Northern leopard frog reintroduction to the Columbia Marshes

Report on Year 2

Penny Ohanjanian

for

Columbia Wetlands Stewardship Partners

Kootenay Conservation Program

Northern Leopard Frog Recovery Team

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EXECUTIVE SUMMARY

In response to the extirpation of the northern leopard frog, *Lithobates pipiens*, from most of its historic range, the Northern Leopard Frog Recovery Team identified reintroduction as one of several recovery actions. Another action was the establishment of a captive assurance population at the Vancouver Aquarium. Reintroductions, using wild stock from Creston, have taken place since 2003 at Bummers Flats on the Upper Kootenay Floodplain (UKF). The first captive-bred tadpoles (approx. 2,000) were moved from the Vancouver Aquarium to Brisco in 2013 where they developed and metamorphosed into froglets. On May 26, 2014 tadpoles (2,226) were again brought to the Brisco Release Pond from the Vancouver Aquarium. This document reports on that translocation and prior and subsequent field activities.

There were 3 distinct sizes of tadpoles released, with the largest approximately 8 mm (body length). Following acclimation to local conditions (through multiple water changes) 65% of the tadpoles were released into the marsh and 35% were held overnight. Overall detectable mortality was very low (1%), with the smallest individuals suffering the highest mortality (2.7%) during the transportation, holding, and release process. Water conditions at the Brisco Release Pond were: pH: 8.3, Conductivity: 449 µS/cm, Temp: 14° C and Depth: 30 cm.

Visual Encounter Surveys (VES) involving 2 or 3 persons began in April and continued weekly between July 22 and October 9. No individuals from the 2013 release were observed. In 2014 metamorphosis occurred in the second week of July. The mean snout-vent-length (SVL) of 79 Young-of-Year (YOY) leopard frogs was 52.4 mm (SD = 6.6, range = 34.4-66.2), mean shank length was 29.5 mm (SD = 3.8, range = 18.0-36.5) and weight was 15.9 g (SD = 6.1, range = 4.0-33.0). Several males developed nuptial pads. They were larger in 2014 than in 2013, probably because of earlier metamorphosis this year *(shank length (unequal variances t-test, $t(53.9) = 5.43$, $p \leq 0.001$)*. They were also significantly bigger than YOY at the UKF in 2014 (*shank length (unequal variances t-test, $t(189.6) = 3.38$, $p \leq 0.001$)*. Preliminary data from 14 recaptured YOY indicate that they grew, on average, 0.24 mm (SD = .15) and 0.12 mm (SD = .06) per day for snout-vent length and shank, respectively. There appeared to be no difference in growth rate between the captive-bred Brisco YOY and the wild-bred UKF YOY in 2014. Nor did intrinsic development rate (as measured by Shank : SVL ratio) differ between the two populations. Movements were highly variable, with some YOY moving towards the river within 3-4 weeks of metamorphosis. Others remained in the Release Pond for the entire summer, with one still present on October 9.

YOY appeared to be in good health. The exceptions to this were one individual with an extra rear foot (possibly from the trematode, *Ribeiroia ondatra*) and another had a broken shank and was consequently underweight.

Failure to locate any individuals from the 2013 release is not unexpected – the success of amphibian reintroductions is highly dependent on large numbers of animals being translocated.

Recommendations:

i. Release a minimum of 8,000 individuals per year over the next four years
ii. Continue to monitor leopard frog sizes, growth rates and health of trans-located individuals
iii. Determine if and where breeding occurs using Songmeters and nocturnal calling surveys
iv. Collect data on sizes and numbers of breeding animals
v. Investigate the feasibility of doing a Mark-Recapture study to estimate population size
vi. Expand the area surveyed to possible dispersal sites downstream, upstream and across the river

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1.0 INTRODUCTION

The recent global amphibian decline has sparked interest in the captive rearing and reintroduction of a variety of species throughout the world. These efforts have met with mixed results (Alberta Environment and Sustainable Resource Development 2012, Griffiths and Pavajeau 2008, Romanchuk and Quinlan 2006). In British Columbia, the extirpation of the northern leopard frog, *Lithobates pipiens*, from most of its historic range led to the formation of the Northern Leopard Frog Recovery Team (NLFRT) in 2001. Among the recovery actions identified by the NLFRT were the establishment of a captive assurance population at the Vancouver Aquarium and reintroduction of the species to historic sites in the Columbia and Kootenay systems (NLFRT 2012). Reintroductions have been ongoing on the Upper Kootenay Floodplain (UKF) since 2003 using wild-bred stock from the only known extant population at Creston. In 2010, focus spread to the Columbia marshes (Ohanjanian and Carli 2010) with the assistance of the Columbia Wetland Stewardship Partners and funding from the Columbia Valley Local Conservation Fund. The first captive-bred tadpoles were moved from the Vancouver Aquarium to a pond at Brisco (the Brisco Release Pond) in 2013 and released (Ohanjanian et al. 2013). Those tadpoles developed and metamorphosed into froglets. This document reports on the 2014 translocation of northern leopard frog tadpoles from the Vancouver Aquarium to the Brisco Release Pond.

2.0 STUDY AREA

The study area is near Brisco, British Columbia[1]. The Brisco Release Pond is a naturally-impounded marsh adjacent to the Columbia River (Figure 1). It is approximately 11.4 ha in size and is connected to the river by two channels. The hydrology of the pond is affected by inflows from small streams on the hillside, as well as the height of the river. A cottonwood-dominated forest lies between the marsh and the river itself and there are several beaver runs between the two bodies of water. There is an abundant and diverse submergent vegetation community in the pond and there are areas of open water and emergent vegetation, primarily bulrush[2].

In 2014, pH values in spring and summer ranged from 8.1 to 9.5, and conductivity was between 267 and 705 µS/cm. Water temperatures were periodically measured and ranged from 13.6° C on May 7 to 26.8° C on Aug 26. Two HOBO temperature loggers were deployed to measure water temperature (No. 10498861) and air temperature (No. 10498862). These will be retrieved following break-up of the ice in March 2015 and re-deployed.

[1] To conform to provincial confidentiality practices regarding endangered species, the precise location of this pond is not included here.

[2] For a detailed description of the study site, including water chemistry and emergent and submergent vegetation species, see Ohanjanian and Isaac (2013).

Figure 1. Northern leopard frog reintroduction site, Brisco Release Pond.

3.0 METHODS

3.1 Tadpole release

Approximately 2,200 tadpoles, hatched at the Vancouver Aquarium, arrived by jet in Cranbrook on May 26, 2014. They were driven to the Brisco Release Pond where they underwent a series of water changes to acclimate them to local water conditions. A portion of the tadpoles (65%) were subsequently preleased into the pond, while others (35%) were kept overnight in mesh cages (Figure 2). As in 2013, the release methodology followed guidelines set out by the IUCN's Guidelines for Reintroduction (IUCN 2013). Techniques developed by Kendall and Prescott (2007) and Adama and Beaucher (2006) were utilized.

Figure 2. Release of tadpoles into Brisco Release Pond on May 27

To avoid excess handling, tadpoles were not measured. During the release the tadpoles were assessed for vigour and gross signs of morbidity, they were counted and percent mortality was calculated.

The release site was visited in mid-July to ascertain tadpole development stage and metamorphosis and to observe hydrology, water depths, and water temperature.

Visual Encounter Surveys (VES) began in April, and were carried out weekly from July 22 to Oct 9 (10 surveys). Total survey effort was 81 person hours. Environmental conditions, including air and water temperature, water pH and conductivity, wind speed (Beaufort Scale) and cloud cover were recorded for each survey. Effort was also recorded, however no attempt to obtain catch per unit effort was made. The British Columbia amphibian hygiene protocol was followed to minimize risk of transferring pathogens (British Columbia 2008). Nets and field gear were only used at the Columbia marsh site and all individuals were handled with nitrile gloves and Ziploc bags that were specific to that animal. Snout-vent length and shank lengths were each measured five times (to the nearest 0.1 mm) to obtain a measure of variability and increase precision. Weights were obtained using a Pesola scale (to the nearest 0.5 g). Body size was compared with frogs at the other reintroduction site, the Upper Kootenay Floodplain (UKF)[3]. All frogs were photographed from above for inclusion in the Brisco Leopard Frog Photo Archive. Dorsal spot patterns were used to identify individuals that were re-captured (n = 14). This allowed data to be obtained on growth rates[4] and movements. One recapture occurred 4 days apart; this individual was not included in growth rate analysis. Locations were geo-referenced using hand held Garmin GPS units. Health was assessed by gross examination of skin condition, anatomy, vigour and righting reflex.

As the Brisco frogs came exclusively from the Vancouver Aquarium the mean SVL, shank, weight and growth rates of YOY were compared with YOY from the UKF to detect potential differences in wild vs captive bred leopard frogs. Scatter plots of body weight as a function of SVL were generated to provide visual comparison of the two

[3] The UKF encompasses Bummers Flats and areas across the river from that site.
[4] SVL and Shank measurements were used only, as wgt is influenced by prey consumption

groups as a whole. Potential differences in frogs' rate of development between the two groups were examined (e.g. shank length in relation to snout-vent length) in full knowledge that local site conditions could result in different development rates.

The presence of other species of amphibian and snakes was recorded and numbers and age class of Columbia spotted frog (*Rana luteiventris*) were tallied during surveys of leopard frogs.

4.0 RESULTS

4.1 Tadpole Release

There were three distinct size classes of tadpole with the largest being approximately 8 mm (body length). Overall detectable mortality was very low (1%), with the smallest tadpoles suffering the highest mortality (2.7%) both during a) acclimation followed by release and b) acclimation followed by holding (Table 1). Others appeared very vigorous and, on gross inspection, showed no visible signs of morbidity.

Table 1. Detectable mortality of 2226 L. pipiens tadpole from the Vancouver Aquarium

	Cage/Bucket 1 (smallest)	Cage/Bucket 2 (medium)	Cage/Bucket 3 (medium)	Bucket 4 (largest)	Total tadpoles
Held overnight	313	240	224	0	777
Released May 26	506	294	481	168	1429
Total tadpoles	819	534	705	168	2226
Mortality May 26	12	1	0	1	
Mortality May 27	10	3	1	--*	
Total mortality (%)	2.7%	0.7%	0.1%	0.6%	1.0%

*All were released on May 26

Water conditions at the release site were within expected values at 10 am on May 27 (Table 2). Water depth was approximately 30 cm.

Table 2. Water conditions at Brisco release pond on May 27, 2014

pH	8.3
Cond.	449 µS/cm
Temp.	14° C

4.2 Visual Encounter Surveys

4.2.1 *L. pipiens* released in 2013

No leopard frogs that had been released in 2013 were encountered during surveys in 2014.

4.2.2 *L. pipiens* released in 2014

The first post-release survey took place on July 22. No leopard frog metamorphs were observed on that date. This was partially due to deep water in the release area that impeded access. Metamorphosis, however, was

underway by that time, likely starting in the second to third week of July. On August 5 Young-of-Year (YOY) without tail stubs and weighing 8 - 9 g were present. The mean SVL of 79 leopard frog YOY was 52.4 mm, and mean shank length was 29.5 mm. Mean weight of 78 individuals was 15.9 g (Table 3)[5].

Table 3. Sizes of leopard frog Young-of-Year at Brisco, Aug 5 to Oct 9, 2014

	Wgt (g)	SVL (mm)	Shank (mm)
Mean	15.9	52.4	29.5
SD	6.1	6.6	3.8
Range	4.0 - 33.0	34.4 - 66.2	18.0 - 36.8
n	79	79	78

Preliminary data from 13 recaptured YOY indicate that they grew at .24 mm (SD = .15) and .12 mm (SD= .06) per day for snout-vent length and shank, respectively (Table 4).

Table 4. Daily growth rate for 13 individual leopard frog YOY at Brisco, 2014.

	Capture interval	SVL (mm/day)	SHANK (mm/day)
Recap 1	7	0.27	0.19
Recap 2	14	0.22	0.1
Recap 3	6	0.44	0.23
Recap 4	14	0.27	0.15
Recap 5	29	0.23	0.13
Recap 6	29	0.27	0.17
Recap 7	21	0.10	0.10
Recap 9	29	0.25	0.12
Recap 10	29	0.11	0.04
Recap 11	8	0.15	0.03
Recap 12	29	0.20	0.10
Recap 13	8	0.03	0.04
Recap 14	8	0.60	0.20

Sample size was too small and variability too great to allow statistical comparison of 2014 growth rate (n = 13) with that of 2013 (n = 5). Similarly, sample size of recaptures of frogs at the UKF reintroduction site was also low

[5] These data include recaptures

(n = 10). With this caveat, preliminary data do appear to show consistency in growth rate between the two sites in 2014 (Table 5).

Table 5. Mean daily growth rates of YOY at Brisco and UKF (2014)

		SVL mm/day	Shank mm/day
Brisco 2014	Mean	0.24	0.12
	SD	0.15	0.06
	n	13	13
UKF 2014	Mean	0.26	0.14
	SD	0.21	0.08
	n	10	9

There was no difference in intrinsic rate of development between captive bred YOY (Brisco) and wild YOY (UKF) as measured by shank length as a function of snout-vent length (Table 6).

Table 6. Mean Shank : SVL ratio of YOY at Brisco and UKF (2014)

	Brisco	UKF
Mean	0.55	0.54
SD	0.02	0.03
n	78	111

Movements of leopard frog YOY were highly variable. Within 4 weeks of metamorphosis leopard frog young of year were observed over 500 m from the release site, at the north end of the Release Pond. Other leopard frogs travelled overland, moving to the river bank to the east and south of the pond relatively early in the season - on August 18, there were ten individuals on the river bank beside the water over a 100 m stretch of the Columbia River. Other YOY remained in one location for up to a month, with one observed still in the Release Pond on October 9.

4.2.3 Health and mortality

With the exception of two individuals, YOY appeared to be in good health. One of these frogs was injured, with a broken shank. This individual was underweight, weighing only 10 g despite having a SVL of 54.8 mm. Others of similar length had weights of 16 to 18 g, suggesting that the injury had affected food intake.

Another individual had a malformation, with an extra right rear foot (Figure 3). This frog, whose tibiale and fibulare bones appeared to be duplicated with extra metatarsals and phalanges[6], was the first of all leopard frogs observed at UKF and Brisco to have a malformation.

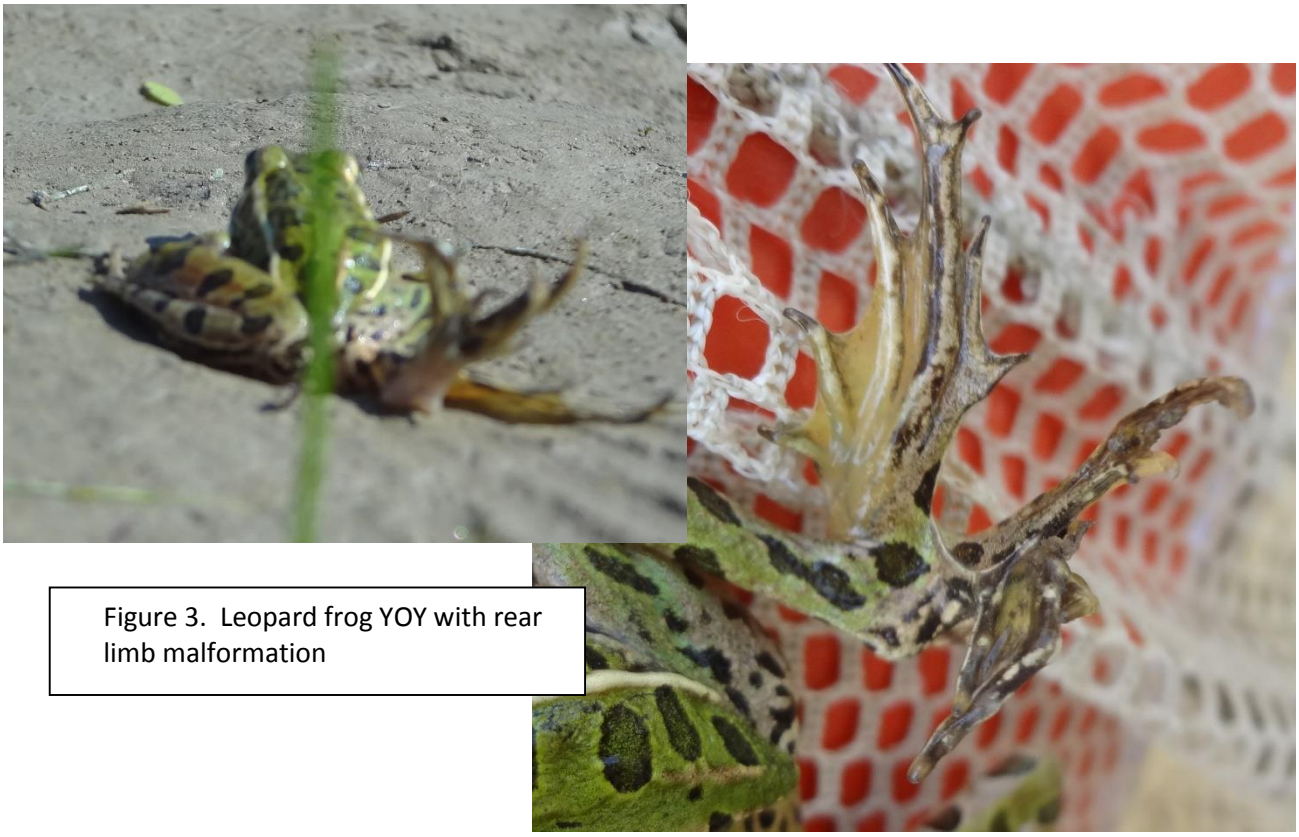

Figure 3. Leopard frog YOY with rear limb malformation

Two other YOY had minor abrasions on their leg and chest. One of these was recaptured four days later. It was vigorous and the abrasion had partly healed.

4.3 Other species

Columbia spotted frogs were observed during every visit. On July 22 tadpoles at Gosner stage 41, with forelimbs about to emerge, were utilizing shallow, warm areas along the east shoreline. Juveniles from the previous year were also present on the, flooded river bank. The maximum number observed during one survey was 30 YOY and 1 adult at the Release Pond in early October.

Long-toed salamander (*Ambystoma macrodactylum*) larvae were present at the Release Pond on July 22.

Common garter snakes (*Thamnophis sirtalis*) were observed twice, on May 7 and July 22.

[6] This specimen was not collected and no radiographs were taken. Therefore, this description is based on gross observation of anatomical irregularities.

4.4 Habitat comments

In 2014 the river did not breach its banks and flood the Release Pond as it did in 2013. On July 22, however, the Columbia River was still high from the freshet, and there were small pools of standing water remaining on the muddy river bank (Figure 4). The release area itself had deepened, from 30 cm on May 27 to more than 100 cm on July 22, as the river water had entered through the two inlets at the north end. Access to much of the release pond was impeded by deep water and few basking areas were visible. Spotted frog juveniles were observed in the riverside pools (Figure 4) on July 22. Early in May the small pond along the shore of the Columbia River near the cabins (Figure 1) was considerably warmer than the Release Pond (18° C vs 12.6° C). Its value as an intermediate staging area should continue to be assessed in future.

Figure 4. Standing water on river bank at Brisco, July 22, 2014

Beaver trails were used by frogs to move from the Release Pond to the river. The creation of travel routes by beavers may be an important habitat component for leopard frogs. It is well established that their dams enhance growth of submergent vegetation and create water bodies for frogs and other wildlife (Bayley and Guimond 2008).

5.0 DISCUSSION

The transportation of recently-hatched leopard frog tadpoles from the Vancouver Aquarium to Brisco appeared to cause very little trauma, as measured by the extremely low mortality at the release. After the release additional tadpole mortality likely occurred that went undetected, but tadpole mortality is typically high throughout the entire larval stage as they are subject to predation by invertebrates, salamander larvae, birds, (such as pied-billed grebes) and fish, all of which were present at Brisco Release Pond. We do not know what percentage reached metamorphosis. The sizes, general health and growth rates of the metamorphs, however, indicated that there was no obvious inherent weakness or defect in the tadpoles that were bred in the Vancouver Aquarium – they achieved metamorphosis and good growth prior to over-wintering. Seven individuals were over 60 mm SVL by September 12-16 and several males had developed nuptial pads. This has been reported elsewhere (Eddy 1976; cited in Seburn and Seburn) but is considered unusual.

Leopard frog YOY were larger in 2014 than in 2013 at Brisco (Figure 5). Mean shank length in 2014 was 29.5 mm vs 25.9 mm in 2013 (unequal variances t-test, $t(53.9) = 5.43$, $p \leq 0.001$). The greatest weight achieved by a YOY in 2013 was 22 g, while several reached 32 g in 2014. This may be a function of low sample size in the previous year, or it may be explained by earlier metamorphosis in 2014. In 2013 it was estimated to have begun at the beginning of August, whereas in 2014, it occurred in early to mid-July, allowing an extra 2-3 weeks of growth. Local temperatures in June, July, Aug and Sept were not significantly different between years (Government of Canada 2014).

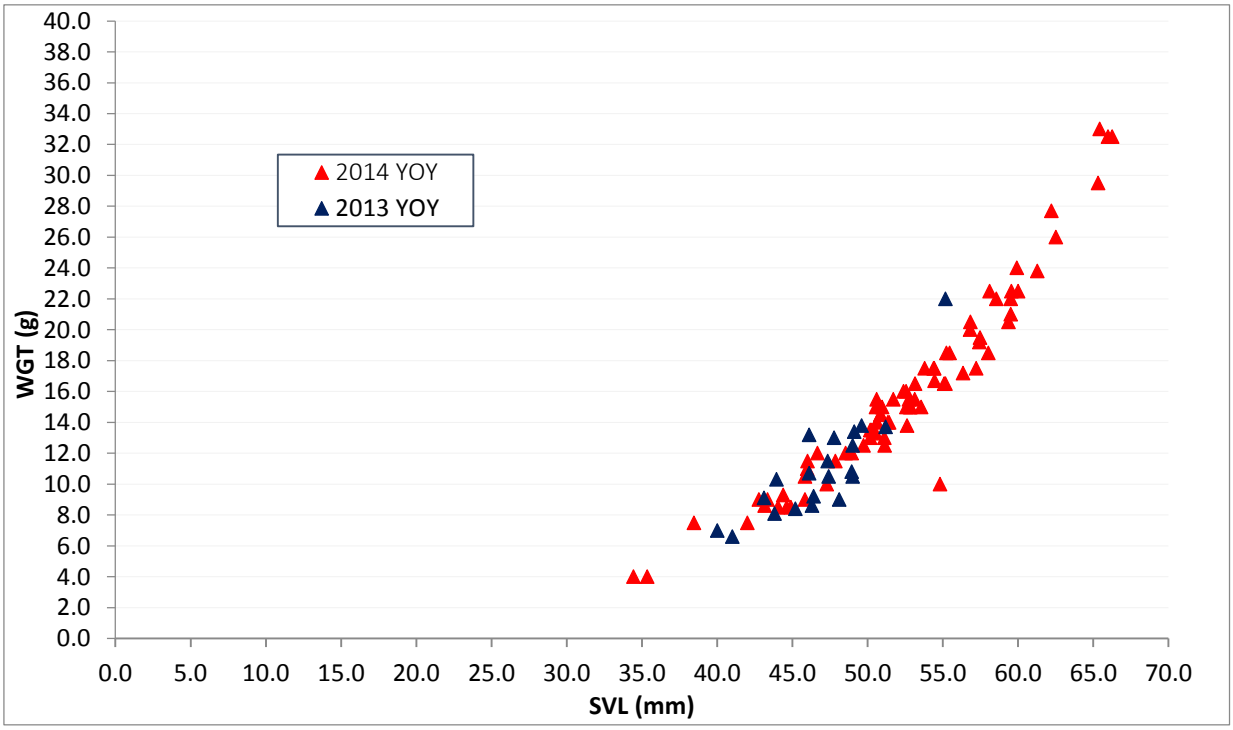

Figure 5. Comparison of SVLs and WGTs of 2013 and 2014 YOY at Brisco

The upper size range and body condition of 2014 Brisco YOY appear to be comparable with the wild bred YOY at UKF (Figure 6). However, the mean body lengths and shank lengths were different, with the Brisco YOY being slightly larger (Table 7) (SVL t-test (equal variance t(188) = 2.19, p = 0.03; Shank length t-test (unequal variance t(189.6) = 3.38, p <= 0.001).

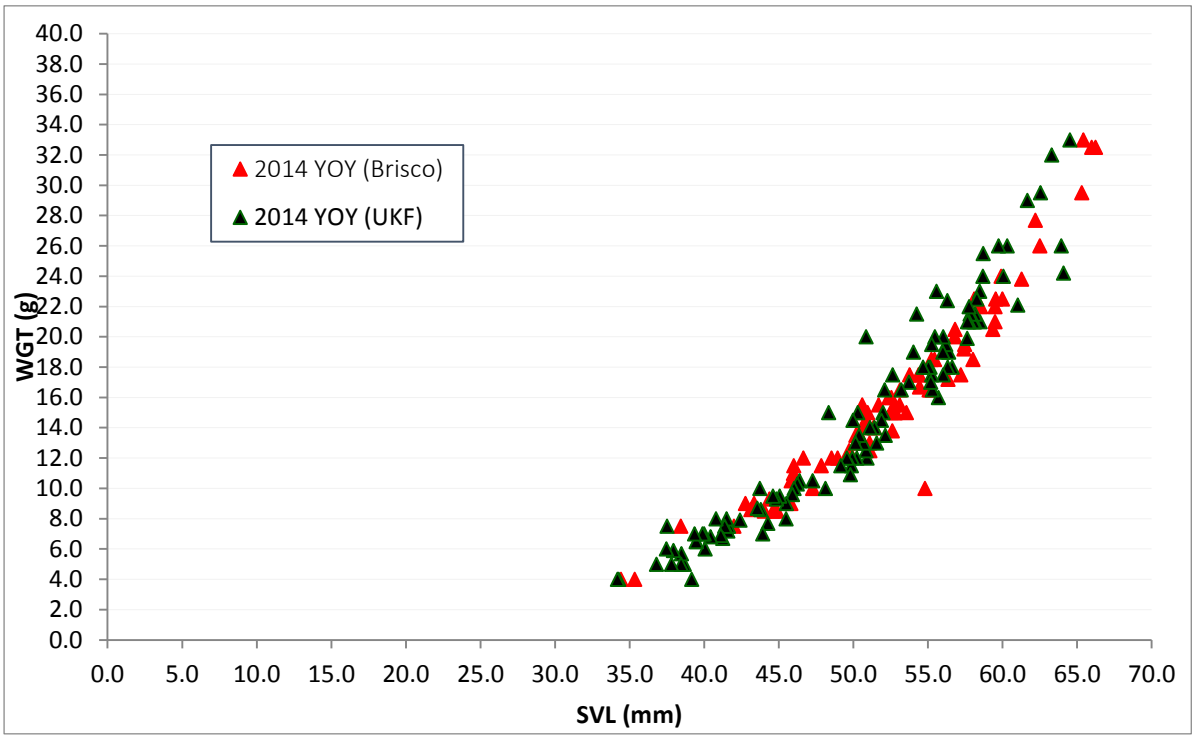

Figure 6. Sizes of YOY leopard frogs at Brisco and UKF in 2014.

Table 7. A comparison of SVL and shank sizes of leopard frog YOY at Brisco and UKF (2014)

		SVL mm	Shank mm
Brisco 2014	Mean	52.4	29.5
	SD	6.6	3.8
	n	79	78
UKF 2014	Mean	50.1	27.3
	SD	7.7	5.3
	n	111	225

These data are likely skewed by the fact that more small individuals were found at UKF later in the season. At that site, 27.5% of frogs captured on or after Sept 16, were $\leq$ 10 g in size (n = 40). At Brisco only one (3.5%) was $\leq$ 10 g (n = 28). Similarly, 22.5% of these later individuals at UKF had SVLs of less than 45 mm while at Brisco only 2.2 % were so. The relatively large number of small, later individuals at UKF has yet to be explained, although it is speculated that a late egg mass may have gone un-detected (Ohanjanian and Isaac 2014). These individuals are at a disadvantage. Ranid tadpoles that metamorphose early are more likely to survive during the early terrestrial phase, their early metamorphosis improves subsequent growth, and compensatory growth is unlikely as growth rates appear to decline as the first terrestrial season progresses (Altwegg and Reyer 2003). These factors make it unlikely that the small, late individuals at either site will reach sexual maturity.

Ficetola and De Bernardi (2006) have shown that there may be a trade-off between growth rate vs development rate in ranid tadpoles. Pressure to leave a pond soon (as a result of competition, predation or low food supply) may manifest itself in smaller shank size in metamorphs. In contrast, food abundance during the tadpole stage leads to longer leg length in ranid metamorphs (Tejedo et al. 2010). This trade-off, resulting from the larval environment, influences subsequent risk on land – shorter rear legs decrease jumping distance and, therefore, lower the probability of escape from predators. If there are differences in rate of development between the UKF and the Brisco frogs in this study, one metric that could illustrate this (and that is measurable in the field) is leg length. In 2014 there was no difference between the UKF and the Brisco YOY in the size of shank relative to body length. As both groups have a similar genetic source we would expect similar developmental paths unless local environmental pressures were dissimilar, resulting in a slower or increased rate of development. The correspondence between the two groups shown here should be compared with the source population at Creston. If a similar correspondence is found, this may provide further evidence, albeit indirectly, that larval habitat quality at both reintroduction sites is good.

The number of recaptures (20 recaptures of 14 individuals, or 25% of total captures) is approaching values needed to estimate population size and survivorship. The use of Mark-Recapture (M-R) techniques should be examined. Emigration and dispersal is a major component of leopard frog post-metamorphic life-history (Seburn and Seburn 1998, Dole 1971), so any application of M-R models must take into account the fact that this is not a closed population.

The causes of limb malformation in ranids are complex and diverse. Factors that have been implicated include ultraviolet radiation, pesticide exposure, predation and parasites (Ouellet et al. 1997, Meteyer et al. 2000, Skelly et al. 2007). Frog malformations due to the trematode *Ribeiroia ondatrae* are reported to account for a significant proportion of those observed in the western US (Schotthoefer et al. 2003). Free-swimming cercariae, released from planorbid snails, penetrate and encyst in tadpoles during the limb development stage, leading to malformations in the metamorphosed individual. *Ribeiroia*-induced malformation is not a new phenomenon - it has been confirmed in museum specimens (Johnson and Sutherland 2003). The individual found on Aug 18 was slightly underweight for its size (shank = 26.2 mm, wgt = 8.5 g). It still retained some tail stump and showed poor muscle development on the right flank. Its chances of survival to sexual maturity are exceedingly low. We did not collect this frog, so the cause of the malformation remains unknown. It is recommended that any individual encountered in future be collected and tested for *Ribeiroia* metacercariae.

As in 2013, leopard frog YOY moved for several hundred meters to the north, east and south of the release area. Some of these movements, including several over-land passages, had occurred before mid-August. Early movements (within three weeks of metamorphosis) have been documented in the Cypress Hills (Seburn et al. 1997), and Dole (1971) observed two metamorphs that moved at least 1.6 km in Manitoba. It is clear, therefore,

that Brisco metamorphs may also have moved further than the scope of this year's surveys. It will become necessary to enlarge the survey area to include sites downstream and across the river in 2015.

Failure to locate any individuals from the 2013 release is not unexpected given the relatively small numbers that had been translocated into the site. In 2013 and in 2014 only 4000 individuals were released into the study site. While it is impossible to know precisely how many (or how few) individuals should be reintroduced, there is a body of evidence that large numbers are needed. With amphibian translocation, the number of animals released significantly affects success (Germano and Bishop 2008). Griffith et al. (1989) identified and evaluated factors that helped determine success of translocations. Although not specific to amphibians, the attributes with which success was highly correlated included large founder populations, multi-year translocations, and good habitat quality. Semlitsch (2002) suggests that the release of 10,000–50,000 eggs over several years is required to reach an adult population of 100 individuals. Over 36,000 tadpoles and metamorphs have been released onto the Upper Kootenay Floodplain since 2003, and there are positive signs, including the establishment of two local breeding sites, that it has been successful up to this point in time. To increase the likelihood of success at Brisco more individuals must be released there also.

It is too early to determine the success of this reintroduction, especially without evidence of over-winter survival. In their meta-analysis of the success or failures of amphibian reintroduction projects Germano and Bishop (2008) determined a project was successful if there was a) "evidence of a substantial addition of new recruits to the adult population due to successful reproduction at the translocation site" and b) "the site had to have been monitored, at the very least, for the amount of time it takes that species to reach maturity". The additional monitoring effort applied to Visual Encounter Surveys in 2014 provided a larger sample size than in 2013. At a minimum, this level of effort (weekly surveys) should be continued at the Brisco Release Pond, and additional work in an expanded study area should be undertaken to locate dispersed individuals.

6.0 RECOMMENDATIONS

1. Release a minimum of 8,000[7] individuals per year over the next four years at Brisco. Tadpoles from the wild source population at Creston should supplement those from the Vancouver Aquarium if needed to reach that number, provided that the source population is not jeopardized.

2. Continue to monitor leopard frog sizes, growth rates and health of trans-located individuals.

3. Determine if and where breeding occurs in the next years using a) remote sensing (Songmeters SM2+, *Wildlife Acoustics*) at sites difficult to reach and b) on-site Nocturnal Calling Surveys at the Brisco Release Pond beginning in April and continuing until early June.

4. Collect data on sizes and numbers of breeding animals at breeding locales. This will provide information not only on breeding, but on over-winter survivorship.

5. Investigate the feasibility of doing a Mark-Recapture study to estimate population size and survivorship

6. Expand the area surveyed to possible dispersal sites downstream, upstream and across the river

[7] Approximately 8,000 leopard frog tadpoles per year were moved to UKF between 2011 and 2014 (B. Houston pers. comm)

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