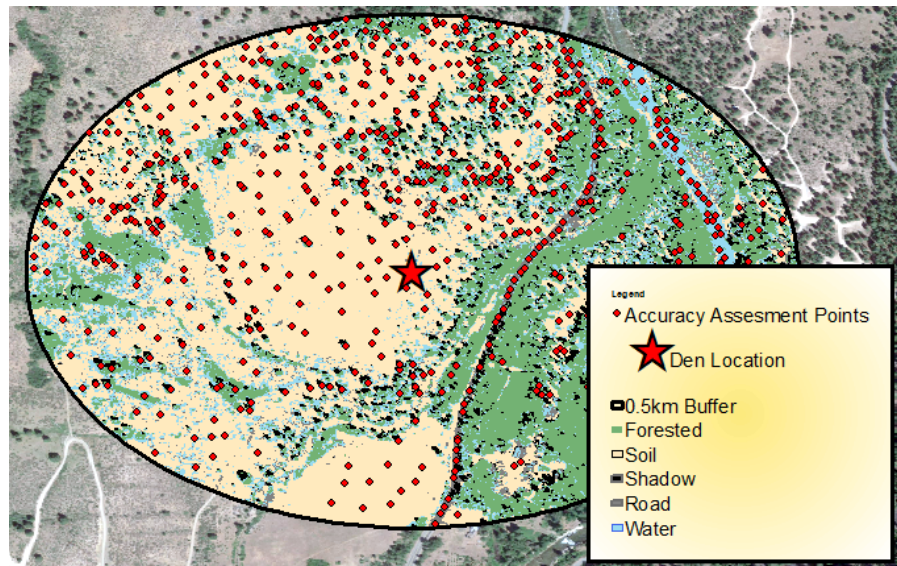


Figure 31: A land cover classification resulting from the SMLC, showing the different classes and accuracy assessment points used for the Uphill (UP) den in the Methow region, WA.

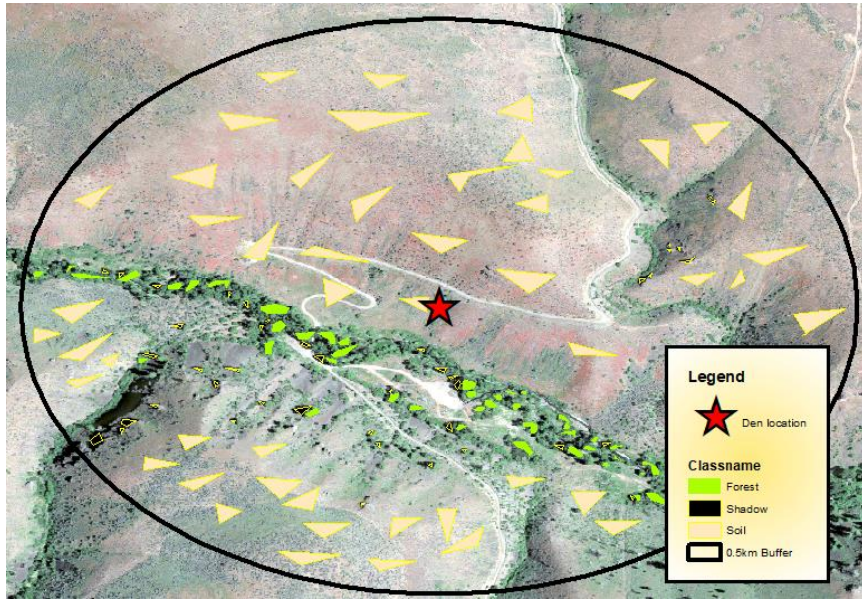


Figure 32: A Supervised Maximum Likelihood Classification scheme (SMLC), showing the training polygons used for the Upper Umptanum (UU) den in the Ellensburg region, WA.

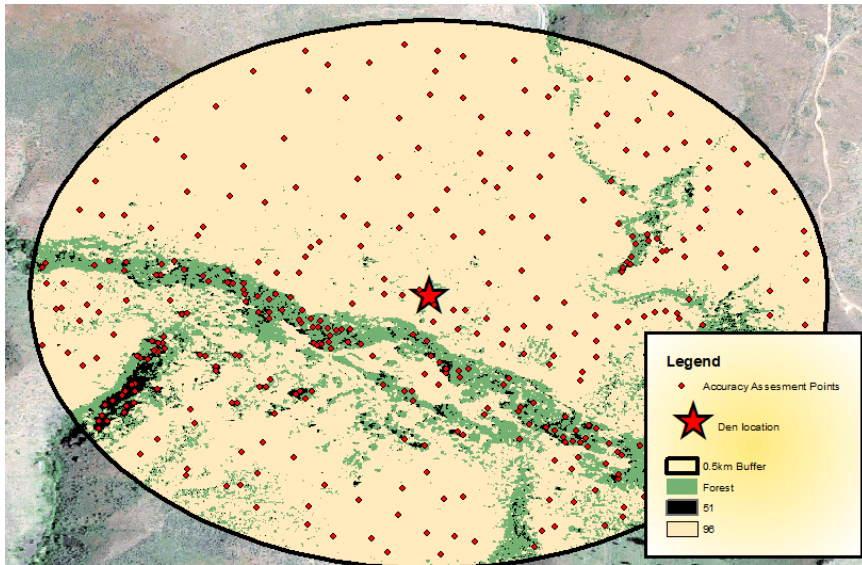


Figure 33: A land cover classification resulting from the SMLC, showing the different classes and accuracy assessment points used for the Upper Umptanum (UU) den in the Ellensburg region, WA.

Table 7: Confusion Matrix for the Bear Creek den. Actual classification of accuracy assessment points were determined by visually referencing the original satellite image. User's Accuracy represents the error of overestimation in the class. Producer's Accuracy represents the error of underestimation in the class.

	Actual Forested	Actual Soil	Actual Shade	Total	User's Accuracy (%)
Classified Forest	117	0	2	119	98.32
Classified Soil	0	97	0	97	100
Classified Shadow	0	0	100	100	100
Total	117	97	102	316	--
Producer's Accuracy (%)	100	100	98	--	--

Table 8: Confusion Matrix for the Falls Creek den. Actual classification of accuracy assessment points were determined by visually referencing the original satellite image. User's Accuracy represents the error of overestimation in the class. Producer's Accuracy represents the error of underestimation in the class.

	Actual Forest	Actual Soil	Actual Water	Actual Shadow	Total	User's Accuracy (%)
Classified Forest	113	1	1	0	115	98.26
Classified Soil	0	112	3	0	115	97.39
Classified Water	0	2	50	0	52	96.15
Classified Shadow	0	0	0	99	99	100
Total	113	115	54	99	381	--
Producer's Accuracy (%)	100	97.39	92.59	100	--	--

Table 9: Confusion Matrix for the Frenchman Coulee den. Actual classification of accuracy assessment points were determined by visually referencing the original satellite image. User's Accuracy represents the error of overestimation in the class. Producer's Accuracy represents the error of underestimation in the class.

	Actual Forest	Actual Vegetation	Actual Soil	Actual Shadow	Actual Road	Actual Water	Total	User's Accuracy (%)
Classified Forest	18	1	12	0	0	0	31	58.06
Classified Vegetation	0	192	1	0	0	0	193	99.48
Classified Soil	0	23	75	1	5	0	104	72.12
Classified Shadow	0	0	0	59	12	0	71	83.09
Classified Road	0	0	0	6	10	0	16	62.50
Classified Water	1	8	1	0	0	91	101	90.10
Total	19	224	89	66	27	91	516	--
Producer's Accuracy (%)	94.74	85.71	84.27	89.39	37.04	100	--	--

Table 10: Confusion Matrix for the Matrix den. Actual classification of accuracy assessment points were determined by visually referencing the original satellite image. User's Accuracy represents the error of overestimation in the class. Producer's Accuracy represents the error of underestimation in the class.

	Actual Shadow	Actual Forest	Actual Soil	Actual Road	Total	User's Accuracy (%)
Classified Shadow	120	2	0	0	122	98.36
Classified Forest	5	135	2	0	142	95.07
Classified Soil	0	1	138	0	139	99.28
Classified Road	0	0	0	46	46	100
Total	125	138	140	46	449	--
Producer's Accuracy (%)	96	97.83	98.57	100	--	--

Table 11: Confusion Matrix for the Uphill den. Actual classification of accuracy assessment points were determined by visually referencing the original satellite image. User's Accuracy represents the error of overestimation in the class. Producer's Accuracy represents the error of underestimation in the class.

	Actual Forest	Actual Soil	Actual Shadow	Actual Road	Actual Water	Total	User's Accuracy
Classified Forest	196	0	3	0	2	201	97.51
Classified Soil	1	135	0	0	1	137	98.54
Classified Shadow	2	0	189	0	0	191	98.95
Classified Road	0	6	0	40	1	47	85.12
Classified Water	10	8	0	5	49	72	68.06
Total	209	149	192	45	53	648	--
Producer's Accuracy (%)	93.78	90.60	98.44	88.89	92.45	--	--

Table 12: Confusion Matrix for the Upper Umptanum den. Actual classification of accuracy assessment points were determined by visually referencing the original satellite image. User's Accuracy represents the error of overestimation in the class. Producer's Accuracy represents the error of underestimation in the class.

	Actual Forest	Actual Shadow	Actual Soil	Total	User's Accuracy (%)
Classified Forest	91	2	6	99	91.91
Classified Shadow	1	67	0	68	98.53
Classified Soil	0	0	209	209	100
Total	92	69	215	376	--
Producer's Accuracy (%)	98.91	97.10	97.21	--	--

Table 13: Confusion Matrix for the Lost River den. Actual classification of accuracy assessment points were determined by visually referencing the original satellite image. User's Accuracy represents the error of overestimation in the class. Producer's Accuracy represents the error of underestimation in the class.

	Actual Forest	Actual Soil	Actual Shadow	Total	User's Accuracy (%)
Classified Forest	118	2	2	122	96.72
Classified Soil	1	167	0	168	99.40
Classified Shadow	1	0	106	107	99.06
Total	120	169	108	397	--
Producer's Accuracy (%)	98.33	98.81	98.15	--	--