

## Reproduction and Diet of the Brown Frog *Rana longicrus* in Taiwan

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**Yeong-Choy Kam, Chin-Shian Wang and Yao-Sung Lin (1995)** Reproduction and diet of the brown frog *Rana longicrus* in Taiwan. *Zoological Studies* 34(3): 193-201. The natural history of the brown frog *Rana longicrus* was studied for twelve months in the Aoti area, northern Taiwan. *Rana longicrus* is a diurnal, terrestrial species that lives mainly in hill areas during the non-breeding season. However, during the breeding season, frogs move to lowland areas, particularly ricefields, to breed. Adult females are larger than males. Reproductive data from males and females suggest that the breeding season is from November to March. Froglets were abundant in March, and they reach adult sizes by September. Desiccation and disturbances from human activities were the leading causes of tadpole mortality. Diets consisted of invertebrates of the classes Gastropoda, Oligochaeta, Arachnida, Crustacea, Insecta, and Chilopoda. Seasonal, sexual, and ontogenetic differences in stomach contents were described.

**Key words:** Amphibian, Diet, Reproduction.

Little information is available on the biology of the brown frog *Rana longicrus*. Current knowledge of this species is limited mostly to systematics and distribution (Pope 1931, Yuan 1950, Liu and Hu 1961, Kuramoto 1974, Lue 1990). *R. Longicrus* is currently found on the island of Taiwan and in Fujian Province (Zhao and Adler 1993). *R. longicrus* and *Rana japonica* are so similar in size and shape that Pope (1931) and Liu and Hu (1961) placed *R. longicrus* in the synonymy of *R. japonica*. However, Kuramoto (1974) concluded from hybridization experiments that *R. longicrus* is a valid species. A similar conclusion was also made by Yuan (1950) who studied the development of tadpoles and the morphology of adults.

*R. longicrus* is a lowland species mainly distributed in the northern part of the island of Taiwan (Lue 1990). Yuan (1950) has briefly described morphological characters of adults, tadpoles, and eggs of *R. longicrus* and also he has reported that these frogs breed during the coldest months; however, detailed information on reproduction has

not been available. In fact, of the twelve ranids found in Taiwan, only *R. longicrus* breeds solely in the winter (Lue 1990, pers. obs.).

Why *R. longicrus* breeds only during winter is an ecological and physiological puzzle. A cold environment poses a severe threat to the frogs, particularly with respect to the development and growth of embryos and tadpoles. Because amphibians are poikilotherms, cold temperatures depress the metabolism, growth, and development of embryos and tadpoles. A further drop of temperature, which exceeds the thermal tolerance of embryos or tadpoles, will cause mortality. Another important factor is that cold temperatures inhibit the metamorphic process; thus, tadpoles may continue to grow but are unable to metamorphose (Dodd and Dodd 1976). Thus, a full understanding of the biology of *R. longicrus* is needed to shed light on how this species adapts to its environment.

In this study, we examined the natural history of *R. longicrus*, including its reproductive biology, morphology, and diet. This is part of a project

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designed to understand the reproductive physiology and ecology of anurans found in Taiwan.

## MATERIALS AND METHODS

The study site is located in the Aoti area (approximately 25°00'40" N, 121°50'40" E) which is characterized by low terrain, with hills no more than 300 m in height, and a lowland area principally used for growing rice. The hillsides are mainly covered with primary and secondary forests. Four major vegetation communities were recognized in the hill regions: bamboo, *Pinus*, *Acacia*, and primary broadleaf forests. The dominant species of the primary forests include *Psychotria rubra*, *Glochidion rubrum*, and *Persea thunbergii*.

Climate in the Aoti area (Fig. 1) (provided by the Central Weather Bureau) is similar to that in much of western Taiwan with the exception of the precipitation pattern (Alexander et al. 1979, Lin 1979). Air temperatures and photoperiod reached their maximums in August and June, and dropped to their minimum values in January and December, respectively. However, there were two major rainy seasons. From May to September was the first rainy season in which most rains were associated with typhoons and southwest monsoons (Alexander et al. 1979). From November to December was the second rainy season in which most rains were

probably caused by gusty northeast monsoons and cold fronts.

Frogs were collected biweekly along a transect line between 0900-1530 hours from January 1981 to December 1981. This transect line, about 3 km long, passed through all major habitats, including ricefields, creeks, marshes, open areas and woods (primary and secondary forests). Frogs were killed and preserved in 10% formalin soon after capture. The snout-vent length (SVL) and body mass of some frogs were measured before they were killed. Autopsies were then performed in the laboratory: the testes in long axis (males) and ova and oviducts (females) were removed and measured. Gross ovarian morphology was recorded to monitor the reproductive status of females. The method developed by Alexander et al. (1979) was modified for this study. Briefly, a total of five reproductive classes were recognized: classes 1-3 represent non-reproductive stages, while classes 4-5 represent reproductive stages. Class 1 was characterized by clear and colorless ovaries. Oocytes were small, no more than about 0.4 mm in diameter. Oviducts were hardly visible to the naked eye. Class 2 was characterized by yellowish ovaries. The largest oocytes were about 0.4-0.6 mm in diameter and were beginning to accumulate pigment. Oviduct diameter was no more than 0.7 mm. Class 3 was characterized by uniformly dark ovaries. The largest oocytes were about 0.6-0.9 mm in diameter and pigmented without distinct vegetal hemispheres. Oviduct diameter was about 0.7-0.9 mm. Class 4 was characterized by dark ovaries. Oocytes diameters were 0.9-1.3 mm and many had distinct vegetal hemispheres. Oviduct diameter was about 0.9-1.47 mm. Class 5 was characterized by bright ovaries. Oocytes were wellpolarized with diameters greater than 1.3 mm. Oviduct diameter was greater than 1.47 mm.

For dietary analyses, the stomachs were opened, and the contents were carefully removed and identified to a reliable taxonomic level. Most food items were identified to class but insects were further identified to orders. The proportion of each food item and the percent of frogs containing the items were calculated (Vitt and Ohmart 1975). The degree of dietary overlap was derived from stomach contents using the following equation:

$$D = [1.0 - 0.5 \text{ Sum } |P_{x,i} - P_{y,i}|] \times 100$$

where D is the percent of overlap and  $P_{x,i}$  and  $P_{y,i}$  are the proportions of the number of items

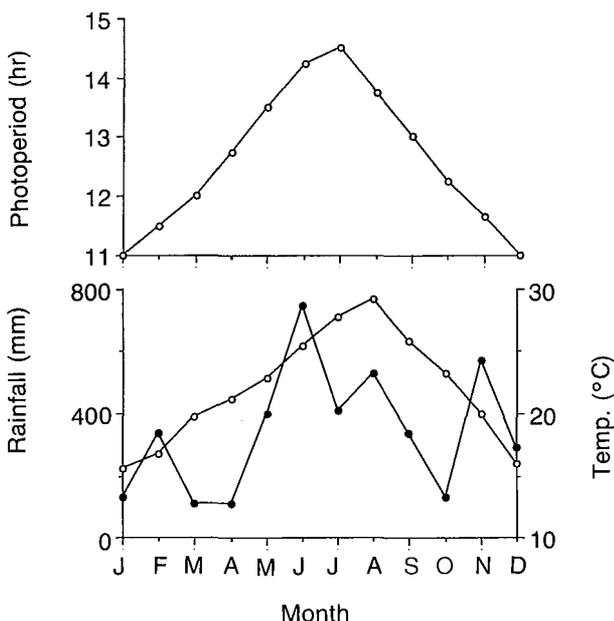


Fig. 1. Photoperiod, temperature (empty circles), and rainfall (solid circles) for the Aoti area.

individuals (or groups)  $x$  and  $y$  utilized in resource category  $i$  (Schoener 1970, Rathcke 1976). Dietary overlap was compared between (a) males and females, (b) juveniles and adults, (c) seasons, and (d) habitats. Prey items between groups were statistically compared using a G test (Sokal and Rohlf 1980). Data were analyzed by a SAS program (SAS Institute Inc. 1988), and a significant difference was declared when  $p < 0.05$  or less.

## RESULTS AND DISCUSSION

### Size and morphology

*R. longicrus* showed sexual dimorphism in that the SVL of females ( $41.76 \pm 4.60$  mm, mean  $\pm$  SD,  $n = 119$ ) was significantly greater than that of males ( $38.22 \pm 2.51$  mm,  $n = 53$ ,  $t = 3.69$ ,  $p < 0.001$ ) (Fig. 2). Females attained SVL as large as 55 mm and weighed 13.3 g, but the largest SVL of a male measured only 42.8 mm with a body weight of 5.5 g. The smallest gravid female measured 32.2 mm SVL and weighed 2.32 g, while the smallest sexually mature male (judged by the well-developed nuptial pad) measured 33.0 mm SVL. Body size and weight were well correlated in males and females ( $r = 0.9$ ,  $n = 71$ ,  $p < 0.01$ ).

### Reproduction

*R. longicrus* is a diurnal terrestrial species and

can be found in lowland and hill regions. During the breeding season, frogs migrated to rice fields in the lowlands and aggregated in particular sites to breed. Breeding activities were probably initiated by heavy rains. No eggs or tadpoles were found in the hill regions where streams, creeks, and a permanent marsh were available, which suggests that *R. longicrus* breeds only in the lowland regions, particularly, in ricefields.

The reproductive status of male frogs can be assessed from the presence of a nuptial pad and vocal sac and from testes morphology. Nuptial pads and vocal sacs, which are major secondary characteristics, were used as indicator of reproductive status in *R. rugulosa* (Kao et al. 1994). Nuptial pads, enlarged testes, and convoluted epididymides were observed in all male frogs from October to March suggesting that the breeding season for males extended at least from October to March. Also, males reached sexual maturity within the first year. The smallest male collected during the breeding season was 33 mm in SVL and had enlarged testes and convoluted epididymides.

The ovarian morphology of female frogs is summarized in Fig. 4. Vitellogenesis began in September, most females became gravid in December, indicating that the breeding peak was in December, and all females reproduced synchronously (Fig. 4). Most likely, only one clutch per female was produced in each breeding season. Ova were smallest in August and were largest in

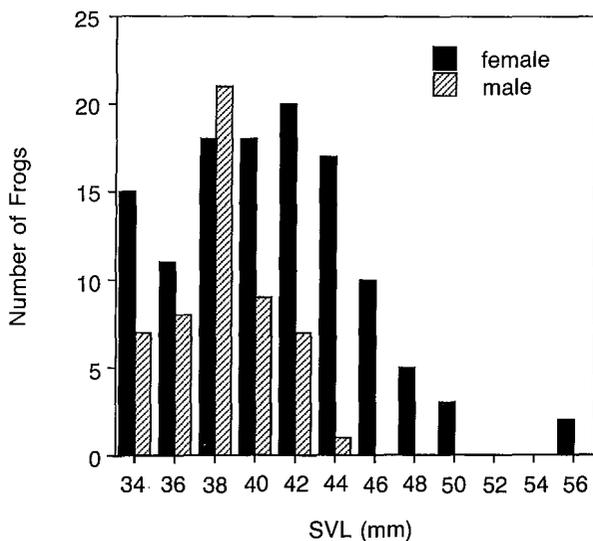


Fig. 2. Size (SVL) distribution of female and male *Rana longicrus*.

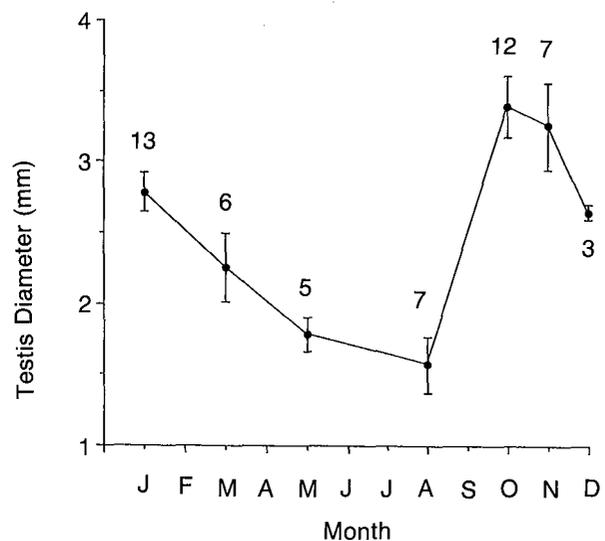


Fig. 3. Monthly changes (mean values  $\pm$  SE) in testis diameter of *Rana longicrus*. Sample size is indicated by numbers.

December ( $0.45 \pm 0.06$  mm and  $1.53 \pm 0.17$  mm, respectively;  $t = 12.1, p < 0.001$ ).

Seasonal changes of male and female reproductive organs and accessory organs show that the reproduction of *R. longicrus* is cyclic. The reproductive mode of *R. longicrus* belongs to the most primitive type of breeding, according to the classification of reproductive modes by Duellman and Trueb (1986), i.e., mode 1: eggs are deposited in water, and eggs and feeding tadpoles are in lentic water. It is most likely that the breeding season is from November to March with the breeding peak in December, but the exact timing can be influenced by precipitation or human agricultural activities. Most eggs were oviposited in water of ricefields prior to the cultivation, and the water in the ricefield was either from rainfall or irrigation. Yuan (1950) reported that *R. longicrus* found in

Mu-chan and Tsao-shan bred a month earlier than frogs found in the ricefields near the campus of National Taiwan University. He speculated that the delay in the breeding season was due to the shortage of water in the ricefield which was caused by the delay of the rice harvest. A similar close relationship between anuran reproduction and human agricultural activities was also reported for *Rana limnocharis* (Alexander et al. 1979).

The thermal ecology of *R. longicrus* has not been studied, thus, the physiological effects of low temperatures on the development of embryos and tadpoles are not known. The duration of development of *R. longicrus* from eggs after fertilization to newly metamorphosed adults is about 50-60 days and it is comparable to other ranids (Yuan 1950, Duellman and Trueb 1986). This suggests that development of embryos and tadpoles

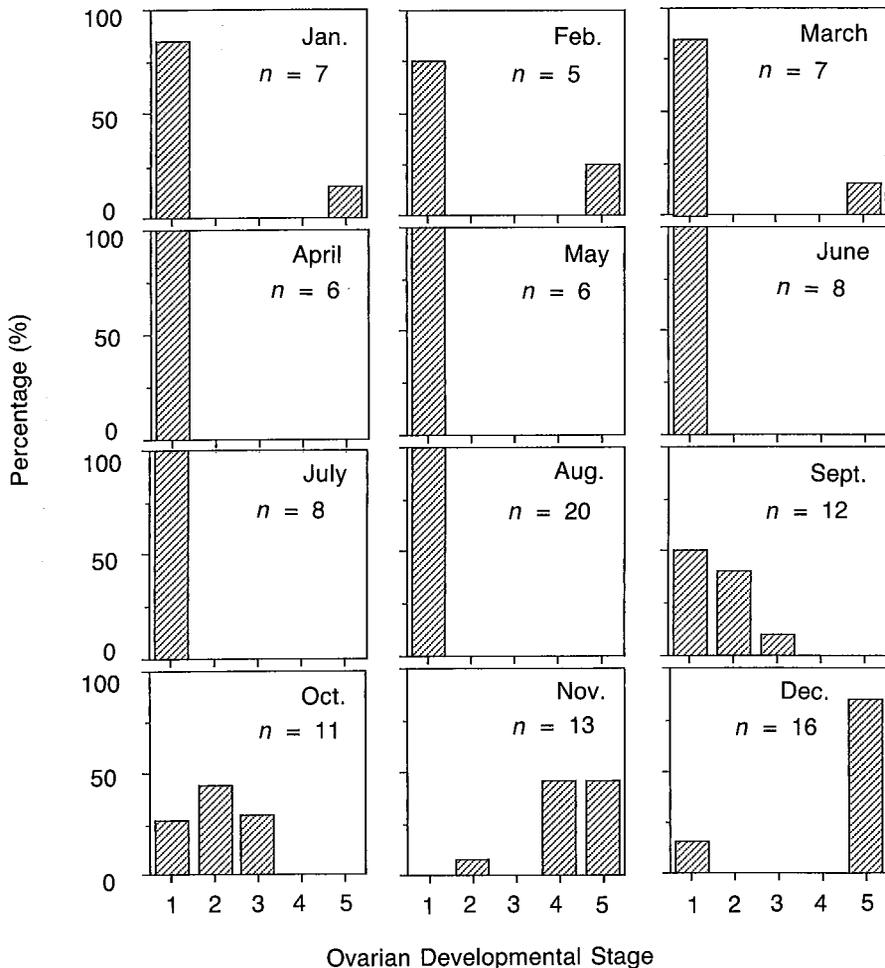


Fig. 4. Proportion of each ovarian developmental stage in monthly collections of female *Rana longicrus*. Samples size is indicated by *n*.

of this species is not affected by local cold temperatures. Early studies on the thermal relations of amphibians have shown that individuals which breed in cold environments exhibit physiological plasticities which allowed them to adapt well to those environments. These adaptations include a low temperature tolerance, a high rate of development, and a high developmental  $Q_{10}$  at low temperatures (Herreid and Kinney 1967, Bachmann 1969, Brown 1975 1976, Duellman and Trueb 1986). Herreid and Kinney (1967) have shown that eggs of *Rana sylvatica* had a survival

rate of at least 50% when water temperature was 6-29°C, while the preferred temperature of tadpoles ranged from 9° to 29°C. Anurans that breed in extreme northwestern Washington, U.S.A., had embryonic temperature tolerances of 4-21°C (*Rana aurora*), and 6-28°C (*Hyla regilla*) (Brown 1975 1976).

Winter temperatures at Aoti are not that cold as the lowest monthly temperature was about 16°C in December. Thus, temperature may not pose a severe threat to the eggs or embryos. However, when a cold front moves in, ambient

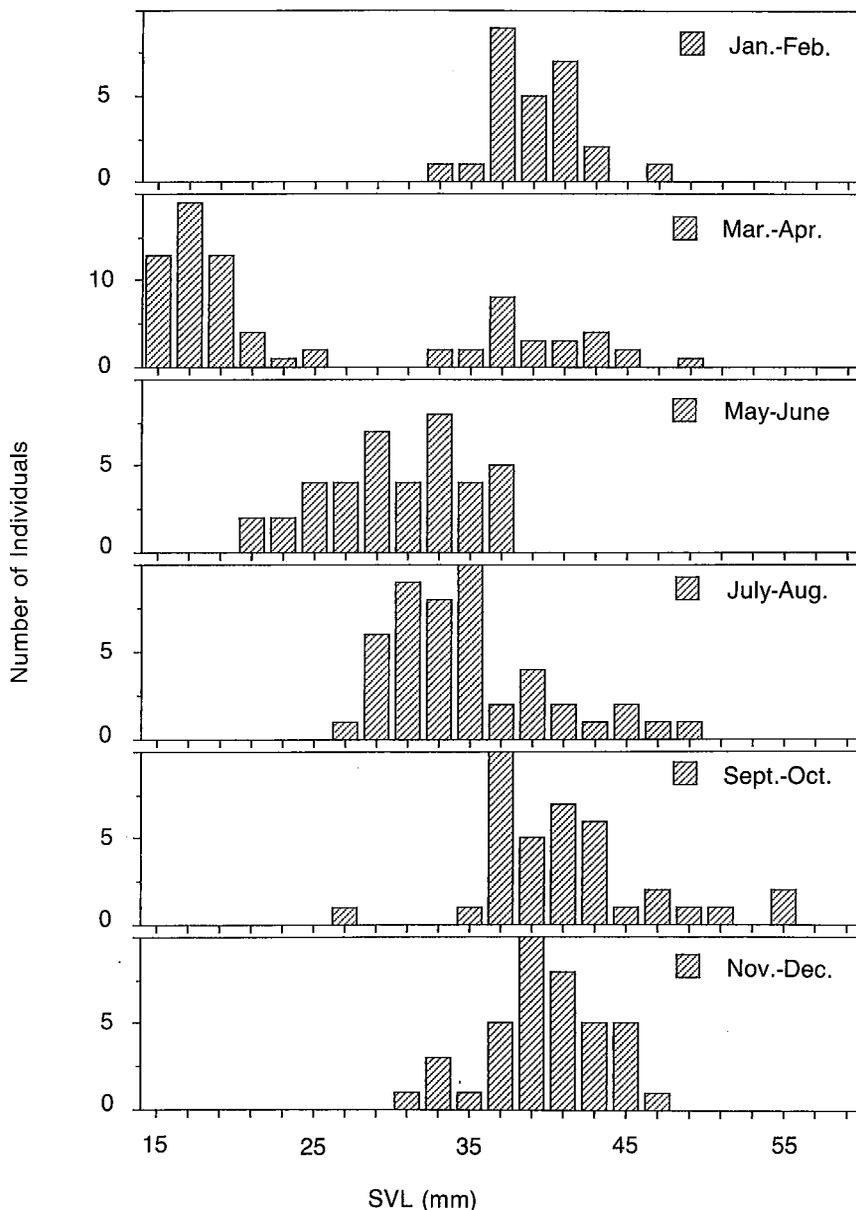


Fig. 5. Bimonthly size distribution of *Rana longicrus*.

temperatures have been recorded well below 10°C. The duration of cold shock may be short, yet critical to the development and growth of embryos. Thus, further studies are needed to reveal possible mechanisms by which embryos and tadpoles survive in cold environments.

What are the advantages of being a winter breeder? Aggregations of frogs at a particular site during the breeding season attract predators, and a corresponding high predation rates. However, less predation can be expected for winter breeders because many poikilothermic predators, such as snakes, may enter hibernation. In addition, breeding in winter provides a temporal partitioning of breeding sites which reduces interspecific competition for space and food. Of the nine species of anurans found in the Aoti area, seven species are spring or summer breeders, and four species (*Rana limnocharis*, *Hyla chinensis*, *Bufo melanostictus*, and *Microhyla ornata*) use ricefields as their main oviposition sites. Thus, seasonally heavy competition for space and food in both adults and tadpoles is unavoidable. In addition, increased densities also attract more predators, resulting in even higher predation rates. Temporal partitioning of breeding sites is common within tadpole communities (Heyer 1976, Seale 1980, Wiest 1982).

### Egg, embryonic, and tadpole development

Eggs were fertilized externally, and egg clutches with 600-2,000 eggs were then deposited into water. No parental care was observed. Field observations showed that tadpoles continued to grow and develop in water pools after hatching. The appearance of froglets in March suggests that this development took about two months. Our field observations agree with an earlier study where the duration of development from an egg after fertilization to a newly metamorphosed froglet took about two months under laboratory conditions (water temperature 19-20°C) and 50 days in the field (water temperature 19-20°C) (Yuan 1950).

Froglets were abundant in March, reaching adult size in September (Fig. 5). Frogs collected from September to February had almost identical size distributions, indicating that froglets grew rapidly during spring and summer and reached adult size within one year (Fig. 5). During breeding season, the ovaries in most of the collected females contained vitellogenic follicles, indicating that *R. longicrus* reaches sexual maturity within one year.

### Tadpole mortality

Eggs and tadpoles in the ephemeral flooded ricefields before plowing were subjected to heavy mortality due to disturbance by human activities and by the desiccation of habitats. Field observations have confirmed that the plowing of ricefields is the single most important cause of mortality of eggs and tadpoles of *R. longicrus*. Six weeks prior to plowing (from 11/29/1981 to 1/20/1982), a total of 34 egg clutches were deposited at a particular site in a ricefield; however, all the eggs and tadpoles disappeared soon after plowing. It is believed that the turning over of the soil simply buried all the eggs and tadpoles.

Field observations also confirmed that desiccation is another major cause of tadpole mortality in *R. longicrus*. Ephemeral ponds in ricefields and depressions on dirt roads were favorite spots for egg deposition. However, high evaporation during sunny days caused rapid drying of the

**Table 1.** Summary of the diet of *Rana longicrus*. Total number of stomachs examined was 148

Prey category	<i>n</i>	% total	% frequency <sup>a</sup>
Mollusca			
Gastropoda	2	0.6	1.4
Annelida			
Oligochaeta	2	0.6	1.4
Arthropoda			
Crustacea	43	13.2	11.5
Arachnida	40	12.3	21.6
Chilopoda	11	3.4	6.1
Insecta			
Coleoptera	35	10.8	18.2
Hymenoptera	90	27.7	32.4
Orthoptera	10	3.1	6.8
Diptera	13	4.0	6.8
Isoptera	13	4.0	2.0
Lepidoptera	1	0.3	0.7
Homoptera	10	3.0	6.1
Hemiptera	9	2.8	4.7
Dermoptera	1	0.3	0.7
Collembola	5	1.5	1.4
Others			
Insect larvae	40	12.2	22.3
TOTAL	325	100	—

<sup>a</sup>Percent of frogs containing the item.

<sup>b</sup>Number of unknown prey species = 42; number of stomachs with vegetation and/or seeds = 27; number of empty stomachs = 23.

water pools. On many occasions, we found thousand of tadpoles trapped in drying and choked water pools. No fish predators were observed in these breeding ponds.

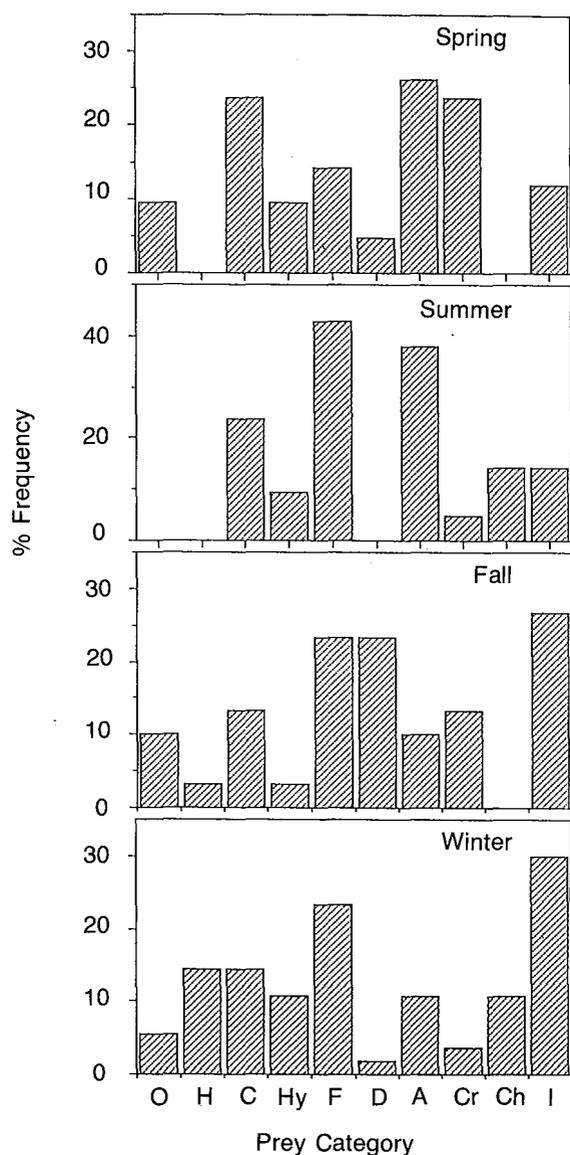
### Dietary analyses

The diet of 148 individuals of *R. longicrus* consisted primarily of invertebrates of the classes Gastropoda, Oligochaeta, Arachnida, Crustacea,

Insecta (adults and larvae), and Chilopoda (Table 1). Arachnids and larval and adult insects were the most abundant items in their diets. Within class Insecta, the orders Hymenoptera and Coleoptera comprised over 85% of the total diet item. The family Formicidae (ants) were particularly abundant within the order Hymenoptera, consisting of 86% of the diet item of this order. Thirty-three of 148 frogs had empty stomachs, and half of these were collected during the summer season. The stomachs of 27 frogs contained detritus and soil particles.

Seasonal differences in diet were apparent in *R. longicrus* (Fig. 6, Table 2). During the winter season, frogs consumed mainly coleopterans, spiders, and crustaceans. Frogs spent more time in or near water, thus, a high frequency of crustaceans was expected (Fig. 6). However, the diet shifted to ants and spiders in the spring. During summer and autumn, ants and insect larvae became the dominant diet items. Seasonal differences in diet most likely reflect the availability of prey in different seasons (Toft 1980a, b).

An  $X^2$  test of independence showed that the number of male and female frogs collected was independent of location, i.e., hill or lowland region ( $X^2 = 0.18$ ,  $n = 1$ ,  $p = 0.74$ ), thus, data were pooled for subsequent statistical analyses. Intra-specifically, the diet between females and males and hill and lowland are significantly different (Table 3). Frogs collected from the hill and lowland habitats had the lowest diet overlap probably because prey availability from these habitats was different. Similar findings were found in earlier studies of anurans and newts (Elliott and



**Fig. 6.** Seasonal importance in prey frequency of the ten most commonly recorded food items in the diet of *Rana longicrus*. O, H, C, Hy and D are the orders Orthoptera, Homoptera, Coleoptera, Hymenoptera, and Diptera, respectively; A, Cr, and Ch are the classes Arachnida, Crustacea, and Chilopoda, respectively; F is the family Formicidae; I is insect larvae.

**Table 2.** Seasonal diet comparisons for *Rana longicrus*

	Summer	Fall	Winter
Spring	52 <sup>a</sup> 69.7*** <sup>b</sup> <i>df</i> = 17 <sup>c</sup>	68 38.7*** <i>df</i> = 16	52 66.6*** <i>df</i> = 14
Summer		63 52.9*** <i>df</i> = 18	47 67.4*** <i>df</i> = 15
Fall			38 93.2*** <i>df</i> = 16

<sup>a</sup>is a measure of overlap index (%).

<sup>b</sup>is a G value with significant probability; \*\*\* represents  $p < 0.0001$ .

<sup>c</sup>is degrees of freedom.

**Table 3.** Diet comparisons between females/males, froglets/adults, and hill/lowland in *Rana longicrus*

Comparison	<i>n</i>	D	G	<i>p</i>
Females/males	20	74	27.8	0.04
Hill/lowland	20	60	49.4	0.0001
Froglets/adults	20	71	31.1	0.03

*n* = the number of prey categories compared. D = percentage overlap in diet. G is the G test value. *p* = significance probability.

Karunakaran 1974). Ontogenetic changes in the number and size of prey items in *R. longicrus* were also found in many anurans and salamanders (Table 3). As an individual grows larger, the morphological changes in its feeding apparatus, including the number of teeth and gape size, allow a wider selection of prey items (Bell 1975, Labanick 1976, Toft 1980a, Christian 1982).

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## 長腳赤蛙生殖及食性之研究

關永才<sup>1</sup> 王慶讓<sup>2</sup> 林曜松<sup>2</sup>

本文報告澳底地區長腳赤蛙的自然史。長腳赤蛙是陸上白天活動的種類，在非生殖季節主要棲息在山林中；當生殖季節來臨時，青蛙即遷移到稻田中繁殖。雌蛙的吻肛長大於雄蛙。生殖資料顯示：長腳赤蛙的繁殖時間是從11月至翌年3月，在3月開始發現小蛙，同年9月吻肛長已和成蛙相同。水池早枯及人類干擾是蝌蚪死亡的主要原因。青蛙的食物包括腹足、貧毛、蛛形、甲殼、唇足、及昆蟲綱的種類。青蛙的食物在季節、性別及成長過程中有顯著的不同。

關鍵詞：長腳赤蛙, 無尾兩棲類, 食性, 生殖。

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