FWCP Northern Leopard Frog Project: 2015 Field Season Report

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Submitted to:
Fish and Wildlife Compensation Program-Columbia Basin

Submitted by:
Barb Houston B.Sc.

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EXECUTIVE SUMMARY

Once widespread and common throughout south-eastern British Columbia (B.C.), the Northern leopard frog (*Lithobates pipiens*) has declined in recent decades and as of the 1990's was believed to be restricted to a single extant remnant native population located in the Creston Valley Wildlife Management Area (CVWMA) near Creston, B.C.

The Fish and Wildlife Compensation Program – Columbia (FWCP – Col.) has been working on recovery efforts for the Endangered Northern leopard frog since 2000. This report summarizes the results of the 2015 field season program for the Northern leopard frog project administered by the FWCP - Col.

The main objectives for the 2015 field season were to monitor the extant remnant native population of B.C. *Lithobates pipiens* (LIPI) in the CVWMA, locate and protect egg masses, bolster the reintroduced population at Bummer's Flats by completing year 5 of phase 2 reintroductions, provide tadpoles from Creston to increase the number of tadpoles being reintroduced at the Columbia Marsh reintroduction site at Brisco and supply additional founder tadpoles to enhance the captive assurance colony at the Vancouver Aquarium.

In order to meet the project objectives, fieldwork was carried out from April 21 to October 16, 2015 and 6 types of survey methods were utilized: Songmeters, nocturnal calling surveys, egg mass surveys, tadpole trapping, visual encounter surveys and road surveys.

During the field season calling males were detected in the main breeding area at DLNA on the first night of NCS on April 21 through May 26. Due to high winds 1/3 of NCS were cancelled so it was not possible to determine the maximum number of calling males, but 38 were detected on April 21-22 at WDLNA where the highest levels of calling were detected. Calling levels at the East ponds were lower, with 12 calling males detected on April 30.

Although analysis of 80 hours of recordings from 2 Songmeters deployed at Leach Lake from May 13 – June 1 failed to indicate any LIPI calling occurred, calling was detected during an in person survey in Pond 1 on May 20 when 12 calling males were detected. This was a very significant result as calling has not been detected in pond 1 since 2006.

A total of 26 egg masses were detected in the DLNA breeding area (23 in WDLNA and 3 in EDLNA). Of these, 15 were protected from predators using cages until the free-swimming stage, at which point hatchling counts were done. Overall health and hatching success of the egg masses varied and hatchling counts ranged from 0 to 3937 hatchlings per egg mass. Overall it is approximated that a grand total of 41,239 hatchlings were produced in the DLNA during the spring of 2015, of these 8,226 (19.9%) were removed as part of the reintroduction program and an estimated 33,013 (80.1%) were released in situ at DLNA.

The first sign of metamorphosis was detected on July 9 during tadpole trapping at DLNA, at which point a variety of developmental stages of tadpoles were detected, ranging from Gosner stage 26 to 42. Although tadpole trapping was conducted at Leach Lake ponds 1 and 4 to search for evidence of successful breeding none were detected during 224:34 trap-hours.

Visual encounter surveys during the summer and fall failed to provide any evidence of successful breeding at Leach Lake.

Road mortality has been well documented during the late-summer and fall migration period between seasonal habitats since surveys began in 2009. During the 2015 surveys 25.0% of the LIPI detections were road mortalities. It is recommended that mitigation measures be discussed and implemented.

The majority (84.8%) of LIPI detections throughout the year were deemed to be in good health by visual inspection and there were few animals exhibiting overt signs of Chytridiomycosis (4.5%). Of concern were 5 dead male juvenile LIPI detected in the breeding area during the spring.

A total of 144 dorsal spot pattern photos were taken for captures during the 2015 field season and a digital image library created; however there are no results to report as it was decided that the recognition would not be completed due to the considerable amount of time required, combined with the fact that in the past there have been relatively few between-year recaptures which are required for population estimate work. Since it was not done there is no recapture information available for the 2015 field season so all reported detections are observations in time and do not indicate individuals as recaptures have not been removed.

Morphometrics indicate that LIPI YOY detections made during the fall of 2015 were statistically significantly smaller in both weight and snout vent length (SVL) when compared to 2014 fall YOY detections, they also had the smallest mean weight and mean SVL when compared to fall YOY detections made from 2010 – 2013.

For the final year of phase 2 reintroductions at Bummers Flats 5,279 free-swimming tadpoles were transferred from 4 egg masses from the source population in Creston. This brings the grand total to 33,958 tadpoles released since 2011.

In response to the British Columbia Northern Leopard Frog Recovery Team (BC-NLFRT) request for tadpoles from the source population in Creston to supplement the Columbia Marsh reintroduction project at Brisco, a total of 2947 free-swimming hatchlings were transferred. An additional 616 were released from the Vancouver Aquarium captive breeding program on June 10, bringing the 2015 total to 3533, for a grand total of 7533 reintroduced since 2013.

This year marked the 7th year since the captive assurance colony was established at the Vancouver Aquarium in 2009, during which time a total of 312 tadpoles from the wild population in Creston have been provided. In 2015 only 15 tadpoles were requested due to a lack of space, as of the last update in December 2015, there were 12 survivors from the 2015 brood, all of which had successfully metamorphosed prior to brumation.

ACKNOWLEDGEMENTS

Thanks to the Fish and Wildlife Compensation Program (FWCP) – Columbia Basin for administering this project, to Irene Manley for project support, Amy Waterhouse for GIS support and Crystal Klym for providing valuable direction as the FWCP Columbia region manager. Special thanks to the field staff, Emily Ackroyd, Steph Whyte and Thomas Hill for many long hours, late nights and dedication in the field and to Kat McGlynn for her help during the tadpole transfer to the Columbia Wetlands. Lea Ramsay and Josée-Anne Otis of the Calgary Zoo provided help surveying for 10 days during the spring breeding season which was much appreciated. Thanks also to Vince Nitchie for his volunteer efforts throughout the field season. Acknowledgements also to Marc-André Beaucher and the Creston Valley Wildlife Management Area (CVWMA) for providing support and allowing us to carry out our fieldwork on the WMA. I would also like to thank Dennis Thoney, Darren Smy and Kris Rossing for their dedication in caring for the Northern leopard frog captive assurance colony housed at the Vancouver Aquarium and for providing me with pre-brumation morphometric data they collected in the fall of 2015 on captive animals.

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1. INTRODUCTION

Once widespread and common throughout south-eastern British Columbia (B.C.), the Northern leopard frog (*Lithobates pipiens*) has declined in recent decades and as of the 1990's was believed to be restricted to a single extant remnant native population located in the Creston Valley Wildlife Management Area (CVWMA) near Creston, B.C. Currently the B.C. population referred to as the Rocky Mountain population is listed as Endangered nationally by the Committee On the Status of Endangered Species In Canada (COSEWIC, 2009) and is on Schedule 1 of the *Species at Risk Act* (SARA, 2006); provincially, it is ranked S1 (critically imperiled; 2010) and it is on the British Columbia Red list (B.C. Conservation Data Centre, 2016). For additional information on species status, reasons for decline, current threats and general species information see the Recovery plan for the Northern Leopard Frog (*Lithobates pipiens*) in British Columbia (Northern Leopard Frog Recovery Team, 2012).

The Fish and Wildlife Compensation Program – Columbia (FWCP – Col.) has been working on recovery efforts for the Endangered Northern leopard frog since 2000. A captive-rearing and reintroduction program ran from 2001-2005 and since then the focus has been monitoring the Creston population, protecting wild egg masses from predators, helping to establish a captive assurance colony at the Vancouver Aquarium and conducting reintroductions.

This report summarizes the results of the 2015 field season program for the Northern leopard frog project administered by the FWCP - Col.

The main objectives for the 2015 field season were to monitor the extant remnant native population of B.C. *Lithobates pipiens* (LIPI) in the CVWMA, locate and protect egg masses, bolster the reintroduced population at Bummer's Flats by completing year 5 of phase 2 reintroductions, provide tadpoles from Creston to increase the number of tadpoles being reintroduced at the Columbia Marsh reintroduction site at Brisco and supply additional founder tadpoles to enhance the captive assurance colony at the Vancouver Aquarium.

2. STUDY AREA

The main study area is the CVWMA located in the Creston Valley within the Kootenay region of south-eastern B.C. The area is located within the very dry warm (xw) subzone of the Interior Cedar – Hemlock (ICH) Biogeoclimatic Ecosystem Classification (BEC) zone, which is characterized by warm dry summers and wet, cool winters with high snowfall (Ketcheson et al.,1991). The managed wetland complex is a 7,000 ha (17,000 acre) area of provincial Crown land located along the Kootenay River system near the town of Creston. The wetland complex is predominantly composed of shallow warm water wetlands dominated by cattails which support a large amount of biodiversity.

Secondary study areas include the 2 reintroduction sites:

1.) Bummers Flats, located just north of Cranbrook, B.C. on the Upper Kootenay Floodplain (UKF) which lies within the dry hot (dh) subzone of the Ponderosa Pine (PP) BEC zone
2.) Columbia Wetlands - Brisco site, located near the town of Brisco B.C. within the dry cool subzone of the Interior Douglas-fir (IDF) BEC zone.

See map in Figure 1 for locations.

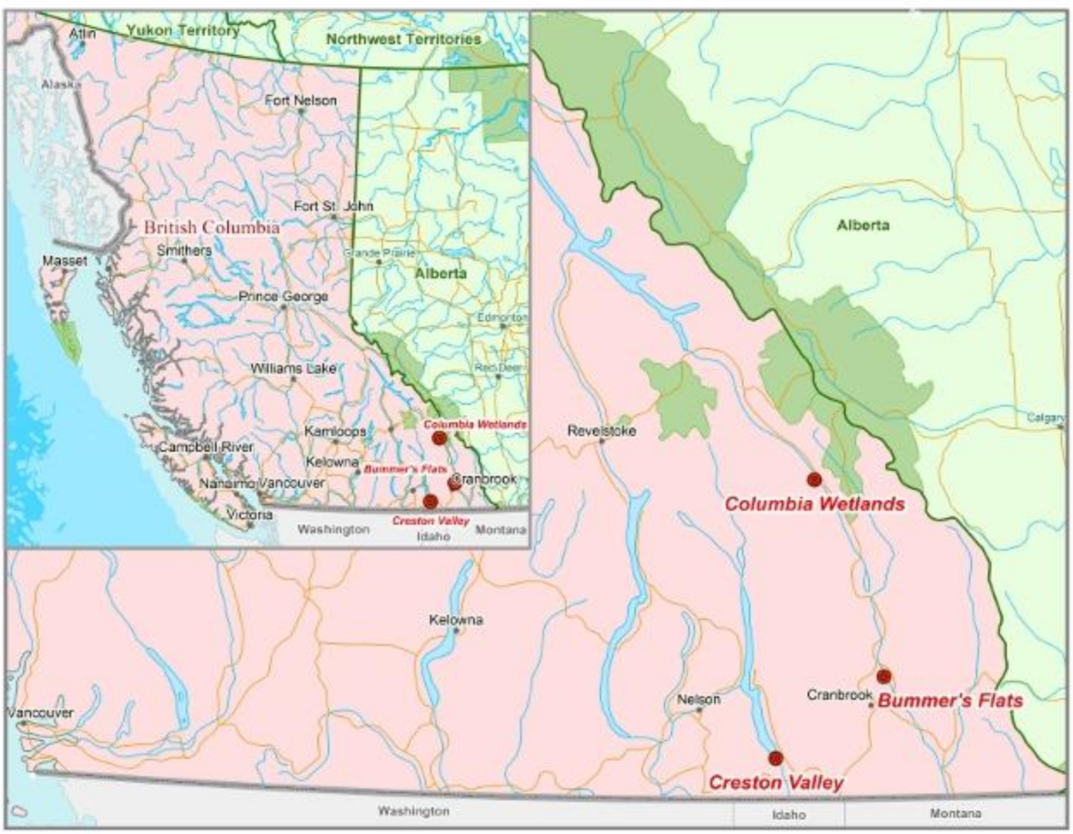


Figure 1. Map of main study area in the Creston Valley and the secondary study areas at the 2 reintroduction sites (Bummer's Flats and the Columbia Wetlands near Brisco).

3. METHODS

In order to meet the objectives for the project, fieldwork was carried out from April 21 through October 16, 2015 and 6 types of survey methods were utilized: Songmeters, nocturnal calling surveys, egg mass surveys, visual encounter surveys, road surveys and tadpole trapping. All data summaries are based on detections, they are observations in time and should not be assumed to represent individuals since recaptures have not been removed.

3.1 Songmeters

To gather information on whether or not LIPI were calling at 2 sites within the Leach Lake wetland complex, 2 SM2+ Songmeter remote recording devices produced by Wildlife Acoustics Inc. were deployed. The units were set up in shallow open water areas within suitable breeding habitat during the peak of the spring breeding season to monitor at the presence/not-detected level. They were each deployed from May 13 to June 1 and set to record daily at 22:00 for 2 hours. The map in Appendix 1 shows the deployment sites of the Songmeters in 2015.

Data analysis (presence/not-detected) was carried out using Songscope software, a spectrogram viewing program developed by Wildlife Acoustics Inc.; .wav files were scanned at high speed visually searching for the digital signature of a LIPI call.

3.2 Nocturnal Calling Surveys (NCS)

To monitor breeding activity NCS were used, methodology was based on that outlined by the Resource Inventory Committee (MELP 1998a) and discussed in the book entitled Measuring and Monitoring Biological Diversity - Standard Methods for Amphibians (Heyer et al. 1994) with modifications. NCS are carried out as night falls during the spring breeding season (late April to early June), see map in Appendix 1 for location of calling stations.

Three types of NCS are utilized:

1.) standardized auditory surveys
2.) blitz style surveys
3.) reconnaissance level 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. 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 large wetlands such as Leach Lake where crews are not able to frequently visit the site and 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 5 minutes at each fixed station and record the number of LIPI detected calling.

Reconnaissance Level Surveys

At less frequently visited sites reconnaissance level surveys are carried out to try and detect calling male LIPI in areas of suitable habitat when environmental conditions are optimal. Surveys are usually done by travelling along a predetermined survey transect route via canoe listening for calling males.

Methodology

Efforts are made to conduct SAS at each of the main breeding areas in DLNA at least once per week, and blitz style surveys and reconnaissance level surveys at other areas at least once during the peak of the breeding season; all surveys are weather permitting. Surveys are cancelled when winds are excessive or there is heavy rain, as it is impossible to detect calling activity under these conditions. Surveys are also cancelled if the air temperature drops below 5ºC, because previous experience has shown that LIPI do not call strongly, if at all, under these types of unfavourable conditions

For all 3 types of NCS, the survey begins by noting the start time, GPS location, and environmental conditions, including: air temperature, water temperature, cloud cover, precipitation (current and last 24 hours), approximate wind speed, pH, and conductivity. Surveyors then listen for calling males, after determining the approximate location calling groups, efforts are made to determine the specific location of each calling LIPI by going to locate them; 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 survey time is calculated (which includes time listening as well as time to locate, capture and process frogs). If time permits, the surveyor attempts to capture and processes calling male LIPI, starting with the core calling groups in the area (see section 2.8 Animal Capture and Tissue Collection for methods). Capture is important as it not only enables surveyors to pinpoint the exact location of the calling males, which allows them 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. Determining the age class (whether juvenile or adult) indicates if they are of reproductive age or not and is important information taken into account when prioritizing search areas for follow up egg mass searches. By capturing the animals, surveyors are also able to collect morphometrics, assess the animal's health, collect a swab for *Chytridiomycosis* testing and take a dorsal spot pattern photo for the dorsal spot pattern recognition image library.

3.3 Egg Mass Surveys (EMS)

To monitor and quantify the breeding activity of *L. Pipiens* during the spring breeding season (late April to early June) egg mass surveys are conducted. It is through these surveys (in combination with NCS) that egg masses are located, the hatchlings of which are the source for the reintroduction program and the additional founders provided yearly to the Vancouver Aquarium captive assurance colony.

It has been found that the most efficient way to conduct EMS is to return to the specific breeding site where a NCS had been recently conducted, as this enables the surveyor to focus on key areas of concentrated calling; due to the vast amount of habitat there is not enough time to search all areas so this method increases efficiency. 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 on foot. Weather is a very important factor in conducting EMS, as wind, rain, and cloud cover can obscure the surveyor's visibility, even with the use of polarized sunglasses. When an egg mass is detected, surveyors immediately make every effort to neutralize disturbance to the area, to prevent siltation of the egg mass. Once that is completed, 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 condition of the egg mass, percent fertilization and estimated age (based on development) is noted, and finally, the area is marked by a ring of flagged stakes driven into the substrate (at a radial distance of 2 meters from the egg mass). When possible, egg mass enclosures (see photo in Figure 2) are utilized to facilitate collection of hatchlings for the Vancouver Aquarium captive assurance colony, the reintroduction program and to provide protection from predators until free-swimming. Whether or not egg masses are caged depends on numerous factors, including the developmental stage, condition, position in the water column, etc. When caged, native aquatic vegetation is added to the enclosure and any predatory aquatic invertebrates removed.

Figure 2. Egg mass enclosure (design by Barb Houston)

Since capturing and processing LIPI is not the main objective of an EMS, LIPI that are observed during these surveys are generally just recorded as an auditory or visual observation with a UTM to mark the location and are not captured and fully processed as this would take away time from the main priority of the EMS which is searching for egg masses.

3.4 Tadpole Trapping

To gather presence/not detected data on tadpoles and monitor developmental stage tadpole trapping is conducted. The primary focal area within the CVWMA in 2015 was Leach Lake pond 1 to determine if there was any evidence of breeding since calling was detected during the spring breeding season; trapping was also done in Leach Lake pond 4 to determine if any breeding had taken place even though no calling was detected in the spring. Tadpole trapping was also conducted at EDLNA ponds and WDLNA, known breeding sites, to monitor the developmental stage of tadpoles which provides an estimated date for metamorphosis. Trapping was also conducted at the release site at North Bummers Flats where hatchlings were released in spring as part of the reintroduction program. See Appendix 1 for location of tadpole traps within the CVWMA and Appendix 9 for tadpole trap locations in Bummers Flats.

Tadpoles are trapped using un-baited 21 inch wire minnow traps (shown in Figure 3). The traps are partially submerged in warm shallow areas with high levels of submergent aquatic 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 were deployed overnight and checked within 8-12 hours to decrease the likelihood of predation on trapped tadpoles; everything trapped is released on site.

Tadpoles were identified to the species level, total length (TL) was measured and they were aged using the Gosner Stage (Gosner, 1960). If handling tadpoles, Nitrile gloves were used as there is evidence latex gloves can be harmful to tadpoles (Gutleb, 2001). In most cases surveyors are easily able to identify tadpoles to the species level but when necessary the United States Geological Survey (USGS) document entitled *Tadpoles of the United States and Canada: A Tutorial and Key tadpole key* (Altig et al, 1998) or the field guide *Amphibians of Oregon, Washington and British Columbia: a field identification guide* (Corkran and Thoms, 1996) were consulted; these guides are especially useful when identifying small early stage tadpoles to the species level.

Figure 3. Wire minnow traps used for tadpole trapping

3.5 Visual Encounter Surveys (VES)

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 and to monitor habitat quality VES are conducted. 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 summer, daytime VES are carried out in an effort to target *L. pipiens* young of year (YOY) in their preferred habitat within natal ponds before late summer and fall dispersal. During late summer and fall, migration corridors and over-wintering areas are surveyed during the daytime in the CVWMA; methodology usually involves walking the perimeter of the water body searching for LIPI, but in some cases surveys are conducted by canoe.

LIPI are captured and processed using methods outlined in section 2.8 Animal Capture and Tissue Collection.

3.6 Late-summer and Fall Road Surveys

To gather information on migrating frogs and associated road mortality as they cross over the Duck Lake dike while moving from summer foraging grounds to the primary over-wintering area in late-summer and fall road surveys are conducted. 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 using methods outlined in section 2.8 Animal Capture and Tissue Collection; if time is restricted a UTM location is taken at a minimum and if possible a dorsal spot pattern photo taken. Dead frogs are removed from the roadway to ensure they are not double counted and mortally wounded animals are euthanized.

3.7 Animal Capture and Health Assessment

During all survey types, when time permits effort is made to capture *L. pipiens* detected for full processing (recorded as a *capture)*. When the surveyor is unable to make a capture, or time prohibits it, the observation is noted as a *visual*; if it is heard calling and a location noted, but not seen or captured, it is recorded as an *auditory* detection; if only a photo and UTM are taken it is recorded as a *photo only* observation. 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 any longer, 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 of all observations is marked by GPS, the habitat features 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 and the length of time the animal is in the bag is as minimal as possible). Photos documenting the process of weighing, SVL measurement and swabbing are provided in Figure 4.

The health of each *L. pipiens* 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, vascularisation, 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 animal's 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 major signs of disease, such as *Chytridiomycosis*. If *Chytridiomycosis* is suspected, details of the symptoms are noted. If health is deemed to be anything other than good, the reason for the designation is noted and photos are taken where possible.

To test for the presence of *Batrachochytrium dendrobatidis (Bd),* which causes the disease *Chytridiomycosis,* sterile Mediwire MW100 rayon tipped swabs stored in a dry labelled test tube are used to collect body swabs. The process involves swabbing the animal a total of 33 times, in the following order to minimize the spread of *Bd*: 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.

Swabs are stored in the refrigerator until 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. In 2015 there was no budget allocated to lab analysis of swabs to test for the presence of *Bd,* however a subset of swabs were taken from high priority animals including all post-metamorphic and late-season YOY captured during the field season; samples will be stored in the event that future analysis is required. Lab analysis of the swabs is done using quantitative PCR methodology as described by Boyle et al (2004) to test for the presence of *Bd*.

If a recently deceased animal is found in relatively good body condition (i.e., decomposition is not in advanced stages) it is immediately submitted to 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.

Fieldwork methods follow the Ministry of Environment Standard Operating Procedures: Interim Hygiene Protocols for Amphibian field staff and researchers (MoE 2008). The Live Animal Capture and Handling Guidelines for Wild Mammals, Birds, Amphibians and Reptiles (MELP, 1998b) and the Canadian Council on Animal Care Species Specific Recommendations on Amphibians and Reptiles (CCAC, 2011) were consulted during field program development to ensure proper standards were being followed; see Northern Leopard Frog Project Description for Wildlife Act Permit (Houston, 2012).

Figure 4 (left to right): the process of weighing, measuring SVL and swabbing LIPI for *Batrachochytrium dendrobatidis (Bd),* which causes the disease Chytridiomycosis.

3.8 Dorsal Spot Pattern Recognition

To facilitate recapture analysis dorsal spot pattern photos were taken for all LIPI captures during the field season and a 2015 digital image library was created by naming each .jpg file with the observation number and cataloguing it by detection. These photos were then added to the master digital image library containing dorsal spot pattern photos of all LIPI captures dating back to 2006.

Due to the amount of time required to do the recapture analysis by manually comparing the photos, along with the fact that there have been relatively few between-year recaptures in previous years (required for population estimate work) it was decided the analysis was not a priority so it was not done in 2015. Since all photos are catalogued in the digital image library the dorsal spot pattern recognition could be done at any time in the future if needed.

Given that the recapture analysis was not done, all data summaries within this report are based on detections of LIPI at a point in time, they are observations, and do not indicate the number of individuals (as recaptures are included).

3.9 Vancouver Aquarium Captive Assurance Colony

To provide additional founders for the captive assurance colony, 15 tadpoles were collected from 3 different egg masses. Tadpoles were held and cared for in 3 different egg mass enclosures in situ at DLNA natal sites until they reached the free-swimming stage and were ready for transfer to Vancouver. Native vegetation with algal growth for grazing was added to each enclosure to provide a food source and shade for the developing hatchlings and predatory aquatic invertebrates were removed from the cages. Enclosures were carefully monitored; native vegetation replaced as needed and efforts made to keep it free of large amounts of waste build up. Once ready for transfer to the Vancouver Aquarium, tadpoles were collected from the natal site in 2 gallon thermos coolers 2/3 full of water and transported as quickly as possible with care to the CVWMA office where they were held overnight awaiting transfer to Vancouver. The following morning they were packaged in plastic bags used for live fish transport with 1/3 fresh water and 2/3 air tied off by elastic band (Figure 5); no food was packed in the bags for transport to prevent the buildup of wastes as recommended by the Vancouver Aquarium (Lee Newman, pers. comm.).

Figure 5. Photos of hatchling transfer to Vancouver Aquarium (left to right): egg mass enclosure used to house animals in situ until ready for transfer; hatchlings packaged in plastic bags and packed in cooler; Pacific Coastal staff at Trail Regional Airport (YZZ) with packages for air transport.

3.10 Bummers Flats Reintroduction Program

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. Methods for transport and acclimatization developed by Kendell and Prescott (2007) in the Northern leopard frog reintroduction strategy for Alberta were utilized with some modifications.

It was decided by the British Columbia Northern Leopard Frog Recovery Team (BC-NLFRT) that if greater than 10 egg masses were detected in Creston, that where logistically feasible, a portion of each egg mass above that level would be moved to Bummers Flats. In 2015 the source of all animals was WDLNA and all reintroduced animals were moved just after reaching the free-swimming hatchling stage. Where logistically feasible a portion (target = 50%) of each caged and healthy egg mass detected over and above the threshold level of 10, were moved in insulated 2 gallon thermos containers. Pond water was placed in each container to approximately the 2/3 level and hatchlings were transferred from egg mass enclosures to thermos containers using stainless steel strainers; care was taken to minimize collection of waste products from the cages and aquatic vegetation was removed. Once collected, hatchling containers were placed in the canoe under the shade of a large umbrella without lids and transported to land where they were removed and carefully walked to the transport vehicle. Lids were placed on the containers for the short trip (~20 minutes) to walk the coolers from the marsh to the vehicle for transport to the recipient site, once at the vehicle containers were placed in the shade and water quality tested. Once loaded in the vehicles, lids were again placed on the containers for the approximately 15-20 minute trip along a rough dirt road until pavement was reached; lids were then removed to allow for oxygen exchange for the remainder of the trip. Air temperature, water temperature, pH and conductivity were tested 3 times during transport (at the end of tadpole collection, at the transport vehicle prior to travel and at approximately the half-way point along the highway) and compared to in situ conditions prior to tadpole collection to ensure conditions remained within a safe range.

Once at the release site, in situ water quality was tested and compared to that in the thermos containers holding the hatchlings and if required hatchlings were acclimatized by gradually adding water from the release site. Once acclimatized hatchlings were released into enclosures set up to hold them overnight; if available, vegetation native to the site was added (bladderwort genus Utricularia was not used as it can cause mortality), with special attention to ensure no invertebrates were added to the enclosures. The following day, hatchlings were counted and released at various locations along the length of the release ditch. Figure 6 shows photos of some of the steps in the reintroduction process. During the entire reintroduction process, extreme care was taken to ensure the overall safety of the hatchlings.

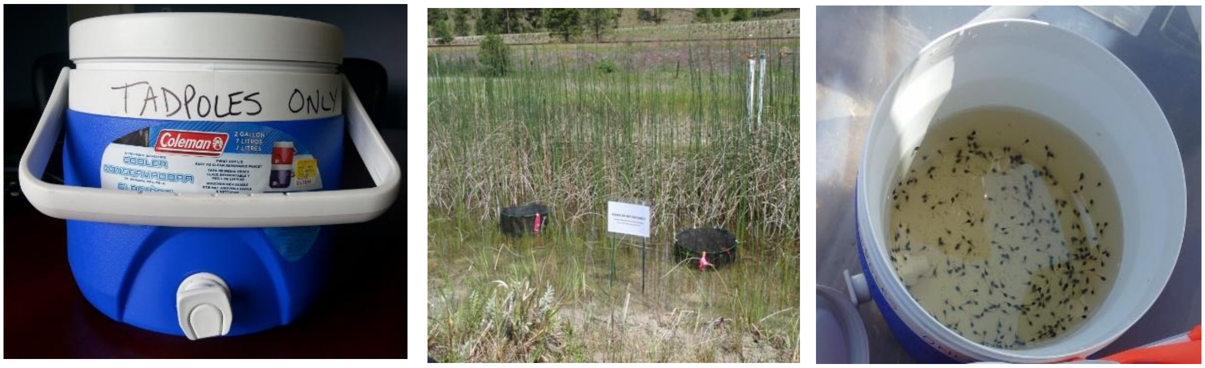


Figure 6 (left to right): 2 gallon Thermos container used to transport tadpoles, Bummer's Flats reintroduction site with caged animals and *please do not disturb* signage, Thermos container with ~300 hatchlings for transport to reintroduction site.

3.11 Columbia Marsh Reintroduction Program

Tadpoles were collected, packaged up and walked out to the road for the trip to the Columbia Marsh release site near Brisco by the FWCP crew using the same methodology as described above for the Bummers Flat's reintroduction; they were then picked up by the Brisco crew funded by the Columbia Valley Local Conservation Fund and transported by vehicle to the release site where they were held overnight in enclosures in the marsh, then counted and released the following day.

3.12 Data Management

Data collected in the field, including survey information, observation data, egg mass data and tissue data is recorded on survey datasheets and entered into the custom designed Northern Leopard Frog Project FileMaker Pro 13.0v3 master database where it is managed along with all other records dating back to 2000. The new data is exported annually to Wildlife Species Inventory (WSI) managed by the Knowledge Management Branch in the Ministry of Environment using the General Survey template with the addition of some user-defined fields.

During the 2015 field season work began on the development of a FileMaker Go form for use on iPad to enable field data collection from which data would be exported directly to the master Northern Leopard Frog Project FileMaker Pro 13.0v3 database; work is ongoing on this project.

4. RESULTS AND OBSERVATIONS

4.1 Songmeters

During the spring breeding season a total of 80 hours of .wav files were recorded from Leach Lake on the 2 Songmeters deployed nightly from 22:00 to 24:00 for 20 days from May 13 – June 1 to survey for calling LIPI at the presence/not-detected level. Analysis by visually scanning .wav file spectrograms at high speed using Songscope Software was completed in approximately 16 - 20 person-hours. No LIPI calling was detected during analysis, however, calling was detected by surveyors during a reconnaissance level NCS in Leach Lake pond 1 approximately 500 m away from the location of a deployed Songmeter; this calling was not picked up by the Songmeter. See map in Appendix 1 for location of 2 deployment sites at Leach Lake.

4.2 Nocturnal Calling Surveys (NCS)

In the CVWMA, 78:14 person-hours were dedicated to NCS (n=14 surveys) from April 21 to May 26. In addition to standardized auditory surveys (SAS) carried out at fixed stations within the core DLNA breeding area (WDLNA and East Pond), blitz style surveys at Leach Lake and reconnaissance level surveys at southwest DLNA, Duck Lake and Leach Lake pond 1, were completed. High winds caused the cancellation of 7 NCS (1/3 of all NCS) from May 5-19, 2015.

During the spring breeding season calling was detected at WDLNA, the East Pond of DLNA, south-west corner of DLNA, Duck Lake and in Leach Lake pond 1; see Table 1 for details. Although blitz style surveys were conducted throughout Leach Lake calling was not detected in any areas other than pond 1. The map in Appendix 1 shows the location of calling stations and the maps in Appendices 2, 4, 5, 6 and 7 document the locations of all detected calling males.

Table 1. LIPI calling detected (number of surveys) by site during NCS in CVWMA

Site Survey type	WDLNA SAS	East Pond SAS	Leach Lk Blitz	SW DLNA Recon	Duck Lk Recon	Leach 1 Recon
April 21-25	yes (n=2)	yes (n=1)				
Apr 26-May 2	yes (n=2)	yes (n=1)	no (n=1)			
May 3-9	yes (n=1)*	*	*	yes (n=1)		
May 10-16	* *	*	*	*		
May 17-23	yes (n=1)	*	yes (n=1)		yes (n=1)	yes (n=1)
May 24-26	n/a	n/a	yes (n=1)	n/a	n/a	n/a

*surveys cancelled due to poor weather
n/a: NCS at DLNA no longer required to inform EMS as egg mass detection became impossible with dense ingrowth of emergent vegetation and field program switched gears to focus on reintroductions

Of the sites surveyed, WDLNA had the greatest amount of calling activity. During the survey period the maximum level of detected calling was 38 calling males which were detected April 21-22 (dates pooled since it is not possible to survey the entire WDLNA in one night); however, due to the cancellation of 3 NCS over a 2 week period at this site it is unknown whether calling levels peaked above this level or when the peak occurred. Calling was widespread and numerous calling groups were clustered throughout the area as in previous years. Calling was detected on the first NCS on April 21 through the last survey on May 20. It is likely that calling at WDLNA continued through to early June but surveys were wrapped up earlier than usual as they were no longer required to inform egg mass searches since the emergent vegetation was so dense it was no longer possible to search for egg masses.

At the East ponds calling was detected during NCS in late April but since surveys were cancelled at the site for the following 3 weeks due to high winds it is unknown how long calling continued, but based on observations from previous years it likely continued into late May or early June. The maximum number of calling males detected at the East ponds during NCS was on April 30, when a minimum of 12 individuals were detected calling but due to the limited number of surveys at this site it is not possible to determine what the maximum calling levels were or when the peak occurred. The majority of the calling was clustered in one area within the East ponds.

At Leach Lake pond 1, calling was detected during the only reconnaissance level NCS in the area on May 20, at which time at least 12 individual males were detected calling. At Duck Lake, 1 reconnaissance level NCS resulted in the detection of 5 males calling on May 20.

Since a large number of NCS (1/3) had to be cancelled due to high winds the calling estimates are based on the best available data but likely do not represent maximum levels of calling or the peak dates of calling.

During spring NCS a total of 150 *L. Pipiens* detections were made, a detection rate of 1.92 LIPI per person-hour. Of the total observations made during NCS, the majority (94%) were male (n=141), 2 were female and 7 were visual observations of unknown sex (Table 2). The majority (n=86, 61.0%) of the male detections were undetermined (unknown) stage as they were auditory detections; of the 63 male detections of known stage, 24 were adult (38.1%) and 39 were juvenile (61.9%). It should be noted that the reported number of male and female detections does not represent the sex ratio of the breeding population. For size data on calling males see Table 16 and 17 in Section 4.10 on Morphometrics.

Table 2. LIPI observations by stage and sex during NCS at CVWMA

	Adult	Juvenile	Unknown*	Total
Male	20	35	86	141
Female	2	0	0	2
Unknown	2	4	1	7
Total	24	39	87	150

*not possible to determine stage with auditory detection so *unknown* assigned

Of the 150 total LIPI observations made during NCS the majority, 86 (57.3%) were auditory detections, as expected, since the focus of the survey program is to identify the location of as many calling males as possible to guide EMS; 26 were captures, 2 were photos and 36 were visual detections. It should be noted that this data cannot be assumed to be a direct representation of the actual breeding male population and furthermore it should not be used as an estimate of the adult to juvenile calling male ratio; see section 5.2 for a discussion.

4.3 Egg Mass Surveys (EMS)

In the CVWMA, there were 176:49 person-hours (n=22 surveys) dedicated to EMS between April 21 and June 5, 2015. EMS were primarily conducted at WDLNA and EDLNA in 2015 (Table 3); no EMS were conducted at Leach Lake. In total, there were 26 egg masses detected in the CVWMA, 3 at EDLNA pond and 23 at WDLNA (see Table 3 for details and Appendix 1 for map of egg mass locations). The egg mass detection rate was 6.8 person-hours of survey-effort for each egg mass detected.

Table 3. Egg mass detection rate by site in the CVWMA in spring 2015

	WDLNA	EDLNA pond	East Ditch	All Sites
Number of surveys	14	7	1	22
Effort (person-hours)	147:42	28:53	00:14	176:49
# of egg masses detected	23*	3	0	26
Person-hours/egg mass	**6.4**	**9.6**	**0**	**6.8**

*includes 1 egg mass detected during NCS (detection rate 6.7 person-hours/egg mass if removed)

In 2015, it took 6.8 person-hours of survey effort to detect each egg mass, which is slightly higher than the mean for the preceding 5 year period from 2010-2014, which was 6.0 (n=5, SD=2.2, range 3.1 – 8.35), for a summary see Table 4 and Figure 7.

Table 4. Summary of egg mass detection rate in the CVWMA (all sites); 2010-2015

	2010	2011	2012	2013	2014	2015
Number of surveys	17	23	29	26	16	22
Effort (person-hours)	58:26	97:22	104:17	167:42	120:52	176:49
# Egg masses detected	7	17	22	21	39	26
Survey effort/egg mass	**8.3**	**5.7**	**4.7**	**8.0**	**3.1**	**6.8**

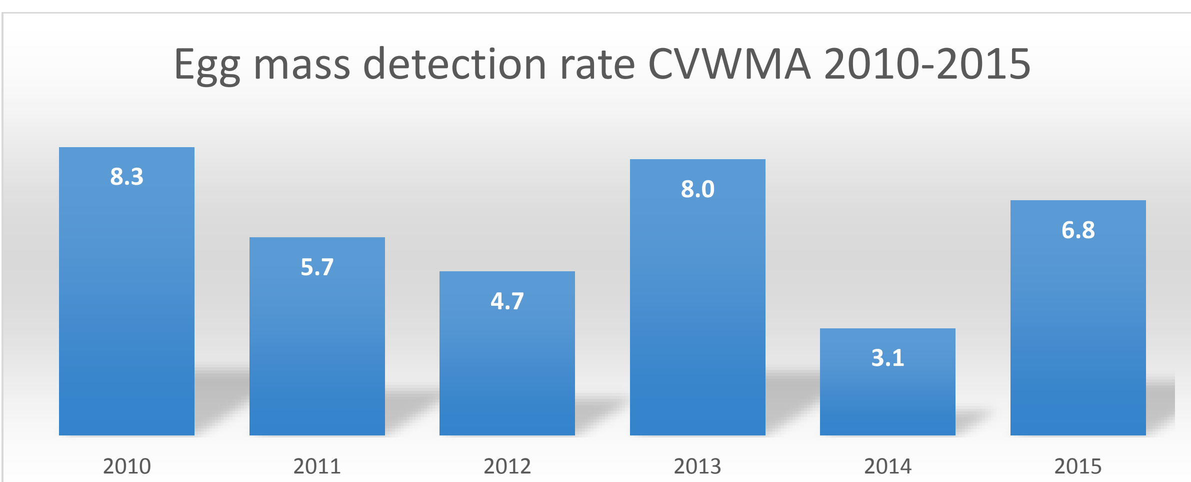


Figure 7. Survey effort (person-hours) per LIPI egg mass detected in the CVWMA (all sites combined) from 2010-2015

Egg masses were detected between April 23 and May 20 and were estimated to have been laid between April 21 and May 19, 2015; egg masses were estimated to be between 1 and 6 days old upon detection. The majority (n=15, 57.7 %) were laid during the 10 day period between May 1 to 10. For photos of egg masses and development see Figure 8.

Of the 26 egg masses detected in 2015, the majority (n=24, 92.3%) were detected prior to hatching; 2 were detected in the process of hatching (7.7%). Of the 24 pre-hatch egg masses the majority (n=17, 70.8%) were deemed to be in good condition at the time of detection, 5 (20.8%) were in fair condition and 2 (8.3%) in poor condition. Of the 2 detected in the process of hatching, 1 was deemed to be in good condition while the other was in fair condition. Estimated percent fertilization upon detection ranged from 15% to 99%. Of the egg masses deemed to be in fair or poor condition, issues included: small overall egg mass size, poor fertilization, greater than average number of dead eggs, predation (see photo in Figure 8 of leech eating eggs), egg mass detachment (slumped onto substrate), siltation on surface of egg mass and poor overall hatch-out.

Of the 23 egg masses detected at WDLNA, 15 were caged and monitored closely, this includes 13 that were good candidates for caging and 2 that were in poor condition when detected so caged as an attempt to salvage them; none of the egg masses at the East ponds were caged. Of the 15 caged egg masses, 13 were counted (11 regular and 2 salvaged); of the non-caged egg masses there were no precise counts except for one which was not viable and had no hatchlings successfully hatch out.

Overall hatching success of the egg masses was variable. Precise hatchling counts were done on 14 egg masses, all at WDLNA (11 caged, 2 salvaged, 1 not caged as not viable) during release from cages at the free-swimming stage; of these the totals ranged from 0 to 3937 hatchlings per egg mass (mean=1795, n=14, SD=1474 and sum=25,129). The majority, 8 of the 14 (57.1%) counted had a below average number of hatchlings (mean=662, SD=625 range 0-1709) and 6 were above average (mean=3306, SD=553, range 2500-3937). For the remaining 12 egg masses detected at DLNA (9 at WDLNA, 3 EDLNA) a rough estimate of the number of hatchlings per egg mass was made based on visual observations during hatch-out (10 uncaged and 2 caged but not counted); of these, estimated hatchling counts ranged from 150 to 1795 (mean=1343, n=12, SD=698 and sum=16,110).

Of the egg masses with precise hatchling counts, there was a total of 25,129 hatchlings, of which 8226 (32.7%) were removed for the reintroduction program (5279 to Bummers Flats and 2947 to the Brisco in the Columbia Marshes) and 16,903 (67.3%) released in situ at DLNA. In addition to those, it is approximated that another 16,110 hatchlings were released in situ at DLNA from the egg masses where hatchling counts were not counted precisely but were estimated; no hatchlings were removed from these for the reintroduction program. Overall it is approximated that there was a grand total of 41,239 hatchlings produced in the DLNA during the spring of 2015, of these 8226 were removed (19.9%) as part of the reintroduction program and an estimated 33,013 (80.1%) were released in situ at DLNA.

The hatchlings that were removed for the reintroduction program came from 8 different egg masses. The proportion of each egg mass removed ranged from 39.6% to 65.4%, the majority

(5 of 8 or 62.5%) were below 50%; efforts were made to keep the portion removed to less than 50% of each egg mass, however this can be challenging when dealing with thousands of tiny swimming hatchlings.

Development rate is temperature dependant but the majority of the egg masses hatched within 7-10 days from the date laid and hatchlings reached the free-swimming stage after approximately another week in most cases.

Figure 8. Clockwise (from top left): freshly laid LIPI egg mass, leech (Subclass Hirudinea) eating eggs, developing egg mass, hatchlings

Table 5. LIPI egg masses detected in the CVWMA in 2015; precise hatchling counts (c) were made for those in the blue section, while those in the red section were roughly estimated (e).

Egg mass #	Site	~Laid	Cond	% Fert	Caged	Hatch-out	Removed	Released in situ	Total
EM150423-EA01	WDLNA	21 April	poor	15%	no	below average	0 (0%)	0 (0%)	0 c
EM150506-BH06	WDLNA	1 May	good	99%	Yes	above average	0 (0%)	3653 (100.0%)	3653 c
EM150504-BH05	WDLNA	2 May	good	99%	yes	above average	0 (0%)	3937 (100.0%)	3937 c
EM150508-LR15	WDLNA	4 May	good	99%	yes	above average	1328 (39.6%)	2025 (60.4%)	3353 c
EM150508-LR14	WDLNA	5 May	fair	98%	yes	below average	539 (48.3%)	577 (51.7%)	1116 c
EM150508-LR12	WDLNA	6 May	good	99%	yes	above average	1496 (53.7%)	1291 (46.3%)	2787 c
EM150508-LR13	WDLNA	6 May	good	99%	yes	above average	1783 (49.4%)	1824 (50.6%)	3607 c
EM150512-EA17	WDLNA	9 May	good	99%	yes	above average	1000 (40.0%)	1500 (60.0%)	2500 c
EM150512-BH16	WDLNA	10 May	good	99%	yes	below average	1000 (58.5%)	709 (41.5%)	1709 c
EM150514-EA19	WDLNA	11 May	fair	98%	salvage	below average	0 (0%)	409 (100.0%)	409 c
EM150519-EA22	WDLNA	15 May	poor	85%	salvage	below average	0 (0%)	155 (100.0%)	155 c
EM150520-EA24	WDLNA	19 May	good	95%	yes	below average	0 (0%)	68 (100.0%)	68 c
EM150520-SW25	WDLNA	19 May	good	94%	yes	below average	279 (45.7%)	332 (54.3%)	611 c
EM150520-EA23	WDLNA	19 May	good	94%	yes	below average	801 (65.4%)	423 (34.6%)	1224 c
EM150428-TH03	WDLNA	23 April	good	99%	yes	average	0 (0%)	1795 (100.0%)	~1795 e
EM150429-TH04	WDLNA	23 April	good	98%	yes	average	0 (0%)	1795 (100.0%)	~1795 e
EM150428-TH02	WDLNA	25 April	fair	97%	no	below average	0 (0%)	1000 (100.0%)	~1000 e
EM150506-TH08	WDLNA	2 May	good	99%	no	average	0 (0%)	1795 (100.0%)	~1795 e
EM150506-EA10	WDLNA	3 May	fair	99%	no	below average	0 (0%)	350 (100.0%)	~350 e
EM150506-EA11	WDLNA	3 May	good	97%	no	average	0 (0%)	1795 (100.0%)	~1795 e
EM150506-EA07	WDLNA	4 May	fair	99%	no	average	0 (0%)	1795 (100.0%)	~1795 e
EM150506-SW09	WDLNA	5 May	fair	99%	no	below average	0 (0%)	250 (100.0%)	~250 e
EM150512-SW18	WDLNA	6 May	fair	n/a	no	below average	0 (0%)	150 (100.0%)	~150 e
EM150515-TH21	EDLNA	10 May	good	99%	no	average	0 (0%)	1795 (100.0%)	~1795 e
EM150515-TH20	EDLNA	11 May	good	99%	no	average	0 (0%)	1795 (100.0%)	~1795 e
EM150522-TH26	EDLNA	15 May	good	n/a	no	average	0 (0%)	1795 (100.0%)	~1795 e
TOTAL							**8,226 (19.9%)**	**33,013 (80.1%)**	**41,239**

A summary of the number of egg masses detected by site each year from 2000 to 2015 is provided in Table 6. In 2015, as in the previous 4 years, the greatest proportion were detected at WDLNA. This year marked the fifth consecutive year of above average egg mass detections, compared to a mean of 8.2 (SD 3.8, range 4 – 16) between 2000 and 2010.

Table 6. LIPI egg masses detected in the CVWMA 2000-2015

Year	EDLNA pond	East 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
2014	6	0	33	***	39
2015	3	0	23	**	26
Total	**62**	**5**	**138**	**10**	**215**

* indicates area not surveyed

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

***YOY observed in summer so possible undetected breeding occurred

As shown in Figure 9, air temperatures recorded on the hour, 24 hours per day at DLNA by HOBO temperature data logger #5 for the period of April 13-June 1, 2015 ranged from a low of -2.9 °C on April 16 at 06:00 to a high of 31.5 on May 23 at 15:00; mean for the period was 12.9 °C (SD=7.1, n=1200) and the first peak was on April 17 at 15:00 when it reached 23.6 °C. Water temperatures recorded at DLNA by HOBO temperature data logger #6 show that the water temperature for the same period ranged from a low of 4.2 °C on April 15 at 07:00 to a high of 22.5 °C on May 27 at 17:00; mean for the period was 14.0 °C (SD=3.5, n=1200) and the first peak was on April 16 at 18:00 when it reached 15.6 °C (Figure 9).

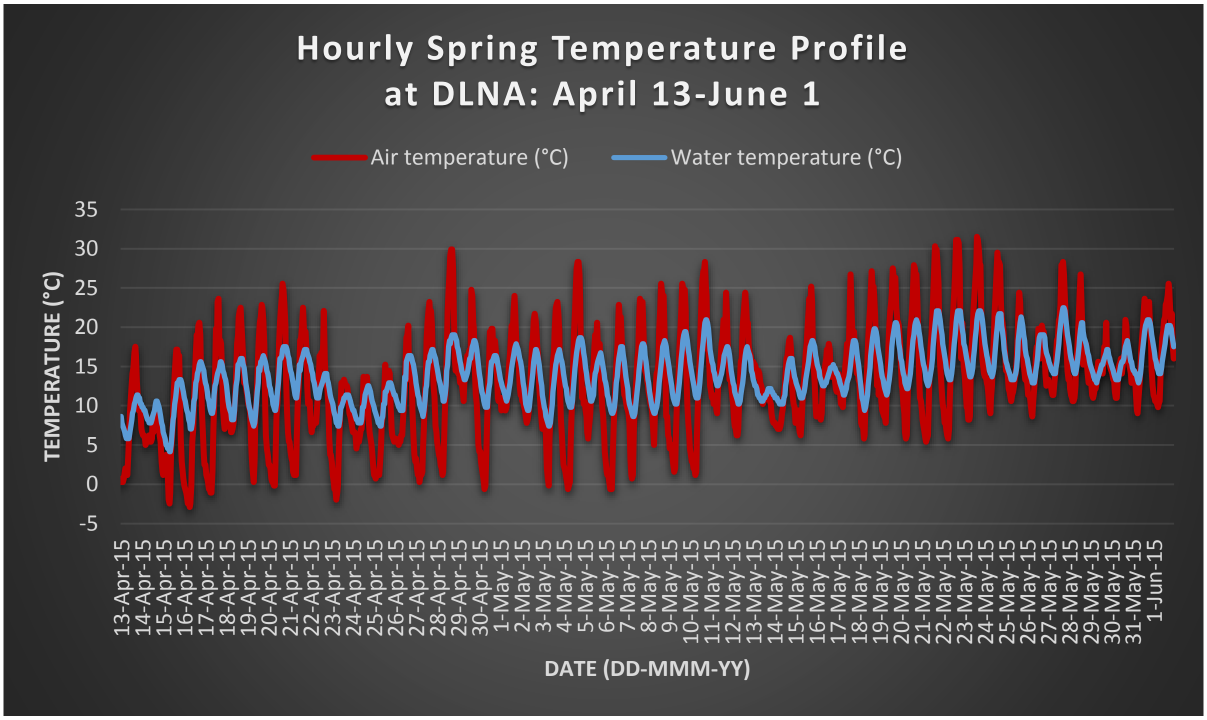


Figure 9. Daily air and water temperatures recorded 24 hours per day at DLNA for the spring breeding season.

As shown in Figure 10, the nightly air temperature profile spans several degrees during the spring breeding season; data source: HOBO temperature data logger #5 at DLNA breeding site, temperatures at 22:00 nightly. Nightly air temperatures at 22:00 ranged from a minimum of 1.6 °C on April 15 to a maximum of 20.6 °C on May 22; the first peak in air temperature within the April 13 to June 1 time period was recorded on April 18 when the temperature reached 12.9 °C. Nightly water temperatures at 22:00 ranged from a minimum of 7.8 °C on April 14 to a maximum of 19.8 °C on May 22 and 23; the first peak in water temperature within the April 13 to June 1 time period was recorded on April 20 when the temperature reached16.4 °C. Egg mass deposition dates ranged from April 21 to May 19.

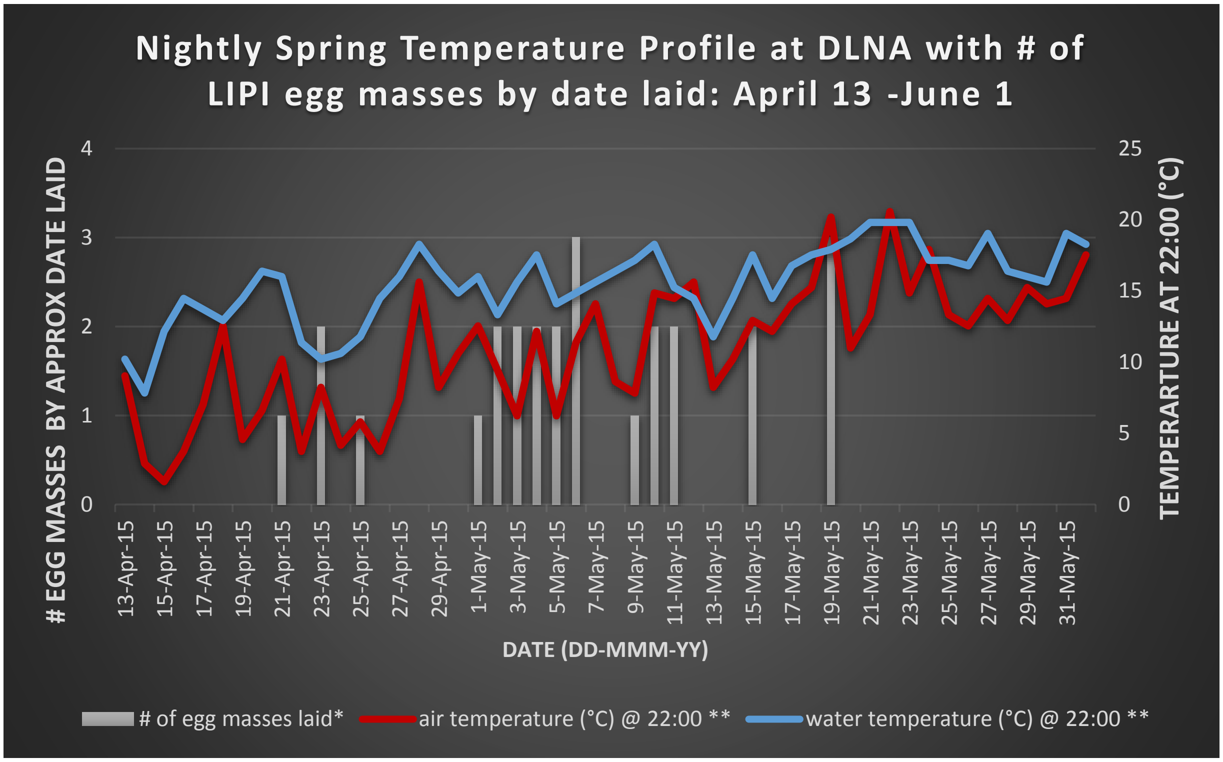


Figure 10. LIPI egg masses by date laid with air and water temperature (°C) at 22:00
*indicates # of egg masses detected (by date laid, not date of detection)
**source for temperature data is HOBO data loggers (#5 for air and #6 for water), both at DLNA breeding area; temperature at 22:00 nightly for period shown

Although capturing animals is not the primary objective of EMS, some observations were made; Table 7 summarizes the EMS LIPI observations by stage and sex. Of the 34 total observations, 10 were adults, the majority (n=15, 44.1%) were juvenile and 9 were unknown age class. The majority of the observations were male (n=29, 85.3%). These observations are shown on the map in Appendix 2.

Table 7. 2015 CVWMA LIPI EMS observations by stage and sex

Stage	Female	Male	Unknown*	Total
Adult	2	8	0	10
Juvenile	0	13	2	15
Unknown*	0	8	1	9
Total	2	29	3	34

*unknown because auditory or visual detections

4.4 Tadpole Trapping

Tadpole trapping was conducted at the main breeding areas in DLNA (East pond and WDLNA) to monitor the developmental stage of tadpoles and get an estimated date of metamorphosis, it was also conducted at Leach Lake ponds 1 and 4 to try and determine if breeding occurred at these suspected breeding sites. Trapping was conducted from July 7-9; a total of 23 traps were used and tadpole trapping was done for a total of 435:00 trap-hours, with an overall trap-rate of 0.014. LIPI tadpoles were detected in the East pond and WDLNA (n=6 trapped, n=11 visuals, Gosner stages 26-42), see photos in Figure 10; no tadpoles were detected at Leach Lake in ponds 1 or 4. The greatest number of tadpoles were trapped at WDLNA and the most successful trapping period was overnight. This level of trapping took 9:30 person-hours of effort to deploy, check and remove the tadpole traps. The first sign of metamorphosis was at WDLNA on July 9 when a metamorph with a 5.0 mm tail stub was detected hopping across the surface of the pond while surveyors were travelling between tadpole traps, see photo in Figure 11. Given that the first detected viable egg mass was estimated to have been laid on April 23, minimum development time from egg to metamorphosis was 77 days. Table 8 provides a summary of tadpole trapping results by site; see map in Appendix 1 for trap locations and map in Appendix 2 for location of tadpole observations.

Table 8. Summary of 2015 LIPI tadpole trapping effort by site at CVWMA

	East Pond	WDLNA	Leach 1	Leach 4	Overall
# Traps used	7	7	6	3	23
# Trapped LIPI	1	5	0	0	6
Trap-hours	117:38	92:48	150:08	74:26	435:00
Trap-rate	0.009	0.054	0	0	0.014

Figure 11. July 9 observations (left to right): trapped tadpole with hind limbs (~GS 39-40), metamorphosing LIPI with long tail (visual observation, not trapped) and first sign of metamorphosis (YOY with ~5.0 mm tail stub).

4.5 Visual Encounter Surveys (VES)

In 2015 at the CVWMA 84:52 person-hours were dedicated to visual encounter surveys (n=55 surveys) in summer and fall, Table 9 provides a summary of VES by season. Locations of LIPI observations in the CVWMA are provided in maps in Appendices 2, 4, 5, 6 and 7.

Table 9. Summary of VES efforts in the CVWMA for 2015

	Summer	Fall	Total
Number of surveys	32	23	55
Survey effort (person-hours)	52:29	32:23	84:52
Number of LIPI observations	115	95	210
LIPI catch/effort during NCS	2.19	2.93	2.47

During summer 52:29 person-hours were spent on VES (n=32 surveys) from July 9 to September 18. Summer VES were done in known breeding areas to search for YOY, in suspected breeding areas to search for YOY to provide evidence of successful breeding and in the migration corridors to search for all age classes as they move between seasonal habitats. Surveyed areas include: Duck Lake, East ditch, East ponds, Kootenay River (main channel and inlets at Leach Lake), Leach Lake (compartments 1, 4 and 5), Pumphouse channel, Southwest ditch, Squiggle channel and WDLNA. Attempts were made to search the frog-bear channel but it has become too overgrown with weeds to survey; however the frog-bear field between the East ditch and the frog-bear channel were surveyed to look for usage during the migration period. See Table 10 for survey details.

During summer VES a total of 115 LIPI detections were made (Table 10), a catch per effort of 2.19 LIPI per person-hour of survey effort. As expected, the majority of the summer VES detections were YOY of unknown age class (n=104, 90.4%), most of which were detected in the known breeding areas of DLNA (n=101). Unfortunately summer VES did not provide any evidence of successful breeding at Leach Lake or in Duck Lake. LIPI were detected in some of the known migration areas in the DLNA but none were detected in Leach Lake or utilizing the frog-bear property (however there was not a considerable amount of survey effort put forth on the frog-bear property); see Table 10 for survey details and LIPI detection data.

Table 10. Summer VES sites in CVWMA (n=#surveys) with LIPI observations by stage

Summer VES (n=32)	Adult	Juvenile	YOY	Other	TOTAL
Duck Lake (n=1)	X	X	X	X	**X**
East Ditch (n=3)	1	0	1	0	**2**
East Pond (n=4)	0	0	27	2 T	**29**
Frog-bear (n=2)	X	X	X	X	**X**
Kootenay River – Leach Lake (n=3)	X	X	X	X	**X**
Leach Lake 1 (n=2)	0	1*	0	0	**1**
Leach Lake 4 (n=6)	X	X	X	X	**X**
Leach Lake 5 (n=1)	X	X	X	X	**X**
Pumphouse channel (n=2)	X	X	X	X	**X**
Southwest ditch (n=2)	X	X	X	X	**X**
Squiggle channel (n=3)	0	0	2	1 U	**3**
WDLNA (n=3)	3	1	74	2 T	**80**
TOTAL	**4**	**2**	**104**	**4**	**115**

X = site was surveyed but no LIPI detected. T = tadpole, U = unknown age class
*dead LIPI detected July 15 (too large to be a metamorphosing YOY)

During fall VES a total of 32:23 person hours (n=23 surveys) were spent surveying for LIPI in the migration corridors (including areas in and around suspected breeding sites where YOY would likely be moving through) and over-wintering areas between Sept 21 and October 16 (see Table 11 for details). Areas surveyed: Duck Lake (south shoreline), East ditch, Goat channel, Kootenay River adjacent to DLNA (aka the east channel), Kootenay River main channel and inlets at Leach Lake, Leach Lake Ponds 1 and 5 (surveys had been planned for pond 4 as well but due to the fact that the area had been dry since mid-summer they were cancelled) and the Pumphouse channel; attempts were made to survey the frog-bear channel but it has become too overgrown with weeds to survey.

During fall VES, a total of 95 LIPI detections were made, a catch per effort of 2.4 LIPI per person-hour of survey effort. By survey area, the majority of the fall VES detections were made in the Old Goat channel overwintering area (n=68, 71.6%), of these most were YOY of unclassified sex (n=43), 23 were adults (15 females, 5 males, 3 unknown age class), and 2 were juvenile males. By age class, the majority of the fall VES detections were YOY of unclassified sex (n=64, 67.4%), 28 were adults, of which the majority were females (n=19, 67.9%), 5 were male and 4 were unclassified sex. There were no fall VES LIPI detections in Duck Lake, or any part of Leach Lake, including the main stem of the Kootenay River adjacent to Leach Lake so there is no detected evidence of successful breeding at either of the suspected breeding sites of Leach Lake or Duck Lake; see Table 11 for survey details and observation data by age class.

Table 11. Fall VES sites in CVWMA (n=#surveys) with LIPI observations by stage

Fall VES (n=23)	Adult	Juvenile	YOY	Total
Duck Lake (n=1)	X	X	X	**X**
East Ditch (n=3)	3	1	13	**17**
Old Goat channel (n=9)	23	2	43	**68**
Kootenay River - DLNA (n=2)	0	0	4	**4**
Kootenay River – Leach Lake (n=2)	X	X	X	**X**
Leach Lake 1 (n=1)	X	X	X	**X**
Leach Lake 5 (n=1)	X	X	X	**X**
Pumphouse channel (n=4)	2	0	4	**6**
Total	**28**	**3**	**64**	**95**

X =site was surveyed but no LIPI detected

4.6 Late-summer and Fall Road Surveys

Night road surveys during the late-summer and fall migration period were conducted along the Duck Lake dike between August 29 and October 10 (n=11 night surveys). A total of 20:07 person-hours of survey effort was dedicated to these surveys; with a combined total of 20 LIPI observations, a catch per effort of 0.99 LIPI/person-hour of survey effort (Table 12). See maps in Appendices 2, 4 and 5 for locations of LIPI observations made during road surveys. Incidental observations on the road at other times are not included in this summary; they are provided in Section 4.8 Incidental LIPI Observations.

Table 12. Summary of 2015 late-summer & fall night road survey effort

	Road surveys
Number of surveys	11
Survey effort (person-hours)	20:07
Number of LIPI observations (all YOY)	20
LIPI catch/effort during road surveys	0.99

Of the 20 LIPI observations made during night road surveys all were YOY, 15 were alive (75.0% of detections) and 5 were dead animals killed by vehicles (25.0% of detections), see Table 13. During night road surveys the pumphouse channel and several points along the south shoreline of Duck Lake were surveyed for eyeshine but none was detected.

Other herptile observations during late summer and fall night road surveys along Duck Lake dike included: 1 live YOY Pacific chorus frog *(Pseudacris regilla)* and 1 dead juvenile Common garter snake *(Thamnophis sirtalis).*

Table 13. Late-summer and fall night road survey results at Duck Lake dike 2015

Species (age class)	Live	Dead	Total	Proportion Live	Proportion Dead
LIPI (all YOY)	15	5	20	75.0%	25.0%
Pacific chorus frog	1	0	1	100.0%	0%
Common garter snake	0	1	1	0%	100.0%
Total	16	6	22	72.7%	27.3%

Mean air temperature at dusk for the subset of night surveys where LIPI were encountered was 12.5°C (n=5, SD=2.0, range 9.9°C to 15.3°C). LIPI were encountered in 5 of the 11 night road surveys (45.5%); detection dates were September 17, 19, 22, 23 and October 8. Movement onto the road surface seemed to begin at dusk, and as darkness set in the number of encounters increased. Some of the frogs moved across the road quite quickly, but the majority seemed to be resting on the road, likely for the main purpose of thermoregulating and possibly to opportunistically forage as well. In many cases the frogs did not even flee when approached.

4.7 Incidental LIPI Observations

During the 2015 field season there were 8 incidental LIPI detections made outside of regular surveys, all of which were YOY in the fall. Five of the 8 were on the Duck lake dike road at dusk on October 6, incidentally detected while travelling back from a day survey; all were alive on the road. The other 3 were incidentally detected while collecting HOBO data loggers and checking water levels in the breeding area. The observations included 1 in fair health at the East pond on September 21 which was exhibiting signs of *Chytridiomycosis,* 1 in the East ditch on September 21 which was dead, and 1 in good health at WDLNA on September 23.

4.8 Animal Health

Of the captured LIPI whose health was assessed visually in the field (n=151), the majority were deemed to be in good health (n=128, 84.8%), 6.6% in fair health (n=10), 0.7 % in poor health (n=1) and 7.9% were found dead (n=12). See Table 14 for a detailed summary of these results by season and age class and Figure 12 for photos.

Table 14. Health assessment of LIPI 2015 CVWMA field observations (n=151)

Season	Age Class	Good	Fair	Poor	Dead	Total
Spring	**Adult**	17	3	1	0	21
	Juvenile	8	2	0	5	15
	Tadpole	2	0	0	0	2
	All age class	27	5	1	5	38
	All age class %	71.1%	13.2%	2.6%	13.2%	100.0%
Summer	**Juvenile**	1	0	0	1	2
	YOY	40	0	0	3	43
	Tadpole	16	0	0	0	16
	All age class	57	0	0	4	61
	All age class %	93.4%	0.0%	0.0%	6.6%	100.0%
Fall	**Adult**	20	3	0	0	23
	Juvenile	3	0	0	0	3
	YOY	21	2	0	3	26
	All age class	44	5	0	3	52
	All age class %	84.6%	9.6%	0.0%	5.8%	100.0%
Over all seasons	**All age class**	128	10	1	12	151
	All age class %	84.8%	6.6%	0.7%	7.9%	100.0%

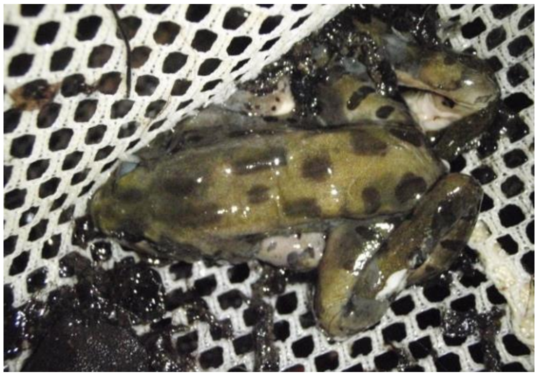
Of the 128 LIPI deemed to be in good health, there were 37 adults, 12 juveniles, 61 YOY and 18 tadpoles.

Of the 10 live LIPI deemed to be in fair condition, there were 6 adults, 2 juveniles and 2 YOY. The animals in fair condition had the following issues: *Chytridiomycosis* suspected (n=5; see Table 15 for details), missing front limb below elbow (n=1 adult female in the fall), dislocated toe (n=1 adult male during spring breeding season), severe grass cuts on dorsal surface (n=1 juvenile male during spring breeding season), prolapse (n=1 adult female in fall) and an eyelid abnormality (n=1 YOY of unknown sex in the fall).

There was 1 LIPI observed in poor condition during the 2015 field season, a male adult detected during the spring breeding season with what appeared to be a dislocation of the upper leg at the hip.

Of the 12 dead LIPI detected, there were 6 juveniles and 6 YOY. Of the 6 dead juveniles, 1 was a summer detection at Leach Lake pond 1 but the carcass was in the advanced stages of decomposition so it was not possible to conclusively determine the cause of death. The remaining 5 dead juveniles were detected at WDLNA during the spring breeding season, 4 of these animals had already begun to decompose so it was difficult to conclusively determine the cause of death and lab analysis was not possible; however 1 carcass had not begun to decompose. When examined it showed signs of Chytridiomycosis with redness on the ventral surface, it was sent to the Animal Health Lab for a full work up and the cause of death was attributed to the cumulative effects of the cutaneous *Zygomycosis* and *Chytridiomycosis*, PCR was negative for *Iridovirus*. Of the 6 dead YOY, 5 were road mortalities caused by vehicle traffic on the DLNA dike road (n=3 in late summer and n=2 in fall) and 1 was a fall detection in the East ditch which was nearly dry at the time, the animal did not exhibit any obvious signs of trauma or disease but was sent to the Animal Health Lab for a full work-up and results indicated that the animal most likely died of complications following oomycete dermatitis and accompanying acute bacteria and toxemia, PCR tests for *Chytridiomycosis* and *Iridovirus* were negative.





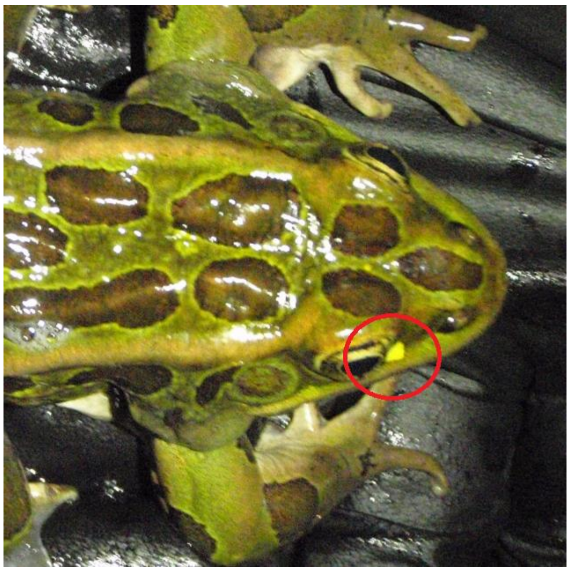



Figure 12. From top row, left to right: (spring) dead juvenile with *Chytridiomycosis*, dislocated toe, advanced stage of decomposition, extensive scratches on dorsal surface, yellow dot (lack of pigmentation) in front of right eye, a 2nd individual with a yellow dot (lack of pigmentation) on left hind limb; (fall) YOY road mortality, YOY road mortality, dead YOY with no obvious signs of trauma or disease, YOY with eyelid abnormality, adult female with prolapse.

In total there were 133 LIPI observations that were visually inspected by surveyors for signs of *Chytridiomycosis* including 12 dead specimens; *Chytridiomycosis* was not suspected in the majority of the 133 observations visually inspected for signs of the disease (n=121, 91.0%). *Chytridiomycosis* was suspected in 6 of the 133 observations (4.5%); it was suspected in 3 of 44 adults (6.8%), 2 of 20 juveniles (10.0%), and 1 of 69 YOY (1.5%). It was not possible to conclusively determine whether or not *Chytridiomycosis* was suspected in 6 observations (4.5%); 5 of these were carcasses that were too decomposed and 1 was an injured animal that was not assessed for signs of *Chytridiomycosis* as it would have caused unnecessary stress to the animal. Table 15 provides a breakdown of these results by season and age class.

Table 15. Field observer's determination if *Chytridiomycosis* suspected by visual inspection of CVWMA LIPI detections in 2015 (n=133*) by season and stage

		Chytridiomycosis Suspected			
Season	Stage	No	Yes	Unknown	Total
Spring	Adult	18	2	1	21
	Juvenile	9	2	4	15
	All age class	27	4	5	36
	All age class %	75.0%	11.1%	13.9%	100.0%
Summer	Juvenile	1	0	1	2
	YOY	43	0	0	43
	All age class	44	0	1	45
	All age class %	97.8%	0.0%	2.2%	100.0%
Fall	Adult	22	1	0	23
	Juvenile	3	0	0	3
	YOY	25	1	0	26
	All age class	50	2	0	52
	All age class %	96.2%	3.8%	0.0%	100.0%
Over all seasons	All age class	121	6	6	133
	All age class %	91.0%	4.5%	4.5%	100.0%

*includes dead specimens (n=12; n=6 unknown cause, n=5 road mortality, n=1 Bd suspected

A total of 60 LIPI detections were swabbed for Chytridiomycosis testing during the field season. Samples were collected on a priority basis and include 36 adults (16 spring and 20 fall detections), 11 juveniles (7 spring, 1 summer and 3 fall detections) and 13 YOY (1 late-summer and 12 fall detections). Swabs have been stored for future analysis if deemed necessary as lab analysis costs were not budgeted for this year.

4.9 Dorsal Spot Pattern Recognition and Recapture Analysis

A total of 144 dorsal spot pattern photos were taken for captures during the 2015 field season and a digital image library created; however there are no results to report as it was decided that the recognition would not be completed due to the considerable amount of time required combined with the fact that in the past there have been relatively few between-year recaptures which are required for population estimate work.

Based on the total number of dorsal spot pattern photos in the digital image library it is estimated that it would take approximately 50 hours (6.25 days @ 8 hours per day) for an experienced person to complete the full analysis; this includes within-year recapture analysis in addition to between-year analysis for the previous 2 years. Since the digital image library is on file a full or partial analysis could be done at any time in the future if it is deemed necessary.

Since the photo recognition was not done there is no recapture information available for the 2015 field season so all reported detections are observations in time and do not indicate individuals.

4.10 Morphometrics of Creston LIPI

Size data was gathered on 114 LIPI observations during the 2015 field season, this data includes male and female field captures where both a weight and snout vent length were measured, outliers were removed (n=2); recaptures included. Throughout the course of the entire field season, there were measurements taken on 63 YOY (40 in summer, 23 in fall), 13 juveniles (9 in spring, 1 in summer, 3 in fall) and 38 adult captures (20 in spring, 18 in fall), detailed morphometrics are presented in Table 16 and 17.

Table 16. Weight of 2015 CVWMA LIPI captures by stage and sex

Weight (g)	Stage - sex	n	Mean	SD	Range
Spring	Juvenile - male	9	27.2	4.5	22.0 - 34.0
	Adult - male	17	58.6	3.5	53.0 - 64.0
	Adult - female	3	90.7	12.7	77.0 - 102.0
	Total (combined)	29	52.2	20.2	22.0 - 102.0
Summer	YOY - sex unknown	40	4.1	1.6	2.0 - 11.0
	Juvenile - female	1	32.0	n/a	32.0 - 32.0
	Total (combined)	41	4.7	4.7	2.0 - 32.0
Fall	YOY - sex unknown	23	11.4	5.0	6.0 - 27.0
	juvenile	3	45.0	3.0	42.0 - 48.0
	adult - male	2	65.5	17.7	53.0 - 78.0
	adult - female	16	78.6	18.5	50.0 - 112.0
	Total (combined)	44	40.6	34.1	6.0 - 112.0

Table 17. SVL of 2015 CVWMA LIPI captures by stage and sex

SVL (mm)	Stage - sex	n	Mean	SD	Range
Spring	Juvenile - male	9	61.6	3.5	55.9 – 67.7
	Adult - male	17	78.8	3.4	72.8 - 83.4
	Adult - female	3	89.6	2.8	86.9 - 92.5
	Total (combined)	29	74.6	10.0	55.9 - 92.5
Summer	YOY - sex unknown	40	33.7	3.4	29.4 - 48.0
	Juvenile - female	1	65.7	n/a	65.7 – 65.7
	Total (combined)	41	34.5	6.0	29.4 - 65.7
Fall	YOY - sex unknown	23	46.6	5.7	39.1 - 57.0
	Juvenile	3	75.2	0.8	74.2 - 75.7
	Adult - male	2	79.9	7.3	74.7 - 85.0
	Adult - female	16	86.1	5.3	79.0 -96.4
	Total (combined)	44	64.4	19.8	39.1 - 96.4

During the 2015 field season it was noted that YOY appeared to be smaller than usual, to test this theory, size data for fall 2015 YOY observations was compared to 2014 data. Results of a non-paired t-test show that the difference between the means for both size metrics (weight and SVL) was statistically significant at the 0.05 level (weight p-value =6.29153 E-08, SVL p-value=2.44203 E-07). The fall 2015 YOY had the lowest mean weight and smallest mean SVL of any of the previous 5 years; see Table 18 for details.

Table 18. Size data for fall YOY LIPI detections in the CVWMA 2010 – 2015

Year	Date	n	$\bar{x}$ Weight (g)	SD	Range	$\bar{x}$ SVL (mm)	SD	Range
2010	9/28 - 10/20	25	20.2	7.4	8.0 - 33.0	57.6	6.8	46.1 - 67.2
2011	9/24 - 10/19	9	13.2	7.4	7.5 - 32.0	53.3	7.9	45.1 - 69.5
2012	9/26 – 10/11	52	19.3	6.1	9.0 – 32.0	56.8	6.2	44.7 – 66.1
2013	9/21 - 10/18	10	18.3	9.0	9.0 - 37.0	52.6	8.9	41.1 - 68.1
2014	9/22 - 10/20	58	21.0	9.0	6.0 - 39.0	56.1	8.8	37.6 - 70.8
2015	9/21 - 10/16	23	11.4	5.0	6.0 - 27.0	46.5	5.7	39.1 - 57.0

Figure 13 provides a visual representation of the morphometrics of Creston LIPI. During the spring (blue markers), there are 3 main clusters: 1) the smallest animals are males with weights ranging from 22.0 – 34.0 g and SVL from 55.9 mm to 67.7 mm, 2) large males with weights from 53.0 – 64.0 g and SVL ranging from 72.8 – 83.2 mm, and 3) the large females with weights from 77.0 – 102.0 and SVL from 86.9 – 92.5. In the summer (red markers), the majority of the observations are below 6.0 g in weight and 38.0 mm SVL with only 2 larger animals detected. During the fall (grey markers), there are 3 clusters: 1) the smaller LIPI with weights of 6.0 – 27.0 g and SVL measurements of 39.1 – 57.0 mm, 2) the medium sized LIPI, with males ranging in size from weights of 42.0 – 78.0 g and SVL measurements of 74.2 – 85.0 and the females ranging in size from weights of 50.0 – 76.0 g and SVL measurements of 79.0 – 84.0 mm, and 3) the largest LIPI, all females which ranged in size from weights of 88.0 – 112.0 g and SVL measurements of 86.0 - 94.2 mm. The majority of the fall observations are greater than 50 grams in weight (see dashed line in Figure 13) and 72.8 mm SVL.

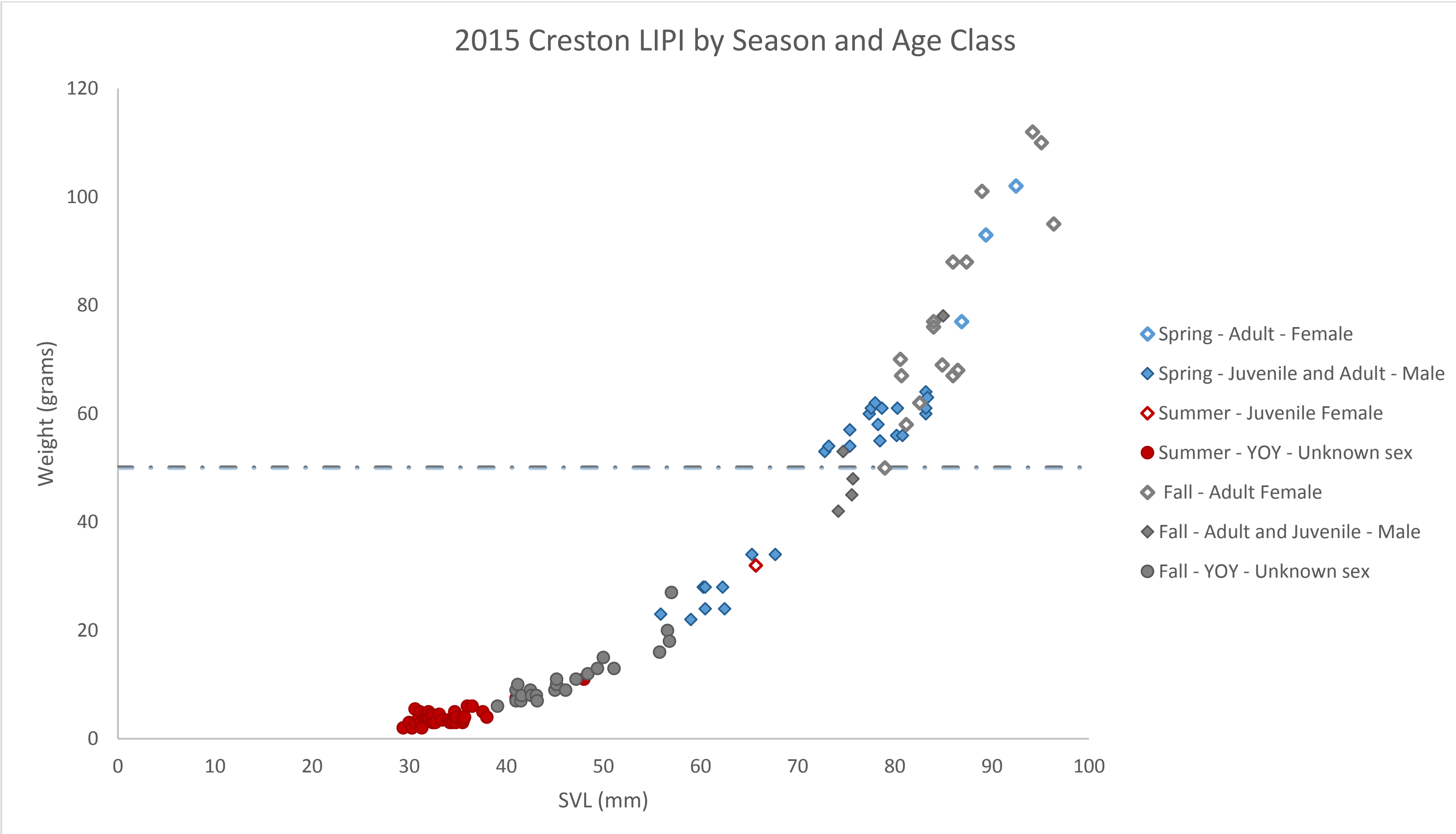


Figure 13. Size Chart: 2015 CVWMA LIPI Captures (n=114) by Season and Age Class (both sexes)

4.11 Vancouver Aquarium Captive Assurance Colony

In 2015, due to a lack of space at the Vancouver Aquarium a lower number of tadpoles than usual was sent from Creston to bolster the captive assurance colony; a total of 15 free-swimming hatchling tadpoles, from 3 different egg masses (5 tadpoles from each) were transferred to the Vancouver Aquarium May 27, 2015. Tadpoles were collected from the breeding site ponds where they had been housed in situ in egg mass cages on May 26, 2015, housed overnight in tanks at the office and packaged up the following morning and driven to the Trail Airport (YZZ) where they were flown to Vancouver the same day via Pacific Coastal Airlines. Tadpoles were collected from DLNA egg masses EM150508-LR14, EM150508-LR15 and EM150515-TH21; these egg masses were estimated to have been laid May 4-10. Sizes ranged from a total length (TL) of 10.9 to 14.0 mm (Table 19).

Table 19. Summary of tadpoles transferred to captive assurance colony in 2015

Transfer Date	Source Site	Egg mass #	~Date laid	TL (mm) range	# Transferred
May 27, 2015	WDLNA	EM150508-LR14	5 May	10.9 – 12.9	5
May 27, 2015	WDLNA	EM150508-LR15	4 May	12.8 – 13.7	5
May 27, 2015	EDLNA	EM150515-TH21	10 May	13.7 – 14.0	5
Grand Total					**15**

As of the last update received from the Vancouver Aquarium in December 2015, when pre-brumation morphometrics were taken, there were 12 survivors of the original 15 Creston source tadpoles transferred from Creston in 2015; all of which successfully metamorphosed into YOY prior to brumation.

As part of the captive assurance colony, there is an in-house captive breeding program, although a number of egg masses were laid in 2015, there were few viable hatchlings and only 616 were sent to Brisco for the Columbia Marsh reintroduction program. In addition to this, there was a small subset held back and maintained at the aquarium and as of December 2015 there were 23 captive bred animals from the egg masses produced at the aquarium.

Table 20 shows the fall 2015 pre-brumation morphometrics of the Creston source and the captive bred animals. The mean weight of the Creston source YOY at the Vancouver Aquarium is 8.11 grams compared to 6.18 grams for the captive bred animals, a difference of 1.93 grams; unpaired t-test results show that the difference between these 2 means is statistically significant at the 95% level (p-value = 0.0009). Mean SVL for the Creston source YOY was 39.2 mm compared to 34.9 mm for the captive bred animals, a difference of 4.3 mm; unpaired t-test results show that the difference between these 2 means is statistically significant at the 95% level (p-value = 0.0002).

Table 20. Pre-brumation morphometrics of 2015 Vancouver Aquarium LIPI YOY

Morphometric	Mean	n	SD	Range
Creston source weight (g)	8.11	12	1.26	6.73 – 10.14
VA captive bred weight (g)	6.18	23	1.78	1.79 – 8.97
Creston source SVL (mm)	39.2	12	2.5	35.5 – 42.3
VA captive bred SVL (mm)	34.9	23	3.5	26.0 – 38.8

Figure 14 shows the size difference between the 2 sets of 2015 YOY at the Vancouver Aquarium captive assurance colony (captive bred and Creston source) and the wild Creston YOY; the wild Creston YOY are the largest of the 3 groups. Size data for the Vancouver Aquarium captive assurance colony YOY is summarized in Table 20 and the size data for the wild Creston YOY field observations made between September 21 and October 16 is provided in the previous section on morphometrics in Table 16 and 17.

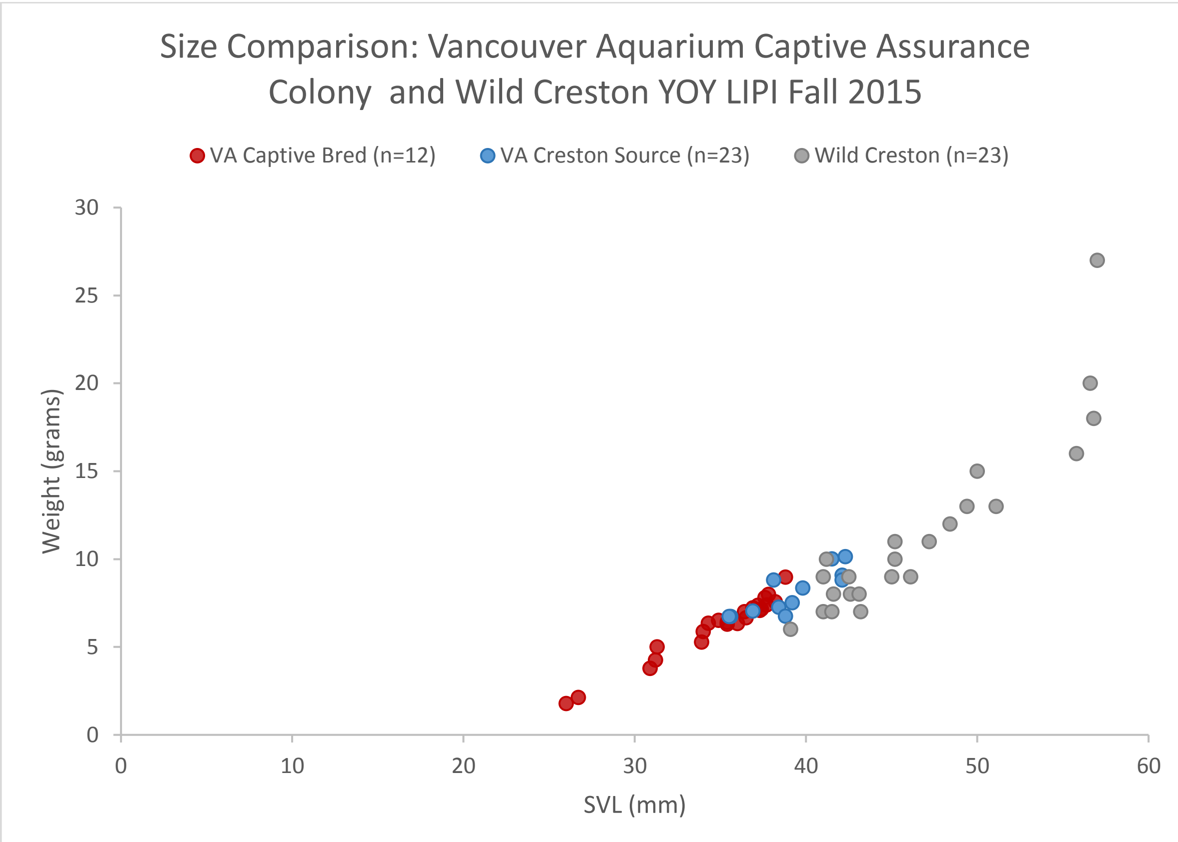


Figure 14. Size Comparison of 2015 Vancouver Aquarium captive LIPI YOY (captive bred and Creston source LIPI) and Creston wild LIPI YOY fall detections

4.12 Bummers Flats Reintroduction Program

In 2015, year 5 of phase 2 reintroductions at Bummers Flats, a total of 5279 free-swimming hatchlings were transferred from 4 different egg masses from the source population in Creston; total length (TL) of hatchlings transferred ranged from 8.3 to 14.0 mm (Table 20, Figure 15). The source egg masses were from WDLNA and were estimated to have been laid between May 6 and 10, 2015. Transfers were done using hatchlings that had recently become free-swimming and were carried out between 16 – 19 days from the date the egg masses were laid. Targets were to transfer approximately half of the hatchlings from the 4 egg masses, actual counts came in between 40.0 % and 58.5 % (mean 49.8%); transfer mortality was very minimal. Appendix 9 shows the location of the release ditch at North Bummers Flats.

Table 20. Summary of 2015 Bummers Flats Reintroduction with Creston hatchlings

Transfer Date	Source Site	Egg mass #	~Date laid	# Moved*	%**	TL (mm)
22-May-15	WDLNA	EM150508-LR12	06-May	1496	53.7%	9.7 - 12.5
22-May-15	WDLNA	EM150508-LR13	06-May	1783	49.4%	8.3 - 11.2
28-May-15	WDLNA	EM150512-BH16	10-May	1000	58.5%	10.0 - 13.0
28-May-15	WDLNA	EM150512-EA17	09-May	1000	40.0%	11.0 - 14.0
Grand Total				**5279**	**49.8%**	**8.3 - 14.0**

*number transferred to Bummers Flats; number reported is live animals counted upon release
**percent of total hatchlings moved to Bummers Flats (remainder released in situ at breeding site)
All animals transferred as free-swimming hatchlings

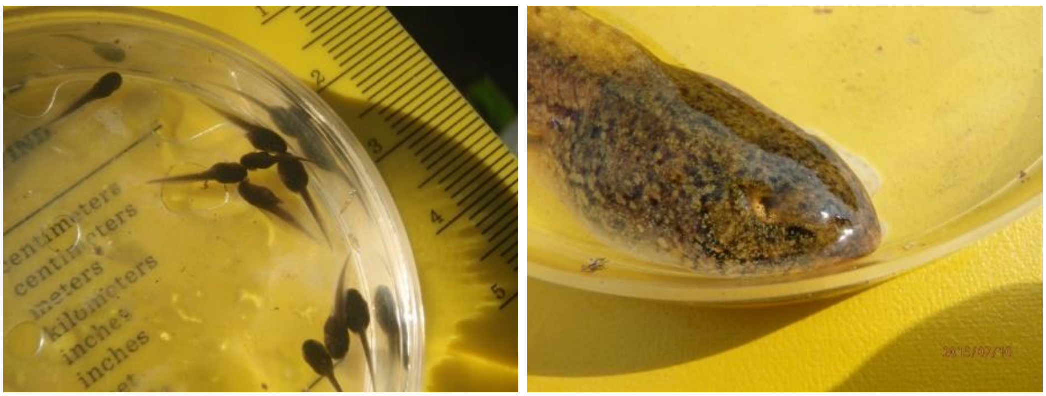


Figure 15. Size range of reintroduced hatchlings (L), tadpole with malformed eye (R)

Since 2011, when phase 2 reintroductions at Bummers Flats began a total of 33,958 LIPI hatchlings from Creston's WDLNA have been released, see details by year in Table 21. The number of animals transferred to Bummers Flats in 2015 (n=5279) was slightly less than the mean of 6791.6 (n=5, SD=1651.2, range 4948-8878); this is due to the fact that animals were also being transferred from Creston to the Columbia Marsh release site.

Table 21. Summary of phase 2 reintroductions at Bummers Flats 2011-2015

Year	Source	Release Site	# NLF released
2011	WDLNA	North Bummers release ditch	4948
2012	WDLNA	North Bummers release ditch	7600
2013	WDLNA	North Bummers release ditch	8878
2014	WDLNA	North Bummers release ditch	7253
2015	WDLNA	North Bummers release ditch	5279
Total	WDLNA	North Bummers release ditch	**33, 958**

In order to follow up on the reintroductions 6:47 person-hours were spent surveying at the North Bummers Flats release site (n=2 tadpole trapping surveys and n=2 VES); this does not include time associated with the reintroduction itself or time FWCP crew helped Bummers Flats crew funded by Columbia Basin Trust (CBT) with summer VES for YOY (the results of which are summarized in the monitoring report by Ohanjanian, 2016).

Tadpole trapping was conducted at North Bummers Flats at the site of the spring 2015 tadpole release. A total of 4:27 person-hours were spent deploying, checking and collecting tadpole traps on July 9 and 10 during which time 11 LIPI tadpoles were trapped during 129:15 hours of total trap-effort using 10 traps. Animals ranged from Gosner stages 26-40; all tadpoles appeared to be in good health except for one which had a deformed eye (see photo in Figure 14). Trap locations are mapped in Appendix 9 and the map in Appendix 10 shows the location of tadpole observations.

At North Bummer's Flats 2:33 person-hours of survey effort were put into VES. The first VES was on July 16, 2015 at the site where 5279 hatchling tadpoles were released in the spring; the objective of this survey was to determine the stage of development and to determine if metamorphosis had begun to occur. During this survey there were 3 detections, 1 tadpole in the process of metamorphosing and 2 large tadpoles which had not begun to metamorphose (approximately Gosner stage 38); it is estimated that the date of first metamorphosis was approximately July 18 - 20. The second VES was done on October 9, 2015 while on site to retrieve HOBO temperature data loggers during which time a small portion of the north end of the release ditch at North Bummer's Flats was surveyed to determine if any YOY were still present; 2 small YOY were detected. A map of observations is provided in Appendix 10.

4.13 Columbia Marsh Reintroduction Program

In response to the BC-NLFRT request for tadpoles from the source population in Creston to supplement the Columbia Marsh reintroduction project at Brisco (which has until now relied exclusively on Vancouver Aquarium captive bred animals), a total of 2947 free-swimming hatchlings were transferred. They were taken from 4 different egg masses; total length (TL) of hatchlings transferred ranged from 8.5 to 14.0 mm (Table 22). The source egg masses were all wild laid in WDLNA and were estimated to have been laid between May 4 and 19, 2015. Transfers were done using hatchlings that had recently become free-swimming and were carried out between 16 – 22 days from the date of egg mass deposition. The target was to transfer approximately half of the hatchlings from 4 egg masses, actual counts came in between 39.6 % and 65.4 % (mean 46.7%). Hatchlings were packaged up in Creston by the FWCP team and transported to the release site by the Brisco team with members from the BC-NLFRT, where they were held overnight then counted and released the following day. Overall transfer mortality was very low (n=30, 1.0%), so 2917 live animals were released. Table 22 provides a summary.

Table 22. Summary of 2015 Columbia Marsh Reintroduction using Creston hatchlings

Transfer Date	Source Site	Egg mass #	~Date laid	# Moved*	%**	TL (mm)
May 26, 2015	WDLNA	EM150508-LR14	5 May	539	48.3%	10.9 - 12.9
May 26, 2015	WDLNA	EM150508-LR15	4 May	1328	39.6%	12.8 - 13.7
June 4, 2015	WDLNA	EM150520-EA23	19 May	801	65.4%	9.2 - 12.2
June 4, 2015	WDLNA	EM150520-SW25	19 May	279	45.7%	8.5 - 14.0
Grand Total				**2947**	**46.7%**	**8.5 - 14.0**

*number live tadpoles transferred to Columbia Marshes

**percent of total hatchlings moved to Columbia Marshes (remainder released in situ at breeding site)

In addition to the 2917 live free-swimming hatchlings released from Creston for the first time, 616 were released from the Vancouver Aquarium captive assurance colony breeding program on June 10 by the BC-NLFRT for a total of 3533 reintroduced during the 3rd year of the project; a grand total of approximately 7533 since 2013.

5. DISCUSSION

5.1 Songmeters

Analysis of the 80 hours of recordings from the 2 Songmeters deployed in Leach Lake failed to provide any evidence of LIPI calling. However, field results of an in-person survey during the Songmeter deployment period show that at least 2 LIPI were calling approximately 500 meters away from the Songmeter deployed in Leach Lake pond 1. Since the weather conditions were optimal and all of the Songmeter settings, including the gain were configured correctly, the non-detection result indicates that either there were complications encountered during analysis or the LIPI call did not reach the microphone. While it is possible that both of these explanations are relevant, it is more likely that in this situation the non-detection is a result of the LIPI call not reaching the microphone.

During analysis, a large number of vocalizations from Canada Geese (*Branta canadensis*) were detected in the same general time frame as the LIPI which could cause complications with analysis. This complicates the analysis because the vocalization of the Canada goose is in the 281 – 1750 Hz frequency so it overlaps the 218 -1093 Hz LIPI call on the spectrogram. Since it is not possible to separate 2 sounds that occur at the same time in the same frequency the quieter LIPI call could go undetected. However, it is not likely that the geese would be vocalizing at exactly the same point in time during 100% of the LIPI calls, so it is unlikely that this accounts for the non-detection. The non-detection was more likely a result of the LIPI call not reaching the microphone due to habitat characteristics and detection distance.

Orthophoto interpretation indicates that while the majority of habitat between the Songmeter deployment site in Leach Lake pond 1 and the calling LIPI detected in person approximately 500 m away is shallow open water, there are some significant patches (totalling approximately 75 m) of tall emergent vegetation including cattails (*Typha latifolia*) which could account for LIPI calls not reaching the Songmeter microphone. It is also quite possible that 500 m is outside the maximum detection distance of the Songmeter.

Detection distance of LIPI using the Songmeter SM2+ was previously thought to be somewhere in the 500 m range (depending on habitat and environmental conditions) based on the fact that Wildlife Acoustics, who produces the Songmeter, indicates that the rule of thumb is "if you can hear it with your ears, the microphone will also hear it". Therefore the estimated 500 m detection distance was based on the fact that the author has detected LIPI calling from 615 m away (across a field) by ear under optimal conditions. However, that is being reconsidered not only due to the non-detection results from this year but results of the 2014 Songmeter analysis also indicate there was a non-detection at Leach Lake pond 4 where calling was detected in person 386 m away from where a Songmeter was recording. In this situation the non-detection may have been a result of habitat characteristics which impaired the LIPI call from reaching the Songmeter as the weather was optimal and there was not a large amount of other vocalizations recorded in the same frequency which might impair detection during analysis (Houston, 2015). Since there is not much in the literature citing detection distance for amphibians as they are so situationally specific and the results of 2 years of Songmeter analysis have shown some non-detections, it is recommended that detection distance be re-evaluated and field tested.

A field test of detection distance would involve manual testing of the Songmeter within a known breeding area. Testing would be done along a transect starting at the maximum test distance and working towards the calling LIPI by establishing test stations at 100 m intervals. Testing should be done at night during the spring breeding season when optimally only 1 calling male LIPI (or 1 geographically isolated group) is present to ensure that is the only calling the Songmeter is picking up on so other calls do not confound the results. Environmental conditions should be optimal with no wind or rain and little environmental noise to ensure a clean test recording for analysis. During the test a variety of habitats on a scale from shallow open water to thick dense stands of cattails should be tested to determine how different habitats impact detection distance. Analysis would involve mapping the test stations and calling LIPI and analyzing the Songmeter results to determine detection distances in the variety of habitats tested.

A discussion of analysis time was presented in the 2014 field season report (Houston, 2015) but given that our results indicate it is quite time consuming, it is recommend that Songmeters not be relied upon as the only means of detection at sites where calling is suspected; in some situations in-person surveys would be more effective and a better use of resources. For sites that are of particular interest, such as Leach Lake, it is recommended that in-person reconnaissance level NCS by canoe be conducted during the peak of the breeding season during optimal weather conditions as was done in 2015. This is thought to be a better use of resources and more effective since results are immediate (no analysis required) and there is the additional benefit that larger geographic areas can be sampled so results are not limited to the detection distance of a stationary Songmeter.

Songmeters can be a useful tool in some situations, and calling LIPI have been detected in new areas using this method in the past (Houston, 2015) so are recommended for use on the project at the presence/not-detected level at smaller sites where the entire area is within the detection distance or sites which are not suitable for in-person reconnaissance level NCS by canoe due to access issues or other limitations.

5.2 Nocturnal Calling Surveys (NCS)

Calling male LIPI were already present when the first NCS was conducted on April 21, an indication that the critical temperatures required to trigger male LIPI calling had already been reached. Since the literature indicates that males tend to begin calling when water temperatures reach 10°C and air temperatures reach 15°C (Seburn, 1992), the temperature profile for the month of April was examined. Results show that while water temperatures hovered at or above 10°C for the majority of the beginning of April, it wasn’t until April 16 that early evening air temperatures reached 15°C. Results during the 2014 field season for the DLNA population showed that calling began the night after evening temperatures for both air and water first reached the peak temperatures Seburn indicated trigger male calling (Houston, 2015). This indicates that both air and water temperature together are important in triggering calling and that calling in 2015 likely began on or within a few days of April 16. Although this is 5 days prior to the start of NCS it is not believed that any successful breeding occurred during this period as females don't tend to arrive until after the males start calling.

It was not possible to get an accurate estimate of calling levels or determine when the peak of calling occurred at the DLNA breeding area due to the fact that a significant portion (1/3) of all NCS had to be cancelled due to extreme winds as they impede the ability of the surveyor to detect calling. As a result it is not possible to do a between year comparison which would indicate whether calling levels increased or decreased over the previous year. However, our results do indicate that there were at least 38 males calling at the WDLNA during the first week of surveys on April 21-25. While this is below the maximum level of 53 individuals detected calling in 2014 (Houston, 2015) it does not conclusively indicate that calling levels decreased from the previous year since it was not possible to conduct surveys during the typical peak in calling which usually occurs during the first or second week in May (Adama, 2006; Houston, 2015). Since it was not possible to conduct surveys during this period, it is unknown what the maximum was for 2015, but it is unlikely that 38 was the maximum.

Due to the fact that only a small portion of calling males are actually captured during NCS due to limited resources, the reported number of adult and juvenile male detections cannot be assumed to be a direct representation of the actual breeding male population. Furthermore, this data should not be used as an estimate of the adult to juvenile calling male ratio as it does not represent the number of individuals calling as recaptures have not been removed. Requests for this type of estimate have been made by the BC-NLFRT, while it is possible to collect this type of data it would require a change in the survey program. If this is a priority it is recommended that the survey program objectives return to a focus of capturing as many males as possible (as in previous years); photo recognition would also have to be completed so that recaptures could be removed from the analysis as has been done in the past.

Dedicated NCS are no longer being conducted at the East Ditch of EDLNA since calling has not been documented there since 2012 and there have been no egg masses detected since 2007 so it was thought that it would be more efficient to utilize survey effort elsewhere. However, since surveyors walk past the site while accessing the East ponds of DLNA it is expected that if any significant calling were occurring it would be detected.

The calling detected at Leach Lake pond 1 during a reconnaissance level NCS by canoe was very significant as calling has not been detected in this area since 2006. Our results show that a minimum of 12 individual males were calling in one night, which suggests that this could be a fairly significant breeding site. Calling activity was documented but it was not possible to capture all animals due to limited resources, however there was at least one adult present. This indicates that it is possible that even though calling has not been detected in pond 1 for 9 years, it is quite possible that calling has been occurring at satellite breeding sites within Leach Lake for many years but has gone undetected. This is likely a result of the extremely vast size of the Leach Lake wetland complex which is approximately 700 ha in size in combination with the limited resources allocated to NCS in the area and the fact that detection distances during Blitz style surveys are limited. Detection distances are limited to approximately 500 metres under optimal conditions from survey stations which are located on the roadway adjacent to extensive wetlands. These wetlands can be up to 1500 meters across, and as a result there are large areas not surveyed as they are outside of the detection distance covered by Blitz style surveys at Leach Lake.

Since calling has now been detected in Leach Lake ponds 1, 3 and 4 over the past 2 years (Houston, 2015) it is recommended that NCS continue and additional effort be allocated to survey during the spring of 2016. If it is a priority of the program to detect all breeding activity, effort should be increased in the Leach Lake area by allocating additional resources to reconnaissance-level canoe surveys. As spring is a very busy time of year and there is not much spare time it is likely that this would mean a trade off or decrease in survey time at DLNA. It is recommended that during the 2016 field season, reconnaissance-level canoe surveys be conducted at Leach Lake at least once per week (weather permitting) which should allow all 4 compartments to be surveyed during the breeding season.

Unfortunately there was no calling detected at Leach Lake pond 4, the site of habitat restoration in 2013. Although calling was documented in 2014 following habitat restoration, no calling was detected in 2015 despite numerous blitz style surveys in the southwest corner of the unit where calling historically occurred. As calling was documented in other areas of this unit in 2014 via Songmeters it is recommended that reconnaissance level canoe surveys be tried in 2016, however it is possible that habitat will make canoe access a challenge.

With the changes to the NCS program this year from a focus on catching as many calling males as possible to simply recording location information on as many calling males as possible to guide EMS, this resulted in less information gathered on the state of the population. Without capturing animals, data such as morphometrics, health, and age class are not collected and between-year comparisons are limited. This is evident by the fact that only half the number of LIPI were captured in the spring of 2015 (n=36) as compared to 2014 (n=72; Houston, 2015). As a result of these changes to the program, the majority of the male detections were of unknown age class as they were auditory detections; without seeing the animal it is not possible to determine the age class. If the FWCP or BC-NLFRT would like to gather additional information on the state of the male breeding population, it is recommended that the survey program return to a focus on capturing as many animals as possible, however with finite resources, this would mean that less time would be dedicated to egg mass surveys which could impact the reintroduction program.

The majority of the NCS detections were male, as expected as there is a bias towards male detections as they are the target of the survey program. There were only 3 females detected during the spring breeding season at DLNA aside from the pair detected in amplexus which is similar to previous years and as expected since females are elusive during this time of year, often concealing themselves in the vegetation in the water near calling males (Merrell, 1977) in addition to the fact that detecting them is not the primary objective of the survey program. As mentioned in the results, the reported number of male and female detections does not represent the sex ratio of the breeding population.

As in previous years the captured males detected calling during the spring breeding season at DLNA ranged in size and age class. In 2015, weights ranged from 22.0 – 64.0 grams and 55.9 – 83.4 mm SVL, forming 2 distinct clusters; see Size Chart in Figure 12. Since size data can be used to approximate age this data indicates that the calling male population at DLNA in 2015 was composed of animals ranging in age from those that have overwintered once (1 year olds) to those that have overwintered twice (2 year olds). Approximate ages are based on the author's observations and data summarized from VIE marked individuals (Houston, 2013). A comparison with the 2014 size charts indicates that there is a small cluster of larger males (71.0 -73.0 g weight and 82.3 – 88.8 mm SVL) believed to be 3 years old that was present in 2014 but is missing in 2015; this could be explained by the fact that the 2015 sample sizes is nearly half the size of the 2014 data so it is possible that a few larger animals were present but went undetected.

5.3 Egg Mass Surveys (EMS)

Since females usually tend to arrive at the breeding pond 3-14 (usually 5-7) days after male calling begins (Hine et al., 1981), which is estimated to have been on April 16 based on temperatures, females likely didn't arrive until approximately April 19-21. Since the first detected egg mass was laid on approximately April 21 this estimate of female arrival time is supported by field results. It is not believed that any successful breeding occurred before the spring field surveys began as there was no detected evidence of it in the form of egg masses or hatchlings.

As shown in the temperature profiles, there were multiple peaks and troughs in both the air and water temperatures spanning several degrees during the spring breeding season. This series of peaks and troughs appears to trigger egg mass deposition as temperatures begin to warm after a night or more of colder temperatures at dusk. These results are supported by observations made by Adama and Beaucher during survey efforts in Creston from 2001-2005, who noted that many egg masses were found immediately after temperatures increased following a cold spell, (Adama and Beaucher, 2006)

This year, egg masses were estimated to have been laid between April 21 and May 19, this is very similar to the date range of April 24 – May 20 for detected egg masses in 2014.
While the overall number of egg masses detected in 2015 (n=26) was lower than in 2014 (n=39), these are still very positive results as it was the second highest number of egg masses detected on record for this population.

In 2015, the overall EMS survey-effort was higher than in the previous 5 years, this is due to the fact that we had the extra help of Lea Randall from the BC–NLFRT and her field assistant Josee-Anne Otis for 10 days. The fact that a number of NCS had to be cancelled due to poor weather also enabled us to reallocate some survey time to EMS.

Although the amount of survey-effort was considerable, egg mass detectability was impacted by 2 factors and as a result it is quite likely there were egg masses that went undetected at DLNA. The first factor that impacted detectability was a difference in vegetation. Typically, watershield (*Brasenia schreberi*), a floating-leaved perennial aquatic plant that is widespread in the DLNA, dies back each winter and while it does begin to grow back in the spring, it does not usually impair egg mass detectability until late May, when the coverage of its floating leaves becomes so great that it is impossible to see into the water to detect egg masses. However, in 2015, for the first time since the author has been working in the area since 2005 it was observed that during the winter the watershield did not die back. As a result the vegetation cover was much greater than it typically is during the first 3 weeks of EMS which most likely had impacts on detectability of egg masses. The second factor that impaired egg mass detectability was the fact that 1/3 of all NCS had to be cancelled due to high winds as they limit auditory detectability of calling males. Since the DLNA is so large and it is not possible to survey the entire area each week (within the time frame of egg mass development), NCS data on the location and numbers of calling males is utilized to prioritize which areas to focus EMS efforts on. Without this important information, it was not possible to identify priority search areas during a large portion of the survey period. As a result, the only information we had to base our delineation of search areas on was the limited sporadic calling of a few males that occasionally called during the day. As a result of these 2 factors, it is highly probably that detectability was impacted and accounted for the higher than usual amount of survey-effort required to detect each egg mass, as each one was harder to find, as shown by the detection rate of 6.8 person-hours of survey-effort per egg mass, which was higher than the mean of 6.0 for the previous 5 year period. It should be noted that between-year comparisons of the egg mass detection rate are presented for a general comparison only, for a precise comparison it would be necessary to compare values between years by specific site within the CVWMA as survey effort by site varies from year to year.

The condition of egg masses upon detection ranged from good to poor and hatch-out was variable. Hatchling counts ranged from 0 – 3937 hatchlings per egg mass. The literature states that in some areas up to 7000 eggs have been deposited per female however, 3500 eggs per female is more common (Corn and Livo, 1989). By comparison only a portion of our egg masses are within this range; these are likely being deposited by the older females, and the smaller egg masses are likely being deposited by the younger females which have just reached sexual maturity. Since the number of eggs deposited per female is positively correlated with SVL (Gilhen, 1984; Gilbert et al, 1994) and SVL is an indication of age, our results indicate that we have at least 2 age classes of females depositing eggs.

Since it is not possible to obtain a viable population estimate with the data we currently have, an estimate of the minimum size of the breeding population can be calculated by doubling the number of egg masses detected to account for the breeding pair. This gives us 52 for 2015, but it should be noted that this is believed to be a very conservative estimate given that the male to female ratio is not thought to be 1:1.

5.4 Tadpole Trapping

Tadpole trapping can be an effective technique to monitor tadpoles with relatively little survey-effort as traps are set up and left deployed for up to 12 hours resulting in a large numbers of trap-hours with minimal person-hours of effort. While the effective trap-rate is low it does enable us to monitor tadpole development and get an estimated date of metamorphosis. Trap placement and timing seem to be key factors influencing the success of tadpole trapping. Our results indicate that traps should be deployed in warm shallow areas with high levels of aquatic vegetation along the perimeter of the marsh. Our test results of trap type and optimal deployment times done during the 2014 field season indicated that metal wire cages had better results than the fabric mesh type and that the most successful trapping period was overnight (Houston, 2015).

There were a range of developmental stages detected during tadpole trapping at DLNA. Trapped LIPI ranged from Gosner stage 26-42 and recently metamorphosed YOY were also detected as surveyors moved throughout the wetlands checking traps; this range in developmental stages is as expected since detected egg mass deposition dates ranged from 23 April to May 19. The minimum observed development time from egg fertilization to metamorphosis occurred in 77 days, which is similar to the results observed at DLNA in 2014 (Houston, 2015) but is on the rapid end of the scale of 75-90 days reported in the literature (Hill, 2016). For a precise evaluation, accumulated degree-days since hatching would have to be examined, but this apparent advanced rate of development could be due to the extremely low water levels observed in 2015 which can have negative impacts on tadpole development.

Tadpole trapping at Leach Lake failed to provide any evidence of successful breeding at ponds 1 or 4 even though a minimum of 12 calling male LIPI individuals were detected during the spring breeding season in pond 1. Since tadpole trapping is only done at the presence/not-detected level, non-detection of tadpoles does not provide conclusive evidence that breeding did not occur. Non-detection could be a result of the fact that this is a very large area and tadpole density would be very low across the landscape, therefore decreasing the probability of detection. Since tadpole trapping is not a conclusive method to determine whether or not breeding occurred in an area, it is recommended that when looking for evidence of successful breeding at suspected breeding sites it be combined with YOY VES during summer and fall, as was done in 2015.

It is recommended that tadpole trapping be continued in future years as it provides valuable information and enables us to monitor tadpole development at known breeding sites and get an estimated date of metamorphosis.

5.5 Visual Encounter Surveys (VES)

There were no detections of LIPI during summer or fall VES in the Leach Lake area, unfortunately this suggests that the males detected calling in the spring in pond 1 did not breed successfully since no YOY were detected. However, the area is vast and due to access issues it is difficult to survey the southern region of pond 1 so it is possible that YOY were present but went undetected. All that can be concluded is that there was no detected evidence of successful breeding.

In the main over-wintering area at the Old Goat channel, LIPI were detected on the first survey on September 23, this suggests that the migration to the over-wintering area had already started, even though large numbers of animals were not detected during road surveys. This is not completely out of the ordinary as it is expected that the timing of the migration is somewhat variable depending on environmental conditions; in 2014 LIPI were also detected in the area during the first fall VES on September 10 (Houston, 2015). Surveys in the secondary over-wintering channel in the Kootenay River East Channel resulted in few detections, this is similar to previous years and is likely a function of the habitat complexity along the shoreline which limits detectability, especially when compared to the main overwintering area where the habitat is quite different and detectability is better.

5.6 Late-summer and Fall Road Surveys

LIPI road mortalities on the Duck Lake dike roadway have been well documented since surveys began in 2009 so the objective of survey program in 2015 changed as it was felt that sufficient detection data has already been collected. The road surveys that were done in 2015 started earlier than normal with the objective of gathering information to indicate what environmental cues trigger the start of the migration. While Merrell indicated that in Minnesota, migration began on a warm night following a cold spell during or after a rain (Merrell, 1977) it has not been determined if this is the case with the Creston population since in the majority of the surveys, LIPI have been detected on the first night of road surveys.

Unfortunately, during the 2015 field season it was not possible to gain any additional information to indicate what environmental cues are triggering the movement of LIPI during this seasonal migration between habitats. Although surveys began early in an effort to try and catch the start of the migration and determine what factors trigger movement it is believed that due to abnormal weather conditions movements were atypical.

Detected levels of road mortality (25.0 % of LIPI detections were dead) were similar to those observed during the 2011 surveys when the most effort was put forth. During this time 45 surveys were conducted and 19.0% of detected LIPI were road mortalities (Houston and Hill, 2012). It should be noted that the low number of detections should not be misinterpreted to mean that fewer animals are using the roadway, as this is a function of the change in objectives. It is recommended that the BC-NLFRT work with land managers to develop a suitable plan for implementation to mitigate this ongoing source of mortality.

5.7 Animal Health

While the majority (84.8%) of the LIPI detections throughout the year were deemed to be in good health it should be noted that due to a change in methodology for the spring 2015 field program fewer animals were captured to enable surveyors to focus on pinpointing the locations of as many LIPI as possible to inform egg mass searches (the new priority); as a result health was only assessed in half (n=36) the amount as in the spring of 2014 (n=72, Houston, 2015). Since very few animals within the breeding population were captured in the spring of 2015, caution should be exercised when attempting to draw conclusions about the observed health trends in the population, especially when looking at between year comparisons.

The large number of dead juveniles detected at the WDLNA breeding area in the spring was a concern since this is the largest number of dead animals detected during the spring breeding season in the last 5 years. From 2010 to 2014 between 0 and 2 dead were detected yearly during spring surveys (mean=1, n=5, SD=1). Due to the fact that most of the animals were in

the advanced stages of decomposition at the time of detection it was not possible to conclusively determine the cause of death for all animals. However the lab results for the 1 carcass that was sent to the lab for a full work-up indicate cause of death was attributed to cutaneous *Zygomycosis* and *Chytridiomycosis*, PCR was negative for *Iridovirus.* It is recommended that surveyors watch for any signs of a similar situation in future years and where possible have specimens tested to determine if there is cause for concern.
Two health related observations were detected in 2015 that have never been detected in this population in the past; neither of which are believed to be significant health issues. The first, a case of bright yellow dots detected in the skin of 2 individuals during the spring breeding season at WDLNA are believed to be a lack of pigment, cause unknown. The second health related observation was a prolapse in an adult female detected during the fall, it is unknown what the cause was, but since it was detected in the fall it is not believed to be related to egg mass deposition; staff at the Vancouver Aquarium noted that they have seen this in amphibians and it is related to constipation (pers. comm. Kris Rossing).

There were no detected cases of frog chiggers infection (genus Hannemania) or necrotic tissue, (2 health issues that were observed in the population in the past) during the spring of 2015 however as noted earlier there were fewer captures made in the spring of 2015 compared to previous years so it is possible these issues could have gone undetected. Frog chiggers was first detected in the population in the spring of 2011 at the East pond of DLNA when one individual exhibited a large number of tiny red bumps on the ventral surface. Since then there have been 15 additional detections: 7 in 2012 (in 5 individuals), 0 in 2013 and 8 in 2014. The majority of these (75.0%) were detected at the East pond of DLNA (25.0% at WDLNA); ventral body swabs were collected for Chytridiomycosis testing from these detections and 87.5% tested positive for Bd. Necrotic tissue was mainly detected in the spring of 2012 at the East Pond of DLNA when 6 detections were made (4 individuals), of these 5 of the 6 tested positive for Bd; and aside from 1 detection in the fall of 2014 has not been detected since. After consulting various specialists it was thought that this was likely caused by freezing conditions during migration.

As body swabs were not submitted to the lab for analysis due to the fact that there was no funding allocated for it by the FWCP in 2015 it is not possible to do between year comparisons of the Chytridiomycosis results.

Since the health of the breeding population likely plays an important role in recovery, it is suggested that changes to the spring survey program which resulted in fewer captures and therefore a move away from monitoring the health of the population be reconsidered by the FWCP.

5.8 Doral Spot pattern recognition

Since recapture analysis using dorsal spot pattern photos was not done for the 2014 or 2015 field season as it is not seen as a priority given the amount of time required to complete it, it is not possible to identify unique individuals and determine within year or between year recaptures. Without this information data summaries are limited to analysis using observations (detections in time); since recaptures have not been identified the number of unique individuals within the observations is unknown. As a result, it is not possible to get an estimate of the number of individuals captured during the field season, or do summaries such as juvenile to adult calling male ratios, or calculate growth rates, etc. It also prevents comparisons of data between sites such as morphometrics because without recapture information if you have 10 observations, it is unknown if you are comparing the same animal caught 10 different times or 10 different animals. Between-year comparisons of data such as morphometrics or numbers of calling male LIPI are also limited as recapture analysis has only been done on data collected prior to 2014.

The digital image library containing dorsal spot pattern photos from the 2015 field season has been added to the master library which is on file so a full or partial analysis could be done at any time in future. If data summaries such as a juvenile to adult male calling ratios, YOY growth rate, comparisons of YOY morphometrics between years or between sites is requested by the BC-NLFRT then resources should be allocated to at least within year recapture analysis to facilitate this.

For a discussion of how to increase between year recaptures to facilitate obtaining a population estimate see discussion in 2014 field season report (Houston, 2015).

5.9 Morphometrics of Creston LIPI

YOY observed in the migration corridors and overwintering areas during the fall of 2015 were statistically significantly smaller at the 0.05 level than those observed in the fall of 2014, they were also the smallest in terms of both weight and SVL when compared to the 2010 - 2013 data. While this data does include recaptures and sample sizes vary from year to year, detections were all from migration corridors and overwintering areas and detection dates were within the same general time period (September 21 – October 20). There could be a number of plausible explanations for these observed differences including weather and productivity differences between years but it is possible that extremely low water levels at the DLNA during tadpole development played a role. In addition to an expected increase in water temperature and increased exposure to UV (which can cause deformity) as well as potential water quality issues, low water levels result in increased density during development which not only increases competition but has been known to cause anuran tadpoles to metamorphose earlier and at a smaller size (Darren Smy, Vancouver Aquarium, pers. comm.). It is not likely that the egg mass enclosures contributed to this observed size difference as they have been used for many years and a marked difference in size has not been observed until now and additionally, egg masses at the east ponds of DLNA were not caged and these animals were also small, indicating it has nothing to do with caging.

It is suggested that if morphometrics of Creston YOY or any other age class be compared with morphometrics of animals captured elsewhere, such as at the reintroduction sites, the data will have to be standardized to correct for detection dates, time since metamorphosis, etc. Seasonal habitat type should also be factored in as YOY detected on the same date in breeding sites, migration corridors and over-wintering areas have been observed to be very different sizes due to the exhibited behaviour of leaving the breeding site once a certain size is reached. It should also be noted that there would likely be some regional variation in rate of development in YOY due to site specific differences in habitat and foraging quality but data such as size at metamorphosis and growth rates could be compared to provide an index of site suitability.

5.10 Vancouver Aquarium Captive Assurance Colony

The results of a size comparison between captive bred YOY and Creston source YOY both housed at the Vancouver Aquarium as part of captive assurance colony indicates that the captive bred animals are smaller; this difference in statistically significant at the 95 % level. In Addition to this, both sets of animals at the Vancouver Aquarium are smaller than wild caught YOY in Creston. This appears to be a trend as results obtained in 2014 (Houston, 2015) indicate the same thing. It is recommended that the BC-NLFRT work with the Vancouver Aquarium to investigate the possible reasons for the size differences and determine if there are any corrective actions that could be implemented.

5.11 Bummers Flats Reintroduction Program

After 5 years of phase 2 reintroductions of LIPI to North Bummers Flats (during which time a total of 33,958 tadpoles were released), with very low numbers of calling males detected returning each spring, a new level of success was reached in the spring of 2015. The Bummers Flats crew, with funding through CBT, detected 19 calling males at the release site (Ohanjanian, 2016); this is a very significant result as it is the largest number of calling males detected there since reintroductions began at the site in 2003.

The reintroduction methods utilized seem to work well and success as measured by survival to metamorphosis was achieved in 2015. During YOY surveys a total of 54 individual YOY were identified by the Bummers Flats crew funded by CBT (Ohanjanian, 2016). As 5279 tadpoles were released in 2015, this provides evidence that at least 1.0 % survival was achieved. This is a conservative estimate since not all YOY were captured.

Since 2015 was year 5 of a 5-year commitment made by the FWCP to carry out phase 2 reintroductions at North Bummers Flats it is recommended that in 2016 reintroductions be put on hold in order to assess the effectiveness of the program. This would be done by monitoring survivorship and determining whether or not wild breeding occurs by conducting nocturnal calling surveys at the release site in the spring, followed by egg mass searches and summer YOY surveys.

It is also recommended that if Bummers Flats reintroductions continue in the future, suitability of the current release site should be re-evaluated as the condition of the habitat has declined over recent years (shoreline habitat containing open areas required for basking has been replaced by dense monotypic stands of bulrush), availability of food sources may be limited and linkages between seasonal habitats are restricted; wetland restoration may be a solution.

5.12 Columbia Marsh Reintroduction Program at Brisco

The total number of tadpoles transferred from the wild LIPI population in Creston in 2015 was limited by a number of factors.

It is not possible to plan transfer dates well in advance as there are many factors involved and it requires a great deal of coordination. Some of these factors include: timing of tadpole development, necessity of tadpoles to be released upon reaching the free-swimming stage (holding tadpoles for extended periods of time in the enclosures in high densities is not recommended as it can lead to stunted growth), inability to predict weather conditions on collection day (collection is not possible in some weather conditions) and availability of the Creston FWCP crew. As a result the Brisco crew must be available to pick up tadpoles with very limited notice if tadpoles are requested in 2016.

The number of tadpoles moved per transfer is also limited by a number of factors. Factors involved include not only the number of available tadpoles which have reached the critical stage required for transport on a specific date, but also the number of experienced people on hand to estimate numbers and quickly but safely collect and package them as well as the total number of hands available to carry the Thermos's containing the tadpoles from the collection site in the wetlands to the vehicles. Due to these factors, among others, the total number of animals available for transport in 2015 was limited. Depending on the total number of tadpoles requested by the BC-NLFRT, the Brisco crew may have to make numerous trips to Creston to achieve the target numbers. In 2015, due to careful planning and coordination, nearly 3000 tadpoles were transferred during just 2 trips, depending on timing of tadpole development and other factors it should not be assumed that this rate of transfer will be possible in 2016.

A greater number than preferred (65.4%) was taken from EM150520-EA23 as it was found out in early June that the number of hatchlings coming from the captive breeding program at the Vancouver Aquarium was lower than expected so the BC-NLFRT wanted to see as many tadpoles transferred as possible during the final transfer from Creston. Since there were a limited number of egg mass enclosures containing tadpoles at this late stage in the breeding season and this was the last opportunity to collect hatchlings for 2016 a greater number than was planned were collected. In the future it would be preferable if the number of animals being requested by the BC-NLFRT is communicated well in advance, optimally by the beginning of the breeding season in late April to facilitate proper planning and required resources.

The low overall transfer mortality of 1.0% seems to indicate that the transfer method which has been utilized for the Bummers Flats reintroduction program seemed to work well to transport the tadpoles from the Creston source population to Brisco even though the total transport time was longer.

5.13 Habitat Concerns

Low water levels in the breeding areas became a concern during the 2015 field season. During the spring breeding season, water levels at the main breeding site of DLNA (measured at the East Pond staff gauge) dropped by 114 mm over a 6 week period from April 21 and June 2; this is a significant drop of approximately 1/3 for this shallow wetland. This is likely a result of low amounts of precipitation and warmer than average weather in addition to the fact that there was a below average snowpack resulting in little runoff. In the future it is recommended that communication around water levels with the CVWMA continue as has been done since the project started; however to facilitate site specific water level readings and changes it is recommended that a staff gauge be installed at WDLNA as there is not one at the site currently. All staff gauges being monitored by the NLF project should be calibrated yearly to ensure consistency from year to year as winter conditions can cause them to move.

During the summer of 2015 during the tadpole development period water levels in the CVWMA became very low and many areas became dry mudflats as a result of the below average snowpack and resulting minimal run off. This was of particular concern at the known breeding area of DLNA as low water levels can have impacts on tadpole development. The site was monitored regularly during the summer of 2015 to ensure it did not dry out but it should be noted that even low water levels can have impacts on tadpole development. During low water years it is recommended that water levels be closely monitored at known breeding areas during the entire tadpole development period as part of the FWCP NLF project and any concerns communicated to land managers at the CVWMA as was done in 2015. It is also recommended that a contingency plan for bringing water in during an emergency situation be discussed and developed by the NLFRT. This is necessary since the DLNA breeding area water levels are currently managed by passive flow through a series of culverts with gates, it is not actively pumped so water can only be brought in through the extant water control structures if the source water level is higher than the breeding area (which was not the case during the summer of 2015). An easily executed plan which may involve active pumping should be developed for implementation should the need arise in the future.

Due to the rapid ingrowth of the aquatic perennial plant watershield (*Brasenia schreberi*), and observed difference in growth pattern this year (no die back over the winter) it is highly recommended that the BC-NLFRT and land managers assess the situation and determine if there are any feasible restoration options as this ingrowth is likely impacting the quality of the habitat, and may be having affects not only the northern leopard frog but many other species that rely on this important shallow open water wetland habitat.

In addition to the habitat concerns discussed above, a number of habitat-related concerns that could potentially have negative impacts on the Creston LIPI population have been identified and brought to the attention of the BC-NLFRT and land managers over the years but to date remain largely unresolved; see Discussion in 2014 field season report (Houston, 2015). It is recommended that the BC-NLFRT and land managers review these concerns and discuss a course of action.

5.14 Additional Recommendations

In addition to the recommendations outlined in the Discussion, below is a list of some others for consideration:

- Health, condition and hatch-out of wild laid egg masses sourced from Creston should be factored into the BC-NLFRT decision matrix for egg mass translocation (based on a threshold number of egg masses required prior to initiating translocation); egg masses that are not in good condition or have poor hatch out should not be included in the count
- HOBO temperature data loggers are outdated and the associated software is no longer compatible with current computers, as a result they will need to be upgraded in order to continue collecting temperature data.
- Continue working with the Nature Conservancy of Canada on the Frog-bear property management and farm planning
- Research Topics:
    - Population estimate; for discussion of recommended methods see 2014 field season report (Houston, 2015)
    - Egg mass caging efficacy study
    - Telemetry project to determine timing and movement patterns between spring breeding and over-wintering areas

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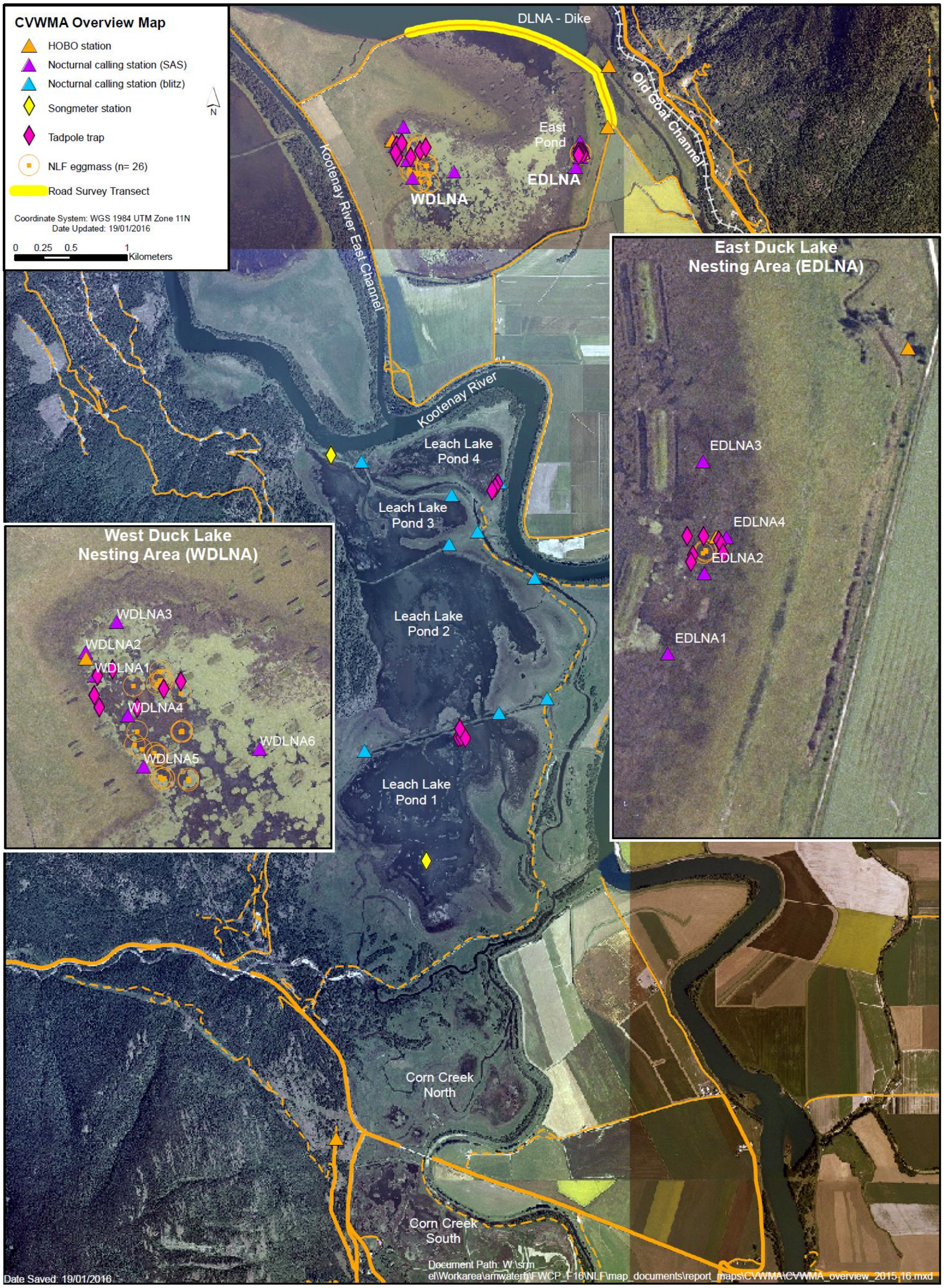
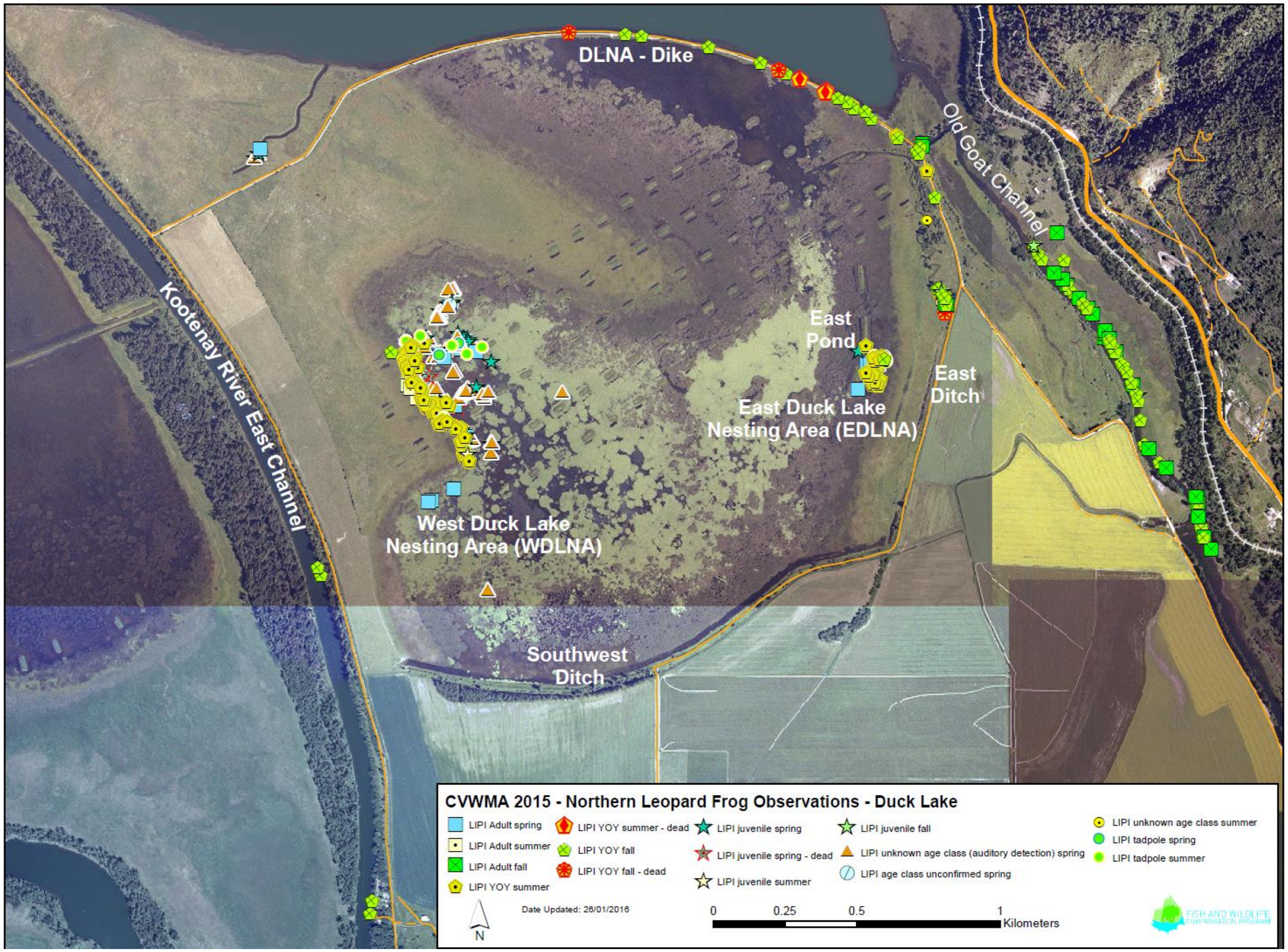
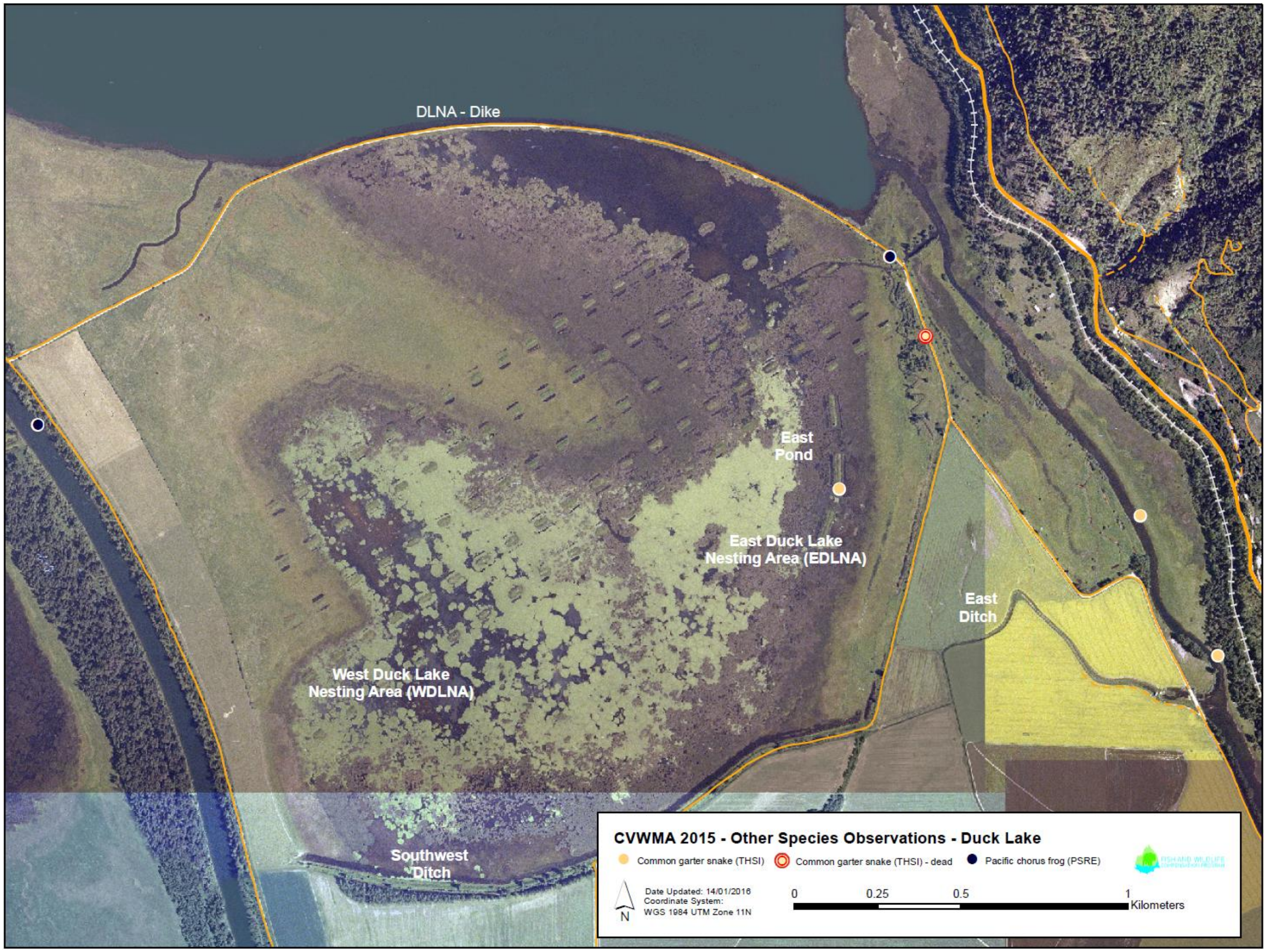
Appendix 1. CVWMA Overview Map



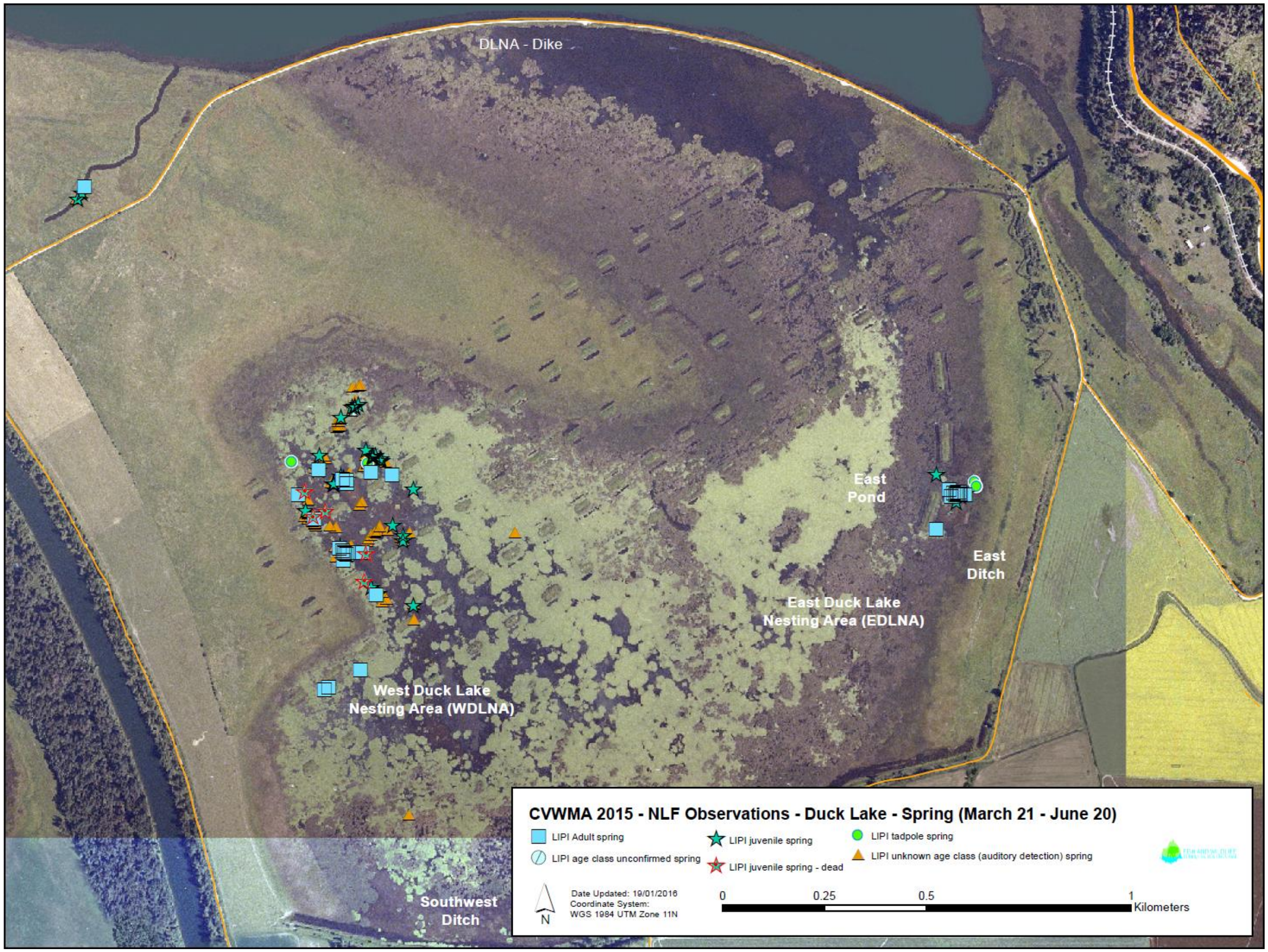
Appendix 2. Duck Lake LIPI Observations



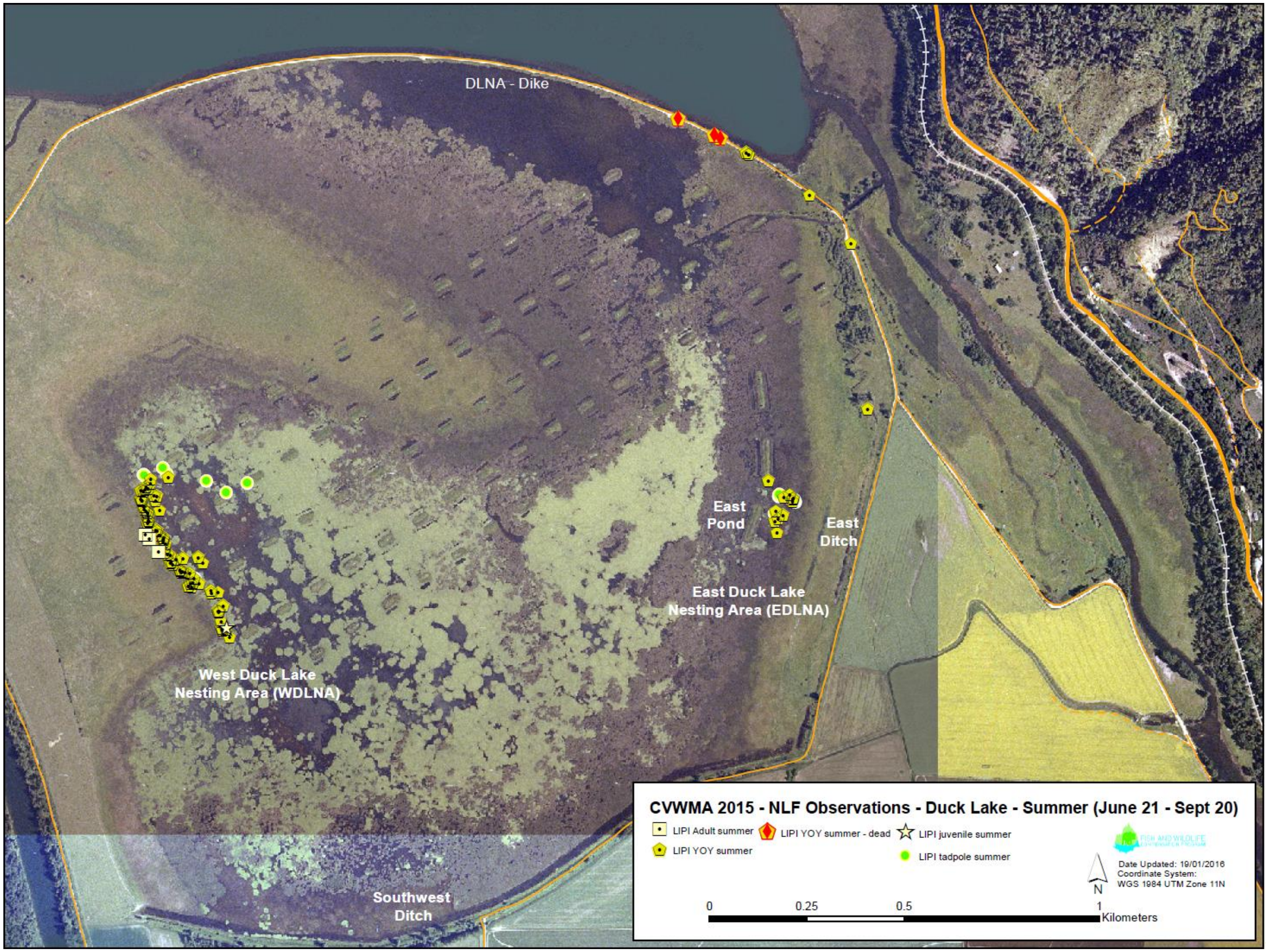
Appendix 3. Duck Lake Other Herptile Species Observations



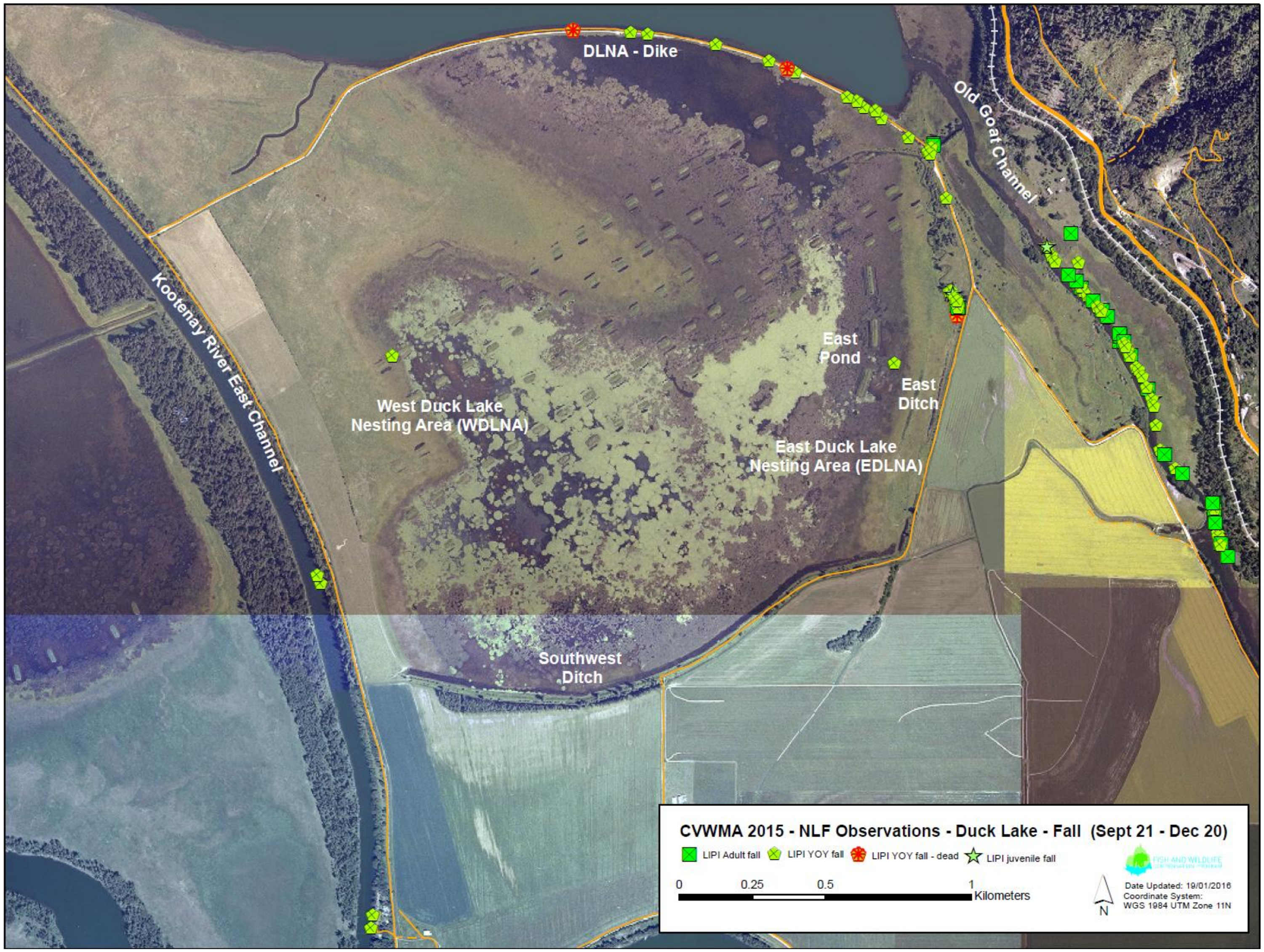
Appendix 4. Duck Lake LIPI Observations by Season- Spring



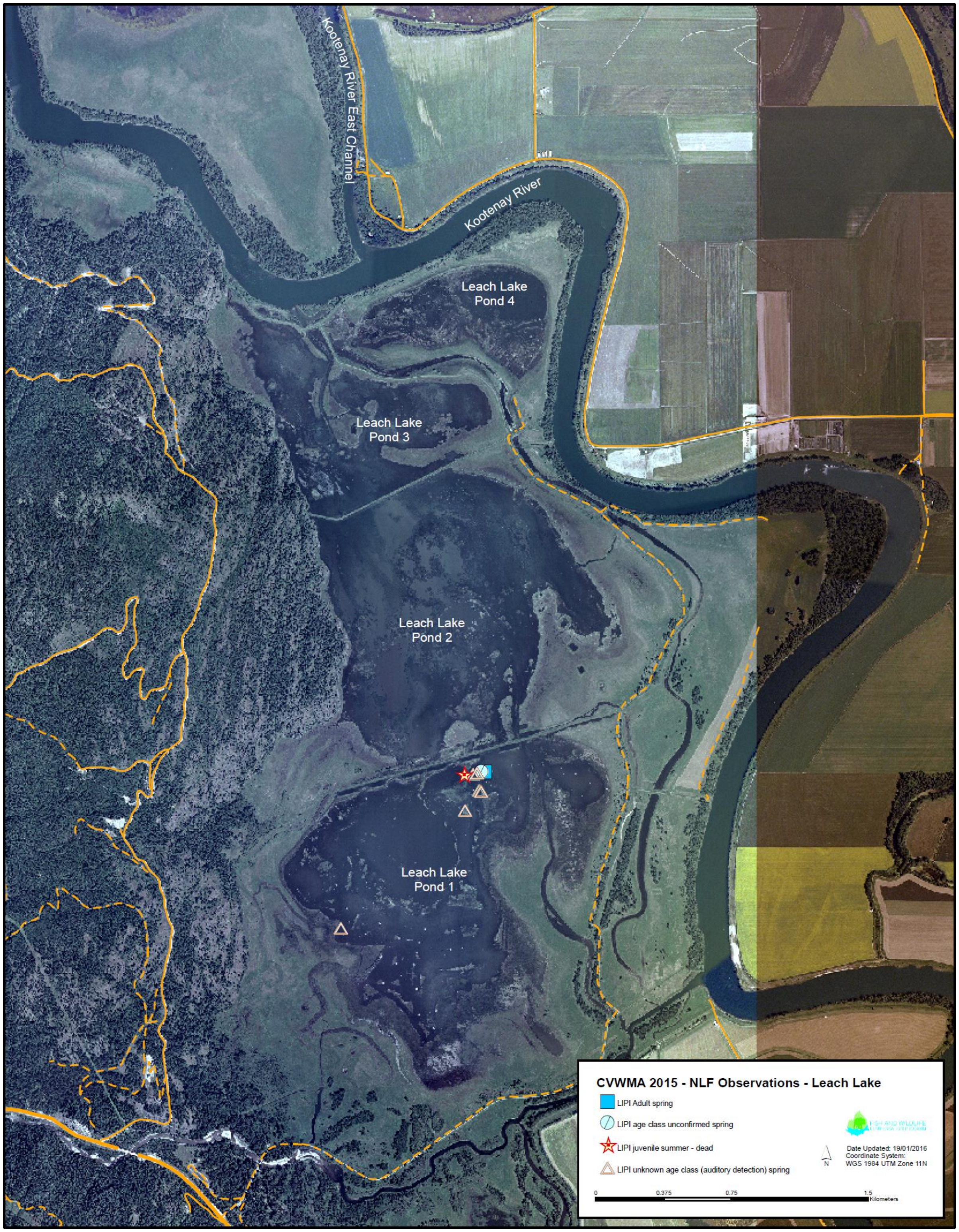
Appendix 5. Duck Lake LIPI Observations by Season- Summer



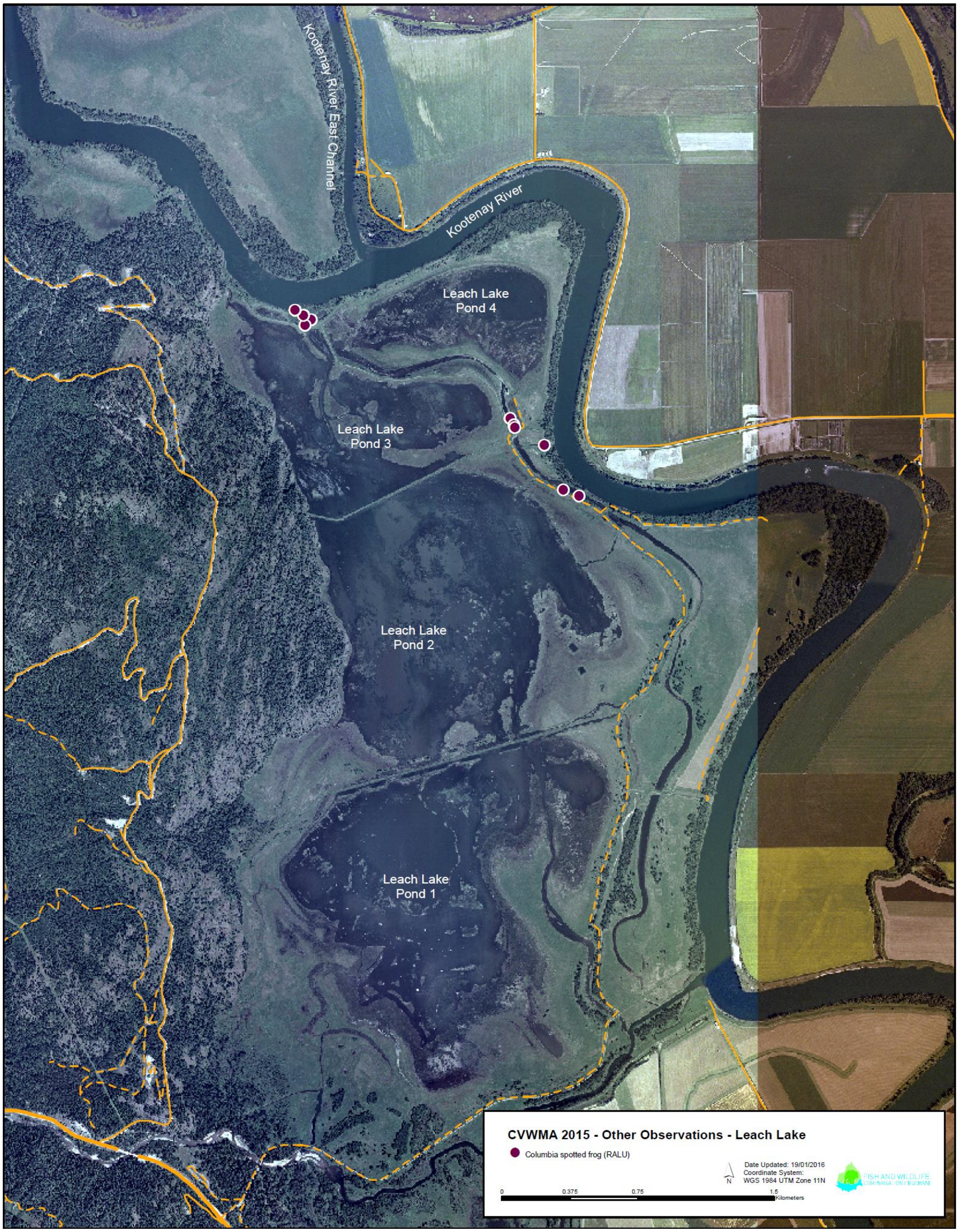
Appendix 6. Duck Lake LIPI Observations by Season- Fall



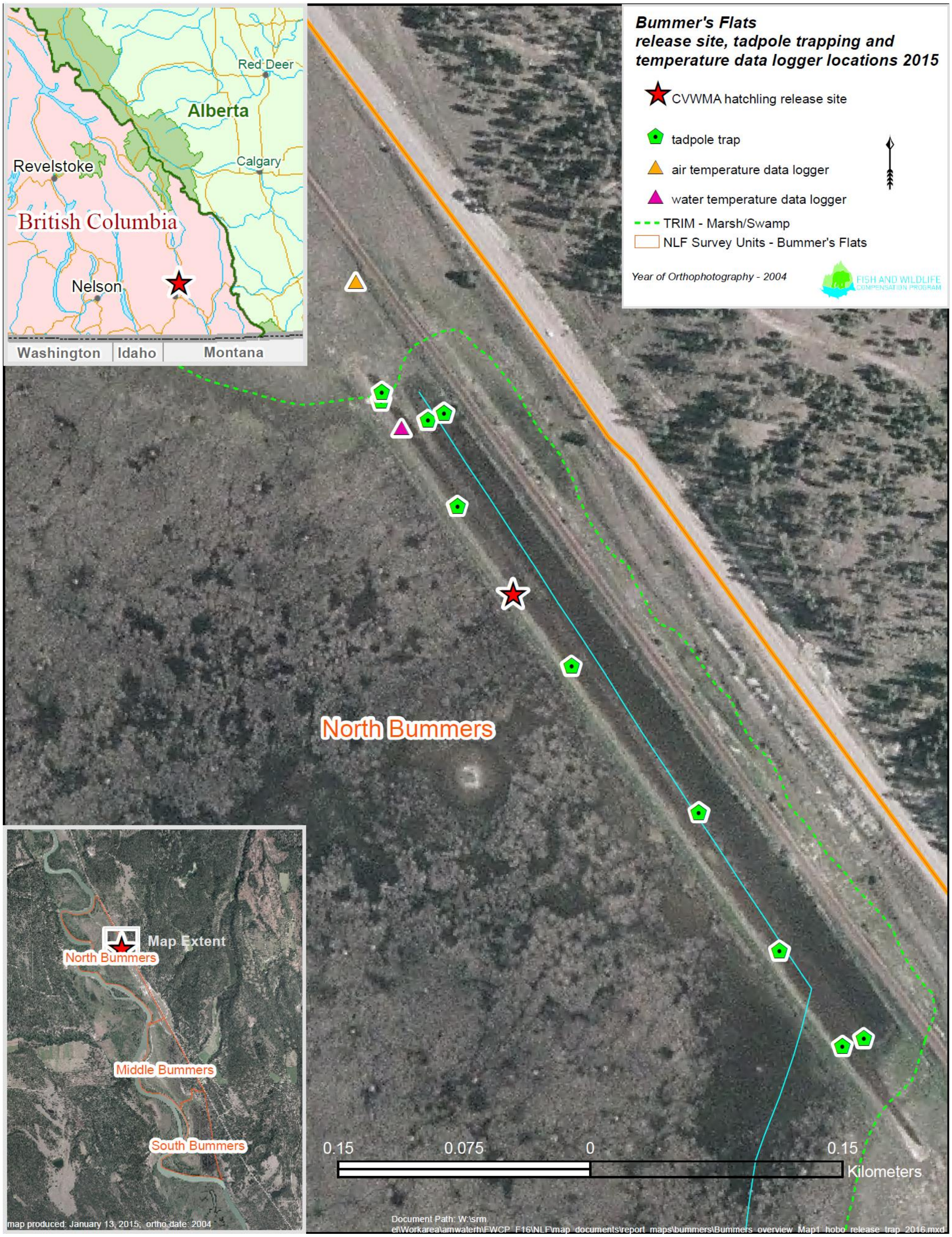
Appendix 7. Leach Lake LIPI Observations



Appendix 8. Leach Lake Other Herptile Species Observations



Appendix 9. Bummer's Flats Overview Map



Appendix 10. Bummer's Flats LIPI Observations