Chapter IV

Materials and Methods

4.1 Materials

4.1.1 Vernier caliper (15 cm)

4.1.2 Spring balance (3 kg)

4.1.3 Operation materials

4.1.4 Vial bottles

4.1.5 Head lamp

4.1.6 GPS

4.1.7 Compass

4.1.8 Thermometer

4.1.9 Wet-dry hygrometer

4.1.10 Latex gloves

4.1.11 10% Formaldehyde

4.1.12 Antibiotic medicine

4.2 Collection of Data

A total of 15 surveys were carried out monthly from July 2001 to June 2002. Due to the high water level and the danger of entering the cave in rainy season, the survey in October 2001 and May 2002 were done only 5 and 13 times, respectively.

In the cave, the area inside was divided into 9 sections (30 m interval) along the length of the cave (270 m) for the study of habitat utilization (Appendix A).

The toads that were found in any survey were collected by hand or sweep net and individually marked using toe-clipping method. All of them were weighed to the nearest 5 g with a spring balance and the snout to vent length was measured to the nearest 0.1 mm with a vernier caliper. Sex, age, breeding behavior, and location were recorded for all toads.

The toads could be sexed and aged using their sizes (snout to vent length), secondary sexual characters (Figure 4.1 to Figure 4.3), and breeding behavior. Because of smaller size and had no secondary sexual character or breeding behavior, juveniles could be separated from adults. Male toads could be identified using the appearance of nuptial pads on their thumbs and the opening of vocal sac. In contrast, female toads had neither vocal sac opening nor nuptial pad and were larger than male toads.

Figure 4.1 Male with vocal sac opening.

In any survey, every time a toad was found, it was caught to check its number, location and breeding behavior. Normally, a marked toad was weighed and measured once a month. After the toad had been examined, it was released at the point of capture. Moreover, eggs and tadpoles were also searched to examine their breeding season from March 2001 to July 2002.

Environmental factors including air temperature, soil surface temperature, water temperature, relative humidity, and relative humidity at soil surface were recorded for both inside and outside the cave every time the survey was done.

Figure 4.2 Male with dark nuptial pads on the base of thumbs.

Figure 4.3 Female without nuptial pad.

4.3 Data Analysis

4.3.1 Population Study

4.3.1.1 Population Estimation

Population parameters including proportion of marked toads, population size, probability of survival, and number of new toads joining the population were estimated using Jolly-Seber Program (Krebs, 1989). The formulas are as follows:

- **Proportion of marked toads**

$$\alpha_t = \frac{m_t+1}{n_t+1}$$

where

α_t = Proportion of marked toads at sample t

m_t = Number of marked toads caught in sample t

n_t = Total number of toads caught in sample t

- **Size of marked population**

$$M_t = \frac{(s_t+1)Z_t}{R_t+1} + m_t$$

Where

M_t = Estimated size of the marked population just before sample time t

s_t = Total number of toads released after sample t

Z_t = Number of toads marked before sample t, not caught in sample t, but caught in some sample after sample t

m_t = Number of marked toads caught in sample t

R_t = Number of the s_t toads released at sample t and caught again in some later sample

- **Population size**

$$N_t = \frac{\text{Size of marked population}}{\text{Proportion of marked toads}}$$

$$N_t = \frac{M_t}{\alpha_t}$$

Where

N_t = Estimated population size just before sample time t

M_t = Estimated size of the marked population just before sample time t

α_t= Proportion of marked toads at sample t

- **Probability of survival**

$$\phi_t = \frac{\text{Size of marked population at start of sample time t+1}}{\text{Size of marked population at end of sample time t}}$$

$$\phi_t = \frac{M_{t+1}}{M_t+(s_t-m_t)}$$

where

ϕ_t = Probability of survival from sample time t to sample time t+1

M_{t+1} = Estimated size of the marked population just before sample time t+1

M_t = Estimated size of the marked population just before sample time t

s_t = Total number of toads released after sample t

m_t = Number of marked toads caught in sample t

- **Number of new toads joining the population**

$$B_t = N_{t+1} - \phi_t(N_t-(n_t-s_t))$$

B_t = Number of new animals joining the population between time t and t+1 and still alive at time t+1

N_{t+1} = Estimated population size just before sample time t

N_t = Estimated population size just before sample time t

ϕ_t = Probability of survival from sample time t to sample time t+1

n_t = Total number of toads caught in sample t

s_t = Total number of toads released after sample t

Calculations were performed using Microsoft Excel version 7.0 for Windows 98. Statistical analysis was performed using SPSS version 10.0 for Windows.

4.3.1.2 Correlation between Population Size and Climatic Factor

The correlation between population size and each climatic factor (temperature and relative humidity) was examined using Spearman's correlation analysis at $P<0.05$ (SPSS version 10.0).

4.3.1.3 Population Structure

- Proportion of population size among sexes and age was calculated.

- Size distribution was examined considering the number of individuals of each size-class.

4.3.2 Habitat Utilization

4.3.2.1 Movement

Movement of the toad was considered from the location in which the toad was found.

4.3.2.2 Area Utilization

The toad of which the location was recorded at least 25 times was used in the study of area utilization.

4.3.2.2.1 Area Fidelity

- Due to the area inside the cave was divided into 9 sections, the difference in appearance of each toad in its utilized sections was tested using Chi-square test at $P<0.05$.

- Shannon-Weiner's diversity index (Krebs, 1989) was used to examine individual niche width in term of area utilization. The difference in the mean of diversity index between sexes and between breeding and non-breeding season was examined using t-test for equality of means. The formula of Shannon-Weiner's diversity index is as follow:

- **Shanon-Weiner index**

$$H = -\sum_{i=1}^{s} Pi \log Pi$$

where

H = Shannon-Weiner Index

= Index of area utilization diversity

S = Number of utilized areas

Pi = Proportion of locations in area i to the total location of each toad

- Calculations were performed using Microsoft Excel version 7.0 for Windows 98. Statistical analysis was performed using SPSS version 10.0 for Windows.

4.3.2.2.2 Favored Area

Chi-square test was used to examine the difference in proportion of toad found in the most frequently utilized area.

4.3.3 Breeding Season

The breeding season of them was determined using the appearance of mating, eggs, and tadpoles.