

Reproductive ecology of *Rana kukunoris* NIKOLSKII, 1918, a high-altitude frog native to the Tibetan Plateau (Anura: Ranidae)

Fortpflanzungsökologie von *Rana kukunoris* NIKOLSKII, 1918,
einem Gebirgsfrosch des tibetischen Hochlandes
(Anura: Ranidae)

XIN LU & XIANHAI ZENG & BO DU & CHUAN NIE

KURZFASSUNG

Wir untersuchten die Laichökologie und Entwicklung des im tibetischen Hochland endemischen Frosches *Rana kukunoris*, NIKOLSKII, 1918 in einem montanen Feuchtbiotop (34°17' N, 102°18' E, 3450 m ü. M.) Westchinas, von April bis Mai 2007. Die Laichzeit im feuchten, von Bächen durchflossenen Sumpfland dauerte von Anfang April bis Anfang Mai. Obwohl die Männchen in starken Chören während der Nacht riefen, waren die Frösche hauptsächlich am frühen Nachmittag an für die Jahreszeit warmen Tagen aktiv. Die Männchen verweilten im Wasser und klammerten jedes Froschweibchen, das zu den Laichplätzen in den Bachläufen anwanderte. Die positive Korrelation zwischen der Größe klammernder Männchen und Weibchen war signifikant aber schwach ($r = 0.22$). Das Paarungssystem ist charakterisiert durch den Wettbewerb zwischen den Männchen, worauf die Zahl atypischer Klammeraktivitäten (Männchen klammert andere Objekte als laichreife Weibchen - 10,1% von 138 untersuchten Klammerungen), Vertreibung des klammernden Rivalen (ein einzelnes Weibchen wird von mehr als einem Männchen geklammert - 1,4%) und Partnerwahl während der relativ langdauernden Laichzeit (ein Monat) hindeuten. Die Eier wurden gemeinschaftlich in Randnähe der Bäche abgelegt und an Wasserpflanzen am Gewässerboden in einer Tiefe von 9,5 (5-27) cm befestigt, vermutlich zum Schutz vor Kälteeinbrüchen und ultravioletter Strahlung. Die Art zeigt einen deutlichen Sexualdimorphismus im Adultstadium, indem die Weibchen größer werden als die Männchen. Die Laichballen beinhalteten 315 bis 1577 (628 ± 277) Eier mit Durchmessern von 1,95 bis 2,62 mm ($2,24 \pm 0,19$); eine Beziehung zwischen Eizahl und Eigröße war nicht nachweisbar. Ein Laichballen wog 28,2% der Masse eines Weibchens; größere Weibchen produzierten schwerere Gelege. Im Vergleich mit Gattungsverwandten im Tiefland investierte *R. kukunoris* vermehrt in die Nachkommenschaft und produzierte größere aber weniger Eier.

ABSTRACT

We investigated the breeding ecology and life history of a Tibetan Plateau endemic brown frog (*Rana kukunoris*, NIKOLSKII, 1918) in an alpine marsh (34°17' N, 102°18' E, 3450 m elevation) of west China, during April to May 2007. Annual spawning activity, which occurred in wet marshes with streams passing through, began in early April and ended by early May. Although male chorused extensively during nights, frogs were active around early afternoon when temperature was highest, and on a seasonal basis, on warm days. Males stayed in water to amplex any females immigrating towards oviposition sites along streams. There was a significant but weak positive relationship ($r = 0.22$) between sizes of amplexing male and female. This mating system could be attributed to male-male competition indicated by exceptional amplexus - male clasping objectives but gravid females (10.1% of 138 clasping events recorded), mate displacement - more than one male embracing a single female (1.4%), and mate choice during the relatively prolonged breeding period (one month). Eggs were laid communally near the margin of streams, and attached to aquatic plants at the bottom with an average water depth of 9.5 (5-27) cm, assuming to take advantages against colder climates and stronger ultraviolet radiation. A significant adult sexual dimorphism was found, with females being larger than males. Clutch size varied between 315 and 1577 (628 ± 277) and egg diameter from 1.95 to 2.62 mm (2.24 ± 0.19), with no trade-off between them present. A clutch weighed 28.2% of the female mass and larger females produced heavier clutches. Compared to its lowland congeners, *R. kukunoris* invested more in egg production and laid larger but fewer eggs.

KEY WORDS

Amphibia: Anura: Ranidae; *Rana kukunoris*, reproductive ecology, breeding ecology, breeding season, life history, mating system, oviposition habitat, Tibetan Plateau, China

INTRODUCTION

Rana kukunoris NIKOLSKII, 1918 is an amphibian species endemic to the eastern Tibetan Plateau (29° to 41° N and 93° to 104°

E in range). It inhabits open alpine marshes from 3000 to 3800 m elevation, representing one of the highest-distributed anurans in the

world. In earlier taxonomic revisions, the frog was considered as a subspecies of *R. temporaria* LINNAEUS, 1758 – the commonest anuran over Europe (POPE & BORING 1940), and then a subspecies of *R. chensiensis* DAVID, 1875 (LIU & HU 1961). Its status as an independent species was recently established based on the difference in morphology (XIE et al. 2000) and mitochondrial DNA sequences from other races (JIANG et al. 2002). Because of the phylogenetic similarity, this group of Palearctic anurans has well been known as “brown frog”.

There have been several studies on the natural history of *R. kukunoris*, including population age structure prior to hibernation

(LI & LI 1991), hibernation habitat use (LIU & SHI 2000), seasonal patterns of body condition (DAI et al. 2004) and post-breeding habitat selection (DAI et al. 2005). However, information regarding reproductive ecology of this species is still lacking. Whereas such information is very useful not only for understanding the evolution of life history of anurans occurring in high altitudes, but also for planning conservation actions since many amphibian populations residing in relatively pristine high-altitude habitats are experiencing a global-scale decline (POUNDS et al. 2006). Evidence showed that *R. kukunoris* populations in Zoige wetlands in west Sichuan have decreased (FELLERS et al. 2003).

STUDY AREA AND METHODS

Field work was conducted at the Gahai wetland natural reserve (34°17'N, 102°18' E) in south Gansu province, China, during April and May of 2007. The wetland belongs to the Zoige wetland system – the largest, turfy marsh in China (HE & ZHAO 1999). Topography characterized the study area as alpine lake marshes with an average altitude of 3,450 m. Vegetation covering the marshes consists of short terrestrial and aquatic grasses, with emergent plants absent. The study site has an annual average temperature of 1.5°C and a total precipitation of about 600 mm, with prevailing wind (annual average speed 2.5 m/s) over the most of the year. The study plot is located in a marsh of about 200 ha, 5 km from the natural reserve.

In our study region, *R. kukunoris* is one of the three anurans (the others are *Nanorana pleskei* GÜNTHER, 1896 and *Bufo minshanicus* STEJNEGER, 1926) native to the eastern Tibetan Plateau. The breeding habitat was an open marsh through which a stream system flows (most < 10 m in width and between 10 and 20 cm in depth). We randomly caught frogs by hand in streams or neighboring meadows during daytime throughout the spawning period. Capture was alternatively made in different streams to avoid repeated sampling. For each specimen we estimated its sex (by nuptial pads on the first finger for male, eggs readily visible through the skin of the abdomen for female), breeding status (in amplexus or not, and for female, already-laid

or not), measured body length (from snout to vent, SVL) through holding it in normal posture with calipers (to the nearest 0.1 mm) and body mass with an electronic balance (to 0.01 g). After measuring, the frogs were released at their home sites. To investigate daily and seasonal patterns of frog activity and breeding chronology, we selected a 150 meter long section of a focal stream and counted the frogs (as solitary or in pair) encountered and ambient temperature (both in air and water) hourly from 9:00 to 19:00 when walking along the stream. When analyzing, we also used climate data obtained from the natural reserve. During these field days, we also observed behavioral interactions between individual frogs.

Spawning habitats for 10 sites were described in the late breeding period with respect to number of egg clutches, hydrological features (permanent or temporary), water depth and distance to the stream bank at the center of egg mass aggregation, thickness of aquatic glass layer at the water bottom.

Clutch size of 33 newly-laid egg masses deposited in nature ponds was calculated by partially weighing and counting each egg mass. Six eggs were randomly chosen from each clutch to measure their diameters using a caliper (to the nearest 0.01 mm). In addition, thirteen gravid females were preserved as museum specimens and their ova were removed and weighed to assess reproductive investment.

RESULTS

Breeding season

On 13 April when we arrived at the study plot, several egg clutches were already present in oviposition sites; some of the clutches hatched on 22 April. Based on features of the egg masses, of known spawning time and estimated hatching period (approximately two weeks), we calculated that the frogs had initiated spawning in early April. No newly laid clutches were found during the following three days (14 to 17 April). Later, egg clutches were laid in great numbers almost every day from 18 to 27 April, but production became rare (daily deposition of egg masses less than five) from 28 April through 2 May. Therefore, we estimated that oviposition of the frogs was initiated in early April and terminated in early May, lasting 4-5 weeks.

Temporal pattern
of individual activities

Among 1,523 frogs detected along the 120 m long stream over the breeding period, 1,381 (90.7%) were unpaired and the remaining 142 (9.3%) in amplexus. Daily proportion of mating pairs among the population ranged from 0 to 16.7% (4.3 ± 6.0 , $n = 14$), varying consistently with the probability of detecting solitary individuals ($r_s = 0.84$, $p < 0.001$, Fig. 1). We had no data on frog abundance in the early breeding period. According to a general observation on the abundance of egg masses, we expected a relatively low intensity of activity during this stage. The reduced daily occurrence after 20 April might be associated with completion of reproduction of more individuals as the season progressed.

For both solitary and pairing frogs, there were high daily encounter rates on warmer days (Fig. 1). During daytime, water temperature was highly correlated with air temperature ($r_s = 0.96$, $n = 11$, $p < 0.001$), both being highest around 15:00 (Fig. 2). The numbers of frogs observed active in the stream were significantly associated with air temperature (solitary frog, $r_s = 0.94$, $p < 0.001$; amplexing frogs, $r_s = 0.78$, $p = 0.005$, Fig. 2).

Social behavior

General observations showed that strong choruses began late in the afternoon (16:00-17:00, $n = 5$ field days), reached a high level around 19:00 with dusk darkness and continued through the night. Male calls were occasionally heard in the morning.

During daytime, both male and female frogs were frequently seen floating down the streams. Males stayed at or near by the streams initiating amplexing attempts towards any frogs passing by regardless of sexes. A male released his grip at once if the frog he clasped was of the same sex. When encountering an amplexing pair, the male struggled to displace the male already fixed to the female's back ($n = 11$ instances). The male firstly clasped at the leg or wrist of the female and then got his head into the primary male's abdomen to push him off; while the primary male kicked the attacker with hind limbs. Among 138 male clasping events detected, there were two instances in which two males were clasping a single female. Of the 138 clasps recorded, the majority occurred normally, i.e. a male clasped a gravid female in an axillary posture. Males were also seen clasping other objects rather than gravid females (Table 1). The high probability of detecting pairings during daytime and nocturnal spawning behavior (see below) indicated that mating pairs might remain in amplexus for at least one night before oviposition.

Table 1: Occurrence of normal or abnormal amplexus of *Rana kukunoris* NIKOLSKII, 1918 breeding in an alpine marsh, west China.

Tab. 1: Normale und abnorme Verpaarungen bei *Rana kukunoris* NIKOLSKII, 1918 in einem Gebirgs-sumpf in Westchina.

	n	%
Multiple clasp / Knäuelbildung	2	1.4
A male clasping / Ein Männchen klammert		
gravid female / laichreifes Weibchen	122	88.4
dead conspecific female / totes Weibchen	4	2.9
conspecific male / artgleiches Männchen	2	1.4
<i>Bufo minshanicus</i> male / Krötenmännchen	3	2.2
<i>Triplophysa</i> fish / Fisch	5	3.6
Σ	138	100

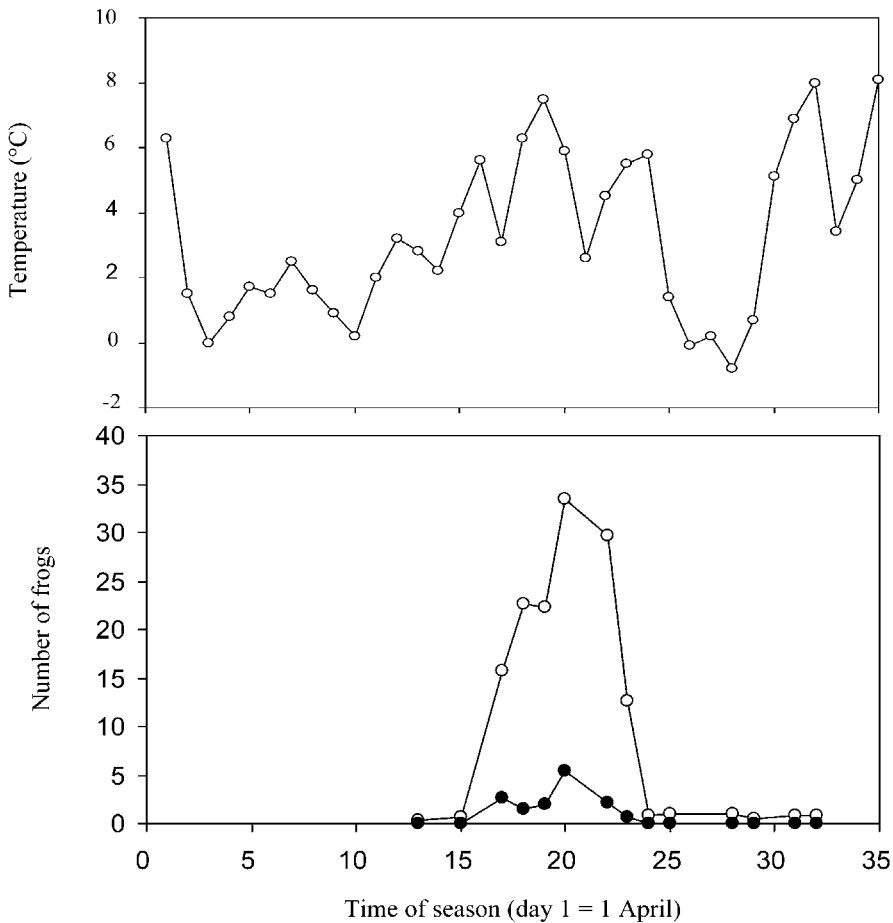


Fig. 1: Seasonal pattern of daily average air temperature ($^{\circ}\text{C}$) and daytime number of adult *Rana kukunoris* NIKOLSKII, 1918 along a 120 m long stream transect during daytime throughout the breeding season (day 1 = 1 April, day 35 = 5 May) in an alpine marsh, west China. Temperature data were obtained from the weather station 5 km from the study site. Open circle - solitary individual, closed circle - amplexing pair.

Abb. 1: Jahreszeitliches Muster der mittleren Tagestemperaturen ($^{\circ}\text{C}$) und tägliche Zahlen adulter *Rana kukunoris* NIKOLSKII, 1918 entlang eines 120 m langen Bach-Transsektes während der Laichzeit (Tag 1 = 1. April, Tag 35 = 5. Mai) in einem montanen Feuchtbioptop Westchinas. Temperaturdaten stammen von einer 5 km vom Untersuchungsstandort entfernten Wetterstation. Leerer Kreis - einzelnes Individuum, voller Kreis - Pärchen.

Body size and sex selection

Adult males were significantly smaller in SVL than females for all samples examined in this study (male, 47.8 ± 3.5 mm, $37.8\text{--}58.4$, $n = 228$; female, 54.3 ± 4.4 mm, $43.5\text{--}65.6$, $n = 244$; $t = 17.86$, $p < 0.001$, Fig. 3). The female-biased size dimorphism was also observed in the members of mating pairs (male, 48.6 ± 3.3 ,

$39.5\text{--}58.0$, $n = 122$; female, 54.5 ± 4.2 , $45.9\text{--}64.7$; paired-sample t -test, $t = 13.78$, $p < 0.001$, Fig. 3). The same case was true for body mass (male, 9.7 ± 2.4 g, $4.1\text{--}21.6$, $n = 98$; female, 16.6 ± 4.5 g, $9.8\text{--}33.0$, $n = 87$; $t = 13.16$, $p < 0.001$). In fact, there was a positive correlation between body length and body mass either for male ($r = 0.70$, $p < 0.001$) or for female ($r = 0.81$, $p < 0.001$).

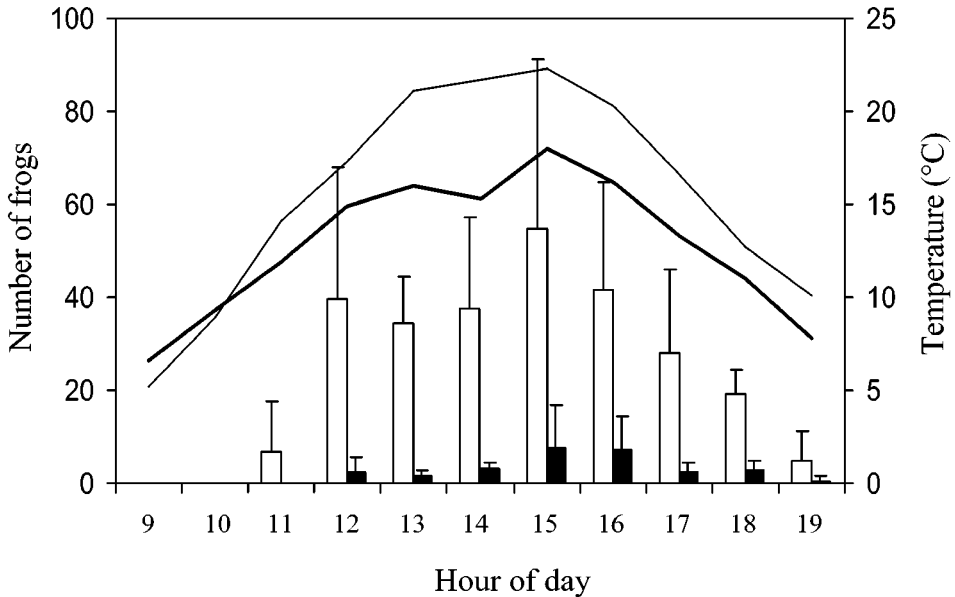


Fig. 2: Diurnal pattern of ambient temperatures and number of adult *Rana kukunoris* NIKOLSKII, 1918 (counts and standard deviation) along a 120 m long stream transect during daytime throughout the breeding season in an alpine marsh, west China. Thick line - air temperature, thin line - water temperature, open bar - solitary individuals, closed bar - amplexing pairs.

Abb. 2: Die Umgebungstemperaturen und die Anzahl adulter *Rana kukunoris* NIKOLSKII, 1918 (Zählwerte und Standardabweichung) im Tagesverlauf entlang eines 120 m langen Bach-Transsektes während der Laichzeit in einem montanen Feuchtbiotop Westchinas. Dicke Linie - Lufttemperatur, dünne Linie - Wassertemperatur, weiße Säule - einzelne Individuen, schwarze Säule - Pärchen.

There was a significantly positive relationship between SVLs of amplexing males and females ($r = 0.22$, $p = 0.014$, $n = 122$, not including those where male scramble competitions were occurring, Fig. 4). Mating males were significantly larger than non-mating males (47.0 ± 3.5 mm, 37.8 - 58.4 , $n = 105$; $t = 3.60$, $df = 225$, $p < 0.001$), but females were statistically similar in size to those found not in amplexus (54.3 ± 4.6 mm, 43.5 - 65.6 , $n = 106$; $t = 0.32$, $df = 226$, $p = 0.75$).

Oviposition site

A female released her entire clutch in a single spawning event with the male that clasped her. Oviposition took place in the night except one case which was at 11:50. All adult females participated in reproduction, since they all were found gravid before, and empty after, spawning period. Fresh spawnings were spherical, compact, and

detritus-free capsules, distinguishable from those laid earlier (more than five hours), which swelled to some times their initial volume.

Females tended to lay communally, with the aggregation number of the egg clutches varying between 10 and 70 (30.3 ± 23.5 , $n = 10$). The oviposition sites were situated in the sections where water flowed slowly and relatively shallow (water depth at egg mass aggregation 6.7 ± 1.3 cm, 5 - 9 , $n = 10$) and close to the edge of streams (57.0 ± 34.6 cm, 20 - 120 , Fig. 5). Water depth at which a clutch was present ranged from 5 to 27 cm (9.5 ± 7.2 , $n = 15$). Egg masses usually were located in the bottom of streams and attached to the layer of aquatic grasses that had a thickness of 2 - 23 cm (6.9 ± 7.1). The water where egg masses were deposited was covered by ice (0.5 - 5 cm in thickness) every morning throughout the spawning period.

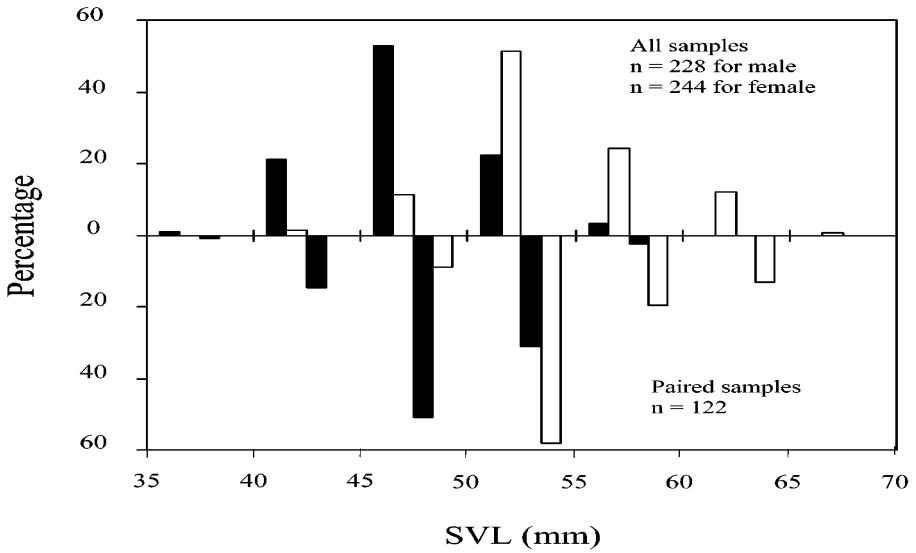


Fig. 3: Frequency distribution (%) of snout-vent length (SVL, mm) of adult male (closed bar) and female (open bar) *Rana kukunoris* NIKOLSKII, 1918 found at breeding habitats in an alpine marsh, west China. All samples (above), paired samples (below).

Abb. 3: Häufigkeitsverteilung (%) der Kopf-Rumpf-Längen (SVL, mm) bei adulten Männchen (schwarze Balken) und Weibchen (weiße Balken) von *Rana kukunoris* NIKOLSKII, 1918 an Laichplätzen in einem montanen Feuchtbiotop Westchinas. Betrachtet werden sowohl die Individuen einzeln (oben) als auch als Paar (unten).

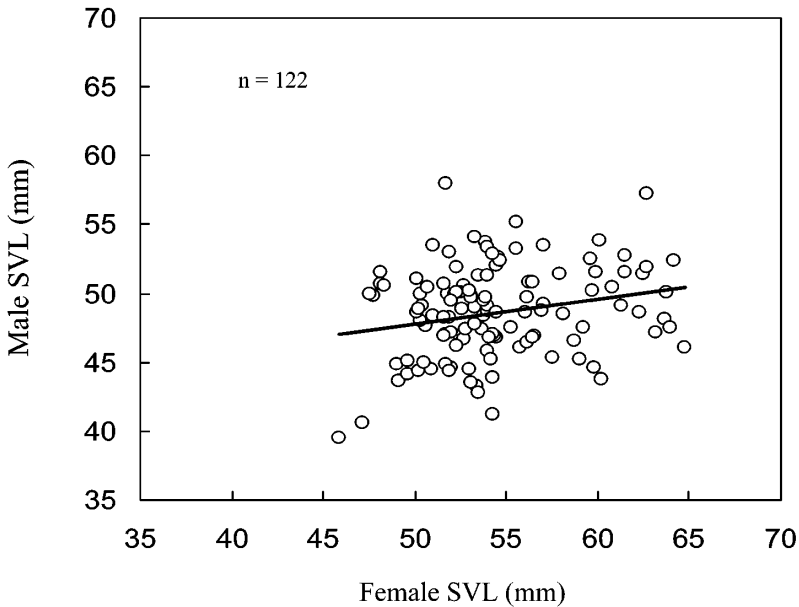


Fig. 4: Plots of snout-vent lengths (mm) of amplexing male and female *Rana kukunoris* NIKOLSKII, 1918 breeding in an alpine marsh, west China.

Abb. 4: Streudiagramm der Kopf-Rumpf-Längen (mm) verpaarter *Rana kukunoris* NIKOLSKII, 1918 (x-Achse: Weibchen, y-Achse: Männchen) an Laichplätzen in einem montanen Feuchtbiotop Westchinas.

Table 2: A comparison of life history parameters between *Rana kukunoris* NIKOLSKII, 1918 and phylogenetically close species. 1 - This study; 2 - XIN LU unpubl. data; 3 - LU (2004); 4 - MA (1982); 5 - KOSKELA & PASANEN (1975); 6 - CUMMINS (1986); 7 - SMITH (1950).

Tab. 2: Kenngrößen der Fortpflanzung bei *Rana kukunoris* NIKOLSKII, 1918 und nahe verwandten Arten. A - Mittlerer Prozentanteil der Eimasse an der Körpermasse des Weibchens; B - Mittlere Fekundität (Anzahl der Eier) je mm Kopf-Rumpf-Länge des Weibchens; C - Mittleres Verhältnis von Ei-Durchmesser zu Kopf-Rumpf-Länge des Weibchens. 1 - diese Untersuchung; 2 - XIN LU unpubl. Daten; 3 - LU (2004); 4 - MA (1982); 5 - KOSKELA & PASANEN (1975); 6 - CUMMINS (1986); 7 - SMITH (1950).

Species	Location	% ovarian mass (g) per unit female	Fecundity per unit female	Egg diameter (mm) per unit female	Source
Art	Fundort	mass (g) A	SVL (mm) B	SVL (mm) C	Quelle
<i>R. kukunoris</i>	W China, 3450 m	28.2	11.5	0.04	1
<i>R. chensinensis</i>	N China, 40° N, 1400-2000 m	-	13.1	0.03	2
	N China, 37° N, 760 m	24.0	16.3	0.04	3
<i>R. dybowskii</i>	NE China, 43° N, 800 m	16.3	23.8	0.03	4
<i>R. temporaria</i>	N Finland, 64° N, 100 m	10.9	17.6	0.03	5
	S England, 52°-53° N, < 100 m	16.0	16.6	0.03	6, 7

Clutch parameters

The number of eggs per clutch ranged from 315 to 1577 (628 ± 277 , $n = 33$) and average egg diameters of different clutches from 1.95 to 2.62 mm (2.24 ± 0.19 , $n = 198$, 6 individual eggs in each clutch were measured). There was a positive relationship be-

tween egg number and size within a clutch ($r_s = 0.42$, $n = 33$, $P = 0.02$). Clutch mass accounted for 28.2% (± 3.1 , 22.4-32.2, $n = 13$) of female mass. Longer females tended to lay heavier clutches ($r_s = 0.73$, $p = 0.004$), but clutch masses per unit body length were independent of their body size ($r_s = 0.24$, $p = 0.43$).

DISCUSSION

Rana kukunoris had an annual spawning period of 4-5 weeks, and according to WELLS (1977), is neither a typical explosive breeder nor a typical prolonged breeder. The highland frogs began laying in early April before the first rainfall came, suggesting that the species' oviposition phenology is solely determined by ambient temperature, as observed in its close relative *R. chensinensis* (XIN LU, unpubl. data) and other high-altitude or -latitude taxa (DUELLMAN & TRUEB 1986; BEEBEE 1995; READING 1998; BLAUSTEIN et al. 2001). Egg-laying was completed within a single ovulating event and one clutch was laid per season, models similar to those performed by other brown frogs such as European *R. temporaria* (e.g. BEATTIE 1985, 1987; RYSER 1989), northeastern China *R. dybowskii* GÜNTHER, 1876 (MA 1982) and northern China *R. chensinensis* (LU 1994), but typically different from that by sympatric *Nanorana pleskei* GÜNTHER, 1896 (observation during this

study) and *N. parkeri* (STEJNEGER, 1927) in southern Tibet (3000-4700 m, our unpubl. data), which disperse aquatic eggs singly or in small group (2-20 eggs) over a relatively prolonged period of time (several days). If the later ovipositing strategy, which may increase surface exposure of embryos (WARKENTIN et al. 2005), is an adaptation to low availability of oxygen in the Plateau, the mechanism by which egg clutches of *R. kukunoris* survive the oxygen-poor developmental conditions remains to be explored.

In the night, like most anuran taxa (DUELLMAN & TRUEB 1986), male *R. kukunoris* chorused strongly and both sexes were involved in mating activities more frequently. However, they also remained active (moving, chorusing and mating) throughout daytime, with a high detectability in early afternoon when ambient temperature was high (Fig. 2). It was likely that the high temperature excited more individuals to leave their hibernation sites. The asynchronous emer-



Fig. 5: Breeding habitats (above) and oviposition sites (below) of *Rana kukunoris* NIKOLSKII, 1918 in an alpine marsh, west China.

Abb. 5: Fortpflanzungsbiotop (oben) und Laichplatz (unten) von *Rana kukunoris* NIKOLSKII, 1918 in einem montanen Feuchtbiotop Westchinas.

gence of breeding individuals may be associated with the relatively long duration of the spawning period. In contrast, typically explosive breeding species congregate in breeding ponds to mate and spawn within one week (WELLS 1977).

We found that *R. kukunoris* preferred to communally oviposit along the edges of streams, and in a suitable regime of water depth with a grass layer at the bottom. This selectivity can be related with potential thermal benefits for development of embryos since cold weathers in the study area frequently returned after eggs had been laid, leading to water surface of oviposition sites being frozen again. Embryos may receive sustained heat supply both from permanent stream water, and from sunlight through being deposited in relative shallow water. Through attaching to aquatic plants at the water bottom, as do some amphibians such as *Ambystoma jeffersonianum* (GREEN, 1827) and *A. maculatum* (SHAW, 1802) (FRISBIE et al. 2000), the embryos were also protected from damage by freezing, prevailing wind and ultraviolet radiation (KIESECKER et al. 2001). Moreover, developing individual embryos could gain thermally from the aggregation, an adaptive strategy adopted by many anurans breeding in cold climates (e.g. WALDMAN 1983; CALDWELL 1986; HÅKANSSON & LOMAN 2004).

We detected a significant but low positive size collection between mating male and female ($r = 0.22$, Fig. 4), suggesting that about 5% of variation in size of amplexing members may be explained by the assortative mating pattern. Sex selection in which either sex or both prefer bigger mates and large individuals win intra-sexual competition has been demonstrated to be the underlying mechanisms (e.g. HÖGLUND 1989; TELFORD & SICKLE 1989; SULLIVAN et al. 1995). Among the frogs detected in the field, there were 105 unpaired males and 106 gravid females. However, we were unable to determine sex ratio of the breeding population relying on these figures since our capture could be greatly affected by behaviors of different sexes. For a population of adult *R. kukunoris* studied in autumn in northern Qinghai, LI & LI (1991) reported an excess of males (64 vs. 33). In our population, the observations of excep-

tional male clasping and male-male takeover attempts implied a male biased sex ratio and presence of male-male competition. Being fecund, large females should be more favorable. However, other mechanisms such as length of breeding seasons (SULLIVAN et al. 1995) rather than sex selection should be responsible for the dominant component of random mating observed in this species. Further quantitative assessments on factors determining the intensity of sex selection are needed.

Reduced investments in egg production with increasing latitudes have been observed in temperate anurans, assuming as a result of restrictions of short seasonal time and harsh climates experienced by northern animals (review in MIAUD et al. 1999; LU 2004). Because of similar environmental constraints encountered by high-altitude animals, their life-history strategies are expected to mimic high-latitude ones (MIAUD et al. 1999). It is the case in Polish *R. temporaria* populations (KOZŁOWSKA 1971). However, inconsistency to the predication has been observed in several species: *R. temporaria*, ELMBERG (1991); *Hyla labialis* PETERS, 1863, LÜDDECKE (2002); *R. chensinensis* XIN LU (unpubl. data). It was true for our species when compared with its lowland congeners (Table 2). Female *R. kukunoris* need three years to attain reproductive maturation (LI & LI 1991), and it is one or two years for *R. chensinensis* (LU et al. 2006) and for *R. temporaria* breeding in lowland (RYSER 1988). Delayed sexual maturity for high-altitude *R. kukunoris* could allow them to grow larger and thus invest reproductively more. Compared to its lowland relatives, female *R. kukunoris* laid relatively small clutches but large eggs, following the general pattern performed by most high-latitude or -altitude anurans (review in MORRISON & HERO 2003). The K-selection strategy should be adaptive to the colder, unpredictable high-altitude climates, because of the potential advantage of large eggs (e.g. KAPLAN & KING 1997; LAUGEN et al. 2003; ALTWEGG & REYER 2003). In the population studied, we failed to detect a significant relationship between the number and size of eggs within a clutch. This result is consistent with that observed in several anuran species (TEJADO 1992; CASTELLANO et al.

2004; DZIMINSKI & ALFORD 2005) including *R. chensinensis* (XIN LU unpubl. data), but different from some others (CUMMINS 1986; BERVEN 1988; LÜDDECKE 2002).

Our field observations showed that *R. kukunoris* at our study site still are at a good status. We detected 13 dead individuals (11 females and 2 males) over the study period. The post-spawning mortality, as has commonly been seen in several *R. chensinensis*

populations (XIN LU unpubl. data), is not an indicator of population decline. Observed decline of *R. kukunoris* population in Zoige wetlands is mainly a result of overall habitat loss (FELLERS et al. 2003). As argued by several authors e. g. BEEBEE (1995), BLAUSTEIN et al. (2001), POUNDS et al. (2006), however, it is necessary to monitor high-altitude anurans since they could be more sensitive to climate change.

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Corresponding editor: Heinz Grillitsch

AUTHORS: Xin LU, Xianhai ZENG, Bo DU, Chuan NIE, Department of Zoology, College of Life Sciences, Wuhan University, Wuhan 430072, China < luxinwh@163.com >