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OREGON SPOTTED FROG *(RANA PRETIOSA)* RESPONSE TO ENHANCEMENT OF OVIPOSITION HABITAT DEGRADED BY INVASIVE REED CANARY GRASS (*PHALARIS ARUNDINACEA*)

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Abstract.—**Invasive Reed Canarygrass (*Phalaris arundinacea*) is widespread in the Pacific Northwest, USA and develops dense, tall stands in shallow wetland habitats. Oregon Spotted Frogs (*Rana pretiosa*) are a species of conservation concern, and lay eggs in clusters in seasonally flooded margins of emergent wetlands. We hypothesized that reducing Reed Canarygrass might favor Oregon Spotted Frog oviposition in invaded shallows. In a Reed Canarygrass-dominated marsh, we examined probability of oviposition and thermal attributes in 32 pairs of mowed and unmowed plots. Oregon Spotted Frogs laid one cluster of egg masses in each of two mowed plots but no egg masses in unmowed plots, an unlikely result based on a binomial function (*P* = 0.006). We also recorded three separate Oregon Spotted Frog egg mass clusters outside of study plots, but exclusively in habitat that appeared structurally similar to mowed plots. We conclude that mowing may enhance oviposition habitat for Oregon Spotted Frogs in Reed Canarygrass-dominated wetlands. However, to have confidence in the response given our limited data, this manipulation should be repeated before this management strategy is broadly applied. Future manipulations of this system should consider how such treatments influence other Oregon Spotted Frog life stages and co-occurring species.**

Key Words.—enhancement; habitat; Oregon Spotted Frog; oviposition; *Phalaris arundinacea*; *Rana pretiosa*; Reed Canarygrass; Washington

INTRODUCTION

Habitat loss and alteration remain prominent among the factors responsible for the decline of amphibians worldwide (Dodd and Smith 2003; Stuart et al. 2004; Cushman 2006). Invasive plants are among the factors generally known to contribute to habitat loss. Studies have examined invasive plant effects on amphibians, such as the potential impediments to food resources, ingestion and growth (Maerz et al. 2005a, b; Brown et al. 2006; Watling et al. 2011a, b). The influence of invasive plants on the structure of amphibian habitat, however, has been largely ignored.

Reed Canarygrass (*Phalaris arundinacea*) is an invasive plant well known to develop persistent, dense, tall, and often monotypic stands that reduce or eliminate the low vegetation structure in wetlands (Maurer et al. 2003; Lavergne and Molofsky 2004; Reinhardt Adams and Galatowitsch 2005), a condition common in many Pacific Northwest USA wetlands (Paveglio and Kilbride 2000; Fierke and Kauffman 2006; Kim et al. 2006). The extent of its alteration of Pacific Northwest wetlands makes *P. arundinacea* a good candidate to evaluate to determine whether such changes influence native amphibians.

The Oregon Spotted Frog (*Rana pretiosa*) is a federal candidate for listing and a species listed as Endangered by Washington State. This species typically deposits egg masses communally (i.e., in groups in direct contact) in the seasonally flooded shallows of emergent marshes in areas with low vegetation that does not shade the eggs (McAllister and Leonard 1997; Watson et al. 2003; Fig. 1). Frogs tend to breed in the same areas every year and, depending on local topography and water levels, may even use the same oviposition locations each year (Licht 1969, Watson et al. 2003). Oviposition sites are in shallow water, typically around 15 cm in depth and rarely exceeding 30 cm deep (Licht 1969; Pearl et al. 2009). Apparent avoidance of dense stands of Reed Canarygrass for oviposition and observations of oviposition in microhabitats opened by disturbance (i.e., vehicle ruts) in an otherwise Reed Canarygrass-dominated wetland led us to design an experiment to evaluate Oregon Spotted Frog oviposition response to change in vegetation structure. At the time this study was implemented in 2000, this was one of only five sites known to be occupied by Oregon Spotted Frogs in Washington and the only site without management that prevented overgrowth of Reed Canarygrass (e.g., grazing

FIGURE 1. Communal oviposition by Oregon Spotted Frog (*Rana pretiosa*). A group of > 50 egg masses is depicted in a shallow oviposition site along Dempsey Creek, Thurston County, Washington. (Photographed by Kelly R. McAllister).

or haying).

MATERIALS AND METHODS

Study area.—The study area was in the low relief headwaters of Allen Creek, a secondary tributary of the Black River, northwest of Tenino (town), Thurston County, Washington, USA (Fig. 2). The study site is a 12-ha wetland at about 60 m elevation MSL. It is currently located within the 324-ha West Rocky Prairie Wildlife Area managed by the Washington Department of Fish and Wildlife (WDFW), but at the time of this study, the land was privately owned by Citifor Corporation. Examination of historical aerial photographs revealed that the study site had been an agricultural field that was actively cultivated until the mid-1980s. Two parallel ditches were located near the east and west margins of this wetland (Fig. 2). They joined, flowed north as Allen Creek, which ultimately flows into Beaver Creek and drains to the Black River ca. 9 km west of the study site.

This wetland was herbaceous marsh and part of a mosaic that includes riparian and upland habitats. Reed Canarygrass dominated the marsh vegetation, with Slough and Beaked Sedges (*Carex obnupta, C. utriculata*), and Soft Rush (*Juncus effusus*) as subdominants. Fringing areas graded into Douglas' Spiraea (*Spiraea douglasii*) and shrubby willows (*Salix* spp.). Red Alder (*Alnus rubra*) and Oregon Ash (*Fraxinus latifolia*) dominated the higher riparian areas, and uplands were a mosaic of second-growth Douglas-fir (*Pseudotsuga menziesii*) forest, Oregon White Oak (*Quercus garryana*) woodland, and glacial outwash prairie.

Pre-experimental assessment.—Because Oregon Spotted Frogs tend to reuse ovipostion sites across years (Licht 1969; McAllister and Leonard 1997), it was necessary to locate the breeding areas prior to designating study plots. We systematically searched the entire study wetland for egg masses every day during February and March 2000. Surveys consisted of slow walks along parallel transects separated by 2-3 m, a spacing we know allows adjacent surveyors to easily detect egg masses (unpubl. data). We marked each oviposition location with a fiberglass rod, obtained its coordinates using a global positioning system unit, and counted the number of egg masses in the cluster. We resurveyed the wetland until no new breeding was found. Overall, we identified 107 egg masses across seven oviposition sites.

Experimental approach.—We installed study plots in late summer 2000 when the seasonally inundated breeding areas were dry. From the seven possible oviposition sites available, we used four as experimental areas (Fig. 2). Three of the four sites were selected because 89% of 107 Oregon Spotted Frog egg masses observed in 2000 were deposited at those sites. The fourth site was chosen because of its proximity to the other three.

We defined the experimental areas by centering a 30-m diameter circle on each of the four chosen sites (Fig. 2). This 30-m size encompassed enough habitat to include breeding shallows over a range of potential water levels the following spring. Within each 30-m circle, we identified random locations for eight pairs of non-overlapping 3-m diameter circular plots (Fig. 3). The center of the first plot in each pair was located a random distance and direction from the center of the 30-m circle, with the constraint that the distance had to be ≤ 28.5 m. Each succeeding plot could touch, but not overlap the edge of any previously defined plot. We located the second plot of each pair in a random direction from the center of the first plot. Its center had to be located 1.5-m from the edge of its pair. We purposely placed pairs abutting one another to limit spatially induced variation for monitoring water temperature between mowed and unmowed treatments, and to make it easier for researchers conducting surveys to avoid excessive trampling of vegetation in unmowed circles. We chose a 3-m circle for plots based on our previous observations that Oregon Spotted Frogs will use similarly sized pools for egg deposition (unpublished data). We also restricted selection to areas with some chance of inundation based on water levels from 2000.

For each of the eight 3-m paired plots, we randomly chose one for mowing (Fig. 3). The other circle was used for reference temperature monitoring locations. We mowed the circles as close to the ground as possible

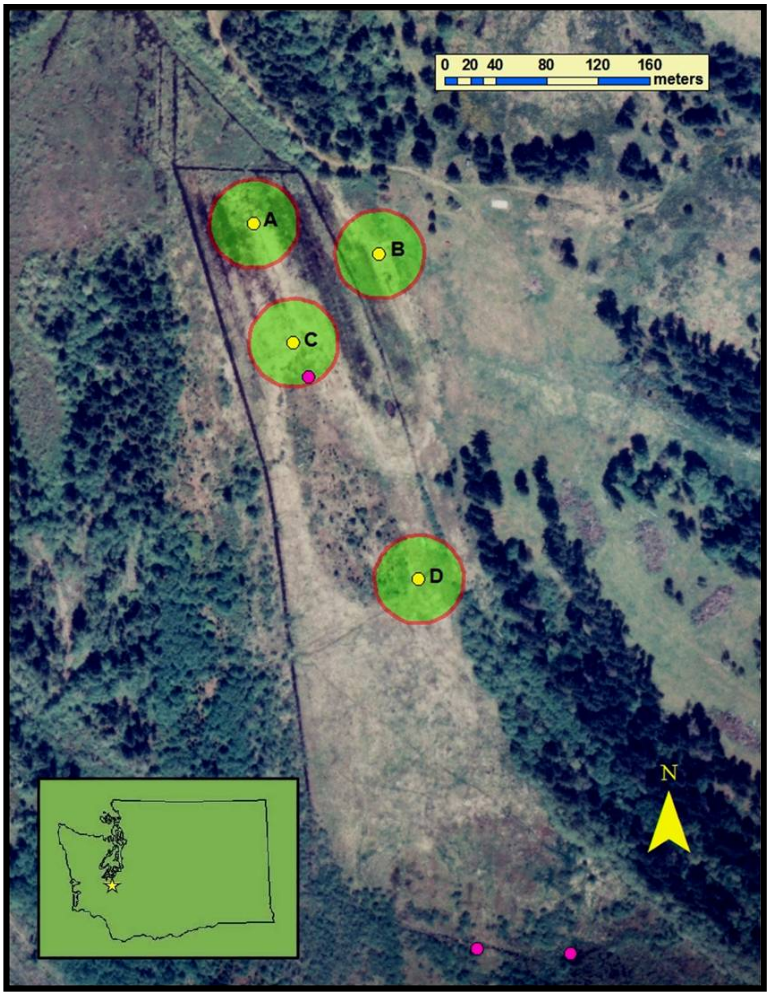

FIGURE 2. Aerial photograph of the study wetland showing the seven Oregon Spotted Frog (*Rana pretiosa*) oviposition sites in the pre-treatment year 2000 (yellow and magenta dots). We conducted the experiment within the four green 30-m diameter circles centered on the four 2000 oviposition sites indicated by the letter-labeled yellow dots.

with a gas-powered, hand-held brush cutter between 16 August and 13 September 2000 (Fig. 4).

We used dataloggers to monitor potential differences in surface water temperatures between mowed and unmowed treatments during the oviposition season. We centered one Stowaway Tidbit® temperature dataloggers (Onset Computer Corporation, Pocasset, Massachusetts, USA; accuracy 0.2 °C) on 19 January 2001 within each plot for 16 of the mowed-unmowed plot pairs. Selected plot pairs were those deep enough to ensure that dataloggers had adequate contact with water. We set dataloggers to record hourly. We monitored Oregon Spotted Frog breeding in 2001 with the same visual encounter search method as during the pre-experimental assessment phase.

Analyses.—We assessed the likelihood that the mowed circular plots had no effect on choice of oviposition location by testing the following null (H_o) hypothesis:

H_o = Egg mass cluster placement is random
H_a = Egg mass cluster placement is non-random
(potentially favoring mowed plots)

We evaluated this hypothesis as a binomial function (Zar 1999), where the likelihood of x "successful" occurrences of one of two possible outcomes in n trials in which the probability of a "success" on each trial P_s is given by:

$$B(x,n,P_s) = \left({n!}/{x!\,(n-x)!} \right) {P_s}^x (1-P_s)^{n-x}$$

Our test statistic was the appearance of each egg mass cluster (or trial) in the model, rather than each individual egg mass. An egg mass cluster consisted of a group of egg masses where each egg mass was in physical contact

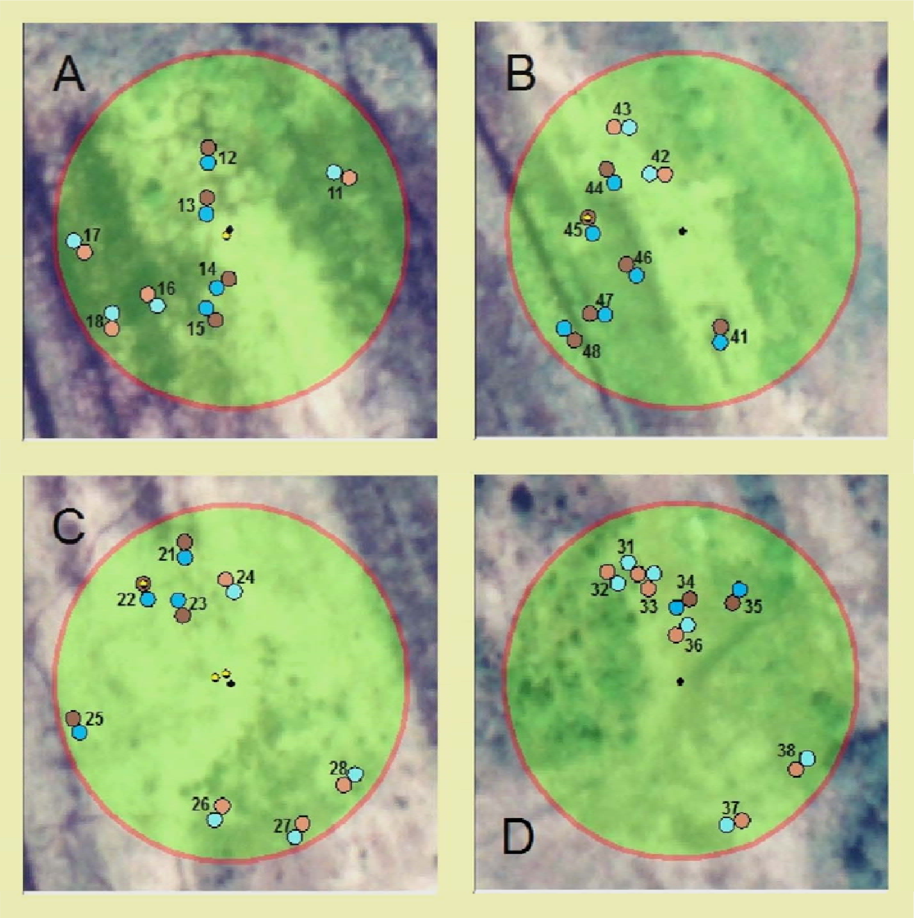

FIGURE 3. Detail of the experimental configuration of mowed and treatment plots within the 30-m circles. The panel labels correspond to the letter-labeled areas in Figure 2; here a small black dot identifies the center of each 30-m diameter circle. Brown circles are mowed 3-m plots; blue circles are unmowed plots. Darker colors indicate plot pairs that had temperature dataloggers; a number identifies each plot pair and corresponds to the plot pair numbers in Tables 1 and 2. Small yellow dots within 30-m circles A, B, and C are the five oviposition sites (location of egg mass clusters) recorded in the post-treatment year (2001). Note that the only two oviposition sites located in experimental plots are located in the mowed plots of pairs 22 (in circle B) and 45 (in circle C).

with at least one other egg mass in that same group. We used egg mass cluster as our unit of interest (rather than individual egg mass), because females appear to cue on egg masses already deposited as the locus where they will lay eggs (unpubl. data). Such a cue would make laying of individual egg masses in a cluster non-independent. Assuming the entirety of the four experimental areas (30-m circle) was available for oviposition, the probability (P_s) of "success", defined as a groups of frogs randomly laying their eggs at an oviposition site within one of the treatment plots, would be:

$$P_s = \frac{4*(8*\pi*(1.5\text{ m})^2)}{4*(\pi*(30\text{ m})^2)} = 0.02 \qquad \text{(Equation 1)}$$

Thus, $1 - P_s = 0.980$.

However, 0.06 to 0.39 of each experimental area was unavailable for oviposition (unwatered), so we orrected the denominator in Equation 1 by subtracting the mean proportion of unwatered area (0.21) for the four 30-m circles. Hence, the corrected probability (P_c) of “success” would be:

$$P_c = \frac{4*(8*\pi*(1.5\text{ m})^2)}{4*(\pi*(30\text{ m})^2-(0.21[\pi*(30\text{ m})^2]))} = 0.025 \qquad \text{(Equation 2)}$$

Thus, $1 - P_c = 0.975$

Second, we examined temperatures between mowed and unmowed plots. We analyzed temperature data beginning three weeks before the first oviposition date

FIGURE 4. Kelly R. McAllister mowing one of the 3-m diameter treatment plots. Non-flowering portions of this Reed Canarygrass (*Phalaris arundinacea*) stand exceeded 1 m in height. (Photographed by Heather Q. W. Kapust)

FIGURE 5. Vegetation structure of mowed (treatment) and unmowed (reference) plots on 7 February 2001 (about four weeks prior to first breeding). Fiberglass rods identify plot centers. Note green new-season growth in the mowed plot (foreground); the unmowed plot (back and to the left) and most of the untreated wetland are covered by a dense light-brown thatch of dead Reed Canarygrass (*Phalaris arundinacea*). (Photographed by Heather Q. W. Kapust)

(13 February 2001) and extending through the completion of hatching (11 April 2001; total = 58 days).

Our basis for this choice was that if temperature differences existed between mowed and unmowed plots, they were most likely to reflect either the generally non-freezing interval prior to adult frogs selecting a favorable oviposition site, or habitat conditions during embryonic development. Hourly temperature data are highly autocorrelated, so we examined lag intervals to determine which interval was most useful to reduce the data to the least autocorrelated subset. We also desired a subset of data where every hour of the day had near equal representation, which required us to use a lag interval of hours involving a prime number less than 24. Distributions of differences in temperature between some pairs of plots were either strongly negatively skewed or multimodal, so we used Spearman Rank correlations to examine the data using lag intervals based on each of the nine qualifying primes. Sensitivity analysis using all 32 dataloggers revealed that the data culled to a 7-h interval was the least autocorrelated. Specifically, a 7-h interval had the fewest significant correlations (n = 3) for the 16 pairs of plots among qualifying primes, and the remaining three significantly correlated pairs of plots all had Spearman coefficients (ρ) ≤ 0.315. Though the latter were significant based on an adjusted α using Sidak (1967), we regarded their contribution to variability as too small to warrant not using the 7-hr interval in subsequent analyses. We then examined potential differences with a Wilcoxon signed-rank test.

We also considered whether the basis of a difference in temperature between mowed and unmowed plots might be greater during the day than at night, so we analyzed temperature differences by partitioning diel data into two 12-h intervals: 0600–1759 (day) and 1800–0559 (night). Day length increased slightly over the 58-day data interval, but these equal-length intra-diel intervals provided an advantage in analysis that exceeded the small undesirable contribution to the variance resulting from few misclassified day or night points because of the 7-hr interval used in selecting points for analysis. For this comparison, we used a Mann-Whitney U test. For both the latter tests, we also adjusted α for 16 comparisons based on Sidak (1967). We conducted all statistical analyses using JMP® Version 8 (SAS Institute Inc. 2008).

RESULTS

Treatment landscape.—Structural differences between mowed and unmowed plots were evident through the duration of the study (Fig. 5). Mowed plots exhibited little growth of vegetation (new emergent vegetation height above the water surface was ≤ 15 cm) between the time of fall mowing in 2000 and when egg masses were laid in late winter 2001. In contrast, dense Reed Canarygrass dominated nearly every reference plot, and though its structure had collapsed considerably from winter rain and snow by February 2001, it was typically > 15 cm high (Fig. 5).

Oviposition patterns.—Our surveys in February-March

TABLE 1. Comparison of water temperatures in 16 treatment (mowed) and reference (unmowed) plot pairs in the study marsh at the headwaters of Allen Creek, 2001. Data are the medians ($\tilde{x}$) and interquartile ranges (*IQR*) summarizing 197 7-hr interval points obtained from an hourly record on each plot taken from 0000 on 13 February to 2300 on 11 April. Based on Sidak (1967), the 16 contrasts were adjusted to α = 0.003; significant probabilities have an asterisk. Treatment Plots 22 and 45 had Oregon Spotted Frog oviposition in them in 2001.

	Water Temperatures (ºC)						Wilcoxon Signed-Rank Probability
	Treatment		Reference		Difference		
Plot Identifier	$\tilde{x}$	*IQR*	$\tilde{x}$	*IQR*	$\tilde{x}$	*IQR*	*P*
12	8.1	3.4	7.3	3.5	0.7	1.6	< 0.001*
13	8.3	3.4	6.7	3.3	1.5	1.2	< 0.001*
14	7.8	3.6	6.9	3.6	0.9	1.2	< 0.001*
15	7.6	3.9	7.3	2.3	-0.2	1.9	0.692
21	7.6	2.5	6.2	2.4	1.2	0.8	< 0.001*
22	7.1	2.5	5.7	2.3	1.4	0.8	< 0.001*
23	7.3	2.3	6.2	2.7	1.0	0.9	< 0.001*
25	7.2	3.3	5.7	3.0	1.4	0.6	< 0.001*
34	5.7	3.6	3.8	3.8	1.5	1.5	< 0.001*
35	4.9	3.9	4.9	2.8	0.0	1.6	0.177
41	6.8	2.8	5.4	2.5	1.3	0.9	< 0.001*
44	6.3	3.3	5.9	3.3	0.6	1.4	< 0.001*
45	6.9	2.8	5.8	2.5	1.4	1.1	< 0.001*
46	6.9	2.8	5.8	2.5	1.4	1.1	< 0.001*
47	6.9	3.1	5.9	2.7	0.9	1.4	< 0.001*
48	5.1	2.7	4.7	2.9	0.1	0.5	< 0.001*

TABLE 2. Day and night water temperature differences in 16 treatment (mowed) and reference (unmowed) plot pairs in the study marsh at the headwaters of Allen Creek, 2001. Data are medians ($\tilde{x}$) and interquartile ranges (*IQR*) summarizing 197 7-hr interval points (day: n = 99; night: n = 98) obtained from an hourly record on each plot taken from 0000 on 13 February to 2300 on 11 April. Based on Sidak (1967), the 16 comparisons were adjusted to α = 0.003; significant probabilities have an asterisk. Treatment Plots 22 and 45 had Oregon Spotted Frog oviposition in them in 2001.

	Water Temperature (ºC) Differences (Treatment - Reference)					Mann-Whitney U Probability in Medians
	Day		Night		Day-Night Difference	
Plot Identifier	$\tilde{x}$	*IQR*	$\tilde{x}$	*IQR*	*IQR*	*P*
12	1.5	2.1	0.4	0.9	1.1	< 0.001*
13	1.7	1.8	1.5	0.9	0.2	0.038
14	1.4	2.0	0.8	0.8	0.6	< 0.001*
15	0.1	2.1	-0.5	1.6	0.6	0.002
21	1.1	0.9	1.4	0.8	0.3	0.018
22	1.2	0.9	1.4	0.8	0.2	0.147
23	0.8	0.6	1.1	0.8	0.3	< 0.001*
25	1.4	0.7	1.3	0.6	0.1	0.088
34	2.0	2.4	1.3	0.9	0.7	< 0.001*
35	0.3	2.0	-0.2	1.3	0.5	0.004
41	1.4	0.9	1.1	0.8	0.3	0.009
44	1.1	1.4	0.3	1.1	0.8	< 0.001*
45	1.5	1.2	1.3	1.0	0.2	0.251
46	1.9	0.8	1.9	0.8	< 0.1	0.650
47	1.6	1.8	0.6	0.6	1.0	< 0.001*
48	0.4	0.6	-0.1	0.5	0.5	< 0.001*

2001 found five Oregon Spotted Frog egg mass clusters (Fig. 3). All egg mass clusters were found within the 30-m experimental areas surrounding year 2000 oviposition locations. Two of these clusters (containing 1 and 18 egg masses) were located in the mowed plots (plot pairs: 22 and 45; Fig. 3). Based on a binomial function, the likelihood that Oregon Spotted Frogs would place at least two of five egg clusters within mowed circles if egg mass clusters were randomly placed is highly unlikely (*P* = 0.006). The three other egg mass clusters found outside of any study plots consisted of 20 and 5 egg masses in circle C and a lone egg mass in circle D (Fig. 3).

Two of the three clusters (those in circle C; Fig. 3) were in an area that we used as a 'model' for the mowed areas. Both were in tire track ruts consisting of exposed shallow water surrounded by vegetation of reduced height. Only one egg mass cluster (in circle A; Fig. 3) was located in relatively undisturbed vegetation, but even this location had a relatively open, low vegetation structure. Lastly, all three of egg mass clusters in circles C and D were within 3 m of oviposition sites from the previous year.

Temperature patterns.—Median water temperatures in mowed plots were significantly higher than those in unmowed plots for 14 of 16 monitored plot pairs (Table 1). In six of these 14 pairs, the median difference in water temperature between mowed and unmowed plots was ≥ 1.4 °C. The two mowed plots used for oviposition were among these six pairs. In 12 of 16 plot pairs, variation in water temperature in mowed plots (based on interquartile range) was greater or equal to that in unmowed plots. Additionally, plots where oviposition occurred were among the seven mowed plots with lower variation in water temperature (interquartile range ≤ 2.8 °C; Table 1).

Differences in temperature between mowed and unmowed plots were significantly greater during the day than at night in half the 16 plot pairs, but not significantly different between day and night in the remaining eight pairs (Table 2). Plot pairs where oviposition occurred in the treatment plot were among the eight pairs that lacked a difference between day and night, and based on differences in day versus night medians and probability values, were among the four plot pairs that exhibited the least variation in temperature.

DISCUSSION

Oregon Spotted Frog response to our mowing experiment suggests choice of oviposition location is non-random and a vegetation structure similar to that found in mowed plots appears favored. We found two egg mass clusters in mowed plots, but mowed plots averaged only 2.6% of the area available for oviposition within our four 30-m experimental areas. Further, even if potential oviposition area was two-fold too large, in which case mowed plots would represent 5.2% of potential oviposition area, our result would remain significant with these data (i.e., $P = 0.022$). Equally important, no egg masses were laid in unmowed reference plots. This pattern, combined with the fact that the other three detected egg mass clusters were in locations with structure more similar to mowed than to unmowed plots, imply that vegetation structure similar to that of mowed plots was associated with the observed response.

Our water temperature data identify a potential advantage of ovipositing in low, open microhabitat: a more favorable thermal environment for embryonic development than nearby more vegetated sites. Though we have too few data to approach such an assessment systematically, mowed plots where oviposition occurred were among those with the greatest difference in water temperature from their unmowed counterparts and among those that had the lowest variation in water temperature among mowed plots. Embryonic development in amphibians can occur disproportionately more rapidly with small increases in temperature, a situation that has been documented in Oregon Spotted Frogs (Licht 1971). Indeed, based on Licht (1971), an increment of only 1 °C over the 10–13 °C temperature range, as we observed in unmowed plots, could decrease time to hatching by at least four days. Though it remains to be investigated whether Oregon Spotted Frogs adults actually select oviposition sites based on the available water temperature, these data imply that this line of investigation would be worthwhile.

The results of this experiment suggest an opportunity to enhance oviposition habitat for Oregon spotted frogs where Reed Canarygrass limits habitat quality, the typical pattern at occupied sites in the lowlands of western Washington. This possibility is encouraging, but we suggest caution prior to implementation. First, our ability to quantify response was limited by the small size of the local Oregon Spotted Frog population and the typical use by this species of a small number of oviposition sites. Hence, further research is required to ensure that our results were not simply anomalies. Second, we regarded all watered area within the 30-m circles as suitable for oviposition. We recognize that plots having water deeper than typical oviposition depth had the potential to reduce the area that Oregon Spotted Frogs might view as suitable for oviposition. However, our qualitative observations of water depth over the area used in this experimental area being within the range reported for Oregon Spotted Frogs (Pearl et al. 2009) reduce this possibility. Third, the treatment scale in this experiment (3-m plots) was small. Increasing treatment scale might make temperature profiles in treatment plots more favorable for Oregon Spotted Frog oviposition if treatment size does not unfavorably reduce temperatures at night. Lastly, though mowed treatments appear to enhance Oregon Spotted Frog oviposition habitat, such treatments may not favor all species that co-occur with Oregon Spotted Frogs. For this reason, evaluating the potential range of effects of mowing treatments on co-occurring species would provide a perspective to determine under what circumstances such treatments might be applied.

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KELLY R. MCALLISTER is a Wildlife Biologist with the Washington Department of Transportation where he works on wildlife-friendly highways for maintaining and improving habitat connectivity. He received a B.S. with a major in Fisheries from the University of Washington. His early career was devoted to work in the Nongame Program of the Washington Department of Wildlife, including the development of the department's wildlife data system and writing some of Washington's first status reports and recovery plans for listed species. For nine years, he was a District Wildlife Biologist, responsible for wildlife surveys and a variety of wildlife management activities. Oregon Spotted Frogs have been one of Kelly's long standing interests. He was involved in efforts to find extant populations in Washington starting in 1989 and found most of the known populations in the Black River drainage of Thurston County. (Photographed by Michael Crawford)

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