THERMAL TOLERANCE AND THERMOREGULATION BY TAIWANESE RHACOPHORID TADPOLES (*BUERGERIA JAPONICA*) LIVING IN GEOTHERMAL HOT SPRINGS AND STREAMS

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ABSTRACT: We measured the critical thermal maxima (CTMax) of tadpoles of *Buergeria japonica* inhabiting streams and geothermal hot springs. The CTMax of all *B. japonica* tadpoles was over 41 C, indicating that the tadpoles are heat-tolerant. At Rushan, we monitored the temporal and spatial distribution of tadpoles over a 24-h period in a 220 cm × 70 cm plot that is fed by a geothermal hot spring. The thermal profiles of the plot varied seasonally, but the highest water temperature selected by tadpoles remained similar (ca. 37 C). In the winter, the thermal range of the plot was lower (29.5–40.6 C), and tadpoles could be found in the majority of the plot. In contrast, during the spring and summer the temperature ranges of the plot were higher (33.1–43.8 C and 33.0–42.4 C, respectively), and tadpoles could be found only in the cooler sections of the plot, especially around 1000 h with direct solar radiation. These results suggest that extreme heat tolerance and behavioral thermoregulation (i.e., heat avoidance behavior) are the main means by which *B. japonica* tadpoles cope with the high temperatures in geothermal hot springs.

Key words: Anura; *Buergeria japonica*; Heat tolerance; Hot Spring; Thermal Physiology; Thermoregulation

ANURANS possess physiological, behavioral, and morphological plasticity that enables them to explore and survive in many unusual habitats such as arid environments, high elevations, and arboreal pools (Ultsch et al., 1999). The rhacophorid tree frog, *Buergeria japonica*, is common and widely distributed in unforested lowlands and forests in Taiwan up to 1500 m (Lue et al., 1999; Zhao and Alder, 1993). This species usually breeds in slow-moving water in

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TABLE 1.—The unadjusted and adjusted Critical Thermal Maxima (CTMax) of tadpoles of *Buergeria japonica*. Locations of collection, elevation, sample size, and the maximum water temperature were also noted. Means with the same letter are not statistically different.

| Species | Locations | Latitude Longtitude | Elevation (m) | Sample size | Max[1] water temp | CTMax (Mean ± SD) | |
						unadjusted	Adjusted
Buergeria japonica	Wulai hot spring	24° 52′ 5″ N, 121° 32′ 39″ E	70	9	42.2	43.2 ± 0.3[a]	42.7 ± 0.6 [ab]
B. japonica	Rushan hot spring	24° 1′ 36″ N, 121° 11′ 12″ E	980	14	44	43.2 ± 0.5[a]	42.5 ± 0.7[ab]
B. japonica	Guoshin	24° 3′ 46″ N, 120° 48′ 40″ E	360	9	32.9	42.2 ± 0.3[b]	42.7 ± 0.5[a]
B. japonica	Chukou	23° 26′ 5″ N, 120° 38′ 8″ E	500	18	30.8	41.2 ± 0.6[c]	41.8 ± 0.6[b]

[1] Maximum water temperature was the highest water temperature recorded by a Hi-low thermometer at the collection sites for four weeks.

ditches and small streams (Chou and Lin, 1997). However, *B. japonica* may also breed in an unusual habitat, geothermal hot springs, which are carbonate-rich and slightly alkaline. To our knowledge, *B. japonica* is one of few anuran species known to breed in such unusual habitats (Straw, 1958; Ultsch et al., 1999). Although geothermal hot springs are found throughout Taiwan, they occur at comparatively low densities. As a result, only certain populations of *B. japonica* have access to hot springs to breed. One of the most distinct differences between the two breeding sites of *B. japonica* is the thermal environment where tadpoles live. The temperature of a geothermal hot spring is unusually high, whereas that of stream is usually low and readily affected by the surroundings. Chen et al. (2001) compared the reproduction and ecology of populations that breed in geothermal hot springs and streams. Their results suggest the advantages of breeding in geothermal hot springs are a prolonged breeding season, rapid larval growth and development, and either reduction of intra- and inter-specific competition or reduced predation. However, the disadvantage of breeding in geothermal hot springs is that adult frogs, and particularly the tadpoles, must cope with the high water temperatures.

The purpose of this study was to evaluate the physiological and behavioral adaptations of tadpoles of *B. japonica* that live in geothermal hot springs. Specifically, we compared the thermal tolerance of tadpoles from geothermal hot springs and streams. In addition, we studied the spatial and temporal distribution of tadpoles in a geothermal hot spring in different seasons. Finally, we studied thermoregulation by tadpoles from geothermal hot springs and streams in the laboratory.

MATERIALS AND METHODS

Sites of Tadpole Collection and Study Areas

We collected tadpoles of *B. japonica* from two hot springs (Rushan and Wulai) and two streams (Guoshin and Chukou; Table 1). In Rushan and Wulai, we commonly found hot spring water seeping from the rocks to be as hot as 60–70 C; as the water flows into ditches or depressions, it cools gradually. As a result, water temperatures varied depending on the distance from the source, ranging from 70 C to local air temperature (5–35 C), and tadpoles were commonly observed. We also collected tadpoles from roadside ditches at Guoshin and Chukou where geothermal hot springs were absent. The ditches were about 40–100 cm wide, and water was from 1–30 cm in depth, depending on rainfall. The ditch in Guoshin dried up during the dry season whereas the ditch in Chukou contained water year round, but the water volume was lower during the dry season. The stream's bed usually contained a thin layer of sandy substrates, with fallen leaves and twigs forming shelters for the tadpoles. Other anuran species such as *Rana sauteri*, *R. kuhlii*, *R. swinhoana*, *R. latouchii* and *Rhacophorus moltrechti* have been found in the ditches of Chukou, and the latter two are also present in that of Guoshin.

We selected a geothermal hot spring in Rushan and a roadside ditch in Guoshin for a year-round survey. At Rushan, we located a geothermal hot spring where water seeped from the rocks at temperatures of about 47–49 C. As water flowed into depressions, it cooled

gradually, forming a thermal gradient. Water depth was about 3 cm and was rather uniform throughout. *Buergeria japonica* was the only anuran species breeding in this water, with tadpoles present throughout the year. No macro-invertebrates except a few damselfly larvae were observed during the study period. From October 2000 to September 2001, we put a Hi-Low thermometer (Megaview EM-N276) in the middle of the thermal gradient and in a shaded area nearby each to monitor the water and air temperature, respectively. Monthly precipitation was obtained from the Rushan weather station, which was about 2 km from the study site.

At Guoshin, we located a roadside ditch where tadpoles of *B. japonica* were abundant during the breeding season. The ditch is a typical breeding habitat for *B. japonica*, being about 45 cm wide and fed by underground water that seeped from the rocks. The ditch contained water during the spring and summer but dried up during the fall and winter. The water depth was about 8 cm, and no geothermal hot spring was nearby. The streambed contained a thin layer of sandy substrates, with fallen leaves and twigs providing shelter for the tadpoles. From December 2000 to September 2001, we put a Hi-Low thermometer in the water of the ditch and a shaded area nearby to monitor the water and air temperatures, respectively. Because the ditch dried up for part of the year, we were only able to record water temperatures for five months. Monthly precipitation was obtained from the Peishan weather station, which was about 1 km from the study site.

Reproductive Phenology

Each month, from June 2000 to September 2001, we recorded the reproductive activities of *B. japonica* in the geothermal hot spring at Rushan and in the stream at Guoshin. During each visit, we recorded the presence of calling frogs, amplectant pairs, egg clutches and tadpoles. Because the water is rather shallow at both sites, we surveyed tadpoles visually.

Critical Thermal Maxima (CTMax) Measurements

We conducted the CTMax measurements on tadpoles between June 2000 and July 2001.

Tadpoles were collected from two hot springs (Wulai and Rushan) and two streams (Guoshin and Chukou). At the beginning of the breeding season, we put a Hi-Low thermometer (Megaview EM-N276) at the collection sites to determine the maximal and minimal water temperatures. We visited all sites once a month to collect tadpoles of Gosner stages 26–28 (Gosner, 1960) for the experiment. We transported tadpoles back to the laboratory in a Styrofoam box to avoid a rapid increase in water temperature. Tadpoles were maintained in an aerated tank at water temperatures similar to the collection site, under a LD 12:12 light cycle, and the CTMax of tadpoles was determined between 1000 and 1500 h within three days of collection.

The procedure used to determine CTMax was similar to that of Hutchison (1961) except for the endpoint. In tadpoles, the occurrence of spasms was hard to determine, and thus we decided to use the losing of righting response of tadpoles as endpoint (Cupp, 1980; Lutterschmidt and Hutchison, 1997*a,b*; Manis and Claussen, 1986). The losing of righting response was defined as the inability of the animal to swim or respond to mechanical stimulation with a probe. For the CTMax measurement, tadpoles were placed in a flask with 200 ml of water which was then placed in a heated water bath set to increase the water temperature at a rate of about 0.25 C per min. The initial temperature in the water bath was set at 30 or 25 C for tadpoles that were collected from hot springs or streams, respectively; these temperatures represented the average water temperature experienced by tadpoles in the field. Air was bubbled into the flask to distribute the heat evenly in the water. Deep body temperatures were not taken. We assumed that, because of the small body size of the tadpoles and the low heating rate employed, there was no measurable lag between change in water temperature and change in body temperature (Dunlap, 1968; Hutchison, 1961).

Because each specimen was tested in a separate flask, a CTMax was recorded for each individual. We removed tadpoles from the flask soon after CTMax was reached. Before tadpoles recovered, we weighed and staged them.

Spatial and Temporal Distribution of Tadpoles in the Field

In each season, we studied the spatial and temporal distribution of *B. japonica* tadpoles in the sites during a 24-h period. In the geothermal hot spring at Rushan, we conducted the experiment two separate times each season (winter: 9–10 December 2000 and 14–15 January 2001; spring: 2–3 and 7–8 April 2001; summer: 3–4 and 15–16 July 2001). First, we set up a 220 cm × 70 cm plot with a hot spring source at one end. At the other end (i.e., cool end), we installed a wire screen to prevent the escape of tadpoles. We used threads that were held 5 cm above the water to divide the plot into a grid of 10 sections. The plot contained a thin layer of sandy substrates with a low density of blue-green algae.

A day prior to the experiment, we carefully removed all tadpoles found in the plot and put back only 100 tadpoles, which were at stage 30–34 and were about 2.5–3 cm in total length. If there were not sufficient tadpoles at the proper stage, we collected additional tadpoles from nearby hot springs. We chose tadpoles of this stage because they were abundant in the field and large enough to be counted easily. We removed any fallen leaves and damselfly larvae present in the plot. We started the experiment the next morning (0800 h), and it lasted 24 h. We took measurements once every two hours, recording the abundance of tadpoles, water temperature in each grid, water temperature at the hot spring source, and air temperature. Water temperature was measured by placing a thermocouple in the center of each section of the grid.

We ran the same experiment in the roadside ditch at Guoshin on 26–27 May 2001. We could not do the experiment earlier because we found no tadpoles in the ditch until April when the ditch started to fill with water. Briefly, we set up a 220 cm × 45 cm plot in the ditch and divided it into a grid of 10 sections. At the downstream end, we installed a wire screen to prevent the escape of tadpoles. Other methods were identical to those used at the Rushan site. Our results indicated that no thermal gradient was in the plot (See Results), and thus we did not conduct further field study for the rest of the season.

Selections of Temperature by Tadpoles in the Laboratory

From October 2000 to August 2001, we collected tadpoles of stage 30–34 monthly from the geothermal hot spring (Rushan) and stream (Guoshin), and thermal selection experiments were done within 3 d of collection. We collected tadpoles from Rushan monthly, but we were only able to collect tadpoles from Guoshin from April to August due to the restricted breeding season. After tadpole collection, we transported them back to the laboratory in a Styrofoam box to avoid a rapid increase in water temperature. Tadpoles were maintained in an aerated tank at water temperatures similar to the field, under a light cycle of LD 12:12. Experiments were conducted between 0800 and 2000 h when the effect of diel cycles, if present, was minimal. Field observations have indicated that tadpoles from Guoshin were active all day, whereas tadpoles from Rushan were active and behaved similarly during most of the day except during the early morning when tadpoles aggregated at the cooler end of the plot.

We determined the thermal selection of tadpoles in an aquatic thermal gradient (Lucas and Reynolds, 1967; Wollmuth et al., 1987). This thermal gradient was achieved by setting up a water lane (150 cm × 20 cm × 4 cm deep) with heat exchangers coupled to either a hot or cold source by circulating pumps (Julabo F10). The water lane was divided into 11 chambers by baffles, and each chamber was aerated. About 1.5 to 2 h after the gradient was turned on, a linear thermal gradient from 23.2 ± 0.4 C to 40.6 ± 0.3 C (mean ± SD) was established.

At the onset of the experiment, we placed a tadpole randomly in a chamber at either the cold end (40.3 ± 0.3 C), hot end (23.3 ± 0.3 C), or the middle chamber (32.7 ± 0.6 C) (mean ± SD). We then continuously monitored the position of the tadpole and water temperature once every 15 min for 3.5 h. Preliminary experiments showed that tadpoles might have moved around at the beginning (Hutchison and Dupre, 1992), but they settled down after 2–2.5 h. We therefore averaged the water temperature of the chambers where the tadpoles were located during the last hour and designated that as the temperature selected by the tadpoles. We terminated the experiment

if the tadpoles were in thermal spasm or lost their righting response, and the data were excluded from subsequent analyses. We also excluded trials in which tadpoles consistently positioned themselves at either the extreme cold or warm end of the thermal gradient; this was because we were not sure that these tadpoles preferred those particular water temperatures, or whether they might have wanted to select even lower or higher water temperatures (Nie et al., 1999).

Statistical Analyses

We analyzed the data using the SAS program (SAS Institute, 1996). Because the CTMax of tadpoles is related to their thermal history, especially the extreme temperatures that tadpoles have experienced (Brown, 1969; Cupp, 1980; Heatwole et al., 1968), we used analysis of covariance (ANCOVA) to analyze CTMax, with the maximum water temperature collected in the field as a covariate. Because tadpoles were at early developmental stages (Gosner 26–28), we assumed that a four-week period of water temperature measurement in the field by Hi-Low thermometers was a reasonable estimation of thermal history of tadpoles.

We used a chi-sqaure test to determine spatial distributions of tadpoles at each sampling time (every 2 h) for a 24-h period by comparing the expected frequency in each grid based on an equal probability of events to the observed frequency. Because the variances of some data were not homogeneous, even after the transformations, we used the Kruskal-Wallis test to analyze the air temperature and water temperature at the hot spring source, the cool end of the plot, the warm end of the plot, the different temperature between adjacent grids (i.e., inter-grid temperature difference), the maximal water temperature selected by tadpoles, and the coefficient of variation (CV) of water temperature selected by tadpoles. If measurements were significantly different between treatments, we conducted multiple comparisons between sampling days (Sokal and Rohlf, 1994). We used a *t*-test to compare the water temperature selected by tadpoles collected from Rushan and Guoshin. All values are expressed as means ± SD unless otherwise noted. A significant difference was declared when $P \leq 0.05$.
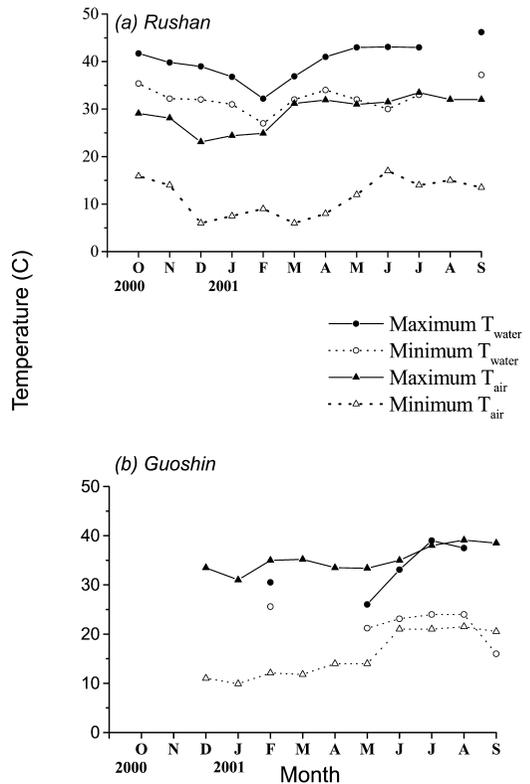

FIG. 1.—Maximal and minimal water and air temperatures at (a) Rushan and (b) Guoshin. At Rushan, measurements were taken from October 2000 to September 2001. Water temperature from August 2001 are missing because the thermometer was swept away by a typhoon. At Guoshin, measurements were taken from December 2000 to October 2001. Water temperature data from December 2000, and January, March, April, and September 2001 were excluded because the roadside ditch was either completely or partially dry during that time span.

RESULTS

Precipitation, Air, Water Temperatures, and Reproductive Phenology

The pattern of precipitation in Rushan and Guoshin was similar, characterized by a wet and dry season, with the wet season ocurring from February to September. In the spring, heavy rains were mainly associated with the "Mei" rain season, characterized by continuous light rain for days with occasional heavy thunderstorms. In the summer, heavy rains were associated with thunderstorms or typhoons, which occurred most often from June through September.

TABLE 2.—Seasonal differences in water and air temperatures, upper selected water temperature by *Buergeria japonica* tadpoles, and tadpole dispersion in an experimental plot with thermal gradient in Rushan. Measurement unit of all variables is degree in centigrade (C) unless noted. The sample size of all variables is 12 unless noted. *P* is significant level. T_w and T_a represent water and air temperature, respectively.

Variables[1]	Date (year, month, day)						Chi- square	P
	2000	2001						
	12/9–12/10	1/14–1/15	4/3–4/4	4/7–4/8	7/3–7/4	7/15–7/16		
T_w at hot spring source	48.1 ± 0.4^{ab}	48.5 ± 0.8^{bc}	48.9 ± 0.9^{c}	47.9 ± 0.6^{bc}	47.1 ± 0.3^{a}	48.5 ± 0.7^{bc}	32.00	0.0001
T_a	13.8 ± 2.0^{a}	13.7 ± 2.0^{a}	18.5 ± 1.7^{b}	17.5 ± 1.7^{b}	25 ± 3.8^{c}	23.2 ± 4.3^{c}	59.67	0.0001
T_w at warm end of the plot	40.9 ± 0.6^{ad}	40.2 ± 0.5^{a}	43.8 ± 0.4^{b}	43.8 ± 1.0^{b}	41.4 ± 0.9^{cd}	43.3 ± 0.8^{b}	58.73	0.0001
T_w at cool end of the plot	30.1 ± 1.0^{a}	29 ± 0.8^{a}	32.7 ± 0.4^{b}	33.5 ± 0.7^{b}	32.8 ± 2.0^{b}	33.2 ± 1.9^{b}	51.98	0.0001
Inter-grid T_w difference[2] (n = 9)	1.2 ± 0.8	1.3 ± 0.8	1.3 ± 1.1	1.2 ± 0.9	1.0 ± 0.8	1.1 ± 1.1	0.69	0.9834
Upper T_w selected by tadpoles[3]	36.3 ± 1.7	38.2 ± 1.5	36.5 ± 1.5	37 ± 1.4	36.8 ± 2.7	36.7 ± 2.1	8.79	0.1179
CV of T_w selected by tadpoles[4] (%)	6.6 ± 2.0^{a}	10.5 ± 1.7^{b}	3.9 ± 1.5^{a}	3.2 ± 0.8^{a}	4.3 ± 2.2^{a}	3.6 ± 0.8^{a}	43.47	0.0001

[1] Within a row, means with the same letters are not statistically different.
[2] There are 10 grids in the experimental plot. Temperature differences between adjacent grids (i.e., inter-grid temperature difference) were calculated at each sampling time (every 2 h) for a 24-h period, then averaged. The overall mean of inter-grid temperature difference were calculated from the means of the 9 inter-grid temperature differences.
[3] Highest water temperature selected by tadpoles was recorded at each sampling time for a 24-h period, then averaged.
[4] Water temperatures selected by all tadpoles was recorded, and the mean and coefficient of variation (CV) of temperature selected were calculated for each sampling time for a 24-h period where mean = $(\Sigma T_i \times N_i) / \Sigma N_i$, where T_i and N_i is water temperature and tadpole number in the grid i. At the end of experiment, the CVs of all sampling times were averaged.

In Rushan, hot springs seeped from the rock constantly and were present throughout the study period. The maximal and minimal water temperatures were rather high, and the minimal water temperature was 27 C. Water temperatures were consistently higher than air temperatures (Fig. 1). In Guoshin, water was present only during the wet season, and the ditch dried up for some part of the year. Both the maximal and minimal water temperatures were lower than the maximal air temperature but higher than the minimal air temperature (Fig. 1).

At Rushan, newly hatched tadpoles and calling frogs were recorded throughout the year. We also observed amplectant pairs each time we conducted a 24-h experiment. In Guoshin, tadpoles were abundant in April when the rain started, and we observed newly hatched tadpoles each month. The number of tadpoles started to decrease in late July, and by September no tadpoles were seen.

Comparisons of CTMax of Tadpoles

We measured the CTMax of tadpoles of *B. japonica* from four populations (*n* = 50; Table 1). The CTMax of all tadpoles, unadjusted for water temperature, was over 41 C regardless of collection locality. The unadjusted CTMax of tadpoles from hot springs (Wulai and Rushan) were statistically higher than that of tadpoles from streams (Guoshin and Chukou; Table 1). However, after adjusting the CTMax of tadpoles using the maximum water temperature in the field, the CTMax of tadpoles from hot springs did not differ statistically from that of streams (Table 1).

Thermal Selection of Tadpoles in the Field

Air temperatures, water temperatures at the hot spring source, and water temperatures at the warm and cool ends of the plot varied seasonally (Table 2). Post-hoc comparisons showed that air temperature was highest in the summer and was lowest in the winter (Table 2). A slightly different pattern in water temperature was seen at the warm and cool ends of the plot, in that temperature at spring and summer were generally higher than that of winter. In contrast, the water temperature at the hot spring source varied from day to day but did not show a clear pattern.
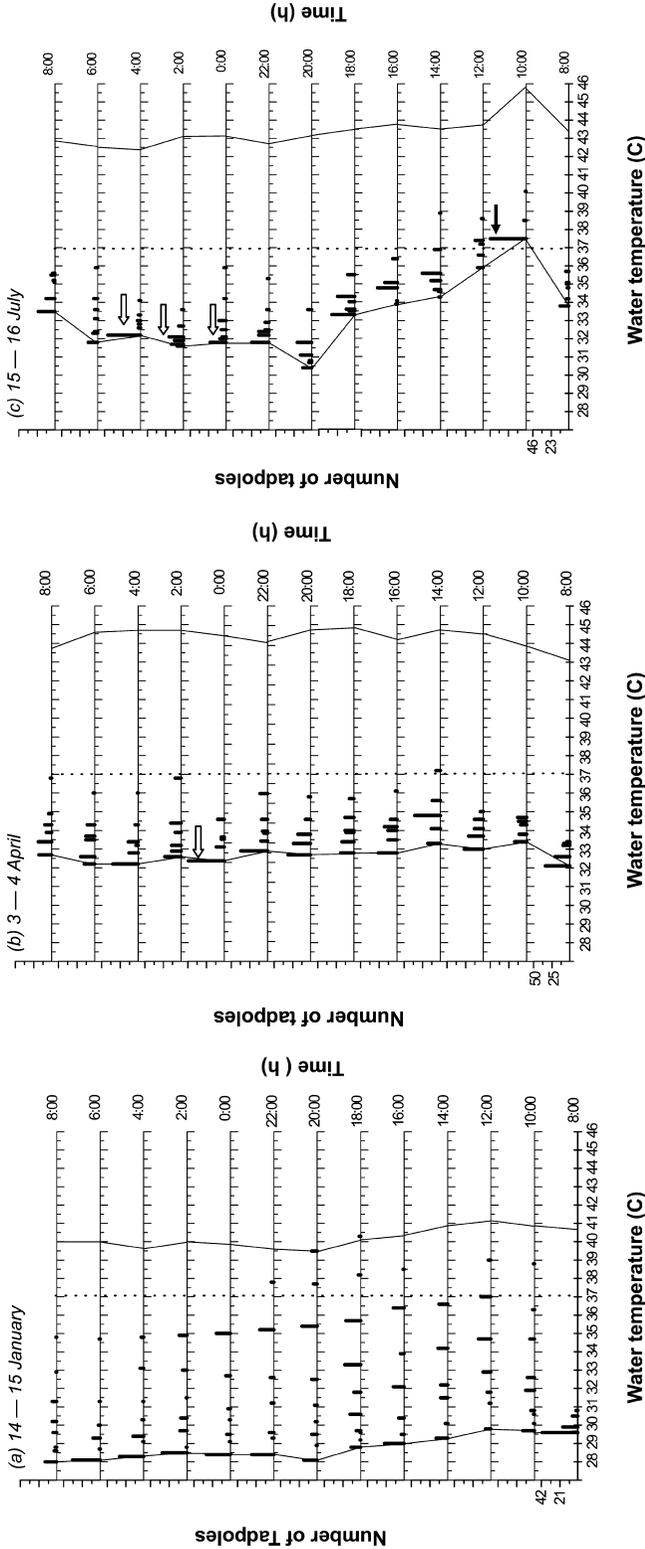
Fɪɢ. 2.—The temporal and spatial distribution of *Buergeria japonica* tadpoles on (a) 14–15 January, (b) 3–4 April, and (c) 15–16 July 2001, in an experimental plot in Rushan. Measurements were taken from 0800 h to 0800 h of the next day. The solid lines to the left and right represent water temperatures at the cool and warm end of the plot, respectively. A dashed line represents the average upper water temperature selected by tadpoles based on data from all seasons. An open arrow indicates tadpoles aggregated and showed little movement. A solid arrow indicates tadpoles aggregated at the cool end of the plot to avoid high water temperature.
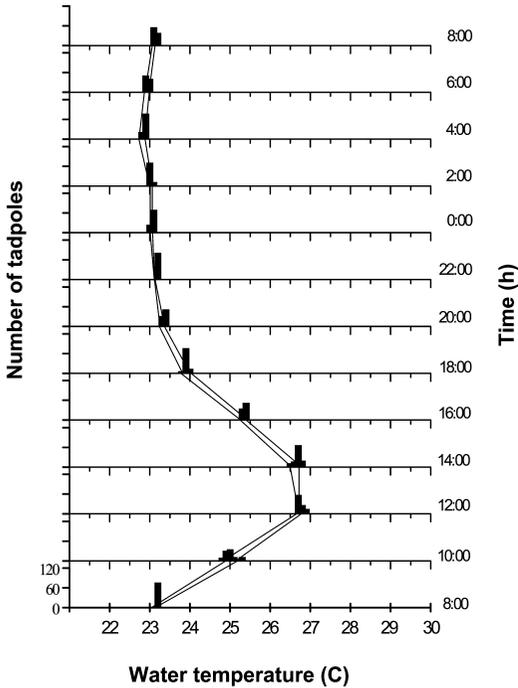
FIG. 3.—The temporal and spatial distribution of *Buergeria japonica* tadpoles in an experimental plot in Guoshin. Measurements were taken from 0800 h on 25 May to 0800 h on 26 May 2001. See Fig. 2 for an explanation of the symbols.

TABLE 3.—Temperature selected (mean $\pm$ SD) by *Buergeria japonica* tadpoles from Rushan and Guoshin in the laboratory. T_w and CV represent water temperature and coefficient of variation, respectively.

	Month	Selected T_w (C)	CV (%)	Sample size
Rushan	10–11	30.1 ± 2.1	7.1	5
	12–2	28.5 ± 2.1	7.4	18
	3–5	27.9 ± 2.4	8.8	14
	6–8	27.3 ± 2.6	9.7	10
	Overall	28.2 ± 2.3	8.4	47
Guoshin[1]	4–5	25.7 ± 4.6	17.9	9
	6–8	23.9 ± 4.0	16.9	10
	Overall	24.8 ± 4.3	17.3	19

[1] We were unable to collect tadpoles at Guoshin from September to March because of the short breeding season.

Every 2 h during the 24-h experiment, we also measured the water temperature of each grid and recorded the tadpole abundance in each grid. Although there were 100 tadpoles in the plot, we could not locate all of them at each sampling time (means: 63 ± 13, range: 33–86, $n = 77$), this was because some tadpoles hid at the edge of the plot, and it was difficult to detect them without disturbance. Results showed that the upper water temperature selected by tadpoles was statistically similar, between 36–38 C. The temperature difference between adjacent grids was rather uniform, ranging from 1–1.3 C (Table 2); in other words, the drop in water temperature along the thermal gradient in the plot was linear.

In winter, the water temperature was lower, and tadpoles were scattered in many grids. Figure 2a represents the temporal and spatial distribution of tadpoles in the plot on 14–15 January 2001, the majority of the tadpoles did not move beyond 37 C. From 1000 to 2000 h,

tadpoles were more evenly distributed. Tadpoles swam actively and foraged by scraping algae from the substrate bed (Fig. 2a). From 0400–0800 h, many tadpoles aggregated at the cool end of the plot.

In the spring, water temperatures were higher, and the thermal gradient of the plot was shifted to higher temperatures (Fig. 2b). The majority of tadpoles did not swim beyond 37 C and were restricted to the cool end of the plot all day. The furthest the tadpoles dispersed was to the third grid from the hot end of the plot at 1400 h. At 0000 h, we observed the majority of tadpoles crowded at the cool end of the plot and immobile with minimal lateral movement. In the summer, the thermal gradient varied more than in the spring, because of direct solar radiation. Figure 2c illustrates the temporal and spatial distribution of tadpoles in the plot on 3–4 July 2001. Starting at 0700 h, the plot received direct solar radiation, and the water temperature of all grids increased, with water temperature at the cool end of the plot rising dramatically to about 37.5 C by 1000 h (Fig. 2c). At that time, all tadpoles aggregated at the cool end of the plot. A similar scenario occurred on July 15–16, 2001. Tadpoles appeared active at all times, although on some occasions (i.e., at 0000 h of 4 April 2001 and 0000 to 0400 h of 15 July 2001) tadpoles were found aggregated at the cool end of the plots and appeared inactive.

Field measurements indicated that the thermal gradient profiles in the plot shifted seasonally, however, the majority of tadpoles did not swim beyond 37 C. Assuming equal

probabilities of distribution of tadpoles along the thermal gradient of the plot, chi-square tests of independence of the frequency of tadpoles along the plot at every sampling time of six experimental days gave significant values ($P < 0.05$). Further analyses revealed that the coefficient of variation of the temperature selected by tadpoles was smaller in the spring and summer than in the winter, and the former two were statistically similar (Table 2).

At Guoshin, we conducted the same 24-h observation on 26–27 May, 2001. Results showed that, although water temperature fluctuated daily between 23–27 C, a thermal gradient was virtually absent in the plot, as seen in the very small differences in water temperatures between the warm and cool ends of the plot at each time of measurement (0.14 ± 0.12 C, range 0–0.5 C, $n = 12$; Fig. 3). Tadpoles scattered all over the plot during the 24-h period and foraged on the substrate bed. We did not see any form of aggregation as in the geothermal hot spring.

Thermal Selection of Tadpoles in the Laboratory

We collected 55 and 24 tadpoles from Rushan and Guoshin, respectively, to conduct the thermal selection experiments. Two and three tadpoles from these respective localities lost their righting responses, and eight and five tadpoles from these respective localities stayed at an extreme end of the thermal gradient. We subsequently excluded these data from further analyses.

At Rushan, tadpoles collected at different seasons selected similar temperatures (ANOVA: $F_{3,43} = 1.75$, $P = 0.172$; Table 3). At Guoshin, tadpoles were available during spring and summer due to the restricted breeding season. Tadpoles collected in spring and summer also selected similar temperatures (ANOVA: $F_{1,17} = 0.83$, $P = 0.374$; Table 3). We compared the temperatures selected by tadpoles collected from both localities in the same months (i.e., April to August) and found that Rushan tadpoles selected higher temperatures (27.8 ± 2.3 C) than Guoshin tadpoles (24.8 ± 4.3 C; $t = 2.64$, df $= 28.6$, $P = 0.013$). In addition, the coefficient of variation of temperature selected by Rushan tadpoles (8.4%) was smaller than that of Guoshin tadpoles (17.3%).

DISCUSSION

The population of *B. japonica* at Rushan breeds year-round whereas those at Guoshin breed seasonally, which agrees with the results of an earlier study conducted in these habitats in other parts of the island (Chen et al., 2001). Likely, the persistence of the warm environment in the geothermal hot spring allows *B. japonica* to prolong the breeding season. On the other hand, low temperature probably inhibits *B. japonica* from breeding in streams or ditches during the winter.

Tadpoles of *B. japonica*, whether collected from geothermal hot springs or streams, all had a CTMax over 41 C, and some even reached 43 C, indicating that they tolerate heat well (Ultsch et al., 1999). Tadpoles collected from another geothermal hot spring (i.e., Jentse, Ilan county) also exhibited high thermal tolerance (Chen et al., 2001). Apparently, the high thermal tolerance of tadpoles enables them to cope with the high water temperature in the geothermal hot spring. Our field measurements revealed that, with the exception of winter, tadpoles at Rushan routinely encounter water temperatures over 40 C, particularly in summer. Our findings are consistent with the contention that the thermal tolerance of amphibian larvae is correlated with their geographical distribution and breeding habits; for example, larvae that live in desert habitats, temporary ponds or shallow pools, in which high temperatures are common, usually have a high thermal tolerance (Brown, 1969; Cupp, 1980; Herreid and Kinney, 1967; Hoppe, 1978; Miller and Packard, 1977; Ultsch et al., 1999). Tadpoles of *B. japonica* from Chukou and Guoshin also exhibited a high CTMax even though they live in streams with a water temperature less than 30 C most of the time, and there were no geothermal hot springs nearby (Chen et al., 2001). This finding suggests that the high thermal tolerance of tadpoles of *B. japonica* is probably genetically fixed.

The temporal and spatial distribution of tadpoles was synchronized with changes of the thermal gradient in the experimental plot, suggesting that tadpoles thermoregulate behaviorally to avoid heat (Hutchison and Spriestersbach, 1986). Field measurements indicated that the thermal gradient in the experimental plot was shaped by the temperature of water emerging from the hot spring

and air temperature, further modified by direct solar radiation. Our results strongly suggest that the dispersion of tadpoles was affected by the profile of the thermal gradient that changed daily and seasonally. Field measurements at different seasons showed that tadpoles consistently selected water temperatures lower than 37 C. In the winter, water temperature cooled down more rapidly because of the lower air temperature, which allowed tadpoles to disperse to sections of the plot that physiologically were inaccessible previously. In contrast, water temperature in the summer was high on all days, and fewer sections of plots were physiologically accessible to tadpoles, which resulted in a clumped distribution of tadpoles. One of the field observations that best illustrates the heat avoidance behavior occurred at 1000 h on 3–4 and 15–16 July, when the plot was under direct solar radiation and the water heated up dramatically. As water temperature elevated, there were fewer and fewer sections of the plot that were suitable for the tadpoles, and most of the tadpoles appeared to avoid high water temperature and took refuge in the cooler end of the plot, which was about 37.5 C. Tadpoles were basically clumped together in the cool end, but still more tadpoles kept swimming and scrambling toward that end. As soon as the water temperature dropped, tadpoles dispersed to other grids.

The temperature selected by tadpoles in the laboratory (ca. 28 C) was significantly lower than that selected in the field (34 C), suggesting that the temporal and spatial distribution of tadpoles in the field cannot be explained solely by heat avoidance behavior. The temporal and spatial distribution of tadpoles also may be affected by other biotic and abiotic factors such as dissolved oxygen (Noland and Ultsch, 1981), rheotaxis (Hutchison and Dupre, 1992), food (Anderson and Graham, 1967; Holomuzki, 1986), predators (Holomuzki, 1986; Stange and Semlitsch, 1987), and intraspecific interactions (Alford and Crump, 1982; Beiswenger, 1975). Tadpoles of *B. japonica* are mainly benthic scrapers (Chou and Lin, 1997). Our observations suggest that the distribution of food (algae) in the plot and intraspecific interactions probably account for the difference in the temperature selected by tadpoles in the

laboratory and field. Based on the thermal selection in the laboratory, the preferred temperature of tadpoles is about 28 C. We contend that a high density of tadpoles in the cooler end of the plot resulted in depletion of algae, and increased intraspecific competition forced tadpoles to disperse to less favored, warmer microhabitats that were less exploited and contained more abundant algae on the substrate. Field measurements indicated that the voluntary maximum temperature (ca. 37 C) of tadpoles varied little seasonally. Thus, whenever water in the plot cooled down, tadpoles would forage further toward the warmer end of plot. On several occasions, we observed tadpoles foraging on the substrate turning back immediately when they encountered high temperatures. We also observed on one occasion (from 0000 to 0200 h on 20 December 2000) that tadpoles aggregated in a warmer area (ca. 34 C) to scavenge dead tadpoles. As soon as the carcass was consumed, they returned immediately to a cooler area. With regard to other factors, the effect of predation was negligible because predators were removed prior to experiments. Also, the water was very shallow (3 cm deep) and slow moving, so the effects of dissolved oxygen and rheotaxis should have been minimal.

Rushan tadpoles selected a higher temperature than Guoshin tadpoles, which is consistent with earlier findings that the thermal preference of amphibian larvae is affected by their recent thermal history (Hutchison and Dupre, 1992). Tadpoles acclimated in higher water temperatures had a higher thermal preference (Wollmuth et al., 1987; Wollmuth and Crawshaw, 1988). The coefficient of variation of temperature selected by Rushan tadpoles was smaller than for Guoshin tadpoles, suggesting that the former is a better thermoregulator (Dupre and Petranka, 1985), assuming the tadpoles of Rushan and Guoshin populations are similar in mobility. In the experimental plot at Rushan, the temperature difference between the two ends of the plot (ca. 2 m in length) was about 10 C. Thus, a strong thermal selector would enable the tadpoles to detect rapid changes in water temperature in the plot to avoid becoming overheated and possibly select the temperatures that maxi-

mize their growth and development (Dupre and Petranka, 1985; Hutchison and Hill, 1977; Wollmuth and Crawshaw, 1988).

In conclusion, hot springs represent unique resource-rich breeding sites; *B. japonica* that breed in geothermal hot springs have a prolonged breeding season due to stable thermal aquatic environments, and more importantly, tadpoles that live in this habitat experience rapid growth, high food (algal) availability, reduced predation and inter-specific competition, and low risk of habitat desiccation (Chen et al., 2001). *Buergeria japonica* represents one of few amphibian species that has successfully explored this resource-rich yet hostile environment. Interestingly, although six Taiwanese species of frogs, like *B. japonica*, are stream breeders, island-wide field observations suggest that *B. japonica* is the only frog species to use this unusual habitat even though some frog species, like *R. limnocharis*, which is a common and abundant species, can also tolerate heat well (Y.-C. Kam, unpublished data). Thus, we contend that other ecological, physiological, or behavioral variables might have been involved in limiting colonization of frogs other than *B. japonica* to the hot springs.

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