Northern Leopard Frog Project: 2016 Field Season Report

Submitted to:
Ministry of Forests, Lands and Natural Resource Operations

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Submitted by:
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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. CDC, 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 supported 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 2016 field season program for the Northern leopard frog project administered by FLNRO

The main objectives for the 2016 field season were to monitor the extant remnant native population of B.C. *Lithobates pipiens* (LIPI) in the CVWMA, locate and protect egg masses, 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. PIT tagging was introduced this year to begin population monitoring

2. STUDY AREA

The main study area is the CVWMA located in the Creston Valley within the Kootenay region of south-eastern B.C. (Figure 1). 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. For site locations within CVWMA used in this report see Appendix 1

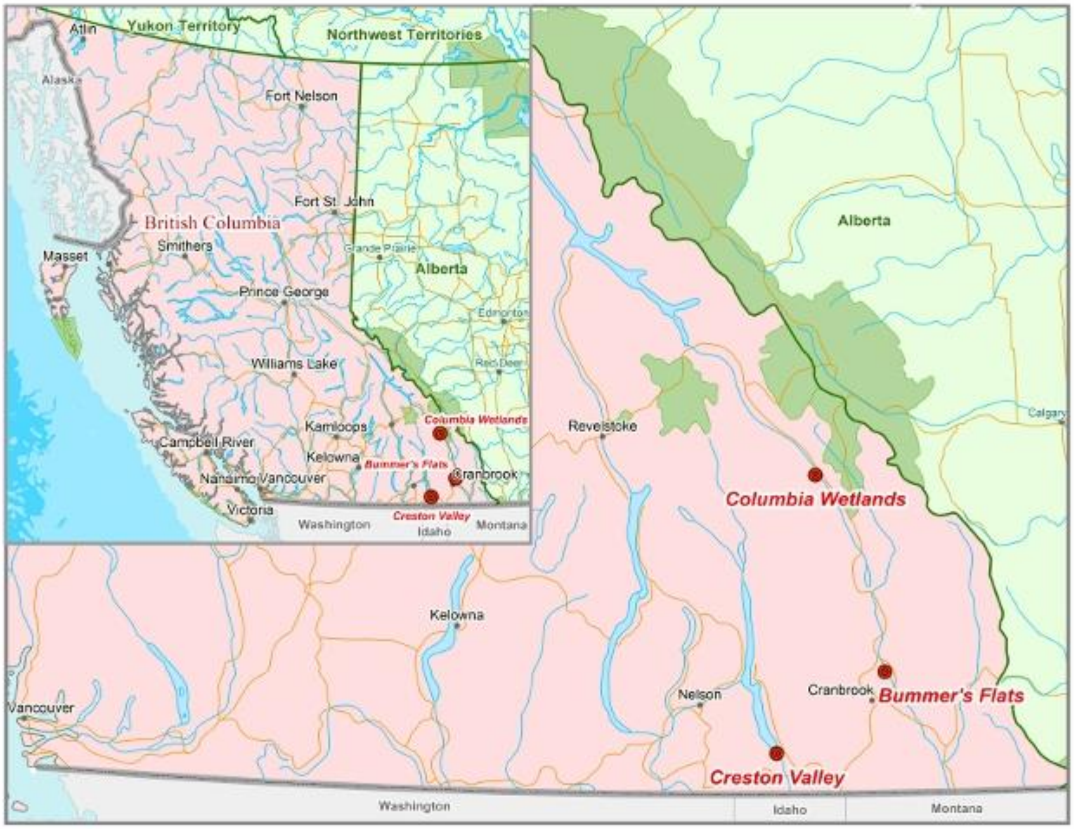

Figure 1. Map of main study area in the Creston Valley and the 2 reintroduction sites (Bummer's Flats and the Columbia Wetlands near Brisco).

3. METHODS

In order to meet the objectives for the project, fieldwork was carried out from April 19 through November 15, 2016 and 5 types of survey methods were utilized: 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

Songmeters were not used on this project in 2016 as the units were on loan to the bullfrog team as part of the early detection rapid response (EDRR) project.

3.2 Nocturnal Calling Surveys (NCS)

To monitor breeding activity NCS were used, methodology followed that outlined by the Resource Inventory Committee (Heyer et al. 1994, MELP, 1998) 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), however with the increase in numbers of calling males over the years and the shift in focus to prioritizing egg mass detection this is becoming increasingly difficult to fit in.

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, it is determined whether or not it is cageable, 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 (Figure 2) are utilized to provide protection from predators until free-swimming and facilitate collection of hatchlings for the reintroduction program and the Vancouver Aquarium captive assurance colony. 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 potential predatory aquatic invertebrates are 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 at possible breeding sites and monitor developmental stage at known breeding sites tadpole trapping is conducted. Tadpole trapping was conducted at Leach Lake pond 4 to determine if there was any evidence of breeding since this is a historic breeding site (even though calling was not detected during the spring breeding season). 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. See Appendix 1 for location of tadpole traps within the CVWMA.

Tadpoles are trapped using un-baited 21 inch wire minnow traps (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). Tadpoles are not usually handled directly but if handling was required, vinyl gloves were used as there is evidence that latex (Gutleb, 2001) and nitrile gloves can be harmful to tadpoles of some species (Cashins et al, 2008). In most cases surveyors are able to easily 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. As shown in the photo, water levels at DLNA were very low in some areas it was difficult to find enough water depth to deploy the traps; photo taken July 6 at WDLNA.

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 in various seasons 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 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.

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

3.6 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. In 2016, FLNRO Ecosystems staff completed the majority of the road surveys. Dorsal spot pattern photos from all road surveys were added to the image library. The results of all road surveys conducted in 2016 were added to the master NLF Filemaker Pro database and a summary of results is provided in this report.

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. 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 minimal). 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 of the specific issue 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. Since 2015 funds were not available for 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, B.C. 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 (MoE, 1998) 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. Photos (left to right): weighing, measuring SVL and swabbing LIPI for *Batrachochytrium dendrobatidis (Bd),* which causes the disease Chytridiomycosis.

3.8 Passive Integrated Transponder (PIT) Tagging

In an attempt to begin a mark-recapture population estimate, PIT tagging was initiated during late-summer and fall field surveys in 2016. With input from the British Columbia Northern Leopard Frog Recovery Team (BC-NLFRT) it was decided that all LIPI 25.0 grams in weight and greater would be PIT tagged. Prior to PIT tagging any animals, all field staff participated in a hands-on amphibian PIT tag training session in the field led by Jakob Dulisse who has extensive experience in PIT tagging amphibians.

The PIT tagging procedure was carried out using Biomark 8 mm PIT tags (#HPT8) which were inserted after all other processing was completed (weight, SVL, swab, photo, etc.), using a single use sterile syringe-style implanter (Biomark model MK165) with a sterile stainless steel needle (Biomark model N165). They were inserted just under the surface of the skin by pinching a flap of the dorsum skin and injecting the needle horizontally. The syringe needle tip was inserted on the upper dorsal section of the body behind the head and once inserted the PIT tag was gently massaged back towards the pelvic bones. Single use gloves were worn when handling the animal. Every effort was made to safely process animals as quickly as possible to minimize stress and animals were released at the site of capture immediately after the procedure. After PIT tag insertion animals were scanned by a handheld PIT tag reader (Biomark model 601) to read the unique number, which was recorded on the datasheet. PIT tagging equipment was shared with the road survey team.

3.9 Dorsal Spot Pattern Recognition

To add to the master digital image library of all LIPI captures dating back to 2006, dorsal spot pattern photos were taken for all post-metamorphic LIPI captures during the field season. A 2016 digital image library was created by naming each .jpg file with the observation number and cataloguing it by detection site.

Photo analysis has not been completed since the 2013 field season due to the large amount of time required to manually compare the photos, along with the fact that there have been relatively few between-year recaptures in previous years (required for population estimate work). 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. PIT tagging will replace photo analysis once fully implemented.

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).

3.10 Vancouver Aquarium Captive Assurance Colony

To provide additional founders for the captive assurance colony, 10 tadpoles were collected from 4 different egg masses for a total of 40 tadpoles. They were held and cared for in 4 different egg mass enclosures in situ at WDLNA natal site 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 and transported with care as quickly as possible 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 an 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 at DLNA until ready for transfer; hatchlings packaged in plastic bags (10/bag) and packed in cooler for transport.

3.11 Columbia Marsh Reintroduction Program

It was decided by the BC-NLFRT that a target of 8000 tadpoles should be released at Brisco in 2016. Since very low numbers were produced at the Vancouver Aquarium captive assurance colony breeding program (originally intended to be the main source of tadpoles), a request was made to collect a large number from wild laid egg masses in Creston. In response, tadpoles were collected from the DLNA breeding site in Creston and packaged up by the Creston FLNRO crew and subsequently picked up by the Brisco crew funded by the Columbia Valley Local Conservation Fund with help from Lea Randall of the Calgary Zoo and transported by vehicle to the release site near Brisco. 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 made by the author after years of experience transporting tadpoles to Bummers Flats for reintroduction.

The source of all animals was WDLNA and all reintroduced animals were moved just after reaching the free-swimming hatchling stage. If logistically feasible only a portion (target ~ 50%) of each caged and healthy egg mass detected over and above the threshold level of 10, was moved in insulated 2 gallon thermos containers. Predator free pond water was placed in each container to approximately the 2/3 level and an estimated 300 hatchlings were quickly collected and transferred from egg mass enclosures to each thermos container using stainless steel strainers; care was taken to minimize collection of waste products from the cages and aquatic vegetation was removed. Once collected, thermos's containing hatchlings were placed in the canoe (under the shade of a large umbrella as required depending on weather conditions) without lids and transported to land (~15 – 30 minute trip depending on location of egg mass being collected from) 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, lids removed 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; Thermos lids were then removed again (and mesh lids placed on each Thermos) to allow for oxygen exchange for the remainder of the trip. Air temperature, water temperature, pH and conductivity were tested at the end of tadpole collection, at the transport vehicle prior to travel and at numerous times during the trip to the recipient site and compared to in situ conditions prior to tadpole collection to ensure conditions remained within a safe range (Figure 6).

Once at the release site, the Brisco team acclimatized the tadpoles and released the majority the same day, a subset was held overnight and counted the following day prior to release to get precise counts and monitor mortality. During the entire reintroduction process, extreme care was taken to ensure the overall safety of the hatchlings.

Figure 6. Photos (left to right): crew carrying containers with tadpoles from WDLNA to vehicle for transport; 2 gallon Thermos container with ~300 tadpoles for transport to reintroduction site; water quality testing at vehicles prior to transport to recipient site.

3.12 Habitat

Water Levels

DLNA water levels are monitored regularly by the CVWMA by recording the water level readings at the Pumphouse channel staff gauge, which is located adjacent to the wetlands and is the source of the water via a passive water control gate connected to the Old Goat Channel and ultimately Duck Lake. Since water levels at the LIPI breeding sites in the DLNA wetland are not typically monitored specifically by the CVWMA on a regular basis, our crew records water levels readings from the EDLNA and newly installed WDLNA staff gauge (Figure 7) while on site conducting surveys during the breeding season (late-April to early June), doing tadpole trapping (typically in early July) and YOY VES surveys (typically in mid-July and intermittently through early August) and communicates any concerns with the CVWMA.

As a result of the observed issues in the summer of 2016 with extremely low water levels and water retention issues at the DLNA the author prepared a summary document prior to wetland specialist Tom Biebighauser's fall visit to the CVWMA (Houston, 2016b). While at the CVWMA Tom completed a wetland assessment and dike inspection with the author and Marc-Andre Beaucher (head of CVWMA operations) to try and determine possible causes for the issues observed in the summer of 2016, as well as determine a course of action to correct the issues.

Figure 7. New staff gauge installed at WDLNA on June 1, 2016 to monitor water levels.

Watershield

Over the last several years, Canadian watershield (*Brasenia schreberi)* ingrowth has been dramatic at the DLNA, percent cover has been increasing and it now covers a very large portion of the once shallow open water wetland, resulting in very few remaining open water areas. Since it seems to have implications for breeding LIPI, as they seem to prefer shallow open water areas of the DLNA for egg mass deposition, a literature review was done to try and determine what options might be available for control. The majority of the information sourced indicated that chemicals are the main approach to control the plant, which although native, can become extremely weedy and a nuisance as it becomes very dense, and even kills off competing vegetation due to its phytotoxic properties (Elakovich and Wooten, 1987). Since chemical treatment is not an option at the DLNA, a small pilot project to test the effectiveness of hand-pulling was initiated in the spring of 2016.

Figure 8. Photos (left to right): dense in-growth of watershield at WDLNA (May 19); majority of detected egg masses are clustered around last remaining opening in watershield cover (note egg mass cages around open area free of watershield).

In an effort to test whether or not hand-pulling may be an effective method to create small openings in dense watershield cover and determine the rate of revegetation a small test project was completed in the spring of 2016 at WDLNA. This involved removal of watershield by hand-pulling. All parts of the plant were removed including the root balls and rhizomatous stem using bow and straight edge rakes; spoil piles were created on an adjacent terrestrial nest island to kill the vegetation.

Prior to wetland specialist Tom Biebighauser's fall visit to the CVWMA the author created a document to summarize the watershield problem at the DLNA breeding site. As a result Tom visited the site and completed an assessment of the situation with the author and Marc-Andre Beaucher, his conclusions are provided in the report entitled, Proposed Wetland Projects: Creston Valley Wildlife Management Area (Biebighauser, 2016), a summary is provided in Section 4.12.

New Wetlands Created
A number of new wetlands were designed at the CVWMA with input from Marc-Andre Beaucher and the author during wetland specialist Tom Biebighauser's visit in the fall of 2016. Six of the wetlands designed for LIPI use in the Duck Lake area were constructed in late November-early December of 2016 (for details see Biebighauser, 2016); funding was provided by the CVWMA and FWCP.

3.13 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. Project data is available on WSI website at: http://a100.gov.bc.ca/pub/siwe/details.do?id=4955

4. RESULTS AND OBSERVATIONS

4.1 Nocturnal Calling Surveys (NCS)

In the CVWMA, 82:03 person-hours were dedicated to NCS (n=19 surveys) from April 19 to June 1. 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 a reconnaissance level canoe survey at Leach Lake pond 1 were completed. It was not possible to do any reconnaissance level canoe surveys in the south-west portion of DLNA due to the dense ingrowth of watershield which has made navigation by canoe impossible. Poor weather caused the cancellation of 3 SAS at DLNA and 3 blitz style surveys at Leach Lake. Surveys from May 17 onward in the southern portion of WDLNA (where the majority of the calling and detected egg masses had been found) were called off due to the fact that the watershield had become so dense that entering the area of high probability of new egg masses at night with poor visibility would have meant potentially trampling egg masses.

During the spring breeding season calling was detected at WDLNA and the East Pond of DLNA; see Table 1 for details. Although blitz style surveys were conducted throughout Leach Lake no calling was detected. The map in Appendix 1 shows the location of calling stations and the maps in Appendices 2, 4, 5 and 6 document the locations of all detected calling males.

Table 1. Number of nocturnal calling surveys at 5 sites in CVWMA in spring of 2016. Bold numbers indicate Northern Leopard frogs were detected calling.

Site	WDLNA	East Pond	Leach Lk	SW DLNA	Leach 1
Survey type	SAS	SAS	Blitz	Recon	Recon
April 19-20	**1**	0	0	0	0
April 24-30	**2**	**1**	1	0	0
May 1-7	**2**	**1**	1	*	*
May 8-14	**2**	*	*	*	0
May 15-21	**2**	**	**	0	0
May 22-28	**1**	**1**	1	0	0
May 29-June 1	**1**	**1**	0	0	1

Each * represents a survey that had to be cancelled due to poor weather
Note: from May 15 onwards NCS were limited in main calling areas by watershield density

Of the sites surveyed, WDLNA had the greatest amount of calling activity. During the survey period the maximum level of detected calling was 49 males which were detected May 2-3 (dates pooled since it is not possible to survey the entire WDLNA in one night). Calling was widespread and numerous calling groups were clustered throughout the area as in previous years but the majority of calling was centered around the last remaining opening in watershield in the area adjacent to station 5 in the southern portion of WDLNA (Appendix 4). Calling was detected on the first NCS on April 19 through the last survey on June 1 at DLNA (Table 1). It is likely that calling at DLNA continued through early June but surveys were wrapped up June 1 as the emergent vegetation was too dense to locate egg masses.

At the EDLNA ponds calling was detected during all 4 NCS conducted between April 27 and June 1 (Table 1); calling levels ranged from 3 – 7 individuals per night surveyed. The maximum number of calling males detected during NCS at the East ponds was 7 on May 24, this is followed closely by 6 individuals detected on May 4. However it is unknown what calling levels were from May 8 – 21 as surveys had to be cancelled due to poor weather. The majority of the calling was clustered in one general area within the East ponds with a few individuals calling to the west behind the nest island (Appendix 4).

During spring NCS a total of 139 *L. Pipiens* detections were made, all at DLNA; a detection rate of 1.69 LIPI per person-hour. Of the total LIPI detections made during NCS, the majority (95%) were male (n=132), 3 were female and 4 were visual observations of unknown sex (Table 2). The majority of the male detections (n=105, 79.6%) were undetermined (unknown) stage as they were auditory detections. Of the 27 male detections of known stage, 21 were juveniles (15.9%) and 6 were adults (4.5%). All detected females were adults. For size data on calling males see Section 4.10 on Morphometrics.

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

	Adult	Juvenile	Unknown*	Total
Male	6	21	105	132
Female	3	0	0	3
Unknown	1	3	0	4
Total	10	24	105	139

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

4.2 Egg Mass Surveys (EMS)

In the CVWMA, there were 132:58 person-hours (n=27 surveys) dedicated to EMS between April 19 and May 31. EMS were primarily conducted at WDLNA and EDLNA in 2016, with minimal surveys at the East Ditch (Table 3); no EMS were conducted at Leach Lake as no calling was detected. In total, there were 23 egg masses detected in the CVWMA, 4 at EDLNA pond and 19 at WDLNA (Table 3, Appendix 1). The egg mass detection rate was 5.8 person-hours of survey-effort for each egg mass detected.

Table 3. Egg mass detection rate by site in the CVWMA in spring 2016

	WDLNA	**EDLNA pond**	**East Ditch**	**All Sites**
Number of surveys	17	7	3	27
Effort (person-hours)	107:03	25:09	0:46	132:58
# of egg masses detected	19	4	0	23
Person-hours/egg mass	**5.6***	**6.3**	**0.0**	**5.8***

*includes 3 egg masses detected during NCS (detection rate 6.7 person-hours/egg mass if removed)

The egg mass detection rate was lower than documented in 2015 (6.8 person-hours) but similar to the mean of 5.7 person-hours (n=5, SD=1.9, range 3.1 – 8.0) for 2011-2015 (Table 4).

Table 4. Summary of egg mass detection rate in the CVWMA (all sites); 2011-2016

	2011	2012	2013	2014	2015	2016
Number of surveys	23	29	26	16	22	27
Effort (person-hours)	97:22	104:17	167:42	120:52	176:49	132:58
# Egg masses detected	17	22	21	39	26	23
Survey effort/egg mass	**5.7**	**4.7**	**8.0**	**3.1**	**6.8**	**5.8**

Egg masses were detected between April 19 and May 30 and were estimated to have been laid between April 12 and May 29; egg masses were estimated to be between 1 and 10 days old upon detection. The majority (n=18, 78.3 %) were laid during the 11 day period between April 30 and May 11 and the greatest number laid in one day was 4, which occurred on both May 7 and 10 (Table 5 and Figure 9).

Of the 23 egg masses detected in 2016, the majority (n=12, 52.2%) were hatching or had hatched already upon detection. Of these, 5 (41.7%) were deemed to be in good condition at the time of detection, 5 (41.7%) were in fair condition and 2 (16.6%) were in poor condition. Of the 11 egg masses detected prior to hatching, the majority were in good condition (n=6, 54.5%), 4 (36.4%) were in fair condition and 1 (9.1%) was in poor condition. Estimated percent fertilization upon detection ranged from 85% 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, egg mass detached/slumped onto substrate and siltation on surface of egg mass.

Of the 23 egg masses detected only 5 were deemed to be cageable as it is not possible to cage those detected late in development, in the process of hatching, or those detached or slumped onto the substrate. All 5 cageable egg masses were detected in good condition at an early stage of development at WDLNA and were subsequently caged; development was monitored closely and hatchlings counted upon release. All 5 of the caged egg masses had excellent hatch out, precise hatchling counts were done at the free-swimming stage upon release and totals ranged from 2680 to 4072 hatchlings per egg mass (mean=3247, n=5, SD=542 and sum=16,233; Table 5).

Of the 5 healthy egg masses with precise hatchling counts, there was a total of 16,223 hatchlings, of which 7640 (47.1%) were removed (7600 to the Columbia Marsh reintroduction site and 40 to the Vancouver Aquarium captive assurance colony) and 8583 (52.9%) released in situ at DLNA. The hatchlings that were removed for the Columbia Marsh reintroduction program came from 3 different egg masses laid in WDLNA and the hatchlings removed for the Vancouver Aquarium captive assurance colony came from 4 different egg masses laid in WDLNA (Table 5). The proportion of each egg mass removed ranged from 0.3% to 95.2%, leaving 99.6% to 4.8% to be released in situ at WDLNA (Table 5).

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.

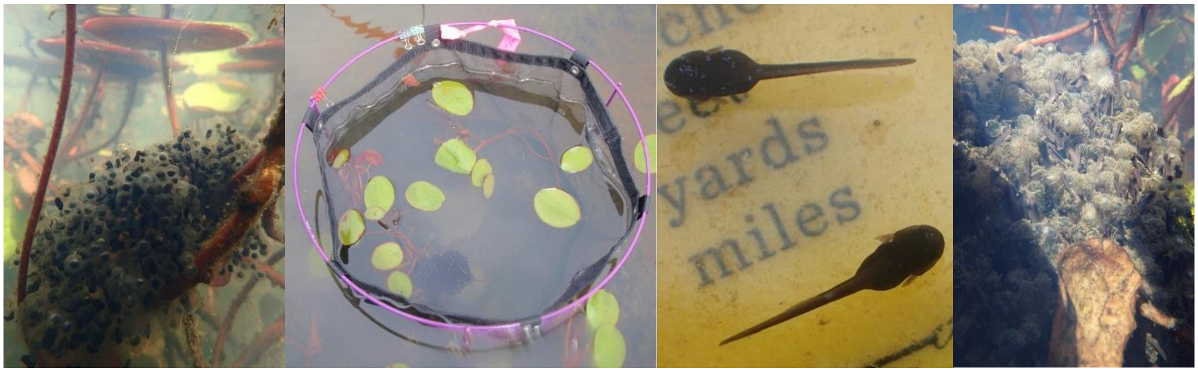

Figure 8. Photos (left to right): developing egg mass showing variation in stages of development between eggs within the egg mass, caged egg mass, hatchling tadpoles with external gills visible, hatched egg mass showing fungal growth on egg at top right.

Table 5. LIPI egg masses detected in the CVWMA in 2016 with # removed and hatchling counts for 5 caged egg masses

Egg mass #	Site	Date laid	Condition	% Fertilized	Caged	Number (%) of hatchlings Removed*	Number (%) of hatchlings Released in situ	Total
EM160419-TH01	EDLNA	12-Apr	good	n/a	No	0	not counted	not counted
EM160502-SW02	WDLNA	01-May	good	98	Yes	10 (0.4%) VA	2660 (99.6%)	2670
EM160502-LP03	WDLNA	30-Apr	poor	n/a	No	0	not counted	not counted
EM160503-BH04	WDLNA	02-May	good	99	Yes	10 (0.3%) VA	3353 (99.7%)	3363
EM160504-BH05	WDLNA	30-Apr	good	99	No	0	not counted	not counted
EM160510-LP06	WDLNA	06-May	good	99	Yes	3010 (73.9%) VA/CM	1062 (26.1%)	4072
EM160510-BH07	WDLNA	07-May	good	98	Yes	3110 (95.2%) VA/CM	158 (4.8%)	3268
EM160510-BH08	WDLNA	04-May	fair	96	No	0	not counted	not counted
EM160510-LP09	WDLNA	07-May	fair	85	No	0	not counted	not counted
EM160510-SW10	WDLNA	04-May	fair	99	No	0	not counted	not counted
EM160511-SW11	WDLNA	10-May	good	99	Yes	1500 (52.6%) CM	1350 (47.4%)	2850
EM160512-LP12	WDLNA	06-May	good	96	No	0	not counted	not counted
EM160513-BH13	EDLNA	07-May	good	n/a	No	0	not counted	not counted
EM160513-SW14	EDLNA	06-May	poor	n/a	No	0	not counted	not counted
EM160517-SW15	WDLNA	07-May	good	n/a	No	0	not counted	not counted
EM160518-SW16	EDLNA	10-May	fair	n/a	No	0	not counted	not counted
EM160518-SW17	WDLNA	11-May	fair	n/a	No	0	not counted	not counted
EM160520-SW18	WDLNA	10-May	fair	n/a	No	0	not counted	not counted
EM160520-LP19	WDLNA	10-May	poor	n/a	No	0	not counted	not counted
EM160525-SW20	WDLNA	22-May	fair	98	No	0	not counted	not counted
EM160525-BH21	WDLNA	21-May	good	96	No	0	not counted	not counted
EM160525-TH22	WDLNA	23-May	fair	95	No	0	not counted	not counted
EM160530-BH23	WDLNA	29-May	fair	99	No	0	not counted	not counted

* VA indicates tadpoles removed for Vancouver Aquarium captive assurance colony (n=10 tadpoles)

* CM indicates tadpoles removed for Columbia Marsh reintroduction program

The 2016 field season marked the sixth consecutive year of above average egg mass detections (Table 6).

Table 6. LIPI egg masses detected in the CVWMA 2000-2016

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	*	23	**	26
2016	4	*	19	**	23
Total	66	5	157	10	238

* indicates area not surveyed

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

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

Spring air temperatures at DLNA ranged from a low of -1.0°C on April 1 at 07:00 to a high of 32.3°C on April 20 at 17:00 (Figure 9). Water temperature for the same period ranged from a low of 7.8°C on April 4 at 08:00 to a high of 24.0°C on May 7 at 18:00. The first major peak in air temperature was on April 20 when it reached 32.3°C and the first major peak in water temperature was on April 21 when it reached 21.3°C. During the peak egg laying period of 2016 (April 30 – May 11) temperatures ranged from 1.6°C - 30.7°C for air and 10.6°C to 24.0°C for water.

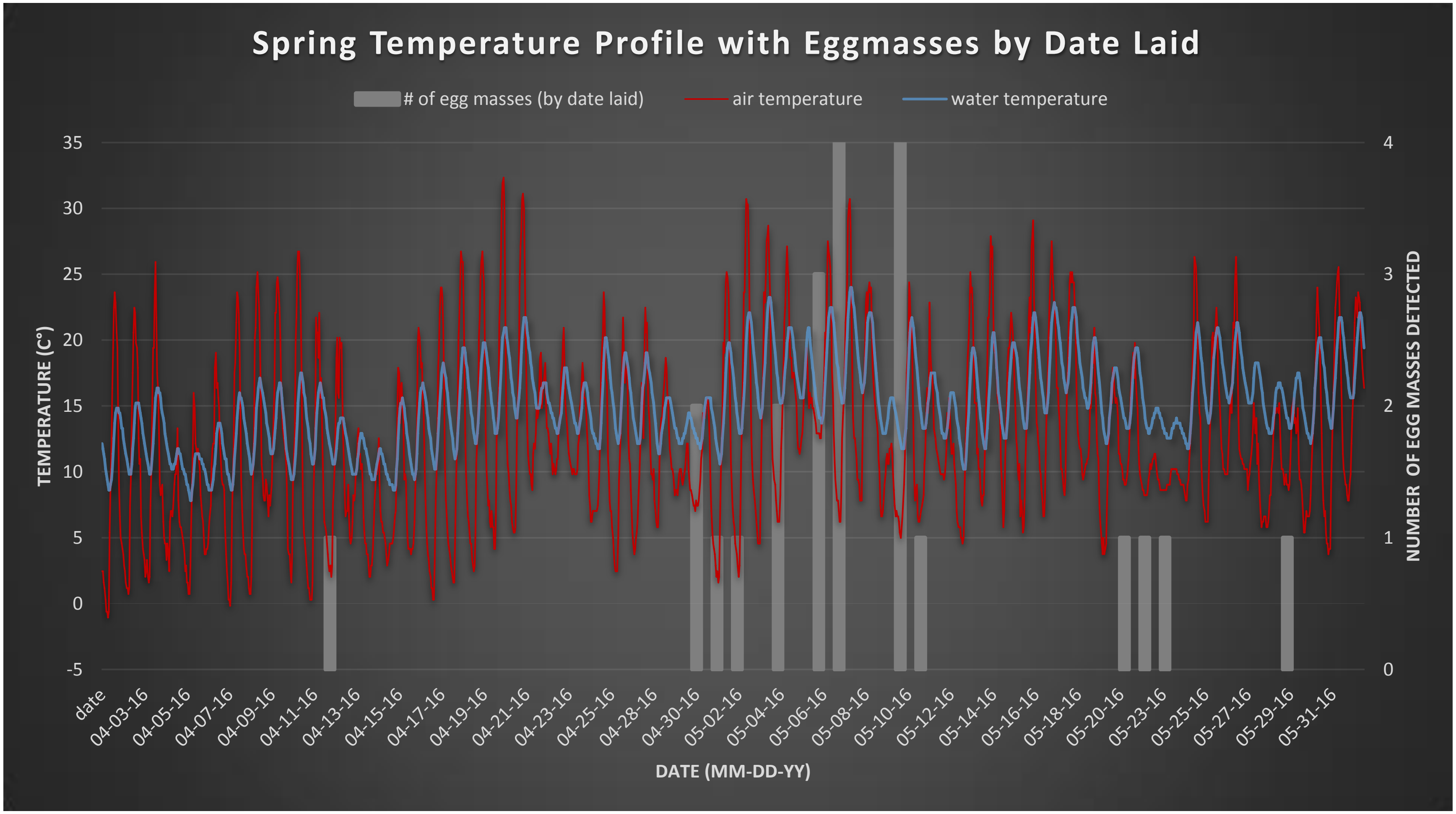

Figure 9. Hourly air and water temperatures at DLNA from April 1 – June 1 with number of detected egg masses by date laid. Air temperature data from HOBO #5 and water temperature data from HOBO#7 at EDLNA.

4.3 Tadpole Trapping

Trapping was conducted at 3 sites from July 6-9; a total of 32 traps were used and tadpole trapping was done for a total of 661:22 trap-hours, with an overall trap-rate of 0.015 (Table 7; Appendix 1, 2). LIPI tadpoles were detected in the East pond (n=3 trapped, n=11 other observations, Gosner stages 25-40) and WDLNA (n=7 trapped, n=2 other observations, Gosner stages 28-41; Table 7, Figure 10). No tadpoles were detected at Leach Lake. All tadpoles were deemed to be in good health, with the exception of 2 that were missing a tail portion.

The first sign of metamorphosis (defined by the detection of a metamorph with a tail stub <5.0 mm long) was at EDLNA on July 12 (Figure 10), however the majority of the other detections during that week were tadpoles of varying stages.

Table 7. Summary of 2016 LIPI tadpole trapping effort by site at CVWMA

	East Pond	WDLNA	Leach Pond 4	Overall
# Traps used	8	16	8	32
# Trapped LIPI	3	7	0	10
Trap-hours	174:16	316:58	170:08	661:22
Trap-rate	0.017	0.022	0.000	0.015

Figure 10. Photos (clockwise from top left): tadpole missing part of its tail, very small tadpole (TL 41.1 mm) detected on July 6 (Gosner stage 31), metamorphosing LIPI with long tail (35.4 mm) and first sign of metamorphosis (YOY with 3.7 mm tail on July 12).

4.4 Visual Encounter Surveys (VES)

In 2016 at the CVWMA 123:47 person-hours were dedicated to visual encounter surveys (n=84 surveys) from July 11 to November 15 (Table 8). Locations of LIPI observations in the CVWMA are provided in maps in Appendices 2, 4, 5 and 6.

Table 8. Summary of VES efforts in the CVWMA for 2016

	Summer	Fall	Total
Number of surveys	63	21	84
Survey effort (person-hours)	86:27	37:20	123:47
Number of LIPI observations	161	59	220
LIPI catch/effort during NCS	1.86	1.58	1.78

During VES a total of 161 LIPI detections were made from July 11 - November 3, a catch per effort of 1.78 LIPI per person-hour of survey effort. As expected, the majority of detections were YOY (n=135, 61.4%), of those most (n=64, 47.4%) were detected during the summer in the known breeding areas of DLNA (Table 9). LIPI were detected in all areas surveyed except for Leach Lake, so there was no evidence of successful breeding at Leach Lake in 2016.

Table 9. Summer VES sites (n=#surveys) with number of LIPI observations by stage

CVWMA VES (n=84 surveys)	Adult	YOY	Tadpole	Unknown	TOTAL
Duck Lake (n=2)	0	4	0	0	**4**
East Ditch (n=16)	4	8	0	0	**12**
ELDNA Pond (n=5)*	0	27	35	0	**62**
Goat channel (n=19)	13	37	0	1	**51**
Kootenay River – East channel (n=4)	1	7	0	0	**8**
Kootenay River – Leach Lake (n=3)	0	0	0	0	**0**
Leach Lake 2 (n=1)	0	0	0	0	**0**
Leach Lake 4 (n=5)	0	0	0	0	**0**
Leach Lake 5 (n=1)	0	0	0	0	**0**
Leach Lake 6 (n=1)	0	0	0	0	**0**
Pumphouse channel (n=11)	2	0	0	2	**4**
Southwest ditch (n=4)	5	15	0	0	**20**
Squiggle channel (n=5)	0	0	0	1	**1**
WDLNA (n=6)*	2	37	19	0	**58**
TOTAL	**27**	**135**	**54**	**4**	**220**

*****known breeding area

4.5 Road Surveys

Night road surveys during the late-summer and fall migration period were conducted along the Duck Lake dike between August 31 and October 6 (n=19 night surveys). A total of 70:09 person-hours of survey effort was dedicated to these surveys, with a combined total of 24 LIPI observations, a catch per effort of 0.34 LIPI/person-hour of survey effort (Table 10). See maps in Appendices 2, 4 and 5 for locations of LIPI observations made during road surveys.

Table 10. Summary of 2016 late-summer & fall night road survey effort

	Road surveys
Number of surveys	19
Number of LIPI observations	24
Survey effort (person-hours)	70:09
LIPI catch/effort during road surveys	0.34

In 2016 road surveys documented overall 29.2% road mortality LIPI detections (Table 11). Of the 24 total LIPI observations 4 were adults and 20 were YOY (Table 11; Figure 11). In addition to this there were some animals detected on the south shoreline of Duck Lake during the migration period, these detections are summarized in the VES section (Section 4.5).

Other herptile observations during late summer and fall night road surveys along Duck Lake dike included: Pacific chorus frogs *(Pseudacris regilla*), long-toed salamanders *(Ambystoma macrodactylum)* and 1 dead Common garter snake *(Thamnophis sirtalis).*

Table 11. Late-summer and fall night road survey results on Duck Lake dike 2016

Species -Age class	Live	Dead	Total	Proportion Live	Proportion Dead
LIPI - Adult	3	1	4	75.0%	25.0%
LIPI - YOY	14	6	20	70.0%	30.0%
LIPI Total	**17**	**7**	**24**	**70.8%**	**29.2%**
Pacific chorus frog	5	0	5	100.0%	0.0%
Long-toed salamander	8	2	10	80.0%	20.0%
Common garter snake	0	1	1	0.0%	100.0%
Grand Total (all species)	**30**	**10**	**40**	**75.0%**	**25.0%**

Mean air temperature at dusk for the subset of night surveys where LIPI were encountered was 13.0°C (n=8, SD=2.5, range 9.6°C to 16.3°C). LIPI were encountered in 8 of the 18 night road surveys (38.9%); detection dates were September 19, 23, 28, 29, 30 and October 1, 2 and 6. 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.

4.6 Animal Health

Of the captured post-metamorphic LIPI whose health was assessed visually in the field the majority were deemed to be in good health (97.2%), 2.7% in fair health (n=3) and none in poor health (Table 12, Figure 7). No mortalities were documented other than the road mortalities described previously (Table 11).

Table 12. Health assessment of LIPI 2016 CVWMA field observations (n=113)

Season	Age Class	Good	Fair	Poor	Dead	Total
Spring	**Adult**	3	0	0	0	3
	Juvenile	13	0	0	0	13
	All age class	16	0	0	0	16
	All age class %	100.0%				
Summer	**Adult**	11	0	0	0	11
	YOY	32	1	0	0	33
	All age class	43	1	0	0	44
	All age class %	97.7%	2.3%			
Fall	**Adult**	6	1	0	0	7
	YOY	38	1	0	0	39
	All age class	44	2	0	0	46
	All age class %	95.7%	4.3%		0	
Over all seasons	**All age class**	103	3	0	0	106
	All age class %	97.2%	2.8%			

Of the 103 LIPI deemed to be in good health, there were 20 adults, 13 juveniles and 70 YOY.

Of the 3 live LIPI deemed to be in fair condition, there was 1 adult and 2 YOY. The adult and 1 YOY (captured in fall) had sloughing skin, a symptom of Chytridiomycosis. The other YOY was captured in the summer and was missing its hind right foot, likely from a predator attack (Figure 11).

Figure 11. Photos (clockwise from top left): live YOY in fair health (summer detection) missing right hind foot (likely predator attack), live YOY in fair health (fall detection) with sloughing skin (possibly from Chytridiomycosis), dead YOY in fall from road mortality at Duck Lake, dead adult female in fall from road mortality at Duck Lake.

In total there were 133 post-metamorphic LIPI observations that were visually inspected by surveyors for signs of Chytridiomycosis during the 2016 field season, this includes 7 dead specimens (cause of death was road mortality). Chytridiomycosis was suspected in 2 of the 113 observations (1.8%), 1 adult and 1 YOY during the fall survey period (Table 13).

Table 13. Field observer's determination if Chytridiomycosis suspected by visual inspection of CVWMA LIPI detections in 2016 (n=113*) by season and stage

		Chytridiomycosis Suspected		
Season	Stage	No	Yes	Total
Spring	Adult	3	0	3
	Juvenile	13	0	13
	All age class	16	0	16
	All age class %	100.0%		
Summer	Adult	11	0	11
	YOY	34	0	34
	All age class	45	0	45
	All age class %	100.0%		
Fall	Adult	7	1	8
	YOY	43	1	44
	All age class	50	2	52
	All age class %	96.2%	3.8%	
Over all seasons	All age class	111	2	113
	All age class %	98.2%	1.8%	

*includes dead specimens (n=7, all road mortalities)

A total of 28 post-metamorphic LIPI detections were swabbed for Chytridiomycosis testing during the field season. Samples were collected on a priority basis and include 14 adults (3 spring, 9 summer and 2 fall detections), 13 juveniles (all spring detections) and 1 YOY (fall detection with sloughing skin on the ventral surface). Swabs have been stored for future analysis.

4.7 Passive Integrated Transponder (PIT) Tagging

A total of 15 LIPI individuals were PIT tagged during the late summer and fall field season between September 8 and November 3. There were 10 adults tagged, with weights ranging from 53.0 g to 97.0 g and 5 YOY tagged, with weights ranging from 25.0 g to 30.0 g. No recaptures were made during this first field season of tagging. All PIT tag injections went well and there were no signs of stress detected in the animals during the procedure.

4.8 Dorsal Spot Pattern Recognition

A total of 120 dorsal spot pattern photos were taken of post-metamorphic LIPI captures during the 2016 field season and stored in the digital image library.

4.9 Morphometrics of Creston LIPI

Size data was gathered on 98 LIPI detections during the 2016 field season, this data includes live male and female field captures where both a weight and snout vent length were measured (Tables 14, 15, Figure 12).

Table 14. Weight of 2016 CVWMA LIPI captures by stage and sex

Weight (grams)	Stage - sex	n	Mean	SD	Range
Spring	Juvenile - male	13	31.8	12.3	15.0 - 46.0
	Adult - male	2	54.0	1.4	53.0 - 55.0
	Adult - female	1	53.0	n/a	n/a
	Total (combined)	16	35.9	14.1	15.0 - 55.0
Summer	YOY - sex unknown	31	9.0	7.4	2.0 - 28.0
	Adult - male	5	61.4	6.9	53 - 68.0
	Adult - female	6	69.8	19.8	51.0 - 97.0
	Total (combined)	42	24.0	27.2	2.0 - 97.0
Fall	YOY - sex unknown	33	14.5	6.5	5.0 - 30.0
	adult - male	2	62.0	17.0	50.0 - 74.0
	adult - female	5	71.4	12.1	61.0 - 85.0
	Total (combined)	40	24.0	22.3	5.0 -85.0

Table 15. Snout-Vent Length (SVL) of 2016 CVWMA LIPI captures by stage and sex

SVL (mm)	Stage - sex	n	Mean	SD	Range
Spring	Juvenile - male	13	64.7	7.9	52.7 - 74.5
	Adult - male	2	77.3	6.9	72.4 - 82.1
	Adult - female	1	79.5	n/a	n/a
	Total (combined)	16	67.2	9.1	52.7 - 82.1
Summer	YOY - sex unknown	31	42.8	11.2	26.1 - 70.8
	Adult - male	5	80.4	4.8	75.4 - 87.2
	Adult - female	6	82.4	8	74.0 - 95.4
	Total (combined)	42	53.0	19.9	26.1 - 95.4
Fall	YOY - sex unknown	33	51.2	8.6	37.1 - 69.3
	adult - male	2	79.3	4.8	75.9 -82.7
	adult - female	5	78.7	6	69.2 - 83.8
	Total (combined)	40	56.1	13.3	37.1 - 83.8

During the spring (blue markers), there are 3 main clusters: 1) the smallest animals are males with weights ranging from 15.0 – 24.0 g 2) medium size males with weights from 38.0 – 46.0 g; and 3) the larger adults (53.0 g, -55.0 g (Figure 12).

In the summer (red markers), the majority of the observations are YOY (n=31) between 2.5 - 28.0 grams in weight and 26.1 - 62.9 mm SVL. There are an additional 2 clusters: 1) medium size males and females between 51.0 - 68.0 g, 2) females (n=2) that weighed 92.0 - 97.0 g.

During the fall (grey markers), there are 2 clusters: 1) the smaller LIPI with weights of 5.0 – 30.0 g, and 2) a loose cluster of larger animals that includes both male and female animals ranging in size from 50.0 to 85.0 g, 83.5 mm. The majority of the fall observations were less than 50 grams in weight (see dashed line in Figure 12).

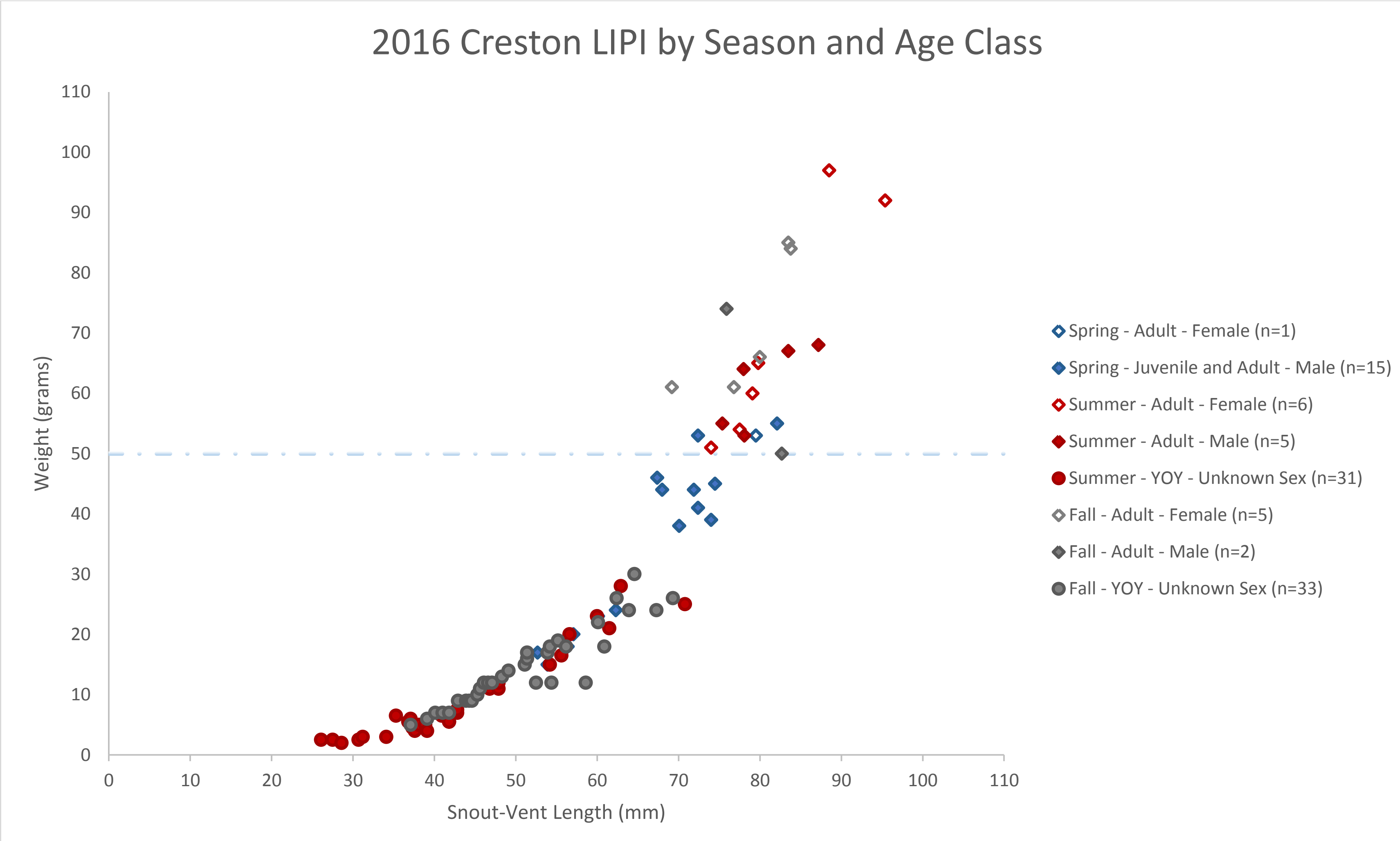

Figure 12. Size Chart: 2016 CVWMA LIPI Captures (n=98) by Season, Age Class and Sex

A multi-year comparison of size data of YOY captured in the fall shows that 2016 YOY were smaller than observed in 4/6 previous years, only 2015 and 2011 had smaller mean weights for YOY (Table 16).

Table 16. Size data for fall YOY LIPI detections in the CVWMA 2010 – 2016

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
2016	9/21 – 10/25	32	14.5	6.6	5.0 – 30.0	51.2	8.7	37.1 – 69.3

*captures made in 2012 and 2016 in November omitted for date range consistency between years

4.10 Vancouver Aquarium Captive Assurance Colony

In 2016, the Vancouver Aquarium requested 10 tadpoles from 4 wild laid Creston egg masses to bolster the captive assurance colony that was initiated in 2009. In response to this request, a total of 40 free-swimming hatchling tadpoles were transferred to the Vancouver Aquarium June 1, 2016. Tadpoles were collected from the WDLNA breeding site on May 31, where they had been housed in situ in egg mass cages. They were then housed overnight in tanks at the office and packaged up the following morning, driven to the Trail Airport (YZZ) and flown to Vancouver the same day via Pacific Coastal Airlines. See Table 5 for details of egg masses.

As of the last update received from the Vancouver Aquarium in December 2016, there were 24 survivors of the original 40 wild source tadpoles transferred from Creston in the spring of 2016; all of which successfully metamorphosed into YOY prior to brumation.

As part of the captive assurance colony, a number of animals from the in-house captive breeding program are held back from the Columbia Marsh reintroduction and maintained at the aquarium; as of December 2015 there were 23 captive bred animals from the egg masses produced at the aquarium in 2016.

Captive bred YOY are smaller and weigh less than Creston source YOY and wild YOY (Figure 13). The mean weight of the Creston source YOY at the Vancouver Aquarium is 7.8 grams (n=24, SD=1.1, Range 4.4 – 10.0) compared to 6.0 grams (n=23, SD=1.7, Range 3.4 – 9.6) for the captive bred animals, a difference of 1.8 grams; unpaired t-test results show that the difference between these 2 means is statistically significant at the 95% level (p-value = 0.0001). Mean SVL for the Creston source YOY was 36.8 mm (n=24, SD=2.4, Range 33.5 – 44.3) compared to 34.4 mm (n=23, SD=3.6, Range 28.4 – 39.4) for the captive bred animals, a difference of 2.4 mm; unpaired t-test results show that the difference between these 2 means is statistically significant at the 95% level (p-value = 0.0110).

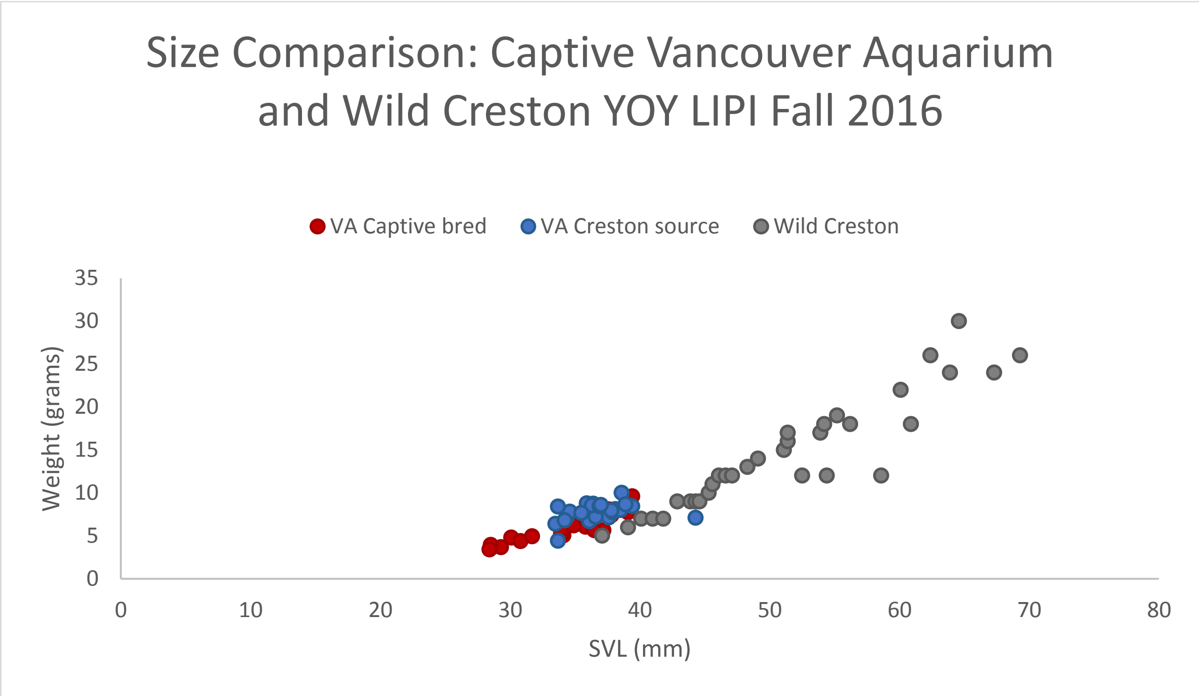

Figure 13. Size Comparison of 2016 Vancouver Aquarium captive LIPI YOY (captive bred and Creston source LIPI) and Creston wild LIPI YOY fall detections.

4.11 Columbia Marsh Reintroduction Program

The BC-NLFRT set a target of releasing 8000 tadpoles at the Columbia Marsh reintroduction site at Brisco in 2016. Since the Vancouver Aquarium could only provide 500, a request was made in late May for the remainder to be collected from Creston. In response to this request, approximately 7600 tadpoles were transferred during 3 sessions (May 24, 25 and 27).

Tadpoles were taken from 3 different egg masses (Table 5). Transfers were carried out between 17 – 18 days from the date the egg masses were laid and all hatchlings had recently reached the free-swimming stage. Between 52.6 % and 94.9% of these 3 egg masses were transferred to Brisco (Table 5). These proportions are greater than what would ideally be removed per egg mass, for a discussion, see Section 5.12.

An estimated 7600 hatchlings were collected and packaged up in Creston by the FLNRO team and picked up by the Brisco team with members from the BC-NLFRT for transfer and release in Brisco. In total it took 25 Thermos containers (2 gallon capacity each) to house the estimated 7600 tadpoles for transfer. Estimates by the FLNRO team during collection and packaging are consistently very accurate, of the subset that was counted at the recipient site upon release, the FLNRO estimate 1000 tadpoles, which turned out to be only 8 tadpoles off at 1008, so it is likely that the estimated total of 7600 transferred is very close even though they were not all counted with accuracy upon release.

Of the subset of 1008 hatchlings that were held overnight at the recipient site prior to release only 1 died.

An additional 500 hatchlings from the Vancouver Aquarium captive assurance colony breeding program were released in Brisco on June 1 by the BC-NLFRT for a total of 8100 reintroduced during the 4th year of the project; a grand total of approximately 15,633 since 2013.

4.12 Habitat

Water Levels

At both East and West DLNA there were very low water levels detected, as well as problems getting water into DLNA and water retention issues during the summer of 2016. For more details see the summary document prepared by the author in 2016 (Houston, 2016b).

The water levels dropped considerably between the last day of spring breeding surveys and arrival to do tadpole trapping. At East DLNA, the staff gauge read 285 mm on May 26, and had dropped by 205 mm to read 80 mm by July 7 when we returned to do tadpole trapping (see photos in Figure 14). Water levels also dropped at the WDLNA site, the staff gauge read 560 mm when it was installed June 1, and had dropped to 465 mm by July 7, a decrease of 95 mm. CVWMA managers were alerted to the situation and the water control gates at the Pumphouse channel were immediately opened to bring water into DLNA. By July 12 it was up to 203 mm at East DLNA and 491 mm at WDLNA. Gates remained open until water levels reached those considered to be within the range of what they would normally be for that time of year, (248 mm at EDLNA, 520 at WDLNA on July 18) and were then closed to prevent too much water coming in.

Figure 14. Photos (left to right): Conditions July 7, 2016 EDLNA staff gauge reading at 80 mm (very low for this time of year); habitat at EDLNA on July 12, 2016 (during tadpole development period, prior to majority of metamorphosis), showing low water, mostly mud with very little standing water.

In an effort to keep an eye on the situation for the remainder of the tadpole development period, additional site visits to the DLNA were added to the field schedule so that water levels could be monitored on a weekly basis.

While water levels appeared suitable when they were checked July 26 (220 mm at EDLNA, 507 mm at WDLNA), they dropped considerably again in a short time frame between July 27 and August 5. Over the 9 day period, levels at East DLNA dropped 60 mm and at WDLNA dropped 49 mm. Again, the CVWMA opened the water control gates but this time the water levels did not rise, and instead dropped another 10 mm at EDLNA (to 150 mm) by August 10 even though the water control gates had been open since August 4 (see photos of dry conditions in Figure 15).

Figure 15. All 3 photos taken at EDLNA August 10 highlighting very dry conditions (no standing water, just mud) due to problems getting water in and retaining it.

As of August 25, water levels had decreased further, to 130 mm at EDLNA, resulting in cracked dry substrate in some areas (Figure 16) and 490 mm at WDLNA as a result of problems getting water in and retaining it; water levels did not rise, and actually decreased even though water gates were open since August 4.

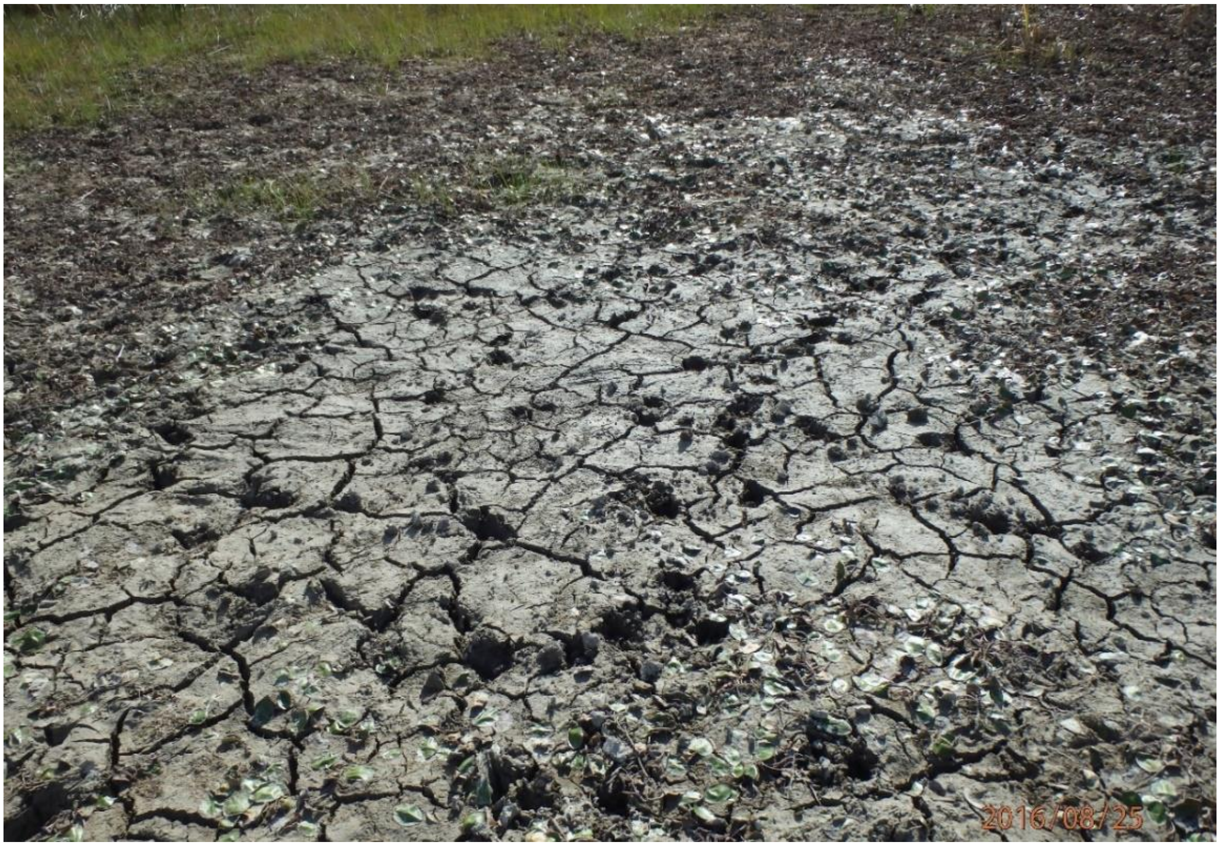

Figure 16. Conditions on August 25 at EDLNA, showing extremely dry conditions.

Results of a careful inspection of the south cross dyke of DLNA on October 30, 2016 by Tom Biebighauser, Marc-Andre Beaucher and the author to try and identify potential causes of wetland water retention issues, resulted in a number of potential causes, requiring further investigation. Potential causes including numerous muskrat holes and a 50 cm deep beaver run adjacent to the large beaver lodge that was detected in the southwest ditch of DLNA several years ago (Figure 17). The theory is that if beavers or muskrats have burrowed through the dike and impacted the structural integrity, it could result in seepage. Water levels were too high in the fall when the inspection was completed to determine if water was seeping through the dyke, so CVWMA staff will follow up on it when conditions improve.

Figure 17. Photos (left to right): DLNA south cross dike inspection October 30, 2016, showing large beaver lodge (potential cause of wetland water retention issues, if beavers have burrowed through dike and impacted structural integrity of dike); Marc-Andre Beaucher standing in beaver tunnel (~50 cm deep) which appears to go into the dyke.

Watershield

In the watershield removal test project a circular patch approximately 5 m in diameter was cleared of watershield at WDLNA (Figure 18). In total it took 9 person-hours to complete this work. As a result of the removal of the rhizomatous mat in the test area, the substrate was lowered by at least 10 centimetres (in some areas of the DLNA, the rhizomatous mat averages 20 cm thick).

Figure 18. Photo of watershield removal test plot upon completion May 19, 2016.

The watershield ingrowth problem was assessed by Tom Biebighauser in the fall of 2016 and potential control options were discussed. Tom indicated that it is very possible that the thick rhizomatous mat (which averages 10-20 cm thick in some areas of DLNA) will likely become thicker over time, further reducing the depth of water in the wetland (Biebighauser, 2016). He noted that there are a number of techniques theoretically available for controlling watershield in a wetland (although they might not all be applicable in this situation) and provided a summary with some potential drawbacks in his 2016 report, techniques include:

1) Watershield may be controlled by periodically drying the wetland, it does not appear to grow in wetlands with fluctuating water levels
2) Use heavy equipment to greatly increase the depth of water in the wetland. The waters in the wetland can be expected to become turbid if deep areas are dug while water is present.
3) Herbicide application. A number of herbicides are available for controlling watershield.
4) Grass Carp. Introducing triploid grass carp is another way of controlling watershield.
5) Build ephemeral wetlands that would not become dominated by watershield.

New Wetlands Created

A total of 6 new wetlands were constructed in November – December 2016 in the Duck Lake area, 2 were created on the periphery of the DLNA (EDLNA-1 and EDLNA-2) and 4 were created east of the road (OGCS 1-4), adjacent to the Old Goat Channel over-wintering area, (Figure 19). The newly constructed wetlands range in size from 0.35 – 0.76 ha, and total 3.41 ha all together.

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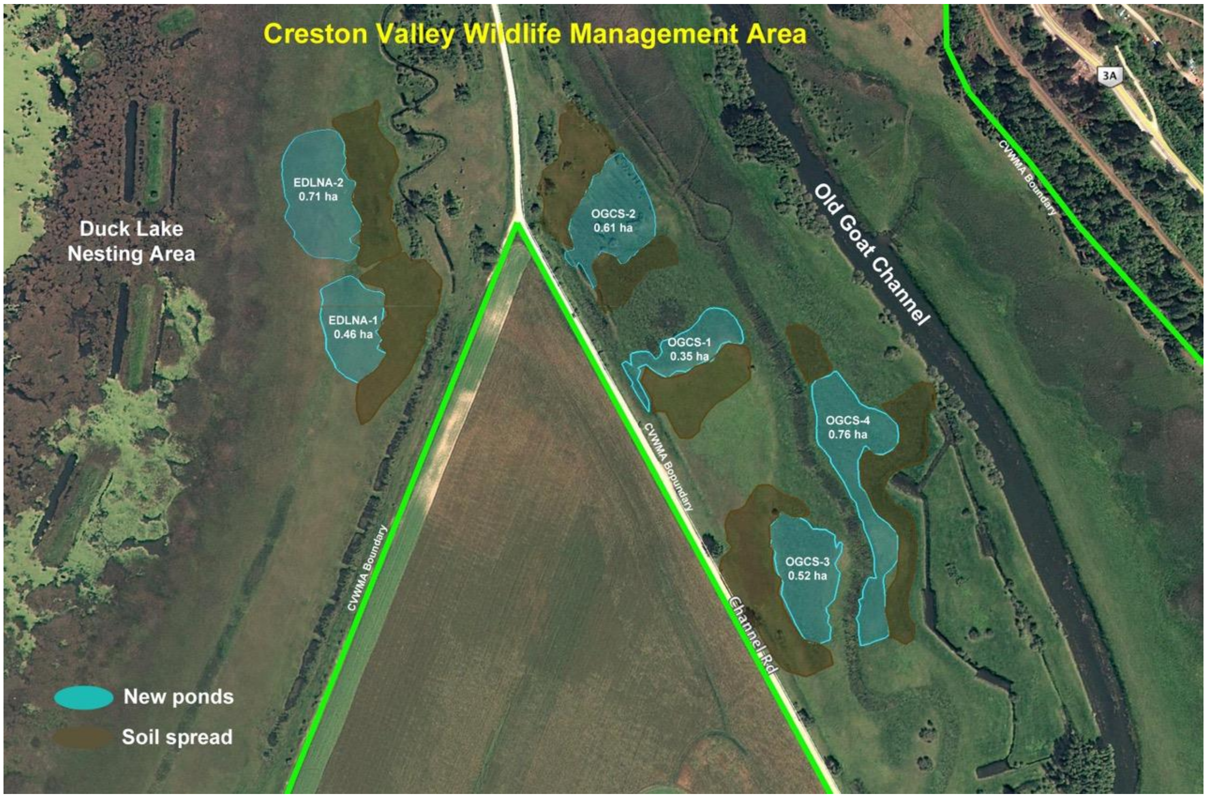

Figure 19. Map showing newly constructed wetlands (courtesy of CVWMA).

5. DISCUSSION

5.1 Songmeters

As bullfrogs are a significant threat to LIPI it is recommended that the NLF project continue to loan their Songmeters to the American bullfrog surveillance team. It should be possible to use them for LIPI work at the CVWMA during the peak calling period in late April through early June since bullfrogs likely don't begin calling in the region until mid-June at the earliest.

5.2 Nocturnal Calling Surveys (NCS)

Calling male LIPI were already present at DLNA when the first NCS was conducted on April 19, an indication that the critical temperatures required to trigger male LIPI calling had already been reached. Males tend to begin calling when water temperatures reach 10°C and air temperatures reach 15°C (Seburn, 1992) and this pattern has been confirmed for the Creston LIPI population (Houston 2015). April 2016 temperature data from HOBO temperature data loggers at DLNA show that these thresholds were not reached until April 7 and then only lasted a few days. Cooler weather lasted until April 16 when air temperatures again approached the critical temperatures required to trigger male LIPI calling, as observed by the 14.5°C air and 16.4°C water temperatures, this was followed by temperatures of 17.0°C air and 17.9 °C water the following day. As a result it is thought that while males may have had conditions favorable for calling in early April (April 7-8-9-10), calling would have been sporadic until things warmed up again on April 16-17. This theory is supported by the observed egg mass deposition dates as there was one laid on April 12, but no others were detected until April 30.

The maximum number of LIPI detected calling during the survey period was 49 individuals, which occurred May 2-3 at WDLNA. This is greater than the reported maximum of 38 detected in 2015 (Houston 2016a). However comparisons between years are constrained, as peak calling numbers were not determined in 2015 due to the large number of surveys cancelled because of high winds.

Due to the fact that only a small portion of calling males are actually captured during NCS as a result of the change in priority of the survey program, the reported number of adult and juvenile male detections cannot be assumed to be a direct representation of the actual breeding male population. Additionally, 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 occurred prior to 2015); photo recognition or PIT tagging would need to be done to identify recaptures

Unfortunately there was no calling detected at Leach Lake in 2016, the majority of the surveys were blitz style surveys conducted from the dykes at Leach Lake. With the resources allocated, and marginal opportunities because of weather windows, it was not possible to get out into the heart of the large wetlands (up to 1500 meters across, and therefore out of the audible detection distance of the blitz survey stations) to conduct more than 1 reconnaissance-level NCS via canoe at Leach Lake in 2016. However it is believed that there are likely calling groups at Leach Lake that have gone undetected due to the enormous size of the wetland complex at Leach lake, which is approximately 700 ha, and very difficult to survey without additional resources. Even though there were no detections of calling male LIPI at Leach Lake in 2016, it is recommended that NCS continue to be carried out in the area and that as recommended in previous years (Houston, 2016a), if possible, calling surveys be expanded to include reconnaissance-level NCS via canoe in all compartments to increase detection probability.

It is somewhat surprising that LIPI have not been detected calling on a regular basis in pond 4 of Leach Lake since the drawdown was completed in 2013; they were only detected in 2014, the first year after the drawdown. In an effort to try and determine why, aquatic migration corridors believed to previously link spring breeding habitat in pond 4 with overwintering habitat in the Kootenay River, were assessed in the fall of 2016 to determine if there were any potential issues with connectivity, preventing LIPI from effectively "finding" pond 4. It was noted that one of the small side channels which used to provide a direct link between the main aquatic migration corridor and pond 4 had been altered. It no longer contains standing open water and is choked with dense stands of cattails, in effect breaking the aquatic linkage between these 2 seasonal habitats. It appears that a section of high ground at the wetted end of the small side channel has effectively cut it off from the main channel. It is recommended that this area be dredged to reopen it and restore the aquatic migration corridor linkage zone between seasonal habitats to determine if this may restore pond 4 as a LIPI breeding area, which would be very beneficial to the structure of the CVWMA LIPI population. At one point in time Leach Lake accounted for 5 of the total 13 egg masses detected (see Table 6, year 2007). This is a very small scale restoration project that could potentially have a significant reward. Since it would be fairly easy to do, as the area is accessible by road and the banks surrounding the ditch that would need to be dug out appear to be dry during many parts of the year, it is recommended that if possible the BC-NLFRT obtains funding to do it as soon as possible.

With the changes to the NCS program in 2015, 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 the result was less information gathering on the state of the population. Without capturing animals, data such as morphometrics, health, and age class are not possible and between-year comparisons are limited. This is evident by the fact that only a fraction of the number of LIPI were captured in the spring of 2016 (n=13) 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.

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 2 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 during the spring breeding season at DLNA ranged in size and age class. In 2016, weights ranged from 15.0 – 55.0 grams and SVL ranged from 55.9 – 83.4 mm, smaller than documented in 2015 (53-64 grams in weight, 72.8 – 83.4 mm SVL, Houston 2016). These detections form 2 distinct clusters, a group of smaller animals (15.0 – 24.0 g), and a group of larger animals (38.0 – 55.0 g) (Figure 12). Since size data can be used to approximate age this data indicates that the calling male population at DLNA in 2016 was composed of 1 year olds and 2 year old animals. Age classes are estimated based on data from marked individuals (Houston, 2013). A comparison with the 2015 size charts shows similar results (2 main clusters of calling males, 1 and 2 year olds), but upon comparison with 2014 data (from a time when a much greater number of calling males were captured) 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 both 2015 (Houston, 2016a) and 2016. This could be explained by the fact that the 2015 sample size (n=36) is half the size of the 2014 sample size (n=72) and the 2016 sample size is even smaller yet (at n=15), so it is possible that a few larger animals were present but went undetected. Alternatively, this cohort may be missing; without capturing a larger percentage of calling males in the spring it is not possible to determine or make meaningful conclusions about the age class structure of the spring breeding male population.

5.3 Egg Mass Surveys (EMS)

It was not possible to do a rough estimate of condition or hatch out success on any other egg masses due to the fact that most were detected in the process of hatching or had already hatched. As a result it was not possible to estimate the total number of hatchlings produced this year, estimate the overall percentage removed for reintroduction or determine relative hatch out rates. Furthermore, since it was only possible to get hatchling counts on the 5 caged egg masses which were all in good condition upon detection and had excellent hatch out it is not possible to compare the means between years, as in 2015 the overall mean number of hatchlings included a number of egg masses which were in poor or fair health with variable hatch out success so the comparison of means would not be accurate.

Although the amount of survey-effort was considerable, egg mass detectability was impacted by ingrowth of watershield and as a result it is possible that were egg masses that went undetected at DLNA. Normally, prior to spring 2015, watershield (*Brasenia schreberi*), a floating-leaved 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 typically 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, again in 2016 (as in 2015), 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.

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 46 for 2016, 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

The level of tadpole trapping in 2016 exceeded that in 2015 (435:00 trap-hours) by 226:22 trap-hours, the reason for this increase was to monitor the development of tadpoles due to concerns for their health during the alarmingly low water level situation at DLNA. Interestingly the trap-rate was very similar between the 2 years at 0.015 in 2016 and 0.014 in 2015 (Houston, 2016a). It appears that the number of LIPI trapped is proportional to the trap-time.

The number of person-hours devoted to tadpole trapping in 2016 (18:19) was almost double of that in 2015 (9:30), a difference of 8:49. This increase in effort was a result of the extremely low water levels detected in DLNA in the summer, so effort was increased to try and monitor developmental effects associated with this.

There were a range of developmental stages detected during tadpole trapping at DLNA, trapped LIPI ranged from Gosner stage 25-40, this is as expected since detected egg mass deposition dates ranged from April 12 through May 29 so it follows reason that there would be a wide range of developmental stages on any given date.

The first sign of metamorphosis was a YOY with a 3.7 m tail stub detected on July 12 at EDLNA ponds. In comparison to the results of tadpole trapping in 2015, done over the same time frame, Gosner stages detected were slightly more advanced in 2015 at 26-42, and the first sign of metamorphosis was earlier in 2015, with the detection of a YOY with a 5.0 mm tail stub on July 9. In addition to this, there were numerous YOY observed on the date of first detection of metamorphosis in 2015, where as in 2016 there was only one YOY detected on July 12. This apparent delay in development could be attributed to differences in weather between the 2 years; 2016 was much cooler than 2015 during the tadpole development period, but it could also be due to the extremely low water levels which could possibly cause a shortage in food availability and an increase in tadpole density or other factors.

The minimum observed development time from egg fertilization to metamorphosis in 2016 occurred in 91 days, which is considerably longer than the approximately 77 days estimated for 2014 and 2015 (Houston, 2016a), and is at the maximum end of the range 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 lag in developmental rate could be due cooler weather conditions in 2016 as temperatures are the main factor that controls developmental rate. It is unknown whether or not extremely low water levels, such as those observed in the summer of 2016, could have a negative impact on developmental rate, in most cases it is believed to increase the rate of development, but a lack of food and increased density as a result of low water levels could plausibly result in a decreased developmental rate.

Tadpole trapping at the main breeding area of DLNA proved to be a very important part of the field survey program in 2016, not only to monitor the development of tadpoles and determine the date of first metamorphosis but it alerted us to the extremely low water level situation. If tadpole trapping had not been conducted, the low water level would have gone undetected. It is recommended that tadpole trapping remain a high priority of the field survey program in future years to monitor water levels, determine date of metamorphosis and monitor size at metamorphosis.

Tadpole trapping at Leach Lake failed to provide any evidence of successful breeding at pond 4, even though 8 traps were deployed for a total of 170:08 trap-hours no LIPI tadpoles were trapped. This is not surprising since no calling was detected at the site during NCS in the spring. However, since tadpole trapping is only done at the presence/not-detected level, non-detection of tadpoles alone does not provide conclusive evidence that breeding did not occur but coupled with the fact that no YOY were detected during summer VES at Leach Lake it is unlikely that there was an successful breeding at Leach Lake pond 4 in 2016.

5.5 Visual Encounter Surveys (VES)

There were no detections of LIPI during summer or fall VES in the Leach Lake area. However this does not necessarily indicate no breeding occurred as the area is vast and not all areas were surveyed extensively. As a result, 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 2, this suggests that the migration was underway even though large numbers of animals were not yet detected during road surveys. In previous years LIPI were detected in the overwintering area September 10, 2014 and September 23, 2015 (Houston 2015, 2016a). Surveys in the secondary over-wintering 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. However, of note was that LIPI were detected for the first time on the west shoreline of the Kootenay River east channel. Also of note is the fact that Columbia spotted frog (*Rana luteiventris*) YOY were detected in the DLNA for the first time on record in the fall of 2016 at the southwest ditch.

5.6 Road Surveys

Road mortality is still occurring on the dyke road at Duck Lake (documented at 29.2% of LIPI detections during the fall migration period of 2016) despite signage indicating a road closure. Traffic data was collected by the CVWMA, which could be reviewed by the BC-NLFRT to determine how effective the signage was at deterring drivers. It is recommended that the BC-NLFRT review the issue and potential solutions to come up with a solution so it can be implemented prior to the late summer- fall migration period of 2017. For a list of potential solutions see recommendations in the report entitled *Northern leopard frog (Lithobates pipiens) road mortality assessment during the fall migration period of 2011 at Duck Lake, British Columbia* (Houston and Hill, 2012).

If the road does remain open in 2017, it is recommended that the new signage (Figure 20) installed by the Province of British Columbia be reworded. It would be beneficial to state specific times instead of using vague language like dusk to dawn as this can be interpreted differently by different people. As the goal is to have no vehicles on the road at dusk (as this is the time when the LIPI come out onto the road) it is suggested the road closure run from 6pm to 6am. It is also recommended that the seasonal closure period be extended until at least October 15, based on the fact that LIPI have been detected on the road as late as October 11, which is the latest date surveyed, so unknown if they use the road later than this.

Figure 20. New road closure signage produced by the BC government.

5.7 Animal Health

While the majority (97.2%) of the LIPI detections throughout the year were deemed to be in good health by visual inspection, it should be noted that due to a change in methodology implemented in 2015 fewer animals are now being captured in the spring to enable surveyors to focus on pinpointing the locations of as many LIPI as possible to inform egg mass searches. As a result in the spring of 2016, health was only assessed in a small fraction (n=16) of the amount as in the spring of 2014 (before the priority changed; n=72, Houston, 2015). Since very few animals within the breeding population were captured in the spring of 2016, caution should be exercised when attempting to draw any conclusions about health trends in the population, especially when looking at between year comparisons. Although no new health issues were detected, and there were no signs of frog chiggers or necrotic tissue detected (both issues detected previously in the spring breeding population at DLNA), this could simply be an artifact of the small sample size of LIPI whose health was assessed in the spring of 2016, as most of these issues tend to show up in the spring. Therefore it is not possible to draw any conclusions on whether or not these health issues are still present in the spring breeding population.

As body swabs were not submitted to the lab for Bd analysis due to the fact that it is no longer seen as a priority and there was no funding allocated for it for the 2016 samples collected it is not possible to do between year comparisons of the Chytridiomycosis results. The fact that only a very small portion of animals throughout the year showed overt symptoms of Chytridiomycosis (n=2 of 113 LIPI, 1.8%) does not indicate the true prevalence of the disease for 2 reasons: 1) a large portion of the animals whose health was visually inspected for symptoms of Chytridiomycosis were YOY during the summer and fall (78 of 113, 69.0%), which is not a cohort that would be expected to have the disease; and 2) the only way to determine prevalence is by testing for Bd DNA. In previous years when LIPI were sampled for Bd, animals often tested positive for Bd even though they were not exhibiting any overt symptoms (Voordouw et al, 2010). It should also be noted that some of the symptoms regularly attributed to Chytridiomycosis are not exclusive to that disease, for example, sloughing skin can be a result of changes in water chemistry.

All of the dead LIPI detected in 2016 were road mortalities, including 1 adult and 6 YOY.

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 managers.

5.8 Passive Integrated Transponder (PIT) Tagging

The total number of animals implanted with PIT tags was limited in 2016 by 2 factors:

1) Equipment was being shared with the FLNRO road survey crew who was given priority as the main objective of the project was to take advantage of the large numbers of LIPI moving across the road during the late-summer and fall migration to get as many animals PIT tagged as possible
2) A permit amendment had to be filed and the wait time was longer than anticipated.

5.9 Doral Spot Pattern Recognition

Since recapture analysis using dorsal spot pattern photos was not done for the 2016 field season given the amount of time required to complete it, and the fact that the PIT tagging project has not been fully implemented, 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 it is not possible to standardize the data, for example, 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 2016 field season have 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.

It is anticipated that once the PIT tagging project is fully implemented it will replace dorsal spot pattern photo analysis as it is a much more efficient way of identifying individuals and recaptures.

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).

5.10 Morphometrics of Creston LIPI

While YOY observed in the migration corridors and overwintering areas during the fall of 2016 had larger mean weight and mean SVL than those in 2015, the difference in weight (3.1 g) was not statistically significant, but the difference in SVL (4.7 mm) was statistically significant at the 0.05 level. This indicates that on average the 2016 animals were longer but did not weigh more (which would be expected if they were longer), which could be an indication that overall body condition was less than optimal. This is a concern as animals are heading into the over-wintering period as it may put them at a disadvantage as they may not have the body condition to get them through the winter. This difference in size can potentially be partially explained by the fact that the majority of the animals metamorphosed later in 2016 than they did in 2015, and therefore would have had fewer total days to forage and put on weight in 2016, but this does not explain the difference in SVL.

A comparison of 2016 data with a larger dataset, that includes YOY detected in the migration corridors and overwintering areas during the fall seasons of 2010-2015, shows that while the mean weight of 2016 YOY (14.5 g) was greater than 2015 (11.4 g), it was less than all other years in the dataset (range=18.3 – 21.0 g), except for 2011 (13.2 g). A comparison of mean SVL for the same dataset, shows similar results, 2016 YOY (51.2 mm) had greater SVL than 2015 (46.5 mm), but were the smallest out of all the other years (range=52.6 - 57.6 mm). It should be noted that while the dates of detection have been standardized in the above multi-year comparisons, time since metamorphosis was not. These results indicate that in general the YOY detected in the migration corridors and overwintering areas in the fall of 2015 and 2016 appear to be smaller for both size metrics than in most other recent years. Some potential reasons for this difference may include variability in weather, productivity differences, food availability and water level differences between years.

It is recommended that a multi-year statistical analysis be completed to determine if these apparent differences in size between years are statistically significant. Due to the fact that water levels during the tadpole development period were extremely low in both 2015 and 2016 it is quite possible that this has had an effect on YOY size. As noted in the 2015 NLF Field Season Report (Houston, 2016a) low water levels can have many impacts on developing tadpoles, including: increased water temperature and increased exposure to UV (which can cause deformity) as well as potential water quality issues, increased competition because of density and earlier metamorphosis at a smaller size. If animals are metamorphosing earlier in response to diminishing habitat, it is expected that they will be smaller at metamorphosis and will likely continue to be smaller into the fall as well, which puts them at a disadvantage going into the over-wintering period. To test this theory, it would be necessary to do a multi-year comparison of fall YOY sizes, with time since metamorphosis factored in to increase accuracy. To differentiate whether this apparent difference in size during the fall is more likely a function of size at metamorphosis or a result of something else (such as low food availability post-metamorphosis, cold weather limiting activity and foraging patterns in the fall, etc.), a multi-year comparison of size at metamorphosis should also be conducted.

5.11 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 during the fall. This appears to be a trend as results obtained in 2014 (Houston, 2015) and 2015 (Houston, 2016a) indicate the same thing. It is recommended that solutions to this problem be investigated by the BC-NLFRT. This is also something that the Calgary Zoo should be aware of if they decide to proceed with establishing a captive assurance colony and captive breeding program as well, that way they can strive to mitigate the sources of this problem from the onset.

During a conversation with Vancouver Aquarium staff it has recently come to light that LIPI housed at the Vancouver Aquarium as part of the captive assurance colony have been observed active during a period when they should be hibernating. This could be due to the fact that winter temperatures in Vancouver are much warmer than in Creston, and even though air and water temperatures from DLNA were provided to the VA at the onset and have been emulated as close as possible, the animals are still active. It has been well documented that a variety of amphibian species adapted to cold environments in northern latitudes require a period of cold temperatures for egg maturation and sperm production. Since a lack of exposure to cold temperatures can prevent maturation of eggs in females and sperm production in males it is possible that this is the root of the problem which has resulted in low numbers of viable eggs from the captive breeding program at the aquarium. It is recommended that this problem be corrected as soon as possible by ensuring the animals have the required period of cold temperatures while hibernating annually. If this does not result in an increase in viable offspring, other potential improvements should be looked at, including development of more natural habitat conditions, with more space (and decreased density of housed animals), as well as possible dietary changes.

5.12 Columbia Marsh Reintroduction Program

Due to the fact that a limited number of tadpoles can be moved per container and the considerable amount of time and effort required for the Creston crew to capture, collect, package and transport a large number of hatchling tadpoles out of the wetland (approximately 7600 in 2016), it is recommended that if the Vancouver Aquarium cannot produce enough tadpoles to fulfil the target of 8000 again in 2017, and as a result the BC-NLFRT requests additional LIPI from the CVWMA, they be moved as portions of egg masses and not tadpoles. Removal of any egg masses should only be considered if the threshold outlined in the translocation matrix is surpassed. No more than half of any given egg mass should be moved to ensure that some of the genetics from each egg mass remain in the CVWMA; the priority should always be to maintain a healthy viable population at the CVWMA first and foremost. Prior to giving any consideration to moving eggs, water quality and water temperature at the recipient site should be closely compared to the conditions at the CVWMA collection site to ensure the best possible chances of successful development. It is recommended that if the Brisco team has not already done so, they should install HOBO Pendant waterproof temperature data loggers (model UA-001 by Onset) to collect air and water temperatures every hour on a daily basis starting mid-April. This data can then be compared accurately to the data collected by the same temperature data loggers in place at the CVWMA to determine whether or not conditions are suitable to move eggs between sites.

If eggs are moved they should be taken from egg masses that have been recently laid in order to reduce the risk of damaging embryos further along in development and the process should be done with great care to ensure the portion of the egg mass remaining in situ at the CVWMA is not damaged or compromised in any way. As noted previously (Houston, 2016a) 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, as a result, the Brisco team will have to be prepared to come pick up the packaged eggs with very short notice, in most cases it will likely be with less than 24-hour's notice as freshly laid egg masses (target for reintroduction) are very rarely detected, they are most often detected at various levels of development within the 7-10 day development period or even after hatch out has begun. Depending on the total number of eggs requested, the Brisco team will also have to be prepared to make multiple trips to pick up the packaged eggs as not all egg masses will be laid on the same date, egg mass deposition dates can be spread out over more than a 1 month period. For planning purposes, the Creston FLNRO team will need to know well in advance (by mid-April at the latest) what number of animals the BC-NLFRT is requesting.

The portion of each egg masses removed were higher than what would ideally be removed under normal circumstances, however in this situation, since the BC-NLFRT did not find out until late May that the Vancouver Aquarium could only provide 500 of the target 8000 tadpoles they decided that it was necessary. Ideally no more than half of any one egg mass should be removed but since it was the end of the breeding season the number of tadpoles available was limited to those from only the 3 egg masses left, as a result in order to get 7600 a greater than ideal portion of each had to be taken.

5.13 Habitat

Water Levels

Water levels dropped to alarmingly low levels in the DLNA in the summer of 2016. This likely had an effect on tadpole development and resulted in some mortality as large areas were reduced to thick mud prior to tadpole metamorphosis in many areas of the main breeding areas at DLNA. In addition to low water levels, there were problems getting water into the DLNA once the control gates were opened, as well as keeping water in the area (even though water was available from the source; which was the problem in 2015). Water levels were also low in 2015, but not to the same degree. It is recommended that the problems associated with water retention and filling be resolved by the CVWMA as soon as possible and that water levels continue to be monitored closely.

Watershield

It is recommended that the level of regrowth of watershield in the test plot created in May 2016 be measured early in the 2017 growing season. If the 2016 test project which resulted in the removal of a 10-20 cm dense rhizomatous mat, associated plant and floating leaves, which effectively lowered the wetland bottom by 10-20 cm in the treated area was successful, consideration should be given to creating additional openings. If additional test plots are created they should be monitored to determine if LIPI select them for breeding over adjacent areas that have become densely infested with the plant.

It is also recommended that the BC-NLFRT review the potential solutions to the watershield ingrowth problem at DLNA identified by Tom Biebighauser during his site visit (Biebighauser, 2016) and discuss potential solutions.

New Wetlands Created

It is recommended that the wetlands newly created in November-December of 2016 at DLNA be monitored for LIPI use in 2017 by conducting NCS in the spring. If calling is detected, and time permits, EMS and VES for YOY should also be conducted with the goal of determining whether or not successful breeding occurred.

Hobo temperature data loggers and permanent water level monitoring staff gauges should be installed to monitor water temperatures and water levels throughout the year. In the first year, it is especially important to monitor the hydro-period of these newly created wetlands to determine how they perform, and whether or not they stay wetted for long enough to allow for amphibian metamorphosis.

In order to monitor vegetation growth and changes in the plant community within the newly created wetlands over time, photo plots should also be established. Additional sampling that would be beneficial, but is not a high priority, includes invertebrate sampling to determine the quality of the new wetlands for foraging, however it is not anticipated that this will be a limiting factor, given the rich wetlands and invertebrate life in the surrounding areas.

5.14 Additional Recommendations

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

- Follow up on Frog-bear farm plan and recommendations submitted to NCC
- The BCNLF-RT should work with land managers to come up with a solution for the cattle impacted over-wintering areas

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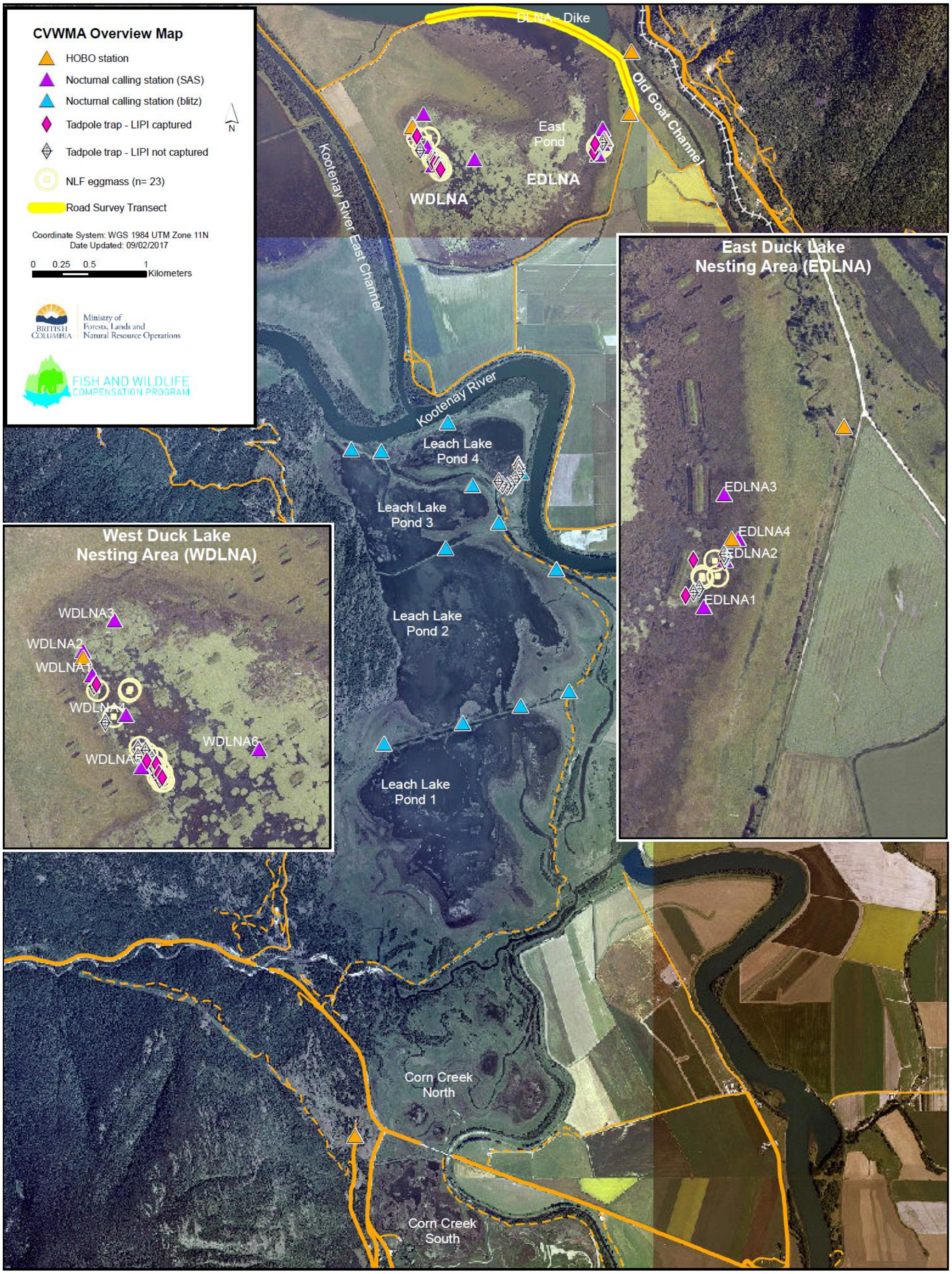
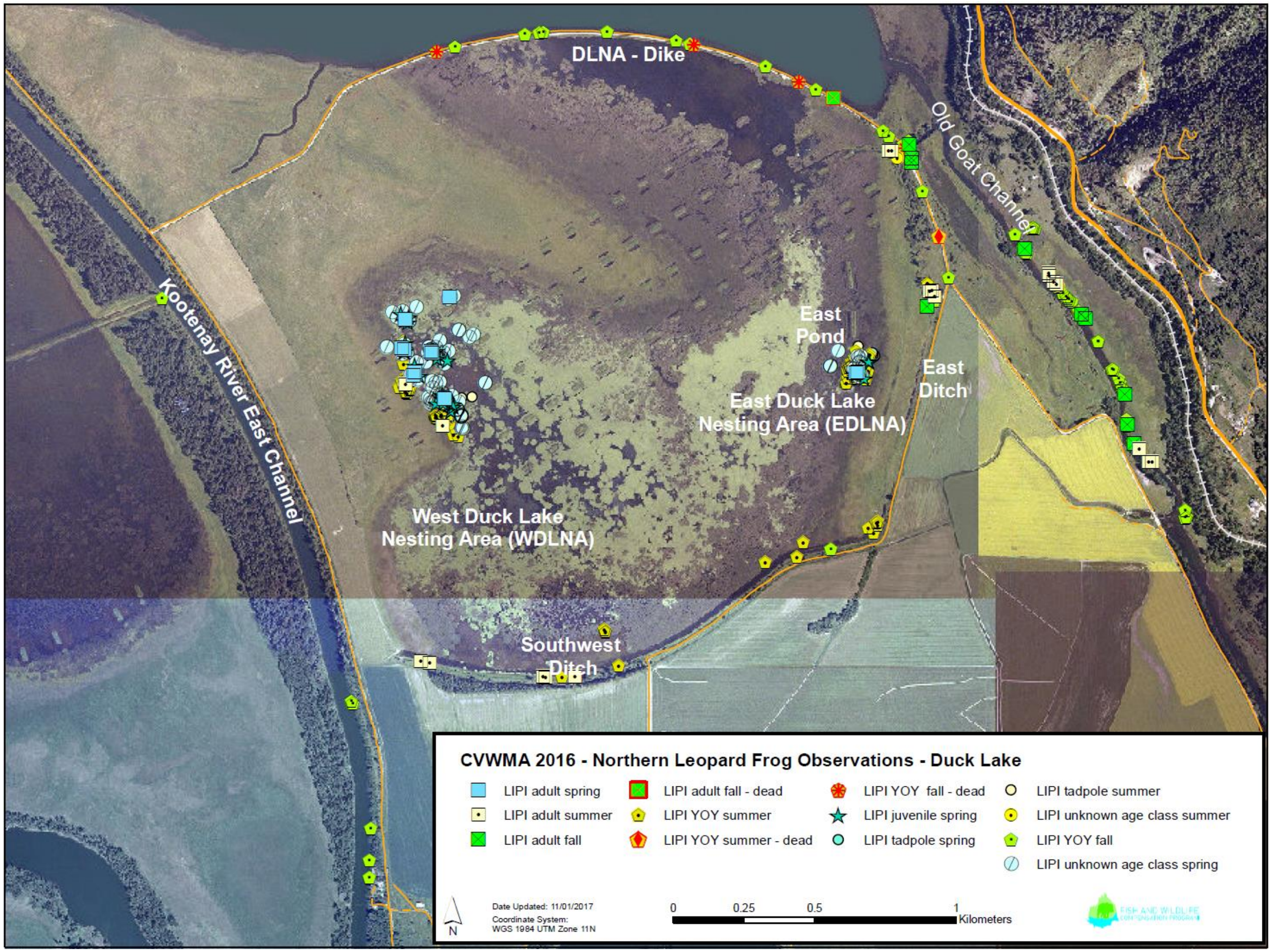
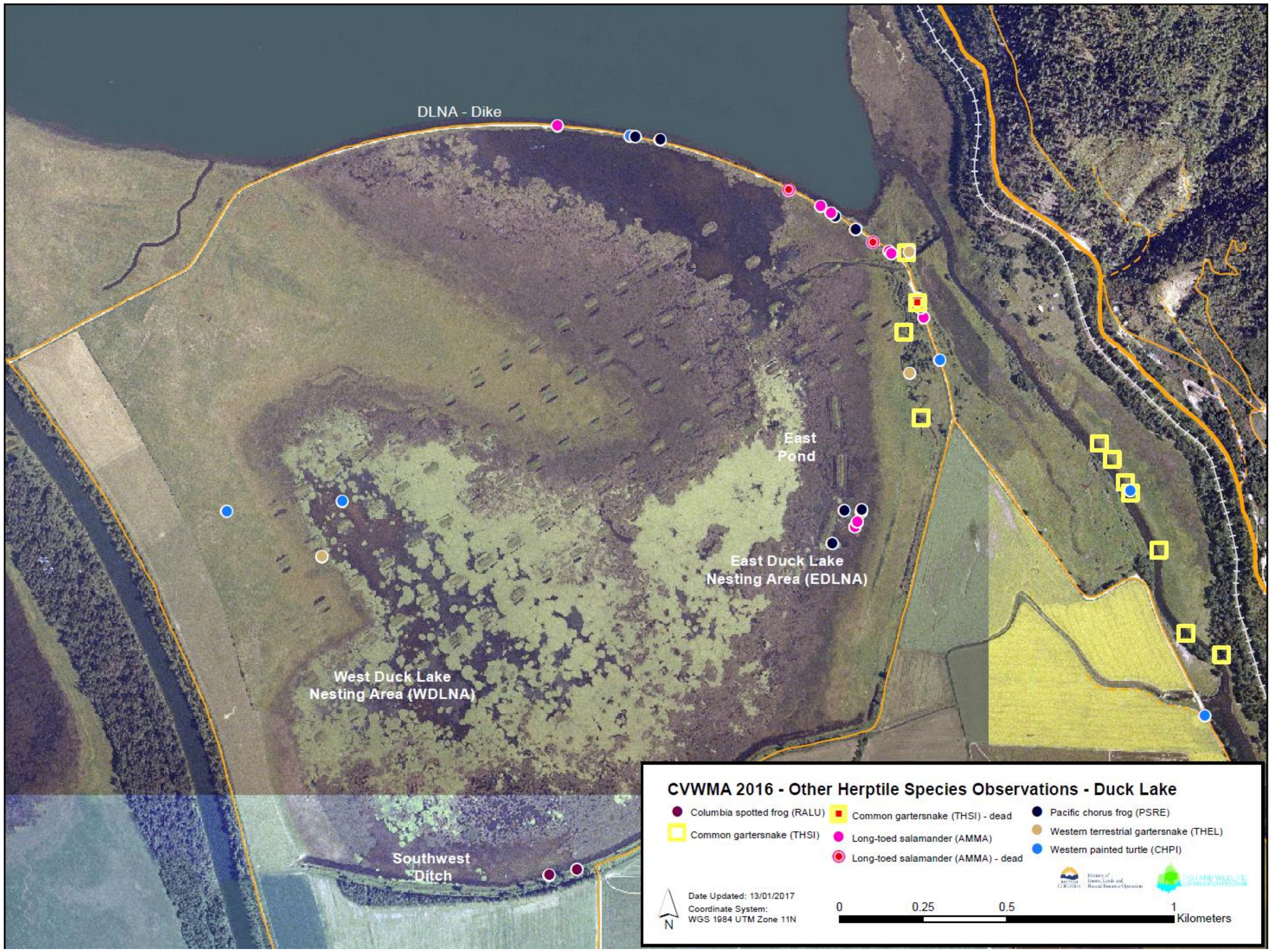
Appendix 1. CVWMA Overview Map



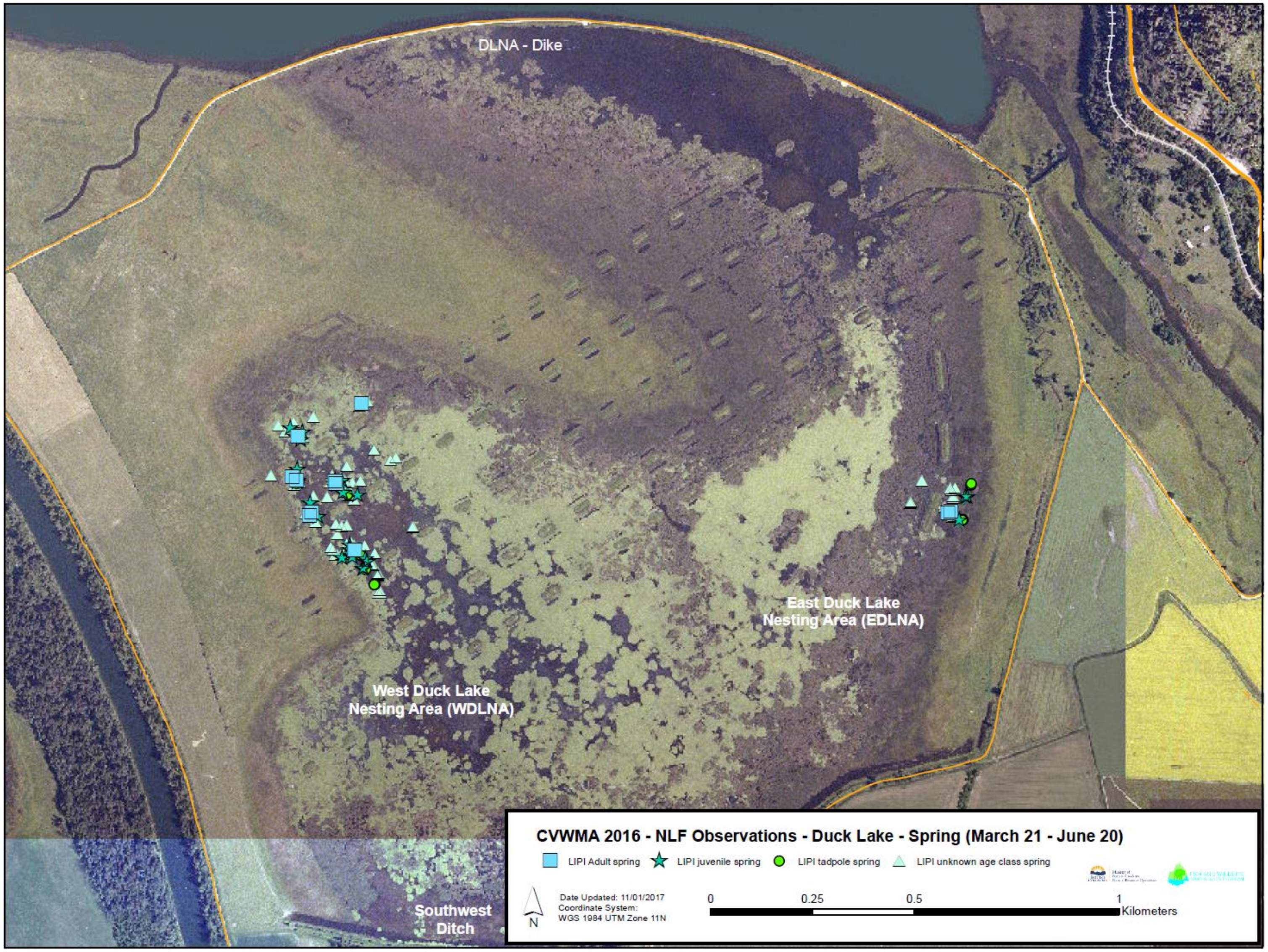
Appendix 2. Duck Lake LIPI Observations



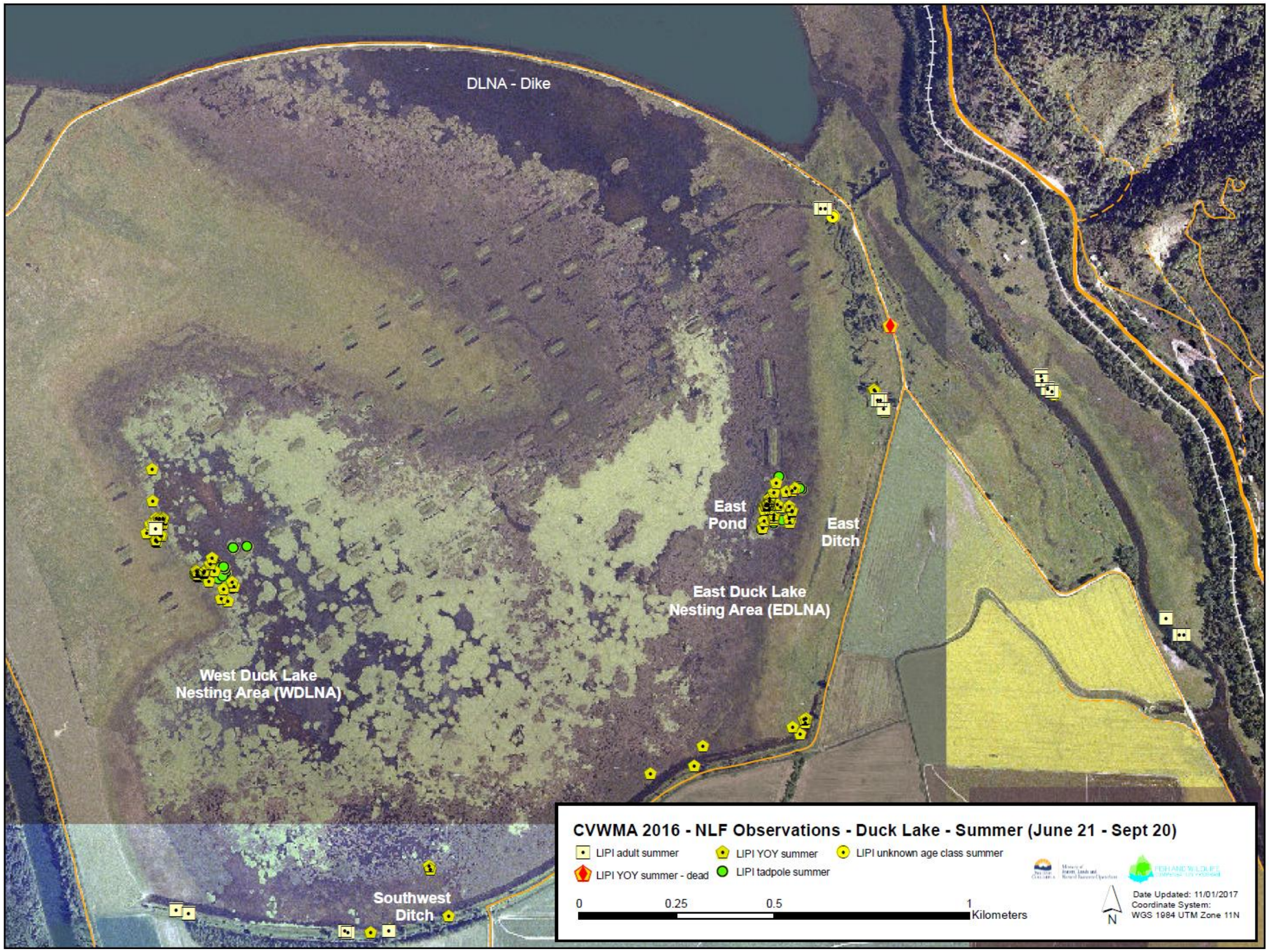
Appendix 3. Duck Lake Other Herptile Species Observations



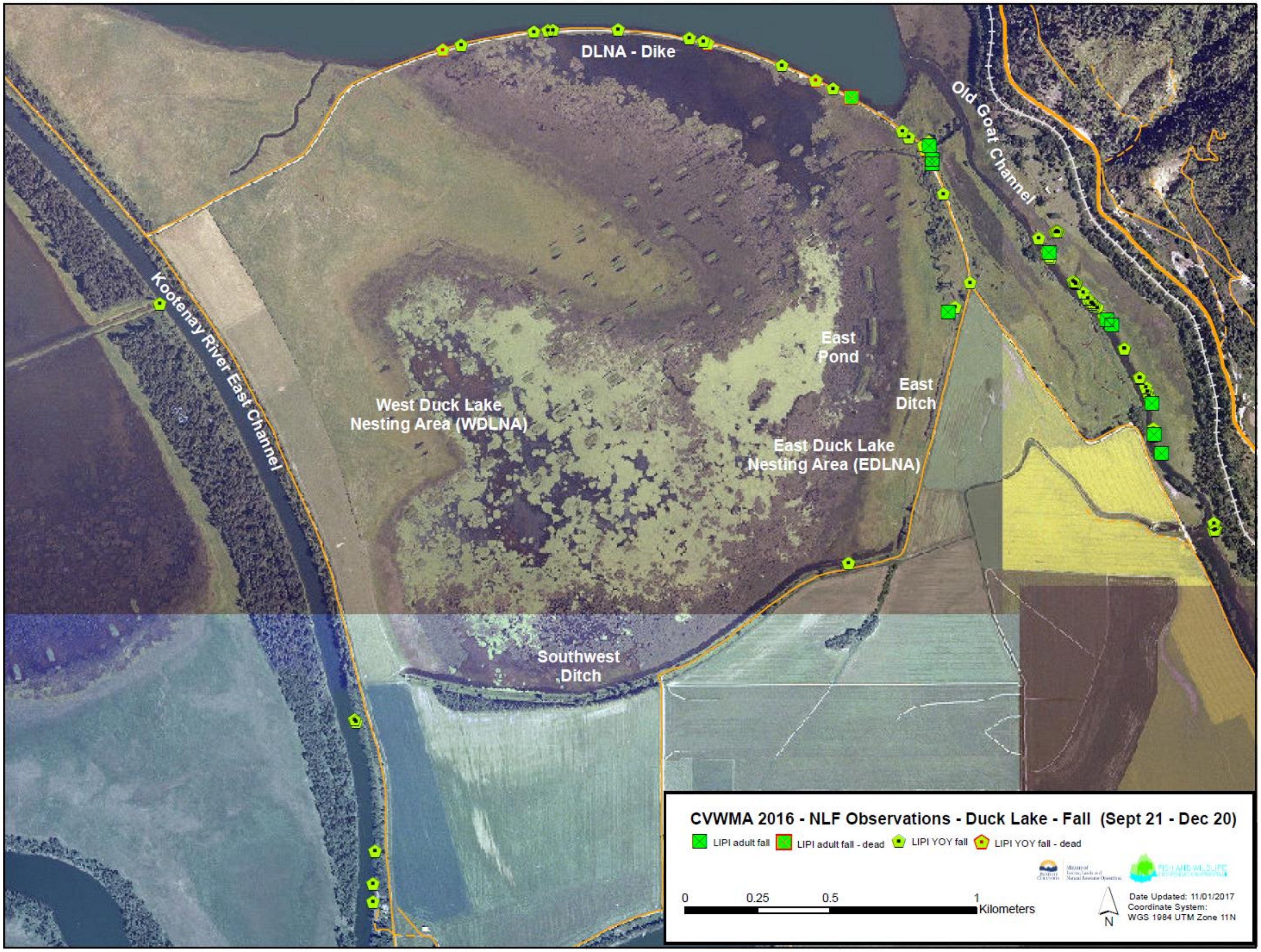
Appendix 4. Duck Lake LIPI Observations by Season- Spring



Appendix 5. Duck Lake LIPI Observations by Season- Summer



Appendix 6. Duck Lake LIPI Observations by Season- Fall



Appendix 7. Leach Lake Other Herptile Species Observations (no LIPI detected)