



Squeeze me, please me: the reproductive biology of the Amazonian bamboo frog *Nyctimantis rugiceps* (Anura: Hylidae), with notes on possible interspecific brood parasitism and skin secretions

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Manuscript received: 23 February 2023

Accepted: 27 June 2023 by JÖRN KÖHLER

Abstract. Reproduction of the western Amazonian tree frog *Nyctimantis rugiceps*, a species breeding in bamboo internodes and tree holes, was studied in the field and laboratory. Once a male had attracted a female, the pair attached 206–553 (mean = 357.4) eggs to the phytotelme wall at water level. Subsequently, the male left the site, while the female regularly returned to the hatched larvae after 2–9 (mean = 5.1) days and deposited 211–878 (mean = 446.6) unfertilized nutritive eggs that the larvae bit open and consumed when feeding for the first time, and subsequently swallowed wholly. Time to metamorphosis depended on the number of tadpoles present, but the numbers that hatched and reached metamorphosis were low (2–15 larvae). A possible case of egg parasitism by a *Ranitomeya variabilis* larva was observed. Skin secretions of a dying frog were probably a toxin known to be harmful even to humans. Some morphological features of *N. rugiceps* are discussed, especially with respect to breeding in phytotelmes and oophagy.

Key words. Amphibia, reproduction, parental care, oophagy, phytotelmes, egg parasitism, Dendrobatidae, *Ranitomeya variabilis* larva, skin toxin, Ecuador.

Introduction

Studying reproduction in Neotropical anurans is not excessively difficult in species that congregate in ponds or streams and are encountered on the ground or in vegetation overhanging breeding sites, even if these frogs are strictly arboreal otherwise (e.g., KLUGE 1981, MARTINS 1993, BASTOS & HADDAD 1996, BORGES et al. 2018). Observations become more challenging in species that specialize in using phytotelmes such as tree holes or bromeliads, firstly because they are typically not encountered in well-delimited areas, such as a pond or a stretch of stream, but scattered in the forest, usually with no more than one calling male or one pair present at one site; and secondly, because they are often located in the canopy, in some instances as high up as 20 m above the ground (e.g., LANNOO et al. 1987, SCHIESARI et al. 2003, PÉREZ-VILLOTA et al. 2009). It is therefore no surprise that few data, especially behavioural ones, have been gathered for those species.

Nyctimantis rugiceps BOULENGER, 1882, an Amazonian species whose males attain 67.6 mm (DUELLMAN & TRUEB 1976) and females 68.5 mm in snout–vent length (pers. obs.), is one of those frogs for which little published information has become available, even though it is peculiar not only for its calling from phytotelmes, but also for cranial

co-ossification, an unusual pupil shape (best described as diamond-shaped, Fig. 1), and irritant skin secretions (BOULENGER 1882, DUELLMAN & TRUEB 1976, CAMARGO DE SOUZA et al. 2018). Apart from inhabiting a restricted area in Amazonian Ecuador, the species is known only from two localities in Colombia and the vicinity of Iquitos, Loreto, Peru (MORAVEC et al. 2002, LYNCH 2005, PÉREZ-VILLOTA et al. 2009, IUCN SSC Amphibian Specialist Group 2018, METCALF et al. 2020). Supposing that it also occurs in the area in between, covering roughly 186,000 km², the species has been hiding most successfully from researchers.

When describing *N. rugiceps*, BOULENGER (1882) had no information available on the habitat of the species. More than 90 years later, CRUMP (1974), DUELLMAN & TRUEB (1976) and DUELLMAN (1978) were the first to associate calling *N. rugiceps*, and possibly eggs, with phytotelmes, namely open bamboo internodes and tree cavities. Subsequent authors substantiated these findings (MORAVEC et al. 2002, PÉREZ-VILLOTA et al. 2009, CAMARGO DE SOUZA et al. 2018). Here, I present some field observations on the reproduction in phytotelmes of this species. However, given the frogs' secretive nature and inaccessibility of their breeding sites, more insights into the behaviour were gathered from observing individuals in the laboratory. Several Neotropical hylid frogs whose reproduction takes place in

phytotelmes, namely five species of *Osteocephalus*, *Osteopilus ocellatus*, *Nyctimantis arapapa*, and *Tripurion spinosus*, exhibit some degree of parental care by providing their tadpoles with nutritive eggs (JUNGFER 1996, THOMPSON 1996, JUNGFER & WEYGOLDT 1999, JUNGFER et al. 2000, 2013, HAUGEN 2002, MORAVEC et al. 2009, LOURENÇO-DE-MORAES et al. 2013, MELO-SAMPAIO et al. 2021). Initial field observations suggested that *N. rugiceps* was also among those species.

Materials and methods

Fieldwork was carried out at the biological reserve of Jatun Sacha, Provincia Napo, Ecuador, ca. 01°04' S, 77°37' W, during four stays of about two weeks each, between August 1994 and December 1997. A detailed description of the reserve and its herpetofauna was provided by VIGLE (2008). The main study area was the alluvial floodplain of the Río Napo (about 400 m a.s.l.) with stands of giant bamboo (*Guadua superba*) up to 20 m tall and having diameters of up to 13 cm at the base and internodes up to 40 cm long. Both live and dead bamboo canes, the latter often lying horizontally among vegetation, had water-holding capacities that were suitable for *N. rugiceps*. Additionally, trails were followed in primary and mature secondary forests on

rolling hills, about 450 m a.s.l., in search of suitable tree cavities.

Male frogs were usually encountered at night by following their calls. Tadpoles were sought by inspecting possible breeding sites (open bamboo internodes) during the day by means of a torch or by widening an aperture in an internode. Females were encountered by night-long vigils (19:00–7:00 h) for several consecutive nights near bamboo internodes after tadpoles had been discovered there. A small twig or straw, which could easily be removed by a frog, was squeezed into the aperture of the internode. In this manner, it was possible to inspect the entrance without having to approach it closer than a few metres in order to not deter a frog possibly moving towards the breeding site. Sites higher than about breast height were inspected using ladders and in few instances bamboo stalks were cut. Measurements were taken in the late morning or late afternoon. Water oxygen concentrations were measured with an oxygen meter (Greisinger Oxymeter), acidity with a pocket pH meter pen (Etercycle), and temperature with a digital thermometer with an external sensor (Greisinger GTH 175).

In order to substantiate reproductive behaviour and parental care, two adults (one female, one male) and two tadpoles were collected. These four individuals, together with seven of their offspring hatched in the laboratory (three females and eight males altogether), were used in this study. They were housed in different terraria of 50 × 50 × 60 (wide × deep × high) to 80 × 60 × 80 cm that were outfitted with plants, twigs, and hideouts made of baked cork bark boards. Up to three small glass aquaria per terrarium, about 20 × 15 × 18 cm, lined with dark brown cork bark boards on three sides and at the bottom, served as artificial tree cavities and held about 3.0–3.5 l of water. Most of the time, one male and one female were kept together, while additional males were kept singly for most of the time. For experiments, individuals were placed together in different combinations. Frogs were studied by direct observation. Whether tadpoles had received nutritive eggs during the night was often found out the following day when their guts were filled with yolk or eggs. By assessing the state of digestion of eggs, it was also possible to date the previous two or three nights as feeding times. Tadpoles were staged according to GOSNER (1960). The reproductive period (i.e., the time from deposition of fertilized eggs to metamorphosis of juveniles) was regarded as being completed when the froglets left the water with a tail bud (Stage 46). Total length of larvae is abbreviated TL. Apart from their calling activity, males in breeding condition were identified by the presence of grey nuptial pads on the first finger. Snout-vent length of frogs is abbreviated SVL.

Results

Calling and breeding sites

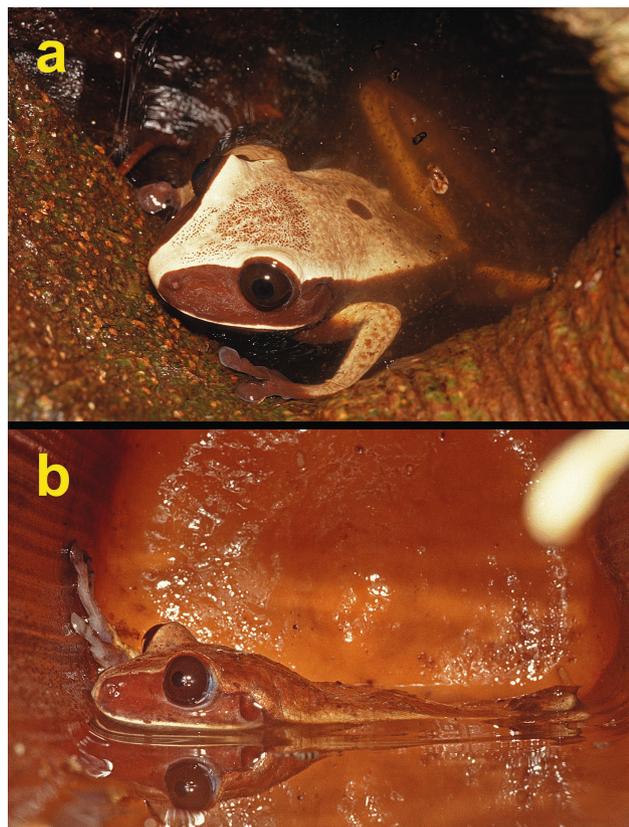


Figure 1. *Nyctimantis rugiceps* males occupying (a) a tree cavity, (b) a vertical bamboo internode, as calling sites.

Male *N. rugiceps* have a dark brown, moderately distensible subgular vocal sac. One male was found calling from a

Table 1. Measurements of phytotelmata with *N. rugiceps* present. Height – Height above ground; Aperture – aperture measurements and type; Length – internode length; Ø – internode outer/inner Ø; Level – lower level of aperture opening in internodes; Depth – water depth; Volume – water volume; Temp – Temperature; Time – time oxygen measured; Oxygen – dissolved oxygen; Individuals – individuals present (m – calling male, f – female, l – larvae).

Phytotelm type	Height (cm)	Aperture (mm)	Length (cm)	Ø (mm)	Level (cm)	Depth (mm)	Volume (ml)	Temp (°C)	Time (h:min)	Oxygen (ml/l)	pH	Individuals
1. Tree hole	120	80 Ø horizontal, round				30 (+80 soft organic residue)	150	23.8	10:30	2.8	6.2	m
2. Vertical bamboo stump (dead), top of internode open	497	80 Ø horizontal, round	32.5	103/80	32.5	120	603	28.6	16:00	2.6		m
3. Vertical bamboo internode (dead)	220	105×12 vertical slit	30.5	88/70	17.5	40	154	24.7	10:00	1.9	5.8	m
4. Vertical bamboo internode (alive)	ca. 700	vertical slit										m
5. Vertical bamboo internode (alive)	163	93×64 vertical, cordiform, animal-made	28	100/75	13	33	146	26.0	17:30	1.1		2 l, f
6. Horizontal bamboo (dead)	141	33×14 horizontal slit	34.5	92/70	7.5	30	540	24.6	18:00	1.2		15+1 l, f
7. Almost vertical, slightly inclined bamboo (alive)	1106	110×16 vertical slit	33	80/-	24							m
8. Horizontal piece of bamboo (dead)	160	63×14 horizontal slit	33	90/68	4	30	510					m
9. Vertical bamboo internode (alive)	874	110×15 vertical slit	34	110/85	20							m

cavity in a small tree 1.2 m above the ground (Fig. 1a). The hole had a round aperture about 8 cm wide and held water about 3 cm deep with an 8 cm layer of decaying organic matter on the bottom. The oxygen concentration of the water was low (2.8 ml/l). For more measurements of the phytotelmes see Table 1.

Six males were located calling from internodes of the bamboo *Guadua superba* at heights between 1.6 and 11.06 m (Fig. 1b). Many of the latter had openings, e.g., when a cane had cracked open at the top of an internode. Others had slits or rounded apertures that looked as though they had been pecked or gnawed open by animals (Fig. 2).

More males were heard calling from bamboo (as was confirmed by putting the ear to the stem) at heights between 8 and 14 m, but since I assumed that these were without females or tadpoles, no attempts were made to exactly locate them. On two different occasions outside the study period and site, one male each was heard calling from a tree in excess of 20 m in Reserva Nacional Allpahuayo Mishana, Loreto, Peru, in 2012 and 2018.

Calling sites occupied at night and checked during the day did not contain any frogs during the day (n = 4 sites; 15 observations). Likewise, terrarium-kept males never stayed at their calling sites during the day.

Six of the seven observed males and one not seen that had selected a tadpole-holding breeding site exhibited a

preference for very narrow slit-like apertures 12–16 mm wide in bamboo. Six out of eight preserved adults had maximum heights of 17.2–18.6 mm at midbody or the posterior end of the occiput. Therefore, the frogs really must have had a tight squeeze to enter the cavities through those slits.

Two internodes holding tadpoles presumed to be *N. rugiceps* were discovered during the day on 25 and 26 December 1996 (Table 1). The first one, in a live bamboo stalk with an aperture 163 cm above ground, harboured two tadpoles of different sizes (40 and 48 mm TL) and stages (34 and 37). The gut of the larger one was filled with undigested eggs visible through the abdominal skin. No eggs were seen in the other individual. In order to check the tadpoles, the aperture was widened. After returning the larvae, a piece of bamboo was used to partially reseal the aperture (Figs 2a, b).

The second site was a dead vertical bamboo 141 cm above ground (Fig. 2d). In one of the internodes there was a narrow slit of 33 × 14 mm. A “lid” was sawed out next to it to allow inspections. It held 15 larvae of different sizes and stages (about 27–37) and one *Ranitomeya variabilis* larva at Stage 40 (see Discussion below). We left the larvae in situ. The oxygen concentrations of the water were very low (1.1 and 1.2 ml/l), but the water was clear. Some other sites in which only males were present were found to contain a murky viscous liquid instead (Fig. 3) and may have been contain-

ing even less oxygen. By comparison, some authors (cited in DIAZ & BREITBURG 2009) consider oxygen levels of 5–6 ml/l a critical threshold for the survival of freshwater fish.

Adult presence at breeding sites with larvae

During the following nights no males returned to breeding sites containing tadpoles, but on the third night after discovery of each site a female frog entered the internode. The first female arrived between 19:40 and 19:55 h, while the second one entered the cavity between 20:05 and 20:20 h. The female and larvae of Site 1 were collected. The adult laid eggs while in a plastic bag; these were given to the tadpoles and they ingested them wholly within less than five

minutes. At Site 2, the female remained with the tadpoles for about 40 minutes and then left. At least some of the tadpoles appeared fuller than before, and no eggs remained in the water. Only the *R. variabilis* tadpole was collected the next morning and kept for a few days until the dorsal colour pattern appeared and allowed identification. No further observations were made at Site 2 because our field period terminated.

Lab observations on reproductive behaviour

Within six years of captive keeping, three females laid eggs with five different males. Eleven reproductive periods could be observed at least in part.

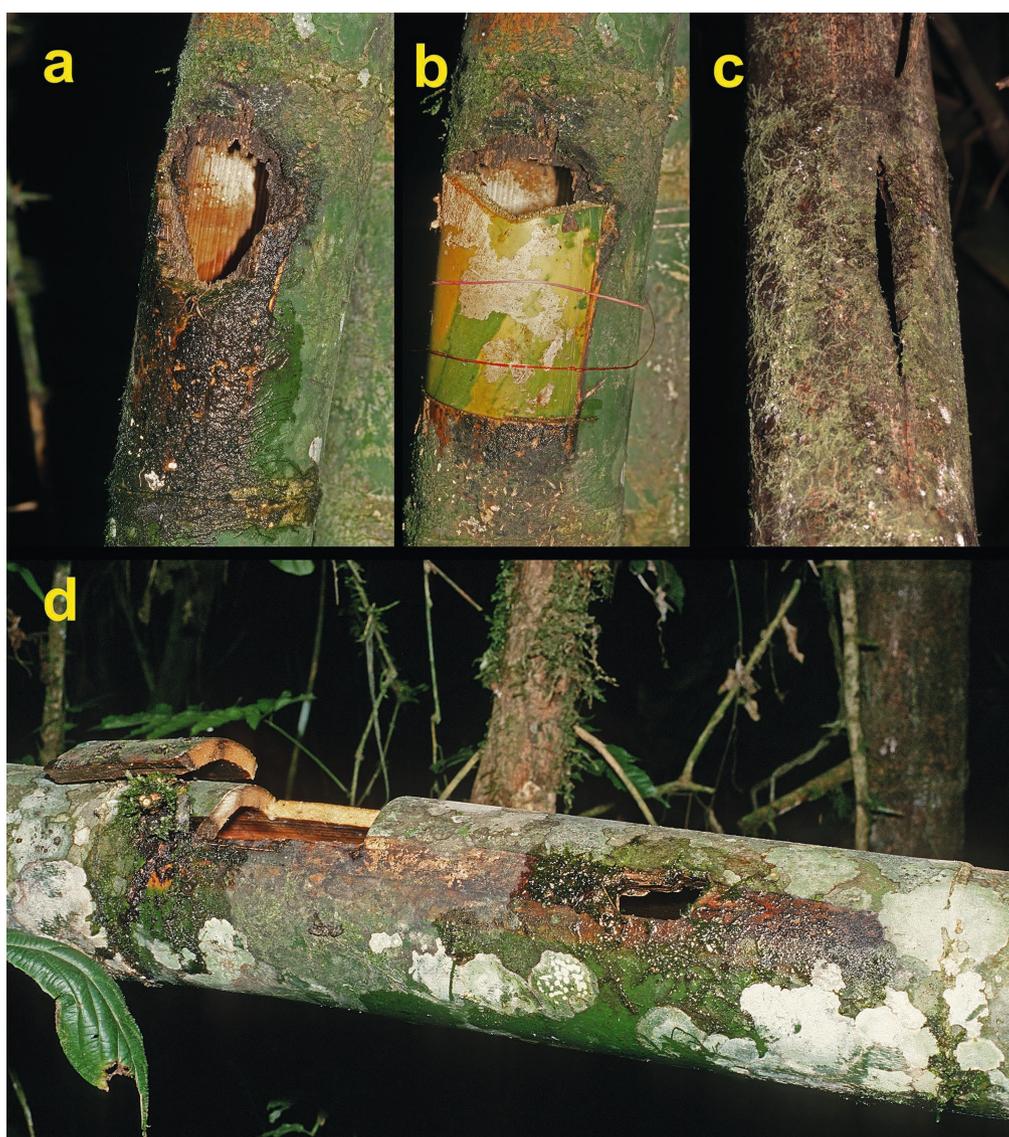


Figure 2. Breeding and calling sites in giant bamboo (*Guadua superba*). (a, b) Site of two tadpoles, original state, and after inspection (Site 5 in Tab. 2); (c) vertical dead bamboo with slit-like opening (Site 3); (d) dead bamboo stalk lying horizontally, internode harbouring 15 *N. rugiceps* and one *Ranitomeya variabilis* larvae (Note the “lid” made for inspection) (Site 6).

Deposition and fate of fertilized egg clutches: Once a male had selected an artificial tree hole, he called from there with only the head above the water surface for several hours almost every night. A female ready to mate would enter the tree hole about three to five hours after the lights were switched off at 20:30 h. The male would clasp the female, holding her with his hands on the proximal parts of her upper arms or on the body above the arm insertions (Fig. 4). Egg were laid during the second half of the night, the latest observation occurring between 8:04 and 8:07 h in the morning. The pair would dive with the heads lowest and the cloacae at about the water surface, the female's cloacae touching the wall. As the eggs started to be extruded, the female would move to the right or left in the same position, the pair keeping their cloacae as high as before. In this manner, the clutch was spread over 1–3 patches and affixed to the wall slightly above or under the water line (Fig. 4). Egg deposition took 2–3 minutes. Eleven fertilized

clutches comprised between 206 and 553 (mean = 357) eggs (Tab. 2) that were arranged in single or partly double layers. Ten eggs measured had diameters of 1.11–1.13 (mean = 1.12) mm and were light grey with large white vegetal poles, subsequently turning grey with further cleavages (Fig. 5). After deposition, the male would release the amplexus. In some cases, the female stayed within the “tree hole” for at least another hour, but both frogs would leave in the early



Figure 3. Inside of a bamboo internode calling site, 80 mm in diameter, 4.97 m above the ground (Site 2): on the left with water removed; note the dragonfly larva, 28 mm long, a potential predator of tadpoles. Inset: The water surface before removal, with detritus, arthropod remains, and culicid pupa.

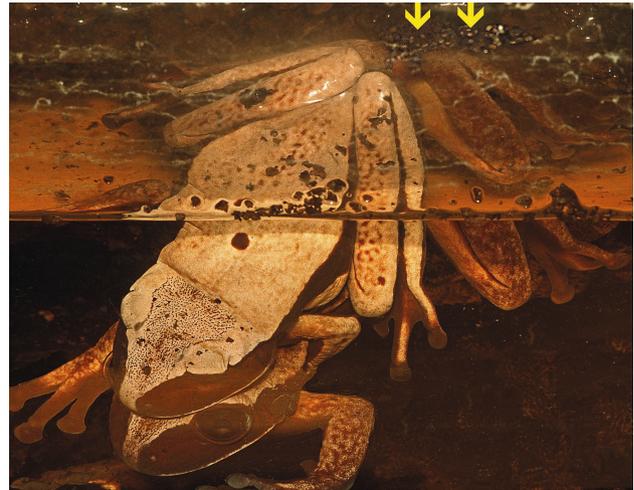


Figure 4. A pair of *N. rugiceps* depositing eggs (arrows) above the water surface.

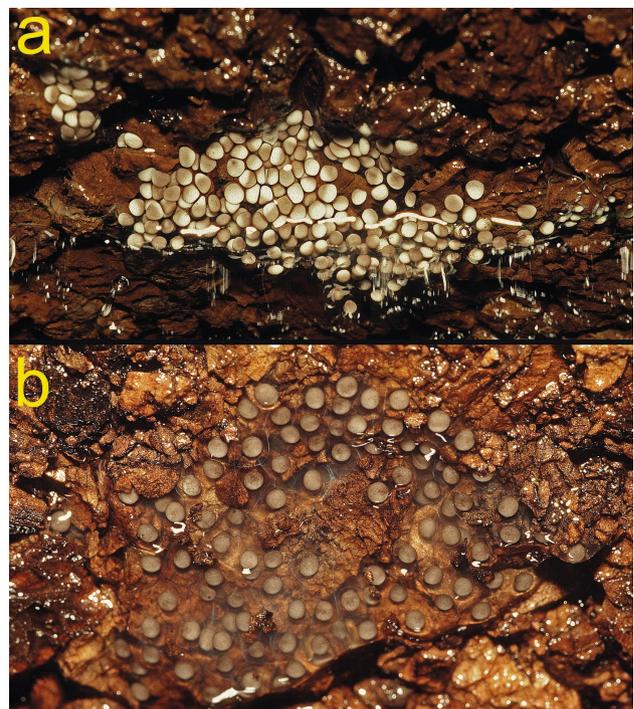


Figure 5. Fertilized egg clutches of *N. rugiceps*: (a) a fresh clutch with about one third of the eggs submerged; (b) a clutch about 10 h old, slightly above the water surface. Not to scale.

morning. Thereafter, the males did not return to the sites. They were usually found calling from an another, unoccupied site 2–3 nights later. Only a few tadpoles (9–75, mean = 24.1, $n = 11$) hatched after 80–86 hours at a TL of 5–6 mm, exhibiting small external gills, and clung to the water surface soon after hatching.

Nutritive egg clutches, larval behaviour, and female–larvae interactions: Upon depositing a fertile clutch females returned to their respective breeding sites after 3–5 days (mean = 4.5, $n = 8$) during the night and deposited clumps of new eggs without raising the cloaca above the water line. Eggs of nutritive clutches could not always be counted precisely, because especially older larvae consumed them rapidly. My impression was, however, that first nutritive clutches, when larvae were still small and had probably eaten no more than one egg each, were about the same size as fertilized ones. Hatchlings consumed the nutritive eggs by first destroying the gelatinous layer and then ingesting the contents. When they received eggs a second time, they were already able to ingest 2–4 of them whole. Older larvae were full of eggs (Fig. 6b). Females returned to deposit nutritive clutches every 2–9 days (mean = 5.1, $n = 81$). Juveniles ($n = 23$) completed metamorphosis after 79–112 (mean = 93.4) days when they had received eggs 18–22 times. Larger numbers of tadpoles per site required longer periods of time to reach metamorphosis. In four reproduc-

tive periods observed, 3–9 tadpoles (mean = 5.8, $n = 23$) reached metamorphosis; details are given in Table 2.

Tadpoles exclusively ate eggs. Attempts at feeding older larvae (Stages 30–36) with commercial fish food flakes, culicid or psychodid fly larvae (dead or alive) failed. Tadpoles never attacked one another, not even if a female failed to return after four nutritive clutches, as happened in Reproductive Period 2 (Table 2).

During the female's presence, tadpoles swam round her closely, especially close to her cloacal area, often touching her with their mouths. At least sometimes they bit her. Larger tadpoles were then vigorously wiped off by her with one of her hind feet. A female never deposited any more eggs after the last juvenile had left the water. Since tactile interactions of old tadpoles were intense, missing touches probably indicated to her the absence of larvae after they had completed metamorphosis.

Agonistic behaviour

Defending territories is a common trait in anurans, especially when they include breeding sites that constitute limited resources (in hylid frogs; e.g., LUTZ 1960, KLUGE 1981, WEYGOLDT 1981, MARTINS 1993, MARTINS et al. 1998, SCHIESARI et al. 2003).



Figure 6. (a, b) Lateral and ventral views of a larva of *N. rugiceps* at Stage 40, 50 mm TL, 21 mm head–body length, three days after egg consumption; (c) recent metamorph, 25 mm SVL.

Table 2. Reproductive periods of captive *N. rugiceps*.

Period	1	2	3	4	5	6	7	8	9	10	11	mean
Number of eggs in fertilized clutches	235	219	355	206	452	337		553		488	372	357.4
Number of hatched larvae	75	9	22	10	46	14			12	12	17	24.1
Number of nutritive egg clutches	8	5		18	3	6				20	22	
Number of eggs in first nutritive clutch	211			344	878	293				412	388	446.6
Intervals between clutches (d)	(n=8)	(n=4)		(n=18)	(n=3)	(n=6)				(n=20)	(n=22)	(n=81)
range	2–5	4–6		4–7	2–5	4–9				3–7	4–7	2–9
mean	4.1	4.8		5.2	4	5.8				5.1	5.3	5.1
Number of metamorphosed frogs				4			3			7	9	
Metamorphosis after (d)												
range				84–92			79–97			83–104	88–112	79–112
mean				88			88			93	98	93.4

Experiments using two male *N. rugiceps* in one terrarium with one “tree hole” regularly ended up in fights between them. When one was inside the cavity, he tried to block the competitors’ way in with its head. When both were inside, one of them would try to dive beneath the other frog and push it out with his head. Once outside, wrestling began with opponents trying to clasp each other wherever possible, sometimes in an amplexus-like grip, venter-to-venter (Fig. 7), or one would hold the other’s leg with one or both hands until the latter fled. Usually, the winner then pursued the opponent over 40–50 cm away from the cavity and then returned.

Discussion

Possible heterospecific egg parasitism

Among the 15 tadpoles of *N. rugiceps* found in the second bamboo internode in the field (see above) we found one



Figure 7. Two male *N. rugiceps* fighting. The upper individual had already driven its competitor out of the artificial tree hole, but then followed it for about 30 cm, engaging it in another fight before the subdued male could escape.

tadpole of the lowland morph of *Ranitomeya variabilis* (taxonomy fide BROWN et al. 2011). Larvae of the latter are commonly transported on the father’s back to phytotelmes (BROWN et al. 2008a). These tadpoles are cannibalistic and eat conspecific eggs and younger tadpoles if they get hold of them (SUMMERS 1999, POELMAN & DICKE 2007). Adults can detect tadpoles, both con- and heterospecific, by means of chemical cues in phytotelmes and avoid depositing their larvae in pools where a conspecific larva is already present. In contrast, they show a high preference for pools containing heterospecific tadpoles, because these represent a possible source of food for their own larvae (SCHULTE et al. 2011, SCHULTE & LÖTTERS 2014). Tadpoles of *R. variabilis* consumed heterospecific larvae in field experiments (SCHULTE & LÖTTERS 2014), but also under natural conditions, e.g., those of the phytotelme-breeding hyliid *Osteocephalus planiceps* (HAUGEN 2002).

In the bamboo internode at the study site both species appeared to coexist. The *N. rugiceps* larvae were much larger than the *R. variabilis* larva and did not exhibit any bite marks or other injuries. Although the *N. rugiceps* larvae varied in size, neither they nor the *R. variabilis* larva appeared to be undernourished. The *N. rugiceps* larvae were at Stage 27 and later, while the *R. variabilis* one already was at Stage 40. Nonetheless, the former most likely were older, not only because of their larger sizes, but also because the lab-raised tadpoles of similar group size took much longer to metamorphosis (Reproductive Period 11 with nine metamorphosing larvae took 88–112 days, see Table 2) than a *Ranitomeya* larva does. Field data on larval development periods in *R. variabilis* are not available, but a single tadpole of a related species that also lives in bamboo internodes, *R. sirensis*, is known to have taken 58 days to complete metamorphosis (WALDRAM 2008). Most likely, the *R. variabilis* tadpole was deposited there by a male frog when the *N. rugiceps* larvae were already present, but too large to be eaten. The good shape the larva was in can best be explained by assuming that it fed on the *N. rugiceps* eggs regularly provided by the female. Apparently, *R. variabilis* larvae not only cannibalize heterospecific tadpoles, but also parasitize nutritive eggs.

Observations of skin secretions

Numerous species in the tribe Lophyohylini, to which *N. rugiceps* belongs, under stress excrete toxic substances from serous glands (reviewed by BLOTTO et al. 2000). The white secretions are sometimes so obvious that one species, *Trachycephalus typhonius*, is locally known as rana lechera (“milk frog”). At least two species in the genus *Nyctimantis* are exceedingly toxic. The skin secretion of *Nyctimantis brunoi* is known to be 25 times more lethal than *Bothrops* venom, and the frog is able to deliver its toxin from skin glands by means of cranial spines to potential predators (JARED et al. 2015). Toxicity is known in *N. rugiceps* as well. CAMARGO DE SOUZA et al. (2018), after having handled an individual and passing the hand across the forehead, described a sensation that felt like receiving a strong blow with a hard object, making tears and mucus flow, and continuous pain for two days. I inadvertently killed three *Pristimantis* sp. when I placed them in a plastic bag with an individual of *N. rugiceps* for two hours. The latter species, despite its cranial co-ossification, does not have any spines to actively deliver any venom. Skin glands are not visible upon superficial inspection and their location is unknown. But an observation of a captive specimen indicates that toxin might be secreted from various parts of the body, when a six-year-old individual was found dying in a terrarium. It obviously was in agony, for its lungs were inflated and it did not exhibit its normal light dorsal coloration, but was tan, and hardly able to move. When it was dead two hours later (without heart and lymph heart beats), it was immersed in 70% ethanol. During the next minutes the frog released a whitish substance in fine hair-like strands, especially from the posterolateral areas of the throat, from the posttympanic area to above the arm insertion, in the interorbital and occipital areas, mid- and posterior area of the dorsum, and the dorsal surfaces of lower arm, shank, and tarsus (Fig. 8). I assume that this substance represented the toxin released under stress.

Evolutionary and adaptive aspects

Cranial hyper-ossification: BLOTTO et al. (2020) discussed in detail cranial hyper-ossification and phragmosis, the behaviour of closing a shelter with the head, in the Lophyohylini. Evaluating existing literature, they found that the adaptive significance of both was the reduction of evaporative water loss and antipredator behaviour. While the former most likely is of little importance to a frog living in a moist habitat as in the case of *N. rugiceps*, closing up a retreat by means of lowering the head, especially in combination with the capability of excreting toxin (see above), may be an effective defence mechanism. Unfortunately, we know nothing about the diurnal retreats of *N. rugiceps* in the wild, but it is obvious that its co-ossified skull is advantageous for several purposes. It is a valuable tool in fights between males and also in the

search for suitable breeding sites, in particular those with narrow entrances to phytotelmes such as bamboo internodes.

Male and female SVL: In general, female frogs are larger than males (SHINE 1979, MONNET & CHERRY 2002), especially when females produce large amounts of eggs (NALI et al. 2014). Selection may favour smaller females (not larger than males) in species that deposit only small numbers of eggs per clutch, especially in small water bodies, such as *Triprion spinosus* (DUELLMAN 2001) and some phytotelme-breeding dendrobatid frogs like *Oophaga* spp. (SILVERSTONE 1975) or *Ranitomeya summersi* (BROWN et al. 2008b), whose sexes are more or less the same size. Larger-sized males may benefit from a competitive advantage when defending space for oviposition against conspecific males, as in the nest-building hylid Gladiator Frogs *Boana faber* (LUTZ 1960, MARTINS 1993) or *B. rosenbergi* (KLUGE 1981), but also in non-hylid frogs (e.g., SHINE 1979, EMERSON 1992, KATSIKAROS & SHINE 1997). Both adaptations may be represented in *N. rugiceps*: clutches are small, not so much with respect to fecundity, but to the extremely small size of the eggs, and large sizes in males are advantageous in defending a breeding site against conspecific males.

Adaptations to breeding in phytotelmes: phytotelme-breeding frogs exhibit numerous adaptations at different degrees to their breeding sites. In *N. rugiceps*, apart from the co-ossified skull and unusual sexual size dimensions (see above), a moderately distensible vocal sac in calling males (pers. obs., but see DUELLMAN & TRUEB 1976, who noted a large vocal sac) may be an adaptive advantage in a confined space such as a phytotelme. Some other, both morphological and behavioural features demonstrate that the parental care of *N. rugiceps* is highly evolved in comparison to other phytotelme-breeding frogs of the family Hylidae.



Figure 8. Hair-like skin secretions, possibly toxin, emanating from a freshly dead specimen in preservative.

In *Osteocephalus*, where phytotelm-breeding and oophagy have evolved twice independently (JUNGFER et al. 2013), a pair will return to the breeding site together and the eggs are fertilized by the male. A pair returns to this site to lay more eggs, no matter if the initial larvae have metamorphosed, indicating that communication between adults and larvae does not play a major role in parental care (although the presence of larvae may indicate to the female that it is a good site). In *N. rugiceps*, the female not only is independent of the male after the first fertilized egg clutch, but terminates depositing eggs after metamorphosis of the offspring. Most likely, the female responds to missing tactile cues from her tadpoles. Moreover, the female does not deposit the eggs invariably in the same place, but at or above the water surface (i.e., outside of the oxygen-deprived water) when the eggs are to be fertilized, and below, within the tadpoles' reach, when they are to serve for nutritive purposes.

Clutch sizes of a mean of 357 eggs are considerably smaller than those of most non-phytotelm-breeding hylids from the same general area (CRUMP 1974), but especially striking is the small size of the eggs, making the overall egg mass even smaller compared to traditionally breeding hylids. This may enable the female to produce more clutches at short intervals, which is necessary for effective maternal care through oophagy. On the other hand, small egg sizes enable the tadpoles to ingest the eggs wholly from early stages on. The trend to relatively small eggs is discernible in other oophagous hylid (JUNGFER 1996, THOMPSON 1996) and non-hylid species as well, e.g., in the obligatorily oophagous dendrobatid genus *Oophaga*, and is manifest in the tiny tadpoles that female *Oophaga* carry to phytotelms (e.g., LÖTTTERS et al. 2007). This is in contrast to findings that species exhibiting parental care tend to have relatively large eggs (CRUMP 1996, SUMMERS et al. 2006).

Acknowledgements

I am greatly indebted to STEFFEN REICHLER and OLIVER PISKUREK for spending long nights in search of the needle in the haystack with me. Collecting and export permits were issued by INEFAN (No. 063-IC INEFAN/DANVS/VS and 37-IC DANVS/VS). I am grateful to the staff of the Estación biológica Jatun Sacha for providing space and equipment, without which some of the calling sites would have been inaccessible.

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