



**CULTIVATION OF RANA TIGERINA AND R. CATESBEIANA  
USING ARTIFICIAL FEED**

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**A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF  
THE REQUIREMENTS FOR THE DEGREE OF  
MASTER OF SCIENCE  
(ENVIRONMENTAL BIOLOGY)**



**IN**

**FACULTY OF GRADUATE STUDIES  
MAHIDOL UNIVERSITY**

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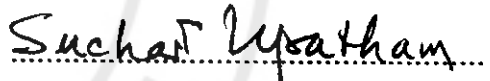
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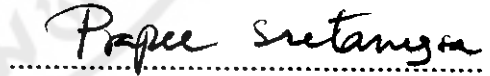
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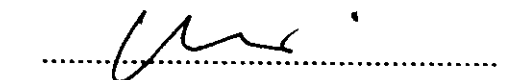
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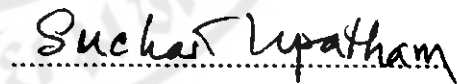
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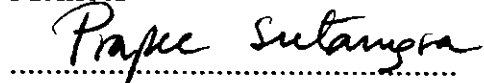
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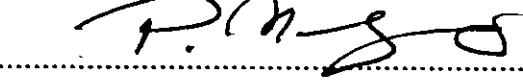
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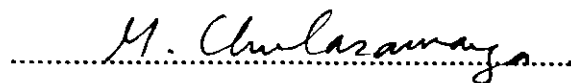


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### บทคัดย่อ

ในการทดลองเลี้ยงกบนา (*Rana tigerina*) และกบบูลฟรอก (*R. catesbeiana*) ที่ภาควิชาชีววิทยา มหาวิทยาลัยมหิดล มีวัตถุประสงค์ที่ต้องการศึกษาวงจรชีวิตของกบนาและกบบูลฟรอก และอาหารโปรตีนที่มีผลต่อการเจริญเติบโตของกบในระยะต่างๆ จากการศึกษาพบว่าในกบนา ไข่ฟักตัวเป็นลูกอ๊อดและเจริญเติบโตไปเป็นกบเล็กภายในเวลา 30-40 วัน และเจริญเติบโตเป็นกบเต็มวัยภายในเวลา 12 เดือน สำหรับกบบูลฟรอก ไข่ฟักตัวเป็นลูกอ๊อดและเจริญเติบโตไปเป็นกบเล็กภายในเวลา 60-80 วัน และเจริญเติบโตเป็นกบเต็มวัยภายในเวลา 18 เดือน จากการทดลองเรื่องอาหารในกบนาและกบบูลฟรอกพบว่าอาหารที่มีโปรตีน 35% เหมาะสำหรับการเลี้ยงลูกอ๊อดจนเป็นกบเล็กโดยมีการเจริญเติบโตดีที่สุด ส่วนในการเลี้ยงกบเล็กจนเป็นกบโตเต็มวัยนั้น อาหารที่มีโปรตีน 21% ให้ผลดีที่สุด

Thesis Title            Cultivation of *Rana tigerina* and *R. catesbeiana*  
                                 Using Artificial Feed

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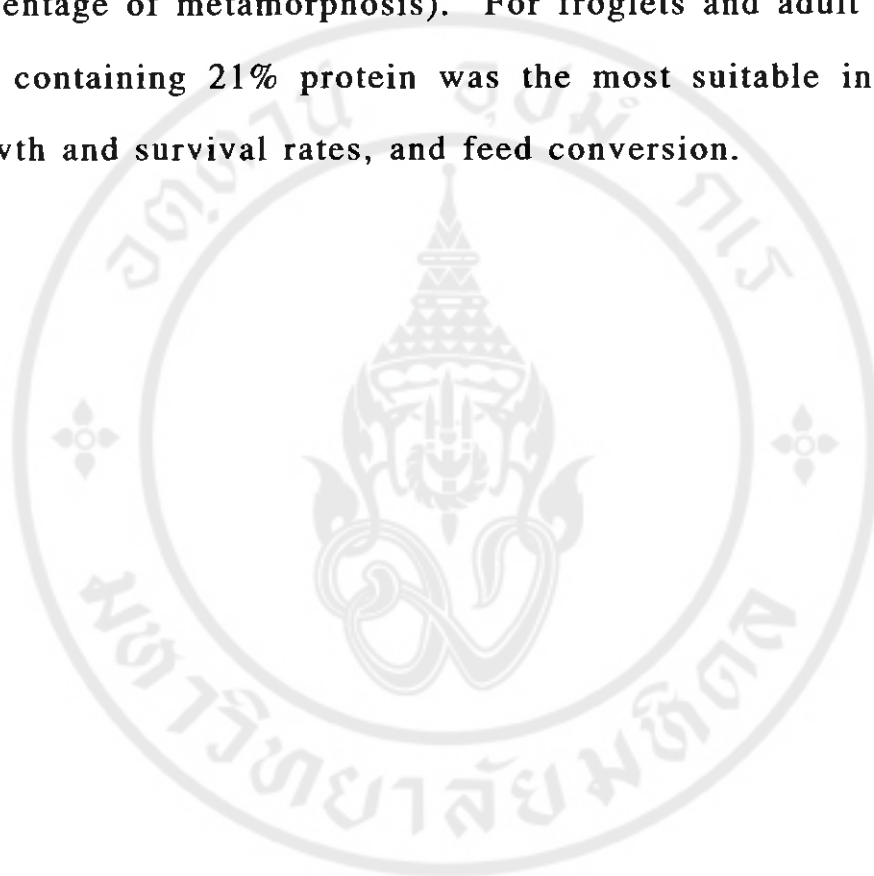
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### ABSTRACT

Frog cultures of *R. tigerina* and *R. catesbeiana* using the outdoor facility was performed in the Biology Department, Mahidol University. The objectives of this investigation is to study the life cycles of *R. tigerina* and *R. catesbeiana*, and the effects of diets varying in protein concentrations on frogs at various stages. It was observed that *R. tigerina* should be held for a minimum of 30-40 days, during the eggs hatched into tadpoles and froglets, and they completed their reproductive maturity within 12 months. For *R. catesbeiana*, they should be held for a minimum of 60-80 days, during the eggs hatched into tadpoles and froglets, and reproductive maturity was completed within 18 months. Both *R. tigerina* and *R. catesbeiana* tadpoles

fed with diet containing 35% protein concentration achieved the best results with regard to growth (weight gain, growth rate, feed conversion) and development (length of larval period, percentage of metamorphosis). For froglets and adult frogs, the diet containing 21% protein was the most suitable in terms of growth and survival rates, and feed conversion.



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# CHAPTER I

## INTRODUCTION

Traditionally, frog has been one of the major protein diets consumed by Thai people, and recently it also becomes one of the potential export items, although still in a comparatively small quantity. Most of frogs consumed locally are obtained by capturing from nature, which is limited by many constraints, i.e. uncertain weather condition, hibernation, slow reproductive and growth rates, diseases and high mortality. Commercial frog culture appears to be the better way to keep up with the significantly increasing demands for both local consumption and for export. However, there are insufficient supplies for consumption and food market because of inefficient culture techniques in commercial system and many problems including the farming methods, diseases, insufficient quantity of living food for feeding, hibernation and cannibalism.

## 1. Taxonomy and classification

The word "frog" is a popular term, that it is a member of a major group or class of vertebrate animals called the amphibian whose body temperature is dependent upon the external environment. The amphibians have soft glandular skin without scales. The egg lacks a shell and, to prevent the developing embryo from desiccating, it must be laid in water or in humid surrounding.

Frogs are distinguished from other vertebrates in their double life, i.e. the tadpole larva which hatches from the egg and lives in the water and the adult frog which usually lives on land. Frogs constitute a separate and easily definable order of the class Amphibia called the Anura, or they simply are referred as "anurans". The principle feature that distinguishes the Anura from other amphibian orders is the absence of a tail in the adult stage (Taylor, 1962).

Most of the edible frogs belong to the family Ranidae and genus *Rana*. In Thailand, forty - four species of *Rana* are found. There are 4-5 species which are popularly edible. They are the common rice-field frogs, *Rana rugulosa*, *R. tigerina*, *R. tigrina*; the mountain frogs, *R. blythii*, *R. macrodon*; and the imported American bullfrog, *R. catesbeiana*. Frog cultures are mostly concentrated on two species, *R. tigerina* and *R. catesbeiana*.

*R. tigerina* can be found in all parts of Thailand. They are large frogs with the mean length of 160 mm (snout to vent) and weigh 240-400 g. They differ from the typical local frogs in having shorter front legs and scarcely overlapping heels. They have smooth and brownish olive skin with numerous small black spots and the lips with large dark spots separated by cream color. The male frogs have two lateral vocal sacs by folding of skin on underside of the head. The dorsal surface of the legs is heavily covered with small tubercles, while the entire ventral surface is smooth (Taylor, 1962). The females *R. tigerina* are larger than the males.

The American bullfrog, *R. catesbeiana* had the origin in the Rocky Mountains in North America. They are large frogs, 200 mm long (snout to vent) and weigh 250-480 g, with the smooth skin with some rough parts. The mature male frogs have larger tympanum than that of the females and are larger in size. The skin of the ventral surface is smooth. The head is greenish in color and contains brown spots on the sides (Witschi, 1930).

At present, *R. catesbeiana* dominates the production for human consumption. It is so highly esteemed that it has been introduced into Mexico, South America, Pacific Islands, Europe, and several Asian countries, such as China, Japan and Thailand.

## 2. Reproduction, development and growth

During the breeding season, the female frog lays eggs in the water. These eggs are fertilized externally by the sperms released from the male frogs. The emerging eggs are coated with a material which swells up on coated with water to form the jelly-like mass. The eggs hatch into young tadpole larvae within 18-28 hours for *R. tigerina* (Pariyanonth et al., 1985) and approximately 3 days for *R. catesbeiana* (Culley et al., 1977) at the water temperature of 25-27°C. The tadpoles generally spend their lives feeding and their rate of growth is really rapid.

The first sign of metamorphosis is the development of the hind limb buds which lie against the tail at the end of the body. From the hind limb buds, the entire limbs grow. The front legs develop in partnership with the back ones but are hidden from view and appear later in development. At the completion of their development, the limbs bulge against the body wall. The tadpoles thrust one of the front legs through the spiracle or aperture from the gills, and the other through a specially weakened area of skin on the opposite side of the body. The tadpole shrinks in size rapidly and the tail is absorbed into the body. The head and body become smaller as the soft tissues collapse and tighten around the bones beneath. The metamorphos turn from tadpoles to juvenile frogs or

froglets in 28-36 days for *R. tigerina* (Pariyanonth *et al.*, 1985) and 70-80 days for *R. catesbeiana* (Culley *et al.*, 1977).

The frog growth and adult body size are characteristically plastic and indeterminate, implying that environmental factors substantially affect the genetically fixed growth pattern. The most important environmental factors are temperature, water and food availability, which may vary seasonally depending upon latitude and altitude of the geographical location of the habitat. For *R. tigerina* in culture, the female frogs usually lay 1,000-3,000 eggs at one time (Tarnchalanukit, 1976). The adult frogs reach marketable size weighing 300-400 g in female and 200-300 g in male within 4 months (Pariyanonth *et al.*, 1985). They reach reproductive maturity within 6-8 months for male and 18 months for female (Nootprapan *et al.*, 1989). For *R. catesbeiana* in culture, the female frogs lay a clutch of more than 80,000 eggs (Duellman, 1986). The adult frogs reach marketable size weighing 300-400 g within 10 months (Priddy & Culley, 1971). They reach reproductive maturity within 2-3 years for male and 3 years for female (Duellman, 1986).

### **3. Frog culture**

Although frog culture and farming have been introduced into Thailand and the other countries for more than a decade, the success is still marginal. Frogs in culture are faced with many problems such as (1) predation by mammals, birds, reptiles and small aquatic insects, (2) cannibalism, (3) diseases, (4) inadequate food supply, (5) poor water quality, (6) sporadic egg production, (7) fluctuating temperature, (8) poor sanitation and (9) inadequate nutrition (Culley, 1989).

#### **3.1 Culture of *R. tigerina***

The culture of the common rice-field frog, *R. tigerina* is well documented by Pariyanonth et al. (1985). They described the culture facilities, diet, growth, breeding, diseases, and other aspects of culture. They used the concrete pond in Rayong Province, the mud pond in Chantaburi and Chachoenasao Provinces. Typically, the culture area is approximately 15-100 m<sup>2</sup> with the low density of 20 frogs/m<sup>2</sup>. Both concrete pond and mud pond contain the netting support to prevent the frogs from escaping. However, the mud ponds, while more close to natural conditions, are difficult to clean and drain. The frogs are more exposed to predators and difficult to collect for sale. The concrete pond, while easy to feed, clean and

drain, is too dry with low humidity and high temperature. The water temperature is 34-38°C with the pH of 6.0 (Pariyanonth *et al.*, 1985).

From the experiment of Pariyanonth *et al.*(1985), fertilized eggs developed and hatched into tadpoles within 18-24 hours which later metamorphosed into young or juvenile frogs within 28-36 days. At 18<sup>th</sup> day, the tadpoles which began their feeding activities were fed with boiled egg yolk and protein supplement. After one month, the tadpoles were transferred into new pond where they were fed with minced fresh fish, chopped fish, living termites, live insects from night light-trap, or boiled rice as food supplement, only once in the evening or only once every two days for frogs of four months old. Marketable size was reached approximately within 4 months with the weights of 300-400 g in female and 200-300 g in male (Pariyanonth *et al.*, 1985).

### **3.2 Culture of *R. catesbeiana***

The culture of bullfrogs or *R. catesbeiana* is well documented by many authors (Modzelewski & Culley, 1974; Glorioso *et al.*, 1974a, 1974b; Culley, 1976; Culley *et al.*, 1977, 1978; Easley *et al.*, 1979; Leibovitz *et al.*, 1982; Amborski *et al.*, 1983; Culley & Sotiaridis, 1984). These authors described the culture facilities, diets,

growth, breeding, diseases, and other aspects of culture. It appeared that culture of the bullfrog indoors or in outdoor concrete system had been more successful (Culley, 1989).

**Artificial ponds (concrete ponds)** The use of artificial pond to control variables may be correlated in nature with size of pond, including surface area and depth. Culley(1989) described different types of ponds for breeding and rearing *R. catesbeiana*. The breeding pen was concrete pond of 0.5 x 2.0 m in size and 0.15 m in depth. Water plants, for example *Eichhornia crassipes* were provided as source of oxygen and site of egg-laying. After the eggs were deposited at these sites, the plants were removed and the eggs collected. The breeding pens were chlorinated daily and cleaned weekly (Culley, 1989). Commonly the tadpole hatchery was a covered, screened building containing 1 or 2 m<sup>2</sup> concrete ponds with a depth of 15 cm. The building roof contained clear roofing panels (such as fiberglass) every 2-3 m for adequate sunlight which was needed for algae production in the ponds (Culley, 1989).

**Density and water volume** Optimum densities for egg incubation and tadpole culture are about one per 2 cm<sup>2</sup>, or 5,000 eggs/m<sup>2</sup> and low tadpole densities of 100-200 tadpoles/m<sup>2</sup> (Culley,1989). Water is changed every 24

hours and flows continuously. Higher tadpole densities are possible (Culley et al., 1977). However, frequently these tadpoles are stunted because of poor management practices (low water flow, inadequate food and poor quality water).

For the juvenile frog to adult, the densities are 100 frogs/m<sup>2</sup> or after metamorphosis up to 50 frogs/m<sup>2</sup>. As they grow, the density should be reduced to 20 frogs/m<sup>2</sup> (Culley, 1989).

**Pathology** Enough food should be given to the frogs so there is always an excess present. With practice, it is easy to judge the correct amount for tadpoles or adult frogs of different sizes so that little feed remains after 12 hours. If excess feed is evident at the subsequent scheduled feeding, additional feed is withheld or reduced. Spoiled feed reduces water quality because it induces microbial infestation and frog disease. Microbial infestation is best controlled by maintaining the animals in an optimal nutritional state, by isolation, and, when necessary, by the use of appropriate antibiotics, e.g. Pfizer's cosaterramycin, choramphenical, and sulfadiazine (Nace, 1968). In advanced cases of infection, especially when accompanied by inflammation and edema, it is also advisable to keep the animals in a salt solution (approximating 25-30% frog Ringer solution).

The syndrome called "red-leg" caused by Aeromonas bacteria is frequently cited as a serious disease in laboratory colonies of amphibians. It can be avoided and even cured by the measures mandatory to cage-mates. The "red-leg" which gives rise to complaints when seen in newly received frogs is usually simple irritation which develops in transport when frogs sit on dry surfaces. The lesions may afford entry to microbial organisms if not adequately treated, but are not inherently dangerous (Nace, 1968). Other microbial diseases have been identified in frog colony on several occasions (Joiner & Abrams, 1967).

**Feeding** The choice of food is determined by the tadpoles, juvenile or adult frogs. Anuran tadpoles may be raised on a variety of foodstuffs (Hamberger, 1960; Rugh, 1965; Di Berardino, 1967). The food must not float to the surface or produce a scum which inhibits gas exchange. It must be available throughout the year in uniform quality. Spinach must be avoided because it causes kidney stone in a number of species (Berns, 1965). Romain or escarole lettuce has been found to be most suitable. It is readily softened in a pressure cooker, if desired, may be stored in the frozen stage. Protein supplement is provided by feeding cubes of raw or boiled

liver 2-3 times per week (Nace, 1968) or fed with Purina Rabbit Chow, which is a pressed, partially dehydrated combination of plant parts furnished in the form of pellets (Rose, 1960; Richards, 1962; Newman, 1988).

For juvenile frogs or froglets, living insects must be provided. They are mealworms (*Drosophila*), a bird mosquito, eschew flying insects or maggot and immature crickets. This has resulted in growth rate as high as those reported for froglets in nature (Ryan, 1953). The adult frogs are fed with living insects such as meatfly (*Phoenicia sericata*), field cricket (*Acheta domestica*). Such insects appear among the stomach contents of almost every frog. A mature *R. pipiens*, approximately 8 cm long consumes 25-50 *Sarcophaga bullata* or crickets every other day (Nace, 1968). The analysis of *R. tigrina* stomach contents of 272 frog samples collected from 31 districts of Bangladesh contained arthropods, paddy, annelids, amphibia, mollusca and mammalia (Barua & Islam, 1987). The procedures used for fly culture differ in some important details from methods reported in the literature (Whiting, 1958).

#### 4. Parasites

Frogs possess an active and effective immunity system (Evans, 1963; Evans *et al.*, 1965; Baculi *et al.*,

1970; Marchalonis, 1970; Cohen, 1970). Little is known about the endemic levels of bacterial or viral infections, with the exception of the kidney tumor virus (Rafferty, 1964).

There is considerably more documentation on the types of parasites which infect wild frogs (Frandsen & Grundmann, 1960; Lehmann, 1960; Lees, 1962; Walton, 1964, 1966, 1967), but unfortunately, very little is known about the effect of parasites on the frogs (Brockelman, 1969). Of course, it is likely that many parasites, bacteria or viruses do not improve the frog's health although some symbiotic relationships may exist. However, parasitic helminth, one genus of fluke; Glypthelmins sp. and one genus of round worm, Spinitectus sp. were found in farm frogs (Meckvichai et al., 1989). Four genera of flukes : Glypthelmins sp., Diplodiscus sp., Geneo sp., Pleurogenoides sp. and two genera of round worms: Cosmocerca sp., Zanclophorus sp. were found in wild frogs, R. tigerina (Meckvichai et al., 1989). For the study of Protozoan in frogs (R. tigerina), four species of Protozoan; Nyctotherus cordiformis, Opalina obtrigonoidea, O. hylaxena and Balantidiu duodeni were found in alimentary canals especially in the lower part of the hind gut and rectum of frogs in farms and natural habitats (Rasmidatta, 1989).

## 5. Feed formulation

Feeding is one of the most important problems encountered in commercial culturing. Usually the frog feed can be divided into three types according to the age of the frog :

1. A variety of feed has been used in feeding amphibian larvae (Cairns et al., 1967; Merrell, 1963; Hirschfeld et al., 1970). The food for tadpoles usually consists of artemia, boiled egg yolk, boiled fish, rice bran, boiled lettuce and steamed egg.

2. The food for froglets consists of flies' worms. Culturing of flies' worms can be done by using a wooden tray containing animals' wastes mixed with rotten fish as a culture medium. After the eggs hatch into worms, the froglets will feed on the emerging worms.

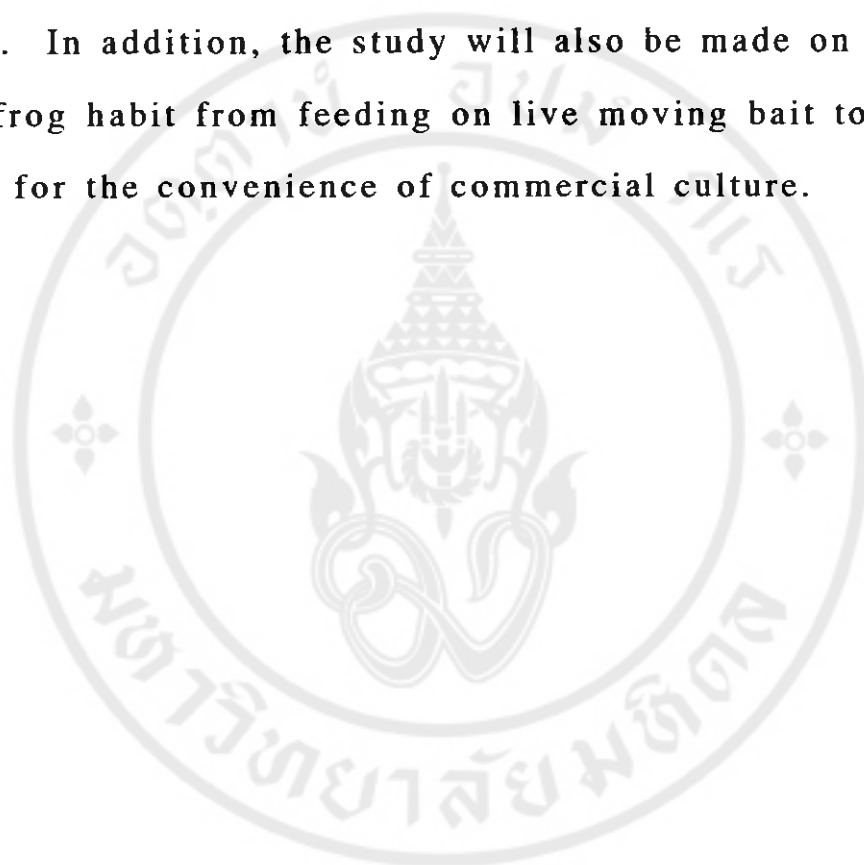
3. The food for adult frogs, are insects which are the naturally preferred bait. In addition, Culley (1989) suggested that tadpoles, fish and crawfish were the best food for growing frogs. In large commercial farms, pelleted fish and puppy food can be used as feed for growing frogs. Some farmers prepared their own recipes such as the mixture of fish meal, rice bran, ground peanut, glutinous rice flour, fish oil, vitamins and minerals of variable proportions. For *R. catesbeiana* or bullfrog

tadpoles and adults, different formulation of artificial feed had been introduced (Culley *et al.*, 1977; Schafer, 1982; Fontanello *et al.*, 1985; Barua *et al.*, 1986; Mundriyanto, 1988; Subania *et al.*, 1988). The standard diet formulation of Culley *et al.* (1977) consisted of shrimp meal, fish meal, soy and yeast protein, rice bran, whey, fish oil, fish solubles, vitamin premix, linoleic acid, sodium hexametaphosphate and Kelgin as binder. The formulation of Fontanello (1985) consisted of crude protein of vegetable (soybean, wheat and maize meals) or animal (fish and meat meal and powdered milk).

Effects of additive substances in diet on the development of frogs had also been studied. Marshall *et al.* (1980) reported that calcium in diet (0.5-0.9%) was required for the normal development of skeleton in *R. catesbeiana* larvae. In addition, diets containing 2-8% vitamin C were reported to reduce the incidence of scoliosis in larvae of *R. catesbeiana* (Leibovitz *et al.*, 1982). The ingredients consisted of fish meal, shrimp meal, soybean meal, yeast protein and defatted rice bran, and the additives contained vitamin mix, calcium phosphate monobasic and coated ascorbic acid (Schafer, 1982).

From current literature and local practice, it is believed that there has not yet been any systematic

scientific investigation on the types of food, especially pelleted feed, that are most economically and practically suitable for frogs at various stages. Therefore, the aim of the present study is to establish the most suitable feed formulation for *R. tigerina* and *R. catesbeiana* of various ages. In addition, the study will also be made on changing the frog habit from feeding on live moving bait to pelleted feed for the convenience of commercial culture.



## CHAPTER II

### OBJECTIVES

The objectives of the present study are as follows:

1. To study the life cycles of *R. tigerina* and *R. catesbeiana*.
2. To establish the best culture methods for *R. tigerina* and *R. catesbeiana*.
3. To investigate the effects of various protein concentrations in pelleted feed on growth, metamorphosis, survival, feed consumption and conversion in *R. tigerina* and *R. catesbeiana*.
4. To establish the most suitable feed formulation for *R. tigerina* and *R. catesbeiana* of various stages.

# **CHAPTER III**

## **MATERIALS AND METHODS**

### **1. Artificial pelleted feed**

The basic formulae for artificial pelleted feed in frog culture was modified from catfish pelleted feed (Sitasitt, 1982). Different formulae were developed to obtain various total protein contents of 21%, 25%, 29%, 33%, 35%, 39%, 43% and 47% (Table 1).

**Table 1** Different formulae of artificial pelleted feed with total protein contents of

21%, 25%, 29%, 33%, 35%, 39%, 43% and 47%.

Ingredients	Formulae for froglets (g)				Formulae for tadpoles (g)			
	I	II	III	IV	V	VI	VII	VIII
Fish meal	14.6	19.87	25.13	30.4	33.03	38.29	43.56	41.74
Rice bran	30.4	25.13	19.87	14.6	11.97	6.72	1.45	3.26
Soybean meal	14.6	19.87	25.13	30.4	33.03	38.29	43.56	41.74
Glutinous rice flour	30.4	25.13	19.87	14.6	11.97	6.72	1.45	3.26
Fish oil	5	5	5	5	5	5	5	5
Vitamin C	3	3	3	3	3	3	3	3
Vitamins and minerals	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Binders	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Sodium benzoate	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
<b>Total protein content</b>	<b>21%</b>	<b>25%</b>	<b>29%</b>	<b>33%</b>	<b>35%</b>	<b>39%</b>	<b>43%</b>	<b>47%</b>

Fish meal, rice bran, soybean meal and glutinous rice flour were mixed with horizontal mixer, then, water was added (approximately 25% of the total weight of the ingredients). The ingredients were mixed immediately with vitamin C, vitamins and minerals, binders (rice powder), sodium benzoate and fish oil. Next, the ingredients were poured into the pelleting machine. The pellets consisted of 3 sizes with different diameters, i.e. 0.20 mm for tadpoles, 0.52 mm for froglets and 0.71 mm for adult frogs (Fig. 1). The pellets were put in the oven (60°C) for 2 hours, packed and stored at room temperature.

## **2. Cultivation of R. tigerina**

R. tigerina selected for broodstock were obtained from Narathorn frog farm at Chonburi Province. The frogs were 12-18 months old; the females weighed 265.3g and the males weighed 158.5g (Fig. 2).



Fig. 1 Artificial pelleted feed of three sizes with different diameters. A. 0.71 mm for adult frogs. B. 0.52 mm for froglets. C. 0.20 mm for tadpoles.

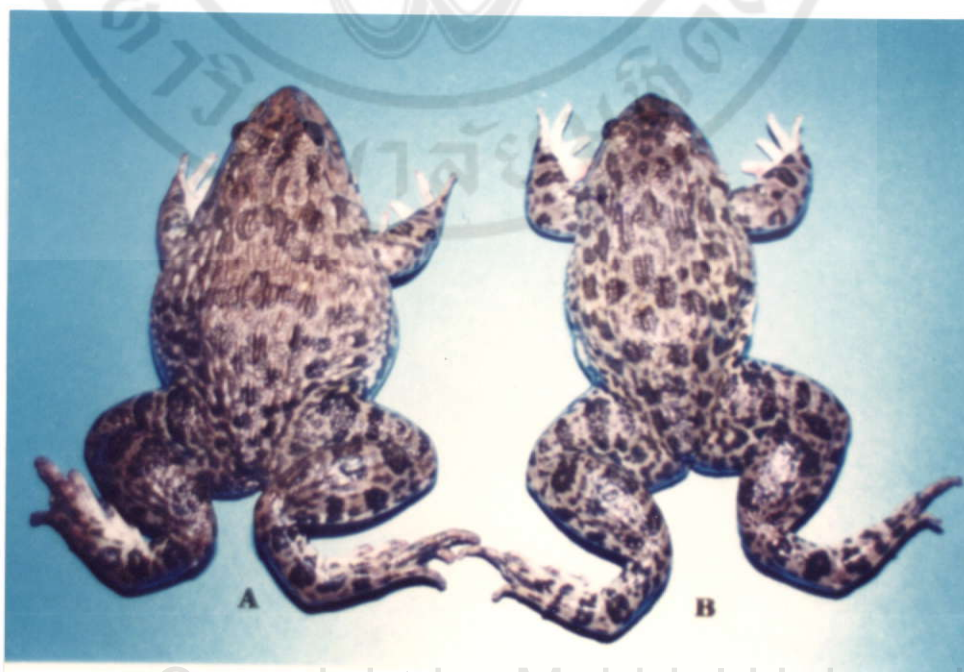


Fig. 2 *R. tigerina* selected for broodstock. A. Female. B. Male.

## 2.1 Breeding culture

In commercial production, natural breeding predominates. The frog commonly breed in large temporary ponds, 3 x 4 x 1.2 m in size, with the water depth of 0.20-0.30 m and slope floor (Fig. 3). Building roof with the netting support at 3.0 m above the ponds was supplied for adequate sunlight (Fig. 4). The water had been dechlorinated by standing overnight. The water temperature was 25 - 30°C with the pH of 7.1 - 7.5. The photoperiod was 12 D : 12 L. The sex ratio of the male to female was 1:1.

## 2.2 Culture of tadpoles and froglets

After the breeding and separation of brooders, eggs were left in the breeding pond to be hatched. The eggs hatched into tadpoles within 24 hours.

The concrete ponds, 1 x 1 x 0.5 m in size, with the water depth of 0.20-0.30 m and slope floor were used for tadpole culture. The water temperature was 25 - 30°C with the pH of 7.1 - 7.5.

On the 14<sup>th</sup> day, the tadpoles which began their feeding activities were fed with an artificial feed containing protein, adjusted to 20% of their body weight



Fig. 3 Breeding pond for *R. tigerina*.



Fig. 4 Culture and breeding ponds for frogs supplied with the netting support.

daily. After the emergence of forelimbs or metamorphosis which took approximately 1 month, froglets were reared in ponds (2 x 3 x 1.2 m) with slope floor with the density of 130 froglets/m<sup>2</sup>.

To prevent the froglets from harmful diseases, artificial feed were mixed with antibiotics (chlortetracycline HCl or aureomycin) and fed to the froglets for five consecutive days. Froglets were fed once in the evening. The quantity of feed was 5-20% of body weight or indicated by demand. The water in the pond was changed every other day and the ponds were cleaned once a week. Selection of size was done once a week and froglets of different sizes were put in different ponds at the density of 40 frogs/m<sup>2</sup>. The froglets were reared until they reached marketable size and completed their reproductive maturity.

### 3. Cultivation of R. catesbeiana

R. catesbeiana selected for broodstock were obtained from the Fisheries Station at Mae Hongson Province. The frogs were 18 - 24 months old; the females weighed 439.9 g and the males weighed 401.3 g (Fig. 5).

#### 3.1 Breeding culture

In commercial production, natural breeding predominates. The frogs commonly breed in temporary ponds 2 x 2 x 1.2 m in size with 0.30 m water depth and slope floor. Water plants (morning glory) were provided as source of oxygen and sites of egg-laying (Fig. 6). The water had been dechlorinated by standing overnight before putting in the breeding pond. The water temperature was maintained at 25 - 30°C with the pH of 7.1 - 7.5. The photoperiod was 12 D : 12 L. The sex ratio of male to female was 1 : 1. After the eggs were laid, the brooders were removed. The ponds were supplied with oxygen by air pump system for 10 - 15 days.

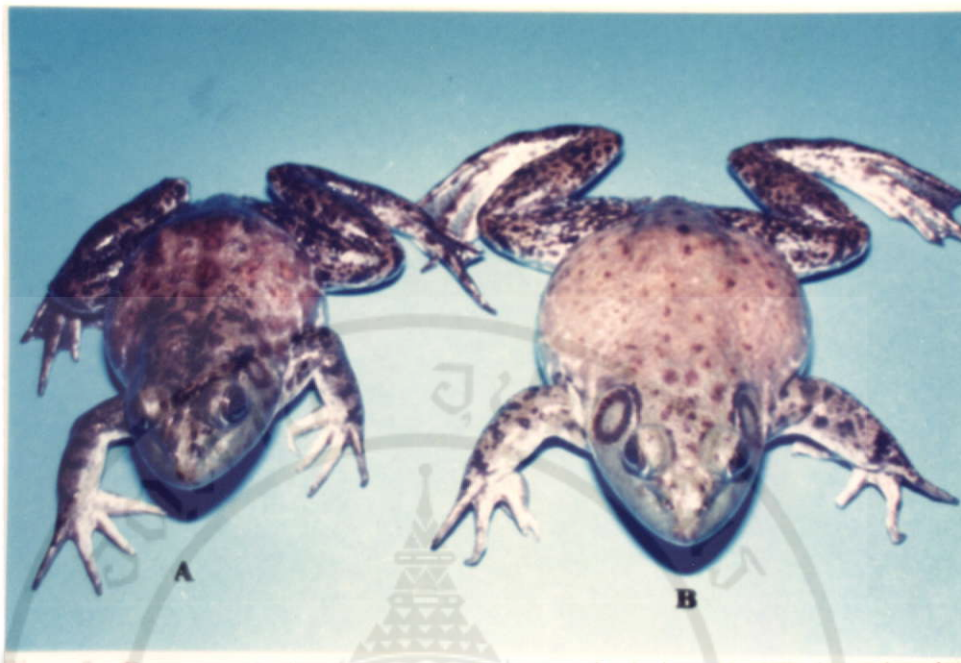


Fig. 5 *R. catesbeiana* male and female.

A. Female.

B. Male.



Fig. 6 *R. catesbeiana* male and female in breeding pond.

### 3.2 Culture of tadpoles and froglets

After the breeding and separation of brooders, eggs were left in the breeding pond to be hatched. The eggs hatched into tadpoles within 2 - 3 days.

The concrete ponds, 1 x 1 x 0.5 m in size, with the water depth of 0.20-0.30 m and slope floor were used for tadpole culture. The water temperature was 25 - 30°C with the pH of 7.1 - 7.5.

On the 15<sup>th</sup> day, the tadpoles which began their feeding activities were fed with an artificial feed containing protein, adjusted to 20% of their body weight daily. After the emergence of forelimbs or metamorphosis which took approximately 2 - 3 months, froglets were reared in concrete ponds (2 x 3 x 1.2 m) at the density of 130 froglets/m<sup>2</sup>. To prevent the froglets from harmful diseases, artificial feed were mixed with antibiotics (chlortetracycline HCl or aureomycin) and fed to the froglets for five consecutive days. Froglets were fed once in the evening. The quantity of feed was 5 - 20% of body weight or indicated by demand. The water in the pond was changed every other day. Selection of size was done once a week and froglets of different sizes were put in different ponds at the density of 40 frogs/m<sup>2</sup>.

## 4. Study on various protein concentrations in pelleted feed on growth, survival and metamorphosis

### 4.1 Growth, survival and metamorphosis of tadpoles

Four round concrete tanks (0.80m in diameter) with the water depth of 0.10 - 0.20 m were used for rearing tadpoles of *R. tigerina* and *R. catesbeiana* (Fig. 7). The water temperature was 25 - 30°C with the pH of 7.1 - 7.5. One hundred tadpoles of each species were placed in each tank. There were four experimental groups of tadpoles fed with different formulae of pelleted feed.

Group 1 : fed with formula of 35% protein

Group 2 : fed with formula of 39% protein

Group 3 : fed with formula of 43% protein

Group 4 : fed with formula of 47% protein

Tadpoles were fed once in the evening. The quantity of feed was 10 - 30% of body weight or indicated by demand. The tanks were supplied with oxygen from air pump. The water was changed every other day. Tadpoles were weighed once every 15 days. Dead tadpoles were removed and the numbers recorded every day. Food conversion data were gathered at the beginning of 7<sup>th</sup> day.



Fig. 7 Round concrete tanks used for rearing tadpoles in the dietary protein experiments.

#### 4.2 Growth and survival of froglets and frogs

After metamorphosis, froglets of *R. tigerina* and *R. catesbeiana* were transferred into the concrete ponds, 3 x 4 x 1.2m in size with water depth of 0.03 - 0.15 m. Then, they were selected for weight or size (15 - 25 g). Four concrete ponds (2 x 2 x 1.2 m, water depth of 0.03 - 0.20 m) were prepared. The water temperature generally varied between 25 - 30°C, with the pH of 7.1 - 7.5 and humidity range was 80 - 90%. One hundred froglets were placed in each pond. The froglets were divided into four groups and fed with different formulae of pelleted feed.

Group 1 : fed with formula of 21% protein

Group 2 : fed with formula of 25% protein

Group 3 : fed with formula of 29% protein

Group 4 : fed with formula of 33% protein

To prevent the froglets from harmful diseases, pelleted feed were mixed with antibiotics (chlortetracycline HCl or aureomycin) and fed to the froglets for five consecutive days. Froglets were fed once in the evening. The quantity of feed was 5 - 20% of body weight or indicated by demand. The water in the pond was changed every other day and the ponds were cleaned once a week. Selection of size was done once a week and

froglets of different sizes were put in different ponds to prevent cannibalism. Dead froglets were removed and numbers recorded everyday. Froglets were weighed once a month until they were fully grown and sexually mature. Food conversion data were gathered at the beginning of 7<sup>th</sup> day after froglet culture.

## 5. Food conversion

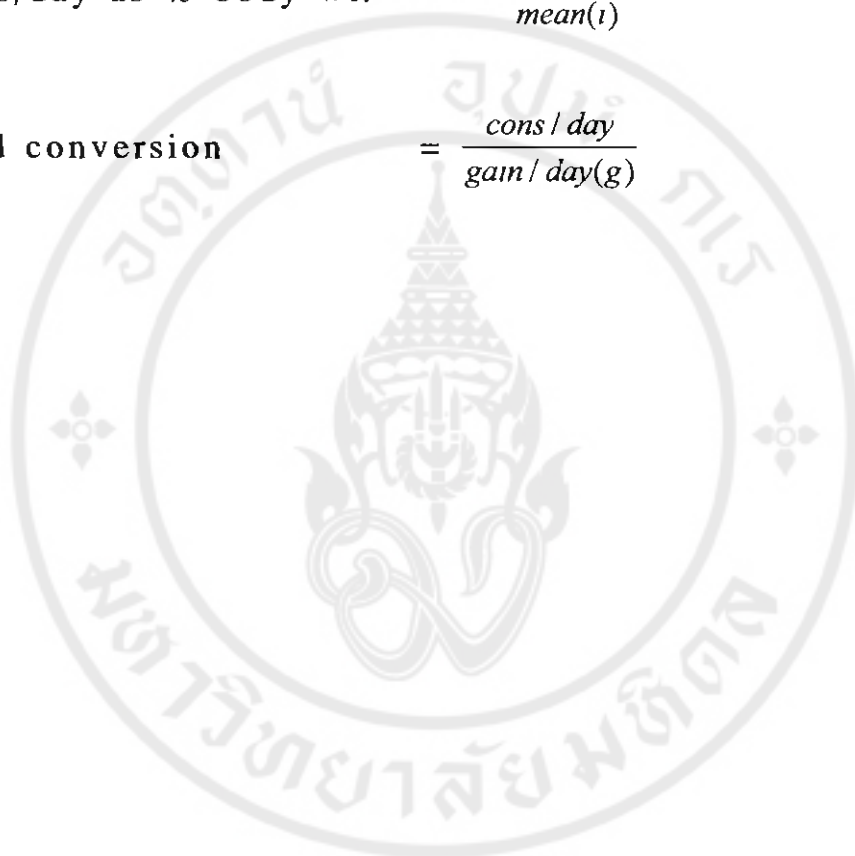
Food conversion data were obtained by weighing the food before and after feeding. The weights of tadpoles, froglets or frogs were taken prior to feeding. The feed was weighed daily as described to determine food consumption. Food conversion values were obtained by dividing the total food consumed wet weight by the total gain of each group. A dietary protein somatic index was obtained by dividing the dry weight of protein consumed by the total wet weight gain of each group. This value is an index of protein consumption related to growth. Analyses of diets were performed using standard Association of official Agriculture Chemists(A.O.A.C., 1970) method to determine composition percents. The following parameters were estimated :

$$\text{Gain/day(g)} = \frac{\text{mean}(f) - \text{mean}(i)}{\text{number of day}}$$

$$\text{Gain/day as \% body wt.} = \frac{\text{gain / day(g)} \times 100}{\text{mean}(i)}$$

$$\text{Cons/day as \% body wt.} = \frac{\text{cons / day} \times 100}{\text{mean}(i)}$$

$$\text{Feed conversion} = \frac{\text{cons / day}}{\text{gain / day(g)}}$$



# CHAPTER IV

## RESULTS

### 1. Life cycles

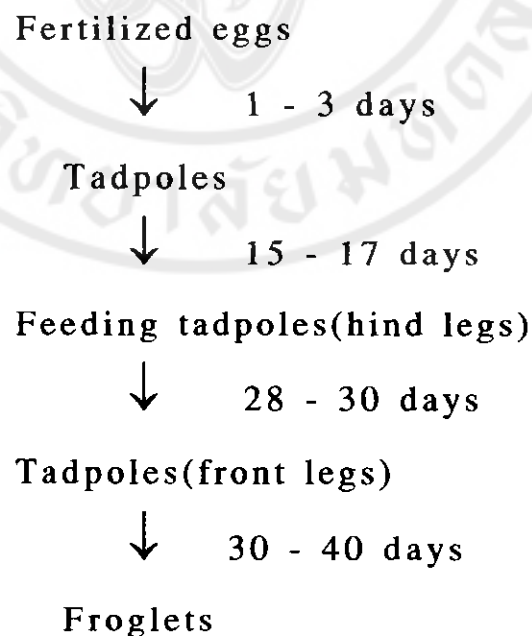
#### 1.1 *R. tigerina*

*R. tigerina* usually bred at night or early in the morning. The female frog laid 3,000-5,000 eggs at one time. Fig. 8 shows the egg mass of *R. tigerina*.

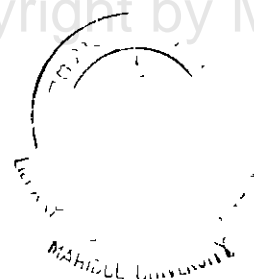
After the breeding and separation of brooders, eggs were left in the breeding pond to be hatched. Air pump (source of oxygen) was supplied in each pond. The eggs hatched into tadpoles within 1-3 days (Fig. 9). The hatching rate was approximately 75%.

When the tadpoles hatched out of their eggs, they had round head and body, and a tail, but they had no legs (Fig. 10). The tadpoles' hind legs appeared later after 14 days (Fig. 11). They swam about using their tails, little hind legs, and breathed by means of gills. The front legs developed in partnership with the back ones but were hidden from view and appeared later in development. At the completion of their development, the limbs bulged

against the body wall. The tadpoles thrust one of the front legs through the spiracle or aperture from the gills and the other through a specially weakened area of skin on the opposite side of the body which appeared later after 30-35 days (Fig. 12.). After that the tadpoles shrank in size rapidly and the tail was absorbed into the body (approximately 3-5 days). The head and body became smaller as the soft tissues collapsed and tightened around the bone beneath. They metamorphosed into juvenile or froglets after 30 days (Fig.13). Developmental stages of *R. tigerina* tadpoles are shown below:



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Frogllets grew into adult frogs and reached marketable size approximately within 4-5 months with the weights of 150-200g in female and 100-200g in male (Figs.14-16). They completed their reproductive maturity within 12 months.





Fig. 8 The egg mass of *R. tigerina*.

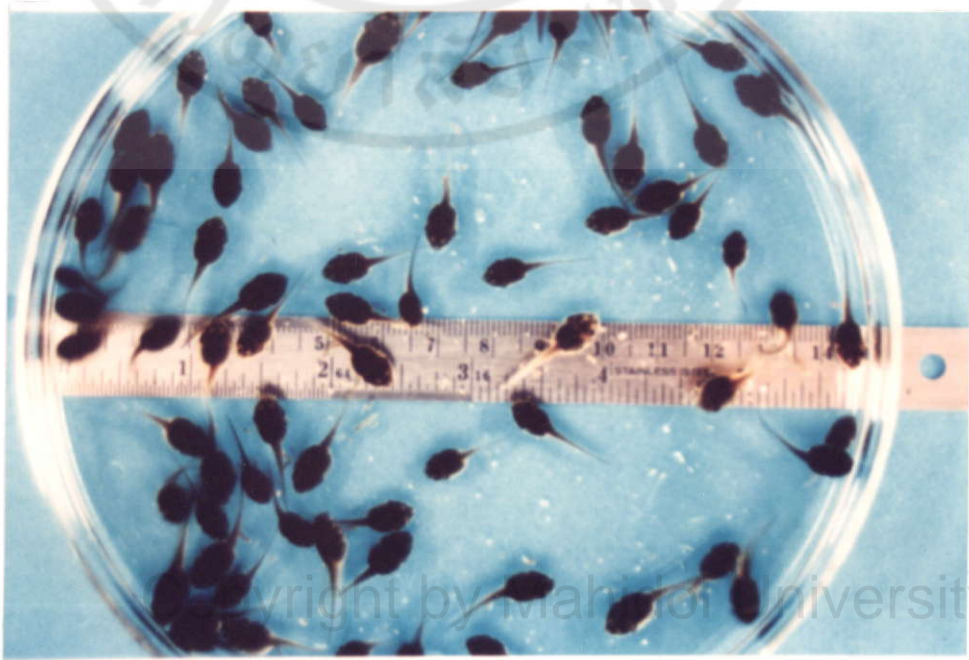


Fig. 9 Tadpoles of *R. tigerina*.



Fig. 10 Tadpoles of *R. tigerina* of 14 days old.  
Note the emergence of hind legs.



Fig. 11 Tadpoles of *R. tigerina* of 20 days old.



Fig. 12 Tadpole of *R. tigerina* of 30 days old.  
Note the emergence of front legs.

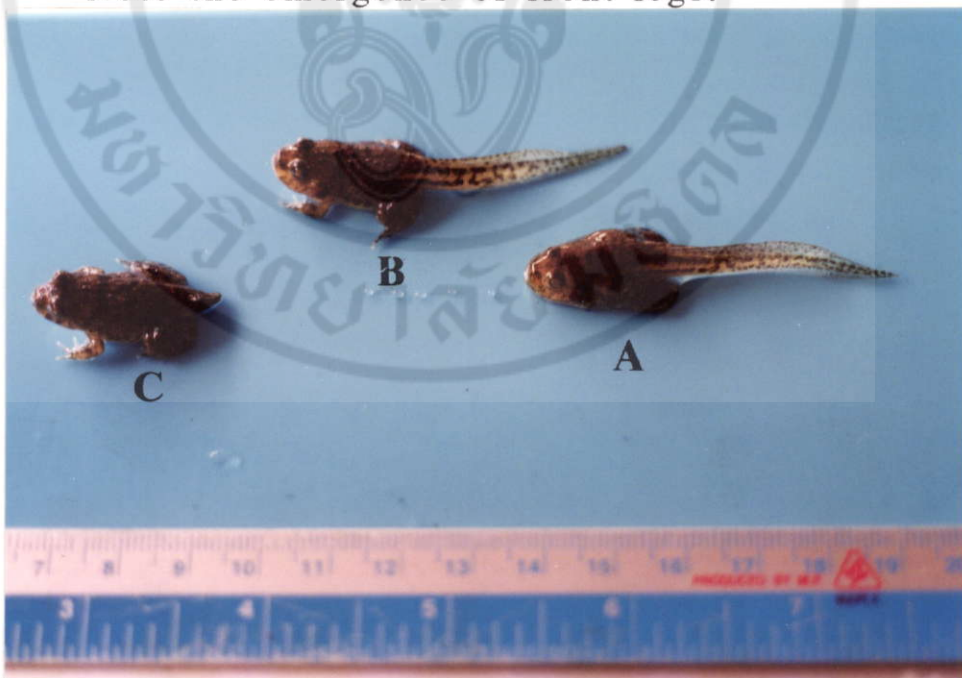


Fig. 13 Tadpoles and froglet of *R. tigerina* at various ages. A. Tadpole of 20 days old. B. Tadpole of 30 days old. C. Froglet of 35 days old.



Fig. 14 Concrete ponds with slope floor for rearing froglets and frogs.



Fig. 15 *R. tigerina* froglets of 1 1/2 months old.

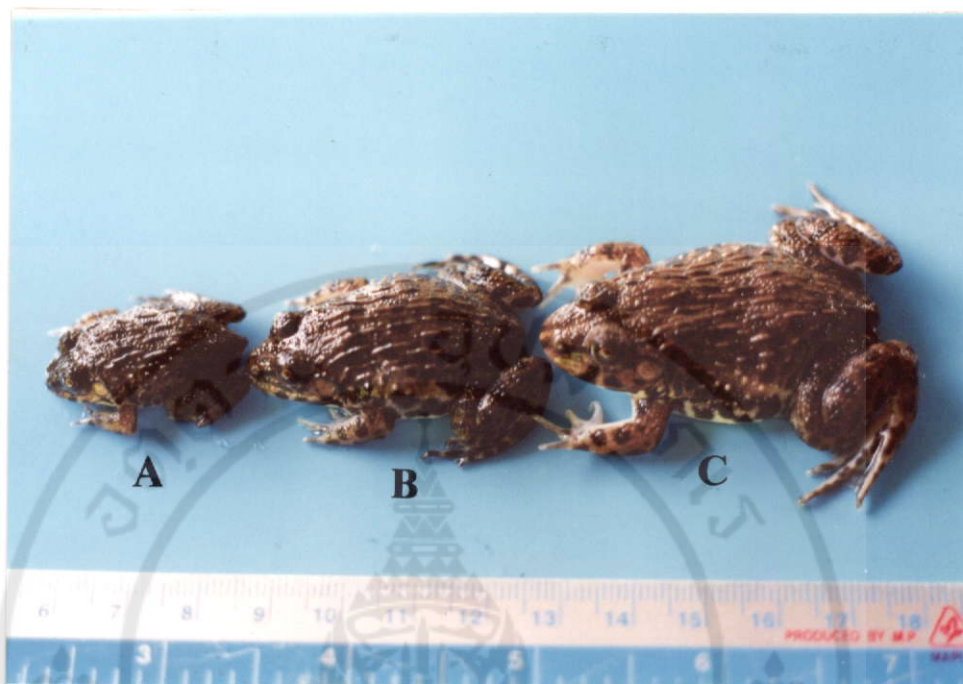
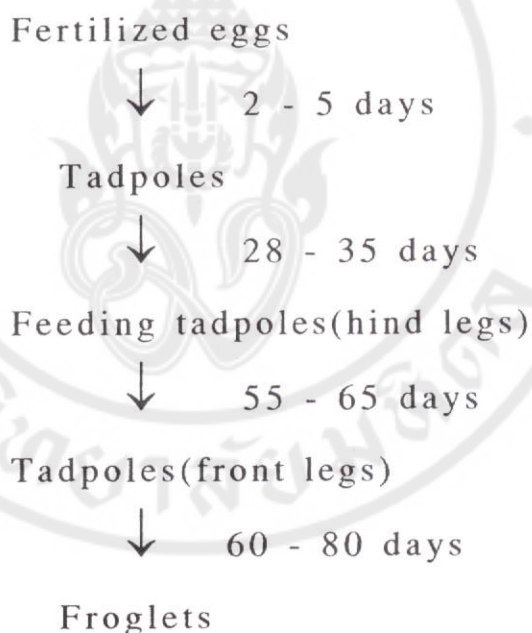


Fig. 16 *R. tigerina* froglets at various ages. A. Two-month-old froglet. B. Three-month-old frog. C. Five-month-old frog.

### 1.2 *R. catesbeiana*

*R. catesbeiana* usually bred at night or early in the morning. The female frog laid 6,000-8,000 eggs at one time. Fig. 17 shows the egg mass of *R. catesbeiana*. After the breeding and separation of brooders, eggs were left in the breeding pond to be hatched. Air pump (source of oxygen) was supplied in each pond. The eggs hatched into tadpoles within 2 - 5 days (Fig. 18). The hatching rate was approximately 70%.

When the tadpoles hatched out of their eggs, they had a round head and body, and a tail (Figs. 19,20), but they had no legs. The tadpoles' hind legs appeared later on 30<sup>th</sup> - 40<sup>th</sup> days (Fig. 21) while the front legs appeared later on 60 - 75 days (Fig.22). They became juveniles or froglets within 60-80 days (Fig.23). Developmental stages of *R. catesbeiana* tadpoles are shown below :



Froglets grew into adult frogs and reached marketable size approximately within 6-8 months with the weights of 100-300g (Figs.24,25). They completed their reproductive maturity within 18 months.



Fig. 17 The egg mass of *R. catesbeiana*.



Fig. 18 Newly hatched tadpoles of *R. catesbeiana* of 2-5 days old.



Fig. 19 Tadpoles of *R. catesbeiana* of 7-9 days old.

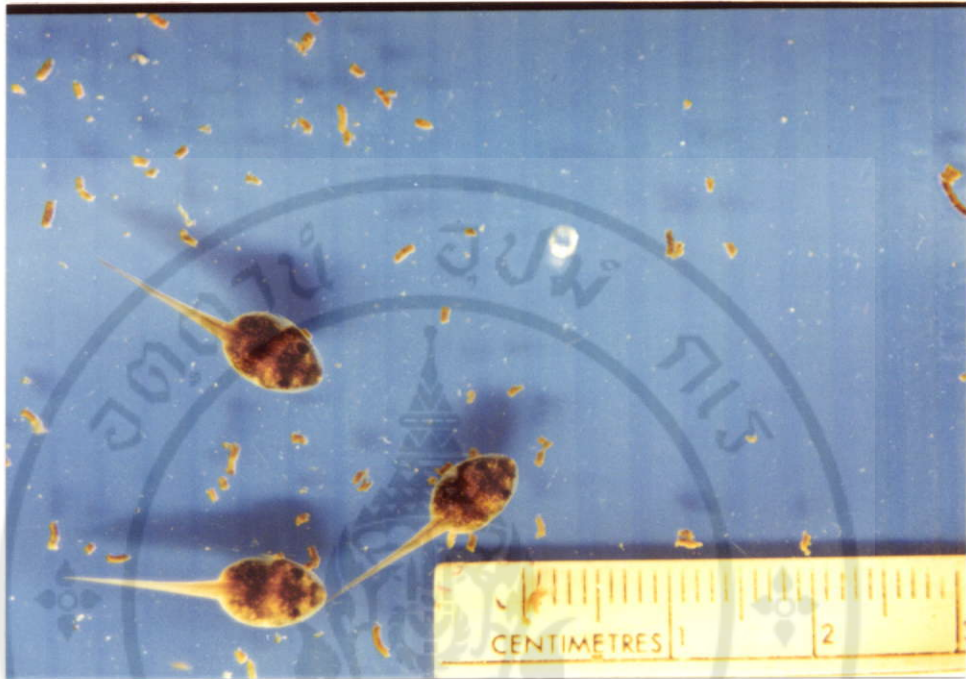


Fig. 20 Tadpoles of *R. catesbeiana* of 13-15 days old.



Fig. 21 Tadpole of *R. catesbeiana* of 42 days old.

Note the emergence of hind legs.



Fig. 22 Tadpole of *R. catesbeiana* of 75 days old.  
Note the emergence of front legs.

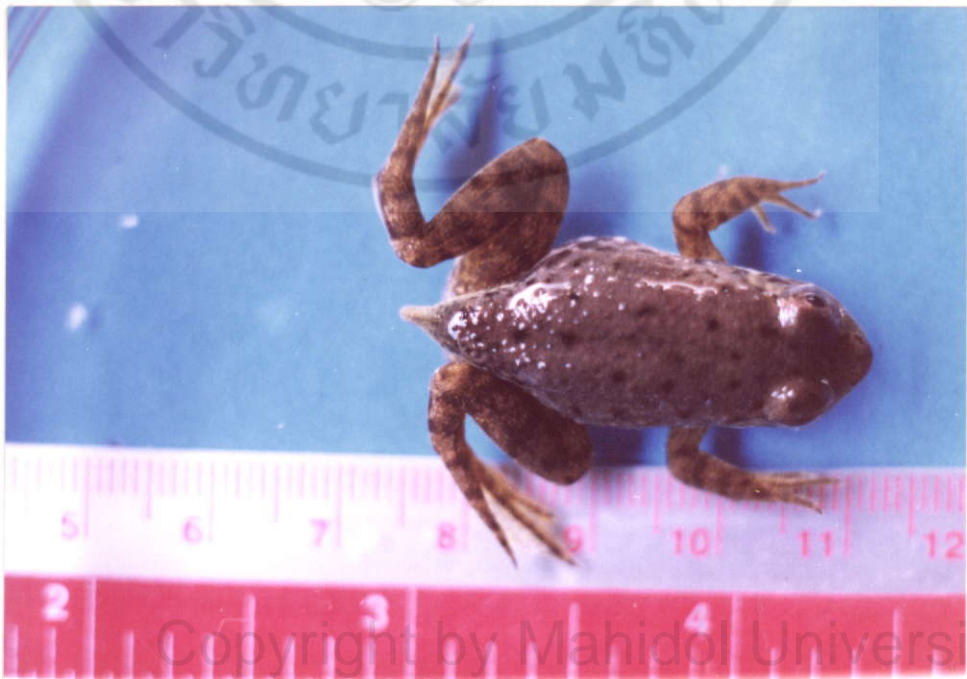


Fig. 23 Froglet of *R. catesbeiana* of 80 days old.



Fig. 24 Concrete ponds for rearing *R. catesbeiana* froglets and frogs.



Fig. 25 *R. catesbeiana* frogs at various ages. A. Four-month-old frog. B. Five-month-old frog. C. Eight-month-old frog.

## **2. The effects of various protein concentrations in pelleted feed on growth, survival and metamorphosis**

### **2.1 *R. tigerina***

#### **2.1.1 Tadpole stage**

##### **Growth**

The tadpoles of *R. tigerina* were fed with different formulae of pelleted feed of varying protein contents (35%, 39%, 43%, 47%). The use of diets displayed simplified preparation and acceptable stability without altering water quality (Table 1). Growth curves summarized in Table 2 and Fig. 26 show the average body weights of the tadpoles fed on various diets. They suggested that higher protein levels were promoting better growth. Results from the comparison of average body weights of the tadpoles throughout the experiment indicated that at the end of the fourth week, tadpoles receiving higher dietary protein (43%, 47%) increased their body weights more than those of the two lower protein groups (35%, 39%). Over the following two week period, the diet with 43% protein promoted the best tadpoles' growth (1.95g).

Daily consumption did not differ between groups throughout the experimental period. The amount of food given was the same in all groups, although there were differences in the amount of protein taken in by the tadpoles (Table 3). The results obtained from the food consumed daily (% body weight) showed that as the level of protein increased, the tadpoles consumed less. The maximum percentages of consumption (from 8.05% to 13.80%) were registered throughout the third fortnight, when tadpoles reached the metamorphic phase. The values for the indices of nutritive used, feed conversion was shown in Table 3. Feed conversions for tadpoles were increased from 0.912 to 1.790 while the percentages of protein tended to decline. Thus, feed conversion for tadpoles fed with diets containing 35% and 39% protein (1.790, 1.415) were better than those fed with diets containing 43% and 47% protein (0.912, 1.020).

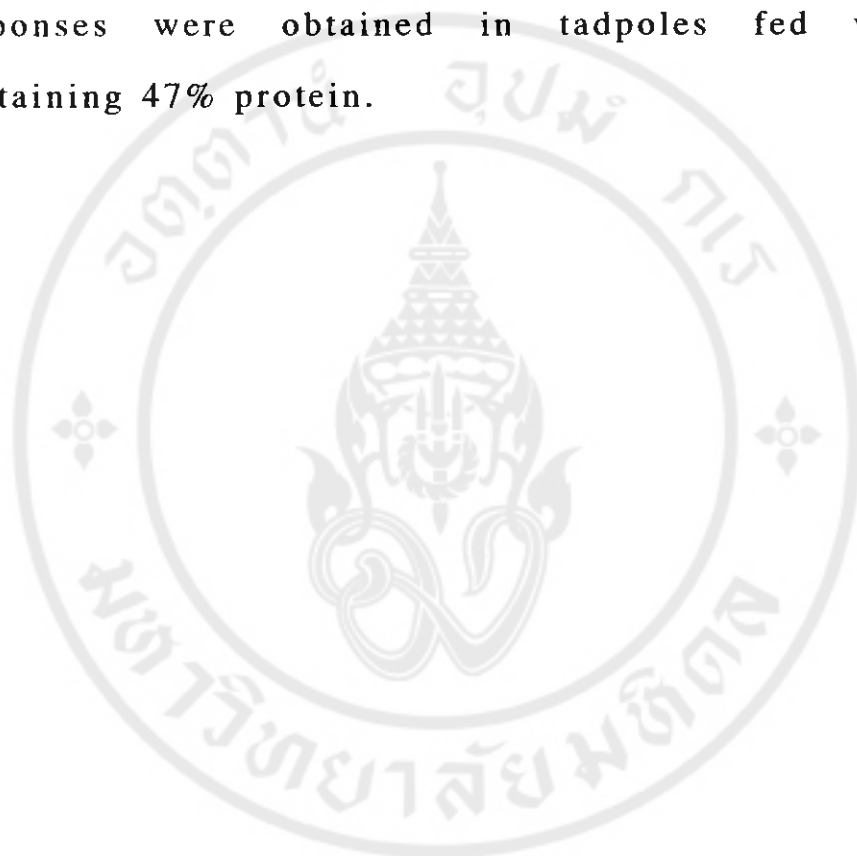
## Survival

The results obtained from the comparison of percentages of survival for tadpoles indicated that the percentage of survival in tadpoles fed with 43% protein diet was the lowest (80%) after the first three weeks. However, mortality values were averaged at 7.5% as the average percentage of survival was very high (92.5%) through the end of the experiment. In late metamorphosis, diets of 47%, 35% and 39% protein resulted in percentages of survival of 99%, 97% and 94%, respectively (Table 4, Fig.27).

## Metamorphosis

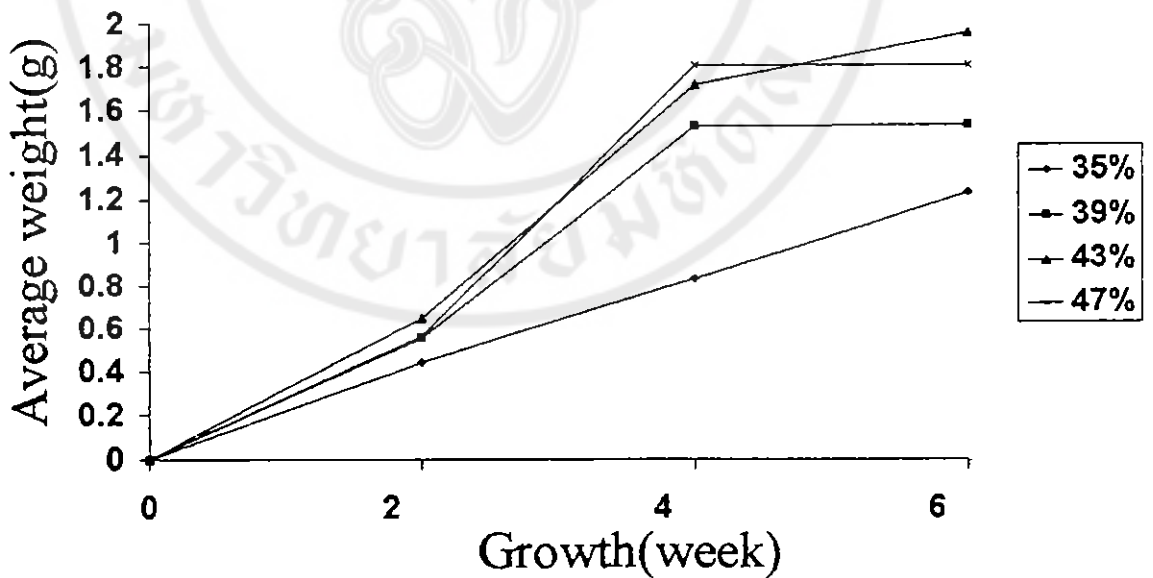
Metamorphosis occurred in all groups. Tadpoles gradually changed into froglets. On the third week, they developed hind-legs which lay against the tail at the end of the body. Then, on the fourth week, some became froglets. On the fifth week, 89% of tadpoles fed with diet containing 47% protein became froglets. The lower percentages of metamorphosis occurred in tadpoles fed with diets containing 39% protein (80%), 43% protein (70%) and 35% protein (54%). By the sixth week, 99% of tadpoles fed with diet containing 47% became froglets. The lower percentages of metamorphosis occurred in tadpoles fed with diets containing 39% protein (93%), 35% protein (84%) and 43% protein (80%) (Table 5, Fig.28).

Table 6 summarized the growth, survival and metamorphic responses of *R. tigerina* tadpoles fed with diets varying in protein concentrations. Tadpoles fed with diet containing 43% protein reached the highest average weight (1.95 g). The best survival and metamorphic responses were obtained in tadpoles fed with diet containing 47% protein.



**Table 2** The average weights of *R. tigerina* tadpoles fed with diets varying in protein concentrations.

Age (week)	Average weight (g)			
	35%	39%	43%	47%
2	0.442	0.552	0.646	0.562
4	0.830	1.526	1.709	1.800
6	1.230	1.530	1.950	1.800



**Fig. 26** Growth curves of *R. tigerina* tadpoles fed with diets varying in protein concentrations (35%, 39%, 43%, 47%).

**Table 3** Weight, growth responses and food utilization by *R. tigerina* tadpoles fed with diets varying in protein concentrations.

	Protein concentration			
	35%	39%	43%	47%
Mean initial wt.(g)	0.442	0.552	0.646	0.562
Mean final wt.(g)	1.230	1.530	1.950	1.800
Gain/day(g)	0.034	0.043	0.057	0.054
Gain/day as % body wt.	7.692	7.790	8.824	9.609
Consumed/day(g dry wt.)	0.061	0.061	0.052	0.055
Cons/day as % body wt.	13.800	11.050	8.050	9.780
Feed conversion	1.790	1.415	0.912	1.020

**Table 4** Percentages of survival for *R. tigerina* tadpoles fed with diets varying in protein concentrations.

Age (week)	% survival			
	35%	39%	43%	47%
1	100	100	100	100
2	100	100	100	100
3	100	100	100	100
4	100	99	84	100
5	97	99	80	100
6	97	94	80	99

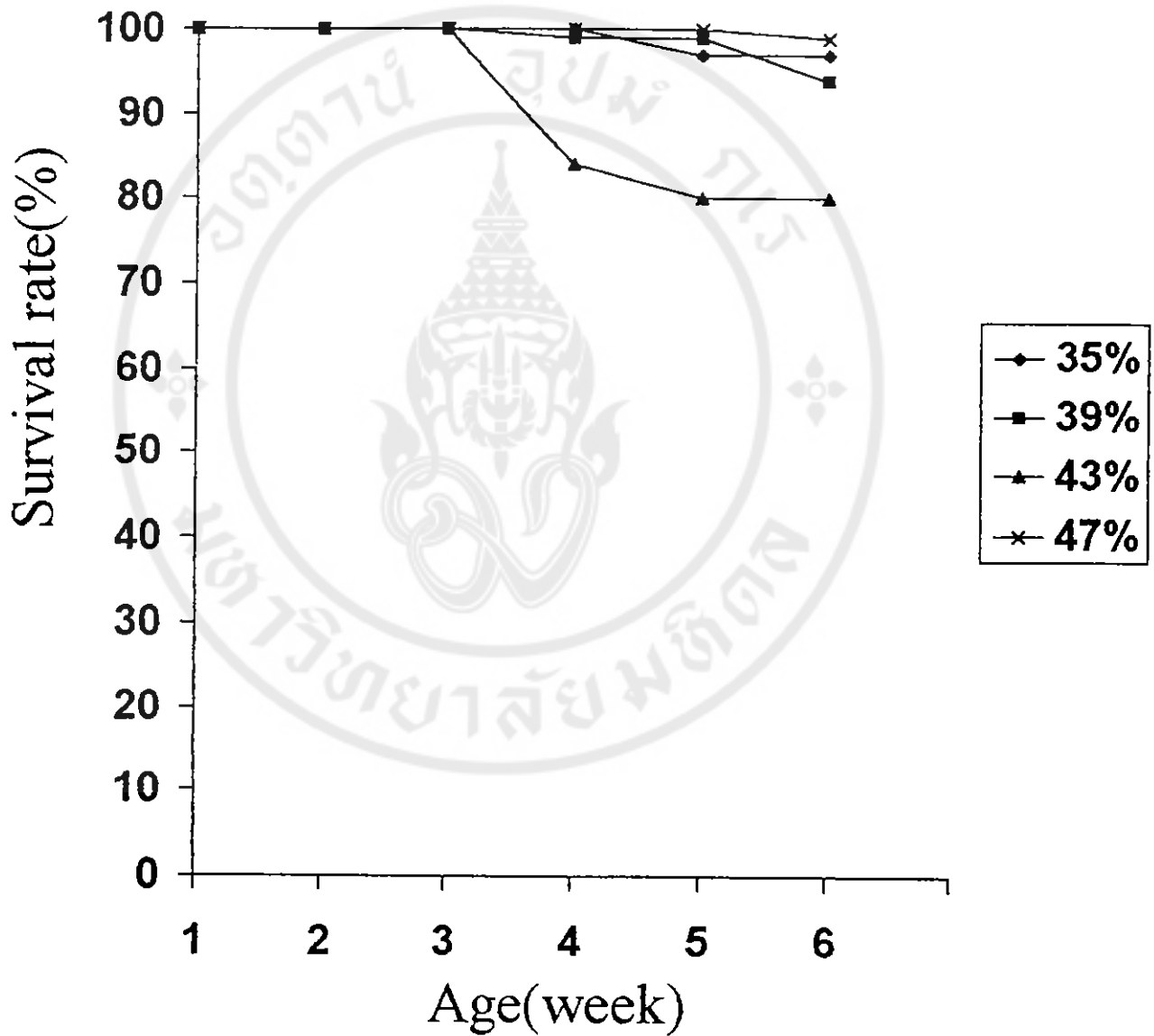
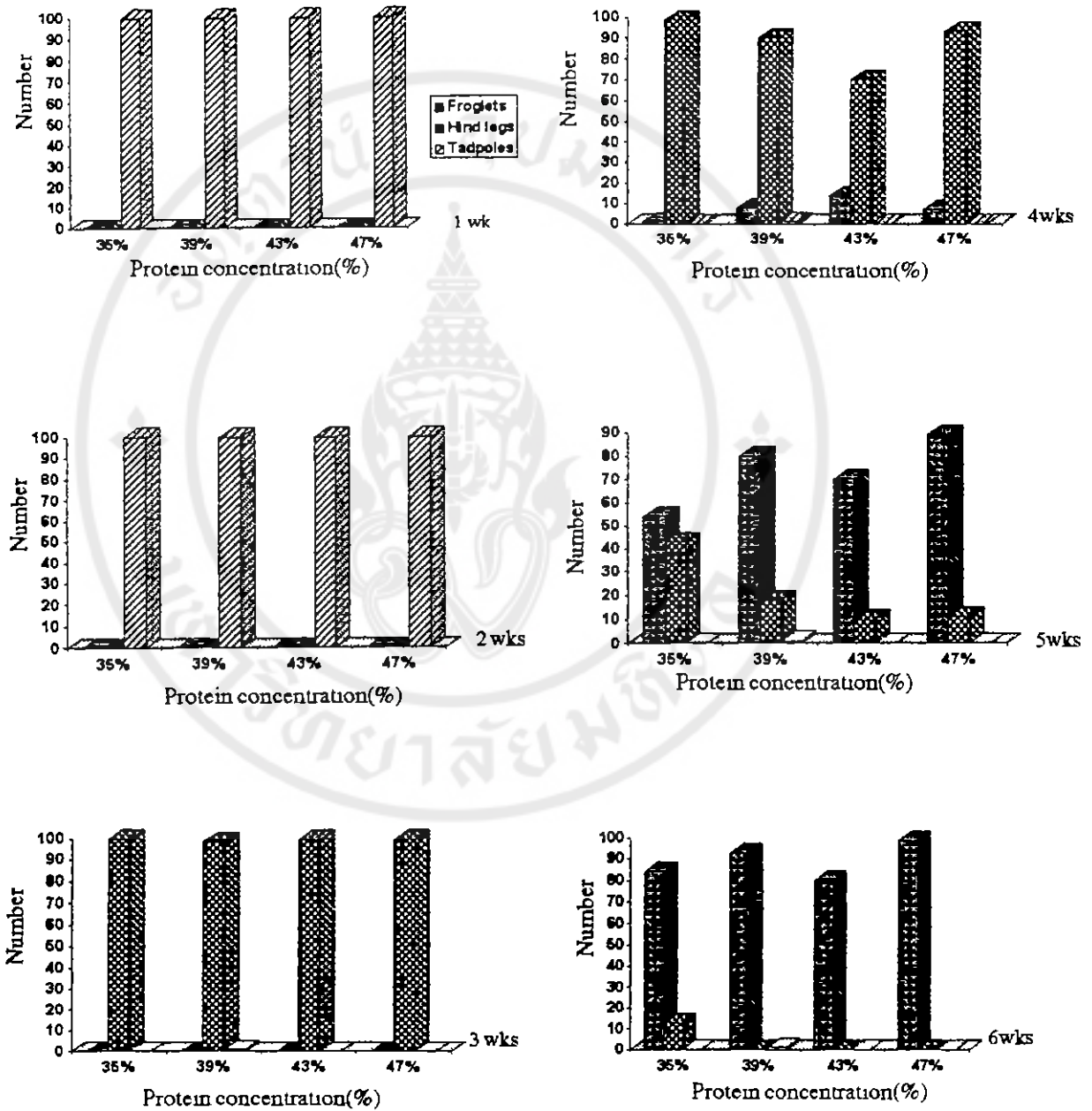


Fig. 27 Percentages of survival in *R. tigerina* tadpoles fed with diets varying in protein concentrations (35%, 39%, 43%, 47%).

**Table 5** Metamorphosis in *R. tigrina* tadpoles fed with diets varying in protein concentrations.

Age (week)	% metamorphosis							
	35%		39%		43%		47%	
	Froglets	Hind-legs Tadpoles	Froglets	Hind-legs Tadpoles	Froglets	Hind-legs Tadpoles	Froglets	Hind-legs Tadpoles
1	0	0	100	100	0	0	0	100
2	0	0	100	100	0	0	0	100
3	0	100	0	99	0	100	0	100
4	1	99	8	90	14	70	7	93
5	54	43	80	18	70	10	89	11
6	84	13	93	0	80	0	99	0



**Fig. 28** Histograms showing rates of metamorphosis in *R. tigerina* tadpoles fed with diets varying in protein concentrations (35%, 39%, 43%, 47%).

**Table 6** Growth, survival and metamorphic responses of *R. tigerina* tadpoles fed with diets varying in protein concentrations.

	Protein concentrations			
	35%	39%	43%	47%
(1) Initial number	100	100	100	100
(2) Final number	97	94	80	99
* (3) % Survival	97	94	80	99
* (4) % Metamorphs	84	93	80	99
(5) Average weight(g)				
of (4)	1.23	1.53	1.95	1.80
* (6) % of hind-legged tadpoles remain undeveloped	13	0	0	0
(7) Average weight(g)				
of (6)	1.43	0	0	0
* (8) % of undeveloped tadpoles	0	1	0	0
(9) Average weight(g)				
of (8)	0	3.09	0	0

Average weight of  
all animals at the  
end of experiment

1.83

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\*The percentages of (3), (4), (6) and (8) were calculated from the initial number.

### **2.1.2 Froglet and frog stages**

#### **Growth**

Froglets of *R. tigrina* were fed with different formulae of pelleted feed varying in protein concentrations (21%, 25%, 29%, 33%). The average body weight values from the growth curves showed that protein was necessary early in the first three months. After that, the average body weights were quite different. The results from the comparison of average body weights of the froglets throughout the experiment indicated that froglets fed with diet of 29% protein showed the best growth (198.10g body weight) comparing to those fed with diets containing 25% protein (190.20 g), 21% protein (177.0 g) and 33% protein (177.0 g) (Table 7, Fig. 29). The highest weight gain per day (0.77 g) was also observed in froglets fed with diet of 29% protein (Table 8). The growth rates throughout the study are shown in Table 9 and Fig. 30. The weights of froglets increased rapidly during the first three months. Froglets fed with diet containing 29% protein showed the highest average growth rate.

Daily consumption did not differ among groups throughout the experimental period, although there were differences in the amount of protein taken in by the frogs (Table 8). The results obtained from the food consumed daily (% body weight) showed that different

groups of frogs consumed different amount of food (Table 8). It can be seen that the feed conversion values also are different in frogs fed with diets containing varying protein concentrations (1.47 for 29% protein, 1.53 for 25% protein, 1.64 for 33% protein and 1.66 for 21% protein). The diet of 21% protein promoted the best feed conversion.

### **Survival**

The results obtained from the comparison of percentages of survival for froglets indicated that the mortality was very low. Mortality values were averaged less than 4% through the end of the experiment. The percentages of survival are not different significantly among the experimental groups, i.e. 99% in froglets fed with diet of 25% protein, 98% in those fed with diet of 21% protein, 97% in those fed with diet of 29% protein and 93% in those fed with diet of 33% protein (Table 10, Fig.31).

**Table 7** The average weight of *R. tigerina* froglets fed with diets varying in protein concentrations.

Age (month)	Average weight (g)			
	21%	25%	29%	33%
1	19.90	18.50	19.70	17.30
2	67.80	69.00	61.20	67.40
3	147.80	139.20	149.60	132.40
4	148.40	163.20	158.60	168.40
5	153.00	165.80	194.20	172.50
6	173.60	170.50	194.80	176.20
7	175.00	183.60	195.30	176.50
8	177.00	190.20	198.10	177.00

**Table 8** Weight, growth responses and food utilization for 8 months period in *R. tigerina* froglets fed with diets varying in protein concentrations.

	21%	25%	29%	33%
Mean initial wt.(g)	19.90	18.50	19.70	17.30
Mean final wt.(g)	177.00	190.20	198.10	177.00
Gain/day(g)	0.68	0.74	0.77	0.69
Gain/day as % body wt.	3.42	4.00	3.91	3.99
Consumed/day (g dry wt.)	1.13	1.13	1.13	1.13
Consumed/day as % body wt.	5.68	6.11	5.74	6.53
Feed conversion	1.66	1.53	1.47	1.64

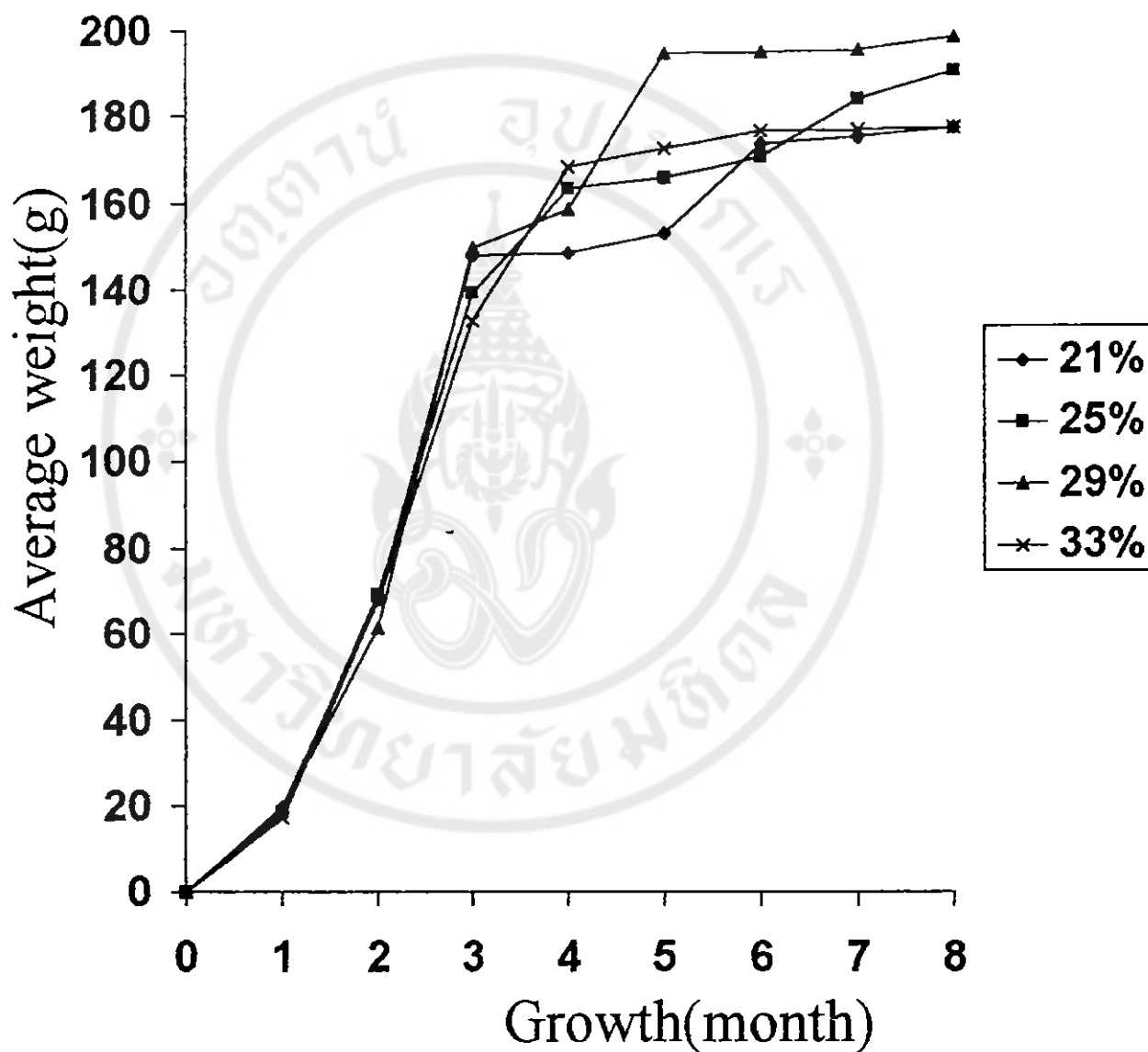


Fig. 29 Growth curves of *R. tigerina* froglets fed with diets varying in protein concentrations (21%, 25%, 29%, 33%).

**Table 9** Growth rates (average wt/ month) of *R. tigrina* froglets fed with diets varying in protein concentrations.

Age (month)	Average weight (g)			
	21%	25%	29%	33%
2	47.50	50.50	41.50	50.10
3	80.00	70.20	88.40	65.00
4	0.60	24.00	9.00	36.00
5	4.60	2.60	35.60	4.10
6	20.60	4.70	0.60	3.70
7	1.40	13.10	0.50	0.30
8	2.00	6.60	2.80	0.50

**Table 10** Percentages of survival in *R. tigrina* froglets fed with diets varying in protein concentrations.

Age (month)	% survival			
	21%	25%	29%	33%
1	100	100	100	100
2	100	100	100	100
3	100	100	100	100
4	100	100	100	100
5	100	100	100	100
6	100	100	100	100
7	100	100	100	100
8	98	99	97	93

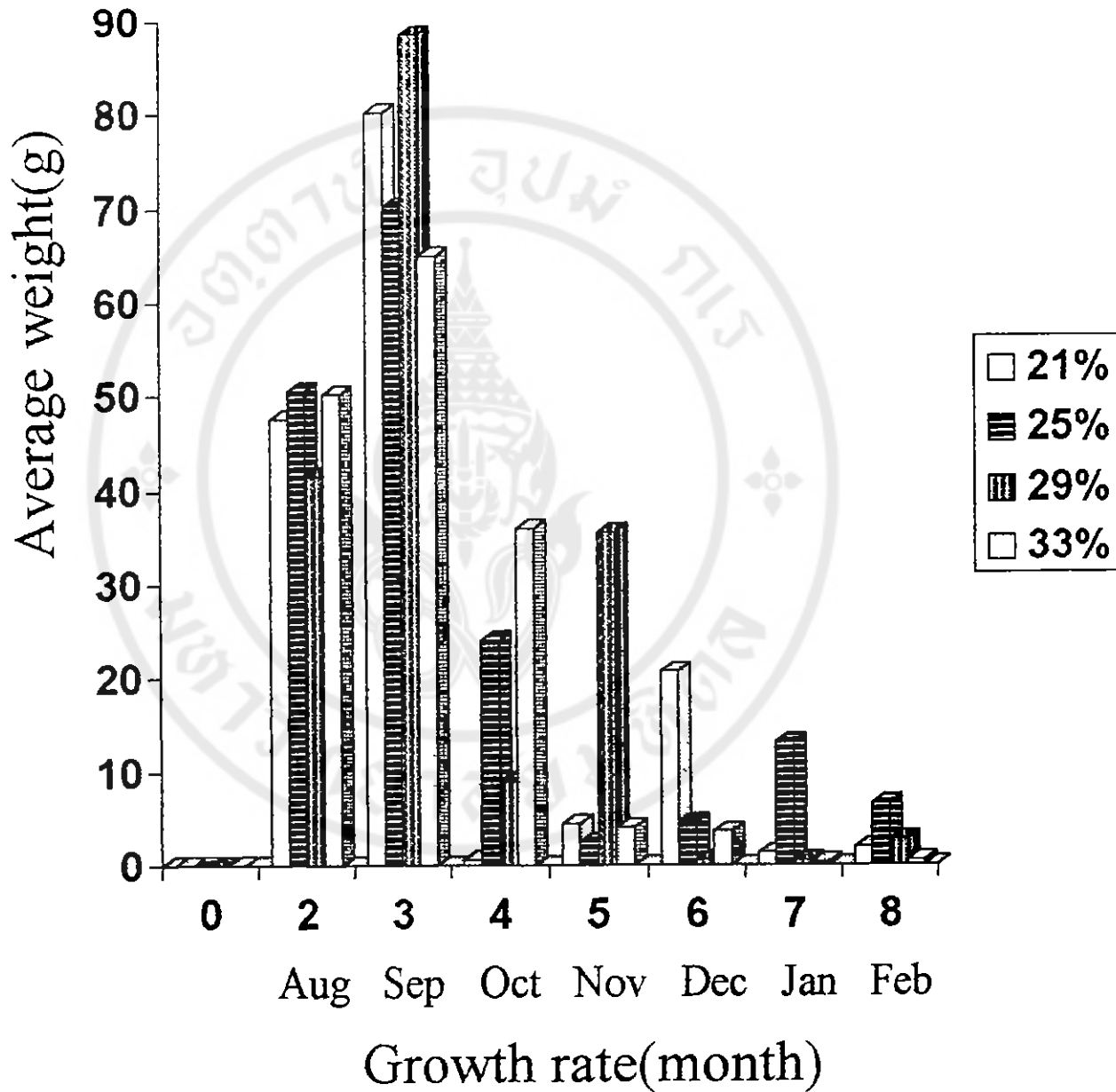


Fig. 30 Growth rates of *R. tigerina* froglets fed with diets varying in protein concentrations (21%, 25%, 29%, 33%).

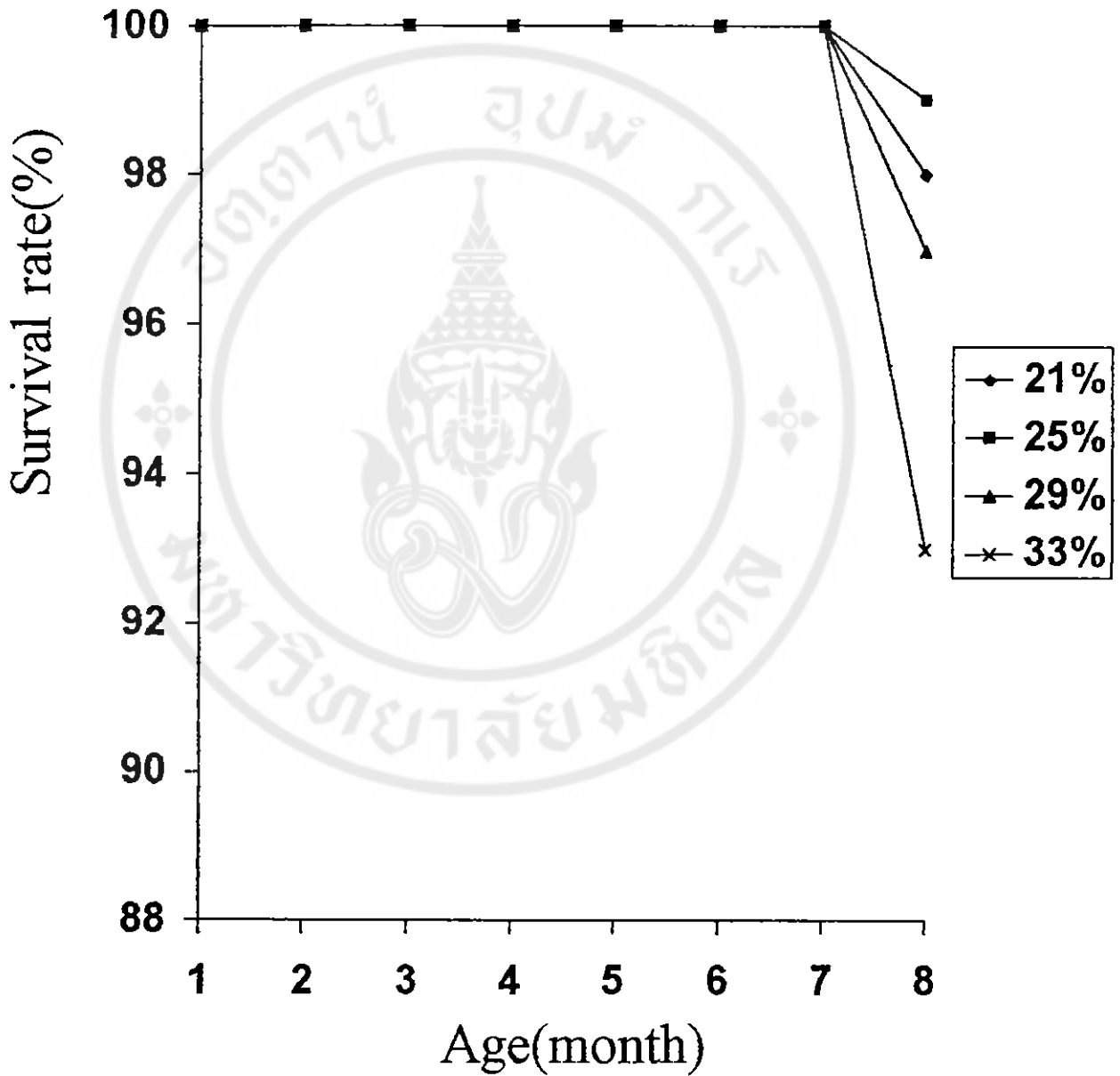


Fig. 31 Percentages of survival in *R. tigerina* froglets fed with diets varying in protein concentrations (21%, 25%, 29%, 33%).

## **2.2 *R. catesbeiana***

### **2.2.1 Tadpole stage**

#### **Growth**

The tadpoles of *R. catesbeiana* were fed with different formulae of pelleted feed of varying protein concentrations (35%, 39%, 43%, 47%) (Table 1). Growth curves summarized in Table 11 and Fig. 32 showed the average body weights of the tadpoles fed on various diets. They suggested that higher protein levels promoted better growth. Results from the comparison of average body weights of the tadpoles throughout the experiment indicated that at the end of the tenth week, tadpoles receiving higher dietary protein (43%, 47%) had increased in their body weights (4.95 g, 5.50 g respectively) more than those receiving lower dietary protein (4.55 g in 39% protein, 3.20 g in 35% protein) (Table 11).

Daily consumption did not differ between groups at the end of the experimental period, although there were differences in the amount of protein taken in by the tadpoles (Table 12). The results obtained from food consumed daily (% body weight) showed that as the level of protein increased (43%, 47%), the tadpoles consumed less. The maximum percentages of consumption (from 29.67% to 32.76%) were registered throughout twelve weeks, when tadpoles reached the metamorphic phase. The

values for the indices of nutritive used and feed conversion are shown in Table 12. Feed conversion values increased from 1.23 to 1.79 while the percentages of protein tended to decline. Thus, feed conversion for tadpoles fed with diets of 35% and 39% protein (1.79, 1.54 respectively) were better than those fed with diets of 43% and 47% protein (1.23, 1.29 respectively).

Table 11 The average weights of *R. catesbeiana* tadpoles fed with diets varying in protein concentrations.

Age (week)	Average weight (g)			
	35%	39%	43%	47%
4	0.29	0.29	0.29	0.29
6	1.40	3.37	1.43	2.80
8	1.50	3.83	2.67	3.93
10	3.20	4.55	4.95	5.50
12	3.63	4.13	4.72	4.71

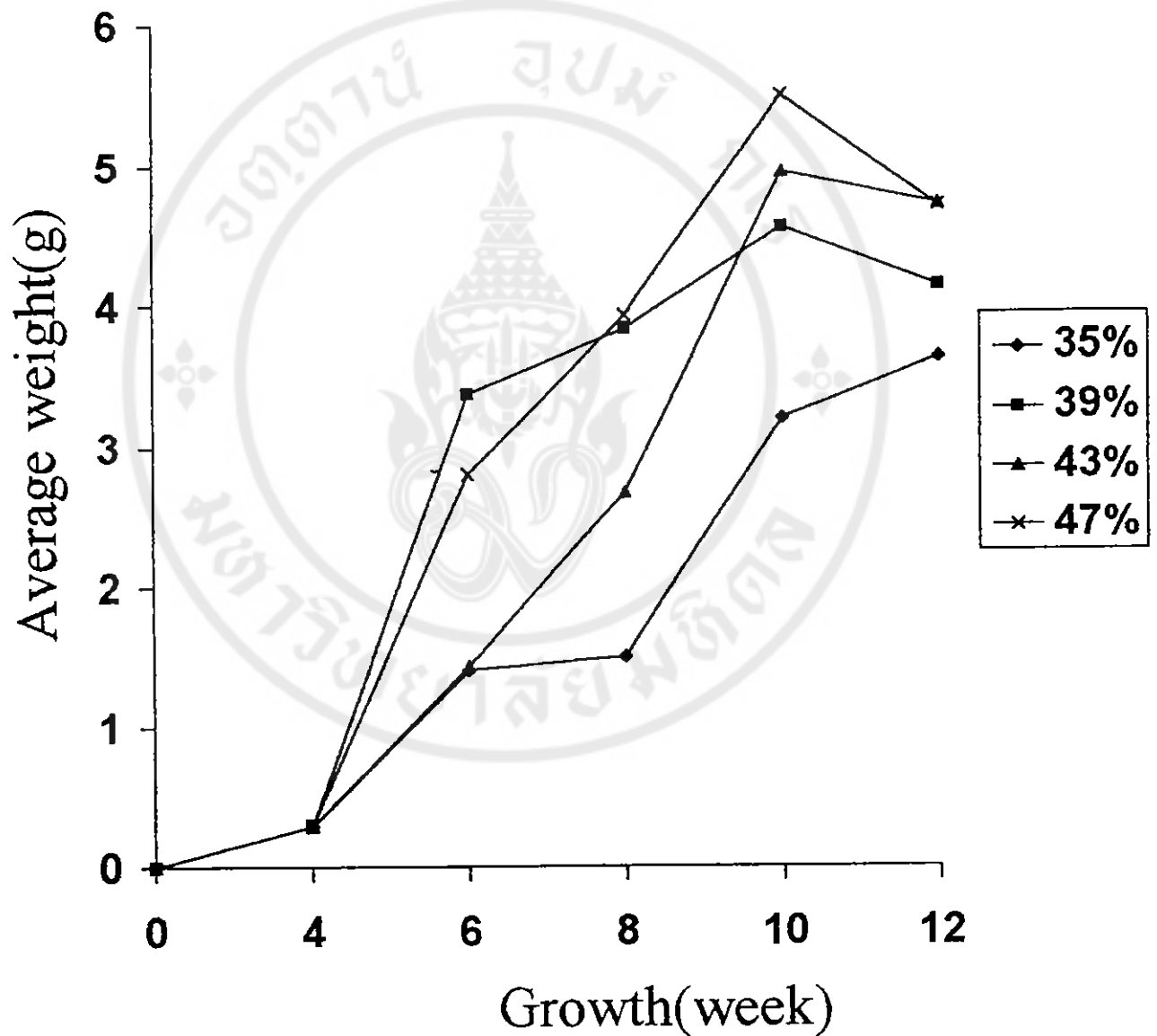


Fig. 32 Growth curves of *R. catesbeiana* tadpoles fed with diets varying in protein concentrations (35%, 39%, 43%, 47%).

**Table 12** Weight, growth responses and food utilization by *R. catesbeiana* tadpoles fed with diets varying in protein concentrations.

	Protein concentration			
	35%	39%	43%	47%
Mean initial wt.(g)	0.29	0.29	0.29	0.29
Mean final wt.(g)	3.63	4.13	4.72	4.71
Gain/day(g)	0.053	0.061	0.070	0.070
Gain/day as % body wt.	18.28	21.03	24.14	24.14
Consumed/day(g dry wt.)	0.095	0.094	0.086	0.090
Cons/day as % body wt.	32.76	32.41	29.67	31.03
Feed conversion	1.79	1.54	1.23	1.29

### **Survival**

The results obtained from the comparison of percentages of survival for tadpoles fed with diets varying in protein concentrations indicated similarities on the first six weeks, and a little death rate throughout the end of the experiment. This compared well with percentages of survival in tadpoles fed with diets of 35%, 39%, 43% and 47% protein i.e. 87%, 93%, 92% and 90%, respectively (Table 13, Fig.33). Mortality values of all groups were averaged 9.5% as the average percentage of survival was very high (90.5%).

### **Metamorphosis**

Metamorphosis occurred in all groups. The tadpoles gradually changed into froglets. On the fourth week, they developed the hind-legs which lay against the tail at the end of the body. Then, on the ninth week, some became froglets. On the tenth week, 96% of tadpoles fed with diet containing 39% protein became froglets. The lower percentages of metamorphosis occurred in tadpoles fed with diets containing 47% protein (92%), 43% protein (69%) and 35% protein (31%). By the 12th week, 100% of tadpoles fed with diet containing 39% protein became froglets. Lower percentages of metamorphosis occurred in tadpoles fed with diets

containing 43% protein (99%), 47% protein (97%) and 35% protein (95%)(Table 14, Fig.34).

Table 15 summarized the growth, survival and metamorphic responses of *R. catesbeiana* tadpoles fed with diets varying in protein concentrations. Tadpoles fed with diet containing 43% protein reached the highest average weight (4.72 g). The best survival and metamorphic responses were obtained in tadpoles fed with diet containing 39% protein.

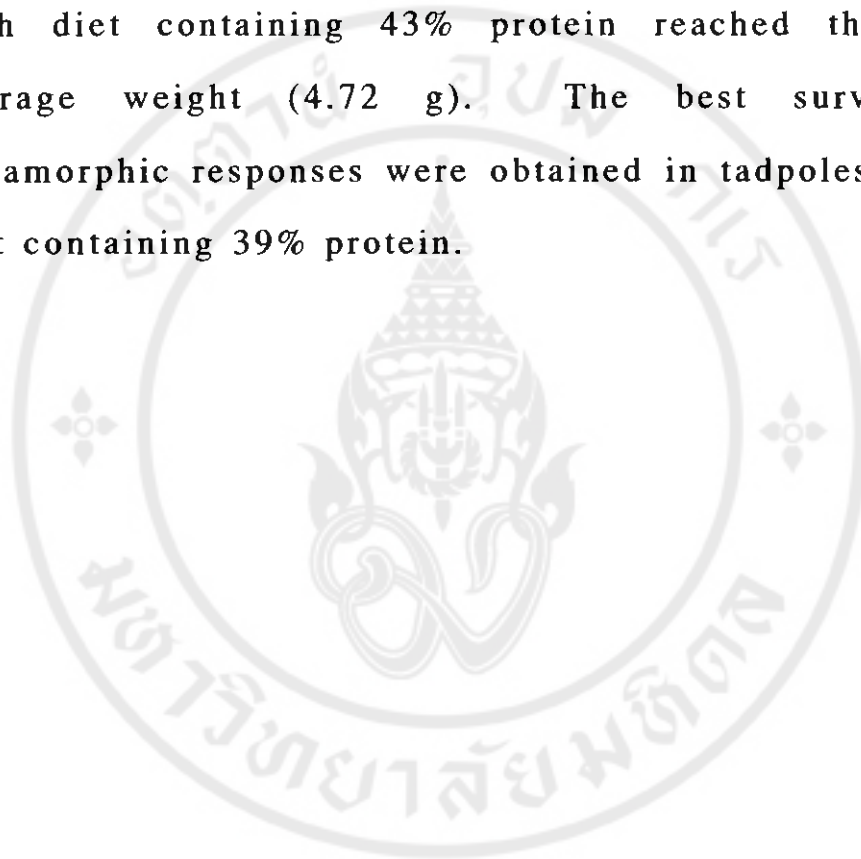


Table 13 Percentages of survival for *R. catesbeiana* tadpoles fed with diets varying in protein concentrations.

Age (week)	% survival			
	35%	39%	43%	47%
3	100	100	100	100
4	100	100	100	100
5	100	100	100	100
6	97	100	100	100
7	95	100	99	98
8	95	100	99	97
9	95	100	99	97
10	95	100	99	97
11	95	100	99	97
12	95	100	99	97
13	87	93	92	90

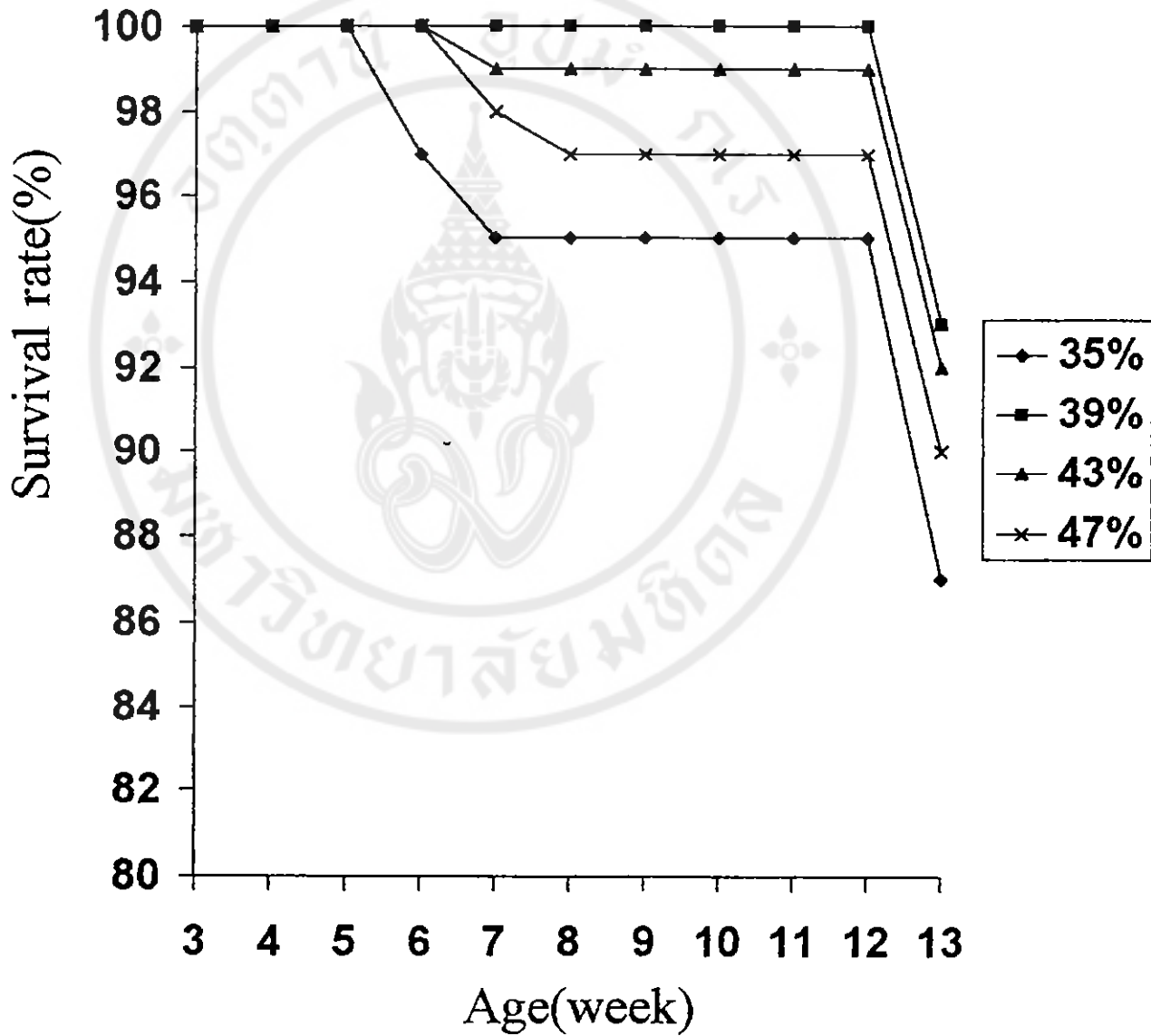
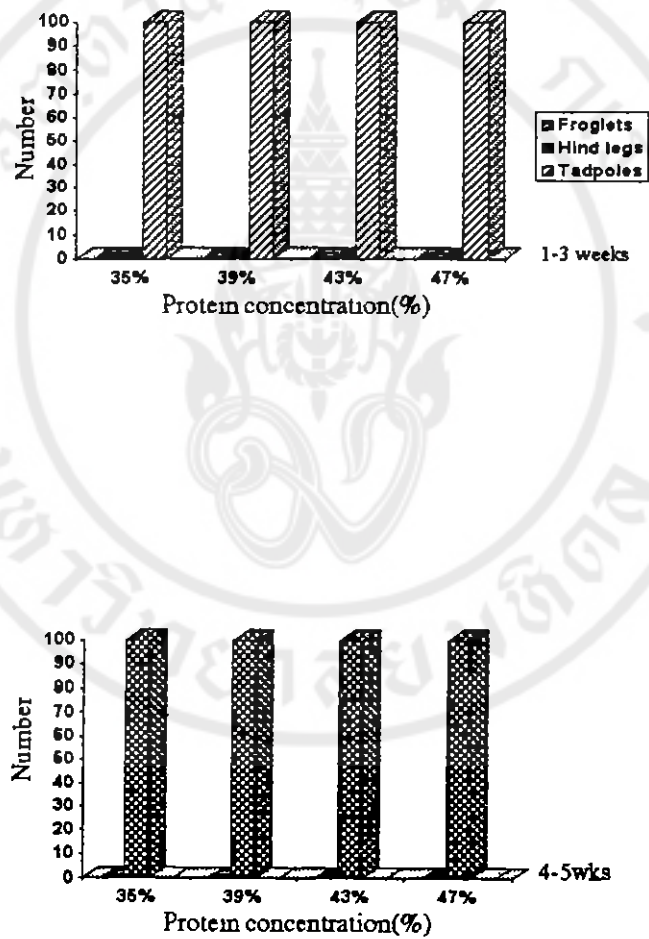


Fig. 33 Percentages of survival in *R. catesbeiana* tadpoles fed with diets varying in protein concentrations (35%, 39%, 43%, 47%).

**Table 14** Metamorphosis in *R. catesbeiana* tadpoles fed with diets varying in protein concentrations.

Age (week)	% metamorphosis			
	35%	39%	43%	47%
	Froglets Hind-legs Tadpoles	Froglets Hind-legs Tadpoles	Froglets Hind-legs Tadpoles	Froglets Hind-legs Tadpoles
3	0 0 100	0 0 100	0 0 100	0 0 100
4	0 0 100	0 0 100	0 0 100	0 0 100
5	0 0 100	0 0 100	0 0 100	0 0 100
6	0 0 97	0 0 100	0 0 100	0 0 100
7	0 0 95	0 0 100	0 0 99	0 0 98
8	0 0 95	0 0 100	0 0 99	0 0 97
9	6 6 89	55 45 0	24 75 0	70 27 0
10	31 31 64	96 4 0	69 30 0	92 5 0
11	57 57 38	97 3 0	92 7 0	96 1 0
12	78 78 17	100 0 0	99 0 0	97 0 0



**Fig. 34** Histograms showing rates of metamorphosis in *R. catesbeiana* tadpoles fed with diets varying in protein concentrations (35%, 39%, 43%, 47%).

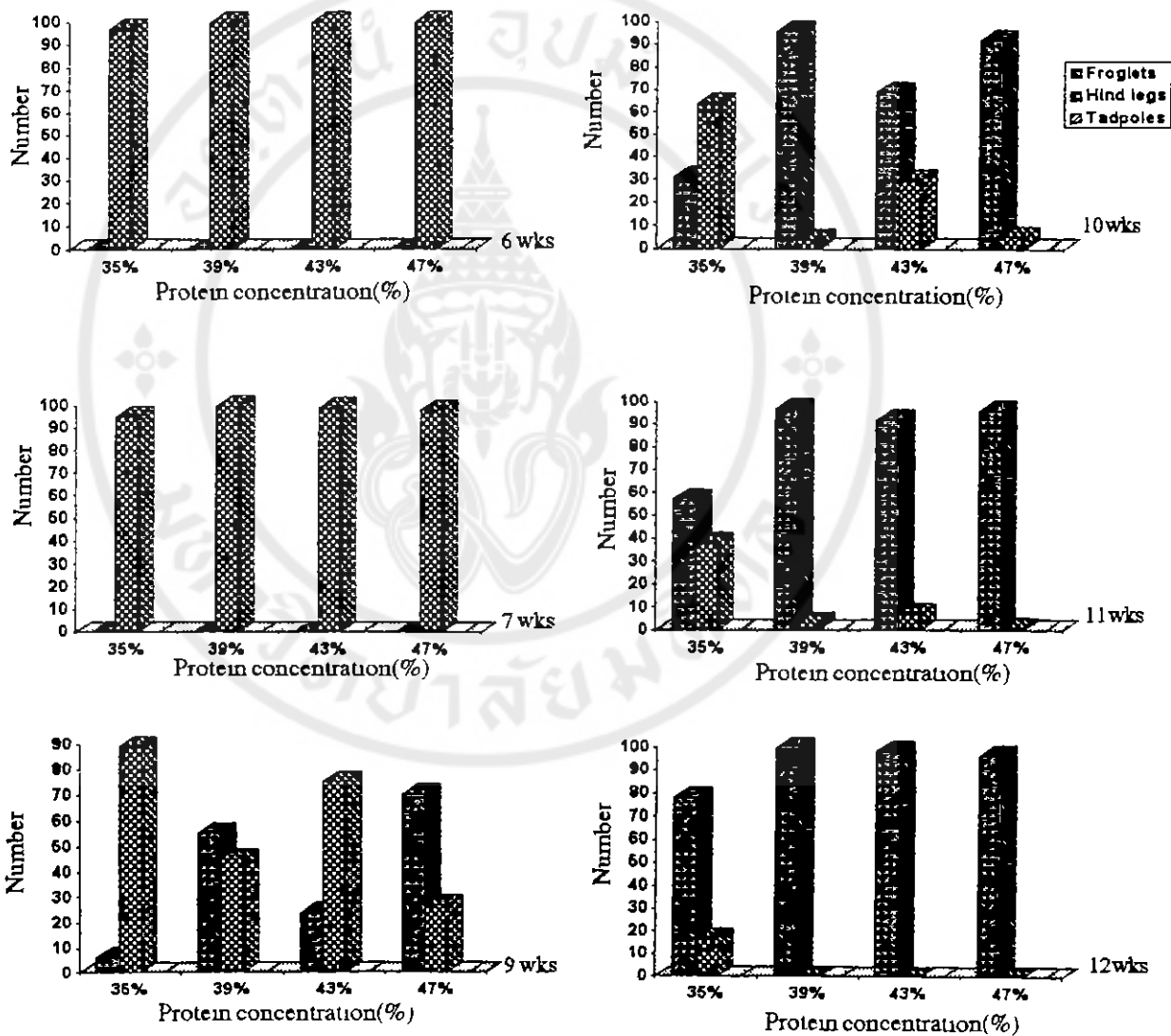


Fig. 34(continue)

**Table 15** Growth, survival and metamorphic responses of *R. catesbeiana* tadpoles fed with diets varying in protein concentrations.

	Protein concentrations			
	35%	39%	43%	47%
(1) Initial number	100	100	100	100
(2) Final number	87	93	92	90
*(3) % Survival	87	93	92	90
* (4) % Metamorphs	78	100	99	97
(5) Average weight(g) of (4)	3.63	4.13	4.72	4.71
* (6) % of hind-legged tadpoles remained undeveloped	17	0	0	0
(7) Average weight(g) of (6)	3.59	0	0	0
* (8) % of undeveloped tadpoles	0	0	0	0
(9) Average weight(g) of (8)	0	0	0	0

Average weight of

all animals at the end

of experiment

4.16

\*The percentages of (3), (4), (6) and (8) were calculated from the initial number.

### **2.2.2 Froglet and frog stages**

#### **Growth**

Froglets of *R. catesbeiana* were fed with different formulae of pelleted feed varying in protein concentrations (21%, 25%, 29%, 33%). The average body weight values from growth curves in Table 16 and Fig. 35 showed that protein was necessary early in the first four months. After that, the average body weights were quite different. The results from the comparison of average body weights of froglets throughout the experiment indicated that froglets fed with diet of 29% showed the best growth (325 g body weight) comparing to those fed with diets containing 33% protein (305 g), 25% protein (294.40 g) and 21% protein (278.80 g). The highest weight gain (1.31 g) was also observed in froglets fed with diet of 29% protein (Table 17). The growth rates throughout the study are shown in Table 18 and Fig.36. The weights of froglets increased rapidly during the fifth month. Froglets fed with diet containing 29% protein showed the highest average growth rate.

Daily consumption did not differ between groups throughout the experimental period, except for those fed with diet of 21% protein (1.15 g), although there were differences in the amount of protein taken in by the froglets (Table 17). The results obtained from the food

consumed daily (% body weight) showed that different groups of froglets consumed different amount of food (Table 17). The percentages of consumption in all groups were 4.97 in froglets fed with diet of 21% protein, 5.37 in those fed with diet of 25% protein, 6.00 in those fed with diet of 29% protein and 7.29 in those fed with diet of 33% protein. It can be seen that the feed conversion values also are not significantly different (1.04 for 21% protein, 1.05 for 25% protein, 0.93 for 29% protein and 0.99 for 33% protein) (Table 17).

### Survival

The result obtained from the comparison of percentages of survival in froglets indicated that the mortality increased throughout the experiment. After the first seven months, the percentage of mortality was very high (47%) in froglets fed with 21% protein due to diseases. Mortality values were averaged less than 22%. The percentages of survival are different among the experimental groups, i.e. 93% in froglets fed with diet of 25% protein, 86% in those fed with diet of 33% protein, 83% in those fed with diet of 29% protein and 53% in those fed with diet of 21% protein (Table 19, Fig.37).

### **2.3 Comparison of growth rates of R. tigerina and R. catesbeiana**

The comparison of growth of the froglets into frog stage of R. tigerina and R. catesbeiana indicated that on the first four months, froglets of R. tigerina fed with diet containing 29% protein showed the higher average body weight than those of of R. catesbeiana. After that, the froglets of R. catesbeiana fed with diet containing 29% protein showed the best growth(Fig. 38).

**Table 16** The average weights of *R. catesbeiana* froglets fed with diets varying in protein concentrations.

Age (month)	Average weight (g)			
	21%	25%	29%	33%
1	23.10	22.80	20.40	16.80
2	63.00	62.20	46.20	39.60
3	101.32	90.70	92.65	78.08
4	114.00	101.80	99.00	101.03
5	205.80	188.80	225.80	176.60
6	220.60	258.20	289.60	210.20
7	276.60	293.80	314.00	300.00
8	278.80	294.40	325.00	305.00

**Table 17** Weight, growth responses and food utilization for 8 months period in *R. catesbeiana* froglets fed with diets varying in protein concentrations.

	21%	25%	29%	33%
Mean initial wt.(g)	23.10	22.80	20.40	16.80
Mean final wt.(g)	278.8	294.4	325.0	305.0
Gain/day(g)	1.10	1.17	1.31	1.24
Gain/day as % body wt.	4.77	7.50	6.44	7.39
Consumed/day(g dry wt.)	1.15	1.23	1.23	1.23
Consumed/day as % body wt.	4.97	5.37	6.00	7.29
Feed conversion	1.04	1.05	0.93	0.99

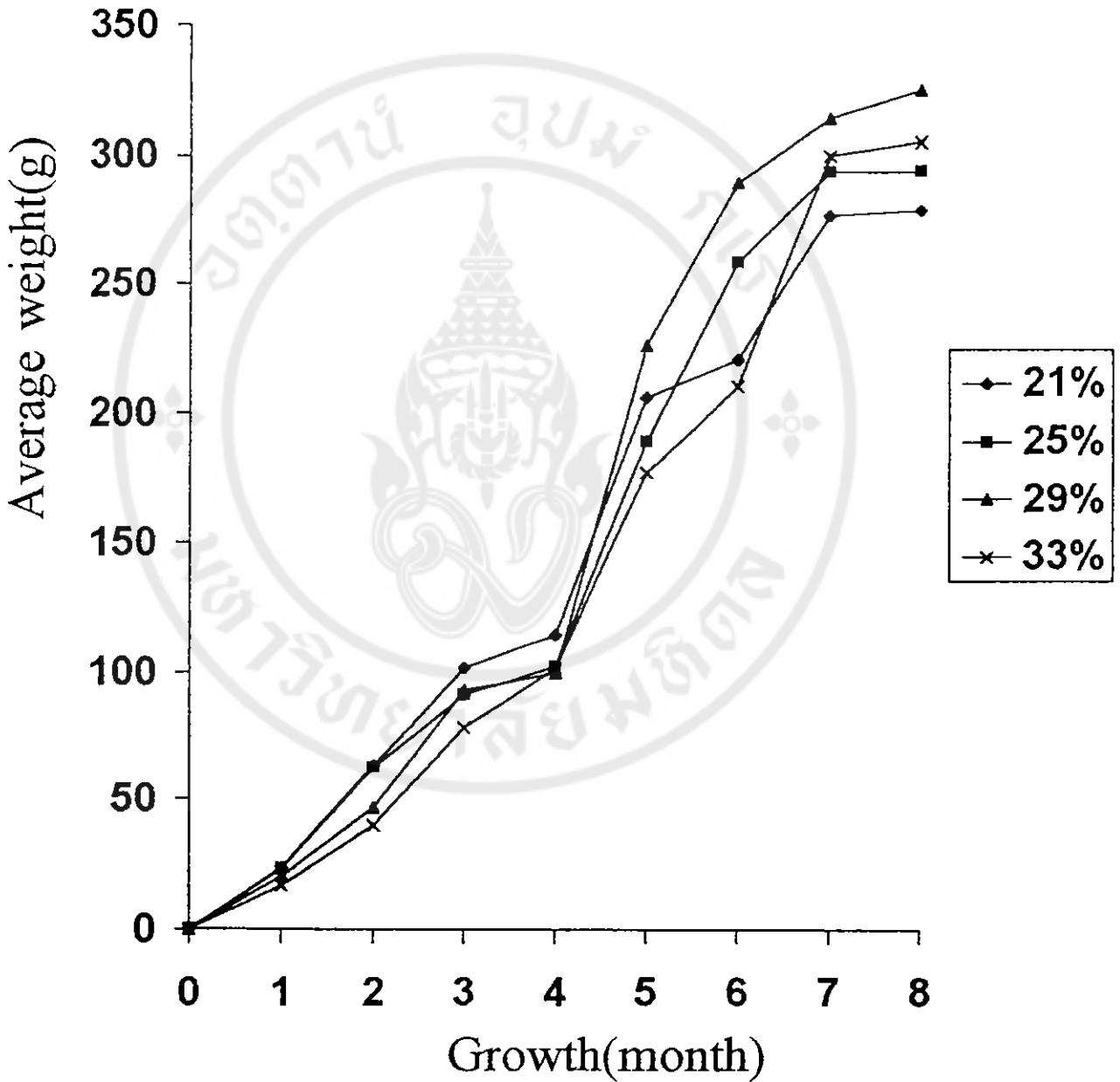


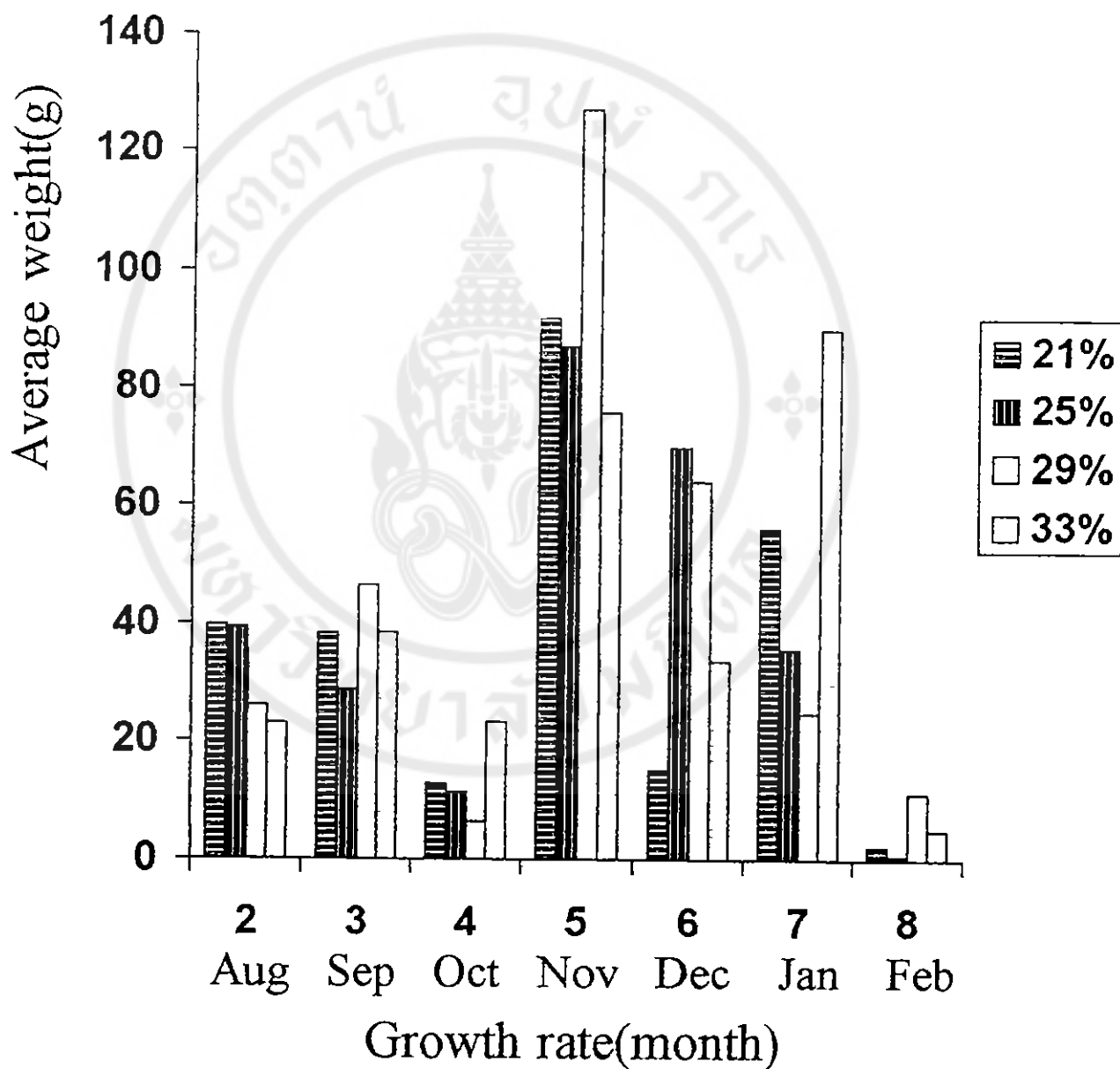
Fig. 35 Growth curves of *R. catesbeiana* froglets fed with diets varying in protein concentrations (35%, 39%, 43%, 47%).

**Table 18** The growth rates (average wt/month) of *R. catesbeiana* froglets fed with diets varying in protein concentrations.

Age (month)	Average weight (g)			
	21%	25%	29%	33%
2	39.90	39.40	25.80	22.80
3	38.32	28.50	46.45	38.48
4	12.68	11.10	6.35	22.95
5	91.80	87.00	126.80	75.57
6	14.80	69.40	63.80	33.60
7	56.00	35.60	24.40	89.80
8	2.20	0.60	11.00	5.00

**Table 19** Percentages of survival in *R. catesbeiana* froglets fed with diets varying in protein concentrations.

Age (month)	% survival			
	21%	25%	29%	33%
1	100	100	100	100
2	99	97	100	98
3	99	93	99	93
4	98	93	99	88
5	98	93	99	88
6	86	93	86	88
7	79	93	83	87
8	53	93	83	86



**Fig. 36** Growth rates of *R. catesbeiana* froglets fed with diets varying in protein concentrations (21%, 25%, 27%, 33%).

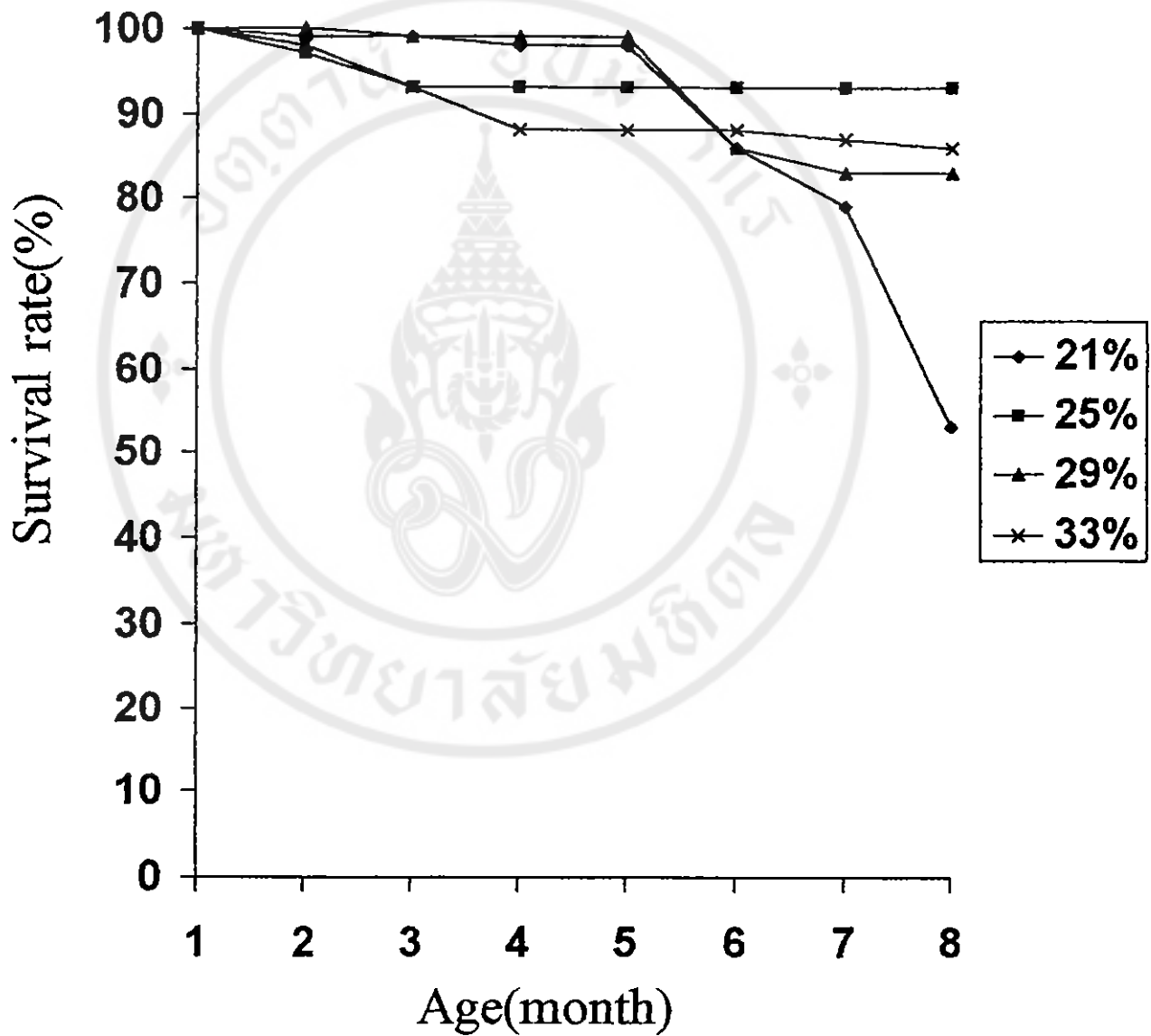


Fig. 37 Percentages of survival in *R. catesbeiana* froglets fed with diets varying in protein concentrations (21%, 25%, 29%, 33%).

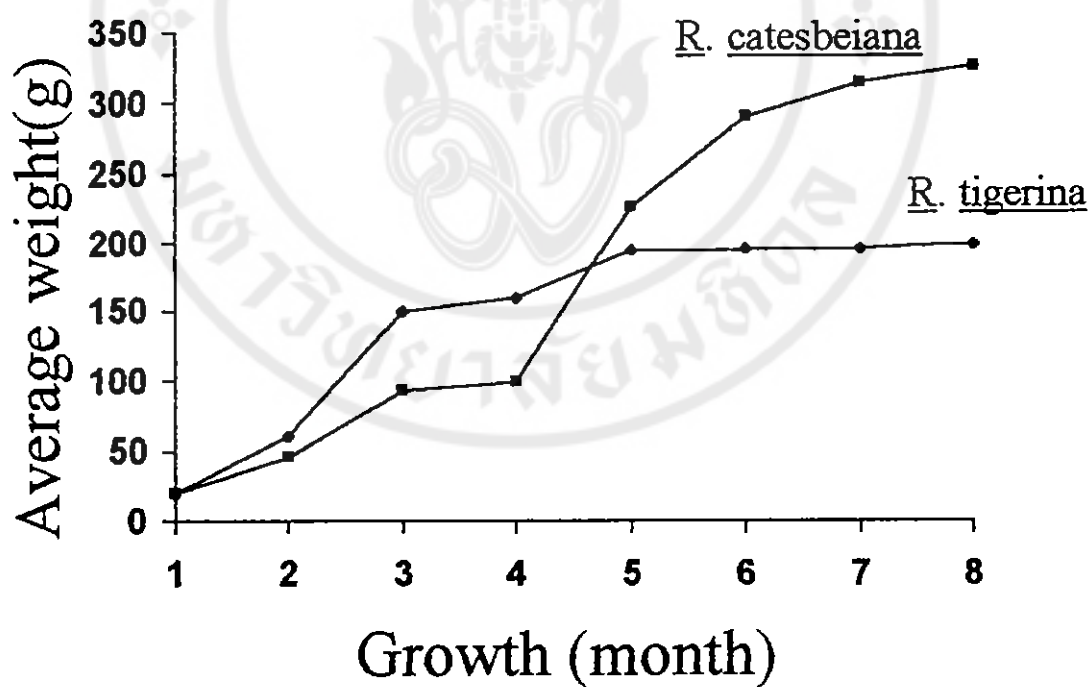


Fig.38 Growth curves of *R. tigerina* and *R. catesbeiana* froglets fed with diet containing protein concentration of 29%.

## DISCUSSION

The culture of any animal species must make use of a balance habitat and source of food. Both food quality and food levels have significant effects on growth rate, size at metamorphosis, and length of larval period (Wilbur & Collins, 1973; Berven & Chadra, 1988; Murray, 1990). In the case of amphibian larvae, they are affected by: handling, water quality, photoperiod, water currents, genetic variability, physical characteristics of the food (Culley & Sotiariadis, 1984).

Controlled culturing of frogs similar to those of other animals, is very complex because of the different environmental requirements at different stages in their life cycles. In their tadpole stages, they live entirely in an aquatic environment; upon metamorphosis they become partially or wholly terrestrial (froglet or adult frog stages). Thus, any frog culture must design housing facilities to provide for this dual requirement of aquatic and terrestrial environments. For *R. tigerina* and *R. catesbeiana* culture, although many problems have been solved, investigation of this systematic study should still continue so as to yield more efficient culture techniques.

Sanitation and waste removal are important as the materials often are soluble and may directly expose tadpoles.

Therefore, water quality must be rigidly controlled, as shown by the previous studies (Richards, 1958; Culley, 1973; Mohanty & Dash, 1986; Martinez *et al.*, 1993).

Pond designs are important to guide the planning of developing facilities for amphibians. The serious planner should visit one or more of these facilities before investing heavily in equipment for the care of frogs. The housing and management system used in this facility is thoroughly described in Nace (1968); although improvements have changed some specific operations, that document remains essentially current.

From the experiment, the ponds used to house tadpoles from the initiation of feeding until the emergence of forelimbs were concrete ponds with 1 x 1 x 0.5 m in size. Each pond held 100 tadpoles initially, and this density was suitable until the time of metamorphosis. Besides density, other factors have to be taken into consideration for tadpole development, such as control of water flow (Mohanty & Dash, 1986), aeration of the water which enhanced the growth and rate of metamorphosis, easy cleaning by frequent flushing, ready access for servicing and efficiency of space utilization. The growth characteristics of tadpoles in these ponds closely resemble those of larvae in nature.

The ponds for juveniles and adults used in the experiment were concrete ponds with 2 x 3 x 1.2 m in size. Each pond contained up to 100-130 froglets/m<sup>2</sup>. The smaller number of

occupants was used from 20 to 100 adults/m<sup>2</sup> when they became fully mature. However, three-fourths of the floor area of the pond should be covered with water with a depth of 0.03-0.20m with smooth surface, which allowed frogs to move between the aquatic environment and the terrestrial environment. Access to the animals was possible around the pond. For ready access, water control valves were placed at the front immediately above each pond but the drain tubes for removing waste water were placed at the bottom.

Temperature is a critical environmental parameter in the rearing of poikilothermic species, as the metabolic rates decrease or increase in an interval of preferential temperature. It has been shown that in the fish, Salmonidae, both the rates of metabolism and ingestion of food increased with a rise in the environmental temperature (Brett, 1972). These affirmations coincide with our observations on the consumption of food by *R. tigerina*, and *R. catesbeiana*. In the winter with the water temperatures of 25-28°C, the frogs consumed less and there was a decrease in their growth rate. The temperatures for optimum growth and development have not been well established for most amphibians. The investigation at the Louisiana State University amphibian facility indicated that frogs of the same species collected from different geographical regions separated by 4° latitude and 458 m elevation had different growth responses at a given temperature. Below 21°C, larvae and juvenile *R.*

*catesbeiana* collected at 30° latitude and 9.15 m elevation (Baton Rouge, Louisiana) did not grow as rapidly as those collected at 34° latitude and 460 m elevation (south central Arkansas).

Cannibalism is common amongst tadpoles and froglets, but little was observed in this culture, because they had enough food and selection of size was done once a week. Moore (1961) suggested that the tadpoles of *Lechriodus fletcheri* in New South Wales might be habitually carnivorous and cannibalistic, but it could appear to be just the result of too many tadpoles and not enough food, and that it was not a normal habit.

Microbial infestation is one of the major problems in frog culture. In this study, the mild inflammation of the skin was observed in the froglets of *R. catesbeiana*. However, an essential step in the study is the isolation of normal frogs from sick animals. Then, it is also advisable to keep the sick animals in a potassium permanganate ( $\text{KMnO}_4$ ) solution (approximately 0.01%) for 5-7 days. Artificial feed were mixed with antibiotic (chlortetracycline HCl or aureomycin, 100g/1000kg of artificial feed) and fed to the froglets for 7 consecutive days. Tetracycline HCl was selected as the drug of choice on the basis of sensitivity and wide tissue distribution observed in other animals. Soluble tetracycline HCl should be made up at known concentrations of about 25mg/ml in distilled water so that the injection volume can be kept to approximately 0.2ml for *R.*

pipiens, as larger quantities are easily regurgitated. Approximately 1ml may be given to R. catesbeiana without a serious risk of regurgitation (Volpe,1971).

It is often necessary to develop special feed formulation for frogs since conventional feed may rapidly deteriorate and contaminate the environment when added to the water. Artificial pelleted feed of 3 sizes (0.20, 0.52, 0.71 mm) were used for tadpoles, froglets and adult frogs in this study. Mandelli et al.(1985) studied the effect of particle size of the feed on weight gain in intensively reared tadpoles of R. catesbeiana and showed that pelleted feed of 0.21mm in diameter was most suitable for tadpole stage. In addition to the size of the pelleted feed, other factors have to be taken into consideration. Pelleted feed should stay solid above the water for 18-24 hours so that frogs can consume at longer time. The artificial feed should contain only 25% of water. Culley & Sotiaridis (1984) recommended that the moisture in the feed should not exceed 75%. At a higher level of water content, the digestive activity of the tadpoles would increase greatly. Hence, the food was less consumed. Martinez et al. (1993) reported that with the high moisture content in pelleted feed, R. perezi grew slowly and took a long time to reach metamorphosis. Mohanty & Dash (1986) registered a slow growth rate and a delay in metamorphosis in R. tigrina larvae fed on vegetables or meat. Culley & Sotiaridis (1984)

considered that these food stuffs (lettuce, liver, etc.), which were so frequently used on the non-commercial rearing of amphibians would never meet the nutritional requirements of tadpoles. It is agreed that larvae of the genus *Rana* are omnivorous - detritivorous (Kenny, 1969) . Currently, results obtained with fresh diets showed that they were not suitable for the intensive culture of anuran larvae (Berger & Pniewski, 1981; Culley & Sotiariadis, 1984; Mohanty & Dash, 1986; Martinez et al., 1993).

From the experiment, the artificial pelleted feed in frog culture was modified from catfish pelleted feed which was quite similar to the standard diet formulation of Culley et al. (1977). Vitamin C is an important ingredient in the diet formula. Previous studies indicated that scoliosis occurred in larvae of *R. catesbeiana* because of vitamin C deficiencies. However, the skeleton deformities did not appear in *R. tigerina* and *R. catesbeiana* tadpoles in the present study. Thus, diets varying in protein concentrations containing 3% vitamin C might reduce the incidence of scoliosis in frog larvae. This percentage agreed with that of Leibovitz et al. (1982).

In nutritional studies, the evaluation of the indices of nutritive use is important. The values of feed conversion and dietary protein concentration of *R. tigerina* and *R. catesbeiana* obtained in this study are quite similar to those of the other studies. *R. tigerina* and *R. catesbeiana* tadpoles provided with

diet of 35% protein achieved the best results with regard to growth (weight gain, growth rate, feed conversion) and development (length of larval period, percentage of metamorphosis). According to Marschall (1978), as the level of protein increased, feed conversion was more efficient. Marschall (1978) found that a content of 35-43% protein in diet for *R. catesbeiana* larvae was sufficient to achieve efficient growth, allowing metamorphosis to take place. These percentages agreed with those of Lima & Agostinho (1984) (40%), Chim-Figueiredo & Gallassini (1988) (40%), Fontenelle et al.(1988) (33-40%) and Monteiro et al.(1988) (40%). High dietary protein levels are counterproductive because animals must expend energy to metabolize excess protein, and unless compensated for by fat and carbohydrates, they must use protein as an energy source, reducing the performance of protein growth purposes (Cowey, 1979). Moreover, the proportion of other macronutrients (lipids and carbohydrates) present in the diet influences the use of the protein (Garcia et al.,1990). Up to a certain point, fat improves growth considerably and causes a saving in proteins (Garcia et al.,1981).

In this kind of study, it is important to determine the appropriate quantities of feed that should be given to the animals because the amount of food consumed determines the primary availability of nutrients and energy. In the present study, the quantity of food daily offered to tadpoles of *R.*

tigerina and R. catesbeiana was 20% body weight. In other experiments, the quantity of food daily offered to R. catesbeiana larvae varied from 13% to 3% body weight (Fontanello et al., 1982 ; Justo et al.,1985). In this experiment, the feed intake of R. tigerina larvae was from 8% to 14%, but the feed intake of R. catesbeiana larvae was from 30% to 33% during late metamorphosis. Culley et al. (1978) found that daily consumption of R. catesbeiana larvae prior to metamorphic climax was at 7% body weight. However, Lima & Agostinho (1989) registered the daily consumption to be only 3% or 4%.

Studies carried out with R. catesbeiana larvae fed with diets containing protein levels of 20-70% indicated that significant differences observed in daily consumption were related to protein concentrations (Marschall, 1978). Feed intake increased as protein percentage decreased. Similar correlation was also observed in the present study. This fact is related to the lower average weights of the larvae fed with poorer diets, therefore, the larvae were able to regulate the quantity of feed consumed according to dietary protein levels. In fishes, feed intake is conditioned mainly by available energy in the diet (Page & Andrews, 1973). Protein level alone has relative importance, taking into account that lipids and carbohydrates also act as energy sources (Lovell, 1979).

Every living animal has a need for protein. There is a certain minimum level of dietary protein recommended for each

class of animals. Protein is the most expensive component of the diet, so that the use of it should be carefully analysed in the selection of any particular diet. The protein nutrition should cover the requirements for essential amino acids plus non-essential nitrogen for growth. Little is known on the types of food consumed by anuran larvae in their natural state (Jenssen, 1967; Parra *et al.*, 1974; Sabnis & Kolhatkar, 1977; Diaz-Paniagua, 1985) and even less on the proportion of food digested and assimilated (Altig *et al.*, 1975). However, it is estimated that amongst the protein levels tested, 35% dietary protein is the most suitable for *R. tigerina* and *R. catesbeiana* tadpoles, being very similar to those found for *R. catesbeiana* larvae (Culley *et al.*, 1978). No benefits were found in protein levels higher than 40%.

In the froglet stage, diet with 21% protein is the most suitable for both *R. tigerina* and *R. catesbeiana* with the feed conversion values of 1.66 for *R. tigerina* and 1.04 for *R. catesbeiana*. Sarwono (1992) reported that in rearing *R. catesbeiana*, the frog weighed at 5-10 g after 4 months could reach 100-150g each as a consumption size with feeding conversion ratio of 1-2.

## CHAPTER V

### CONCLUSION

Based on the results of this study, the following conclusions were made.

1. it was observed that *R. tigerina* should be held for a minimum of 30-40 days, during the eggs hatched into tadpoles and froglets, and they completed their reproductive maturity within 12 months. The percentages of survival were very high on the average of 92.5% for tadpoles and more than 96% for froglets. Metamorphosis occurred in all groups but indicated that the highest percentage (99%) was obtained from tadpoles fed with diet of 47% protein.

2. It was observed that *R.catesbeiana* should be held for a minimum of 60-80 days, during the eggs hatched into tadpoles and froglets, and reproductive maturity was completed within 18 months. The percentages of survival were very high on the average of 90.5% for tadpoles and more than 78% for froglets. Metamorphosis occurred in all groups but indicated that the highest percentage (100%) was obtained from tadpoles fed with diet of 39% protein.

3. The indices of nutritive use were obtained in protein diets on *R. tigerina*. Diet containing 35% protein showed the best feed conversion value(1.79) and it should be considered as suitable feed for tadpoles until they became froglets. For froglet stage, the best feed conversion value(1.66) was obtained in froglets fed with diet containing 21% protein. Hence, pelleted feed with 21% protein concentration should be considered to be suitable for froglets until they became adult frogs.

4. The indices of nutritive use were obtained in protein diets on *R. catesbeiana*. Diet containing 35% protein showed the best feed conversion value(1.79) and it should be considered as suitable feed for tadpoles until they became froglets. For froglet stage, the best feed conversion value(1.04) was obtained in froglets fed with diet containing 21% protein. Hence, pelleted feed with 21% protein concentration should be considered to be suitable for froglets until they became adult frogs.

#### 5. Suggestions :

a. Both cultures of *R. tigerina* and *R. catesbeiana* should be considered as suitable for commercial system.

b. Pelleted feed was suitable for the intensive cultures of *R. tigerina* and *R. catesbeiana*.

c. The best growth of *R. tigerina* and *R. catesbeiana* froglets into adult frogs was obtained during the summer (March - May) and the rainy season (July - September).

d. Mortality values could be increased easily from harmful diseases, poor condition and cannibalism, especially in the *R. tigerina* culture.



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