PROJECT REPORT 2016

LONG-TERM MONITORING OF NORTHERN RED-LEGGED FROG (*RANA AURORA AURORA*) IN THE LITTLE CAMPBELL RIVER WATERSHED – 2009-2014

A ROCHA CANADA CONSERVATION SCIENCE SERIES

November 2016

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Acknowledgements

We thank the considerable list of A Rocha staff, interns, volunteers and friends who helped with egg mass surveys over the six years reported. Thank you to all landowners and park staff for allowing us to observe amphibians on their properties and sites. Throughout the project, science advisory members contributed to project management and oversight.

Recommended Citation:

Bunnell, C., F. Bunnell, A. Baylis, and C. Juteau. 2016. Long-term monitoring of northern red-legged frog in the Little Campbell River watershed – 2009-2014. A Rocha Canada Conservation Science Series. A Rocha Canada, Surrey, B.C., Canada.

Cover illustration:

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Executive Summary

A Rocha Canada began conducting annual amphibian egg mass surveys throughout the Little Campbell River (LCR) watershed in 2009, with a particular emphasis on northern red-legged frog (*Rana aurora aurora*). Northern red-legged frog is a species of Special Concern under COSEWIC and is blue-listed, ranking 1 (of highest conservation concern) within British Columbia's Conservation Framework. This species is directly threatened by habitat loss and fragmentation resulting from anthropogenic activities and development, as well as competition and predation by exotic species. Our goal was to increase knowledge of current distribution of northern red-legged frog within the LCR watershed, to document when peak egg mass abundance occurs, and to investigate associations with potential limiting factors such as exotic species and land use.

This report summarizes findings from the first six years of monitoring the northern red-legged frog in the LCR watershed. During that period, 58 sites covering a wide variety of conditions were sampled at varying intensities. That work was largely exploratory. This report summarizes analyses intended to evaluate points noted above and to refine subsequent sampling.

Northern red-legged frog egg masses were found at most sites surveyed, suggesting that the species is fairly widespread within the watershed. Some sites hosted higher relative abundance of eggs across the study period, particularly those in parks or surrounded by a forested buffer. Egg laying was concentrated in a 2-week period centered around March 28, similar to other studies of the species. Of 239 site visits, northern red-legged frogs were observed without accompanying bullfrogs only 31% of the time. Forest cover was a major factor determining the favourability of sites for northern red-legged frogs.

Features of the environment having clear negative effects on the frogs were documented and include: forest removal, drying and warming of the water (e.g., climate change and declining aquifers), bullfrogs, expansion of *Phalaris*, and several indices of pollution. The northern red-legged frog is a useful sentinel of the health of the watershed.

Given the species' apparent vulnerability in the province, it is important to continue monitoring northern red-legged frogs where we can. Suggestions for refining the design are summarized in the Discussion. They devolve primarily to the importance of repeated measures from a consistent but expanded range of sites during the period of peak egg laying. We anticipate the next full update on our monitoring in the LCR watershed in about five years.

Introduction

This report summarizes findings from the first six years of monitoring the northern red-legged frog in the Little Campbell River (LCR) watershed. During that period 58 sites covering a wide variety of conditions were sampled at varying intensities. That work was largely exploratory. This report summarizes analyses intended to refine subsequent sampling and expose limiting factors.

Biology of Northern Red-legged Frogs

The northern red-legged frog is a medium-sized frog of the family of “true” frogs (Ranidae). It is presently listed as Special Concern under Canada’s Species At Risk Act (COSEWIC, 2004) and is blue-listed with Conservation Priority 1 in British Columbia. Of the two subspecies recognized, *Rana aurora aurora*, the northern red-legged frog (see cover), occurs in British Columbia. About one-third of northern red-legged frog range is in Canada, on Vancouver Island and the southwest coast of British Columbia.

Northern red-legged frog typically have a brown or reddish-brown back marked with black speckles and spots, dark bands on the back legs and black dorsal speckles or blotches. The lower belly and underside of the legs are translucent red, more evident on adults than juveniles. Prominent dorsolateral folds run parallel down the back and a dark eye mask is usually present. Male frogs typically grow to 50 – 70 mm, while females are typically larger, growing up to 100 mm (COSEWIC 2004; Matsuda *et al*. 2006).

The onset of breeding activity is dependent on temperature (Licht 1969). Licht completed his study in the LCR watershed and observed the first northern red-legged frog of the year immediately following the melting of snow and ice from fields. Males began moving to breeding ponds as early as late February to early March and egg laying occurred from March to early April when water temperatures were at least 4°C (Licht 1969, 1971). Grapefruit-sized, gelatinous masses of about 600 eggs are laid in wetlands with little to no water flow and are attached to vegetation or sticks 30 to 90 cm beneath the surface (Licht 1969, Corkran and Thoms 1996). Literature indicates a preference for wetlands with ample emergent and floating aquatic vegetation and forest habitat within a 200-m buffer (Beasley et al. 2000, Cary and Marks 2009, Englund 2007, Ostergaard 2001). Woody vegetation at wetland margins is not as favourable to oviposition as aquatic vegetation (Cary and Marks 2009). Dense forest canopy at the wetland margin blocks sunlight and decreases growth of algae, the tadpoles’ primary food (Nussbaum et al. 1983).

While wetlands are critical for breeding, northern red-legged frogs spend most of their life in upland habitats. Metamorphosis occurs in June or July (Licht 1974) and sexual maturity is usually reached after 3 years (Licht 1974), at a snout-to-vent length of 50 to 60 mm (Storm 1960). BC Ministry of Environment (2015) provides a useful summary of upland habitat noting closed canopy to maintain cool and moist microclimate, uncompacted soil, coarse woody debris, and undisturbed leaf litter (Aubry and Hall 1991;

Haggard 2000; Schuett-Hames 2004) and that juveniles often occupy relatively moist, densely vegetated riparian microhabitats (Licht 1986; Twedt 1993).

Threats

Environment Canada (2016) lists the northern red-legged frog as a taxon of Special Concern. BC Ministry of Environment (2015: Table 2) lists 22 specific threats to northern red-legged frog in British Columbia. These range from those with certain and documented impact, such as road mortality and American bullfrogs (*Lithobates catesbeianus*), to those with strong but poorly unpredictable effects, such as climate change. The reason the Ministry of Environment lists 22 specific threats is that the northern red-legged frog belongs to a group of species that is particularly exposed to threats – species requiring more than one distinct and separated habitat type.

Habitat of northern red-legged frog in British Columbia has been characterized (Beasley *et al*. 2000, Orchard 1984, Matsuda *et al*. 2006, Wind 2003, 2012). It is only broadly similar across studies and better documentation is required for the Lower Fraser Valley where the human population is growing and the threats are significant (COSEWIC 2004). A recent study surveyed wetlands within the City of Surrey to gather baseline distribution data. It correlated successful breeding sub-populations with specific habitat factors (Englund 2007).

Research Objectives

Given that its life style exposes the northern red-legged frog to many different kinds of threats, it is important that the species trend in abundance and use of habitat be documented and related to habitat variables believed to be significant to the species. Because consequences of environmental change are long term and may not be immediately observable, it is helpful to assess how various factors could affect populations. Ideally evaluation should occur while frog populations appear to be stable and widespread enough for a practical and economically feasible study.

The purpose of this study was to increase knowledge of current distribution of northern red-legged frog within the LCR watershed, to document timing of peak egg mass abundance and associations of egg mass with major habitat factors, including exotic species and land use. At its outset, the project was intended to augment Englund's (2007) surveys of park wetlands for the City of Surrey. That study was one year in duration. We report 6 years of measurements with the objectives of refining survey approaches, exposing factors that affected northern red-legged frog egg mass abundance, and evaluating potential threats.

Methods

Study Area

The Little Campbell River (LCR) watershed covers 7,580 hectares of land (Juteau 2008) consisting of primarily agricultural areas, urban areas and Semiahmoo First Nations land. An area of roughly 7.25% of the watershed is already under protection as the Campbell Valley Regional Park. The LCR watershed is important for northern red-legged frog populations because it provides many interconnected wetlands and terrestrial habitats while having a relatively low human density. Over the period 2009 to 2014 a total of 58 wetlands were surveyed (see maps in Figures 1 and 2).

Site Selection

A Rocha Canada began amphibian egg mass surveys in the LCR watershed in 2009 and has continued annually since then. The number of sites surveyed each year ranged in number from 9 in 2009 to 55 in 2013. After the 2013 field season, when five full seasons were complete, the study was re-evaluated and the number of sites was narrowed down to 31, with the intent of maintaining consistency and increasing utility of future surveys. Those 31 wetlands were selected based on accessibility, ability to help reveal influential habitat features and the presence of northern red-legged frog egg masses during previous surveys. Those were subsequently reduced to 11 sites in 2014.

Surveys

During 2009 to 2014, surveys were conducted by one to six observers, each equipped with chest waders, measuring poles and polarized sunglasses. Other equipment taken on these surveys included two Garmin GPSmap 60 units, one Eutech Instruments pH Testr 2 meter, one YSI Professional Series Pro 2030 meter (dissolved oxygen and conductivity), high-visibility vests, a waterproof digital camera and a clipboard with survey permit, datasheets, maps and identification guides. Data were recorded on a datasheet (modified from Englund 2007) printed on waterproof paper. Data recorded included egg mass counts for amphibian species, water quality measurements, surrounding habitat type, presence/absence data for American bullfrog at any life stage, numbers of photos used to identify the sites, UTM coordinates (NAD Zone 10) for each site and weather conditions including air temperature. In addition to these data, duration of surveys was recorded and GPS tracks were measured to estimate effort. The latter proved unreliable.

Amphibian egg masses

All observed amphibian egg masses were identified and counted along the edges of each wetland site, as deep as was accessible by observers on foot wearing chest waders. When multiple observers were counting egg masses, the sites were divided to ensure effective and complete survey of the site. In the case of particularly large and accessible wetlands, single-person kayaks were used to access deeper areas.

Water quality

At each site, the following water quality measurements were taken at four locations spread around the perimeter of the wetland: water temperature (ºC), pH, dissolved oxygen and conductivity (µS/cm). After the survey, an average value of each variable was calculated based on the four measurements that were taken at each site.

Habitat assessment

A set of habitat classification data was collected at each site that was visited. Cowardin class (Cowardin et al. 1979) was defined in two parts: system and class (e.g., 'Palustrine – Open Water'). Percent forested shore (proportion of shoreline within 5m of the water's edge supporting trees and shrubs) and percent emergent vegetation (proportion of wetted area with current emergent vegetation) were recorded in classes of 0-10%, 10-25%, 25-50%, 50-75% and 75-100%. Dominant forest types were identified as Deciduous, Coniferous, Mixed, Scrub or None. The relative abundance of coarse woody debris (>20 cm in diameter and >150 cm in length) was recorded as the average number per 10 m of shoreline, and classed as being High (>3 pieces/10 m shoreline), Medium (1-3 pieces/10 m shoreline), or Low (<1 piece/10 m shoreline). Hydroperiod was classified as Permanent or Ephemeral. Soft and hard depths (in cm) were measured at the deepest accessible part of the wetland. Any measurement deeper than 200 cm exceeded the length of the measuring poles and was recorded as '>200 cm'. The amount of reed canary grass (*Phalaris arundinacea*) present was classified as not detected, uncommon, common, or dominant.

Land Use Comparison

Using ArcMap, all of the sites were placed on a map of the LCR watershed. A 50-m buffer was drawn around each wetland to compare surrounding land cover between each site. Each buffer was divided into land use categories, using 2012 land cover data. At the outset these included: Agricultural Herb and Grass, Agricultural Row Crops, Freshwater Lake, Freshwater River, Marsh, Turf Grass, Unmanaged Herb and Grass, Unmanaged Shrub, Urban Suburban/High Density, Urban Suburban/Low Density, Urban Suburban/Moderate Density, Urban Trees, Young Deciduous Forests (5-80 yrs), Young Evergreen Forests (5-80 yrs) and Young Mixed Forests (5-80 yrs). Once analyses were complete it was apparent that Unmanaged Shrub was not present in the buffers for areas sampled at least three times. Freshwater Lake and Freshwater River were aggregated into one class.

Statistical Analysis

The sample design is unbalanced for two reasons: 1) the number of science interns visiting A Rocha differed each term which affected sampling effort and 2) as funding allowed acquisition of new equipment more variables were sampled. To ask questions of interest, variables assumed a variety of forms including simple counts (e.g., northern red-legged frog egg masses), simple measurements (e.g., water temperature, dissolved oxygen %), estimated cover classes (e.g. *Phalaris*, emergents) and binary or presence:absence data (e.g., adult bullfrogs).

The primary variable of interest is egg mass of northern red-legged frogs. The other variables are intended to describe the frog's habitat and reveal the frog's relations with that habitat. Three broad groupings of data are employed: all sample sites (when an overview of a specific variable is sought), the 9 original sites sampled in all subsequent years (when repeated measures are potentially revealing) and site-years (when individual sites in a specific year are potentially revealing). Many sites were sampled more than once a year. In those cases, environmental variables are averaged across visits for that year.

The variety of measures used to assess potential influential variables and the range of questions asked required both parametric and non-parametric forms of analysis. Tests employed include t-tests of differences between means, Kruskall-Wallis analysis of variance, correlated ANOVA coupled with Tukey tests, Spearman's rho, z-tests of proportions and false discovery rates (Verhoeven *et al*. 2005).

Results

Findings are grouped under three headings: spatial distribution of egg mass abundance, temporal distribution of egg mass abundance and habitat variables potentially influencing northern red-legged frogs and egg mass abundance. For the remainder of the report, northern red-legged frogs will be referred to as red-legged frogs and American bullfrogs will be referred to as bullfrogs.

Spatial Distribution of Peak Egg Mass Abundance

Survey effort was most intensive in 2013 and 2014. Figure 1 shows 55 sites surveyed between 2009 and 2014 and found occupied by red-legged frog. Size classes of peak egg masses found at each site are shown for the 2013 survey. Thirty of these sites were selected for the following season (Figure 2). Both years display higher abundance values in the same three areas: 1) southwest Campbell Valley Regional Park, 2) near the Kingfisher Farm and Glades properties and 3) at the downstream end of the watershed at the Meridian Par 3 Golf Course. It is apparent in the figures that higher densities are associated with forest cover.

The two figures reveal inter-annual differences. In 2013, relatively high abundance of egg masses were observed at the sites in northeast Campbell Valley Regional Park, but in 2014, low or null numbers were observed. Among other factors, differences in peak abundance between years could be caused by changes in breeding behavior, changes in site characteristics or a change in the timing of surveys between the years. Analyses following attempt to expose causal factors.

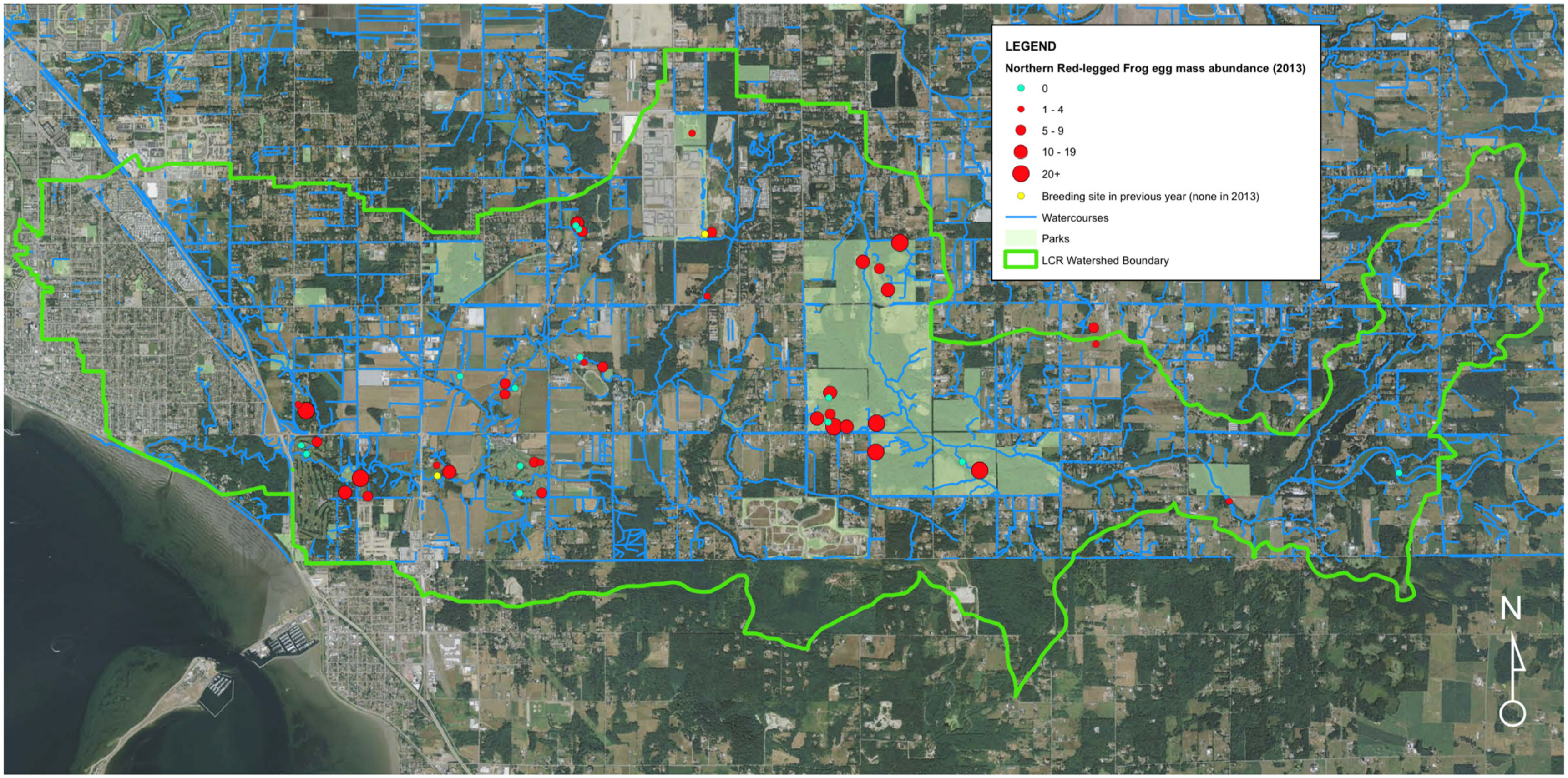

Figure 1. Little Campbell River watershed map showing 55 sites occupied by northern red-legged frog and peak egg mass abundance for 2013.

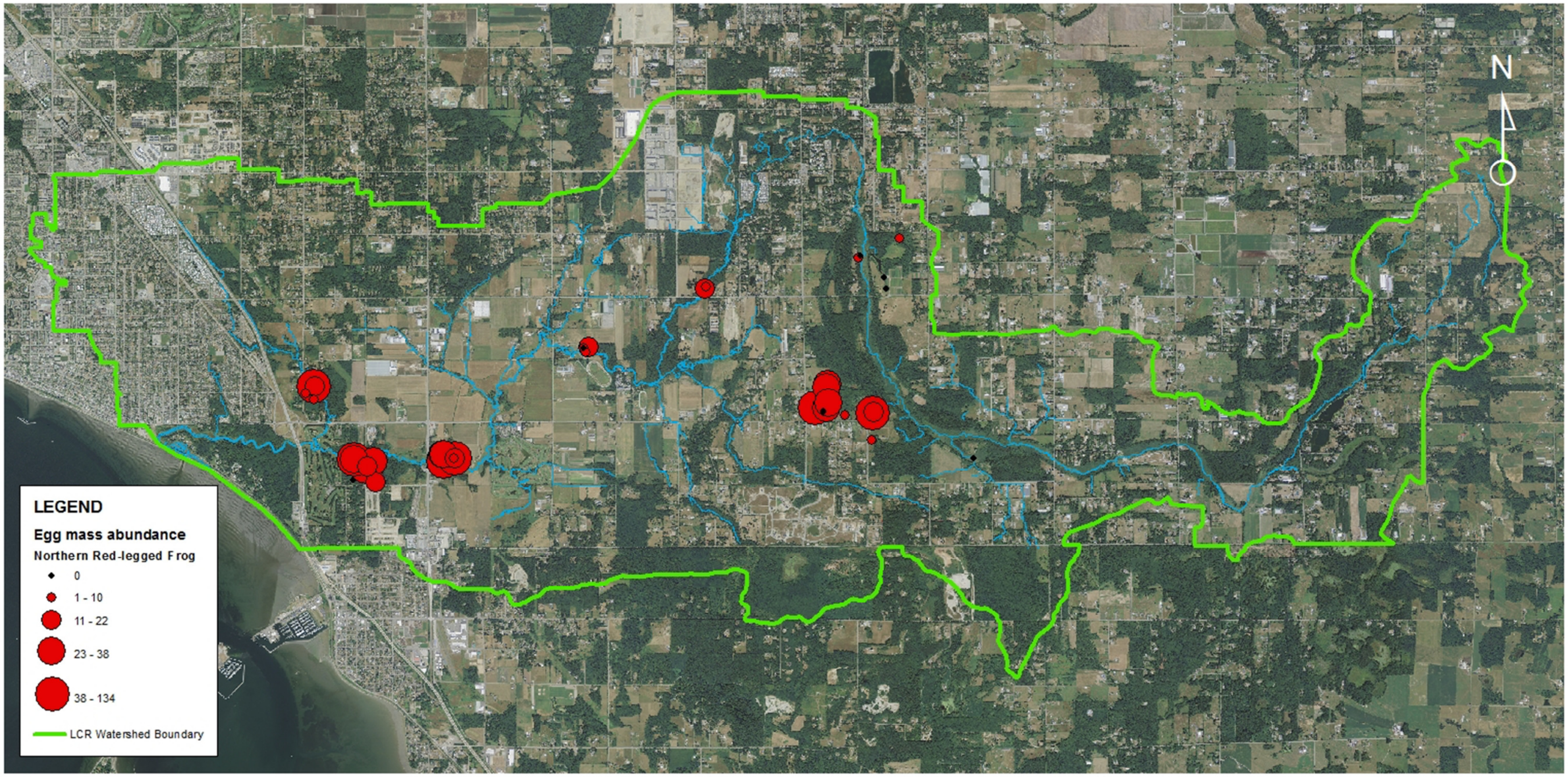

Figure 2. Little Campbell River watershed map showing peak egg mass abundance for 30 sites with northern red-legged frogs in 2014.

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Temporal Distribution of Peak Egg Mass Abundance

Annual pattern of peak egg mass

Figure 3 shows the cumulative frequency distribution of the daily mean egg masses of red-legged frog aggregated over all sites surveyed from 2009 to 2014.

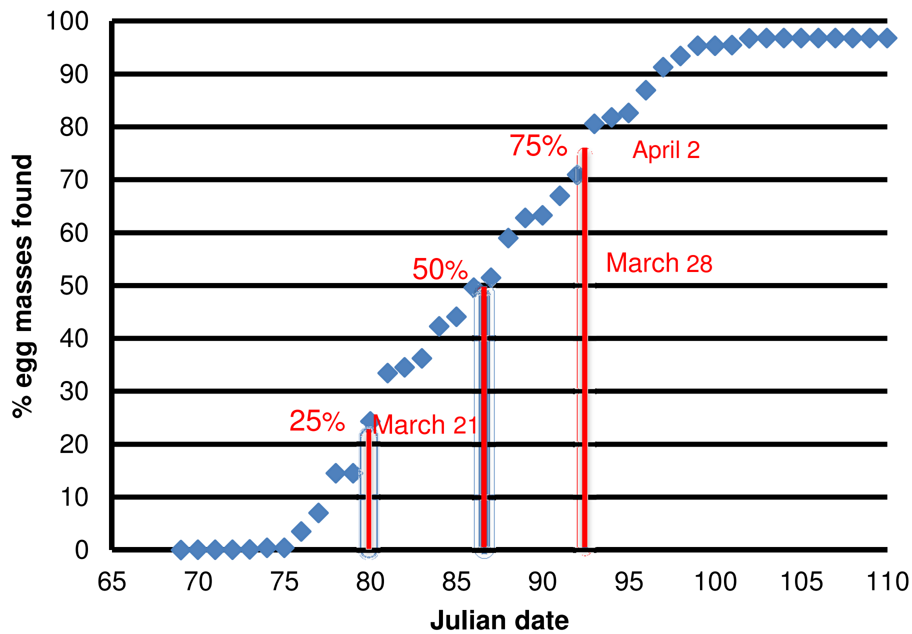

Figure 3. Cumulative frequency distribution of egg masses observed over all sites in 2009 to 2014.

When summed across all years, total egg masses did not exceed 10 until March 21. Overall, 25 to 75% of the egg masses were observed in the 2-week period from March 21 to April 3. The accumulation of egg mass is near linear from March 18 to April 8; 50% of egg mass over all sites and years is attained by March 28.

Information relative to start date for surveys is summarized in Figures 3 and 4. The first egg masses were encountered on March 16. Surveys on March 9 through 15 revealed no egg masses (Figure 3). Only four sampling dates recorded water temperatures less than 5^{o} C, the lowest being 4.4^{o} C (Figure 4). Mean number of egg masses on those dates was 18.25.

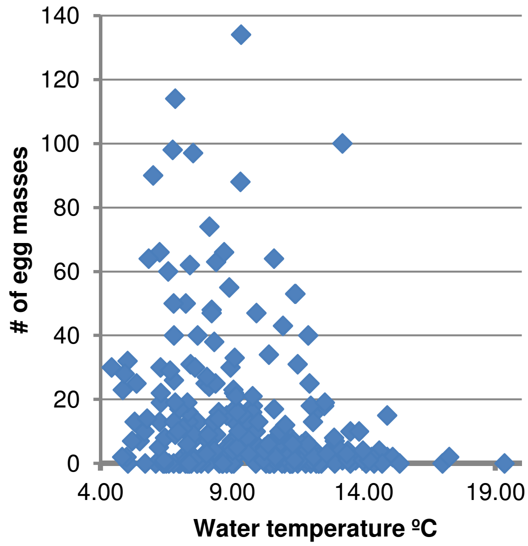

Figure 4. Egg mass versus water temperature.

Year effect

Because sampling effort varied among years, a year effect was expected. It was found (Figure 5 and Table 1). The year effect was not strong enough to eliminate the site effect. Figure 5 illustrates both a

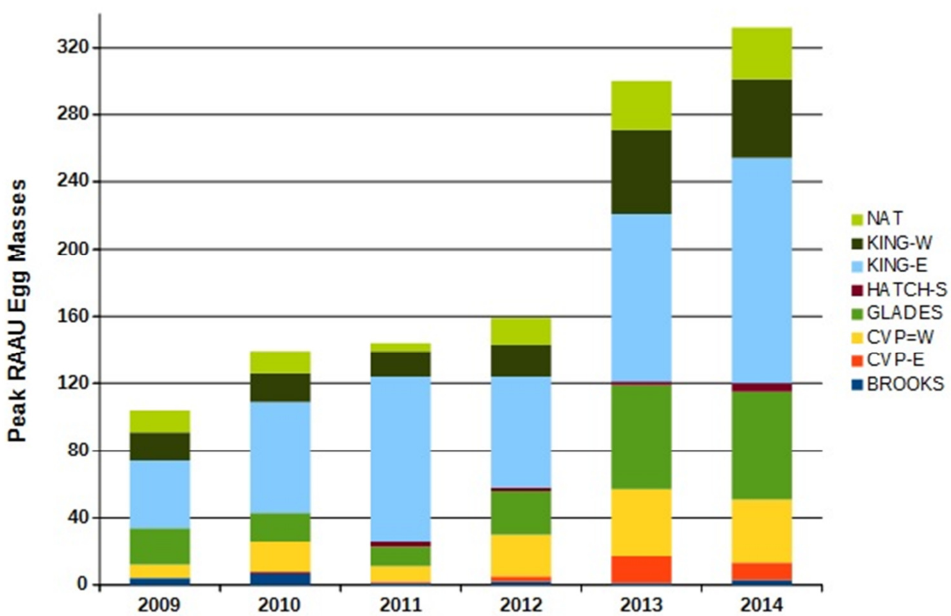

Figure 5. Peak egg mass abundance for northern red-legged frog at 8 sites from 2009 to 2014. No egg masses were found at Hatchery-North during 14 visits over 6 years.

year effect and considerable consistency in relative abundance of peak egg mass across the 9 sites consistently sampled. That is, ranking of egg mass abundance at the 9 sites varied relatively little across years. Site rankings across years were not consistent ($p<0.15$; Kruskall-Wallis analysis of variance), but for consistency to be expressed in ranking across so many sites, the governing signal must be very strong. Test results suggest a strong element of ranking with some inconsistencies among sites in a given year. Such inconsistencies are likely because some sites had few egg masses so ranks of peak egg mass could easily switch between years. As expected, Figure 5 indicates that shifts in rank occurred primarily among sites where frog abundance was low.

Both environmental variables (habitat suitability) and sampling effort could create the apparent year effect. We examined sampling effort by two indices: best estimates of the number of surveyors involved each year and total days or site visits undertaken each year for the nine sites examined each year (Table 1). The two indices are largely independent. Relative effort in Table 1 is based on the number of surveyors involved, but number of surveyors is inversely correlated with number of days sites were visited (fewer observers required more days to sample sites). For example, in 2011 there was only a single surveyor and in 2014, five surveyors were consistently involved; the numbers of days or visits were 21 and 12, respectively (Table 1).

Table 1. Egg mass by relative effort and number of visits across years for nine sites sampled each year.

Year	Total days	Relative Effort	Average Mean egg mass [a]	Average Peak Egg Mass [b]
2009	26	Low	7.38	11.56
2010	36	Low	12.14	15.55
2011	21	Low	7.05	16.00
2012	25	Moderate	18.24	17.63
2013	11	Moderate	39.73	33.33
2014	12	High	32.75	36.88

[a] calculated across all site visits [b] calculated across all sites

Mean egg mass is higher than peak egg mass in 2013 because the mean is based on more values than the peak and the two most productive sites were visited twice near peak breeding; other sites were visited only once in 2013. The number of survey days (Table 1) is not responsible for the higher mean egg mass abundance or peak egg masses observed in 2013 and 2014 (Figure 5). Across 131 survey days, there is a negative relationship between mean egg mass and the number of survey days ($r^2 = 0.60$) and between peak egg mass and number of days ($r^2 = 0.76$). Both relations are significant ($p < 0.05$, one tailed) and are expected, provided years of few measures are timed to encompass the peak. The analysis reveals that frequent visits are less important than well-timed visits to estimate peak egg mass. Data of Table 1 also suggest a relationship between egg masses counted and relative effort based on number of observers. Sites were grouped into three year-groups: 2009-2010 (low effort), 2011-2012 (low to moderate effort) and 2013-2014 (moderate to high effort). Correlated ANOVA was used because six observations from each specific site over six years are not independent. For any given year and site combination, only one value was used. So if in year X site A had two observations of 23 and 31, and site C had one observation of 17, the two values entered would be 27 for site A and 17 for site C.

Using ANOVA with post-hoc Tukey tests reveals the three year-groups were significantly different for both mean egg mass and peak egg mass at 0.0015 and <0.001, respectively. Post-hoc Tukey tests revealed that 2009-2010 and 2011-2012 did not differ significantly from each other for either mean egg mass or peak egg mass. Mean egg mass was significantly less than 2013-2014 ($p < 0.01$ for 2009-10; $p < 0.05$ for 2011-2012); peak egg mass was significantly less than 2013-2014 ($p < 0.01$ for both 2009-10 and 2011-2012). In short, well-timed visits are more important than number of visits, but relative effort based on number of surveyors was significantly related to estimates of egg mass reported for a site (Table **1**).

Somewhat stronger relationships were evident with peak egg mass than with mean egg mass. Multiple visits to a site generally lower estimated mean egg mass (Table 1), but do not change peak egg mass estimates. In analyses following we use peak egg mass observed.

Habitat Variables

Figures 1 and 2 illustrate potential effects of site-specific habitat variables on egg mass. Tests of rank correlation of egg abundance by site (Figure 5) indicate moderate consistency of ranking across sites, despite the strong year effect (Table 1). We assessed potential influences of environmental or habitat variables on frog presence and egg masses at three levels: surrounding land use and vegetative cover, features of the wetland margin and features of the wetland itself. Both the uneven spatial distribution (Figures 1 and 2) and the year effect on observed egg mass (Figure 5) could result from influences of environmental variables. Similarly, a significant year effect resulting from variable surveyor effort could obscure environmental effects. For environmental variables assessed, we found that when all sites and visits are analyzed there were no significant associations between habitat structure variables and egg mass. Thirteen sites were visited only once. Subsequent analyses used peak egg mass for sites visited three or more times and examined potential differences across four classes of peak egg mass (Table 3). Of the 58 sites sampled, only 33 were visited at least three times. Note that sites could change density strata between years and have different measures for some habitat variables between years. Even sites sampled only three times may have missed peak egg mass. For these and other reasons, there are few clear differences among strata in Tables 3 and 4.

Surrounding land use

Potential influences of surrounding land use were evaluated by GIS analysis of features in a 50-m buffer around candidate wetlands. All wetlands sampled were analyzed, but statistical analysis was restricted to the 33 wetlands visited at least 3 times. These were stratified into three equal classes of mean egg mass containing 11 sites each (Table 2).

Table 2. Mean proportions of habitat types within three classes of egg density.

	Mean Egg Mass		
	0 - 2	**3 - 16**	**17- 60**
Total water area (m)	797	3335.4	1105.5
% any forest type	23.4	41.3	59.7
% agriculture herb/grass	8.2	16.2	7.7
% unmanaged herb/grass	4.2	3.2	2.3
% marsh	8.1	7.2	9.9
% water	1.3	0.8	1.0
% urban-suburban low density	3.3	7.9	5.7
% planted crop rows	0	3.0	0
% turf grass	44.9	20.1	11.9
% urban-suburban high density	4.1	0	0
% urban trees	1.7	0.3	1.5

Sizes of the wetlands were sufficiently variable that mean size did not differ significantly between the lowest and highest egg mass classes ($p < 0.08$). Variables other than size had a more profound effect. Wetlands hosting the lowest egg density were surrounded by significantly less forest cover than the other two classes ($p < 0.012$), and proportions of turf grass were higher ($p < 0.086$ for the middle class and $p < 0.023$ for the highest density class). The apparent effects of high urban-suburban density on egg mass was a product of only two sites so the difference is not highly significant ($p = 0.077$) although the effect is strong. Frequency of low-density urban-suburban development was likewise low, so on average differences between egg density classes were insignificant ($p = 0.22$ and 0.27). Low occurrence of the effect masks the magnitude of impact over larger areas. The relative values across egg mass classes of Table 2 provide a better estimate of degree of local impact.

Features of wetland margins

Features of wetland margins summarized in tables following include:

- Dominant forest cover: % conifer / % hardwood / % mixed / % scrub
- % of shore forested class: 1 = 0-10%, 2 = 10-25%, 3 = 25-50%, 4 = 50-75%, 5 = 75-100%
- Woody debris as mean number of pieces >20 cm in diameter and 150 cm in length per 10 m of shoreline: 1 = <1, 2 = 1-3, 3 = >3
- Emergent class: 1 = 0-10%, 2 = 10-25%, 3 = 25-50%, 4 =50-75%, 5 = 75-100%.
- Canary reed grass (*Phalaris*) abundance class: 1 = not detected, 2 = uncommon, 3 = common, 4 = dominant.

Table 3. Mean values for variables describing wetland margins by four strata of peak egg mass (2009-2014). Means are by sites receiving three or more visits in a year.

	Peak # eggs/site-year			
Variable	<1	1-3	>3-20	>20
Number of sites	5	6	13	9
Number of visits	40	53	90	67
Mean visits/site over 6 years	8.0	8.83	6.50	7.44
% visits in 2013 & 2014	0.0	5.7	6.7	26.9
Dominant forest cover[a]	0/20/80/0	25/25/50/0	9/18/64/9	25/25/37.5/12.5
% shore forested class	2.10	1.00	2.58	3.06
Downed wood class	1.00	1.00	1.12	1.00
Emergent class	2.00	2.00	1.63	1.64
Phalaris class	-	2.13	2.77	2.21
% of visits adult frogs present[b]	33.0	62.8	88.20	96.20
% of visits bullfrogs present[b]	42.5	24.50	96.7	22.4

[a] Forest cover classes ordered as above (p. 12) from conifer through scrub.
[b] Mean % of visits to a specific site in which a northern red-legged frog or bullfrog was seen.

Other than presence/absence of red-legged frogs and bullfrogs, which were assessed in all years, variables of Table 3 were collected from 2011 through 2014. Eliminating sites where <3 egg masses were observed approximately doubles the number of mean visits per site over the six years (stronger samples) and makes the visits per site much more equitable across density classes. When sites visited <3 times are excluded, sites having zero to three egg masses decline from 30 to 11; sites having greater than 3 egg masses decline much less, from to 28 to 22. The fact that sites having the least egg masses were dropped from sampling in 2013 and 2014, when numbers of observers were high, indicates a conscious effort to sample the most productive sites in later sampling. Sites appearing less productive were dropped from sampling early, often after one visit. Sites with the greatest number of egg masses were visited more frequently in 2013 and 2014 (Table 3) when sampling effort was greater (Table 1), affirming that many of the most productive sites were discovered early and consistently sampled. Counts were more gratifying but representation was reduced.

Variables describing sites were not consistently measured so number of visits does not consistently reflect the number of measures used to create site means. When sites having maximum numbers of egg masses of three or less are compared to sites with egg masses >3, the % of forested shoreline is significantly higher for sites with greater egg mass counts ($p < 0.02$). Conifer dominant cover was absent from the lowest density class and scrub was present only at the highest density classes. Those data are visual estimates by surveyors. Within the 50-m buffers, no conifer cover was found at sites with fewer than three egg masses. There were few significant trends between egg density and other features of wetland margins. We expected that the abundance of emergents would influence egg laying, but there was no statistical difference between the two lowest and two highest density classes ($p > 0.15$). Nor was there evidence that abundant *Phalaris* at the margins negatively influenced egg laying. Lack of strong distinctions in variables describing wetland margins across egg density classes is encouraged by the fact that measures are subjective and surveyors differed across and within years. Among variables in Table 3, the relation most strongly expressed is that red-legged frog eggs are more abundant where red-legged frogs are more abundant. Bullfrogs, however, showed no trend with red-legged frog egg density.

Properties of wetland waters

Properties of wetland waters included temperature, oxygen concentration, conductivity and depth. These are even more likely to change between visits, so exploration of site effects on egg density are again restricted to sites visited at least three times a year (Table 4). Strata of Table 4 were chosen to contain reasonable representation in each stratum, while treating sites with egg masses with <1 egg mass per site year as a separate class. For example, the apparent effects of oxygenation would disappear if the two lowest classes were combined.

Table 4. Means of wetland variables by four strata of peak egg mass (2009-2014). Means are by sites for sites visited at least three times in a year.

	Peak # eggs masses per site-year			
Variable	<1	1-3	>3-20	>20
Number of sites	5	6	13	9
Cowardin System[a]	100/0/0	100/0/0	84.6/7.7/7.7	100/0/0
Mean water temperature °C	8.90	10.64	9.51	8.11
Mean O mg/l	7.86	10.98	9.14	9.03
Mean DO %	67.28	97.68	72.94	77.21
Mean pH	7.45	7.80	7.43	7.03
Mean conductivity (µS/cm)	261.1	266.0	171.4	103.8
Mean soft depth (cm) [b]	142.1	140.5	144.88	90.89
Mean hard depth (cm) [c]	162.7	124.5	159.80	131.28
Mean % visits with bullfrogs	44.6	25	82.2	22.2

[a] Cowardin System: %Palustrine, % Lacustrine, % Riverine
[b] Soft depth is that depth at which resistance to the measuring stick is first encountered.
[c] Hard depth is that depth at which it is difficult to penetrate the substrate.

Palustrine wetlands are non-tidal wetlands, usually substantially covered with emergent vegetation. Lacustrine wetlands are inland water bodies situated in topographic depressions; they lack emergent trees and shrubs and typically have less than 30% vegetation cover. Riverine wetlands are freshwater, perennial streams with deep water habitat contained within a channel. Of the 33 sites visited more than three times a year only two were not palustrine.

Estimates of properties of wetland waters employed instrumentation, so are less likely to vary among observers but more likely to differ between visits. When all 58 sites and individual visits instead of means are analyzed the variance is large and r^2 values never exceed 0.07. Regressions of site means of egg mass on site means of wetland water measurements for the 33 sites visited at least three times are shown in Figure 6. As expected, the scatter is significantly less across sites with more visits, but values for mean peak egg mass remain scattered indicating little predictability with weakly predictive linear relations for only pH, water temperature and soft depth (Figure 6). All other variables of Table 4 showed wider scatter. The pattern of the variables tested showed the possibility of parabolic relation. When tested, linear relations provided a better fit to the observations.

Repeatedly testing the same variable against several other variables in the same database incurs the likelihood of 'false discovery' (Verhoeven et al. 2005). The probability value for testing false discovery rates is conventionally designated 'q' rather than 'p'. The probability values for tests of slope = 0 (see Figure 6) are corrected for the false discovery rate. Mean egg mass declined broadly linearly but weakly with increasing pH ($r^2 = 0.165$; $q = 0.05$), with decreasing water temperature ($r^2 = 0.341$; $q = 0.017$) and with increasing soft depth ($r^2 = 0.211$; $q = 0.033$). In short, we can feel confident in the direction of the response, but the actual magnitude of response is variable.

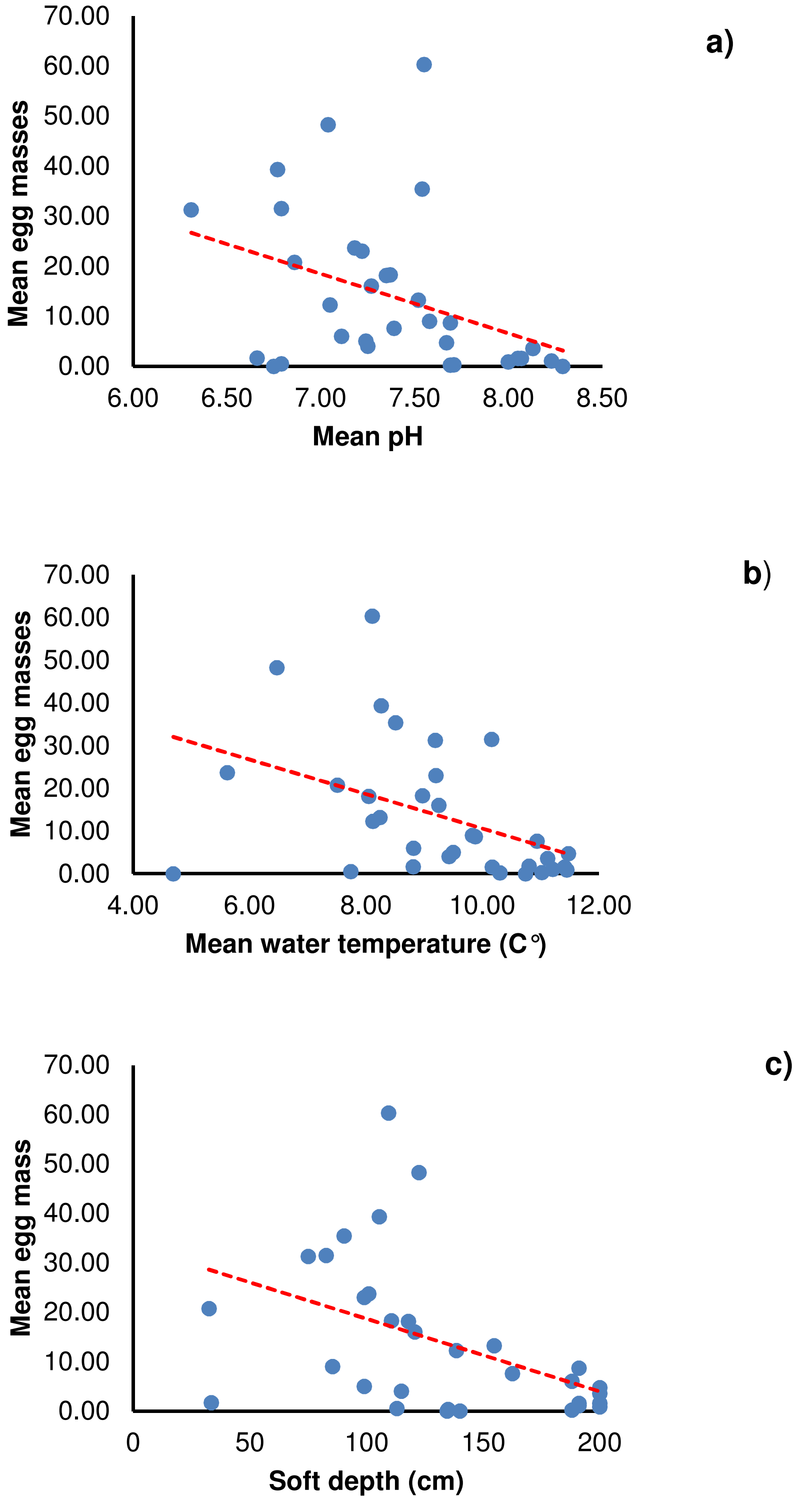

Figure 6. Mean egg mass as a function of means of habitat variables for sites with >3 visits.
a) pH; y =101.59 – 11.87 pH, r^2 = 0.165 b) water temperature; y = 50.98 – 4.04 °C, r^2 = 0.34
c) water depth to soft substrate; y = 33.43 – 0.147 cm; r^2 = 0.21.

Potential Vulnerabilities

Ideally the surveys expose potential vulnerabilities that can be evaluated in more detail. Data of Tables 2, 3 and 4 expose sensitivities that may signal vulnerabilities. To assess potential vulnerabilities of red-legged frogs to environmental variables it is important to know whether the strong year effect on egg mass abundance (Figure 5, Table 1) is primarily a product of relative effort across years or changes in environmental variables across years. There are significant differences in some habitat variables between density classes (Tables 3 and 4), but those differences are also covariate with sampling intensity as indexed by number of visits, with the lowest density classes being visited least during the years of highest effort (Table 1). The proportion of visits that occurred in 2013 and 2014, when observer effort was greatest, is markedly higher in the stratum hosting most frogs (Table 3). The role of differential effort is ambiguous, because some sites yielding few frogs were dropped from sampling in later years. An alternative way of evaluating the relative roles of effort and environment is to restrict analyses to the nine sites that were sampled in all six years to see if there is a rank order of peak egg mass with any of the environmental variables found to have marked effects on egg density (Table 5).

Table 5. Means of peak egg mass and environmental variables for those sites measured in all six years. Ranked lowest to highest peak egg mass.

Site	Peak Egg Mass	Water temperature	Standardized Conductivity	pH	Shoreline forest cover[a]	Emergent Class[b]
Hatch-N	0.0	10.73	0.91	8.31	2.33	1.00
Hatch-S	2.0	11.73	0.301	8.09	1.0	1.00
Brooks	3.0	11.51	0.190	7.07	1.0	1.00
CVP-east	5.2	9.28	0.044	7.05	2.0	1.00
Nathouse	17.8	8.47	0.033	7.34	1.67	1.00
CVP-west	23.0	8.8	0.040	7.02	4.67	2.33
Glades	33.8	8.3	0.112	7.52	2.67	1.00
King-W	27.5	8.51	0.217	7.42	3.0	1.00
King-E	84.0	8.35	0.115	7.61	1.0	2.00

[a] % shore forested classes: 1=0-10%, 2=10-25%, 3=25-50%, 4=50-75%, 5=75-100%

[b] Emergent class: 1=0-10%, 2=10-25%, 3=25-50%, 4=50-75%, 5=75-100%.

Using Spearman's rho, tests of rank correlation showed only one significant relationship between descriptor variables for sites and peak egg mass: water temperature. Peak egg mass tended to increase with decreasing water temperature ($r_s = 0.80$; $p < 0.015$). All other p values were >0.20. Not included in

Table 5, and also showing no relationship with peak egg mass, are the cover classes of down wood or *Phalaris* and the probability of bullfrog presence (tested by z-test of proportions).

Tests employing linear regression or rank correlation evaluate whether there is a clear directional trend in response to changes in a variable. Tests across egg density classes do not assume continuity of effect, but can reveal impacts of habitat variables on red-legged frogs (e.g., Tables 3 and 4). We repeated the latter using fewer classes to better expose extremes.

Table 6. Means and t-tests of habitat variables for two strata of peak egg mass density.[a]

Variable means	<3 egg masses	≥3 egg masses	p value
# egg masses	1.25	19.99	**<0.0001**
Visits/site	8.45	7.14	<0.43
% shore forested class	1.65	2.78	**<0.07**
Downed wood class	1.00	1.07	<0.36
Emergent class	1.15	2.63	**<0.11**
Phalaris class	2.13	2.54	<0.36
Water temperature ^{o}C	9.25	8.94	**<0.15**
DO mg/l	9.42	9.09	<0.71
DO %	82.48	74.79	<0.44
pH	7.72	7.27	**<0.06**
Conductivity (μS/cm)	0.2673	0.1437	**<0.11**
Soft depth (cm)	152.6	90.88	**<0.11**
Hard depth (cm)	148.21	148.13	<0.999
% visits with adult RAAU[b]	49.0	89.7	**<0.001**
% visits with bullfrogs	36.2	66.0	<0.31

[a] Variable metrics as in Tables 3 and 4. [b] RAAU = northern red-legged frogs

As expected, the differences in peak egg mass and presence of adult red-legged frogs are highly significant between density strata. Comparing broad groups changes the interpretation of potential sensitivities only modestly. The importance of forested shoreline is more clearly revealed, as is the presence of emergents. There is little apparent effect of *Phalaris* across sites surveyed. Sensitivity to water temperature, pH, conductivity and soft depth are affirmed. In short, sites with higher egg masses had a greater percentage of the shoreline forested, more emergent vegetation, slightly cooler water, lower pH, lower standard conductivity and were less deep to soft, penetrable depth. Hard depths of the two classes were very nearly identical. To a large extent that reflects the fact that rods used to measure depth offered readings to no more than 180 cm so all deeper depths were truncated at the same point.

These sensitivities may expose vulnerabilities of the red-legged frog to environmental change. Bullfrogs are another potential threat, so it is insightful to examine relative habitat use of the two species. Records on presence of bullfrogs during surveys were not kept consistently, which may undermine inference. Records available are summarized in Table 7.

Table 7. Means of wetland features by strata of frog species presence. Means are by site visits within each stratum. Note: the presence or absence of a frog species at a site can change between visits.

	Frog species present			
Variable	Neither	Bullfrog	*RAAU*[d]	Both
Number of visits	30	12	74	123
Mean *RAAU*[d] egg mass	0	0	21.11	15.15
% visits in 2013 & 2014[a]	46.7	58.3	60.8	26.6
Dominant forest cover[b]	5/9/10/0/6	0/6/6/0/0	8/19/33/0/15	4/10/24/4/10
% shore forested class	2.63	3.00	2.64	2.56
Downed wood class	1.13	1.00	1.13	1.10
Emergent class	1.86	2.00	2.00	1.25
Phalaris class	1.84	2.92	3.04	2.71
Cowardin class[c]	22/2/6	10/0/2	64/1/10	48/1/3
Water temperature ^{o}C	8.93	9.21	8.94	9.73
O_2 mg/l	9.49	8.97	9.88	10.06
O_2 %	84.32	79.27	86.13	86.77
Conductivity	0.1916	0.4752	0.1602	0.1646
pH	7.57	7.55	7.18	7.48
Soft depth (cm)	104.4	112.3	99.92	133.4+
Hard depth (cm)	137.0	>161.75	>124.1	>155.1

[a] Mean % of visits to a specific site in which a northern red-legged frog or bullfrog was seen
[b] Dominant forest cover: conifer/hardwood/mixed/scrub/no record
[c] Cowardin system: palustrine/lacustrine/riverine
[d] RAAU=northern red-legged frog

For this analysis sites could appear in more than one stratum and have only bullfrogs present on one visit and both frog species present on another. There were an additional 45 visits from which neither frog species was recorded but habitat measures are lacking. Sites hosting neither frog on a specific visit had the lowest abundance of *Phalaris*, lowest water temperature and highest pH. Site visits reporting only bullfrogs differed from sites with only red-legged frog or both species only for conductivity ($p < 0.0001$). Values for oxygen content were lower in sites with only bullfrogs, but not significantly ($p < 0.25$). Means for hard depth were rounded up when depths exceeded the measuring rods (180 cm). Hard depths of sites where only bullfrogs were seen were greater than sites with only red-legged frog ($p < 0.025$) but were not when sites with both frogs present were included in the comparison ($p < 0.124$). It is potentially noteworthy that none of the 12 bullfrog only site visits had predominantly conifer forest.

Discussion

Northern red-legged frogs are considered a Species of Special Concern in California and Oregon (California Department of Fish and Game 1999, Oregon Biodiversity Information Center 2013) but have no status designation in Washington (Washington Department of Natural Resources, Access Washington website). It is designated as a species of Special Concern in Canada (COSEWIC 2002). In British Columbia, the taxon is assigned a conservation priority of 1, the highest priority within a six-tiered provincial system (BC Conservation Framework 2014).

There is ample evidence of amphibian declines in the Pacific Northwest (Lannoo 2005). Pearl (2005) reported that thorough field studies documenting declines in red-legged frogs are lacking, but a broad array of potential stressors may affect the species. Modeling studies suggest stressors that impact larval and young, juvenile red-legged frogs have the greatest potential to influence population fluctuations (Biek et al. 2002). That, in turn, implies that studies of the places where eggs are laid can be revealing. Juveniles, however, often remain around edges of breeding ponds for only short periods (days to weeks) before dispersing, and cues for emigration are not well known (Licht 1986, Twedt 1993).

These analyses were undertaken for three broad reasons: 1) to refine surveys based on information in hand, 2) to evaluate land use and habitat variables potentially affecting red-legged frogs, and 3) to assess potential vulnerabilities of red-legged frogs in the watershed. Initial analyses were completed in time to guide surveys after 2014.

Refining Surveying

Onset of egg mass production reported here is similar to that documented by others for southwestern BC (Licht 1969, Calef 1973b). Over the six-year period 2009 to 2014 in the LCR watershed the first egg masses appeared on March 15 and 50% occurred between March 18 and April 2 (Figure 3). Over the six sample years only 5% of egg masses were observed after April 9 (Figure 3). Maxcy (2004) reviewed data of Storm (1960), Licht (969), Calef (1973b) and Brown (1975) and concluded that frogs become active and move towards breeding sites during rainy periods when daytime temperatures are >4–5 ^{o}C. Other workers have observed that for egg laying to occur, water temperatures must be at least 4 ^{o}C (Licht 1969, 1971; Calef 1973a). The coldest water temperature at which we found eggs was 4.4 ^{o}C (Figure 4).

The strong year effect on the number of egg masses found (Figure 5) appears to result largely from differences in surveys among years. For example, most of the surveys from 2009 through 2011 were by a single observer. Observer numbers since have largely stabilized at a higher number. We evaluated the stability of estimated egg mass for the three years of greater effort: 2013, 2014, 2015. Correlated ANOVA was used across those sites surveyed in all three years and revealed no significant differences among years ($p > 0.08$). We believe it is possible to maintain a largely consistent number of observers in the future so that inter-annual differences will no longer be correlated with observer effort and will better reveal effects of environmental variables.

Analyses of data for the period 2009 to 2014 (Figure 5, Table 1) reveal not only the impact of past survey approaches on estimated egg mass, but that fewer, well-timed visits with more surveyors is more revealing than many visits. That is, survey groups should not be divided in an effort to complete surveys in a shorter period. These findings influenced the approach to surveys after 2014. A Rocha has refined its sampling approach to employ a consistent group of 11 sites (further information could modify site selection). A subgroup of these sites can be sampled more frequently each season, to permit better quantification of vulnerability factors such as drying.

Environmental Influences

One longer-term goal of this study is to expose potential vulnerabilities of red-legged frogs in the study area. A first step in doing that is to evaluate potential environmental influences. Our ability to do that with current data is hampered by the unbalanced sample design. Much of the imbalance has since been corrected. Even though the design was initially unbalanced, it has permitted insights.

Although survey effort influenced numbers of egg masses observed (Table 1), effort was largely constant within a year across sites so it is potentially informative across sites. To reduce impacts of the survey approach on interpretation of habitat effects on egg mass, analyses used site means across years for both habitat variables and egg mass. Multiple visits to a site generally lowers calculated mean egg mass as compared to peak egg mass (Table 1). The effect is variable, however, and dependent on the timing of visits. We considered peak egg mass more revealing of potential site effects than estimates anywhere along the accumulation curve, so most analyses of potential habitat variables on egg mass employ peak egg mass. Comparison of habitat use by red-legged frogs and bullfrogs was an exception (see Potential vulnerabilities).

Surrounding land use

The density of red-legged frog egg mass varies spatially across the watershed (Figures 1 and 2). We evaluated potential impacts of surrounding land use on egg mass using GIS (Table 2). Search effort was higher in later years, but did not differ between locations in a given year. We averaged egg densities across years.

Englund (2007) performed a broadly similar GIS analysis for 12 park areas in the City of Surrey, but the purpose and scale were much different. Our analyses differ from those of Englund (2007) in three major ways: 1) Englund had no repeatable measure of red-legged frog abundance, 2) Englund used a 200-m buffer centered on the wetland, and 3) habitat classes of Englund included six types of forested habitat, shrubland and grassland. Englund relied on amounts of favourable habitat as estimated from aerial photographs within a 200 m buffer to estimate habitat quality. We used surveys of egg mass. Englund's choice of a 200 m buffer was based on a literature review by Semlitsh and Bodie (2003) who reviewed existing research on terrestrial habitat use by 19 frog species associated with wetlands to determine core habitat requirements. The red-legged frog was not one of the species. Core habitat for the 19 species was found to range between 159-290 m from the wetland. Our approach focused on demonstrably negative or positive features of wetlands themselves and their immediate environment. For that reason, measured features of the buffer zone also differed and included a wider range of land uses than Englund encountered in parks. Our measures are more detailed and do not compare readily to Englund's; e.g., his shrubland includes trees up to 10 m.

Maxcy (2004) estimated that up to 90% of an adult red-legged frog's life is spent in forested habitat. Table 2 reveals that egg densities were significantly higher in wetlands buffered by more forest cover, affirming the importance of forest cover for the species. Conversely, turf grass and golf courses supported limited frog reproduction, but were unreliable sources of habitat. Only six of 22 sites having more than two egg masses had turf grass. Some habitat types of interest appeared sufficiently sporadically in the buffers that statistical tests were unreliable. However, it is apparent that neither high- nor low-density urban and suburban areas are compatible with good frog breeding habitat. It is intriguing to find that unmanaged herb/grass areas in wetland buffers are less favourable to breeding than agricultural herb/grass areas.

Hermann et al. (2005) evaluated the influence of landscape and wetland characteristics on pond-breeding amphibian assemblages in south-central New Hampshire. Assemblages were influenced primarily by forest cover and wetland hydroperiod. Though species responded positively to different buffer widths, species richness was most strongly influenced by the proportion of forest cover within 1000 m of the wetland. They argued that in the northeast US, wetlands with <40% forest cover within a 1000 m radius may have depauperate larval amphibian assemblages, but forest cover above 60% within a 1000 m radius is likely to ensure species richness and abundant larval amphibian assemblages. They note that their findings suggest that regulations focusing amphibian protection efforts on narrow terrestrial buffers surrounding wetlands are likely to be inadequate. We are aware of no similar analyses in the PNW and note that analyses here simply document that red-legged frog egg mass abundance increases with forest cover up to 96% within a 50-m buffer.

Features of wetlands and water

Relying on largely the same sources, Maxcy (2004) and BC Ministry of Environment (2015) offer very similar descriptions of preferred habitat for red-legged frogs: wetlands with both emergent and open water near abundant forest canopy with downed wood and litter in a suitable spatial configuration to complete its life cycle. Suitable spatial configuration refers to the species' movement between breeding ponds and upland sites. None of the studies cited by these sources, nor more recent studies, illustrate apparent thresholds other than eggs aren't laid until water temperature exceeds 4 ^{o}C. Nor does this study, though strong trends are evident that are congruent with the descriptions of preferred habitat noted. Wetlands with less than 10% of the shore forested were significantly less productive (Table 3). When sites having mean peak egg masses $\leq$3 were compared to sites having means of >3, sites with higher egg mass had significantly greater forest cover by visual estimates ($p < 0.02$).

Abundance of emergent vegetation (rushes and sedges) was expected to have an impact on egg masses simply because it provides both egg-laying sites and rearing habitat for developing tadpoles. Markedly higher egg masses were found only where emergent cover exceeded 25% (Tables 3 and 6). Dense, tall stands of reed canary grass appeared to limit oviposition habitat of Oregon spotted frog (*Rana pretiosa*) in Washington (Kapust *et al.* 2012). Large amounts of decaying grass, which can be typical in dense stands of reed canary grass, reduced survival of wood frog (*Lithobates sylvatica*), pickerel frog (*Lithobates palustris*), American toad (*Anaxyrus americanus*) and Cope's gray treefrog (*Hyla chrysoscelis*) in mesocosm or controlled environment studies (Rittenhouse 2011). Data for the LCR watershed reveal no apparent effect on egg mass by *Phalaris* cover up to about 50%. Mean egg mass for all sites receiving at least 3 visits was 12.64. For the 3 sites with >50% *Phalaris* cover, the mean was 11.02.

Only three of the 33 sites receiving at least three visits had estimates of mean downed wood class exceeding 1.0. Downed wood class 1 represents a mean of <1 pieces of woody debris in pieces >20 cm in diameter and 150 cm in length per 10 m of shoreline. Small amounts of woody debris are apparently sufficient.

The situation is largely similar for measures characterizing wetland waters. We found strong, though highly variable, influences of water attributes on egg mass abundance primarily through pH, conductivity, water temperature and depth (Figure 6, Table 6). Egg masses declined with increasing pH (p of zero slope is q =0.05). It is possible that the two sites consistently having zero egg masses and visited at least three times had unfavourable values of water characteristics. Removing those two from analysis altered the relationship almost imperceptibly. Average pH for those 2 sites was 7.52 and was 7.37 for all other sites. In Portland, Oregon, Young (2010) reported almost no egg masses at pH values >6 for ponds surveyed for red-legged frogs and noted that a negative relationship with increasing pH had held in 4 of the last 5 surveys. In the LCR watershed a negative relationship was evident, but was not consistently near zero until pH exceeded 7.75 (Figure 6).

Conductivity and pH are related. However, conductivity is a measure of all ions present in the water, both cations and anions, so both strongly acidic and strongly basic solutions create high conductivity. In the wetlands sampled, there was a tendency for conductivity to increase with increasing pH ($r^2 = 0.24$, p of 0 slope <0.005) suggesting that the dominating agent of change in conductivity was increases in abundance of hydroxyl ions (OH^-). The number of egg masses showed a weak tendency to decline as conductivity increased and water became more basic. In the Greater Yellowstone Ecosystem higher conductivity was positively associated with boreal toad (*Anaxyrus boreas*) occupancy but negatively associated with boreal chorus frog (*Pseudacris maculata*) occupancy. Occupancy of Columbia spotted frog (*Rana luteiventris*) and barred tiger salamander (*Ambystoma mavortium*) was little influenced by water conductivity (Klaver et al. 2013). Because conductivity reflects all ions, it fingerprints no particular 'culprit'. In studies of amphibians it is most often used as a proxy for salinity. In the LCR watershed, more basic waters clearly depress the number of egg masses (Figure 6) and it would be helpful to know the sources of hydroxide ions. Some studies have used pH as a proxy for dissolved oxygen. The relationship is evident in these data but is 'sloppy' with $r^2 = 0.27$.

COSEWIC (2002) citing Brown (1975) reported that male red-legged frogs call in water that is 4 to 9 °C, but the minimum water temperature for egg-laying is 6 ºC (Brown 1975). We found egg masses at water temperatures of 4.4 °C. Mean egg mass declined with increasing mean water temperature (Figure 6b). We again redid the regression removing the two sites that consistently had zero egg masses. The linear fit increased to $r^2 = 0.34$ and q of 0 slope declined to 0.017 (the regression is then y = 71.60- 6.11X). There are four factors that could encourage a decrease in number of egg masses with increasing water temperature. Red-legged frog oviposition and larval development are dependent on water temperature (Storm 1960, Licht 1971, Calef 1973a, Brown 1975) with periods being shortened by warmer water. Eggs aren't around as long. Warmer water also contains less oxygen, which might cause egg masses to die with increasing water temperature. Warmer water also encourages bullfrogs (McKercher and Gregoire 2011) that consume red-legged frogs at all stages of their life cycle. As well, when comparing simple means, warmer temperatures occur later in the season when eggs are more likely to have hatched. These factors occur simultaneously and cannot be neatly separated within the data, but generally red-legged frog eggs are cited as being more susceptible to overheating than most other frog eggs, so require more stable water temperatures. That relation likely explains the affinity for shoreline forest cover.

Mean egg mass also declined with increasing soft water depth (depth of first resistance; Figure 6c; q of 0 slope = 0.033). Removing the two sites without egg masses changed the relationship insignificantly. The decline of egg mass with increasing water temperature and depth is consistent with the increase in surface water temperature with soft depth ($r^2 = 0.25$). Egg masses decrease with increasing surface water temperature and surface water temperature increases with wetland depth. Why this relationship should occur is unclear. It is possible that deeper water reduces mixing, so increases near-surface temperature. Sources describing red-legged frog biology repeatedly note that their eggs are more susceptible to overheating than most other frog eggs and so they require stable water temperatures (e.g., Frogwatch; www.naturewatch.ca/frogwatch/red-legged-frog). We found no primary source for the statement, nor estimates of the optimal temperature for red-legged frog eggs. Egg clusters are typically attached to submerged vegetation or rotting logs, 7 to 15 cm below the pond surface, though Calef (1973b) reported egg masses deposited from 5 cm to 5 m deep.

American Bullfrogs

Non-native bullfrogs are now established throughout much of the lowland range of red-legged frogs west of the Cascades, and have been hypothesized as displacing red-legged frogs there (Nussbaum et al., 1983; St. John, 1987; Kiesecker and Blaustein, 1997, 1998). In Oregon, red-legged frog larvae have been found to compete poorly with American bullfrog larvae when food resources are concentrated (Kiesecker and Blaustein, 1997,1998; Kiesecker et al., 2001). Conversely, studies conducted in Washington have failed to identify direct effects of competition on red-legged frog larvae or exclusion from wetlands supporting American bullfrogs (Richter and Azous, 1995; Adams 2000). Our data for the LCR watershed suggest findings similar to those in Washington. Sites hosting only red-legged frogs differed from sites where both frog species were observed (Table 7).

Adult bullfrogs are carnivorous, aggressive and eat just about anything they can fit into their mouths. Second year bullfrog tadpoles likely eat red-legged frog tadpoles. In terms of predation on red-legged frogs, published data are equivocal about the major threat – non-native fish or bullfrogs. Several authors observe that predation by both introduced game-fish and bullfrogs may represent important threats to red-legged frogs throughout their range (Hayes and Jennings 1986; Twedt, 1993; Kiesecker and Blaustein 1997, 1998). Adams (1999, 2000) observed that effects of non-native fish appear to be of greater importance. Kieseker and Blaustein (1998) noted that the interactive effects of fishes and bullfrogs may be greater than either separately. Our data show red-legged frog egg mass densities are depressed 28% when bullfrogs are present (Table 7). The pumpkinseed sunfish (*Lepomis gibbosus*) is in the watershed but we cannot distinguish potential effects of its presence.

T-tests of pond selection (Table 7) show that ponds with only red-legged frogs are significantly less deep than those hosting both red-legged and bullfrogs ($p = 0.06$ and $p < 0.05$ for soft and hard depths respectively). Bullfrogs were expected to require deeper depths simply because red-legged frog tadpoles require less time to metamorphose than do bullfrog tadpoles, so wetlands need not be as durable. In nearby northwestern Washington, red-legged frog larva metamorphosed in 110 days on average (Brown 1975); bullfrog metamorphosis ranges from a few months in the southern part of the range to three years in the north where colder water slows development (Casper and Hendricks 2005). It is likely about two years in the LCR watershed. Selection for deeper water by bullfrogs (Table 7) also may be a product of them laying eggs later in the summer than do red-legged frogs. Despite having deeper water, sites with bullfrogs only had higher water temperatures and lower oxygen concentrations than sites with only red-legged frogs (Table 7). Water at bullfrog only sites also had much higher conductivity ($p < 0.001$) and higher pH ($p < 0.067$).

More generally, continued surveys and analyses will strengthen interpretations and permit stronger statements on potential vulnerabilities. That is particularly true because drier periods are likely to be more frequent. Currently, the lack of repeated measures prohibits analyses of potential drying on red-legged frogs, but the fact that they favour the more shallow ponds suggests they could be susceptible as climate continues to warm.

Exposed Vulnerabilities

Sensitivity to particular environmental features noted above reveals vulnerabilities of the red-legged frog. Of these features, several clearly merit response and continued tracking: bullfrogs, forest cover, extent of *Phalaris*, indicators of pollution (conductivity, pH and oxygen content) plus water temperature and depth.

Bullfrogs: In Stanley Park and other well-surveyed parts of southern BC, bullfrogs have completely displaced red-legged frogs (e.g., Stanley Park, Stanley Park Ecology Society 2010). In other places, such as Delta (Rithaler 2002) and southern Vancouver Island (Anthony 2013), bullfrogs have eliminated red-legged frogs from some, but not all wetlands. In the LCR watershed bullfrogs already have occupied most wetlands visited. In sites where bullfrogs occur, red-legged frog egg mass count is reduced 28% (Table 7). On the plus side, red-legged frogs can breed in ephemeral or temporary pools, while bullfrogs and introduced fish are restricted to permanent waterbodies. On the down side, bullfrogs have a greater fondness for warmth than any native amphibian. McKercher and Gregoire (2011) report that bullfrog embryos develop best at water temperatures between 24 and 30 ℃ and hatch in three to five days. Our limited efforts so far have done little to slow global warming. Some biologists, we among them, expect that, left to their own devices, bullfrogs will continue to increase, expand and locally extirpate native frog species. S. Orchard (who runs a company that kills bullfrogs) has described two instances of at least temporary success at controlling bullfrogs (Orchard 2011). In both cases, eradication began shortly after colonization by bullfrogs (one spawning season). Orchard (2011:218) noted “There are few published case studies of bullfrog eradication, and the few successful examples were laborious and costly.” One problem is that adult bullfrogs are prolific and, although they prefer to stay in water, can move well over 1 km so eradication may be temporary.

There is relatively little that can be done. Encouraging habitat features sought by native amphibians is an obvious step. Eradication of bullfrogs buys time and is simple enough but messy. Every opportunity to thwart bullfrogs should be taken. Education of local residents about identification and negative impacts of the bullfrog should reduce the likelihood of residents moving them about. The six years of data reviewed show little difference between sites used by bullfrogs and red-legged frogs, other than bullfrogs exploit deeper water and are much tolerant of impurities in the water (Table 7). Continued measurements may reveal small differences that can be exploited.

Forest cover is important to all life stages of red-legged frogs. Greater amounts around the shore encourage significantly higher egg masses (Tables 2 and 3). In Washington State, Ostergaard (2001) found that egg masses were most numerous in ponds with over 30% forest cover within 200 m from the shore. Outside the breeding season the species is often in forests well removed from wetlands. Dispersal distances of several km from aquatic environments into adjacent riparian and upland forests have been observed, but are more often 300 to 500 m (Nussbaum et al. 1983; Licht 1986; Gomez and Anthony 1996; Hayes et al. 2001). Narrow wetland buffers are inadequate to meet their needs. Like the western toad, this species displays high fidelity to breeding sites and many individuals may utilize the same migration pathways. Maintenance of forest cover is critical. Documenting pathways and communicating them to area residents would be helpful.

Phalaris: *Phalaris arundinacea* or reed canary grass is a highly competitive and assertive species. It is listed as native in North America by the USDA and is found across the continent in most states and provinces. Cultivars brought in for ornamental use and as pasture grasses have been introduced from Europe and Asia. These hybridize with native populations, producing aggressive offspring in the central and western regions of the continent. The result is a highly competitive, rapidly spreading, assertive species. In the watershed it has expanded rapidly. Across the 58 sites visited, 90% host some reed canary grass. There is no reason to assume it will not continue to expand.

In the struggle between red-legged frogs and bullfrogs, *Phalaris arundinacea* favours bullfrogs. Its tall stature (50 cm to 2 m) and robust leaves create a dense vegetative mat that suppresses other plant species and can be a barrier to small amphibians, like red-legged frogs. Bullfrogs are sufficiently robust to be unimpeded. Abundant *Phalaris* apparently constrains success of small frogs beyond limiting movement (Hayes 1994; Platt 2014). Rittenhouse (2011) found that the pulse of organic matter created by large stands can create anoxia. Bullfrogs appear to cope better at lower levels of oxygen than do red-legged frogs (Table 7). Left unchecked, expanding *Phalaris* will encourage a decline in red-legged frogs. Patient restoration can change that. Experience at A Rocha Canada's Brooksdale Environmental Centre indicates that cutting down *Phalaris* and planting red alder will eventually reduce *Phalaris* significantly. So far amounts of *Phalaris* present show no relation to oxygen content of the water.

Pollution: Conductivity, pH and oxygen content are each indices of pollution. Currently only conductivity and pH are correlated with a strong reduction in egg mass count, particularly conductivity (Table 4, Figure 6). Many studies have assessed the impact of different pollutants on amphibians across a variety of experimental venues (laboratory, mesocosm and enclosure conditions). Egea-Serrano et al. (2012) provide a meta-analysis of studies. Their meta-analysis revealed a 14.3% decrease in survival, a 7.5% decrease in mass and a 535% increase in abnormality frequency across all studies. In contrast, no overall effect of pollutants on time to hatching and time to metamorphosis was documented. The large increase in abnormalities is significant. Activity level, habitat use, courtship and swimming performance are all affected by pollution. Thus, the ability of individuals to escape from predators (or other stressors such as ultraviolet-B radiation), to feed or reproduce can be affected. That means the impact of pollutants is likely higher than reported from counts near wetlands. Unlike LCR watershed data, Egea-Serrano et al. reported no effects on egg mass. However, it is as eggs that the large impact on abnormalities begins. It is likely that the negative effects reported for LCR watershed are greater than a simple reduction in numbers of eggs.

It is important that measures of these variables be continued. As climate continues to warm and water depth declines, all pollutants will become more concentrated and anoxia will increase. As negative effects strengthen, sources of effects will become more evident.

Water depth and temperature: Mean number of egg masses at a wetland declines significantly with increasing near-surface water temperature and with increasing soft depth (Figure 6). The latter relation likely occurs because near-surface water temperature increases with increasing soft depth ($r^2 = 0.25$). As noted earlier, the association of warmer surface water with increased depth is likely a product of reduced mixing in deeper waters. The rate of global warming appears far more likely to increase than to decrease for decades yet. Documented patterns indicate that egg masses will decline with increased warming. Unfortunately, bullfrogs prefer warmer temperatures than do red-legged frogs. Given that continued warming is inevitable for some time yet, efforts to maintain red-legged frogs need to be focused on the possible: retaining and encouraging forest cover, focused efforts to replace *Phalaris* with cover such as red alder, localized efforts to reduce bullfrog numbers and continued diligence regarding pollutants. During summer the LCR already flows both ways, to and away from the ocean, as it dries out part way along. Unlike rivers that supply Vancouver's drinking water, the Little Campbell is not replenished by deep snowfalls each year. Aquifers play a much larger role in water flow of the Little Campbell. All water is connected. Currently, the sources of the Little Campbell are being depleted. Observation well #7 in Langley shows an average decline in aquifer water level of 0.225 m per year (see http://www.env.gov.bc.ca/soe/indicators/water/groundwater-levels.html), but the rate has been increasing since 1983. Actions to slow the decline are unlikely to occur until things become much worse and drinking water declines.

This simple summary of apparent vulnerabilities reveals that the red-legged frog is a readily measurable sentinel for the state of the LCR watershed and all organisms dependent on it. Continued assessment of the variables revealing vulnerabilities will help document both the direction and rate of change. In addition to continued sampling of the current sites, it will likely be revealing to monitor several of the sites from which no egg masses were reported. For example, in the first six years no egg masses were reported from Hatchery North and it also had an uncommonly high pH. Of the 58 sites sampled in the first six years, 11 had a mean egg mass of 0.0. Seven of those, however, received only one visit.

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Appendix 1: Datasheet used for 2014 surveys

RED LEGGED FROG SURVEYS - Spring 2014

Observers ____________________ Date ______________ Jul Date _________

Site Code _____________ Site Name ________________ Start_____End_____

Cloud __________ Wind (1-8) __________Weather/Precip _________________

Air Temp ________ UTM(10) E ________________ UTM N _________________

Waypoint# ________________________ Photo#s ____________________

Habitat Data

Cowardin System _____________

Cowardin Class _______________

%ForestShore _______________

Forest type _______________

CWD (H/M/L) _______________

%Emerg Veg _______________

Hydroperiod _______________

Depth (S/H) _______________

Phalaris _______________

Detection Data

	Y/N	Sign	Number
RAAU	_____	______	___________
RAPR	_____	______	___________
AMGR	_____	______	___________
AMMA	_____	______	___________
PSRE	_____	______	___________
ANBO	_____	______	___________
LICA	_____	______	___________
LICL	_____	______	___________

Water	Temp @5cm	DO (mg/L)	%DO	Conduc'ty	Sp. Cond.	pH
1						
2						
3						
4						

Search Effort Duration (min) __________ Distance (m) __________

Notes

Bullfrogs present?

Y N

Kayak?

Y N