Movement, Habitat Use, and Dispersal of Juvenile

Oregon Spotted Frogs (Rana pretiosa)

on Joint Base Lewis McChord

by

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ABSTRACT

Movement, Habitat Use, and Dispersal of Juvenile Oregon Spotted Frogs (Rana pretiosa) on Joint Base Lewis McChord

John Richardson

The dispersal, habitat use and movements of juvenile R. pretiosa on Joint Base Lewis McChord (JBLM) were monitored using radio telemetry (telemetry). Telemetry occurred in four sessions. In the first fall 2008 session, six frogs were tracked for 11 to 25 days (\bar{x} =16.66) from 22 September to 17 October. In the second session ten frogs were tracked for 14 to 25 days (\bar{x} =22.7) from 14 November to 9 December. In spring 2009 12 frogs were tracked from 4 April to 17 June for 9 to 70 days (\bar{x} = 35.25). In fall 2009 12 frogs were tracked for 13 to 34 days (\bar{x} =23.33) from 29 October to 2 December. Movement of juvenile frogs was influenced by habitat conditions. Aquatic connectivity during the spring allowed for frogs to make larger moves. Average daily distance traveled was greater in the spring than the fall (F=16.38 p=.0001). In the fall, average daily distance was not significantly different between tracking sessions despite frogs from 2009 weighing four times as much as those in 2008 and reaching near adult sizes. Further, frogs on JBLM used terrestrial habitats at a higher rate (36.5% of the time) than previously reported. Finally, terrestrial habitat use was higher during the fall (47% in 2008 and 42.45% in 2009) compared to the spring (10.9%). Increased terrestrial use is likely the result of behavior differences between adult and juvenile R. pretiosa.

Secondary objectives of this study were to improve on telemetry transmitter attachment techniques for ranid frogs and examine the impacts of frequent handling associated with telemetry on frog movements. Results suggest that 7mm silk ribbon had the greatest reduction in injury rates and allowed frogs to break free of transmitters if the frogs could not be recovered and the transmitters removed by the investigator. Impacts of handling include the observation that frogs moved further following capture (p=.0067); this increased movement may have influenced data on habitat use and dispersal.

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Introduction

Historically, the Oregon spotted frog (Rana pretiosa) inhabited shallow warm water wetlands from northern California to southern British Columbia and was widely distributed throughout much of the lowlands of the Puget Trough and Willamette Valley. Over the last 150 years, declines in quantity and quality of habitat have led to the loss of R. pretiosa from 78% to 90% of its historic range (McAllister and Leonard 1997). Populations of R. pretiosa at the edges of its range and those that inhabited the lower elevations, such as the Puget Trough and Willamette Valley, have been most heavily impacted (Pearl and Hayes 2004). R. pretiosa have been extirpated from the Willamette Valley as well as Northern California and only one population remains in the Puget Trough (Pearl and Hayes 2004; McAllister and Leonard 1997). Range-wide declines have led to legal protections for R. pretiosa under federal, state and provincial law. R. pretiosa is listed as an endangered species in the state of Washington and is a candidate for listing under the federal Endangered Species Act (ESA). Canada, Oregon and California also provide legal protections to R. pretiosa under their respective endangered species laws.

Compliance with legal protections and the concern over the future viability of *R. pretiosa* populations have led to an increase in research into the ecology and habitat use of *R. pretiosa* over the last 15 years. Despite this increase in research, much is still unknown about the ecology of *R. pretiosa*. Much of what is known about the ecology and habitat use of *R. pretiosa* comes from studies conducted in British Columbia during the seventies and eighties (Lict 1974; Lict 1986a; Lict 1986b). More recent studies have

been conducted on the habitat selection and movements of *R. pretiosa* in Oregon and Washington, however these studies are focused on adult behavior and habitat needs (Watson et al 2003; White 2002; McAllister et al 2004). The focus on adult *R. pretiosa* has left a gap in the knowledge of juvenile behavior and habitat needs that limit recovery. Increasing our understanding of the habitat requirements and behavior of sub-adult *R. pretiosa* is important for shaping future recovery of the species. By further understanding the needs of juvenile *R. pretiosa*, management strategies can be shaped to benefit all life stages and therefore have a more positive impact on recovery.

Recovery efforts in the state of Washington have primarily focused on the protection of existing populations through restoration of oviposition sites. Restoration projects have focused on removing invasive vegetation such as reed canary grass (*Phalaris arundinacea*) and establishing consistent water levels during breeding to prevent stranding of egg masses. More recent efforts have focused on the establishment of new populations of *R. pretiosa* within its historic range. In 2007 Washington created an Oregon spotted frog recovery team consisting of state, federal, and private organizations to help establish and reach recovery goals. In 2008, the recovery team started a captive rearing and reintroduction program in order to establish new populations of *R. pretiosa*. In October of 2008, the first of five planned releases of captive reared *R. pretiosa* occurred at Dailman Lake on Joint Base Lewis McChord (JBLM). Monitoring of released frogs was begun as part of the reintroduction program. In 2008 and 2009, radio telemetry (telemetry) was used to monitor dispersal,

movements, and habitat use of juvenile *R. pretiosa*. Transmitter attachment methods and the impact of frequent handling of frogs were also studied.

Telemetry was selected for monitoring frogs post-release because it offered numerous advantages to other monitoring techniques, such as visual encounter surveys or the use of passive integrated transponders (PIT tags). Telemetry has two key advantages over other methods of monitoring: the ability to individually identify study animals without capture and the ability to reliably relocate study animals.

Prior telemetry studies have focused on adult *R. pretiosa*, because of the need to maintain a transmitter to bodyweight ratio of less than 10%. Transmitters available in the past were too heavy to attach to juveniles, but recent advances in technology have produced transmitters that weigh approximately half a gram allowing them to be attached to juvenile *R. pretiosa*. These smaller transmitters make it possible to gather information on movements and habitat use of sub-adult *R. pretiosa*. Information gathered from telemetry will be used to help guide future management of *R. pretiosa* on and off JBLM as well as aid in selecting future reintroduction sites.

While telemetry has many advantages over other monitoring techniques, the impact of the transmitter attachment is a major weakness. The delicate skin of ranid frogs can make safe telemetry particularly difficult. Improperly attached transmitters can cause severe injury and even death. Currently, an insufficient body of literature exists on transmitter attachment methods for ranid frogs making it difficult to select an appropriate attachment method. Many studies involving telemetry either fail to describe the attachment technique in detail or fail to report results on how successful

the selected attachment method was in preventing injuries or dropped transmitters. In an effort to expand the knowledge on transmitter attachment methods for ranid frogs, three attachment methods were used during telemetry on JBLM and injury rates were recorded.

Due to the delicate nature of the skin of ranid frogs, great care must be taken to prevent injury from transmitter attachments. The need to prevent injury led to frequent capture and handling of study animals. Little is known about the impact frequent handling has on the behavior of transmittered individuals. Studies of amphibians have found that habitat selection is influenced by many factors including the need for protection from predators (Bloomquist and Hunter 2010). If frogs perceive researchers as predators, it is possible that habitat selection will be different for those study animals. If capture and handling influences the behavior of transmittered frogs, it is possible that data gathered via telemetry are biased. During the fall of 2009, in an effort to shed light on the impacts of frequent handling on movements of transmittered frogs, data were gathered on the daily distance traveled following capture and compared to the daily distance traveled without capture.

Background

Global Amphibian Decline

Researchers across the world have reported rapid, large- scale declines in amphibian populations. The first reports of large scale amphibian declines surfaced during the late 1980's, although many declines began in the 1970's (Blaustein and Wake

1990). Recent publications (IUCN; Kriger and Hero 2007; Lips et al. 2008) give ample evidence of worldwide decline: 1) one third of the world's 6,140 amphibian species are classified as threatened, and 43% of all species are in decline; 2) there is little or no evidence that populations have begun to rebound from recent losses; 3) currently less than one percent of amphibian species are increasing. In many cases, declines have been extremely rapid with populations collapsing in a matter of months (Lips et al. 2006). In the last 50 years, rates of amphibian extinctions are up to 97 times higher compared to the previous 500 years (Kriger and Hero 2007B). The relatively compressed time scale of global declines coupled with the rapid increase in extinction events is unprecedented in current ecological history (Collins and Storfer 2003).

The loss of amphibians will have far reaching ecological impacts on the ecosystems they inhabit. In some ecosystems, larval amphibians make up the majority of herbivores and their loss could lead to increased algal growth. An increase in algal growth could in turn lead to eutrophication and hypoxia. Loss of amphibians will also alter higher trophic level food webs. Many adult amphibians are major predators of insects, and amphibians provide an important food source for many other species in the ecosystems they inhabit (Johnson 2006).

Amphibians also serve as indicator species for the overall health of ecosystems. In general, amphibians are highly sensitive to changes to their environments making them excellent indicators of increased environmental stresses including pollution and habitat degradation (Blaustein and Wake 1994). The global collapse of amphibian

species has led many researchers to question the overall health of the biosphere and to fear that amphibians are just the first of many genera to suffer declines.

In the United States, 21% of the 272 native species of amphibians are threatened or extinct. Declines have been most severe in the western part of the USA. In the Sierra Nevada of California, a 98% decrease in populations of yellow legged frogs (*Rana muscosa*) has been reported between the mid 1970's and late 1980's. Other amphibian species in the west have undergone similar declines. In Oregon, 80% of the thirty populations of cascade frogs (*Rana cascadae*) that have been monitored since the mid 1970's were extirpated by 1990 (Blaustein and Wake 1990).

Declines are driven by a multitude of factors that are species and context dependent (Collins and Storfer 2003; Rollins-Smith and Conlon 2005). Causes of amphibian decline can be grouped into six major categories, including habitat alterations and destruction, overexploitation, impact of exotic species, global environmental changes, chemical exposure, and emerging infectious diseases (Collins and Storfer 2003). A graph of the major causes of global amphibian decline can be found in Figure 3. As can be seen from the graph, habitat loss has by far the largest impact on amphibian species declines followed by pollution. Disease only accounts for a small portion of the overall impact on amphibian species, although the majority of species affected by disease are also threatened.

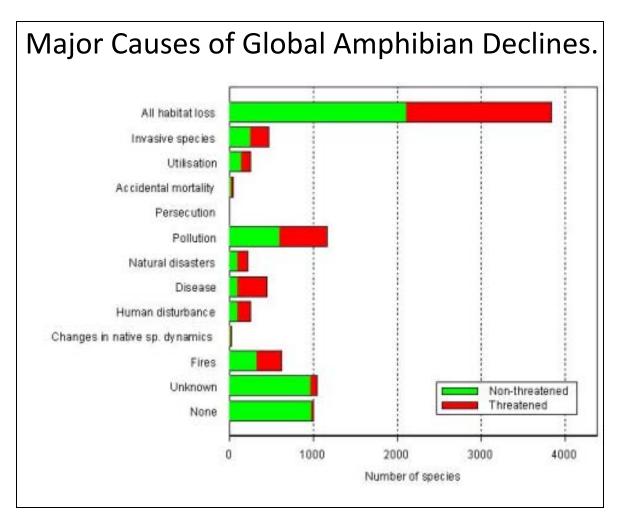


Figure 1: Major causes of global amphibian decline and the number of species affected by each cause. Threatened species are represented by the red portion of the graph. Source IUCN http://www.iucnredlist.org/initiatives/amphibians/analysis/major-threats

Decline of Oregon Spotted Frogs

R. pretiosa has been extirpated from between 78% and 90% of their original range. Much of this decline is the result of habitat loss and fragmentation. Over the last 150 years, much of the habitat used by spotted frogs was converted to agriculture or developed. This is especially true of lowland habitats such as those found in the Puget Trough (Pearl and Hayes 2004). R. pretiosa rely on early successional wetlands. Many of these wetlands have been altered through human activity and natural succession, both

of which reduce available habitat. European settlers diked and drained many emergent wetlands that provided ideal habitat for *R. pretiosa* for use in agriculture and urban development. Not all habitat loss can be attributed to human activity; natural succession of wetlands from emergent to scrub shrub has led to a decrease in quality and quantity of available habitat for *R. pretiosa* (McAlister and Leonard 1997). Invasive species, both plant and animal, also play a role in the reduction of *R. pretiosa* through increased predation, competition and changes in habitat structure. The extent of the impacts of invasive species on populations of *R. pretiosa* is unclear.

In the late 1990's and early 2000's, populations of *R. pretiosa* in Washington exhibited multi-year declines in the number of egg masses laid. These declines led biologists to become increasingly concerned about the continued viability of *R. pretiosa* in Washington. At Conboy Lake, the largest known population of *R. pretiosa* in Washington, the number of egg masses plummeted from just over 7,000in 1998 to slightly under 1,500 in 2002 (USFWS 2010). Furthering the concern over declines of *R. pretiosa* was the fact that specimens collected at Conboy Lake following an observed die off of frogs tested positive for the amphibian chytrid fungus *Batrachochytrium dendrobatidis* (Bd). Subsequent testing found Bd at the three known *R. pretiosa* sites in Washington. The presence of Bd at all sites led to concerns that Bd was the proximate cause of declines throughout the region and that Bd would drive *R. pretiosa* to extinction. Concerns over Bd are now being reduced after preliminary research indicated that *R. pretiosa* from Conboy Lake are very tolerant of Bd exposure and are capable of shedding the infection (Padgett-Flohr and Hayes 2011). Despite these test

results, it is possible that Bd caused the die offs of *R. pretiosa* observed at Conboy Lake leaving only resistant animals to test. Even if Bd caused the die off at Conboy Lake, the results of Padgett-Flohr and Hayes (2011) provide hope that the remaining resistant frogs will rebound.

While there are many contributing causes of recent declines, the specific cause of recent decline is not yet known. It is likely that all listed impacts have played some role in recent population declines of *R. pretiosa* in Washington.

Conservation Status

The Oregon spotted frog has suffered significant population declines throughout its entire range resulting in legal protections for the species in all three states where it occurs and in Canada. *R. pretiosa* is designated as Sensitive-Vulnerable in Oregon, State Endangered in Washington, Species of Special Concern in California, and Endangered in Canada.

In 1993, prior to splitting the spotted frog complex into distinct species, the United States Fish and Wildlife Service (USFWS) determined that four distinct populations of spotted frogs within the larger spotted frog complex warranted listing under the Endangered Species Act (ESA). Included in these sub-populations was the pacific coast sub-population, later given full species status as *R. pretiosa*. Despite the determination of a need for listing, *R. pretiosa* was precluded from listing due to higher priority species and lack of funding (McAllister and Leonard 1997). In 1997, following the elevation of *R. pretiosa* to full species status, a USFWS review of *R. pretiosa* concluded that listing under the ESA was still warranted but once again was precluded due to lack

of funding and other higher priority species. Currently the Oregon spotted frog remains a federal candidate species.

Current Conservation Actions:

Conservation actions are underway throughout R. pretiosa's entire range. Much of the conservation efforts for R. pretiosa have been focused on habitat restoration and captive rearing. Captive rearing projects to augment existing populations as well as establish new populations are part of the R. pretiosa recovery plans for Washington and British Columbia. Captive rearing projects collect eggs from the wild and rear them through metamorphosis after which they are released. Rearing protocols differ between the two countries. The differences are focused on how to deal with predation on released frogs. In Washington, size is favored over numbers while the opposite is true in Canada. In Washington frogs are exposed to elevated temperatures in an effort to head-start their growth before release. This head-starting, which often produces adultsized frogs by the time of release, is designed to help reduce predation on newly released individuals by reducing the number of predators capable of consuming released frogs. In Canada, frogs are reared without artificial heat and therefore are much smaller at release. The lack of heat reduces husbandry costs and allows for greater numbers of frogs to be reared which compensates for increased predation rates (Andrea Gielens personal communication 2010). In addition to this difference in rearing protocols, the two programs differ with respect to where captive reared frogs are released. In Canada, captive reared frogs are used to augment existing populations, while in Washington efforts are directed towards establishing new populations (Andrea

Gielens personal communication 2010). Biologists in Washington believe that expanding the number of localities with *R. pretiosa* reduces chances of regional extirpation. The captive rearing program in Washington is entering its fourth year with a total of 2,363 frogs released into Dailman Lake and Muck Creek on JBLM. Releases on JBLM are expected to continue through 2012, at which time the project will be assessed and the need for additional releases will be determined. If additional releases are not needed, then a second release site will be selected. In the spring of 2011, biologists located 11 *R. pretiosa* egg masses at JBLM which represented the first signs of breeding as a result of the project. The fact that breeding has occurred at the first reintroduction site is a positive sign. Conservation partners are thus cautiously optimistic that reintroduction efforts have been successful.

Substantial effort has been put into *R. pretiosa* recovery throughout its range. In recent years, populations of spotted frogs in Washington have shown signs of recovery with egg masses trending upward for all three populations where long term monitoring has occurred; however, none of the populations have reached the peak pre-crash levels.

Geographical Distribution

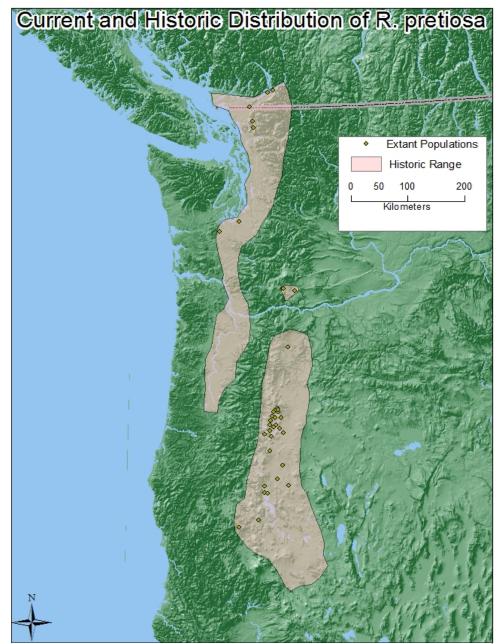


Figure 2: Historic and Current Distribution of *R. pretiosa*. Data are from Cushman and Pearl 2007. and McAllister and Leonard 1997.

Historically *R. pretiosa* ranged from Southwestern British Columbia to

Northeastern California and was widely distributed throughout the *Puget Trough* and *Willamette Valley* (Figure 2).

Habitat loss and fragmentation has resulted in *R. pretiosa* being lost from between 78% and 90% of their historic range (McAllister and Leonard 1997). The literature on *R. pretiosa* gives varying numbers of current locations ranging from 33 to 43. The range in the number of locations of *R. pretiosa* is caused by how breeding sites are counted. Some publications combine sites based on what are believed to be populations while others include all known breeding sites (Pearl et al 2009, White 2002, USFWS 2010). While the exact number of extant populations of *R. pretiosa* is unclear it is known that the majority occur along the Cascades in Central Oregon. As much as two thirds of known populations of *R. pretiosa* are located along the cascades in Central Oregon (Pearl et al 2009). The number of locations for *R. pretiosa* is difficult to determine because increased survey efforts have found several new populations in recent years that are not reflected in this total.

Currently, five populations of *R. pretiosa* are known to exist in Washington, two of which were discovered in 2011. Populations of *R. pretiosa* are known to occur along the Black River in Thurston County, at Trout Lake and Conboy Lake in Klickitat County and within the Nooksack and Samish watersheds in Whatcom County. The discovery of *R. pretiosa* in Whatcom County is the result of increases in survey efforts that led to the discovery of four oviposition sites. These four sites represent two additional populations, but the exact size of these populations has yet to be determined (Jennifer

Bohannon personal communication 2011). Early signs indicate that a sixth population may have established on JBLM as a result of re-introduction efforts. Despite these recent positive trends, populations of *R. pretiosa* in Washington remain in a perilous position. The majority of *R. pretiosa* occur at three sites which makes them vulnerable to stochastic events and extirpation.

Taxonomy and Genetics

R. pretiosa is a member of the order anura, the family ranidae and the genus Rana, or true frogs. The genus Rana incorporates the majority of North America's larger frogs (McAllister and Leonard 1997).

R. pretiosa was first described in 1853 by Baird and Girarad from specimens collected near the site of Fort Nisqually (McAllister and Leonard 1997). When first classified taxonomically, the species Rana pretiosa included what are now three distinct species, Oregon spotted frog (R. pretiosa), Columbia spotted frog (R. luteiventris) and Cascades frog (R. cascadae). The first of these species was separated from R. pretiosa in 1939 by Slater when he described the cascade frog (R. cascadae). The Columbia and Oregon spotted frogs remained as subspecies of R. pretiosa until 1995 when genetic analysis was used to elevate these two subspecies to species status (Green et al 1996).

Historic references and museum samples of *R. pretiosa* can be misleading due to the above noted taxonomic changes. Further complicating the historic record is the fact that *R. pretiosa* and *R. luteiventris* are not simply subspecies that have been elevated to full species status. The work conducted by Green et al. (1996) divides the two species differently than the subspecies were divided. This confusion can make it difficult to

determine historic ranges and localities for these three species which makes it difficult to determine the exact level of declines for *R. pretiosa*.

When compared to other species of ranids, R. pretiosa exhibit low genetic diversity throughout their range. R. pretiosa likely exhibit high levels of genetic isolation because of their highly aquatic life history which limits dispersal between watersheds (Blouin et al 2010). Recent genetic analysis revealed three major groupings of R. pretiosa: the Northern group, which includes populations from British Columbia to Camas prairie in northern Oregon, the Central Cascades group in Central Oregon, and the Klamath Basin group in southern Oregon (Blouin et al 2010). The Northern group can be further broken down into four unique subgroups: British Columbia, Chehalis River, Columbia River and Camas prairie, making for a total of six genetically distinct populations of R. pretiosa throughout its range. Isolation of these six groups occurred prior to European influence and the lack of genetic diversity is not the result of habitat fragmentation caused by European settlement (Blouin et al 2010). More recently, habitat fragmentation has further isolated the majority of remaining R. pretiosa populations and further reduced genetic exchange. Meta-population dynamics only exist in three locations: the Chehalis drainage, Central Cascades and the Klamath River basin (Blouin et al 2010). The lack of meta-populations leads to an increased risk of local extinctions.

Species Description

Adult *R. pretiosa* range in size from 44 to 100mm snout to vent length, with females attaining larger sizes than males (Lict 1974; McAllister and Leonard 1997).

Individuals over 100mm are rare, but do occur. The largest *R. pretiosa* on record, a female from Conboy Lake measured 107.5mm and weighed 100.5 g (Rombough et al 2006). A study of three populations of *R. pretiosa* in Washington found that females were an average of 14mm larger than males (McAllister and Leonard 1997).

Coloration of *R. pretiosa* varies with age and location. Dorsal coloration of juvenile *R. pretiosa* ranges from olive green to reddish brown. As individuals age they tend to develop more reddish coloration on their dorsal side. Older individuals are often brick red on their backs with ragged black spots (Figure 3).



Figure 3: Dorsal view of *R. pretiosa*. Note the presence of a telemetry attachment belt around the hips.

Black spots with light centers are present on the head and back and become darker and more ragged edged in appearance with age. Dorsal lateral folds are present and extend from the eye approximately half way down the back. *R. pretiosa* have a white to tanish

throat and chest which transitions to red on the belly and legs (Figure 4). Older individuals tend to have more extensive red colorations on their under parts (McAllister and Leonard 1997).



Figure 4: Ventral view of *R. pretiosa*: Photo by Gary Nafis Source: http://www.californiaherps.com/frogs/pages/r.pretiosa.html

Male and female *R. pretiosa* are similar in coloration and can be difficult to distinguish, especially as juveniles. Distinguishing the sexes of *R. pretiosa* becomes easier as they reach adulthood. Sexually mature males can be distinguished from females by the presence of nuptial pads on their thumbs and stouter forelimbs.

Several species of ranid frogs are very similar in appearance and have ranges which are adjacent to, or overlap with, *R. pretiosa* making field identification of individuals difficult. *R. pretiosa* are similar in appearance to the cascades frog, Columbia

spotted frog and the northern red-legged frog (*R. aurora*). *R. pretiosa* in western Washington are most likely to be confused with the northern red-legged frog due to their sympatric existence. *R. pretiosa* can be distinguished from northern red-legged frogs by the presence of full webbing on their hind feet, up turned eyes, and lack of mottling on the groin patch (Figure 5). For an extensive explanation of how to differentiate all life stages of similar species to *R. pretiosa* see McAllister and Leonard 1997.



Figure 5: Comparison of groin patch for *R. pretiosa and R. aurora. R. pretiosa* is on the right: Source Washington Herp Atlas Photo by D. Hagin

Natural History

R. pretiosa are a highly aquatic amphibian spending little time outside of wetlands. In one study using radio telemetry, frogs were found in areas without measurable water 1.4% of the time (n=295), and over land movement was only observed once (n=645) (Watson et al 2003). Populations of R. pretiosa are associated with wetlands greater than four hectares in size where some form of permanent water is present (Pearl and Hayes 2004). Habitat use by adult R. pretiosa varies with season and directed seasonal movements occur between habitat use areas (Pearl and Hayes 2004). During breeding season, R. pretiosa use shallow seasonally inundated areas while during the summer they retreat to areas of permanent water (Watson et al 2003). Overwintering generally occurs in lotic habitats or areas of ground water up-welling (Pearl and Hayes 2004). The need for permanent water greatly limits the amount of suitable wetlands within the range of R. pretiosa (Pearl and Hayes 2004). When in deeper water, R. pretiosa prefer areas with floating aquatic vegetation which provide refuge from predators as well as concealment from potential prey (Lict 1986, Pearl et al 2005).

R. pretiosa reach sexual maturity two to three years after metamorphosis with females maturing later than males (Lict 1974; McAllister and Leonard 1997). In Canada, females reach maturity at 63mm while males are mature at 46 mm (Lict 1974). R. pretiosa breed explosively during the late winter to early spring. The timing of breeding is closely correlated to water temperature so the exact timing is variable throughout their range (Chelgren et al 2008; White 2002; McAllister and Leonard 1997). Water

temperatures at time of first oviposition for populations along the Black River in Thurston country ranged from 47 to 52 degrees Fahrenheit (McAllister and Leonard 1997).

Eggs are laid in communal oviposition sites where as many as 75 egg masses are laid in a single cluster (McAllister and Leonard 1997). The average fecundity of R. pretiosa is around 600 eggs per egg mass (McAllister and Leonard 1997; Lict 1974). Males arrive at communal breeding sites first and begin calling to attract females (McAllister and Leonard 1997). The call of R. pretiosa is a soft clucking sound and has been described as similar to a woodpecker tapping on a distant tree. R. pretiosa oviposition sites are generally located along the shallow margins of seasonal ponds and stream edges. Eggs are typically deposited in water between 5.9 and 25.6 cm in depth (Pearl and Hayes 2004), but breeding has been observed on top of reed canary grass mats in areas of deep water (pers. obs). Deeper water will be used for oviposition if mats of vegetation are present that allow the frogs to place eggs in the top of the water column (personal observation). R. pretiosa use of shallow seasonal pools leaves eggs vulnerable to desiccation and freezing, making egg survival rates highly variable from year to year. In drought years, it is possible for an entire year's breeding to fail (Lict 1974). R. pretiosa appear to show site fidelity with communal oviposition sites being used for multiple years.

Eggs usually hatch within 18-30 days of oviposition, depending on water temperature. Once hatched, tadpoles remain attached to the jelly for several days

before becoming free swimming. As free swimming tadpoles, *R. pretiosa* are primarily herbivores, feeding extensively on algae.

Until metamorphosis is complete, *R. pretiosa* remain vulnerable to receding water levels. In typical years, tadpoles metamorphose 13-16 weeks after hatching. At this point, young of the year (YOY) *R. pretiosa* are capable of dispersing from seasonal pools to more permanent water bodies (McAllister and Leonard 1997). Once metamorphosis is complete, *R. pretiosa* become primarily carnivorous, feeding on insects, macro invertebrates, and other amphibians.

R. pretiosa are primarily ambush predators who feed opportunistically on available prey items. R. pretiosa have been known to eat juvenile western toads and hatchling tadpoles of their own kind (Pearl and Hayes 2002). During captive rearing, R. pretiosa were observed cannibalizing smaller individuals in their holding tanks. This cannibalistic feeding is not uncommon amongst ranid frogs.

Post-metamorphic *R. pretiosa* are known to feed on a variety of organisms including: leaf beetles (*Chrysomelidae*), ground beetles (*Carabidae*), spiders (*Arachnidae*), rove beetles (*Staphylinidae*), syrphid flies (*Syrphidae*), long-legged flies (*Dolichopodidae*), ants (*Formicidae*), water striders (*Gerridae*), dragon flies (*Odanata*) and damselfly (*Coenagrionidae*) (McAllister 1997; Pearl et al 2005).

R. pretiosa use two methods for capturing prey. Frogs will either sit and ambush prey that comes within range of attack or actively stalk prey. *R. pretiosa* have been observed stalking prey by slowly moving towards food items with only their eyes above

water (Pearl et al 2005). Frogs will also locate a prey item and dive, surfacing close to the prey. In either case, prey is attacked with a lunging motion.

Different life stages of *R. pretiosa* are predated by different organisms. Primary predators of adult *R. pretiosa* include garter snakes (*Thamnophis spp*), bullfrogs (*Lithobates catesbeianus*), great blue herons (*Ardea herodias*), green herons (*Butorides virescens*), raccoons (*Procyon lotor*), belted kingfishers (*Ceryle alcyon*), sandhill cranes (*Grus canadensis*), coyotes (*Canis latrans*), striped skunks (*Mephitis mephitis*), mink (*Mustela vison*), and river otters (*Lutra canadensis*)(Lict 1974).

Study Area:

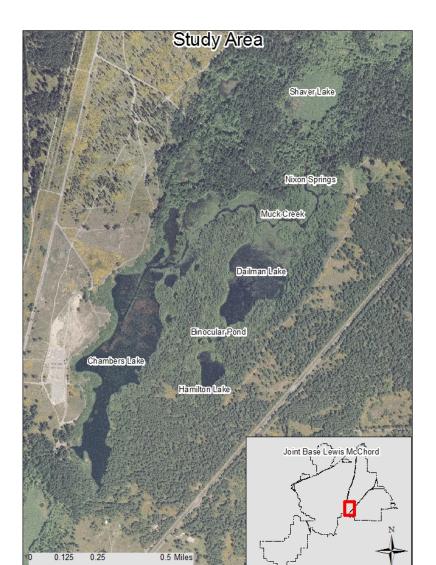


Figure 6: Radio telemetry study area on JBLM. Releases of *R. pretiosa* for telemetry occurred at Dailman Lake

Dailman Lake and associated wetlands cover approximately 300 acres on Joint Base Lewis McChord and are part of the Muck Creek drainage. The wetland complex consists of five distinct water bodies which are hydrologically connected on a seasonal basis via Muck Creek and other small channels (Figure 6). During summer, these wetlands become isolated as water levels drop. Wetlands in this complex are dominated by emergent vegetation with cattail (Typha latifolia), hard stemmed bulrush (Schoenoplectus acutus), reed canary grass (Phalaris arundinacea), pond shield (Potamogeton spp), sedges (Carex spp), rushes (Juncus spp), and Eurasian watermilfoil (Myriophyllum spicatum) accounting for the primary vegetation. Water levels in the study area have large seasonal fluctuations of several meters fed by ground water. Water levels generally begin to rise significantly in December and remain high through May before beginning to recede. Low water generally occurs in October(personal observation).

Telemetry

Remote tracking, which includes radio telemetry, is increasingly becoming a cornerstone of wildlife biology. Remote tracking allows biologists to gather information such as movements, habitat selection, home ranges, and migrations of cryptic species

that would otherwise be extremely difficult to gather (Rowely and Alford 2007). Telemetry is similar to a high-tech game of hide and seek which works on the same concept as household radios. Transmitters emit a signal that is picked up by a receiver as an audible beep. Each transmitter has its own specific frequency which allows individual animals to be identified. Individual animals are tracked by tuning the receiver to their frequency much like changing stations on a radio. Animals are tracked using directional antennas that allow researchers to determine the direction of the strongest signal. By following the strongest signal, exact locations of animals can be discovered. Like all monitoring techniques, telemetry has its weaknesses. The largest drawback is transmitter weight which limits the size of animals that can be used in telemetry studies. Research has found that to prevent negatively impacting the fitness and behavior of study animals, the weight of transmitter should not exceed 10% of the body weight of animals studied (Richards et al 1994). A second drawback to telemetry is the risk of injury to study animals. Attachment of telemetry transmitters can open sores to develop.

Methods:

In this study, habitat use, movement, and dispersal of juvenile *R. pretiosa* were monitored using radio telemetry. Analysis of movements and dispersal is based on average daily movements recorded during telemetry. Habitat use was assessed by determining the habitat type used at the time frogs were located.

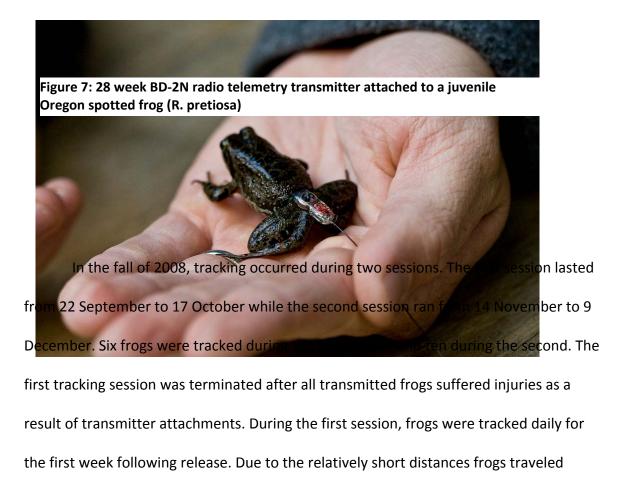
Statistical analysis

Statistical analyses were run using version 8.0 of JMP by SAS, Microsoft Office Excel 2007 and re-sampling stats add-on for Microsoft Office Excel 2007 by Statistics.com LLC.

Telemetry

During fall 2008, spring 2009 and fall 2009, juvenile R. pretiosa were tracked using radio telemetry following their release into Dailman Lake on JBLM. Telemetry was conducted by biologists from Washington Department of Fish and Wildlife and Joint Base Lewis McChord Fish and Wildlife. BD-2N transmitters from Holohil Systems Ltd (http://www.holohil.com/) were used throughout the course of telemetry. During the fall of 2008, telemetry occurred over two sessions with six frogs tracked the first session and ten frogs tracked the second. All frogs were fitted with BD-2N transmitters with a 28 day battery life. During the spring of 2009, eight frogs were fitted with BD-2N transmitters with a 28 day battery life and four were fitted with BD-2N transmitters with between 12 and 16 week battery life. In the fall of 2009, all 12 frogs were fitted with BD-2N transmitters with between 12 and 16 week battery life. A total of 40 frogs were tracked over four tracking sessions. Transmitters with a 28 day battery life weighed between .52 and .56 grams, and transmitters with a 12-16 week life weighed between 1.4 and 1.5 grams. Minimum body weight for frogs fitted with transmitters was six grams for units with 28 days of battery and 15 grams for the 12-16 week units. For telemetry on JBLM, transmitters were attached to frogs using three different materials. Attachment methods were adapted from Lynch 2006. Transmitters were attached with

silk thread, 4mm or 7mm silk ribbon. Silk was selected as the attachment material because it is a natural fiber that will break down in the environment over time allowing frogs to eventually break free of transmitter attachments if they could not be recaptured (Lynch 2006). Attachment material was threaded through a tube on the transmitter and secured around the hips of a frog using an overhand knot (Figure 7). Transmitters were secured snuggly but not tight enough to cause compression to the abdomen.



on each tracking day in order to assess belt condition and determine if the frog was still living. Mass and snout to vent length were recorded once a week.

During the spring of 2009, telemetry occurred from 8 April to 17 June. At the beginning transmitters were attached to 12 frogs. This number was reduced on 17 April to eight after it was determined that tracking 12 frogs in a day was not feasible due to time and staffing constraints. Transmitters were removed from the first four frogs captured. Frogs were tracked three days a week. Again, attempts were made to recover frogs on each tracking day in order to assess belt condition and determine if the frog was still living. Mass and snout to vent length were recorded once a week.

During the fall of 2009, 12 frogs were tracked following telemetry protocols established during the spring of 2009. Telemetry started on 29 October and did not end until 12 February. Frogs 331, 333, 336, 346 and 348 dropped transmitters within 15 days of the start of tracking. These five frogs were replaced with frogs still held at rearing facilities. These frogs were fitted with transmitters and released on 17 November. The locations of frogs that dropped their transmitter prior to 17 November were dropped from analysis of movements and number of tracking days but kept when analyzing habitat use and dispersal. An extended freeze at the beginning of December caused Dailman Lake to ice over which prevented tracking during December. Tracking was resumed in January in an attempt to remove transmitters. Data on movement from during January and February is excluded from analysis of movements. Frog 337 was dropped from all analyses because of discrepancies in the data. During fall 2009, frogs

were only handled once a week in order to record belt condition, snout to vent length, and mass.

During all telemetry sessions, attempts were made to locate all frogs each tracking day; however, this was not always possible, so some frogs were located less often. If a frog was not located on a tracking day it was tracked first the following scheduled tracking day.

For all radio telemetry tracking sessions, locations of frogs were recorded using a Trimble GEO XH hand held data logger and GPS. Locations were recorded to sub-meter accuracy

Movement

The distance moved by transmittered frogs was determined using the measure tool in ArcGIS, and recorded to the nearest tenth of a meter. All distances were measured in a straight line between locations and represent the shortest possible distance traveled. The daily distance from last tracking was calculated by dividing the distance traveled between trackings by the number of days between trackings. Average daily movement was calculated by dividing the total distance traveled during the tracking period by the number of days the frogs had transmitters. To compare movement between tracking sessions the pooled average of the daily distance from last tracking was used. Comparisons of daily movements within tracking sessions and average daily movement between tracking sessions were carried out using ANOVA in JMP.

Habitat Use

When frogs were located during radio telemetry, the primary macro habitat being used was recorded. Habitats were classified into 15 categories: emergent, emergent aquatic, emergent terrestrial, floating aquatic, forested wetland, muck, muck edge, mud puddle, open water, open water>1m, scrub shrub, upland, wetland edge, wetland edge aquatic, wetland edge no water, and wetland edge terrestrial. Habitat categories are defined in Appendix A. If a frog was found using a habitat that did not meet the definition of an existing habitat category a new habitat category was created. Along with the primary macro habitat the dominant vegetation of the area was recorded. Vegetation was grouped into categories based on structure. If vegetation species could be identified it was recorded and categorized. Vegetation groups included: low groundcover, floating aquatic, rush, sedge, reed canary grass, pond shield, pond lily, bull rush, and grass.

Habitat classification varied between years. As experience with tracking increased so did the detail of habitat categories. During 2008, many frogs were classified as wetland edge that would have been classified as emergent in 2009. This was due to the fact that in 2008, the emergent category was not included as a habitat type so frogs not near the edge of standing water, yet still well within the wetland boundary, were classified as wetland edge. During 2008, no distinction was made between frogs utilizing aquatic or terrestrial habitats along wetland edges and in emergent areas. In an effort to create a more consistent data set, habitats were changed to match those of 2009 when enough information was available to do so. Conversions were completed based on

notes taken at the time of tracking, presence of standing water at frog locations and the dominant vegetation. For example, the habitat for a frog classified as wetland edge in 2008 which was located within the wetland boundary with a dominant vegetation of rush with no standing water was reclassified as emergent terrestrial for data analysis purposes.

During spring and fall of 2009, frog locations that occurred within a half meter of the water line were classified as either wetland edge terrestrial or wetland edge aquatic. Frogs more than half a meter from water and outside the wetland boundary were classified as upland. If emergent vegetation was present and frogs were located more than a half meter from the water line they were considered to be using emergent terrestrial or emergent aquatic.

Available habitat during each tracking session was not determined due to fluctuating water levels which influenced the amount and types of available habitats. This large change in habitat type and availability within tracking periods made analysis of habitat selection unreliable. Thus, comparisons of habitat use between and within tracking sessions were not conducted.

Transmitter attachment materials

During the first tracking session of fall 2008, all transmitters were attached using silk thread (n=6). In an effort to create a thicker attachment material and reduce the risk of injury, 8 strands of thread were used on each attachment. This failed to prevent injury as all animals suffered abrasions within 25 days of release, with the majority of animals suffering injuries sooner. In an effort to reduce injury, thread was dropped as a

transmitter attachment material and only 4mm and 7mm silk ribbon was used during the second session. This greatly reduced injury with only one animal with a 4mm belt showing signs of abrasion. Unfortunately data on how many frogs were fitted with each type of belt were not recorded. During the spring of 2009, an effort was made to determine if injury rates differed for transmitters attached with 4mm or 7mm ribbon. Transmitters were attached to six frogs using 4mm ribbon and six frogs using 7mm ribbon. Animals were captured as often as possible to assess if injury had occurred. Any signs of abrasion were recorded. The first signs of abrasion were defined as white patches of skin under belt attachments. Transmitters were removed once abrasion was observed. Data were analyzed using a Chi-square test.

Affects of capture on frog movement

The affect of frequent capture on frog movements was studied during the fall of 2009. Frogs were only captured once a week and efforts were made to reduce habitat disturbance on days frogs were not captured. A frog was considered to be captured if its habitat was disturbed enough to alter the structure or if the frog was handled. Resampling statistics for Microsoft Excel 2007 was used to create a bootstrap t-test to determine if the average daily movement following capture differed from the average daily movement without capture. Frogs were pooled and treated as a single frog.

Analyses of movements following capture were run with and without frog 335. Frog 335 was excluded from analysis because of its use of a mud puddle which potentially acted as a trap to keep it in a confined area masking the impacts of capture.

Results and Discussion

Habitat Use

Habitat use data are presented separately for all three tracking periods. Results were not combined due to differing habitat conditions encountered during each tracking period. For example, during the fall of 2008, frogs were observed using muck 33 times (n=138), but in the spring and fall of 2009 frogs were observed using muck only once (n=188). The reduction in the use of muck was the result of the lack of available muck habitat during the spring and fall of 2009. During both these tracking sessions, water levels were elevated to the point that muck was not available or its availability was greatly reduced. Frogs utilized 15 habitat types during radio telemetry with variations of emergent and wetland edge accounting for the majority of all frog locations (Table 1).

Table 1: Number of observations and % use of 15 habitats recorded during radio telemetry. * indicates those habitat categories not used during all three tracking sessions.

Oregon Spotted Frog Habitat Use			
Habitat Type	Fall 2008 Number of Observations / % Use	Spring 2009 Number of Observations / % Use	Fall 2009 Number of Observations / % Use
Emergent	1/0.7%	0	1/0.9%
Emergent Aquatic	1/0.7%	37/45.1%	14/13.2%
Emergent Terrestrial	27/19.6%	1/1.2%	3/.9%
Floating Aquatic	1/0.7%	0	0
Forested Wetland	0	8/9.7%	0
Muck	33/23.9%	0	1/0.9%
Muck Edge*	2/1.4%	0	0
Mud Puddle*	0	0	5/4.7%
Open Water	27/19.5%	1/1.2%	26/24.5%
Open Water>1m	0	0	3/2.8%
Scrub Shrub	0	1/1.2%	4/3.7%
Upland	2/1.4%	1/1.2%	24/22.64%
Wetland Edge*	6/4.3%	2/2.4%	0
Wetland Edge Aquatic	0	24/29.2%	7/6.6%
Wetland Edge No Water*	40/28.9%	0	0

Wetland Edge Terrestrial	0	7/8.5%	18/16.9%
Total Observations	138	82	106

Fall 2008

During the first session, six frogs were tracked for an average of 16 days. Fifty-six locations with macro habitat data were recorded during the first session. During the second session, ten frogs were tracked for an average of 20 days with 82 locations recorded. When both tracking sessions are combined, 138 frog locations were recorded during telemetry in the fall of 2008. Throughout tracking, all frogs remained within or in very close proximity to Dailman Lake. Frogs used 11 different habitat types. Wetland edge no water (26%), muck (24%), open water (20%) and emergent terrestrial (14%) accounted for 84% of the total habitats used (Table 1). Frogs were recorded using habitats without measurable water 47% of the time, with the majority of these locations occurring within the wetland boundary (Table 2). Frogs in habitat classified as muck were considered to be in aquatic habitats even if standing water wasn't recorded.

Table 2: Terrestrial habitat use recorded during radio telemetry. Habitats without measurable standing water were considered to be terrestrial with the exception of muck.

Terrestrial Habitat Use			
Tracking Session	Terrestrial observations/Total observations	%Terrestrial habitat use	

Fall 2008	65/138	47.10%
Spring 2009	9/82	10.90%
Fall 2009	45/106	42.45%
Total	119/326	36.50%

The use of terrestrial habitats was observed during both tracking sessions in the fall of 2008. During the first tracking session, frogs 602 and 607 moved away from standing water into emergent terrestrial habitat. These frogs were between 10 and 50 meters away from standing water for multiple tracking days and remained away from water until transmitters were removed. Frogs during the second tracking session utilized terrestrial habitat within and outside the wetland boundary. Frog 615 was observed at the base of a large stump buried in leaf litter in upland habitat outside of the wetland boundary. On two occasions, frog 617 was located elevated above the wetland in vegetation. Frog 617 was located in bull-rush 24 cm above the surface of the wetland and a second time 10 cm above the wetlands surface in reed canary-grass stems.

Spring 2009

During the spring of 2009, 12 frogs were tracked for an average of 32 days.

Between 8 April and 17 June, 82 frog locations in nine different habitats were recorded.

Of the nine habitats used, emergent aquatic (45%) and wetland edge aquatic (29%) accounted for 74% of habitats used by frogs. No other habitats were used more than 9% of the time (See Table 1). Frogs were located in terrestrial habitats 10.9% of the time (see Table 2). A single upland location was recorded during spring tracking.

On April 17, frog 612 was found concealed under duff within six inches of a small garter snake, a known predator of *R. pretiosa*. Both frog 612 and the small garter snake were on top of an ECO-block that is part of the culvert over Muck Creek at Nixon Springs.

Fall 2009

In the fall of 2009, 12 frogs were fitted with transmitters and tracked for an average of 23 days. Between 29 October and 30 November, 106 frog locations in 11 different habitat types were recorded. Open water (24.5%), upland (22.64%), wetland edge terrestrial (16.9%) and emergent aquatic (13.2%) accounted for 77.24% of the habitats used (See Table 1) Frogs were recorded using terrestrial habitats 42.34% of the time during the fall of 2009 (see Table 2). This is comparable to the amount of terrestrial habitats used in fall, 2008.

The increase in use of upland habitat observed during the fall of 2009 is particularly interesting. Multiple frogs were observed using habitats outside of the wetland boundary for multiple days. Six frogs were located in upland habitats at least one time during tracking. Frogs 349, 348, 347,335,334, and 331 all used upland habitat to varying degrees.

On November 6, frog 335 moved upland to a large puddle in the dirt road that leads to Dailman Lake and remained near this puddle for the duration of tracking. Prior to taking up residence in the puddle, frog 335 had occupied several other upland habitats including a roadside ditch and leaf litter under a big leaf maple (Figure 9).

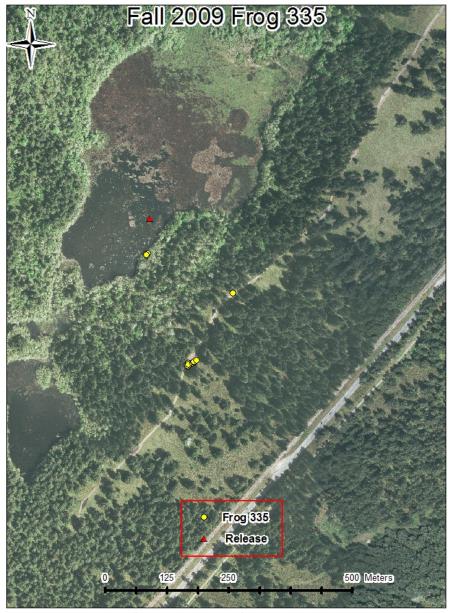


Figure 8: Locations of Frog 335 during fall 2009 telemetry. Note the clustering of points around a mud puddle in the lower part of the map.

During all three tracking periods telemetered frogs favored edge habitats over those in the central part of the wetland. When found in open water, frogs were associated with some form of floating aquatic vegetation that provided refuge from predators. In the spring, frogs used terrestrial habitats at a lower rate than during the fall tracking sessions.

R. pretiosa is considered a highly aquatic species that seldom ventures far from standing water. The close association of *R. pretiosa* with standing water is thought to be the result of feeding and escape behavior (Lict 1986b: Pearl et al 2005). Terrestrial habitat use data from telemetry on JBLM is anomalous when compared to other habitat use studies of *R. pretiosa* which found little or no overland movement and limited use of terrestrial habitats (Watson et al 2003; Shovlain 2006).

During telemetry on JBLM, frogs were observed away from standing water 47.1%, 10.9% and 42.45% of the time during the fall of 2008, spring of 2009 and fall of 2009 respectively. When all tracking sessions are combined, as they were for Watson et al 2003, terrestrial habitat was used 36.5% of the time (see Table 2). Five overland movements were observed during telemetry. The number of overland movements recorded only accounts for the initial move from aquatic habitat and does not account for repeated overland moves of frogs that were using terrestrial habitats. If these movements are included then the total overland movement is substantially higher with 31 probable overland movements (n=326). The substantial use of terrestrial habitats by frogs released at Dailman Lake was in stark contrast to Watson et al 2003 which found

frogs away from standing water only 1.4% of the time (n=295) and only had one out of 645 observations that may have represented overland movements.

The increase in use of terrestrial habitats in this study compared to other habitat use studies may be due to the fact that the study involved dispersal of juvenile frogs. All other studies of *R. pretiosa* habitat use and movement have used adult frogs. The observed increase in terrestrial habitat use and overland movement is possibly caused by a specific behavior of juvenile *R. pretiosa*.

The increase in terrestrial habitat use during fall telemetry compared to spring may be the result of increased availability of terrestrial habitats within wetland boundaries during the fall. Water levels at the time of the fall releases are very near yearly lows and aquatic connectivity between water bodies was greatly reduced while availability of emergent terrestrial habitat was substantially increased which would allow for greater use of this habitat. While fall telemetry was being conducted, aquatic connectivity was only present between Hamilton Lake, Binocular Pond, and Dailman Lake during both years. The lack of aquatic connectivity may have led frogs to disperse terrestrially. During the spring of 2009, when water levels were up and aquatic connectivity existed between all wetlands, the use of terrestrial habitats was greatly reduced and the number of observed overland movements was much lower. The reduced use of terrestrial habitats and overland movements is likely the result of frogs using aquatic corridors for dispersal.

The use of terrestrial habitats by sub-adult *R. pretiosa* warrants further research.

The hypothesis that aquatic refuge is the primary escape response of *R. pretiosa*

suggests that juveniles would potentially be more likely to remain near the safety of standing water. Understanding the habitat requirements of sub-adult *R. pretiosa* is vital for future recovery efforts of the species.

Movement

Telemetered frogs made the largest movements during the spring of 2009, when the average daily distance traveled was 53.46 m. In the spring of 2009, Frog 337 moved 330 meters upstream of the confluence of Muck Creek and Dailman Lake which marked the furthest documented movement of a frog away from Dailman Lake during this study (Figure 10).

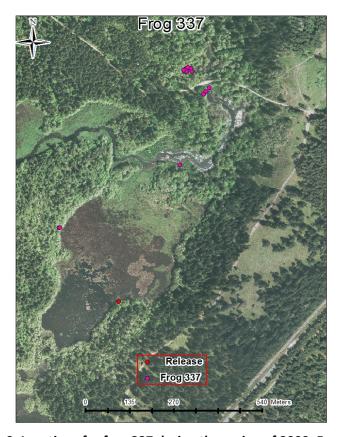


Figure 9: Locations for frog 337 during the spring of 2009. Frog 337 moved the furthest distance away from the release point of any frog tracked during this study.

Not all frogs made large movements during the spring of 2009. Frog 354 remained within several meters of the same location from 22 April to 17 June. During this time, the average daily movement of frog 354 was 5.9m. The lack of movement suggested a slipped transmitter or mortality, but frog 354 was recovered alive on June 17. The lack of movement may have been caused by injuries sustained from the transmitter attachment. On April 15, frog 354 began to exhibit signs of abrasion from the transmitter attachment. When Frog 354 was re-captured on June 17th, open sores were present in the groin area under the belt. The presence of wounds may account for the lack of mobility, but it is possible that frog 354 simply found an area of high quality habitat and remained until capture.

Comparisons of frog movements within tracking sessions did not reveal any statistical significant differences in the average daily movements of individual frogs.

Comparisons of pooled average daily movements between tracking sessions found an interesting connection between fall 2008 tracking and the 2009 fall tracking. Results from ANOVA found that the average daily distance moved during the spring was greater than movements in the fall 2008 and 2009 (F= 16.38, p=.0001) yet there was no difference in movements for fall 2008 and fall 2009 telemetry (Table 3).

Table 3: Summary of daily distance traveled by frogs during radio telemetry. * Distances traveled immediately following release were excluded from analysis of maximum distance traveled. Numbers in red are statistically significant.

Tracking Session	Pooled Average Daily Distance Traveled (m).	Minimum/Maximum daily distance traveled (m)*.	
Fall 2008 1st Release	6.86	0.026/63.5	
Fall 2008 2nd Release	16.92	0.066/102.35	
Spring 2009	53.46	0.166/277.5	
Fall 2009	18.19	0.033/137	

Water level appeared to relate to the distance that frogs moved. During the spring when water levels were higher and aquatic connectivity existed between all water bodies, frogs moved greater distances. This increased movement is likely the result of the ease with which spotted frogs can travel through water compared to overland.

Furthering the link between habitat conditions and movements of *R. pretiosa* is the fact that frogs from fall of 2008 and fall of 2009 did not have significantly different average daily movements despite a large difference in average size. Frogs released in the fall of 2008 had an average mass of 7.33g while frogs released in the fall of 2009 had an average mass of 31.5g. The fact that frogs tracked over similar time periods exhibited similar movement despite a considerable size difference suggests that size does not play

a significant role in movements of captive reared frogs. This indicates that habitat and other environmental conditions are the driving force behind movements of individual frogs.

Dispersal

The dispersal of released frogs was mapped in an effort to determine the availability of suitable habitat at the Dailman Lake release site as well as to see if released frogs were clustering in any particular locations. During all tracking sessions, frogs dispersed throughout the wetland complex and exhibited limited signs of clustering. Frogs did cluster around the margins of wetlands, and during the spring frogs clustered at the north end of Dailman Lake along the margins of Muck Creek. This clustering along the margins of wetlands and streams fits with the expected behavior of *R. pretiosa*. Dispersal appeared to be limited by available aquatic connectivity. Maps of dispersal for all transmittered frogs can be found in Appendix B.

While frogs were detected using the majority of the available shoreline, frogs didn't appear to disperse evenly to all available habitats. During radio telemetry, frogs were found in Hamilton Lake, Dailman Lake, Muck Creek, and Nixon Springs. Egg mass surveys in the spring of 2011 found *R. pretiosa* egg masses in Shaver Lake. When egg mass survey data are combined with telemetry data from all telemetry sessions, frogs were located in all areas of the study site except Chambers Lake.

Frogs exhibited different dispersal patterns in the spring and fall. Frogs released during the spring moved out of Dailman Lake and into Muck Creek where they traveled upstream. A single location was recorded downstream of the confluence of Muck Creek

and Dailman Lake but that frog moved upstream by the next tracking day. Transmittered frogs from the spring release did not move downstream into Chambers Lake despite a perceived abundance of available habitat. In the fall of 2008 and 2009, the majority of frogs remained within Dailman Lake. Several frogs moved into Binocular Pond and Hamilton Lake but none entered Muck Creek. This dispersal pattern is likely the result of water levels. In the fall Dailman Lake is hydrologically isolated from Muck Creek but remains connected to Binocular Pond and Hamilton Lake. Frogs likely used this aquatic connectivity while dispersing.

Juvenile dispersal may account for the high degree of terrestrial habitat use observed during telemetry. During telemetry in the fall of 2008 and 2009 frogs were observed making overland movements on multiple occasions. It is likely that these overland movements were part of natural juvenile dispersal. While prior studies of *R. pretiosa* have found little or no cross land movement, those studies were focused on adult frogs (Watson et al 2003).

In many species the YOY are the primary drivers of dispersal to new habitats. It is important for conservation actions to determine if YOY *R. pretiosa* are dispersing terrestrially during the fall as a mechanism for colonizing new habitats. Further understanding of how *R. pretiosa* move between wetlands will aid in creating corridors to facilitate meta-population dynamics in currently isolated sites.

Affects of capture on frog movement

Throughout the course of telemetry, frogs were captured on a regular basis to determine if injuries were being caused by the attachment material. Analysis of distance

traveled following handling revealed that the frequent capture of frogs influenced the rates of movement for telemetered individuals. The average daily distance traveled following capture was 28.25m compared to 14.72m for un-captured frogs. When the average daily movements were analyzed using re-sampling stats, the average distance moved by frogs with and without capture was not statistically different (p=.068). However when frog 335 was removed from analysis the average distance traveled following capture changed to 32.58m compared to 12.78m without capture. When the same re-sampling stats were run without frog 335 the results indicated a significant difference between the average movement with and without capture (p<.01). Frog 335 was removed from analysis of movements following capture because of its use of a large mud puddle in a logging road. The use of this mud puddle by frog 335 indicated a potential limited ability of that frog to move following capture. Frog 335 would have had to make a large overland movement before it would reach another area with standing water.

Further evidence of an impact of disturbance on frog movements can be found in the data from spring 2009. Of the top ten daily distances traveled, nine occurred immediately following release. Only two frogs, (611 and 618), did not make their largest move between release and the first day of tracking.

The results of statistical analysis and daily distance traveled in spring 2009 suggest that researchers need to be exceedingly cautious when conducting telemetry studies where study animals are repeatedly captured. Analysis of habitat use and movements are potentially biased by increased movement caused by frequent capture.

During telemetry on JBLM, frogs appeared to exhibit a form of predator evasion by making longer movements following capture. The need to evade a perceived predator could have altered the habitat selection of telemetered individuals. It is also possible that frogs were utilizing less than ideal habitats because they perceived that they had been driven from preferred habitat by capture. Habitat selection in amphibians is determined by many factors including predator evasion. If researchers were perceived as predators it is likely they influenced habitat selection.

Due to the small sample size and lack of formal controls, results of this analysis should be viewed as preliminary findings. Further research is needed to confirm the results of this preliminary study.

Telemetry Attachment Material

Due to the delicate skin of amphibians, abrasions caused by transmitter attachments can be a major concern for researchers (Muths 2003). Despite the abundance of research using radio telemetry few papers detail how transmitters are attached or the impacts of the selected transmitter attachment on research animals. This lack of information makes it difficult for researchers to make the important decision of what material to use and how to attach transmitters.

Reported attachment methods used on amphibians are variable and range from ribbon tied around the hips of animals to belts sewn around the forelimbs. Both synthetic and natural fibers are used for transmitter attachment, and include bead chains, silk ribbon, cotton thread, medical adhesive strips and other stretchy material (Watson et al 2003; Muths 2003; Shovlain 2006; Lynch 2006). While synthetic materials

offer a variety of properties that are useful for attachment, including elasticity, they do not biodegrade which means researchers must recapture transmittered individuals and remove transmitters. Natural materials such as silk will biodegrade, which allows animals to break free of transmitter attachments and self release if researchers are unable to recapture them or transmitters fail. The major drawback to using natural materials is the fact that the majority of available natural materials lack elasticity which makes sizing belts difficult. Improperly sized belts can negatively impact telemetry studies in two ways. A belt that is too tight may constrict frogs and cause damage which will affect behavior, while a belt that is too loose has an increased risk of prematurely falling off resulting in a lack of data.

Of the attachment materials used, 7mm silk ribbon resulted in the fewest injuries to frogs. Of six frogs with 7mm ribbon attachments, only one showed signs of abrasion compared to three of six frogs injured with 4mm ribbon and six of six injured with thread. A chi-square test found a significant difference between injury rates for transmitters attached with thread but not those attached with ribbon. While there was no statistical difference between injury rates caused by 4mm vs. 7mm ribbon, the sample size was very low. If the sample size were increased, it is possible that the difference in injury rates would become statistically significant. The observed reduction in injuries with 7mm ribbon is likely the result of the increased width of the attachment material which helps prevent abrasion by providing a greater surface area where skin contact is made reducing the ability of the material to constrict and cut into delicate skin.

Injuries to frogs as a result of transmitter attachments could often be identified prior to formation of open sores formed. Injuries followed a predictable pattern: first the skin under the attachment belt would darken, then the skin would develop white patches; finally open sores would develop. If the condition of frogs were monitored closely, belts could be removed once white patches appeared and prevent the occurrences of sores. The difficulty with this approach is that it required handling frogs on a regular basis. Analysis of movements following handling shows that frogs made larger movements following capture than if not handled. By using an attachment method that reduces risks of injury, the need to capture frogs is reduced and therefore so is the potential bias on movements caused by capture.

Silk was selected for attachment material because we believed it would biodegrade and allow frogs to break free of transmitters if they could not be recaptured. Based on observations made during the spring of 2009, silk ribbon performed as expected with three transmitters breaking loose prior to removal. All three transmitters were on animals for 70 days prior to the discovery of belt failure with all three belt failures discovered on the same day. The silk that remained on the transmitter was extremely brittle and broke easily when stressed. The grouping of this phenomenon suggests that in aquatic conditions, attachments using silk ribbon will last about 10 weeks before animals can break free. The ability of frogs to self release is a major strength of the use of silk belts. Animals often elude capture or transmitters unexpectedly fail during telemetry making removal of transmitters difficult. The use of

natural fibers allows animals to survive even if transmitters fail or animals take up residence in inaccessible areas.

Conclusion and Future Research Needs

This thesis results in three key conclusions regarding *R. pretiosa* and leads to two key questions that warrant further research: 1) available habitat on JBLM appears to be suitable to *R. pretiosa*, 2) habitat connectivity and variability is key for future reintroductions, and 3) the use of wider natural fiber materials for transmitter attachment has the least negative impact on study animals. This thesis also leads to two questions that warrant further research: 1) the cause of increased utilization of terrestrial habitats, and 2) the impact of frequent handling on behavior of study animals during telemetry.

The use of captive breeding to recover *R. pretiosa* in Washington State appears on the path towards success. Habitat use and dispersal patterns of post-release *R. pretiosa* indicate that Dailman Lake and the surrounding wetland complex provide suitable habitat for sub-adult *R. pretiosa*. The discovery of 11 *R. pretiosa* egg masses on JBLM during the spring of 2011 indicates that breeding habitat is also available within this wetland complex.

Gathering specific information on the ecology of all life stages of *R. pretiosa* is important to furthering the recovery of this species. By gaining a better understanding of habitat use, movements, and dispersal of a reintroduced population of juvenile *R. pretiosa*, conservationists will be able to more easily manage existing populations and

select future reintroduction sites. Dispersal patterns at JBLM indicate that connectivity of wetland habitats is key to successful reintroduction. By providing multiple interconnected wetlands, released juvenile frogs were able to seek out suitable habitat for later life stages. All oviposition sites found in 2011 were located outside of Dailman Lake, which has been the primary release site.

Preliminary results from telemetry indicate that the use of relatively wide natural fibers for transmitter attachment had the least negative impact on study animals. Silk attachments performed as expected by allowing frogs to break free of transmitters if they could not be captured. Telemetry is an inherently invasive monitoring technique but researchers can reduce their impacts on study animals by selecting appropriate attachment methods and taking care to properly attach transmitters. Using material that provides both a relatively large surface area and is biodegradable is a good way to protect study animals from harm.

Telemetry on JBLM produced multiple questions that warrant further investigation. More research is needed into the utilization of terrestrial habitats by juvenile *R. pretiosa*. Telemetry data indicates that sub-adult frogs are utilizing a much greater range of terrestrial habitats than previously documented. The role that terrestrial habitats play in the overall ecology of *R. pretiosa* is unknown. It is possible that the high use of terrestrial habitats at JBLM was caused by reared frogs being introduced to a novel environment and that through the course of dispersal they moved into areas that they would not have otherwise occupied. If the frogs had been reared natively at this site, or if adult frogs had been released rather than young of the year, it

is possible that frogs would not have spent as much time in these habitats as was recorded. Several frogs in this study spent the majority of their time in terrestrial environments. A possible explanation for this use of terrestrial habitat is the need for juveniles to disperse to new locations in order to establish new populations. If terrestrial habitats are used by juvenile *R. pretiosa* for dispersal, then corridors can be designed between existing populations and potential habitats in an effort to expand *R. pretiosa* meta-population dynamics.

Further research is needed to determine if the observed terrestrial habitat use is indeed the result of juvenile dispersal. Anecdotal evidence collected during telemetry suggests that the initial movement of frogs into terrestrial habitats was preceded by a rain event. There is ample evidence in the literature of other amphibian species dispersing terrestrially following rains. Efforts should be made to compare the timing of overland movements with precipitation records to determine if rain fall is linked to terrestrial habitat use.

The impacts of frequent handling on movements of telemetered frogs warrant further study. The data for analysis were limited and patchy. Frogs were not captured on a consistent schedule, which reduced the number of data points for each frog. Due to a lack of data points for individual frogs, data for movements with and without capture had to be pooled. By pooling data, the behavior of individual frogs could not be determined and the movements of a single frog could sway results. As evidenced by the removal of frog 335 from analysis, a single frog could have a large influence on the

outcome of this analysis. Future studies should strive to collect more data points for individual frogs so data do not need to be pooled.

In our final conclusion, we note that the future of *R. pretiosa* in Washington looks promising. The three previously known populations of *R. pretiosa* appear to be rebounding from losses sustained in the late 1990's and early 2000's. The discovery of two new populations in North Puget Sound coupled with the positive signs of success at JBLM provides three new geographically distinct populations which reduces the risk of extirpation. However, even given these positive trends a lot of work remains before *R. pretiosa* could be deemed "recovered". Even if recovery goals are met, active management must be continued in order to maintain populations of *R. pretiosa*. This study bolsters prior findings that *R. pretiosa* is dependent on early successional wetlands that will require continued restoration activities to maintain. By applying the knowledge reported in this thesis – knowledge gained through telemetry on JBLM, these restoration activities can be designed to account for juvenile as well as adult habitat needs.

Appendix A Definitions of Habitat Categories

Emergent: acquatigen Strangistragionate represente into breat leadoit act to do strain alteg lovaterner gent vegetation

Emerggrademestrial billed **sympoling divertely pine 2008**. Do in ingstably were figured in expectation. water with a large amount of floating aquatic vegetation.

Upland: Outside of the wetland boundary. Based on the lack of standing water and wetland vegetation.

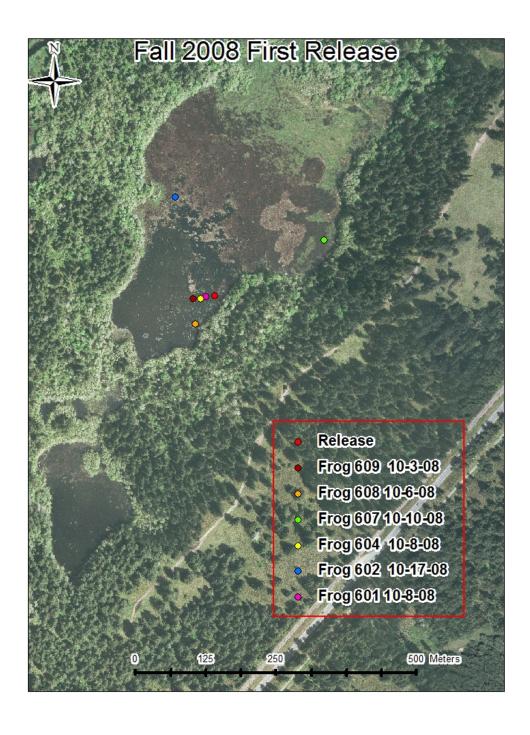
Wetland edge: Within 1m of standing water. Does not distinguish between terrestrial and aquatic habitats. Used during fall 2008 and part of spring 2009

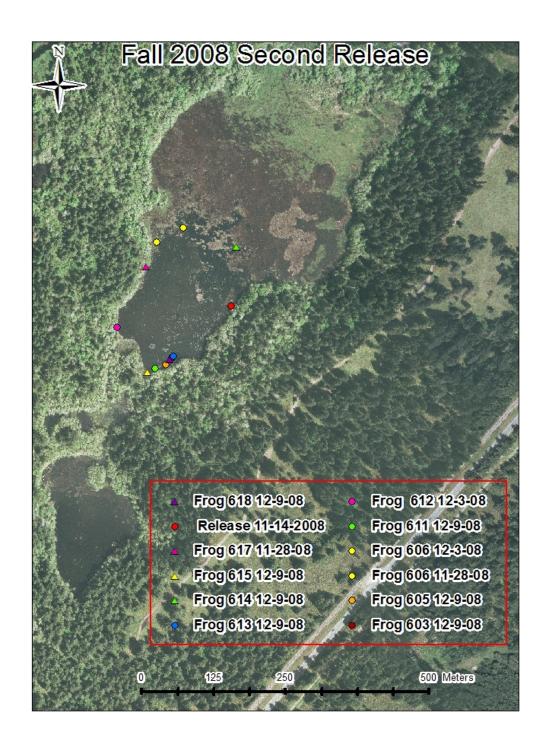
Wetland edge aquativer within 1120008 fithis stateging watersied aquatischiabeitates that were within the wetland boundary but utilizing terrestrial habitat. These frogs would be classified as emergent terrestrial or wetland edge terrestrial in later years.

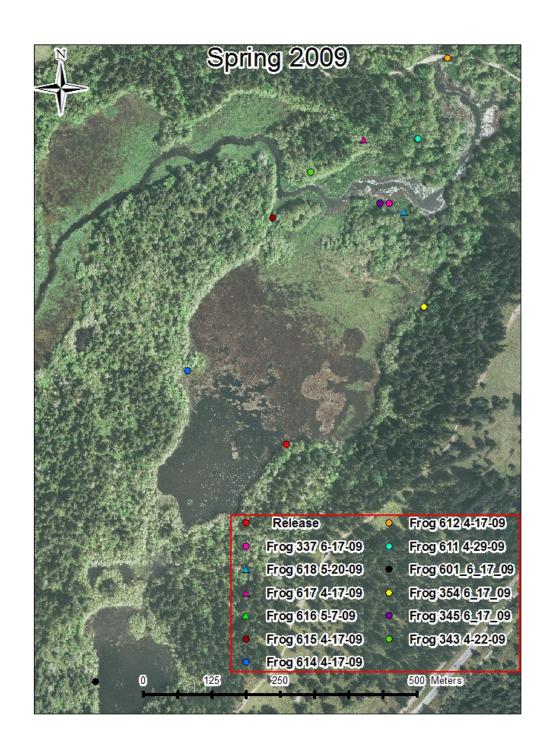
Wetland edge terrestrial: Within 1m of standing water in terrestrial habitat. Terrestrial habitat could be within or outside the wetland boundary.

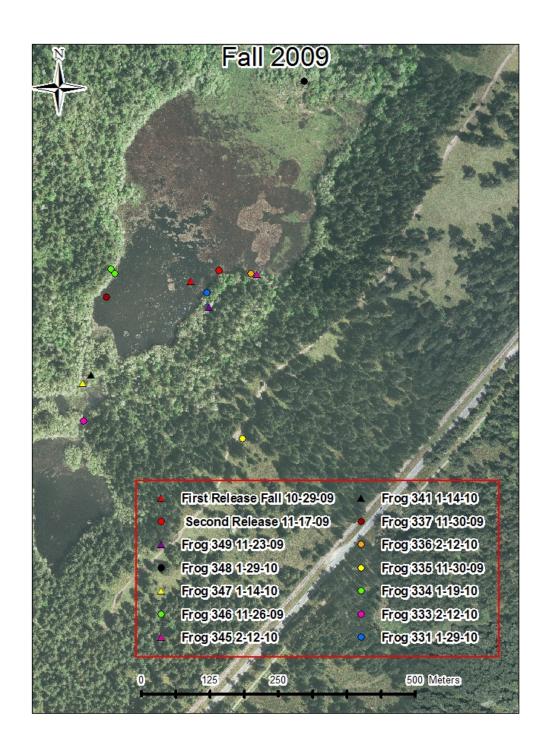
Appendix B: Dispersal of Frogs during telemetry.

Maps depict locations of frogs at the time telemetry transmitters were removed. For the fall of 2009 symbol shapes coincide with the release date. Frogs symbolized with a triangle were released on October 29 and those with a circle were released on November 17.









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