FWCP Northern Leopard Frog Project: 2013 Field Season Report

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Submitted to:
Fish and Wildlife Compensation Program-Columbia Basin
401-333 Victoria Street
Nelson, BC V1L 4K3

Submitted by:
Barb Houston B.Sc.
Houston Ecological

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SENSITIVE SPECIES

Since the northern leopard frog (*Lithobates pipiens*) is listed on the *Provincial List of Species Considered Data Sensitive*, and *all sites* are considered sensitive site information for this species due to *the potential for indirect harassment*, all geographic location information is secure (locked) and is not openly available to the public. This report contains specific geographic information and maps of northern leopard frog locations as it was prepared for internal use only for the FWCP and members of the BC-NLFRT and is not intended to be distributed beyond that audience.

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1. INTRODUCTION

The main objectives for the 2013 field season were to monitor the British Columbia remnant wild population of northern leopard frogs (*Lithobates pipiens*), in the Creston Valley Wildlife Management Area (CVWMA), continue to bolster the reintroduced population at Bummer's Flats, provide additional founder individuals to enhance the captive assurance colony at the Vancouver Aquarium and support the British Columbia Northern Leopard Frog Recovery Team (BC-NLFRT) efforts to establish a new population in the Columbia Marshes near Brisco.

The monitoring program was developed to assess the overall health, chytridiomycosis prevalence, habitat use, road mortality, breeding activity, reproductive success, and survivorship of the different life stages. During 2013, the fifth year of the captive assurance colony at the Vancouver Aquarium, efforts were made for the first time to breed the 2009 adults in captivity and 2013 marked the third year of phase 2 reintroductions at Bummers Flats.

2. METHODS

In order to meet the objectives listed above, seven types of survey methods were used: nocturnal calling surveys, Songmeters, egg mass surveys, visual encounter surveys, road surveys, tadpole trapping and dip-netting.

2.1 Nocturnal Calling Surveys (NCS)

The main objective of the NCS is to monitor breeding activity and determine the number of calling *Lithobates pipiens* (LIPI) in each area during the breeding season. The methodology for calling surveys follows that outlined by the Resource Inventory Committee (Heyer et al. 1994, MELP, 1998) with some modifications. Calling surveys were primarily carried out at the CVWMA; surveys at Bummer's Flats were largely conducted under a separate contract set up by the FWCP to increase survey effort at the site since it is difficult for the Creston crew to find time during the busy spring breeding season to effectively survey both areas.

NCS are carried out as night falls during the breeding season (late April to early June) at fixed calling stations (for CVWMA NCS stations see map in Appendix 1, for Bummers Flats NCS stations see map in Appendix 10). Two types of NCS are utilized: standardized auditory surveys and blitz style surveys.

Standardized Auditory Surveys (SAS)

The SAS methodology is used at fixed calling stations in known breeding areas to approximate the relative abundance of calling males and determine their locations for follow up egg mass searches. Surveys begin with one minute of silence to ensure that any disruptions created by the surveyor reaching the site do not impact the calling activity (Adama and Beaucher, 2006). The survey itself consists of three 3-minute intervals, each separated by 1 minute. The surveyor listens and estimates the number of calling LIPI during each interval, and at the end of the survey also estimates the minimum and maximum number of calling LIPI individuals heard during the entire survey.

Blitz Style Surveys

Blitz style surveys are used in areas where breeding is not documented regularly, but either has been in the past or the area contains suitable habitat so it is reasonable to think that breeding may occur. Surveyors listen for a minimum of 7 minutes at each station and record the number of LIPI detected calling.

Efforts are made to survey each area at least once per week, weather permitting. Surveys are cancelled when winds are excessive or there is heavy rain, as it would be impossible to detect calling activity under these conditions. Surveys are also cancelled if air temperature drops below 5ºC, as it is not likely LIPI would be calling.

Upon arrival at the first station within a breeding site the start time is recorded, GPS location noted, and environmental conditions documented, including: air temperature, water temperature, cloud cover, precipitation (current and last 24 hours), approximate wind speed, pH, and conductivity. NCS are then carried out and special attention is given to determine the exact location of the calling LIPI, as this enables the surveyors to pinpoint the area in which they should focus their efforts during the egg mass survey the next day. Once the NCS is completed, the end time, end air temperature and end water temperature are noted, and if time permits, the surveyor captures and processes any LIPI in the area (see details under animal capture section below), which is important to not only enable surveyors to determine the focal areas for follow up egg mass searches, but it also enables them to identify the age class of the calling males and collect information on health and size from captured frogs.

2.2 Songmeters

Songmeters produced by Wildlife Acoustics Inc. were utilized during the peak spring breeding season at areas that are difficult to reach and/or monitor on a regular basis. In 2013, 3 additional units (SM2+) were purchased by the FWCP to add to the 1 SM1 unit already owned. They were placed at 4 sites within the CVWMA (Leach lake cross dike 1, Leach lake cross dike 2 and at 2 locations in north Corn Creek; see Appendix 1 for map of locations). They were set to record daily from May 19 to June 11 starting at 17:30 for 8 hours.

In an effort to determine when males begin calling in the spring a Songmeter was set up in the EDLNA ponds on April 2, it was set to record daily from 8 pm to 1 am.

Data analysis (for calling LIPI presence/not-detected) is done using Songscope software, a spectrogram viewing program developed by Wildlife Acoustics Inc. by visual scanning .wav file spectrograms at high speed searching for the digital signature of a LIPI call.

2.3 Egg Mass Surveys (EMS)

Egg mass surveys are conducted to investigate the breeding activity of LIPI. It has been found that the most efficient way to conduct EMS is to return to the breeding site where a NCS was conducted recently, as this enables the surveyor to focus on areas of concentrated calling, before surveying other areas. At the beginning of the survey environmental conditions as described above (for NCS) are recorded and a GPS tracklog is started, then the surveyors begin searching the area systematically. After the area of concentrated calling is surveyed, if time permits other suitable habitat within the area is surveyed. Weather is a very important factor

in conducting EMS, as any wind, rain, or cloud cover can obscure the surveyor's visibility, even though polarized sunglasses are always used. When an egg mass is detected, surveyors immediately make every effort to neutralize disturbance to the area, to prevent siltation of the egg mass. The location is marked by GPS, egg mass volume and water depth measurements recorded, and the vegetation species of attachment noted; a photo is also taken when possible. The health of the egg mass, percent fertilization and approximated age is noted, and finally, the area is marked by a ring of flagged cattail stems driven into the substrate (at a radial distance of 2 meters away) to mark the area. When possible, lidded egg mass enclosures are utilized to facilitate collection of hatchlings for the Vancouver Aquarium captive assurance colony, the reintroduction at Bummers Flats and to provide protection from predators until free swimming. Egg mass enclosures are made of black fibreglass window screening attached to a wire frame made from a tomato plant cage (Figure 2). Native vegetation with good amounts of algae for hatchling grazing was added to the enclosure to provide shade and a food source and care was taken to ensure potential predators were removed. Enclosures remained in situ until hatchlings were free swimming and no longer clinging to the egg mass (approximately 1 week after hatching). Once tadpoles reached the free-swimming stage they were released in situ except for a small number that were retained for the Vancouver Aquarium captive assurance colony. Each enclosure was carefully monitored, native vegetation replaced as needed and efforts were made to keep it free of excessive waste build up.

2.4 Visual Encounter Survey (VES)

The main objective of the VES is to gather information on the health and status of the northern leopard frog population, as well as to get an indication of habitat use, dispersal patterns and migration corridors. As habitat use for the northern leopard frog varies by life stage and season, VES are targeted towards specific life stages during different seasons. During the spring migration between over-wintering habitat and spring breeding habitat, in addition to completing road surveys, VES are done along the shores of Duck Lake and associated channels surveying for migrating frogs. During the summer, VES are carried out in an effort to target LIPI young of year (YOY) in natal ponds before late summer and fall dispersal. During fall, migration corridors and over-wintering areas are surveyed in the CVWMA; methodology usually involves walking the perimeter of the water body searching for LIPI, but in some cases surveys are conducted by boat. LIPI are captured and processed using methods outlined in Section 2.8 Animal Capture and Tissue Collection.

2.5 Tadpole Trapping

The main objective of tadpole trapping is to gather presence/not detected data in areas where breeding has occurred in the past but was not detected in 2013. This year, Leach Lake and the East Ditch were the target areas. Traps were set up in Leach Lake ponds 1, 2, 3 and 4 as well as in various locations throughout the East ditch (see Appendix 1 for map of trap locations). Trapping was also done in the EDLNA ponds, a known breeding site, to determine what stage of development the tadpoles were at during the trapping period.

Tadpoles are trapped using un-baited 21 inch wire minnow traps. The traps are partially submerged in warm shallow areas with high levels of emergent vegetation along the perimeter of the marsh. They are set up so that at least a portion of the trap is up out of the water to prevent drowning of post-metamorphic individuals even though there is a very small

probability they would enter and not escape on their own. Traps are checked within 8-12 hours to decrease the likelihood of predation on trapped tadpoles and everything trapped is released on site. If handling tadpoles for identification to species level Nitrile gloves are used as there is evidence latex gloves can be harmful to tadpoles (Gutleb, 2001).

2.6 Spring Migration Surveys

In addition to VES already discussed, survey methods included road surveys and a variety of experimental trapping methods (camera traps, terrestrial drift nets with pitfalls and aquatic fencing with minnow traps). Road surveys were carried out between March 15 and April 19 by a variety of people including the FWCP crew as well as some BC-NLFRT members and followed methods established previously (Houston and Hill, 2012) and those utilized in the fall, see Section 2.7. LIPI are captured and processed using methods outlined in Section 2.8 Animal Capture and Tissue Collection. Trapping was conducted outside the scope of the FWCP project by BC-NLFRT members, so detailed methodology is not provided in this report.

2.7 Fall Migration Road Surveys

The objective of fall migration road surveys is to gather information on migrating frogs and associated road mortality as they cross over the dyke road while moving from summer foraging grounds in the DLNA to the primary over-wintering area at the Old Goat channel. To avoid causing any road mortality, surveyors park their vehicle outside the migration corridor and begin walking or very slowly cycling the roadway starting at dusk and note the location of all LIPI encountered (live or dead) by GPS. If time permits, live animals are captured and processed (see animal capture details in the following Section); if time is restricted a dorsal spot pattern photo is taken at a minimum. Dead frogs are removed from the roadway to ensure they are not double counted and mortally wounded animals are euthanized.

2.8 Animal Capture and Tissue Collection

During all surveys, every effort is made to capture any LIPI detected. When the surveyor is unable to make a capture, or time prohibits it, the species and location is noted as a visual observation; if it is heard calling, but not seen or captured, it is recorded as an auditory detection. Each animal that is captured is processed to collect information about the physical attributes, including: snout to vent length (SVL), weight, health (good, fair, poor, dead), visible signs of chytridiomycosis, age class (YOY, juvenile, adult), and sex. Animals are not usually checked for visual implant elastomer (VIE) marks anymore, unless they are very large individuals, since the last detection of a VIE marked individual from the 2001-2005 captive rearing program was in 2008 and it is not believed that any of the VIE marked frogs are still alive. The UTM location is marked by GPS, the habitat features and distance to shore noted, and a digital photo of the animal's dorsal spot pattern is taken for identification. When an animal is captured, the surveyor puts on a pair of single-use disposable gloves, and places the animal into a one-time use Ziploc bag. The animal is then weighed, measured, visually assessed for health and any signs of chytridiomycosis, swabbed, put back in the net for a photo and then released as quickly as possible to minimize stress (processing occurs in order listed). Photos of weighing, SVL measurement and swabbing are provided in Figure 1.

The health of each LIPI captured is assessed in the field by visual inspection. The surveyor looks for any abnormalities or injuries and for signs of chytridiomycosis such as sloughing

skin, redness, vascularization, lethargy, abnormal body positioning, or loss of righting reflex. General health and whether or not an animal is suspected of a chytridiomycosis infection is noted. An animals general health is defined to be *good* if it has no injury or signs of illness; it is deemed to be *fair* if it has a minor injury such as a wound, which it is expected to fully recover from, or minor symptoms suspected to be from chytridiomycosis; and it is considered to be in *poor* health if it has a major injury, that will likely cause death, or if it is showing signs of disease, such as chytridiomycosis. If chytridiomycosis is suspected, details of the symptoms are noted. When possible, a photo is taken of any health related issues.

Tissue samples are collected to test for the presence of *Batrachochytrium dendrobatidis (Bd),* which causes the disease chytridiomycosis. Body swabs are taken using sterile Mediwire MW100 rayon tipped swabs stored in a dry labelled test tube. The animal is swabbed a total of 33 times, in the following order to minimize the spread of *Bd* if present; 5 times on each side, 5 times on the ventral surface, 5 times on each thigh, and once on the webbing between each toe. Once swabbing is completed the swab is put back into the sterile, dry test tube, labelled with observation number, species, sex, age class, site, health and whether or not *Bd* was suspected; it is stored in the refrigerator until it is submitted to the lab for testing. The swab is not stored in any type of fixative, as it impairs the DNA extraction process, and is not required to maintain the integrity of the *Bd* DNA.

If a recently deceased animal is found in relatively good body condition (i.e., decomposition has not begun) and the cause of death is unknown it is immediately submitted to Dr Stephen Raverty of the Animal Health Centre of the Ministry of Agriculture Fisheries and Food Lab in Abbotsford, BC for a full work up to determine the cause of death and general condition.

Tissue samples are analyzed by the Animal Health Centre of the Ministry of Agriculture Fisheries and Food Lab in Abbotsford, BC for the presence of *Bd* DNA using quantitative Polymerase Chain Reaction (qPCR) assay under the direction of Dr Tomy Joseph using methods developed by Boyle (Boyle, 2004).

Figure 1. Photos (left to right): Weighing; measuring SVL; and swabbing LIPI for *Batrachochytrium dendrobatidis (Bd),* which causes the disease chytridiomycosis.

2.9 Dorsal Spot Pattern Recognition

At the end of the field season dorsal spot pattern recognition is carried out to determine the number of individual LIPI and recaptures based on photos of the dorsal spot pattern of LIPI photographed; this is possible since each individual has a unique spot pattern that it retains for life. Photos of all captures are labelled by observation number, cropped, resized, and organized by site. Once organized, photos from both within sites and between sites (if dispersal possible) for 2013 are compared manually to identify matching spot patterns. Normally, photos dating back 2 years would also be compared to the current year, however in 2013 due to the very large number of photos taken, there was not sufficient time available to include this analysis.

2.10 Captive Assurance Colony at Vancouver Aquarium

There was a request from the Vancouver Aquarium for another 50 founder tadpoles from as many wild laid egg masses as possible this year to continue to bolster the captive assurance colony that was started in 2009. To facilitate collection for the captive assurance colony (as well as to protect the eggs and hatchlings from predators), where possible wild laid egg masses are enclosed with a lidded egg mass enclosure in situ as soon as possible after detection (Figure 2, Section 2.3). Once hatchlings reach the free-swimming stage the majority are released but a small number were held in the enclosure in situ at the natal site until the end of the breeding season when they were collected for transport to the aquarium.

In 2013, 5 tadpoles from each of 10 different egg masses, for a total of 50 tadpoles were collected and housed temporarily at the CVWMA office until they were driven to the aquarium in early June by the author. Tadpoles from each distinct egg mass were kept separate and transported in plastic bags (the same as those used by pet stores to transfer goldfish) filled with 1/3 water and 2/3 air. The bags were packaged carefully in an open top Styrofoam cooler to prevent overheating and transported carefully to minimize disturbance and prevent mortality. Upon arrival at the aquarium husbandry staff transferred them to the rooftop aquaria they are housed in, following biosecurity and acclimation protocols.

Natural breeding and rearing habitat data was provided to the aquarium when the captive assurance colony was started in 2009 to enable them to set up appropriate conditions for housing the animals. Habitat photos and environmental data were provided, including water temperature, air temperature, pH, dissolved oxygen and conductivity measurements.

Figure 2. Photos (left to right): egg mass cage, temporary housing for tadpoles awaiting transfer, tadpoles packaged up for transport to Vancouver Aquarium.

2.11 Bummers Flats Reintroductions

In order to bolster the reintroduced population at Bummers Flats that was started in 2003 a second phase of reintroductions was started in 2011, using eggs and/or hatchlings from wild laid egg masses in Creston. It was decided by the BC-NLFRT that if greater than 10 egg masses were detected in Creston, a portion above that level would be moved to Bummers Flats. This year all reintroduced animals were moved at the hatchling stage; where logistically feasible a portion (ideally not more than half) of the healthy egg masses detected over and above the threshold level of 10 were moved in insulated thermos containers. Pond water was placed in each container to approximately the 1/3 level and hatchlings were collected from egg mass enclosures using stainless steel strainers and quickly transferred into the Thermos for transport to the recipient site (Figure 3). Once at the recipient site, acclimation methods utilized by the Alberta Northern Leopard Frog Recovery Team with some modifications were carried out (Kendell and Prescott, 2007). Cages were set up at the recipient site, vegetation from the site was added and they were held overnight. Tadpoles were counted and released at the recipient site the following day.

Figure 3. Photos (left to right): Field technician Claire Schadeli preparing containers to collect hatchlings for transport, Thermos container with hatchlings, example of size range of hatchlings (both from same egg mass) released at Bummer's Flats.

2.12 Columbia Marsh Reintroduction

Since the captive breeding program at the Vancouver Aquarium successfully bred LIPI from the 2009 cohort of animals brought from Creston to originally start the captive assurance colony, the BC-NLFRT was able to use the progeny to start a reintroduction program in the historically occupied Columbia Marshes at a site near Brisco. Our crew helped with the release and conducted some VES but the majority of post-release monitoring was completed by Penny Ohanjanian's crew with funding from The Columbia Valley Local Conservation Fund, for results see Ohanjanian et al, 2013.

3. RESULTS AND OBSERVATIONS

Unless otherwise noted the summarized results are of LIPI detections in time, they do not necessarily reflect the number of unique individuals, but rather just observations in time as recaptures (if any) have not been removed.

3.1 Survey Effort

In total 629:07 person-hours were spent surveying for northern leopard frogs in the CVWMA, Columbia Marsh release site and Bummer's Flats (n=217 surveys) between March 15 and November 10, 2013. Of this total, 585:44 person-hours were spent surveying at the CVWMA (n=197 surveys); this total includes 157:16 person-hours by volunteer BC-NLFRT members (and crew) to conduct road, trapping and shoreline VES during the spring migration (n=73 surveys between March 15 and April 14). Table 1 outlines the effort put forth by survey type at the CVWMA during the 2013 field season and Table 2 indicates the number of surveys completed. These calculations are based on the amount of time spent surveying multiplied by the number of surveyors and are reported in person-hours (survey-time multiplied by the number of surveyors).

Table 1. Survey effort in person-hours (hh:mm) by survey type at the CVWMA in 2013

Season	NCS	EMS	Incidental	Road	Tadpole	Trapping	VES	Total
Spring	71:12	167:42	1:30	140:54	*	21:04	31:03	433:25
Summer	*	*	*	46:14	11:29	*	34:53	92:36
Fall	*	*	00:05	4:14	*	*	55:24	59:43
Total	**71:12**	**167:42**	**1:35**	**191:22**	**11:29**	**21:04**	**121:20**	**585:44**

* indicates that no surveys of this type were carried out during this time period

Table 2. Number of surveys in the CVWMA by survey type per season in 2013

Season	NCS	EMS	Incidental	Road	Tadpole	Trapping	VES	Total
Spring	21	26	1	42	*	37	19	146
Summer	*	*	*	10	6	*	13	29
Fall	*	*	1	2	*	*	19	22
Total	**21**	**26**	**2**	**54**	**6**	**37**	**51**	**197**

* indicates that no surveys of this type were carried out during this time period

At the Columbia Marsh release site 7:55 person-hours were spent (n=3 surveys) on visual encounter surveys for YOY.

At Bummer's Flats 35:28 person-hours were spent surveying (n=17 surveys). Of the total, 5:46 person hours were spent on spring NCS (including a visit to the Axelrod property to follow up on Songmeter detection), 1:29 person-hours on a dipnet survey and 28:13 on VES (n=13 surveys).

3.2 Nocturnal Calling Surveys (NCS)

In the CVWMA, 71:12 person-hours were dedicated to NCS (n=21 surveys) during the peak breeding season from April 24 to June 4; this tally of survey hours includes time spent conducting NCS and time spent locating and capturing calling males. During these surveys 128 LIPI detections were made (including all detection types: auditory, captures, photo visuals and simple visuals (no photo). Catch per effort was 1.8 LIPI per person-hour of NCS (Table 3).

Table 3. Summary of NCS efforts in the CVWMA for 2013

	NCS
Number of surveys	21
Survey effort (person-hours)	71:12
Number of LIPI observations	128
LIPI catch/effort during NCS	1.8

Table 4 summarizes the LIPI observations by stage and detection type during NCS. Of the 128 total observations, 23 were adults (21 male, 1 female, 1 sex unknown), 74 were juvenile (58 male, 0 female, 16 sex unknown) and 31 were males of unknown age class (auditory detections therefore unable to determine age class). Of the observations, 23 (18%) were auditory, 48 (37.5%) were captures and 57 (44.5%) were visuals (see maps in Appendices 2-3).

Table 4. LIPI observations by stage and detection code during NCS at CVWMA in 2013

Detection code	adult	juvenile	unknown	total
Auditory	*	*	23	23
Capture	18	30	0	48
Photo only	0	0	0	0
Visual (no photo)	5	44	8	57
Total	23	74	31	128

*Auditory detection so impossible to determine if adult or juvenile so assigned to age class unknown

Within the CVWMA calling surveys were conducted at permanent SAS stations in WDLNA (n=9), where 2 new stations were added this year (station 5 and 6), EDLNA ponds (n=6), East ditch (n=5) and Leach lake (limited to one blitz style survey because construction on the main dike prevented access for much of the spring). Calling was detected at WDLNA and EDLNA ponds but none was detected at Leach Lake or the East ditch. WDLNA had the greatest proportion of calling activity with 35 calling males detected in the south end of the unit (in the area of NCS station 5) during a NCS on May 7; the north end of the unit (station 1, 2, 3 and 4) was not surveyed the same night so this is only a tally of partial calling at WDLNA. The peak of calling activity at EDLNA ponds was during the first week of surveys on April 25 when 23 males were detected calling at the site.

It should be noted that due to the large area of WDLNA not all stations were surveyed during each survey so it is difficult to compare calling activity between survey nights, but the range for surveyed nights was 0-35 individuals, mean 17.8, SD=10.9, n=8. There was also a strong amount of calling detected at the EDLNA ponds (mean 6.3 individuals, SD=8.5, n= 6, range 0- 23 individuals); same stations surveyed each survey night so direct comparisons possible.

At the CVWMA the greatest proportion of calling was detected during the week of May 5-11 (Table 5, Figure 4.).

Table 5. Maximum *LIPI* calling detected in the CVWMA during the spring of 2013

Week	WDLNA**	EDLNA	East Ditch	Leach Lake	Grand Total
April 21-27	16	23	0	*	39
April 28-May 4	0	4	*	*	4
May 5-11	35	7	0	*	42
May 12-18	15	1	0	0	16
May19-25	0	*	*	*	0
May 26-June 1	31	3	0	*	34
June 2-8	15	*	*	*	15

* indicates the area was not surveyed

**WDLNA is a very large area with 6 survey stations; not every station is surveyed during each NCS so direct comparisons in calling between surveys cannot be made

Appendix 3 shows the location of captured LIPI during spring and Appendix 1 shows the location of egg masses detected at CVWMA.

There was a major peak in calling detected at the CVWMA (all sites combined) during the week of May 5-11 when night time air temperatures (recorded at the start of NCS) hit the first peak of the season at 12.4°C (Figure 4). This peak in calling corresponds with the peak in 24 hour daily recorded air temperatures for the spring breeding season observed on May 7 at 17:00 of 37.4°C (HOBO temperature logger data from DLNA). Air temperatures recorded at the beginning of NCS were fairly consistent (mean=11.9°C, SD=0.5, minimum 11.3°C, maximum 12.4°C) from the first night of surveys on April 24 until May 18 but temperatures dropped considerably the following week, on May 22 a low of 0.7°C was recorded at 20:00 and it snowed. A NCS was conducted at the WDLNA the night after the cold spell and no calling was detected. Temperatures climbed again the following week and continued to climb as surveys wrapped up June 4 but calling tapered off.

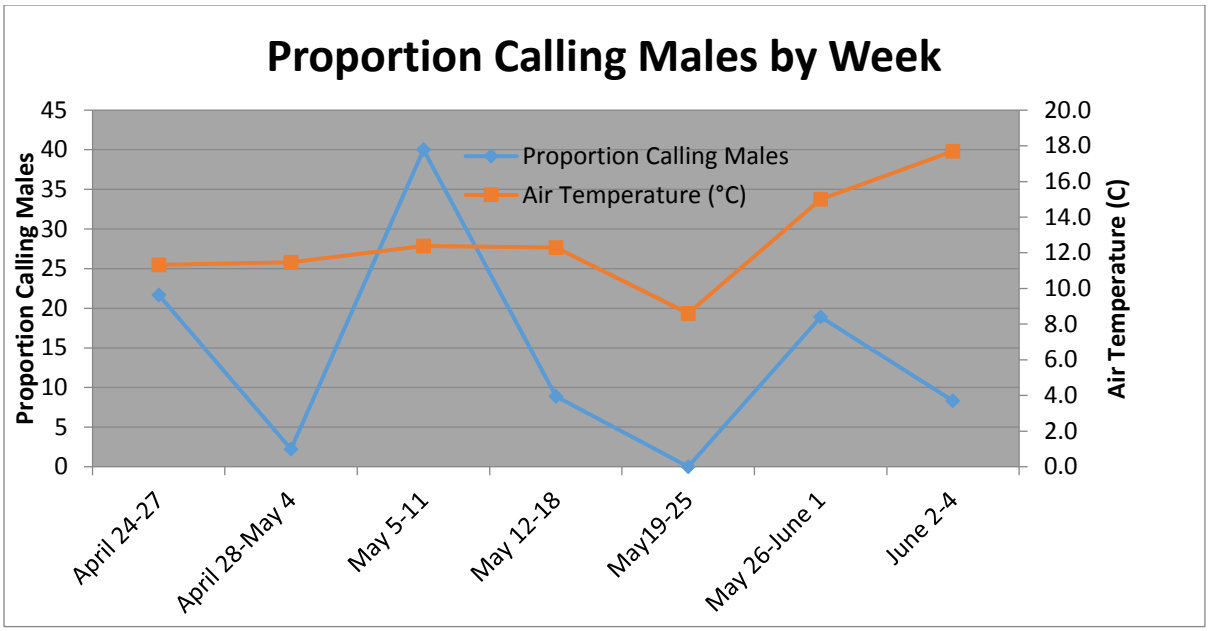

Figure 4. Calling activity at the CVWMA for spring 2013 with mean air temperatures for the week (measured by surveyors at the beginning of NCS)

At Bummer's Flats in spring of 2013, 5:46 person-hours were spent on NCS conducted by our team; n=3 surveys on June 6. Surveys were conducted at North Bummer's, South Bummer's and at Todd's pond on private land north of Bummer's Flats, across the river. No calling was detected.

3.3 Songmeters

Due to time constraints for analysis and the fact that the Songmeter filter/recognizer we had built did not function as had been anticipated (resulting in an enormous number of false positives), it was not possible to complete the analysis of the large amount of data collected during the peak of the spring breeding season. However, the full length of the recording for the experimental unit set up in the EDLNA pond in early spring to determine when calling began was analyzed and at least 1 LIPI call was detected on the recording the first night it was set up, on April 2.

3.4 Egg Mass Surveys (EMS)

In the CVWMA, there were 167:42 person-hours (n=26 surveys) dedicated to EMS at WDLNA (n=17 surveys), EDLNA ponds (n=6 surveys), East ditch (n=2 surveys) and Pumphouse channel (n=1 survey) between April 18 and June 5, 2013 (no EMS were conducted at Leach Lake as construction on the main dike prevented access for much of the spring season and water levels were very low due to the drawdown occurring). In total, there were 21 egg masses detected in the CVWMA (see Appendix 1 for map of egg mass locations). This amount of effort translates to a catch per effort of 0.13 egg masses per person-hour of survey-effort (Table 6). The greatest proportion of egg masses were detected at WDLNA, where 19 egg masses were found, (May 1 (n=6), May 6 (n=2), May 7 (n=1), May 9 (n=4), May 14 (n=1), May 15 (n=1), May 21 (n=3) and May 23 (n=1). There were 2 egg masses detected at EDLNA ponds, both on May 10.

Table 6. Summary of EMS efforts in the CVWMA for 2013

	EMS
Number of surveys	26
Survey effort (person-hours)	167:42
Number of LIPI observations	93
LIPI catch/effort	0.56
Number of egg masses detected	21
Egg mass catch/effort	0.13

Table 7 summarizes the LIPI observations by stage and detection type during EMS; although captures are not the main objective of the EMS there was a greater than normal amount of day time calling this year so many animals were captured. Of the 93 total observations, 40 were adults (38 male, 1 female, 1 sex unknown), 45 were juvenile (40 male, 0 female, 5 sex unknown), 2 were tadpoles and 6 were unknown age class (2 males and 4 unknown sex). Of the observations, 33 (35.5%) were captures, 28 (30.1%) had photos taken only and 32 (34.4%) were visuals. A map of LIPI observations is provided in Appendices 2-3.

Table 7. EMS LIPI observations by stage and detection code at CVWMA in 2013

Detection code	adult	juvenile	tadpoles	Unknown*	total
Capture	21	9	2	1	33
Photo only	9	19	0	0	28
Visual (no photo)	10	17	0	5	32
Total	40	45	2	6	93

* Visual detection (not in hand) therefore unable to determine if adult or juvenile

Table 8 provides a detailed summary of egg masses detected. Of the 21 egg masses detected, 16 were hatched out when found. Of the 15 detected before hatch-out, 11 appeared to be in good health (99% fertilized), 3 were not as well fertilized (75-80-90% fertilized) and one did not hatch-out (EM130501-CS06). The reason for the poor hatch out in EM130501-CS06 is unknown however it was not believed to be related to the caging process since it was not caged until part way through development at which time it was noted that the embryos did not appear to be developing; it was caged anyway to determine the hatching success but less than 10 hatchlings emerged.

While egg mass volume data is still being collected upon detection, it is no longer being reported since it is not possible to gather any meaningful conclusions by comparing volumes of egg masses at different developmental stages.

Table 8. LIPI egg masses found in the CVWMA during the spring of 2013

Egg Mass #*	~Date Laid**	Site	Health	Comments
EM130510-BH14	26/04/2013	EDLNA-pond	Good	Hatched when found
EM130507-BH09	27/04/2013	WDLNA	Good	Hatched when found
EM130501-BH01	29/04/2013	WDLNA	Good	99% fertilized
EM130501-CS02	29/04/2013	WDLNA	Good	99% fertilized
EM130501-TH03	29/04/2013	WDLNA	Good	99% fertilized
EM130501-TH04	29/04/2013	WDLNA	Good	99% fertilized
EM130501-BH05	29/04/2013	WDLNA	Good	99% fertilized
EM130501-CS06	30/04/2013	WDLNA	Poor	did not hatch
EM130509-BH11	01/05/2013	WDLNA	Fair	75% fertilized
EM130506-BH08	02/05/2013	WDLNA	Good	99% fertilized
EM130509-TH13	02/05/2013	WDLNA	Good	Hatched when found
EM130506-BH07	03/05/2013	WDLNA	Good	99% fertilized
EM130514-BH16	04/05/2013	WDLNA	Good	Hatched when found
EM130509-CS12	06/05/2013	WDLNA	Fair	only 80% fertilized
EM130509-CS10	07/05/2013	WDLNA	Good	99% fertilized
EM130510-BH15	07/05/2013	EDLNA-pond	Good	99% fertilized
EM130521-CS20	09/05/2013	WDLNA	Good	Hatched when found
EM130515-CS17	14/05/2013	WDLNA	Good	90% fertilized
EM130521-JM18	14/05/2013	WDLNA	Good	Hatched when found
EM130521-BH19	19/05/2013	WDLNA	Good	99% fertilized
EM130523-JM21	21/05/2013	WDLNA	Good	99% fertilized

*Number format is based on date of detection (YYMMDD)
**Date laid is approximated based on developmental stage of egg mass when detected

Air temperatures recorded hourly at DLNA by HOBO temperature data loggers show that the temperature for the surveyed spring breeding season (April 24-June 4) ranged from -3.85°C to 37.44 °C (mean=13.0°C, SD=7.2); the first major peak in air temperatures for the spring occurred on April 26, 2013 (27.9°C at 15:00; Figure 5). During the 7 days that followed this peak more than half of the egg masses detected for the year were deposited (12 of 21 or 57.1%), including 11 at WDLNA and 1 at EDLNA ponds. The greatest number of egg masses detected in one day was at WDLNA on May 1 when 6 egg masses were detected (an all-time record), with 5 of them estimated to have been laid on one night (April 29).

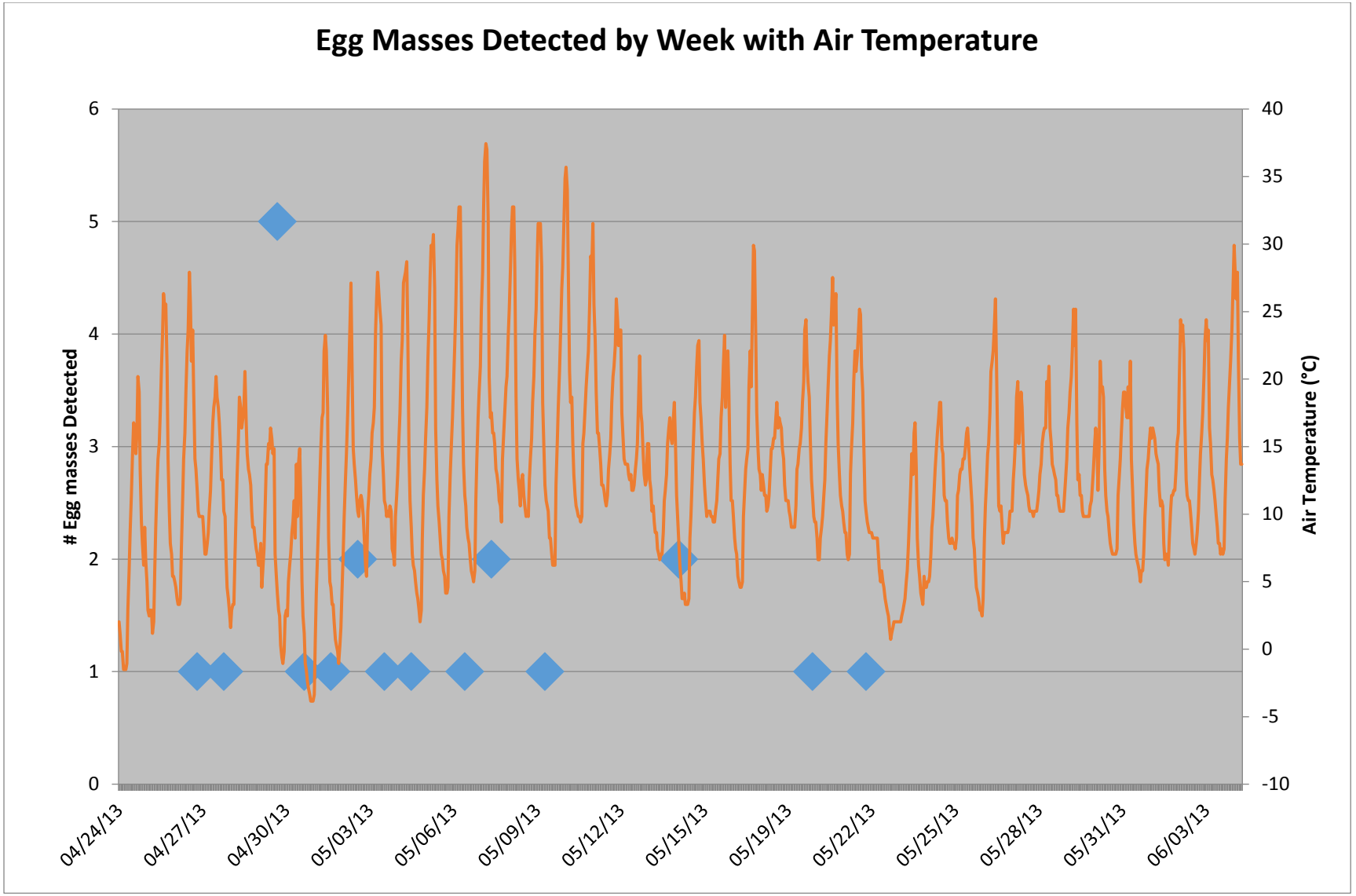

Figure 5. Number of detected egg masses by date laid (in blue) with hourly air temperature April 24 – June 4 (in orange).

It appears that the weekly mean air temperature (calculated from temperatures recorded at the beginning of NCS) at DLNA was fairly constant throughout the first 4 weeks of surveys (April 24-May 18) when 19 of the total 21 egg masses for the season were detected. After this, air temperatures dipped down during the week of May 19-25 and only 2 egg masses were detected, after which it climbed again during the last 2 weeks of surveys (May 26-June 4), during which time no egg masses were detected (Figure 6).

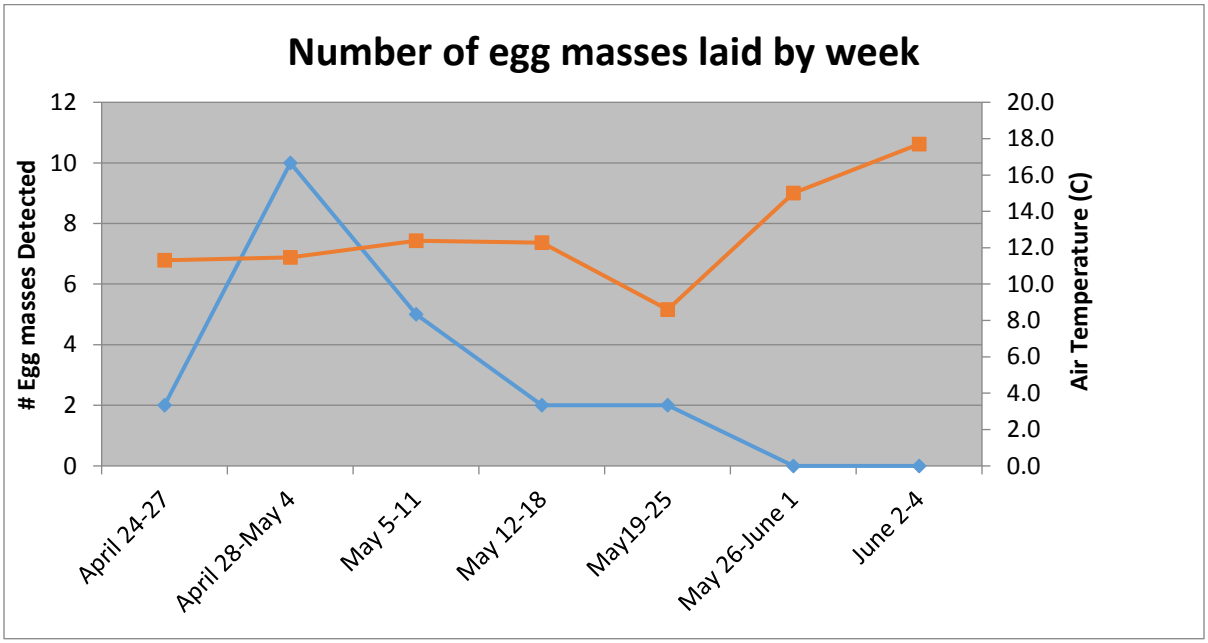

Figure 6. Number of detected egg masses by date laid (in blue) with mean air temperature for the week measured by surveyors at the beginning of NCS (in orange).

A summary of the number of egg masses detected by site each year from 2000 to 2013 is provided in Table 9. This year marked the third consecutive year of above average egg mass detections: 17 in 2011, 22 in 2012 (record) and 21 in 2013, compared to a mean of 8.2, SD 3.8, minimum 4, maximum 16 between 2000 and 2010.

Table 9. LIPI egg masses detected in the CVWMA from 2000-2013

Year	EDLNA pond	EDLNA ditch	WDLNA	Leach #4	Total
2000	8	0	8	*	**16**
2001	12	*	0	*	**12**
2002	1	2	2	*	**5**
2003	4	0	2	*	**6**
2004	3	0	1	*	**4**
2005	0	0	4	3	**7**
2006	3	2	2	0	**7**
2007	3	1	4	5	**13**
2008	3	0	1	2	**6**
2009	4	0	3	0	**7**
2010	5	**	2	**	**7**
2011	2	0	15	**	**17**
2012	3	0	19	**	**22**
2013	2	0	19	**	**21**
Total	51	5	63	10	150

* indicates area not surveyed

**indicates no EMS in area because no calling detected during NCS

In Bummer's flats there were 2:42 person-hours spent looking for signs of wild breeding (egg masses or hatchlings) on May 27 at the North Bummers Flats release ditch. This was done prior to releasing the tadpoles brought from Creston as part of the reintroduction program to determine if the male detected calling (as part of a separate FWCP contract) in the spring was successful at breeding; no signs of wild breeding were detected.

3.5 Visual Encounter Surveys (VES)

In 2013 at the CVWMA 121:20 person-hours were dedicated to visual encounter surveys (n=51 surveys) throughout the year, Table 10 provides a summary of VES catch per effort by season. Appendices 2-11 contain maps of observations.

Table 10. Summary of VES efforts in the CVWMA for 2013

	Spring	Summer	Fall	Total
Number of surveys	19	13	19	51
Survey effort (person-hours)	31:03	34:53	55:24	121:20
Number of LIPI observations	123	33	39	195
LIPI catch/effort during NCS	4.0	1.0	0.7	1.6

Spring VES during the migration period accounted for 31:03 person-hours (n=19 surveys), the majority of this time was spent surveying the south shore of Duck Lake during both day and night surveys (n=14 surveys), but surveys were also done at Pumphouse channel (n=2 surveys) and the East ditch (n=2 surveys). These surveys were carried out between March 28 and April 19. During these spring VES, 123 LIPI observations were made, a catch per effort of 4 LIPI per person-hour of survey time. Of the total 123 observations made, 16 (8F/4M/4U) were adult, 72 were juvenile (17F/20M/35U) and 35 were unknown age class (and unknown sex) as they were visuals so surveyors were unable to verify specific age class and sex without having them in hand (Table 11). For the results of other survey types (road and trapping) during the spring migration period, see Section 3.7.

During summer 34:53 person-hours were spent on VES (n=13 surveys) from July 30 to September 20. Early summer surveys were done in known breeding ponds (*) to search for YOY and late summer surveys focused on searching for all age classes in the migration corridors and overwintering areas. Surveyed areas include: WDLNA*, EDLNA ponds*, East ditch, old goat channel, Kootenay River east channel, Pumphouse channel, south-west ditch, Leach lake and frog-bear channel. During these summer VES, a total of 33 LIPI observations were made, a catch per effort of 1 LIPI per person-hour of surveying. Of the total 33 observations 4 were adult (2M/2U) and 29 were YOY of unknown sex since it is impossible to sex recently metamorphosed YOY by visual inspection (Table 11).

During fall VES a total of 55:24 person hours (n=19 surveys) were spent surveying for LIPI in the migration corridors and over-wintering areas between Sept 21 and October 29. Areas surveyed include: old goat channel, Kootenay River east channel, Pumphouse channel, south-west ditch, frog-bear channel, Duck Lake (south shoreline) and south shore of Duck Lake dike within DLNA. During fall VES, a total of 39 LIPI observations were made, a catch per effort of 0.7 LIPI per person-hour of survey effort. Of the total 39 observations, 26 were adults (7F/13M/6U), 11 were YOY (1M/10U) and 2 were unknown age class and sex (Table 11).

Table 11. LIPI observations by stage and season during VES at CVWMA in 2013 (sex is noted in brackets below totals)

	adult	juvenile	YOY	unknown	total
Spring	16 (8F/4M/4U)	72 (17F/20M/35U)	0 n/a	35 (35U)	123 (25F/24M/74U)
Summer	4 (2M/2U)	0 n/a	29 (29U)	0 n/a	33 (2M/31U)
Fall	26 (7F/13M/6U)	0 n/a	11 (1M/10U)	2 (2U)	39 (7F/14M/18U)
Total	46 (15F/19M/12U)	72 (17F/20M/35U)	40 (1M/39U)	37 (37U)	195 (32F/40M/123U)

Table 12 summarizes the LIPI observations made during VES throughout the year by stage and detection type. Of the 195 total observations, 69 (35.4%) were captured (32 adult, 29 juvenile and 8 YOY), 18 (9.2%) had photos taken only (2 adults, 15 juvenile and 1 unknown stage) and 108 (55.4%) were visuals (12 adult, 28 juvenile, 32 YOY and 36 unknown stage).

Table 12. 2013 VES LIPI observations by stage and detection code at CVWMA

Detection code	adult	juvenile	YOY	unknown	total
Capture	32	29	8	0	69
Photo only	2	15	0	1	18
Visual (no photo)	12	28	32	36	108
Total	46	72	40	37	195

A total of 7:55 person-hours were spent on VES at Brisco by our crew, surveys were done on August 30 and September 26. On August 30, 10 YOY were detected (8 in the release pond and 2 on the river bank) and on September 26, 3 YOY were detected (1 in the release pond, 1 on the river bank and 1 in a small discrete pond south of the release pond below cabin (Appendix 8, 9).

At Bummer's Flats a total of 25:31 person hours were spent on visual encounter surveys throughout the year (n=12 surveys). Spring VES accounted for 17:43 person-hours (n=6 surveys), surveys were conducted on June 6 and 7. Two sites were surveyed: North Bummers Flats (station #6/release ditch, pond at station 7 and the north-south channel north of the access road) and The Nature Trust Cherry Creek Property (2 person FWCP crew provided to Penny Ohanjanian to follow up on her incidental visual detection of 1 LIPI during Songmeter maintenance on May 23); no LIPI were detected (Appendix 12). Summer-fall VES accounted for 10:30 person-hours (n=6 surveys) between August 9 and September 25 during which time North Bummers Flats (station #6/release ditch, south-west ditch, pond at station 7 and the north-south channel north of the access road) was surveyed to follow up on reintroduced tadpoles and ensure they had metamorphosed. Detections were made on each visit; all were YOY, Table 13 provides a summary by survey date and site, and a map of observations is provided in Appendix 11.

Table 13. LIPI YOY observations during summer-fall VES at North Bummers Flats

Date	N-S ditch	Release Ditch	South-west Ditch	Total
August 8	*	15	*	15
August 29	1	12	2	15
September 25	*	2	0	2
Total	1	29	2	32

*indicates area not surveyed on that specific date

3.6 Tadpole Trapping

In the CVWMA 11:29 person-hours were spent tadpole trapping (n=6 surveys). The primary focal areas were Leach Lake (ponds 1, 3 and 4) and the East ditch to determine if there was any evidence of breeding since no calling was detected during the spring breeding season (see map in Appendix 1 for trap locations). Tadpole trapping was also conducted at EDLNA ponds to determine the stage of developing tadpoles. No LIPI tadpoles were trapped at any of the sites, but tadpoles were observed free swimming at the EDLNA ponds.

Tadpole trapping was not conducted at the Brisco release site by our crew.

Tadpole trapping was not conducted at Bummers Flats but 1:29 person-hours of dip-netting was done on July 10; 5 tadpoles in good health were detected (Gosner stage 33-42).

3.7 Spring Migration Surveys

During the spring migration period a total of 161:58 person-hours were spent doing road surveys and trapping; 79 surveys were completed between 15 March and 19 April, 2013 (the majority of which were done by members of the BC-NLFRT and their crew). During this time 63 LIPI observations were made, a catch per effort of 0.4 LIPI per person-hour (Table 14). A map of observations during this period is provided in Appendix 3. Spring migration VES results from Duck Lake shoreline are summarized in Section 3.5.

Road surveys accounted for 140:54 person-hours of survey effort during 42 surveys between 15 March and 19 April, 2013. A total of 58 LIPI detections were made, a catch per effort of 0.4 LIPI/person-hour.

BC-NLFRT members and their crew spent 21:04 person-hours of survey time (n=37 surveys) between March 19 and 31 doing trapping, this included: checking camera traps, terrestrial drift nets with pitfall traps and aquatic fencing with minnow traps (Figure 7). There were 5 LIPI trapped in minnow traps, all juveniles (2F/1M/2U); trap line A2 trapped 3 LIPI (and 1 long-toed salamander) and trap line A5 trapped 2 LIPI. Of the trapping methods, the aquatic fencing with minnow traps was the only method to successfully trap LIPI during the time they were installed, no LIPI (or other species) were detected in the terrestrial drift net fencing with pitfalls or the camera traps.

Table 14. Summary of 2013 spring migration survey efforts in the CVWMA

	Road surveys*	Trapping**	Total
Number of surveys	42	37	79
Survey effort (person-hours)	140:54	21:04	161:58
Number of LIPI observations	58	5	63
LIPI catch/effort during NCS	0.4	0.2	0.4

*road survey results only; results of Duck Lake south shoreline surveys from spring migration period compiled separately as VES (reported in Section 3.5 of this report)
**trapping methods include: camera traps, terrestrial drift net with pitfalls and aquatic fencing with minnow traps

Figure 7. Trapping photos (clockwise from top left): Full terrestrial drift net with pitfall array leading into aquatic fencing with minnow traps located on Pumphouse channel west of the roadway; terrestrial drift net with pitfall; close up of pitfall trap with knotted string to allow small mammals inadvertently trapped to escape; terrestrial drift net fence with pitfall located on Pumphouse channel east of the roadway showing aquatic fencing with minnow traps in the background; close up of minnow trap; aquatic fencing with 4 minnow traps per transect.

Of the total 58 LIPI detections made during spring migration surveys, 7 were adults and 51 were juveniles (Table 15).

Table 15. LIPI observations by stage and detection code during spring road surveys

Detection code	adult	juvenile	total
Capture	7 (4F/3M)	14 (4F/5M/5U)	21
Photo only	0	35 (16F/16M/3U)	35
Visual (no photo)	0	2 (1M/1U)	2
Total	7 (4F/3M)	51 (20F/22M/9U)	58

In total, during the spring migration period, including all survey types (VES, road and trapping) there were a total of 186 LIPI observations. Of these, 118 had dorsal spot pattern photos and it was determined that there were107 unique individuals detected via photo recognition. Recapture events were between 1 and 6 days apart. The majority of the movement appears to have occurred within a few days, so it is likely the spring migration is more of a punctuated movement compared to the fall.

Other amphibian observations included long-toed salamanders *(Ambystoma macrodactylum),* Western toads *(Anaxyrus boreas)* and Pacific tree frogs *(Pseudacris regilla).*

3.8 Fall Migration Road Surveys

A total of 50:28 person-hours were spent on road surveys from September 5-26, when 12 surveys were completed. During this time there were 200 LIPI detections, a catch per effort of 4.0 LIPI per person-hour (Table 16).

Table 16. Summary of 2013 fall road survey efforts

	Road surveys
Number of surveys	12
Survey effort (person-hours)	50:28
Number of LIPI observations	200
LIPI catch/effort during NCS	4.0

Of the 200 LIPI observations made during fall road surveys, 58 were adults and 142 were YOY (Table 17).

Table 17. LIPI observations by stage and detection code during 2013 fall road surveys

Detection code	adult	YOY	total
Capture	39 (30F/8M/1U)	54 (17M/37U)	93 (30F/25M/38U)
Photo only	6 (4F/1M/1U)	45 (5M/40U)	51(4F/6M/41U)
Visual (no photo)	13 (13U)	43 (43U)	56 (56U)
Total	58 (34F/9M/15U)	142(22M/120U)	200(34F/31M/135U)

Of the 200 total LIPI detected on the road, 10 (5.0%) were road mortalities (Table 18). Other amphibian observations during road surveys included long-toed salamanders *(Ambystoma macrodactylum),* Western toads *(Anaxyrus boreas)* and Pacific tree frogs *(Pseudacris regilla).*

Table 18. Summary of LIPI detections during 2013 fall road surveys on Duck Lake dike

Stage	Total	Live	Dead	Proportion Live	Proportion Dead
Adult	58 (34F/9M/15U)	57 (33F/9M/15U)	1 (1F)	98.3%	1.7%
YOY	142 (22M/120U)	133 (22M/111U)	9 (9U)	93.7%	6.3%
Total	**200 (34F/31M/135U)**	**190 (33F/31M/126U)**	**10 (1F/9U)**	**95.0% (33F/31M/126U)**	**5.0% (1F/9U)**

Of the total 200 LIPI detections on the road, 144 had dorsal spot pattern photos and it was determined through photo recognition that of these, 105 were unique LIPI individuals (33 adults, 71 YOY and 1 unknown). Recaptures were seen between 2 and 5 times each and up to 11 days apart indicating that this is not a quick one-way movement across the road, LIPI are using the road. There were 24 unique individuals seen more than once (7 adults and 17 YOY). Most (18) recapture events were within a few meters, but overall the range was 78 – 378 m apart.

3.9 Animal Health

Of the captured LIPI whose health was assessed visually in the field (n=272), 79.4% were deemed to be in good health, 15.1% in fair health, 0 % in poor health and 5.5% were found dead (Table 19).

Table 19. Health assessment *of* LIPI field observations in the CVWMA (n=272)

	Good	Fair	Poor	Dead	Total
Spring	111	34	0	4	149
Summer	82	1	0	10	93
Fall	23	6	0	1	30
Total	216	41	0	15	272
Percentage	79.4%	15.1%	0%	5.5%	100%

Of the 41 live LIPI deemed to be in fair condition, there were 22 adults (1 female, 21 male) and 19 juvenile (6 female, 11 male and 2 unknown sex). chytridiomycosis was suspected in 19 (46.3%) of the live observations, 19 (46.3%) had non-life threatening wounds (cuts, digit abnormality, burst pupil, eye filled with blood, vascularisation of eye lid, broken leg, predator attack), 2 were in poor body condition (thin) and 1 was covered in a red-orange substance (which has never been observed before by the author); see photos in Figure 8.

Of the 15 dead LIPI detected, there were 2 adults (both female; 1 fall road mortality, 1 fall chytridiomycosis), 3 juvenile (1 male, 2 unknown sex; all found dead on road during spring but only one believed to be spring road mortality), 1 unknown age class found during the spring breeding season at WDLNA (likely adult based on size but due to advanced decomposition could not confirm sex or age class) and 9 YOY (unknown sex) all fall migration road mortality. See photos in Figure 8.

Figure 8. Photos of health issues (left to right from top): spring juvenile covered in red-orange substance (dorsal); ventral of previous; burst pupil in adult at WDLNA in spring; predator attack; missing distal portion of toe; missing distal portion of finger; decomposing dead LIPI in spring at WDLNA; dead adult female in fall at Old Goat Channel (chytridiomycosis confirmed).

Of the 272 LIPI observations which were visually inspected by surveyors, chytridiomycosis was suspected in 21 (7.7%) of the frogs; it was suspected in 5.9% of adults, 1.5% of juveniles, 0.4% of unknown age class (decomposing carcass so undetermined whether adult or large juvenile) and 0% of the YOY observations (Table 20). Symptoms the surveyors looked for to assess whether or not chytridiomycosis was suspected were redness, sloughing skin, lethargy, loss of righting-reflex and vascularization.

Table 20. Surveyor's determination if chytridiomycosis suspected by visual inspection of LIPI captured at CVWMA in 2013; includes dead specimens (2 of 15 Bd suspected)

	***Chytridiomycosis* Suspected**		
Stage	**No**	**Yes**	**Grand Total**
Adult	102 (37.4%)	16 (5.9%)	118 (43.2%)
Juvenile	87 (31.9%)	4 (1.5%)	91 (33.3%)
Unknown[a]	0	1 (0.4%)	1 (0.4%)
YOY	62 (22.8%)	0	62 (22.8%)
Grand Total	**251 (92.3%)**	**21 (7.7%)**	**272**
Percentage	**92.3%**	**7.7%**	**100%**

[a] unknown age class because dead carcass decomposing (most likely an adult)

Lab results indicate that 53.9% (n=103) of the northern leopard frogs which were swabbed (n=191) during the 2013 field season tested positive for *Batrachochytrium dendrobatidis (Bd)* DNA via PCR (Table 21). Especially important is the breakdown by age class: 62.5% of adults tested positive, 80.4% of juveniles tested positive and 4.6% of YOY tested positive.

Table 21. 2013 qPCR Results for *Bd* on LIPI in the CVWMA

Age class	Total # sampled	Total # positive	Total % positive
Adult	96	60	62.5%
Juvenile	51	41	80.4%
YOY	44	2	4.6%
Total	191	103	53.9%

Of the LIPI tested for *Bd* during the spring (n=95), 84.2% tested positive (n=80), including 88.6% of adults (n=39) and 80.4% (n=41) of juveniles. Of the LIPI tested for *Bd* during the summer (n=67), 10.5% tested positive (n=7), including 18.2% of adults (n=6) and 2.9% (n=1) of YOY. Of the LIPI tested for *Bd* during the fall (n=29), 55.2% tested positive

Table 22. Number (%) of swabs that tested positive for *Bd* out of 191 LIPI at CVWMA

Stage	Spring	Summer	Fall
Adult	39 of 44 (88.6%)	6 of 33 (18.2%)	15 of 19 (79.0%)
Juvenile	41 of 51 (80.4%)	*	*
YOY	*	1 of 34 (2.9%)	1 of 10 (10.0%)
Total	**80 of 95 (84.2%)**	**7 of 67 (10.5%)**	**16 of 29 (55.2%)**

Analysis of the lab results shows that visual inspection of LIPI in the field for overt symptoms of chytridiomycosis is not always indicative of Bd presence. In 50% (86 of 172) of the samples where chytridiomycosis was not suspected, LIPI tested positive for *Bd* even though no overt symptoms were observed during field inspection (Table 23). This indicates that chytridiomycosis does not always show obvious signs of infection, many animals that appear to be healthy, test positive for *Bd* via swabs, which reinforces the importance of adhering to strict safe-handling procedures and disinfection protocol.

Interestingly not all of the frogs that were suspected to have chytridiomycosis tested positive for *Bd* DNA via qPCR at the lab. Lab results indicate that of the 19 frogs that were suspected of having chytridiomycosis (because they were showing overt symptoms typically associated with the disease) only 17 (89.5%) tested positive, the other 2 (10.5%) actually tested negative. They were showing symptoms, but did not test positive for Bd; the cause of their symptoms is unknown.

Table 23. 2013 Results of field observer's health classification* compared to results of *Bd* lab testing for live LIPI captured in the CVWMA (n=191 total samples tested)

	Chytrid suspected: No (n=172)		Chytrid suspected: Yes (n=19)		
Stage	Bd lab test: Negative	Bd lab test: Positive	Bd lab test: Negative	Bd lab test: Positive	Total
Adult	35	45	1	15	96
Juvenile	9	39	1	2	51
YOY	42	2	0	0	44
Grand Total	**86**	**86**	**2**	**17**	**191**
% of category*	**50.0%**	**50.0%**	**10.5%**	**89.5%**	**n/a**
% of Total	**45.0%**	**45.0%**	**1.1%**	**8.9%**	**100.0%**

*2 categories: chytridiomycosis suspected yes or no

At the Bummers Flats release site, 6 YOY were swabbed for *Bd* and lab analysis by qPCR determined that 100% were negative, as suspected since no symptoms were observed.

At the Brisco release site, 6 YOY were swabbed for *Bd* and lab analysis by qPCR determined that 100% were negative, as suspected since no symptoms were observed.

3.10 Dorsal Spot Pattern Recognition and Recapture Information

Dorsal spot pattern recognition via photo analysis for the 2013 field season was completed and a total of 53 recaptured individuals were identified, these include: 51 at CVWMA and 2 at Columbia Marsh. Each individual was captured between 2 and 5 times (mean=2.45; SD=0.7) during the field season. All recaptured individuals were detected within the same season and most were within the same site. There were no recaptures detected between-seasons. No between-year photo analysis was completed this year due to the extremely large number of capture photos for the year so it is unknown if there were any between-year recaptures. For a detailed Table of recapture results including weight, SVL and time between captures, see Appendix 13. Growth rate for the recaptured animals could be calculated from this data.

Of the 685 LIPI observations made in Creston during all surveys carried out in the 2013 field season, photos were taken of 400 observations which enabled dorsal spot pattern recognition via photo analysis. It was determined that of the 400 observations with photos, 325 were unique individuals (Table 24).

Table 24. LIPI unique individuals (recaptures removed) detected in the CVWMA in 2013

Age Class	Spring	Summer	Fall	Total
Adult	45	35	20	100
Juvenile	147	0	0	147
Unknown	0	0	1	1
YOY	0	68	9	77
Total	192	103	30	325

Of the 325 unique individuals detected in the CVWMA in 2013, the majority were in the spring (192 of 325; 59.1%). The majority of the spring observations (105 unique individuals; 54.7%) were made in the Duck Lake area (south shoreline of Duck Lake, Pumphouse channel or on the DLNA dike road) during the spring migration period, the remainder, 87 unique individuals, were detected in the spring breeding areas (East Ditch, EDLNA ponds and WDLNA) during NCS or EMS (Table 25.). It should be noted that since not all animals were captured caution should be used when drawing conclusions about the actual population size; however, information about the size of the *observed* male breeding population at DLNA, along with morphometrics for this group, and *observed* ratios of juvenile to adult calling males can be summarized.

By sex, the majority of the unique individuals detected in spring were male (123; 64.1%) and of these 81 were detected at the DLNA spring breeding area (East Ditch, EDLNA ponds and WDLNA; Table 25). While not all individuals in the population were caught, this indicates that the size of the *observed* male breeding population at the spring breeding area at DLNA, based on the number of unique individual males captured during EMS and NCS is 81, with an *observed* juvenile to adult ratio of 31 adults to 50 juveniles. Of these, size measurements were taken on a subset of 58 males (26 adults, 32 juveniles); average weight for this subset was 41.2 g and ranged from 18.0 – 69.0 g (n=58, SD=15.9) and average SVL was 70.6 mm and ranged from 53.8 – 85.8 mm (n=58, SD=9.8).

Table 25. Unique LIPI individuals detected in the CVWMA in spring of 2013 (n=192)

Age Class	Spring Migration Period	Spring Breeding Area	Total
Female	45 (9 adults, 36 juv)	1 (adult)	46 (10 adults, 36 juv)
Male	42 (3 adults, 39 juv)	81 (31 adult, 50 juv)	123 (34 adults, 89 juv)
Unknown	18 (1 adult, 17 juv)	5 (all juv)	23 (1 adult, 22 juv)
Total	105 (13 adults, 92 juv)	87 (32 adult, 55 juv)	192 (45 adult, 147 juv)

At the Columbia Marsh release site 2 LIPI were recaptures, each frog was seen twice. The first, RC 13034 was first observed on August 30 in the release pond and was observed again on September 26 on the river bank, 164 metres away at a bearing of 155 degrees (growth rate over 27 day period between capture events: mass 0.11 g/day and SVL 0.12 mm/day). The second recapture, RC 13035 was first observed on August 20 along the river bank and was observed again on September 26 on the river bank 93 metres away at a bearing of 181 degrees. Growth rate for RC13035 over the 27 day period between capture events was: mass 0.26 g/day and SVL 0.22 mm/day.

There were no recaptures detected at Bummers Flats this year.

3.11 Morphometrics of Creston LIPI

Table 26 and 27 summarize the size data, by season and age class for all LIPI captured and measured in the CVWMA during the 2013 field season and Figures 9 – 11 provide a visual representation.

Table 26. Weight of 2013 LIPI observations at CVWMA (recaptures, included)

Weight (grams)		n	mean	SD	range (min)	range (max)
spring						
(March 21-June 20)	all age classes	126	41.9	24.5	9.0	130.5
	juvenile	73	25.3	9.9	9.0	49.0
	adult	53	64.9	19.6	50.0	130.5
summer						
(June 21-Sept 20)	all age classes	77	47.4	25.5	3.0	106.0
	YOY	37	22.6	5.8	3.0	33.0
	adult	40	70.3	10.9	50.0	106.0
fall						
(Sept 21-Dec 20)	all age classes	30	54.8	29.5	9.0	98.0
	YOY	10	18.3	8.9	9.0	37.0
	adult	20	73.1	15.5	50.0	98.0

Table 27. SVL of 2013 LIPI observations at CVWMA (recaptures included)

SVL (mm)		n	mean	SD	range (min)	range (max)
spring						
(March 21-June 20)	all age classes	126	70.0	12.4	44.6	105.0
	juvenile	73	61.6	7.6	44.6	80.0
	adult	53	81.6	7.2	66.0	105.0
summer						
(June 21-Sept 20)	all age classes	77	70.7	13.3	30.4	90.0
	YOY	37	58.1	6.3	30.4	68.0
	adult	40	82.3	4.6	69.4	90.0
fall						
(Sept 21-Dec 20)	all age classes	30	72.3	15.7	41.1	92.0
	YOY	10	52.6	8.9	41.1	68.1
	adult	20	82.1	5.7	74.5	92.0

The size chart of spring 2013 LIPI observations (Figure 9) shows 3 distinct groupings:

1. Smaller animals (less than 35.0 g) which are mostly male. These represent the group born in 2012 (1 year old), most of the males will be too small to successfully breed but they do call at the breeding site.

2. Medium sized animals (43.0 - 77.0 g) which are all male. These represent the group of males which should be sexually mature and able to successfully breed.

3. Largest animals (75.0 - 130.5 g) which are all females and represent reproductively mature breeding females (likely all gravid).

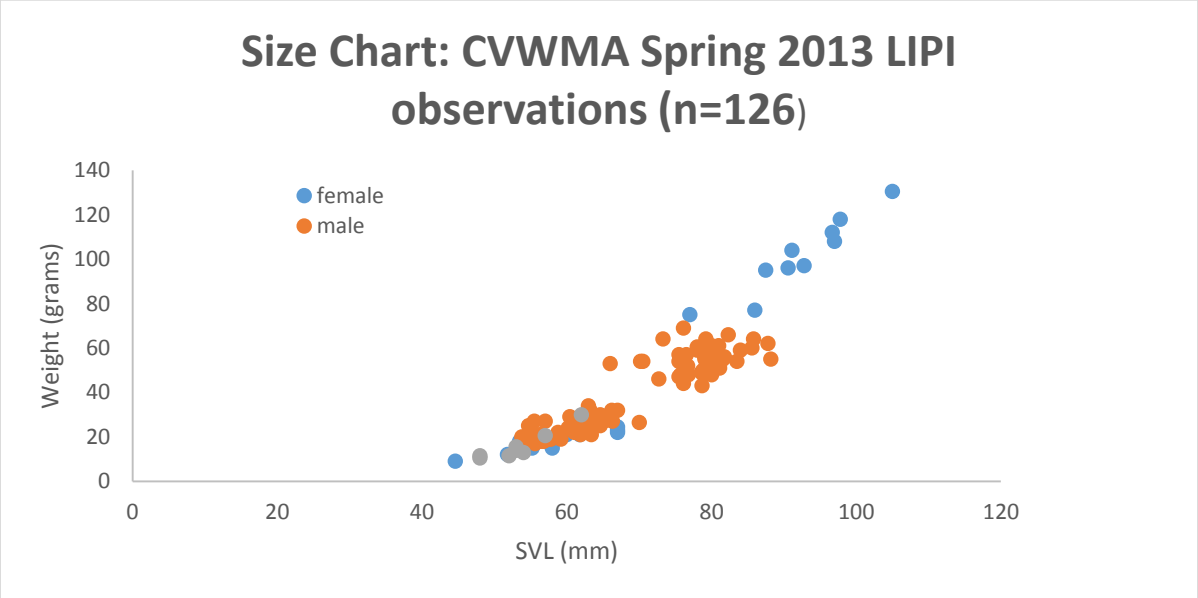

Figure 9. Size Chart (weight versus snout vent length) for CVWMA: Spring (March 21-June 20) 2013 LIPI captures. Recaptures included.

The size chart of summer 2013 LIPI observations (Figure 10) shows 2 main groupings:

1. The smaller animals (16.0 – 33.0 g, with the exception of one outlier, a 3.0 g YOY measured in July), these are the YOY. Of note is the fact that we were able to sex approximately half of the larger sized YOY (all greater than 20.0 g) as they had already developed the secondary sexual characteristic of swollen thumbs and nuptial pads; the remainder (who did not have the swollen thumbs) were classified as unknown sex as they could either be female, or simply males which had not yet developed this secondary sexual characteristic.

2. The larger animals (including both male and female) which are all 50 g and greater (up to 106.0 g).

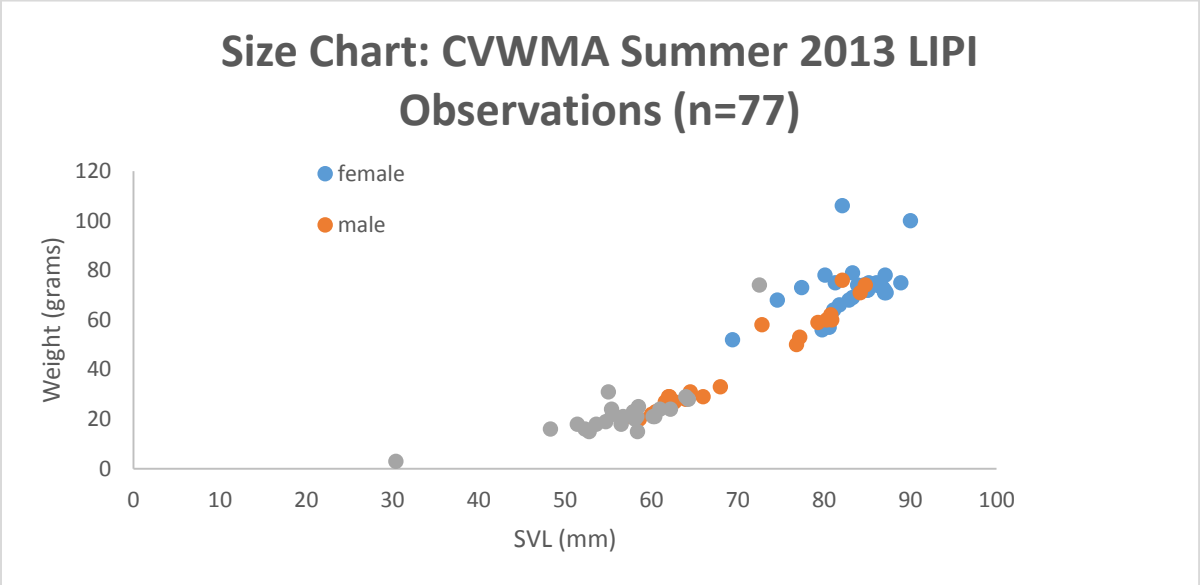

Figure 10. Size Chart (weight versus snout vent length) for CVWMA: Summer (June 21-Sept 20) 2013 LIPI captures. Recaptures included.

The size chart of fall 2013 LIPI observations at the CVWMA (Figure 11) shows 2 main groupings, those smaller than 50.0 grams, and those larger than 50.0 grams:

1. The animals less than 50.0 g are the YOY, the majority of which range in size from 9.0 – 26.0 g their first fall; only one animal is larger at 37.0 g, which may be a larger than average YOY or perhaps a smaller than average 1 year old.

2. The animals equal to or greater than 50.0 g (50.0 – 98.0 g), seem to fall into 2 subsets:
    a. The group of predominantly male animals which are smaller in size, ranging in weight from 50.0 -74.0 g (except 1 female at 72.0 g).
    b. The group of predominantly female animals which are larger in size, ranging in weight from 85.0 – 98.0 g (except 1 male at 95.0 g).

As expected, since most amphibians exhibit female-biased sexual size dimorphism, the data shows that of the detections greater than or equal to 50.0 g made in the fall of 2013, the females are generally larger than males. Of the 6 female detections greater than 50.0 grams, there was only one less than 85.0 grams, compared to 12 of the 13 male detections which were less than 75.0 grams.

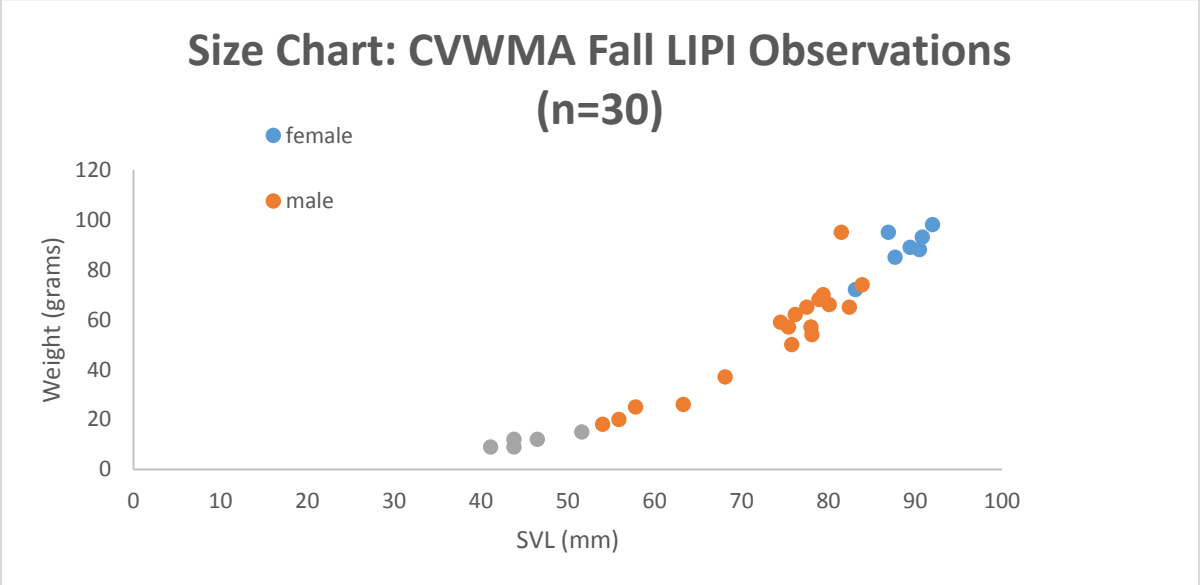

Figure 11. Size Chart (weight versus snout vent length) for CVWMA: Fall (Sept 21-Dec 20) 2013 LIPI captures. Recaptures included.

For additional data and a discussion on age classification based on weight, see the information paper entitled *FWCP Northern Leopard Frog Project: Summary of Select Data from 2006 -2011* written for the FWCP and the BC-NLFRT (Houston, 2013).

3.12 Captive Assurance Colony at Vancouver Aquarium

During the spring of 2013, the 5th year of the captive assurance colony, 50 tadpoles (5 from each of 10 egg masses) were collected from the wild for the Vancouver Aquarium to bolster the captive population. They were packaged up and driven to Vancouver by the author on June 13, 2013. All tadpoles were collected from the WDLNA breeding site from egg masses estimated to have been deposited between April 29 and May 21 (Table 28).

Table 28. Details of LIPI tadpoles transferred in 2013 to Vancouver Aquarium

Egg Mass Number	Breeding Site	Estimated Date Laid	# Tadpoles Transferred
EM130501-CS02	WDLNA	2013-04-29	5
EM130501-TH03	WDLNA	2013-04-29	5
EM130501-TH04	WDLNA	2013-04-29	5
EM130501-BH05	WDLNA	2013-04-29	5
EM130506-BH07	WDLNA	2013-05-03	5
EM130506-BH08	WDLNA	2013-05-02	5
EM130509-CS10	WDLNA	2013-05-07	5
EM130515-CS17	WDLNA	2013-05-15	5
EM130521-JM18	WDLNA	2013-05-14	5
EM130523-JM21	WDLNA	2013-05-21	5
TOTAL			50

3.13 Bummers Flats Reintroductions

This was the third year of phase 2 reintroductions at Bummer's Flats, during which time 8878 hatchlings were released at the North Bummers Flats Release Ditch (Appendix 10). This brings the total number of LIPI hatchlings released at the Bummer's Flats Release Ditch to date during Phase 2 to 21,426.

3.14 Columbia Marsh Reintroduction

During the first year of reintroductions at the Brisco release site within the Columbia Marshes, approximately 2000 hatchling tadpoles were released from the Vancouver Aquarium Captive Assurance Colony. This marks the first year LIPI from the assurance colony were successfully bred. Hatchling tadpoles were flown to Cranbrook where they were picked up by BC-NLFRT members, driven to the release site at Brisco, acclimatized, held overnight in cages in the wetland and counted and released the following day.

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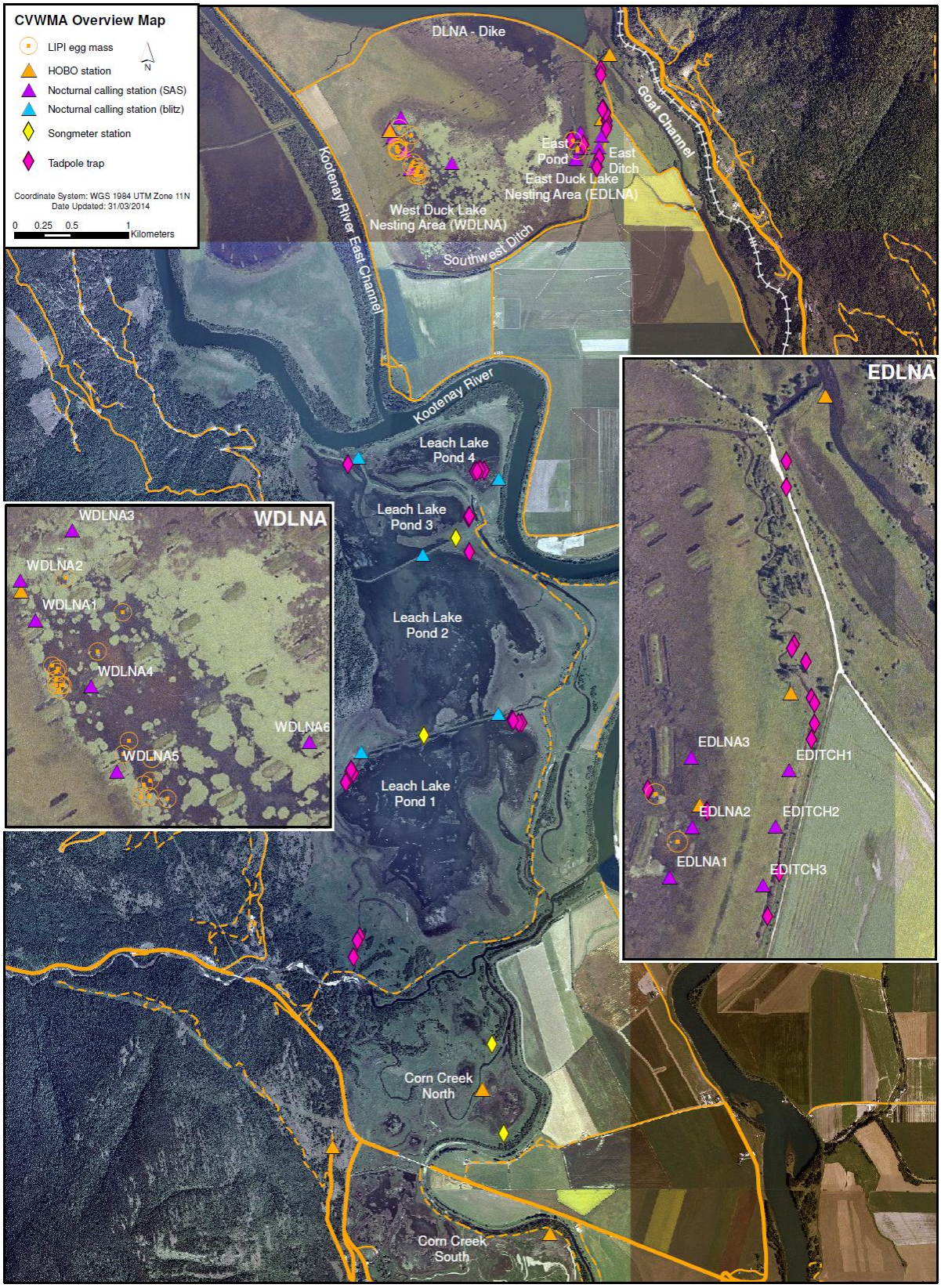
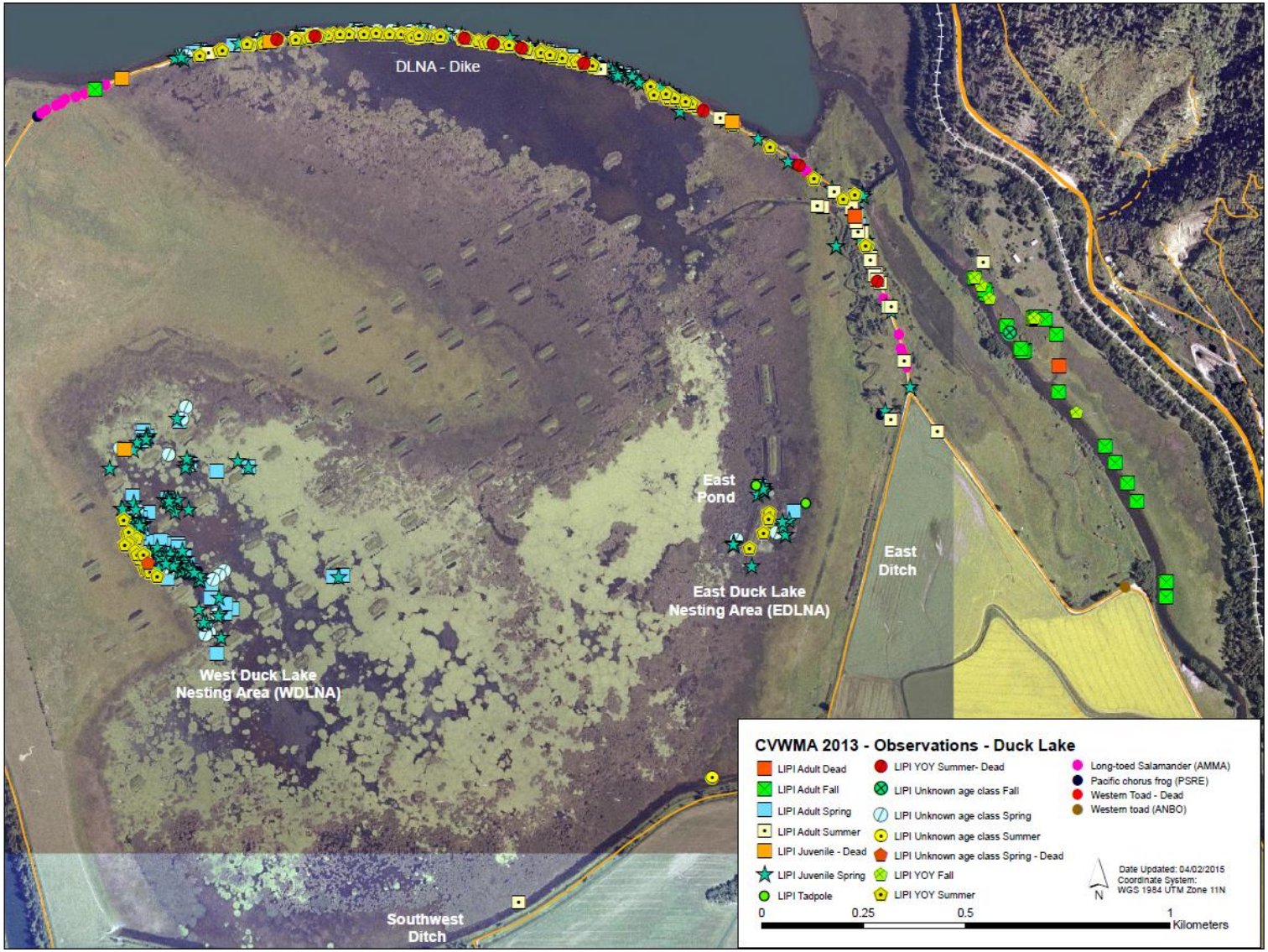
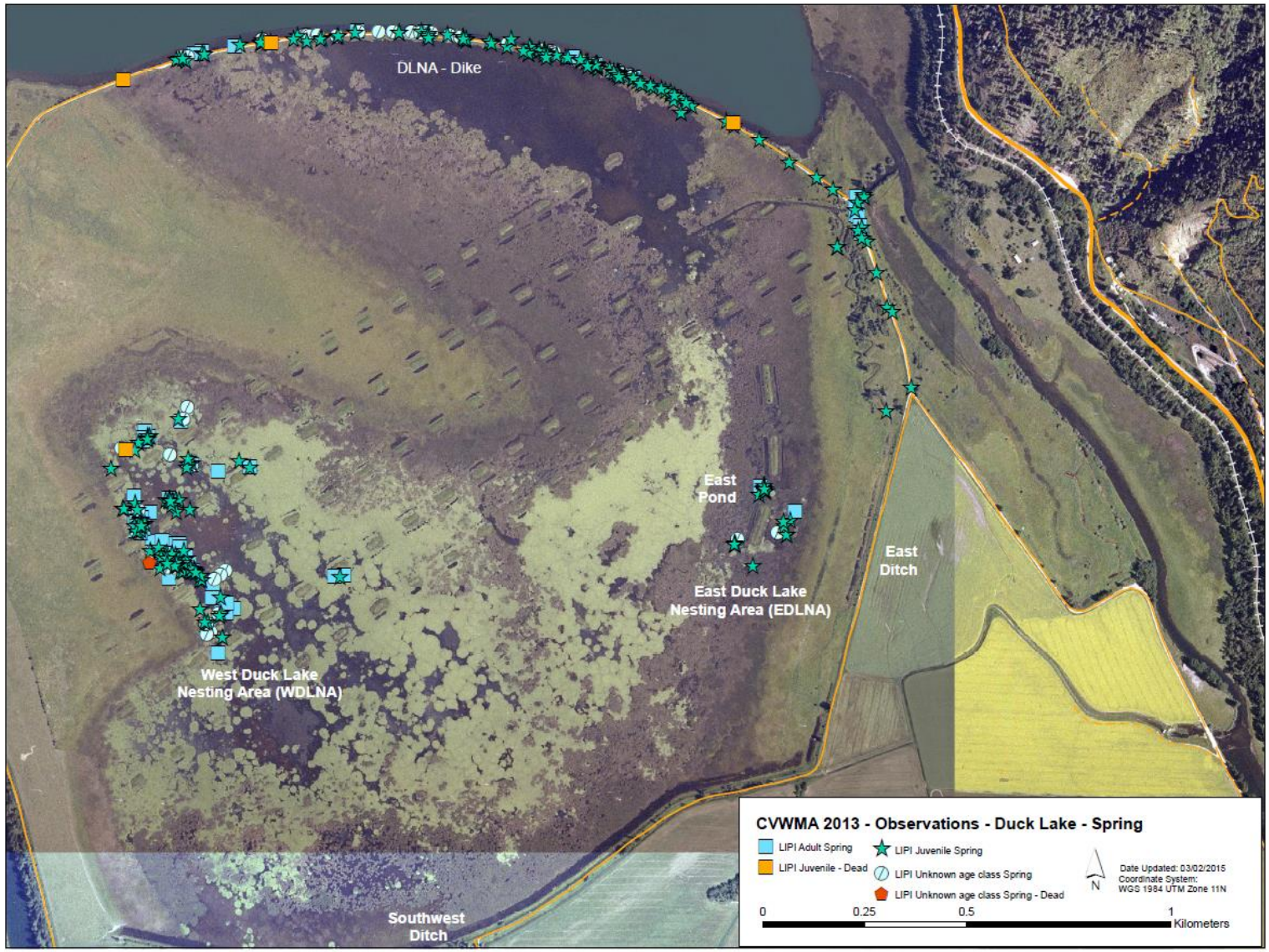
Appendix 1: CVWMA overview map



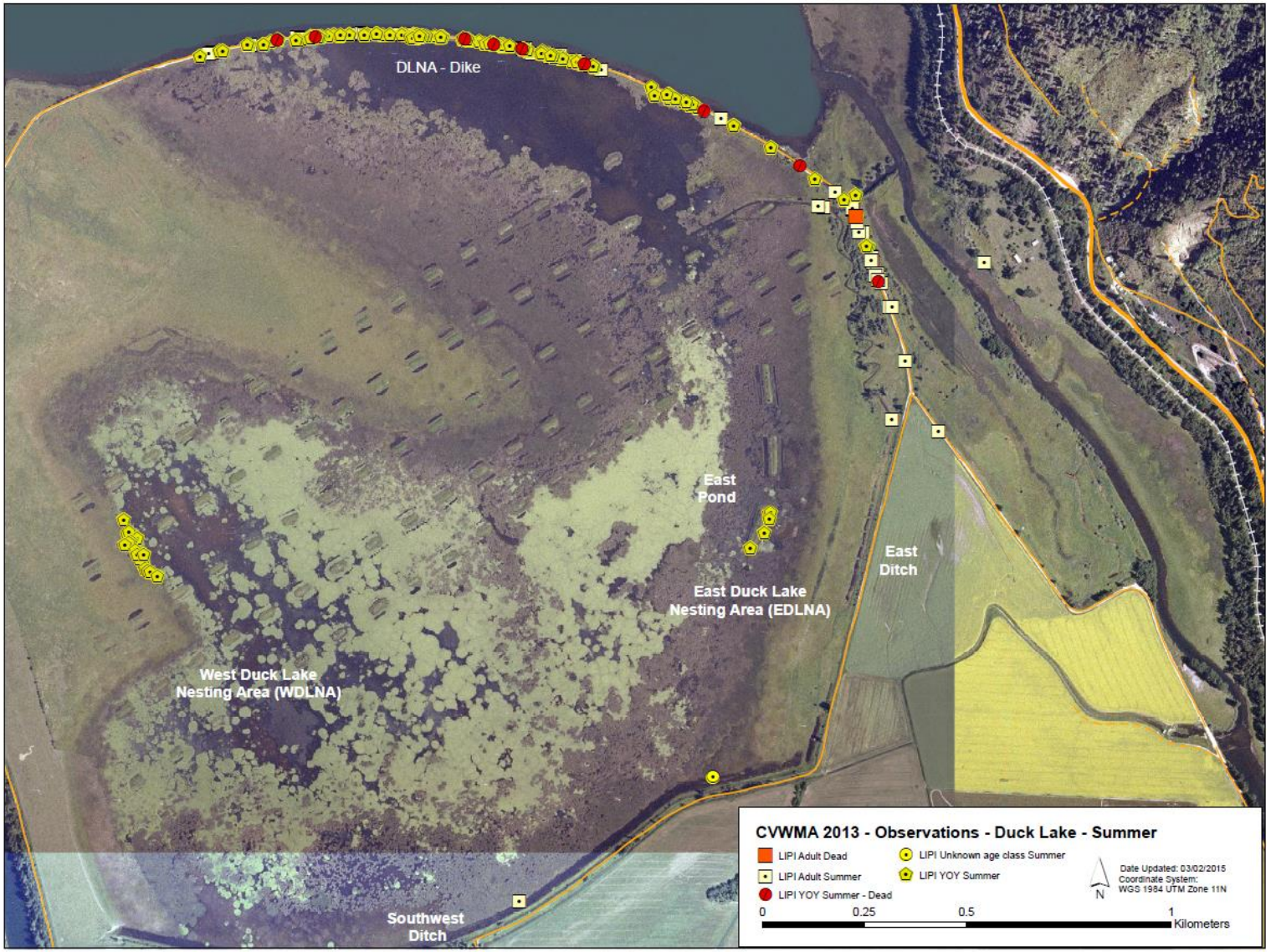
Appendix 2: Map of Duck Lake LIPI observations – all seasons



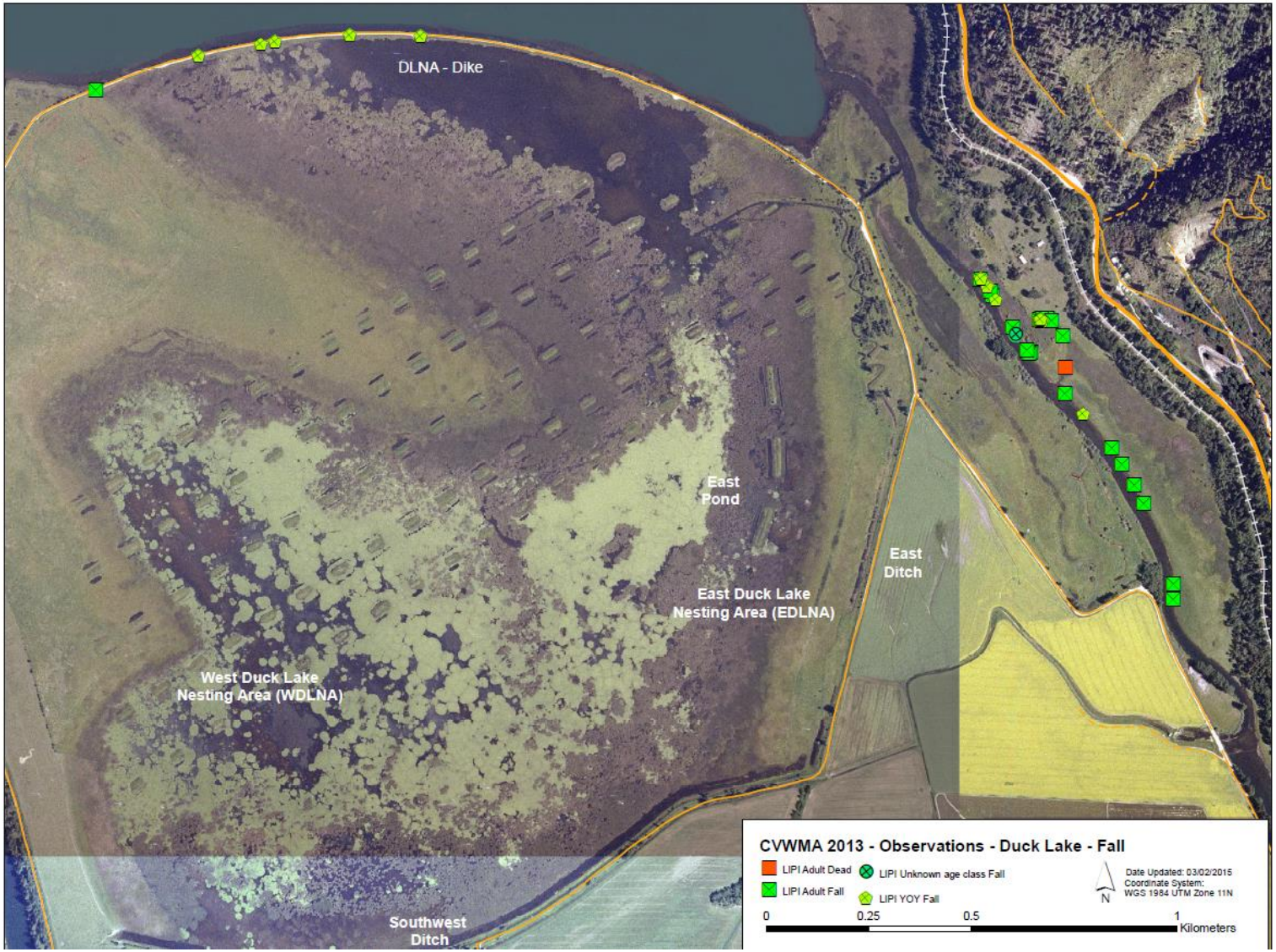
Appendix 3: Map of Duck Lake LIPI observations by season - spring



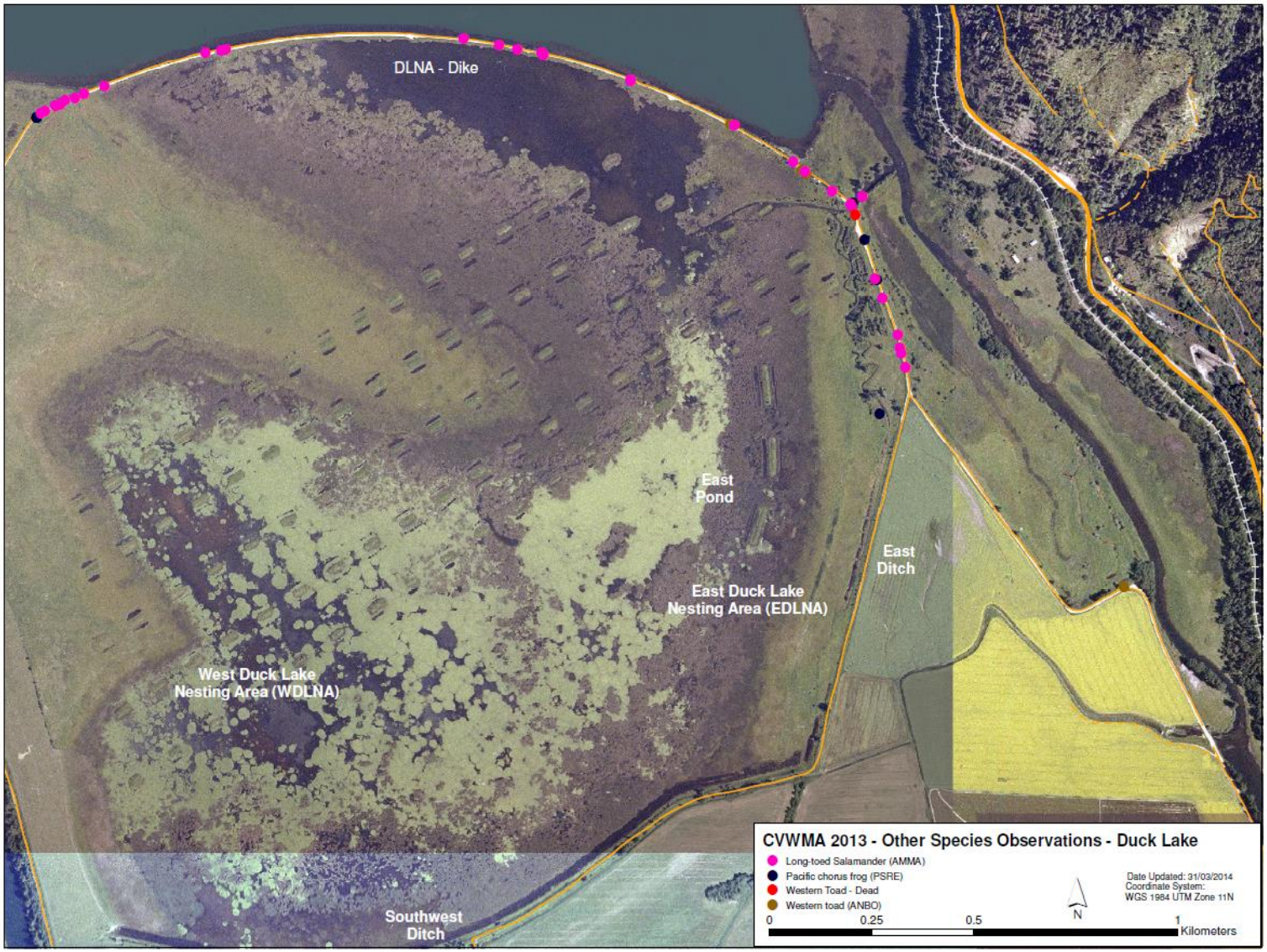
Appendix 4: Map of Duck Lake LIPI observations by season – summer



Appendix 5: Map of Duck Lake LIPI observations by season – fall

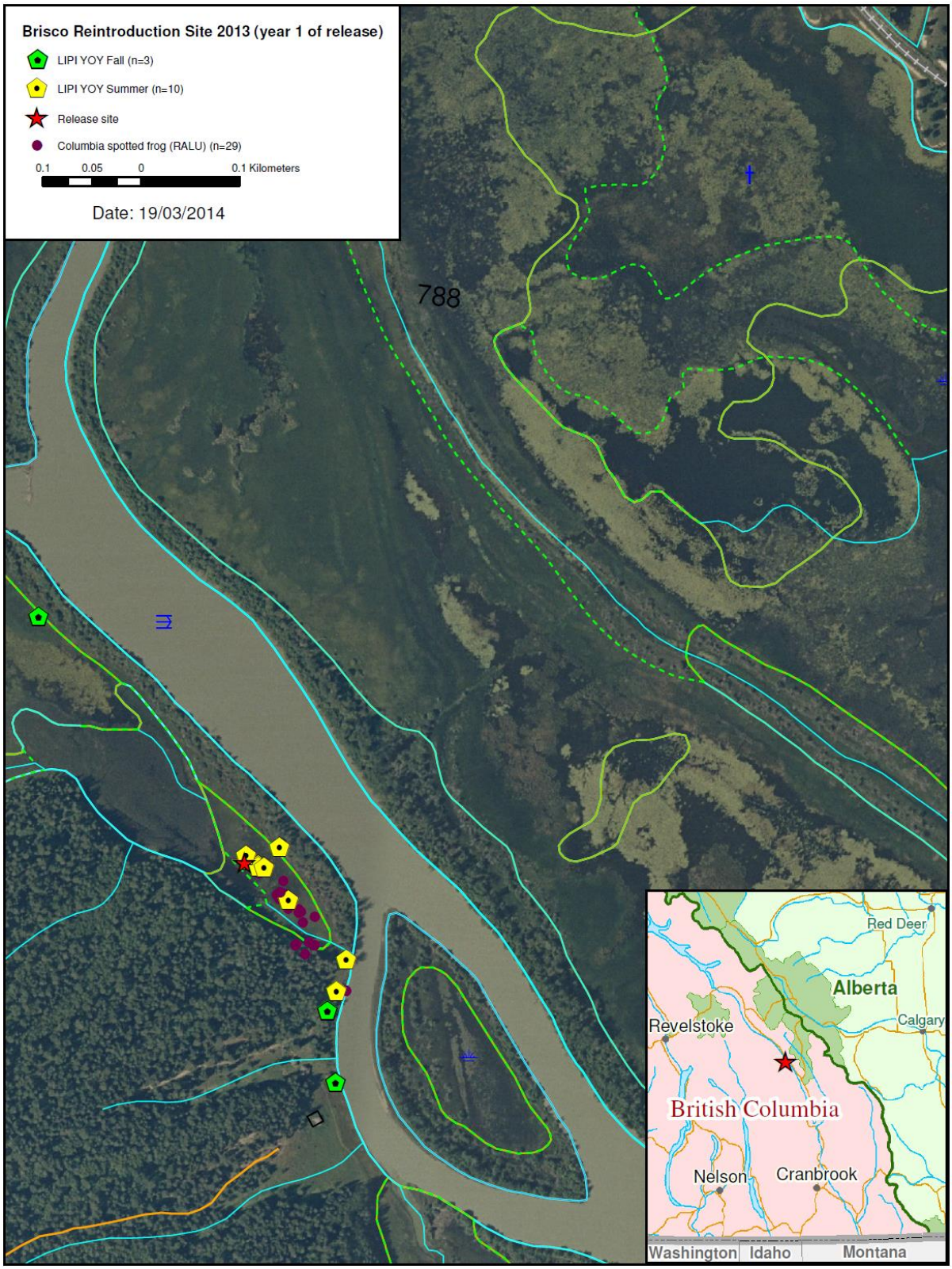


Appendix 6: Map of Other Herptile observations at Duck Lake

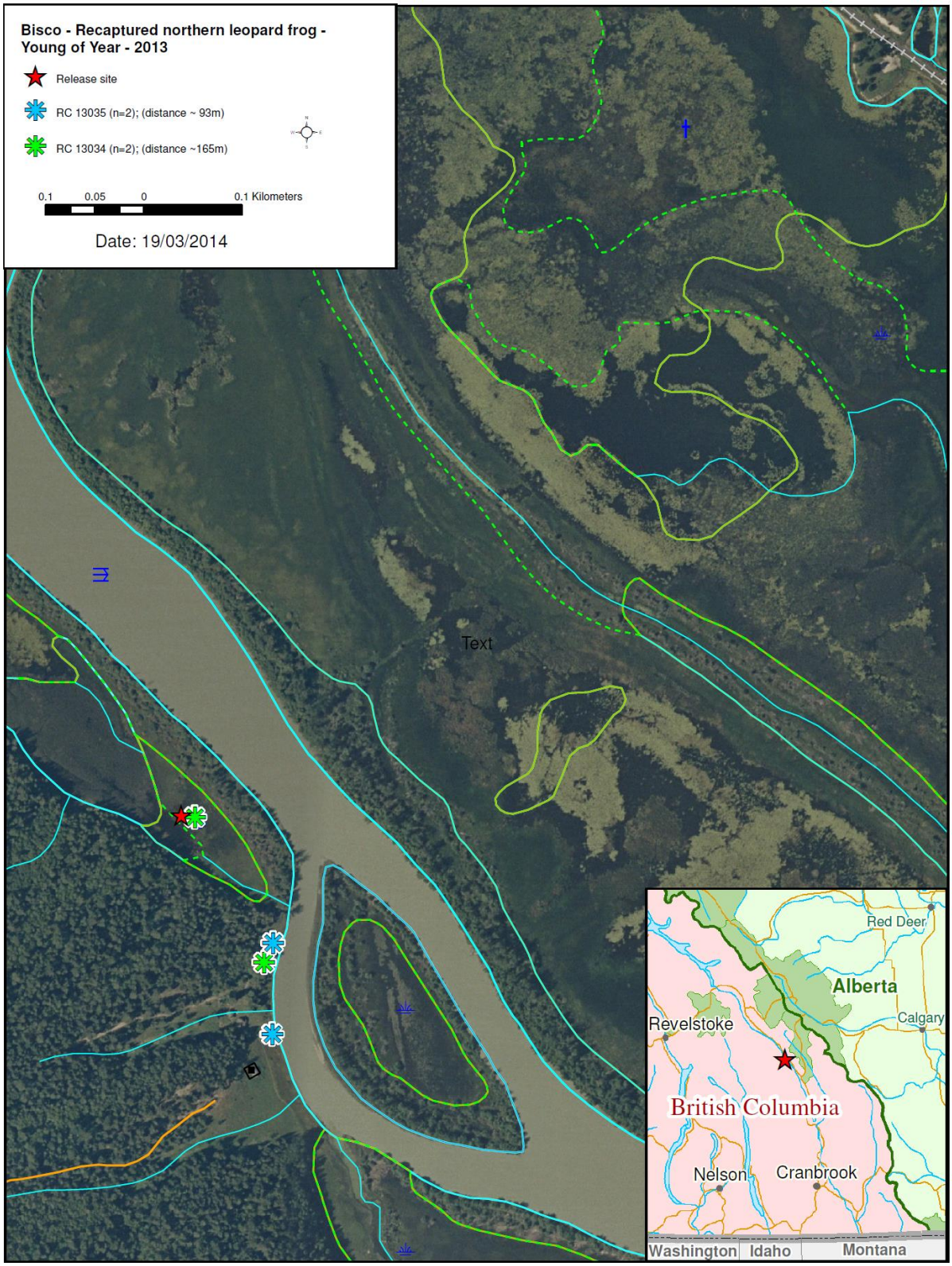


Appendix 7: Map of Leach Lake observations

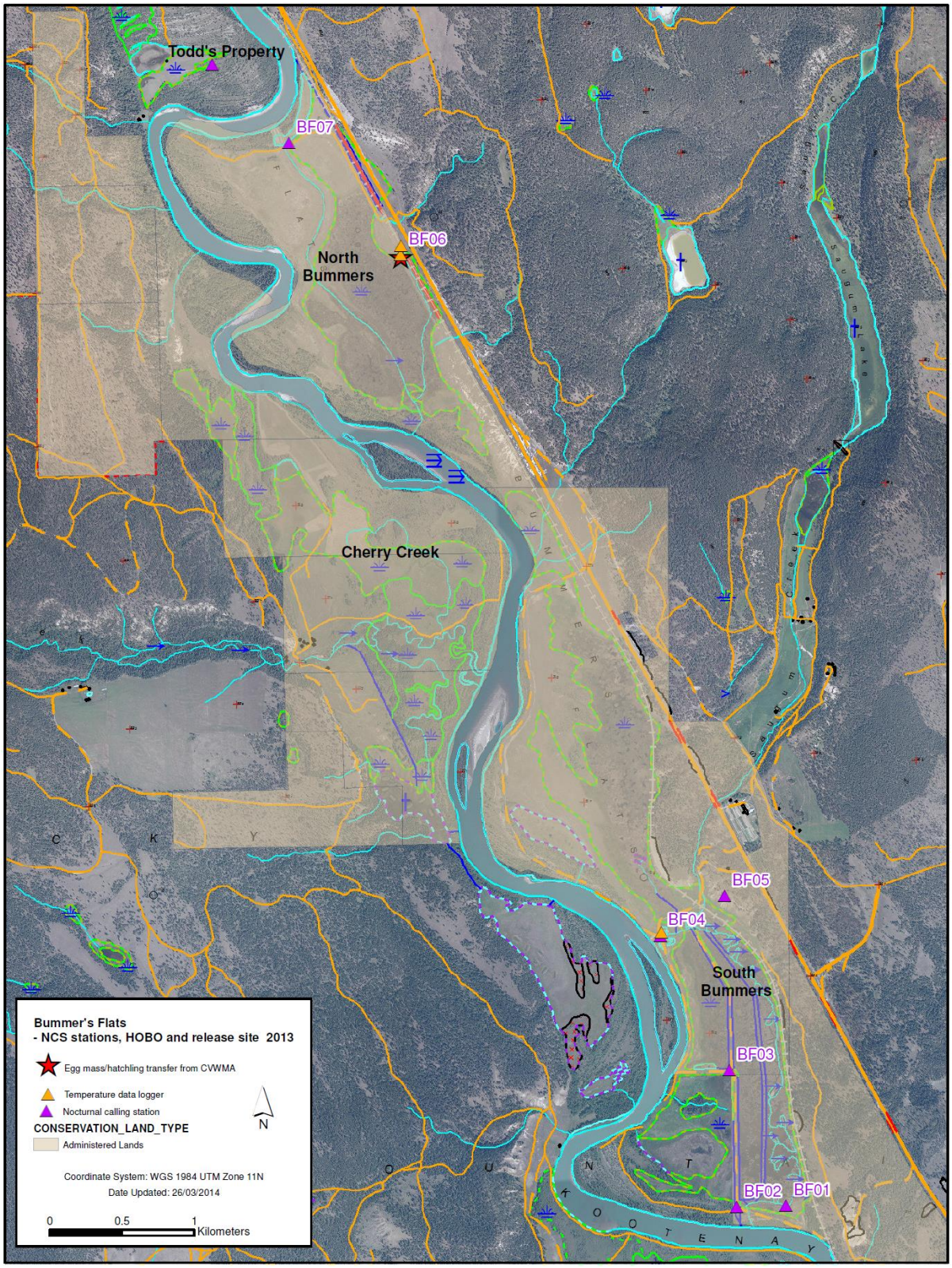
Appendix 8: Map of Columbia Marsh – Brisco observations



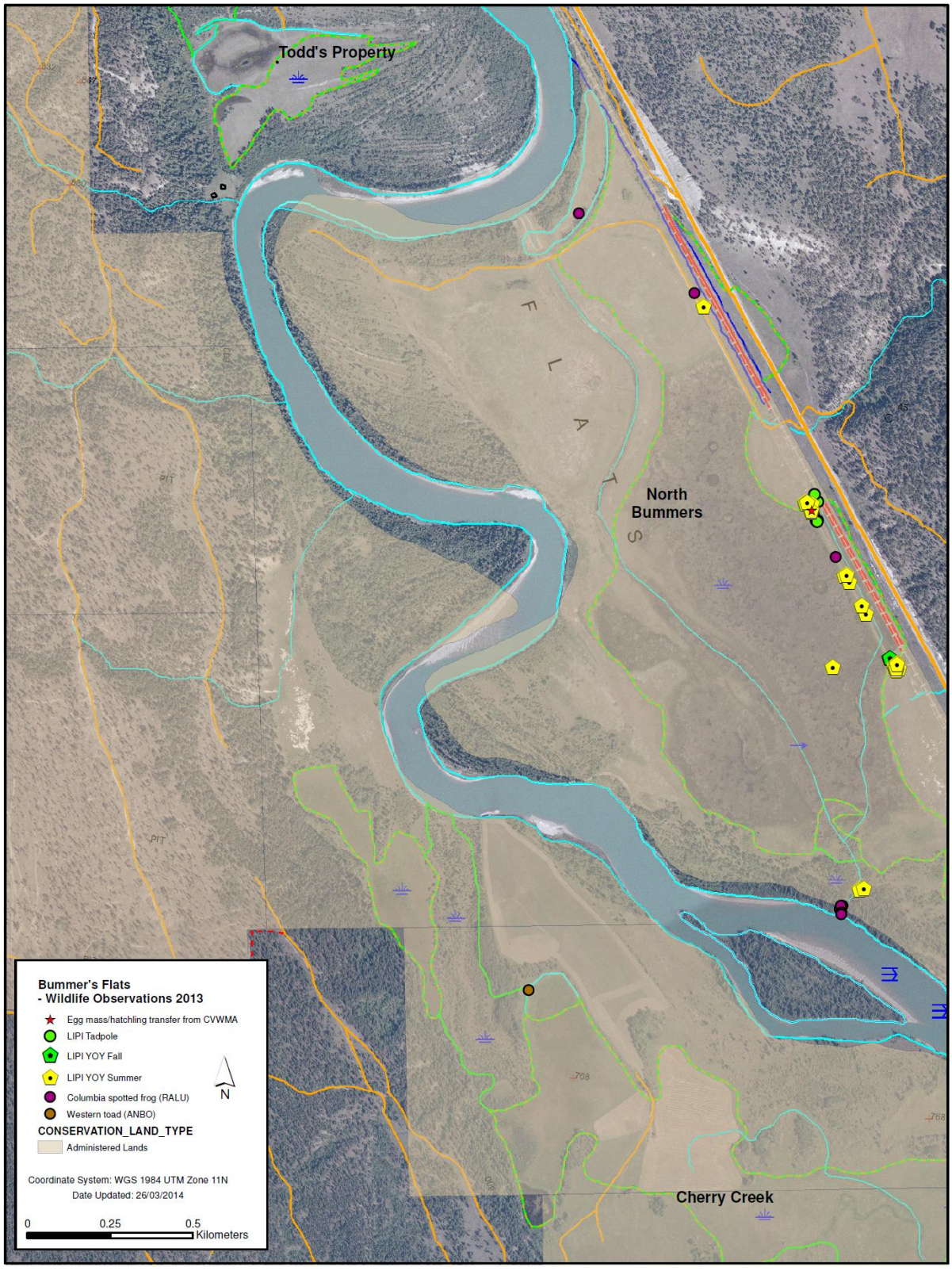
Appendix 9: Map of Columbia Marsh- Brisco LIPI recaptures



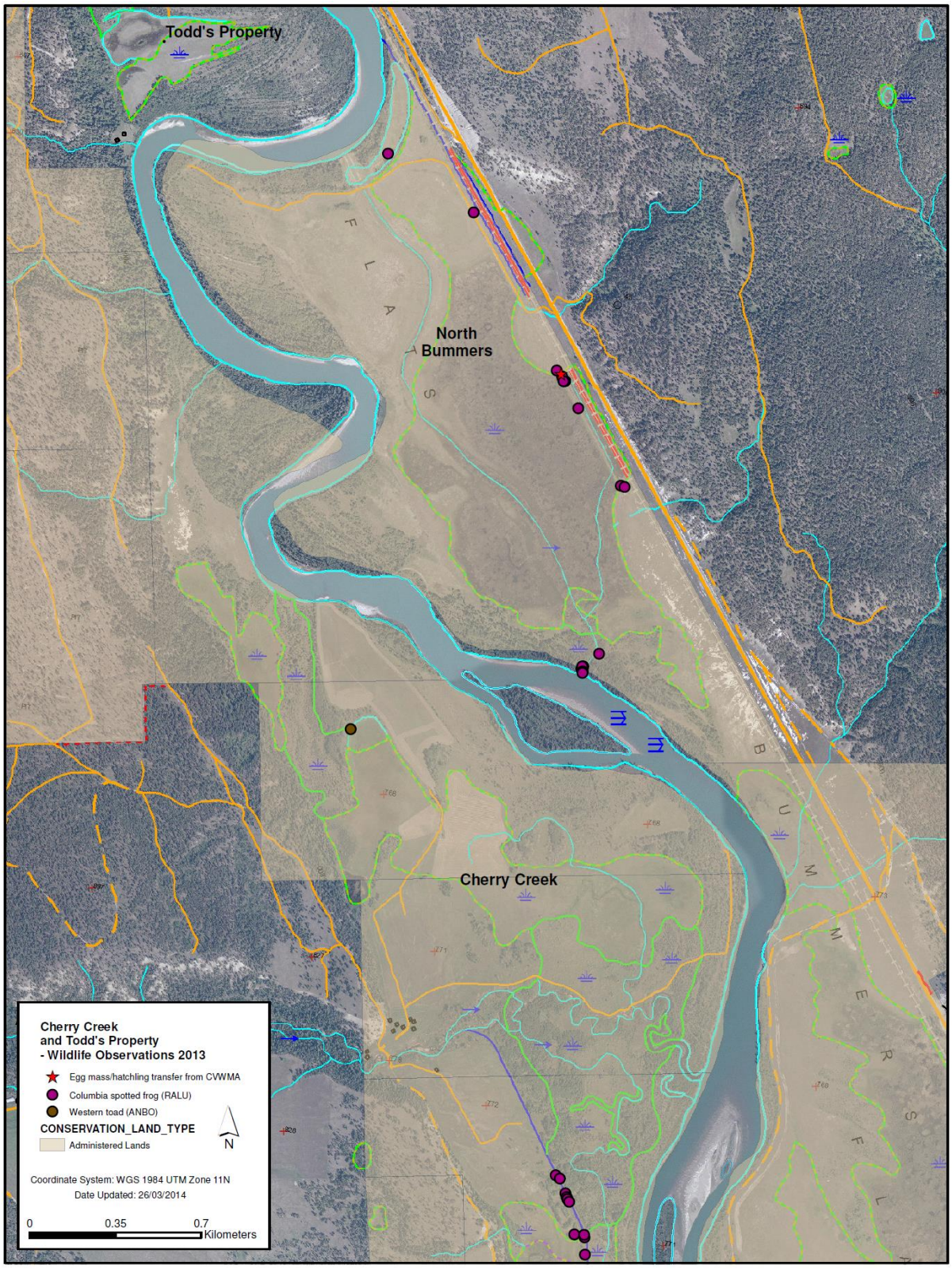
Appendix 10: Bummer's Flats overview map



Appendix 11: Map of Bummer's Flats observations



Appendix 12: Map of other Upper Kootenay Floodplain observations



Appendix 13. Table of recaptures identified by photo analysis for 2013 field season at all sites surveyed (CVWMA, Bummers Flats and Columbia Marsh reintroduction site)

Site[1]	Frog_Id[2]	Obs_Number[3]	Stage[4]	Sex[5]	Weight[6]	SVL[7]	Code[8]
Duck Lake	RC 13001	C130329-LAI07	Juv	M	32	67	Ws
road	RC 13001	C130330-LAI03	Juv	U	30	62	Ws
Duck Lake	RC 13002	C130331-BH05	Adult	M	66	82.3	Ws
Duck Lake	RC 13002	C130401-BH22	Adult	M	64	79.2	Ws
Duck Lake	RC 13003	C130331-BH14	Adult	F	108	97	Ws
Duck Lake	RC 13003	C130401-BH14	Adult	F	112	96.7	Ws
Duck Lake	RC 13004	C130331-BH15	Juv	M	26	61	Ws
Duck Lake	RC 13004	C130401-BH16	Juv	M			Ws
Duck Lake	RC 13005	C130331-BH16	Juv	F	24	61.5	Ws
Duck Lake	RC 13005	C130401-BH19	Juv	F			Ws
Duck Lake	RC 13006	C130331-BH17	Adult	F	97	92.8	Ws
Duck Lake	RC 13006	C130401-BH08	Adult	F	96	90.6	Ws
Duck Lake	RC 13006	C130402-BH08	Adult	U			Ws
Duck Lake	RC 13007	C130331-BH18	Juv	M	22	61.1	Ws
Duck Lake	RC 13007	C130401-BH10	Juv	M	21	55.5	Ws
Duck Lake	RC 13008	C130328-LR17	Juv	F	n/a	61	Ws
Duck Lake	RC 13008	C130331-BH10	Juv	F	17	54.7	Ws
road	RC 13008	C130402-MAB02	Juv	F			Ws
Duck Lake	RC 13009	C130328-LR30	Adult	M	n/a	67	Ws
Duck Lake	RC 13009	C130401-BH06	Juv	M			Ws
road	RC 13010	C130905-TH03	YOY	U	24	55.4	Ws
road	RC 13010	C130907-BH28	YOY	U			Ws
road	RC 13010	C130910-BH01	YOY	U			Ws
road	RC 13011	C130906-BH08	Adult	M	71	84.2	Ws
road	RC 13011	C130909-BH11	Adult	M	74	84.8	Ws
road	RC 13011	C130909-BH23	Adult	M			Ws
road	RC 13012	C130906-BH09	YOY	U	29	64	Ws
road	RC 13012	C130907-BH15	YOY	M	29	66	Ws
road	RC 13012	C130909-BH22	YOY	U			Ws
road	RC 13012	C130909-CS08	YOY	M	29	62.1	Ws
road	RC 13012	C130910-BH07	YOY	U			Ws
road	RC 13013	C130907-BH09	Adult	F	74	84.6	Ws
road	RC 13013	C130908-BH06	Adult	F	74	86.1	Ws
road	RC 13013	C130909-CS03	Adult	F	75	81.3	Ws
road	RC 13014	C130907-BH11	Adult	F	56	79.8	Ws
road	RC 13014	C130907-BH27	Adult	F			Ws
road	RC 13015	C130907-BH16	YOY	M	27	61.6	Ws
road	RC 13015	C130907-BH25	YOY	M			Ws
road	RC 13015	C130909-BH13	YOY	M	28	64	Ws
road	RC 13015	C130909-BH20	YOY	U			Ws
road	RC 13016	C130907-BH23	Adult	F	75	85.2	Ws
road	RC 13016	C130908-BH10	Adult	F	73	86.8	Ws
road	RC 13016	C130909-CS12	Adult	F	73	77.4	Ws
road	RC 13017	C130907-BH24	YOY	U			Ws
road	RC 13017	C130911-TH16	YOY	U			Ws
road	RC 13018	C130907-BH30	Adult	F	72	85	Ws

road	RC 13018	C130908-BH01	Adult	F			Ws
road	RC 13018	C130908-BH14	Adult	F			Ws
road	RC 13019	C130908-BH04	YOY	M	23	58	Ws
road	RC 13019	C130909-CS01	YOY	M	23	60.5	Ws
road	RC 13019	C130910-BH03	YOY	U			Ws
road	RC 13019	C130911-TH03	YOY	U	25	58.5	Ws
road	RC 13020	C130908-BH08	Adult	F	75	88.9	Ws
road	RC 13020	C130909-CS04	Adult	F	74	83.9	Ws
road	RC 13020	C130912-TH05	Adult	F	79	83.3	Ws
road	RC 13021	C130909-CS02	YOY	U	28	64.3	Ws
road	RC 13021	C130911-TH04	YOY	U	28	n/a	Ws
road	RC 13022	C130909-BH07	YOY	U	21	60.2	Ws
road	RC 13022	C130911-TH22	YOY	U			Ws
road	RC 13023	C130909-CS06	YOY	M	29	62	Ws
road	RC 13023	C130920-BH14	YOY	M	33	68	Ws
road	RC 13024	C130909-BH12	YOY	U	18	56.5	Ws
road	RC 13024	C130912-TH08	YOY	U			Ws
road	RC 13025	C130909-CS10	YOY	U	16	52.3	Ws
road	RC 13025	C130911-TH14	YOY	U	16	n/a	Ws
road	RC 13026	C130910-BH05	Adult	F	64	81.1	Ws
road	RC 13026	C130910-BH15	Adult	F			Ws
road	RC 13027	C130910-BH06	YOY	U			Ws
road	RC 13027	C130910-BH13	YOY	U			Ws
road	RC 13027	C130911-TH08	YOY	U	19	n/a	Ws
road	RC 13028	C130910-BH11	YOY	U			Ws
road	RC 13028	C130912-TH19	YOY	U			Ws
road	RC 13029	C130911-TH10	YOY	U	23	n/a	Ws
road	RC 13029	C130912-TH10	YOY	U			Ws
road	RC 13030	C130912-TH16	YOY	U			Ws
road	RC 13030	C130912-TH24	YOY	U			Ws
road	RC 13030	C130920-BH11	YOY	M			Ws
road	RC 13031	C130920-BH08	YOY	M	22	60	Ws
road	RC 13031	C130920-BH13	YOY	U			Ws
road	RC 13032	C130920-BH12	YOY	M			Ws
road	RC 13032	C130921-BH01	YOY	M	20	55.9	Ws
road	RC 13033	C130919-TH01	YOY	U	22	55.5	Ws
road	RC 13033	C130920-BH05	YOY	M	21	57.6	Ws
WDLNA	RC 13036	C130424-TH01	Adult	M	58	78.9	Ws
East Pond	RC 13036	C130530-CS01	Adult	M	54	70.5	Ws
WDLNA	RC 13037	C130424-TH03	Adult	M	57	76.5	Ws
WDLNA	RC 13037	C130507-JM05	Adult	M	51	80.2	Ws
WDLNA	RC 13038	C130425-BH03	Adult	M	61	81	Ws
WDLNA	RC 13038	C130502-CS02	Adult	M			Ws
WDLNA	RC 13039	C130508-TH05	Adult	M			Ws
WDLNA	RC 13039	C130509-BH04	Adult	M	56	81.5	Ws
WDLNA	RC 13039	C130529-CS01	Adult	M			Ws
WDLNA	RC 13040	C130425-BH07	Adult	M	56	81.8	Ws
WDLNA	RC 13040	C130506-CS02	Adult	M	51	81.1	Ws
WDLNA	RC 13041	C130425-TH03	Adult	M			Ws
WDLNA	RC 13041	C130501-BH02	Adult	M			Ws

WDLNA	RC 13041	C130507-CS10	Adult	M	50	76.6	Ws
WDLNA	RC 13042	C130506-JM03	Juv	M	48	75.7	Ws
WDLNA	RC 13042	C130507-BH06	Adult	M	50	79.4	Ws
road	RC 13043	C130404-MAB11	Adult	M	53	66	Wd
WDLNA	RC 13043	C130514-BH02	Juv	M	48	76.8	Wd
WDLNA	RC 13043	C130523-BH01	Juv	M	43	78.7	Wd
WDLNA	RC 13044	C130506-CS07	Juv	M			Ws
WDLNA	RC 13044	C130507-BH05	Juv	M	25	63.4	Ws
WDLNA	RC 13045	C130430-BH02	Adult	M	62	87.8	Ws
WDLNA	RC 13045	C130506-BH10	Adult	M	56	79.1	Ws
WDLNA	RC 13046	C130501-TH02	Juv	M			Ws
WDLNA	RC 13046	C130506-JM07	Juv	M	29	60.4	Ws
WDLNA	RC 13046	C130507-BH03	Juv	M	27	66.3	Ws
WDLNA	RC 13047	C130501-CS02	Adult	M			Ws
WDLNA	RC 13047	C130506-JM05	Adult	M	55	80.5	Ws
WDLNA	RC 13047	C130507-BH01	Adult	M	55	88.2	Ws
WDLNA	RC 13048	C130506-CS03	Juv	M			Ws
WDLNA	RC 13048	C130507-BH07	Juv	M	49	78.6	Ws
WDLNA	RC 13048	C130509-BH03	Adult	M			Ws
WDLNA	RC 13049	C130502-BH02	Juv	M			Ws
WDLNA	RC 13049	C130502-JM01	Juv	M			Ws
road	RC 13050	C130404-MAB01	Adult	M	57	75.5	Wd
WDLNA	RC 13050	C130506-BH07	Juv	M	49	79	Wd
WDLNA	RC 13050	C130509-CS04	Juv	M	46	72.7	Wd
WDLNA	RC 13051	C130425-TH05	Adult	M	60	78.4	Ws
WDLNA	RC 13051	C130502-CS03	Adult	M	57	79	Ws
WDLNA	RC 13052	C130502-JM03	Adult	M	53	76	Ws
WDLNA	RC 13052	C130506-JM04	Adult	M			Ws
WDLNA	RC 13053	C130506-BH04	Adult	M	60	85.6	Ws
WDLNA	RC 13053	C130508-TH01	Adult	M	58	80.2	Ws
Columbia Marsh	RC 13034	C130830-BH19	YOY	U	10	46	Ws
Columbia Marsh	RC 13034	C130926-BH01	YOY	U	13	49.3	Ws
Columbia Marsh	RC 13035	C130830-BH24	YOY	U	14	51.3	Ws
Columbia Marsh	RC 13035	C130926-PO01	YOY	M	21	57.1	Ws

[1] all sites are within the CVWMA except the "Columbia Marsh' sites; no recaptures were detected at Bummers Flats

[2] Frog id is a unique number assigned to each individual determined to be a recapture through photo recognition

[3] Observation number is a unique number assigned to each detection in time; format is CYYMMDD-followed by surveyor initials and observation # (consecutive by day per person)

[4] Stage is the age class assigned: adult, juvenile (juv), young of year (YOY)

[5] Sex (male, female, unknown)

[6] Weight (measured in grams)

[7] Snout vent length (SVL) measured in mm

[8] Code assigned to each recapture (Ws = within season, same site; Wd = within season, different site)