Performance of Wood frog tadpoles (*Rana sylvatica*) on three soybean meal–corn meal rations

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INTRODUCTION

Amphibian conservation has been an important issue as early as 1869 (WALLACE, 1869) but received little attention in North America until the early 1980's (BLAUSTEIN & WAKE, 1990). Interest arose amidst apparent global and local declines of amphibians (BLAUSTEIN & WAKE, 1995). With declining populations the trend, it is surprising that more studies on the nutritional requirements of amphibians have not been performed. Reintroduction may become a common strategy to renew populations that disappear. It may be necessary to raise some species in captivity in order to avoid total extinction. UTESHEV et al. (1986) indicated that the three most important questions regarding captive rearing of amphibians are as follows:

(1) Finding the most reliable and effective methods of getting offspring from adult animals captured in the wild
(2) Determining the optimum conditions for spawning, incubation, and raising larvae through metamorphosis
(3) Determining optimal conditions for raising amphibians to the terrestrial stage of their life cycle.

In order to maintain captive rearing projects it is essential that balanced rations be constructed which establish a balance between production costs and animal health. Protein is typically the most expensive and limiting component of animal rations (JURGENS, 1988). Consequently, determining the optimal protein requirement for captive raised amphibians is essential to lower production feed costs. It is also important to utilize rations that reduce time to metamorphosis since the longer the colonies remain in captivity, the higher the maintenance costs incurred due to facilities operations, etc. (JURGENS, 1988). Rations must be nutritious enough to ensure adequate body size at metamorphosis, an important factor to survivorship in amphibians (CLARKE, 1977).
Few studies have investigated ration development for amphibians. *Xenopus laevis* performed well on various combinations of commercial fish foods (BROWN & ROSATI, 1997) but these studies indicate little about the true nutritional requirements of anuran larvae. An additional problem with this particular study is that many commercial feeds, especially tropical fish flakes (a key component in the study), are rather expensive. Finely-ground

Adult Wood frog, *Rana sylvatica*.

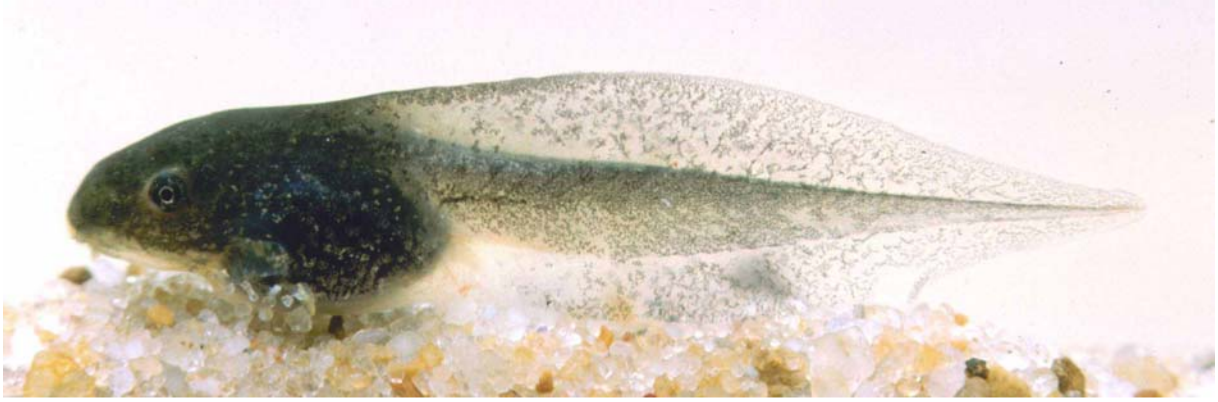
commercial goldfish food, trout chow, and algae have been recommended as tadpole rations but supporting data are lacking (ANONYMOUS, 1992). A mixture of 1 part Gerber high-protein baby cereal, 1 part tropical fish flakes, and 2 parts guinea pig pellets was fed in several studies, again without supporting data to indicate if nutritional requirements were being met (HARKEY & SEMLITSCH, 1988). Commercial frog producers have been advised to rely on algal, fungal, and bacterial growth present in aquaculture ponds as tadpole food (NASH, 1991). Fertilizing these ponds to encourage further algal growth has been further suggested (NASH, 1991). While this strategy may be useful in uncontrolled rearing, it is of little use for laboratory housing and indoor production of protected species. Diet supplementation with lettuce, cereals, brine shrimp, dried shrimp, rabbit pellets, fish meal, liver, cabbage (NASH, 1991; JOHNSON, 1994) has also appeared without experimental justification with the admission that none of these diets was nutritionally balanced for bullfrog tadpoles (NASH, 1991).

Eggs of the Wood frog, *Rana sylvatica*.

Realising that ration development is in its infancy for amphibians, we chose to begin development of a simple ration for use with ranid frogs. We selected wood frog (*Rana sylvatica*) tadpoles because of their availability, relatedness to some protected ranids, and relatedness to traditional laboratory species. Wood frogs are also of interest because of die-offs of unknown causation we have observed in the Arkansas Ozarks (TRAUTH et al. 2000). Most translocations of anuran eggs for reintroduction purposes have not been successful (SEIGEL & DODD 2002). This makes development of techniques to head start anuran larvae useful for reintroduction efforts. These methods may be of additional interest to the pet trade to produce these animals for commercial purposes. The goal of this initial screening of tadpole rations was to determine if the ratio of the ration's high-protein to low-protein component played a significant role in wood frog tadpole performance and survivorship.

MATERIALS AND METHODS

Newly hatched *Rana sylvatica* tadpoles were collected from near Fifty-Six (Stone County, AR) on 19 March 2000. Tadpoles were placed into each of six aerated 40 L aquaria, each containing 30 L of reverse osmosis water. One teaspoon of iodized salt per gallon of water was added to reduce the osmotic pressure experienced by the tadpoles. Fifty tadpoles were housed in each aquarium and maintained at approximately 20°C throughout the



A tadpole of the Wood frog, *Rana sylvatica*.

study. This started with 300 tadpoles with 100 per regiment. Water quality was monitored daily and maintained by frequent water changes.
Larvae were reared on one of three rations: 100% soybean meal (44% protein), 50% soybean meal – 50% corn meal (25% protein) or 100% corn meal (10% protein) by weight. We chose corn meal because it is commonly recommended as a staple diet for tadpoles. The use of soybean meal in combination with corn meal was derived from the common practice of balancing livestock rations using grain corn and soybean meal (Jurgens, 1988). In livestock diets corn is typically used as a carbohydrate source, whereas soybean meal is the staple protein supplement. Rations were stored at 0°C. Rations were allowed to warm to room temperature prior to feeding. Each aquarium was provided enough of the selected ration once a week, that little if any remained the next day. Tadpoles were started on diets 20 March 2000. Because we did not track population levels in each treatment throughout the study, but only recorded the ending numbers for each treatment, survivorship was analysed using Chi square.

A premetamorph of the Wood frog, *Rana sylvatica*.

Once metamorphs had all four legs, they were allowed one week to absorb the tail. Upon absorbing the tail or at the end of the week should they not absorb it, the froglets were tabulated as emerged. If the tail was not absorbed within one week of reaching the froglet stage the individual was recorded as possessing a retained tail. Survivorship was defined as any froglet that lived until its tail was absorbed, or one week after all four legs appeared. Froglets were anaesthetised in dilute chloretone (chlorobutanol, 1,1,1-trichloro-2-methyl-2-propanol, commonly used as an amphibian anesthetic (ETHERIDGE,1958)), fixed in 10% buffered formalin, and preserved in 70% ethanol. Although fixing and preserving the animals prior to measurement probably dehydrates the specimens considerably, we were more interested in the differential performance between diets than the actual performance on any one of the three during this initial screening study. In future investigations to refine the ration, it will become necessary to measure live, preserved, and dried frogs. Date of first emergence and last emergence was recorded for each treatment group. Preserved specimens were massed on an analytical balance to the nearest 0.01 g, and their snout-vent lengths were obtained with callipers to the nearest 0.1 mm. Mean snout-vent length, mass, percent survivorship, and percent of total metamorphs that were without abnormalities were recorded for each treatment group.

A sample of 25 froglets was collected at the source pond in Stone County during May 2000 for comparison to captive-reared specimens. These were preserved as previously described. A Kolmogorov-Smirnov normality test was applied to snout-vent length and body mass data that indicated they were not normally distributed (snout-vent length $P = 0.023$, body mass $P < 0.01$) so a Kruskal-Wallis Test was applied to compare snout-vent lengths and masses of each treatment group with that of the wild caught sample. We used a nonparametric analogue of the Bonferroni *t* test for paired comparisons (GLOVER & MITCHELL, 2002).

RESULTS

The survivorship of *Rana sylvatica* tadpoles on each ration is shown in Table 1. Survivorship to metamorphosis was 40% (121/300) when all treatments were pooled. Of these, 44 specimens evenly distributed among treatments and over time were preserved in 95% ethanol without formalin fixation for use in an unrelated molecular study. Because the different preservation techniques could bias the data, they were not included in the data set.

Treatment	Survivorship	Modified survivorship
100% SBM (soybean meal)	60% (60/100)	47% (47/100)
50% SBM	47% (47/100)	47% (47/100)
0% SBM	14% (14/100)	14% (13/100)

Table 1. Survivorship of *Rana sylvatica* reared on each ration. The column titled modified survivorship represents the number of froglets produced discounting abnormal individuals.

There was a significant increase in survivorship as percent soybean meal increased ($X^2 = 27.54$, df = 2, $P << 0.001$). Survivorship was highest in the 100% soybean meal ration (60%), and lowest in the pure corn meal ration (14%). Thirteen percent of tadpoles reared on the 100% soybean meal ration possessed abnormalities such as lymphedema, retained tails, and 'stiff' legs (immovable at the knee/hock). The other two treatments did not produce noticeable abnormalities. The time it took for *Rana sylvatica* tadpoles to reach metamorphosis on each ration is shown in Table 2. Tadpoles metamorphosed fastest on the 100% soybean diet, and slowest on the 100% corn meal diet. Snout-vent length increased with an increasing soybean meal component (Fig. 1, Kruskal-Wallis, H = 17.69, df = 3, $P = 0.001$). There was no significant difference between the snout-vent lengths of wild-caught metamorphs and those raised on the 50% soybean meal – corn meal diet ($Z_{0.99585} > 1.26$). All other paired comparisons had significantly different snout-vent lengths (wild vs. 100% corn: $Z_{0.99585} < 2.94$; 100% corn vs. 50% soybean meal: $Z_{0.99585} < 4.73$; 100% soybean meal vs. wild: $Z_{0.99585} < 6.50$). Mass increased with increasing soybean meal component (Fig. 2, Kruskal-Wallis, H = 34.28, df = 3, $P = 0.000$). All paired comparisons for body mass were significantly different (wild vs. 100% corn: $Z_{0.99585} < 7.03$; 100% corn vs. 50% soybean meal: $Z_{0.99585} < 5.41$; 100% soybean meal vs. 50% soybean meal: $Z_{0.99585} < 4.44$). Wild caught *Rana sylvatica* metamorphs showed less variance in both snout-vent length (Fig. 1) and mass (Fig. 2) than captive reared populations. Although some results were both outliers and influential they were retained in the analysis because we have no evidence these observations resulted from any stimuli other than the treatments (NETER et al., 1996). Removal of the large outlier in the 100% soybean meal ration still suggested large differences between treatments (H = 15.65, df = 3, $P = 0.001$).

Ration (by weight SBM)	First emergence	Last emergence	Days to last emergence
100%	20 May 2000	31 July 2000	133
50%	2 June 2000	21 Aug 2000	154
0%	29 June 2000	18 Oct 2000	212

Table 2. Time required for *Rana sylvatica* larvae to reach metamorphosis under three feeding regimes. All treatments began March 20, 2000.

DISCUSSION

Survivorship is an important parameter to monitor when devising animal rations, since the ultimate goal is to produce a crop of livestock for dissemination. Although *Rana sylvatica* survivorship to metamorphosis was 33% higher in the 100% soybean meal ration than the 50% ration, and 47% higher than the pure corn meal ration, 13% of the metamorphs produced using the 100% soy ration possessed various forms of abnormalities. The most common abnormality was retained tail buds. Retained tails are a significant impediment to metamorphosing froglets. These tails easily dry to the substrate where the froglets can desiccate in the summer heat, or become easy prey for other animals. Other abnormalities such as stiff legs observed would be equally detrimental to the survival of early metamorphs. This diet produced the same number of normal tadpoles as the 50% soybean meal diet but has significantly higher dollar costs than the 50% soybean meal diet. The high abnormality frequencies in frogs fed this ration combined with its higher dollar cost make it an unfavourable diet for raising *Rana sylvatica* tadpoles for either reintroduction or commercial purposes.

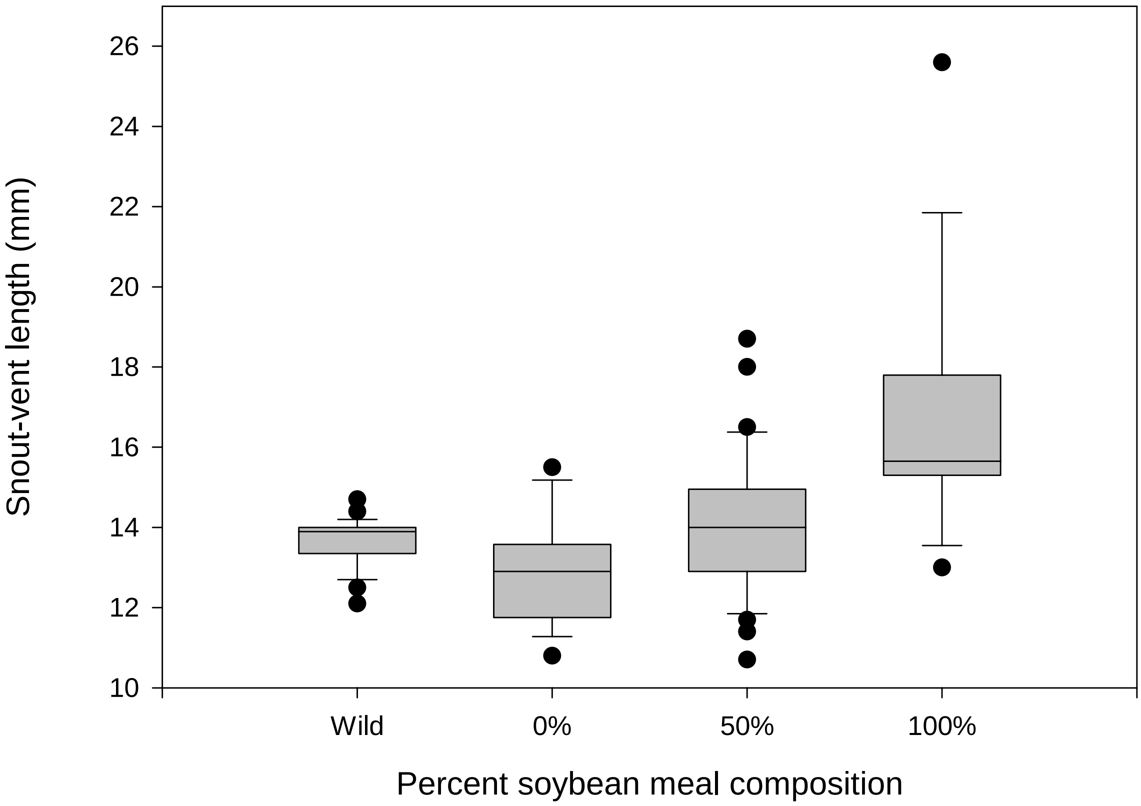


Figure 1. *Rana sylvatica* snout-vent length at metamorphosis. 0% soybean meal resulted in the lowest snout-vent-lengths with the wild-caught, 50% treatment, and 100% treatments possessing incrementally larger snout-vent lengths. Dark circles denote outliers, boxes are the 95% confidence intervals with the median represented as a horizontal line, and error bars show standard error.

The 100% corn meal ration was a poor diet for *Rana sylvatica* tadpoles. Only 14% survivorship was observed when feeding this ration. These results are probably due to the low protein content of this diet. Corn meal is approximately 10% protein (JURGENS, 1988). *Rana sylvatica* tadpoles apparently have a protein requirement that exceeds this level. This fact might also provide inferences on this species' diet in the wild. Little is known about tadpole diet selection in the wild, but this species' apparent high protein requirement might indicate that *Rana sylvatica* tadpoles may spend more time feeding on invertebrates/bacteria than they do on the traditionally accepted diet of phytoplankton.

SEMLITSCH (2002) recommended introduction of between 10,000 and 50,000 eggs to establish a population of 100 adult amphibians. Numbers of this magnitude would be in line with commercial production. At these production scales food, personnel, and equipment costs could become substantial. Food costs are a major component of production expense, and also the most easily reducible by the use of alternate foods (JURGENS, 1988). Anytime an expensive ration can be replaced by a cheaper but equal or better quality feed, the efficiency of production for any purpose becomes more efficient and more desirable.
The more time animals spend in the production facility, the higher the monetary expenses, such as interest and operating expenses, incurred by the operation (KAY & EDWARDS, 1994). This is an important factor to address when implementing large-scale production of anurans for either commercial purposes or reintroduction to the wild. Financial investment is a real-world problem that must be considered in these kinds of studies. Our data suggest an inverse relationship between soybean meal content in the diet and the time to metamorphosis. As the high-protein soybean ration percent increases, larvae reach metamorphosis faster. High protein diets tend to be higher cost than that of low protein diets (JURGENS, 1988). Therefore, it is important to balance the dollar cost of the high-protein soybean meal component with the dollar costs incurred through lengthier stays in the production facility. The low protein 100% corn meal diet, although less expensive, does not provide enough benefits in either resident time as a larva or survivorship to metamorphosis to make it a viable diet for *Rana sylvatica* froglet production.
Rana sylvatica metamorphs had longer snout-vent lengths and higher body masses when raised on 100% soybean meal than wild caught metamorphs or than the other treatment groups. The variance in snout-vent length was least in the froglets raised on corn meal. Froglets raised on 100% corn meal emerged with slightly smaller body lengths and lighter body masses than wild individuals. The mean snout-vent length of froglets raised on the 50% ration was very near that of the wild caught animals and had intermediate body masses. It is apparent that releasing captive reared froglets should be more successful when the animals are larger than if small. Larger froglets should have fewer predators, be more adept at fleeing attack, and be more competitive at procuring prey. For these reasons it would be beneficial to produce froglets that are larger than average when conducting a release program. Such a practice can balance the disadvantage captive reared froglets should have to wild individuals through their inexperience with predators and potential prey, and their unfamiliarity with their surroundings. Larger froglet sizes also make it easier to feed the froglets while in captivity. Since the corn meal ration produced metamorphs that were on the average shorter than their wild caught counterparts, these animals would be at a decided disadvantage upon release to the wild, and very difficult to feed in captivity. Overall, the corn meal diet does not appear to be very useful as a production ration. Iodine is known to play a role in growth of anurans. Soybean meal contains between 0.131 and 0.147 mg/kg Iodine. Corn meal is much lower in Iodine; however, our addition of iodized salt to improve the ionic balance of the water would be expected to negate any influences these differences might have.
Based on this study, it appears that *Rana sylvatica* tadpoles perform best on a 50% soybean meal – corn meal ration. They may perform better on a formula intermediate between the 100% and 50% rations, but this needs further investigation. Neither soybean meal nor corn meal by itself is an acceptable diet for rearing *Rana sylvatica*. Considering the current global declines in anuran populations, we are in a very compromised position regarding captive propagation for the conservation management of these species.

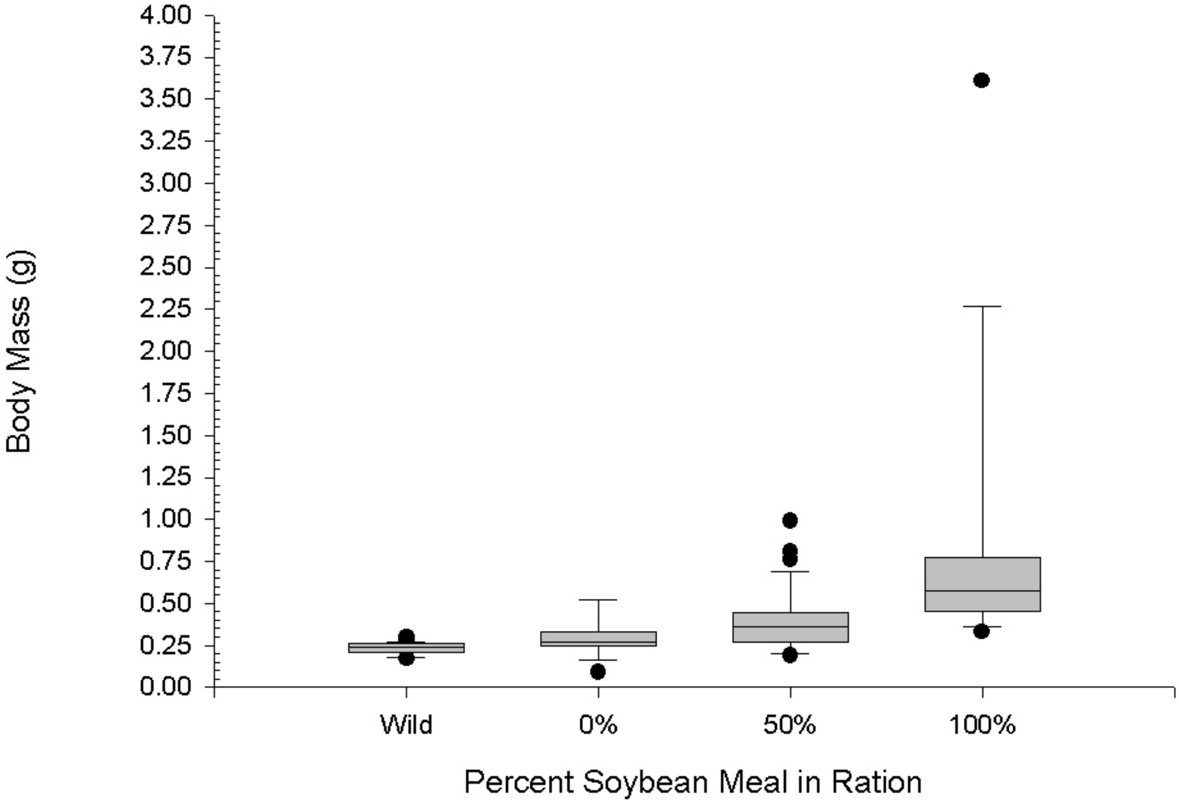


Figure 2. Body masses of *Rana sylvatica* metamorphs reared on three soybean meal – corn meal rations and body masses of wild caught *Rana sylvatica* metamorphs. Largest body masses were observed in the 100% soybean meal treatment while smallest masses were observed in the wild-caught sample. Dark circles denote outliers, boxes are the 95% confidence intervals, and error bars show standard error.

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SUMMARY

Newly hatched *Rana sylvatica* larvae were reared on one of three soybean meal-corn meal diets (100% soybean meal, 50% soybean meal – 50% corn meal, 100% corn meal). Our results indicate that snout-vent length and body mass at metamorphosis were greater when raised on diets with larger components of soybean meal. Metamorphs produced on increasing levels of soybean meal were larger than wild caught metamorphs. Time to metamorphosis decreased as soybean meal component increased, but all rations resulted in an extended time to metamorphosis compared to *Rana sylvatica* that metamorphosed in the wild. The 50/50 diet was the most acceptable ration because corn meal costs less than soybean meal, and production of normal froglets was comparable to that of soybean meal, while better than that of corn meal. Although this study is limited, it does represent the only known attempt to economically balance energy and protein requirements of tadpoles in larvae ration formulation.

LITERATURE

ANONYMOUS, 1992. Care in captivity: Husbandry techniques for amphibians and reptiles. Chicago Herpetol. Soc. Chicago, IL.

BLAUSTEIN, A.R. & D.B. WAKE, 1990. Declining amphibian populations. A global phenomenon? Trends Ecol. Evol. (TREE) 5: 203-204.

BLAUSTEIN, A.R. & D.B. WAKE, 1995. The puzzle of declining amphibian populations. Scient. Amer. 272: 56-61.

BROWN, L.E. & R. ROSATI, 1997. Effects of three different diets on survival and growth of larvae of the African clawed frog *Xenopus laevis*. Progress. Fish-Cult. 59: 54-58.

CLARKE, R.D., 1977. Postmetamorphic survivorship of Fowler's toad, *Bufo woodhousei fowleri*. Copeia 1977: 594-597.

ETHERIDGE, R.E., 1958. Methods for preserving amphibians and reptiles for scientific study. Univ. Michigan Mus. Publ., 18 pp.

GLOVER, T. & K. MITCHELL, 2002. An Introduction to Biostatistics. McGraw-Hill, St. Louis, Missouri.

HARKEY, G.A. & R.D. SEMLITSCH, 1988. Effects of temperature on growth, development, and color polymorphism in the ornate chorus frog, *Pseudacris ornata*. Copeia 1988: 1001-1007.

JOHNSON, R.R., 1994. Conservation of toads of the family Bufonidae. In: MURPHY, J.B., K. ADLER & J.T. COLLINS (eds.). Captive management and conservation of Amphibians and Reptiles: 243-254. Society for the Study of Amphibians and Reptiles, Ithaca, New York.

JURGENS, M.H., 1988. Animal Feeding and Nutrition. Kendall/Hunt Publishing Company. Dubuque, Iowa.

KAY, R.D. & W.M. EDWARDS, 1994. Farm Management. McGraw-Hill Inc., St. Louis, Missouri.

NASH, C.E., 1991. Production of aquatic animals: crustaceans, molluscs, amphibians, and reptiles. Elsevier Science Publishers, New York.

NETER, J., M.H. KUTNER, C.J. NACTSHEIM, & W. WASSERMAN, 1996. Applied Linear Statistical Models, 4th ed. McGraw Hill Publishers. St. Louis, MO.

SEMLITSCH, R.D., 2002. Critical elements for biologically based recovery plans of aquatic-breeding amphibians. Conserv. Biol. 16(3): 619-629.

SEIGEL, R.A. & C.K. DODD, 2002 Translocations of amphibians: Proven management method or experimental technique? Conserv. Biol. 16(2): 552-554.

TRAUTH, S.E., M.L. MCCALLUM & M.E. CARTWRIGHT, 2000. Breeding mortality in the wood frog, *Rana sylvatica* (Anura: Ranidae), from Northcentral Arkansas. J. Arkansas Acad. Sci. 54: 154-156.

UTESHEV, V.K., O.I. SHUBRAVYI, I.A. SERBINOVA & B.F. GONCHAROV, 1986. Breeding of rare and endangered amphibian species in captivity. Stud. Herpetol. 1986: 731-734.

WALLACE, A.R., 1869. The Malay Archipelago. Harper, New York.