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## RECENT DEVELOPMENTS IN FROG CULTURE

By DUDLEY D. CULLEY, JR.

*Assistant Professor  
Department of Forestry and Wildlife Management  
Louisiana State University  
Baton Rouge, Louisiana*

and

CLAUDE GRAVOIS, JR.

*Graduate Assistant  
Department of Forestry and Wildlife Management  
Louisiana State University  
Baton Rouge, Louisiana*

### ABSTRACT

Effects of crowding were investigated with respect to growth, feeding, food conversion, mortality, and health of bullfrogs (*Rana catesbeiana*) up to 9 months of age. No statistically significant differences were found with regard to growth, food consumption, food conversion, mortality, or health.

The average food conversion (C) was less than 2.6 during the first two months of growth and values between 1.65 and 1.99 were obtained. By the third month most values approached 3.00. A high correlation was found between gain and food conversion.

Daily consumption as high as 35% was recorded. The fastest growing bullfrogs approached or exceeded 226 g ( $\frac{1}{2}$  lb.) in 8 months from metamorphosis.

Analysis of 68 frogs for sexual maturity showed that males had motile sperm by 4 months of age and a weight of 32 g. Analysis of females indicated eggs approaching maturity may be produced as early as 6 months from metamorphosis.

Under natural conditions, crayfish make up a large portion of the bullfrog diet. Given a choice they prefer tadpoles, fish and crayfish in that order.

## INTRODUCTION

Since World War I great strides have been made in understanding, correcting and controlling human diseases, primarily through the use of laboratory animals. Noteworthy among these laboratory animals are rats, mice, rabbits, guinea pigs, dogs, chickens, frogs, toads, and fruit flies. With the exception of frogs and toads, laboratory standards for the most common laboratory animals have been established, and well-defined strains of these animals have been developed.

Development of specialized strains has resulted in our ability to conduct highly sophisticated research; yet today numerous biomedical research projects are being conducted with amphibians that are not standardized for any of the basic characters such as age, source, nutrition, species, physiology, health, or genetics (Nace, 1968). Reliable estimates from various researchers and biological supply houses indicate that only 20% of wild-caught frogs needed for experimentation survive from the time they are caught until they are successfully used for research or education purposes. Because of the large demand for species of *Rana*, the suppliers have not been able to fulfill the research and educational needs of the biological community and they state that the situation is deteriorating. There is reason to believe that in ten years the supply of bullfrogs will dwindle so low that the biological supply industry may well have to discontinue sale of bullfrogs except on a limited supply basis.

A recent article published in *Business Week* (Anonymous, 1971) points out the increased demand for laboratory-reared animals. Long-term and tougher drug-testing rules and stepped-up research has created a booming business (already estimated at \$80 million a year.) The government's stricter drug-testing regulations and support of biomedical research have placed a premium on animals that are free from infection or other defects that could ruin long-term research projects. Several million dollars are spent annually on research involving *Rana* sp., particularly *Rana catesbeiana* and *R. pipiens*. Very few of these projects utilize frogs with any known characteristics. The value of much of the data collected on these animals will continue to be questionable until standards for laboratory amphibians are defined and maintained.

Our amphibian research over the past 2 years at Louisiana State University, has shown that bullfrogs, contrary to popular belief, can be reared quickly and in large numbers under crowded conditions (Culley and Gravois, 1970). Much of our work has been conducted to obtain answers on basic biological characteristics of the bullfrog. Other work has dealt with development of rearing and housing techniques, identification and control of disease, testing methods for artificial breeding, selection of defined strains, and working out nutritional requirements. This paper presents our findings on growth characteristics, the effects of crowding on growth, food preferences, food consumption, food conversion, and sexual maturity.

## MATERIALS AND METHODS

Two separate growth studies were conducted using bullfrogs within one population, but with different parents. The first study (Test 1) was conducted from September, 1969 to July, 1970. Newly metamorphosed frogs were placed in test containers in groups of 5, 10, 15, and 20 per container. Three replicates for each of the four groups were set up. Weight variation within each test container was 1 g or less.

The second study (Test 2) was conducted from February, 1970 through May, 1970. In this test 30 newly metamorphosed frogs were placed in test containers in groups of 1 and 5 per container. Fifteen frogs were isolated by placing one frog in each of 15 test containers. The remaining 15 frogs were divided into three containers with five

frogs each. Weight variation within the individually housed frogs and the three groups of five frogs varied as much as 7 g.

In both tests, the following methods were used. Tadpoles in various stages of metamorphosis were seined from the fisheries research ponds of Louisiana State University and maintained in plastic containers in the laboratory until the fore limbs emerged. They were then held in inclined plastic dishpans with water in the lower end and a dry sheltered area at the elevated end. At the end of metamorphosis (1-2 weeks), the young frogs were weighed and placed in the test containers.

Polyethylene plastic dishpans, measuring  $12\frac{3}{4} \times 10 \times 5\frac{1}{2}$  inches and covered with  $\frac{1}{4}$  inch hardware cloth, were used as test containers. Floor area was 127.5 square inches. The floor area per frog was 127.5, 25.5, 12.8, 8.5, and 6.4 square inches for the respective groups of 1, 5, 10, 15, and 20 frogs per container. The pans were rested at an angle so that wet and dry areas were created in a manner similar to the method employed for maintaining emerging new frogs. A piece of plywood ( $11\frac{1}{2} \times 5$  inches) was stapled in each container over the dry end to provide seclusion, and rubber matting was placed on the bottom of the same area to provide footing. A stoppered hole was provided at the lower end to enable flushing out of the container. Approximately  $\frac{1}{2}$  of the bottom area was kept covered with water, with a depth of about one inch at the deepest point.

Water obtained from a 17 acre lake adjacent to the laboratory was treated with aluminum sulfate to remove the suspended material before adding the water to the test containers.

The temperature range in the laboratory was 16 C to 27 C during Test 1 and 23-27 C during Test 2. Due to inadequate building insulation a narrower range of temperature could not be maintained during the winter months.

Food was first supplied about 3 days after the frogs completed metamorphosis. In Test 1, the frogs up to 5 months of age received live mosquitofish (*Gambusia affinis*) as food. The test was then terminated due to an insufficient supply of the fish. The largest frogs at the conclusion of the test were then utilized in a second test that was continued until the frogs were 9 months old. Their diet consisted solely of crayfish (*Procambarus* sp.). During this test, the slower growing frogs were removed at the end of each month so that the results would reflect only the growth responses of the most rapid growing individuals in a population.

The frogs in Test 2 were tested for 3 months and received only mosquitofish.

In tests utilizing mosquitofish as food, small (0.1-0.2g) mosquitofish collected from ponds were supplied daily (alive and in excess). As the frogs grew, larger fish were provided. Fish supplied each group were weighed daily, and any that remained on the following day were removed and their weight recorded. The containers were flushed out daily and refilled before food was supplied.

All groups were weighed monthly. Each frog was dried of excess water and its weight recorded. Data were recorded for calculation of total weight, weight gain, weight range, food consumption, food conversion, food consumed daily and expressed as percent body weight, and percent increase in body weight. Observations were made on mortality and general health.

Animals which died during the first 10 days were replaced with extra frogs being maintained under the same conditions. Mortalities after the tenth day were allowed for by subtracting their proportionate share of the food consumed from the total group consumption up to the day the mortality occurred. Occasionally an animal escaped. Calculations were handled in the same manner as for mortalities.

When a loss occurred in containers with more than one frog it was necessary to subtract the average weight of one frog and the average weight of food consumed by one frog from each preceding month's totals in order to compute totals at the end of the 5 month test period.

TABLE 1. Monthly and five month average weights (g) and percent weight increase \*per frog of combined replicates of bullfrogs fed live mosquito fish and held at various crowding levels.

Number per container	Day 1	1	2	Month 3	4	5	Fifth-month average	Total % weight inc. from Day 1
5	5.8	12.0 (107)	15.1 (26)	20.1 (33)	26.8 (34)	34.5 (29)	34.5 ± 6.9	496
10	3.2	9.5 (197)	15.6 (64)	20.1 (29)	25.2 (25)	34.1 (35)	34.1 ± 7.9	955
15	3.5	10.5 (200)	16.7 (59)	20.4 (22)	25.8 (27)	32.8 (27)	32.8 ± 10.3	811
20	2.9	8.8 (203)	14.5 (65)	19.6 (36)	30.1 (54)	38.6 (29)	38.6 ± 14.4	1245

\* Percent weight increase shown in parentheses.

The food preference tests were set up in a manner similar to the growth tests. In each container the frogs were given a choice of three foods; mosquitofish, crayfish, and bullfrog tadpoles. Observations on preference were recorded.

Sexual development was recorded for 68 frogs of various sizes and ages. The males were analyzed only for motile sperm. In the females data were recorded on egg color and weight of the egg mass, oviduct size, and presence of animal and vegetal poles in the eggs. Eggs were considered mature or approaching maturity if distinct animal and vegetal poles were present.

## RESULTS AND DISCUSSION

### TEST ONE

Table 1 shows that frogs at all crowding levels responded similarly in growth from month to month for 5 months. No significant difference in growth ( $P < .05$ ) could be detected after 5 months.

The data show that the percent increase in body weight was uniformly higher in the first month of growth for all crowding levels, with lower but similar values for the remaining 4 months. The low value obtained in the 5 per container group was due to sampling variations. All groups more than doubled their weight during the first month; on the average they increased their weight by about 50% the second month, and nearly all groups increased their weight by more than 25% for each of the last 3 months.

Food conversion is a function of weight gain and food consumption. In Table 2 the food conversion value (C) increased (less efficient use of food) over the 5 month period for nearly all crowding levels. Food conversion values were similar for each crowding level each month. Food conversion was more efficient during the first 2 months of growth. No significant differences ( $P < .05$ ) could be detected for the 5 month average food conversion values. Taking into account the rise in food conversion values throughout the test, the greater increase in food consumption over the weight gain that occurred is to be expected and

TABLE 2. Average gain and food consumption (g), and food conversion per month of combined replicates of bullfrogs fed live mosquitofish and held at various crowding levels.

Number per container	Average wt. on Day 1	Month					Five month averages	
		1	2	3	4	5		
5	AG	5.8	6.2	3.1	5.0	6.8	7.7	5.8
	AFC		16.5	8.0	14.0	23.4	29.7	18.3
	C		2.7	2.6	2.8	3.5	3.9	3.2
10	AG	3.2	6.3	6.1	4.5	5.1	8.9	6.2
	AFC		13.0	13.7	15.0	20.1	33.5	19.1
	C		2.1	2.2	3.3	4.0	3.8	3.1
15	AG	3.5	7.0	6.2	3.8	5.4	6.8	5.8
	AFC		14.2	15.0	13.8	22.5	28.6	18.8
	C		2.4	2.4	3.8	4.2	4.2	3.2
20	AG	2.9	5.9	5.8	5.0	10.7	8.5	7.1
	AFC		11.7	14.6	18.2	34.3	33.4	22.4
	C		2.0	2.6	3.7	3.2	3.9	3.1

AG = Average gain

AFC = Average food consumption

C = Food conversion =  $\frac{\text{Average food consumption}}{\text{Average gain}}$

probably is a reflection of changing metabolism and deceleration of growth with ageing.

The food consumption expressed as per cent body weight consumed per day is shown in Table 3. Food consumption as percent body weight during the first month was higher than the other 4 months at all levels of crowding. After the first month no difference could be detected in the average daily consumption rate. A more detailed analysis is made in the food consumption section.

TABLE 3. Average percent body weight of food consumed per frog per day in each month of combined replicates of bullfrogs fed live mosquitofish and held at various crowding levels.

Number per container	Month				
	1	2	3	4	5
5 .....	6.2	2.0	2.7	3.3	3.2
10 .....	5.3	3.6	2.7	3.0	3.8
15 .....	6.7	3.7	2.5	3.3	3.3
20 .....	6.6	4.2	3.6	4.6	3.2

Table 4 shows the weight distribution in 10-gram intervals for each crowding level at the conclusion of the test. Over 80% of the frogs in all crowding levels weighed from 20 to 50 g. Although the upper limits of the weight range increased as the crowding levels increased, the difference falls within expected natural variation. The lower limits were the same for each crowding level.

TABLE 4. Weight frequency and distribution after five months of combined replicates of bullfrogs fed live mosquitofish and held at various crowding levels.

Weight interval grams	5 per container		10 per container		15 per container		20 per container	
	no frogs	% of total	no frogs	% of total	no frogs	% of total	no frogs	% of total
10-20	0	...	1	3.4	4	10.3	1	2.0
20-30	5	33.3	11	37.9	12	30.8	13	26.5
30-40	5	33.3	11	37.9	15	38.5	17	34.7
40-50	5	33.3	5	17.2	6	15.4	10	20.4
50-60	0	...	1	3.4	1	2.6	4	8.2
60-70	0	...	0	..	1	2.6	1	2.0
70-80	0	...	0	..	0	..	2	4.1
80-90	0	...	0	..	0	..	1	2.0
Totals	15	99.9	29	99.8	39	100.2	49	99.9

The total number of frogs at each crowding level shows that some losses occurred. A total of seven frogs died from starvation in the 15 and 20-per-container groups. These deaths occurred early during the test as the frogs never started eating. Five died from suspected poisoning, all in the 20-per-container groups, and six frogs escaped during the study. The poisoning was attributed to lack of flowing water to keep the containers clean during feeding. The frogs feed and release waste at

night. It was impossible to clean the container at night because any disturbance would disrupt feeding for a day or two. Ingestion of contaminated water while feeding was the apparent cause of death. It has since been shown that by using a continuous flow, or periodically flushing the containers every few hours, no mortality of a similar nature occurs.

The mortalities that occurred from starvation, may be related to a crowding stress since those that died did so in the containers with the highest densities. Deaths from starvation occurred in only four of the six most crowded containers, which indicates that a stress due to crowding may be linked with certain frogs that are unable to cope with crowded conditions. The feeding response of these frogs may have been different if placed in less crowded containers.

Table 5 shows the food conversion from 5 to 9 months of age of the most rapidly growing frogs at the end of the 5 month test period. Because of the unavailability of mosquitofish the frogs were fed a diet of crayfish (*Procambarus* sp.). At the end of each month, and after all data were recorded for the groups, the smaller frogs were removed from the test. Thus the data reflect the food conversion of only the larger frogs for each succeeding month. Results are not indicative of growth responses that would occur in the population as a whole.

TABLE 5. Average food conversion (C) of rapid growing bullfrogs from five to nine months of age grown under various crowding conditions, and fed a diet of crayfish.

Age (mos.)	Group									
	1		2		3		4		5	
	No.	C	No.	C	No.	C	No.	C	No.	C
6	22	6.79	15	wet. loss	25	5.37	48	4.86	15	3.45
7	22	4.73	15	2.87	25	4.76	48	5.86	15	4.81
8	8	4.94	9	4.18	13	4.50	22	5.09	12	6.02
9	5	4.35	6	5.85	6	7.98	9	14.01	10	13.08

As with previous tests crowding had no effect on food conversion during any month. The food conversion was variable as the bullfrogs aged, but with some indication that the frogs at 9 months of age were less efficient in utilizing the food than 5 months of age. Because the frogs were fed a different diet from metamorphosis to 5 months of age than from 5 to 9 months of age no comparison of the C value can be made. It is evident, however, that even the fastest growing bullfrogs over five months of age do not utilize crayfish as a food very efficiently. The high values are due at least in part to the large amount of crayfish exoskeleton.

It is of particular interest to note the weights of the fastest growing frogs at 9 months of age (Table 6). Five of the seven females ranged from 213-283 g and four males weighed 175, 184, 214, and 220 grams. These frogs ranged from 115 to 140 mm in snout-vent length. Although most growth data (Anonymous, 1938; Ryan, 1953; Turner, 1960; Cochran, 1967; Schroeder and Baskett, 1968) are based on estimates of growth in natural conditions, our data show that reported growth of frogs under natural conditions is much slower and is not representative of potential growth of the most rapid growing frogs when maintained under optimum conditions as in a laboratory. Of paramount significance from the growth data of the fastest growing frogs is the potential to selectively breed for rapid growth. Matings of these frogs have already begun.

Four frogs reached a size of 150 g (one-third pound) in 7 months from metamorphosis. Although small, this size frog is acceptable for

TABLE 6. Individual growth (g) of rapid-growing bullfrogs\* from five to nine months of age and fed a diet of crayfish.

Frog Number	Age (months)					Fold increase	Sex
	5	6	7	8	9		
1	52.9	66.4	106.4	157.3	244.6	4.6	F
2	48.4	64.0	82.7	121.8	184.2	3.8	M
3	40.1	61.3	82.2	106.0	154.4	3.9	F
4	47.6	51.3	94.8	183.8	263.9	5.6	F
5	47.9	41.7	74.7	140.9	195.5	4.1	F
6	63.5	93.2	119.5	176.5	237.0	3.7	F
7	46.8	67.2	87.2	152.2	175.1	4.5	M
8	53.6	101.5	185.0	307.5	282.6	5.2	F
9	83.0	130.4	181.4	213.4	220.0	2.7	M
10	79.7	125.9	169.3	212.6	214.0	2.7	M
11	75.0	116.2	156.6	188.3	213.0	2.8	F

\* Fastest growing frogs from as original stock of 138.

food. A 225 g (½ lb.) frog is definitely marketable for food. At 8 months of age two frogs approached this weight and one greatly exceeded the weight (307 g). Tadpoles obtained from eggs and maintained in the laboratory have been carried through metamorphosis in 3 to 4 months. Through selective breeding it seems quite possible to produce frogs from egg to marketable food size in 11 months. As data are accumulated on nutritional and environmental requirements for the tadpoles and frogs the time may be reduced. However, until frogs can be raised more economically market outlets will continue to be in the field of biological and medical research rather than for food.

#### TEST 2

Table 7 shows the average growth and gain, food conversion, and food consumption of 15 bullfrogs, each reared in isolation and 15 reared in 3 groups of 5 for 3 months (two frogs died in each test series). Although the results in each of the categories indicate that frogs reared in isolation out-performed the grouped frogs, the difference is not significant ( $P < .05$ ). The number of frogs used in this test was not sufficient to indicate conclusive results, but does suggest that crowding may affect some growth responses (gain and food consumption). However, a close

TABLE 7. Growth, food conversion and consumption of bullfrogs caged individually and in groups of five and fed a diet of mosquitofish for three months.

	No.	Ave. start. wt. (g)	Ave. gain (g)	Ave. food consump. (g)	Ave. food conversion
Individually caged frogs	13*	6.7	37.2	89	2.40
Grouped frogs					
A	5	5.4	33.8	77	2.28
B	5	7.3	26.6	78	2.89
C	3*	6.0	28.1	72	2.32
Average		6.2	29.5	76	2.48

\* Two mortalities during test.



look at the food conversion shows that the frogs in Groups A and C, on the average, were actually more efficient in their food utilization than the isolated frogs. Since the average gain of Groups A and C was less than that of the isolated frogs, then the grouped frogs must have consumer less food and gained less since their C values were similar to the isolated frogs. It is conceivable that crowding may have suppressed feeding and growth, but peculiar that if a stress resulted from crowding it would not also be manifest in food utilization.

Several of the grouped frogs were marked in order to follow the response of individuals under crowding. The authors believe their performance adds additional support to the hypothesis that crowding does not affect growth responses, if other environmental conditions are not limiting. Of 6 marked frogs, 3 improved their position with regard to size during the test. For example, 2 frogs, each in different groups and ranking fourth in size after 1 month growth improved their positions by the end of the third month. One ranked first in size and the other second. The position change was due to a combination of increased growth rate of the tagged frogs and a slower growth rate of the other frogs in the 2 groups. This variation in growth by individual frogs for any month was not uncommon even among the isolated frogs as shown in Table 9.

Table 8 is included to show the distribution of weights at the end of 3 months to point out further the apparent natural variation in growth, particularly when compared with the growth variation shown by the frogs in Test 1 (Table 4). The growth response in Table 8 was reversed, i.e., the greater the level of crowding, the greater the number of small frogs. The apparent superior performance of isolated frogs was not significant ( $P < .05$ ).

Close analysis of Tables 4 and 8 shows that many frogs in Table 8 were as large and larger at 3 months of age than the 5-month-old frogs in Table 4. The cause for this is not apparent but could be due to factors unrelated to laboratory conditions. For one thing the frogs in Table 4 were collected in August as the metamorphic process was under way. Fall was rapidly approaching and growth of the newly metamorphosed frogs would have been short-lived under natural conditions. Metabolic processes may have been initiated which functioned to retard growth and prepare the young frogs for hibernation. Laboratory conditioning may not have interrupted these processes fully. The frogs in

TABLE 8. Weight frequency and distribution after five months of bullfrogs caged individually and in three groups of five and fed a diet of mosquitofish.

Weight Interval, grams	Isolated * frogs	Grouped * frogs
10-15	.	2
16-20	.	.
21-25	.	1
26-30	.	2
31-35	3	2
36-40	3	.
41-45	1	3
46-50	2	1
51-55	3	1
56-60	1	1
61-65	.	.

\* Two died during test.

TABLE 9. Growth, per cent gain, food consumption, and food conversion of bullfrogs caged individually and fed a diet of mosquitofish for three months.

No.	1	2	3	4	5	6	7	8	9	10	11	12	13*	14	15*	Ave.
Growth start.																
Weight (g)	9.0	6.7	7.2	8.1	5.1	10.1	5.3	6.9	5.1	3.2	5.8	4.6	9.8	7.6	4.2	6.7
Mo. 1	15.7	10.0	19.0	20.6	8.2	20.3	15.8	12.4	17.0	10.2	10.8	12.6	17.2	12.3	14.4	14.4
Mo. 2	35.0	23.6	39.5	41.6	20.9	31.0	33.6	24.8	28.2	21.0	26.6	28.3	29.5	25.5	29.2	29.2
Mo. 3	51.8	57.2	54.7	52.4	40.2	37.5	46.9	36.3	43.9	30.6	34.2	48.9	...	31.8	...	43.9
Gain (%)																
Mo. 1	74	49	164	154	61	101	198	79	233	219	86	174	76	63	347	131
Mo. 2	123	136	108	102	155	53	113	100	66	105	146	125	71	107	...	108
Mo. 3	48	143	38	26	92	21	39	46	56	46	29	73	...	25	...	52
Average	83	109	103	94	102	58	117	75	118	123	87	124	...	65	...	95
Total	476	754	660	547	688	266	785	426	761	856	490	963	...	318	...	474
Food Consumed (g)																
Mo. 1	14.6	12.7	22.8	23.5	8.9	22.3	18.0	14.0	24.8	15.9	15.6	13.2	19.3	10.2	21.6	17.2
Mo. 2	44.2	32.4	37.6	42.5	37.6	31.2	34.7	27.7	24.8	31.4	34.9	37.3	31.3	26.0	...	33.2
Mo. 3	55.0	68.9	47.3	38.4	42.1	22.6	44.8	26.3	31.8	32.0	34.7	42.6	...	21.0	...	39.0
Total	113.8	114.0	107.3	104.4	79.3	76.1	97.5	68.0	81.4	79.3	85.2	93.1	...	57.2	...	...
Food Conversion																
Mo. 1	2.18	3.85	1.89	1.88	2.87	2.29	1.71	2.54	2.08	2.27	3.10	1.65	2.61	2.17	2.02	2.34
Mo. 2	2.29	2.38	1.83	2.02	2.23	2.91	1.95	2.23	2.21	2.91	2.21	2.37	2.54	1.97	...	2.28
Mo. 3	3.27	2.05	3.11	3.56	2.18	3.48	3.37	2.29	2.03	3.33	4.57	2.07	...	3.33	...	2.97
Average	2.66	2.26	2.26	2.36	2.26	2.83	2.34	2.31	2.10	2.89	3.00	2.10	...	2.36	...	...

\* Died during test.

Table 8 were collected as tadpoles that over-wintered, and brought into the laboratory in January. The warm laboratory conditions may have triggered the metamorphic process and set into motion metabolic responses conducive to rapid growth.

Table 9 shows the average and individual growth performance of the 13 surviving frogs reared in isolation. An average weight of 43.9 g and a range of 30.6 to 57.2 g is typical of the growth response obtained in previous laboratory tests. Gains exceeding 100% for each of the first 2 months following metamorphosis is normal in our laboratory and gain above 200% for the first month is not uncommon. By the third month only one frog increased its weight by better than 100%. A 52% gain for the third month is similar to past work conducted at our laboratory.

Food conversion ranged from 1.65 to 4.57. Average values for all frogs for each month was less than 3.00. Bullfrogs may have a more efficient food conversion in the third month rather than the first or second month (see 2, 5, and 9 of Table 9).

Analysis of individual frogs showed that no correlations could be found between food conversion and food consumption or food consumption and gain. However, comparing gain of all frogs for any month or any frog for all months with their food conversion for that same time showed an inverse correlation. Thus the greater the gain the lower the C value (greater conversion efficiency). Only one frog had its most efficient food conversion at its period of lowest percent gain (9). Nine of the frogs showed an uninterrupted inverse relationship over the 3 months, i.e., as the percent gain increased the C value decreased. The 5 frogs that deviated from this pattern did so in only one month (1, 5, 8, 9, 12).

Caution must be exercised in the above interpretation, however, because three factors mask the true percent gain and C values. When weights were taken each month some frogs had eaten the previous day and subsequent tests indicated they undoubtedly retained some food in their digestive tract for at least 24 hours. A second factor is related to uptake of water, possibly ingested while feeding or absorbed by contacting water (Lillywhite, 1970). When known weights of fish were ingested, often the gain exceeded the total weight of food ingested. A third factor affecting the percent gain is the volume of urine retained during weighing. Weights taken before and after urination varied as much as 3 g for frogs weighing between 30 and 60 g.

Daily records were maintained on food consumption, and analysis of these data indicated that if a frog did eat the day before weighing, its recorded gain for the month could be 35% higher than it should have been. If the frog retained urine the gain figure could be over-estimated as much as 10%. Most frogs did not eat the day before weighing but the weight of one frog could have been over-estimated by 45% for 1 month. Subsequent data on 7 frogs (30 to 60 g) analyzed showed an over-estimation of weight not exceeding 8% of the true weight and 15% of the monthly gain. Adjusted calculations for percent gain and food conversion resulted in lowering the percent gain and slightly raising the food conversion value for each month, but strengthened the correlation when compared to the original data. For calculations on corrected data Month 1, 2, and 3 r values were  $-.676$ ;  $-.611$ ;  $-.617$  and were significant at the  $P < .05$  level. The r value over the three months was  $-.653$  and was significant at the  $P < .01$  level. In the original data (as shown in Table 9) only Month 2 was non-significant at the .05 level ( $r = .294$ ) and the r value ( $-.674$ ) for the three months was significant at the .01 level. Although the accuracy of the data must be questioned the authors feel that the correlation between percent gain and food conversion is valid since some over-estimation probably occurred each month for all frogs.

Assuming a constant error in the data, then it seems reasonable that efficient food conversion is related to physiological state of each

frog that produces spurts of growth, rather than an even but declining growth rate as the frogs age. If the frog is not physiologically ready for a growth phase, then high consumption of food is a useless behavioral response as related to growth. The significance of such behavior is intriguing from an evolutionary point of view.

There was some feeling in the planning of these tests that the larger the newly metamorphosed frogs the sooner the frogs would obtain a larger desired size. The data indicate this size-growth relationship is not so. Comparison of frogs 1, 4, 6, 14 (largest at post metamorphosis) with frogs 5, 7, 9, 10 and 12 (smallest at post metamorphosis) shows that by the end of the third month the smaller frogs at the start of the test equaled or exceeded some of the larger frogs in size. Subsequent tests have supported this growth response.

Comparison of frog size with food conversion shows that the larger frogs (1, 2, 3, 4) do not utilize food more efficiently than small frogs (6, 8, 10, 11, 14) for any month or after three months growth. These data indicate that smaller frogs simply eat less, but tend to convert their food to tissue as efficiently as the larger frogs for any month. Apparently the efficiency of food conversion is an individual characteristic with each frog and may vary greatly from month to month.

Comparing frogs having the greatest food consumption over 3 months (1, 2, 3, 4) with frogs having the least (5, 6, 8) shows that high food consumption cannot be correlated with food conversion. If a frog consumes more food it does not necessarily follow that it utilizes the food more efficiently. The variability between consumption and conversion holds for any single month as well as for the 3 month totals.

Total percent gain over the 3 month period (Table 9) gives an idea of the rate of gain over an extended period. Only one frog (6) did not at least triple its original weight in three months. Seven frogs increased their original weight between 3 and 7 times, and 5 between 7 and 10 times. Obviously the growth rates of some frogs were quite rapid (2, 7, 9, 10 and 12).

One point must be stressed with regard to the temperature range reported in Tests 1 and 2. Although we have conducted no tests measuring growth responses to various temperatures, observation in the laboratory indicate that below 21 C, feeding declines. During the months of January and February on 3 or 4 occasions the temperature in the laboratory dropped as low as 16 C at night. Food consumption and possibly growth responses may have been altered. Other than these few times temperatures ranged from 23 to 27 C, and any effects on feeding and growth responses within this temperature range were probably minimal. However, caution must be exercised in comparing food consumption and growth data from month to month for the first five months of Test 1. Data comparisons within any one month or all months should reflect accurate responses within the temperature ranges for the time interval interpreted. Effects of low temperatures should have lowered feeding and growth responses, and the data given in Test 1 probably are lower than could have been obtained had the temperature been maintained between 23-27 C.

#### TADPOLE GROWTH

Under laboratory conditions tadpoles averaged  $11 \pm 1$  g at the onset of metamorphosis and the young frogs average  $6 \pm 1$  g at the end of metamorphosis. Successful metamorphosis occurred in tadpoles weighing 6 g and the young frogs averaged 2.5 to 3.0 g after metamorphosis.

#### GROWTH AND SEXUAL MATURITY

Thirty females and 38 males reared under laboratory conditions were analyzed for egg and sperm development. Egg maturation appears to be more a function of size rather than age. One 4-month-old female (from metamorphosis) weighing 105 g contained about 50% pigmented

eggs (melanin), but no animal or vegetal poles were visible. Seven-month-old females ranging from 25 to 40 g showed little egg development, but some enlargement of the ovaries and oviducts. Females ranging from 8 to 18 months and 69 to 186 g all had melanin-pigmented eggs and distinct poles in some eggs.

Males, apparently produce mature sperm at an earlier age and smaller size than females produce mature eggs. No males were found to have motile sperm at 2 months of age, but no 2-month-old males over 17 g were checked. Two of three 4-month-old males weighing 44 to 66 g had motile sperm. Three, 7 month males weighing between 32 and 42 g had motile sperm. All males between 8 and 18 months weighing 61 to 294 g had motile sperm.

It is interesting to note the early age and small size at which egg and sperm reach maturity. We know of no study indicating that bullfrogs mate successfully at these sizes and ages, although laboratory-reared frogs have been reported to reach sexual maturity earlier than wild-caught frogs \* (T. Kawamura and G. Nace, personal communication). Thus it seems that both sexes are capable of reaching sexual maturity in less than 1 year from the egg since tadpoles can metamorphose in 3 to 4 months from the egg. Under optimum conditions it seems that bullfrogs could successfully reproduce in less than 1 year, at least in the deep South where water temperatures may not drop below 38 C until December or January. However, other environmental and/or physiological factors may come into play and inhibit mating behavior. Attempts have not been made to obtain eggs and sperm from these small laboratory-reared frogs to determine if fertilization can occur, nor have similar data been collected from wild-caught frogs. The evolutionary significance of early maturation of egg and sperm is unclear at this time.

#### FOOD CONSUMPTION

Food consumption data for all frogs tested indicate an average daily intake of about 3% of the body weight, from 2 to 9 months of age. However, analysis of individual frogs showed that daily consumption varied considerably. A daily consumption as high as 35% of the body weight was recorded, and a daily consumption varying from 10 to 20% for several successive days was not uncommon. No clear-cut patterns of food consumption were detected, other than the frogs tend to feed in spurts.

#### FOOD PREFERENCE

Although crayfish make up a substantial portion of a bullfrog's diet under natural conditions (Korschgen & Moyle, 1955), the crayfish apparently is not a preferred food. Given a choice between crayfish, fish and tadpoles, the bullfrogs preferred tadpoles first, fish second, and crayfish third. Under natural conditions the crayfish is probably an easy food for the frog to catch. In confinement and shallow water the bullfrogs took fish and tadpoles rather than crayfish. When placed on a diet of tadpoles first and then switched to fish or crayfish the frogs often would not eat for several days. When placed on a diet of fish or crayfish first and then switched to tadpoles, most frogs continued eating.

If the water depth exceeded 2 inches, frogs had difficulty in catching fish and tadpoles and began taking crayfish or reverted to cannibalism if mixed-sized frogs were together. Although bullfrogs are reported to be cannibalistic (Dickerson, 1969), laboratory observations showed that this behavior only occurred on bullfrog tadpoles or when other food was not obtainable.

#### SUMMARY

1. Crowding in bullfrogs produced no significant ( $P < .05$ ) effects on growth, food consumption, food conversion, mortality or general health.

\* Dr. T. Kawamura, President Emeritus, Hiroshima University, Hiroshima, Japan. Dr. G. Nace, Professor of Zoology and Director of the Amphibian Facility, University of Michigan, Ann Arbor, Michigan.

2. During the first month of growth, average weight increases ranged from 107 to 203%. Individual gains for the first month were as high as 247% and as low as 47%. During the second month, average gains of 54% were recorded, but weight increases exceeding 100% were not uncommon. By the third month most gains did not exceed 50%, but a few increased their weight by 100%.

3. The average food conversion (C) was less than 2.6 during the first two months of growth, but values between 1.65 and 1.99 were recorded. By Month 3, average C values approached 3.0. A few frogs had their best food conversion in the third month and C values less than 2.1 were not uncommon.

4. Small, newly metamorphosed bullfrogs overtake larger newly metamorphosed ones by the third month of growth.

5. No correlations were found between frog size and food conversion, food consumption and food conversion, or food consumption and gain. A high correlation was found between gain and food conversion.

6. Average daily intake of food is about 3% of the body weight. Daily consumption as high as 35% was recorded, and rates of 10-20% were recorded for several successive days.

7. The fastest growing bullfrogs approached or exceeded 226 g ( $\frac{1}{2}$  lb.) in 8 months from metamorphosis. Three months were required for the tadpoles to grow from the egg through metamorphosis. Selective breeding may result in producing rapid-growing strains of bullfrogs capable of being raised cheaply enough to compete on the food market.

8. Tadpole metamorphosis normally occurred when the tadpoles weighed 10-12 g, but successful metamorphosis occurred in tadpoles weighing 5-6 g. The resulting young frogs weighed 5-6 g and 2-3 g respectively.

9. Male bullfrogs 4 months of age and as small as 32 g had motile sperm. Some females 6 months old from metamorphosis had eggs approaching maturity (animal and vegetal poles visible).

10. No difference was detected between the rate of growth of male and female bullfrogs.

11. Bullfrogs showed a food preference for tadpoles, fish and crayfish in that order.

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## THE FROG CULTURE INDUSTRY, PAST AND PRESENT

By JOHN M. PRIDDY,  
*President, Southern Frog Company*  
*Dumas, Arkansas*

and

DUDLEY D. CULLEY, JR.  
*School of Forestry and Wildlife Management*  
*Louisiana State University*  
*Baton Rouge, Louisiana*

### ABSTRACT

A brief review on the history of bullfrog (*Rana catesbeiana*) culture is covered. Present research programs in frog culture are reviewed.

The need for developing culture techniques are paramount due to the demand for bullfrogs in biological and medical research, education and dwindling of native stocks.

Bullfrogs are reared in 20-foot diameter concrete tanks, housed indoors with controlled temperature. Up to 5,000 young frogs placed in each tank are thinned to about 1,000 as they grow. Crickets, worms, fish, and tadpoles are used for food.

Research needs include the development of a commercial feed, genetically defined strains, simple and effective breeding techniques, methods for disease control, and modification of rearing tanks for more efficient cleaning and temperature control.

### INTRODUCTION

It is not known just when culture, or attempted culture, of the bullfrog (*Rana catesbeiana*) originated. The earliest literature found is an article published by F. M. Chamberlain in 1897, in which he briefly touches on methods of propagation. Additional information on early accounts of frog culture is covered by Cobb, (1903); Meehan, (1913); Herriman, (1933); Anonymous (1938); and Stearns, (1939). These growers collected metamorphosing tadpoles or young frogs from the wilds, placed them in protected ponds and provided them with care and food. In most cases success was marginal due to the inability of growers to prevent disease, control predators, provide sufficient food, and prevent cannibalism. The approach taken today by the authors considers outdoor rearing obsolete and believes that environmental control is essential in every phase of culture, at least until proven differently.

Brief accounts of frog culture in Japan are available. Breeding stock of bullfrogs were shipped to Japan from the United States during the early 1900's. Reasonably successful culture techniques were developed, and the bullfrog has been a favored food in Japan since its introduction. The basic rearing techniques involved confinement and concentrated feeding with fly larvae, silkworm pupae, crayfish, table scraps, etc. Increased use of the bullfrog as food, habitat destruction, high exportation, and DDT contamination has severely reduced the collection and sale of bullfrogs in Japan today. Although the United States still received large imports of froglegs from Japan, most of these legs come from India via Japan. Over-exploitation and DDT contamination in the frogs have seriously threatened the frog population in India today.