EXAMINING THE RELATIONSHIP BETWEEN LANDSCAPE CONNECTIVITY AND THE BREEDING EFFORT OF THE RED-LEGGED FROG (*Rana aurora*) IN WESTERN WASHINGTON WETLANDS

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ABSTRACT

Examining the Relationship Between Landscape Connectivity and the Breeding Effort of the Red-Legged Frog (*Rana aurora)* in Western Washington Wetlands

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Amphibians provide valuable ecosystem services in many environments. However, over the last 30 years, populations of many amphibian species have been declining, largely due to habitat destruction and fragmentation. The Red-Legged Frog, *Rana aurora*, favors mature forests for the non-breeding portion of the year and utilizes forests at relatively far distances from the wetlands and ponds in which it breeds. Without careful planning and landscape stewardship, the expected levels of human development may cause significant declines of *R. aurora* in the Puget Sound lowlands. An estimate of the level of upland habitat loss and fragmentation that *R. aurora* can tolerate is an important area for research. This study contributes to the understanding of the effects of habitat fragmentation on *R. aurora*. I analyzed 14 sites, each of which included a wetland with habitat considered to be ideal for *R. aurora* breeding: physical characteristics of these wetlands included seasonal or semi-permanent hydrology and dominance by emergent vegetation or partial dominance by small shrubs. The sites varied from each other with respect to upland connectivity characteristics when land covers within 2 km of each wetland in the sample were considered. Using *R. aurora* egg mass counts in each wetland as an index for the breeding population size, I found a positive relationship between breeding effort and more extensive, well-connected habitats on all sides of the study wetland. There was a strong correlation (r^2=.79) between egg mass quantities and the size of the forest patch that was physically connected to each study wetland in each site. In addition, I observed a significant difference in the average quantity of egg masses in sites that were near a road and those that were farther away. Sites that were located within .25km of a road averaged 60 egg masses while those that were farther away from roads averaged 268 egg masses ($p<0.05$). Other connectivity factors were analyzed qualitatively; higher traffic levels on nearby roads coincided with lower population size. Easier access to secondary forest patches coincided with higher population size. Higher population numbers coincided with landscapes devoted to wilderness preservation, second growth forest preservation, and timber production while urban landscapes and those featuring mixtures of forestry, rural residential development, agriculture and highways coincided with smaller populations. Suggestions for further research include increasing the sample size and analyzing the connectivity that surrounds each wetland with a least cost analysis in GIS. Least cost analysis assigns numbers that represent energy expenditure and risk of death to various land covers in a landscape and models a species success at crossing such landscapes.

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Chapter 1: INTRODUCTION

Overview

Amphibians occupy valuable niches in aquatic and terrestrial environments but are decreasing in abundance throughout the world. According to Wells (2007), amphibians' highly permeable skin, small size, ectothermic metabolism, dependence on aquatic habitats and dependence on interconnected habitats make them particularly susceptible to a variety of human impacts. Some research has examined the impacts to many amphibian species over broad landscapes (e.g. Rubbo and Kiesecker 2005; Skids et al.2007; Egan and Paton, 2008) while other research has focused on very specific impacts to one or a few species (e.g. Chan-McLeod 2003; Schuytema and Nebeker, 1999, Deguise and Richardson 2009). This research indicates that some threats figure more prominently in the lives of each species, genus or order than other threats.

The Red Legged Frog (*Rana aurora*) formally the Northern Red Legged Frog (*Rana aurora aurora*) is a medium sized frog that favors coniferous or mixed coniferous / deciduous forests ranging from southwestern British Columbia to coastal Northern California (Jones et al 2005). *R. aurora* appears to be less tolerant of heavily urbanized areas than other endemic amphibian species, most notably the Pacific Treefrog (*Pseudacris regilla*) (Nussbaum, 1983; Richter et al. 2008). This study will examine how a group of closely related habitat connectivity characteristics relate to populations of *R. aurora*. Such as the case with many frogs, *Rana aurora* is much more adept at crossing less than ideal habitat areas than salamanders. Although it migrates similar distances from its breeding grounds as the Western Toad (*Anaxarus boreas*) it appears to be tolerating human land use changes better than this species (Adams et al. 1998). Also, *R. aurora* is persisting much better in western Washington than its closely related cousin, the Oregon Spotted Frog (*Rana pretiosa*) (Adams et al. 1999). This species was once widespread in western Washington but currently is documented in only several breeding sites in the state. Although *R. aurora* appears to be coping with human impacts better than these other native amphibians, it is important to better understand its landscape habitat requirements and to consider future impacts to this species in the face of the anticipated increases in human population and development in western Washington.

Observed Declines and Official Listings

Researchers started realizing that *R. aurora* was not present in landscapes that are heavily urbanized or devoted to agriculture in the early 1980's. Allan D. St John conducted a series of amphibian and reptile surveys throughout Oregon in the 1980s and observed that *R. aurora* were not present in urban areas or expansive areas devoted to agriculture, even if wetlands were present (St. John, 1982, 1984, 1985, 1987). In their 1983 field guide, Nussbaum and others stated that the *R. aurora* 'seem[ed] to be less common than it once was' in Oregon's Willamette Valley. This area has been heavily devoted to agriculture, is occupied by Interstate 5 and has been steadily increasing in human population for much of Oregon's history (Bury, 2008). More recent surveys have documented similar *R. aurora* declines (Blaustein and Wake, 1990; Jennings and Hayes, 1994; COSEWIC[1] 2006, 2012).

Because of observed declines, *R. aurora* has been regarded by 4 of the 6 governments that are responsible for managing it as being comparatively abundant but necessary to monitor. In California, it is considered a species of Special Concern (DFG, 2011). The Oregon Department of Fish and Wildlife places *R. aurora* in its least concern category which is 'SV' for 'sensitive vulnerable' in the Willamette Valley area (ODFW, 2011). The Washington Department of Fish and Wildlife does not grant *Rana aurora* a designation (WDFW, 2012). The

[1] COSEWIC means 'Committee on the Status of Endangered Wildlife in Canada'. This body publishes reports assessing status on many species.

British Columbia Ministry of the Environment includes *Rana aurora* on its 'Blue list'[2] (BC Ministry of the Environment, 2011). On the United States federal level, the US Fish and Wildlife Service does not list *Rana aurora* as being 'Endangered', 'Threatened', Sensitive' or 'Candidate' (USFWS, 2012). The Council on Sensitive and Endangered Wildlife in Canada (COSEWIC) has designated it as a 'Species of Special Concern' (2006, 2012).

The Ecological Significance of Amphibians and *R. aurora*

Lentic breeding amphibians are important components of aquatic and terrestrial ecosystems. Being generally high-fecundity animals, amphibians in isolated wetlands have been shown to produce 1400kg of amphibian biomass in a breeding season (Gibbons, 2006). Tadpoles significantly control algae and periphyton (Mallory and Richardson, 2005).Without tadpoles, extreme algae growth can cause eutrophication, which reduces biodiversity (Bedford et al. 2001). Tadpoles also serve as a food source for native fishes, other amphibians and certain insects (Calef, 1973; Licht 1974) (See Table 1: Rana Aurora Predators). Once metamorphs develop into frogs and leave the wetland, they transfer energy and nutrients from the aquatic habitat to the terrestrial habitat (Register et al., 2005). Adult amphibians mainly feed on detritivorous insects on the forest floor and therefore slow down rates vegetative decomposition (Davic and Welsh, 2004). Finally, amphibians are colorful and cryptic providing an aesthetic value and encouraging people to connect with the natural environment.

Because of their life history, *Rana aurora* offer these services to a specific part of the ecosystem at a specific time. *R. aurora* has one of the highest fecundities among local amphibians, with each egg mass containing between 750- 2000 eggs (Jones et al., 2005). This results in a large supply of tadpoles in the early spring which consume algae and periphyton and constitute a significant food source for predators. Since *R. aurora* migrate comparatively far

[2] A 'Blue list' species is defined as 'at risk but not extirpated, endangered or threatened'

distances from the breeding area , they bring their ecosystem services (transfer of aquatic area nutrients, food for larger animals and consumption of insects) to forests located far from aquatic areas. While *Pseudacris regilla* can also be found on the forest floor, *R. aurora* are markedly larger and therefore consume more insects and different species of insects.

Common Name	**Scientific Name**	**Red Legged Frog Life Stage that it Preys On**
Northwestern Salamander	*Ambystoma gracile*	Tadpole
Bullfrog *	*Rana catesbeiana*	Adult
Giant waterbug	*Belostomatidae spp.*	Tadpole
Laval diving beetle	*Dytiscidae spp.*	Tadpole
Dragon and damselfly larvae	*Odanata spp.*	Tadpole
Giant diving beetle	*Lethocerus americanus*	Tadpole
Cutthroat trout	*Salmo clarkia*	tadpole, adult
Rainbow trout	*Salmo gairdneri*	tadpole, adult
Bluegill*	*Lepomis macrochirus*	Tadpole
Western Gartersnake	*Thamnophis sirtalis*	adult, eggs
Belted Kingfisher	*Megaceryle alcyon*	Adult
Raccoon	*Procyon lotor*	adult, eggs
Great Blue Heron	*Ardea Herodias*	Adult

Table 1 Rana aurora Predators

Human Impacts to *Rana aurora*

Studies done in the field and lab have shown that a variety of human activities impact *R. aurora*. This is due to vulnerable amphibian physiology and its dependence on both aquatic and adjoining upland habitats. Impacts can therefore be grouped into toxics, hydrological impacts, disease, parasites, introduced species and habitat loss. Some factors may take a toll on a population over time while others such as road building and land clearing carry immediate impacts. Finally, some impacts are facilitated by others. Habitat fragmentation, for example, not only renders habitat less accessible but facilitates the spread of introduced species that compete and depredate the species in question.

Many studies on amphibian landscape impacts work with the concept of *urbanization* which encapsulates several impacts. 'Urbanization' is an imprecise term but Marzluff (2008) defines it as an increase in 'cities, suburbs and their surrounding built areas' and McDonnell and Picket (1990) define 'urban' as an area with 'high human population density coupled with increased energy use and extensive alteration of the landscape'. In this thesis I will consider urbanization to be a land development trend that includes both of these definitions. Landscapes dedicated to agriculture and timber production will *not* be considered 'urban' while landscapes devoted to other forms of commerce as well as housing and transportation *will be* considered urban. Urbanization generally results in habitat loss, habitat fragmentation, hydrological impacts to aquatic areas, the increased presence of toxics, increased noise and light pollution and the spread of alien species (Mitchell and Brown, 2008).

Loss of aquatic habitat for breeding has occurred as Washington became industrialized but this trend has been significantly slowed in the past 20 years. Lane and Taylor (1996) have estimated that by 1988, 39% of Washington State's wetland area had been eliminated. This trend slowed around 1990 with the 'No Net Loss' doctrine which enforced the sections 301 (a) and 404 of the Clean Water Act more vigorously. Although a court battle eliminated hydrologically isolated wetlands from the Clean Water Act protections, growth management regulations in Washington and California in the early 1990s have been helpful in protecting isolated wetlands (WDOE 2001; CSWRCB 2005) many of which are ideal *R. aurora* habitat. Isolated wetlands in Oregon remain less protected since state growth management regulations were essentially overturned. Nonetheless, *R. aurora* in western Washington continue to suffer from the legacy of wetland loss in many areas that were developed first in the state. These areas generally include river valleys, deltas, the eastern margin of Puget Sound and areas that were first settled and dedicated to commerce and industry. Many of these areas are now intensively urbanized or devoted to agriculture (van Stavaren et al 2006).

An increase in impermeable surfaces over the landscape has been shown to affect hydrology in ways that are adverse to *R. aurora*. Increased impermeable surface area in the surrounding landscape leads to more pronounced changes in water levels and more permanent inundation of aquatic areas (Holland et al.,1995; Thom et al., 2001;Azous and Horner, 2001; Kentula et al. 2004). Rapid decreases in water level have been shown to strand *R. aurora* and *Ambystoma gracile* (Northwest Salamander) egg masses above the water level (Klaus Richter, pers. observation). This stranding can expose egg masses to freezing or desiccation. Additionally, permanent inundation facilitates predatory fish and introduced frog (American Bullfrog, *Lithobates catesbeinus*, and Green Frog, *Lithobates clamatans*) populations (Adams 1999; Ostergaard, 2001). It also facilitates the development of shrub-dominated communities that are not as amenable to *R. aurora* breeding as wetlands that are dominated by emergent vegetation or shallow open water (Reinelt et al. 1998). For these reasons, urbanization in an area has the potential make the area's emergent wetlands less suitable even adjacent forests are also preserved.

Since amphibians have semipermeable skin and are associated with low-lying aquatic habitats that drain wide areas, they are impacted by toxic substances that are applied over the surrounding landscape. Substances originating from a host of sources have been proven to impact *R. aurora* or its close cousin the California red legged frog (*Rana draytonii*). It is important to remember that fertilizers and biocides are used for commercial and residential landscaping in addition to agriculture and timber production, thus making these substances wide-spread throughout the *R. aurora* range. Laboratory experiments have revealed that *R. aurora* embryos can be negatively affected by even small amounts of ammonium sulfate (NH_3SO_4) and ammonium nitrate (NH_3NO_3), which are common components of fertilizers (Schuytema and Nebecker 1999, 2000). They are sensitive to doses that are much lower than are commonly applied (Marco et al., 1990). Few field studies have analyzed the affects of biocides[3] but Hayes and others (2008) contend that these substances could pose a problem given their ubiquity in many parts of the *R. aurora* range. Various biocides have been implicated as factors endangering *Rana draytonii* (Davidson et al. 2001) and this could be a harbinger for *R. aurora*.

A wide variety of industrial and consumer products contain endocrine disrupting compounds which cause male frogs to develop female characteristics. These compounds are a particularly large concern because even small dosages of them can adversely affect amphibians. Bettaso and others (2002) documented the presence of a biomarker in male *R. aurora* at several northwestern California sites that indicated that they had been exposed to endocrine disrupters. This finding suggests that populations in even rural areas throughout the range are being exposed to endocrine disrupters.

Scientists have long suspected that expanding *Lithobates catesbeinus* populations have been a factor in native amphibian declines but more study is required and to date no actual evidence for this has been documented (Hayes et al. 2008). Part of this is due to the fact that it is difficult to select sites to experiment with bullfrogs since other habitat-related factors come into play (Hayes et al., 2008). By conducting field experiments, Kieseker and Blaustein (1998, 1999) have found that when both *R. aurora* and *Lithobates catesbeinus* occupy the same habitat, *R. aurora* are seemingly forced into deeper habitat that is less optimal for them. Cook and Jennings (2007) point out that *Rana draytonii* breeds about 2.5 months earlier than *Lithobates catesbeinus* so presence of large adult populations or developing larvae do not overlap. Since *R. aurora* breeds at the same time of year, these results could plausibly be extended to it. This collective research suggests that adults of these two species may compete for resources to *R. aurora's* detriment but that more research is required to determine if *Lithobates catesbeinus* is significantly impacting *R. aurora* populations.

[3] The term 'biocides' includes insecticides, fungicides and herbicides.

Fish have been shown to impact *R. aurora* populations by predation and or working in concert with bullfrogs. Kiesecker and Blaustein (1998) demonstrated that if smallmouth bass (*Microperus dolomieui*) are present in water bodies with *Lithobates catesbeinus* larvae *R. aurora* growth and survivorship was negatively affected, possibly because *R. aurora* are forced into deeper water with more fish. Trout (*Oncorehynchus* spp.) have been shown to prey on native amphibians (McGarvie-Hirner and Cox, 2007). Bluegill (*Leponis macrochirus*) encourage *Lithobates catesbeinus* survival (Adams et al., 2003). While introduced fish negatively affect native amphibians, it should be noted that fish depend on areas with permanent inundation which are only one type of aquatic area that *R. aurora* utilize for breeding. At present, introduced warm water fish appear to be a greater threat to *R. aurora* than bullfrogs and greenfrogs. This threat is more significant in areas undergoing increased urbanization since urbanization leads to more permanent hydroperiods (Holland et al, 1995; Thom et al, 2001).

Habitat and Connectivity

In their assessment of all of the threats confronting *R. aurora*, Hayes and others (2008) contend that loss and fragmentation of terrestrial habitat may be the greatest threat to the species. When they are away from breeding habitat, *R. aurora*s utilize forest landscapes almost exclusively (Haggard, 2000; Chan-McLeod, 2003; Jones et al., 2005). In addition, *R. aurora* has been shown to migrate as far as 4.8 km from breeding areas (Hayes, 2004), meaning that the species may require extensive connectivity more than other native amphibians. Some studies have found a positive relationship between *R. aurora* abundance in aquatic areas and the amount of forest cover within 1 or 2km (Richter and Azous, 2001; Ostergaard 2001; Ostergaard et al. 2008). While patches of forests may exist near aquatic areas, they are of little use to amphibians if they cannot be reached or are particularly difficult to reach by ranid frogs[4] (Fahrig 1997;

[4] 'Ranid frogs' are all frogs belonging to the genus Rana, or 'true frogs'. This is a world wide genus of frogs and conclusions about the biology and ecology of many of them can be extended to *Rana aurora*.

Stevens and Baguette, 2008). Roads are particularly treacherous barriers and their capacity to fragment habitat increases with the level of traffic on them (Gibbs 1998; Cushman 2006; Eigenbrod et al., 2007). They have also been shown to kill large numbers of migrating *R. aurora* in one study (Beasely, 2002). The concepts of 'functional connectivity' and 'landscape complementation' deal with the degree to which a given species can utilize the broader landscape, when the species' habitat requirements and the landscape's fragmentation are considered (Crooks, 2007). Mathias (2008) used GIS friction analysis of land cover maps to assess functional connectivity for *R. aurora* in King County, Washington. Although she did not incorporate field data, she assessed the landscape based on *R. aurora's* ability and the risk the species incurred to cross most land covers. She found that the more urbanized western part of the county was less connected than the central part of the county. Her research however, did not take into account actual abundance from field data.

Justification for this Study

Although *R. aurora* populations can be observed in suburban and exurban areas, the fact that it cannot be found in more intensely urban areas suggest that this species has limits as to how much human development it can tolerate. The species has been clearly decimated in the urban core of not only large cities but smaller towns. *Pseudacris regilla*, by contrast, is frequently observed in such areas. *R. aurora* is closely associated with forest habitats but the expansive forests that have covered the species' habitat for most of its history are no longer extant. *R. aurora* is now living in landscapes that are covered with a patchwork of forest (of varying age classes), pastures, clear cuts, residential development, business districts and roads. Forest patches vary with respect to size, connectivity to breeding habitat and connectivity to other forest patches.

Despite its relatively high ability to cross sub-optimal habitat and utilize forests patches across the landscape, local scientists have expressed concern that *R. aurora* will decline as western Washington increases in population over the upcoming decades (Shuett-Hames et al, 2007; Hayes et al. 2008). Hayes and others (2008) have expressed a need to examine the importance of habitat connectivity for *R. aurora*. Using breeding effort as an index of population size, this research analyzes how the size of the forest patch adjacent to the breeding area and the proximity of busy roads to the breeding area affect *R. aurora* breeding effort. Egg mass censuses were taken on thirty wetlands but many of these were thrown out of the study because it was believed that other factors besides surrounding connectivity were affecting populations. This resulted in a sample size of 14 selected wetlands that reflected a range of connectivity to forest within 2 kilometers. The study was guided by 4 research questions. From these questions I have developed a series of alternate hypothesis (Table 2).

Research Questions

1). How does the size of the immediate forest patch affect breeding effort?

2). How does the presence of roads within .25 km of the wetland affect breeding effort?

3). How does the level of traffic on nearby roads affect breeding effort?

4). How does connectivity between the immediate patch and the neighboring patches affect breeding effort?

Alternate Hypotheses
Breeding effort will be positively related to the size of the immediate patch because larger patches represent a larger degree of continuous ideal habitat.
Breeding effort will be negatively related to the presence of roads within .25 km of the wetland edge.
Breeding effort will be inversely related to traffic intensity on neighboring roads.
Breeding effort will be positively related to the ease at which frogs can travel between the immediate patch and neighboring forest patches.

Table 2 Alternate Hypothesis

Chapter 2 LITERATURE REVIEW

In order to learn about the accrued scientific knowledge on *R. aurora* life history, ecology and the threats relating to the species, I conducted searches for peer-reviewed journal articles on data bases specializing in ecology, zoology and biology. I used key words such as ‘red legged frog’, ‘Rana aurora’, ‘amphibians, ‘habitat connectivity’, ‘urbanization’ and ‘dispersal’ and ‘migration’ to select articles that had these topics in their abstracts. I reviewed recent books on amphibians. I surveyed thesis work that had been done at universities in the *R. aurora* range. I surveyed unpublished US Forest Service research on *R. aurora* and amphibians. Finally, I surveyed the bibliographies of some of these written works to gather other relevant sources.

The Red Legged Frog: Summary of Biology and Ecology

R. aurora is a medium-sized (50-100 SVL[5]), lentic (still water) breeding frog. Most individuals have a dark patch around an eye with brown irises and a red groin patch. The back can be tan, brown or reddish-brown and spots are usually present (Fig. 1)(Jones et al., 2005). The *R. aurora* range extends from Mendocino County, California in the south northward through all of Vancouver Island to the Margaret Bay area of British Columbia, Canada. In California, the range is close to the coast but in Oregon and Washington it extends to mid elevations (up to 365 m or 1200 ft) of the Cascades. In mainland British Columbia it extends inland from the Straights of Georgia roughly 200 miles in the south to about 100 miles in the Margaret Bay area. The species is absent from the higher elevations of the Olympic Mountains (Jones et al., 2005; Pearl, 2005). Because of urbanization, it is absent from heavily urbanized areas on the east shore of the Puget Sound, the Portland, Oregon metropolitan area and the metropolitan areas in southwest British Columbia (St John 1982, 1984, 1985, 1987; Nussbaum et al. 1983; Jennings and Hayes 1994; COSIWIC 2011).

[5] SVL means ‘snout vent length’. It is the length from the animals snout to its vent (anus).

Figure 1: Red legged frog (*Rana aurora*) identification.

The coloring is variable throughout the range. Reddish markings on the ventral side of the legs are usually diagnostic. LEFT: specimen from Multnomah Co. Oregon, CENTER: Humboldt Co. California, RIGHT: ventral view of adult. All photos by Gary Nafis, californiaherps.org.

Breeding and Larval Development

Rana aurora generally gather en masse at the same aquatic area to breed every winter, commonly in February (Licht 1969). Small wetlands with semi-permanent inundation and ample amounts of emergent or aquatic-bed vegetation constitute ideal breeding habitat (Pearl et al., 2005) but *Rana aurora* also breed in lakes and slow-flowing water (under 5cm/second) (Klaus Richter, personal obs.). Prolific breeding does not occur in forested or shrub dominated wetlands, most likely due to lack of sunlight and nutrients (Shelley 2002; Shelley and Golon 2003). Figures 2 and 3 show a wetland that typifies ideal breeding habitat.

Figure 2 A breeding wetland in mid March,

2011

Figure 3 The same breeding wetland in late June, 2011.

Photos above are the same view of Site 11. Figure 2 shows the area inundated to 60 cm and Figure 3 shows the same area inundated to 38cm several months later. This is ideal R. Aurora breeding habitat; it is interspersed with common cattail (*Typha latifolia*) in the foreground and background, slough sedge (*Carex obnupta*) in the foreground, and Douglas spiraea (*Spiraea douglasii*) which is in the background and reddish-colored in the spring. The National Wetland Inventory classifies this as a palustrine emergent wetland with seasonal inundation (PEMC). 278 egg masses were observed here. Photo by Chris Holcomb

Male frogs arrive at breeding areas first. Populations at lower elevations and at lower latitudes tend to breed earlier, probably due to temperature. Storm (1960) observed that frogs in the Corvalis, Oregon area arrived at breeding sites on December 8 while Licht (1969) observed that frogs in British Columbia did not arrive at breeding sites until February or March when air temperatures reached 10°C. In conducting fieldwork for this thesis, I observed that oviposition had started earlier at sites in west Pierce County, Washington than at higher elevation sites in the central part of the county. Once couples form, the male and female undergo amplexus and the female then deposits a globular mass of 530-830 eggs. The capsules around each egg quickly absorb water causing the mass to have a jelly-like consistency and to grow to the size of a large cantelope (Figure 4). Egg masses are attached to aquatic vegetation, usually in water that is 48 to 70 cm deep (Storm, 1960; Licht, 1969; Calef 1973). I observed that masses are generally in the upper 36 cm of the water column. Breeding activity is often concentrated in the northern part of t he aquatic area, probably because this area has the most sunlight exposure.

Figure 4 Rana Aurora egg mass

Embryos develop over the course of 10-30 days. As the embryonic stage progresses, the egg mass becomes less spherical and becomes laced with algae and sediment (Figure 4, photo on right). Once the tadpoles hatch they tend to stay on or near the egg mass for a short time. Tadpoles reach metamorphosis 11-14 weeks after hatching. Juvenile frogs tend to stay in the wetland anywhere between 2 weeks and 2 months after they reach metamorphosis (Storm 1960; Licht 1974; Brown 1975).

The Importance of Upland Habitat

Adult *R. aurora* usually leave the breeding area in spring and spend a solitary life in uplands. They have been shown to select forested areas when leaving breeding ponds (Rothermel, 2004) and are found in greater abundance in forests (Haggard 2000; Aubry 2000; Chan-McCleod 2003). However, they are capable of crossing more open habitats like clear cuts (Chan-Mcleod 2003; Chan-McCleod and Moy, 2006) and roads (Beasely, 2002). They have also been seen in low-density residential areas (Holcomb, personal obs.) if such areas are small enough and include such features as wetlands, ditches, and forest patches. Studies in different parts of the range suggest a variety of seasonal travel distances. Hayes (2007) has demonstrated that *R. aurora* can travel up to 4.8 km from the breeding wetland in the central Oregon Cascades, which is comparatively far for many local amphibians. Haggard (2000) found that frogs only moved 80 m at her study site on the northern California coast. Most researchers feel that between 2 and 3 kilometers is an average one-way migration distance for *R. aurora* (Mathias, 2008). Semlitsch (2008) has stated particularly far movements (such as Hayes's 4.8 km observation) likely represent extreme distances that are undertaken by very few individuals. Semlitsch (2008) has stated that for all lentic-breeding amphibians, dispersal and migration are different processes that are done at different times of the life history. He defines migration as seasonal movements generally by *adults* from breeding areas into adjacent upland which are followed by returns to the same aquatic area to breed. Dispersal is often undertaken by juvenile frogs and constitutes movements from their breeding area over the upland to new breeding areas (Rothermel, 2004). These dispersal movements may be done over the course of two or three years until the animal is sexually mature. Since radio telemetry techniques can only be used on adult frogs, there is sparse information on *R. aurora* juvenile movements in uplands, but they are presumed to serve a large role in dispersal, as Rothermel (2004) has described.

Due to the expense and challenges of radio telemetry work on small animals, movement data is sparse. However, radio telemetry research has been undertaken in a wide range of habitats and places within the *R.aurora* range and it is probable that average dispersing and migrating distances vary with habitat and location. Haggard (2001) analyzed movement from breeding ponds on the northern California coast. Hayes et al (2001, 2007) analyzed the Umpqua Basin of Oregon. Serra-Shean (2001) analyzed movement out of a large wetland in western Washington. Semlitsch (2008) has theorized that ranid frogs migrate by making sustained trips, triggered by nocturnal rainfall, before they stop in an area and remain comparatively sedentary for long periods. Several bodies of research suggest that frogs move under 10m a day at times (Haggard 2000; Ritson and Hayes 2000; Schuett-Hames 2004). These shorter movements may take place after periods of far sustained movement when frogs have found a good place to forage. Schuett-Hames (2004) employed video recording to document frog behavior and determined that adults spend long periods of time under complex understory feeding. Such behavior likely enables them to stay concealed from predators, conserve energy and water and build up energy reserves for later travel. Shuett-Hames's observations possibly describe Semlitsch's idea of frogs remaining relatively stationary for periods lasting months after periods of sustained travel. How *R. aurora* overwinter is one of the least understood aspects of their life history (Hayes et al. 2008). Post metamorphic individuals have been observed to spend the winter in breeding ponds (Ritson and Hayes 2000) but it is believed that the majority of adults overwinter in uplands.

The collective research over the past 35 years has given us a moderately-clear picture of the types of forest habitats and features favorable to *R. aurora*. There is little information on amphibian use of the extensive old-growth forests that predated American influence (Mathias, 2008) but there has been some research on old growth patches that currently exist (eg. Gilbert and Allawine, 1991). *R. aurora* favor mature forests that have at least some understory and are either dominated by conifers, deciduous trees or are mixed (Aubry, 2000; Haggard 2000; Schuett-Hames, 2004; Gomez and Anthony, 1996).

The US Forest Service examined Pacific Northwest native forest amphibian communities in the 1980s over three different study areas of western Washington and Oregon. This research by Gillbert and Allawine, 1991, Aubry and Hall, 1991, Bury et al 1991 stated the importance of large woody debris, recognizing unique and botanically diverse microhabitats, and the proximity of aquatic areas. This research however, did not consider clear cuts or aspects related to connectivity; it only studied habitat aspects in unmanaged Douglas fir forests.

Keith Aubry conducted research on amphibian presence in managed forests in the early 1990's and considered additional aspects of forest structure. The study took place on private forest lands southeast of Eatonville, Washington in Central Pierce County, near the edge of the *R. aurora* range. Unlike the aforementioned USFS work, this research included an analysis of amphibian use of clear cuts. Aubry also analyzed second growth forests that were dominated by Douglas fir but included other conifers and broadleaf trees. *R. aurora* were most abundant in the oldest age class in which they comprised 5.3% of all amphibian captures. Far fewer *R. aurora* were captured in the clear cut plots and the pre canopy plots and none were caught in the closed canopy plots. In addition, the Aubry study concluded that *R. aurora* abundance was positively associated with leaf litter depth and the abundance of shrubbery and negatively associated with elevation and cover of exposed rock. The elevation relationship is not surprising given that *R. aurora* do not inhabit areas above 1200 m (Pearl 2005) and parts of the study area were close to that elevation.

Martin and McComb (2003) analyzed amphibian associations in second growth patchwork landscapes that typify commercial logging areas in Oregon's Coast Range. The study area was influenced by an expansive wildfire in the mid 1800's but had been used for commercial timber production for 40 years prior to the study. The forests in the study were dominated by Douglas fir but also included Sitka spruce (*Picea sitchensis*), western hemlock (*Tsuga heterophyla*), red alder (*Alnus rubra*) and big leaf maple (*Acer macrophyllum*). They delimited 13 forest types based on tree age, level of tree type dominance (deciduous or conifer) and amount of canopy closure and concluded that *R. aurora* prefer '*mixed, large sawtimber*'. This forest type is defined as being: '*<70% conifer or hardwood composition, > 20% cover, > 53.3 cm dbh*'[6] (Martin and McComb 2003). Gomez and Anthony (1996) conducted a similar study but with only 5 forest types in Oregon and concluded that *R. aurora* were more abundant in deciduous

[6] 'dbh' means diameter at breast height

forests. This is probably due to the fact that red alder forests produce substrates with more nutrient levels and hence more invertebrate prey species for amphibians (Shirley, 2004).

The most recent research recognizes how far *R.aurora* travel and the realities of the silviculture landscape. This leads to the question, under what conditions will *R. aurora* cross clear cuts and can small residual forest patches facilitate migrations? Chan-McCleod has researched *R. aurora* movement in fragmented forest landscapes in British Columbia and came to similar conclusions that Schuett-Hames (2004) did: frogs do travel through open habitat but seem to prefer forests. She concluded that clear cuts under 12 years old pose significant barriers to *R.aurora* movement but those frogs will be more likely to enter and move through them under certain conditions. The study was undertaken from August through October when both rain and high temperatures are extant. Compared with forest habitats, frogs permeated clear cuts at a rate of 16.7% when rain was absent but temperatures and humidity measurements were at their average level during the trial period. However, under the maximum observed noon temperature, the rate of entry into clear cuts dropped to 2.3%. Additionally, streams 3 m wide seemed to encourage entry into clear cuts while streams under 1.5 meters did not significantly affect this (Chan-McCleod, 2003). In another study that evaluated *R. aurora* use of residual tree patches left in clear cuts, Chan-McCleod and Moy (2006) determined that: 1) when travelling through clear cuts, frogs intercepted patches largely by chance and were not likely to gravitate toward such patches unless they were 5-20 meters from them and 2) frogs tended to select patches that were over .8 ha in area.

To conclude, in the second growth forest landscape, *R. aurora* appear to select mature forests that are either dominated by deciduous trees or are a mixture of deciduous trees and conifers with complex understory. They will move through clear cuts but this behavior is facilitated by rainfall and cooler temperatures and is more often undertaken by larger individuals that can more easily withstand environmental pressures. *R. aurora* will also utilize small patches of forest but do not appear to seek out such areas unless they are within 20 meter of them and they are larger- over .8 ha in area. Given their physiological constraints, clear cuts and open areas pose significant challenges to *R. aurora* and highlight problems with habitat fragmentation.

Connectivity Definition and Overview

'Connectivity'pertains to the geographic size of habitats and the magnitude and nature to which they are linked to other habitats (Groom, 2008). Sanjayan (2007) states that connectivity is related to the degree of movement of organisms and processes. Talley and others (2007) also provide a broad definition, stating that connectivity is not just about animals going across the landscape spreading genes, it relates to material and energy moving across landscapes. Adriaensen and others (2003) state that the inverse of habitat connectivity is 'landscape resistance' or 'isolation'. 'Fragmentation' is the process of separating contiguous expanses of habitats into disparate parts. Fragmentation makes it more difficult for organisms to utilize the entire habitat that was originally available to them. Fahrig (2003) emphasizes that the concepts of *fragmentation* and *habitat loss* should be separated. She states that while fragmentation in and of itself results of loss in habitat, it also renders existing habitat blocks less accessible to organisms and thus has unique effects on the species in question.

While some organisms may persist in these patches of habitat soon after they are fragmented, they may suffer ill effects over time. Some reasons for this are that other pre-fragmentation components of the ecosystem may disappear while native organisms that are better adapted for the new landscape may proliferate. Invasive species may enter the system. If native organisms are not as adept at leaving the patch, they may suffer the effects of a limited gene pool.

With respect to lentic breeding amphibians, two types of connectivity are important; the first type of connectivity is landscape complementation, or the arrangement of two important but different habitat types, specifically breeding habitat and upland habitat (Dunning et al., 1992).

This link is important because both habitat types are essential to their life cycle and animals transfer materials between the two habitats and (Talley et al. 2006; Kupferberg, 1997; Anderson et al, 1991). If the landscape is too fragmented, this transfer cannot take place. The second type of connectivity relates to connections with other breeding areas and amounts of contiguous upland habitat for wide ranging animals to utilize. This thesis generally deals with this second type of connectivity. The almost universal assumption underlying discussions of connectivity is that habitats were well connected prior to the very recent influences related to human agriculture, industrialization and urbanization.

The Importance of Habitat Connectivity to Amphibians

Connectivity, including links from the aquatic area to key upland habitats as well as linkages between such habitats enables amphibians to utilize upland habitat. This enables them to take advantage of food and cover resources that uplands have an abundance of. In turn connectivity for amphibians makes it possible for upland habitats and human communities to be shaped by amphibian ecosystem services. Amphibians are a significant consumer of forest floor invertebrates as well as being a significant food source for larger carnivores (Wells, 2005).

Amphibian populations in individual aquatic areas periodically crash and are dependent on being ‘rescued’ by colonization from the broader ‘metapopulation’. Such crashes occur due to insufficient reproduction and immigration, habitat succession, the proliferation of a predator and long term drought. Metapopulations are comprised of many separate populations, each breeding in its own pond year after year, but that are each close enough to be contacted by individuals from other populations (Marsh and Trenham, 2001). Metapopulations are identified by genetic analysis (Marc Hayes, personal communication). Metapopulation theory originated from the assumption that all lentic breeding amphibian populations were highly philopatric and did not disperse far. Smith and Green (2005) questioned this, noting that many species are less philopatric than previously thought, can travel further than previously thought and that females may be more selective in choosing breeding grounds.

Aquatic areas need to be close enough to be occasionally reached by dispersing juveniles to be part of metapopulations.   In addition, sufficient levels of habitat connectivity need to be in place to make colonization possible and for this reason researchers concerned with amphibian conservation have devoted time to metapopulation studies in recent years (Trenham and Shaffer 2005, Trenham et al. 2003, Trenham, 1998, Skelly and Meir 1997, Driscoll 1997). Metapopulation studies on amphibians consistently suggest that the more common and well-distributed breeding wetlands are throughout the landscape, the higher the probability that turnovers can be prevented. Constant colonization and population can be restored in the event of a population die off in any given wetland (Trenham et al. 2003).

Whether or not *R. aurora* have a metapopulation structure is unclear (Hayes et al., 2008). If breeding areas are within 500 m of each other, they may have a more patchy population structure as described by Petranka and Hollbrook (2006). In addition, Hayes and others (2008) suggest that the species may be able to survive population crashes because it has relatively high fecundity and is long lived (8 to 12 years). This means that populations at breeding sites could eventually make up for bad reproductive years. However, Hayes and others also speculate that *R. aurora* populations may function as metapopulations due to their far migration tendencies. Whether or not *R. aurora* have a metapopulation structure, more of a patchy population structure in certain areas, or can overcome occasional population crashes due to lifespan and fecundity is unknown. Nonetheless, in management efforts it is probably wise to consider how well members of one population can contact those of another population.

In contrast with more sedentary lungless salamander species, ranid frogs tend to be more susceptible to negative genetic effects if different populations cannot occasionally exchange genetic material (Wells, 2005). In addressing genetic issues for all animals, Frankam (2006) listed seven factors that determine the susceptibility that a population of one species may have to negative genetic effects. These include 1) the number of population fragments[7], 2) the geographic distribution of the population fragments, 3) the dispersal ability of the species, 4) migration rates between fragments, 5) degree of connectivity between fragments, 6) the time (in generations of the species) that the fragmentation took place and 6) the susceptibility of the species to inbreeding depression. Many of these points are relevant to ranid frogs since they are small, slow moving animals with narrow habitat requirements.

No research has investigated how *R.aurora* genetics has been affected by human caused habitat fragmentation but the species is persisting in many areas that have had low surrounding functional connectivity for decades. This suggests that a species' ability to cross adverse land covers does not particularly give it an advantage at exchanging genes with other populations. Studies on other ranid frog species suggest that human disturbance may already be affecting these isolated *R. aurora* populations. Reh and Seitz (1990) and Hitchings and Beebee (1997) found significant differences in genetic differentiation with increasing pond distance, suggesting that isolation will not only prevent a rescue of a crashed population but eventual inbreeding depression. The Reh and Seitz study as well as 2 other studies in Europe on ranid frog species found that roads, railways and urbanization caused increased genetic difference or distinct genetic groups (Vos et al. 2001; Sefner et al. 2011). Metapopulation studies at sites with low human fragmentation and habitat destruction between other had little genetic difference between sites (eg Gill 1978; Berven 1995; Trenham 1998; Seppa and Laurila 1999; Skelly et al. 1999). These results suggest roads, railways and cities can result in greater genetic difference among their populations of *Rana aurora* and possibly cause inbreeding depression within these populations.

[7] In the case of lentic breeding amphibians, a 'population fragment' would mean an aquatic area with a population of a species. Large aquatic areas with separated habitat areas may have a distinct population fragment in each habitat area.

Assessment of Connectivity: Formal Approaches

In many amphibian environments, including *R. aurora's* Puget Sound basin, vast contiguous forest habitat areas are no longer in existence and animals are often left to travel between patches of suitable habitat via territory that is less amenable to amphibian's survival. Originally, connectivity was assessed based on the size, shape and arrangement of patches of ideal habitat. Since ecologists had some idea of the distances that different species traveled every year, studies evaluated the size and distances of patches of ideal habitat in the landscape that fell under a given distance from breading ponds (e.g. 1km, 2km, 3km).

While large expanses of ideal habitat were naturally deemed the most optimal levels of connectivity, the size of patches and their arrangement were also considered. Generally this was done by incorporating circular buffer functions in GIS programs with the breeding pond at the center of the circle. Additionally 'Nearest neighbor' functions in GIS programs considered the distance of patches of ideal habitat from each other. Prugh (2009) determined that nearest neighbor patches were particularly poor predictors of abundance and occupancy by a target species and that buffer functions were not much better. This is due to the fact that land covers between patches were not considered. In recent years, connectivity studies have been divided into two categories. *Physical* or *structural connectivity* considers and arrangement of habitat and all other land cover types. Functional connectivity considers how a species behaves in all land covers in addition to its ideal habitat.

Assessment of Connectivity: More Recent Approaches

Recently more connectivity studies have adapted new approaches that address other land covers in the species migration and dispersal zone in addition to ideal habitat. *Functional connectivity* is the degree to which a landscape can be crossed by an individual of a given species and is based on behavior. It is largely based on the behavior that animals exhibit on different land covers (Stevens et al, 2006, With et al. 1999, Goodwin and Fahrig, 2002) and is comprised of 2 components. *Patch resistance* is the level of difficulty that an area poses for a given species to cross. *Boundary permeability* is the degree to which one habitat type can be crossed by a given species (Stamps et al. 1987, Wiens et al. 1997). The functional connectivity approach has a distinct advantage over earlier approaches to assessing connectivity in the sense that it more accurately embraces the realities of human-impacted landscapes. Even frogs -- small, slow-moving ectotherms-- will cross adverse landscapes to utilize more ideal habitat types. Figure 4 illustrates an example. If a relatively far-dispersing animal like *R. aurora* wants to reach other patches of forest beyond the one that surrounds its breeding pond, a road or heavily urbanized area separating the 'initial patch' from, say, 'patch A' will be more consequential than rural-residential land with lightly traveled roads, even if Patch B on the other side of such land is farther than Patch A.

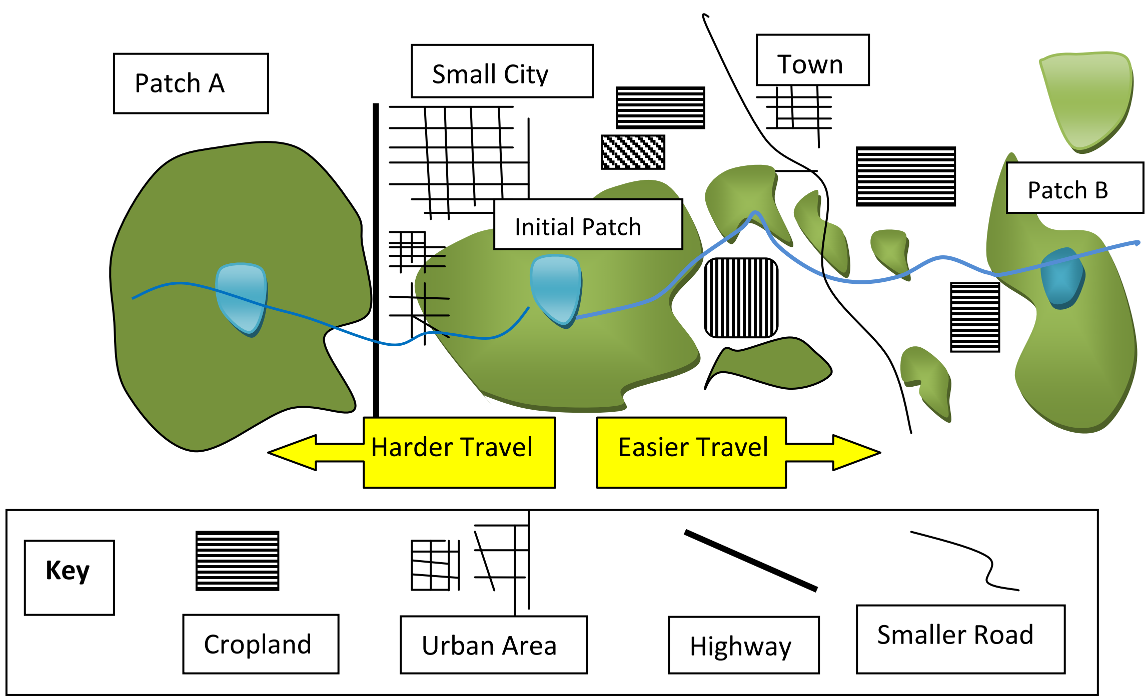

Figure 5 Functional connectivity diagram

Functional connectivity of a landscape for a given species is assessed by creating 'permeability models' using GIS applications. Ray and others (2002) employed this method to assess habitat connectivity for two lentic breeding amphibians in Switzerland. This research comprehensively describes the process, has served as a model for subsequent work and is the source for information in this paragraph. ESRI's software (eg. ArcMap, ArcView, ArcInfo) is the most commonly used GIS tool. In this software, aerial photographs are used to construct a grid-based landscape layer which codes different land covers. Grids are constructed in GIS using the raster format which characterizes landscapes in square-shaped cells instead of lines and polygons. The resolution (cell size) of raster-based values' are then assigned to each cell based on land cover type. The friction values indicate both the *risk of mortality* and *energy expenditure* that a given species incurs for crossing a particular land cover and are based on previous studies of the species and similar species as well as professional judgment. For forest amphibians, such as *R. aurora*, more open artificial habitats lead to water loss or changes in optimal body temperature and may involve more dangers such as maps can be increased or decreased depending on objectives. Numbers or 'friction cars or a higher chance of predation due to lower cover. Within these areas, animals may move more quickly in order to reach a more amenable land cover. Frogs can often be observed quickly crossing roads by hopping. These physiological effects and behavioral responses to increased threats in areas devoid of forest cover translate into higher energy expenditures. Once land cover and friction layers are created, the 'cost distance function' (in ArcMap) is employed to calculate the 'maximum cost of migration' (MCM) for crossing each cell in the model. This is done by multiplying the friction value of a land cover by the 'maximum distance of migration' (MDM). The MDM is considered to be the distance in meters that the species generally travels in ideal circumstances without habitat fragmentation and thus characterizes the species inherent migration or dispersal tendencies. Here is a summary for determining the MCM:

$$MCM = MDM * (\text{friction value of land cover type})$$

For studies of lentic-breeding amphibians, the cost distance function can be centered around each breeding pond in the landscape and set to calculate the MCM of a species moving away from the breeding pond in all directions, thus simulating dispersal or seasonal migration. As a virtual animal moves over cells within this GIS model, the friction value assigned to each cell is subtracted from the animals MCM. The migration ends when the virtual animal has lost all its energy equal to its MCM. The collective MCM for each pond is then averaged: higher percentages of ideal habitat connected to the pond would therefore result in greater average MCM for a species leaving the pond. Ray and others then used 'generalized additive models' to measure relationships between each species to land covers. These models were used because of their ability to handle non-linear relationships between dependent and independent variables.

Mathias (2008) applied the methods outlined by Ray and others (2002) to model functional connectivity for *R. au*rora in King County, Washington. The Mathias study is particularly valuable not only because it was the first functional connectivity study for *R. aurora* but because central and western King County is one of the most urbanized areas in the state and this development trend is expected to continue. Consulting regional amphibian experts, namely Marc Hayes, Joanne Schuett-Hames, Klaus Richter and Ken Jacobsen, Mathias developed friction values for the western Washington landscape. These are given in Appendix A.

Mathias produced a landscape map for central and western King County at 30 m resolution (one cell representing 90 sq meters of land). Using this landscape map, she incorporated the least cost function to create friction maps that assumed a 1000m MDM and a 3000m MDM. This was done because research has shown that *R. aurora* generally travel between 1000 m and 3000m. Mathias also created 2m (one cell representing 4 sq meters of land) resolution landscape and friction maps for the Bear Creek basin in northwest king county, also illustrating functional connectivity under 1000m and 3000m scenarios. She did not incorporate data from real frog movements but general patterns on land cover and connectivity were obvious.

Mathias' work elucidates not only on *R. aurora* habitat connectivity but also on least cost modeling. First, she found that western King County—characterized by Seattle, Bellevue, Renton and Interstate 5-- is significantly less connected than central King County which is more rural. Secondly, connectivity correlated strongly with mature forests and lower road density. Finally, the landscape was significantly more connected for *R. aurora* if the 3000 m MDM is assumed for both the broad part of the county and the Bear Creek Basin. In other words, if we assume that *R. aurora* typically migrates 3000 m one way each year, it is better able to handle the adverse affects of urbanization because it is more likely to encounter new breeding ponds and utilize other forest patches. The finer resolution of the Bear Creek Basin maps revealed less connectivity because it is able to pick up roads which are significant barriers. However, the lower resolution maps of broader geographical areas are valuable because they reveal connectivity patterns over broad regions. Additionally, a portion of animals *do* cross roads so these lower resolution maps evaluate broader connectivity for this segment of the population.

Lowland Forest Connectivity in Western Washington

Landscape History: Pre-European and American Influences

Since the latest retreat of the Puget Lobe of the Vashon Glacier 13 thousand years ago climate changes and human influence have shaped the ecosystem. Originally, much of the Puget Sound Lowlands were dominated by prairies and oak (Quercus spp.) woodlands. Climate change about 8000 years ago started a trend toward temperate coniferous forests. Native Americans preserved prairies and oak woodlands in many places. Recently American and European land use practices have profoundly altered the biome.

Prairies and oak woodlands took a foothold due to a 5000-year period of warmer weather that followed the glacial retreat (Bowcutt, 2009). When the climate began to cool about 8000 years ago, Douglas fir (*Pseudotsuga menziezii*) colonized much of the prairies. Native Americans, however, controlled this succession in some areas in order to conduct agriculture, hunt and gather acorns. Crawford and Hall (1997) estimate that at the time of European and American contact, prairies and oak savannah covered 150,000 acres on areas abutting the southern Puget Sound and scattered portions of the Chehalis River basin. Currently, prairies and oak woodlands persist most notably in what is now the low-lying areas of Lewis, Thurston and Pierce Counties (Kruckeberg, 1991; Duer 1999).

Much of the Puget Sound lowlands became dominated by coniferous forests. This is largely due to a cooling climate 8000 years ago. Kruckeberg (1999) mentions that both Captain Vancouver and Malaspina, sailing for Britain and Spain respectively in the late 1700's, observed mature conifer forests growing to the Puget Sound's shore in many areas. This provides a picture of the Puget Sound Lowland landscape prior to European and American contact. This forest type typically involves a succession of red alder (*Alnus rubra*) colonizing barren or recently-burned areas, Douglas fir (*Pseudostuga menziezii*) and finally western hemlock (*Tsuga heterophylla*) dominating at the end of the succession. Western red cedar (*Thuja plicata*) grows in wetter or more shaded areas often forming groves. Bigleaf maple (*Acer macrophylum*) is often interspersed within conifer forests. Sitka spruce (*Picea sitchensis*) favors coastal areas, valleys with much precipitation or wetter areas. A plethora of additional tree species are present in the region, each favoring specific moisture, shade, altitude and soil characteristics (Kruckeberg, 1991). The succession cycle is generally restarted by wildfires started by lightning (Garman et al. 1990).

American Influences: 1850 to Present

European and American settlement generally began in the 1850s and steadily expanded. American settlers from the east mainly settled along the Puget Sound, in prairies and along rivers. Native Americans had originally established permanent settlements along the coasts and in river valleys but shifted to permanent settlements along the coast as American settlement expanded. Originally, settlers came with the intention of practicing agriculture on prairies and converting Native Americans to Christianity. Settlements soon became more sophisticated and timber extraction was gradually expanded(Cox, 1999).

Timber production expanded as the market for lumber expanded and technological innovations came into play. Not only was the wood used for northwest towns but it was exported to create urban centers in California during the gold rush. Wood was also used for steam energy to drive ships. Up until the early 1900's, logging was restricted to taking place along rivers that could be used to transport the timber to Puget Sound. In addition, the market and available technology resulted in selective logging. Douglas Fir (*Pseudostuga menziezii)* was the only species targeted and larger firs, along with all the other species, were left since the saw blades in lumber mills were too small to process them. Railroads and the 'Steam Donkey', a machine for yarding timber, enabled timber extractors to efficiently clear land well beyond rivers. Western Red Cedar (*Thuja plicata*) also became a valuable commodity. Aided by a network of railroads, the extraction of old growth timber continued but at farther and farther distances from the Puget Sound and large rivers. Trucks and more advanced yarding machines started to be widely utilized in the 1940's – indicative that old growth timber was being extracted at more rugged areas beyond the reach of railroads (Cox, 1999).

Since the time of American settlement, timber extraction, agriculture and urbanization eliminated forest and altered the forest structure of the Puget Sound Lowlands and lower elevations of the Cascade and Olympic Ranges- the *R. aurora* range. Much of the Puget Sound Lowlands was logged by the 1920s and by the mid 1930's most of the lowlands of western Washington had been logged at least once (Andrews and Cowlin, 1940). Agriculture was carried out in river valleys and deltas and small towns developed along the coast and large rivers and established trade routes. Up until the 1930s, artificial replanting was generally not practiced and clear cut areas were left to reforest naturally. As a result, Red Alder became more widespread; bog soil samples from the mid 20th century reflect a greater percentage of red alder pollen than that of other species (Cox, 1999).

The conservation ethic grew and in the 1960s and North Cascades National Park and wilderness areas were created, thus preserving some old growth forests[8] in the Cascades and Olympic Ranges (Franklin, 2007). National parks often preserved old growth in valleys in addition to alpine and subalpine areas while wilderness areas were generally established at higher elevations. Some river valleys in the three national parks protected lowland old growth forests but this was limited. Since many lower elevations in the Cascade and Olympic Ranges were reserved for silviculture, connections between old growth patches in protected areas was dependent on the configuration of mature conifer or deciduous forests. In addition, commercial forestry led to biologically simple forests. Patches of replanted single-species Douglas fir became increasingly common since it was the most valuable species for lumber, pulp and paper (Franklin, 2007).

In addition to timber extraction, American land use beliefs and practices changed the fire regime. The mid to late 1800's were characterized by expansive forest fires, usually set by

[8] The term 'old growth forests' is has many different meanings but in this paper it is used to describe Pacific Northwest temperate rainforests that feature a variety of tree ages, average age of dominant species approaching half the maximum longevity (about 150 years for shade tolerant species), some old trees with ages 300 years or older, natural regeneration of dominant tree species within canopy gaps and presence of standing dead or dying trees. This definition was devised by Moseler, Thompson and Pendrel in 2003.

settlers (Garman 1990). The rapid rate of logging in the early 1900s also increased the amount of 'slash' or unwanted wood on the ground which facilitated powerful fires. As the 20th century progressed, the US forest service and other agencies carried out a policy of attempting to prevent and fight *any* fire. This led to further fuel accumulations which resulted in powerful fires that left much more flora, fauna and soil micro organisms in the landscape dead than the less intense fires of an earlier age. Since the 1990s, forest managers have attempted to ameliorate decades of fire suppression by controlled burning (Puettman et al 2008).

Starting in the 1940s, construction of state and federal highways and a gradual influx of people accelerated forest habitat loss and fragmentation. As environmental sentiments started to grow in the 1960s, old growth forests were preserved. Western Washington, for example typically has relatively intact old growth forest habitat patches in the larger valleys of National Parks and wilderness areas, the I-90 corridor and Department of Natural Resources (DNR) land on the western Olympic Peninsula. These old growth fragments are largely isolated from one another and border lands covered by a mosaics of different age classes of timber as well as agricultural land and land that is steadily becoming more urbanized in certain areas (Cox, 1999). In the 1991, timber extraction slowed somewhat in the national forest lands of the Cascades and Olympics when the Northern Spotted Owl (*Strix occidentalis*) and the Marbled Murrelet (*Brachyramphus mamarotus*) were both listed as threatened under the Endangered Species Act (USFWS, 2004; 2009). As a result of this ruling, logging on National Forest Service lands has been greatly reduced.

Urban development has been the principle habitat fragmentation force in the Puget Sound Lowlands over the past 40 years. This is mainly true in areas nearest the Puget Sound and federal and state highways. Agriculture persists in the river valleys and industry tends to stay in established urban areas. The region continues to attract people from around the country and is expected to increase in population by 1.5 million people by 2020. In 1990, the state legislature passed the Growth Management (GMA) which incorporates various mechanisms to discourage housing sprawl. Nonetheless, landowners often have some economic incentive for converting forest and agricultural areas to residential housing, especially if such areas are within driving distance of an urban or suburban center.

Even though data is limited on the spatial patterns of forest clearing, it is possible that much of the lowlands were adequately well connected for *R. aurora* in the midst of timber extraction. This can be attributed to two factors. First, people generally cleared areas that were small enough to allow for frog movement. Second, the species' has shown an ability to utilize stands of deciduous trees, which naturally took root after humans clear cut land.

Chapter 3 Methods

Methodology was based on the literature review, which provided valuable information on the biology and ecology of *R. aurora* as well as on current accepted sampling and analysis procedures.

Wetland Selection

In selecting aquatic areas to study, I considered wetland type, presence of forest buffers around wetlands and levels of surrounding human impact to the landscape. Ultimately, property owner approval played a role in determining what wetlands were studied. I refer to the final 14 wetlands that I chose to compare as 'Study Wetlands'. While selection commenced in December 2010 and lasted through March of 2011, I continued to search for more sites until the end of my study. Initially, I attempted to focus on wetlands in Thurston and Pierce Counties but as the study progressed I obtained some in King, Skagit and Whatcom Counties. Mainly, I selected wetlands by utilizing Geographical Information Systems (GIS) software and publicly-available GIS websites but I also relied on the advice of regional experts and land managers.

Many wetlands were selected using Arc Map 9.3.1 software (Environmental Systems Research Institute, Redlands, California) or county GIS systems available online. I created 4 separate maps for Thurston, Pierce, Skagit and Whatcom Counties. In each map, I first added a 2009 aerial photo that was created from the US Department of Agriculture (USDA) National Agricultural Inventory Program (NAIP). Specifically, I visited the USDA Geospatial Data Gateway website (http://datagateway.nrcs.usda.gov/) and downloaded compressed NAIP county mosaics for the appropriate Washington county. This data had a coordinate system of Universal Transverse Mercator (UTM) and was projected in the North American Datum (NAD) of 1983. Since it was raster data, this coordinate and projection, UTM NAD 83, was adapted for each wetland selection map. I then overlaid the most current National Wetland Inventory (NWI) shapefile from the NWI website (http://www.fws.gov/wetlands/ Data/Data Download.html). It is widely known that NWI data is incomplete and inaccurate (Gale and Kudray, 2000; Johnston and Maysembourg, 2002) but it is generally effective at showing the inundated and non-forested wetlands that are the most ideal breeding habitat for *R. aurora*. Additionally, NWI classifies wetlands by the Cowardin system (Cowardin et al., 1979) which gives substantial information on vegetation and water regime and this enabled me to identify appropriate wetlands.

I then downloaded road shapefiles from county GIS agency websites and acquired parcel ownership data from county assessor offices. I utilized 2006 Thurston County Parcel data owned by the Evergreen State College. For Pierce County, I purchased 2011 parcel data shapefile from the Pierce County Assessor's office. In the case of King County, I relied exclusively on the online King County IMap service to select wetlands and determine ownership (http://www.kingcounty.gov/operations /gis/Maps/iMAP.aspx). This tool included orthophotos, links to assessor data and information on King County wetlands in addition to those recognized by NWI.

Study Site Selection Criteria

Random sampling was *not* employed to arrive at the final selection of study wetlands. This was due to the fact that, considering my budget and time limitations, it was not realistic to accumulate a large number of wetlands to randomly select from. Instead, I relied on selecting wetlands that were similar with respect to several physical parameters but differed with respect to the dependent variables which were the size of the initial forest patch and the distance to the nearest paved road.

Initially, there were 211 wetlands that I was interested in including in the study based on plant communities, water regime, water quality and size. I then sought permission to enter as many of these as I could. In order to qualify as being good *R. aurora* breeding habitat, the water needed to have sufficient light exposure to allow embryonic development and food resources (Storm, 1960; Licht, 1969; Calef 1973; Brown, 1975), it needed to have relatively thin-stemmed vegetation for egg masses to adhere to (Storm, 1960; Licht, 1969; Calef 1973), and it needed to have a hydroperiod of at least 6 months which is sufficient to allow animals to complete metamorphosis (Richter and Azous, 1995; Hayes et al., 2008). Considering these requirements, I used GIS applications to find *palustrine emergent wetlands with seasonal inundation* (PEMC) and *palustrine emergent wetlands with semipermanent flooding* (PEMF). These are categories of freshwater wetlands that are generally under 6 feet in depth and include non-woody vegetation protruding from the water surface (Cowardin et. al. 1979). I included wetlands with lower-case modifiers; a common example was 'PEMFb' wetlands which are influenced by American beavers (*Castor Canadensis*). I also included PEMC or PEMF *portions* of larger wetland complexes. Although wetlands like this can be found along the margins of lakes and ponds, I avoided deepwater habitats because I wanted to avoid the confounding variables of predatory fish and bullfrogs (*Rana catesbiana*) that are associated with more permanent water bodies. In addition to selecting wetlands with these parameters, some wetlands on Joint Base Lewis McChord were recommended to me by biologists experienced with the area.

In addition to water regime and plant community, I based my selection on wetland size and distance to other wetlands that would be appropriate breeding areas. I selected wetlands between ½ acre and 10 acres in area. Marc Hayes of WDFW recommended this range because wetlands smaller than ½ acre may not adequately reflect surrounding habitat characteristics and wetlands over 10 acres would be too difficult to census in a reasonable time frame. Marc Hayes also suggested that wetlands should be separated from other potential breeding sites by at least 400 meters so that they can represent distinct *R. aurora* populations. Popescu and Gibbs (2010) and Petranka and others (2004) also stipulate this.

In an attempt to limit the variable of poor water quality, I attempted to select wetlands that were surrounded by forests or grassy areas that were not lawns or cultivation. This was the case in all but three study wetlands. Study wetland 5 in Puyallup was bordered by an office property and a road. Study wetland 6 in King County was partially bordered by a lawn. Study wetland 12 in Skagit County was partially bordered by a hay field. Despite the fact that these land features were adjacent to these wetlands, none of them demonstrated high levels of algae growth and they all had pH values from the 6 to 7 range. I therefore included these three sites because they were representative of a certain level of surrounding development.

Once I obtained ownership information for a wetland that I was interested in analyzing, I sent a letter describing my study and requesting entry to the landowner. With the input of Dr. Martha Henderson of the Evergreen State College, I developed a letter template which I used for a while and then shortened. Both letter templates are included in Appendix B. I sent out a total of 236 letters to landowners in Thurston, Pierce and King Counties. I ultimately got permission to ender 29 properties, some of which were owned by the same land owner. Fifteen people contacted me to decline my request and 14 letters were returned by the Postal Service. Earlier in the study, I attempted to call about 15 people who had not responded but either could not find phone numbers for them or got very negative responses.

Several professionals gave me suggestions on appropriate wetlands to survey and permission to do so. Joint Base Lewis McChord (JBLM), owned by the US Department of Defense, straddles Thurston and Pierce Counties and holds large expanses of relatively undisturbed Puget Sound Lowland habitat (Adams and Bury, 1998). JBLM biologists Jim Lynch and John Richardson suggested what areas to survey on the base and gave me permission to enter. I selected wetlands in Whatcom and Skagit Counties toward the end of the *Rana aurora* embryonic phase and time was therefore short. For this reason I almost exclusively surveyed wetlands on either public land or land trust land and relied on the guidance of others. The

Washington Department of Natural Resources (DNR) granted me permission to enter their lands throughout the state. US Forest Service (USFS) biologist Ron Gay facilitated surveys in North Cascades National Park and adjacent Mount Baker-Snoqualmie National Forest lands. Steve Walker and Karen Grimland of the Whatcom Land Trust gave me permission and assistance in surveying their lands. Jennifer Bohanon of WDFW also gave me site suggestions and landowner contacts in Whatcom County.

For two of the study site wetlands, I analyzed fieldwork results that were obtained by other people. Egg mass count data for site 12 was done by Ron Tressler of Seattle City Light. Egg mass count data for Site 14 was conducted by Cedar River Watershed biologists Heidy Barnett and Shelly Nickelson. The site 14 data reflects a five year average for Deep Lake (Barnett and Nickelson, 2008).

Final Selection of Sites

Even after carefully pre-selecting sites, it is necessary to visit them in order to determine if they can be considered appropriate *R.aurora* breeding areas. Ultimately, I surveyed 27 sites but rejected 14 because I felt that other factors at them besides those relating to connectivity affected *R.aurora* breeding. In some cases, I felt that I was surveying the area too late and that egg masses had hatched and disintegrated. In other cases, I arrived at a site to find that the area appropriate for breeding was smaller than .5 acres. Additionally, I omitted other sites because they were excessively shaded , were impacted by water quality issues, were dominated by *Ambystoma gracile*, or I suspected that it had an excessive fish or bullfrog presence. I finally chose to use 14 sites that were all of sufficient size, had the appropriate plant community characteristics and lacked influence by other species.

Ultimately my sites were spread from Thurston County to the Canadian border (Fig 6). The majority of sites were located in the lowlands of Thurston and Pierce Counties and this is probably representative since these areas have a large number of palustrine wetlands. Property owner permission strongly determined what sites were available to me and for this reason there was likely a bias to my selection. Most of the owners that I contacted were residential property owners and 12 of them gave me permission to enter. People who granted me permission did not seem to be politically opposed to what I was doing and were even interested in my results. I also contacted corporate owners although this was a very small subset of the total people I contacted. The majority of them, including timber companies, gave me permission. One timber company denied my request on the basis that I did not have adequate insurance. A few agribusinesses cited food security concerns and denied my request. Land trusts and city utility companies allowed me to enter. I entered public land without seeking permission.

Figure 6 Locations of Selected Study Sites

Number, Name	General Location	UTM	Tax parcel # or Ownership	Size (ha)	Characteristcs
1. Bald Hill Road	East Thurston Co.	535121.14mE 5193718.70mN	22603310000	1.73	-most of wetland was about 50 cm -Dominated by Phalaris arundinacea; patches of Typha latifolia, Carex obnupta, Scirpus microcarpus
2. Rainier #1	North of the town of Rainier, Thurston Co. , East of Hubbard street	523792.79mE 5193923.11mN	21604310000	.52	-about 48cm -East section; *Spiraea douglassii* dominates on the northern edge, ---sedge dominate central portion, -West section; Spirea douglassii around edges, interior comprised of Scirpus microcarpus, Carex obnupta, Juncus effusus
3. Rainier #2	North of the Town of Rainier, Thurston Co.	524065.28mE 5194600.56mN	63550006800 (Vincent) 63550007000 (Miller)	.17	-Spiraea grows around t he
4. Veckved	NW Whatcom Co.	528938.81mE 5427509.27mN		.19	-Wetland generally has about 45 cm of inundation -Alnus Rubra around edges, Phalaris arundinacea dominates, also Lysichiton americanum and Carex obnupta
5. Puyallup	Puyallup, directly East of Pierce Co. Airport	554794.83mE 5217256.77mN	0419275011	.05	-About 80 cm of inundation -*Spiraea douglasii* around edges and in a strip in the middle, Labrador tea, *Scirpus subterminalis* in the open water areas, also *Juncus effusus*
6. Lake Youngs area	SE King County, unincorporatded area between Kent and Covington	567032.06mE 5250383.71mN	1222059015	.64	-Averages 70 cm -Dominated by Phalaris arundinacea. Spiraea douglasii and Salix spp. along edge
7. Taylor family LP	Central Thurston Co., north of Tenino adjacent to private timber land	511922.99mE 5192765.28mN	11608230000 (Taylor Family LP)	.75	-70 cm water depth; this is a beaver pond with a dam at the southern end and another in the middle. Southern section avg. 70 cm depth -wide variety of emergent plants
8. Clear Lake	Western Pierce Co., NW of Eatonville	545313.71mE 5193018.20mN	Manke Timber Co.	.24	-About 80 cm deep in February -East 2/3 of wetland is emergent and dominated by *Phalaris arundinacea*
9. Baker River	North Cascades National Park, on the Baker River trail about 2 miles from end of road at Baker Lake	606962.83mE 5403125.41mN	National Park Service	1.69	-About 65 cm in early April 2011 -Plants not carefully recorded, relatively open water, sedges, rushes
10. Pipeline	West side of the Rainier Training Area, JBLM, SW of Rainier Road	517378.66mE 5197618.83mN	US Army	2.99	-About 5 feet of water in February 2011 -The north half of the lake is open water and dominated by emergent plants,while the southern and southwest portions are dominated by *Salix spp*. The northern portion was considered habitat. Much of this area is dominated by *Typha latifolia* but there are some patches of *Spiraea douglasii*
11. Stringtown Rd	Rapjohn Lake area, NW of Eatonville, N of Stringtown Rd	551502.24mE 5193018.20mN	Manke Timber Co.	.38	-About 30-40 inches of water During the winter of 2011 - The eastern half of the wetland is appropriate for RAAU breeding while the western half is scrub-shrub or forested. This is a very scenic wetland with a variety of plant communities.
12. Harrison Slough	Skagit River Valley, east of Rockport, between the river and	608261.13mE 5371407.76mN	Seattle City Light	.63	-Oxbow pond with up to 10 feet of inundation - Pond is dominated by Nuphar luteum and Potomegeton natans. Typha latifolia and Spiraea douglasii on edges.
13. No Name Lake	Central Rainier Training Area, JBLM, east of Rainier Rd.	520951.60mE 5198344.71mN	US Army	1.31	-Circular depression that gradually increases in depth to about 80 cm in the center. -Completely dominated by *Phalaris arundinacea*
14. Deep Lake	Part of ’14 Lakes cluster; Western portion of Cedar River Watershed, south King County	583756.20mE 5249761.29mN	Seattle Public Utilities	1.2	-Lake was relatively devoid of emergent vegetation due to recently increased water levels; most of the egg masses were found attached to the branches of dead, submerged trees that had been installed to increase amphibian breeding

Table 3 Descriptions of Selected Sites

Site Name	Location	UTM	Tax Parcel #	RAAU Egg Mass Count	Reason for Rejection
Ballard	Central Pierce Co	550252.72mE 5205588.49mN	0418314039	9	-Under .167 ha -Water quality issues from being in a cow pasture -Part of area is shaded by trees
Park 1	Baker River Road, Skagit Co	593282.19mE 5388356.98mN		40	-Directly bordered by paved road, which would constitute a unique disturbance not represented by the other Study Wetlands. -Other large, unsurveyed breeding ponds in area so the role of this wetland in RAAU breeding was unknown
Sumas Mountain Beaver Pond	NW Whatcom Co,	559469.86mE 5419226.76mN		19	-This is mainly an AMGR pond. 95 AMGR egg masses were found.
Wickerhsam 1	SW Whatcom County off SR 9	558703.61mE 5389293.49mN		13	Forested wetland, not ideal
Wickersham 2	SW Whatcom County, off SR9	558324.04mE 5388720.75mN		10	-Actual area where RAAU were breeding was under .0836 -45 AMGR masses in surrounding areas
Potters Pond	North Whatcom Co.	576336.62mE 5416169.83mN		0	Was visited in April, season likely passed
Ranger Lake	JBLM[1], west RTA[2]	516429.36mE 5197752.44mN		0	Good habitat. Possibly visited too late
Springer Lake Beaver pond	Central Thurston Co.	509705.87mE 5198601.88mN		5	-This is mainly an AMGR breeding area.
Springer Lake Stormwater pond, adjacent beaver pond	Central Thurston Co.	509693.35mE 5199172.15mN		12	-area under .167 ha - This was mainly research on natural wetlands, not stormwater ponds, hence this site would have been unique. -The beaver pond was unique in other ways; it was heavily shaded
Pond, NE of No Name Lake	JBLM, Central RTA	521125.67mE 5198897.35mN	521180.76mE 5201099.67mN	12	-area under .167 ha -Heavily shaded; in the same forest patch as site 13.
Paine Jr (Trustee)	SW of Yelm, Thurston Co.	532468.61mE 5194618.08mN	22605140000	0	-The owners informed me that this was once used to raise bullfrogs
Studabaker	West Thurston Co., off of 140th Ave, SW	500649.54mE 5191803.90mN	12618220000	0	-pond as deep as 6 ft (according to the owner) -water was not clear and it was stormy. Owner informed me that he had stocked it with trout, much algae- probably from a Christmas tree farm on opposite shore
Kehoe Clearcut	Clearcut Parcel	512398.15mE 5193047.50mN		33	-Wetland was in the middle of a clearcut, so it was different than all of the other sites. It was surrounded by the same forest patch as site # 7 so I chose to use that site instead.
Kehoe Beaver Pond 1	Clearcut parcel	512486.28mE 5193120.34mN		10	Mainly a AMGR breeding area: 45 egg masses counted
PB Lumber 1	In forest, east of Kehoe clearcut	513020.80mE		0	-Pretty good habitat; large, shallow pond with islands. Possibly surveyed too late
PB Lumber 2	Large pond N of PB Lumber 1	513441.37mE 5193161.11mN		0	-Good habitat around edges; beaver pond. Possible fish presence: I heard a loud splash. Possibly surveyed too late;
PB Lumber 3	East of 'PB lumber2'	513441.37mE 5193226.10mN		0	-pretty good habitat; beaver pond. Deep but with many islands of sedges -Possibly surveyed too late

Table 4 Descriptions of Rejected Sites

Data Collection Methods

Egg mass counting was employed because it indicated the level of breeding effort at a given wetland. Breeding effort can be used as an index to estimate population levels of adult frogs breeding at the wetland and gives an idea of the amount of frogs that are able to utilize the surrounding landscape. Egg mass counts provide a more accurate estimate of population trends than other methods (Patton and Harris, 2010). Per information by Patton and Harris (2010), *R.aurora* is well suited for this method because the species is a relatively explosive breeder, individual masses are clearly separated, egg masses persist for about 5 weeks, masses are large and tend to be found in predictable areas (Jones et al, 2005).

The 2011 breeding season was unique but I attempted to obtain a sufficient sample size of wetlands by expanding my surveys beyond Thurston and Pierce Counties and by utilizing data from other researchers. I visited each wetland once from February 10 to April 5, 2011. *R.aurora* throughout the region had bred particularly early in 2011, probably due to a period of warmer weather in January (Jennifer Bohanon, WDFW, personal communication). As a result, I believe that I surveyed some sites too late to obtain an accurate count of *R. aurora* breeding activity. I omitted these sites from the selection. I therefore visited sites in King County in mid March and sites in Skagit and Whatcom Counties in early April. I incorporated 3 sites from this part of the state; all seemed to reflect later breeding, probably due to a later date at which the water reached high enough temperatures. I also included 2 sites that had been surveyed by other investigators.

I conducted a census of egg masses for each wetland. I chose a census over sampling because I learned that *R. aurora* often deposit egg masses unevenly throughout wetlands, even if conditions throughout the entire wetland are equally favorable with respect to sunlight exposure, emergent vegetation and water depth. I attempted to visually search the entire wetland, by viewing it from the shore, walking on logs that extended into the water and by wading through it while wearing chest waders. I used an inflatable raft to observe some sites. I wore polarizing sunglasses in order to see through the Sun's glare on the water surface. I tallied each egg mass in a field notebook. I tallied Northwestern Salamander (*Ambystoma gracile*) egg masses in order to ascertain if this species was dominating the wetland as a breeding site. *Ambystoma gracile* and *R. aurora* favor similar wetlands. *Ambystoma gracile* prey on *Rana aurora* tadpoles so I considered a large proportion of *Ambystoma gracile* masses to be a confounding variable and did not include wetlands with this characteristic in the selection.

Qualitative Landscape Characterization

I arranged the sites from lowest egg mass counts to highest and then used aerial photo layers on Arc Map 10 to ascertain patterns of connectivity and fragmentation based on information in the literature. I considered the arrangement, quantity and size of 1) the primary forest patch touching the wetland, 2) nearby forest patches, 3) roads, 4) neighborhoods, 5) pastures, 6) clear cuts and 7) business areas. I observed a general relationship involving the size of the primary forest patch and roads and decided to study this quantitatively. I also observed relationships between egg mass counts and 1) the degree of edge effects in the primary forest patch, 2) the size and accessibility of secondary forest patches and habitat and 3) the broader land use zoning in the broader landscape.

After a quick assessment of the sites, it was apparent to me that sites that were at the edge of forest patches had lower egg mass counts, regardless of how extensive the primary forest patch was. I chose to examine roads that lay within .25 km of the site because previous studies have shown that roads or deforestation at roughly this distance have the strongest effect on populations. Eigenbrod and others (2008) concluded that the strongest effects on anuran abundance and biodiversity in wetlands occurred when roads or deforestation was within 500 m of the wetland edge. Semlitsch and Bodie (2003) found that 95% of the population of a given species utilizes upland habitat within 159 – 290 m from the wetland.

Quantitative Analysis

I used Arc Map 10 to measure the forest patch sizes and degree of road disturbance. First, I created maps of each county that included NAIP 2009 aerial photos, 2010 NWI data and layers for roads. For each wetland, I created a 2km wide buffer around the wetland because Hayes and others (2008) state that seasonal movements exceeding 1 km may be typical (Mathias, 2008). I then used the area tool to measure the size of the primary forest patch and the area of the buffer. In order to find the percentage of the buffer that was covered by the primary forest patch, I divided the later by the former. I plotted ‘Egg mass counts’ against ‘percentage of buffer covered by primary forest patch’ in Excel. I tested the relationship for significance by importing the graph into the program JMP (Statistical Analysis Systems, Cary, North Carolina) and used a chi squared test to test for significance.

Based on my observations of sites at the edge of forest patches and on the literature, I used Excel to compare sites that had at least one road within .25 km of the wetland edge. Since this was count data and non-parametric, I used the JMP program to subject the data to the Wilcoxon / Kruskal-Wallis test s (rank sums) to test for significance. The literature states that the traffic level of nearby roads is a significant factor in amphibian diversity and abundance in aquatic areas (eg. Fahrig et al. 1995; Eigenbrod et al. 2008; Mazerolle 2004). To address traffic intensity, I obtained as much traffic count data as I could for roads that were near each study site from county and Washington Department of Transportation reports, web tools and conversations with officials. Based on the literature, I classified roads as having low, moderate, or heavy traffic and speculated on their affects at the sites.

Chapter 4 RESULTS

I numbered the sites in ascending order based on egg mass counts (Site 1 having the lowest count and Site 14 having the highest count) and, after looking at maps surrounding sites, observed landscape connectivity patterns. I observed that egg mass counts seemed to increase as the size of the primary forest patch increased. Many of the sites in the middle of this selection were on the edge of the primary forest patch and within .25 km of a road. I analyzed the sites quantitatively by calculating the percentage of the area within the 2 km buffer that was covered by the primary forest patch and tallying up the sites that had a busy road within .25 km of the study wetland. I compared both of these quantities with egg mass counts.

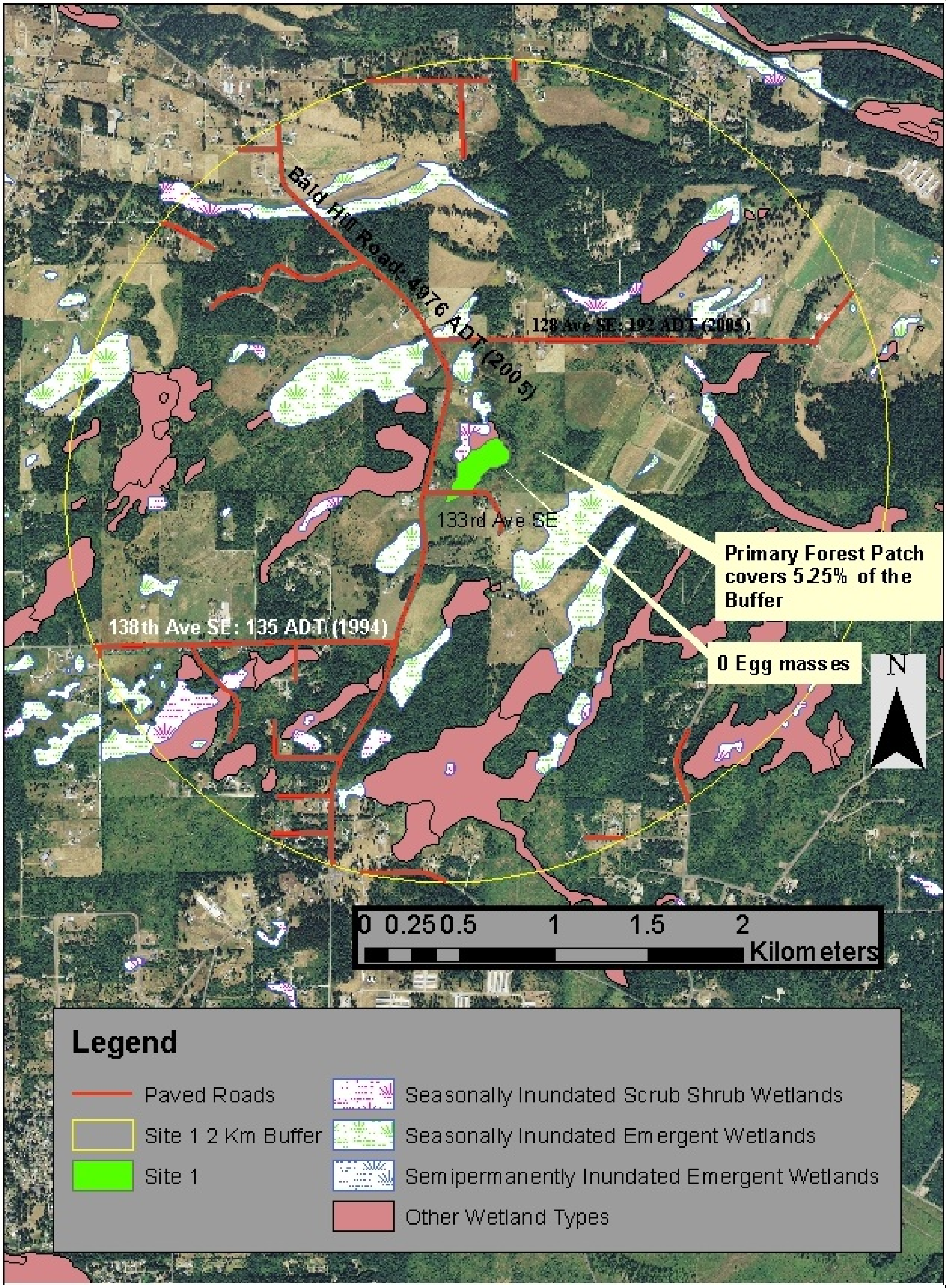

Figure 7 Study Site 1, Eastern Thurston County

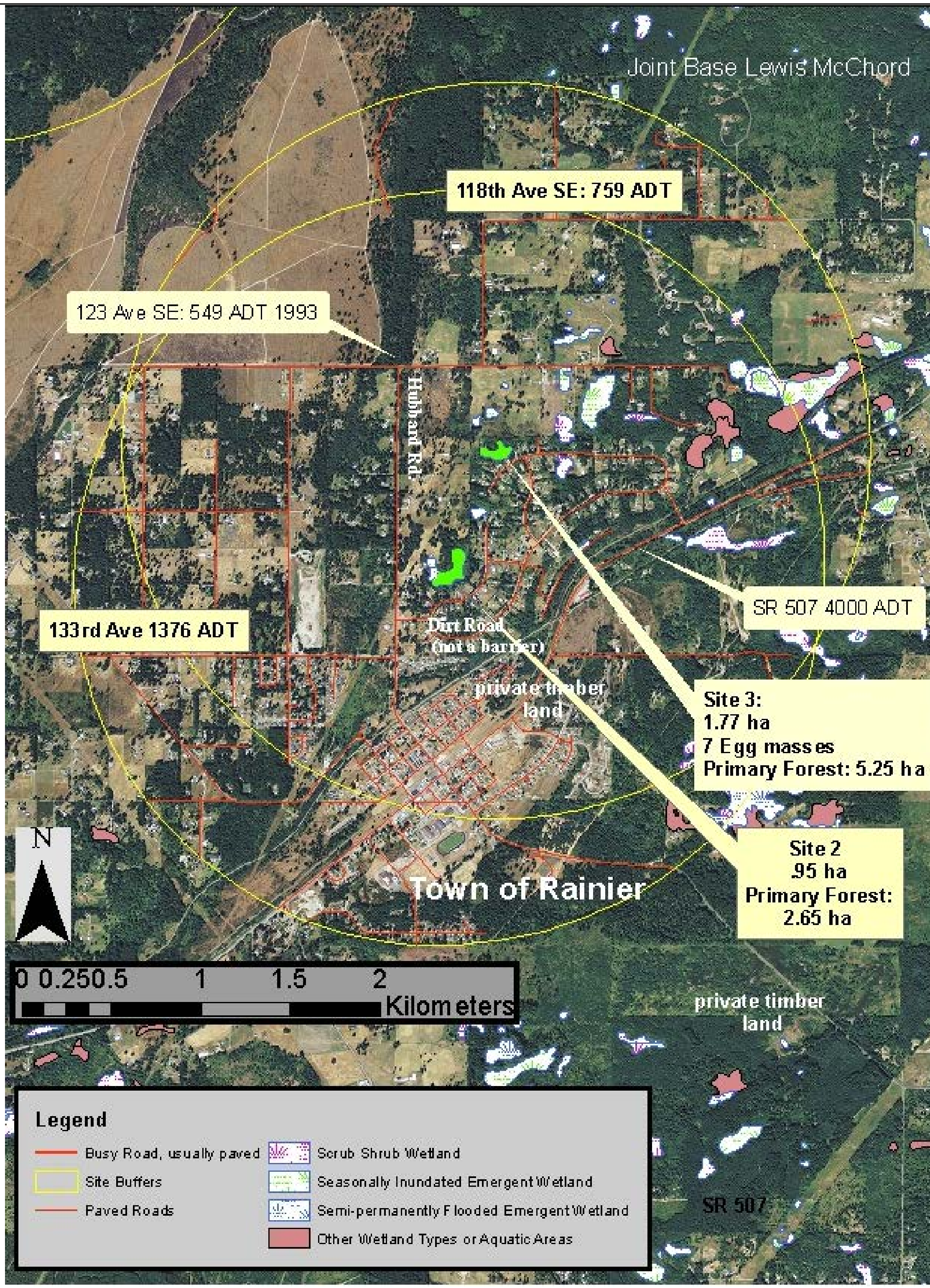

Figure 8 Sites 2 and 3, in the Town of Rainier

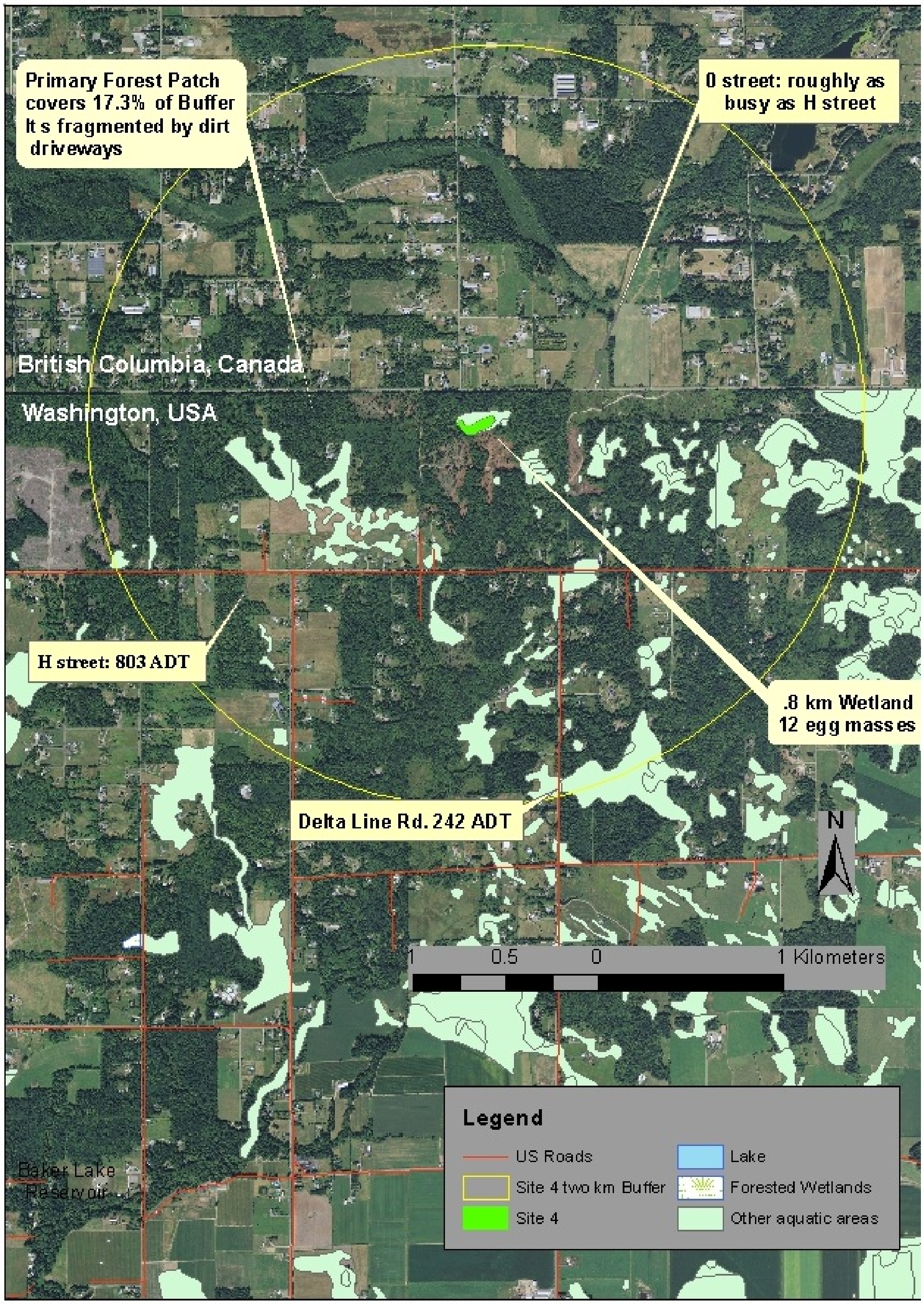

Figure 9 Site 4, Northwest Whatcom County

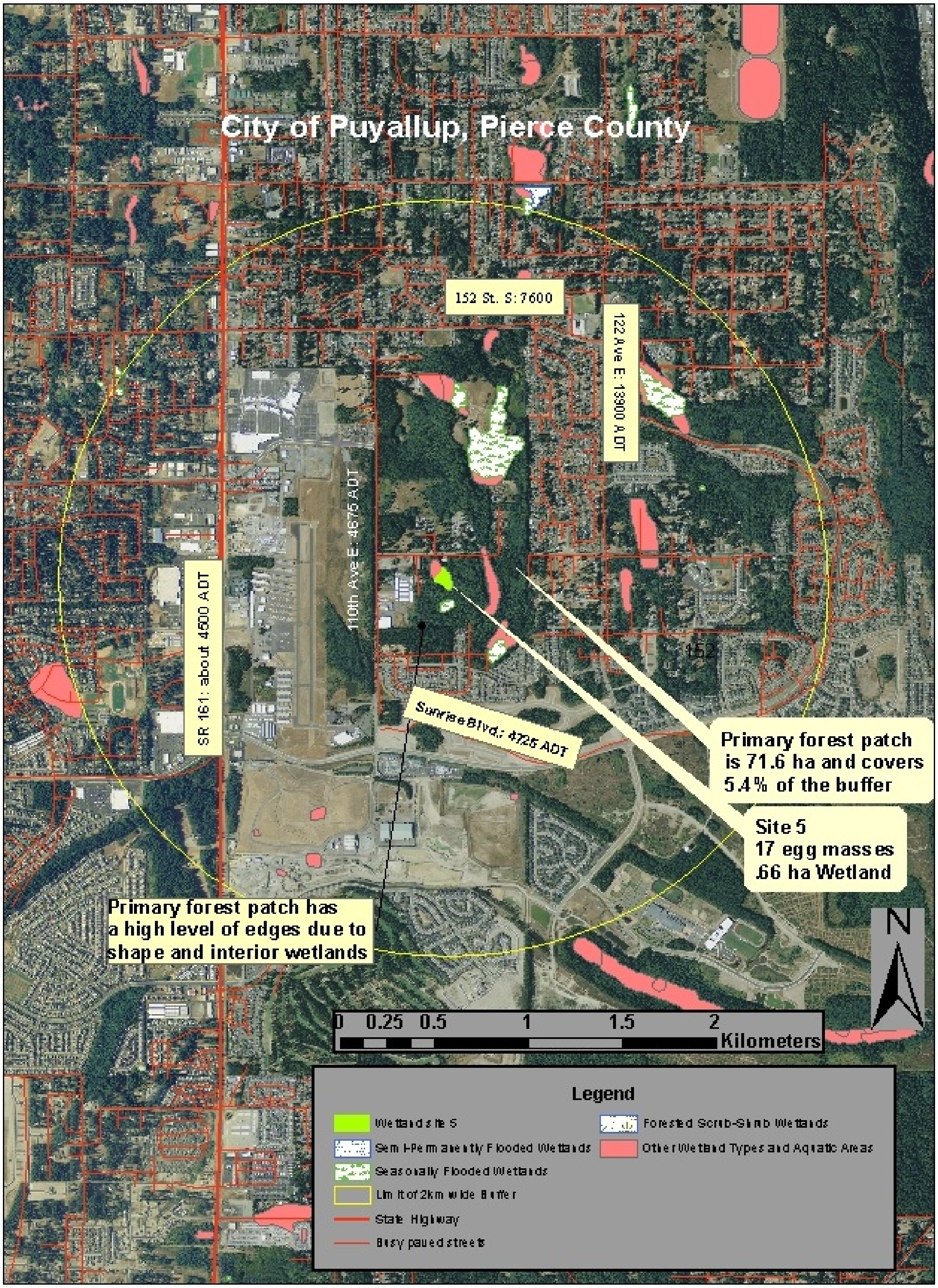

Figure 10 Site 5, in the City of Puyallup

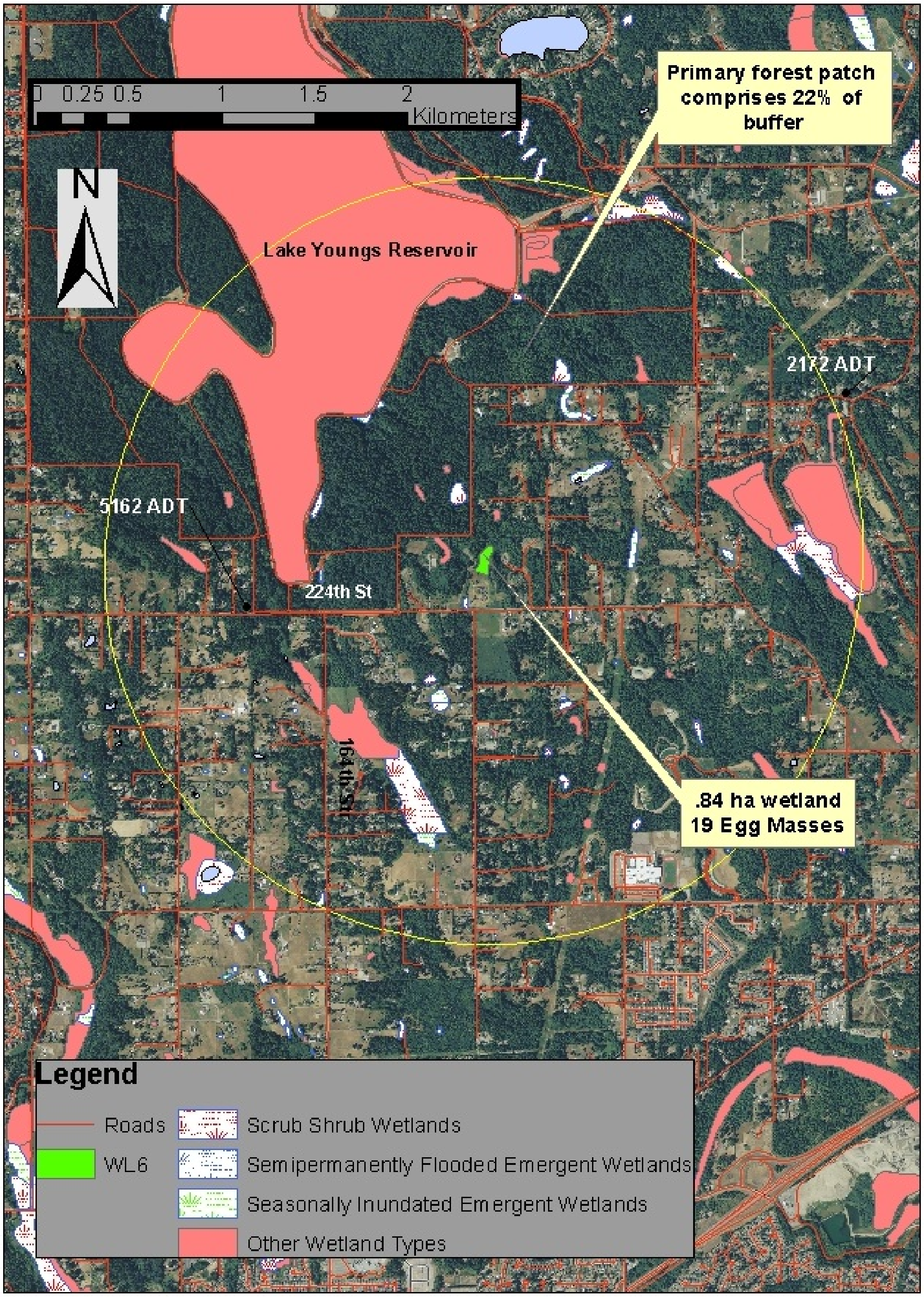

Figure 11 Site 6, Between Kent and Covington, King County

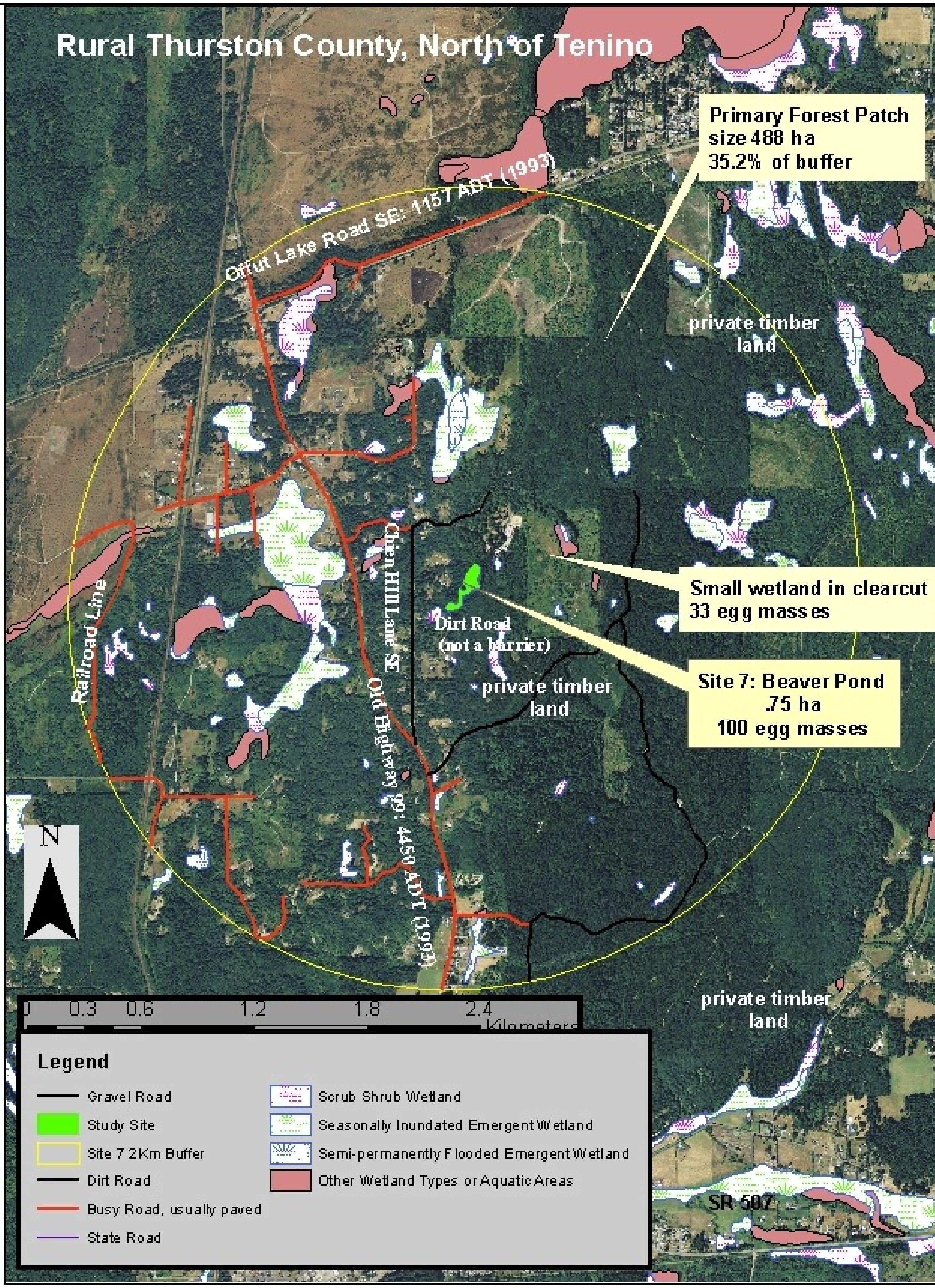

Figure 12 Site 7 North of Tenino, Thurston County

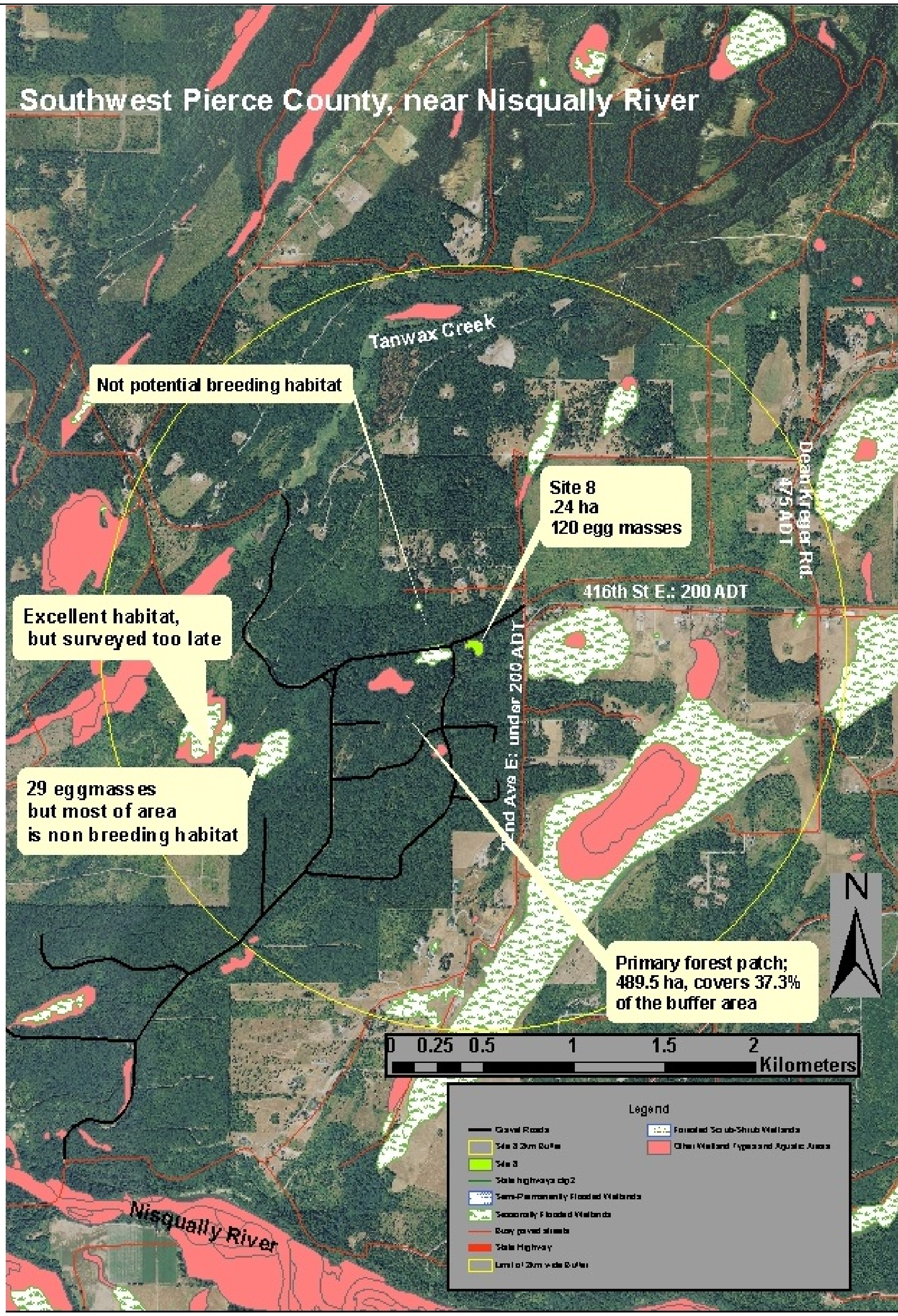

Figure 13 Site 8 Northeast of Eatonville, Pierce County

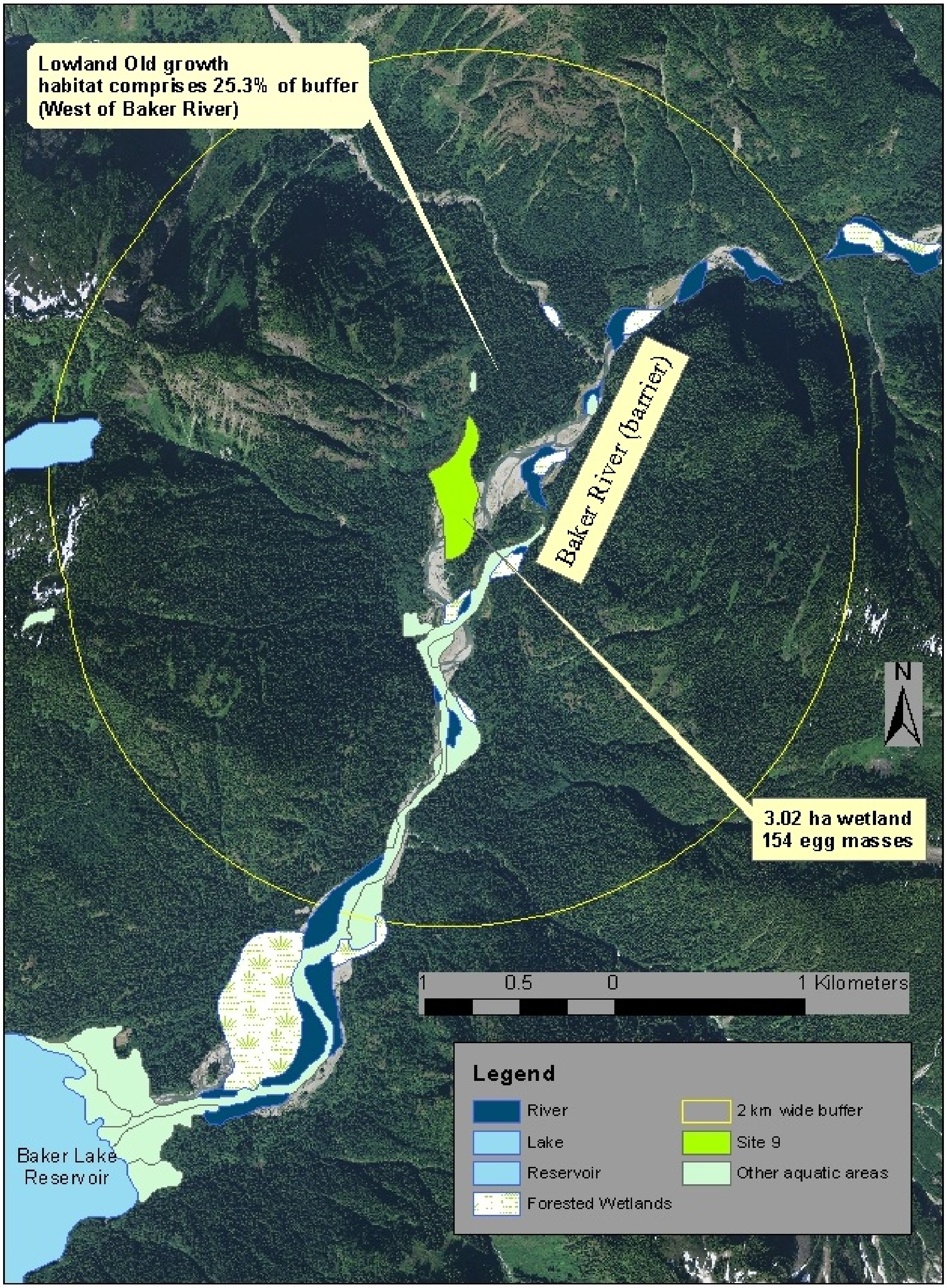

Figure 14 Site 9, Baker River area, North Cascades National Park

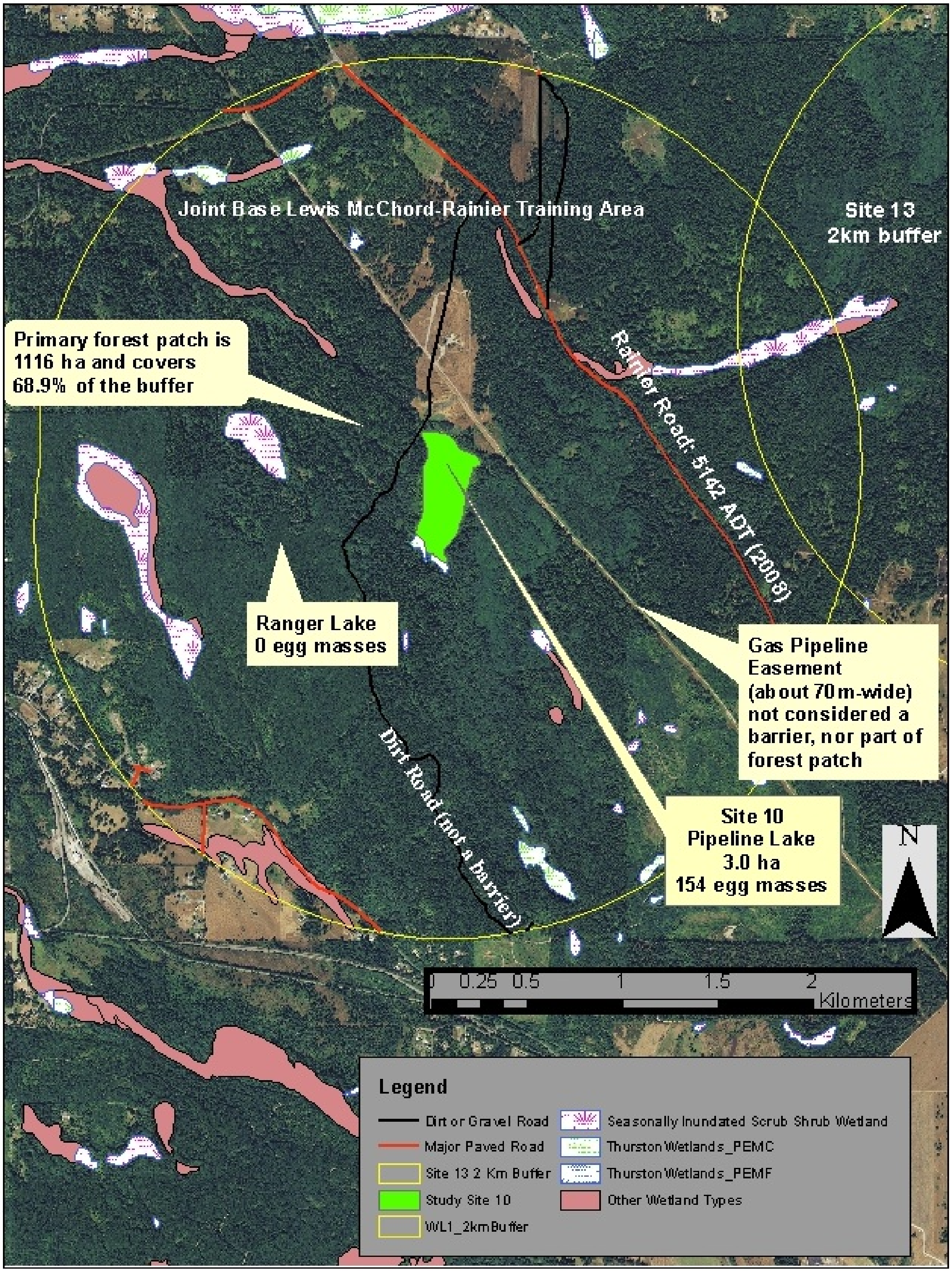

Figure 15 Site 10 Rainier Training Area, Joint Base Lewis-McChord

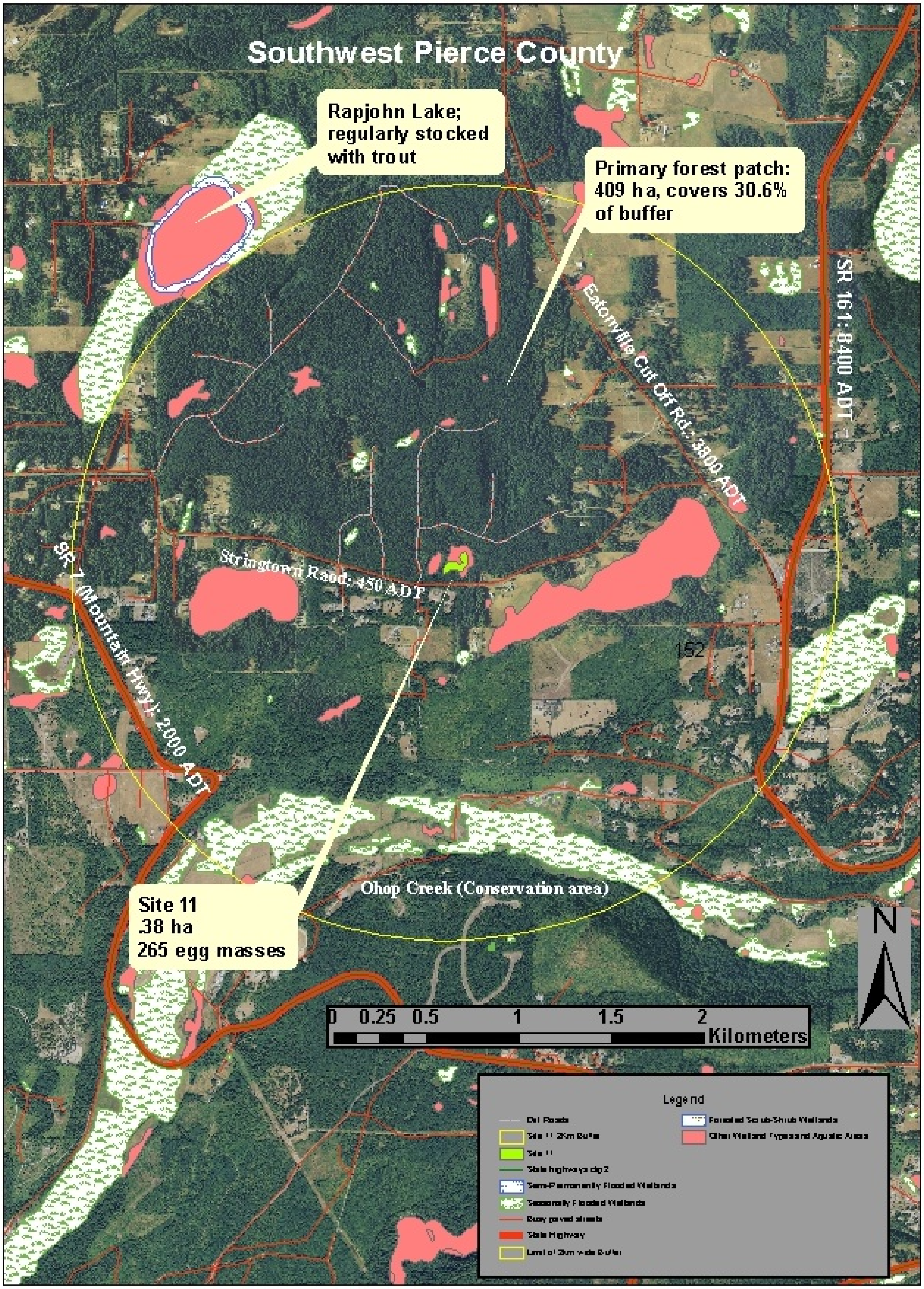

Figure 16 Site 11 Northwest of Eatonville, Pierce County

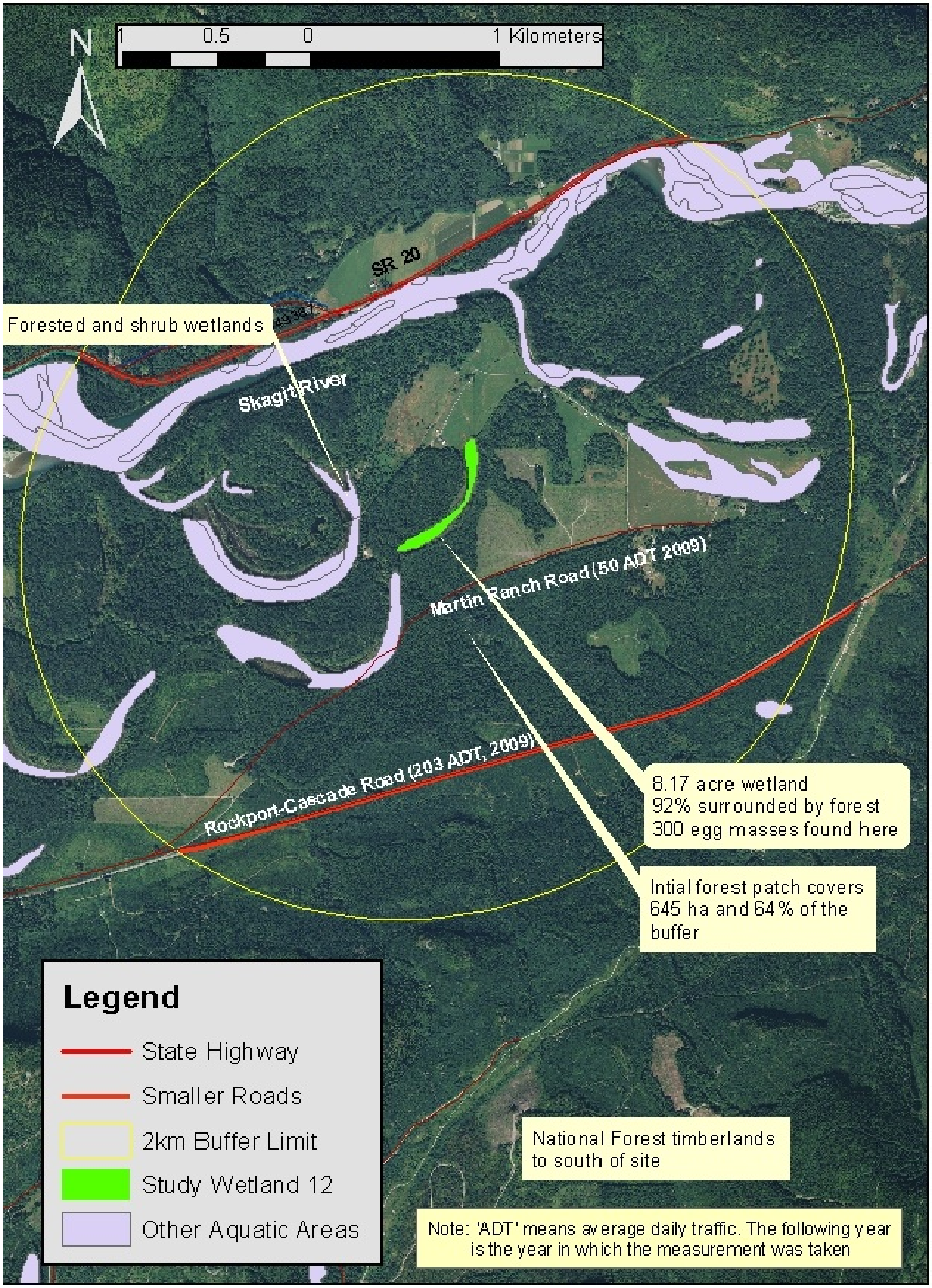

Figure 17 Site 12 Skagit River Valley, Skagit County

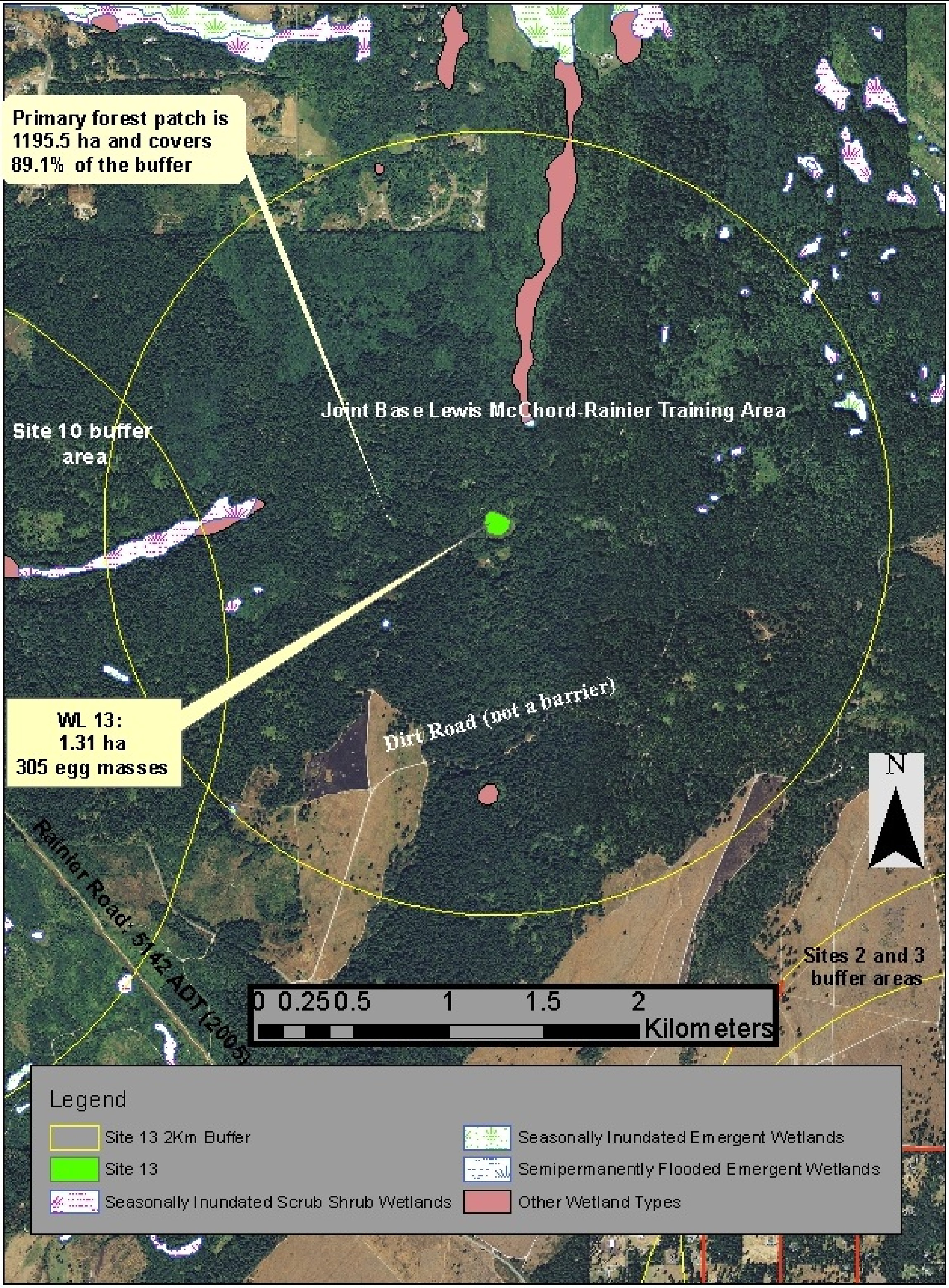

Figure 18 Site 13, Rainier Training Area, Joint Base Lewis-McChord

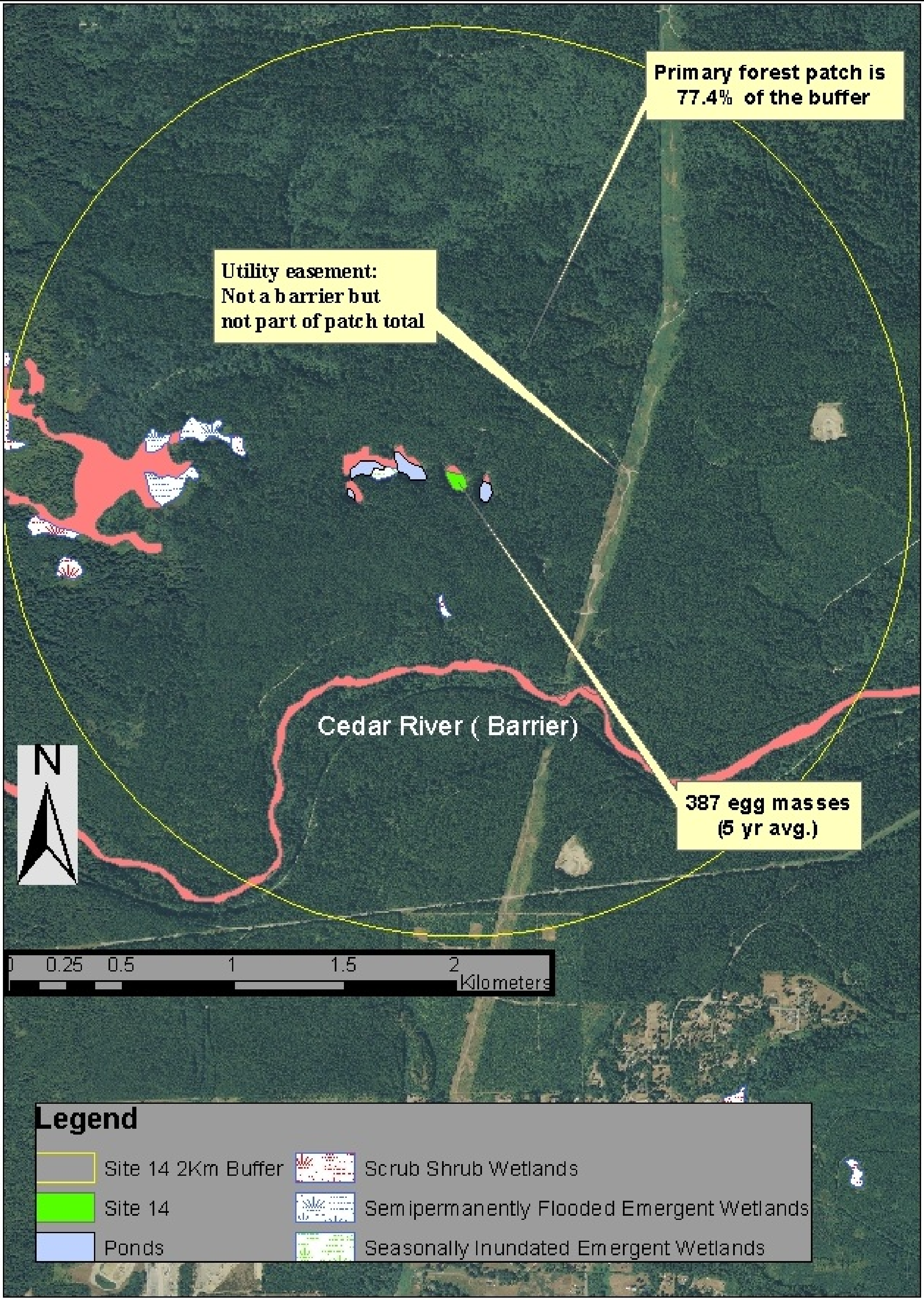

Figure 19 Site 14 Cedar River Watershed, central King County

Primary Forest Patch Size within 2 Km and Breeding Effort

I calculated the percentage of the area within 2 km that was covered by the primary forest (the contiguous forest patch touching each study wetland) (Table 5). I then plotted breeding effort (egg mass counts) against the size of the initial patch (Figure 7). There was a significant ($p=0.0001$) and strong positive relationship: as the primary forest patch increased in size within 2 km, breeding effort increased. TheR^2 value of the regression analysis was .79, indicating that 79% of the variation in breeding effort could be predicted by patch size.

Site Number	Egg Mass Count	Primary Forest Patch Size, Including Area Beyond 2km radius Buffer	Primary Patch size within 2km radius buffer	Percentage of Buffer filled by Primary Forest Patch	Busy Paved Roads within .25km of wetland
1	0	6.88	6.88	5.25	Yes
2	4	5.25	5.25	0.37	Yes
3	7	14.65	14.65	0.01	Yes
4	12	234.8	191.2	14.1	Yes
5	17	71.6	71.6	5.4	Yes
6	19	220	293.8	21.9	Yes
7	100	561	488.9	35.2	Yes
8	120	538	489.5	37.3	Yes
9	154	863.8	333.1	25.3	No
10	198	1725	1116	68.9	No
11	265	431	409	30.6	Yes
12	300	2000	645	64.7	No
13	305	4683.49	1195.5	89.1	No
14	387.7	48227	1020.5	77.4	No

Table 5 Quantitative Results

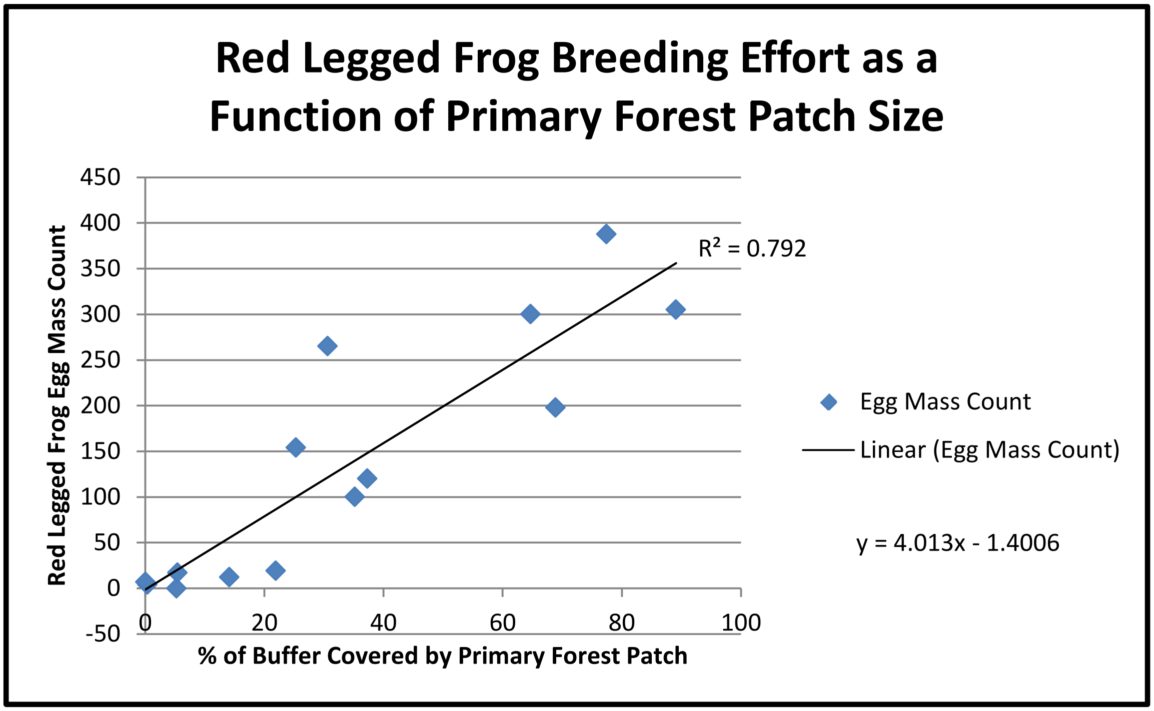

Figure 20 *Rana Aurora* Breeding Effort as a Function of Primary Forest Patch Size

The Effect of Roads and Traffic on Breeding

Road effects were analyzed quantitatively and qualitatively. Eggs mass counts was significantly less in areas near busy roads (<.25 km). This is shown graphically (Figure 19). Egg mass counts in areas < 0.25 km from road were also compared to those >.25 km from a road using the Wilcoxon/Krustal Wallis rank sums test. This test showed that there was a significant difference in egg mass counts between those sites (p<0.01). The effect of traffic levels was analyzed more qualitatively (Table 6). Sites 1 through 7 were moderately or heavily impacted by traffic levels and Sites 8 through 14 were either not impacted by traffic levels or incurred very low impacts from traffic levels. This reflected a general relationship between traffic levels and breeding effort.

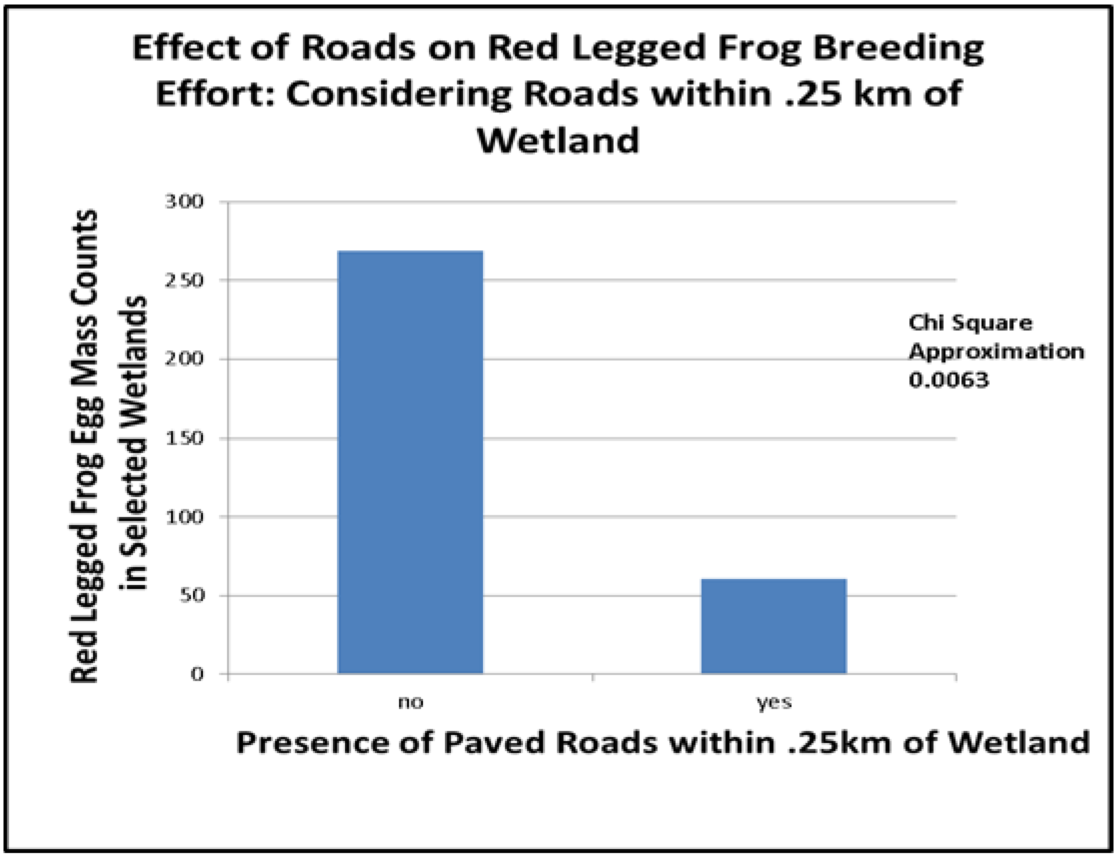

Figure 21 Effect of Nearby Roads on *Rana Aurora* Breeding Effort

Site	**Nearby Roads** (road closest to site is given first)	**Average Daily Traffic (ADT)**[9]	**Traffic Intensity Rating**[10]	**Year of Traffic Count**	**Comments**
1	-Bald Hill Rd -128th -138th	4976 192 135	Medium Low Low	2005 2005 1994	Moderately impacted by roads and traffic. There are few roads in the area but Bald Hill road is directly adjacent to the site and separates it from a large forest patch. Other forest patches to the east are smaller and more distant
2 and 3	-118th -127th 133	756 549 1376	Low Low Low	2001	Heavily impacted by roads and traffic. Many small residential roads in the area for which data isn't available. Traffic intensity for them are probably 'low' as they are similar to 118th and 127th. 133rd Avenue lies between the site and the vast forest areas of JBLM.
4	-'H' Street -Delta Line Rd	803 242	Low Low	2007 2001	Moderately impacted by roads and traffic. Few roads in the area. The '0 road' which is in British Columbia and runs along the border appears to be moderately busy but no traffic count data is available for it.
5	-SR 161 -110th Ave E -152nd St S. -122nd Ave E.	4500 4675 7600 8150	Medium Medium High High	2004 2010 2010 2010	Heavily impacted by roads and traffic. The primary forest patch is surrounded on all sides by either dense urban development or moderately to heavily traveled roads. Beyond these land covers are additional busy roads and development.
6	-224th	5162	Medium	2011	Heavily impacted by roads and traffic. A contiguous 3/5 of the buffer area is characterized by residential area with feeder streets. 224th is immediately south of the site. Traffic levels are likely the same on other similarly sized roads but traffic count data is sparse.
7	-Old Highway 99 -Offut Lake Road	4450 1157	Medium Low	2004 1999	Moderately impacted by roads and traffic. Highway 99 is within .25 km to the west of the site. Much of the land east of the site is not bounded by paved roads.
8	-22nd Ave -416th Ave -Dean Kreger Road	<200 200 425	Low Low Low	estimated 2004 2006	Low Impact by Roads and traffic. The roads nearest the site have low traffic counts. Much of the primary forest area is not bounded by roads.
9					No road or traffic impacts. The entire buffer area is in North Cascades National Park.
10	-Rainier Road	5142	Medium	2008	Low to Medium impact. Rainier road lies close to the site but virtually no other roads are in the buffer area.
11	-Stringtown Road -Eatonville Cutoff -SR 7 -SR 161	450 3800 2000 8400	Low Medium Low High	2001	Low to medium impact. Stringtown road is within 200 meters of the wetland but is not highly traveled. The other highways are more distant.
12	-Rockport-Cascade -Martin Ranch Rd.	203 50	Low Low	2009	Low Impact. These two roads are lightly travelled and do not isolate populations from forested areas to the south
13					No Road Impacts. There are no paved roads in the buffer area
14					No Road Impacts. There are no paved roads in the buffer area.

Table 6 Effect of Traffic Levels on Breeding Effort: Qualitative Analysis

[9] Traffic counts are given for the portions of the road nearest the site.

[10] Traffic Intensity Ratings are taken loosely from those devised by Fahrig et al. (1995): Low: 500-3500, Med: 5000-6000 and High:8500-13,000. I am considering low to mean 0-3500, Med: 3500-8500 and high: >8500.

Chapter 5 DISCUSSION AND RECOMMENDATIONS

Part 1: Scientific Conclusions

Summary

This study quantifies the population of *R. aurora, (as estimated by eggs counts)* that breed in a selection of wetlands and attempts to explain these quantities based on the surrounding landscape characteristics of each wetland. An underlying concept in many connectivity and habitat principles around wetlands is that lentic breeding amphibians that favor forest habitats use visual or other sensory clues to gravitate toward forest when leaving the wetland (Semlitsch, 1998; Walston and Mullin, 2008). Animals will therefore move toward any area around the wetland that is forested and then continue to exploit appropriate habitat or migrate from there. They will even attempt to cross roads, some of the most adverse land covers. This study has found 1) significant positive correlations with the breeding population represented in study wetlands and the size of the primary forest patch adjacent to the wetland and significant negative correlations with breeding population size and the presence of roads within a quarter mile of some of the study wetlands.

In order to fully characterize the surrounding landscape more exact quantitative methods should be applied to landscapes that are comprised of a mosaic of land covers and that lie within the 2km radius of the wetland. This study quantifies 1) the area of the primary forest and 2) the affect of roads within .25 km of the breeding site. Within the 2km buffer, this study addresses landscape characteristics beyond the impacting road in a way that is more qualitative and incompletely quantitative. Beyond the initial impacting road, which *R. aurora* attempt to cross, lie secondary forest patches, pastures and other roads. The collective impacts of all these land covers need to be assessed. The shape of primary forest patches also needs to be assessed with more quantitative methodology to determine the degree of edge effects. The most current developments in amphibian landscape ecology suggest that conducting a GIS least cost analysis for the area within 3km of each study site would accomplish this. Additionally, the sample size of 14 selected sites is small; a minimum of 20 sites is recommended to draw stronger conclusions (Hayes, personal communication). As a result of these factors, this study is preliminary and only suggests ideas for future investigations and provides justification for more exact methodology.

Forest Patch Size and Breeding Effort

As evidenced by Fig 6, breeding effort was positively correlated with the size of the primary forest patch. In the sample of 14 sites, primary forest patch sizes ranged in size from covering .01% of the 2km-wide buffer (study wetland 3) to covering 89.1% of the buffer (study wetland 13). Generally, the sites reflected an increase in breeding effort as the primary forest patch increased in size. Forest habitats in western Washington preserve moisture, maintain a more constant microclimate, protect animals from radiation and provide invertebrates for food. These results are consistent with conclusions reached by Aubry, (2001), Chan-McCloed (2003), Chan-McCleod and Moy (2006), Haggard (2000), and Schuett-Hames (2004) that *Rana aurora* favor precanopy and mature forests. On an intuitive level, larger forest patches come closer to resembling the large expanses of old growth forests that *Rana aurora* evolved in.

Breeding Effort and Nearby Busy Roads

As evidenced by Figure 7, the 4 sites with the most egg masses did not have a busy road within .25 km while all the others did. In addition to resulting in habitat loss, creating edge effects and altering local hydrology, roads are significant barriers to dispersal and migration (Eigenbrod et al., 2008; Fahrig et al. 1995; Beasely 2000) This study did not analyze land cover mosaics in the more disturbed areas around the sites but in many cases a busy paved road lies within .25 km of the site. For sites 1, 2, 3, 4, 5, 6 and 7 (Figures 8-13) the initial road is merely the first obstacle for reaching other smaller habitat areas. For Sites 8, 9, 10 and 11 (Figures 14-17) this road is all that separates animals on study sites from secondary forest patches and other aquatic areas. In the case of study wetland 12 (Figure 18), a paved road, Martin Ranch Road, does exist south of the site but this road is probably not sufficiently busy (50 ADT) to create a sizeable barrier to forest patches south of it.

Access to Secondary Forest Patches

In this study, 'secondary forest patches' are defined as other forest patches within 2 km from the study wetlands that are not contiguous with the primary forest patch that is adjacent to the study wetland. Although primary patches provide the best habitat and the best corridors radiating out from breeding sites, *R. aurora* do attempt to cross deforested areas (Haggard, 2001; Chan-McCleod 2004) so considering other forest patches is important. This study suggested that some breeding populations have easier access to secondary forest patches based on the type of the intervening landscape cover and the size of such covers.

Trends were observed over the study sites that suggest a positive relationship between breeding effort and the population's access to secondary forest patches. Study wetlands 2 and 3 are within 600 m of each other, have very small primary forest patches and are similar with respect to size and characteristics (Figure 8). Study wetland 3 however, is separated from a forest and wetland area by about 500 m of pasture. Site 2 is surrounded on three sides by rural residential development. Factors that separate wetlands from secondary forest patches may also play a role in the increasing egg mass numbers in sites with larger numbers and larger primary forest patch sizes.

Many of the sites in the selection that had middling values for breeding effort were located at the *edge* of large forest patches, thus rendering a large part of their 2 km buffers to be substandard habitat in the form of mosaics of roads, pastures, residential development and small patches of forest. Animals in site 7 (100 egg masses) could exploit wetland, streams and forest patches to the west but a residential road (Chein Hill Road), Old Highway 99, and railroad tracks separate Site 7 from these areas (Figure 12). Study wetland 8 (120 egg masses) lies to the west of smaller forest patches and aquatic areas that lie within pastures (Figure 14). Only one road separates these areas. Site 11 (265 egg masses) is similarly separated by a single road (Stringtown Road) from upland forests and aquatic areas associated with Ohop Creek to the south (Figure 16).

Edge Effects within Forest Patches

Edge effects may have played a role in reducing habitat at some sites. Where the forest patch meets a pasture, clear cut, residential lawn or road, the forest habitat along this edge is degraded. Edges result in an increase in sunlight penetrating the forest which leads to denser understory vegetation and a more variable microclimate (Weyrauch and Grubb, 2004). Edge effects probably play a significant role in the sites with the smallest primary forest patches -- Sites 1, 2 and 3 – because the forest patches associated with these sites are so small and separated that light easily penetrates them (Figures 8 and 9). Sites 4 and 5 had markedly larger primary forest patches than the first 3 sites but the egg mass counts (12 and 17 masses, respectively) did not correspond as tightly with this additional habitat (Figure 10 and 11). Site 4's primary forest patch is interspersed by 7 gravel driveways and rural residences and 3 clear cuts. It also has a comparatively circuitous shape. Site 5's primary forest patch is circuitous in shape and the southern section is interspersed by 4 large dense scrub wetlands. These areas are less than ideal habitat and also create edges in the adjacent forests. Edge effects may affect population sizes on these sites because they reduce habitat quality. Sites with greater egg mass counts simply have a larger primary patch size which mitigates edge effects since the ratio of area to circumference is larger. Additionally, some of the larger sites have primary forest patches that are more circular and not as circuitous which also results in a greater circumference to area ratio.

Position of the Wetland within the Primary Forest Patch

Sites that were positioned more in the middle of the primary forest patch generally had higher egg mass counts than those that were positioned more toward the edge. Although it was constrained by natural features of steep gradients and the Baker River, Site 9 (154 egg masses) was located in the middle of a long forest patch (Figure 14). Site 10 (198 egg masses) was also in the middle of a large forest patch but the fact that a prairie lied to the north and that a deforested 70 m-wide gas line easement / dirt road to the northeast bisected this patch may serve to lessen the *Rana aurora* population associated with Site 10 (Figure 15). Site 13 is almost completely surrounded by contiguous mature forests within 2 km from the breeding area and this area is only broken up by narrow dirt roads (Figure 18). Site 14 (387 egg masses) is surrounded by at least 1 km of mature forests on all sides. The Cedar River forms a barrier about 1.25 km to the south, and a utility easement is located about 1km east of it but mature forests exist beyond the easement (Figure 19).

General Land Use Objectives of the Area Surrounding the Site

This study suggested the broader land use objective of the landscape surrounding the site is a factor in *R.aurora* population levels. Land use is largely governed by economics, government objectives (e.g. national defense) geography and cultural values and is generally organized by ownership, regulations and zoning. Broad land use objectives and zoning can shape habitat use over a large scale which can have a unique affect on habitat, depending on the objective. Some objectives may include factors already discussed such as ‘Edge effects’ and ‘Accessibility of Secondary Forest Patches’. The 14 sites fall into 5 land use objectives; urban, rural mixed use, timberland, second growth forest preservation and wilderness protection

<u>1. Urban</u>

Site 5 (17 egg masses) exists in a relatively large forest patch but this patch is within the City of Puyallup, where the landscape is dedicated to dense residential development, commerce, and the transportation infrastructure that accompanies these activities. The amount of pavement, buildings and traffic severely isolate Site 5 from other habitat beyond 2km (Figure 10). Sites 2 and 3 lie within a small town, Rainier, and are surrounded by residential development. Site 6, (Figure 11) is impacted by suburban development, even though it is in unincorporated King County. Over half of its 2 km buffer area is covered by suburban residential areas and, probably more importantly, many moderately travelled roads that separate small forest patches. While this area is in unincorporated King County, it

<u>2. Rural Mixed Use</u>

Four of the sites exist in unincorporated, flat parts of the Puget Sound Lowlands with mixed land use objectives. Sites 1, 4, 13, and 14 (Figures 7, 9, 18 and 14 respectively) fall into this category. They have a mosaic of commercial forestry holdings; pasture land, rural residential areas and rural roads that are traveled to varying degrees. *R. aurora* populations across all these sites reflect the size of the primary forest patch and the ease to which *R. aurora* can move to secondary patches.

<u>3. Protected Wilderness</u>

Site 9 (154 egg masses) is within North Cascades National Park, an area that is maintained for biodiversity and other environmental values as well as recreation (Figure 14). Although the area is highly constrained by steep topography and the Baker River and much of the buffer area is comprised of alpine meadows, rock and glaciers, the preserved status has prevented many of the human disturbances existent at the other sites. This site is unique in that it is comprised of old growth forests. Old growth forests may have a higher carrying capacity than mature second growth, which would allow a greater density of frogs.

4. Timber Production

Site 12 (300 egg masses) exists at the edge of an area almost exclusively devoted to timber production in the Cascade Mountains (Figure 17). Some pastures intersperse the primary forest patch and the Skagit River, a barrier, lies 1km to the north. The Washington Department of Natural Resources manages lands south of the site and this area is different than timber lands in the 'Rural Mixed Use' category. These timberlands are much more expansive and are a mosaic of clearcuts, old growth, mature and other intermediary forest age classes. Site 12 is bordered on the north by pasture and the primary forest patch is smaller than other primary forest patches in the selection but the high egg mass count suggests that this landscape is more conducive to *R. aurora* populations than the 'Rural Mixed Use' landscape *R. aurora* may be able to tolerate these landscapes better than 'Rural Mixed Use' landscapes because of the lack of moderately or heavily-travelled roads and the fact that even very young forests provide better habitat than pastures and residential development. Although 2 roads are present south of the site, they are comparatively lightly travelled, most likely due to few residences and businesses to the east. Martin Ranch Road has 50 ADT and Rockport Cascade Road has 203 ADT. Since these roads are lightly travelled, frogs can probably reach forested areas south of them relatively easily.

5. Second Growth Forests Preservation

Two of the highest egg mass counts in the selection Site 13 (305 egg masses) (Figure 18) and Site 14 (387 egg masses) (Figure 19) exist in large patches of mature second growth coniferous forests that are preserved or very lightly logged. This land use type reflects territory that, like much of western Washington, was initially logged but subsequently reforested and then preserved under other objectives besides timber production. JBLM maintains forests for military training purposes. JBLM occasionally logs areas but timber sales are limited in size and subjected to more stringent environmental standards than many timber harvesting operations

(McAllister, 2001). JBLM managers are actually attempting to replicate some old growth forest characteristics throughout the base (Adams 2000). Site 10 is also on JBLM but has less connectivity than Site 13, owing, in part to other land use objectives beyond those of the base. Site 10 is bisected by a moderately-busy road (Rainier Road) and a gas line easement (Figure 15). The City of Seattle maintains the Cedar River Watershed for high quality drinking water and therefore does not log it. While these forests have lower carrying capacities than the old growth forests at Site 9, the sheer size of the forest patches are conducive to relatively large *Rana aurora* populations.

Part 1 Conclusion

From 32 surveyed sites, this study selected 14 that were similar to each other with respect to being appropriate *R. aurora* breeding sites. These 14 sites reflected a range of conditions with respect to connectivity in upland forest habitats and this appeared to be reflected in the *R. aurora* breeding effort, represented by egg mass counts. Breeding effort is proportional to the population and the population levels reflect accessibility to appropriate habitat. Egg mass counts can therefore be used to gauge the levels of upland habitat connectivity.

This study found statistically significant relationships between the size of the primary forest patch and the presence of busy roads within .25km of the breeding wetland. The larger the forest patch is and the more it surrounds the site, the more amenable upland habitat *R. auroa* populations have at their disposal that is directly connected to the places that they breed in. Crossing roads pose a substantial risk to *R. aurora* and other anurans so the closer a road is to a breeding site, the more it fragments the landscape for *R. aurora* breeding in the wetland.

This study also observed relationships with other connectivity measures although it only analyzed them qualitatively. 1) The higher the proportion of edge on the forest patch, the lower the level of habitat quality within forest patch. Small primary forest patches or those with very circuitous shapes had lower egg mass counts. 2) Non-forested areas such as utility easements and maintained prairies adjacent to the breeding site may limit *R. aurora* breeding effort, even if the breeding site is in the middle of a broad forest area. 3) The broader regional land use objective appears to have an effect on *R. aurora* populations. Urban areas have lower populations, even if the forest patch is comparatively large. Higher populations are associated with: 1) protected wilderness, 2) large areas of preserved second growth and 3) landscapes that are strictly devoted to timber production. These three relationships often overlapped with *other* relationships but I speculated what factors were influencing *R. aurora* populations given the knowledge reflected in the literature and what I observed within the selection. For example, Site 11 was located at the edge of a landscape devoted to timber production and had one of the highest egg mass counts in the selection. It also was bordered on the north by a pasture and was connected to a primary forest patch that was much smaller than sites within the selection that had between 300-387 egg masses, two factors that are inconsistent with such a high egg mass count. This led me to speculate that the sparsely-populated landscape in the Cascades reserved primarily for timber production was more conducive to *R. aurora* populations than rural mixed use landscapes with a greater human presence.

Part 2: Recommendations for Further Research

Habitat Use

A study with similar objectives to this one should be undertaken with a greater sample size, more narrow selection parameters and analyzed with GIS methods. Marc Hayes, amphibian expert with WDFW has recommended that a selection of 30 sites, selected from an original set of 50 wetlands, would be ideal but that ’20 would work’ (Hayes, personal communication). A larger sample size would likely result in more robust results and enable us to make better generalizations about *Rana aurora* habitat requirements. I would also recommend that the selection focus on the Puget Sound Lowlands and *not* include sites in the National Parks and areas devoted to logging in the Cascades. These landscapes each have variables that the Puget

Sound lowlands lack. National Parks have old growth forests and natural barriers. Logging landscapes are characterized by vast areas that are uninhabited by people, only have a few paved roads and are covered in a patchwork of different age classes of trees.

A ‘least cost’ analyses with GIS should be used to analyze the results. Such an analysis would account for all of the land covers and the different metrics associated with connectivity such as edge effects, and summarize all of them into how appropriate the area is for a given species. Molly Mathias-Levitt has already conducted a least cost analysis for King County, and the Bear Creek Basin in particular (Mathias, 2006) but without field data. It would be valuable to obtain breeding effort data for a selection within King County and observe how the results correspond with Mathias least cost map.

Conduct a Similar Study in the Cascades and Olympic Ranges

R. aurora range also includes lower elevations of the Cascades and Olympics as well as the lowlands between Olympia and Portland and lower mountains such as the Willapa Hills. A study similar to this one in these areas would provide data on how *R. aurora* is doing in landscapes that are relatively uninhabited and devoted to timber extraction and wilderness protection. Such a study could provide insight on forest management and possible perspective on how the species is doing in the more populated Puget Sound Lowlands.

Conduct Genetic Research to Determine Possible

R. aurora populations may be impacted over the long term if the forest patch that they occupy is too isolated to allow for genetic exchange with other populations outside the patch. Because of the embeddednes of human settlement and infrastructure in the Puget Sound Lowlands, *R. aurora* populations depend on habitat islands of varying sizes and varying levels of connectivity with other habitat islands. Research on. The level of habitat fragmentation that leads to inbreeding would long term conservation strategies for *R. aurora*.

Conduct Research on Road Impacts

While it is generally assumed that roads cause a sizeable impact on amphibian populations, only one study, Beasley (2002), that directly addresses road impacts has been conducted in the Pacific Northwest. Much of the work has been done in southern Ontario, Canada by Lenore Fahrig and fellow researchers. A greater understanding on how *R. aurora* are able to negotiate a variety of roads would provide more information on how they can survive in heavily impacted landscapes. Site 11 would be a good subject for this study since it is a strong breeding area and Stringtown Road (450 ADT) is within 200 meters of much of its southern end.

Use Radio Telemetry to Research Habitat Preferences and Migration Distances

Although radio telemetry research is expensive, a clearer picture of how far *R. aurora* typically travel is still needed. Such information would better inform researchers and policy makers on what scale *R. aurora* travel. It would be especially valuable to gain more information migration in ‘rural mixed use areas’. This land cover type is at the fringe of exurban development. Up to this point, radio telemetry work has been done on animals in timber production landscapes.

Use GIS to Inventory Forest Patches in the Puget Sound Lowlands

This research will be valuable for forecasting habitat levels in the future. Such an analysis should take into account whether or not areas are on private timber land: it is possible that market conditions will lead to logging on these lands at roughly the same time and therefore markedly reducing patches of mature forests. This analysis should also take into account whether or not breeding wetlands are adjacent to patches of mature forest. This question would suggest areas for wetland creation. A third aspect of this analysis would be to determine if appropriate *Rana aurora* breeding habitat exists within or adjacent to these forest patches. This information would be helpful for selecting wetland mitigation and restoration sites.

Part III: Management Recommendations

Background

As stated in Chapter 1, *R. aurora* occupies a range extending from the northern California Coast to in British Columbia. In western Oregon and Washington, they inhabit areas from sea level to 1100m (3400 ft) elevation. Although populations should be monitored, *R. aurora* may be more impacted by urbanization and fragmentation within the parts of its range that are closer to cities than areas that are dedicated to timber extraction and wilderness protection. Within this range, the Portland-Eugene metropolitan area, Olympia to Everett metropolitan area and southwest British Columbia are currently highly populated and will continue to grow in population. Rural areas near these population centers will also become more populated.

The Puget Sound Lowlands are expected to increase by 1.5 million people by 2020 and this poses a significant threat to the abundance of *Rana aurora* within the exurban fringe (Shuett-Hames, 2006). Forest patches of varying sizes dot the Puget Sound Lowland landscape. Some of these forest patches are preserved for the sake of watershed protection, public recreational land, conservation easements and conservation trust lands. Others are on private forest land and land that could be converted to housing developments. The continued existence of these forests is therefore more tenuous.

The state of Washington has enacted the Growth management Act (GMA) to a) enhance established cities and towns and b) to facilitate the rural industries such as logging and agriculture and c) to preserve important natural resource areas. The GMA mandates that local jurisdictions establish codes to promote these three objectives. GMA codes that protect aquatic areas by mandating that they have buffers and that impacts to them be mitigated will continue to ameliorate impacts to *R. aurora*. Additionally, GMA laws that encourage forestry and seek to concentrate additional residential and commercial development in urban growth areas will benefit *Rana aurora* populations in the Puget Sound Lowlands. *Transfer of development rights* programs allow rural land owners to sell their development rights to developers of urban areas are currently being practiced in King County and to a lesser extent Pierce and Snohomish Counties because the demand is the strongest in those areas. TDR programs will also benefit *R. aurora*.

While GMA legislation is beneficial to *R. aurora* population persistence, we must realize that it is not enough. Private land owners still have the opportunity to develop forest lands to some extent in unincorporated areas under GMA so more deforestation will continue to occur. Additionally, although GMA mandates the preservation of lands for forestry market conditions may result in harvests in roughly similar time frames. More research is required on private forest lands in the Puget Sound Lowlands and the expected timeframes that they will be harvested. Growing cities will result in increased transportation *between* cities and this will result in increased traffic volumes, require the construction of more roads and the expansion of some existing roads. Road impacts are therefore likely to increase.

In addition to concerns about the future availability of forest lands and increased road impacts, the fact that *R. aurora* will be subjected to surviving on an array of habitat islands in the Puget Sound Lowlands may have long term ill effects. The isolation could result in inbreeding. In bread individuals have been shown to be smaller, more lethargic, less resistant to many threats, and less able to reproduce. The risk of inbreeding would increase with the degree of isolation.

Conservation Recommendations

Recommendations for *R. aurora* management are multidisciplinary; take into account the most prescient needs and the realities of an increasing human population and changing landscape. Many of these recommendations require further research and are thus linked to the previous section.

<u>Continue to Conserve Aquatic Habitat</u>

The GMA's mandate to conserve and protect critical areas is important for one aspect of *R. aurora's* life cycle and should thus be continued. Through buffers and mitigation, Critical Areas Laws help to maintain water quality and regulate flows. Wetland typing --commonly employed in consulting for determining wetland regulations-- enables the land owner and municipal employees to realize how surrounding landscapes would be amendable to wildlife passage.

Promote Compact Human Communities and Minimize Road Development

Laws designed under this paradigm including GMA measures and TDR programs -- will encourage the preservation of larger tracks of landscapes that are relatively amenable to *R. aurora*. This thesis and other research suggest that larger patches of upland habitat contribute to larger populations. The Growth Management Act seeks to concentrate development in or just outside of established cities and towns. Urban areas are some of the most hostile landcovers to *Rana auora* and other amphibians so it is better to concentrate human housing and commerce in these areas and stem the tide of development in more rural areas. This would have the added benefit of slowing the construction of roads throughout the countryside and stemming the increase in traffic flow on at least some existing roads.

The GMA will also help to preserve wetland habitat, forests that surround wetlands and 'greenbelts' within urban areas. Protecting the amphibian aquatic and upland habitat within urban areas would contribute to the gene pool of these species throughout their range and enable urban amphibians to provide urban wetlands and forests with their ecological services as well as educational opportunities for urban residents. Such populations may run the risk of becoming geographically isolated, however, so artificially supplementing them with animals or egg masses from neighboring areas may be required to maintain genetically healthy populations.

Transfer of development right (TDR) programs should continue to further channel development into established urban areas while financially benefiting rural landowners. These programs have been established in 5 of the Puget Sound's most populous counties. The King County TDR program is currently the most active, due to King County having the most demand for housing. Hopefully the continued influx of new people will bring TDR programs into wider use.

Creation and Restoration of Aquatic Habitat

Freshwater wetlands with appropriate amphibian habitat attributes should be established in appropriate places. Many of these wetlands were lost in the decades that preceded the CWA. Some were lost due to the chanellization of rivers while others were drained and filled to facilitate agriculture and urban development in river valleys and deltas. The Puget Sound lowlands features large blocks of forest bordered by urbanized areas that had wetlands at one time, a prime example being Tiger and Cougar Mountain areas adjacent to Bellevue, Newcastle and Issaquah. Wetlands with seasonal and semipermanent hydroperiods and emergent vegetation should be established near these large forest blocks so that amphibian populations can take advantage of these forest reserves and shape their ecology with their ecosystem services. Large forests patches with only one or a few appropriate ponds would also be good locales for additional ponds so that metapopulation structure can be enhanced.

Rannap and others (2009) recommend establishing new wetlands in clusters and adding wetlands to areas where there are just a few natural wetlands. Fostering hydroperiods that are short enough to discourage fish presence yet long enough to allow for amphibian larval development is currently a challenging goal due to limited scientific results. Creating clusters of wetlands with each wetland in a cluster being designed to have a different hydroperiod and plant community, has increased chances of the probability of the 'right' hydropriod and plant community being present within the cluster for a given species. If a variety of hydroperiod and plant community conditions are made available in one area, each of the native amphibian species can utilize the breeding habitat that it is best suited for (Rannap et al. 2009).

'Wetland mitigation' is a well established societal endeavor for creating, enhancing and restoring wetlands and natural areas adjacent to them, but the success rate of this process needs to be raised substantially. It is generally carried out (or supposed to be carried out) by developers who are proposing impacts to wetlands or their buffers and need to fulfill permit requirements for doing so (Hough and Robertson, 2007). The Clean Water Act, GMA and shoreline management (SMA) all require wetland mitigation if wetland functions and values are impacted by a project. The three types of compensatory wetland mitigation are 1) on site projects, 2) in lieu fee programs and 3) wetland mitigation banking. Since the 1970s, on site mitigation has been the most common strategy, but this has resulted in failure over half the time (Murphy et al. 2009; Johnson et al, 2004). In lieu fee programs allow the developer to compensate for proposed impacts by paying a fee to a government agency which in turn puts these funds toward mitigation projects (Ecology, 2006). Under the approval of government agencies, Wetland mitigation banks sell credits to developers applying to do things that impact wetlands so that they can fulfill permit requirements. The banks can sell additional credits as their projects gain success. Washington State has established WAC 173 700 to standardize mitigation bank creation. At the time of writing, Washington State has twelve banks operating and five under review by the Department of Ecology (DOE, 2012).

Both in lieu fee programs and banks allow regional planners and ecologists to coordinate mitigation efforts that compensate for wetland losses most appropriately and address regional needs (such as amphibian breeding habitat). Wetland mitigation banks have the added benefit of using market forces to encourage mitigation success. The more traditional on site approach is still widely used. On site projects are successful when sufficient knowledge about site hydrology guides design, when goals and objectives are realistic, and when sufficient monitoring and maintenance is undertaken (Ecology, 2006). Hopefully wetland mitigation can be harnessed to establish new breeding sites in appropriate places.

All land development codes mandate the establishment of stormwater ponds and amphibians have been shown to utilize them for breeding (Ostergaard et al. 2008). It is important that such ponds have vegetation structures that facilitate egg mass establishment,that they have hydroperiods that allow for larval development yet inhibit occupancy by fish and that surrounding areas have sufficient levels of forest habitat and functional connectivity. It would be beneficial for ecologists to identify existing stormwater ponds that, because of surrounding landscape, serve as good breeding sites. Once this is known, they could be modified and managed. It would also be beneficial for ecologists to work with transportation agencies to identify proposed stormwater ponds that have potential for complementing large forests blocks as breeding sites. Such sites could therefore be designed to encourage amphibian use.

<u>Establish and Protect Upland Habitat</u>

In landscapes that are near urban areas and that are subjected to conversion to suburban or urban land use, cluster zoning should be enacted to consolidate housing. While cluster developments are a good tool, it is best to leave conservation areas physically connected to other natural areas as opposed to establishing ‘habitat islands’ in the middle of developed areas (Abercrombie, 2004). Establishing conservation easements is an even better strategy because planners can identify broad areas that are particularly good habitat attributes and designate them as protection zones.

R. aurora are capable of crossing many land covers but the most preferable are either mature or old growth forests (Haggard, 2000; Chan-McCleod, 2004). National forests and national parks occupy interior areas of the Cascade and Olympic Ranges and this includes areas that are low enough to be considered *R. aurora* habitat. Blocks of state forest lands are distributed in lower elevations of the major mountain ranges.

Encouraging large interconnected blocks of mature forests, leaving islands of trees in clear cuts, and attempting to keep clearcuts smaller and maintaining connectivity between forest and appropriate breeding wetlands would all greatly improve habitat for *R. aurora* on timber lands. Forest lands in the lower elevations of the Cascade Ranges, Olympic Range and most of the Willipa Hills and Black Hills are generally distributed between large timber producers and small forest land owners. There is little that can be done to encourage or mandate *R. aurora* habitat conservation measures among private foresters, largely because *R. aurora* is not threatened or endangered under the Endangered Species Act.

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Appendix A

Land cover Class	**Description**	**Friction Cost**
Non-forested wetland	Wetlands associated with open water	5
Deciduous & Mixed Forest	>80% Deciduous Trees, or 10-90% each Deciduous and Coniferous Trees	5
Coniferous Forest	>80% Coniferous Trees	5
Small Open Water	Small lakes, small reservoirs, small streams	20
Regenerating Forest	Forest replanted after logging	20
Grass	Developed Grass and Grasslands	40
Clearcut Forest	Cleared forest without significant regrowth and very dry grass	40
Agriculture	Row Crops, Pastures	50
Cleared for Development	Cleared Land	50
Shoreline	Marine Shoreline	50
Light Intensity Urban	20-50% Impervious Area	60
Local Roads	Not designated as arterials	60
Medium Intensity Urban	50-80% Impervious Area	80
Collector Arterials	Collectors that serve very little through traffic and serve a high proportion of the local traffic	80
Heavy Intensity Urban	>80% Impervious Area	Barrier: Infinite
Large Open Water	Large lakes, large rivers	Barrier: Infinite
High Traffic Roads	Freeways, Principal arterials, Minor arterials. Serve " through traffic" and are busy roads	Barrier: Infinite

Table 7 Rana aurora Friction Values for Different Land Covers

Appendix B

Landowner Contact Letter Template

Chris Holcomb
-Address-
Olympia, Washington 98502
December 28, 2010

[Name}
[Address}

Dear _______:

I am writing you because I would like to ask your permission to briefly enter your property (on 123rd Avenue SE, Yelm) on a few days this winter and spring for the purposes of ecological research. I am a student in the Masters of Environmental Studies program at the Evergreen State College and am doing research on two of our native amphibians, the Red Legged Frog and the Northwestern Salamander. I am studying the breeding activity of these two animals on 30 wetlands throughout Thurston County so that I can get some idea of how the level of development around the wetland affects breeding. After having had worked in the wetland consulting field for years, I can attest that if I were allowed on your land to conduct this research, it would *not* result in any additional constraints on what you can do on your property.

Why your property? I am interested in including a wetland on your property in my research because it is the appropriate size and type. I learned about your wetland by studying the National Wetland Inventory website's 'Wetland Mapper' feature. This information was generated from satellite infrared photography. The Thurston County Geodata website provided me with property ownership information.

What is involved in me coming on your property to look at your wetland? First, I would like to visit the area to make sure that it is good habitat and appropriate to include in my study. I was hoping to do a visit at whatever time is permissible for you from January 22 through the 30th. If it seems like good habitat, I would like to visit again anytime from March 26th to April 3rd to make sure that these two animals are actually breeding in the wetland. I would ascertain this by wading through the wetland and looking for their egg masses. It is possible that I or someone else would be interested in visiting your property a few times in 2012 and actually counting the egg masses, but this is uncertain at this point. Of course, I would be more than happy to make arrangements with you on when would be a good times to visit. If these dates are not good, I can come on other dates. I would be glad to meet you or if you would rather I can just come and do what I have to do without bothering you. I can follow any important instructions that you may have such as shutting livestock fences. If you are renting the land out, I would be more than happy to communicate with tenants.

Who will get this information? The results of this study will be contained in my master's thesis and possibly a scientific journal article. Neither document will include property ownership information, wetland categories, ratings, or buffer widths. My research would not provide any additional information to government agencies about your property: a master's thesis is *not* a valid document for permit applications. Amphibian activity does not affect wetland buffer widths. Finally, everyone already has access to the websites that I mentioned, so the wetland is already known to the world.

I hope that you will grant me access to your property a few times this winter and spring. Again, I would be glad to meet you, notify you in advance of my visit and follow any special instructions. I would also be interested in any information that you have on seasonal water levels in the wetland, land use history or amphibians that you have observed. I can be reached by mail at the above address, by phone at [phone number] or by e-mail at [email address]. I would really appreciate knowing your thoughts on this by January 10th 2011, but feel free to contact me at your convenience.

Thank you very much for your consideration.

Sincerely,

Chris Holcomb
Evergreen MES student