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Film C 2219

Arboreal oviposition in the neotropical treefrogs Hyla brevifrons and Hyla sarayacuensis (Anura: Hylidae)

W. HÖDL

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Arboreal oviposition in the neotropical treefrogs Hyla brevifrons and Hyla sarayacuensis (Anura: Hylidae)

Walter HÖDL, Institute of Zoology, University of Vienna, Austria

Contents of the film
Habitat, advertisement calls, amplexus, and oviposition in the treefrogs *Hyla brevifrons* DUELLMAN & CRUMP 1974 and *Hyla sarayacuensis* SHREVE 1935. Sequences taken in the field at the "Panguana" study site (9°37'S, 74°56'W, 260 m; lowland Amazonia, Peru) during the onset of the rainy season (November/December).

Filminhalt
Eiablage der neotropischen Baumfrösche *Hyla brevifrons* und *Hyla sarayacuensis* (Anura: Hylidae). - Lebensraum, Anzeigerufe, Amplexus und Eiablage der Baumfroscharten *Hyla brevifrons* DUELLMAN & CRUMP 1974 und *Hyla sarayacuensis* SHREVE 1935. Freilandaufnahmen, aufgezeichnet im Studiengebiet "Panguana" (9°37'S, 74°56'W, 260 m; Amazonastiefland, Peru) zu Beginn der Regenzeit (November/Dezember).

Contenido de la pelicula
Desove de las ranas neotropicales *Hyla brevifrons* y *Hyla sarayacuensis* (Anura: Hylidae). - Habitat, cantos de cortejo, amplexo y desove de las ranas arbóreas *Hyla brevifrons* DUELLMAN & CRUMP 1974 y *Hyla sarayacuensis* SHREVE 1935; hecho en el campo en la localidad "Panguana" (9°37'S, 74°56'W, 260 m; selva amazonica, Perú) durante de la estación de lluvias (noviembre/diciembre).

Mediographic data for film C 2219 of ÖWF
C 2219 Arboreal oviposition in the neotropical treefrogs *Hyla brevifrons* and *Hyla sarayacuensis* (Anura: Hylidae).
16 mm film, optical sound, colour, 12 minutes, English commentary.
This film is for use in research and university teaching.
Institute: Institute of Zoology, University of Vienna.
Scientific author: Walter Hödl.
Produced by Austrian Federal Institute of Scientific Film, Vienna.
A film by W. Hödl and E. Pavlousek. Taken 1988, published 1990. Camera: E. Pavlousek; sound recording: A. Valenzuela Bossmayer; editing: S. Thomas.

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Introduction

Selective pressures on eggs and early larval stages - such as predation and the habitat unpredictability of aquatic environments - have led to the attainment of partial or total independence from water bodies in many frog species inhabiting the humid tropics (McDIARMID 1978, DUELLMAN 1989). CRUMP (1974) considered aquatic sites at the Amazonian study area Santa Cecilia (Ecuador) a potentially limiting resource and suggested "that reproductive diversity enables the coexistence of many frog species through partitioning of breeding sites".

Forty-seven (36.2%) of 130 Amazonian frog species analysed undergo embryonic development out of water and larval development in water (HÖDL 1990). Eighteen of these semiterrestrially reproducing Amazonian anuran species oviposit on vegetation above water bodies. Egg clutches of glass frogs (Centrolenidae) are found on the underside of arboreal foliage above streams. With the exception of the exposed clutches of *Phyllomedusa palliata*, the unpigmented eggs of the Amazonian phyllomedusid treefrogs are deposited in leaves folded during the oviposition process. Eggs of *Hyla* ssp. with semiterrestrial development [*H. bokermanni, H. brevifrons*, and the *H. leucophyllata*-group (*H. bifurca, H. leucophyllata, H. sarayacuensis, H. triangulum*)] are laid on the upper side of leaves of emergent herbs or on leaves, trunks, and branches of bushes or trees overhanging lentic waters (DUELLMAN 1978, HÖDL 1990).

Previous field observations on *H. brevifrons* (AICHINGER 1987a) and *H. sarayacuensis* (HENZL 1987) and the present film study were undertaken in primary forest at Panguana, Río Llullapichis, (9°37'S, 74°56'W, 260 m) in the Amazonian lowland of Peru. At Panguana, *H. brevifrons* and *H. sarayacuensis* show reproductive activities (calling males and/or gravid females) throughout the six month rainy season (AICHINGER 1987b). The nocturnal calling activity (SCHLÜTER 1979, Figs. 1 and 2, Table 1) and oviposition is closely associated with rainfall (AICHINGER 1987a, HENZL 1987).

Oviposition in Hyla brevifrons

Calling males grasped approaching females in axillary embrace. Immediately after amplexus occurred, the female visited leaves overhanging water within a range of a few meters from the male's calling station. After arriving at the tip of a leaf in a head down position the male-carrying female turned around and clasped the edge of the leaf with both hindlegs. She then remained motionless in an upward position (Fig. 3 A) between 18 and 125 s (mean, 46; n = 7) before egg deposition (Fig. 3 B,C) started.

Table 1. Advertisement call characteristics of *Hyla brevifrons* (15 calls / 3 individuals analysed) and *Hyla sarayacuensis* (20/3) (Panguana, Peru, 24 °C). Ranges given below means.

	Dominant frequency [Hz]	pulse rate [s^{-1}]	pulses per call	call duration [ms]	call rate [min^{-1}]
Hyla brevifrons	4585 (4490-4654)	35.3 (32.7-39.3)	12.7 (10-16)	365 (327-411)	5.44 (4.36-5.44)
Hyla sarayacuensis	2768 (2614-2812)	62.3 (57.3-68.9)	6.3 (4-7)	85 (72-108)	4.16 (1.93-7.55)

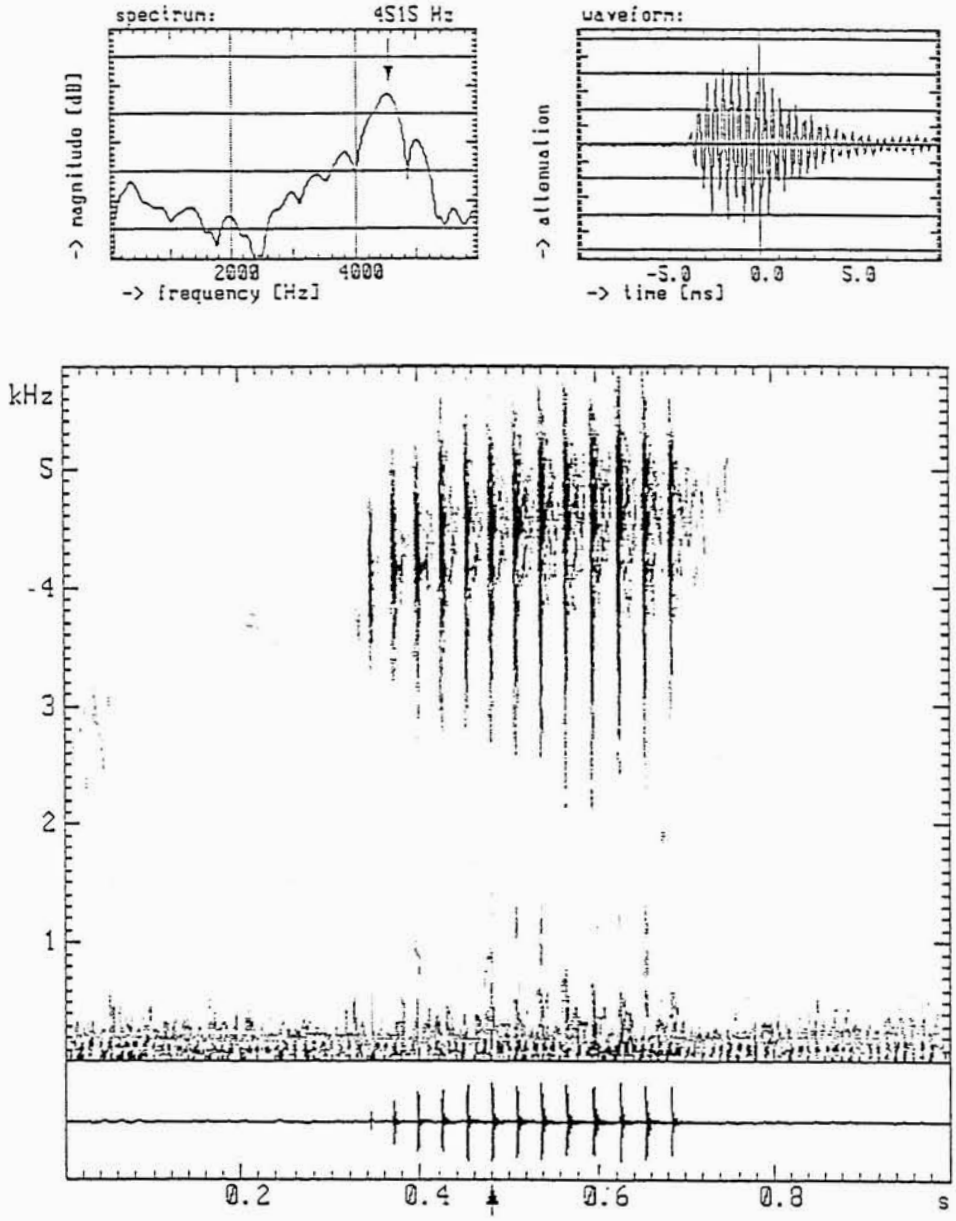

Fig. 1. Advertisement call of *Hyla brevifrons* (Panguana, Peru; 24 °C). Sonagram and oscillogram. Section (above left) and expanded time base display (above right) taken of the period between 10 ms before and 10 ms after peak intensity of arrow-marked pulse.

Oviposition was characterized by alternating phases of activity and rest. Bouts of activity were repeated at a mean interval of 14.2 s [range, 8 - 32 ; SD, 4.61; n = 57 (3 matings analysed)] and lasted 7 s (range, 3.5 - 9). Each activity sequence began with the female lifting her head and taking on a position with a slightly lordotically arched back. During this so-called signal position of the female, the amplecting male pressed his knees inwardly into the female's body and inseminated the extruded eggs (Fig.3 B). From 0 to 14 (mean, 4.37; SD, 4.26) eggs were laid during each acti-

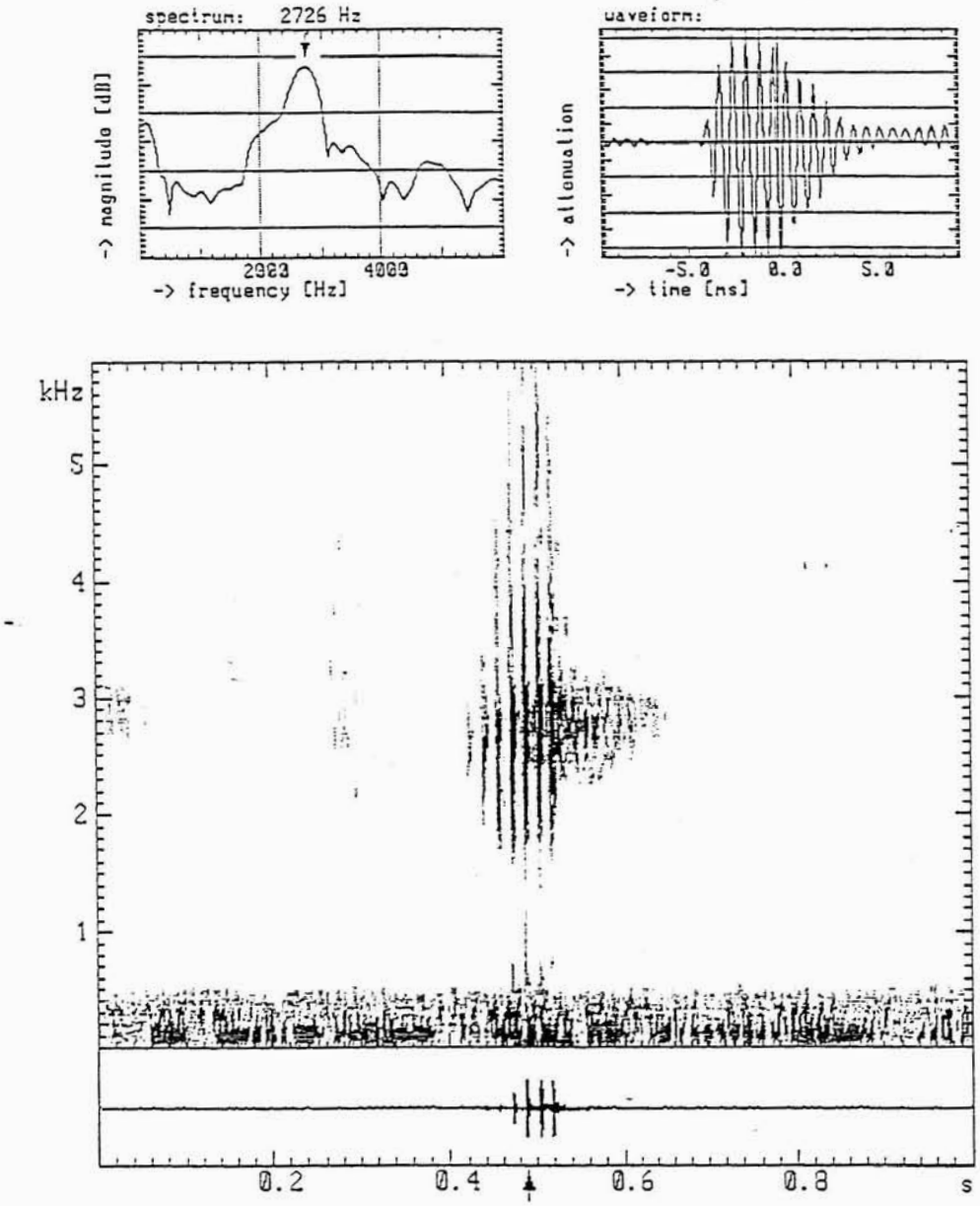

Fig 2. Advertisement call of *Hyla sarayacuensis* (Panguana, Peru; 24 °C). Section (above left) and expanded time base display (above right) taken of the period between 10 ms before and 10 ms after peak intensity of arrow-marked pulse.

vity bout (Table 2). Immediately after insemination the male slid forward and rested with his knees at the widest part of the female's body (Fig. 3 C) until the onset of the next bout. After discharge of each egg batch, the female spread the clutch out into a single layer with her hind legs (Fig. 3 D, situation after the third ovipositional bout). The actual oviposition process (beginning of first until end of last activity bout) of three matings filmed lasted 5:08, 5:10 and 4:04 min respectively. The female oviposition

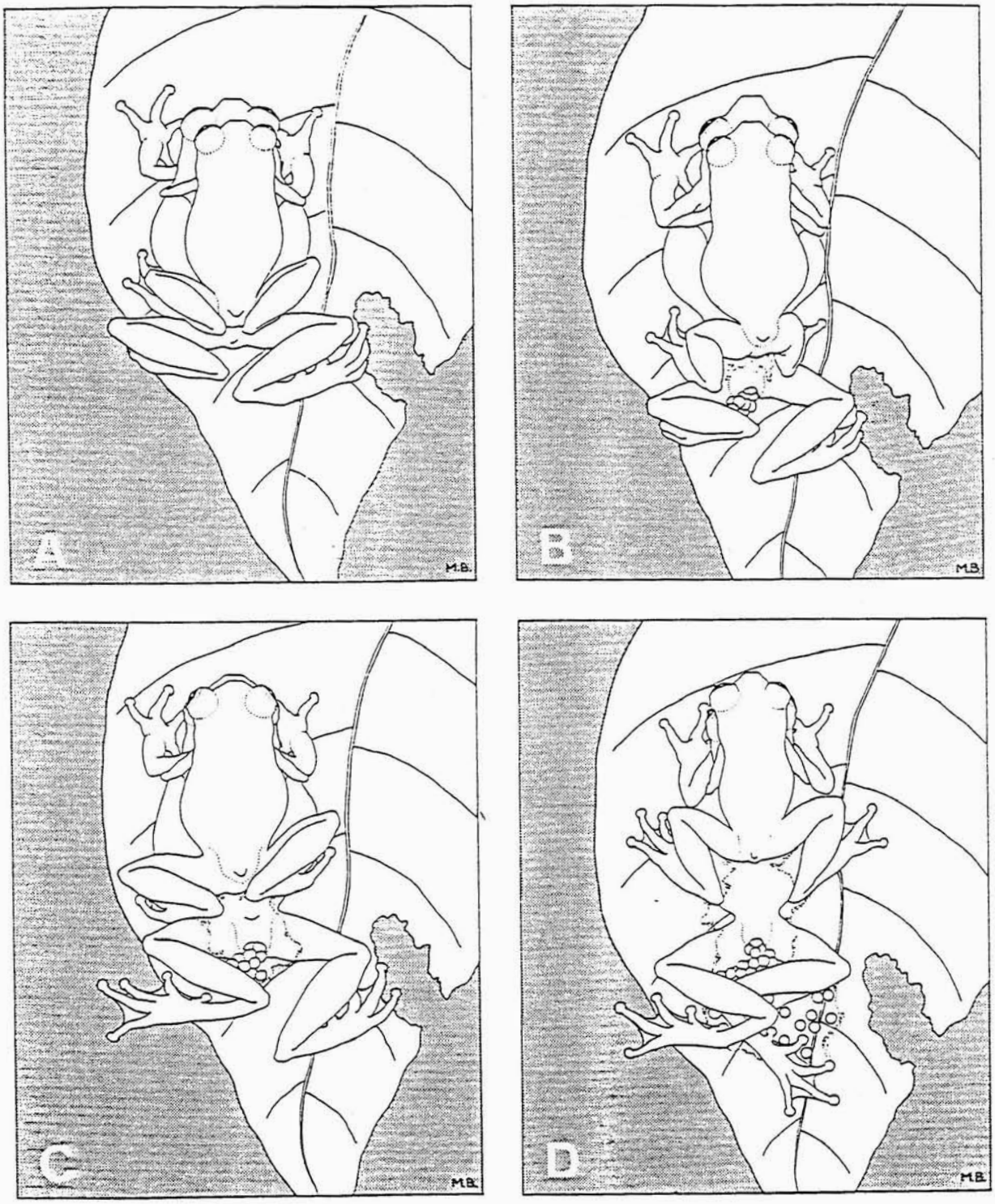

Fig. 3. Oviposition in *Hyla brevifrons*. Position prior to first egg-laying bout (A). Following the female signal position (head lifting, lordotic arching), the amplectant male inseminates the emerging eggs (B) and returns to his original position, resting with his knees at the widest part of the female's body (C). After each egg-laying bout, the female spreads the eggs out into a single layer by brushing motions of her hind legs (D).

Table 2. Film analyses of oviposition in *Hyla brevifrons* (Panguana, Peru). - Onset of ovipositional bouts (min:s; # without male) and number of emerging eggs visible (* unconcealed eggs at beginning of film). (Film sequences of pairs I and III begin 16 s and 14 s, respectively, after the onset of the first bout).

bout number	pair I		pair II		pair III	
1	0:00	- -	0:00	8	0:00	5*
2	- - -	10*	0:08	12	0:20	12
3	0:30	9	0:16	13	0:34	14
4	0:46	7	0:27	13	0:48	11
5	1:00	7	0:37	12	1:02	10
6	1:11	6	0:49	9	1:15	12
7	1:24	7	1:01	7	1:27	10
8	1:37	5	1:12	8	1:42	8
9	1:50	6	1:22	6	1:54	6
10	2:05	3	1:34	5	2:07	3
11	2:20	5	1:44	4	2:34	0
12	2:29	4	1:56	4	2:42	0
13	2:44	2	2:06	4	2:55	0
14	2:55	3	2:23	1	3:06	0
15	3:09	2	2:42	0	3:17	0
16	3:22	3	2:54	0	3:29	0
17	3:40	4	3:09	0	3:43	0
18	3:55	1	3:31	0	3:53#	0
19	4:07	0	3:52	0		
20	4:29#	0	4:14	0		
21	5:01#	0	4:32	0		
22			4:53	0		
23			5:04#	0		
eggs concealed during egg-laying process		7		3		12
total number of eggs laid		97		109		103

manoeuvre continued up to 9 bouts after the last eggs appeared and up to two bouts after the male has loosened his embrace and left (Table 2). Repeated egg spreading without preceding ovipositional bouts continued irregularly up to 40 min after the male left the female.

AICHINGER (1987a) analysed 120 oviposition sites of *H. brevifrons*. 85.8% of the clutches were on the upper side of green leaves, 13 on thin branches, three on trunks and one on a dead leaf. All sites were above water. Eggs were regularly eaten by carabid beetles (*Colliuris sp.*) (AICHINGER 1987a, pers. obs.) and conocephalid grasshoppers (pers. obs.). Five to seven days after oviposition the surviving tadpoles drop into the water below. Artificially covered clutches (i.e. unexposed to rainfall) showed a mortality rate of 88 %. Under natural conditions 49 % of the deposited eggs survived (AICHINGER 1987a).

Oviposition in Hyla sarayacuensis

After heavy rains, amplectant pairs of *H. sarayacuensis* approached moss-covered roots and tree trunks close to or above water, where oviposition (Fig. 4) occurred during early morning hours. Seventeen egg-laying pairs were observed between 0150 and 0440 h. Without changing his clasping position the amplectant male inseminated the emerging eggs during each egg-laying bout. A mean number of 141 eggs (range, 110 - 192; SD 21.9; n = 10) was laid during 5 to 8 activity bouts. The male-carrying female ascended 1 to 3 cm during each bout interval without spreading the egg masses with her hind legs; the result was an elongated clutch of successively laid egg masses. The egg-laying processes lasted between 17 and 34 min (mean, 21; n = 17) (see also HENZL 1987). In contrast to *H. brevifrons*, strong illumination necessary for filming lead to interruption or unusually long inter-bout intervals even after two or three egg-laying bouts already occurred. Therefore it was not possible to continuously film a complete oviposition sequence.

Females of *H. brevifrons* (and *H. sarayacuensis*) are significantly larger than conspecific males. Female-to-male snout-vent-length ratios of amplectant pairs found in the field (present study) are similar to ratios calculated from random samples (AICHINGER 1985) (Table 3). Thus, snout-vent-length ratios do not indicate size-assortative mating in these two species.

The egg jelly of *H. sarayacuensis* swells up markedly by taking up water from the humidity-saturated air and the wet substrate. High humidity leads to liquification of the clutch, and larvae drop into the water between 10 and 13 days after oviposition (HENZL 1987).

Methods

Aerial scenes shown at the beginning and at the end of the film were taken on a flight

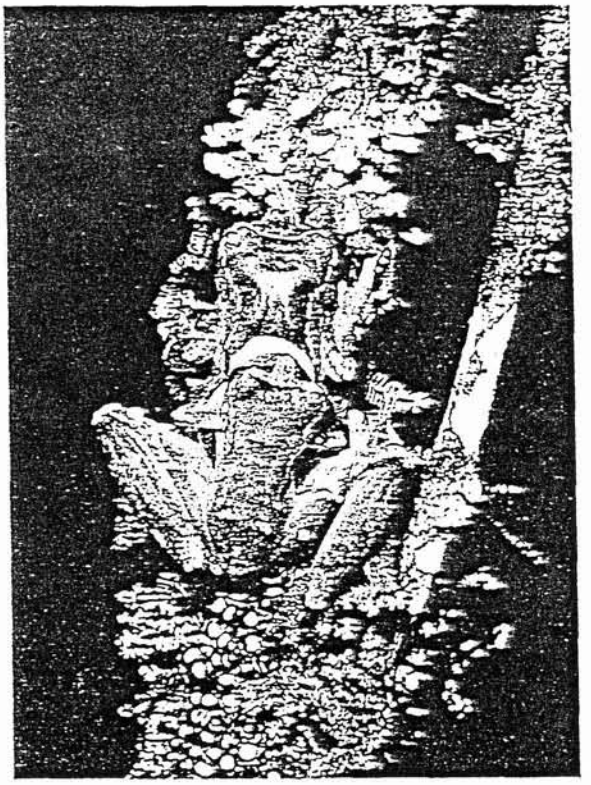

Fig. 4. Oviposition in *Hyla sarayacuensis* (Panguana, Peru).

from Puerto Inca to Llullapichis, the nearest airstrip to the Panguana study area. The reproductive behaviour of *H. brevifrons* and *H. sarayacuensis* was filmed during night hours with a sound-reduced 16 mm film camera (ARRI 16 SR). The original sound was recorded with a SONY Professional Walkman, stereo cassette recorder, WM-D6C, in connection with an AKG D 140 microphone. The frogs were filmed from 4 November to 1 December 1988 at the onset of the rainy season during humidity-saturated conditions or light rainfall at the "Estanque". This pond with up to 1000 m² water surface, is the second largest in the Panguana study area (see AICHINGER 1985, and SCHLÜTER 1984, for a detailled description of the pond site). For illumination, battery-driven 12 V / 100 W lamps and generator- (2000 W) driven 220 V / 300 W spotlights were used. The

Table 3. Reproductive parameters for *Hyla brevifrons* and *Hyla sarayacuensis* at Panguana, Peru. SVL, snout-vent length. * SVL ratio of amplecting partners.

	Hyla brevifrons		n	*Hyla sarayacuensis*		n	source
number of eggs per clutch	75.8	(52-114)	35				AICHINGER 1985
	103.6	(67-142)	16	141.1	(98-192)	12	present study
				90.8	(48-146)	13	HENZL 1987
ovarian egg number	80.3	(35-156)	15	113.1	(89-148)	10	AICHINGER 1985
clutch height above water level (cm)	77.0	(20-190)	35				AICHINGER 1985
	111.0	(40-230)	16	35.0	(10-95)	15	present study
				59.3	(47-130)	13	HENZL 1987
SVL female (mm)	22.0	(20-25)	61	34.1	(32-36.5)	16	AICHINGER 1985
	22.2	(20-24)	12	34.6	(33-36)	10	present study
SVL male (mm)	19.0	(17-21)	50	27.2	(25-30.5)	67	AICHINGER 1985
	18.7	(17-20)	12	27.1	(25-29)	10	present study
SVL ratio female:male	1.16			1.25			AICHINGER 1985
	1.18*	(1.07-1.27)	12	1.27*	(1.2-1.36)	10	present study

generator was stationed at a distance of 200 m to reduce background noise. As oviposition sites could not be predicted and in order to permit immediate filming, several tree trunks close to the shoreline were furnished with waterproofly protected plugs connected to the power source.

Film commentary

Undisturbed rainforests in western Amazonian lowlands are the habitat of the treefrogs *Hyla brevifrons* and *Hyla sarayacuensis*. At the onset of the rainy season these frogs descend from their arboreal sites in order to breed. Unlike most hylid frogs, eggs of *Hyla sarayacuensis* and *Hyla brevifrons* are deposited out of water. Clutches are found exposed on vegetation overhanging permanent or temporary forest ponds. From the arboreal oviposition site, hatching tadpoles drop into the water below, where larval development is completed.

-- During early evening hours of days with saturated humidity, males of *Hyla brevifrons*, a tiny, 2 cm sized species, vocalize in bushes and low trees. -- These advertisement calls announce the male's readiness to mate. Conspecific females attracted by the calls are clasped immediately after physical contact with the vocalizing individual. -- Then, the female selects a suitable oviposition site close to the male calling station. -- Preovipositional clasping may last between several minutes and a few hours. Mating is usually completed before midnight and never exceeds one night. -- Egg laying occurs with both partners orientated vertically, heads up, and starts at the tip of a downward bent leaf overhanging a water body. -- The oviposition process is characterized by highly synchronized movements of both partners. -- Each activity sequence starts with ventral arching of the female's back, whereby she lifts her head and contracts her abdo-

men. -- The axillary embracing male responds to this signal by sliding backwards, placing his vent closer to hers and ejaculating during the onset of the egg discharge. -- After sperm emission the male moves forward and places his knees at the widest part of the female's body. -- While the eggs emerge, the female brushes over the clutch, spreading the ova out into a single layer. She then moves slightly forward, thus enlarging her distance from the tip of the leaf with every oviposition sequence. -- Egg-laying phases with a discharge of up to 14 eggs are repeated at a mean rate of 14 seconds. -- The actual oviposition process lasts from four to five and a half minutes and involves 16 to 24 activity sequences. -- Towards the end of the oviposition process, periods of rest between each activity sequence increase, whereas egg numbers decrease . -- No eggs are laid during the female's last abdominal contractions. By then, the male loosens his embrace and leaves his partner. -- Note that the female carries out her ovipositional manoeuvre without the embracing male. -- The spontaneous ventral arching of the female clearly shows that the egg-laying sequences are not initiated by the male once the oviposition process has started. -- Still, the female performs all ovipositional movements, including subsequent brushing of the clutch, without the presence of the male. -- While the male starts to call again, the female covers the clutch with her body. Up to 40 minutes she repeatedly smoothens the clutch by pressing her body tightly against the eggs. Smoothening as well as brushing over the uncovered ova with the hind legs may improve fertilization and egg adhesion to the leaf surface. -- Lateral bright markings and egg-shaped spots on the outer flanks of the thighs, now clearly visible during the spreading of the clutch, disguise the delicate eggs and may give them the appearance of lateral appendages of the female body. -- Once the female has left the oviposition site the clutch is never visited again by either of the parents. -- The bright yellow eggs are often already taken during the first night by predators such as conocephalid grasshoppers or carabid beetles of the genus *Colliuris*. -- Embryonic development lasts between 5 and 7 days and can only be completed when the humidity is high enough to keep the eggs from drying out. -- Once the clutch swells up and liquifies due to the uptake of atmospheric moisture and rain the larvae drop into the water below.

-- For *Hyla sarayacuensis*, moss-covered roots or tree trunks represent the preferred oviposition sites. Females of this brightly coloured treefrog species with a snout-vent length of up to 3.5 cm are easily identified by their white throat, which lacks the yellowish-brown skin folds of the vocal sac present in adult males. -- Males use twigs or leaves at varying heights as calling stations. -- Attracted females carry the amplecting males to the oviposition site, usually a vertical structure close to or overhanging a water body. -- As in *Hyla brevifrons*, oviposition in *Hyla sarayacuensis* occurs during a pronounced ventral arching of the female. Without changing its clasping position the male releases sperm onto the emerging eggs. Ejaculatory movements are clearly detectable in close-ups. -- Egg laying is occasionally interrupted and portions of clutches are then deposited at different sites. -- In lateral view, the close contact of the vents of both partners is visible. -- Due to the somewhat rearwardly shifted amplexus position, the cloaca of the markedly smaller male is positioned directly above that of the female. This juxtaposition of the cloacae ensures optimal insemination. The good adherence of the eggs on the microepiphytes or well-structured bark may be the functional basis for rendering brushing and smoothing of the clutch unnecessary.

-- Arboreal oviposition - found only in environments with predictable periods of humidity saturation - protects the most vulnerable stages of *Hyla sarayacuensis* and *Hyla brevifrons* from aquatic predation. In humid tropical environments, mass breeding and predator aggregation at aquatic sites have led to partial or total independence from water bodies in many frog species. -- Eggs and embryonic development outside the water may have been the first evolutionary step towards the complete terrestriality found in several Amazonian treefrog species. -- Changes in the water balance are a great threat to the brood of rainforest amphibians: unpredictability of rainfall and low atmospheric humidity increase the problems of maintaining moisture for eggs and offspring. Thus, large-scale forest destruction and subsequent changes of microclimatic conditions highly endanger the tropical frog fauna, which has developed its greatest diversity in the western Amazon basin.

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Address of the author

Dr. Walter Hödl, Institut für Zoologie, Althanstr. 14, A-1090 Wien, Austria.