

# Northern Leopard Frog (*Rana pipiens*) Recovery: Captive rearing and reintroduction in Southeast British Columbia, 2003.



Doug Adama, Adama Consulting  
Kate Lansley  
Marc-Andre Beaucher, Beaucher Wildlife Consulting  
March 31, 2004

Prepared for: Columbia Basin Fish and Wildlife Compensation Program, Columbia Basin Trust, Creston Valley Wildlife Management Area, Ministry of Water, Land and Air Protection, Northern Leopard Frog Recovery Team, and the World Wildlife Fund.



Columbia Basin Fish and  
Wildlife Compensation Program



Environment  
Canada

Environnement  
Canada



## Acknowledgements

Sarah Herring Columbia Basin Fish and Wildlife Compensation Program (CBFWCP),  
BC Hydro  
Brian Stushnoff, Brenda Burns, and Justin Lang  
Creston Valley Wildlife Management Area  
Dr. Stephen Raverty Animal Health Centre, Ministry of Agriculture, Foods, and Fisheries,  
BC Government  
Dr. Trent Bollinger Prairie Diagnostic Services, Western College of Veterinary Medicine  
Beth Woodbridge Columbia Basin Fish and Wildlife Compensation Program, BC Hydro  
John Krebs Columbia Basin Fish and Wildlife Compensation Program, BC Hydro  
Ted Antifeau Ministry of Water, Land and Air Protection, BC Government  
Laura Friis Ministry of Water, Land and Air Protection, BC Government  
Dr. Helen Schwantje Ministry of Water, Land and Air Protection, BC Government  
Ron Ydenberg Simon Fraser University  
Brendan Wilson and participating students, Selkirk College, Castlegar, BC  
Kris Kendell Alberta Conservation Association

Financial and in-kind support for this project was provided by:

Adama Consulting  
Columbia Basin Fish and Wildlife Compensation Program  
Columbia Basin Trust  
Creston Valley Wildlife Management Area  
Ministry of Water, Land and Air Protection, BC Government  
World Wildlife Fund

This publication should be cited as:

Adama, D. B., K. Lansley, and M. A. Beaucher. 2004. Northern Leopard Frogs (*Rana pipiens*)  
Recovery: Captive rearing and reintroduction in southeast British Columbia, 2003. Report to the  
Columbia Basin Fish and Wildlife Compensation Program, Nelson, BC. pp. 26.

For copies of this report, please visit <http://www.cbfishwildlife.org> or contact:

The Columbia Basin Fish and Wildlife Compensation Program  
103 333 Victoria St.  
Nelson, BC  
V1L 4K3  
Phone: (250) 352-6874  
Fax: (250) 352-6178  
URL: <http://www.cbfishwildlife.org>

## EXECUTIVE SUMMARY

*Rana pipiens* have declined across the Pacific Northwest. In British Columbia, a single population remains in the Creston Valley Wildlife Management Area, near Creston. This population is listed as Endangered by the Committee of Status of Endangered Wildlife (COSEWIC) in Canada and protected under schedule 1 of the Federal Species at Risk Act. A recovery team was established in 2001 and a draft recovery strategy has been developed. Since 2001, the captive rearing and release of *R. pipiens* has been the focus of recovery efforts. This report details our recovery efforts in 2003.

We monitored breeding activity using nocturnal calling surveys and visual egg mass surveys at three natural breeding ponds in the Creston Valley Wildlife Management Area from April 11 to June 6, 2003. A total of six egg masses were located and approximately 4.19 males were heard per calling survey hour, both down from previous years. The six egg masses were encased in wire enclosures. Total egg mortality was 25.6% and early larval mortality was 8.8%.

At the request of the recovery team, all surviving larvae (N= 11,873) were brought into captivity. In total, 5,104 *R. pipiens* tadpoles and 5,108 *R. pipiens* metamorphs were released. Sixty-six percent of the animals were released back into source population while 10% and 24% were released into Corn Creek Marsh in the Creston Valley and Doran Marsh in the East Kootenays, both historic sites for this species. Mortality in the captive population was 9.6% and 512 animals were submitted for disease monitoring and necropsy. Average length at metamorphosis was 29.1 mm, an increase over previous years but smaller than animals found in the wild. Time to metamorphosis (64 to 98 days) was similar to that of wild populations.

We found that feeding *R. pipiens* larvae a mixed diet of vegetables (kale and watercress), bloodworm, and native aquatic plants improved water quality and increased size at metamorphosis over completely natural or artificial diets. Post-release monitoring suggests releasing partially developed tadpoles (~ Gosner stage 30) may be more advantageous than releasing completely metamorphosed frogs through reduced costs and increased body size. A single animal released in 2002 was recaptured in the source population in the fall of 2003.

Several recommendations are provided. These include suggestions to improve and simplify husbandry procedures, conduct research on post-metamorphic survival, and re-evaluate pond 1 in Corn Creek Marsh as a reintroduction site. Finally, we strongly recommend that the husbandry manual be updated to reflect current husbandry practices.

# TABLE OF CONTENTS

<b>ACKNOWLEDGEMENTS</b> .....	<b>I</b>
<b>EXECUTIVE SUMMARY</b> .....	<b>II</b>
<b>TABLE OF CONTENTS</b> .....	<b>III</b>
<b>ASSOCIATED DOCUMENTS</b> .....	<b>IV</b>
<b>LIST OF TABLES</b> .....	<b>IV</b>
<b>LIST OF FIGURES</b> .....	<b>V</b>
<b>1. INTRODUCTION</b> .....	<b>1</b>
<b>OUR OBJECTIVES FOR 2003 WERE TO;</b> .....	<b>1</b>
<b>2. STUDY AREA</b> .....	<b>1</b>
<b>3. METHODS</b> .....	<b>2</b>
3.1. FACILITY, COLLECTION AND STOCKING .....	2
3.2. DIET.....	4
3.3. WATER QUALITY MONITORING AND MAINTENANCE.....	5
3.4. DISINFECTION AND DECONTAMINATION .....	5
3.5. DISEASE MONITORING AND MORTALITY .....	5
3.6. HABITAT ASSESSMENT .....	6
3.7. RELEASE .....	6
3.8. POST-RELEASE MONITORING .....	6
<b>4. RESULTS</b> .....	<b>7</b>
4.1. SURVEYS AND TADPOLE COLLECTION .....	7
4.2. HEAD START .....	8
4.3. HABITAT ASSESSMENT .....	9
4.4. CAPTIVE REARING AND RELEASE .....	9
4.5. TEMPERATURE AND WATER QUALITY .....	10
4.6. GROWTH AND DEVELOPMENT .....	11
4.7. MORTALITY AND DISEASE MONITORING .....	13
4.8. POST-RELEASE MONITORING .....	15
<b>5. DISCUSSION</b> .....	<b>18</b>
5.1. BREEDING ACTIVITY .....	18
5.2. TADPOLE COLLECTION AND REARING .....	18
5.3. DIET .....	18
5.4. TEMPERATURE AND WATER QUALITY .....	19
5.5. GROWTH AND METAMORPHOSIS.....	19
5.6. MORTALITY AND DISEASE .....	20
5.7. RELEASE AND REINTRODUCTION .....	20
5.8. POST-RELEASE MONITORING .....	21
<b>6. CONCLUSION</b> .....	<b>21</b>
<b>7. LITERATURE CITED</b> .....	<b>22</b>

## ASSOCIATED DOCUMENTS

- Northern Leopard Frog Recovery Project Plan 2003. (CBFWCP 2003)
- Northern Leopard Frog Recovery Draft Strategy. (Ohanjanian et al. 2004)
- Status of the Northern Leopard Frog (*R. pipiens*) in the Creston Valley Wildlife Management Area. (Waye & Cooper 2000)
- Northern Leopard Frog Husbandry Manual. (Wind 2002)
- A small scale rearing effort for Northern Leopard Frogs (*Rana pipiens*) in the Creston Wildlife Management Area. (Adama et al. 2002)
- Captive rearing and reintroduction of Northern Leopard Frogs (*Rana pipiens*) in the Creston Valley Wildlife Management Area, 2002. (Adama et al. 2003)
- Decontamination Protocol. (Beaucher 2001, draft)

## LIST OF TABLES

Table 1. The amount of food provided to <i>Rana pipiens</i> larvae by diet.....	4
Table 2. Effort expended on egg mass and calling survey effort from April 8 to June 6, 2003 .....	8
Table 3. The number of egg masses found in the CVWMA from 1997 to 2003 .....	8
Table 4. Vital information for six <i>Rana pipiens</i> egg masses found in 2003 .....	8
Table 5. Vital information for <i>Rana pipiens</i> tadpoles reared in captivity between May 22 and July 7, 2003.....	9
Table 6. Vital information and time to metamorphosis for <i>Rana pipiens</i> reared in captivity from July 7 to August 21, 2003.....	9
Table 7. The number of <i>Rana pipiens</i> released at four sites in 2003.....	10
Table 8. The number of <i>Rana pipiens</i> released in 2001, 2002, 2003.....	10
Table 9. Dissolved oxygen (mg/l) in rearing tanks under three dietary regimes. ....	10
Table 10. Average daily temperatures in the breeding ponds and in a rearing tank .....	11
Table 11. Average and cumulative degree-days (C°) for the time to first emergence, 50% metamorphosis and 95% metamorphosis.....	11
Table 12. Mean total length for <i>Rana pipiens</i> tadpoles reared for 45 days in captivity.....	12
Table 13. The size of <i>Rana pipiens</i> reared in 2003 by egg mass and diet .....	12
Table 14. The size of <i>Rana pipiens</i> metamorphs reared in 2001, 2002, and 2003 .....	13
Table 15. The number of <i>Rana pipiens</i> larvae submitted for disease monitoring .....	14
Table 16. Survey effort and results for post-release monitoring surveys.....	15
Table 17. Size of marked and unmarked young-of-year <i>Rana pipiens</i> captured in the fall of 2003.....	15

## LIST OF FIGURES

Figure 1. The location of the Creston Valley Wildlife Management Area and the Bummers Flats Wildlife Management Area.....	2
Figure 2. 4000 litre rearing tanks .....	3
Figure 3. Calling intensity and breeding activity of <i>Rana pipiens</i> plotted against average daily temperatures, April 8 to June 6, 2003 .....	7
Figure 4. Mean daily temperatures at the rearing facility and breeding ponds.....	11
Figure 5. Frequency histograms of <i>Rana pipiens</i> snout-to-vent lengths reared in 2001, 2002 and 2003.....	13
Figure 6. Mean diameters for <i>Rana pipiens</i> tadpoles reared on three diets from July 12 to August 21, 2003.....	13
Figure 7. Cumulative mortality in captive reared <i>Rana pipiens</i> , May 25 to August 17, 2003.....	14
Figure 8. Mean mass of young-of-year <i>Rana pipiens</i> captured in the fall of 2003 by site. ....	16
Figure 9. White VIE markings of a <i>Rana pipiens</i> released in 2002 and recaptured in 2003 .....	16
Figure 10. The location of <i>Rana pipiens</i> and <i>Rana luteiventris</i> captured by Selkirk College students in Corn Creek Marsh, September 25, 2003.....	17

# 1. INTRODUCTION

Northern leopard frogs (*Rana pipiens*) have declined across the western extent of their range (Kendell 2003b; McAllister & Leonard 1996; Seburn & Seburn 1998; Stebbins & Cohen 1995; Wagner 1997; Werner 2003). Prior to the 1990's, *R. pipiens* were found throughout southeastern British Columbia (Seburn & Seburn 1998), however today only a single population is known to occur in the province. This population is located in the Creston Valley Wildlife Management Area (CVWMA), near Creston, BC (Adama et al. in prep; Gillies & Franken 1999; Ohanjanian & Teske 1996; Waye & Cooper 2000). Since 1997, the Columbia Basin Fish and Wildlife Compensation Program and its partners have been monitoring this population, which is precariously close to extirpation (Adama et al. in prep; Waye & Cooper 2000). Listed as endangered (CDC 2001; COSEWIC 2001), the loss, degradation and fragmentation of critical habitats, infectious diseases, predation by introduced fish species, and low recruitment threaten this population (Ohanjanian et al. 2003, draft).

At the request of the Ministry of Water, Land and Air Protection, recovery efforts were initiated in 2001. To date, the captive rearing and release of *R. pipiens* has been the emphasis of recovery efforts. Recognizing that amphibian reintroductions are challenging and often unsuccessful (Denton et al. 1997; Dodd & Seigel 1991; Kendell 2003a) and that little has been published on the rearing of amphibians for conservation (Wright & Whitaker 2001), we have adopted an experimental approach to increase the success of our recovery efforts. In addition, we have pursued several other recovery initiatives including habitat enhancement, a fish inventory (Gebhart & Roberge 2001), and public awareness. The recovery team has also been effective in collaborating with others working on amphibian conservation (Adama & Kendell 2004, in press; Hoffman & Blouin in press-a, b).

Our objectives for 2003 were to;

- 1) increase the capacity of the rearing facility to head start up to 20,000 *R. pipiens* tadpoles for one month and rear 5,000 *R. pipiens* to metamorphosis;
- 2) release animals back into the source population and at two reintroduction sites; one in the Creston Valley and one in the East Kootenays;
- 3) conduct controlled experiments on tadpole diet
- 4) conduct post-release monitoring surveys.

## 2. STUDY AREA

*Rana pipiens* occur in a single wetland compartment in the Creston Valley Wildlife Management Area, a joint Federal and Provincial wildlife management area and designated United Nations RAMSAR site, near Creston, BC (Figure 1). Approximately 430 hectares in size, this compartment is bordered by a system of dykes and agricultural land. Water levels in the compartment are actively managed by the CVWMA, in accordance with the regulations of the International Joint Commission (IJC). The biogeoclimatic designation of the valley floor of the Creston Valley is Interior Cedar Hemlock xw (Braumandl & Curran 1992).

In 2001 and 2002, *R. pipiens* metamorphs were released at three sites; Corn Creek Marsh and near the east and west-breeding ponds of the source population (Adama et al. 2003; Adama et al. 2002). In 2003, following an assessment of potential release sites in the southern East Kootenays, a fourth release site was established in Doran Marsh in the Bummars Flats Wildlife Management Area (BFWMA). The Bummars Flats Wildlife Management Area is actively managed by the Provincial government and Ducks Unlimited for waterfowl production, native ungulates, and cattle grazing (Jamieson 1990). The site is characterized as a Ponderosa Pine–Dry Hot biogeoclimatic subzone/variant (Braumandl & Curran 1992).



**Figure 1. The location of the Creston Valley Wildlife Management Area (triangle) and the Bummers Flats Wildlife Management Area (dot).**

### **3. METHODS**

#### **3.1. Facility, Collection and Stocking**

All activities carried out under this project were conducted under permit of the Ministry of Water, Land and Air Protection and within the boundaries of the Creston Valley Wildlife Management Area and the Bummers Flats Wildlife Management Area. Animals were handled humanely and in accordance with provincial (RIC 1998b) and international guidelines (ASIH 1997; IUCN 1995). Stringent procedures for decontamination were employed to reduce the potential for transferring disease (Beaucher 2001, draft). A husbandry manual Wind (2002) describes many of the protocols and procedures used over the course of the project.

In early May, twenty-six 4,000-litre cattle tanks (258 cm X 129 cm X 76 cm; manufactured by United Farmers Association, Strathmore, Alberta) were set up to house *R. pipiens* tadpoles (Figure 2) at the Simon Fraser University (SFU) field station located in the CVWMA (116-38-4 W, 49-7-5 N). The tanks were set up as described in Adama et al (2003) and filled with approximately 1,000 litres of water. A water drainage system was installed as recommended in Adama et al (2003).





**Figure 2. 4,000 litre rearing tanks.**

Between April 8 and June 6, 2003, a combination of visual egg mass surveys and calling surveys were employed to locate egg masses at the *R. pipiens* breeding sites in the CVWMA. Nocturnal calling surveys were conducted to monitor breeding activity and identify areas of calling activity within the breeding sites. These were followed up with visual surveys the next day to locate potential egg masses. These surveys were conducted one to three times a week at each site, weather permitting. Surveys were aborted and rescheduled due to heavy wind or rain or when air temperatures dropped below 5° Celsius.

Upon locating an egg mass, we estimated its volume and noted the Gosner stage of the developing embryos (Gosner 1960) and the overall health of the egg mass. Egg masses were enclosed in mesh cages to deter depredation and enabled us to easily collect the hatchlings. The egg masses were monitored every two or three days until the eggs had completely hatched out, at which time the hatchlings were brought into captivity. Hatchlings were kept in 109-litre Rubbermaid™ containers for quarantine at a density of 500 hatchlings per container until they reached Gosner stage 25<sup>1</sup>, which took about 7 days. At Gosner stage 25, the tadpoles were transferred to the rearing tanks at a density of 1,000 tadpoles per tank (1.0 tadpole per litre). Over the next month, the stocking density was reduced from 1,000 tadpoles per tank to 250 per tadpoles tank by halving the densities every two weeks. We adopted this stocking regime for several reasons. In 2002, we found that during the first 45 days of development, tadpoles grew more at higher densities which we attributed to the relationship between food availability, tadpole mobility, and the surface area of the tank (Adama et al. 2003). Secondly, a staggered stocking regime enabled us to double our rearing capacity to facilitate the request of the recovery team to head start up to 20,000 tadpoles. Thirdly, as releasing 5,000 metamorphs was our primary objective; a final stocking density of 250 tadpoles per tank (approximately 6,500 tadpoles) was required to accommodate up to 15% for mortality and between 5% and 10% for disease monitoring.

---

<sup>1</sup> Gosner stage 25 marks the stage at which the tadpoles have fully developed mouthparts and are able to feed.

### 3.2. Diet

In 2002, we found that diet had a significant effect on the growth and development of *R. pipiens* larvae. Tadpoles provided an “artificial” diet of spinach, lettuce and bloodworm, grew less and developed more slowly than tadpoles provided a diet of native marsh vegetation, however it was not clear whether this was a result of the nutritional quality of the diet or the quantity of the food provided (Adama et al. 2003). Prior to the start of the project in 2003, concerns were raised about composition of the artificial diet (Jackman, pers. comm.) While several authors have demonstrated that tadpoles grow well on spinach (Briggs & Davidson 1942; Csuti & Seller 2000; Jackman 2002), spinach is high in oxalic acid and can contribute to the formation of kidney stones in *R. pipiens* larvae and may lead to kidney failure (Berns 1965; Briggs & Davidson 1942). Berns (1965) reported almost complete mortality in recently metamorphosed *R. pipiens* reared on a diet of spinach. In response to our findings in 2002 and the concerns raised over spinach, we made several changes to the diet.

In 2003, we reared tadpoles on three diets including 1) an “artificial” diet of kale, watercress, and frozen bloodworm, 2) a “natural” diet of live aquatic plants, which included duckweed (*Spirodela polyrhiza* and *Lemna minor*), yellow waterlily (*Nuphar polysepalum*), pondweed (*Potamogeton spp.*), coontail (*Ceratophyllum demersum*), common bladderwort (*Utricularia vulgaris*) and water-buttercup (*Ranunculus sp.*) and frozen bloodworm, and 3) a mixture of the artificial and natural diets. Partially shredded kale and watercress were fed at a 50:50 ratio in the artificial and mixed diets, once every four days and timed with water changes. Bloodworm was provided daily to all tanks, based on the number of tadpoles in each tank and the developmental stage of the tadpole. The aquatic plants provided in the natural diet were collected from Corn Creek Marsh and hosed down prior to feeding to avoid introducing predacious aquatic insects into the tanks. As the tadpoles grew larger, the amount of food was increased over the course of the project (Table 1). Unfortunately, due to an outbreak of *Saprolegnia* this experiment was abandoned on July 12 and all tanks were moved to a mixed dietary regime.

**Table 1. The amount of food provided to *Rana pipiens* larvae by diet**

Week	Diet						
	Artificial		Mixed			Natural	
	K/WC*	Blood worm**	Marsh veg***	K/WC*	Blood worm*	Marsh veg***	Blood worm**
1 - 2	1	0.15	2	1	0.15	2	0.05
2 - 4	5	0.25	2	5	0.25	2	.25
4 - 6	10	0.25	2	10	0.25	4	
6+	All tanks moved to a mixed diet July 12						

K = Kale WC = Water cress \* grams/tadpole/4 days \*\* grams/tadpole/day \*\*\* 3 gal buckets/week

On July 11, a second experiment employing the three diets described above was set up at a smaller scale. In this experiment, 180 tadpoles were randomly selected from egg masses 1, 2, 5 (Table 4) and reared in 9 Rubbermaid containers filled with approximately 70 litres of water for a density of twenty tadpoles per container or 0.29 tadpoles per litre. Each diet was randomly assigned to three of the nine containers. Every four days, 50 grams each of kale and watercress were provided in the artificial diet trials and 25 grams of each were provided in the mixed diet trials. Aquatic vegetation was fed by volume. Fourteen and seven litres of aquatic vegetation were provided in the natural and mixed diets, respectively and replenished as needed. Frozen bloodworms were added daily at 0.25 grams per tadpole to all containers. Total tadpole lengths were measured at the beginning of the experiment and snout-to-vent length and mass were obtained for the frogs as they completed metamorphs (Gosner stage 46).

### **3.3. Water Quality Monitoring and Maintenance**

Dissolved oxygen (DO; mg/L) and pH were measured frequently over the duration of the project. DO was measured using handheld dissolved oxygen meters (YSI: Model 55 and Model 550A) and pH was monitored using pocket pH meters (Oakton, pH Testr1); each calibrated regularly.

Minimum and maximum water and air temperatures were recorded at the rearing facility using a min-max thermometer. In addition, 10 temperature data loggers (Onset Computer Corporation, Hobo H8™) were placed in the field to record air and water temperatures hourly at the source and release sites. Several temperature data loggers were also located at the rearing facility enabling us to accurately compare air and water temperatures at the rearing facility to the source population and release sites.

Cumulative degree-days were calculated from the estimated date of egg laying to metamorphosis (1<sup>st</sup> emergence, 50% complete metamorphosis and 95% complete metamorphosis). Degree-days were calculated by averaging the hourly temperatures obtained by the temperature data loggers for each day. Lower and upper temperature thresholds of 4° and 30° Celsius for larval growth and development were applied to the temperature data as suggested by Calef (1973) and Eddy (1976). As temperatures did not reach 30° Celsius the upper threshold did not come into effect, however 4° was subtracted from the daily average temperatures as it is assumed that larval development ceases below this temperature.

To maintain water quality complete water changes were conducted every 4 to 6 days in the tanks provided the “artificial” diet and every 8 to 12 days in the tanks provided the “mixed” diet and “natural” diets.

### **3.4. Disinfection and Decontamination**

Chytrid fungus, a disease responsible for the decline of amphibians around the world (Berger et al.; Bosch et al. 2001; Green & Kagarise Sherman 2001; Hopkins & Channing 2003) occurs in the wild population of *R. pipiens* in the CVWMA. Stringent disinfection and decontamination protocols have been developed to prevent the transmission of disease between rearing tanks, and between the rearing facility and the wild population (Beaucher 2001, draft; Wind 2002). A disinfection station was set up at the rearing facility where equipment including nets, pumps, and buckets were disinfected in a bleach solution for a minimum of 15 minutes between uses. Disinfection and decontamination procedures are also employed in the field. A disinfection station has been established at the source population and separate sets of field equipment are used at each site (source population, Corn Creek Marsh, and Doran Marsh).

### **3.5. Disease Monitoring and Mortality**

A rigorous, two-pronged approach was used to monitor disease in the captive population (Adama et al. 2003; Wind 2002). In the first approach, all unhealthy and abnormal animals encountered during daily inspections or during routine husbandry activities (i.e. cleaning tanks) were submitted to either the Animal Health Centre in Abbotsford or to the Wildlife Health Centre in Saskatoon for necropsy. Depending on the circumstance, specimens were frozen or preserved in formalin or ethanol. Each dead animal was packaged separately and labeled with the date of collection, origin (egg mass and tank number), condition and developmental stage. Diagnostic procedures performed under the two veterinarians included histology, bacteriology, toxicology, PCR for chytridiomycosis, and PCR and viral culturing for Iridovirus.

The second approach entailed a random sample of tadpoles taken from each tank. At approximately halfway through development (45 days), 10 tadpoles from each rearing tank were randomly collected. Half of each sample (n=5) was sent to the Wildlife Health Centre and the

other half to the Animal Health Centre (AHC). The tadpoles from each tank (n=5) were packaged live in 4-litre freezer bags and shipped in coolers. Ice packs were added to the coolers to slow metabolic activity and reduce mortality. Each bag was labeled with the date of collection, origin (egg mass and tank number), and developmental stage. The coolers were shipped via Greyhound and careful consideration was given to the timing of shipment to avoid potential delays (e.g. weekend deliveries).

### **3.6. Habitat Assessment**

An assessment of potential reintroduction sites in the southern East Kootenays was conducted by members of the recovery team on June 26, 2003. A simple set of criteria was used to evaluate five wetlands located between Wasa and Kikomun Creek Provincial Park (Appendix A). A site was disqualified if key habitat attributes were lacking.

### **3.7. Release**

Upon completing metamorphosis, *R. pipiens* froglets (Gosner stage 45 and 46) were removed from the rearing tanks and measured. Snout-to-vent (SVL) lengths were obtained with a veneer caliper ( $\pm 0.1$ mm) and weights were obtained with an electron balance (Ohaus;  $\pm 0.1$ g). Animals destined for the source population were marked with a pink Visible Implant Elastomer (VIE; Northwest Marine Technology, Inc.) injected into the toe webbing between the fourth and fifth digits of either the left or right hind limb. The needle was sterilized in ethanol between markings. Nitrile surgical gloves were worn to avoid irritating the frog's skin. After the animals were marked, they were transferred to holding tanks and held for a minimum of 24 hours to ensure that the VIE injections were retained. During this period, the froglets were fed crickets.

We began releasing *R. pipiens* metamorphs (froglets) on July 20, 2004 and releases were conducted several times a week until August 22, 2003. Four release sites were chosen *a priori*. These included the east and west-breeding ponds, Corn Creek Marsh, and Doran Marsh. Metamorphs were transported to the release sites in 60 litre or 109 litre Rubbermaid™ containers. As transportation to Doran Marsh took approximately 3 hours, care was taken to reduce the activity level of the metamorphs and to prevent overheating by providing a small amount of water (approx. 2 litres) in each container and by keeping the temperature in the vehicle below 20 °C using air conditioning.

Two public events were held to publicize the recovery program. On August 6, an event was coordinated in conjunction with the interpretative staff at the CVWMA. On August 7, an event was held in the BFWMA to commemorate the reintroduction of *R. pipiens*, which have been absent from the site for over 20 years.

### **3.8. Post-release monitoring**

We conducted calling surveys and visual surveys (RIC 1998a) opportunistically between April 1 and August 15, 2003 in Corn Creek Marsh and the source population to find *R. pipiens* released in previous years. To monitor the growth and dispersal of animals released in 2003, capture surveys were conducted at all four release sites from September 10 to October 27, 2003. Size and GPS locations were obtained for all animals captured and the presence of VIE markings were noted when present.

On September 25, 2003, 17 students enrolled in the Integrated Environmental Planning Technology Program at Selkirk College, Castlegar, BC, conducted a time constrained visual survey (RIC 1998a). The students were briefed on the procedures and objectives of the recovery project and assigned to six groups of two to four students each. Each group was assigned to a specific area of the marsh, which they surveyed for 4 hours. During this time, the

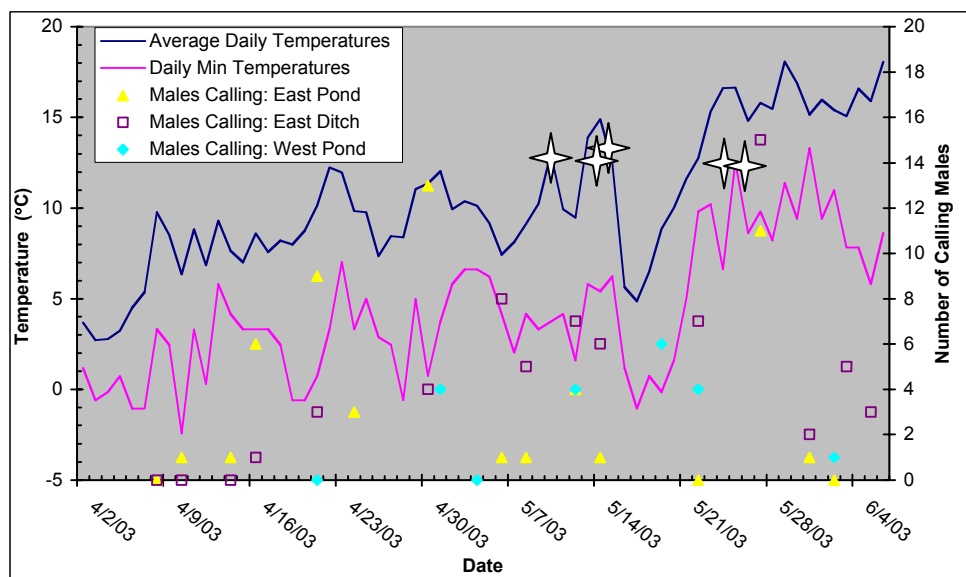
students recorded the species, snout-to-vent (SVL) length, and weight for all amphibians captured. Weight was measured to the nearest gram with 100g Pesola® scales and SVL was measured to the nearest millimetre with flexible plastic rulers. The presence and colour of VIE markings were noted for every *R. pipiens* caught. GPS locations were obtained for each amphibian captured.

## 4. RESULTS

### 4.1. Surveys and Tadpole Collection

To monitor breeding activity, egg mass and calling surveys began on April 8, 2003 and ended on June 6, 2003. *Rana pipiens* were first heard calling on April 10, 2003 in the east ditch and on May 1, 2003 in the west-breeding pond. Figure 3 shows the intensity of calling males at the three breeding areas plotted against daily minimum and average temperatures and the estimated spawn dates for egg masses. Table 2 provides a breakdown of the effort spent conducting surveys by breeding area. Catch per unit effort for calling surveys for all sites was 4.16; similar but slightly less than 2002 (4.87 animals/hour, Adama et al. 2003). In total six egg masses were located, four in the east-breeding pond and two in west-breeding pond. This number is similar to the number of egg masses observed in 2002 but down from previous years (Table 3).

In the spring of 2003, twelve dead or dying adult *R. pipiens* were found in the proximity of the east-breeding area. These animals exhibited sloughing of the epithelia and hemorrhaging at the extremities. Histology and PCR confirmed the presence of chytrid fungus (*Batrachochytrium dendrobatidis*).



**Figure 3.** Calling intensity and breeding activity of *Rana pipiens* plotted against average daily temperatures, April 8 to June 6, 2003. Estimated spawn dates for egg masses denoted by stars. Note that two egg masses were laid on May 14, 2003.

**Table 2. Time expended on egg mass and calling survey effort (hours: minutes) from April 8 to June 6, 2003.**

Breeding Area	Egg Mass Surveys	Calling Survey
East Pond	12:50	10:20
East Ditch	30:05	11:00
West Pond	17:35	15:25
Total	60:30	36:45

**Table 3. The number of egg masses found in the CVWMA from 1997 to 2003. Numbers in brackets indicate the minimum number of egg masses based on the presence of tadpoles found in areas where no egg masses were located.**

	1997	1998	1999	2000	2001	2002	2003
West Calling Site	0	0	0	8	0 (1)	2	2
East Pond	1	3	9	8	12	1	4
East Dyke	0	0	0	0	0 (1)	2	0
Total	1	3	9	16	14+	5	6

We observed substantial egg mortality in two of six egg masses found in 2003 and moderate levels of early hatchling mortality in egg masses 3 and 5 prior to bringing the hatchlings into captivity (Table 4). The combination of egg and early hatchling mortality resulted in a significant reduction in hatching success in comparison to 2002 (65.6% versus 89%, respectively). Specimens submitted in 2003 for pathology were negative for Iridovirus.

**Table 4. Vital information for six *Rana pipiens* egg masses found in 2003.**

Egg Mass	Site	Date Discovered	Collection Date	Estimated Date Laid	Vol (cm <sup>3</sup> )	Estimated number of eggs	Dead Eggs	Dead Larvae *	Surviving Larvae**
1	East Pond	May 10	May 22	May 10	378	3671	1694 46%	177 5%	1800 49%
2	East Pond	May 14	May 26	May 14	360	3332	38 1%	45 1%	3249 98%
3	West Pond	May 15	May 26	May 14	490	3812	368 10%	650 17%	2794 73%
4	West Pond	May 22	May 25	May 15	924	2456	2300 94%	2 0.001 %	154 6 %
5	East Pond	May 25	June 1	May 24	484	3172	36 1%	692 22%	2444 77 %
6	East Pond	May 26	June 3	May 26	120	1662	200 12%	30 2%	1432 86 %
						18105	4636 25.6%	1596 8.8%	11873 65.6%)

\* all larvae brought into captivity

#### 4.2. Head Start

Between May 22, and June 3, 2003, 11,873 *R. pipiens* hatchlings were brought into captivity. All tadpoles were retained in captivity until they reached a minimum of Gosner stage 29, which took between 28 and 38 days and after which 4,464 *R. pipiens* tadpoles were released back into the wild; 493 into the Doran Marsh and 3,971 into the source population. Table 5 provides vital information for the tadpoles during this phase of the project.

**Table 5. Vital information for *Rana pipiens* tadpoles reared in captivity between May 22 and July 7, 2003**

Egg Mass	Breeding Site	Date Brought into Captivity	Tadpoles Collected	Mortality	Disease Monitoring Submissions	Tadpoles Released*	Captive Rearing	Tadpole Release Dates
1	East	22-May	1800	159	10	385	1246	June 19, 2003
2	East	26-May	3249	593	10	1396	1250	June 26, 2003
3	West	26-May	2794	98	10	1403	1283	July 3, 2003
4	West	25-May	154	77	10	0	67	-
5	East	1-Jun	2444	26	10	1138	1270	July 4, 2003
6	East	3-Jun	1432	30	10	142	1250	July 7, 2003
Total			11873	983 (8%)	60 (0.5%)	4464 (38%)	6366 (54%)	

\* Tadpoles released between Gosner stages 29 and 36.

### 4.3. Habitat Assessment

Using the criteria described in Appendix A, Doran Marsh was selected as a potential reintroduction site from five candidate sites assessed in the East Kootenays. Doran Marsh is located in the Bummers Flats Wildlife Management Area, near Fort Steel, and is a site where *R. pipiens* occurred historically (Fred Schueler, pers. comm.).

### 4.4. Captive Rearing and Release

Following the release of 4,464 *R. pipiens* tadpoles, 6,366 were retained and reared to metamorphosis. Vital information and time to metamorphosis is provided in Table 6. The first *R. pipiens* metamorphs emerged on July 18, 2003 and mass emergence occurred between July 29 and August 6, 2003, peaking on August 5, 2003. Mortality during this phase of the project was 2.6 %. By August 21, 2003, 89% of the tadpoles had completed metamorphosis and the remaining 640 tadpoles were released back into the source populations. Table 7 summarizes the numbers of tadpoles and metamorphs released in 2003 by site. In total, 5,108 *R. pipiens* metamorphs and 5,104 tadpoles were released. Table 8 summarizes the number of *R. pipiens* released since 2001.

**Table 6. Vital information and time to metamorphosis for *Rana pipiens* reared in captivity from July 7 to August 21, 2003.**

Egg	Initial stocking	Mortality	Disease Monitoring Submissions	Tadpoles Released*	Metamorphs Released	Days to Metamorphosis		
						1st	50%	95%
1	1246	9	51	23	1163	70	81	98
2	1250	29	128	26	1067	71	83	95
3	1283	42	97	463	681	79	92	NA
4	67	0	51	0	16	80	86	93
5	1270	37	75	18	1140	65	73	85
6	1250	49	50	110	1041	64	71	NA
Total	6366	166 (2.6%)	452 (7.1%)	640 (10.1%)	5108 (80.2%)			

\*Tadpoles released between Gosner stages 30 and 44

**Table 7. The number of *Rana pipiens* released at four sites in 2003. For each site, the numbers of larva released is in the left column and the number of metamorphs in the right.**

Egg Mass	East Breeding Pond		West Breeding Pond		Corn Creek Marsh		Doran Marsh		Totals	
1	408	532	0	0	0	255	0	376	408	1163
2	1422	316	0	0	0	230	0	521	1422	1067
3	0	0	1594	523	0	0	272	158	1866	681
4	0	0	0	13	0	0	0	3	0	16
5	935	358	0	0	0	208	221	574	1156	1140
6	252	403	0	0	0	286	0	352	252	1041
Subtotal	3017	1609	1594	536	0	979	493	1984	5104	5108
Total	4626 45 %		2130 21%		979 10%		2477 24%		10,212	

**Table 8. The number of *Rana pipiens* released at four sites in 2001, 2002, and 2003. For each site, the number of larvae released is provided in the left column and the number of metamorphs in the right column.**

Year	East Breeding Pond		West Breeding Pond		Corn Creek Marsh		Doran Marsh		Totals	
2001	0	0	0	0	0	496	0	0	0	496
2002	0	513	0	48	0*	1364	0	0	1315	1925
2003	3017	1609	1594	536	0	979	493	1984	5104	5108
Subtotal	3017	2122	1594	584	0	2839	493	1984	6419	7529
Total	5139 41%		2178 17%		2839 22%		2477 20%		12,633	

\*1,315 tadpoles released but none were likely to survive

#### 4.5. Temperature and Water Quality

pH values in the rearing tanks ranged between 7.5 and 10.4, and mean pH was 9.0 (N = 378). pH values were slightly lower in the tanks that were provided the artificial diet however the difference was minor (pH 8.8 versus pH 9.1).

Table 9 compares the minimum and average concentrations (mg/l) of dissolved oxygen in the rearing tanks by diet. In the artificial diet, DO levels increased substantially after July 12, 2003 when live marsh vegetation was added. Increases were also observed in the mixed and natural diets but not to the same degree and were likely due to an increase in photosynthesis resulting from warmer, sunnier weather.

**Table 9. Dissolved oxygen (mg/l) in rearing tanks under three dietary regimes.**

	Artificial	Mixed	Natural
Average DO to July 12	5.4	7.3	9.1
Average DO after July 12	10.8	11.4	11.3
Minimum DO	0.38	1.75	5.04

(NDO = 348)

Average daily temperatures at the rearing facility and at the breeding sites are shown in Figure 4. Temperatures in the rearing tanks were comparable to those observed in the breeding ponds (Table 10). Cumulative degree-days for each egg mass from the estimated spawn date to first emergence and to 50% and 95% metamorphosis are provided in Table 11.



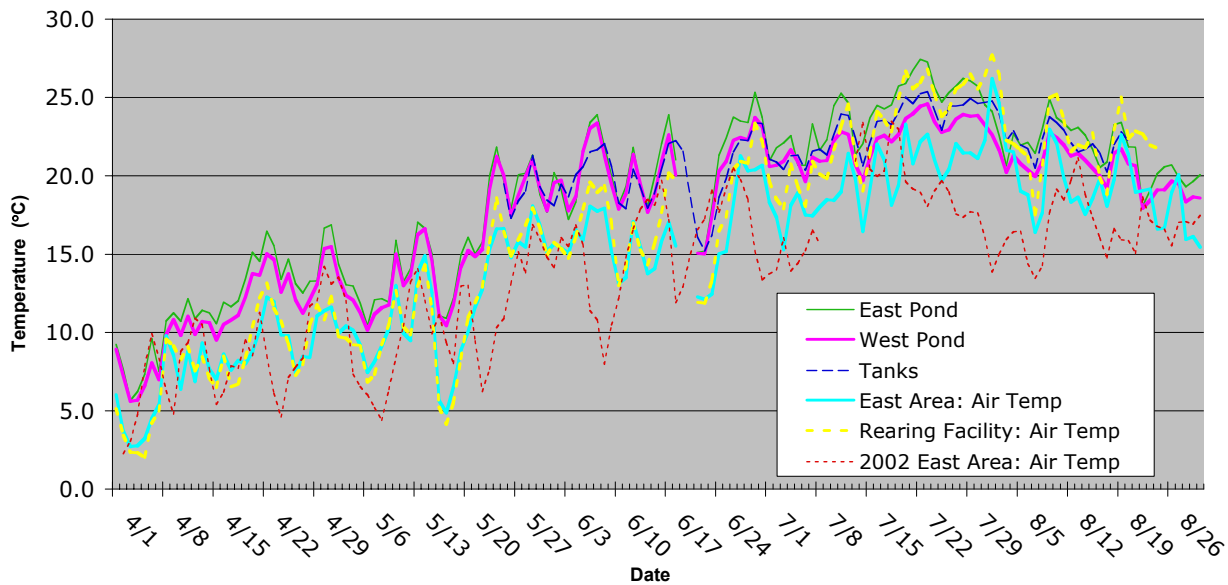


Figure 4. Mean daily air and water temperatures at the rearing facility and at the breeding area, April 1 to August 31, 2003.

Table 10. Average daily temperatures in the breeding ponds and in rearing tank 1.2. The minimum and maximum temperatures recorded during the period are in brackets.

	Month			
	May 25 - 30	June	July	August 1 - 18
Tank 1.2	19.0 (12.9 – 26.3)	20.0 (12.6 – 29.1)	23.1 (15.2-29.9)	22.3 (17.1-28.7)
East Pond	19.4 (11.8 – 30.3)	20.7 (9.8 – 33.6)	24.1 (14.5 – 34.9)	22.5 (16.8 – 31.1)
West Pond	19.2 (13.7-28.2).	20.2 (10.6 – 31.1)	22.3 (14.5 – 29.5)	21.1 (16.0 – 27.5)

Table 11. Cumulative degree-days (C°) from estimated spawn date and the time to first emergence, 50% metamorphosis and 95% metamorphosis.

Egg Mass	Cumulative Degree-days to Metamorphosis		
	1st Emergence	50% Metamorphosis	95% Metamorphosis
1	1091	1317	1634
2	1158	1396	1613
3	1321	1561	NA
4	1346	1456	1596
5	1135	1293	1600
6	1138	1275	NA
Mean and 95 % C.I.	1217 ± 114	1403 ± 120	1611 ± 32

#### 4.6. Growth and Development

Mean total tadpole lengths at day 45 are provided in Table 12. Tadpoles from egg mass 4 were substantially smaller than tadpoles from the other egg masses and were excluded from further analysis. The grand mean of tadpole length at day 45 was 49.0 mm (excluding egg mass 4) a 12% increase over the day 45 measurements from 2002 (43.7 mm; Adama et al. 2003). Median

Gosner stage at day 45 was 36 in 2003, compared to 32 in 2002. Contrary to what we anticipated, mean total tadpole length did not correlate to size at metamorphosis (Table 13; correlation coefficient = 0.15).

**Table 12. Mean total length for *Rana pipiens* tadpoles reared for 45 days in captivity.**

Egg Mass	Tanks (N)	Mean	Std Deviation	95% confidence intervals
1	5	47.0	4.41	41.5 - 52.5
2	5	48.1	4.59	42.4 - 53.8
3	5	44.0	2.10	41.4 - 46.6
4	1	26.8	3.45	25.4 - 28.2
5	5	52.0	3.08	48.2 - 55.8
6	5	54.0	3.25	50.0 - 58.0
Grand Mean*		49.0	4.9	47.0 - 51.0

\* excluding egg mass 4

The size of recently metamorphosed *R. pipiens* are provided in Table 13. SVL differed significantly between egg masses ( $p=0.0009$ ) and between diets ( $p = 0.0029$ ); however there did not appear to be an interaction between the two factors ( $p = 0.59$ ). Tadpoles from egg masses 1 and 2 were significantly larger than tadpoles from egg masses 3 and 6, but not egg mass 5. Tadpoles reared under a natural diet were smaller than tadpoles fed either a mixed or an artificial diet, by 1.51 and 2.04 mm respectively. Due to an outbreak of *Saprolegnia* in tanks containing the natural diet, these results must be interpreted with caution.

**Table 13. The size of *Rana pipiens* reared in 2003 by egg mass and diet.**

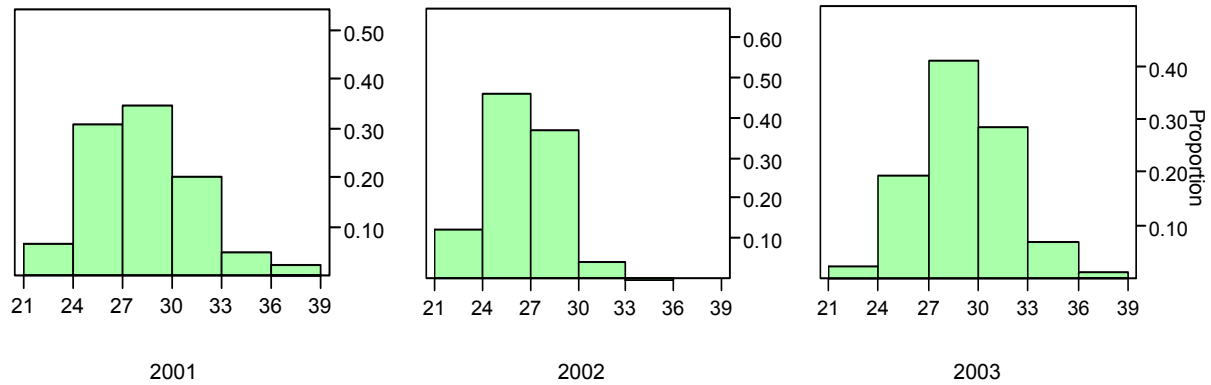
Egg Mass	Tanks (N)	Tadpoles (n)	Mean SVL (mm)	Std Dev	95% CI	Mean Weight (g)	Std Dev	95% CI
1	5	322	30.5	1.30	28.7 - 32.0	2.09	0.31	1.7 - 2.5
2	5	274	30.7	1.39	28.9 - 32.4	2.33	0.27	2.0 - 2.7
3	5	251	27.5	0.84	26.5 - 28.5	1.72	0.16	1.5 - 1.9
4	1	15	28.2	-		1.73	.	
5	5	278	28.9	0.80	27.9 - 29.9	1.83	0.24	1.5 - 2.1
6	5	259	27.3	1.54	25.4 - 29.2	1.65	0.33	1.2 - 2.1
Diet	Tanks (N)	Tadpoles (n)	Mean SVL (mm)	Std Dev	95% CI	Mean Weight (g)	Std Dev	95% CI
Artificial	6	388	30.1	1.50	28.5 - 31.7	2.21	0.30	1.9 - 2.5
Natural	9	465	27.7	1.25	26.8 - 28.7	1.67	0.18	1.5 - 1.8
Mixed	10	546	29.3	1.84	28.0 - 30.7	1.97	0.36	1.7 - 2.2

Captive reared *R. pipiens* were significantly larger at metamorphosis in 2003 than in 2002 and were slightly larger than animals reared in 2001 (Table 14). More importantly, the number of *R. pipiens* metamorphs larger than 30 mm (Figure 5) was much higher in 2003. We estimate that 137 (28%), 80 (4%) and 1,890 (37%) were 30 mm or greater in 2001, 2002, and 2003 respectively.

Time to metamorphosis in 2003 was considerably less than in 2002. In 2002, 68 % of the tadpoles completed metamorphosis in 134 days while in 2003, 89% completed metamorphosis in 91 days.

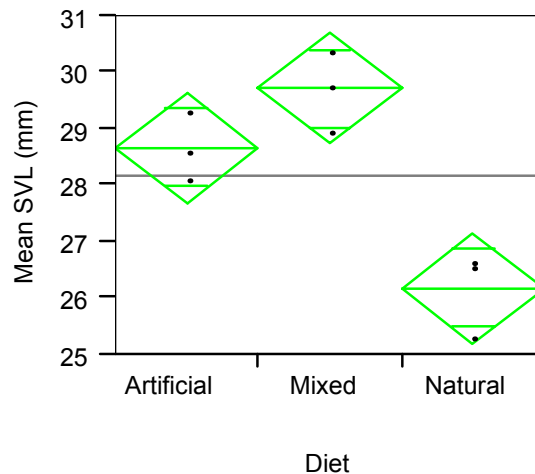
**Table 14. The size of *Rana pipiens* metamorphs reared in 2001, 2002, and 2003.**

Year	Tadpoles (n)	Mean SVL (mm)	Std Dev. SVL	SVL 95% CI	Mean Mass (g)	Std Dev Mass	Mass 95% CI
2001	123	28.4	3.18	27.8 – 28.9	NA	NA	NA
2002	1924	26.3	2.10	26.2 – 26.4	1.61	0.39	1.58 – 1.62
2003	1399	29.1	2.78	28.9 – 29.2	1.95	0.57	1.92 – 1.98



**Figure 5. Frequency histograms of *Rana pipiens* snout-to-vent lengths reared in 2001, 2002 and 2003.**

In an experiment where 180 tadpoles were randomly selected and reared under three diets from July 12 to August 19, 2003, tadpoles reared on a mixed diet of artificial (kale, watercress, and bloodworm) and natural (native aquatic marsh plants) food were larger than tadpoles reared on either the artificial or natural diets (Figure 6).



**Figure 6. Mean diamonds for *Rana pipiens* tadpoles reared on three diets from July 12 to August 21, 2003. 180 tadpoles were allocated randomly to 9 tanks and each diet was replicated 3 times. The middle line represents the mean, and the upper and lower points represent the 95% confidence intervals.**

#### 4.7. Mortality and Disease Monitoring

We documented 790 *R. pipiens* mortalities while they were held in captivity (Table 5 and Table 6). These were categorized as either accidental (n=78), due to abnormalities (n=22), or unknown (n=690). Accidental mortality resulted from improper drainage of the rearing tanks and trauma sustained while cleaning tanks or while handling the animals. Abnormalities were rare,

consisting of incomplete tail development, abnormal gill development or incomplete development of the digestive tract.

The bulk of the mortality occurred during two episodes (Figure 7). The first episode occurred at the end of May and was due to a combination of accidental (n=49) and unknown mortality (n=123). The second episode occurred between June 18 and June 29, 2003, where 484 mortalities were documented, the majority occurring in Tank 2.3 (n=320). Many of these tadpoles were emaciated and infected with *Saprolegnia*. The week prior to this die-off (June 10), the diets in these tanks were changed from an artificial diet to natural vegetation. Due to miscommunication, the natural vegetation was frozen rendering the plants into an organic soup, which resulted in poor water quality and tadpole starvation. This situation was rectified by conducting 100% water changes and by adding live aquatic plants. While mortality subsided shortly afterwards, tadpole growth in these tanks was affected (Table 13).

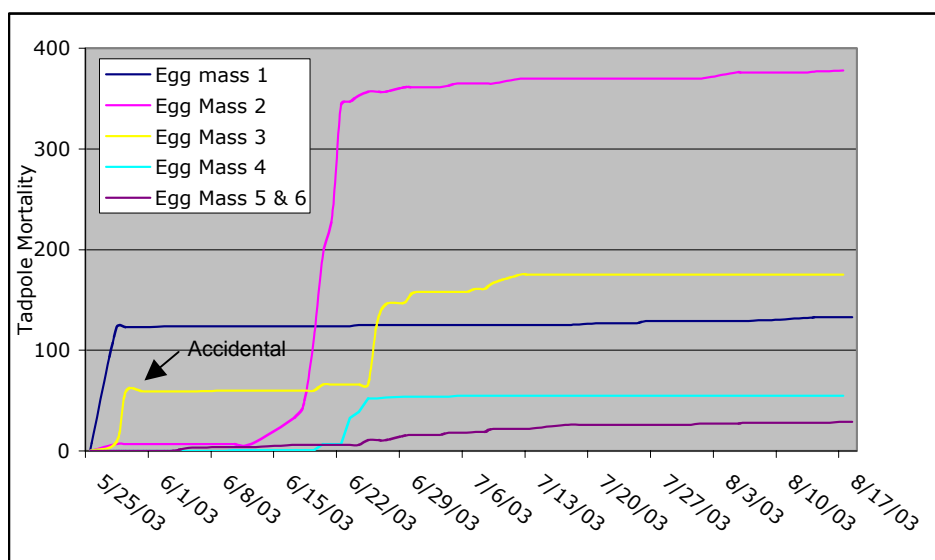


Figure 7. Cumulative mortality in captive reared *Rana pipiens*, May 25 to August 17, 2003.

Table 15 summarizes the number of tadpoles submitted for routine disease monitoring (n = 360) and necropsy (n = 122). All specimens were negative for PCR (Iridovirus and chytrid fungus), toxicology, and viral culturing.

Table 15. The number of *Rana pipiens* larvae submitted for disease monitoring.

Egg Mass	Routine		Diagnostic		Total
	Iridovirus PCR*	Chytrid PCR**	WHC*	AHC**	
1	35	25	0	0	60
2	35	25	65	11	136
3	45	35	0	0	80
4	15	5	31	10	61
5	45	35	0	5	85
6	35	25	0	0	60
<b>Total</b>	<b>210</b>	<b>150</b>	<b>96</b>	<b>26</b>	<b>482</b>

\* Diagnosis conducted by the Wildlife Health Centre, Saskatoon, SK

\*\* Diagnosis conducted by the Animal Health Centre, Abbotsford, BC

#### 4.8. Post-release monitoring

Table 16 summarizes the survey effort and the number of *R. pipiens* and *R. luteiventris* captured between April 1 and October 27, 2003. Unmarked young of the year (YOY) *R. pipiens* captured in the source populations between September 21 and October 24, 2003, were significantly larger ( $p=0.0001$ ) than marked YOY captured in the source population or YOY captured in Corn Creek Marsh and Doran Marsh (Table 17 and Figure 8). This is important as it indicates that the animals reared to metamorphosis were substantially smaller than animals that had completed metamorphosis in the wild. It should be noted that at least some and perhaps even all of the unmarked young of the year (YOY) *R. pipiens* may have been from the 4,464 *R. pipiens* released as tadpoles between June 19 and July 7, 2003 (Table 5).

**Table 16. Survey effort and results for post-release monitoring surveys.**

Location	Survey Type and Period	Survey Days	Time	<i>Rana luteiventris</i>	<i>Rana pipiens</i>
Corn Creek	Spring-Visual	1	2:00	0	0
	Spring-Calling	2	2:50	0	0
	Fall-Visual	4	13:15	n/a	30
	Fall-Selkirk College	1	68:00	34	50
Source Population	Fall Visual	9	38:10	0	104
Doran Marsh	Fall Visual	1	2:00	2	11
<b>Grand Total</b>		18	126:15	36	195

**Table 17. Size of marked and unmarked young-of-year *Rana pipiens* captured in the fall of 2003.**

Site	Description	N	Mean Weight (g)	Stdev Weight (g)	Mean SVL (mm)	Stdev SVL (mm)
Source Population	Marked	15	9.0	2.5	44.5	3.9
	Unmarked	63	12.4	3.9	49.3	5.4
Corn Creek	Staff	15	7.9	2.4	43.3	3.9
	Selkirk College	50	8.2	3.1	38.6	6.3
Bummers Flats		11	7.1	3.1	38.4	7.5

On October 24, 2003, a male leopard frog reared in captivity in 2002 was located near the east-breeding site. This animal appeared healthy and was 45 grams/74.4 mm SVL. The white VIE markings, distinguishing it as an animal reared in 2002, were easily visible (Figure 9). Figure 12 shows the area surveyed and capture locations in Corn Creek from the survey conducted on September 25, 2003 by the Selkirk College students.

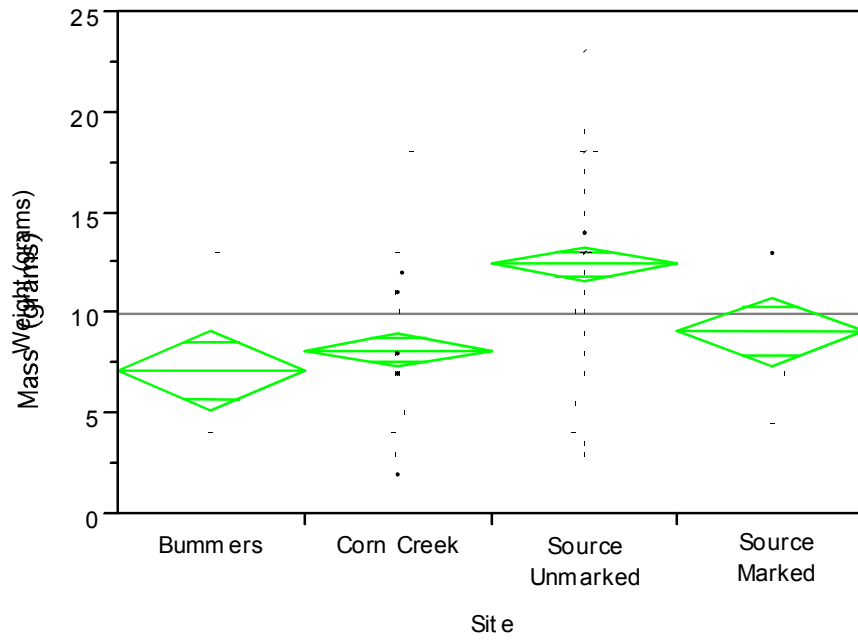


Figure 8. Mean weight of young-of-year *Rana pipiens* caught in the fall of 2003.



Figure 9. White VIE markings of a *Rana pipiens* released in 2002 and recaptured in 2003.

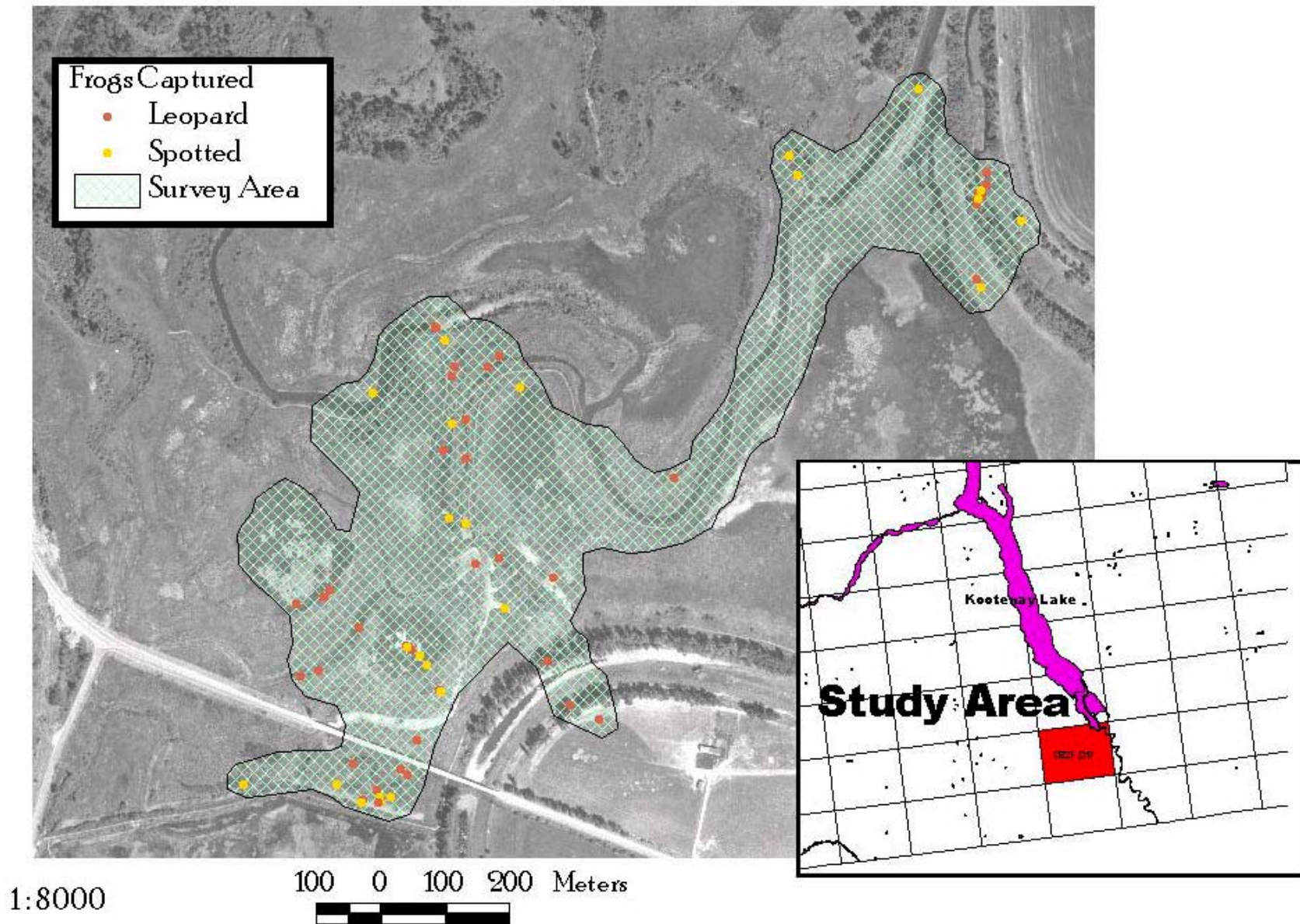


Figure 10. The location of *Rana pipiens* and *Rana luteiventris* captured by Selkirk College students in Corn Creek Marsh, September 25, 2003. Thatching denotes the survey area.

## 5. DISCUSSION

### 5.1. Breeding activity

*Rana pipiens* breed at temperatures above 10°C (Hine et al. 1981; Seburn 1992). In 2002 and 2003, average air temperatures in mid-May were well below 10°C which may explain the decline in calling activity and egg mass production from previous years. While we anticipate amphibian populations to fluctuate in response to environmental stochasticity (Alford & Richards 1999; Pechmann 2003; Pechmann et al. 1991; Pounds 2001), these fluctuations can be determinant to small, isolated populations (Caughley 1994). Due to the precarious status of the population, we recommend that head-starting be continued until the factors suppressing the extant population can be identified and remediated or after viable populations are established in other areas.

### 5.2. Tadpole Collection and Rearing

At the direction of the recovery team, all *R. pipiens* hatchlings from all egg masses located in the source population were brought into captivity. This was facilitated by staggering the stocking regime, which enabled us to initially house almost 12,000 tadpoles. While this strategy worked extremely well, it greatly increased the demands on the rearing staff and hindered our ability to monitor water quality. Often we had to forgo water quality monitoring to keep up with feeding and maintenance. One of the drawbacks of the staggered stocking regime is that it does not lend itself to controlled experiments as the stocking densities were changed every two weeks for the first month. Despite these drawbacks, this strategy was effective in maximizing the capacity of the rearing facility. Although the recovery team provided direction to bring up to 20,000 hatchlings into captivity, a number of approximately 13,000 is more realistic given the existing capacity of the rearing facility.

### 5.3. Diet

The diets adopted in 2003 were a significant improvement over those provided in previous years and resulted in faster growth and increased size at metamorphosis. This improvement was achieved by changing the food items, increasing the food availability and altering the feeding regime. In particular, we found that providing an abundance of shredded kale and watercress every 4 to 5 days was more effective and less time consuming to prepare than providing daily rations.

The results of the diet experiment suggest a mixed diet of kale, watercress, live marsh vegetation and bloodworm offers several advantages over completely natural or artificial diets. Under a mixed diet, size at metamorphosis was comparable to tadpoles reared on an artificial diet but water quality was noticeably better. Providing a mixed diet is also advantageous as it provides additional flexibility in the feeding schedule, as an abundance of food is always available. In future years, we recommend the mixed diet be provided to the 4,000 litre rearing tanks while experiments be confined to smaller aquaria. This will help simplify the feeding regime and reduce the number of water changes required to maintain water quality, ultimately reducing the workload on the husbandry staff.

It should be noted that there are some obvious limitations with our diet experiment, as we did not strictly control factors such as water quality and temperature, nor did we attempt to address the specific nutritional requirements of *R. pipiens* larvae.



#### 5.4. Temperature and Water Quality

Average daily temperatures in the rearing tanks were within the preferred range of 20 to 25 °C reported for *R. pipiens* larvae (Lucas & Reynolds 1967). One of the benefits of the rearing tanks is that they appear to moderate temperature in comparison to the breeding pond. When the temperature in the breeding ponds reached 34.9 °C, temperatures in the rearing tanks did not exceed 30°C (Table 10). This is important as the critical maximum temperature for *R. pipiens* larvae is between 37° and 40 °C (Noland & Ultsch 1981), however they likely begin to experience physiological stressed several degrees lower. In addition to moderating temperatures at the upper extreme, the rearing tanks did not cool down at night as much as the rearing ponds. This is advantageous as cooler temperatures slows both larval growth and development (Alford 1999).

Cumulative degree-day data are not commonly reported for amphibians. In a study on *R. pipiens* in Manitoba, Eddy (1976) compared cumulative degree-days to tadpole growth and found, unsurprisingly, that body length correlated highly with cumulative degree-days, however analysis of covariance found the slopes of the regressions to be unequal suggesting other factors are at play. Based on our experiments conducted in 2002 and 2003, it is apparent that diet can have a large influence on both the growth and developmental rates of *R. pipiens* larvae. While the data collected this year provides some interesting baseline information, cumulative degree-days should be calculated in future years to further investigate it's influence on time to metamorphosis. This could be beneficial by enabling us to predict and prepare for the mass emergence of captive animals.

In many of the rearing tanks, dissolved oxygen in often dropped well below 50% saturation (4.6 mg/l @ 25° C, sea level), however this did not appear to have a negative affect on their growth or development. Noland and Ultsch (1981) found that *R. pipiens* larvae frequently occurred in aquatic habitats below 50 % oxygen saturation, often at levels below 2 ppm (mg/l). While *R. pipiens* tadpoles appear to do well under hypoxic conditions, there are benefits in maintaining higher DO levels. Nitrifying bacteria convert toxic ammonia to nitrite and nitrate require oxygen to thrive. We found that adding native marsh plants increased the DO levels from below 20% to over 80% saturation in just a few days and visual water quality improved noticeably. We did not monitor ammonia levels but we recommend doing so in the future. Given the ability of *R. pipiens* larvae to thrive under hypoxic conditions, DO may not be a useful indicator of water quality for this species, at the later stages of development (Gosner stage 30 and greater). In future, a broader spectrum of water quality parameters should be monitored (Whitaker 2001; Wind 2002).

#### 5.5. Growth and Metamorphosis

Tadpole lengths measured at day 45 provides an indication of how metamorphosis is proceeding (Adama et al. 2003). Although the day 45 measurements observed in 2003 indicated that the tadpoles were larger and developing faster than tadpoles in 2002, they were not useful in predicting size at metamorphosis. Nevertheless, we recommend that these measurements be continued as they provide an opportunity to assess larval growth and correct factors that may be having a negative influence on either growth or development.

In 2002, we identified several challenges in rearing *R. pipiens* in an artificial environment to a size and within the time frame observed in the wild or in semi-natural environments (Adama et al. 2003). In 2003, we were able to partially address these challenges resulting in an increase in size at metamorphosis and decreased developmental times in comparison to our results from

2002. We largely attribute this to an improved diet and more specifically to an increase in food availability as suggested in section 5.3.

## 5.6. Mortality and Disease

Unfortunately, we were not able to determine the cause of the egg and hatchling mortality observed in 2003. Most of the eggs appeared to have been fertilized and we did not observe *Saprolegnia* infections or other disease, although it is possible that the egg and hatchling mortality may have been due to extraneous factors such as water quality, reflect the health of the adult population, or may be a function of inbreeding depression.

In the spring of 2003, we observed a number of adult *R. pipiens* infected with chytridiomycosis, however since this disease requires keratin (Fellers et al. 2001; Longcore et al. 1999), it cannot be passed on to or infect the eggs as they lack keratin. While chytrid may not have directly killed the eggs or the hatchlings, it may have indirectly influenced the viability of the eggs or sperm by reducing the health of the adults. Another plausible factor may be that high egg and hatchling mortality may be a result of inbreeding depression. Hoffman (2001) found the Creston population of *R. pipiens* to be low in genetic diversity in comparison to other populations found in western North America and suggests that the population may be experiencing “substantial genetic drift and inbreeding” and may be at risk of extinction as a result. Inbreeding depression can be particularly risky to small isolated populations (Frankham 1995; Rowe & Beebee 2003) influencing fitness parameters such as fertility and immune function.

Larval mortality in captivity was low despite a *Saprolegnia* infection that resulted from improper feeding and poor water quality. To avoid the error made in 2003, careful instructions and supervision should be provided when critical changes are made to husbandry procedures. Changes to diet should be made slowly, over a week to allow the tadpoles to adjust to the new diet (Dr. Stephen Raverty, pers. comm.).

## 5.7. Release and Reintroduction

In 2003, 5,108 metamorphs and 5,104 tadpoles were released into four sites including three sites in the Creston Valley and into Doran Marsh in the East Kootenays. The release of *R. pipiens* into Doran Marsh is significant as it marks the return of *R. pipiens* to the East Kootenays, where they have not been observed in over 20 years. Two public events were held to commemorate the reintroduction effort generating a great deal of public interest with over 60 children and adults in attendance including many of the project supporters.

In 2001, pond 1 in Corn Creek Marsh was selected as a reintroduction site due to its proximity to potential breeding and over-wintering habitat. While we still considered this one of the best sites reintroduction site in the CVWMA to reintroduce *R. pipiens*, several aspects of this site may hinder a successful reintroduction. First, reed canary grass (*Phalaris arundinacea*) occurs in very dense stands covering much of the upland habitat making it difficult to conduct surveys and rendering much of the upland habitat unsuitable for *R. pipiens*. Second, *R. luteiventris* occurs in this pond and little is known about how the species interact and whether *R. luteiventris* is susceptible to or acts as a carrier for chytridiomycosis. Thirdly, the pond is susceptible to flooding during spring freshet and in the summer of 2002, the marsh was almost completely flooded eliminating almost the entire upland habitat. This is problematic, because flooding lowers water temperature, enables fish to access the isolated ponds, and may force the adult *R. pipiens* out of the compartment. Consequently, we urge the recovery team to re-evaluate Corn Creek Marsh as a reintroduction site.

## 5.8. Post-release Monitoring

To date, relatively little effort was expended searching for animals released in previous years. In a reintroduction program for *R. pipiens* in Alberta, males were not heard calling until the 4<sup>th</sup> year of the project after 4,500 *R. pipiens* metamorphs had been released (Kendell 2003a). The finding of an animal released in 2002 demonstrates that captive reared animals are surviving. We anticipate finding more animals in future years, as more resources will be allocated to post-release monitoring.

Unmarked *R. pipiens* captured in the source population in the fall of 2003 were significantly larger than animals that were reared to metamorphosis in captivity and recaptured in either the source population or at the reintroduction sites during the same period. This comparison is important as it indicates that even after 2 to 3 months in the wild, animals reared in captivity are not able to compensate for their small size at metamorphosis. Unfortunately, we were unable to determine whether the unmarked animals caught in the source population were animals that were head started or part of a wild population. If this was known, it could have important implications for the reintroduction program, particularly if it could determine whether releasing tadpoles at a specific stage is more advantageous than releasing fully transformed metamorphs.

## 6. CONCLUSION

We were successful in meeting our objectives in 2003. 5,108 *R. pipiens* metamorphs and 5,104 *R. pipiens* tadpoles were released at four sites, including a new site established in the East Kootenays, an experiment demonstrated the benefits of using a mixed diet of natural marsh vegetation, kale, watercress and bloodworm, and post-release monitoring surveys provided important information on the growth of captive reared *R. pipiens* in the wild. While these are significant achievements, a number of recommendations are provided that will be valuable in improving the success of the recovery effort. These include suggestions to improve and simplify husbandry procedures and the recommendations that research be conducted on post-metamorphic survival, that the recovery team re-evaluate Corn Creek Marsh as a reintroduction site, and that the husbandry manual be updated to reflect current husbandry practices.

## 7. LITERATURE CITED

- Adama, D., M. A. Beaucher, and K. Lansley. 2003. Captive rearing and reintroduction of northern leopard frogs in British Columbia, 2003. Columbia Basin Fish and Columbia Wildlife Compensation Program, Nelson.
- Adama, D., and K. Kendell. 2004, in press. Rearing *Rana pipiens* for conservation: A discussion paper comparing two approaches to captive rearing. Page 14. Species At Risk 2004: Pathways to Recovery, Victoria, BC.
- Adama, D. B., M. A. Beaucher, and T. Antifeau. in prep. Northern leopard frogs in the Creston Valley Wildlife Management Area : progress report 2000 through 2004. Columbia Basin Fish and Wildlife Compensation Program Area, Nelson.
- Adama, D. B., K. Lansley, and M. A. Beaucher. 2002. A small scale rearing effort for the reintroduction of *Rana pipiens* in the Creston Valley Wildlife Management Area. Columbia Basin Fish and Wildlife Compensation Program, Nelson, B.C.
- Alford, R. A. 1999. Ecology: resource use, competition, and predation. Pages 240-278 in R. W. McDiarmid, and R. Altig, editors. Tadpoles: The Biology of Anuran Larvae. University of Chicago Press, Chicago.
- Alford, R. A., and S. J. Richards. 1999. Global amphibians declines: a problem in applied ecology. Annual Review of Ecology and Systematics **30**:133-165.
- ASIH. 1997. Guidelines for use of live amphibians and reptiles in field research. American Society of Ichthyologists and Herpetologists.
- Beaucher, M. B. A. 2001, draft. Decontamination protocol for the Creston Valley Wildlife Management Area. Columbia Basin Fish and Wildlife Compensation Program.
- Berger, L., R. Speare, P. Daszak, D. E. Green, A. A. Cunningham, C. L. Goggin, R. Slocombe, M. A. Ragan, A. D. Hyatt, K. R. McDonald, H. B. Hines, K. R. Lips, G. Marantelli, and H. Parkes. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. Proc Nat Acad Sci USA **95**:9031 - 9036.
- Berns, M. W. 1965. Mortality caused by kidney stones in spinach-fed frogs (*Rana pipiens*). Bioscience **15**:297-298.
- Bosch, J., I. Martinez-Solano, and M. Garcia-Paris. 2001. Evidence of a chytrid fungus infection involved in the decline of the common midwife toad (*Alytes obstetricans*) in protected areas of central Spain. Biological Conservation **97**:331-337.
- Braumandl, T. F., and M. P. Curran 1992. A field guide for site identification and interpretation for the Nelson Forest Region. British Columbia Ministry of Forests, Victoria.
- Briggs, R., and M. Davidson. 1942. Some effects of spinach feeding on *Rana pipiens* tadpoles. J. Exp. Biol. **90**:401-411.
- Caughley, G. C. 1994. Directions in conservation biology. Journal of Animal Ecology **63**:215-244.
- CDC. 2001. Provincial Red and Blue Lists –2001. Conservation Data Centre, Ministry of Water, Land and Air Protection, BC Government, Victoria, BC.
- COSEWIC. 2001.
- Csuti, B., and B. Seller. 2000. Dietary requirements of larval oregon spotted frogs: final report to the U.S. Fish and Wildlife Service, Pacific Northwest. Page 4 pp. Oregon Zoo, Portland Oregon.
- Denton, J. S., S. P. Hitchings, T. J. C. Beebee, and A. Gent. 1997. A recovery program for the natterjack toad (*Bufo calamital*) in Britain. Conservation Biology **11**:1329-1338.
- Dodd, C. K. J., and R. A. Seigel. 1991. Relocation, repatriation, and translocation of amphibians and reptiles: are they conservation strategies that work? Herpetologica **47**:336-350.
- Fellers, G. M., D. M. Green, and J. E. Longcore. 2001. Oral chytridiomycosis in the mountain yellow-legged frog (*Rana muscosa*). Copeia **2001**:945-953.

- Frankham, R. 1995. Conservation genetics. *Annual Review of Genetics* **29**:305-327.
- Gebhart, D., and M. Roberge. 2001. N. Leopard Frog Recovery Project: CVWMA Fish Inventory. Page 12. Aquatic Resources Limited, Nelson, B.C.
- Gillies, C., and R. Franken. 1999. East Kootenay northern leopard frog project. Living Landscapes Program, Royal British Columbia Museum, Victoria, BC.
- Gosner, K. L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologia* **16**:183-190.
- Green, D. M., and C. Kagarise Sherman. 2001. Diagnostic histological findings in Yosemite toads (*Bufo canorus*) from a die-off in the 1970's. *Journal of Herpetology* **35**:92-103.
- Hine, R. L., B. L. Les, and B. F. Hellmich. 1981. Leopard frog populations and mortality in Wisconsin, 1974-76. Wisconsin Department of Natural Resources, Madison, WI.
- Hoffman, E. A., and M. S. Blouin. in press-a. Evolutionary history of the northern leopard frog: reconstruction of phylogeny, phylogeography, and historical changes in population demography from mitochondrial DNA. *Evolution*.
- Hoffman, E. A., and M. S. Blouin. in press-b. Historical data refute range contraction cause of low genetic diversity in isolated frog population. *Molecular Ecology*.
- Hopkins, S., and A. Channing. 2003. Chytrid fungus in northern and western Cape frog populations, South Africa. *Herpetological Review* **34**:334-336.
- IUCN. 1995. IUCN/SSC Guidelines for re-introduction. International Union for Conservation of Nature and Natural Resources.
- Jackman, P. 2002. unpublished data. Page 25 in E. Wind, editor. Northern Leopard Frog (*Rana pipiens*) Husbandry Manual. Columbia Basin Fish and Wildlife Compensation Program, Nelson, B.C.
- Jamieson, B. 1990. Bumpers Flats Wildlife Management Area: management plan. Ministry of Environment, BC Government, Cranbrook.
- Kendell, K. 2003a. Northern leopard frog reintroduction: Year 4 (2002). Page 15. Alberta Sustainable Resource Development, Edmonton.
- Kendell, K. 2003b. Status of the Northern Leopard Frog (*Rana pipiens*) in Alberta : Update 2003. Page 73. Alberta Conservation Association, Edmonton.
- Longcore, J. E., A. P. Peskier, and D. K. Nichols. 1999. *Batrachochytrium dendrobatidis* gen et sp. nov., a chytrid pathogenic to amphibians. *Mycologia* **91**:219-227.
- Lucas, E. A., and W. A. Reynolds. 1967. Temperature selection by amphibian larvae. *Physiol Zoo* **40**:159-171.
- McAllister, K. R., and W. P. Leonard. 1996. The status of the northern leopard frog (*Rana pipiens*) and the western toad (*Bufo boreas*) in Washington state. Pages 45-46 in E. D. Koch, G. Wood, C. R. Peterson, and P. S. Corn, editors. A summary of the conference on declining and sensitive amphibians in the Rocky Mountains and Pacific Northwest, Boise, Idaho.
- Noland, R., and G. R. Ultsch. 1981. The roles of temperature and dissolved oxygen in microhabitat selection by tadpoles of a frog (*Rana pipiens*) and a toad (*Bufo terrestris*). *Copeia* **1981**:645-652.
- Ohanjanian, I. A., D. Cunnington, D. Adama, T. Antifeau, L. Friis, D. Fraser, M.-A. Beaucher, J. Krebs, and B. Stushnoff. 2004. Northern leopard frog southern mountain population draft recovery strategy. unpublished report to the CBFWCP, Nelson, BC.
- Ohanjanian, I. A., D. Cunnington, and NLFRT. 2003, draft. Northern Leopard Frog Southern Mountain Population Recovery Strategy. unpublished report to the CBFWCP, Nelson, BC.
- Ohanjanian, I. O., and I. E. Teske. 1996. Herpetological surveys of the 87 wetlands in the Columbia Basin Fish and Wildlife Compensation Program area. Columbia Basin Fish and Wildlife Compensation Program, Nelson, B.C.

- Pechmann, J. H. K. 2003. Natural population fluctuations and human influences: null models and interactions. Pages 85-93 in R. D. Semlitsch, editor. Amphibian Conservation. Smithsonian Institution, Washington.
- Pechmann, J. H. K., D. E. Scott, R. D. Semlitsch, J. P. Caldwell, Vitt, L.J., and J. W. Gibson. 1991. Declining amphibian populations: the problem of separating human impacts from natural fluctuations. *Science* **253**:892-895.
- Pounds, J. A. 2001. Climate and amphibian declines. *Nature* **410**:639-640.
- RIC. 1998a. Inventory methods for pond-breeding amphibians and painted turtle: standards for components of British Columbia's biodiversity. Page 94. Resources Inventory Committee, Ministry of Environment, Lands and Parks, Victoria, BC.
- RIC. 1998b. Live animal capture and handling guidelines for wild mammals, birds, amphibians and reptiles. Page 47 pp. Resources Inventory Committee, Ministry of Environment, Lands and Parks., Victoria, BC.
- Rowe, G., and T. J. C. Beebee. 2003. Population on the verge of a mutational meltdown? Fitness costs of genetic load for an amphibian in the wild. *Evolution* **57**:177-181.
- Seburn, C. N. L. 1992. Leopard frog project: field report 1991. Unpublished report. Alberta Fish and Wildlife, Edmonton.
- Seburn, C. N. L., and D. C. Seburn. 1998. Status report on the northern leopard frog (*Rana pipiens*) in Canada (western populations). Committee on the Status of Endangered Wildlife in Canada, Ottawa, Ont.
- Stebbins, R. C., and N. W. Cohen 1995. A Natural History of Amphibians. Princeton University Press, Princeton, NJ.
- Wagner, G. 1997. Status of the Northern Leopard Frog (*Rana pipiens*) in Alberta. Alberta Environmental Protection, Wildlife Management Division, Edmonton, AB.
- Waye, H. L., and J. M. Cooper. 2000. Status of the Northern leopard frog (*Rana pipiens*) in the Creston Valley Wildlife Management Area 1999. Columbia Basin Fish and Wildlife Compensation Program, Nelson, BC.
- Werner, J. K. 2003. Status of the northern leopard frog (*Rana pipiens*) in western Montana. *Northwest Naturalist* **84**:24-30.
- Whitaker, B. R. 2001. Chapter 12: Water Quality. Pages 147-157 in K. M. Wright, and B. R. Whitaker, editors. Amphibian Medicine and Husbandry. Krieger, Malibar, FL.
- Wind, E. 2002. Northern leopard frog (*Rana pipiens*) husbandry manual. Columbia Basin Fish and Wildlife Compensation Program, Nelson, BC.
- Wright, K. M., and B. R. Whitaker 2001. Amphibian Medicine and Captive Husbandry. Krieger Publishing Company, Melbourne, FL.

## Appendix A: Habitat Assessment Criteria

Criteria	Weighting	Description
<b><u>General Habitat</u></b>		
All 3 seasonal habitats present	*	
Movement	*	No barriers between seasonal habitats
Adjacency of habitats	*	Spring and winter habitats less than 1 km apart
	**	Spring and summer habitats less than 200 m apart
Pollution	*	Proximity to agricultural, industrial point sources
<b><u>Summer Habitat</u></b>		
Suitable habitat	*	Grass meadow adjacent water course
Low slope	*	Not too steep: less than 20 degrees
Accessible	*	Vegetation not too dense.
<b><u>Spring Habitat</u></b>		
Suitable habitat	*	Shallow, open water less than 30 cm deep
		Open area greater than 500 m <sup>2</sup>
Water regime	*	Does not dry up before mass emergence August 30
	**	Drys up after mass emergence after August 30
Warm temperature	*	Not cooled by adjacent water sources
Fish free	**	
Aquatic vegetation	*	Food available for tadpoles
Water quality	*	pH over 7
<b><u>Winter</u></b>		
High DO	*	Over 10 mg/l
Does not freeze to bottom	*	
<b><u>Logistical</u></b>		
Water control mechanisms	**	Ability to control water levels
Land status	**	Crown, protected, private, First Nations
	**	Monitoring easily accomplished, site access, sight-ability
Monitoring access		
Historically occupied by <i>R. pipiens</i>	**	

\* Single asterisk indicates critical attributes

\*\* Double asterisk indicates desired but not critical criteria

## Appendix A Continued: Habitat Assessment

Criteria	Site					
	Cherry Creek	Doran Marsh	Penny's Marsh	Stony Lake	Surveyors Lake	
<b>General Habitat</b>						
1) All 3 seasonal habitats present	*	Y	Y	?	N	N
2) No barriers between seasonal habitats	*	Y	Y	Y	?	?
3) Not adjacent agricultural or industrial point sources		Y	Y	Y	Y	Y
4) Spring and winter habitats less than 1 km apart	*	Y	Y	Y	?	?
5) Spring and summer habitats less than 200 m apart	*	Y	Y	Y	?	?
6) Area could potentially support more than one population		Y	Y	Y	N	Y
<b>Summer Habitat</b>						
7) Grass meadow present and adjacent water course	*	Y	Y	Y	Y	N
8) Not too steep: less than 20 degrees	*	Y	Y	Y	Y	Y
9) Vegetation not too dense	*	Y	Y	Y	Y	Y
<b>Spring Habitat</b>						
10) Shallow, open water less than 30 cm deep	*	Y	Y	Y	N	N
11) Open area greater than 500 m <sup>2</sup>		Y	Y	Y	Y	Y
12) Does not dry up before August 15 to 30	*	Y	Y	Y	Y	Y
13) Dries up after August 30th		N	N	N	N	N
14) Water warmer than 20 Celsius	*	N	Y	N	?	?
15) Fish free		N	N	?	N	N
16) Abundant aquatic vegetation	*	Y	Y	N	?	N
17) pH over 7	*	Y	Y	Y	Y	Y
<b>Winter Habitat</b>						
18) Overwinter habitat with high DO	*	Y	Y	Y	Y	Y
19) Does not freeze to bottom	*	Y	Y	Y	Y	Y
<b>Logistical Concerns</b>						
20) Water control mxns: ability to control water levels		N	Y	N	N	N
21) Land status: crown, protected, private, First Nations		Protected	Protected	Private	Crown	Protected
22) Monitoring easily accomplished, site access, sight-ability		Y	Y	?	Y	Y
23) Historically occupied by <i>Rana pipiens</i>		?	Y	?	?	?

\* key criteria