Reintroduction and monitoring of northern leopard frogs (*Lithobates pipiens*) in the Columbia marshes, 2019

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Executive Summary

Northern leopard frog (*Lithobates pipiens*) monitoring activities at the Brisco reintroduction site proceeded as in other years, with deployment of automated recording units (songmeters, n=13) and opportunistic visual encounter surveys (VES) during the breeding season, VES in the summer and fall, and release of tadpoles, metamorphs, and juvenile frogs via wild-to-wild translocation, head-starting, and captive breeding.

No calling was detected on any of the songmeters in 2019. Data collected from a water level logger showed that flooding of the river into the release pond occurred in early June, with water depth increasing significantly over a few days.

The egg mass threshold (5 egg masses) at the source population in the Creston Valley Wildlife Management Area was met this year, allowing for direct wild-to-wild translocation of 2317 tadpoles from 2 egg masses to the Brisco release site. An additional 1797 tadpoles, 59 metamorphs, and 8 juveniles were reintroduced through a combination of head-starting and captive breeding, for a total of 4181 individuals. Although this was short of our 8,000 target, it was more than twice as many as the previous two years. Mortality during translocation was low (0.1%).

Overwinter survival at the reintroduction site was confirmed this year through three observations of either juveniles or adult frogs. We were only able to obtain photos of two of the three, but it is possible that the unphotographed individual was one that was later photographed as it was in the same location. This frog was identified as a YOY that had been captured in 2018.

Visual encounter surveys occurred weekly from July 29 to October 6, over which time 221 young-of-year (YOY) observations were made. Of these, 160 were captured, accounting for 130 individuals as identified through spot patterns. There were 25 YOY captured twice and four captured on three occasions; the use of recapture data allowed for population size calculation, resulting in a population estimate of 293 YOY with a 95% confidence interval of 207 to 427 individuals. Mean weight was 10.5 g (± 3.0 SD) and mean SVL 46.5 mm (± 4.5 SD); body condition was significantly lower in 2019 than in 2018, but higher than in 2016.

Signs of chytridiomycosis (hereafter “chytrid”) were first observed on September 11 and continued until the final survey on October 6. A total of 17 individuals were identified as showing signs of chytrid, of which 15 were swabbed. We also swabbed 3 Columbia spotted frogs (*Rana luteiventris*) with no outward signs of chytrid. Laboratory analysis confirmed the presence of chytrid in all of the swabbed leopard frogs and 2 of the Columbia spotted frogs. Chytrid was about half as common as it was in 2018.

This was the first year since 2016 that we were able to locate enough egg masses in the CVWMA to do wild to wild translocations, allowing us to almost double the number of tadpoles released in the previous two years combined. Despite this effort we failed to meet our target releasing 8,000 tadpoles. There is hope that the number of released tadpoles may increase in the future, pending successful captive breeding at the Calgary Zoo in 2020.

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Introduction

Although the northern leopard frog (*Lithobates pipiens*) is one of the most widely distributed amphibian species in North America, western populations have experienced significant declines in recent decades (reviewed in Rorabaugh, 2005). The genetically distinct Rocky Mountain Population in British Columbia is ranked as *Endangered* federally (Government of Canada 2019) and *Critically Imperiled* (S1) provincially (BC Conservation Data Centre, 2019), with only a single extant population remaining at the Creston Valley Wildlife Management Area (CVWMA). This population has become the source for reintroductions in the province as well as the establishment of captive assurance and breeding populations at the Vancouver Aquarium and Calgary Zoo.

One of the reintroduction sites is located near the community of Brisco in the Columbia marshes, a region where leopard frogs were historically present. For this reason, the region was identified as a high priority location for re-establishment of leopard frog populations in the Federal Recovery Strategy (ECCC, 2017), and recent evidence demonstrates the contemporary presence of suitable habitat and environmental conditions at the site (Ohanjanian & Carli, 2010).

The purpose of this document is to report on the 7th year of release and monitoring of the Brisco reintroduction site. Success at this site is continuously evaluated using the following indicators of success (Randall et al., 2016):

- Indicator 1: Reintroduced tadpoles complete metamorphosis
- Indicator 2: Frogs overwinter successfully
- Indicator 3: Male calling observed during the breeding season
- Indicator 4: Evidence of successful breeding in the wild as indicated by wild-bred eggs, tadpoles, or frogs
- Indicator 5: Evidence of colonization and successful breeding at additional sites
- Indicator 6: Some or all life-stages are detected at least 3-years post-release
- Indicator 7: Persistence of reintroduced or colonized populations for >10 years without supplementation

Metamorphosis of tadpoles was observed in 6 out of 7 years, while a breeding call was recorded on an automated recording unit (songmeter) in 2017 with evidence of overwinter survival seen in the same year. Activities in 2019 included deployment of songmeters and opportunistic visual encounter surveys (VES) during the breeding season, VES in the summer and fall, and release of tadpoles via wild-to-wild translocation, head-starting, and captive breeding.

Methods

Remote Sensing

Songmeters (n = 13; Wildlife Acoustics) were deployed at the release pond and other wetlands in the vicinity during the first week of May (Figure 1). No songmeters were placed on the river island just south of the release pond ("Larry's island") or the wetland north of the release pond as these locations were completely dry in May. Units were programmed to record ten minutes per

hour between 13:00 and 17:00, ten minutes from 21:00 to 21:10, and then continuously from 22:00 to 01:00. Although the highest intensity of calling is widely considered to occur at night, northern leopard frogs have been shown to call extensively during the day when temperatures are cold (Sommers et al., 2017). Songmeters were retrieved in late July, and the spectrograms were visually scanned for the presence of leopard frog "snore" calls.

Water depth and temperature were measured in the release pond from May 8 to October 6 through use of a water level data logger (model U20, Onset Computer Corporation) and water temperature data logger (model UTBI-001, Onset Computer Corporation), respectively. The water level logger, which also records temperature, was placed near the second most southern songmeter in the release pond (11U, 550813 E, 5628497 N; see Figure 1 for songmeter locations), while the temperature logger was placed near the most southern in the release pond (11U, 550942 E, 5628306N).

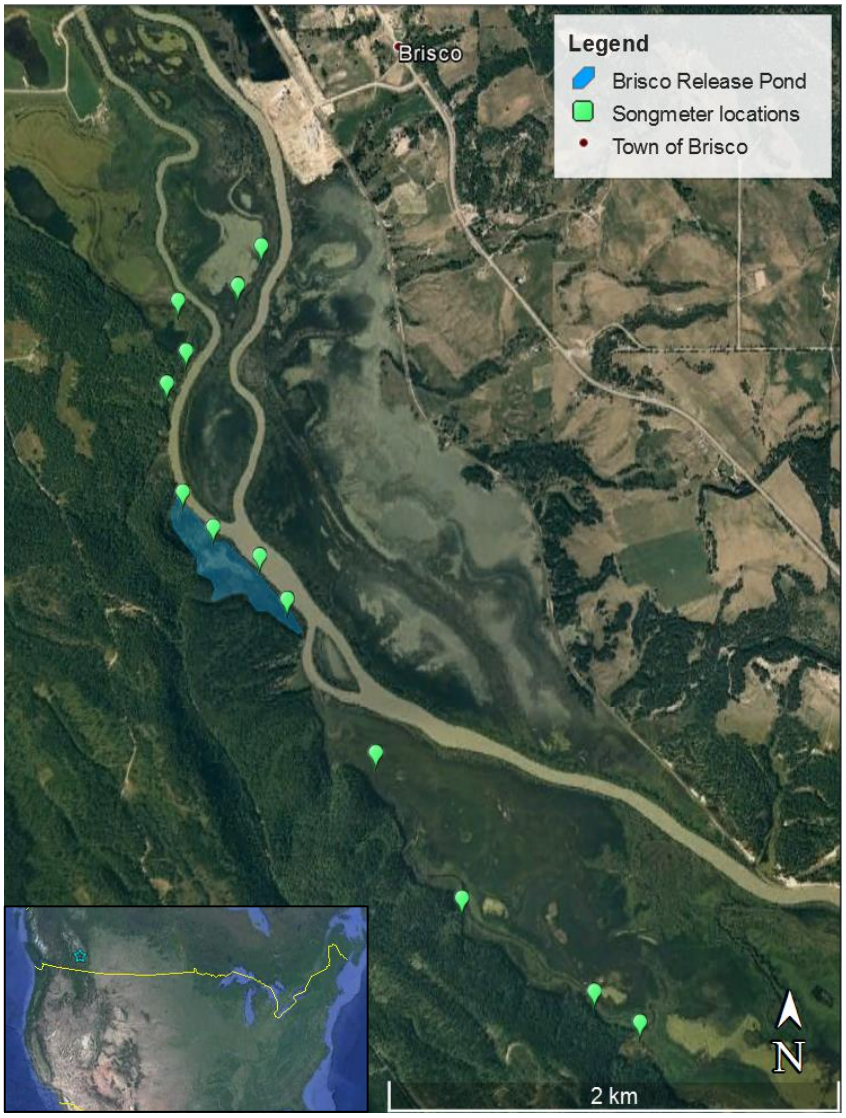

Figure 1: Locations of songmeters deployed at Brisco in 2019.

Egg Mass Surveys, Translocations, and Release

The 2019 threshold for number of egg masses detected at the CVWMA before direct translocations were permitted was set at five by the recovery team. Researchers from the Calgary Zoo joined others at the CVWMA to conduct egg mass surveys and nocturnal calling surveys over 8 days between April 26 and May 18 (additional surveys were conducted at the CVWMA without Zoo researchers). These surveys were to locate eggs and cage them to protect them from predator and for translocation to the wild and captive assurance and breeding programs.

Head-started tadpoles and young-of-year (YOY) from the Calgary Zoo and captive-bred tadpoles, YOY, and juveniles from the Vancouver Aquarium were able to be released at the reintroduction site in 2019. All translocations and releases were conducted using serial water changes and following the translocation guidelines outlined in Kendell and Prescott (2007) and BC Hygiene Protocols (BC Ministry of Environment, 2008)

Visual Encounter Surveys

In an effort to detect post-metamorphic, juvenile, or adult frogs, VES occurred on a weekly basis from July 29 to October 6 and consisted of personnel visually searching for individuals during the warmest part of the day (typically between 11:00-16:00) in appropriate habitat and recording number and location of animals captured or observed. Two additional VES were conducted opportunistically on June 24 and July 16 while personnel were on site for tadpole releases. Additionally, if juveniles or adults were observed outside of survey periods, there was an attempt to get a photograph for identification. Environmental variables (air temperature, cloud cover, wind chill, and water temperature and pH) and survey start and end times were recorded for all VES.

A capture attempt was made on all individuals observed and a waypoint was taken as close to the first observed location as possible. We attempted to take a photo of the juvenile/adults observed in the spring prior to capture. Individuals were captured using a net and transferred to an unused zip-top sandwich bag; this was facilitated by the observer placing their hand inside the bag while the bag is inside out and then picking up the frog with the bagged hand. In cases where frogs needed to be handled outside of the bag (*e.g.* when swabbing for chytridiomycosis, hereafter "chytrid"), individual-specific powder-free nitrile gloves were worn. Once captured, individuals were weighed using a Pesola scale, and snout to vent length (SVL) and shank length measured and recorded. Overall health was noted by visual inspection (checking for vigour, sloughing skin, righting ability, presence of all limbs/digits, and other general health criteria). If chytrid was suspected, a skin swab was collected for analysis. Photos were taken of the dorsal spot pattern of all individuals in order to identify recaptures. Individuals were then released in the vicinity of where they were captured within 5 minutes from the capture time. Net heads were disinfected with Virkon between individuals to avoid spreading chytrid.

Statistical analysis

Body condition of YOY

A linear regression analysis was conducted using the *lm* function in R (version 3.6.1; R Core Team 2019) to investigate differences in YOY body condition between years at the release site and between the release site and the source population at the CVWMA in 2019. A body condition index was established using the residuals from a regression of weight against SVL. Linear regression was also used to look at drivers of body condition at the release site and included effects of date, direction from release location, and recapture status, with model selection completed using Akaike Information Criterion (AIC; Burnham & Anderson, 2002).

Population Estimation

A population estimate for YOY at the reintroduction site was calculated using capture-mark-recapture data and the *FSA* and *FSAdata* packages in R. Populations estimates were derived using the Schnabel estimation method with a Chapman modification (Chapman, 1954), following the equation,

$$\widehat{N} = \frac{\sum_{i=1}^{k} C_i M_i}{\left(\sum_{i=1}^{k} R_i\right) + 1}$$

where $\widehat{\boldsymbol{N}}$ is the estimated population size, $\boldsymbol{k}$ is the number of samples, $\boldsymbol{C_i}$ is the number of individuals captured in sample $\boldsymbol{i}$, $\boldsymbol{M_i}$ is the total number of individuals captured and marked prior to sample $\boldsymbol{i}$, and $\boldsymbol{R_i}$ is the number of marked individuals in sample $\boldsymbol{i}$. The Chapman modification (the addition of "+ 1" in the denominator) is used when the proportion of the total population caught in each sample is less than 10%. The Schnabel method follows the same assumptions as the Petersen population estimation method, being that

- the population is closed and *N* is constant,
- all individuals have the same chance of being captured in subsequent sampling periods,
- marking (or, in this case, capturing and photographing) an individual does not affect future capture probability,
- each sample is random, and
- all marks are recorded correctly (or, in this case, all photographs are identified correctly).

The objective of obtaining a population estimate is that this data can be used along with the number of released tadpoles to estimate survival from the tadpole to metamorph stage at the reintroduction site. For this reason, any recapture events of individuals released as metamorphs are not included in the analysis.

Results

Remote Sensing

No leopard frog calling was detected on any of the songmeters in 2019. Two of the songmeters (at 11U, 550712 E, 5629703 N and 551351 E, 5627637 N) only recorded until May 21; it is uncertain why these stopped recording, as the memory cards were not full and batteries retained

power at time of retrieval. This should not have affected our results as the leopard frog breeding period is generally wrapping up by this time in British Columbia.

Water level data shows a significant increase in water depth over a few days in early June, with levels rising from 0.15 m on June 3 to 0.80 m on June 6 (Figure 2). This change in depth is likely due to the influx of water from the river flooding into the release pond. There is also an observable drop in temperature (22.9°C on June 4 to 14.7°C on June 7) directly following the significant rise in water level, which would be expected given the comparatively colder temperature of the river water. Water temperature required about a week to recover, returning to temperatures above 20°C on June 14.

Water temperature was relatively variable throughout May, June, and July, becoming more stable in late July and early to mid-August. A steady decline in temperature began in late August and continued throughout most of September, before rapidly declining between September 25 and 28 and staying below 9°C thereafter. Temperature readings were nearly identical between the two loggers, which had been placed approximately 230 m apart.

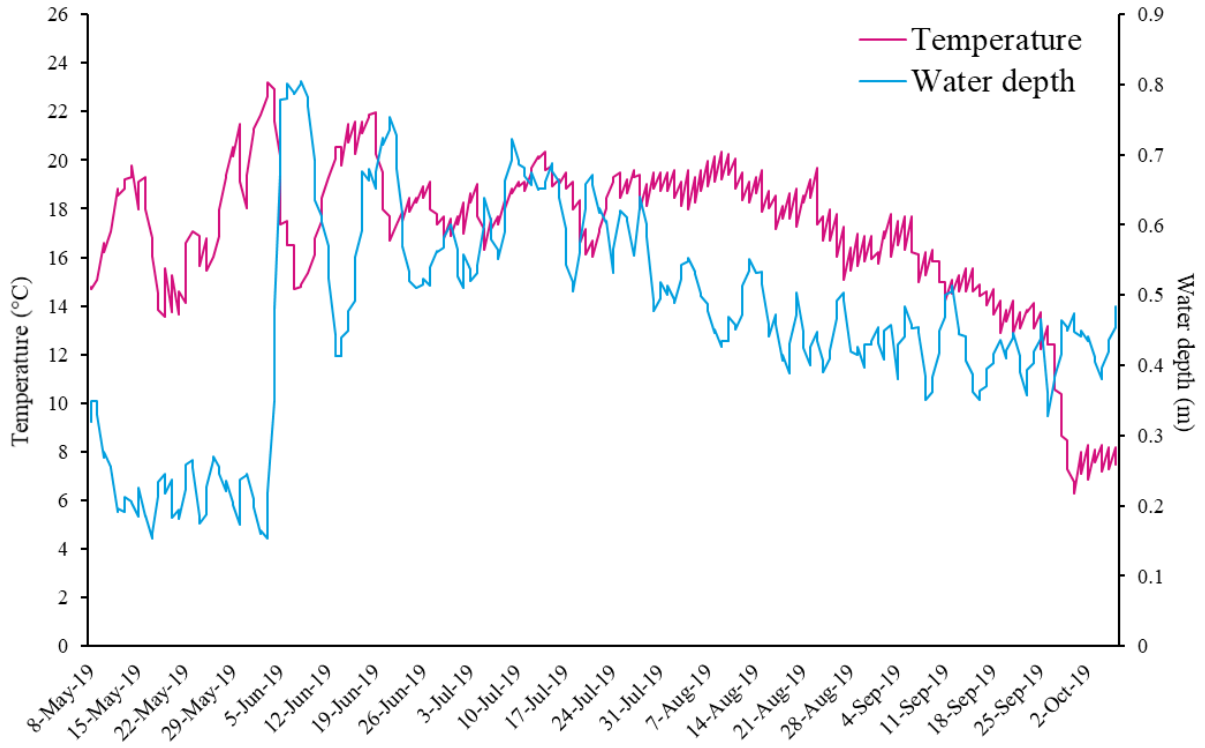

Figure 2: Temperature (°C) and water depth (m) as measured at the Brisco release pond from May 8 to October 6, 2019.

Translocations and Release

Seven egg masses were detected, surpassing the threshold and allowing for direct wild-to-wild translocations for the first time since 2016. Portions of two egg masses were translocated directly from CVWMA to Brisco on May 19; they were temporarily suspended in mesh cages and provided with romaine lettuce for sustenance prior to being released once tadpoles were free-swimming (soft-release). On three occasions between May 24 and 30, 303 tadpoles were released from one egg mass and 2014 from the other. Additionally, portions of 4 egg masses were brought to the zoo for integration into the captive breeding program and for head-starting of tadpoles prior to release

Individuals brought to the Calgary Zoo as eggs and head-started until release were transported to the reintroduction site on 4 occasions – May 30, June 6, July 16, and September 7. This accounted for a total of 596 tadpoles and 13 YOY from four egg masses. Gosner stage of tadpoles at release varied from 26 to 41 and all appeared healthy and vigorous. A total of 1200 captive-bred tadpoles from the Vancouver Aquarium were released on June 24, with an additional 1 tadpole, 8 juveniles (over-wintered as tadpoles), and 46 YOY released on August 29.

Mortality during translocation was low, with a loss of only 4 individuals (all tadpoles) out of 4181 (0.1%).

Visual Encounter Surveys

Survey and body condition

Surveys occurred on 14 occasions for a total of 77:49 survey hours. Surveys were halted after October 6th when weather conditions became unfavorable for frogs. The surveys with the highest catch per unit effort occurred on August 29 and September 17, with a calculated 8.6 and 8.0 YOY captured or observed per hour, respectively. The former survey occurred along the release pond and a small portion of the riverbank south of the release pond, while the latter occurred along the channel at the north end of the release pond as well as the small pond to the north of the release pond. It is worth noting that the total number of observations is limited by the time required to capture and process frogs during the allotted survey time.

In total, 221 YOY observations were made over the season, of which 160 were captured (note: these totals and all calculations hereafter do not include the 59 individuals released as YOY from the Calgary Zoo and Vancouver Aquarium, but it does include 3 recapture occasions of these individuals). The highest density of YOY observations and captures was along the channel at the north end of the release pond, followed by the riverbank south of the release pond (Figure 3).

Mean and range for weight and SVL in 2019 can be found in Table 1. Note that the three smallest values for both measurements were recaptures of individuals released as YOY from the Vancouver Aquarium. Excluding these three individuals, the minimum weight and SVL were 6 g and 37.8 mm, respectively. Frogs released as metamorphs were smaller (mean weight = 3.2 g; mean SVL = 31.0 mm; n = 46) on their release date than those captured on the same date having

been released as tadpoles (mean weight = 8.9 g; mean SVL = 45.1 mm; n = 9). The first observation of potential nuptial pads on a male occurred on August 12 on a 17.5 g frog.

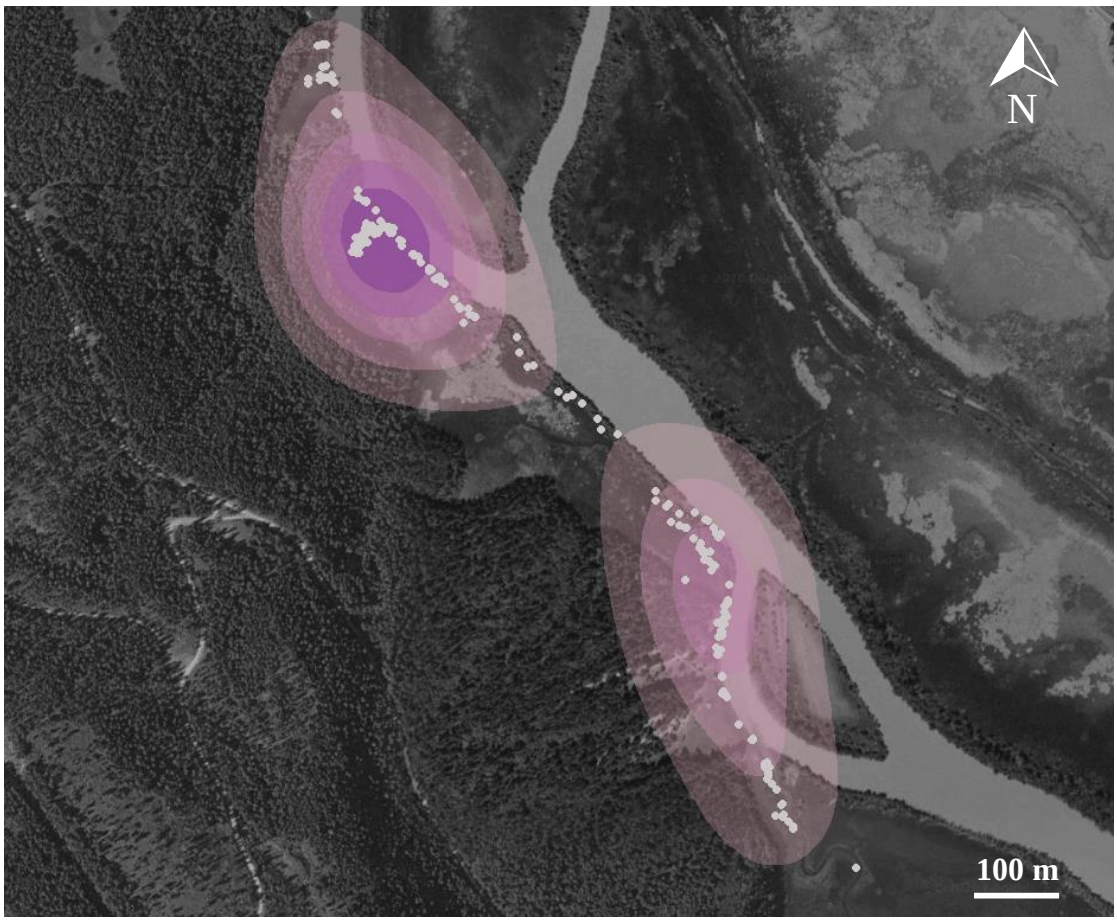

Figure 3: Density plot showing spatial distribution of YOY at the release site, with white points representing individual observation or capture locations.

Table 1: Minimum, average, and maximum weight and SVL observed for YOY in 2019 (n = 160). To allow comparisons with previous years, data from recaptures were included in calculations.

	Min	Mean ± SD	Max
Weight (g)	3.5	10.5 ± 3.0	23.0
SVL (mm)	33.0	46.5 ± 4.5	58.2

Body condition index in 2019 at Brisco was significantly lower than 2018 ($p < 0.001$), but higher than 2016 ($p = 0.04$; Figure 4a). Additionally, body condition of YOY was significantly lower in Brisco as compared to the CVWMA in 2019 ($p < 0.001$; Figure 4b). The best-fit model for drivers of body condition at Brisco in 2019 included direction from the release location ($\beta = 0.41$, $p = 0.04$) and recapture status ($\beta = 0.70$, $p = 0.004$); individuals captured south of the release location and individuals that were recaptures had a higher body condition. There was no relationship between body condition and capture date.

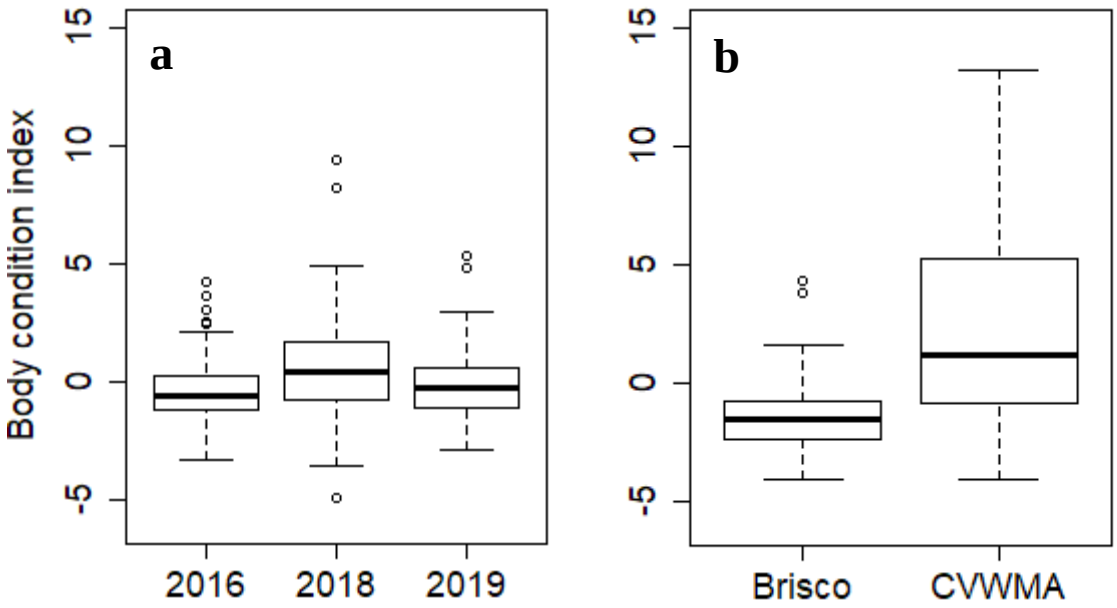

Figure 4: Body condition index by year at Brisco (a) and by site for 2019 (b).

Movements and recaptures

Of the 160 captures that occurred, there were 130 individuals, with 25 captured twice (including 3 YOY from the Vancouver Aquarium, whose release date serves as a proxy for first capture), and 4 captured on three occasions. The maximum distances that YOY were observed from the point of tadpole release was 707 m north (downstream) and 804 m south (upstream). Of the 160 captures, 88 occurred north of the release location and 72 south, indicating a relatively even distribution in the directionality of seasonal movement through and from the release pond. Several areas that were surveyed yielded no observations of leopard frogs (Figure 5).

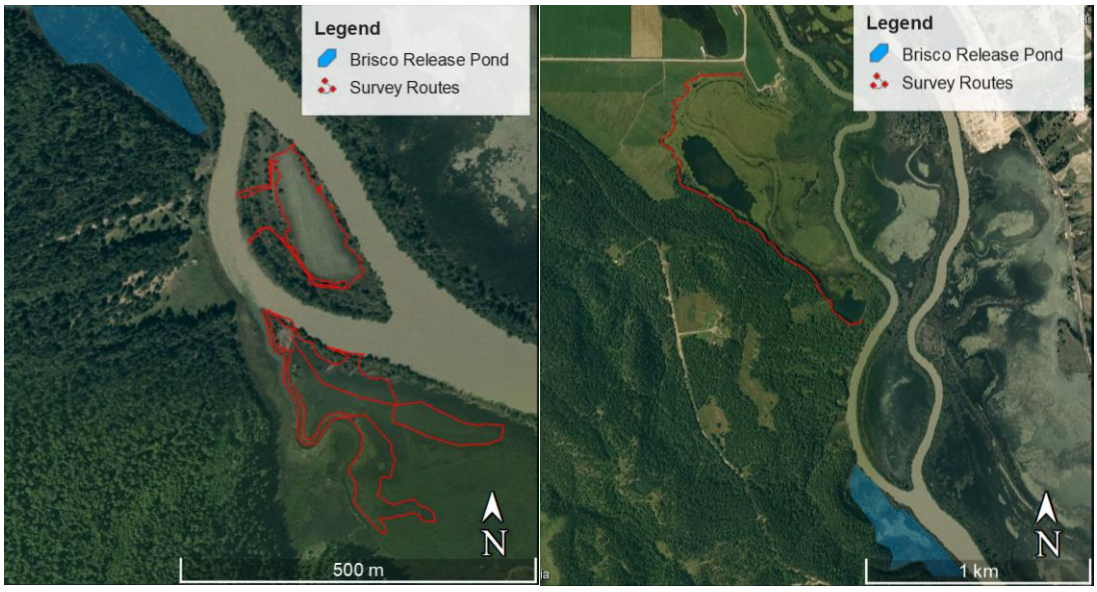

Figure 5: Survey routes along which no leopard frogs were detected.

Movement patterns were more thoroughly examined for the four individuals with three capture occasions (Figure 6). On their final capture, all four individuals were found south of the release location. Interestingly, two of the four individuals were originally captured north of the release location, before turning and heading south at a later date. The furthest cumulative known distance travelled was by BC19-23RS, which was first found 338 m north of the release location, before turning and travelling 221 and 757 m south before the second and third capture events, respectively, ending near the channel south of the release pond.

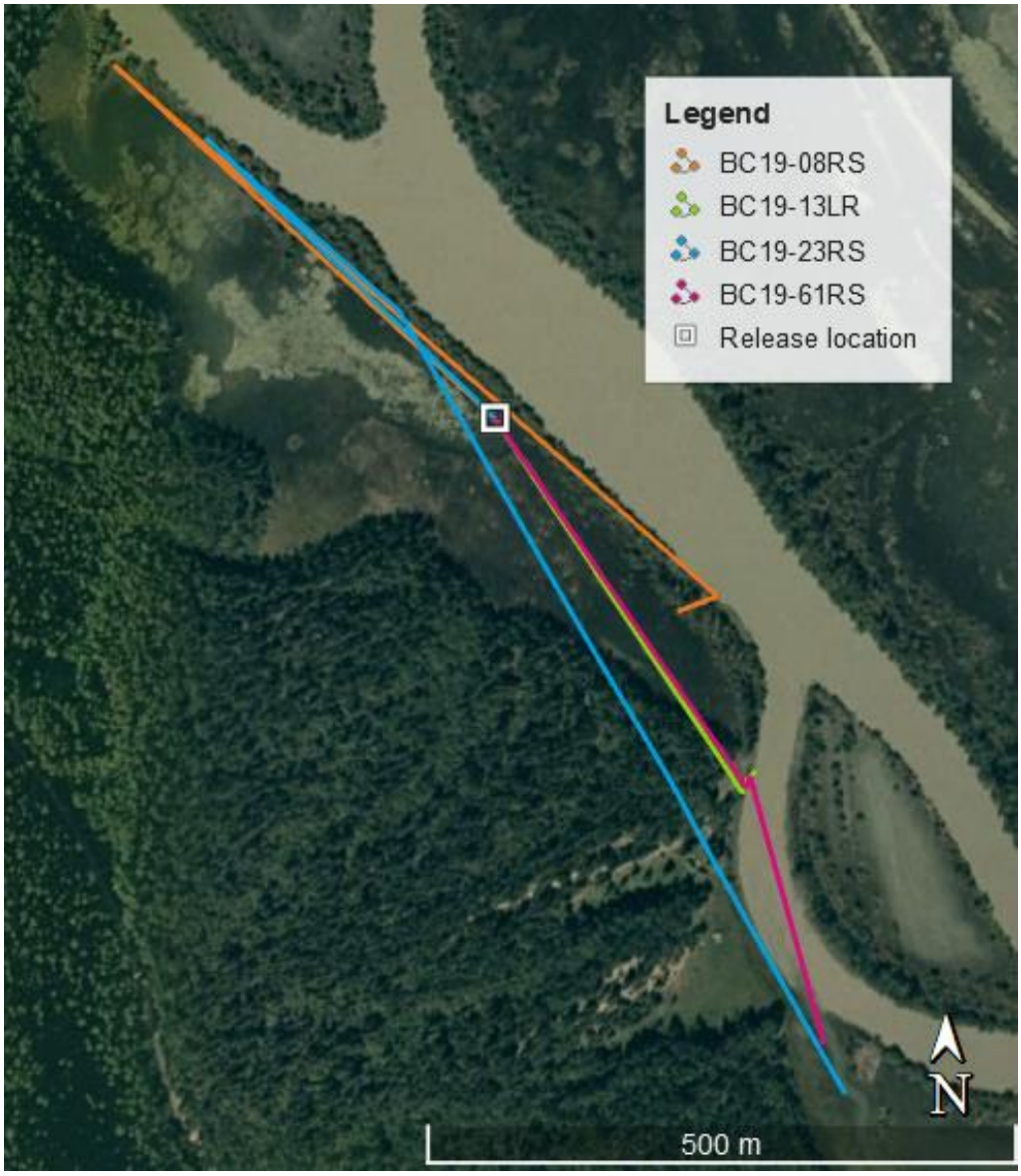

Figure 6: Movement patterns of four YOY that were captured on three occasions. Note that lines do not suggest movement pathways, but simply show connection between capture locations.

Of the 29 YOY with recapture data, 4 lost weight between the first and second capture (0.50-1.75 g decrease), 6 exhibited no change in weight, and 19 gained weight (0.25-11.50 g increase). One of the four that lost weight (1.0 g decrease) was later identified as having chytrid, and

another gained weight (6.75 g) between its second and third capture. Of those that gained weight, there was an average increase of 0.16 g per day. Summary data related to weight change and movement between recapture events can be found in Table 2.

Table 2: Movement and weight change data for YOY between the first and second recapture events.

	Min	Mean	Max	n
Days between recapture events	8	15	36	29
Distance (m)	3	180	695	29
Distance/day	0.4	13.0	60.6	29
Weight gain (g)	0.25	2.43	11.50	19*
Weight gain/day	0.01	0.16	0.50	19*

*weight gain data is only shown for individuals that had a positive weight change between recapture events

A large leopard frog, either a juvenile or adult, was observed on June 6 but eluded capture and photo. Another large leopard frog was photographed on July 29 at the release pond, but eluded capture and was not observed again (Figure 7a). Using the Brisco Photo Archive, we were able to confirm that this individual was a YOY in 2018 that had been previously captured. It is possible that the frog observed on June 6 and on July 29 were the same individual given that they were observed in the same location. Additionally, local landowner and president of the Columbia Wetlands Stewardship Partners, Suzanne Bayley, observed and photographed a leopard frog at the wetland just north of the release pond on September 6, which she estimated to be approximately 75 mm in length (Figure 7b). Assuming accuracy of the estimate, this would indicate successful overwintering of a second juvenile or adult.

Figure 7. Juvenile leopard frog observed on July 29, providing evidence of overwinter survival at the release site (a) and juvenile or adult observed on September 6 (b).

Physical abnormalities and disease

On August 12, a metamorph was captured and observed that had an underdeveloped rear leg and only one eye (Figure 8). This was the only individual observed throughout the season with physical abnormalities.

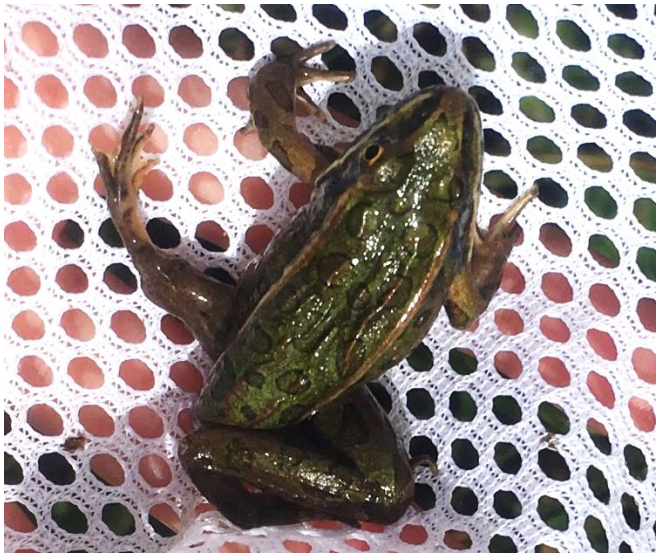

Figure 8: Metamorph observed at the release pond with a limb deformity and only one eye.

Signs of chytrid were observed in 17 frogs, first on September 11 and continuing until the final survey on October 7; these individuals made up 26.6% (17/64) of the captures over this period, and 10.6% (17/160) of all captures. Skin swabs were collected from 15 of the 17 individuals. Laboratory analysis confirmed that all of these swabs were positive for chytrid. Additionally, three Columbia spotted frogs (*Rana luteiventris*) showing no symptoms of chytrid were opportunistically sampled; two of these tested positive for chytrid, demonstrating their potential role as a reservoir for the disease.

The prevalence of chytrid in leopard frogs in 2019 was lower than the 35.2% (31/88) reported over the same time period for 2018 (September 11 to October 17) and the 19.3% (31/161) over the entire 2018 season. This may be a result of warmer water and air temperatures in 2019, given the negative relationship between temperature and rates of chytrid infection (Fernandez-Beaskoetxea et al., 2015).

Population Estimation

The capture history of 127 individuals was included in the population estimate calculation, as the three recaptures of individuals released as metamorphs from the Vancouver Aquarium were omitted. The calculated YOY population estimate for the site using the Schnabel method was 293, with a 95% confidence interval of 207 to 427 individuals.

It is important to acknowledge that these estimates should be viewed with caution, as likely some assumptions of the modelling approach were violated, in addition to the fact that the sampling

location itself was not constant (*e.g*. we did not begin surveying at the wetland just north of the release pond until September 11). Estimates would likely be more accurate if a pre-defined survey route was set and followed for each sampling occasion, but this would also result in a smaller total search area due to logistical and time restrictions. Additionally, even with a set route the amount of area covered between surveys would likely vary depending on number of individuals encountered.

Discussion

Overall, in 2019 we were able to release more tadpoles than in the previous two years combined due to the ability to directly translocate eggs from Creston to the reintroduction site. There were also adequate numbers of captures and recaptures to allow for estimation of population size and survival at the reintroduction site. Looking strictly at known variables, there were 4114 tadpoles released in 2019 and 127 individuals identified and captured as YOY; this would result in a minimum survivorship from tadpole to metamorphosis of 3.1%. Considering that, 1) there were another 62 YOY observations that were not able to be identified due to lack of capture, and 2) that it is unlikely that we observed every surviving YOY, the survival estimate can be assumed to be higher than the calculated minimum. This idea is further supported using population estimates derived from the Schnabel method (95% CI of 207 to 427 individuals), which would result in survivorship ranging between 5.0 to 10.4%. Both these values and the estimate based solely on known variables are within a reasonable range of expected survivorship, as reported for similar species at this life stage; larval survival rates for *Rana aurora* and *Rana temporaria* have been estimated at 0.03 ± 0.01 and 0.06 ± 0.05, respectively (Biek et al., 2002).

While there was no overt evidence that release of small metamorphs in late summer was detrimental, as was seen in 2018 (Ohanjanian, 2019), data may still suggest an advantage for earlier release during the tadpole stage. If the date of release for frogs introduced as metamorphs or juveniles is used as their first capture occasion (since they were identifiable at this point, as compared to individuals released in the tadpole stage), 4.5% of them experienced a recapture at a later date (3 of 67). In comparison, of the 157 captures that occurred of individuals released as tadpoles, 16.6% of them were recaptured (26 of 157). While these calculations cannot be directly compared without accounting for factors such as release date, considering that later released individuals would have fewer opportunities to be recaptured, the large discrepancy of the two recapture rates may provide an argument for prioritizing release during the tadpole stage. It should also be noted that the three recaptures that did occur of frogs released as metamorphs were the three smallest YOY captured throughout the 2019 season. Additionally, when comparing the weight of metamorphs on their release date to YOY captured on the same day that had been released as tadpoles, the latter group of individuals had a mean weight more than twice that of the individuals released as metamorphs. This is an important distinction, given the positive relationship between YOY size and survival (Altwegg & Reyer, 2003).

Another point of concern is the lower body condition at the release site when compared to the source population at the CVWMA. Considering that more than half of the tadpoles released at Brisco in 2019 were direct wild-to-wild translocations from the CVWMA, this may indicate a deficiency in habitat quality or a density issue rather than an effect of captive breeding. Body

condition was also significantly lower at Brisco as compared to Creston in 2016 (p <0.001), another year where the majority of releases were wild-to-wild. Density seems unlikely to be a major problem, however, as the number of tadpoles released would only represent approximately 2 egg masses in a 9 ha pond; mean density of leopard frog egg masses in Wisconsin was 277/ha (Hine et al., 1981) and 58/ha in Quebec (Gilbert et al., 1994). If there is a density concern at the release pond, given that the two years with high releases had YOY with lower body condition (2016 and 2019), it may be possible to release into multiple wetlands in the vicinity of the release pond going forward but local landowners would need to be supportive and agreements would need to be in place.

It is also possible that frogs at Brisco are smaller than those at Creston due to cooler temperatures in this region (amphibians have temperature dependent development) and because the release pond was inundated with cooler water from the river in June this could have delayed development resulting in smaller size. However, water temperatures only took about a week to recover. The habitat quality hypothesis is made more interesting by the result that individuals who travelled south of the release pond had a higher body condition than those who travelled north, potentially indicating more adequate resources in one direction.

While there were at least two juvenile/adult frogs observed this year, providing evidence of overwinter survival, it is nearly impossible to determine whether other individuals from previous years are failing to survive over the winter or are simply dispersing to other locations outside of the primary study area. Perhaps the first step in determining where any surviving juveniles and/or adults may be going would be to locate the overwintering area(s) for YOY frogs. This would provide a good starting point for spring VES in the following season, and potentially provide better information regarding the optimal locations for songmeters. This could be facilitated through use of radio-tagging YOY in late fall to track them to the overwintering area(s).

Although the reintroduction site has met the first three indicators of success (Randall et al. 2016) over the last few years, and this year experienced some successes with a larger number of releases than in each of the previous two years, as well as evidence of overwinter survival, there are still barriers to accomplishing Indicators 4 through 7 (see introduction). There continues to be inadequate numbers of individuals available for release each year; Semlitsch (2002) recommends 10,000-50,000 eggs released over several years. Assuming an embryo survival rate of 0.92 (Biek et al., 2002), this would be equivalent to 9,200-46,000 tadpoles. While >8000 tadpoles were able to be released in 2016, there have only been 6364 individuals released over the last three years combined, falling far short of the recommendation. There is hope that the Calgary Zoo captive breeding facility may begin producing tadpoles in 2020, which could greatly bolster the number of individuals released. Additionally, soft releases, in which tadpoles will first be released into a protective enclosure in the wetland to allow them to orient to the site, are planned for 2020 with the expectation that this may increase survival rate (Mendelson III & Altig, 2016).

Literature Cited

Altwegg, R., & Reyer, H. U. (2003). Patterns of natural selection on size at metamorphosis in water frogs. *Evolution, 57*(4), 872–882. doi:10.1111/j.0014-3820.2003.tb00298.x

BC Conservation Data Centre. 2019. BC Species and Ecosystems Explorer. B.C. Ministry of Environment. Victoria, B.C. Available at: http://a100.gov.bc.ca/pub/eswp/ (accessed August 31, 2019).

BC Ministry of Environment. (2008). Interim Hygiene Protocols for Amphibian field staff and researchers. Retrieved from https://scitech.viu.ca/sites/default/files/interimhygieneprotocols-for-amphibian-field-staff-and-researchers.pdf

Biek, R., Funk, W. C., Maxell, B. A., & Mills, L. S. (2002). What is Missing in Amphibian Decline Research: Insights from Ecological Sensitivity Analysis. *Conservation Biology, 16*(3), 728-734.

Burnham, K. P., & Anderson, D. R. (2002). *Model selection and multi-model inference: a practical information-theoretic approach*. New York: Springer.

Chapman, D. G. (1954). The Estimation of Biological Populations. *Ann. Math. Statist., 25*(1), 1-15. doi:10.1214/aoms/1177728844

ECCC. (2017). *Recovery Strategy for the Northern Leopard Frog (Lithobates pipiens), Rocky Mountain population in Canada*. Ottawa.

Fernandez-Beaskoetxea, S., Carrascal, L. M., Fernandez-Loras, A., Fisher, M. C., & Bosch, J. (2015). Short Term Minimum Water Temperatures Determine Levels of Infection by the Amphibian Chytrid Fungus in Alytes obstetricans Tadpoles. *PLoS ONE, 10*(3). doi:10.1371/journal.pone.0120237

Gilbert, M., Leclair, R., Jr., & Fortin, R. (1994). Reproduction of the Northern Leopard Frog (*Rana pipiens*) in floodplain habitat in the Richelieu River, Quebec, Canada. *Journal of Herpetology, 28*(4), 465–470. doi:10.2307/1564959

Government of Canada. 2019. Species at Risk Public Registry. Available at: https://www.canada.ca/en/environment-climate-change/services/species-risk-public-registry.html (accessed August 31, 2019).

Hine, R. L., Les, B. L., & Hellmich, B. F. (1981). *Leopard Frog populations and mortality in Wisconsin*. (122). Madison, Wisconsin: Wisconsin Dept. of Natural Resources Retrieved from http://digicoll.library.wisc.edu/cgi-bin/EcoNatRes/EcoNatRes-idx?id=EcoNatRes.DNRBull122.

Kendell, K., & Prescott, D. R. C. (2007). *Northern Leopard Frog reintroduction strategy for Alberta*. Sherwood Park, Alberta: Alberta Conservation Association.

Mendelson III, J., & Altig, R. (2016). Tadpoles, froglets, and conservation: A discussion of basic principles of rearing and release procedures. *Amphibian and Reptile Conservation, 10*(1), 20-27.

Ohanjanian, P. (2019). *Translocations and monitoring of Northern Leopard Frogs, Lithobates pipiens, at Brisco, BC – 2018*. Retrieved from

Ohanjanian, P., & Carli, C. (2010). *The Northern Leopard Frog: An assessment of potential reintroduction sites in the Columbia marshes*. Retrieved from

Pechmann, J. H. K., Scott, D. E., Semlitsch, R. D., Caldwell, J. P., Vitt, L. J., & GIibbons, J. W. (1991). Declining Amphibian Populations: The Problem of Separating Human Impacts from Natural Fluctuations. *Science, 253*(5022), 892-895. doi:10.1126/science.253.5022.892

R Core Team 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Randall, L., Kendell, K., Govindarajulu, P., Houston, B., Ohanjanian, P., & Moehrenschlager, A. (2016). Re-introduction of the Northern Leopard Frog (*Lithobates pipiens*) in British Columbia and Alberta, Canada. In P. S. Soorae (Ed.), *Global Re-introduction Perspectives: 2016. Further case studies from around the globe* (pp. 45-50). Gland, Switzerland: IUCN/SSC Re-introduction Specialist Group and Abu Dhabi, UAE: Environment Agency-Abu Dhabi.

Rorabaugh, J. C. (2005). *Rana pipiens* Schreber, 1782. Northern Leopard Frog. In M. J. Lannoo (Ed.), *Amphibian declines: the conservation status of United States species* (pp. 570-577). Berkeley, California: Univ of California Press.

Semlitsch, R. D. (2002). Critical elements for biologically based recovery plans of aquatic-breeding amphibians. *Conservation Biology, 16*(3), 619-629. doi:10.1046/j.1523-1739.2002.00512.x

Sommers, M., Randall, L. A., & Barclay, R. M. (2017). Effects of environmental variables on the calling behaviour of northern leopard frogs (Lithobates pipiens) in Alberta. *Canadian Journal of Zoology*.

Appendix A: Summary of activities at Bummers Flats reintroduction site, 2019

Reintroduction of northern leopard frogs at Bummers Flats on the Upper Kootenay Floodplain occurred from 2003 to 2005 and again from 2011 to 2015. During this latter set of reintroductions, over 35,000 tadpoles were able to be released; consequently, breeding was detected at the site annually from 2014 to 2017, and the population was considered to be self-sustaining. Unfortunately, the pumps responsible for maintaining water levels in the release pond (north Bummers calling ditch) have not been operational for the last couple of years and there has been little to no water in the breeding pond. Activities in 2019 included deployment of automatic recording units (songmeters) during the breeding season, with potential for targeted visual encounter surveys (VES) in late summer and fall depending on the results of the songmeter analysis.

In May, songmeters (n = 11; Wildlife Acoustics) were deployed on private land and Cherry Creek Nature Trust land on the west side of the river, and in the north pond and at south Bummer's on the east side of the river (Figure A-1). No songmeters were placed at the historic calling ditch as it was completely dry in May. Units were programmed to record ten minutes per hour between 13:00 and 17:00, ten minutes from 21:00 to 21:10, and then continuously from 22:00 to 01:00. Songmeters were retrieved in late July, and the spectrograms were visually scanned for the presence of leopard frog "snore" calls.

One songmeter on the west side (second from the north) was knocked down by wildlife sometime in the evening of May 22. It was replaced on May 30, but unfortunately, no data was collected between those dates.

No leopard frog calling was detected on any of the songmeters in 2019, and therefore no targeted VES occurred. No breeding or YOY have been detected at the site or adjacent habitat for the last two years. It is unclear whether this is simply part of the natural fluctuations seen in amphibian populations (Pechmann et al., 1991), or something more significant regarding habitat suitability.

Figure A-1: Locations of songmeters at Bummers Flats in 2019.