Diel Breeding Patterns of the Oregon Spotted Frog (*Rana pretiosa*)

Observed by Camera Traps

By

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ABSTRACT

The decline of species worldwide strongly suggests underlying environmental issues. Amphibians are particularly sensitive to environmental changes and are declining more rapidly than most other taxonomic groups. The Oregon Spotted Frog (*Rana pretiosa*) is a threatened species endemic to the Pacific Northwest that has sustained both population declines and local extirpations across its range. In particular, more information is needed about the environmental parameters that may affect the continued survival of the Oregon Spotted Frog during critical periods in their life cycle, such as breeding. To begin to address these gaps in our understanding of Oregon Spotted Frog reproductive ecology, I deployed professional series Reconyx PC900® Hyperfire Professional infrared wildlife research cameras to study their oviposition behavior. I collected time-lapse photography at oviposition locations so the precise times of egg deposition were delineated and the conditions associated with oviposition could be identified for the following parameters: time of day, water temperature, light intensity, cloud cover, precipitation and moon phase. My data revealed that most Oregon Spotted Frogs deposit eggs between evening and morning civil twilight and when water temperatures are within 4.1-8.0^{0}C. I also found a significant inverse relationship between light intensity and oviposition activity, suggesting that the reproductive success of the Oregon Spotted Frog may be sensitive to environmental changes brought on by selected human impacts on the environment, such as climate change and light pollution from artificial night lights. More data are needed to further examine the relationship between oviposition and cloud cover, moon phase and precipitation. Additional focused studies of Oregon Spotted Frog ecology are recommended to elucidate what other aspects of local weather patterns and hydrology cycles may affect Oregon spotted frog population persistence and recovery. These further studies could also establish important base lines with which to compare future studies to address climate change impacts on threatened amphibians.

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Chapter 1: Literature Review

Background on Diel Behavior

Most organisms inhabiting the Earth have been subjected to the cyclic patterns of light and dark during the course of their evolution. Some organisms require darkness for cell recovery from overstimulation (from photosynthesis, for example) or for damage repair after exposure to ultraviolet light and many of these organisms do not thrive when darkness is not available for recovery (Gerrish et al. 2009). Over time, different organisms have evolved to take advantage of light and dark cycles in ways that optimize their survival, often taking advantage of specific lunar cycles or a narrow range of light intensity to enable them to securely engage in critical behavior patterns, such as breeding, foraging or communication (Gerrish et al. 2009).

Nocturnal behavior patterns can vary as a function of light intensity but when a behavior is associated with daily cycles or with the moon cycle it is called a circadian or circalunar pattern (Gerrish et al. 2009). Circadian patterns can be endogenous time-dependent behaviors that are independent of physical cues or they can be cued purely to physical parameters such as light intensity, and hence represent exogenous behaviors (Gerrish et al. 2009). Both patterns can be interrupted by light pollution, either by altering the timing of the cycle or by preventing darkness thresholds (minimum levels of light for certain critical behavior patterns) from being reached (Gerrish et al. 2009).

Light pollution is a general term addressing the effects of human-created light at night and generally refers to the degradation of the human view of the night sky (Longcore & Rich 2004). Longcore & Rich (2004) identify a series of more specific terms, including ñastronomical light pollution,ò which refers to the tendency for artificial light to obscure or wash out the human perception of the stars. The light reflected back from this is called ñsky glow,ò and how sky glow, a decreased perception of the night sky and how more direct sources of night lighting affect non-human organisms in the environment is known as ñecological light pollutionò (Longcore & Rich 2004). Ecological light pollution has been used to describe the negative effect of light on wildlife, but Longcore & Rich (2004) proposed dividing this up in more specific terms: direct glare, chronically increased illumination and unexpected fluctuations in lighting. Which type(s) of light pollution are detrimental, if any, varies with the species involved, but many believe that all three types of ecological light pollution can negatively affect wildlife (Longcore & Rich 2004).

Ecological light pollution is a global concern: its impacts likely vary with latitude, with tropical communities proposed as being one of the more sensitive ecosystems (Longcore & Rich 2004). A region with relatively consistent and stable light cycles may have species adapted to subtle nuances in light intensity or quality that make them vulnerable to changes in these patterns (Longcore & Rich 2004). Temperate species only active during a specific portion of the year may also be affected, especially if critical behavior patterns are dependent on these seasonal light fluctuations (Longcore & Rich 2004).

Problems with disruption in light patterns can have various effects on organisms: altered light patterns may alter predator-prey interactions and may partly explain decreased foraging intervals among prey species and can disrupt niche partitioning (Longcore & Rich 2004). Niche partitioning based on light occurs when sympatric species (ones that live in the same region) preferentially forage within non-overlapping ranges of light intensity. If some of these light intensity ranges are no longer present, an organism may experience an altered frequency of contact (increase or decrease) with other species as competitors or predators, or an altered ability to detect prey (also either increased or decreased). These changes could affect community structure and ultimately spill over to affect or disrupt entire ecosystem functions (Longcore & Rich 2004). In general, our understanding of such effects is limited and warrants further research.

Diel Behavior Patterns in Amphibians

Many amphibians exhibit differences in behavior based on the light intensity. Many hypotheses exist for the basis of these differences but most appear liked to the distinctive life histories of the species. For example, diurnal species may be dependent on the higher temperatures associated with sunlight such as seen in Wood Frogs (*Rana sylvatica*) in Alaska, which preferentially breed during the warmest period of the day (Herreid & Kinney 1967). Nocturnal species may require certain prey items that come out only at night, or perhaps nocturnal behavior has evolved as a predator-evasion tactic (Perry et al. 2008). Ecological light pollution caused by artificial lighting has the potential to negatively impact species that have evolved behavioral cues based on ambient light cycles. For example, sea turtle hatchlings, which usually move away from the dark silhouettes of dunes and towards the reflective moon and/or starlight off the ocean surface can become disoriented by artificial lights (Longcore & Rich

2004). Sea turtle hatchlings are one of many examples of the negative effects ecological light pollution (Longcore & Rich 2004). Therefore, it is important to determine whether a species is sensitive to or adversely affected by ecological light pollution, particularly if its status is threatened or endangered.

While much remains unknown about the photosensitivity of anurans and other frogs, some frog species feed on nocturnal insects, some of which are known to be drawn to artificial lights at night (Rich & Longcore 2006, Perry et al. 2008). Changes in prey behavior may alter the behavior of the predator and though lights may provide large congregations of prey, increased light levels may also expose them to greater predation, or draw them across roads, increasing the likelihood of mortality (Rich & Longcore 2006, Perry et al. 2008).

Additionally, lighting can influence cycling of hormones such as that of melatonin, which is present in amphibians. In the adult Spotted Salamander (*Ambystoma maculatum*), melatonin slows the metabolic rate and prepares the amphibian for decreased activity and cooler temperatures at night (Perry et al. 2008). A decrease in melatonin production due to artificial lighting could disrupt metabolic cycles during inappropriate times, such as during periods of low food availability, drought or during egg production (Perry et al. 2008), but to date, this is untested in amphibians. Light may also affect other hormones that regulate breeding behavior, so it is important to investigate whether or not a species exhibits photo-sensitive behavior to help understand how changes in natural lighting may affect the species.

In this study, with the Oregon Spotted Frog (*Rana pretiosa*, henceforth OSF), I measure light intensity using data loggers to help establish whether OSFs exhibit a photo-sensitive breeding pattern. This represents a first step in knowing whether or not this species might be adversely affected by ecological light pollution.

General Amphibian Biology and Conservation

Amphibians are a taxonomically diverse group that highlight the evolutionary transition between water and land (Jones 2005). The two most speciose groups of amphibians are the Anura (frogs) and the Caudata (salamanders) and though these two groups have diverged into unique assortments of species, both share a set core traits common to all amphibians. Species from both groups have moist and relatively permeable skin used for gas exchange and are typically dependent on water for at least one or more life stages, making them important biological indicators of environmental degradation (Warkentin et al. 2009). This biphasic lifestyle is ecologically significant in that many amphibians have multiple ecological roles, including roles as predator and prey (Jones et al. 2005, Warkentin et al. 2009).

Many investigators suggest that amphibian species are declining and going extinct at a higher rate than the background extinction rate (Hayes & Jennings 1986; McCallum 2007; Collins 2009). Some potential causes of this accelerated rate of decline include habitat destruction, increased UV radiation, the introduction of non-native species like American Bullfrogs (*Lithobates catesbeianus* [= formerly *Rana catesbeiana*]), diseases such as chytridomycosis, water quality and climate change, among others (Hayes & Jennings 1986; Adams 1999; Cushman & Pearl 2007; Collins 2009). The factors contributing to decline vary according to species and location. The Pacific Northwest is a location with several amphibian species that are declining, with some of the primary causes thought to be the introduction of exotic fishes and habitat destruction associated with land use changes and urbanization (Hayes & Jennings 1986, Boyer & Grue 1995, Aldrich 1998, Adams 1999, Semlitsch 2003, Pearl et al. 2004, Jones et al. 2005, Collins 2009).

While ample research exists focusing on the possible threats to amphibian decline, some of these studies have yielded mixed conclusions or only weakly correlated results. For example, two studies by Adams concluded that the effects of American Bullfrog populations on amphibian species diversity and Northern Red-Legged Frog (*Rana aurora*) distribution were minimal and concluded that the presence of Bullfrogs may not be a primary factor driving the decline of amphibians in the Pacific Northwest (Adams et al. 1998, Adams 1999). Likewise, Richter & Azous (1995) suggested that these predators could actually increase species richness by feeding on ranid frogs that may otherwise outcompete smaller or less competitive amphibian species. These studies were conducted after American Bullfrog invasion had occurred and a temporal baseline with which to compare the current amphibian biodiversity (before the presence of the invasive species) was lacking. Hence, it is difficult to assess the magnitude of species declines attributable to American Bullfrogs.

More recent studies suggest that American Bullfrogs may pose a greater threat to OSFs than previously realized. Using a captive arena study, Pearl et al. (2004) ultimately concluded that American Bullfrogs prey upon OSFs more frequently than Northern Red-Legged Frogs in a quasi-terrestrial circumstance. Oregon Spotted Frogs tended to be more vulnerable to American Bullfrog predation likely because the aquatic life history of OSF is more similar to that of the American Bullfrog (Pearl et al. 2004). A recent study by Rowe and Garcia (2013) showed a negative effect of American Bullfrogs on the post-metamorphic numbers of two native frog species: Pacific Chorus Frogs (*Pseudacris regillis*) and Northern Red-Legged Frogs (*Rana aurora*). Although this study did not include OSFs, the two affected species, like the OSF, were also the species that were most prevalent in habitats preferred by the American Bullfrog (Rowe & Garcia 2013). The discrepancy between these early studies and the later studies highlights how basic natural history can provide insights for species-specific conservation that previous studies may have overlooked. Still, the Pearl et al. (2004) study did not address aquatic predation on OSFs by American Bullfrogs, so the inter-specific interactions between these two species remain incompletely understood. An understanding of a particular speciesô life history, from environmental preferences to breeding behavior, can better inform how a species might react to either the introduction of exotic species or habitat alterations. This is why a detailed examination of OSF breeding behavior in relation to environmental conditions will be valuable to amphibian conservation.

Oregon Spotted Frog Biology and Conservation

It is generally accepted that OSF were once more widespread in stillwater habitats from southwestern British Columbia to northeastern California and that the species has declined to the point where it no longer inhabits 70-90% of its original range (Hayes 1997; Pearl et al. 2005; Pear et al. 2009). In Washington State, Oregon Spotted Frogs currently occupy six watersheds: Sumas, Nooksak, Samish, Black, White Salmon and Klickitat Rivers (USFW 2013, Figure 1). This western form of the Spotted Frog is distinct from, and more restricted in range, than its sister species, the Columbia Spotted Frog (*Rana luteiventris*), that occurs east of the Cascades (Cushman & Pearl 2007). Although these two species are genetically distinct (Blouin et al. 2010), the general aspects of their breeding biology and ecology are similar.

Oregon Spotted Frogs prefer permanent wetland habitats that seasonally inundates into surrounding marshland during the breeding season (February to March in Washington State), when the water temperature exceeds 6^{o}C, although this purportedly varies depending on the population involved (Cushman & Pearl 2007). Temperature is thought to be an important factor cuing the breeding cycle since the eggs are laid in shallow waters and are susceptible to freezing temperatures and yet need to be deposited early enough in the season to minimize chances of desiccation due to stranding as a consequence of the typical seasonal decrease in water levels (Cushman & Pearl 2007).

Aside from temperature and hydrologic factors, other listed threats to the breeding success of the OSF included marsh habitat loss or alteration, plant succession that shades or otherwise eliminates breeding habitat and predation by bullfrogs and possibly introduced fish species (Cushman & Pearl 2007, Pearl et al. 2009). Interestingly, unlike the eggs of some other amphibian species in the Pacific Northwest, OSF eggs appear insensitive to the increasing ambient levels of environmental UV-B radiation (Blaustein et al. 1999). Oregon Spotted Frogs also display resistance to the amphibian chytrid fungus, *Batrachochytrium dendrobatidis*, a fungus that has had catastrophic epizootic effects on other amphibian species (Padgett-Flohr & Hayes 2011).

Although multiple factors have been shown to have negative impacts on OSF populations, the frog populations in decline are usually subject to multiple influences that are difficult to disentangle from one another. In order to better protect this organism and its environment, behavioral studies are also needed to determine the highest priorities in conservation. Since the OSF is considered to be the most aquatic of the native ranid species in the Pacific Northwest (Leonard et al. 1993), future studies focused on the frogôs behavior and habitat characteristics involving the breeding season, when hydrology is at its most critical point of the frogôs lifecycle, would be of value. Key hydrologic issues may be uncovered that clarify priorities for its future conservation.

Amphibian Embryology and Development

Embryology is another central component of reproductive biology, and knowledge of how to promote successful early development can aid in species conservation. Many factors can affect the timing and success rate of amphibian embryos, including their radiation and temperature environments. The effect of

UV-B radiation on embryologic development has been notoriously difficult to assess in the Pacific Northwest because the high level of rainfall usually results in a high level of dissolved organic matter in the water and a low UV-B transmission levels (Palen et al. 2002). Moreover, Blaustein et al. (1999) found that the higher photolyase activity in OSF eggs correlated with a higher resistance to UV-B radiation in the field than for the ova of some other species of amphibians (Blaustein et al. 1999). Collectively, these studies suggest that UV-B is less likely to have an impact on OSF.

Besides UV-B, several other factors can also influence the development rate and survival of amphibian eggs. However, the collective effects of all these factors do not appear as influential as temperature. Bradford (1990) pointed out that temperature strongly influences embryonic development across all amphibian taxa. However, how temperature affects the embryos of a particular species of amphibian is dependent on that speciesô evolutionary temperature tolerances (Bradford 1990). A later study found that this temperature specificity can vary even within populations of a species, such as was found in *Rana temporaria* (Laugen et al. 2003). Embryonic development of *R. temporaria* showed different development rates at the same temperature depending on the population of origin (Laugen et al. 2003).

Embryonic temperature requirements are likely associated with breeding site selection by adult amphibians. Moore (1939) had found that various species had different optimal developmental temperatures that seemed to correspond to the range of environmental conditions experienced during the time breeding for that species and that faster-developing species tended to develop best at warmer temperatures (Moore 1939). Later, Licht (1969) observed that the differences in the temperatures of oviposition (egg laying) sites selected by *Rana pretiosa* and *Rana aurora* were related to the critical temperature range of the respective embryos.

For OSFs, critical thermal limits were determined by maintaining eggs at a constant temperature for the duration of development and observing the development of the embryos and their survivability (Licht 1969). Lichtôs study, among others, shows that small changes in lower temperatures can accelerate development more significantly than similar thermal increases at higher temperatures (Atlas 1935, Licht 1969, Bradford 1990). Lichtôs research did not extensively examine the effects of short-term exposure to sub-optimal temperatures, but did show that some short-term exposure (8 hours or less) of freezing temperatures did not seem to affect the survival or development of *R. pretiosa* embryos (Licht 1969). The critical thermal limits that supported successful growth for the Canadian populations of OSF (*R. pretiosa*) that Licht studied were found to range between 6^{o}C and 29^{o}C when held at a constant temperature over the length embryonic development (Licht 1969).

While these data provide general guidelines about the development of OSFs, they are incomplete at best. First, Licht only dealt with the population of OSFs in the Lower Fraser Valley in British Columbia. Whether all populations of *R. pretiosa* have similar thermal limits is unknown. Second, temperatures are rarely held close to constant under natural circumstances, and short term thermal tolerances in the field may differ from those in a lab setting. In fact, a recent study in 2010 explored the *in-vivo* temperature fluctuations experienced by OSF egg masses in central and eastern Oregon and found that embryos tolerated water temperatures between 0-6 °C for about half their incubation period (Bowerman & Pearl 2010). The lower lethal temperature limit had previously been determined to be 6 °C in constant-temperature lab environments (Licht 1971). These findings underscore the fact that *in-vivo* thermal limits differ from the limits of eggs held at constant temperatures. Since these limits may also vary between populations, information is also needed about the temperature tolerances of other populations of OSF embryos in the field. As breeding behavior tends to correspond to the specific thermal requirements of a population, studies on how temperature relates to behavior during oviposition in more populations could provide valuable insight on the necessary conditions for the successful development of *R. pretiosa* embryos.

Oregon Spotted Frogs and Climate Change

Climate change is thought to have the potential to drive population declines, particularly in species that are sensitive to temperature, either directly through changes in temperature and/or precipitation or indirectly through changes in community structure, food availability and changes in interspecies competition (Cahill et al. 2013). Temporal shifts (shifts in timing) in breeding behavior in response to temperature, for example, can lead to competition for resources between the larvae of species that would otherwise not encounter each other (Parmesan 2007). Shifts in temporal behavior have been observed in several amphibian species, although species vary dramatically in their response to changes in temperature and/or precipitation (Parmesan 2007). Although the response to environmental changes varies according to species, in general amphibians are thought to be one of the most susceptible taxa to climate change due to their relatively high sensitivity to changes in moisture and temperature (Carey & Alexander 2003; Hopkins 2007; Todd et al. 2011).

Climate change is expected to bring changes in temperature and precipitation worldwide, although the type and magnitude of these changes is expected to vary greatly according to region (Erwin 2009). In the Pacific Northwest (PNW), the hydrologic cycle is considered to be vulnerable to changes in climate because of the dominance of snowmelt in its annual water cycle (Elsner et al. 2010). Many Global Climate Models (GCMs) for this region show a trend toward drier summers, an average temperature increase of 0.8 degrees Celsius and wet winters with an increase in extreme precipitation events (Elsner et al. 2010; Mote & Salathe Jr. 2010). At the same time, the contribution of snowmelt (measured as snow water equivalents) has been projected to decrease from 38-46%, which will lead to changes in the timing and volume of streamflow throughout the Pacific Northwest (Elsner et al. 2010).

Since wetland water levels also fluctuate with seasonal hydrological changes, it is likely the timing and amount of water availability in wetland microhabitats will also be affected (Erwin 2009). Changes in the amount and availability of water can impact the community structure and hospitability of these wetland habitats to resident amphibians (Carey & Alexander 2003;

Blaustein et al. 2010). Large increases in water levels can cause mortalities in amphibian larvae and embryos, as can early or rapid drying and subsequent desiccation of larvae or eggs (Carey & Alexander 2003). Populations of Columbia Spotted Frogs in Yellowstone have shown continued population declines in response to warming and drying trends (McMenamin et al. 2008). Lowland populations of OSF may be particularly vulnerable to changing hydrological fluctuations in the PNW because of their tendency to lay in shallow waters and the importance of permanent bodies of water in their summer and winter habitats. However, little is known about how OSF reproductive timing and success may respond to changes in temperature and precipitation. Understanding how these factors drive breeding behavior can lend insight on how climate change might affect OSFꞌs future reproductive success.

Camera Traps and Monitoring

The use of camera traps for relatively unobtrusive observation has been well established as an aid in the study of elusive, shy or nocturnal animals (Silveira, et al. 2003; Maffei & Noss 2008; OꞌConnell et al. 2010). Silveira et al. (2003) evaluated the usefulness of a camera trap in the assessment of species richness and abundance in a vertebrate community in central Brazil, where it was found to be useful to survey mammals. In this instance, camera trapping differentially detected animals it picked up depending on trap set up. Thus, a camera trap could be adjusted to gather information on certain species based on their height/weight/size. Camera traps were also found to adequately assess smaller fauna when properly adjusted (Silveira et al. 2003). Probably one of the more common uses of camera traps has been found to be for those animals that are nocturnal or sensitive to human disturbance, such as ocelots and other carnivores (Maffei & Noss 2008). Techniques and technologies have been developed with these and more sensitive species to reduce the detectability of the camera trap to the monitored animals, such as using a pulsed-infrared triggering device instead of trip lines, treadles or persistent beams (Carthew & Slater 1991). Once an animal crosses the beam, the camera is triggered and a series of photographs or video surveillance is recorded for a set period of time. Some camera traps are capable of simultaneously recording time, temperature, humidity and other data. Whether a photo or video capture is preferred depends on the level of detail desired and how quickly the organism moves. Slower frames often capture more detailed, higher quality images, whereas videos capture more frames at a lower resolution (OôConnell et al. 2010).

For amphibians, camera traps are just beginning to be recognized for their potential to contribute to elucidating life history and behavior. One study involving Gopher Frogs (*Lithobates areolata circulosa*) successfully deployed infrared camera traps and used a time-lapse cycle to capture the 24-hour activities of this species around their burrows (Hoffman et al. 2010). This study revealed that although amphibians may not be a suitable species for the more traditional motion-detection set up, that camera trapping technique can easily be modified using time-lapse photography to capture species that exhibit spatially or temporally predictable behavior. Such behavior is exhibited by the OSF during the late winter and early spring, when aggregated egg laying occurs at specific locations. Once a breeding location has been identified, one or more camera traps may be installed for time-lapse observation of breeding behavior.

In a pilot effort, camera traps were used to capture selected OSF reproductive behavior during a previous breeding season with some success (Hayes 2012). The methods involved using an AcornÊ wildlife camera to photograph a selected location at 10-second time intervals independent of motion detection. Although some photos were obtained that captured revealing snapshots of *R. pretiosa* breeding behavior, the 10-second intervals made it difficult to follow the movements of different frogs and the pictures did not have consistently adequate resolution to identify when frogs were present in the upper quadrants of the photos. In this study, one of my original goals was to increase photographic frequency to one photo every 5 seconds using a higher resolution camera (a ReconyxÊ PC900) in order to refine tracking ability. However, I later found that a 15-second interval was a sufficient resolution to compare breeding behavior to selected environmental covariates such as temperature, precipitation, humidity, light intensity and time of day to determine which of these might influence the behavior.

Chapter 2: Diel Breeding Behavior of the Oregon Spotted Frog (*Rana pretiosa*)

Introduction

The recent global accelerated rate of decline and progressive extinction of species strongly suggests underlying environmental issues. Prominent among declining and disappearing taxa are amphibians (Aldrich 1998; McCallum 2007; Collins 2009). This prominence is thought to be due to their sensitivity to habitat degradation because of their need for different habitats to complete their life cycles, their highly permeable skin used for gas exchange and their unique physiology (Welsh & Ollivier 1998), including salt intolerance that reflects typical amphibian freshwater-shedding kidneys. Among declining amphibian species, Oregon Spotted Frogs (*Rana pretiosa*) have sustained a large reduction in geographic range (Hayes 1997).

Oregon Spotted Frogs (OSFs) are an anuran species found in certain freshwater wetland habitats of the Pacific Northwest. The OSF is highly dependent on standing freshwater for most of its life cycle (Watson et al. 2003). The importance of understanding its specific habitat requirements and reproductive biology has recently become imperative, as the species was recently proposed for Federal protection as a threatened and endangered species (USFWS 2013). OSFs are also listed as endangered in Washington State (McAllister 2012) and in British Columbia (COSEWIC 2000). Their endangered status warrants research to better understand its life history for conservation purposes.

Besides researching OSF life history for their conservation, this speciesô particular sensitivity to changes in vegetation structure and water fluctuations that directly affect the integrity of the wetland (Cushman & Pearl 2007) could make them suitable as an ecological indicator species. The requirements laid out by Carignan and Villard (2002) for a suitable indicator species for any given habitat were suggested to include one or more of the following characteristics: restriction to a certain type of habitat; respond relatively quickly to changes in habitat (i.e. quick generation time); can be accurately and easily assessed; and respond to different types and intensities of stressors. In fact, at least the first three of these criteria apply to the OSF. Some of the other characteristics of OSF that makes them both vulnerable to habitat change and suitable as an indicator species includes: their relatively short generation time and their explosive, communal breeding habits that make population surveys feasible (Pearl et al. 2009; USFWS 2013). Because of these characteristics, further research of this organism is warranted to both inform how to better conserve the OSF, and what the environmental changes that affect their success may indicate for the wetlands they inhabit.

Of particular interest are what environmental habitat changes are most likely to affect OSF breeding success. Reproductive success is critical for the persistence of a species. Embryonic development is one stage of amphibian reproduction that tends to be particularly sensitive to environmental variations such as temperature fluctuations (Bachmann 1969). It follows that if breeding behavior has adapted to maximize reproductive success, then responses to temperature or other cues can provide insight for conservation. The OSF behavior of frequently ovipositing communally with some predictability in timing and location makes this important transitional life stage particularly suitable for further research in that it is easier to locate and identify annual changes in reproductive effort (Pearl et al. 2009). Specifically, OSFs breeding habits make it easier to not only track the conditions under which most oviposition occurs, but also to locate and track changes in the location and breeding populations over time. The importance of breeding ecology for the conservation of the OSF, as an important transitional life stage, and the relative predictability of the location and timing of this stage warrants further research on OSF breeding behavior, its drivers and an assessment of its important environmental parameters.

Current research on the OSF breeding behavior, drivers and environmental parameters is currently incomplete due largely to their cryptic behavior. Oregon Spotted Frogs have been difficult to study without remote sensing devices. Consequently, little is known about the important environmental drivers during critical periods in their life history, such as breeding. Of particular interest would be how temperature, water levels, light, and precipitation may affect or change the habitat preferences at certain life stages or alter incidence of disease, such as prevalence of the oomycete water mold *Saprolegnia* on developing egg masses (USFWS 2010).

My research focused on examination of the diel breeding behavior of the OSFs at West Rocky Prairie, Black River Watershed (Thurston County, Washington latitude 46.89, longitude -122.91) and investigated whether selected covariates (specifically the time of day, temperature, cloud cover, precipitation or twilight range) may influence the timing of oviposition. This aspect of its biology is not well understood for this species, especially the environmental parameters that affect oviposition behavior, making this research valuable for lending key insights on OSF conservation.

Methods

Study site

My study site was the West Marsh at West Rocky Prairie, Thurston County, Washington (Figure 1). West Rocky Prairie is a protected wildlife area managed by the Washington Department of Fish and Wildlife. The West Marsh portion of this reserve includes a marsh-riparian complex that encompasses the headwaters of Allen Creek and a closely abutting reach of Beaver Creek a few kilometers below its headwaters. Beaver and Allen Creek are primary and secondary tributaries of the Black River (Figure 2). The west end of this complex, which encompasses the extreme headwaters of Allen Creek, consists of a 12-ha graminoid marsh that harbors most oviposition areas for the OSF (*Rana pretiosa*) population on West Rocky Prairie. I selected West Rocky Prairie for this study because it supports an accessible and representative population of OSFs in an area where humans were unlikely to disturb deployed camera traps was unlikely to be affected by human disturbance.

Survey Approach

I conducted preliminary assessment surveys to guide the placement of wildlife cameras for oviposition monitoring. Beginning on 5 February 2013, I conducted surveys at least every other day. I surveyed using a systematic back and forth slow walk, visually scanning for evidence of both frogs and oviposition activity. For about 40% of the surveys, I was assisted by one or two people. I focused most of the surveys in proximity to known OSF oviposition locations from the previous year, which were associated with mowed plots (Figure 3) from a Reed Canarygrass (*Phalaris arundinacea*) manipulation study (Hayes 2012). I did this largely due to the demonstration that OSFs show a strong preference for low-stature vegetation in mowed plots (Kapust et al. 2012). I was also on the alert for any adult OSFs, since adult males are often observed in proximity to oviposition locations prior to oviposition occurring (Tyson 2013b). I also conducted surveys of the entire area once or twice a week, which included all known OSF oviposition locations from previous years and over the area with potential suitable oviposition habitat.

When I located a new egg mass, I gave it a unique location mark using a pin flag. Each location was georeferenced using a Garmin GPSMAP 62stÊ handheld unit (Garmin International Inc., Olathe, Kansas) and I subsequently monitored the location for additional oviposition events. At each subsequent survey of flagged locations, I tallied the total number of egg masses. I obtained tallies of egg masses on at least a biweekly basis. Egg mass tallies helped me determine whether new egg masses had been laid, which helped me narrow down which picture series to screen for oviposition activity.

Camera Trap Setup

I set up a total of four camera traps either at locations where I observed aggregating adult male OSFs or where I encountered an egg mass group. I placed three of the cameras on the western half of West Rocky Prairie where the main population and breeding activity occurred (Figure 3). I placed the fourth camera on an oviposition site on the eastern half of West Rocky Prairie to observe a separate subpopulation of OSFs, but this camera was later moved to the western half because of lack of data at the eastern site (Figure 4). I used Reconyx PC900 Hyperfire Professional High Output Covert IRÊ cameras (Reconyx Inc., Suite 2, Holmen, Wisconsin) designed to minimize disturbance to nocturnal animals by emitting a diffuse infrared flash. I programmed the cameras to take high-resolution still photographs at 15-second intervals and continuous 24-hour surveillance. This lower resolution was used rather than 5-second intervals because of limited battery life and card storage capabilities.

I equipped each camera with either a 16- or 32-GB SD card that read and recorded at a minimum speed of 30 MB/second to ensure there would both be enough memory and writing speed to record an uninterrupted photostream. The minimum reading speed also facilitated more rapid uploading to other storage devices. I labeled each SD cards with the camera number in black permanent marker and assigned that card to a specific camera for the duration of the study to ensure that I could unambiguously identify which pictures came from which camera. I used at least two SD cards for each camera so that I could swap out SD cards and still maintain continuous monitoring. Each camera was also equipped with twelve 2600 mAh rechargeable NIMH batteries that were swapped out for a fully charged set, along with the SD cards, no less than every third day. I performed memory card and battery swaps in less than 2 minutes in order to help minimize the entry of rain or water to the interior of the camera and to help maintain the continuity of the photostream. I also replaced the silicon desiccant inserts designed to absorb unwanted moisture during the card and battery swaps.

I had to shut off and reboot cameras at each battery and memory card change, but did not adjust either time or settings as the camerasô internal programming could track of time and settings for at least 48 hours after being shut down. Time flashing across the screen at reboot was compared to satellite time as provided by a cell phone to ensure correct minute-level resolution values. To keep the time string consistent over the study period, I did not switch the cameras to Daylight Savings Time.

I mounted each of four cameras up on 91-cm (3-foot) high stakes approximately 90-120 cm (1-1.5 meters) away from an oviposition location, angled (ñSun or Moon Rise/Set Table for One Yearò 2012)45-70 degrees from horizontal (Figure 5) and aimed towards the center of the male activity if no egg masses were yet present or at the oviposition location if an egg mass group was present. I attached the cameras level with the top of the stakes by running a bungee cord through a hole that provide an anchor point on the camera and wrapping it a few times around the stake and body of the camera, being sure not to obscure the lens (Figure 6). I also used fishing line, as necessary, to bind the camera more firmly. I took care to remove or minimize any obstructing vegetation between the camera lens and the cameraôs picture field. I also recorded the GPS location of each camera at the initial placement and each successive location if a camera was moved.

As soon as the first oviposition was observed, the camera in the location with the least amount of activity was moved to observe subsequent oviposition activity. Once a camera was placed on an oviposition location, I left it to capture all remaining ovipositions until breeding activity ceased.

I left cameras in the field until 16 April 2013. I reduced the picture frequency to once every 5 minutes on 28 March 2013, two weeks after last OSF oviposition event captured on camera. Cameras remained in the field through hatching without further need for battery or memory card changes. During this time, I refreshed the silicon inserts and checked the cameras to determine whether they were still functioning once a week.

Light Meters/ Data Loggers

I used HOBO Pendant Temperature/Light Data Logger 64K-UA-002-64Ê (Onset Computer Corporation, Bourne, MA) to take continuous light and water temperature readings at each camera trap location. Each data logger was fastened to the base of the wooden camera stake by fishing line and left to float in the water with the photosensitive portion oriented horizontally and facing up. I checked the data loggers at each camera trap visits to ensure proper orientation and make sure the indicator lights were still flashing to ensure readings were being taken. I synchronized the data loggers to the same satellite time used for the camera trigger and set to record temperature and light readings at 3-second intervals.

Photo Scoring

Initially, I scored photos by a series of observable behaviors and activities displayed by all of the frogs in a series of frames. The activities included: appearance, movement, reposition, amplexus and oviposition. This range of activities was initially collected because little is known about OSF breeding behavior and certain behavioral patterns specific to mating could shed insight on diel breeding preferences. The pertinent information collected specifically by the camera about these activities included the time, the date and the air temperature during which these activities took place. Later, I narrowed the recorded activity to only confirmed pairs in amplexus, and reduced the recorded activities to the beginning and end of both oviposition and amplexus. I elected to limit the data collection to successful pairs in order to facilitate the completion of scoring in a timely manner.

In order to narrow down the number of photos that needed to be scored, I also reviewed field notes for egg mass counts at specific camera sites to see if oviposition activity occurred in one or more of the given files. If notes about oviposition were lacking, I reviewed some of the photos anyway, but not the entire photostream. Often it was often sufficient to look at 6-10 photos both at the beginning and then at the end of each 12-hour period to determine whether or not any new egg masses had appeared. Fresh egg masses were typically easy to identify, even in the photos, by their smaller size, positioning generally more on top of older egg masses and had a more firmly round, compact appearance. If fresh egg masses were found to be present, photos would be sifted through one by one on a laptop using Windows Photo Viewer by quickly scrolling until either a confirmed pair or an oviposition event was noted.

I defined a confirmed pair as a pair capable of a successful oviposition. Such pairs consisted of a male frog grasping a female frog, with male frogs invariably being somewhat smaller than females, and possessing disproportionately enlarged forearms and a relatively narrower waist than females, which have thinner arms and a fatter waist. Besides oviposition, I also used selected behavioral cues to help determine actual male-female pairs. Males attempting to amplex other males were common events, but were typically associated with a high level of activity, such as leaps, rolls (where the pair rolls laterally, usually with legs outstretched) or dives, until the amplexor (top male) was dislodged from the amplexee (bottom male). Amplexus usually lasted fewer than ten frames (2.5 min) for these male pairs. In contrast, a male and female pair would typically remain in amplexus for more than 10 frames and frequently would return to specific chosen locations and exhibit a rotating behavior, where the pair would remain upright and pivot horizontally around a point near the femaleôs vent often lasting between 3-6 frames (approximately 45-90 seconds). Confirmed amplectic male-female pairs would occasionally dive, but did not exhibit the dislodging techniques of leaps and rolls used by the attempted male-to-male pairings.

I identified most oviposition events by the appearance of small eggs behind a pair in amplexus (Figure 7). Since unfertilized eggs have the animal (dark) pole of the egg rotated in random directions (uniform rotation occurs post-fertilization), emerging eggs are typically easier to identify as a checkerboard of black and white. However, eggs in the process of being laid were not always be observable in the camera trap photos because of the orientation or positioning of the amplectic pair, so I also used distinctive behaviors exhibited by an ovipositing pair to identify the oviposition sequence. These behaviors usually started with a halt in movement by a pair at a single location, where the pair would often rotate horizontally around a point as previously described before the oviposition commenced. The female would then begin to deposit eggs, and often in the process a characteristic bobbing of the pair would occur, where the head of the female would sometimes disappear under the water. A cluster of eggs would then slowly become visible, and the female would extend forward as the eggs were laid until her legs were fully extended. Then there would be a pause in activity and the male would shortly thereafter break amplexus, with the female usually last to leave the visual frame. I used this characteristic progression of behavior to identify pairs that oviposited further away from the camera when it was not possible to view the actual eggs being laid.

Once an oviposition event was recorded, the next step was to trace the pair back to their initial appearance in the cameraƒs field of vision in order to get an estimate on how long amplexus occurred prior to oviposition. For this, the pairƒs movement would be viewed by viewing the photos in reverse from the start of oviposition to the point where the pair could no longer be identified as being in active within the scope of the camera. Often the photographs were detailed enough to allow identifying specific dorsal spot patterns on the maleƒs back of amplectic pairs, making positive identification of a single pair possible, even if they disappeared out of the field of view for a length of time. Occasionally this was not possible, particularly if a pair was far back in the field of view. In this case, amplexus was traced as far back as it was possible to positively track a particular pair based on movement within the visual range of the camera. Photo intervals of 15 seconds provided enough resolution to be able to track most amplectic pairs based on movement alone. A lack of activity in a particular area for 10 frames or more was considered a stopping point for any pairs that were not visually identifiable by back pattern.

Data Handling and Analysis

I extracted data on amplexus and oviposition events through review of the camera trap data. In particular, wherever possible, I extracted start and end times for both categories of events. For those oviposition events for which both start and end times were available, I determined the length of the event. I performed a similar calculation on amplexus events, but because I did not capture the true start of amplexus in any of the ovipositing pairs, amplexus event length was originally recorded to be from the first appearance of the pair in amplexus to when oviposition was complete and the pair separated. Because this made the length of all amplexus times consistently larger than oviposition times, for the purposes of comparison I subtracted the time spent ovipositing from the total length of amplexus. I omitted amplexus events from the analysis where a pair left the field of the photo frame and could not be unambiguously identified as the same pair. I provide descriptive data on variation in length of amplexus and ovipositions events as means (⍰), standard deviations (SD), maxima (Max) and minima (Min). I compared the mean length of amplexus versus oviposition events with a two-tailed Students t-test for independent samples. As the variance of amplexus events was nearly 50-fold that of oviposition events (F test: $F = 47.9$, Critical $F_{df=24/26,0.05}$

= 2.22), I applied the form of this t-test for unequal variances. Because total amplexus events numbered only three more than oviposition events, separated analyses of the relationship to the physical environmental variables was not justified. As a consequence, all analyses of the relationship to the physical environmental variables addressed only oviposition.

Time of Day

To examine the diel oviposition pattern, I partitioned all oviposition events for which I had a start time into hourly blocks by camera beginning at one minute after midnight so that each hourly block ended on the hour (i.e., 0:01-1:00 for the first hour, 1:01-2:00 for the second hour and so forth). On some days, there were time intervals during which one or more cameras were not monitoring. Additionally, one camera operated every day but only between the hours of 13:00-23:59. Hence, to make meaningful comparisons, it was necessary to standardize the data by calculating the rate of oviposition per camera-hour.

To determine the best description of the diel pattern, I partitioned the daily oviposition times into day and night categories using four different classification boundaries: 1) sunset-sunrise, 2) civil twilight, 3) nautical twilight and 3) astronomical twilight (Table 2). I determined these boundaries from data that had been calculated for the latitude of Olympia, WA (ñSun or Moon Rise/Set Table for One Yearò 2012). I then tallied the number of ovipositions that occurred between each of classification boundary.

Light Intensity

Light intensity readings (measured as lux) from the Hobo data loggers were summarized by calculating hourly means and matching them to the hourly data for each corresponding camera. If a camera lacked monitoring data for a given hour, the hourly light data were discarded for that particular camera and left out of the final averaged light intensity. The light data were compared across all three data loggers using a correlation coefficient to ensure that these were highly correlated prior to being averaged together. I obtained means of the light data for the monitoring cameras for all hours when no ovipositions were recorded. For hours in which there was an oviposition, only the hourly light intensity for the camera at which oviposition took place was used for that hour. I had no instances in which ovipositions took place at two or more cameras simultaneously, although an average between the light intensities at those cameras would have been taken had there been such an instance.

I performed a t-test to compare light intensity values of hours when oviposition occurred versus those where no oviposition occurred. Light intensity was also summarized on an hourly basis across all the days ovipositions took place by averaging the hourly light values for all the monitored days and comparing these data to the total number of oviposition rate.

Temperature

Air temperature was recorded by a thermistor imbedded in the wildlife cameras and was included with the time stamp on each photo frame. I averaged the temperature from the first and last minute of each hour block to summarize the data hourly for each camera. An air temperature mean was taken if a camera was monitoring for more than half of a given hour block, or if an oviposition took place during that hour block. I compared hourly air temperature means to hourly water temperature means for each hour using a correlation coefficient. Because of the high degree of correlation (r = 0.900, n = 384), and because frog activity took place almost exclusively in water, I used water temperature data for the rest of this analysis.

Water temperature readings were taken by the Hobo data loggers and were summarized in a fashion similar to the light intensity data. Only readings taken while the camera was monitoring were used. The temperature values for non-oviposition hours were shown to be highly correlated across all three sites and were averaged together. For hours in which oviposition events occurred, only the water temperature from the logger at the oviposition site was used. I used a t-test to compare the mean temperature during oviposition events to the mean temperature when no ovipositions took place.

The number of total ovipositions per degree Celsius was recorded to observe the temperature at which most ovipositions were observed. I had sorted the water temperature data into 2°C temperature blocks from -1.9-18.0 °C that spanned the entire range of water temperatures recorded from the date of the first oviposition to the date of the final oviposition. In order to standardize the data, the number of hours recorded in each degree (effort) was compared to the number of ovipositions that occurred for each temperature block. The effort per degree was graphed with the rate of oviposition per hour corrected for effort for comparison.

Cloud Cover

Cloud cover data came in an hourly format from the nearby Olympia Airport Weather Station data, located approximately 9.7 km N of my study site. For analysis, I grouped these data into one of four categories: 0-25%, 26-50%, 51-75% and 76-100% cloud cover. These relatively large categories were used as an attempt to get some data in each category because the data were very unevenly distributed across the range of cloud cover conditions. The total ovipositions for each category were summed up and divided by the total number of hours within each cloud cover category.

Precipitation

I also retrieved hourly precipitation data from the Olympia Airport Weather Station and converted the values from inches to millimeters. Precipitation was then divided up in 10 different 0.5-mm groupings and was standardized by the number of camera hours in each block in order to graph oviposition rates per camera trap hour. A t-test was performed for the average hourly precipitation to see if there was a difference between oviposition and non-oviposition intervals.

Moon Cycle

Moon illumination data were provided by the Moon Calendar (2013) from Calender-365.com and consisted of the percentage of the moon illuminated at a daily level of resolution. To accommodate a daily resolution, the total number of monitoring hours for all the cameras needed to be converted from hours to trap-days for each illumination percentage value. The number of ovipositions that occurred for each illumination value was then divided by the trap-days to get a daily oviposition rate. I also calculated a correlation coefficient (r) to compare the oviposition rate to the illumination value. Lastly, I ran a t-test to compare the illumination percentages on days where no oviposition occurred to the days with oviposition data.

Results

Descriptive Data on Amplexus and Oviposition

OSFs generally spent more time in amplexus as compared to depositing eggs (Table1). Mean time spent in amplexus was 43.8 min (range: 0.3-111.3 min), significantly longer than oviposition ($p = 0.00003$), which averaged 11.7 min (range: 4.0-22.5 min). I also found that variation in the duration of amplexus ($s^2 = 979$) was an order of magnitude greater than that of oviposition ($s^2 = 20$), a significant difference ($F_{24/26} = 47.9$, $p = 0.00003$).

Time of Day

Oviposition took place mostly at night, between the hours of 20:00 and 6:00 (Figure 8). I recorded the most oviposition between 23:01 and 0:00, when the frequency was 0.141 ovipositions/trap-hour. The immediately preceding hour (22:01-23:00) also had an oviposition rate close to this value, 0.139 ovipositions/trap-hour. I recorded no oviposition activity in the 1:00 hour block, but oviposition resumed between 02:00 and 06:00. Limited oviposition activity also occurred during the day, but the rates were low. Both hour blocks in the only daytime interval in which oviposition occurred (12:00-14:00) had oviposition rates of 0.030 ovipositions/trap-hour.

I partitioned day and night using different boundaries that varied in their information content in describing the diel pattern of oviposition (Table 2). Though all four alternative boundary choices (sunrise-sunset, civil twilight, nautical twilight, and astronomical twilight) for day versus night partitioning showed an unequal distribution in oviposition activity in favor of night category, the sunrise-sunset and civil twilight boundaries were pattern-wise equivalent descriptors and the most asymmetric.

Light Intensity

Though light levels averaged lower during oviposition, I found no significant difference between the mean light intensity that occurred during hours of oviposition ($\bar{X}$= 7270 lux; SD =19429 lux) versus that which occurred during the hours of no oviposition ($\bar{X}$ = 10614 lux; SD = 18384 lux, $p = 0.206$; Table 3). However, examination of the diel oviposition pattern in response to mean light intensity revealed a bimodal condition (Figure 9). Oviposition occurred either during times when I recorded light intensity as zero or infrequently, when light intensities were greatest. I found a modest but significant negative correlation between light intensity and oviposition activity ($r = -0.385$; $r_{\text{critical}(0.05)} = 0.337$).

Water Temperature

I found no significant difference in the mean water temperature during oviposition events versus the mean water temperature during no oviposition ($\bar{X}$ = 6.8 ºC during oviposition; $\bar{X}$= 6.9 ºC during no oviposition; $p = 0.811$), but oviposition did not appear to occur randomly throughout the temperature range (Figure 10). I recorded no oviposition activity below 2 ºC. Additionally, most (22 of 28) ovipositions occurred at temperatures in the 4.1-8.0 ºC range. The 2-degree intervals of 4.1-6.0 ºC and 6.1-8.0 ºC also each had the highest rates of oviposition, 0.119 and 0.181 ovipositions/trap-hour, respectively. The temperature range of 12.1- 14.0 ºC, which contained two daytime oviposition events, also had a relatively high rate, 0.118 ovipositions/trap-hour.

Cloud Cover

I found cloud cover unevenly distributed across the four quartile categories used. Most samples were in the range of 76-100% cloud cover whereas I lacked data in the 51-75% category (Figure 11). Most of the oviposition activity (24 out of 28 events) took place in the 76-100% cloud cover range, but the rate of oviposition in this category was only 0.08 ovipositions/trap-hour due to the large sampling effort in this range. The highest rate of oviposition (0.15 ovipositions/trap-hour) occurred in the 26-50% cloud cover range but this pattern results from only three of the total 28 ovipositions. The 0-25% cloud cover category had the lowest oviposition rate, 0.017 ovipositions/trap-hour. All oviposition events that occurred in the categories with <51% cloud cover occurred during the day, whereas all of the ovipositions that occurred during the night fell within the highest cloud cover range.

Precipitation

I found mean precipitation levels to be less during oviposition (0.25 mm/hour) than for periods with no oviposition (0.28 mm/hour), but this difference was not significant (*p* = 0.784). Most oviposition (16 of 28) occurred during hours without precipitation, but 12 ovipositions occurred during hours with values greater than zero. These values ranged between 0.13-1.78 mm/hour. The highest rate, 0.06 oviposition/ trap-hour, occurred when precipitation was in the 1.6-2.0 mm range and represented two of the 28 oviposition events. The 0-0.5 mm precipitation category contained 23 of the 28 total oviposition events. Though monitoring occurred over a period with up to 4.6 mm of precipitation, I recorded no oviposition when precipitation was >1.8 mm (Figure 12).

Moon Cycle

I recorded a weak correlation between the proportion of moon face illuminated and oviposition events, although this correlation was not significant ($r = 0.299$; $r_{critical(0.05)} = 0.374$). Most oviposition took place at moon illumination values >0.72, but they did not appear to exhibit any kind of close relationship to increasing illumination beyond that (Figure 12). The highest oviposition rate of 2.9 took place both during the waxing cycle the waning cycle at moon face illumination proportions, respectively, of 0.72 and 0.89. Additionally, t-test results did not yield a significant difference between the mean moon face illumination values for days with oviposition versus days without oviposition (*p* = 0.940).

Discussion

Diel Behavior Trends

As indicated by the pilot camera trap study from the previous year, OSFs seemed to exhibit a preference for nocturnal oviposition (Hayes 2012). Based on other research of amphibians showing a preference for nocturnal activity, the most significant difference between nocturnal and diurnal behavior occurs between the civil twilight boundaries for morning and evening (Pechmann & Semlitsch 1986), as we found in this study. As in the case of these other nocturnal amphibian species, the most asymmetric difference between night and day in oviposition activity for OSFs was found using civil twilight and sunrise-sunset boundaries. Civil twilight was selected as the best descriptor because it contained the same asymmetric pattern as sunrise-sunset, but encompassed a more specific window of time.

For the ovipositions occurring at night that did not occur within the astronomical twilight boundaries, they all occurred in the morning rather than the evening, before the morning civil twilight boundary was reached. The lack of ovipositions before the evening astronomical twilight boundary may suggest that oviposition activity typically does not commence until after the evening astronomical twilight has been reached. It is possible that most OSF breeding individuals prefer a threshold of darkness that occurs shortly after the evening astronomical twilight boundary has been reached, but the presence of daytime oviposition suggests this is not an absolute requirement for breeding activity and that other factors may be driving behavior.

Behaviors preceding oviposition exhibited a similar pattern of activity within the twilight boundaries. Male congregational activity around subsequent oviposition sites appeared to begin between evening civil and astronomical twilight. Activities such as when the male frogs first appear and general interactions between the male frogs at the breeding site show much more activity before the evening astronomical twilight could be analyzed with the photostream from this study. Since these male activities are breeding-related behaviors that precede oviposition, it may be worthwhile to quantify the relationship of the patterns to photoperiod and other covariates in future studies.

Light Intensity

Since OSFs exhibited a general nocturnal oviposition pattern, it was not surprising that I found a negative correlation between oviposition and light intensity. Whether or not this negative correlation between light intensity and oviposition activity is a true reflection of light-dependent behavior or a reflection of a circadian rhythm cannot be determined with the present data. The necessary data would require a more sensitive data logger that would be resolved enough to quantify changes in light intensity between the astronomical and civil twilight boundaries. Unfortunately, the data loggers I used were not sensitive enough to pick up changes in light intensity data after even the civil twilight boundary. As a result, much of the oviposition activity is tied to a non-fluctuating lux value of zero and cannot be correlated to small changes in light intensity that may occur between the evening and morning civil twilight boundaries.

Of particular interest are the three ovipositions that did not occur within the civil twilight boundaries. This daytime oviposition activity is particularly interesting because it occurred during some of the brightest light intensities. These ovipositions occurred on sunny days with low cloud cover and tended towards the upper extreme range of the light intensity (62,000 lux range).

Because of the small sample size, it is difficult to say whether these observations indicate a bimodal trend, or if these behaviors are rare and disproportionately represented in this sample. Overall, the predominance of a nocturnal oviposition suggests that this behavior is at least partially driven by either a light dependent circadian rhythm or by light intensity, but the diurnal ovipositions suggest it is not the only factor driving this breeding behavior. It would be useful to investigate whether there is an interaction between temperature and light intensity. Also, this study was done on a lower elevation population. A higher elevation population may respond differently to light and temperature in ways that could lend insight on how these covariates drive breeding timing and behavior.

Water Temperature

Most breeding activity of the OSF seemed to occur around a mean water temperature of 7 ºC, which was expected based on past observations of this species (Hayes 2012). It was interesting that the preferred mean temperature for oviposition was not significantly different the mean temperature during no oviposition, since the average temperature outside of oviposition exhibited greater variation than the temperature during oviposition. There are many possibilities on how breeding behavior might be responding to temperature. A couple examples of such possibilities could be because OSF initiate the breeding cycle when the mean environmental temperature approaches their preferred oviposition temperature range, or because temperature has to reach a minimum temperature for several days. It would be interesting to gather average environmental water temperature data immediately preceding and following oviposition to see if there is a significant difference between these and the mean environmental temperature during oviposition activity. Likewise, it would be valuable to determine if the mean water temperature is consistent for future breeding cycles.

The OSF does seem to exhibit a temperature-dependent breeding activity, which seems to be consistent with the thermal requirements of developing embryos. The lower thermal limit in a constant environment is around 6 C↔ for a few populations of OSFs, but lower temperature fluctuations, and even a small amount of frost do not cause developmental abnormalities in a field setting (Licht 1969; Bowerman & Pearl 2010). Therefore a preferred temperature range for oviposition that supports the earliest normal embryonic development, in this case between 4.1-8.0 C↔, is not surprising. These temperature-related drivers could have important implications for future conservation measures for this species in the face of climate change. If the OSF shifts its breeding behavior in response to changes in temperature as some other amphibian species have (Todd et al. 2011), breeding timing may be shifted to a less optimal period in the hydrologic cycle. Drier summer trends in the Pacific Northwest (Mote & Salathe Jr. 2010) could cause the OSF to face earlier desiccation of their wetland habitat.

Cloud Cover

Except for three oviposition events that occurred during the day under less than 51% cloud cover, most ovipositions occurred in a cloud cover range of 88%-100%. The three ovipositions that occurred during low cloud cover resulted in a higher oviposition frequency than those occurring under Ó88% cloud cover. This higher frequency could reflect an actual preference for low cloud cover, a preference for the higher light or temperature associated with that lower cloud cover during the day, or it could simply reflect a spurious pattern because of small sample size. One surprising observation was that many daylight hours had more than 88% cloud cover, which means daytime in periods with lower cloud cover could reflect an actual preference. This seems to contradict the cloud cover trends associated with nocturnal oviposition.

Unlike daytime oviposition, nighttime oviposition was associated with a cloud cover class greater than 88%. There were a few nights with 50% or less cloud cover, but no oviposition events were recorded during these periods. The association of nighttime oviposition with greater than 88% cloud cover could be a reflection of incidental conditions, or a preference for warmer temperatures or other conditions that are associated with greater cloud cover at night.

Again, similar to light, there seemed to be a bimodal pattern with nocturnal ovipositions occurring during higher cloud cover and diurnal ovipositions occurring during lower cloud cover. The diurnal trend was unexpected as many amphibian species prefer to be active during periods with precipitation and higher humidity (Bellis 1962). Additionally, nocturnal species or those that prefer lower light levels would be expected to prefer more cloud cover because it can block, reflect or refract sunlight, moon illumination or starlight (Rich & Longcore 2006). Based on the bimodal observations, it appears that cloud cover may not be a primary driver in oviposition behavior. For future studies incorporating cloud cover, it would be helpful to have a camera recording sky conditions synchronously with the camera traps recording oviposition, although a specialized camera may be necessary to view nighttime cloud cover. This is particularly important since the resolution of cloud cover data for this study was not resolved to a site level. Overall, the coarse resolution of cloud cover classes that the data demanded and the limited scope of the data make it difficult to come to any meaningful conclusions.

Precipitation

Most ovipositions occurred when little to no precipitation took place, but this could have been incidental because most of the monitored hours had little to no precipitation. Furthermore, when the data was standardized for the hours recorded in each precipitation category, there was a higher rate of oviposition that occurred at a precipitation rate between 1.6-2.0 mm/hour, but the oviposition rate dropped to zero at all precipitation levels above 2.0 mm/hour. The lack of activity at higher precipitation and oviposition activity with no precipitation were unexpected observations, since frog movement at activity is often associated with moist or wet conditions.

Upon my further examination of the photos, fewer total frogs appeared to be active when rain was visibly hitting the water. Additionally, there was no detectable precipitation in the photos during the actual oviposition events. During camera checks on rainy days I noticed there were seldom any OSFs out at the breeding sites. It is difficult to assess whether OSFs preferred to oviposit during intervals with or without precipitation when examined as units were resolved in hour blocks associated with less than 2.0 mm/hour of precipitation, especially since camera trap photographs may not have had high enough resolution to allow detection of low precipitation levels. Based on the available visual evidence, OSF may actually prefer no precipitation during breeding activities. However, if the OSFs do actually prefer low levels of precipitation during oviposition, it is at levels below that which can be detected using a camera trap for graphic evidence.

While camera traps can provide coarse visual evidence of what precipitation may have been doing during oviposition, it would be beneficial if there was another method of recording changes in precipitation at the study location with a more refined ability to detect low precipitation. However, the data gathered was sufficient to identify that OSF seem to prefer low to no precipitation during oviposition. While visual evidence suggests a preference for low to no precipitation during oviposition for OSF, a larger sample size and more resolved data would be necessary to further investigate the actual relationship between precipitation and oviposition activity. Knowledge of how OSFs respond to precipitation may provide insight on how changes in the duration, timing or intensity of precipitation, such as in the face of climate change.

Moon Cycle

Most egg deposition occurred while the moon was in a gibbous or full moon state but there was not an obvious relationship between moon illumination and oviposition frequency. Past studies revealed a tendency for amphibian breeding to be more common in or around a full moon, so OSF may also show a similar trend (Church 1961; Grant et al. 2009). It is possible that there is a minimum illumination of the moon that is correlated to when breeding cycles begin, but this is unlikely since all nocturnal breeding activity took place with cloud cover. In order to effectively examine this relationship, several years of follow-up study are needed. Since breeding typically lasts two weeks at this location and does not extend through an entire lunar cycle, the trend for ovipositions to occur in a mostly full phase of the lunar cycle may not reflect a true pattern. Breeding may have simply coincided with a mostly illuminated moon this year, but it may not do so in subsequent years if other variables influence breeding.

Additionally, moon illumination data resolution was coarser than the other covariates, available on a daily scale rather than an hourly scale. This resolution may have been too coarse to establish a relationship, if one existed, and may not reflect the illumination experienced by an OSF on the overcast nights during which all of the recorded nocturnal ovipositions occurred. Additionally, the daily moon illumination data did not examine the time the moon was actually up each night, which would be useful to look at in the future. A lunar cycle may be more important in initiating the breeding cycle, but based on the current analysis does not seem to be associated with the fluctuations in breeding activity during the breeding cycle.

Whether or not lunar cycles are important in driving oviposition activity could be important for conservation because we would better understand breeding cycle triggers. Additionally, moon illumination could be important to breeding success if the OSF is sensitive to changes in night light intensities. It is also important to be able to distinguish whether the OSF exhibits a circadian rhythm behavioral pattern or one driven by light intensity itself in order to help establish just how important changes in light intensity are in triggering a successful breeding pattern. If, for example, OSF exhibited a circadian rhythm based on average fluctuations in light following a lunar cycle, it is possible that ecological light pollution could interfere with their ability to initiate breeding at a proper time by not allowing for these cyclic fluctuations in light intensity.

Improving Data Acquisition

I had some selected difficulties with maintaining a continuous flow of data with some of the cameras. Camera 9164546 shifted to incorrect time lapse settings shortly after deployment and remained this way until an entire series of photos from this camera was examined and I discovered that only a 12-hour period was being recorded daily. As a result, only activity between the hours of 12:00 pm and 12:00 am were recorded for several days, with more egg masses counted at this site than could be accounted for from the ovipositions observed via the camera trap. Fortunately, this time period still captured oviposition data within a similar range of temperature and light data to contribute to the study. This problem could have been identified and remedied earlier with checking and verifying the proper settings after swapping batteries and memory cards.

Some other relatively easy-to-fix technological issues that interrupted camera data acquisition also occurred. Batteries ran out prematurely a couple of times for each camera, either because they did not receive a full charge before being deployed or due to other unknown factors affecting battery life. In addition, occasionally the SD cards would stop recording, even though there was substantial memory was left on the disk and adequate battery life existed. On two occasions, the SD cards suffered data loss due to corrupted files that were not recovered. As a result, the hours throughout sampling were not represented equally, and the data collected had to be standardized to compensate for the unequal representation of each hour. These problems could have been minimized by more frequent trips out to the cameras for memory card and battery swapping. More than two 32-GB SD data cards per camera would allow more time to recover corrupted data before card re-deployment, and fewer hours of deployment would not stress the storage capacities of the cards. Because some spare memory is required for the card to properly store data, it is possible that cards reaching maximum storage capacity were a contributing factor to why some data was corrupted or lost. Despite these issues, enough oviposition data were gathered to at least preliminarily assess the relationships of each variable to oviposition behavior.

Facilitating Data Transcription

Scoring camera trap photos for OSF breeding activity is a time-consuming process and can significantly extend the length of the project unless certain techniques are used to facilitate this portion of the study. A single photostream of 10,000 photos takes between 4-8 hours to view completely (spending a maximum of 3 seconds per photo) at normal resolution without recording data. Significantly more time is necessary per photo if some breeding or other activity must be recorded. A computer with a fast loading or a rapid photo streaming program would greatly facilitate this portion of data transcription, although sections with a high level of activity would still need to be viewed at a slower pace to ensure oviposition and amplexus activity are properly identified. In this project, I had to handle approximately 500,000 photos, necessitating the implementation of methods to identify whether oviposition activity took place without viewing 100% of the photostreams.

One of the primary techniques I used to facilitate faster data acquisition was to scan through the photos and identify ovipositing pairs first before tracking pairs in amplexus. A successful oviposition became a method of confirming a true pair as opposed to a male to male pair. This saved a significant amount of time, since male to male amplexus events are common and sometimes last for several frames, giving the impression of a true pair until a fight and physical separation occurred or closer proximity to the camera gave visual confirmation of two males. Additionally, field notes detailing new eggs masses can help to identify oviposition data for each camera site during field visits. These notes should be kept in detail to help to reduce the need to search entire photostreams. Photographs can also be scanned at random sections towards the beginning and end of photostream sections to narrow down the times of when fresh egg masses were laid within that photostream. The latter method is not as reliable, however, and should be followed up with a more thorough viewing as time permits because not all new egg masses will be obviously visible in the photos.

Initially, all activity around the breeding site was recorded, including non-pair appearances, movements, repositions and interactions. Each particular frog would be identified and followed throughout the entirety of their appearance on the screen. Oftentimes there were several individual frogs per series of frames to track simultaneously. Such high-level behavioral transcription has the potential to contribute valuable insights to breeding activity, particularly since there is a larger quantity of breeding-related activity data available at oviposition locations if the scope could be expanded to include more than amplexus and ovipositions. However, this more intensive form of data can be daunting in the sheer amount of time it takes to transcribe. For future studies examining this expansion, breeding behavior could be more thoroughly investigated through an assortment of smaller, randomly selected sections of photos instead of entire photostreams. For this project, these data were determined to be outside the scope of this paper in the interest of time.

Limitations to the Research

One limitation to these data are that it is based around a single population of the OSF and cannot be stated as representing the behavior of all populations until data are collected from other populations. Initially, a fourth camera was originally deployed to capture oviposition behavior of a subpopulation on the Eastern half of West Rocky Prairie complex for comparison to the population on the Western half of West Rocky Prairie. However, this fourth camera did not capture any oviposition data and thus was not able to be incorporated into part of the analysis.

Another limitation is that more data are necessary to better understand the trends seen in this exploratory study. In a future study, it would be ideal to set cameras out early and conduct regular full surveys to capture the earliest OSF oviposition activity at any particular point. Capturing the initial ovipositions can help maximize data collection, as well as possibly lend more insight as to what the initial covariates that trigger breeding activity might be. Unfortunately, in this study the very first site of mass oviposition activity data was missed over the course of a weekend because full surveys were not being conducted more than once a week. The breeding cycle of the OSF is fairly rapid, and it is critical to identify and start recording shortly after oviposition is first observed in order to successfully record sufficient data, especially since some analyses, such as the t-test for light intensity, may have revealed a more definitive trend with more oviposition data.

Also to facilitate more data collection, in the future it would be helpful to check the cameras once every other day instead of every third day to exchange batteries and SD cards and to verify that the camera settings and orientations were still correct, to help minimize gaps in data collection. Additionally, a quick scan of the photos after downloading would help identify and correct for any potential problems missed in the field, such as vegetation blocking the lens, adjusting the camera angle to better capture oviposition or to identify programming issues not recognized or corrected for while out in the field.

Conclusions

Despite the limitations faced in this study, enough data were gathered for a preliminary assessment of OSF oviposition trends as they related to the selected environmental variables. Although not all of the results were significant, this study shows behavioral trends that are worth further investigation and suggests what environmental parameters might be the most important in driving OSF breeding activity. There is a significant tendency for OSF to oviposit during night at this location and there may be some interaction between time of day, light, water temperature and precipitation. The primarily nocturnal behavior pattern observed through time of day and light intensity trends suggest this species could be negatively impacted by ecological light pollution during the breeding season, although this would need to be tested through a manipulative study. Additionally, the narrow window of oviposition temperature suggests that changing temperatures could influence future breeding timing or success of this organism. Therefore, these results might be useful for the continued conservation and protection of this species.

Chapter 3: Interdisciplinary Relevance

This study involves the intersection of a biologically important feature (the breeding behavior of the OSF), aspects of the physical environment (temperature, precipitation and other variables), and a social need (conservation of the Federal and Washington State endangered OSF). This topic is relevant to a graduate program in Environmental Studies because OSFs, like many amphibians, are sensitive to diverse anthropogenic alterations, including habitat loss, introduction of exotic species, and changes in climate that may influence temperature and water availability.

Breeding represents a particularly vulnerable time in amphibian life history and it is unlikely that OSFs represent an exception to this pattern. Oregon Spotted Frogs are already limited in their distribution and are continuing to decline. Hence, it is important to understand whether its breeding behavior, which is unstudied, has aspects that may contribute to its vulnerability. Understanding its breeding behavior can offer insights to both improve management of OSFs, other amphibian species, and perhaps even non-amphibian taxa.

The unique methodology used in this study involving the use of camera traps to identify breeding behavior describes a pioneering method for researching OSF and has potential application to other amphibians that display communal oviposition, such as the Cascade Frog (*Rana cascadae*) and the Sierran Yellow-legged frog (*Rana sierrae*). The findings of this study also describe oviposition behavior and provide a base of how that behavior relates to certain environmental factors. This base can be built upon in future studies to aid in the continued monitoring and protection of this species.

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Appendix A: Figures

Figure 1. Current and historical populations of the Oregon Spotted Frog in Washington Adapted for use in this paper from Hallock (2013).

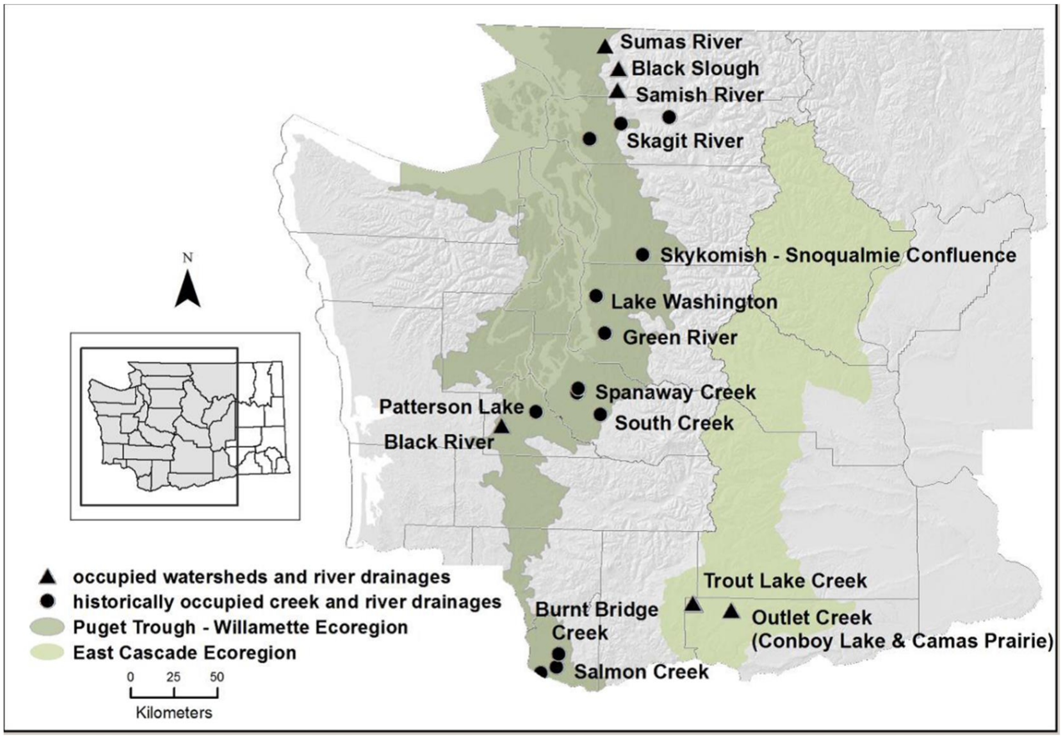


Figure 2. Location of West Rocky Prairie in Washington State, USA Adapted for use in this paper from Tyson (2013a**).**

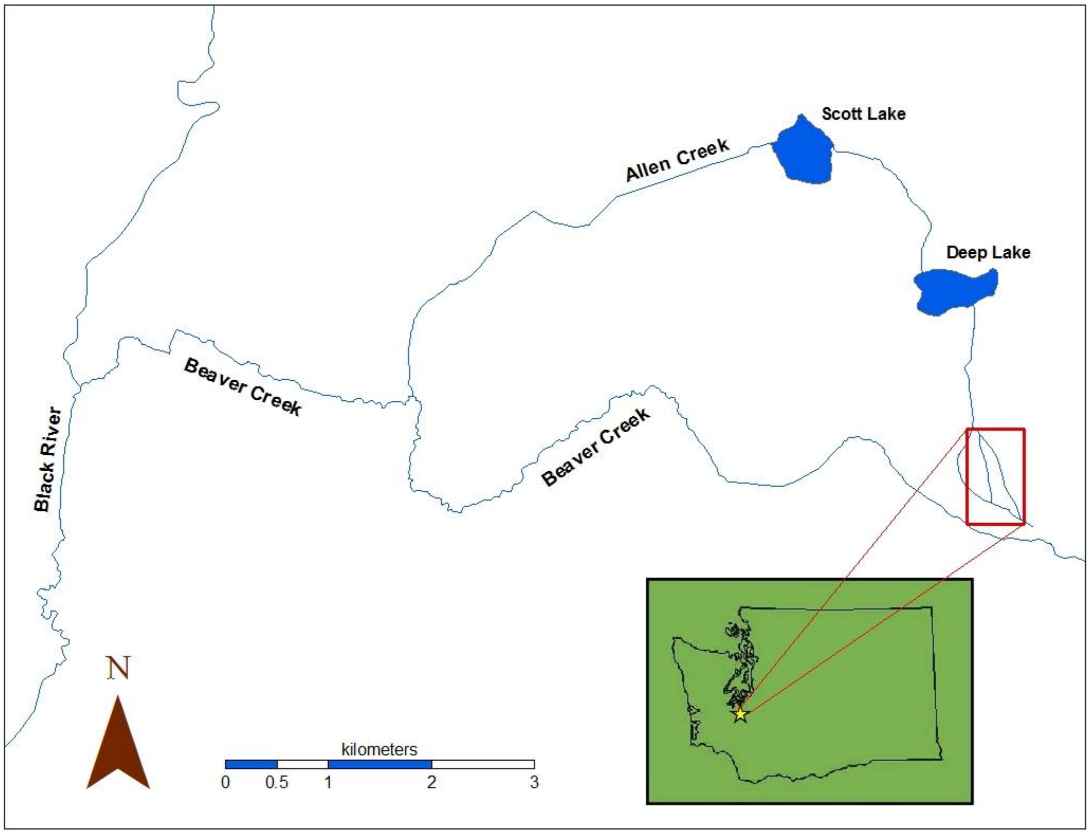


Figure 3. Plot, camera and oviposition locations Adapted for use in this paper from Tyson (2013a). These were the locations of active oviposition in 2013 at which the camera traps were placed on the western half of the West Rocky Prairie Wildlife Area. The numbers next to the yellow triangles correspond to the number of the oviposition site that each camera was monitoring. For data on the egg counts at each of these locations, see Table 4.

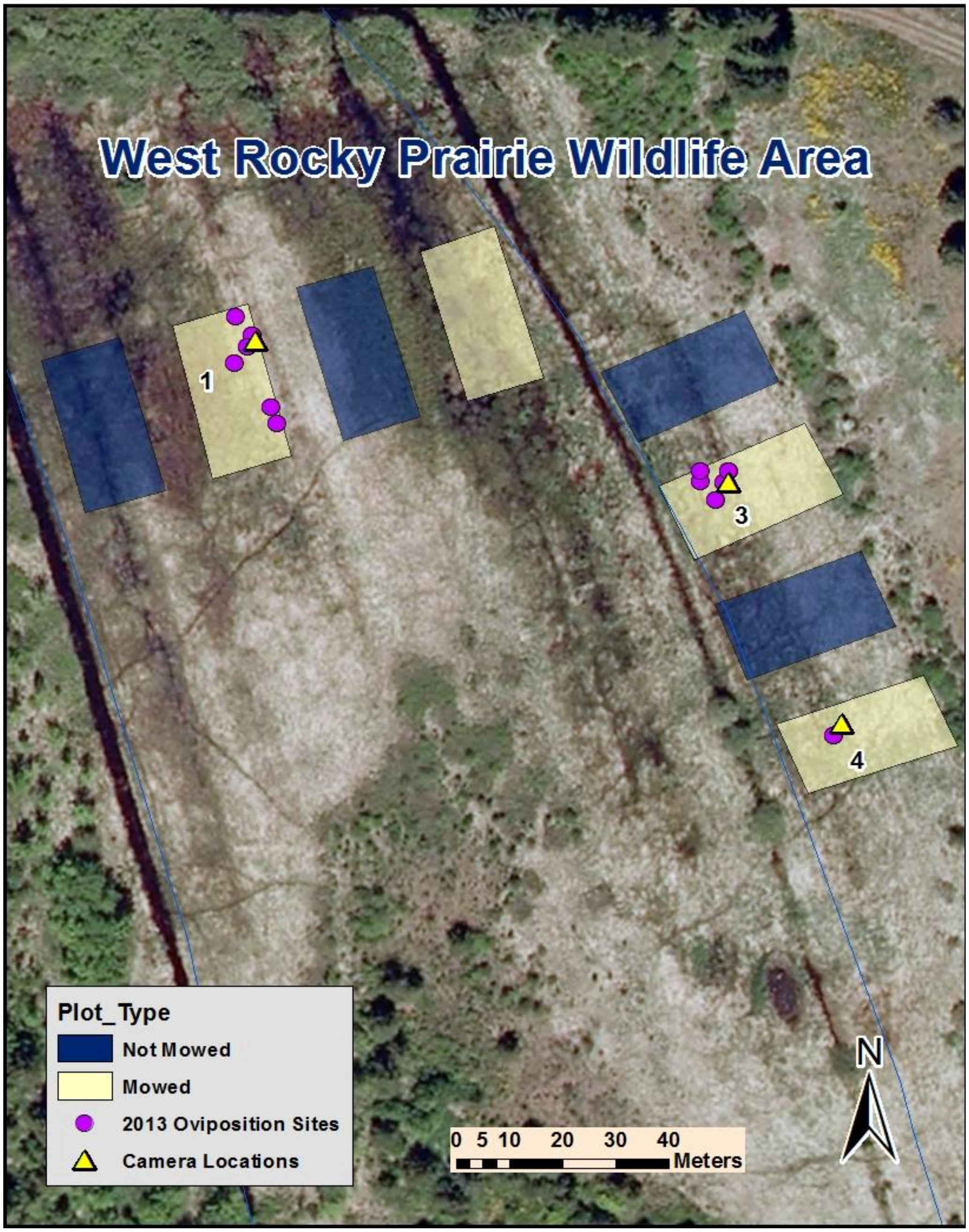


Figure 4. Camera locations on the West Rocky Prairie Wildlife Area Adapted for use in this paper from Tyson (2013a). Three cameras (yellow triangles) were placed on the western plots of the West Rocky Prairie, while one camera was located on the eastern half.

Figure 5. Camera Trap Positioning and Orientation. Cameras were placed on a stake 1-1.5 meters away from the location of oviposition, leaning and facing towards the target area at an angle of approximately 45-70 degrees above horizontal.

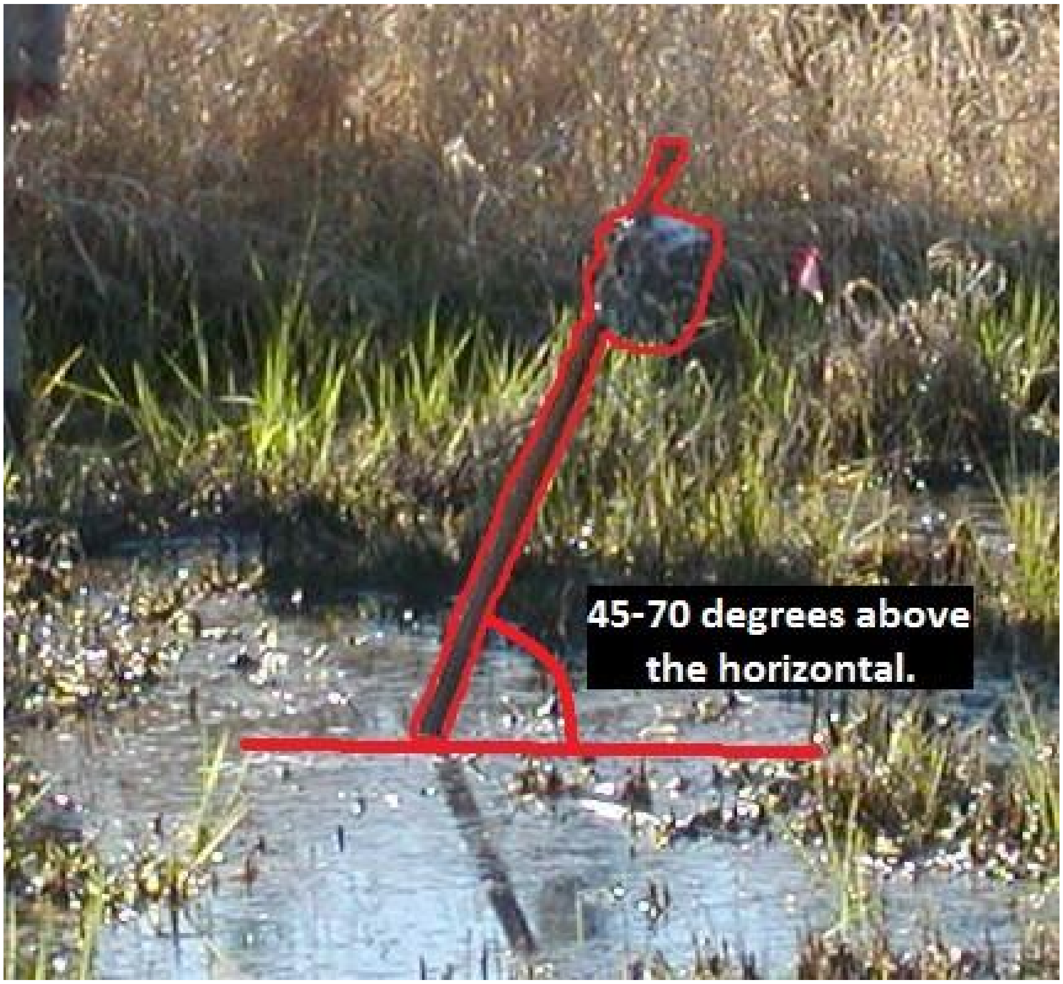


Figure 6. Camera Trap Attachment. The technique used for attaching the camera to the stake involves using the provided bungee (comes attached to the camera) wrapped around the stake and top portion of the camera, taking care not to block the lens.

Figure 7. Camera Trap Photograph This is one of the infrared photos taken by the Reconyx PC900 Hyperfire professional research camera showing two amplectic pairs in oviposition in the lower right quadrant.

Figure 8. Oviposition as a Function of Time of Day. This table presents the standardized frequency of ovipositions per camera trap hour for each hour block in a 24-hour cycle, with the first hour (0) starting at midnight.

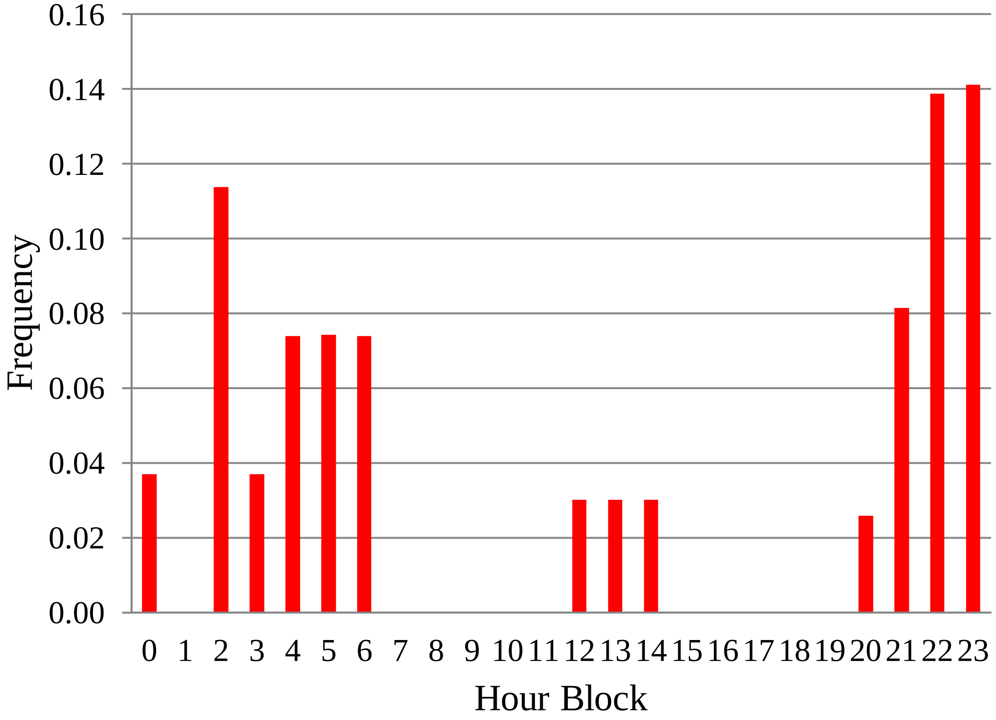


Figure 9. Oviposition as Function of Light Intensity. This shows the number of ovipositions per camera trap hour plotted against mean daily light intensities in lux (blue) for each hour block over a 24 hour cycle. Oviposition rate has been adjusted for easy visual comparison.

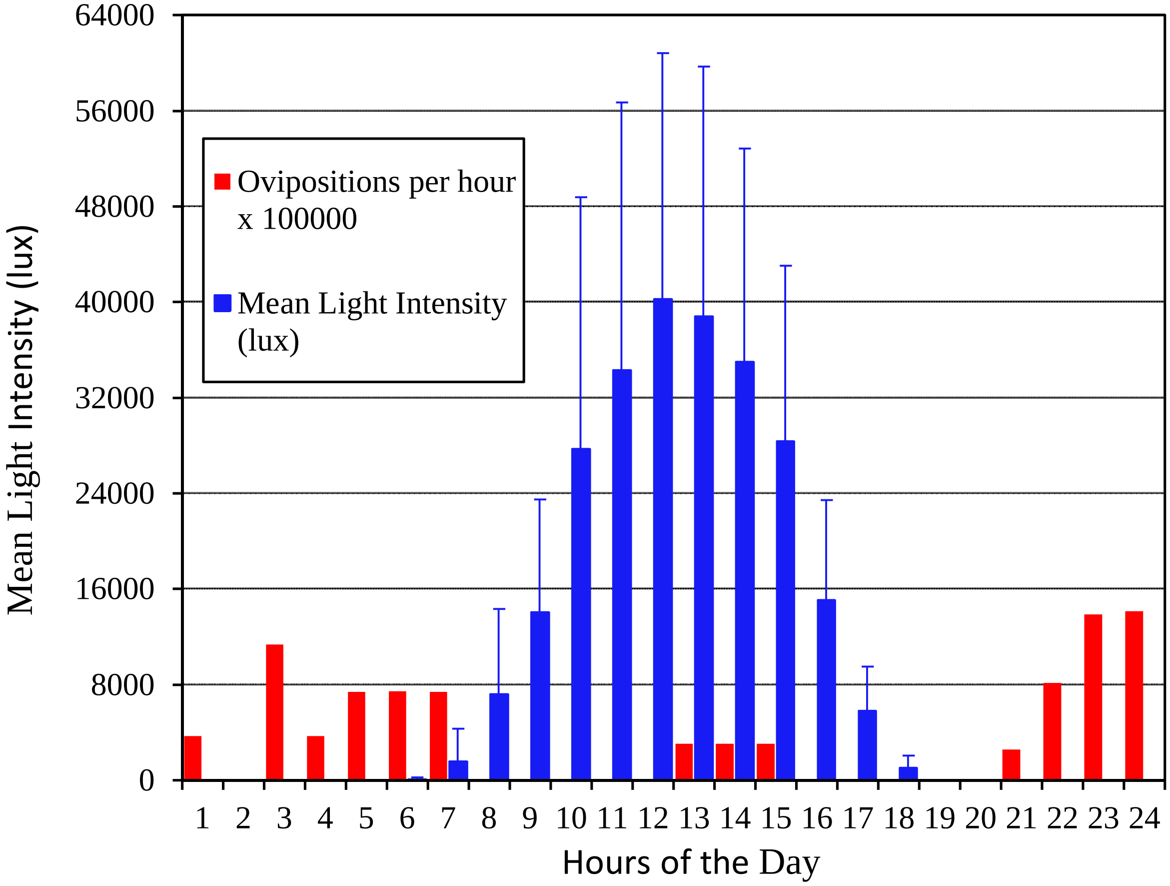

Mean Light Intensity (lux)
64000
56000
48000
40000
32000
24000
16000
8000
0
Ovipositions per hour x 100000
Mean Light Intensity (lux)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Hours of the Day

Figure 10. Oviposition as a Function of Water Temperature. This bar graph shows the rate of oviposition at different water temperatures in both raw (red), and corrected for the total number of camera trap hours at that temperature range (pale blue). The total numbers of camera trap hours are in dark blue. Effort and the corrected oviposition rate have been adjusted for easy visual comparison.

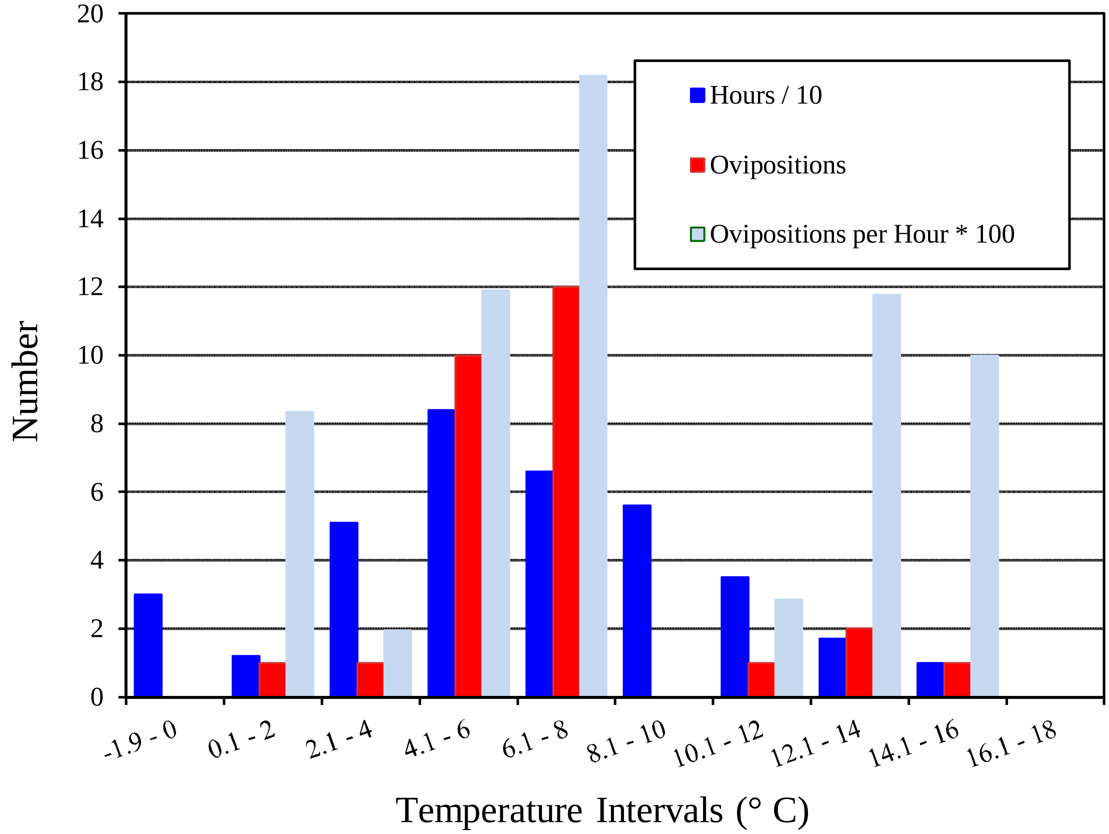


Figure 11. Oviposition as Function of Cloud Cover. This table presents number of ovipositions as a function of cloud cover categories as the raw data (red) or corrected for effort (blue) as ovipositions per camera trap hour. The interval of 51-75% cloud cover category lacks data empty because cloud cover was never in the 51-75% category when camera trap were operating.

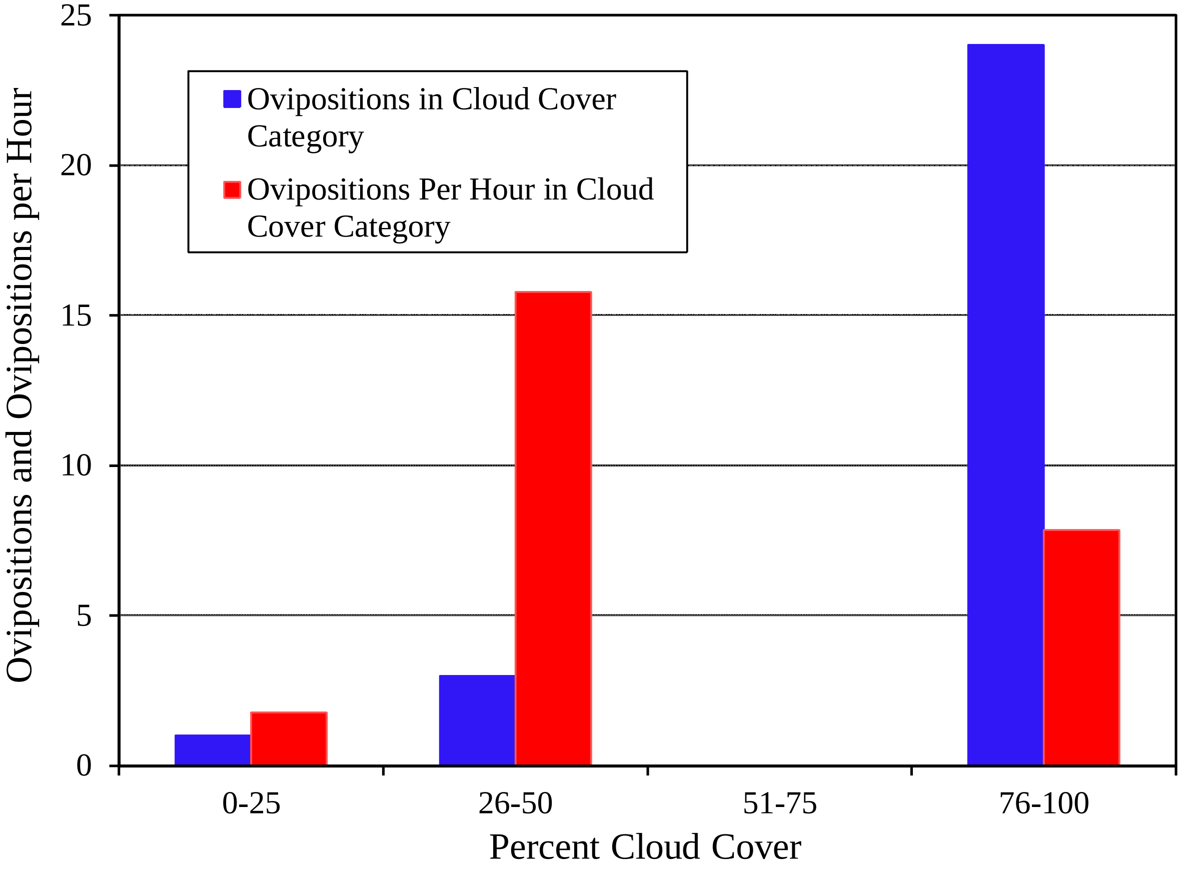


Figure 12. Oviposition as a Function of Precipitation. This bar graph presents ovipositions corrected for effort (red) as function of precipitation rate. Effort in camera trap hours is shown in blue. Oviposition corrected for effort has been adjusted for easy visibility.

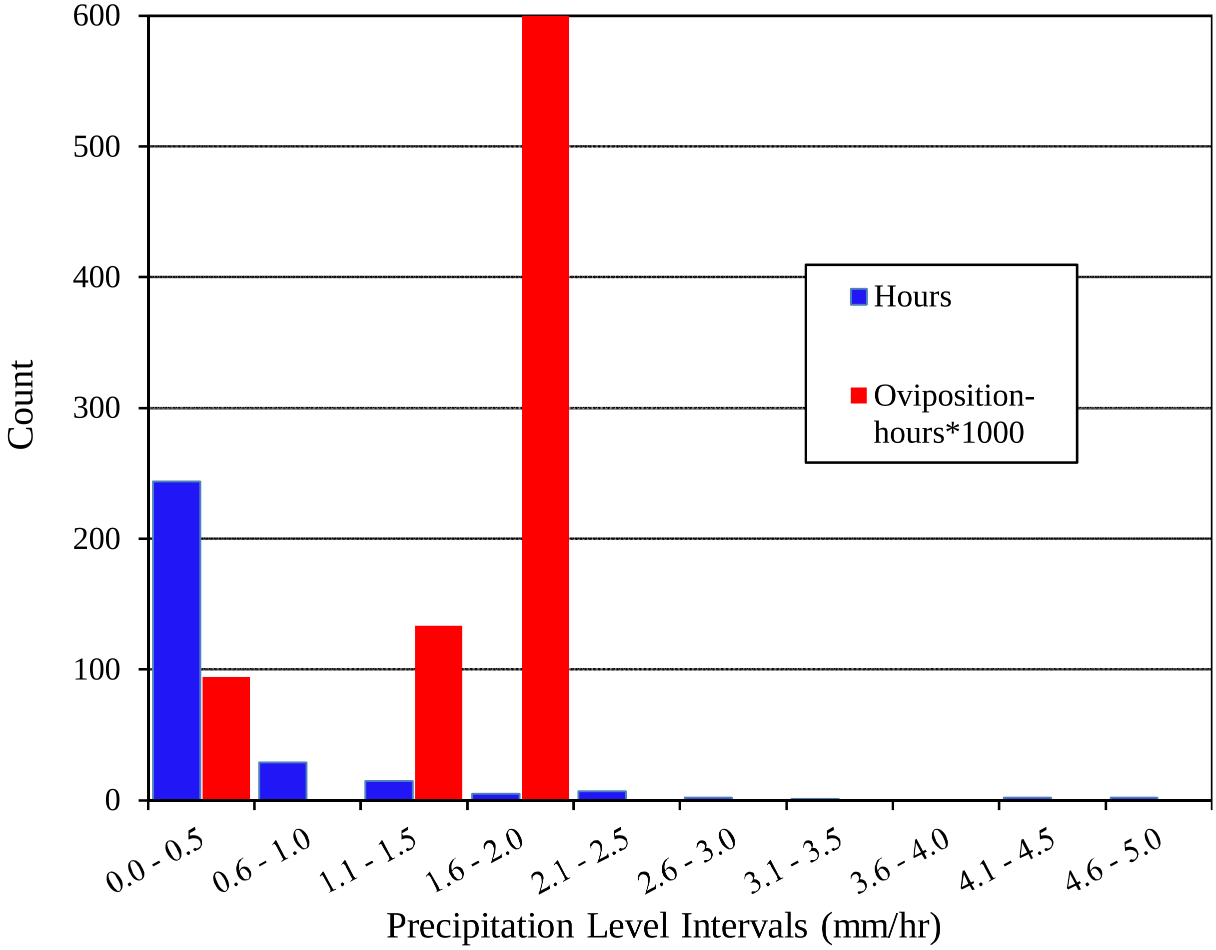
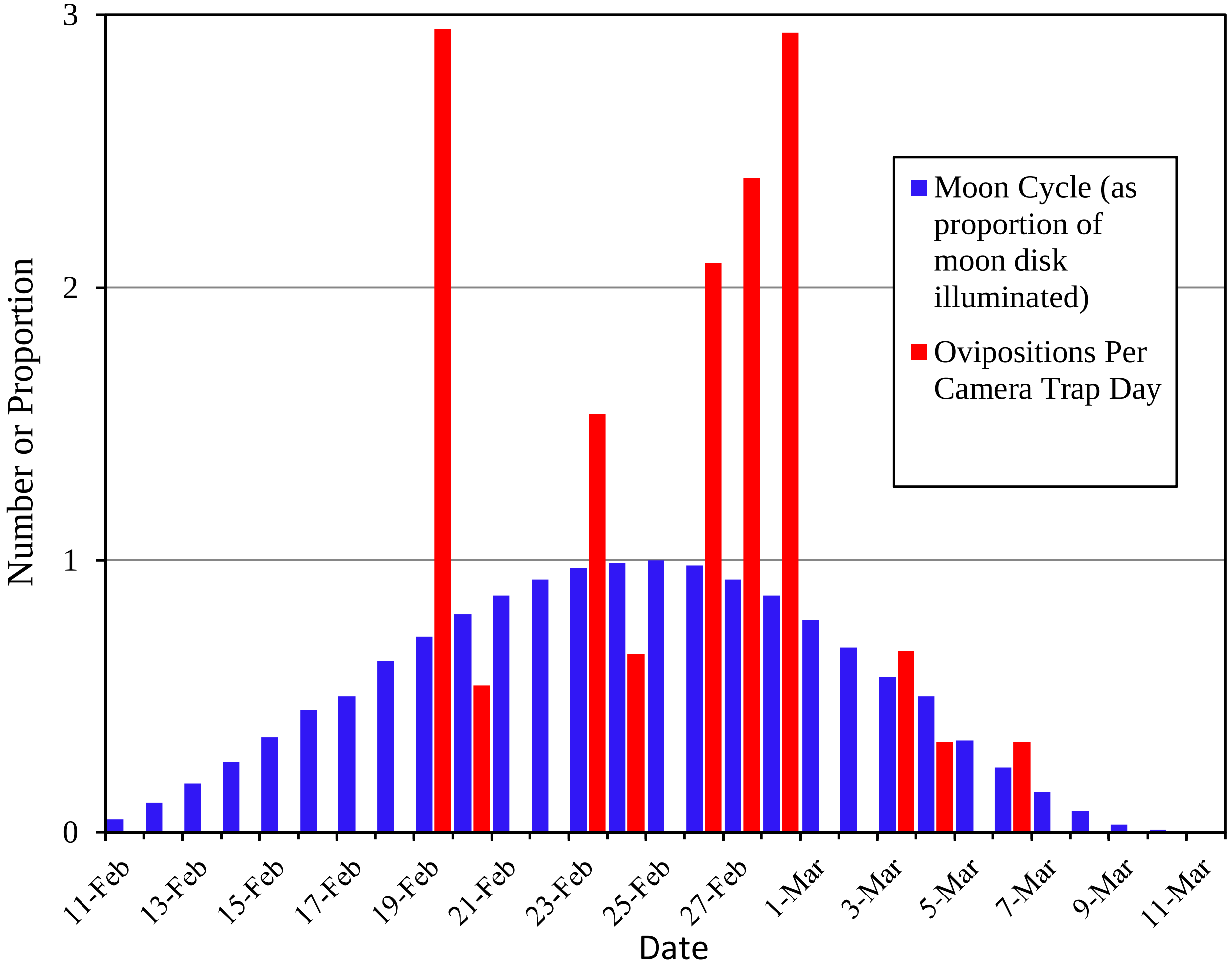


Figure 13. Oviposition and Moon Cycle. This bar graph compares the full lunar cycle (blue) measured as a proportion of moon disk illuminated to the oviposition rate corrected for effort (red) in camera trap days.



Appendix B: Tables

Table 1. Duration of Oviposition and Amplexus. This table provides the descriptive data for duration of amplexus and oviposition (in minutes). Parameters are the sample size (n), mean ($\overline{X}$), standard deviation (SD), maximum (Max), and minimum (Min). Amplexus and oviposition events summarized are only those for which the full length of the event was recorded. All amplexus events are also exclusively those ultimately leading to an oviposition and exclude the time required for oviposition.

Event	n	$\overline{X}$	SD	Max	Min
Oviposition	27	11.72	4.52	22.50	4.00
Amplexus	25	43.80	31.29	111.25	0.25

Table 2. Alternative Partitioning Categories for Day and Night. This table shows how the number of ovipositions varies using different partitioning categories for day and night both as raw data and corrected for effort. Effort was the number of camera traps hours of day or night using the respective partitioning categories.

Partitioning Category	Number of Ovipositions			
	Day		Night	
	Raw	Corrected	Raw	Corrected
Sunrise-Sunset	3	0.009	26	0.06
Civil Twilight	3	0.008	26	0.07
Nautical Twilight	4	0.001	25	0.07
Astronomical Twilight	6	0.01	23	0.08

Table 3. Statistical Summary of Covariates

Parameter	t-test Probability (P)	Significant (y/n)	Correlation Coefficient (r)	Significant (y/n)
Duration of Amplexus vs. Duration of Oviposition	<0.001	Yes	NA	NA
Light Intensity	0.206	No	-0.385	Yes
Water Temperature	0.811	No	NA	NA
Precipitation	0.784	No	NA	NA
Moon Cycle	0.940	No	0.299	No

Table 4. Observational Summary of Covariates

Parameter	Observation
Amplexus and Oviposition	Oviposition duration was significantly shorter than amplexus, with a definitive start and end.
Time of Day	OSF oviposited primarily at night between astronomical and twilight boundaries.
Light Intensity	There was a trend for OSF to oviposit with lower light levels, though t-test was not significant.
Water Temperature	Oviposition mostly occurred in a small range around the overall mean temperature.
Cloud Cover	Night ovipositions tended to have 100% cloud cover, day ovipositions tended to have <50% cloud cover.
Precipitation	Weak trend to oviposit with little to no precipitation.
Moon Cycle	Weak trend to oviposit with moon >50% illuminated.

Table 5. Oviposition Sites and Egg Mass Counts. Sites were numbered according to the chronological order of their appearance. Egg mass counts displayed by site here are the final counts at the end of the breeding season.

Site Number	Final Egg Mass Count
1	106
2	39
3	48
4	30
5	7
6	11
7	14
8	1
9	1
10	1
11	1
12	3
13	1
14	1
15	1
16	1
Total	266