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〈Report〉

Preferred Feeding Sites and Prey of the Adult Gold-spotted Pond Frog, *Rana plancyi chosenica*

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ABSTRACT: To determine the feeding sites preferred by adult gold-spotted pond frogs, *Rana plancyi chosenica*, and the foods that induce favorable growth of the frogs in the laboratory, we conducted two separate experiments between 27 May and 12 July 2007 in a vivarium. In the first experiment, we counted the number of crickets eaten by four gold-spotted pond frogs in a 60 min period at four different feeding sites within the experimental arenas: on the water surface, at the edge of a pond, and at two terrestrial sites. Adult gold-spotted pond frogs ate more crickets on the water surface and at the edge of the pond than the terrestrial sites. In the second experiment, we measured the growth of SVL (snout-vent length) and body mass of adult gold-spotted pond frogs fed crickets, mealworms, maggots, or earthworms in individual experimental boxes over a one month period. The SVL and body mass of the adult gold-spotted pond frogs fed crickets, mealworms, or maggots were greater than those of the frogs that were fed earthworms. These results indicate that providing crickets, mealworms, or maggots on the water or at the edge of a pond should induce favorable growth of captive-reared adult gold-spotted pond frogs.

Key words: Feeding ecology, Gold-spotted pond frog, *Rana plancyi chosenica*, Vivarium

INTRODUCTION

Amphibian populations are declining throughout the world due to climate change, environmental pollution, invasive species, infectious diseases, and habitat destruction (Stebbins and Cohen 1995, Gardner 2001, Walther et al. 2002, Carey and Alexander 2003, Collins and Storfer 2003, Parmesan and Yohe 2003). As amphibians play a critical role in connecting aquatic and terrestrial ecosystems, stable amphibian populations are important for healthy ecosystems and for human well-being (Gardner 2001). For these reasons, many countries, including Korea, have developed conservation and restoration projects for endangered amphibian species (Fog 1995, Seburn and Seburn 2000, Ra et al. 2007) following the conservation and recovery guidelines for amphibians published by the IUCN (The International Union for the Conservation of Natural Resources).

The gold-spotted pond frog, *Rana plancyi chosenica*, inhabits rice-farmland and wetlands along the west coast of the Korean peninsula (Kang and Yoon 1975). The appearance and habitat of the gold-spotted pond frog are similar to those of the black-spotted pond frog, *R. nigromaculata*, but the gold-spotted pond frog is distinguished by the presence of two distinctive golden strips on the dorsal plate, rather than the three white strips found on the plate of the black-spotted pond frog (Kang and Yoon 1975). Tadpoles of the gold-spotted pond frog also have golden strips on both sides of the body, which makes them easy to discriminate from other species' tadpoles. In the field, adult gold-spotted pond frogs mainly eat dipteran and hymenopteran insects (Hirai and Matsui 1999), possibly because they stay mainly in the water. The gold-spotted pond frog is currently listed as vulnerable to extinction by the IUCN and as a second grade near-extinction species by the Korean Ministry of Environment (The Ministry of Environment of the Republic of Korea 2006).

Restoration projects for endangered animal species (e.g., developing protocols for artificial rearing and the recovery of endangered gold-spotted pond frogs; Ra et al. 2007) have been initiated in Korea. One of the most important problems for the development of optimal artificial rearing programs is the identification of appropriate foods for both larval and adult gold-spotted pond frogs. In previous rearing studies, mealworms, cabbage inchworms, and crickets were successfully used for rearing of larval toads, *Bufo woodhousei* (Claussen and Layne 1983), and earthworms and newborn mice were identified as appropriate foods for the horned frog, *Ceratophrys cranwelli* (Grayson et al. 2005). In a previous study of gold-spotted pond frog tadpoles (Ra et al. 2007), we developed appropriate foods for the tadpoles by mixing spirogyra, cabbages, and commercial tropical fish foods (Tetramin, Tetra Inc.). Nevertheless, the feeding sites and food types preferred by adult gold-spotted pond frogs have

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not yet been determined, which may hinder artificial rearing of adult gold-spotted pond frogs.

In this study, we determined the feeding sites preferred by adult gold-spotted pond frogs in a vivarium and the food items that induced the most favorable growth of the frogs between 27 May and 12 July 2007. Our results suggest artificial rearing protocols that may be useful for in the development of successful reinforcement or recovery programs for the gold-spotted pond frog in Korea.

MATERIALS AND METHODS

To determine the feeding sites and prey preferred by the gold-spotted pond frog, we conducted site-preference and prey-feeding experiments in artificial arenas and in plastic experimental boxes, respectively.

Experiment Ⅰ

To determine the feeding sites preferred by the gold-spotted pond frog, we first constructed four rectangular test arenas (250 cm wide × 160 cm long) that contained a pond (100 cm diameter × 60 cm deep) at the center (Fig. 1). We planted *Erigeron annuus* plants in the terrestrial areas of the arena. Within every arena, we placed four plastic feeding boxes (12 cm wide × 18 cm long × 4 cm deep) in a line at 30 cm intervals, starting from the center of the pond and extending through the edge of the pond to the terrestrial areas. To allow the gold-spotted pond frogs to find the prey crickets, *Teleogryllus emma*, easily, we placed the opening of the plastic feeding boxes level to the surface ground. For boxes placed within the pond, we floated the feeding boxes so that their opening was even with the water surface by balancing their buoyancies using pebbles. We then anchored the feeding boxes at the center of the pond using a wire. At the edge of the pond, we buried the boxes with half in the ground and the other half floating above the water.

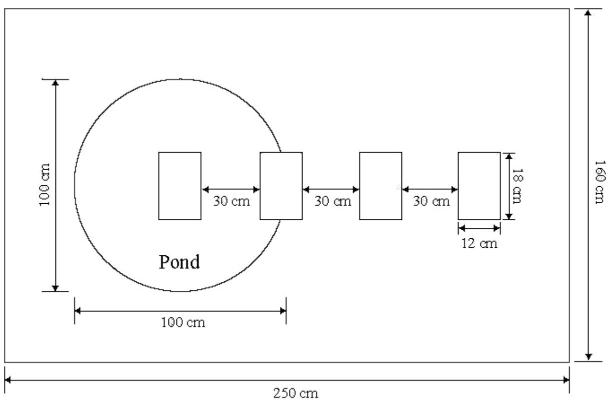

Fig. 1. A diagram showing the test arena for the Experiment I. Each rectangle indicates a feeding plate.

We placed 4 adult gold-spotted pond frogs in every arena 24 hrs before the start of an experiment to allow the frogs to adapt to the experimental situation. During this acclimation period, the frogs were not fed. To start the experiment, we put 8 prey crickets (with hind legs removed to prevent escape) into each feeding box. Then we counted the number of crickets eaten from each feeding box by the four gold-spotted pond frogs every 20 min for an hour. This experiment was conducted from 26 to 30 May 2007.

The number of crickets eaten by four gold-spotted pond frogs was averaged for each experimental box in each trial. To determine whether different numbers of crickets were eaten at different feeding sites, we analyzed the data using the Kruskal-Wallis test, followed by *post-hoc* tests (Siegel and Castellan 1988) when the difference among feeding sites was significant.

Experiment Ⅱ

To determine which prey are most appropriate to promote growth of the gold-spotted pond frog in captivity, we conducted prey-feeding experiments in experimental plastic boxes (28 cm wide × 24 cm long × 18.5 cm deep). We provided captive frogs with four different prey types: crickets (*T. emma*), mealworms (*Tenebrio molitor*), maggots (*Calliphora lata*), and earthworms (*Eisenia foetida*) to study the effects of different foods on frog growth rates. Each of the four experimental groups received only one of the four prey types.

We partially submerged the experimental boxes in a shallow body of water and made holes on the wall of the boxes to allow the water to enter, providing the frogs in the boxes with wet areas. The water temperature varied from 22℃ to 24.8℃ during the experimental period. We also covered part of the bottoms of the boxes with moss to maintain the humidity. To prevent the frogs from escaping from the boxes, we covered the openings of the boxes with plastic mesh. We supplied prey by placing a feeding plate (10 cm diameter × 3 cm deep) in a corner of each experimental box. During the experiment, the average air temperature in the vivarium was 23.33℃ ± 5.75 (range = 11.38～37.00) and the humidity was 77.51% ± 20.72 (range = 23.80～100.60). There was no loss or death of gold-spotted pond frogs in this study.

In this experiment, each experimental group contained 15 gold-spotted pond frogs. To ensure that the frogs in each group had a similar initial body mass, more than 300 stock frogs obtained from our vivarium were initially classified into 0.1～0.2 g, 0.21～0.3 g, 0.31～0.4 g, and 0.41～0.5 g body mass groups. Then we sequentially placed frogs from each body mass group into each experimental group until there were 15 frogs in each of the four experimental groups.

We supplied approximately equivalent amounts of prey to each box based on the average body mass of each prey species. The average body mass was 0.39 g ± 0.07 (range = 0.30～0.50, n = 100) for crickets, 0.86 g ± 0.05 (range = 0.80～0.90, n = 100) for mealworms, 0.46 g ± 0.05 (range = 0.40～0.50, n = 100) for maggots and 1.05 g ± 0.16 (range = 0.90～1.30, n = 50) for earthworms, so 10 crickets, 4 mealworms, 8 maggots, and 4 earthworms had approximately equivalent mass. We used a matched number of prey items to feed each experimental group every three days. Maggots and earthworms were purchased at a local fishing-tackle store. Crickets and mealworms came from our laboratory insectariums.

On every fifth day from the start of the experiments, we measured the SVL (snout-vent length) and body mass of each gold-spotted pond frog in each experimental group to the nearest 0.1 g and 0.01 cm, respectively, using an electronic balance (AND; CB-1200) and a digital caliper (Mitutoyo; CD-15CPX). This experiment was conducted from 11 June to 12 July 2007.

To analyze the data, we first standardized the SVL and body mass growth data to the SVL and body mass data obtained on the starting day of this experiment, 11 June. Since data were repeatedly obtained from more than 2 experimental groups, we used mixed model ANOVA (repeated measure linear model) to determine whether different prey induced differences in growth rates in the gold-spotted pond frogs over the study period, using Tukey *post-hoc* tests when the effects were significant. All statistical analyses were two tailed and were performed using SPSS version 11.0 (SPSS Inc., Chicago Illinois, USA). Values were reported as the mean ± SD, unless otherwise indicated.

RESULTS

Experiment Ⅰ

Sixty-four gold-spotted pond frogs ate 128 of the 512 crickets provided in the feeding boxes during the four experimental days. The number of crickets eaten by gold-spotted pond frogs varied significantly across feeding sites (χ^2 = 160.799, df = 3, p<0.001, Fig. 2). More crickets were eaten at the feeding sites on the water surface (4.81 ± 1.85) and at the edge of the pond (3.28 ± 2.30) than at the terrestrial feeding sites 30 cm (0.21 ± 0.56) and 60 cm (0.13 ± 0.47) from the edge of the pond (*Post-hoc* test, p<0.05). The number of crickets eaten by the frogs did not differ between feeding sites on the water surface and at the edge of the pond, and between the two terrestrial sites (*Post-hoc* test, p>0.05, Fig. 2).

Experiment Ⅱ

The SVL growth of gold-spotted pond frogs significantly differed among groups fed with different prey types during the experimental period (F = 149.470, df = 5, p<0.001, Fig. 3A, B).

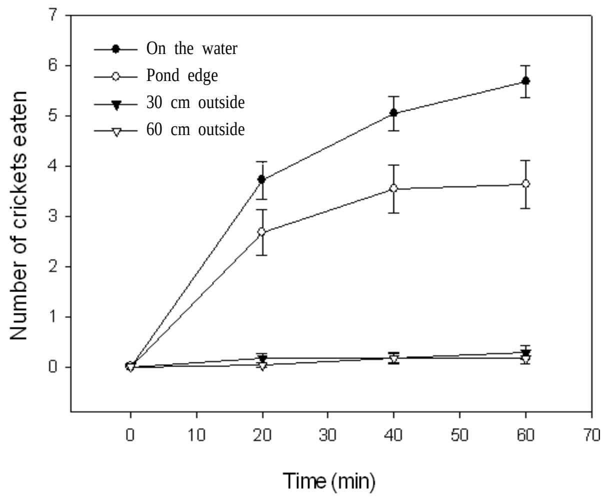

Fig. 2. The mean number of crickets eaten by four gold-spotted pond frogs, *Rana plancyi chosenica,* at each feeding site over a 60 min period.

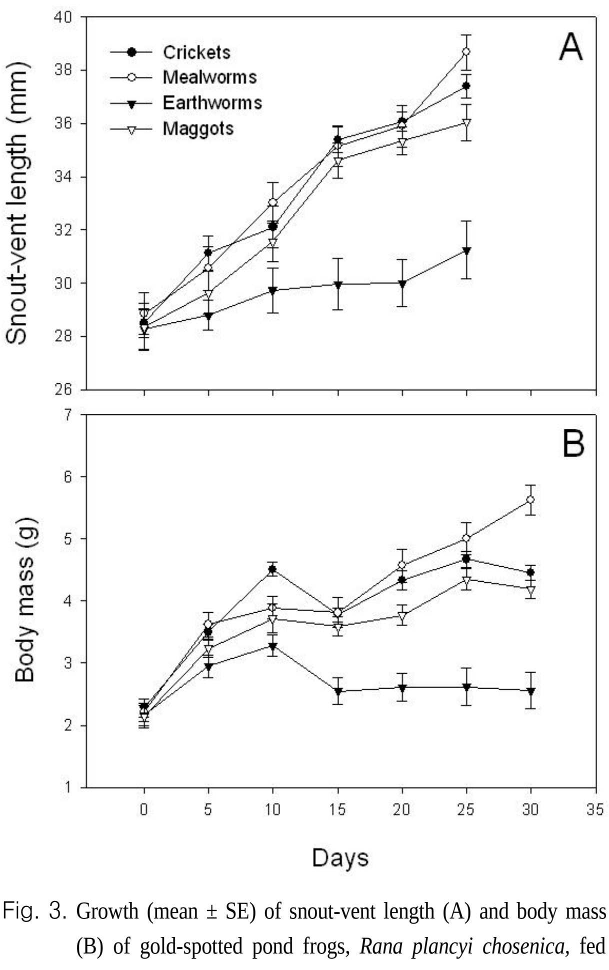

Fig. 3. Growth (mean ± SE) of snout-vent length (A) and body mass (B) of gold-spotted pond frogs, *Rana plancyi chosenica,* fed crickets, mealworms, maggots, or earthworms over the study period.

The effect of the interaction between prey type and time on the SVL growth of the frogs was also significant (F = 5585.951, df = 1, p< 0.001). The SVL growth of the gold-spotted pond frogs fed earthworms was significantly lower than that of the frogs fed the three other prey types (Tukey *post-hoc* test, p<0.05 for each comparison, Fig. 3A), but differences in SVL growth among frogs fed with crickets, mealworms and maggots were not significant (Tukey *post- hoc* test, p>0.05 for all comparisons, Fig. 3A).

The body mass growth of the gold-spotted pond frogs also differed significantly among groups fed with the four different prey types during the experimental period (F = 90.882, df = 6, p<0.001), and the effect of the interaction between prey item and time on the frogs' body mass growth was also significant (F = 2551.453, df = 1, p<0.001). The body mass growth of the gold-spotted pond frogs fed earthworms was significantly lower than that of the frogs fed with all other prey items (Tukey *post-hoc* test, p<0.05 for each comparison, Fig. 3B). However, the body mass growth of the frogs fed crickets, mealworms, and maggots did not significantly different (Tukey *post-hoc* test, p>0.05 for all comparisons, Fig. 3B).

DISCUSSION

Our results indicate that gold-spotted pond frogs preferred to feed on the water surface and at the edge of the pond, rather than in terrestrial areas, and that the most suitable prey for captive-reared frogs were crickets, mealworms, and maggots.

The gold-spotted pond frog and the black-spotted pond frog, *R. nigromaculata*, have relatively strong affinities for water-rich habitats in Korea (Hirai and Matsui 1999, Yang et al. 2001). Despite their similar habitats, however, their main prey are different. The black-spotted pond frog mainly eats coleopteron insects, whereas the gold-spotted pond frog primarily eats dipteran and hymenopteran insects (Yoon et al. 1998). These observations suggest that the two species may prefer to forage in different microhabitats, possibly terrestrial areas for the black-spotted pond frog and aquatic areas for the gold-spotted pond frog. Our current finding that gold-spotted pond frogs prefer to feed on the water surface and at the edge of ponds as opposed to in terrestrial feeding sites is consistent with these previous suggestions.

Gold-spotted pond frogs that were fed crickets, mealworms, and maggots showed better growth than those fed earthworms. This may result from two different aspects of frog foraging behavior. First, since the gold-spotted pond frog can be classified as a sit-and-wait forager (Duellman and Trueb 1986) based on their dipteran and hymenopteran insect diets (Yoon et al. 1998), moving prey may be easily detected and eaten, while relatively cryptic and slow moving prey like earthworms may not be easily found by the gold-spotted pond frog. Indeed, during the experiments, we often found the earthworms, which were placed in the feeding boxes under the moss, behaving in a cryptic manner. The hiding of the earthworms may also hinder effective foraging by the frogs. Second, the length of the earthworms may reduce the foraging efficiency of the frogs. Food size is an important factor affecting whether a particular food is suitable for an anuran species (Houston 1973). Most anuran species do not prefer relatively large food items because they require a high expenditure of energy for handling (Toft 1980). During our experiment, we often observed partially eaten earthworms escaping from the mouths of a gold-spotted pond frog by twisting their long body. These two factors might result in a lower feeding rate for earthworms than other food types, subsequently resulting in the low SVL and body mass growth of the gold-spotted pond frogs. In the field, earthworms are hardly ever seen at the edges of bodies of water such as ponds and reservoirs (personal observation) so they might not be major prey items for wild gold-spotted pond frogs.

Which prey types are the most suitable to promote rapid growth of captive-reared gold-spotted pond frogs? Crickets, mealworms, and maggots of appropriate size for each different size of gold-spotted pond frog appear to be good rearing prey. Among those prey, mealworms are the easiest to breed and keep in the laboratory. For crickets, young crickets are the best prey for recently metamorphosed gold-spotted pond frogs. However, rearing crickets requires a greater expenditure of time and money and a larger space. It is also difficult to keep crickets within the feeding plates due to their jumping ability. In this experiment, a diet of earthworms induced the lowest SVL and body mass growth of gold-spotted pond frogs. However, several previous studies (Donnelly 1991) showed that soft and slender prey such as caterpillars and earthworms were good prey for anurans because of their digestibility and relatively high energy yield per gram. Indeed, the four prey types used in this study might all be useful for rearing gold-spotted pond frogs if prey are carefully selected based on the developmental stages and sizes of the gold-spotted pond frogs. In the future, the presentation of various feeding plates for various prey sizes could promote efficient feeding by allowing the use of a wide range of prey types by frogs in a vivarium. It is also important to keep in mind that frogs to be released in recovery field sites should be reared in such a way as to ensure optimal adaptability to the local prey found in the release sites.

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