Population Monitoring and Recovery of the Northern Leopard Frog (*Rana pipiens*) in Southeast British Columbia, 2000 to 2005

Doug Adama, Adama Consulting
Marc-André Beaucher, Creston Valley Wildlife Management Area
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Executive Summary

In recent decades, the northern leopard frog (*Rana pipiens*) has declined across western North America. In British Columbia, surveys conducted in the 1990's failed to detect leopard frogs outside of a single wetland in the Creston Valley Wildlife Management Area (CVWMA), near Creston, BC. Designated as "Endangered" by the Committee on the Status of Endangered Wildlife in Canada and protected under the *Species at Risk Act*, this population is in imminent threat of extinction.

Between 2000 and 2005, the population of leopard frogs in the CVWMA was monitored using visual encounter surveys, nocturnal calling surveys, egg mass surveys, and Mark-Recapture techniques. Calling activity, egg mass counts, catch-per-survey hour, and Mark-Recapture population estimates indicate that the population of leopard frogs declined approximately 50% over this period. Although there is a high degree of uncertainly with the population estimates, we estimate the population in 2005 to be in the low to mid-hundreds. In part, we attribute the decline observed during this period to an outbreak of *Saprolegnia* that resulted in high egg mortality in 2001. Moreover, we observed chytrid-associated (*Batrachochytrium dendrobatidis*) mortality and observed numerous unhealthy animals with chytridiomycosis, which lead us to believe that chytridiomycosis is suppressing the population.

In 2001, recovery actions for the northern leopard frog were initiated and included a captive rearing and reintroduction program, and to a lesser extent habitat enhancement. Between 2001 and 2005, 30,065 leopard frog hatchlings from 27 egg masses were collected and reared in captivity under artificial conditions. The objectives of captive rearing program were to achieve a survival rate greater than 50% and rear leopard frogs to a mean minimum body size of 30 mm snout-to-vent length (SVL) at metamorphs. To this end, we conducted controlled experiments manipulating diet and stocking density to help refine our husbandry techniques and found that the amount of protein provided in the diet had a profound effect on size at metamorphosis. In the final two years of the project, we exceeded our goal of 30 mm SVL at metamorphosis achieving SLV's of 34.9 mm in 2004 and 32.1 mm and 2005. Average survival of leopard frogs in captivity for all years was 82%. In total, 10,147 leopard frog tadpoles and 14,487 leopard frog metamorphs were released into the wild.

The objectives of the reintroduction program were to establish leopard frogs at two sites in their former range: one in the CVWMA and the second in the Bummer Flats Wildlife Management Area (BFWMA). In the spring of 2005, three egg masses were detected in the Leach Lake management unit in the CVWMA and numerous young-of-year were observed during fall visual encounter surveys. In 2004, restoration was conducted at this site to reduce the emergent vegetation (e.g. *Typha* and *Scirpus*) and create open water habitat for waterfowl. Restoration entailed drawing down the water, mowing the emergent vegetation, tilling the soil, and then re-flooding the wetland in late fall. The establishment of leopard frogs at this site indicates that habitat restoration may assist in recovery.

The results of this project suggest that reintroduction and habitat restoration are viable methods for the recovery of leopard frogs in BC, however the population of leopard frogs in BC remains extremely vulnerable and recovery is confounded by *B. dendrobatidis*. We recommend that future recovery actions should focus on conserving the leopard frog population in the CVWMA and include habitat restoration and monitoring. If this population continues to decline over the next three years, we recommend establishing a captive breeding program. We also recommend that research should be conducted immediately to determine the prevalence and epidemiology of *B. dendrobatidis*. Finally, if a reintroduction program is resumed, we highly recommend that a reintroduction strategy be developed to address population viability, genetics, disease, monitoring, public education, and the facility operations, and that long-term funding be secured at the outset. In conclusion, we suggest that in the absence of recovery, the leopard frog will become extinct in British Columbia.

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1. Introduction

The northern leopard frog (*Rana pipiens*) is a medium bodied frog that varies from 30 mm in size at metamorphosis to approximately 100 mm as an adult. It is characterized by numerous dark spots surrounded by a light coloured halo, which give the leopard frog its name. Body colour may be green (dominant) or brown (recessive) and is determined by a simple two-allele, one locus system of inheritance (Fogleman et al. 1980).

During the breeding season, the male leopard frog emits a series of uniquely evolved advertising calls consisting of snores, chuckles and grunts (Larson 2004). These calls are characteristic of a group of closely related species in the Pantherana complex within the family Ranidae to which leopard frogs belong (Hillis and Wilcox 2005). Taxonomic relationships within this complex are poorly understood (Hillis 1988), however a recent phylogenetic assessment of the evolutionary history of northern leopard frogs concluded that there is sufficient genetic variation between eastern and western haplotypes to warrant a taxonomic review of the species (Hoffman and Blouin 2004).

The range of the northern leopard frog extends from Nova Scotia to west of the Rocky Mountains and from Great Slave Lake to Arizona. Over the past three decades, the northern leopard frog has experienced a dramatic decline, particularly within the western and central portions of its range. Declines have been observed in Colorado, Arizona, Washington Montana, Utah, Alberta and BC (Roberts 1981, Corn and Fogleman 1984, Clarkson and Rorabaugh 1989, Stebbins and Cohen 1995, McAllister and Leonard 1996, Seburn and Seburn 1998, Kendell 2003b, Werner 2003, Rorabaugh 2005). Figure 1 shows the range retraction of northern leopard frogs in western North America.

In British Columbia, declines were first noted in the 1980's (Orchard 1992) and by the mid-1990's it was thought that the leopard frog was extirpated from BC altogether (Ohanjanian and Teske 1996). In 1996, several male leopard frogs were caught in the Creston Valley Wildlife Management Area (CVWMA), near Creston, BC (Ohanjanian 1997) and between 1997 and 1999, a study was conducted to determine the status of this population (Waye and Cooper 2000). Despite several extensive surveys conducted over the past decade (Orchard and Ohanjanian 1995, Ohanjanian and Teske 1996, Gillies and Franken 1999, Ohanjanian et al. 2006), no other populations have been found. As a result, the population of leopard frogs in the British Columbia was designated as Endangered by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC 2002).

In 2001, a recovery team was established and recommended that immediate action be taken to establish two additional leopard frogs populations in the their historic range, one in the CVWMA and a second in the Bummers Flats Wildlife Management Area (BFWMA, Northern Leopard Frog Recovery Team 2006). This paper presents the results of these recovery actions and provides an update on the status of the leopard frogs in British Columbia.

2. Methods

2.1. Study Area

The study area of this project included both the CVWMA and the BFWMA, located in southeast British Columbia (Figure 2). The CVWMA is located at the south end of Kootenay Lake in the Creston Valley, nestled in between the Purcell and Selkirk mountain range at an elevation of 531 meters. The CVWMA occupies 6,885 hectares of the Kootenay River Floodplain and is divided into several large wetland compartments. These compartments are managed for flood control, hydroelectric power generation, and wildlife (Wilson et al. 2004). Water is maintained in these wetlands through a series of stop gates or by active pumping. The CVWMA is characterized by hot dry summers and mild winters and occurs in the very-dry-warm Interior Cedar Hemlock (ICH xw) biogeoclimatic subzone (Braumandl and Curran 1992). Daily average temperature in July is 18.9 ° C and annual precipitation is 572.2 mm (Environment Canada 2006).

The BFWMA is located in the Rocky Mountain Trench approximately 87 km northeast of the CVWMA. It lies along the Kootenay River Floodplain, between the Purcell and the Rocky Mountains at an elevation of 771 meters. The BFWMA is approximately 850 hectares in size and divided into several large wetland compartments. Like the CVWMA, water levels are controlled with stop-gates or through active pumping. The climate of the BVWMA is somewhat cooler and dryer than the CVWMA and is characterized as the dry-mild Interior Douglas Fir zone (IDF dm2) (Braumandl and Curran 1992). Weather data obtained from the nearest weather, reports a daily average temperature in July at 17.9 ° C and an average annual precipitation of 439.1 mm (Environment Canada 2006).

Both the CVWMA and the BFWMA are Wildlife Management Areas (WMA), under the jurisdiction of the BC government. These areas are managed by the Minister of Environment and provided a modest level of habitat protection. Permits to conduct recovery activities and monitor the population were obtained from the Ministry of Environment. At the request of the Ministry, we have refrained from providing the name of the compartment where leopard frogs occur. Throughout this document we refer this compartment as the "source site" and refer to the population in this compartment as the "source population". As leopard frogs breed at three locations, we refer to these sites as the "east-breeding pond", "west-breeding pond", and "east ditch". Maps of the CVWMA and of the BFWMA are provided in Appendix A (*Confidential*).

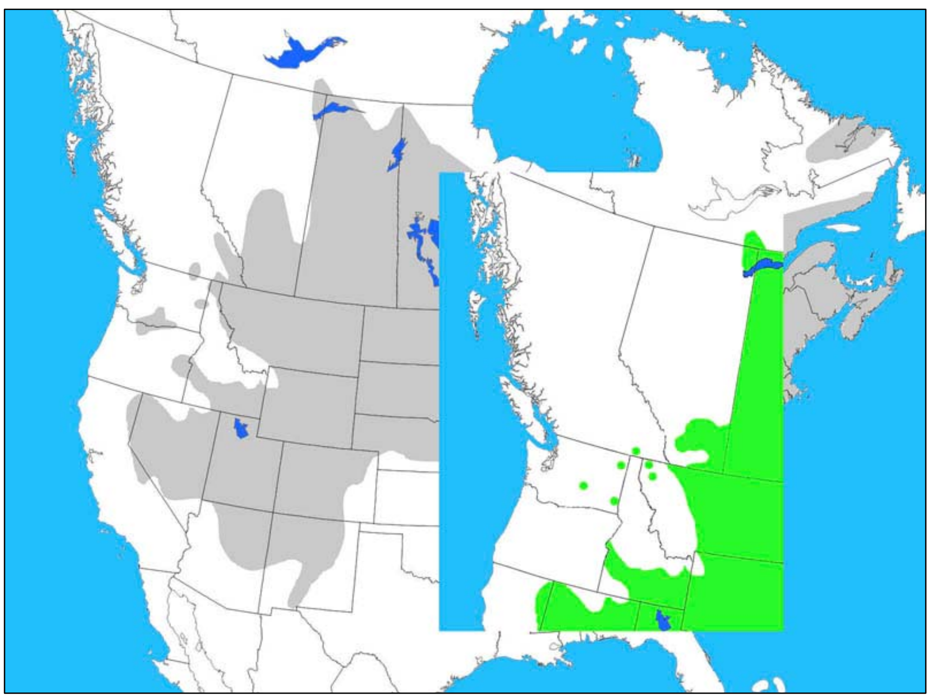

Figure 1. Range retraction of Rana pipiens in western North America (from Kendell 2003).

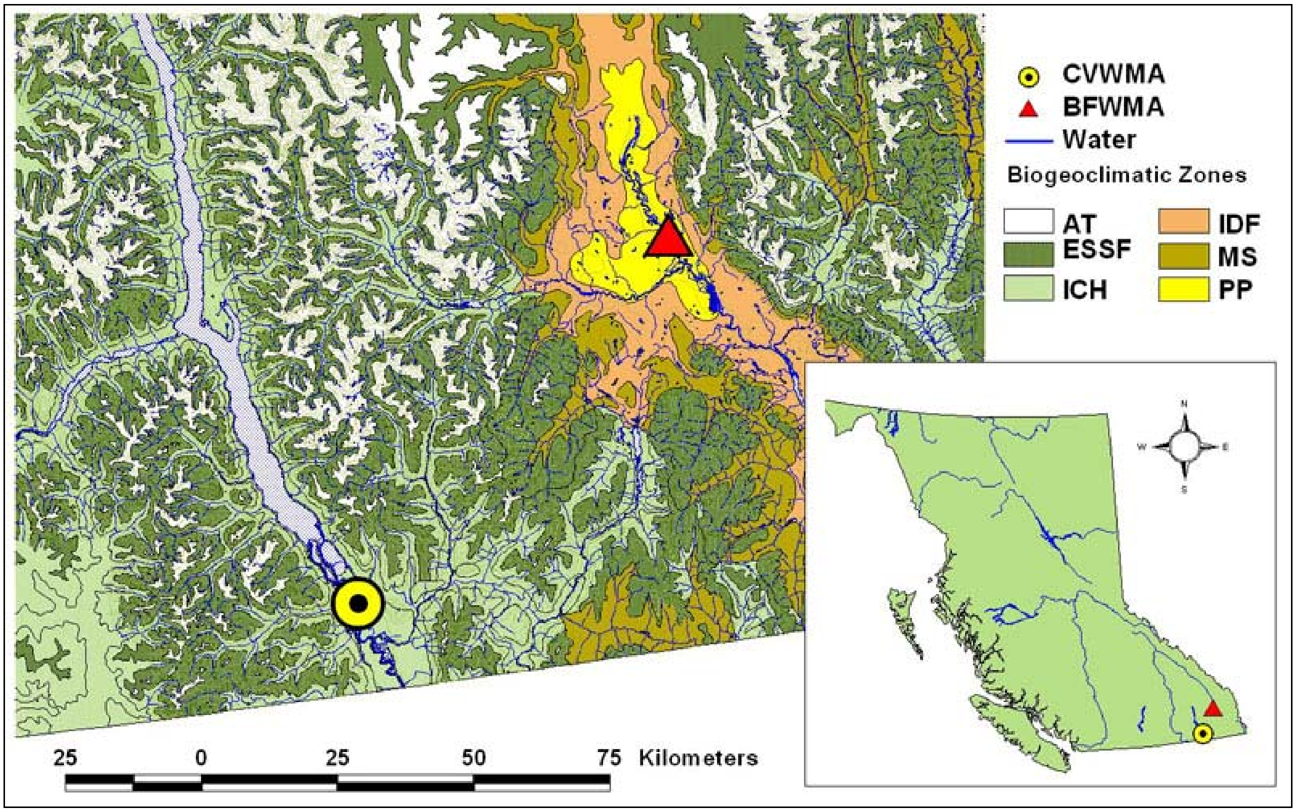

Figure 2. The location of the Creston Valley Wildlife Management Area (CVWMA) and the Bummers Flats Wildlife Management Area (BFWMA).

2.2. Surveys

Nocturnal calling surveys, visual egg mass surveys, and daytime visual surveys were the primary survey methods employed during this project, and were supplemented with road surveys and dipnet surveys. Standardized procedures were followed or adapted for animal handling (ASIH 1997, Resources Inventory Committee 1998b), tissue collection (Berger and Speare 1998, Resources Inventory Committee 1999), and survey methods (Heyer et al. 1994, Resources Inventory Committee 1998a). Survey effort for the various survey types are summarized in Appendix B.

Data collected during each survey included: date, start time, end time, number of persons, surveyor name(s), location, GPS coordinates and datum, elevation, weather conditions, air temperature, water temperature, precipitation, wind, and cloud cover. Occasionally water depth, pH, and conductivity were recorded. Separate Microsoft Excel spreadsheets were maintained for (i) survey data, (ii) animal captures and observation data, (iii) tracking tissue samples, (iv) radio telemetry data, and (iv) habitat data.

2.2.1. Nocturnal Calling Surveys (NCS)

Nocturnal calling surveys (NCS) were conducted from 2000 to 2005 to monitor breeding activity. Initially we followed the technique employed by Waye and Cooper (2000) that entailed capturing as many calling males as possible at a calling site during each survey session. In 2001, we abandoned this procedure in favour of conventional calling surveys methods (Heyer et al. 1994, Resources Inventory Committee 1998a).

Three permanent calling stations, located approximately 100 meters apart, were established in each breeding pond. At each station, we estimated the number of calling male leopard frogs during three 3-minute intervals. Each three-minute interval was separated by a one-minute break to allow for the recording of data. Surveys began shortly after dusk and were conducted from early April and to mid-June. Two NCS surveys were conducted per week at the breeding sites and one NCS survey was conducted per week at the release sites. Surveys were canceled or rescheduled when air temperatures dropped below 5° Celsius or when winds exceeded 15 km/h.

2.2.2. Visual Egg Mass Surveys (VEMS)

Visual egg mass surveys (VEMS) were conducted from April to mid Jun in order to locate egg masses for the reintroduction program and to monitor breeding activity. Typically, VEMS were preceded by a NCS conducted the previous evening to increase the likelihood of detecting egg masses. During a NCS, centers of calling activity were noted, enabling us to anticipate where egg masses might be laid. The next day, a VEMS was conducted in a grid pattern with attention given to the centers of calling activity. Polarized sunglasses were worn while conducted VEMS to reduce the glare from the surface of the water. As the size of the breeding ponds varied, survey times varied accordingly. To assess whether the number of egg masses observed was simply a function of survey time, a Coefficient of Correlation was calculated (Zar 1984, SAS 2001).

When egg masses were encountered, animal capture data was recorded (Section 2.2.3). Where possible the length, height, and width of an egg mass was measured to estimate egg mass volume. To facilitate the collection of tadpoles for captive rearing, the egg masses were encaged in mesh baskets until the eggs hatched out. Within three days of hatching out, the hatchlings[1] were counted and the entire cohort or a portion thereof was brought into captivity (Section 2.3.1). The number of dead eggs and embryos were also counted to determine hatching success.

2.2.3. Visual Encounter Surveys (VES)

Visual encounter surveys (VES) are daytime surveys used to detect conspicuous post-metamorphic amphibians (Heyer et al. 1994, Resources Inventory Committee 1998a). These surveys entailed searching for animals in suitable habitats and counting the number of animals seen or captured during the survey. VES were employed from April to October, however sampling effort varied greatly from year to year in response to varying levels of funding (Appendix B).

During VES, an effort was made to capture every amphibian encountered. Captured frogs were placed in Ziploc™ bags for ease of handling. Data collection included: date, time, surveyor name(s), survey type, detection code (caught, seen, or heard), location, GPS coordinates and datum, elevation, species, development, sex, animal health, markings, activity, habitat type, and environmental data. Every animal captured was weighed to the nearest gram with a Pezola™ spring scale and a snout-to-vent lengths (SVL) measurement was obtained using a caliper to the nearest 0.1 mm. Since dorsal spot patterns of northern leopard frogs are unique (Donnelly et al. 1994), a photograph was taken of each frog to allow us identify individual animals over time (Merrell 1972, Nace et al. 1973). This provided insight into animal movement, growth, and habitat use; and facilitated Mark-Recapture population estimates. Tissue samples were taken to monitor for *Batrachochytrium dendrobatidis* (Section 2.2.5).

[1] Hatchling refers to amphibian tadpoles that are between Gosner development stages 20 and 25. At Gosner stage 25 tadpoles are free swimming and have fully formed mouths (Gosner 1960).

2.2.4. Abundance Estimates

Several indices of relative abundance were used to monitor population trends over time. These included the number of calling males detected per survey, the number of egg masses detected in each breeding pond per breeding season, and catch per survey effort (survey hour).

Population estimates were calculated for the source population in 1999, 2000, 2003, and 2005 using the Petersen Mark-Recapture model (Krebs 1989). The “Marked” sample included the total number of frogs from all age classes caught and photographed in one year and the “Capture” sample included the number of adult and juvenile frogs captured and photographed in the next year. Young-of-year (YOY) animals were excluded from the “Capture” sample of year two, as they were not part of the initial “Marked” sample. Animals that were caught in both years, as identified by their spot patterns were “Recaptures”. We also included Mark-Recapture data collected by Waye and Cooper (2000) but we recalculated the population estimate for 1999 using weight categories rather than SVL, as we found weight to be a better predictor of age class (unpublished data). To distinguish YOY from juveniles and adults in late summer and fall, we considered animals weighing below 35 grams to be YOY and animals weighing 35 grams or more to be either a juvenile or adult. Animals captured in the spring, weighing less than 35 grams, were classified as juveniles. Unhealthy frogs were excluded from the population estimates, as these animals were often lethargic and easy to catch, and would have likely died between capture-recapture session (years).

Population estimates were also calculated for YOY at Leach Lake and Bummers Flats in 2005. This was facilitated by the fact all the animals released from captivity were marked with a Visual Elastomere Implant (VIE, Section 2.4). This allowed us to consider these animals as our “Marked” sample and animals caught during subsequent VES surveys comprised the “Capture” sample. Frogs caught with a VIE were “Recaptures”.

Confidence intervals for all population estimates were calculated using the binomial distribution. This procedure was employed because sample sizes were small and the Recapture/Capture (R/C) ratio exceeded 0.1 in all cases (Krebs 1989). It is important to note that because we violated several of the assumptions of the Petersen model, the estimates should only be considered an index of relative abundance rather than a measure of absolute abundance.

2.2.5. Frog Health and Mortality

All frogs encountered were assessed for animal health and assigned to one of four health categories: good, fair, poor, or dead. Frogs that showed no signs of injury or illness were considered in “good” health; frogs that had a minor injury or exhibited a potential sign of illness such as mild skin discolouration but appeared otherwise healthy and active were considered to be in “fair” health; frogs that had an obvious debilitating injury or exhibited signs of lethargy, excessive skin sloughing, or moderate discolouration were considered to be in “poor” health; and frogs that were not alive were, of course, categorized as “dead”. For an index of animal health, we divided the number of dead and unhealthy frogs (poor) by the total number of individuals observed (Appendix D).

Tissue samples were collected from most unhealthy animals and many healthy animals. Tissue samples consisted of primarily toe clippings, skin swabs, and sloughing skin, although occasionally whole animals were collected. Toe clippings were obtained by removing the first phalange of the 4th digit from one of the hind feet using heavy-duty nail clippers. Swabs were obtained by rubbing the tip of a sterile cotton swab (#018-460 AMG Medical Inc.) on the underbelly ten times. Sloughing skin was obtained by gently rubbing the belly of the frog while the animal was in a Ziploc™ bag. After releasing the animal, the bag was then rinsed with 2 to 5 ml of 95% ethanol, which was then poured into a 10 ml test tube. Whole animals, toe clips and skin swabs were also preserved in 95% ethanol.

Tissue samples and whole animals were sent to the Animal Health Centre (Ministry of Agriculture and Foods, Abbotsford BC) or the Wildlife Health Centre (Prairie Diagnostic Services, Saskatoon). Diagnostic procedures included gross morphology, histology, bacteriology, and PCR for *B. dendrobatidis (*chytridiomycosis*)* and Iridovirus. Since *B. dendrobatidis* was previously detected in the leopard frog population in the CVWMA, much of the emphasis on tissue collection has been for diagnosing this pathogen.

2.3. Captive Rearing and Reintroduction

The second objective of this project was to reintroduce leopard frogs at two sites were they occurred historically: one in the CVWMA and one in the BFWMA. To achieve this, a captive rearing program was developed. Our measures of success for this program were to (1) increase the survival rate of leopard frog tadpoles to metamorphosis to greater than 50%, and (2) to rear leopard frogs to a size greater than 30 mm SVL at the time of metamorphosis. Since body size at metamorphosis is important to post-metamorphic fitness and survival (Semlitsch et al. 1988, John-Alder and Morin 1990, Goater 1994, Beck and Congdon 2000, Altwegg and Reyer 2003), we felt that a high rate of survival to metamorphosis was not by itself a sufficient target. Our measures of success for reintroduction were: (1) successful over-wintering of released animals, and (2) breeding at the release sites.

2.3.1. Captive Rearing

Northern leopard frog egg masses were located using a combination of NCS and VEMS. In 2001, 667 tadpoles were collected from five egg masses and transferred to seven 1,000-litre rearing tanks filled with approximately 700 litres of water. A diet consisting of spinach, lettuce, zucchini and frozen bloodworm was provided every second day and 100% water changes were conducted once a week. At the end of the summer, 496 leopard frog metamorphs were released into Corn Creek Marsh (Appendix A). Given the high survivorship achieved (74%; Appendix A), the rearing program was expanded in subsequent years.

From 2002 to 2005, a combination of NCS and VEMS was employed to locate egg masses. Once the egg masses were detected, they were enclosed in mesh cages to facilitate the collection of hatchlings. Encaging the egg masses also enabled us to count the number of eggs in each egg mass to determine hatching success. Once in the mesh cages, the egg masses were monitored every two or three days until they had hatched out completely, at which time the hatchlings were brought into captivity.

Leopard frog hatchlings were initially kept in 109-litre Rubbermaid™containers at densities up to 5 hatchlings per litre until they reached Gosner stage 25 (Gosner 1960), which took between seven to twelve days. At Gosner stage 25, the tadpoles were transferred to 2.5 m circular rearing tanks (Figure 3; 4,000-litre capacity, 258 cm X 129 cm X 76 cm) filled with 1,000 to 1,500 litres of water. The rearing tanks were initially stocked at a density of 1000 tadpoles per tank (0.67 to 1.0 tadpole per litre) and were reduced to a final stocking density of 200 to 300 tadpoles per tank (0.1 to 0.3 tadpoles per litre) over a 30-day period by halving the densities in each tank every two weeks. These steps were taken because we had found that tadpoles below Gosner stage 30 grow faster at higher stocking densities (Adama et al. 2003a). At 30 days in capivity, 30 to 50% of the tadpoles were released into the wild and the remainder were reared to metamorphosis (Appendix F).

Figure 3. The arrangement of rearing tanks located outside in the CVWMA.

In order to attain a mean body size of 30 mm for newly metamorphosed frogs, experiments were conducted to determine the effects that diet and stocking densities had on tadpole growth and development (Adama et al. 2003a, Adama et al. 2004). With these experiments, we found that diet had significant effect on both size and time to metamorphosis. Furthermore, we found that the most important factor was the amount of protein in the diet (Adama et al. 2003b, Adama et al. 2004). The diet we used in 2004 and 2005 consisted of frozen bloodworm, kale, watercress, and live marsh vegetation. During the height of the growth period (weeks 7 to11), we provided up to 1 gram of bloodworm per tadpole per day as a protein supplement.

Water quality in the tanks was maintained by conducting frequent water changes. Early in the season (May to mid-June), water changes were conducted every seven to twelve days. As temperature and feeding rates increased, the water was changed more frequently. At the height of the rearing season (July), 50 to 75% of the water was changed every two days and the tanks were completely drained and cleaned every four days. Dissolved oxygen, pH, and ammonia were monitored daily and alkalinity, hardness, and nitrites were monitored every two weeks. The chemistry of water used in the rearing tanks and from the east-breeding pond are provided in Table 1 for comparison.

Rearing wild animals in captivity increases the risk of disease to both the animals in captivity and to local populations (Viggers et al. 1993, Cunningham 1996, Snyder et al. 1996). To reduce the risk of transmitting disease between rearing tanks, and between the captive population and the wild population, stringent disinfection and decontamination protocols were developed (Beaucher 2001, draft, Wind 2002). An example of two measures that were taken to reduce the risk of transmitting disease including disinfecting all rearing equipment in a bleach solution for a minimum of 15 minutes between use, and the use of separate sets of field gear for the CVWMA and the BFWMA.

To monitor the health of the captive population and to ensure that we were not releasing diseased animals into the wild, a two-pronged approach for monitor disease in the captive population was employed (Wind 2002, Adama et al. 2003a). The first approach entailed submitting any suspiciously dead and unhealthy animals for diagnosis, as an early warning mechanism. The second approach entailed selecting ten 45-day-old tadpoles at random from each rearing tank and submitting them to the Animal Health Centre for a suite of diagnostic procedures. Diagnostic procedures included histology, bacteriology, toxicology, PCR for *B. dendrobatidis*, PCR for Iridovirus, and viral culturing for Iridovirus.

Table 1. Water chemistry of the east breeding pond and water used for rearing.

Parameter	Units	MDL*	East Pond	Rearing Tank
Biochemical Oxygen Demand (BOD)	mg/L	5	5	5
Chloride (Cl)	mg/L	0.1	1.0	0.3
Fluoride (F)	mg/L	0.01	0.06	0.13
Sulphate (SO4)	mg/L	0.5	< 0.5	7.2
Bromide (Br)	mg/L	0.05	< 0.05	< 0.05
Nitrogen, Nitrate as N	mg/L	0.002	< 0.002	0.089
Nitrogen, Nitrite as N	mg/L	0.005	< 0.005	< 0.005
Phosphorus as P	mg/L	0.05	< 0.05	< 0.05
pH	pH Units	0.01	7.95	7.75
Conductivity	uS/cm	2	316	145
Turbidity	NTU	0.05	0.48	0.24
Nitrogen, Ammonia as N	mg/L	0.005	0.026	0.017
Nitrogen, Total as N	mg/L	0.02	0.71	0.19
Phosphorus, Ortho as P	mg/L	0.001	< 0.001	0.006
Phosphorus, Total Dissolved as P	mg/L	0.002	0.007	0.008
Phosphorus, Total as P	mg/L	0.002	0.012	0.01

* MDL: minimum detectable limit

Upon completing metamorphosis, the leopard frog metamorphs were removed from the tanks and immediately marked and measured. Snout-to-vent (SVL) lengths were measured using a vernier caliper (±0.1mm) and weights were obtained with an electronic balance (Ohaus; ±0.1g). Each metamorph was marked with a Visible Implant Elastomere (VIE, Northwest Marine Technology, Inc.) injected into the toe webbing of the hind feet, either between the fourth and fifth digit or between the third and fourth digit. Every year, different colours of VIE (Appendix F) were used to allow us to identify which year an animal was released. After an animal was marked and measured, it was then transferred to a holding tank and held for a minimum of 24 hours to ensure that the VIE was retained. During this holding period, the metamorphs were provided two-week old crickets for food.

The number of leopard frogs released at each site was decided *a priori*. Typically between one third and one half of the animals were released into the source population and remainder was split between the two-reintroduction sites: Corn Creek Marsh and BFWMA. A breakdown of the number animals reared in captivity and released is provided in Appendix F. Post-release monitoring was conducted at the release sites using VES and NCS to monitor animal growth, determine over-wintering success, and to determine if leopard frogs were breeding.

2.4. Habitat Enhancement

Although habitat enhancement was not a primary recovery approach, two relevant projects are worth noting. In 2001 and 2002, leopard frogs bred in a ditch near the east-breeding site (Appendix A). As this ditch often dies up in mid-summer before tadpoles are able to complete metamorphosis, a channel was created and a stop-gate installed to maintain water level in the ditch.

The second relevant project includes a vegetation management technique used in the CVWMA, referred to as a “drawdown” (Wilson et al. 2004). To maintain wetland productivity and habitat for wildlife, (e.g. waterfowl), emergent vegetation (e.g. *Typha* and *Scirpus spp.*) is controlled by drawing down or pumping out the water in the summer, mowing the emergent vegetation, tilling the soil, and then reflooding the wetland in late fall. Figure 4 provides a before and after look at a “drawdown” conducted in Pond 4 of Leach Lake. In 2004, 45 hectares of this 100-hectare compartment was mowed and tilled to restore the wetland to an earlier succession state in 2004.

Although this approach is not carried out specifically for amphibians, Steven (2002) reports that managing wetland vegetation in an open state is beneficial for many amphibians including northern leopard frogs. Over the past five years, several “drawdowns” have been conducted in the Leach Lake and Corn Creek management units (Stushnoff and Beaucher 2004, Beaucher 2006). As we shall report below, these projects may be highly beneficial to the recovery of leopard frogs in BC.

Figure 4. Before (above) and after (below) of the drawdown conducted in pond 4 of Leach Lake in 2004. Photos provided by the CVWMA.

3. Results

3.1. Breeding Phenology

In the CVWMA, northern leopard frogs began calling in early-April (Figure 5). Initially, calling activity was low but by the third week of April, male calling activity increased significantly. In most years, calling activity peaked at least twice during the breeding season, usually around late-April or early-May and between mid and late-May (Figure 5). Peaks in calling activity were associated with warm temperatures (above 10°C) while the dips in calling activity were associated with colder temperatures (below 5°C).

A stacked frequency histogram of leopard frog egg masses detected during one-week intervals is provided in Figure 6. As with calling activity, egg laying corresponded to temperature fluctuations and in fact, many egg masses were found immediately after temperatures increased following a cold spell. The earliest date that an egg mass was located was April 25, however egg laying may occur as early as the second or third week of April. In 2005, an egg mass was located on April 28, and hatched out 3 days later. Since it takes between 9 and 12 days for eggs to begin hatching (Eddy 1976), this egg mass was likely laid around April 18. An aerial photo showing the location of breeding sites and egg masses laid is provided in Appendix A. The size of the breeding sites were calculated from egg mass locations with home range software (Rodgers and Carr 1998) using 50 and 95% kernel density estimation functions (Table 2).

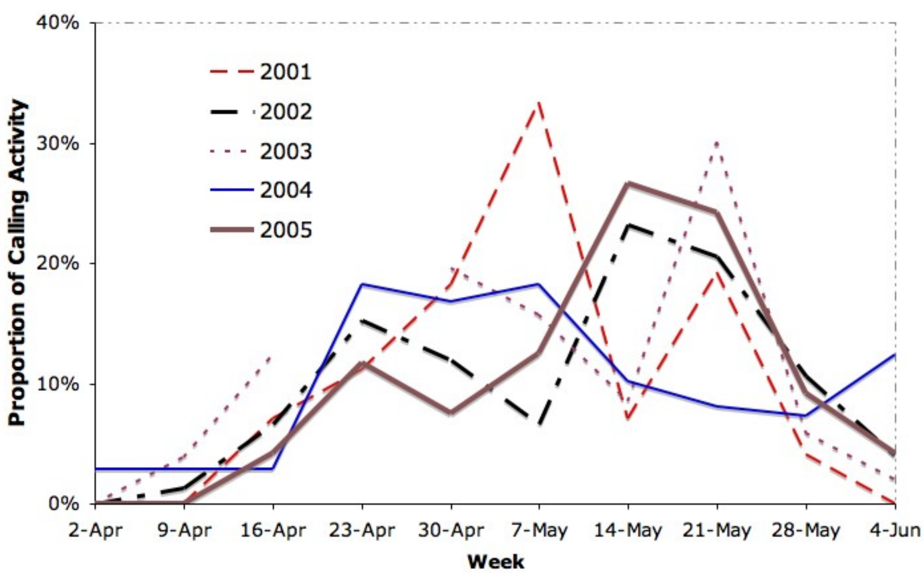

Figure 5. Proportion of male leopard frogs calling activity by week in the CVWMA, 2001 to 2005.

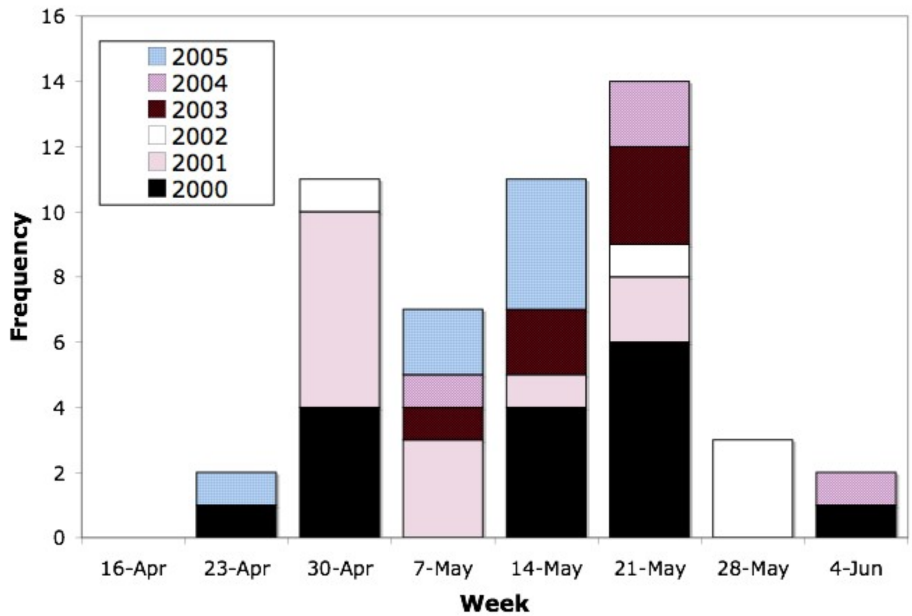

Figure 6. A stacked frequency histogram of northern leopard frog egg masses detected during weekly intervals in the CVWMA, 2000-2005.

Table 2. The size of the leopard frog breeding sites (hectares) calculated using 50 % and 95% kernel density estimators.

Breeding Site	50% Kernel	95% Kernel
East Pond	0.14	0.68
West Pond	0.59	2.16
Leach Lake	0.68	2.44

3.2. Population Monitoring

Figure 7 shows the annual mean calling activity at the east and west breeding sites from 2000 to 2005. During this period, calling activity remained constant in the west-breeding pond but declined dramatically in 2002 at the east-breeding pond. The number of egg masses observed also declined during this period from 16 and 12 egg masses per year in 2000 and 2001 to a mean of 5.5 egg masses per year thereafter (Table 3). The average number of egg masses observed per breeding site in the CVWMA was low (mean = 3.2 ± 3.9) in comparison to data reported elsewhere: Wisconsin, 12.3 ± 7.5 (Hine et al. 1981); Michigan, 40.4±11 (Merrell 1968); Colorado 7.8 ± 8.9, (Corn and Livo 1989); Massachusetts, 11.9±8.4 (Resetarits 2003); and Quebec, 244 egg masses at a single 6-hectare site (Gilbert et al. 1994)

Because survey effort varied from year to year (Appendix B), we calculated a correlation coefficient (*r*) to determine whether the number of egg masses observed was simply a function of search time. An *r-value* of -0.80 was obtained, suggesting that the number of egg masses observed was not simply a function of search effort.

The decline observed in the number of egg masses and calling activity corresponds to similar trends observed in capture rates and to population estimates. Table 4 summarizes the captures per survey hour for adult and juvenile leopard frogs caught during fall VES surveys. In 2003, 2004 and 2005, capture rates were much lower than they were in 2000. A breakdown of all captures is provided in Appendix C.

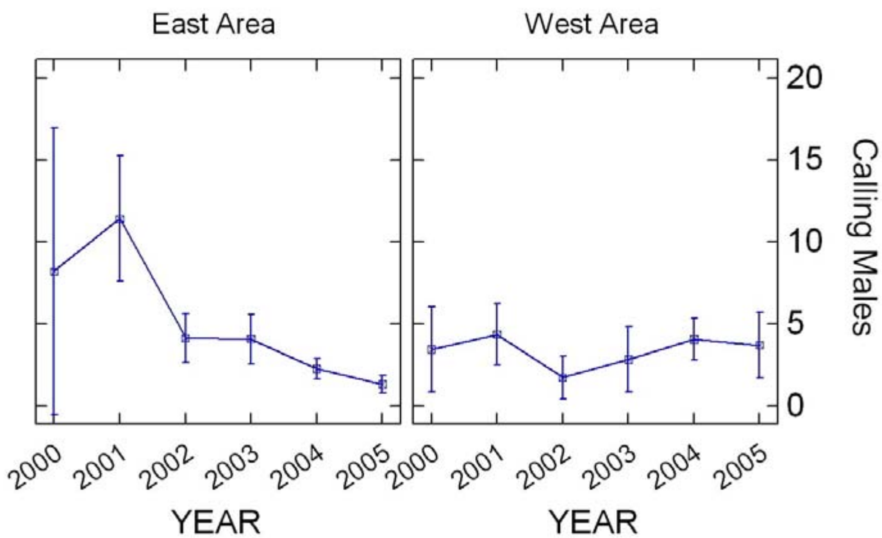

Figure 7. Mean calling activity (± 2 SEM) at the east and west breeding sites.

Table 3. The number of leopard frog egg masses found in breeding ponds in the CVWMA from 2000 to 2005.

Breeding Pond	2000	2001	2002	2003	2004	2005	Total
East Pond	8	12	1	4	3	0	28
East Ditch	0	*	2	0	0	0	2
West Pond	8	*	2	2	1	4	17
Leach Lake	-	0	-	-	-	3	3
Total	**16**	**12**	**5**	**6**	**4**	**7**	**50**

Table 4. Mean capture rates for leopard frogs caught per survey hour in the source site during fall surveys

Year*	2000	2003	2004	2005
Mean captures/survey hour	0.41	0.22	0.14	0.15
Surveys (N)	32	11	13	19
Std. Dev	0.67	0.35	0.26	0.31
Std. Error	0.11	0.10	0.07	0.07

* no surveys were conducted in the fall of 2001 or 2002.

Population estimates were calculated using the Petersen Mark-Recapture model for the source population in 1999, 2000, 2003 and 2005 (Table 5). Again, a declining trend similar to was observed in the number of egg masses, calling activity and catch per effort is apparent. While it is important to note that the use of the Petersen model is inappropriate for estimating the size of an open population, these estimates are valuable as they provide a relative index of abundance. Because animals died during the sampling periods, the population estimates are biased positively and over estimate true population size.

Table 5. Population estimates for northern leopard frogs in source population from 1999 to 2005.

Description	1999*	2000	2003	2005
Mark (M)	102	143	38	81
Capture (C)	88	117	20	26
Recapture (R)	13	13	2	5
Petersen Estimate (N)	**654**	**1213**	**272**	**368**
Lower 95% C.I.	359	660	34	125
Upper 95% C.I	1060	1992	862	753

* Data from Waye and Cooper (2000)

3.3. Animal Health and Mortality

Between 2000 and 2005, 32 leopard frog mortalities and 48 frogs in poor health were observed (Appendix D). The proportion of dead and unhealthy leopard frogs to the total number of individuals observed in the source population was 0.12 (standard error = 0.01). The highest proportion of unhealthy frogs observed in 2001 (0.18)

Batrachochytrium dendrobatidis (Bd), a fungus that causes the disease chytridiomycosis, was diagnosed in four dead and eleven live leopard frogs in the source population. In 2003, two seemingly healthy leopard frogs captured in late April were found dead on May 7 due to chytridiomycosis. Based of visual symptoms, chytridiomycosis was also suspected in twenty-seven leopard frogs in the source population, six in BFWMA, three in Leach Lake, and two in Corn Creek (Appendix D). Symptoms of chytridiomycosis include sloughing of the skin, vascularization of the epidermis (particularly in the extremities and underside), unusual posture, and lethargy (Berger and Speare 1998, Berger et al. 1999, Longcore et al. 1999). Bd is also prevalent in at least one other species in the CVWMA. In 2005, Bd was detected in a pooled sample of toe clippings taken from seven Columbia spotted frogs (*Rana luteiventris*) captured in Corn Creek Marsh. During this project, over 400 tissue samples were collected and PCR assays for Bd will be preformed on this tissue in subsequent study.

In addition to Bd, a second fungus caused leopard frog mortality. In 2001, water levels at the source site were maintained 10 to 20 cm above normal. As a result, most of the egg masses were laid 30 to 50 meters away from the east-breeding pond in a grassy meadow and became heavily infected with *Saprolegnia* (Figure 9). While this fungus is not typically considered to be infectious, outbreaks resulting in catastrophic egg mortality have been reported in amphibians elsewhere (Banks and Beebee 1988, Kiesecker and Blaustein 1997, Robinson et al. 2003). We suspect that as a result of the high water levels, the eggs were laid in decaying upland vegetation that in someway contributed to the *Saprolegnia* outbreak. In addition, tadpoles collected in June were unusually small in size, suggesting that these egg masses were laid in unfavourable habitat. This habitat dried up over the course of the summer, likely resulting in further mortality.

Figure 8. Northern leopard frogs exhibiting signs of chytridiomycosis: (a) subcutaneous vascularization, (b & c) sloughing of the skin, (d) unusual posture and emaciation.

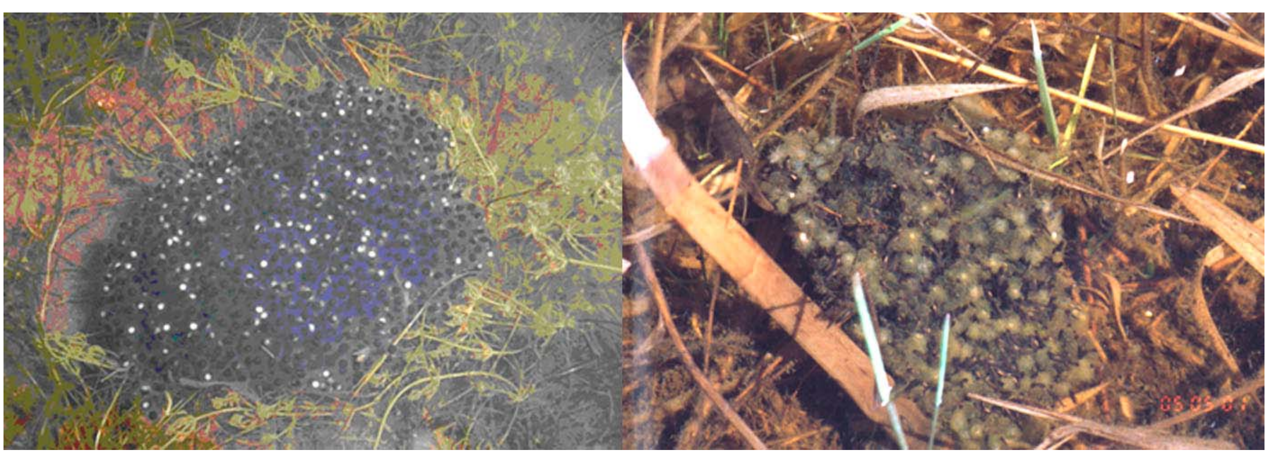

Figure 9. Normal (left) and *Saprolegnia* infected (right) egg masses. White eggs in left photo are unfertilized.

Other sources of mortality observed during this project included road kill and predation. We documented two instances where leopard frogs were killed by vehicle traffic along a dyke that bisects the breeding sites from the main over-wintering channel. Four instances of predation were also documented. Garter snakes were observed eating leopard frogs on two occasions (Figure 10) and avian and mammalian predators were suspected in the other two cases.

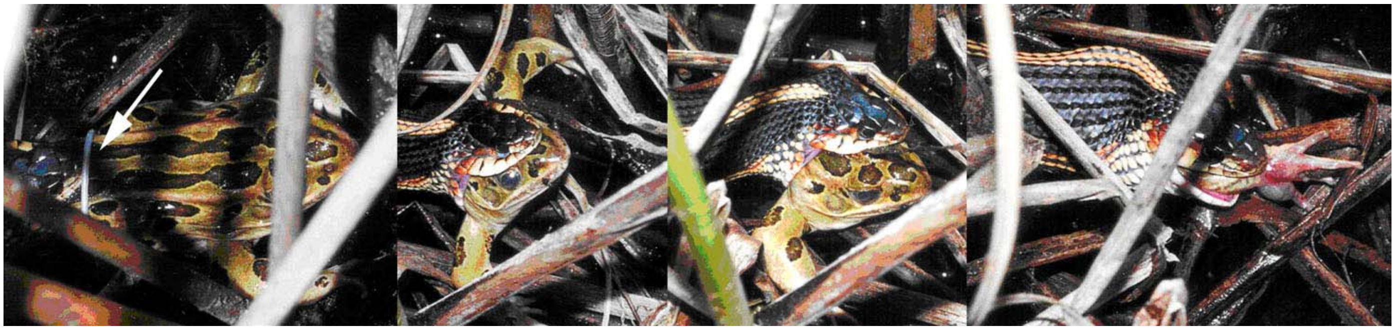

Figure 10. Predation of a radio tagged leopard frog by a common garter snake (*Thamnophis sirtalis*). The radio tag hip belt can be seen in left hand image (arrow).

3.4. Other Amphibian Observations

Other amphibians observed during the course of this project include Columbia spotted frogs (*Rana luteiventris*), western toads (*Bufo boreas*), pacific tree frogs (*Pseudacris regilla)* and long-toed salamanders (*Ambystoma macrodactylum*). All of these species were observed in the CVWMA and only long-toed salamanders and spotted frogs were observed in BFWMA (Appendix E).

Pacific tree frogs were common during calling surveys in the east-breeding site and both tree frogs and long–toed salamanders larvae were observed in the east-ditch. Pacific tree frogs were only occasionally heard in the west-breeding pond and always in low numbers.

Columbia spotted frogs were numerous in Corn Creek Marsh and in BFWMA (Appendix E). As mentioned previously, *B. dendrobatidis* was detected in a pooled sample of toe clips taken from spotted frogs (N=7) captured in Corn Creek Marsh. All seven of these animals appeared healthy.

Western toads were observed in the source compartment and in the Leach Lake management unit. In the spring of 2005, a pair of western toads was observed in amplexus in pond 4 of Leach Lake, where three leopard frogs egg masses were also detected. Although numerous toad tadpoles were observed throughout May, no YOY toads were observed during summer VES.

3.5. Captive rearing

The number of leopard frogs reared in captivity each year and the associated survival rates are provided in Appendix F. Between 2001 and 2005, 30,065 leopard frog hatchlings were collected from 27 egg masses. During this period, 10,147 leopard frog tadpoles and 14,487 leopard frog metamorphs were released back into the wild: 6,288 metamorphs and 6,122 tadpoles were released into the source population, 4,283 metamorphs and 1,928 tadpoles were released into Corn Creek, 3,639 metamorphs and 493 tadpoles were released into BFWMA, and 624 metamorphs were released into Leach Lake. Average survival under the rearing program was 82.0%.

Mortality of captive animals was approximately 15%, of which the cause could not be determined in 42.5 % (Appendix F). Human error accounted for 39.1 % of the mortality and was attributed to poor water quality (31.9%) or simple accidents (7.2%). Because tadpoles are reared at high densities, simple errors or misjudgment such as over-feeding or skipping water changes can result in substantial mortality.

We experienced two episodes of high mortality, one in 2003 and a second in 2004. In both instances, improper feeding led to poor water quality. Even after the water quality problems were rectified, high levels of mortality continued (recorded as "unknown: causes in Appendix F) suggesting that these incidents have longer-term carry-over effects.

Accidental deaths were caused by netting injuries, bleach residue from disinfected equipment, electric water pumps, and trauma sustained while marking. Inadequate rinsing of bleach residue was the main cause of accidental deaths, and resulted in over 200 mortalities in 2005 (Appendix F). Approximately 3 % of the captive population was sacrificed for disease monitoring.

Of the 30,065 tadpoles reared in captivity, only a small number of abnormalities were observed (1.2%); and in fact, most of these would not be considered true malformations (Meteyer 2000, Sessions 2003). The most common abnormality was scoliosis (Figure 11). In a few extreme cases of scoliosis may have contributed to mortality (<10), but in most cases the syndrome completely disappeared after the tail was absorbed. Scoliosis has been reported elsewhere in *R. pipiens* (Merrell 1972) and in other species including *R. perezi* (Martinez et al. 1996), and *Litoria aurrea* (Robinson 1993, Browne et al. 2003). It is thought that scoliosis may be caused by rapid growth (Browne et al. 2003) or improper nutrition (Wright and Whitaker 2001). Other abnormalities observed include limb hyperextension, intestinal gas, and anasarca (Figure 12). None of these abnormalities were common and anasarca was extremely rare, with only four cases observed during the entire project.

In order to rear leopard frog metamorphs to 30mm SVL or greater, we conducted experiments to determine the effects of diet and density. We found that the amount of protein in the diet had a profound effect on both the size at metamorphosis and the time to complete metamorphosis (Adama et al. 2003a, Adama et al. 2004). By increasing the amount of protein in the diet, which was provided as bloodworm, in the final two years of the project, we were successful in increasing the size of the metamorphs to over 30 mm (34.9 mm in 2004 and 32.1 mm and 2005) and reducing the time to metamorphosis to approximately 75 days. These values are within the range observed in wild leopard frog metamorphs (Eddy 1976, Merrell 1977, Hine et al. 1981) and leopard frogs reared under semi-natural settings (DeBenedictis 1974, Kendell 2003a). Figure 13 shows two recently metamorphosed leopard frogs reared under high and low protein diets.

Figure 11. Mild scoliosis in captive reared leopard frogs at Gosner stage 32 (left) and at Gosner stage 44 (right).

Figure 12. Abnormalities in captive reared leopard frogs; A) limb hyperextension B) anasarca, C and D) intestinal gas.

Figure 13. Two leopard frog metamorphs reared under high (left) and low (right) protein diets.

3.6. Post-Release Monitoring

Appendix B and C summarize the monitoring effort and capture data for surveys conducted in Corn Creek Marsh, Leach Lake and BFWMA. Despite having released 4,283 metamorphs and 1,928 tadpoles into Corn Creek Marsh, not a single leopard frog was caught beyond the first winter. Leopard frogs captured in Corn Creek Marsh in the fall were smaller than captive reared YOY found in the source population ($p < 0.0001$, Table 8). This suggests that the habitat in Corn Creek Marsh may not be as suitable as the habitat at the source site and as a result they grow less quickly.

Since 2003, 3639 leopard frog metamorphs and 493 tadpoles have been released into the BFWMA. In 2005, seven juvenile leopards frogs were observed in the spring and three adults were observed in the fall. Although one of the three adult frogs was dead, all three were females weighing in excess of 80 grams and all bore a green VIE indicating that they were released in 2004. In the fall of 2005, thirteen unmarked YOY were captured along the boundary of the BFWMA and the St. Mary's First Nations Reserve at the south end of Doran Marsh (Appendix A). This indicated that leopard frogs bred in or near the BFWMA in the spring of 2005.

In April 2005, leopard frogs were detected during NCS in pond 4 of Leach Lake (Appendix B). Of four males captured, three were marked with pink VIE indicating that these animals were reared in captivity in 2003 and released into the east-breeding pond, 3 km to the north. Three egg masses were subsequently found in this compartment and numerous metamorphs were observed in the summer and fall. Mark-Recapture population estimates were calculated for the population of YOY in Leach Lake and in BFWMA (Table 6).

The weights of wild and captive reared YOY leopard frogs caught in September and October are summarized in Appendix G and in Tables 7, 8 and 9. Overall, wild YOY were significantly larger than captive reared YOY ($p = 0.0001$, Table 7), except in 2002 and 2004 where there was no significant difference (2002: $p = 0.41$; 2004: $p = 0.5$). In comparing the weights of captive reared YOY to wild YOY, the minimum target of rearing leopard frog metamorphs to 30 mm should be re-evaluated. In 2005, YOY reared in captivity were 32.1 mm on average, however when recaptured in the fall these animals were significantly smaller than wild YOY ($p = 0.0001$; reared = 7.4 g ± 2.6, wild = 19.2 ± 2.5). In 2004, leopard frogs were reared to a size of 34.9 mm and in the fall these animals were on par with wild YOY ($p = 0.16$; reared = 12.4 g ± 1.6, wild = 10.8 ± 1.64). Moreover, captive reared YOY caught in the fall of 2005 were smaller than captive reared YOY from 2004 ($p = 0.0016$), despite what appeared to be a better growing season for leopard frogs in 2005 (based on the size of wild YOY caught that year). As post-metamorphic survival is influenced by size at metamorphosis (Semlitsch et al. 1988, Berven 1990, Scott 1994, Morey and Reznick 2001, Altwegg 2002), producing large healthy metamorphs must be a primary objective for a captive rearing project. In future years, a target SVL at metamorphosis closer to 35 mm should be considered.

Location also appeared to have a significant effect of the growth of YOY leopard frogs (Table 8). Captive reared YOY in the source population were significantly larger than captive reared YOY in BFWMA and Corn Creek Marsh ($p = 0.0001$; source = 10.1 grams ± 1.02, BFWMA = 7.9 grams ± 1.4, Corn Cr = 6.7 ± 0.7; all years pooled). This suggests that factors such as habitat quality or climate in BFWMA and Corn Creek Marsh may be less than optimal for leopard frogs.

We also observed a high degree of variation in the size of wild YOY from year to year ($p < 0.0001$). The mean weight of wild YOY pooled across all years was 12.3 grams ± 0.94. In 2005, YOY were significantly larger than YOY from all other years except 2001 (Table 9).

Table 6. Population estimates for YOY northern leopard frogs in Leach Lake and BFWMA, 2005.

Description	Leach Lake	BFWMA
Mark (M)	624	591
Capture (C)	30	22
Recapture (R)	4	9
Petersen Estimate (N)	**3874**	**1361**
Lower 95% C.I.	1093	689
Upper 95% C.I	8926	2117

Table 7. Weight of captive reared and wild YOY captured in September and October. Data is pooled across sites and years, and excludes unhealthy animals.

Origin	N	Mean Weight (g)	SE	95% Confidence Intervals (g)	
Captive reared	264	**7.7**	0.31	7.1	8.3
Wild	157	**13.4**	0.40	12.6	14.2

Table 8. Weight of captive reared and wild YOY captured in September and October by site. Data is pooled across all years and excludes unhealthy animals.

Origin	Site	N	Mean Weight (g)	SE	95% Confidence Intervals (g)	
Captive reared	Bummers Flats	35	**7.9**	0.70	6.5	9.3
	Corn Creek	162	**6.7**	0.33	6.1	7.3
	Source Population	67	**10.1**	0.51	9.2	11.1
	Leach Lake	4	**8.5**	2.06	4.4	12.6
Wild	Bummers Flats (2005)	13	**10.3**	1.55	7.1	13.4
	Source Population (all years)	121	**12.3**	0.47	11.7	13.21
	Source Population (2005)	13	**19.2**	1.23	16.8	21.7
	Leach Lake (2005)	23	**21.0**	1.16	18.7	23.3

Table 9. Weight of wild caught YOY in the source population in September and October by year. Excludes unhealthy animals.

Year	N	Mean Weight (g)	SE	95% Confidence Intervals (g)	
2001	7	**15.4**	1.68	12.0	18.7
2002	4	**5.0**	2.22	0.6	9.4
2003	68	**11.7**	0.54	10.7	12.8
2004	30	**10.7**	0.81	9.04	12.3
2005	13	**19.2**	1.23	16.8	21.7
All years	122	**12.3**	0.47	11.2	13.2

4. Discussion

4.1. Population Status and Decline

Between 2000 and 2005, the leopard frog population in the source site declined. A downward trend was observed in calling activity, egg mass counts, catch per effort and population size. The population estimate for 2005 suggests a population size somewhere in the low to mid-hundreds, however as this estimate was biased positively, the true population size may be considerably lower.

In part, we attribute this decline to an outbreak of *Saprolegnia* that occurred in 2001. While stochastic events such as this are not unusual and contribute to natural fluctuations in population size (Pechmann et al. 1991), it is expected that this population would have dipped for a year or two and then recovered. Instead, breeding activity and abundance remained low, suggesting other factors may be suppressing the population. During this project, we observed chytrid associated mortality and numerous unhealthy animals that were confirmed or suspected to have chytridiomycosis. These observations lead us to believe that chytridiomycosis is not only lethal to leopard frogs but this disease is also suppressing the population.

Chytridiomycosis has been attributed to declines elsewhere. In Spain, chytridiomycosis was responsible episodes of mass mortality in the common midwife toad (*Alytes obstetrians*) where they disappeared from 86% of the sites between 1997 to 1999 (Bosch et al. 2001). In New Zealand, populations of Archey's frog (*Leiopelma archeyi)* crashed between 1996 and 2001 (Bell et al. 2004). In Colorado, adult survival of western toads declined from 78 % in 1994 to 3% in 1999 (Muths et al. 2003). While chytridiomycosis is devastating to many species, there is evidence that some species may persist following an initial decline. Retallick et al. (2004) found that remnant populations of the Eungella torrent frog (*Taudactylus eungellensis*) persist following a catastrophic decline caused by chytridiomycosis. Although the mechanism(s) that allow this species to co-exist with the disease is not known, the authors suggest that either the torrent frogs have developed resistance or a less-pathogenic strain of the disease has evolved. The authors further suggest that a sympatric occurring species, *Litoria wilcoxii/jungguy* may be acting as a reservoir for the disease.

In British Columbia, there is some evidence that Columbia spotted frogs may be a reservoir for *B. dendrobatidis*. First, as we report, spotted frogs carry *B. dendrobatidis* but do not appear to exhibit any sign of disease and chytrid-associated mortality has not been observed. Second, our efforts to reintroduce leopard frogs where spotted frogs are abundant (Corn Creek Marsh) were not successful. Third, spotted frogs have not been detected in the source site, despite over 1000 hours of survey effort. Although speculative, spotted frogs may carry and transmit *B. dendrobatidis* and their presence may exclude leopard frogs due to a higher prevalence of the pathogen in the environment. Bullfrogs and tiger salamanders are also suspected be reservoirs for *B. dendrobatidis* (Davidson et al. 2003, Daszak et al. 2004).

The northern leopard frog still remains very much at risk in British Columbia. While it appears that this population is fluctuating due to natural stochastic events, we suspect that the resiliency of this population in response to these events is suppressed by chytridiomycosis. In the absence of recovery efforts, it is likely this population will disappear.

4.2. Recovery

4.2.1. Captive Rearing and Reintroduction

In assessing our captive rearing program, we were successful in achieving our objectives (Section 2.3). The average rate of survival in captivity was 82% and in the last two years of the project we exceeded our target to raise leopard frog metamorphs to 30 mm SVL and reduced the time to metamorphosis to approximately 75 days.

With respect to the reintroduction program, we demonstrated that reintroduction is a viable way of reestablishing leopard frog populations. We found that captive reared leopard frogs were able to over-winter in the CVWMA and in BFWMA and we documented successful breeding by captive reared animals at two sites, Leach Lake and BFWMA. The lack of success, both in terms of over-wintering and in breeding in Corn Creek Marsh suggests that the recovery of leopard frogs may be challenging. It appears that either the habitat is not suitable or that the presence of spotted frogs may exclude leopard frogs through a reservoir-host disease interaction (Hochachka and Dhondt 2000, Hudson et al. 2001).

Recognizing that this project was very much experimental, a number of improvements and changes must be made if captive rearing and reintroduction is considered in the future. As a first priority, we recommend that a comprehensive strategy be developed to address population viability, genetics, disease, monitoring, public education, and the facility operations. Before embarking reintroduction, long-term funding must be ensured at the outset. This will be challenging, as few funding agencies will commit to projects extending beyond 2 years. Additional recommendations are provided below.

4.2.2. Habitat Enhancement

Although habitat enhancement was not a major element of this project, our findings demonstrate that habitat enhancement can play an important role in the recovery of northern leopard frogs. The discovery of leopard frogs breeding in previously unsuitable habitat suggests that habitat may be a limiting factor. Opportunities for vegetation management and wetland restoration should be explored in both the CVWMA and the BFWMA. In addition, future wetland restoration projects should incorporate monitoring to determine the effects on leopard frogs and other amphibians.

5. Recommendations

Between 2000 and 2005, progress was made towards the recovery of northern leopard frogs in British Columbia. At the conclusion of this project we identified a primary threat to the population, developed a sound captive rearing program, and documented breeding at two new sites. While these accomplishments are noteworthy, leopard frogs are still very much at risk in British Columbia. We present the following recommendation to identify a number of concerns, challenges, and questions that arose from this project.

Conservation Priorities

i. The conservation of the leopard frog population in the CVWMA must be the ***foremost priority*** for recovery. Efforts to conserve and expand this population or improve habitat should be given a higher priority over reintroduction and habitat restoration elsewhere.

ii. If the decline in the leopard frog population does not reverse in the next three years, a captive breeding program should established. In the mean time, a contingency plan for rearing and breeding leopard frogs in captivity should be developed.

Monitoring

iii. Monitoring the population in the CVWMA and at release sites should continue. It will be important to determine if self-sustaining population become established. If they do not become established, it will be important to understand why to allow the recovery team to incorporate this knowledge into the Recovery Strategy.

iv. To ensure monitoring is efficient, cost-effective, and meets the needs of the recovery team, a long-term monitoring plan should be developed to identify monitoring priorities, describe survey procedures, and estimate the required resources.

v. Populations of other amphibian species should be monitored to determine their status and to understand the interactions between species.

vi. Habitat quality should be monitored at the leopard frog breeding sites, over-wintering sites, and at reintroduction sites. Changes to land use patterns or wetland community structure in the CVWMA could be detrimental to the extant population.

Reintroduction

vii. Prior to resuming a reintroduction program, we recommend that a reintroduction strategy be developed to articulate the goals and objectives of the program and to address a number of important scientific and logistic considerations such as population viability, genetics, disease, monitoring, funding, and facility operations. If a second phase of reintroduction is pursued:

   a. Long-term funding should be secured at the outset.

   b. The rearing facility should be set up as a professional facility and reviewed on a regularly basis by a Provincial veterinarian.

   c. Husbandry staff should receive professional training such as the amphibian biology and management course offered annually by the American Zoo and Aquarium Association.

   d. A full-time manager must be dedicated to the program. Due to the responsibilities and workload involved, a reintroduction program cannot be managed on a part-time basis.

   e. Controlled experiments should be continued to further refine husbandry techniques and producing large healthy metamorphs must be a primary objective for captive rearing. We recommend a target SVL at metamorphosis of 35 mm be considered.

Habitat

viii. There is evidence that habitat quality may be a limiting factor for leopard frogs in the CVWMA. Priority should be given to projects that improve wetland habitat in the CVWMA and opportunities for wetland restoration should also be considered in the BFWMA.

ix. Future wetland restoration projects should incorporate monitoring to determine their effects on leopard frogs and other amphibians.

x. Private land adjacent the east-breeding site should be secured (Appendix A-1). This land lies in between the east breeding site and the over-wintering site and is used for commercial agriculture, which may be detrimental to the extant population.

xi. Additional release sites should be identified. In particular, surveys should be conducted in the wetlands in the Lower Kootenay Reserve and in the Kootenai Wildlife Refuge in northern Idaho. If suitable or potentially suitable habitats can be identified, partnerships should be established with these agencies to facilitate the reintroduction of the leopard frogs into these sites. However priority ***should not*** be given to reestablishing populations at these sites over the conservation of leopard frogs in the CVWMA.

Disease

xii. A disease management strategy should be developed to identify measures that will prevent the spread and transmission of chytridiomycosis and decrease the impact chytridiomycosis has on leopard frogs or other species.

xiii. Research should be directed to further our understanding of chytridiomycosis. In particular, it will be important to understand (1) how and where leopard frogs become infected, (2) if some individuals are able to survive an infection, (3) whether spotted frogs and other species are a reservoir for the disease, and (4) determine if leopard frogs from other regions are more resistant to the disease.

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