Rearing *Rana pipiens* for Conservation: Two Approaches to Captive Rearing

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Abstract: The northern leopard frog (*Rana pipiens*), once the most common amphibian in North America, has declined dramatically, particularly across the western portion of its range. In British Columbia and Alberta, recovery efforts are currently underway. The captive rearing of *R. pipiens* larvae for release has been a significant aspect of each province's recovery effort; however, because the availability of resources and the status of *R. pipiens* vary between the two provinces, each jurisdiction has taken a different approach to the rearing of *R. pipiens* larvae in captivity.

In Alberta, *R. pipiens* were reared in two seminatural, outdoor ponds at the Raven Brood Trout Station from 1999 to 2003. Tadpoles were reared at densities of 0.005–0.020 tadpoles per litre. Due to the self-contained nature of the ponds, a natural diet of algae and other wetland vegetation was available to the larvae, and a number of steps were taken to reduce depredation from aquatic insects, American mink (*Mustela vison*), and great blue herons (*Ardea herodias*). Survival to metamorphosis ranged from 14 to 33%, while average size at metamorphosis was consistent with that of wild populations and ranged from 30.6 to 40.7 mm.

In British Columbia, *R. pipiens* were reared in 4000-L tanks from 2001 to 2003. The tanks were located outside and were exposed to ambient environmental conditions. Mixed vegetables and bloodworm were provided to supplement a diet of locally collected marsh vegetation. Depredation was controlled by screening the tanks with mosquito netting and thoroughly cleaning the marsh vegetation to avoid the introduction of predators. Initially, tadpoles were reared at a density of 1.0 tadpoles per litre, but this was reduced to 0.25 tadpoles per litre after 30 days. Mean size at metamorphosis in the rearing tanks ranged from 24.8 to 32.9 mm while survival to metamorphosis ranged from 55 to 97%.

Drawing from eight years of data, we discuss the advantages and disadvantages of the two approaches with respect to survival to metamorphosis, size at metamorphosis, and cost.

Key Words: *Rana pipiens*, northern leopard frog, captive rearing, reintroduction, Alberta, British Columbia, metamorphosis, larvae, survival

1 Introduction

Once common and widespread throughout much of Canada, the northern leopard frog (*Rana pipiens*) has declined or disappeared throughout the western extent of its range (Fig. 1) (Corn and Fogleman 1984; Stebbins and Cohen 1995; McAllister and Leonard 1996; Wagner 1997; Seburn and Seburn 1998; Kendell 2003; Werner 2003). In Alberta, the decline has resulted in a significant range contraction; consequently, the species is now absent from much of its northern and western range in the province (Roberts 1981; Wagner 1997; Seburn and Seburn 1998; Kendell 2003). In British Columbia (B.C.), *R. pipiens* formerly occurred throughout the southeastern portion of the province (Seburn and Seburn 1998), however, today only a single population is known to occur in the Creston Valley Wildlife Management Area near Creston, B.C. (Ohanjanian and Teske 1996; Gillies and Franken 1999; Waye and Cooper 2000; Adama et al., in preparation). This remaining population is threatened by the loss, degradation, and fragmentation of critical habitats; infectious diseases (*Chytridiomycosis*); predation by introduced fish species; and low recruitment (Ohanjanian et al. 2004; Adama et al., in preparation). *R. pipiens* is listed as a species of Special Concern in Alberta and as Endangered in British Columbia (COSEWIC 2002).

Presently, recovery efforts in both provinces involves captive rearing of *R. pipiens* for release in an effort to reestablish populations in historical locations (Ohanjanian et al. 2004; Kendell, unpublished). In the wild, the survival of *R. pipiens* larvae[1] from hatching to metamorphosis ranges from <1 to 7.5% (Seburn and Seburn 1998). Alberta and British Columbia are using captive rearing to increase the survival of *R. pipiens* larvae and thus increase the success of reintroduction efforts. Each province, however, has adopted a different approach to captive rearing based on the status of local *R. pipiens* populations and on available infrastructure and funding resources. In this paper, we present the methods used by each province, discuss their advantages and disadvantages, compare larval survival and size at metamorphosis, and discuss some of the main considerations involved with each method, including cost.

2 Methods

2.1 Seminatural Captive-rearing Approach (Alberta)

A captive-rearing and reintroduction program for *R. pipiens* was initiated in Alberta in 1999. Between 1999 and 2003, *R. pipiens* were reared in two seminatural ponds that were originally outdoor trout raceways at the Raven Brood Trout Station (Fig. 2). Use of the hatchery was provided in kind by the Alberta government.

[1]The terms larvae and tadpoles are used synonymously.

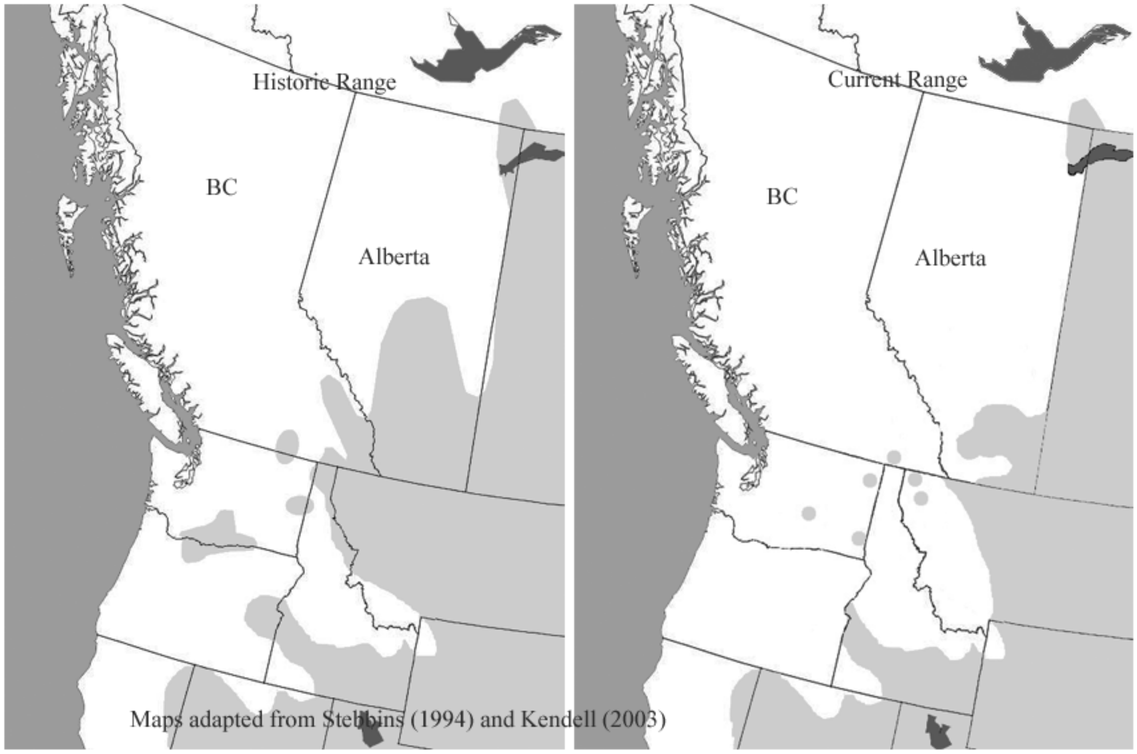

Figure 1. Historic and current ranges of *Rana pipiens* in western North America.

Figure 2. Seminatural captive-rearing facility for *Rana pipiens* (Alberta).

To locate egg masses, a combination of night time calling surveys and visual egg mass surveys were conducted at known breeding ponds in southern Alberta (Kendell 2002b). Immediately upon location, egg masses (three to six per year) were transported to the Raven Brood Trout Station in 2-L Thermos™ containers. Once at the station, the egg masses were gradually acclimatized to water in the rearing ponds over a two-hour period by a series of 20% water changes conducted every 20 minutes. The egg masses were then placed into 35 cm x 25 cm floating predator exclosures constructed of no-see-um mesh. Two exclosures were then placed in each of the rearing ponds. The exclosures were strategically positioned in direct sunlight in the warmest areas of the rearing ponds and were anchored in place by a short length of twine. Once the eggs had hatched out, the hatchling[2] tadpoles were confined to the predator exclosures until Gosner stage 25[3], and then were counted and released into the rearing ponds.

The raceways/ponds were 55 m in length, approximately 7 m wide at the end, and 12 m wide at the center. The ponds were filled to a depth of approximately 95 cm and were maintained by an input of spring water that was used to replace water lost through evaporation. The ponds contained approximately 300,000 L of water. Because the number of tadpoles varied from year to year, they were reared at densities of 0.005–0.020 tadpoles per litre.

Since the ponds were located outdoors, several steps were taken to keep out predators. First, a 60-cm-tall silt fence was installed around each pond to keep out small mammals and to confine the froglets as they completed metamorphosis. To discourage great blue herons (*Ardea herodias*) from foraging in the rearing ponds, three rows of 14-gauge aluminum fence wire were installed along the shoreline at a height of about 75 cm and 40 cm apart, providing approximately 1.2 m of coverage over the shallow water zone and the shore of the ponds. To reduce invertebrate predation, the ponds were drained and allowed to completely freeze during the winter. This prevented adult aquatic invertebrates from overwintering in the ponds and quickly establishing dense populations early in the season while the tadpoles were small and less mobile.

Once the tadpoles were released into the rearing ponds, little work was required as a natural diet of algae and other wetland vegetation was available to the larva and natural processes maintained water quality. General maintenance, basic water quality monitoring (temperature, dissolved oxygen, nitrate, nitrite, pH, ammonia, alkalinity), and topping up water levels was conducted weekly. To reduce the threat of disease transmission between the ponds, equipment was allocated to each rearing pond and disinfected with 10% bleach solution between uses. At metamorphosis, *R. pipiens* froglets were collected using dip nets and specially designed funnel traps (Kendell 2002a). As the frogs were collected, they were marked using a Visible Implant Elastomer (VIE) tagging system (Northwest Marine Technology, Inc. 2003), and snout-vent lengths were obtained for every tenth frog. Once marked and measured, the frogs were transported to a reintroduction site and released (Kendell 2001).

[2]Hatchling refers to recently hatched anuran larvae up to Gosner stage 25.
[3]Gosner stage 25 marks the stage at which the tadpoles have fully developed mouthparts and are able to feed.

2.2 Artificial Captive-rearing Approach (B.C.)

In British Columbia, captive rearing for reintroduction began in 2001 (Adama et al., unpublished). The project was carried out at a field research station owned by Simon Fraser University and located in the Creston Valley Wildlife Management Area. An adaptive management approach was taken each year to assess the role of diet and stocking density on larval growth. Each year the methods were modified to incorporate information gained from the previous year and from other sources. The methods outlined below briefly describe those employed in the third year of the rearing program (Adama et al., unpublished). The procedures employed in years one and two differed mainly with respect to diet and stocking density.

Between mid-April and early June, *R. pipiens* egg masses were located in the Creston Valley Wildlife Management Area by using a combination of calling and egg mass surveys (RIC 1998). After egg masses were located, they were enclosed in a mesh cage to deter predation and were monitored every two or three days until all the tadpoles had hatched, at which time they were brought into captivity. For the first week to 10 days, *R. pipiens* hatchlings were held in quarantine in 109-L Rubbermaid™ tanks at a density of 500 hatchlings per tank until they reached Gosner stage 25. At Gosner stage 25, they were transferred into twenty-six 4000-L rearing tanks (dimensions: 258 cm x 129 cm x 76 cm; manufactured by United Farmers Association, Strathmore, Alberta). The tanks were located outside and were exposed to ambient environmental conditions. The tanks were filled with approximately 1000 L of water, and predator screens were installed on each tank (Fig. 3). Initially, the tanks were stocked at a density of 1000 tadpoles (approximately 1 tadpole per litre); however, those densities were reduced to 250 tadpoles per tank (0.25 tadpoles per litre) over the next month by halving the densities every two weeks. During the final density reduction, approximately 5000 *R. pipiens* tadpoles (Gosner stage 30[4]) were released back into the wild, and 6366 tadpoles were retained and reared to metamorphosis.

While in captivity, the tadpoles were provided with a mixed diet of native marsh plants, leafy vegetables (kale and water cress), and a protein supplement of frozen bloodworm. The marsh vegetation included great and common duckweed (*Spirodela polyrhiza* and *Lemna minor*), yellow pond-lily (*Nuphar lutea*), pondweed (*Potamogeton* spp.), common hornwort (*Ceratophyllum demersum*), common bladderwort (*Utricularia vulgaris*) and water-buttercup (*Ranunculus* spp.). Marsh vegetation was cleaned to avoid introducing aquatic predators into the tanks. Every four days, 125–500 g (0.5 to 2 g per tadpoles) of partially shredded kale and watercress were added to the tanks depending on the size of the tadpoles and on the stocking density.

Over the course of the rearing effort, dissolved oxygen and pH were monitored frequently with handheld meters, and data loggers recorded temperature hourly. Water quality was maintained by frequent water changes in which 100% of the water was replaced every 4 to 12 days.

[4]Gosner stage 30 marks the stage at which toe differentiation begins.

Figure 3. Artificial captive-rearing facility for *Rana pipiens* (British Columbia).

Disease is an important concern when rearing animals in an artificial setting for reintroduction (Langdon 1989; Viggers et al. 1993; Cunningham 1996), so several steps were taken to address this concern. First, stringent disinfection and decontamination protocols were employed to prevent the transmission of disease between rearing tanks and between the rearing facility and the wild population (Beaucher 2001; Wind 2002). Second, all unhealthy and abnormal animals encountered during daily inspections or routine husbandry activities (i.e., cleaning tanks) were submitted to one of two veterinarian labs experienced in amphibian necropsy and pathology. Finally, a rigorous disease-monitoring program was established. At approximately halfway through their development (45 days), 10 tadpoles were randomly collected from each tank and submitted for pathology. Diagnostic procedures included general histology, bacteriology, and toxicology; immunohistochemistry and polymerase chain reaction (PCR) test for chytridiomycosis; and PCR and viral culturing for iridoviruses.

In 2003, 95% of the captive *R. pipiens* completed metamorphosis within 90 days. At metamorphosis, weights and snout-vent length were obtained, and animals were marked with a colored VIE. 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 time, crickets were provided as food, and every second or third day, the newly metamorphosed frogs were transported to the release sites and set free. Once released, surveys were conducted in the fall to monitor the growth and dispersal of the captively-reared animals.

2.3 Comparison of Captive-rearing Approaches

To illustrate the differences between the two captive-rearing approaches, we compare larval survival and size at metamorphosis, and discuss some of the main considerations that each method entails, including cost.

For each approach, survival to metamorphosis was estimated by dividing the number of newly metamorphosed frogs by the original number of larvae. In the artificial rearing tanks used in British Columbia, this was confounded as not all tadpoles were reared to metamorphosis and adjustments were made to account for this.

In both provinces, mean snout-vent lengths were obtained from a subsample of recently metamorphosed *R. pipiens* for each year. In British Columbia, annual mean snout-vent lengths were calculated by averaging the values across the rearing tanks from a subsample of 25 randomly selected animals from each tank. In Alberta, annual mean snout-vent lengths were calculated by averaging the means obtained for the two rearing ponds. For each pond, a randomly selected subsample was obtained by measuring every tenth frog.

To compare costs, we estimated the cost per tadpole based on the operating expenses for each project in 2003. For each project, we simply divided the costs associated with labor, equipment, supplies (i.e., food), and veterinarian services by the number of metamorphs produced. In Alberta, facility costs were not included as the use of the Raven Brood Trout Station was provided in kind by the Alberta government. In British Columbia, the cost of the land and building were provided in kind by the Creston Valley Wildlife Management Area and Simon Fraser University, and the cost of the rearing tanks was amortized over five years.

3 Results

3.1 Seminatural Captive-rearing Approach (Alberta)

Between 1999 and 2003, over 12,000 *R. pipiens* were reared at the Raven Brood Trout Station and released into habitats within the species' historical range in Alberta (Table 1). Approximately 7700 of these frogs were released into the upper headwaters of the Red Deer River near Caroline, 2845 were released at a site along the North Saskatchewan River near Rocky Mountain House, and 1310 were released at a Ducks Unlimited property near Red Deer. Survival to metamorphosis ranged from 14 to 33%, while average size at metamorphosis was consistent with that of wild populations and ranged from 30.6 to 40.7 mm.

Table 1. Results of rearing *Rana pipiens* using a seminatural approach (Alberta).

Year	*No. of egg masses*	*Survival to metamorphosis*		*Mean size at metamorphosis (snout-vent length [mm])*
1999	3	17%		36.0
		No. at start	8292	
		No. released: metamorphs	1430	
2000	4	22%		38.4 (East pond = 36.0; West pond = 40.7)
		No. at start	6692	
		No. released	1477	
2001	6	14%		32.9 (East pond = 32.9; West pond = 32.8)
		No. at start	21036	
		No. released	2983	
2002	4	33%		32.9 (East pond = 30.6; West pond = 35.1)
		No. at start	12676	
		No. released	4191	
2003	4	33%		34.6 (East pond = 32.2; West pond = 37.0)
		No. at start	7380	
		No. released	2491	

3.2 *Artificial Captive-rearing Approach (B.C.)*

In British Columbia, the capacity of the rearing program increased from approximately 500 animals in 2001 to over 10,000 in 2003 (Table 2). Over 7500 metamorphs and over 6400 tadpoles (reared to Gosner stage 30) were released back into the source population and at two reintroduction sites: Bummer Flats and the Creston Valley Wildlife Management Areas. Survival to metamorphosis ranged from 55 to 97%, while mean size at metamorphosis in the rearing tanks ranged from 24.8 to 32.9 mm.

Table 2. Results of rearing *Rana pipiens* using an artificial approach (B.C.).

Year	*No. of egg masses*	*Survival to metamorphosis*[a]		*Mean size at metamorphosis (snout-vent length [mm])*[b]
2001	5	84%		28.4 (range = 25.7–32.9; *n* = 7)
		No. at start	667	
		No. released: metamorphs	496	
2002	5	55–93%		26.3 (range = 24.8–28.7; *n* = 26)
		No. at start	3858	
		No. released: metamorphs	1925	
		tadpoles	1315	
2003	6	75–97%		29.1 (range = 26.0–32.5; *n* = 26)
		No. at start	11873	
		No. released: metamorphs	5108	
		tadpoles	5104	

[a]Excludes animals sacrificed for disease monitoring.
[b]*n* = number of rearing tanks

3.3 Comparison of Captive-rearing Approaches

A comparison of larval survival, size at metamorphosis, and cost per animal for *R. pipiens* reared in seminatural and artificial environments is provided in Table 3. While survival was substantially higher when reared under artificial conditions, *R. pipiens* metamorphs reared in a seminatural setting were larger and operating costs were lower.

Table 3. Comparison between artificial and seminatural approaches for captive rearing of *Rana pipiens*.

	Artificial	*Seminatural*
Mean larval survival rate (%)	85	24
Mean snout-vent length at metamorphosis (mm)	27.9	35.2
Cost per metamorph ($)	15.47	6.00

4 Discussion

While the comparison of captive-rearing approaches illustrates the advantage and disadvantage each approach has with respect to larval survival, size at metamorphosis and cost, each approach is faced with its own set of unique challenges.

4.1 Seminatural Captive-rearing Approach (Alberta)

In Alberta, a seminatural approach to rearing *R. pipiens* was taken due to the availability of the Raven Brood Trout Station. Culturing tadpoles using this approach is challenging as environmental conditions are not easily manipulated, and the enclosure design cannot be readily modified. As a result, controlling predation poses the biggest challenge.

The interactions between tadpoles and their predators have been well studied (for a review see Alford 1999). Field data on survival rates of tadpoles suggest that predation is a major source of mortality from the egg stage through to metamorphosis (Wassersug and Sperry 1977; Arnold and Wassersug 1978; Villa et al. 1982; Alford 1999). In Alberta, *R. pipiens* larvae are vulnerable to a wide range of predators including water beetles ([Dytiscidae sp.] especially their larvae), great blue herons, dragonfly nymphs, fish, American mink (*Mustela vison*), belted kingfishers (*Ceryle alcyon*), and even crustaceans such as *Daphnia* (Merrell 1977). Despite our efforts to control predators, a substantial number of tadpoles were lost, likely due to aquatic invertebrates. Nevertheless, larval survival rates under seminatural conditions were at least double that reported for wild populations (Seburn and Seburn 1998).

In addition to maximizing larval survival, rearing *R. pipiens* in a seminatural setting poses several other challenges. Efforts have been made to determine the optimum carrying capacity of the rearing ponds as a means of minimizing tadpole competition for limited resources, such as food, shelter, and space; however, the inability to strictly control a number of variables within the ponds (such as diet and density), coupled with the fact that there are only two ponds, greatly limits the opportunity to determine an accurate carrying capacity.

Disease and the potential loss of captive-reared frogs through escape are also serious threats. A disease outbreak is an important concern in rearing animals for reintroduction (Viggers et al. 1993; Cunningham 1996). Perhaps the largest drawback to a seminatural setting in this respect is that a disease outbreak would not be immediately identifiable, as sick or dead animals would likely go unnoticed until most of the population was affected. Complicating matters further, a disease outbreak would be difficult to control and impossible to treat. As the entire population was split between two rearing ponds, substantial mortality in just one of the ponds would be a significant loss to the recovery program. This would also be true in the event of equipment failure. For example, a malfunction at one of the gate valves, which results in a loss of water or damage to the silt fence surrounding the ponds, could result in a high loss of release stock.

While a seminatural approach poses several challenges, there are several salient advantages, the most important of which was that the ponds were self-contained and provided a natural diet for the *R. pipiens* larvae. As a result, the size of metamorphosing *R. pipiens* was similar to that observed in the wild. Size at metamorphosis is an important indicator of post-metamorphic fitness for amphibians as it influences survival, fecundity, and age and size at first reproduction (Smith 1987; Berven 1988; Semlitsch et al. 1988; Berven 1990; John-Alder and Morin 1990; Goater 1994; Beck and Congdon 2000; Altwegg and Reyer 2003). In the wild, recently metamorphosed *R. pipiens* average between 33 and 40 mm snout-vent length (Eddy 1976; Merrell 1977). Similar values have been reported in other studies where *R. pipiens* was reared under seminatural conditions (Eddy 1976; Merrell 1977). In addition, because the ponds were essentially self-maintained, little to no maintenance was required; consequently, the operating costs were low.

4.2 Artificial Captive-rearing Approach (B.C.)

Due to the precarious status of *R. pipiens* in British Columbia, the species was reared in artificial ponds to maximize larvae survival while in captivity. As with the seminatural approach, this approach has both its own advantages and disadvantages.

As reported here and by others, rearing *R. pipiens* in an artificial environment results in much smaller metamorphs than those which occur in the wild. Size of metamorphs reared in artificial environments averages between 25 and 32 mm (Briggs and Davidson 1942; Jackman, unpublished data). While much has been published on the factors influencing the growth and development of anuran larvae (for a review see Alford 1999), applying this information to the rearing of amphibians for conservation is highly problematic since animal fitness is rarely addressed, and procedures for rearing amphibians for conservation purposes are lacking (McDiarmid and Altig 1999; Wright and Whitaker 2001). To partially address this challenge, an adaptive experimental approach was taken in this study in an effort to increase animal fitness, and experiments were conducted on diet and stocking density. The results of these experiments were then used to modify husbandry procedures. This approach suggests that diet is important in captive rearing *R. pipiens* under artificial conditions, and that an increase in metamorph size is possible[5].

A second challenge posed by an artificial approach to captive rearing *R. pipiens* is cost. Because this approach is inherently laborious, it is more costly than a seminatural system (Table 3). For example, in the third year of the project, the cost of labor was approximately 72% of the overall costs and was almost double the entire operating cost of the seminatural approach ($15.47 x 72% = $11.14 compared to $6.00 per metamorph).

While the artificial approach does have several drawbacks, there are several advantages including high larval survival, the ability to manage disease, the ability to conduct controlled experiments, and the ability to manipulate factors such as diet, stocking density and water quality. Under an artificial setting, at least a seven-fold increase in larval survival was achieved over the values reported for wild populations. Since each tank is a closed system, the approach lends itself more readily to experimental design, and the array of tanks facilitates the monitoring and management of disease, provided that rigorous protocols are in place. Finally, animals can be head-started to a variety of stages for release. This last advantage is particularly noteworthy as it greatly increases the capacity of the rearing facility while providing the option of releasing larvae at several stages. This has the benefit of increasing the efficiency of the facility by rearing substantially more animals, which should increase the success of reintroduction, while reducing the inherent risk of retaining all animals in captivity to metamorphosis.

5 Summary

Species-specific methods for rearing *R. pipiens* for conservation purposes are lacking. Through a collaborative effort between recovery programs in British Columbia and Alberta, we have compared two approaches to rearing *R. pipiens* larvae in captivity. The seminatural approach employed in Alberta is cost-effective and yields larger frogs at metamorphosis; however managing depredation and disease poses significant challenges. The artificial approach employed in British Columbia attained substantially higher larval survival while enabling a more experimental approach and better disease management; however, decreased size at metamorphosis and increased cost were significant drawbacks of this approach.

Although, at this point we cannot recommend one approach over the other, the method of choice will likely depend on species' status and available resources. In closing, we suggest that additional research should focus on refining captive management techniques to improve animal fitness and post-metamorphic survival.

[5]In 2004, mean size at metamorphosis increased to 34.9 mm by increasing the protein content of the diet (Adama et al., in preparation).

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