Northern Leopard Frog Project Spring 2019 Report

Submitted to:

Ministry of Forests, Lands, Natural Resource Operations & Rural Development

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1. INTRODUCTION

This report summarizes the key survey results of the spring 2019 field program for the Northern Leopard Frog (*Lithobates pipiens*) Recovery Project in the Creston Valley Wildlife Management Area (CVWMA). Work was directed by the MFLNRORD in partnership with the British Columbia Northern Leopard Frog Recovery Team (BCNLFRT). A combination of nocturnal calling surveys (NCS) and egg mass surveys (EMS) were conducted to detect breeding activity of *Lithobates pipiens* (LIPI). Survey areas included: the Duck Lake Nesting Area (DLNA), Constructed wetlands (CW), Six Mile Slough (6MS) and Leach Lake. A limited passive integrated transponder (PIT) tagging session was conducted and samples were collected to support the collaborative genetics study (partners include: Laurentian University, Calgary Zoo, MFLNRORD and BCNLFRT)

2. METHODS

Spring breeding surveys including nocturnal calling surveys (NCS) and egg mass surveys (EMS) were conducted from April 11 through June 3 within the Duck Lake Nesting Area (DLNA), the constructed wetlands (CW), Six Mile Slough (6MS) and Leach Lake (NCS only as no calling detected). For the majority of the surveys, methodology followed that outlined in the *Northern Leopard Frog Project: 2017 Field Season* report (Houston, 2018), with some modifications.

For the first time during the spring, a limited PIT tagging session was conducted. When safe to do so without potential negative effects to breeding or egg mass health, LIPI opportunistically encountered during day time surveys were captured and scanned for a PIT tag. When possible, males not previously PIT tagged were implanted with a PIT tag; to minimize disturbance to breeding, females that appeared gravid were left alone, but if possible a photo was taken of the dorsal spot pattern for identification. PIT tagging methodology followed standard protocols used in previous years (Houston, 2018).

New to the field program in 2019 was the collection of samples for a genetics study. Samples were collected following the MFLNRORD spring workplan (Manley, 2019) and protocols developed by the Calgary Zoo (Randall, 2019a,b). In addition to the standard breeding surveys, some spring visual encounter surveys (VES) were performed in an effort to collect additional samples for this study.

Note that throughout this report, *detections* are defined as point observations (in time) and do not necessarily indicate unique individuals (unless specified as such), as recaptures (if any) have not been removed from general data summaries.

Project data and reports are available at: http://a100.gov.bc.ca/pub/siwe/details.do?id=4955

3. RESULTS

3.1. BREEDING SURVEYS

DUCK LAKE AREA

As part of the spring breeding program survey methodology, nocturnal calling surveys (NCS) and egg mass surveys (EMS) were done at west duck lake nesting area (WDLNA), east duck lake nesting area (EDLNA), the constructed wetlands (CW) and the east ditch/south cross dike (NCS only as no calling detected) between April 11 and June 3 for a total of 237:24 person-hours of survey effort.

A total of 165 LIPI detections were made during these surveys, including 110 calling male detections. Calling was detected at WDLNA, EDLNA and the CW; no calling was detected in the east ditch or south cross dike. Calling was already underway when the first NCS was conducted at WDLNA on April 11. Calling levels ranged from 0-16 individuals per survey night at the DLNA; the greatest level of calling was detected at WDLNA on April 30, when 16 individuals were detected calling; these levels are lower than what has been detected in recent years. The geographic extent of the majority of calling males was very limited at the WDLNA in comparison to other years, with the majority of calling located directly adjacent to the west shoreline (Figure 2). Calling levels at EDLNA were quite low, ranging from 1-3 per survey night (with an additional 4 LIPI of unknown sex detected via eyeshine). At the CW, there were 2 detections of calling males (1 in OGC-4 on April 24 and 1 in OGC-1 on May 13; not captured so it is unknown if they were the same individual).

A total of 6 egg masses were detected at DLNA (5 at WDLNA and 1 at EDLNA; Figure 3); none were detected at the CW (Table 1). In comparison to other years, the number of detected egg masses at WDLNA in 2019 was within the range of 0-8 detected annually from 2000-2010 and above the mean of 2.6 for that period (SD=2.2, n=11), slightly lower than the 7 per year detected in 2017-2018, and considerably lower than the 15-33 egg masses detected at WDLNA per year from 2011-2016 (mean=21.3, SD=6.3, n=6); Table 2. At EDLNA the number of egg masses detected annually since 2000 has been quite variable but appears to be declining in recent years.

The 5 LIPI egg masses detected at WDLNA were estimated to have been laid between April 21-and May 14, the majority appeared to be in good health upon detection and while the number of hatchlings were not counted for all egg masses, there appeared to be an average hatch-out in all. The egg masses at WDLNA were tightly clustered and there were only 2 clusters of detected egg masses, the first 3 egg masses were clustered within 10 meters of each other, the second cluster (containing 2 egg masses) were less than a meter from each other; these locations were respectively 5 and 30 meters of shore (Figure 3). The LIPI egg mass detected at EDLNA was estimated to have been laid on April 14, it was deemed to be in good health upon detection and appeared to have an average hatch-out. Additionally, 2 Columbia spotted frog (*Rana luteiventris*) egg masses were detected at EDLNA (1 within a meter of the LIPI egg mass) as well as numerous Pacific chorus frog (*Pseudacris regilla*) and long-toed salamander (*Ambystoma macrodactylum*) egg masses; this is the first time that a Columbia spotted frog egg mass has been recorded at the DLNA.

Table 1. LIPI egg masses detected within DLNA in 2019 by site with additional associated details

Egg mass #	Site	Estimated date laid	Estimated hatch-out	Tadpoles: Genetics study	Eggs: Brisco Reintroduction	Eggs: Calgary Zoo	Tadpoles: Vancouver Aquarium
EM190417-01	EDLNA	14-April	average	yes (5)	no	n/a*	n/a
EM190424-02	WDLNA	21- April	average	yes (5)	no	yes	yes
EM190424-03	WDLNA	23- April	average	yes (5)	no	yes	yes
EM190428-04	WDLNA	27- April	average	yes (5)	no	yes	n/a
EM190515-06	WDLNA	14-May	average	yes (5)	yes	yes	n/a
EM190515-07	WDLNA	12-May	average	yes (5)	yes	yes	n/a

*n/a because early detection, hatching had begun and Calgary Zoo stipulated eggs only (not hatchlings)

Table 2. Number of egg masses detected annually at DLNA since 2000 (bold are years with highest totals)

Year	WDLNA	EDLNA	Total
2000	8	8	8
2001	0	12	0
2002	2	1	2
2003	2	4	2
2004	1	3	1
2005	4	0	4
2006	2	3	2
2007	4	3	4
2008	1	3	1
2009	3	4	3
2010	2	5	2
2011	**15**	**2**	**15**
2012	**19**	**3**	**19**
2013	**19**	**2**	**19**
2014	**33**	**6**	**33**
2015	**23**	**3**	**23**
2016	**19**	**4**	**19**
2017	7	0	7
2018	7	1	7
2019	5	1	5
Total	**176**	**68**	**176**

Habitat conditions at WDLNA were notably different in comparison to other years. Aside from the near-shore area, submergent vegetation was very sparse (used by LIPI as a point of attachment for egg masses) until mid-May. There was an extensive algal bloom and water levels were significantly lower than usual (Figure 1). Hatchlings from the 6th and 7th egg masses were moved to areas of deeper water due to concerns about future water levels and the potential for stranding or mortality.

Figure 1. WDLNA photos (left to right): caged egg mass in spike rush adjacent to shore, showing the area beyond devoid of emergent vegetation (atypical habitat condition for this site); extensive algal bloom; checking on development of caged egg masses from a canoe to prevent siltation

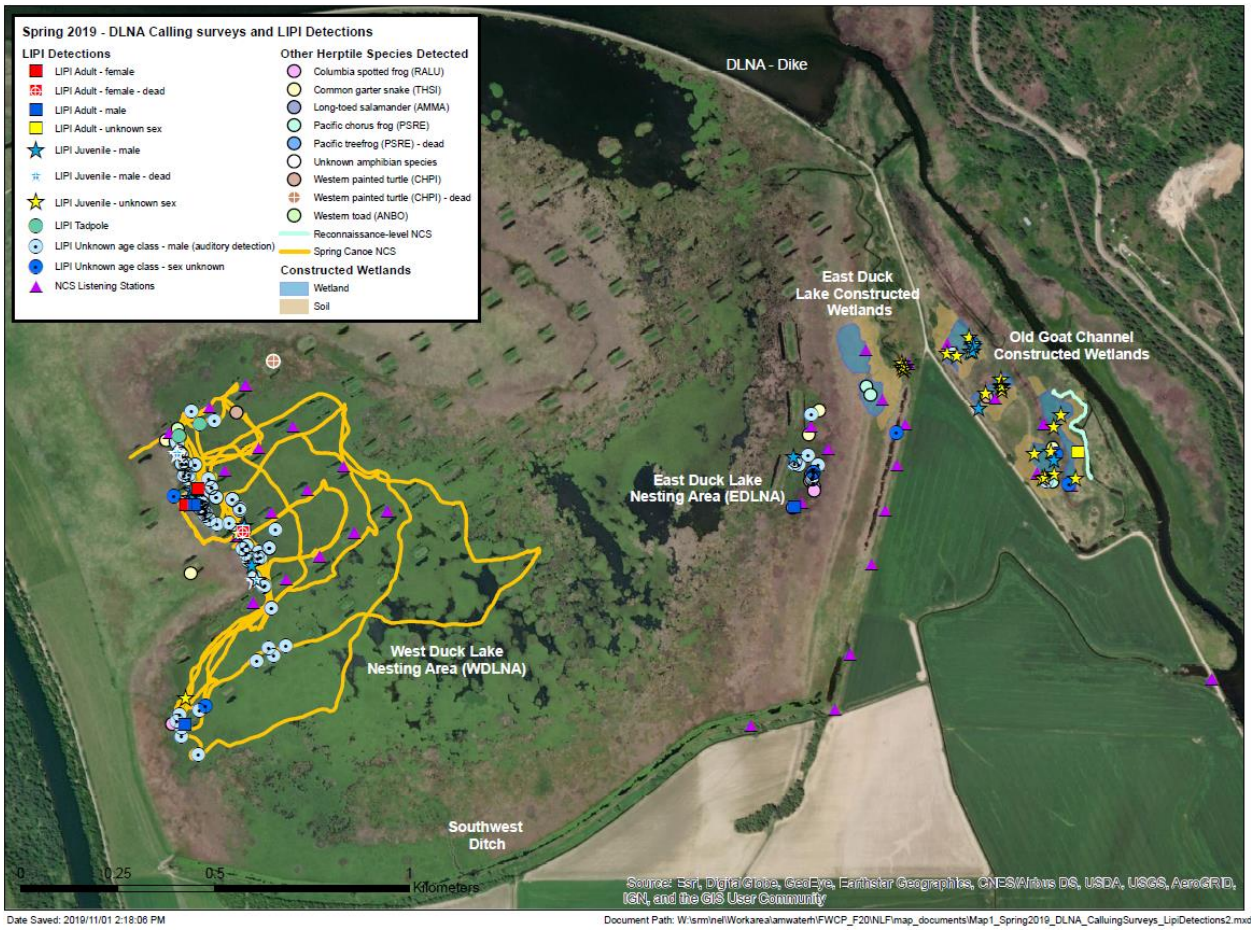

Figure 2. Map of spring 2019 calling survey results and LIPI detections in the Duck Lake area

Figure 3. Map of spring 2019 egg mass survey results in the Duck Lake nesting area

SIX MILE SLOUGH

After initially identifying areas of potentially suitable breeding habitat within six mile slough (6MS) using orthophotographs during consultation with CVWMA staff familiar with the area, a number of prioritized reconnaissance-level habitat suitability surveys were conducted via canoe (totaling 21:08 person-hours) to identify suitable LIPI breeding habitat between May 7 and 22 (Figure 4, 5). Of the surveyed areas, suitable breeding habitat was identified in compartment 4 and 5. Within the areas surveyed in compartments 1-3, surveyors determined that LIPI breeding habitat suitability appeared to be low to non-existent in compartment 1 and 2, and limited to the northwest corner of compartment 3.

As a follow up to the reconnaissance-level habitat suitability surveys, a total of 15:02 person-hours were dedicated to NCS in compartments 4 and 5 at 6MS on 5 nights between May 8 and 29. Calling male LIPI were detected in compartments 4 and 5. In compartment 4, NCS were conducted on 3 nights between May 15 and 29, during which time 2-6 calling males were detected per NCS. In compartment 5, NCS were conducted on 2 nights (May 8, 23); 1-3 calling males were detected per NCS and a pair was detected in amplexus (male believed to be a juvenile). In total there were 18 LIPI detections at 6MS during the spring breeding season (14 auditory male detections, a pair in amplexus, a dead adult male and a healthy juvenile male that was PIT tagged). The majority of the calling males were not captured so it is unknown what the juvenile to adult calling male ratio was or how many unique individuals there were.

In all, 10:36 person-hours of survey effort went into EMS at 6MS in areas where calling was detected, during which time 1 egg mass was detected; located in compartment 5 (Figure 4, 5). The egg mass was broken into 3 portions and unfortunately the majority did not appear to develop properly; none of the eggs within the portion of approximately 100 eggs caged in-situ for the genetics study hatched. Although no egg masses were detected in compartment 4 it is believed that at least one was laid there outside of the survey window as LIPI young of year were detected in the summer in the immediate area where calling was detected.

With limited survey time, it was thought that effort would best be spent in areas with the highest probability of detecting breeding based on habitat suitability (compartments 4 and 5). As a result, follow up surveys were not completed in compartment 1 or 2 (as habitat suitability within surveyed area appeared low to non-existent) or within potentially suitable LIPI breeding habitat in northwest corner of compartment 3 due to extensive travel time to the area along with the fact that the CVWMA had a remote recording device deployed in that area, so it was decided that those results would be relied upon to detect calling (results pending from CVWMA).

Figure 4. Photos from 6MS (left to right): pond 4 where calling detected; EM190514-05 showing cloudy eggs that did not develop and few hatchlings; habitat in pond 5 near where egg mass detected

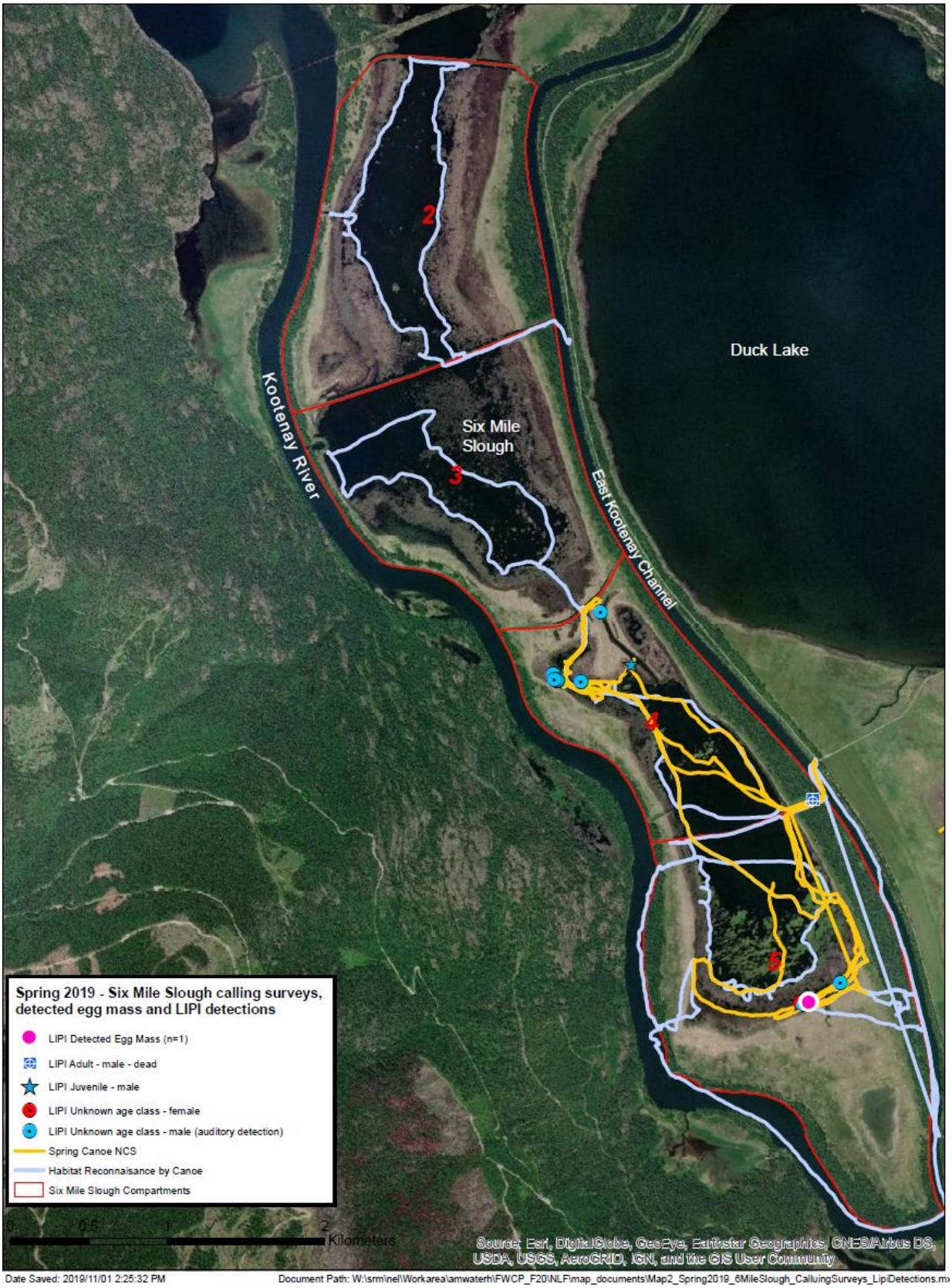

Figure 5. Map of spring 2019 Six Mile Slough surveys and results

LEACH LAKE

At Leach Lake, blitz-style surveys were completed on 3 nights between May 11 and 23 at the stations shown in Figure 6 (circles show limited area of detection probability within the vast wetland complex). In addition to the blitz-style surveys, 2 nights of canoe surveys covering the majority of pond 1 were conducted (May 23, 28). A total of 18:29 person-hours of effort were dedicated to surveys. No LIPI calling was detected during these limited surveys and therefore no follow up EMS were conducted. Due to the vast size of the wetland complex at Leach Lake, it is unknown if LIPI are still actively breeding at the site.

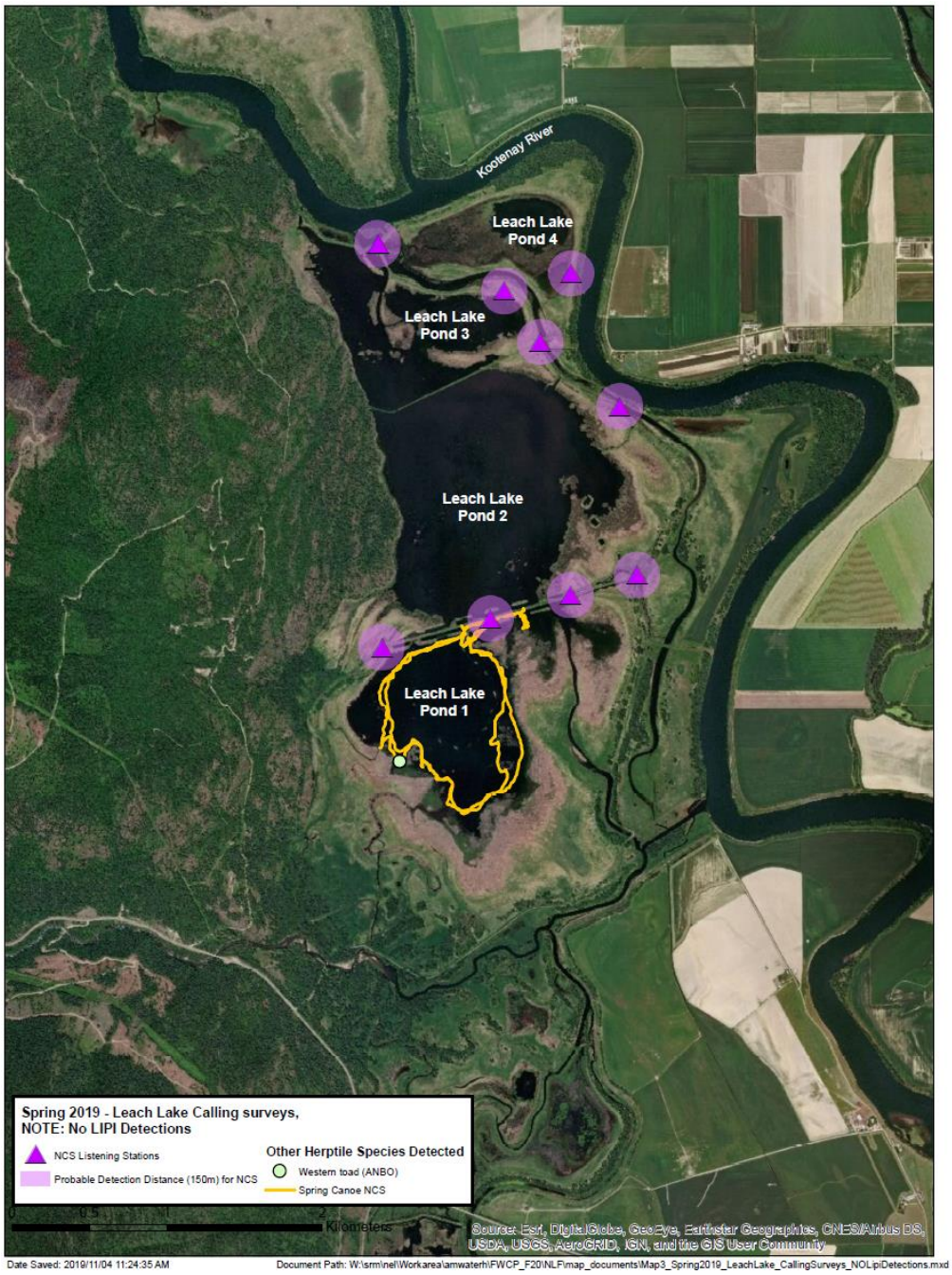

Figure 6. Map of surveyed areas within Leach Lake during spring 2019

3.2. Northern Leopard Frog Detections

Of the 183 detections (165 in Duck Lake area and 18 at 6MS) during the spring breeding season in the CVWMA (includes live and dead LIPI; Figure 2, 5), the majority (144) were males; 4 were females and the sex of remainder was unclassified. Of the male detections, the majority (124) were calling/auditory detections (heard but not seen so not possible to determine age class). Of the 20 male detections that were seen and identified to age class, 4 (20%) were classified as adults and the remainder were juveniles. Morphometrics were collected on a subset of 27 detections (14 male and 13 unclassified sex), the mean weight of the juvenile detections was 21.5 g (n=24, SD=6.5, range=11.0 – 38.0 g) and mean SVL was 60.1 mm (n=24, SD=6.1, range=45.3 – 72.6 mm). Mean weight of adult detections was 57.0 g (n=3, SD=5.2, range=51.0 – 60.0 g) and mean SVL was 80.8 (n=3, SD=1.1, range=79.9 – 82.0 mm).

3.3. Health

Of the 32 live LIPI detections whose health was visually assessed in the field, the majority (n=26) were deemed to be in good condition, 4 in fair condition and 2 in poor condition; there were 3 dead LIPI detections. The health issues noted with the 4 in fair condition included: minor grass cuts on dorsal surface, red raw nose, necrotic digits and blistering tissue (possibly chiggers). Of the 2 in poor condition, one had severe redness and blackened dorsal tissue (possibly frostbite) and the other was a moribund adult female (with a severe leg wound and some moderate redness likely due to chytridiomycosis on the ventral surface). Of the 3 dead males (1 adult and 2 juveniles), the body of 2 was intact, but the 3rd was detected as a skeleton with leg and hand tissue intact. To determine the cause of death in the moribund adult female, a necropsy was performed by veterinarians at the Calgary Zoo but they were not able to conclusively determine the cause of death; necropsy results did indicate that she still had eggs, had a small amount of digesta in the intestines and nothing in the stomach. The sample submitted to the Animal Health Lab of the Ministry of Agriculture tested positive for chytridiomycosis testing and negative for ranavirus.

Figure 7. Health issue photos (clockwise from top left): blackened tissue; carcass; blistering skin; malformation of foot, moribund female with moderate redness on ventral surface; moribund female (same individual as previous photo) showing severe leg wound

3.4. Passive Integrated Transponder Tagging

There were a total of 19 PIT tag events during the spring sampling session, including 12 first-tagged and 7 recaptures (Table 3). All were juveniles, 9 were males and 10 were of undetermined sex (too small to exhibit the secondary sexual characteristics required to definitively sex them). Weights of the measured animals ranged from 16.0 – 38.0 g, SVL ranged from 55.0 – 72.6 mm. The majority (11) were captured at the constructed wetlands. All of the within-year recaptures were detected once after first being tagged and were recaptured within the same site 2 -17 days later. The between-year recaptures were all recaptured once in the spring of 2019 within the constructed wetlands and were first-tagged in 2018, between September 12 and October 3 (Table 4, Figure 8).

Table 3. Summary of spring PIT tag session results by site

PIT tag type	CW	EDLNA	WDLNA	6MS	Total
first-tagged	5	1	5	1	**12**
recapture (within-year)	2	1	0	0	**3**
recapture (between-year)	4	0	0	0	**4**
Total	**11**	**2**	**5**	**1**	**19**

Table 4. Detailed information for 4 LIPI between-year PIT recaptures

PIT tag #	Initial Stage (sex)	Date PIT Tagged (2018)	PIT Tagging Location	Recapture Stage (sex)	Date Recaptured (2019)	Recapture Location	Net Weight Change (g)
985114000054273	YOY (U)	12-Sep	SWD - N	Juv (U)	22-May	CW (OGC-1)	-2.0
985114000070668	YOY (U)	03-Oct	Goat chann.	Juv (M)	24-Apr	CW (OGC-2)	n/a
985114000072009	YOY (U)	28-Sep	CW (OGC-4)	Juv (U)	22-May	CW (OGC-3)	3.0
985114000072027	YOY (U)	28-Sep	CW (OGC-2)	Juv (M)	14-May	CW (OGC-1)	8.0

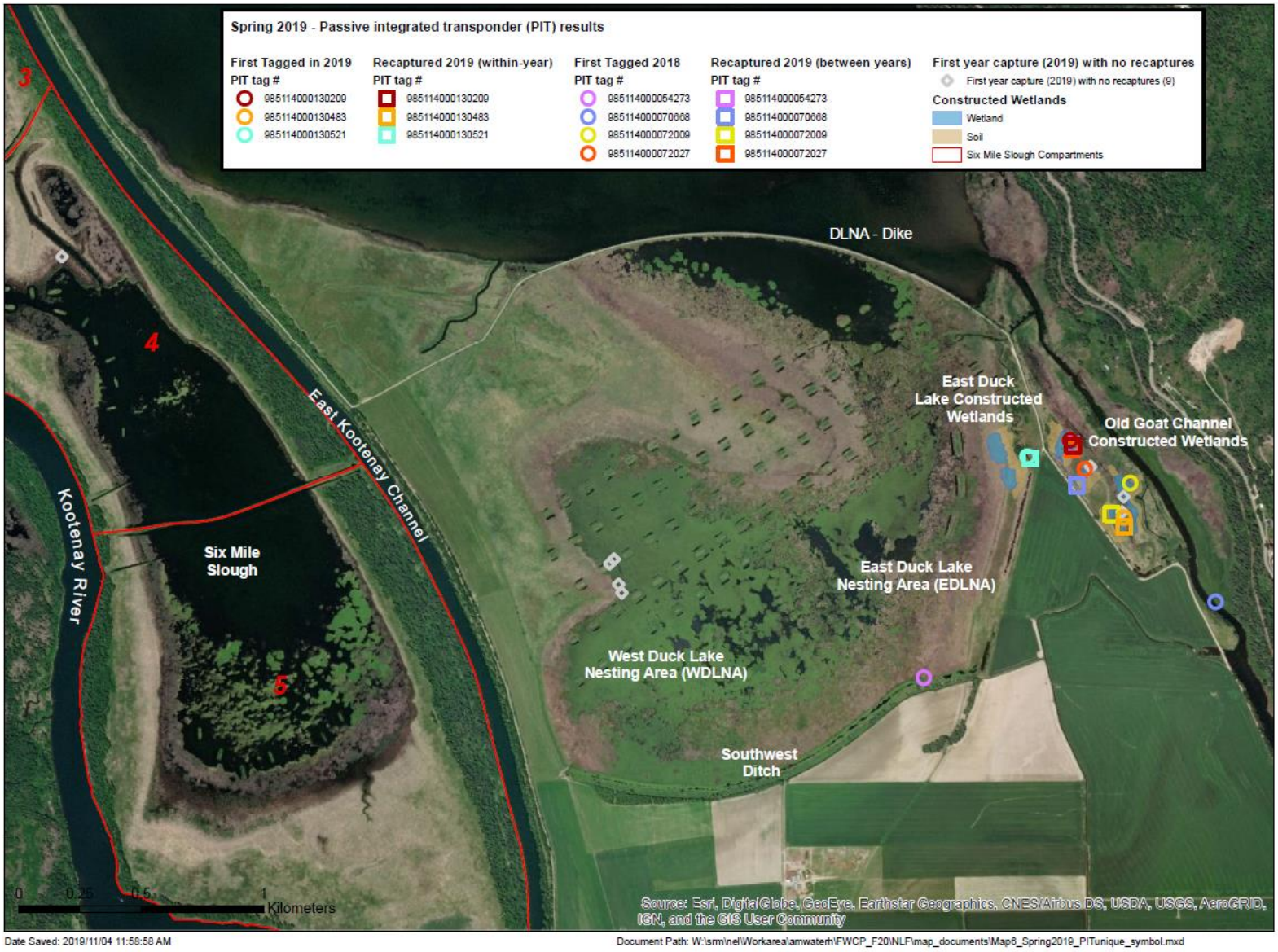

Figure 8. Map of PIT tagging results for spring 2019

3.5. SAMPLES COLLECTED FOR GENETICS STUDY

DNA FROM DETECTED EGG MASSES

To provide DNA from each detected egg mass for the genetics study, 5 tadpoles from each of the 6 viable egg masses were euthanized once tail length was approximately 5-6 mm. Since the egg mass detected at 6MS had limited viability and the portion caged did not hatch, tadpoles were not available so eggs were provided (Table 5, Figure 9, 10).

Table 5. DNA collection from egg masses for genetics study

Egg mass#	Site	# Tadpoles collected	# Eggs (approx.) collected	Total
EM190417-01	EDLNA	5	n/a	5
EM190424-02	WDLNA	5	n/a	5
EM190424-03	WDLNA	5	n/a	5
EM190428-04	WDLNA	5	n/a	5
EM190515-06	WDLNA	5	n/a	5
EM190515-07	WDLNA	5	n/a	5
EM190514-05	6 Mile slough	n/a	10	10
Total		**30**	**10**	**40**

TISSUE

Tissue from all 4 dead LIPI incidentally detected in the field while surveying was provided for the genetics study, including 2 adults and 2 juveniles (Table 6, Figure 10).

BUCCAL SWABBING

In total 13 live juvenile LIPI were buccal swabbed in the spring (Table 6, Figure 10); no adults were opportunistically encountered after the buccal swabbing project began in early May. The majority (n=7) of the buccal swabs were collected from the CW. All buccal swabbed animals were PIT tagged so this prevented duplicate sampling. Based on information provided by the CVWMA of LIPI being observed on nest islands within the DLNA in late spring in the early 2000's, 11 visual encounter surveys (VES) for a total of 23:56 person-hours were conducted to try and collect additional samples.

Table 6. Summary of tissue and buccal samples collected during spring for genetics study

Sample type	Site	Adult female	Adult male	Juvenile male	Juvenile (U)	Total
Tissue (dead LIPI)	**WDLNA**	1	0	2	0	**3**
	6MS	0	1	0	0	**1**
Tissue (dead LIPI)	**Subtotal**	**1**	**1**	**2**	**0**	**4**
Buccal swab (live LIPI)	**CW**	0	0	0	7	**7**
	EDLNA	0	0	0	1	**1**
	WDLNA	0	0	4	1	**5**
Buccal swab (live LIPI)	**Subtotal**	**0**	**0**	**4**	**9**	**13**
All combined	**Grand total**	**1**	**1**	**6**	**9**	**17**

Figure 9. Genetics study field photos (left to right): portion of egg mass caged in-situ at DLNA to facilitate collection of 5 tadpoles per egg mass for DNA; hatchling tadpoles; measuring a tadpole

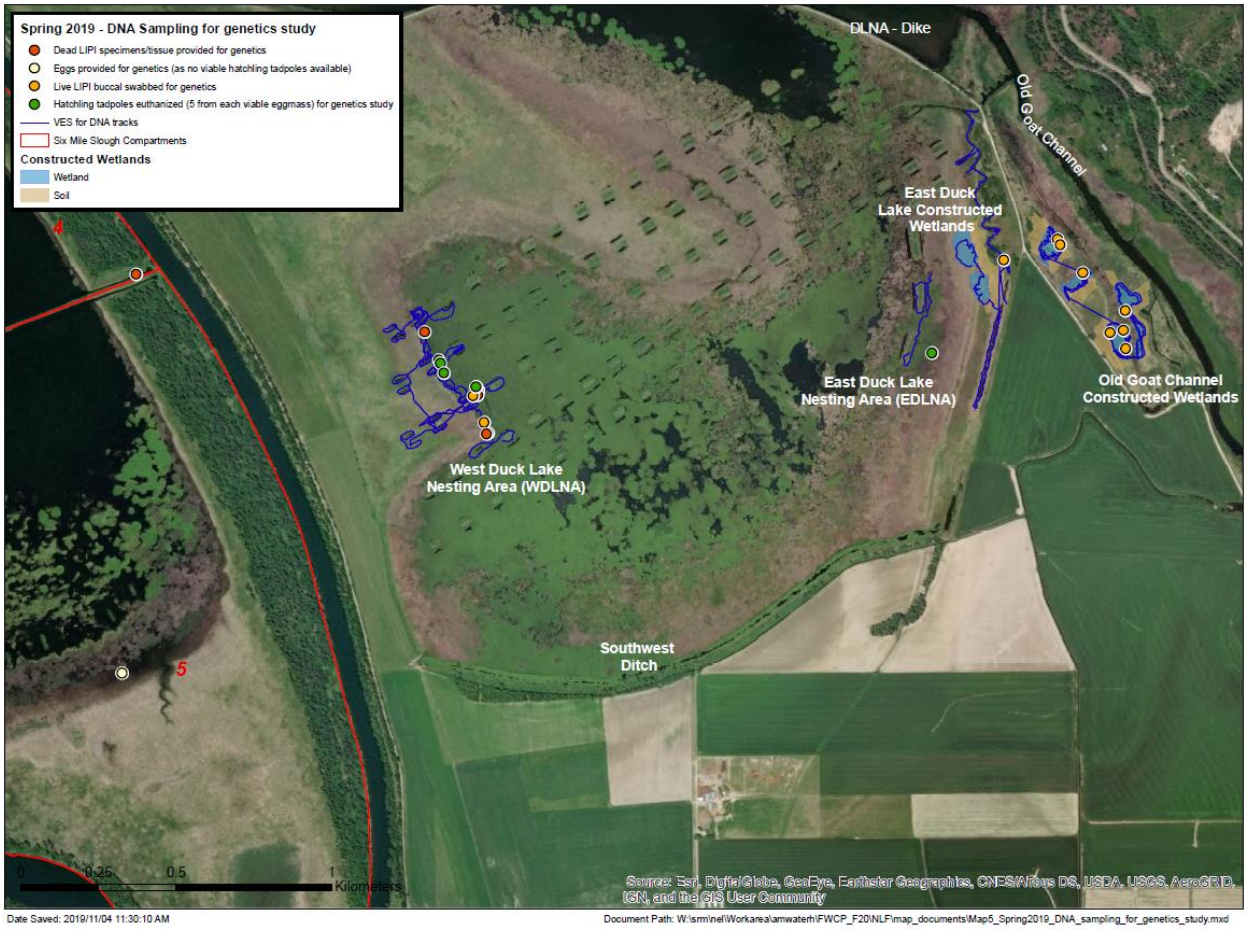

Figure 10. Map of DNA sampling locations for genetics study

3.6. Captive Assurance Colonies

A total of 630 LIPI were collected from the wild at the CVWMA during the spring to supply the captive assurance colonies at the Vancouver Aquarium and Calgary Zoo. As requested, 10 healthy hatchling tadpoles (5 from each of 2 egg masses) were received by the Vancouver Aquarium on May 15. For the captive assurance colony at the Calgary Zoo, it was possible to meet the facilities stated objective of supplying approximately 100 eggs from 5 egg masses by providing eggs from 5 of the 6 viable detected egg masses (Figure 11). With an estimated 66.7% - 85.7% hatch-out they added a total of approximately 620 hatchlings to the facility. Eggs were not provided from the first detected egg mass as it was beginning to hatch so they were not a candidate for transfer since the Calgary Zoo stipulated only eggs (not hatchling tadpoles) and eggs were not supplied from the detected egg mass at 6MS because the majority of it was not viable.

Figure 11. Photos of 2 methods of egg collection for Calgary Zoo (left to right): eggs packaged in cheese cloth, weighted on bottom to ensure remain submerged; trial method using cylindrical perforated thermoplastic fish egg tubes (with eggs from EM06/07 awaiting pickup by Calgary Zoo in egg mass cage)

3.7. Brisco Reintroduction Program

The threshold set by the BCNLFRT of detecting 5 egg masses prior to initiating removal of LIPI for the reintroduction program at Brisco was met so a portion of the last 2 detected eggmasses at WDLNA (EM06 and EM07) were removed and brought to Brisco by the Calgary Zoo team; they reported good hatch-out and a total of 2317 hatchlings were released at the site as free-swimming tadpoles.

4. DISCUSSION

Calling activity at DLNA was lower than in recent years, the greatest number of calling males detected in 1 night in 2019 was 16, compared to 54 in 2018 (Houston, 2019), 35 in 2017 (Houston, 2018) and 49 in 2016 (Houston, 2017). While it is not possible to conclusively determine the cause, this seems to indicate one of 2 things: that the number of individuals returning to the area is down (possibly because they have moved elsewhere) or it could be that there was decreased survival in the cohort from 2017 (which should be reproductively mature adult males in 2019) and/or the 2018 cohort (which would be calling juvenile males in 2019). Without identifying the age class of all calling individuals to determine the ratio of juvenile to adult calling males present at DLNA, it is not possible to speculate which cohort (if either) was lower than expected.

There could be several possible reasons for LIPI to move elsewhere (such as 6MS), including some of the observed changes in the condition of habitat observed in the spring of 2019, such as the lower than average water levels, lack of submergent vegetation (important for cover and egg mass attachment) as well as the extensive algal bloom. While it is unknown if these changes would cause LIPI to move elsewhere it is possible and worth consideration. Since it is not known how long LIPI have been present in 6MS (as this was the first year extensive NCS were conducted), it is impossible to determine whether LIPI detected there this year moved over from DLNA or not. Since LIPI exhibit very strong natal site fidelity, it is possible that LIPI from the 4 egg masses laid in the flooded field west of DLNA in 2017 kept moving west in search of their specific natal site, leaving DLNA behind. In addition to this the number of viable egg masses in 2017 was limited by the conditions so this likely impacted recruitment for the year, which would be apparent in 2019 when the animals would be expected to be reproductively mature; this could also be one of the reasons for the limited number of egg masses.

In comparison to other years, the number of egg masses detected at WDLNA was greater than the number detected annually from 2001-2010, but less than other years on record since 2000. Since there have been significant fluctuations in the annual number of detected egg masses over the years, it is possible that this is a natural fluctuation. In order to make meaningful comparisons, between-year differences in survey effort would have to be factored into the calculations.

The number of egg masses detected at EDLNA has been decreasing in recent years, while there are a number of possible reasons and it is not possible to determine the cause with certainty, a few possible explanations include the following: 1) it could be habitat related (emergent vegetation ingrowth resulting in a decrease in the preferred open water habitat for egg mass deposition) or, 2) related to increased tadpole mortality since the site is slightly higher in elevation so is more susceptible to drying in early summer. Another possibility is related to the recent discovery of Columbia spotted frogs (*Rana luteiventris*) at EDLNA in the past 2 years (one was detected in the immediate area where LIPI were calling in 2018 and 2 RALU egg masses were detected in 2019). Within the CVWMA it has been observed that Columbia spotted frogs and LIPI habitat-use do not overlap, prior to 2018, RALU have not been documented in the DLNA and were not documented using the same compartments as LIPI at Leach Lake in the past so it is possible that this may be a contributing factor to the decreased use by LIPI. Another possible explanation is the widespread chiggers infection detected some years ago which may have led to increased mortality.

While it is unclear if the lack of submergent vegetation early in the season (likely a direct result of the 2018 CVWMA project to control extensive watershield ingrowth) and extensive algal bloom (likely tied to the large nutrient input from decaying vegetation as a result of the previously mentioned project) may have impacted LIPI habitat use, the location of the majority of calling activity and detected egg masses seems to indicate it did as they were detected within areas with vegetation in the water column (near shore), areas which also appeared not to have been as extensively affected by the large algal bloom.

Discovery of LIPI breeding at 6MS for the first time on record was a very positive result, which will be taken into account by the CVWMA during planning for the 6MS wetland restoration project. Due to the limited calling at DLNA, surveyors were available to do reconnaissance-level habitat suitability surveys and follow up breeding surveys (nocturnal calling and egg mass surveys) in prioritized areas of 6MS, which led to the discovery; this may not have been possible if there was extensive widespread calling at DLNA. While it was not possible to conduct extensive surveys in all areas of 6MS due to the vast size of the area and other factors, surveys could be expanded upon in future years if it is deemed a priority by MFLNRORD and the BCNLFRT.

With survey-effort focused on the Duck Lake area and 6MS in 2019, it was not possible to survey Leach Lake thoroughly. While it is useful to conduct surveys at the blitz stations as a way to determine if there is breeding in the immediate area, due to the vast size of the wetlands at Leach Lake and limited detection distance from the stations there are large areas that are not surveyed. Non-detection does not indicate there are no LIPI remaining at Leach Lake. To accurately determine if LIPI are still active at Leach Lake, extensive surveys via canoe would have to be conducted under ideal conditions.

Although no breeding has been detected at the constructed wetlands to date, they do provide habitat that is utilized by LIPI throughout the year.

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