

**Alternative life history strategies in the
West African reed frog,
*Hyperolius nitidulus***

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1 Introduction

Natural selection favours life history traits that maximise individual fitness. Life history theory is concerned with understanding these favoured life histories and with examining the relationships between variation in life history traits and their consequences for individual fitness [Lessels 1991, Stearns 1992; Heino et al. 1997]. The definition of life history normally includes a set of demographic traits that evolved in response to ecological factors [Roff 1992, Stearns 1992, 2000]. It is commonly assumed that the end points of selection are “optimal” in an evolutionary sense [Heino et al. 1997]. Variation in life-histories between different species or populations [MacCallum et al. 1998] can often be explained by different habitats, climatic changes, differing life-style or ancestral bias [Futuyma 1990; Bulmer 1994; Hastings 1997]. Different life-cycle strategies in the same population are much harder to explain.

In a variable environment with unpredictable duration of favourable and unfavourable conditions, differing life-histories can spread the risk of total reproduction failure. Therefore, bet-hedging strategies, for example, that reduce the variance in fitness even at the possible expense of arithmetic mean fitness [Seeger and Brockmann 1987, Phlippi & Seeger 1989] can select for and maintain different life-histories in the same population.

The climate in the West African savannah is characterised by rainy seasons of variable length. Hence, most water bodies are temporary ponds of rather unpredictable filling durations. These ponds are breeding sites of amphibians, which are faced with the problem of changing time periods for their larval development. Such environmental conditions are likely to select for plastic life histories.

The reed frog *Hyperolius nitidulus* is very common in West African savannah regions [Schiøtz 1967, 1999; Rödel 2000]. It is known for its extraordinary aestivation behaviour and physiology. Immature individuals survive the dry season by clinging to dry vegetation exposing themselves fully to the harsh climate prevailing for months above ground. [Geise & Linsenmair 1988, Kobelt & Linsenmair 1986, 1992, 1995; Schmuck & Linsenmair 1988, Schmuck et al. 1988]. Adult frogs usually do not survive the dry season [Kobelt & Linsenmair 1986, Geise & Linsenmair 1986, 1988]. Reproduction, restricted to the wet season, usually takes place from May/June to October. Until now, it was assumed that *H. nitidulus* has a one year - one generation life-cycle (Figure 1): subadult frogs, having survived the dry season, mature during the first part of the rainy season and reproduce subsequently.

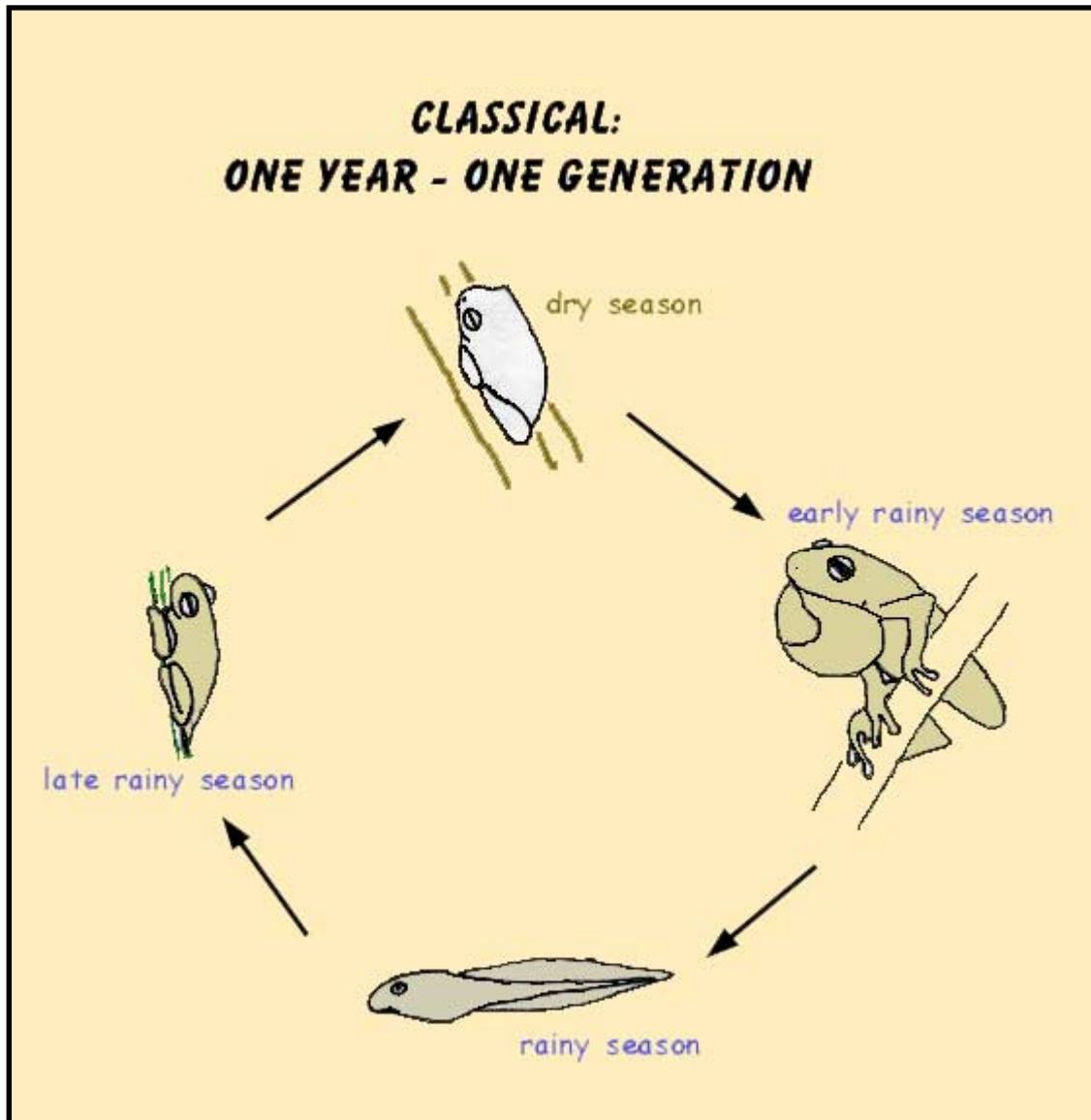


Figure 1: Classical one-year one generation life-cycle of *Hyperolius nitidulus*.

However, frogs metamorphosing during the first two thirds of the breeding season show a very different behaviour compared to those leaving their breeding waters during the last third. Early juveniles hide near the ground, while late juveniles prefer sitting above ground directly exposed to the sunlight. Furthermore, the breeding season is rather long with respect to the minimum time needed for development. Tadpoles take about 8 weeks to metamorphose and fast developing juveniles need no more than two months to fully mature. Therefore, it was assumed that a second, very short-lived summer generation existed (Figure 2), which had not been detected due to the large overlap in generations.

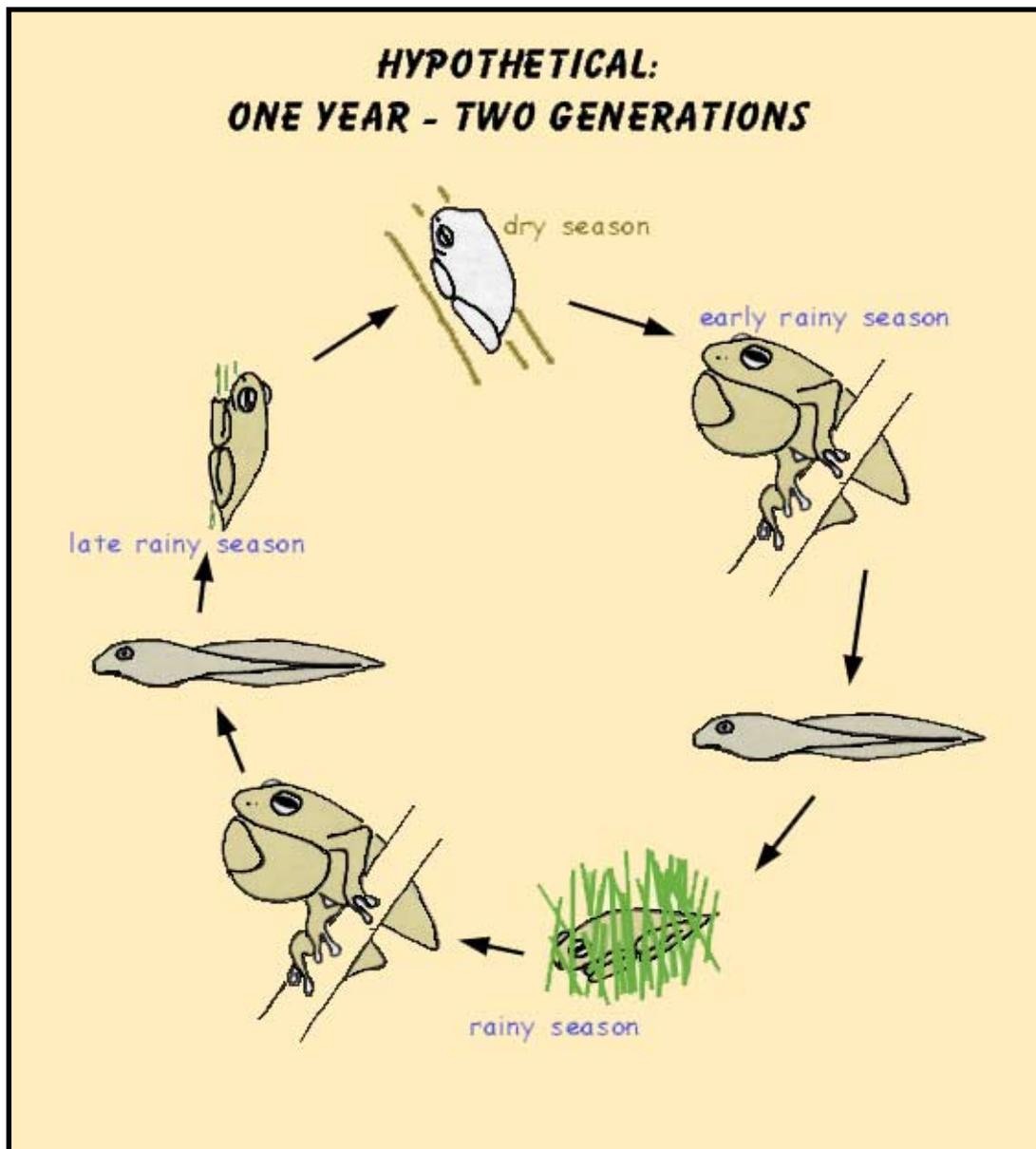


Figure 2: Hypothetical one-year two generations life-cycle of *Hyperolius nitidulus*.

The hypothesis that part of the early summer generation grows most rapidly and does not invest in adaptations for surviving dry periods, was tested. The late summer generation, in contrast, was presumed to differ greatly in growth patterns, behaviour and physiology and to be well adapted to the dry and hot climate conditions which they meet when leaving the water after the commencement of the dry season.

This study was designed to investigate whether different reed frogs of the species *Hyperolius nitidulus* inhabiting the same area show different life-history patterns.

2 General description

2.1 *Hyperolius nitidulus*

Systematic

The genus *Hyperolius* poses some problems for systematic reviews because of its highly variable members. In recent studies *Hyperolius nitidulus* [Drewes 1984; Rödel 2000] is defined as a true species belonging to the African family of the Hyperoliidae (reed frogs). These studies do not follow the superspecies concept [Laurent 1951, Schiøtz 1971, 1999] which classifies *H. nitidulus* as a subspecies of the *Hyperolius viridiflavus* complex. There are many taxonomic problems with the Hyperoliidae [Hess 1995, Richards & Moore 1996], that are of no importance for this study, and are left aside for this reason.

The family Hyperoliidae is restricted to the southern Sahara part of the African continent where it takes on the role of the hylid frogs that are missing here. It is a very variable and successful group, that especially in its marginal distribution areas, still seems to be going through some evolutionary radiation.

Appearance

All members of the genus *Hyperolius* have horizontal pupils and disked finger tips. Males have an external median subgular vocal sac. [Schiøtz 1971]

Hyperolius nitidulus is a medium sized reed frog with a rather blunt snout. Males vary in size between 23 mm and 29 mm (snout-vent length) and weigh about 1g, females are slightly larger and heavier (24 - 32 mm; up to 2 g directly before egg laying).

Juvenile colour is beige with a darker, sometimes lightly flanked stripe at the side running from snout to vent. During the dry season, when day temperatures reach over 36-38°C, juveniles become white (Picture 1). During this period the inside skin of the arms and legs is bright red.

Adult frogs vary in colour. At night, they are all beige; during the day, coloration varies between almost uniformly coloured individuals with white or beige backs and only slightly darker stains at the flanks and quite colourful individuals with yellow backs and numerous big black stains (Picture 2). *Hyperolius nitidulus* are able to change colour very rapidly. They become much darker when stressed. During the day, hydrated juveniles try to match the background colour. [Rödel 1996 and own observation]

Call

During the main rainy season *Hyperolius nitidulus* males call at savannah ponds. Two calling types can be separated very easily: the mating call (“pling”) and the aggressive call (“croak”). The mating call of *Hyperolius nitidulus* is a short metal like “click” of 0.10 – 0.24 seconds. Call frequency varies between 2.04 to 3.43 kHz and seems to correlate with male size. It is used to attract females.

The aggressive call occurs when one male enters the calling site of another and starts calling himself [Rödel 1996]. It is longer (0.28-0.36 s) and deeper (0.98-2.6 kHz) than the mating call and has a much lower sound pressure level.

When up to hundreds male individuals call in choruses at the same pond, the former rather unpleasant sound changes to a sound like a chorus of bells [Rödel 1996].

The main calling time is from dusk to midnight.

Clutch

Female frogs lay between 94 and 800 eggs [Böckheler 1993]. The eggs have a white vegetative and dark brown animal pole and are attached to under water vegetation or pond ground in small portions. Females are able to produce several clutches during one reproductive period [Grafe 1992].

Tadpoles

Tadpoles hatch after 2 to 5 days depending on water temperature. They feed on algae and need about 6 to 8 weeks to metamorphose. Freshly metamorphosed frogs have a snout –vent length between 12 and 15 mm and weigh around 0.2 g.

Biology

Hyperolius nitidulus spends nearly all its live in the savannah. Special adaptations (see below) enable it to survive the harsh climatic conditions during the dry season. *Hyperolius nitidulus* is a prolonged breeder. The start of the reproductive season is very variable and depends mainly on the precipitation patterns at the beginning of the rainy season. If the rainy season starts with regular rainfalls including not too many or too long periods without rainfall, male frogs start calling about two months after the first rains. Calling activity goes on throughout the whole rainy season. While male frogs stay for several days at the ponds and can sometimes be observed over several weeks, female frogs visit the ponds only for oviposition. The tadpoles live in the dense vegetation of the water edges. Freshly metamorphosed young

come out of the water at night and leave the pond during the following nights. Dragonfly larvae, beetle larvae and fish seem to be the most important predators for the tadpoles.

Survival during dry season

Savannah regions are characterised by very distinct seasons: normally a wet rainy season and a hot dry season. Animals inhabiting these regions must adapt to very different living conditions. The wet season may be cool and very humid while during the dry season it gets very hot and may not rain at all for several months.

Due to developmental and morphological reasons amphibians are especially dependent on water. They need very special adaptations to survive dry seasons. Most savannah frogs bury themselves to escape high temperatures and low air humidity, others move to the river beds or use termite mounds to survive the unfavourable dry season conditions [Schjøtz 1967, Rödel 1996].

Hyperolius nitidulus has a very uncommon and extraordinary aestivation strategy. It does not hide at all. Juvenile frogs cling to dry vegetation exposing themselves fully to the sunlight. In the dorsal skin, iridophores filled with purine crystals reflect the sunlight. The frogs turn into a white colour.

Water loss is minimised by a special sitting position, whereby all highly water permeable skin parts in the inside of arms and legs are covered (see Figure 3). During day the juveniles move only when seriously disturbed. Because of their sitting position about one meter above the ground, they are able to use even the smallest amounts of rain and condensed water that would not reach the ground. *Hyperolius nitidulus* is able to spend months above the ground without getting extra water or food.

Adult frogs are unable to adapt fully to the living conditions of a dry season once again. They normally die.

(for more detailed information see [Böckheler 1993, Geise 1987, Geise & Linsenmair 1986, 1988, Kobelt & Linsenmair 1986; 1992; 1995; Linsenmair 1998; Schmuck & Linsenmair 1988, Schmuck et al. 1988, 1989])

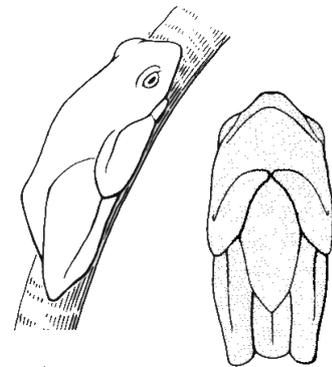


Figure 3:
Aestivating frog's sitting position
[Geise & Linsenmair 1986]

2.2 Study site

Localisation

The study took place in Comoé National Park, Ivory Coast, West Africa (Map 1).

Comoé National Park is the biggest national park in West Africa and the third biggest in Africa. It lies between 8°5' and 9°6' N and 3°1' and 4°4' W [FGU-Kronenberg 1979] in the north-east of the Ivory Coast, very close to Burkina Faso in the north and Ghana in the east. There are no natural barriers to form the park boundaries. Maximum distances in the park are 110 km north-south and 130 km east-west. Comoé National Park contains 11,500 km² of mainly bush land most of it 250-300 m above sea level. The river Comoé, after which the Park was named, runs on a north-south axis through the western part of the park.

The investigation site is located at the south-western part of the Park (Map 2). Starting from Würzburg University's research camp of the (8°45,1'N, 3°49,0'W) and following the main route 5 km to the south, there is one research area at the Gansé Plaine and a second research area, Hyperolius pond, about 5 km to the north. Another research area was further up north, about 25 km from the camp site, but it was no longer used after the first rainy season. Other sampling sites were very close to the camp at Lola pond and following the Bretelle road, but due to irregular frog activity sampling was also irregular.

Landscape

The landscape is dominated by savannah bush land and scattered woodland patches (island forests). Small hills and Inselbergs are rather rare and open fields are also not common, especially in the southern part of the park. The river edges are lined by gallery forests varying in width from 50 m to one kilometre.

Climate

The year is separated very clearly into a dry season (November-March) and a rainy season (April-October). Rainfall is variable in distribution and amount. The mean precipitation between 1992 and 1999 was 1000 mm/year. During the dry season, there is no rainfall for up to 5 months. The only water reservoirs at this time of the year are the Comoé river and the Iringou, a small tributary river.

Mean air temperature varies between 25°C and 28°C. Peaks are reached during the dry season when a maximum temperature of 44°C is possible. The Harmattan, a very dry wind coming

from the Sahara, heavily influences the weather at this time of the year. Temperature can change more during a single day than throughout the year, and maximum day/night differences of 30°C (<10 - 40°C) are possible in the dry season. During the rainy season, day temperatures fluctuates between 20 and 33°C in open savannah.

Air humidity is around 30% in the dry season and mostly higher than 90% in the rainy season.

Flora and Fauna

The park vegetation is typical for the Guinea and Sub-Sudan zone. It consists mainly of bush-tree-savannah (75%), of grass savannah (14%) and forest (11% gallery and island forests). Detailed floral descriptions are given by Poilecot (1991) and Porembski (1991).

The fauna is a mosaic of species from the wetter Guinea zone and the dryer Subsudan zone and, therefore, very diverse. Additionally, there are some abundant rainforest species that live in wider gallery forest patches. For example, 36 different amphibian species have been reported in the research area that contains savannah and forest habitats.

2.3 General methods

2.3.1 Study ponds

All ponds in Comoé National Park are ephemeral and dry out totally during the dry season. They fill with water after the first rains and the larger ones normally do not dry out until the dry season starts. Two main investigation areas were used:

1. The Gansé Plaine about 5 km south of the camp site, which contains several ponds varying in size and vegetation. Our main ponds were the Gansé pond (Picture 3) 12 m in length to 4 m width with a maximum depth of 50 cm, dense vegetation and no connection to the Comoé river; Gansé Bridge pond, a very large and deep pond (maximum size: 70 m x 60 m maximum depth: 1.10 m) with very dense vegetation and a permanent connection to the Comoé river; and Gansé swamp, a flooded grassy savannah part which exists only for some weeks when the majority of rainfall occurs.
2. Hyperolius pond (Picture 4). A large, rather isolated pond about 5 km north of the camp site with a temporary connection to the Comoé river. The oval Hyperolius pond has a maximum diameter of more than 50 m (length) to 35 m (at a right angle). The edges are flat with a very dense vegetation of herbs. In the centre, at a maximum depth of 1.20 m, it is covered with floating vegetation such as waterlilies.

Some smaller ponds at different locations were also used: Lola pond next to the research camp, Afrixalus and Iringou ponds following the road to Gawi and View Point pond (Picture 5) a very attractive, rather shallow pond with uncommonly clear water.

2.3.2 Investigation time

Sampling took place during the rainy seasons of 1997, 1998 and 1999 (mid April to end of October 1997, mid May to end of October 1998, end of April to end of November 1999). 1997 and 1998 sampling started at all ponds equally with the beginning of male calling activity. Sampling started in 1999 at the end of April at the Gansé Plaine, and Hyperolius pond was additionally investigated after 9 July. All frogs were caught during the night at the investigation ponds. At “normal” nights with a moderate number of calling males (estimated under 50) working hours were from 19:30 h to around midnight. When intensive calling was

registered (estimated more than 50) working started directly after dusk (18:00 h) and ended not before 3:00 h in the morning. The goal was always to get as many individuals as possible.

To enable optimal comparisons between the years, the data were subdivided into investigation weeks. The beginning of an investigation week was defined solely by date. For example, every year week 1 from 16 to 22 April, investigation week 2 from 23 to 29 April and so on. With this method direct comparison between the years was possible.

2.3.3 Frog handling

Adult sampling

All adults were caught at night at the investigation ponds.

Catching adult *Hyperolius nitidulus* during the rainy season is easy: most adult males sit in the vegetation at the pond edges at a height between 50 cm and 150 cm. At calling times they do not pay too much attention to potential predators and it is very easy to get near enough to just take them by hand.

Female frogs are mainly found in couples or just sitting in the vegetation. Couples wait for some hours sitting in the vegetation near the pond before spawning and are easy to catch by hand or with a Drosophila glass¹. During nights with moderate mating activity all couples were brought to the laboratory for clutch size measurements. During nights with intense mating activity (1999 only) a maximum of 16 couples was taken into the camp. The rest was measured and toe-clipped. At the beginning of the rainy season 1999, couples were chosen randomly; after the appearance of the second generation, couples with at least one marked individual were preferred.

Adult marking

All adult frogs that were caught for measurements were also marked individually. To mark the adults, they were toe clipped according to a special scheme. (Figure 4): Each hand signifies a position in a four-figure number: a missing toe on the left hand means one, two, three or four thousand; a missing toe on the right hand means one, two, three or four hundred. A missing toe on the left foot means ten, twenty, thirty, forty or fifty; a missing toe on the

¹ A Drosophila glass is a plastic cylinder, closed at one end and with foam stopper at the other end to close it. Originally used to cage Drosophila it is about 7 cm high and has a diameter of 2.5 or 4 cm.

right foot means one, two, three, four or five. Toes are always counted from left to right. Normally just one toe was clipped per hand/foot.

There were not enough numbers for males at the Gansé Plaine in 1999, so double clipping of one hand or foot had to be used to have sufficient numbers available.

Male and female frogs were assigned identical numbers since they could be distinguished. Different numbers were always used for different ponds, except in 1999 when the same numbers were used for ponds that were far enough apart (10 km) to make any exchange extremely unlikely.

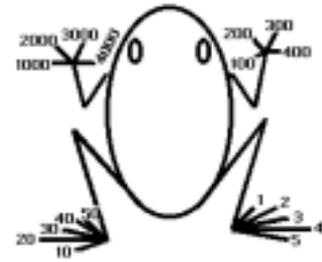


Figure 4:
Toe clipping scheme

Males

In 1997 males were caught at four different sites: at Gansé Plaine, at Hyperolius pond, at Lola pond and at Iringou pond. Since Iringou pond is at quite a distance from the research camp site (around 25 km) and offered a limited number of couples (see below in Table 3), it was abandoned in the following years. Lola pond is quite close to the camp site (0.5 km), but as *Hyperolius nitidulus* calls there very irregularly the main investigations concentrated on the calling sites Hyperolius pond and Gansé Plaine, both at a distance of about 5 km from the camp and around 10 km apart from each other. Toe-clipping was used to mark male calling frogs at the ponds individually.

Each investigated pond was visited at least once a week to catch as many calling individuals as possible. Population size could be estimated with the capture/recapture data using the Jolly-Seber method. The data gave also information about migration events between ponds.

In order to get more detailed information, one very attractive pond was selected and in 1998 visited every night for four weeks, with the aim of catching all calling males. This high sampling frequency resulted in some good data about the time male *Hyperolius nitidulus* spend calling at the pond.

At the same pond in 1997 calling sites were monitored over some nights and marked in a pond map to look at the distribution of individuals. With this map nearest neighbour distances could be determined and calling strategies of individual males, for example site preferences, could be examined. Several calling point parameters like height and width, distance to free water and to edge and surrounding vegetation were recorded. The same data were collected in August 2000 at Hyperolius pond.

Calling activity was measured by counting calling males at the pond.

A successful reproductive male was defined as one that was found in a couple with a female. Since all males were marked individually, determination of the (minimum) number of copulations of the individual males was possible.

Females

The beginning and the end of the reproductive season was defined by the arrival of the first females at the pond and by their disappearance. Females were almost always found in couples that were caught during the male catching times. They were measured and weighed the following day after spawning in order to minimise disturbance before spawning and so avoid influencing spawning. Individual marking facilitated the registering of wandering behaviour and multiple spawning.

Clutches

Couples were taken from all investigation ponds throughout the rainy season. In combination with the resulting clutches, it should be possible to answer questions about female choice and male reproductive success. They were kept in Drosophila glasses for transport and spawned in captivity. 2.5 l aquaria were filled 1/3rd with water and some leaves were put into the water, so that the females could attach the eggs to the leaves and to the aquarium bottom. The aquaria were kept in a silent and fairly dark place to reduce disturbance. The next morning the laid eggs were collected and counted, the developmental stage was determined with a Gosner table [Gosner 1960] (to ensure egg diameter measurements before Gosner stage 12 when eggs start to loose their round shape) and the egg diameter measured using a microscope. In 1997 and 1998 sampling took place from June to October, the eggs stayed in the laboratory until the tadpoles hatched and were then put out into their pond of origin afterwards. In 1999, data were obtained from July to November and the clutches were returned to the ponds immediately after the measurements. Diameters of at least 50 eggs per clutch were measured. To determine hatching rate and time all tadpoles swimming freely in the aquarium were counted daily until the last visibly developing tadpole hatched.

Tadpole development

Tadpoles were raised mainly for behavioural studies after metamorphosis. They came from the clutches laid in captivity to ensure obtaining the right species and equal developmental stages.

Tadpoles were raised in 15x15x30 cm aquaria containing 0.5 or 2.5 l of river or rain water.

They were fed pulverised algae fish food tablets ad libitum. Measurements (body and total length) were taken every fourth day. The water exchange schedule varied with the experimental group. Tests started with tadpoles in Gosner stage 20 (body length 6 mm, total length 12 mm). When the tadpoles reached Gosner stage 42 (four extremities, ready to leave the water) they were taken out of the aquaria and put into large basins containing a dry area and shallow water to allow them to leave the water easily (details in 3.4.2).

In the field *Hyperolius nitidulus* tadpoles were collected by electro-fishing (equipment fabric: Deka 3000 Lord) to obtain information about reproduction activity and pond productivity (predators).

Juvenile behaviour

Freshly metamorphosed frogs were taken from the ponds for the marking experiment (cf. 3.2.3) and to test their behaviour.

Juvenile frogs leave the water by crawling up the vegetation and then sit on the stalks for several hours before leaving the ponds. As they are bright yellow in colour (Picture 9a+b), they are very conspicuous at night and they can easily be caught by hand.

Juveniles for the marking process and for the behavioural tests were kept in large Drosophila glasses and fed every second day with small insects that were attracted to the working place light. As it was not easy to get food for them, captivity periods were kept as short as possible. After a maximum of 5 days they were returned to their ponds of origin.

Juveniles were marked in cohorts with a special dye (see below 3.2.3.1). Individual marking with toe clipping was not possible as juveniles toes are very small and may regenerate in a couple of weeks [Richards 1975]. All other marking possibilities fail because of the daily shedding of skin and the small size of juvenile *Hyperolius*.

Juvenile behaviour was tested in groups of different size depending on how many individuals could be found in the field. Therefore relative values had to be used to compare the results. The monthly changes in behaviour were tested with a nonparametric multiple comparison test in accordance with Zar (1984).

Morphometry

To measure adult and juvenile body and leg length, a frog was taken between thumb and index finger and flattened carefully to obtain its maximum length. Snout-vent length (SVL) measurements were taken with a gauge with an accuracy of 0.5 mm. Leg length (tibia-fibula

length TF) was also measured with a gauge, always using the left hind leg. To weigh the frogs with portable Ohaus balance they had to be put in a closed box to prevent escaping.

2.3.4 Environmental factors

Weather

Data on air temperatures, air humidity, rainfall and light intensity were collected automatically with a weather station (Campbell weather station sensory system: Campbell 50Y Temperature & Relative Humidity Probe @ 2.50m; Campbell ARG Tip Bucket Raingauge @ 1m; Campbell PTB101B Barometric Pressure Sensor @ 1.50m; 05103-5 RM Young Wind Monitor @ ca. 4m; Skye Instruments High Output Light Sensor SKL 2640 @ 3m; Li-Cor Radiation Sensor 200SZ @ 3m; Campbell CR10X Logger). Before the weather station had been installed, these data were recorded daily at the research camp using much more simple equipment: a mercury thermometer, a hygrometer and two rain gauges.

Water quality

Because freshly metamorphosed juveniles were already found to be rainy or dry season adapted, it was thought that the decision whether to aestivate or to reproduce during this season is already made by the tadpoles. As there were many indications of water quality as a possibly important factor for decision making in the tadpoles, several water quality parameters were measured in the field.

Changes in water quality over time were monitored by weekly measuring of conductivity (WTW LF 196 conductivity meter), pH-value (WTW pH 330 meter with integrated thermometer), water depth (ruler) and temperature during the rainy seasons of 1998 and 1999. In 1998 oxygen content was measured and water samples of every third week were analysed for ion content using a photometer. Each sample was tested for nitrogen (nitrite, nitrate, ammonium) and phosphate.

Light intensity and duration

To see whether the light conditions in the field differed significantly between the rainy and dry seasons, light intensity was measured every hour with a luxmeter (Gossen: Mavolux digital) on sunny and cloudy days, every hour from 8a.m. to 6p.m., at two different places in

the savannah and in the forest. These measurements were combined with measurements of water temperature.

To notice changes over the larger time scale of one rainy season the number of sunny hours per day was recorded from July to November in 1999.

3 Research topics

3.1 Reproductive behaviour of *Hyperolius nitidulus*

3.1.1 Introduction

In contrast to the dry season adaptations of *Hyperolius nitidulus*, data on reproduction of *Hyperolius nitidulus* are scarce.

It is known, that *Hyperolius nitidulus* is a prolonged breeder and that the males aggregate in leks. Both sexes show no sign of parental care [Schiøtz 1977, Rödel 2000].

The study concentrated on questions referring to the lek system, focusing on behavioural questions about male distribution and calling habits and female reproductive behaviour, especially female choice. In addition life history data concerning clutches, tadpoles and juvenile frogs were collected to describe the biology of the species in greater detail.

3.1.2 Results

3.1.2.1 Male biology

Male size

Mean male size (snout-vent-length (SVL)) was 27.4 +/- 1.4 mm, mean leg size (Tibia-Fibula-length (TF)) was 12.8 +/- 0.9 mm and male weight lay around 1.1 g. Male snout-vent length correlated significantly with male leg length (Pearson-Product-Moment $p < 0.05$) and weight (Pearson-Product-Moment $p < 0.05$). For this reason SVL was taken as a reliable measure for male size and used for correlations and comparisons.

Males differed in size between the investigation years. A one-way ANOVA with Scheffé Post-hoc test revealed significant ($p < 0.001$) differences between all investigation years for pooled data (Table 1; Figure 5) concerning male body length, male leg length and male weight.

Table 1: Results of the Scheffé test, following the one-way-ANOVA on differences in body size between the investigation years.

<u>Scheffé test</u>	1998 (mean: 27.79)	1999 (mean: 27.29)
1997 (mean: 27.03)	0.00000*	0.00548*
1998	----	0.00000*

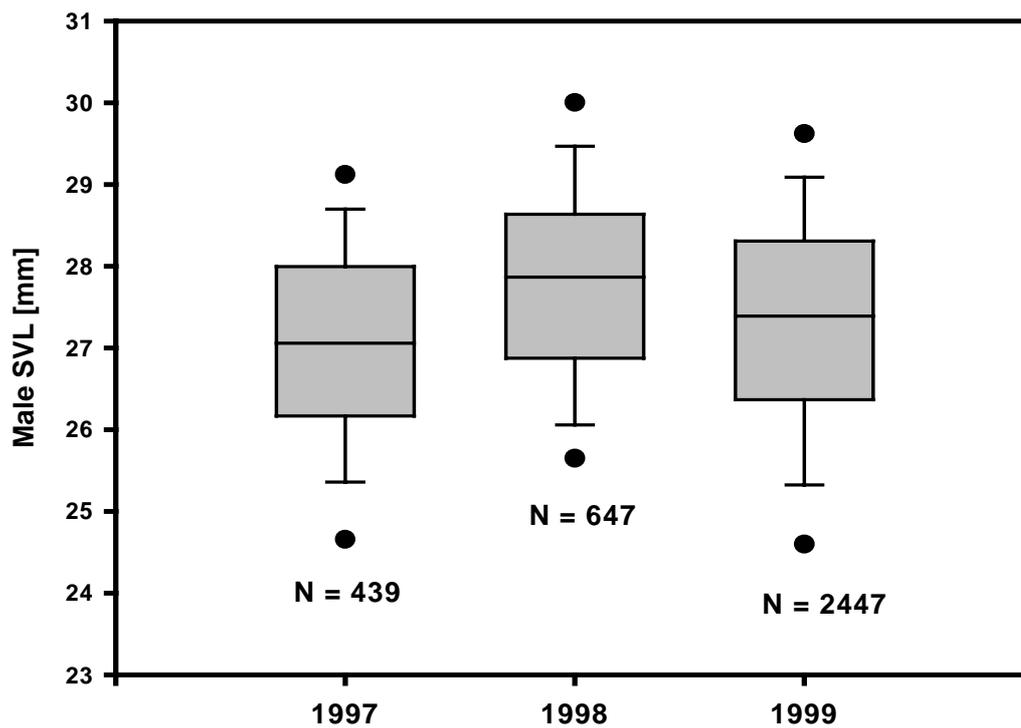


Figure 5: Male size during the reproductive season in different years. Pooled data for all ponds. (Box plots: median (line), 10th, 25th, 75th and 90th percentiles (box with error bars), 5th and 95th (circle))

Hyperolius nitidulus males differed in size depending on the pond they were taken from (Figure 6). Lola pond (L) males were significantly larger than all the other males, Gansé Plaine (G) males differed from all the other males. Only Iringou (I) males did not differ from *Hyperolius* (H) males, all the other differences were highly significant (ANOVA plus Scheffé Post-hoc test $p < 0.05$ – Table 2).

Table 2: Results of the Scheffé test, based on the one-way-ANOVA for differences in body size between the investigated ponds. (Pooled data for all years)

<u>Scheffé test</u>	<u>Lola pond</u> (mean: 28.00)	<u>Hyperolius pond</u> (mean: 27.06)	<u>Iringou pond</u> (mean: 26.94)
<u>Gansé pond</u> (mean: 27.49)	0.00006*	0.00000*	0.00102*
<u>Lola pond</u>	----	0.00000*	0.00000*
<u>Hyperolius pond</u>	0.00000*	----	0.87733

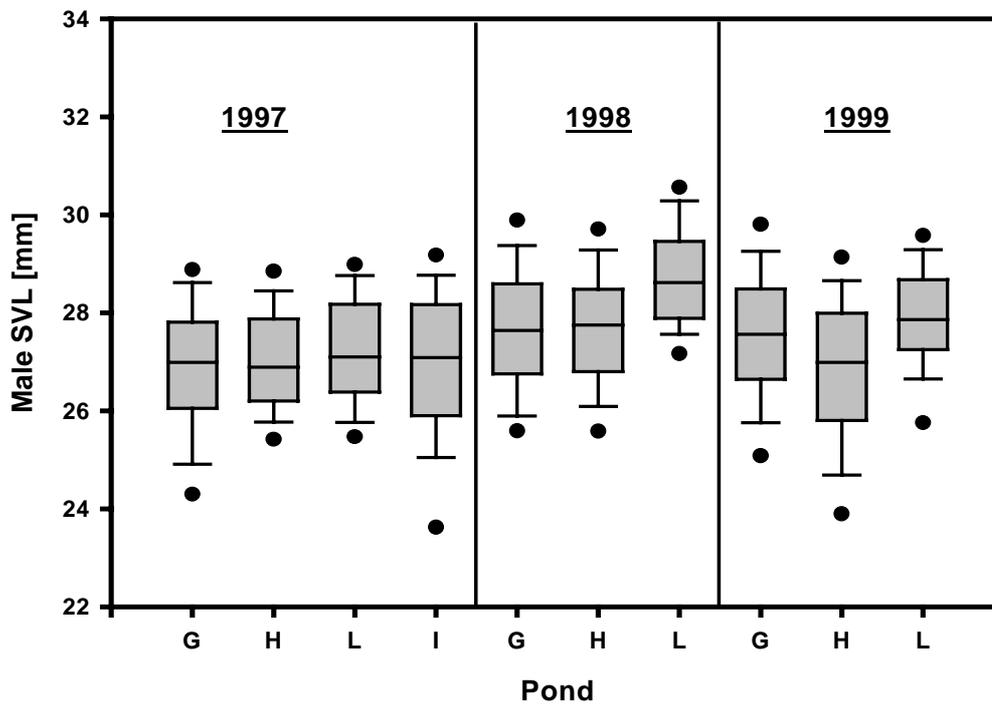


Figure 6: Male size at different ponds in different years. Box plots show: median (line), 10th, 25th, 75th and 90th percentiles (box with error bars) and 5th and 95th percentiles (circle).

Males from different ponds at the Gansé Plaine did not differ in size. There were no differences for either SVL, TF or weight.

Male size varied with time (cf. 3.2.2.2). Males were larger earlier during the rainy season, size decreased towards the end. It was therefore assumed that size differences between males of different years and ponds were mainly due to different sampling times and would vanish when only overlapping times were tested, but the differences found between the years and the ponds investigated remained constant. (ANOVA $p < 0.05$; Scheffé test results: $p < 0.05$ for all groups)

Male activity

The breeding season started in 1997 and 1998 in mid June (1997: 13.6., 1998: 19.6.) and ended at the end of October. In 1998, male frogs started calling three weeks before the first

females were found at the ponds, in 1997 and 1999 reproduction and calling started at the same time. In 1999, the breeding season started already on 21st April and ended near the end of November. During these 7 months of reproduction 2447 males were marked individually. 439 males were found in 1997 and 647 in 1998 (details see Table 3).

Table 3: Numbers of individually marked males during the investigation years 1997 – 1999.

	<u>1997</u>	<u>1998</u>	<u>1999</u>
Total number of Marked males	<u>439</u>	<u>647</u>	<u>2447</u>
Gansé Plaine	167	321	1630
Hyperolius pond	106	265	719
Lola pond	42	61	98
Iringou pond	124	---	---

During some nights, mainly after rainfall, hundreds of frogs called at a breeding pond. Male population size was estimated for the main investigation ponds using the Jolly-Seber method (Figure 7a+b).

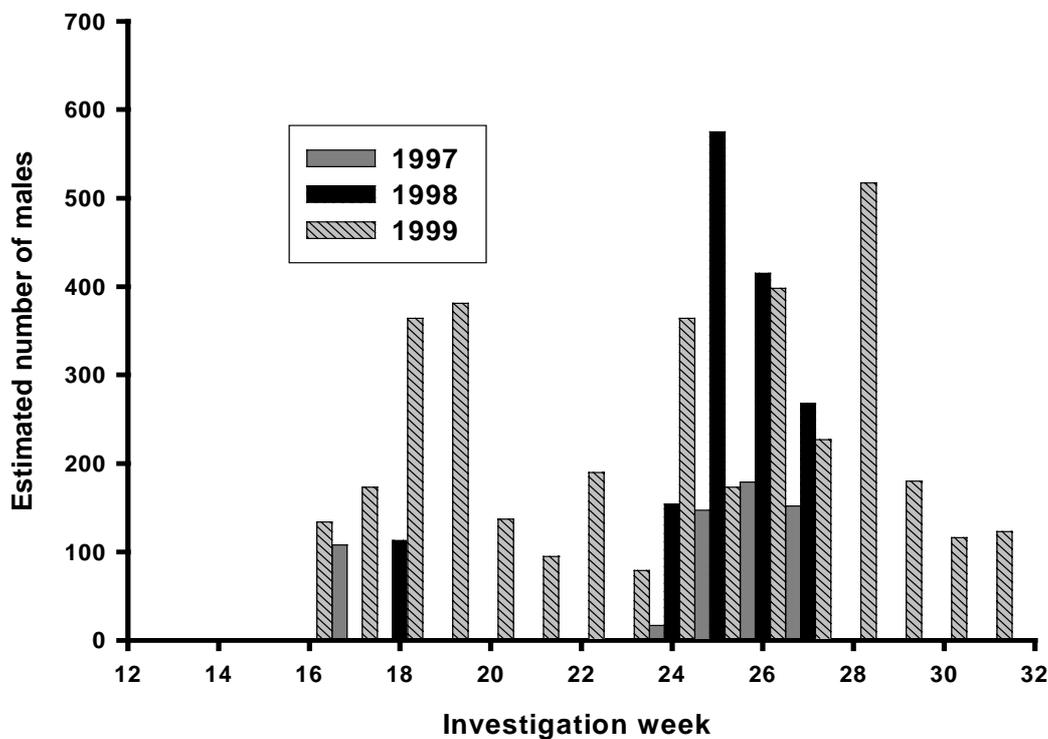


Figure 7a: Estimated population size at the Hyperolius pond.

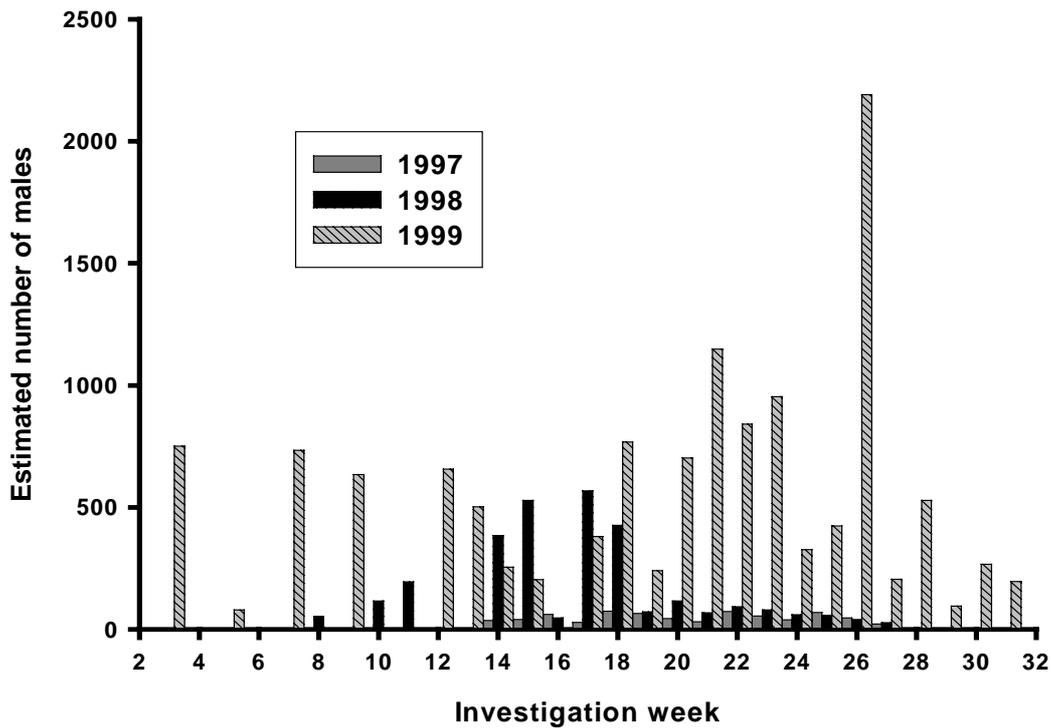


Figure 7b: Estimated population size at the Gansé Plaine.

Patterns of calling activity were very different between ponds but not between years. At Hyperolius pond two activity peaks, interrupted by a phase of low or zero activity, occurred very similarly in 1997 and 1998, but this pattern was not as clear in 1999. At Gansé pond, activity levels fluctuated much more, and in 1999 activity was much higher than in the two previous years.

To investigate possible correlations of male activity with rainfall, the number of calling males and the amount of rainfall were categorised. Categories were built because a stepwise increase in male activity and a relevant limit of precipitation was expected rather than a linear correlation. Eight calling males categories were built: zero males = 0, 1 male to 9 males = 1, 10-19 males = 2, 20-29 males = 3, 30-39 males = 4, 40-49 males = 5, 50-59 males = 6, > 60 males = 7 and precipitation was separated into three categories: zero mm = 0, one to 15 mm = 1 and more than 15 mm = 2. The 15 mm of rainfall was chosen as a relevant limit as many frogs respond only to rains including more than 15 mm of precipitation [Spieler 1997]. A Spearman rank correlation of calling activity and rainfall yielded a significant but weak relationship between rainfall and male calling activity at the same night (Spearman rank

correlation: $r = 0.15$, $p = 0.001$) and a better correlation for rainfall and male calling activity at the following night ($r = 0.25$, $p = 0.001$). Calling activity correlated with rainfall, but it rarely stopped totally (Figure 8 a-c). Activity was higher after rainfall but there was a permanent “background” level of calling activity all the time, which possibly weakens the correlation found. These background callers were investigated more closely to possibly obtain a different calling strategy. Males that called in groups of five or less were summarised in this category of “background” callers, but it were neither all the time the same males showing this behaviour, nor did these males differ in size or weight from the rest off all calling males. The only difference found was that these males stayed longer (were detected during more weeks of investigations) than the rest of all calling males (Mann-Whitney U-test: $U = 8602$; $Z = -3.84$; $p = 0.0001$). In 90% of the observed events when less than 5 males were calling at a pond ($N = 16$) it was found that no new males were calling, but that males called that were already known from a phase of higher calling activity before. It seemed as if these males simply got on calling after the activity peak.

It was also found that in 1999 Gansé Plaine ponds differed in their calling males activity peaks. While at Central Gansé pond frogs called more or less continuously but stopped early, in the southern and northern Gansé Plaine two activity peaks towards the beginning and the end of the rainy season could be found. (Figure 9)

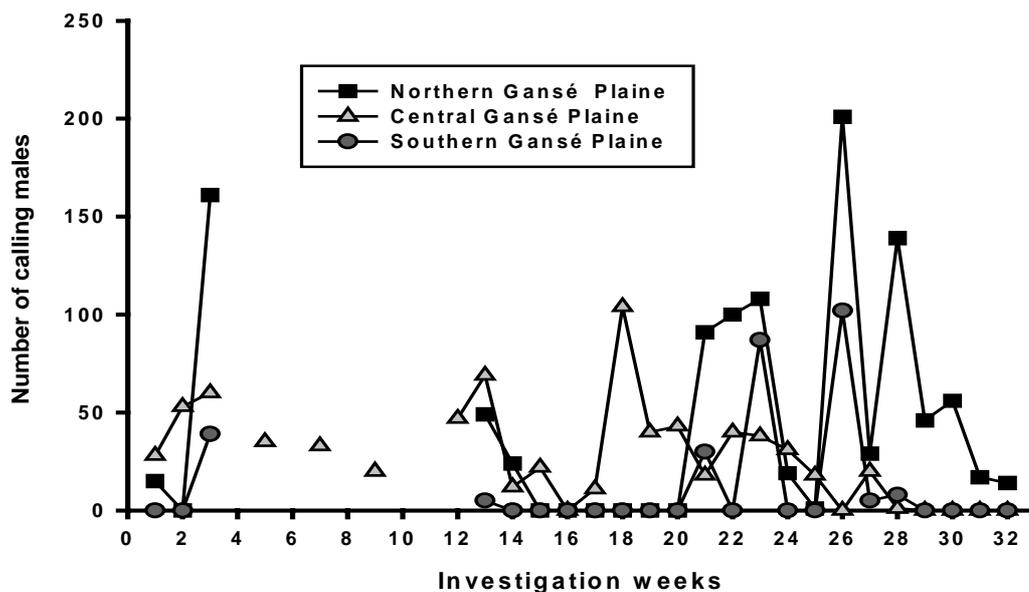


Figure 9: Calling activity at the different Gansé-Plaine areas in 1999. Total number of males calling (newly arriving and already known males) are given.

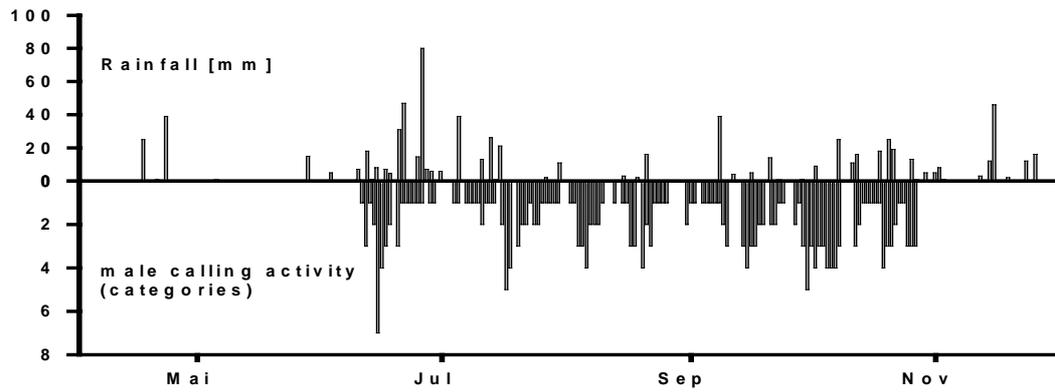


Figure 8a: Rainfall and male activity in 1997.

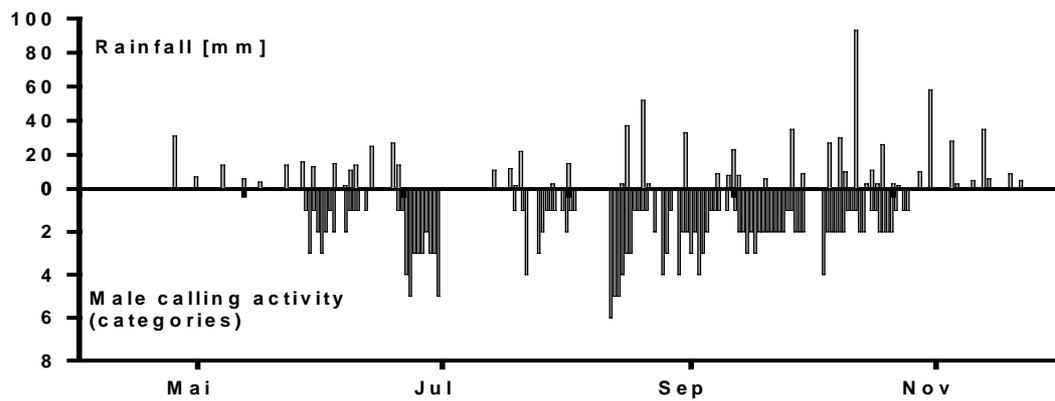


Figure 8b: Rainfall and male activity in 1998.

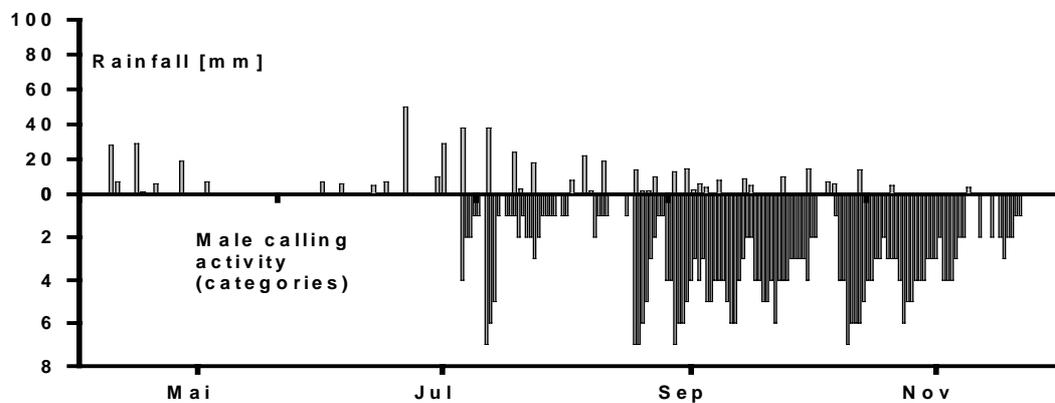


Figure 8c: Rainfall and male activity in 1999.

To further investigate whether the time of day the rain was falling had any influence on the males calling activity day time categories were built (category 1 = rain before 18:00 h, 2 = 18:00 – 20:00, 3 = 20 – 22:00h, 4 = 22 – 0:00h, 0:00 – 6:00 h) and correlated with the number of males calling at the same and at the following night. It was thought that possibly only precipitation events happening before a time limit could increase male activity, because the males need some time to react to the rainfall. Rain falling after the time limit should rather increase male activity at the following night. Data were also tested for any effects of the time during the rainy season. Precipitation timing could be more important as a trigger for male activity at the beginning of the rainy season than at the end of it. However, no correlation of male activity with the time of the rainfall (before or after male activity started) was found (Spearman rank correlations of precipitation timing and male calling activity at the same night: $R = -0.05$, $p = 0.59$; the following night: $R = -0.11$, $p = 0.33$). There is some evidence that time of rainfall is more important at the beginning of the rainy season (Table 4), but due to low numbers only a trend indicating that rainfall between 20:00 and 22:00 h was followed by higher male calling activity that night could be found.

Table 4: Summary of the Median test results, concerning the correlation between day time of precipitation and male calling activity. Pooled data for all years were separated in different months to investigate the influence of the time during the rainy season.

	Number	Chi²	FG	p
June/July	25	6.69	3	0.08
August/September	31	6.17	4	0.18
October/November	26	1.81	4	0.77

Male frogs called (Picture 6) between 18:00 and 2:00 h; the activity peaked around 21:00 h.

Mean calling rate was 2 to 74 calls per minute with a mean of 34 (+/- 28) calls per minute (mean values of 5 minutes counted per frog – total number of frogs: 18). Call rate varied with chorus size, i. e., the more males called, the higher the calling rate of an individual male was (Kruskal-Wallis ANOVA: $p = 0.0025$; $\text{Chi}^2 = 12.0$, $\text{FG} = 2$)

Spacing of the males also changed with the number of frogs calling at one site. The median minimal distance between two calling males was 1.8 m (minimum: 30 cm, maximum: 21.2

m). It varied with chorus size; the more males the smaller the distance (Spearman rank correlation: $N = 30$, $r = -0.55$, $p = 0.0018$). However, two males calling over a longer period of time (several minutes) were never observed closer together than 30 cm. If one male called at a shorter distance both soon called aggressively and sometimes even started to fight. Several fights were observed, always starting with one competitor calling aggressively. After a while the males moved towards each other and started “wrestling”. Kicking and still calling aggressively they tried to push each other away from the calling site (Picture 7). The winner usually started calling shortly after the loser left.

Occasionally several males sat silently next to calling ones. Although this looked like satellite behaviour, mating of “satellite” males was never observed.

Although one single male was heard calling at an investigation site in some cases, normally at least 4 or 5 males were active. A maximum number of callers was evidently not reached at the observation pond. At Hyperolius pond up to 200 individuals were found calling in one night and on some nights at the Gansé Plaine hundreds of males called. The limit had probably not been reached as no males could be observed stopping their activity or leaving the pond to call somewhere else.

Site occupancy

71.5% of all active males appeared in just one investigation week and were never seen again. 17.5% of all individuals were caught in two weeks and 11% could be observed over a period of several weeks. The maximum number of weeks a male could be detected was 7. Males that appeared more than once at the ponds had a median of 2 nights of calling without a break before they went away (= could not be detected anymore). Some of them (50%) returned to the pond after some days to call again for one or several days. The maximum number of calling nights for an individual without a break was 5 (1998 data). The maximum number of calling nights for an individual was 19.

Changing places

Site preferences

On average males called at the water edge at a height of 0.65 m (SD: +/- 0.43 m, range 0-3 m, $N = 98$). They had no favoured calling direction, i. e., males called equally towards and away from the water and in all other possible directions.

A detailed study of the calling site preference of male *Hyperolius nitidulus* in 1997 showed that males differed in their use of calling positions at one pond. Some stayed at the very same point (range about 25 cm) for several days, others changed their calling position from night to night. Two extreme examples are given on Map 4. Male number 4114 (red) had a preferred calling site from which it called for several nights (during day it was gone) and to which it returned for several weeks. Male 231 (blue) wandered around changing its calling place from week to week.

Exchange between ponds

Males were found to change calling ponds if the distances were in the order of several hundred meters. In 1999, 70 out 376 individually marked males that were found more than once were found calling at at least two different ponds (19%). No differences in size (SVL, TF) or weight could be detected between the wandering individuals and other frogs, independent of whether these came only once or more often to the ponds (one way ANOVA $p > 0.15$ for all measurements; Table 5). There was also no higher mating success in the wandering males, wandering (12.7 %) and stationary (14.3 %) individuals had almost the same probability of succeeding in amplexus.

Table 5: Mean sizes of wandering and stationary individuals.

	Number	SVL [mm]	TF [mm]	Weight [g]
Once	1445	27.5 +/- 1.64	12.9 +/- 0.96	1.16 +/- 0.2
More than once (same pond)	306	27.5 +/- 1.4	12.9 +/- 1.2	1.15 +/- 0.2
More than once (different ponds)	70	27.7 +/- 1.5	12.9 +/- 0.7	1.2 +/- 0.2

Towards the end of the rainy season wandering probability for the males changed. In investigation weeks 25 to 32 significantly more of the new males were recaptured during the following weeks ($\text{Chi}^2=12.2$, $\text{FG}=3$, $p < 0.01$) but of these recaptured males significantly less changed ponds ($\text{Chi}^2 = 7.95$ $p < 0.05$) (Figure 10).

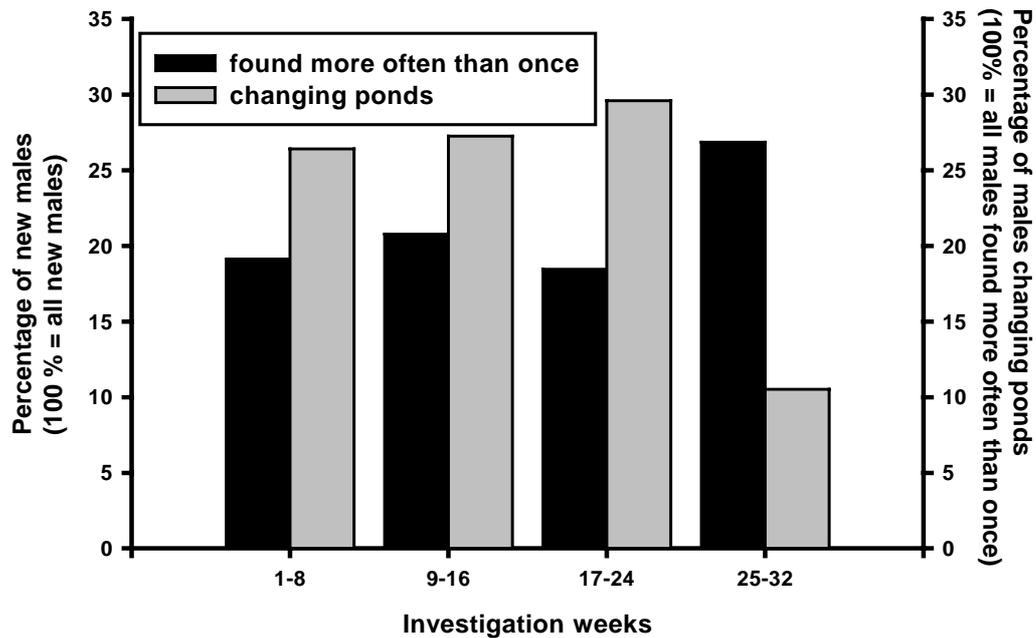


Figure 10: Percentage of new males found more often than once and percentage of wandering males. (Pooled data for 8-week-intervals in 1999)

Although there was some movement, the ponds were not equally attractive. Table 6 shows that many more individuals went to the northern Gansé Plaine ponds (Gansé-Bridge ponds B and B') than to the other ponds. Most of them came from the central Gansé Plaine, approximately 400 m away.

Table 6: Movements of calling males between ponds in 1999.

Gansé Plaine ponds	Leaving the pond	Moving to the pond	Staying at the pond
P (central pond)	22	15	89
B (northern bridge pond)	16	33	63
B' (second northern bridge pond)	15	16	128
S (southern swamp pond)	9	3	20
S' (southern swamp)	8	3	6

It is interesting to note that the attractiveness of a pond seemed to correlate with the pond's capability to store water for a maximum time. Table 7 summarises some pond measurements and the relative values of staying and leaving the ponds. While staying predominates as the male strategy at all larger ponds, 30 % of the frogs left the small swamp pond (S). The Gansé swamp (> 50% leaving) suffered from very high water loss and dried out very, early probably due to its large surface.

Table 7: Relative values of staying and leaving at different Gansé ponds in 1999. (Abbreviations correspond to Table 6)

	Surface [m ²]	Maximum depth [m]	Mean water loss [cm/week]	Calling males*	
				Staying [%]*	Leaving [%]*
P	224	0.5	3.3	85	15
B	450	1.0	4.5	85	15
B'	225	0.9	---	81	19
S	50	0.4	6.9	69	31
S'	2100	0.4	---	43	57

* 100 % = total number of males found more than once

In 1998 wandering behaviour was investigated on a much finer scale looking at some very small ponds in close proximity. On a smaller scale (distances of 5 m to 200 m), changing the calling pond seems to happen more often; 41% of all males caught more than once were found wandering. The males also showed a varying preference for different ponds, but wandering could not be correlated to pond size or fresh filling. Any other obvious advantages like increased access to females could not be detected. Larger chorus seemed more attractive for calling males although no significant result but only a trend was found testing observed male movements towards larger choruses against by chance movements (Chi²: 3.35, p = 0.067).

Another possible explanation for changing ponds was investigated after finding, that several males that already reproduced at the beginning of the rainy season were found again at the ponds later during the rainy season. These long-reproducing males probably face an interesting problem, which could be avoided by changing reproductive ponds: they possibly compete with their own sons for mates (details see chapter 3.2). These males were, therefore, investigated more intensively. For tadpole development a minimum time of approximately 40 days (laboratory results Schmuck et al. 1994) was assumed and the minimum time needed by

a juvenile to mature 45 days was added (85 days ~ 12 weeks). Males that were recaptured over a period of 12 weeks or more were considered to possibly compete with their sons. At the Hyperolius pond 6 males were found (2% of all males found more often than once at Hyperolius pond in 1999) and at Gansé Plaine 16 males were found (4% of all males found more often than once at Gansé Plaine) that met this criterion. The maximum number of days between the first and last capturing of a male were 180 days, while 85 days was the assumed lower limit to possibly encounter own offspring. The number of migrating males (found at another pond than where they were initially marked) was significantly higher in these long reproducing males (69%) than in all males found more than once (19%) ($\text{Chi}^2 = 23.53$ $p < 0.0001$)

Mortality rates

High fluctuation rates were found for the males. Through all rainy seasons many unknown males arrived at the ponds also very late during rainy season, while many marked males were seen just once. Calling breaks of several weeks occurred. Migration between the ponds and towards ponds that were not investigated made it nearly impossible to estimate mortality rates. To get at least a rough estimate of male mortality rates the probability for a male to be recaptured during the following week was calculated. For Gansé Plaine the mean recapture rate from one week to another was 15% +/- 13%, for Hyperolius pond the recapture rate was 23% +/- 15%. The mistake made by estimating recapture rates like this was high. At Gansé Plaine 11 % (sd 7%) of all males were found again not in the first week but after several weeks. At Hyperolius pond 13 % +/- 10% were not found in the following but in a later week. Although the mistake made is large it is speculated that mortality rates for newly arriving males is very high. At least 50% to 75% of the males possibly die before the next week. It was, therefore, especially interesting to note that in 1999 two males were found that definitely already reproduced in 1998. This was evidence for the hypothesis that very scarcely when the conditions are good, a few adults possibly survive the dry season and reproduce a second season.

Reproductive success

As *Hyperolius nitidulus* is a lek breeding species, males face the problem of a heavily shifted operational sex ratio. Hundreds of males are calling but fewer females will come to the ponds to reproduce. The operational sex ratio fluctuated strongly between zero and one female per male and night. The median value was 0.14 females per male and night. Figure 11 shows the

frequencies of observed sex ratios in 1999. It is obvious that relatively low sex ratios (<0.2 females/male) occur most often, which means that male competition for females is high.

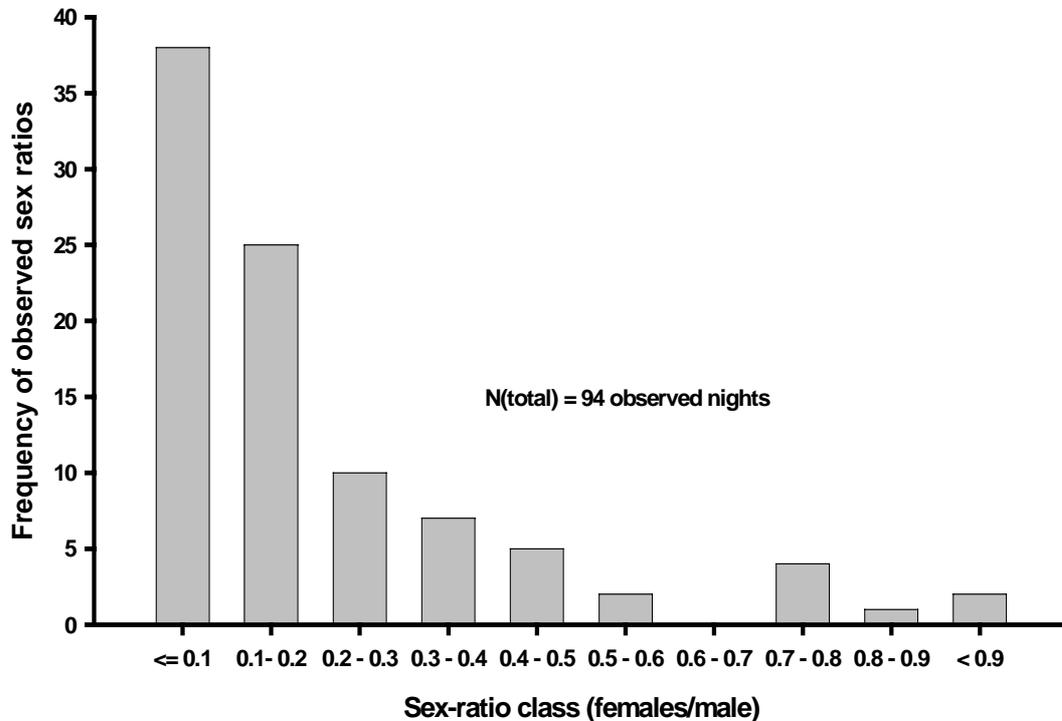


Figure 11: Frequencies of operational sex ratios at all ponds in 1999.

Reproductive success of the males was defined as getting into amplexus. The percentage of males succeeding with more than one copulation did not change significantly between years (1997: 7%, 1998: 11%, 1999: 8% ; Chi² or Fisher's exact test: $p > 0.2$). From 630 couples recorded in 1999, 48 males were in amplexus twice, three were found three times and one male attracted four females for copulation. The main investigation sites: Hyperolius pond (one big pond) and Gansé Plaine (many large and small ponds) differed significantly in the amount of multiple matings of male frogs. Many more males with multiple amplexus were found at Hyperolius pond than at the Gansé Plaine (H: 37 out of 275; G: 15 out of 355; Chi²-test: $p < 0.0001$). This was not observed during the years 1997 and 1998. In 1997, 118 couples were found of which 6 males achieved amplexus twice, in 1998 15 out of 180 successful males reproduced twice and 3 had three reproduction opportunities. In those years no differences between Gansé Plaine and Hyperolius pond were found (Fisher's exact Test: 1997 $p(\text{one-tailed})=0.3491$; 1998 $p(\text{one-tailed})=0.5608$).

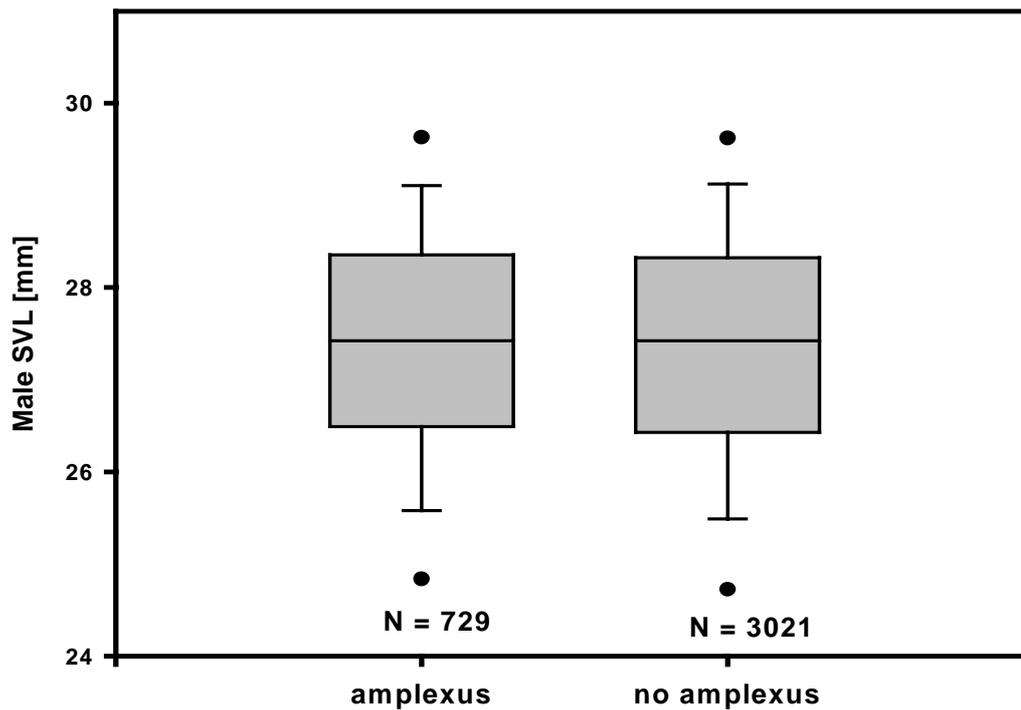


Figure 12: Comparison of male size in couples with unpaired males. Pooled data for all ponds during all investigation years are given. (Box plots: median (line), 10th, 25th, 75th and 90th percentiles (box with error bars), 5th and 95th percentiles (circle))

No investigated individual male feature (e. g. body size) correlated with reproductive success. Males in couples were not bigger than other males. (Figure 12)

Although access to females is probably not controlled by male size, there is evidence that access to females correlated positively with the number of nights spent calling at the ponds. Figure 13 shows that males that stayed several nights at the ponds received more copulations than males leaving directly after a short period of time (1-4 nights). In particular, the proportion of unsuccessful males (no copulation) decreased with longer stays.

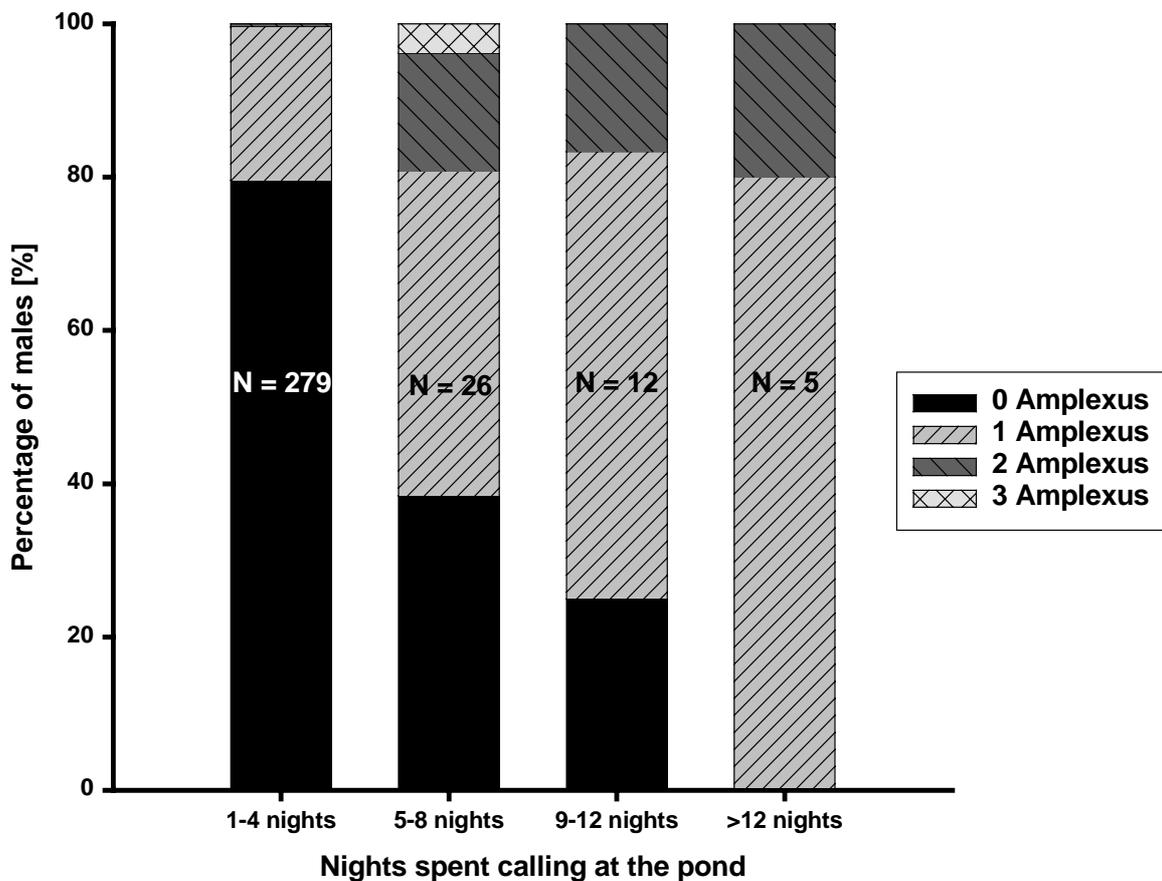


Figure 13: Probability of single and multiple matings for male *Hyperolius nitidulus* calling for different periods of time.

The observed effect seems to be simply additive. No pattern could be detected indicating that after calling for several nights males achieved amplexus more often or that once successful males had a higher probability of also achieving amplexus the following night [cf. Dyson et al. 1998]. The observed effect seems to be due to chance.

Marking effects

Because Clarke (1972) and Golay & Durrer (1994) found negative effects on the survival probability of toe-clipped toads, the percentage of returning males missing one to five toes was calculated (Table 8a).

Table 8a: Toe clipping effects on the survival and reproductive success of male *Hyperolius nitidulus* at Gansé Plaine.

Number of toes missing	Number of marked males	Percentage of returning males	Percentage of males achieving amplexus
one	12	75%	75%
two	124	25%	22%
three	418	21%	21%
four	667	22%	13%
five	401	23%	16%
total number	1622	23%	16%

There were no differences in survival among males missing two to five toes. The high percentage of frogs returning missing only one toe seems more likely to be due to chance, because the total number of males marked in this category was very small (12). Maybe undetected false classification (overlooking of missing toes or an accidentally lost toe) also played a role.

Differences in reproductive success among frogs missing different numbers of toes were calculated using Kruskal-Wallis ANOVA and subsequent Nemenyi test (Table 8b).

Table 8b: Summary of Kruskal-Wallis ANOVA and subsequent Nemenyi test results for differences in reproductive success of males missing different numbers of toes. (Significant results are shown in bold letters.)

	Chi²	df	p	
<u>K-W ANOVA</u>	26.23	4	<0.001	
Nemenyi -test	two (13%)	three (22%)	four (7%)	five (9%)
one (55%)	0.017	0.63	0.001	0.002
two	----	0.8	0.95	0.98
three	----	----	0.03	0.12
four	----	----	----	0.99

(percentage of males found in amplexus)

Significant differences were found between males missing one toe (these seemed to have higher reproductive success) and the rest of all males, but this is probably due to the high recapture rate and low number of animals included in group 1 (see above). Males lacking

three toes seemed to have had higher reproductive success than males lacking four toes but not than males lacking two or five toes.

Since there was no clear pattern showing a negative correlation between numbers of toes missing and reproductive effect or numbers of toes missing and survival probability, it was assumed that toe-clipping had no considerable effect on life history traits in *Hyperolius nitidulus*.

Summary

In three investigation years 3750 male frogs were marked individually. Mean male frog size was 27.5 mm snout-vent-length. Male activity was correlated with rainfall, but did not stop completely during dry intervals. Males differed in calling behaviour: most of them stayed at the same ponds and even at the same place for weeks, but some wandered. Ponds seem to be differently attractive for calling males although a reason for this difference was not obvious. No male features were found that increased reproductive success other than the duration of stay at a pond (the more nights a male calls, the higher the probability of attracting at least one female).

3.1.2.2 Female biology

Female size

Females were found less often at the ponds than males. (cf. sex ratio calculations in 3.1.3.1) In all three investigation years only 1041 females, in contrast to 3750 males were found. The Gansé Plaine ponds yielded the largest numbers, Iringou the lowest due to the fact that it was sampled just one season. 585 females were found at Gansé Plaine, 377 at Hyperolius pond, 59 at Lola pond and 11 at Iringou pond.

Female morphometry and weight are summarised in Table 9.

Table 9: Summary of female measurements.

	total years' means	minimum	maximum
SVL [mm]*	28.7 +/- 1.43	20	33
TF [mm]*	13.5 +/-0.8	10	16
Weight [g]* (after oviposition)	1.36 +/- 0.3	0.7	2.4

***mean +/- standard deviation**

Pearson-Product-Moment-Correlation revealed a strong correlation between female snout-vent length and female tibia-length and slightly weaker correlation for either of these two variables with female weight. All these correlations had lower r^2 then for the males (Table 10).

Table 10: Summary of female size correlations (Pearson-Product-Moment).

	p-values	r²-values	number
SVL versus TF	<0.01	0.58	1021
SVL versus weight	<0.01	0.48	1021
TF versus weight	<0.01	0.28	1021

Female weight correlated strongly with clutch size (Pearson-Product-Moment correlation $p < 0.05$, $r = 0.44$; Figure 14) and correlated weakly with egg size (Pearson-Product-Moment correlation $p < 0.05$, $r = 0.08$; Figure 15). This means bigger females have bigger clutches but not necessarily bigger eggs. Actually a negative correlation between clutch size and egg size was found (see below: clutches).

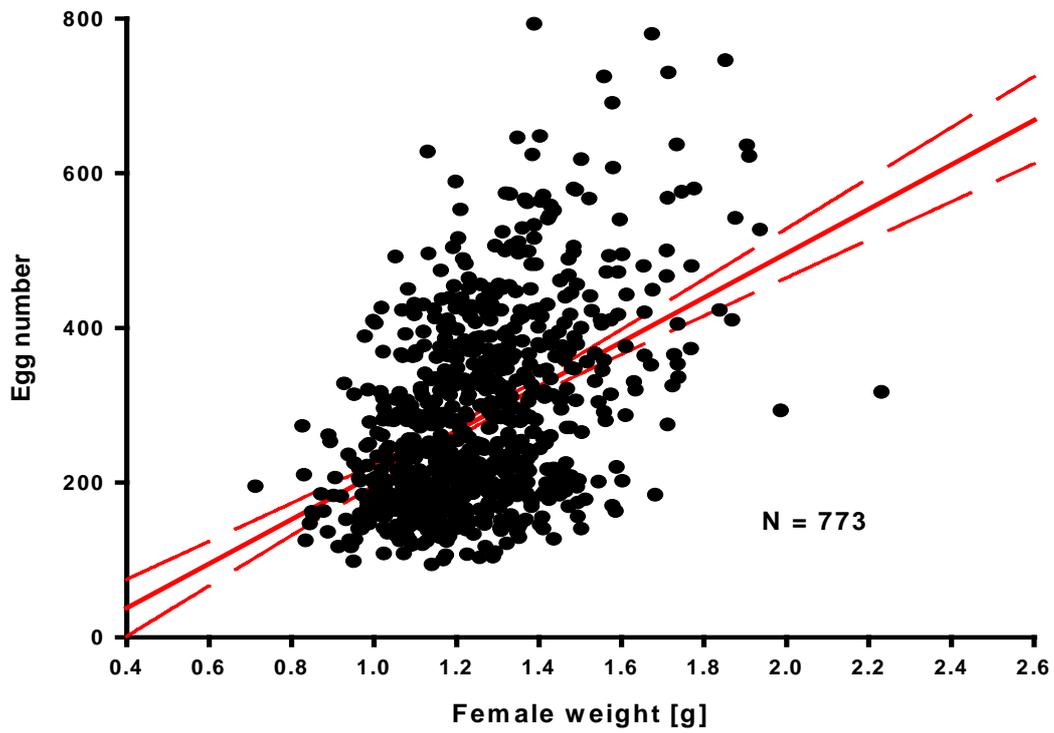


Figure 14: Pearson-Product-Moment correlation of female weight versus clutch size.

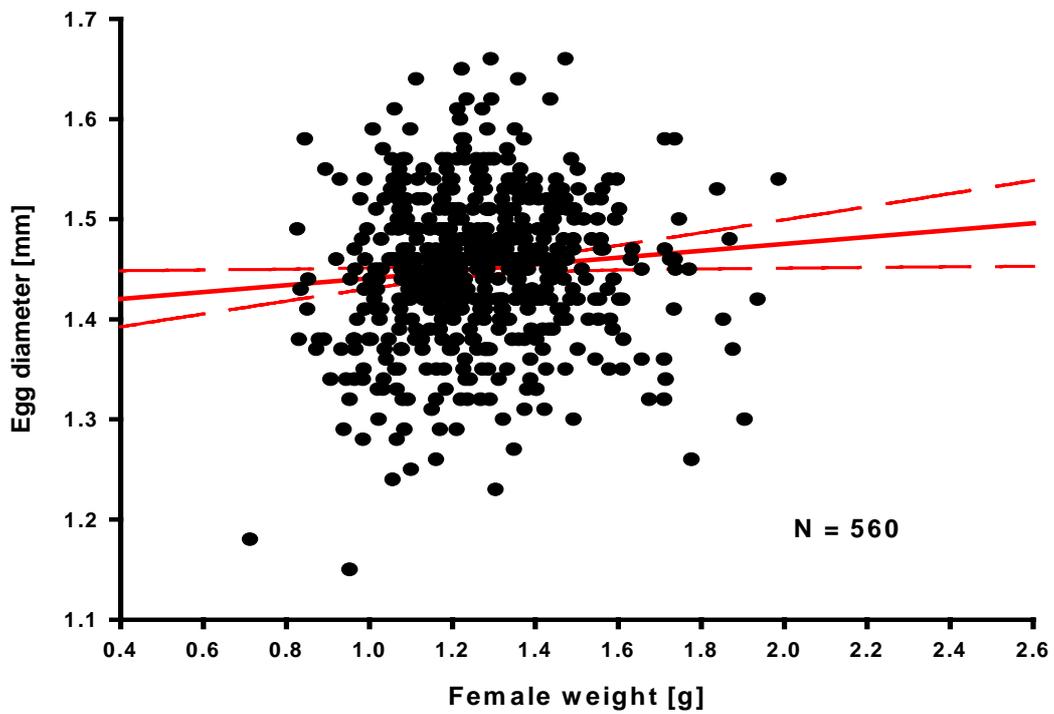


Figure 15: Pearson-Product-Moment correlation of female weight versus egg size.

Females found in 1997 were significantly smaller than females found in the other years (one-way ANOVA followed by Scheffé test $p < 0.01$). In 1998 and 1999 no size differences between the females could be detected (Table 11a/b).

Table 11a: Summary of female measurements comparing 1997 - 1999.

	1997	1998	1999
Mean SVL [mm]*	28.3 +/- 1.5	28.8 +/- 1.5	28.7 +/- 1.5
Mean TF [mm]*	13.4 +/- 1.0	13.7 +/- 1.0	13.5 +/- 1.0
Mean weight [g]*	1.26 +/- 0.2	1.30 +/- 0.2	1.40 +/- 0.3

* +/- standard deviation

Table 11b: Summary of ANOVA followed by Scheffé test for female size differences between years (F=4.83, p=0.008).

Scheffé test	1998	1999
1997	0.0158*	0.0158*
1998	----	0.7979

Between the ponds there were also some differences in size that almost matched the differences for males (Table 12a/b). Lola pond females were significantly larger than Gansé and Hyperolius pond females. Gansé Plaine females were significantly larger than Hyperolius pond females and Iringou males did not differ from any of the other ponds, which could probably be a consequence of the low number of females present.

Table 12a: Summary of female measurements comparing different ponds.

	Gansé Plaine	Hyperolius pond	Iringou pond	Lola pond
Mean SVL [mm]*	28.8 +/- 1.43	28.4 +/- 1.33	28.6 +/- 1.33	29.7 +/- 1.40
Mean TF [mm]*	13.6 +/- 0.76	13.4 +/- 0.73	13.5 +/- 1.10	14.2 +/- 0.73
Mean weight [g]*	1.40 +/- 0.30	1.26 +/- 0.20	1.30 +/- 0.24	1.49 +/- 0.25

* +/- standard deviation

Table 12b: Summary of ANOVA followed by Scheffé test for female size differences between ponds (F=18.06, p=0.00000).

Scheffé test	<u>Hyperolius pond</u> (mean: 28.37)	<u>Iringou pond</u> (mean: 28.62)	<u>Lola pond</u> (mean: 29.71)
<u>Gansé pond</u> (mean: 28.79)	0.00152*	0.98554	0.00004*
<u>Hyperolius pond</u>		0.94998	0.00000*
<u>Iringou pond</u>	0.94998		0.13198

Hyperolius nitidulus females were significantly larger than males. (T-Test $p < 0.01$ for all body measurements – Table 13)

Table 13: Summary of the T-test results comparing all body measurements between male and female *Hyperolius nitidulus*.

	Male	Female	t	P
SVL	27.35 +/- 1.49	28.67 +/- 1.43	-25.61	< 0.0001*
TF	12.86 +/- 0.81	13.53 +/- 0.78	-24.10	< 0.0001*
Weight	1.15 +/- 0.19	1.36 +/- 0.27	-28.09	< 0.0001*

Female activity

Females were found mostly in couples (86%) (Picture 8) and females found individually at the ponds always laid eggs when given a male, hence it was concluded that they visited the ponds only for reproduction. Most coupled females appeared between 20:00 and 21:00 h, single females were found earlier, usually before 20:00 h. In contrast to females of some other frog species (e.g. *Hyperolius nasutus* Rödel 1996) they do not spend several nights at the ponds before oviposition takes place, but spawn the night they come to the pond and leave afterwards.

Population sizes could not be estimated for female frogs, as the recapture rates were too low.

Female and male activity were plotted in relationship with precipitation (Figure 16) to analyse whether female reproductive activity was more closely related to rainfall than male activity. It seemed as if females came to the ponds in higher numbers directly after rainfall, but a correlation between rainfall and male and female activity revealed that female activity correlated strongly with male activity but not with rainfall (Table 14). Male activity explained 43% of the observed variability in female activity.

Table 14: Spearman rank correlation between male and female activity and precipitation in 1999.

	Number	Spearman R	r ²	p
Rainfall versus male activity	94	0.266	0.07	0.009*
Rainfall versus female abundance	88	0.181	-----	0.09
Male versus female activity	88	0.657	0.43	0.0000*

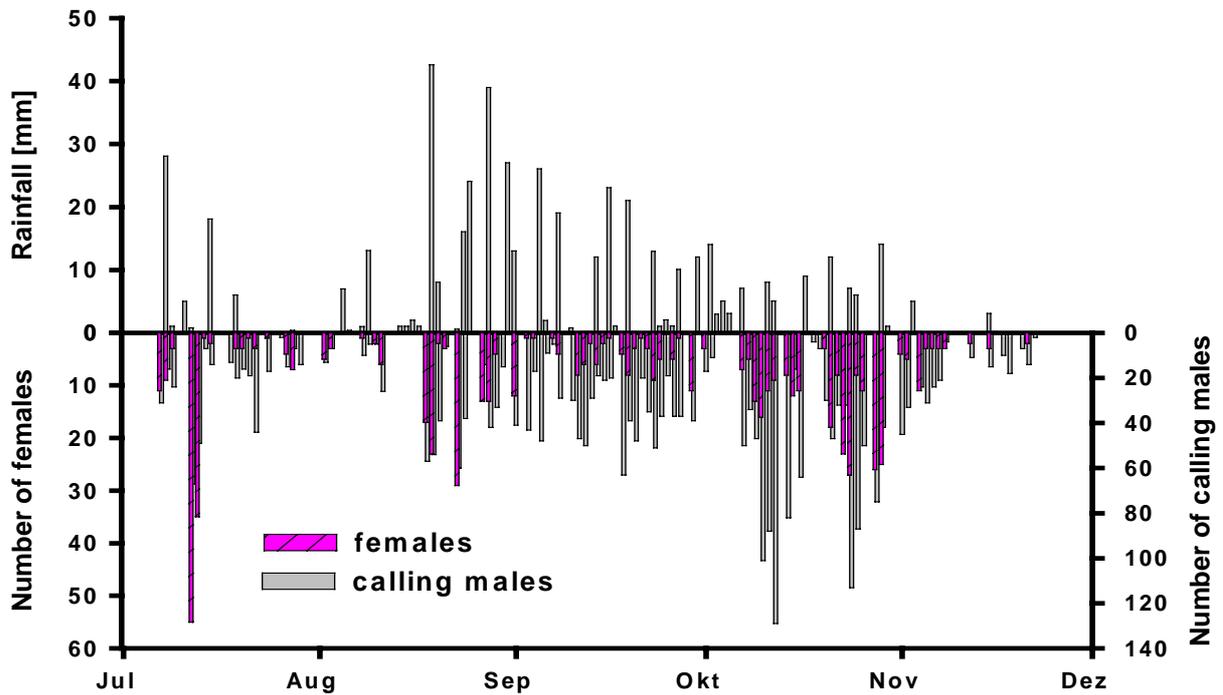


Figure 16: Precipitation, female abundance and male activity at the *Hyperolius* pond in 1999.

Duration of stay and change of ponds

Female frogs were never seen in two successive nights at a pond. The observations point to the following sequence of events: female frogs come to the pond, find a male, sit in amplexus for a while (1-3h), spawn and leave immediately afterwards.

Females were only scarcely found twice or more often at oviposition sites. Of the 1041 females marked individually only 30 individuals (3%) were found more than once, 25 twice, 4 three times and one five times. The clutch size decreased when females came more than once for spawning. On average each following clutch was about 70 eggs smaller than the one before. The minimum time period between two ovipositions was 10 days; on average it was three weeks.

Females did not tend to change the ponds for repeated spawning. Of all 30 females found twice or more often, only one changed its spawning pond (distance: 170 m moving from a

very small (5 m²) to a much larger (250 m²) pond). All the others, even when spawning three or five times, stayed at the ponds they were found first.

Female choice

As very many calling males are confined to a rather small space at the ponds and the sex ratio is heavily biased towards males (cf. male reproductive success), it should be easy for females to choose a partner for spawning. However, no morphological feature that made males preferable to the females could be found. There was no preference for bigger males (Figure 17) as would have been expected, because in other *Hyperolius* species female preference for larger males is known [Dyson & Passmore 1988].

A correlation of male SVL versus female SVL is significant ($p=0.001$), but only because of the very large number of data points ($n = 769$). Only 1.3% of the variability is explained by matching sizes of males and females (Figure 18) (Pearson-Product-Moment correlation: $r = 0.116$).

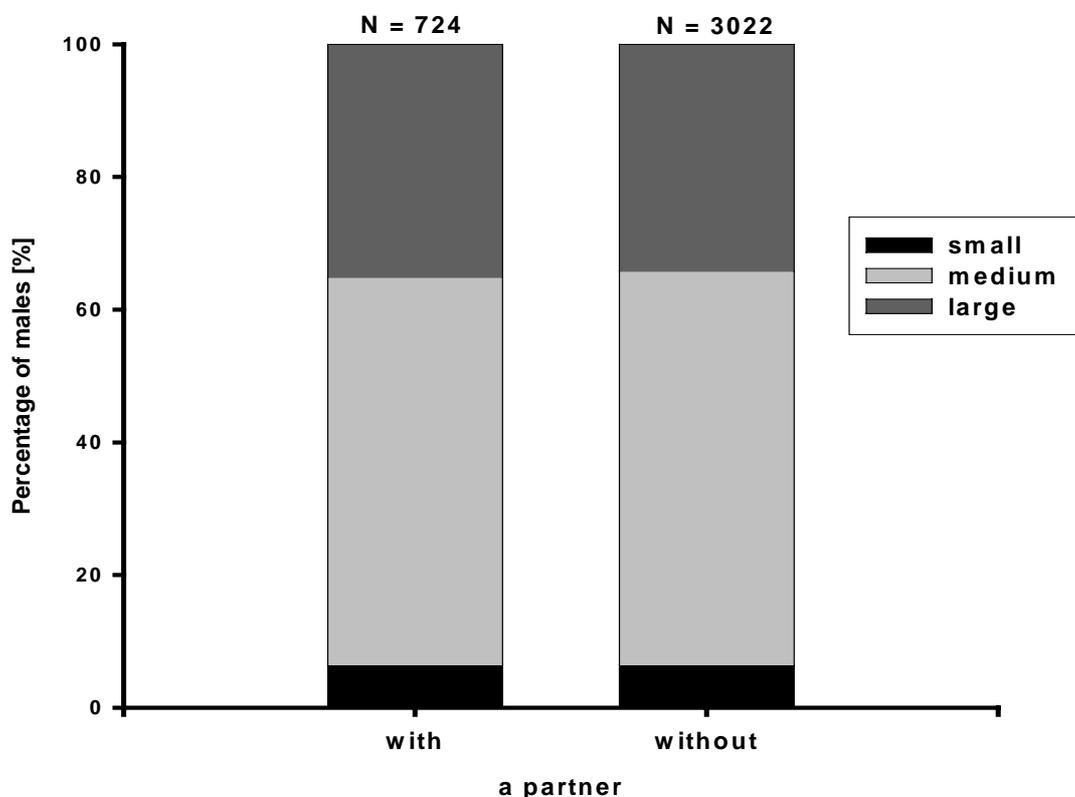


Figure 17: Proportion of small (SVL < 25 mm), medium (SVL 25 - 28 mm) and large (SVL > 28 mm) males found in couples and solitarily at all ponds.

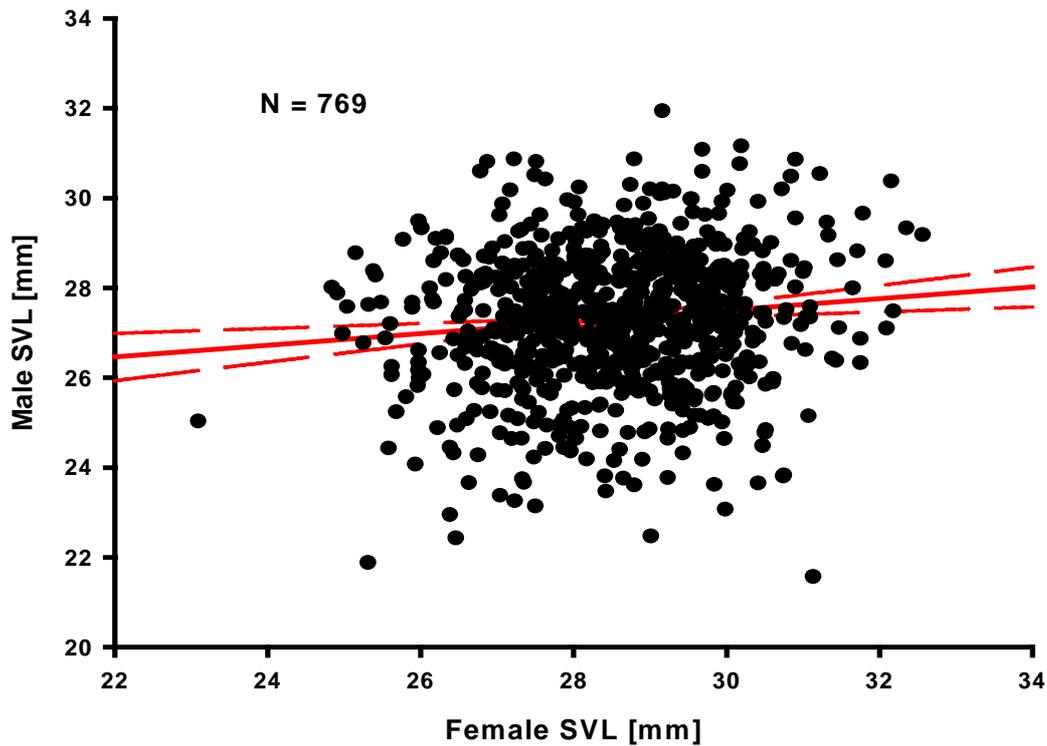


Figure 18: Correlation between coupled male and female sizes.

Summary

Females are bigger than males but found less often at the ponds. They stay at the pond only for spawning. Only a small proportion of females lays multiple clutches. Clutch size decreases when multiple clutches are laid. There is a slight correlation between female and male body size, indicating that females prefer males of their own size, but it remains unclear whether this is a direct size effect or the result of an additional size-related trait.

3.1.2.3 Clutches

Almost all couples found during the years in question accepted the aquaria for spawning. Even couples separated during catching or transport, joined again after less than one hour when left alone. No differences (egg number, fertilisation rate) between these and other clutches could be found. All eggs were deposited under water. Females attached them in small portions between one and 100 eggs (mean: 30 eggs) to the ground and the provided plant material. Eggs found above water level were normally not fertilised.

During the three years of observation a total number of 779 couples was collected. With the exception of one, all females spawned over night in captivity. Of the 288 couples collected in 1997 and 1998 only 7 clutches were not fertilised at all. Fertilisation rate varied between 100% and 1% (1998: mean 82.13 +/-23-51% - median 89.24%, 1997: mean 87.7 +/- 0.199% median: 96%). In 1999 eggs were not kept long enough to tell for sure if they were fertilised.

The following table summarises the results pooled for all investigation years:

Table 15: Number of eggs per clutch and egg diameter [mm]. Polled data for all ponds and years.

	Egg number	Egg diameter
Number of clutches	773	560
Mean +/- sd	285 +/- 125	1.45 +/- 0.07
Minimum	94	1,15
Maximum	793	1,66

The most obvious difference between the year 1999 and the years 1997 and 1998 was the large amount of couples found in the season of 1999. The reproductive season in 1999 started very early and was extremely long. Activity levels were much higher than in 1997 and 1998 (see male behaviour 3.1.2.1).

In 1997 and 1998 every couple found at any pond was collected and its clutch and egg size was measured, but in 1999, 630 couples were found in the field, which were not all kept in the camp. Although sizes of females and males of the couples were measured and the individuals were marked, only a subsample of not more than 16 clutches per night was taken into the laboratory.

There was a slightly negative correlation between clutch size and egg size (Pearson Product Moment $p=0.007$) (Figure 19), indicating that larger clutches contain smaller eggs, but again

the correlation is significant because of the large number of observations. Egg number explains only 1.2% of the variation in egg size.

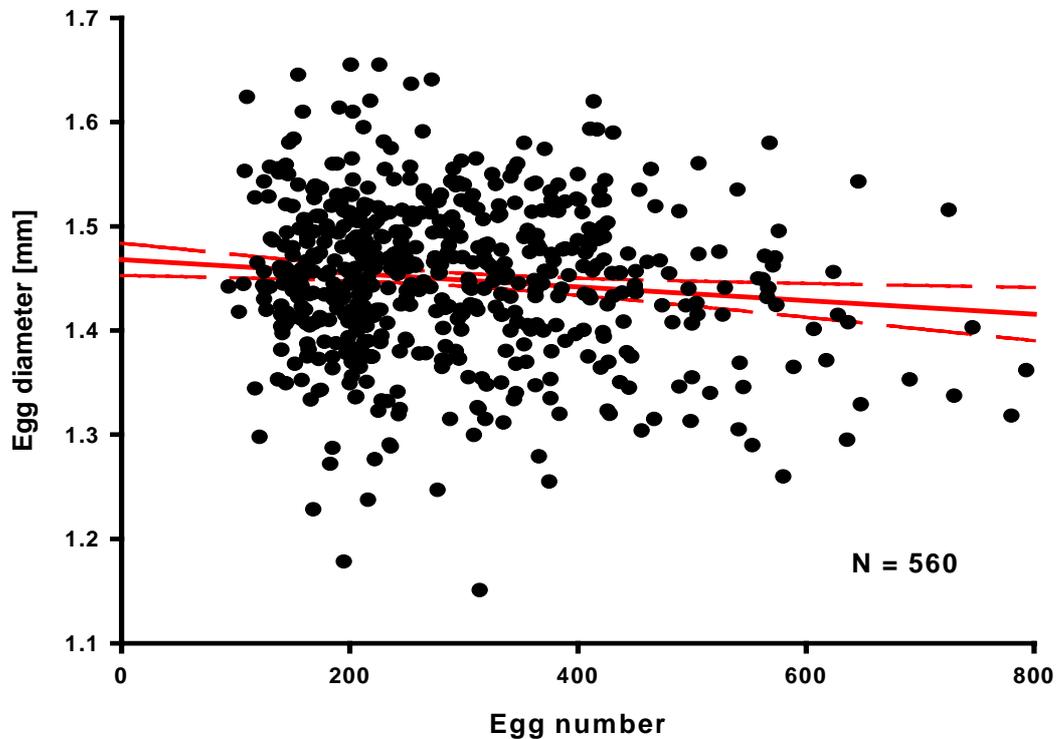


Figure 19: Correlation between clutch size and egg size.

Clutch size showed results parallel to female size considering differences between years and ponds. Table 16 summarises the statistical results of ANOVA calculation using year or pond as factor and female weight as a covariant.

Table 16: Summary of ANOVA and following Scheffé test results for clutch size (differences between years and ponds).

<u>Year</u>	<u>Egg number</u>	<u>Female weight</u>
1997	306.9	1.26
1998	342.1	1.29
1999	249.4	1.24
Scheffé test (clutch size)		
	1998	1999
1997	0.02*	0.00002*
1998	----	0.00000*

(continuation Table 16)			
Pond	Egg number	Female weight	
Gansé	301.1	1.25	
Hyperolius	246.8	1.23	
Iringou	297.8	1.30	
Lola	455.6	1.49	
Scheffé test (clutch size)			
	Hyperolius	Iringou	Lola
Gansé	0.38	0.49	0.0002*
Hyperolius	----	0.24	0.001*
Iringou	----	----	0.002*

Clutch size differed significantly where female size differed significantly, e.g. between the years 1997 and 1999 and between Lola pond and all other ponds.

Egg diameter was also tested for significant differences between years and ponds with an ANOVA and following Scheffé test, using egg number as a covariant this time (Table 17).

Table 17: Summary of year and pond differences in egg diameter.

Year	Egg diameter	Egg number	
1997	1.46	306.9	
1998	1.46	342.1	
1999	1.44	249.4	
Scheffé test (egg size)			
	1998	1999	
1997	0.97	0.02*	
1998	----	0.002*	
Pond	Egg diameter	Egg number	
Gansé	1.46	301.1	
Hyperolius	1.45	246.8	
Iringou	1.49	297.8	
Lola	1.39	455.6	
Scheffé test (egg size)			
	Hyperolius	Iringou	Lola
Gansé	0.39	0.50	0.000009*
Hyperolius	----	0.25	0.0007*
Iringou	0.25	----	0.002*

Egg diameter differed significantly between 1999, 1997 and 1998, this may also be due to the differences of female sizes between these years. The eggs found at Lola pond are significantly smaller than the eggs found at all other ponds. This is interesting in comparison with the clutch sizes, which were significantly larger at Lola pond. Differences between Lola and the other ponds were due to differing sampling times.

Like female size, egg and clutch sizes did not differ significantly between different Gansé Plaine ponds.

Clutch size and egg size were not only related to female but also to male size. This is not surprising if larger females prefer larger males (cf. 3.1.2.2 female choice). However, as for the female weight – clutch size relation, the male weight correlated to a slightly weaker degree of explanation of 16% (females: 20%) (Pearson-Product-Moment correlation: $p < 0.001$) (Figure 20). Male weight did not correlate significantly with egg diameter.

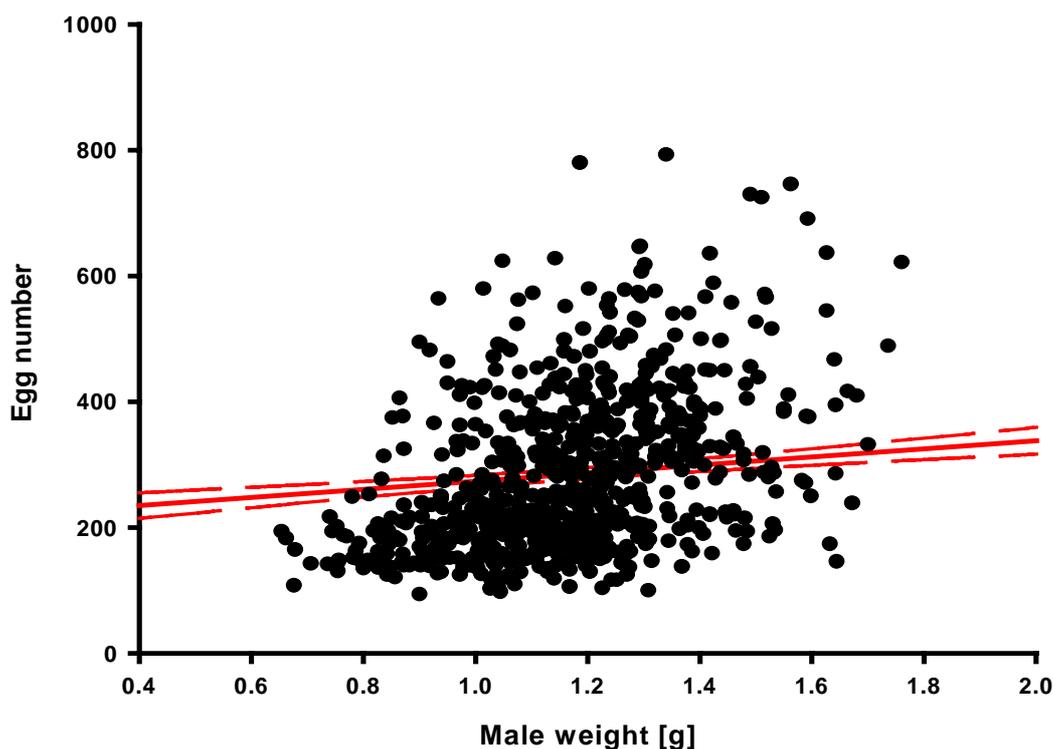


Figure 20: Correlation of male weight versus clutch size.

Summary

Clutch size and egg size are significantly but weakly negatively correlated. Clutch size differed between the years and between all ponds and correlated with female and male size, while egg size was not so variable.

3.1.2.4 Tadpoles

Electrofishing revealed differences between the two main investigation sites. In Hyperolius pond many potential predators like dragonfly larvae and turtles were found, tadpoles, however, were always present in high densities. In Gansé Bridge pond which was the only pond at the Gansé Plaine nearly the size of Hyperolius pond, tadpoles were scarce or could not be found at all. Fish, even big ones, were very common. The differences between the ponds could be observed over several weeks, but sampling was not standardised, therefore, no statistical tests were used on the data.

3.1.2.5 Juveniles

First juveniles (Picture 9) were found in all years (1997-1999) about two months after the start of reproduction. Table 18 summarises juvenile numbers and sizes in all investigation years. Most juveniles were found in 1999 mainly because Hyperolius pond, which almost totally failed the years before, released many juveniles.

Table 18: Juvenile numbers and sizes in 1997, 1998 and 1999.

	1997	1998	1999
Number	41	723	1722
Mean SVL [mm]*	12.8 +/- 0.9	13.2 +/- 0.7	13.8 +/- 0.8
Mean TF [mm]*	5.9 +/- 0.5	6.0 +/- 0.42	6.3 +/- 0.5
Mean weight [g]*	0.15 +/- 0.03	0.17 +/- 0.03	0.21 +/- 0.08

* +/- standard deviation

The sizes of the freshly metamorphosed frogs ranged from 11.5 mm to 16.1 mm body length. The juveniles weighed between 0.09 g and 0.32 g. Juvenile body size differed by 1 mm from 1997 to 1999. In 1999, the numerous juveniles from Hyperolius pond were bigger than those hatched from Gansé Plaine. Figure 21 shows the mean body sizes of freshly metamorphosed juveniles originating from Hyperolius pond and Gansé Plaine, respectively. The size difference between juveniles from Hyperolius pond and those from Gansé pond was significant (Mann-Whitney U-test $p < 0.05$).

Juveniles from Gansé Plaine were consistently smaller than juveniles from Hyperolius pond both in 1998 and 1999. This effected the mean sizes in different years (Table 18) as the recruitment of (larger) juveniles from Hyperolius pond was so high in 1999 (cf. Figure 21).

Juvenile body sizes in individual ponds remained constant in different years.

Mean body length values varied little during the course of the rainy season 1999, but there were differences between the investigated ponds. While juveniles from Hyperolius pond were larger and size seemed to increase at the beginning, to decrease after the break in abundance, those from Gansé Plaine were smaller and sizes seemed to fluctuate in cycles of several weeks (Figure 22).

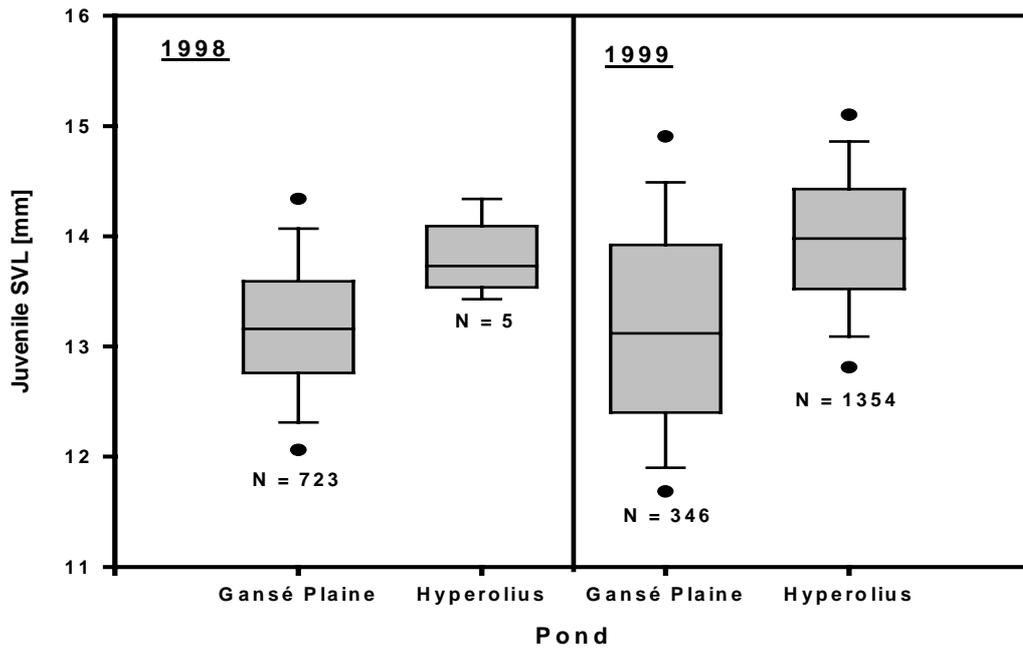


Figure 21: Mean juvenile sizes (SVL) at Hyperolius pond and Gansé Plaine in 1998 and 1999. Box plots give median (line), 10th, 25th, 75th and 90th percentiles (box with error bars) and 5th and 95th percentiles (circle).

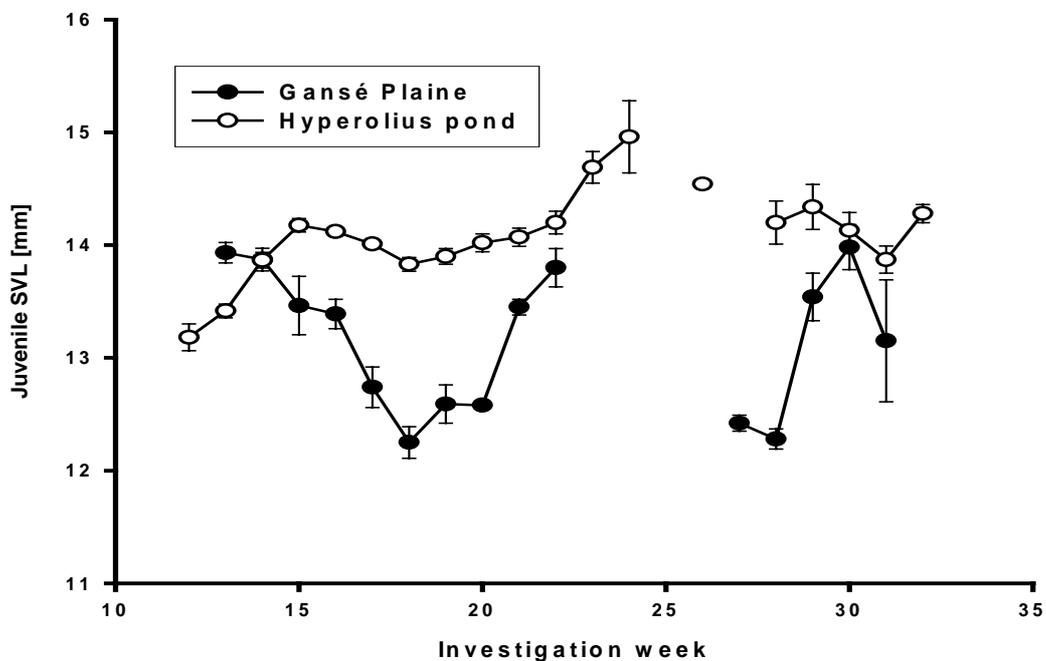


Figure 22: Mean body size plus standard deviation (SVL) of freshly metamorphosed young leaving the ponds in 1999 (mean values, the error bars give the standard errors).

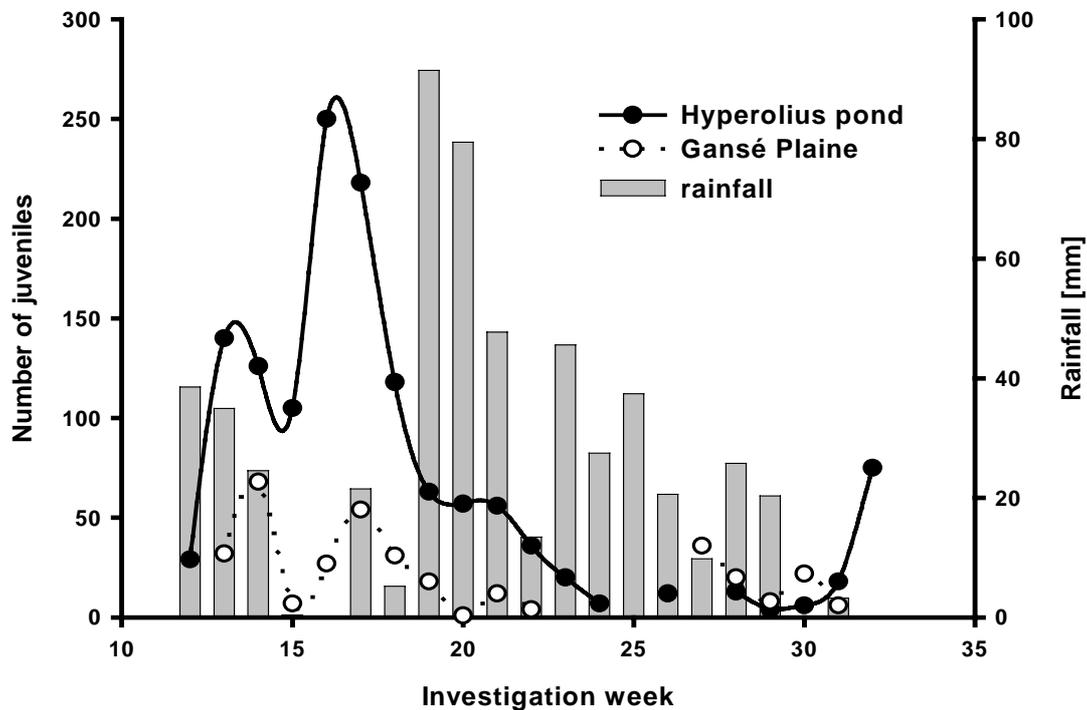


Figure 23: Precipitation and metamorphic rates (juveniles leaving the ponds) in 1999.

Juvenile activity changed in the middle of our investigation time. Figure 23 shows that the number of emerging juveniles decreases despite the rising rainfalls. Probably predators (invertebrates, migrating fish) reduced the number of tadpoles metamorphosing successfully. Numbers of juveniles leaving the pond after metamorphosis peaked in both ponds before the main rainfalls occurred.

There seems to be evidence for serious predation-losses of juveniles at least for the Gansé Bridge pond, as neither *Hyperolius nitidulus* nor any other freshly metamorphosed frog could be found at this pond after investigation week 22. In contrast, although *H. nitidulus* juveniles became scarce in Hyperolius pond in the second half of the rainy season, other species, especially *Afrivalus vittiger*, were still quite common (Figure 24).

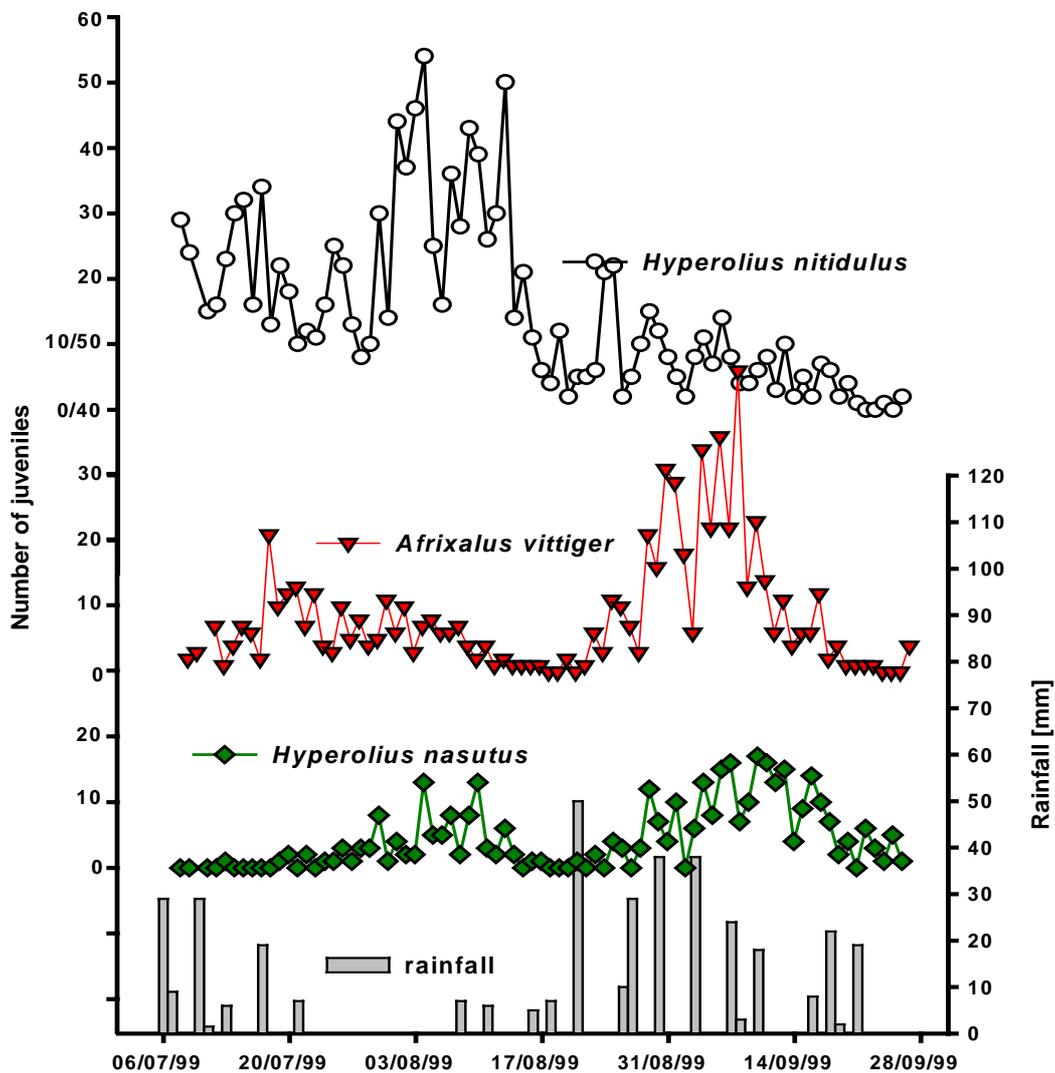


Figure 24: Abundance of freshly metamorphosed juveniles of various frog species during the rainy season of 1999 at Hyperolius pond. Individual zero lines on the y-axis were used for a better species discrimination.

When leaving the water juveniles climbed the vegetation and remained there for about at least one hour (median value: 3 h) before they disappeared. Short time marking with a paint dot on the back revealed that most juveniles left the ponds between 23:00 h and midnight (Figure 25a). Some individuals spent up to 8 hours at the pond (Figure 25b) before leaving.

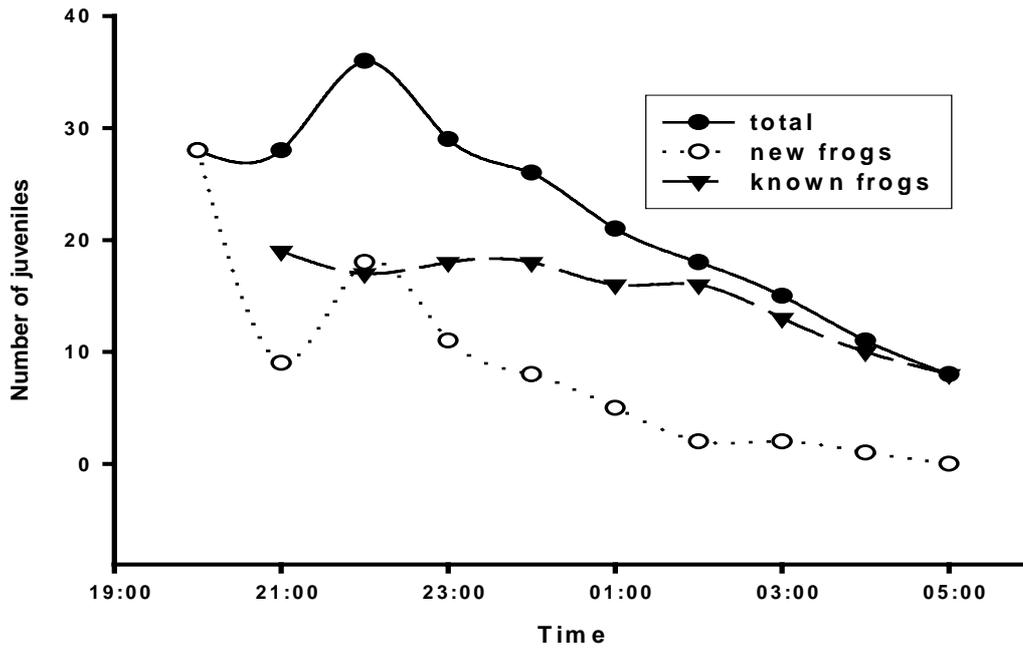


Figure 25a: Abundance of known (marked) and new (unmarked) juveniles at Gansé pond during one night.

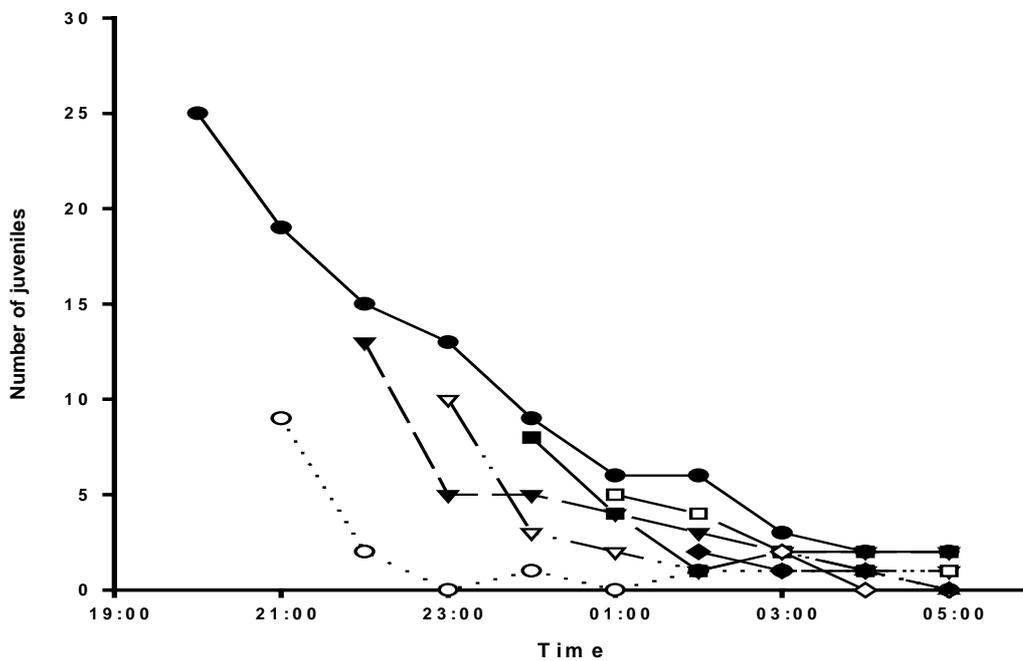


Figure 25b: Abundance of groups of juveniles *H. nitidulus* at the pond after they had left the water. Symbols denote members in groups of individuals that have been marked with a colour code at varying times during the night. The marking time is reflected by the first symbol in every line.

Summary

Different ponds produce juvenile *Hyperolius nitidulus* of different size. During the rainy season, juvenile size in an individual pond does vary but differences among ponds remain constant. The number of juveniles leaving the pond changes dramatically. Juveniles leave the water mainly between 21:00 and 0:00 h and stay at the pond for at least 2-3 hours.

3.1.3 Discussion

Methods

Capturing

Due to the technique used of catching frogs at the different ponds only once a week, a high percentage of individuals was not registered at all or was registered later than their first arrival. This is reflected by the relatively low recapture rates of males, females and juveniles and in the high number of unknown frogs arriving late at the ponds for reproduction. Also male reproductive success was underestimated because not all matings during all nights at all ponds could be observed.

Adults and juveniles were caught by hand simply by grabbing for visible frogs. Few animals escaped, but it cannot be excluded that frogs hid and were overlooked by the collectors. A drift fence, widely used to capture frogs quantitatively when entering ponds [e.g. Berven & Grudzien 1990, Sinsch 1997, Kaminsky et al. 1999], did not seem to be appropriate here because *Hyperolius nitidulus* is easily able to climb over fences even if they are constructed with an overhanging edge. Due to the high number of ponds that had to be visited it was rarely possible to capture frogs at a special pond more often than once a week.

Marking

No clear pattern of negative effects of toe-clipping on the survival probability or the reproductive success of *Hyperolius nitidulus* could be detected in this study. It was, therefore, concluded that toe-clipping had no considerable effect on life history traits in *Hyperolius nitidulus*.

Richards et al. (1975) found that clipped toes of juvenile *Hyperolius* regenerated after a while, therefore making unequivocal recognition of individuals difficult. For the adults marked with toe clipping, regeneration of toes was observed, but the marking always stayed clear and problems with uncertain identification were very scarce (two cases). Thus, toe-clipping appears to be a reliable marking technique for adult *Hyperolius nitidulus*.

Breeding system

The characteristics of the breeding system of *Hyperolius nitidulus* found in this study resemble the reproductive system characteristics of many frog and toad species [Cherry 1992, 1993, Werner 1977, Emlen 1976, Sullivan 1982, 1983, Sullivan & Hinshaw 1992, Arak 1988, Robertson 1986b] including other *Hyperolius* species, for example *H. marmoratus* [Grafe

1997, Rödel 1996, Telford & Dyson 1988, Schiøtz 1977]. Males aggregate and defend small display territories, while female frogs choose a mate from the displaying males but do not utilise any resource within the male's display territory. No resources are defended by the males and couples normally spawn several metres away from the calling site. This behaviour resembles the definition of leks given by Bradbury 1981, Bradbury & Gibson 1983, Höglund & Alatalo (1995), Krebs & Davies (1993) and Sullivan et al. (1995).

With its reproductive period of several months (up to 8 months in 1999), *Hyperolius nitidulus* can clearly be classified as a prolonged breeder [Wells 1977].

Female choice

Female ability to differentiate between call features like call frequency (indicating male size) or call duration is documented for several anuran species [Howard 1988, Robertson 1986, Murphy & Gerhardt 2000] and the adaptive values of female choice are shown [Côté & Hunte 1989, Robertson 1990, Bourne 1993, Böll & Linsenmair 1998]. The lek-breeding system of many *Hyperolius* species is thought to provide ideal conditions for selection [Wells 1977, Janetos 1980, Arak 1983, Duellman & Trueb 1986, Bourne 1992], and the ability of female *Hyperolius* to detect even small differences between male mating calls is well documented [Bishop et al. 1995, Dyson & Passmore 1988a+b, Passmore et al. 1992, Jennions et al. 1995a+b]. However, the results of this study show that female *Hyperolius nitidulus* showed neither a preference for larger males nor size assortative mating. They also did not prefer older mates. The question is: why are *H. nitidulus* females not selective?

Two explanations are possible: 1) There may be constraints on male quality recognition. Females may not be able to make reliable choices in the complex field situation. Phonotactic choice experiments that revealed female preferences were almost always two-loudspeaker experiments with alternating calls. Four-loudspeaker experiments [Bishop et al. 1995] and the testing of simultaneous calls [Dyson & Passmore 1988b] revealed much weaker preferences and even random responses on the part of female frogs. Investigations in the field showed a high percentage of overlapping calls, and the situation is when hundreds of males are calling at the same site much more complicated than in clear-cut laboratory choice experiments.

2) The costs of female choice may outweigh the benefits [Pomiankowski 1987]. Possible benefits of selecting a particular male can be separated in direct and indirect components. In *Hyperolius nitidulus*, where males contribute nothing more than sperm to the offspring, a direct benefit would be to optimise the fertilisation rate, as each unfertilised egg is a loss of

energy for the female. If the female can produce only a limited egg number, egg loss results in reduced reproductive fitness. In some anuran species, small males are not able to fertilise the same amount of eggs as large ones, and large males are favoured [Böll & Linsenmair 1998; Jennions et al. 1995a+b; Mathis 1991]. Assortative mating can also reduce the risk of producing unfertilised eggs [Robertson 1990] as in external fertilisation the proper fit of the cloaca positions may be important for the fertilisation rate. Couples differing very much in size may have a disadvantage, i.e. size matching males should be preferred.

Indirect benefits of female choice can be explained by the “good genes” and “sexy sons” hypothesis, which stress the importance of offspring survival and reproductive success [Fisher 1958, Trivers 1972, Zahavi 1975, Kirkpatrick 1982, 1985, 1986, 1987, 1991, Hamilton & Zuk 1982, Parker 1983]. Male age is often used as an indicator for genetic quality, as it demonstrates survivorship [Passmore & Telford 1983]. Females may prefer older, aestivated males, because they have proven their capability to survive a dry season.

The costs of female choice are possibly a loss of time [Harvey & Bradbury 1991], energetic costs [Slagsvold et al. 1988], high predation risk [Magurran & Nowak 1991, Gibson & Bachman 1992, Rand et al. 1997], high social interference [Clutton Brock et al. 1992, 1993, Nefdt 1995] and high risk of contracting diseases. Adaptation to high predator risk seems most likely to explain that female *Hyperolius nitidulus* did not choose larger males for amplexus. Gong (1996) and Gong and Gibson (1996), for example, found in guppies that the presence of predators changed female preferences. The main predators for adult frogs are spiders and probably snakes [Rödel 1996]. These, however, do not hunt acoustically, but are ambush predators. Moving females thus seem to be far more endangered than sitting males. Calling males are probably detected via vibration. An acoustically frog hunting bat (*Nycteris grandis*) is found in Comoé National Park, but attacks have never been observed. Only a Genette (*Genetta genetta*) was observed once preying on calling *Hyperolius nitidulus* males. Other studies corroborate the conclusion that the absence of female choice is most likely due to high predation pressure (e.g., a study on *Hyperolius marmoratus* by Passmore & Telford (1983) and a study on *Hyla pseudopuma* by Crump & Townsend (1990)).

Although *H. nitidulus* females evidently do not select males on the basis of size, it cannot be excluded that they choose at all. Size assortative mating was observed, but the correlation seemed to be significant mainly because of the large sample size involved (N = 769). The explained percentage of the observed variability in coupled male size ($r^2 = 1.3\%$) was very low. Robertson (1986b and 1990) and Bourne (1993) had much higher explanation values

with much lower numbers of pairs tested (Robertson 1986b/1990 – *Uperoleia rugosa*: N= 15, $r^2 = 94\%$; N = 42, $r^2 = 70\%$ and Bourne 1993 – *Oloolygon rubra*: N = 45, $r^2 = 57\%$).

Unlike in other species, where males are chosen by the number of nights they spend calling rather than for direct call characteristics correlating with size [Jacobson 1985, Cherry 1993, Murphy 1994, Dyson et al. 1998], call duration did not seem to increase reproductive success for the males. Females were never observed staying at the ponds for several days, which could have enabled them to estimate calling nights of different males. However, due to high predation risk they may prefer staying several meters away from the pond where they would remain undetected. It is, however, possible that they estimate males' call durations from the distance and select a male in advance, before approaching the pond.

It is also interesting to note that female choice in another *Hyperolius* species (*Hyperolius marmoratus*) has been reported to be repeatable [Jennions et al. 1995a], which would indicate some kind of choice even if the traits used for this choice are unknown. Female *Hyperolius nasutus* that were separated from the male they had chosen originally, preferred this male when given another choice [Rödel unpublished data]. The same result was obtained in *Hyperolius nitidulus* when kept in aquaria [Linsenmair personal communication].

It is also possible that females use male characteristics that could not be investigated in this study [cf. Kellogg et al. 2000]. Abt & Reyer (1993), for example, reported that females preferred a special male genotype independent of male size or activity.

Since calling is a very costly behaviour for a male frog [MacNally 1981, Grafe et al. 1992], it should contain some information that is favoured by females. The call rate, indicating physiological condition, could be a criterion for which males are selected [Passmore et al. 1992].

In female *Hyperolius nitidulus* no preference for larger or older males and no size assortative mating could be observed. However, it cannot be excluded that females chose males on another trait, for example, call duration or pulse rate.

Male behaviour

Variable reproductive success was observed for the males. Access to zero to 4 females results in a range of an estimated zero to 1200 fertilised eggs. Sullivan and Hinshaw (1992) reported similar results for *Hyla versicolor*; they also observed zero to four matings [see also Gerhardt et al. 1987, Howard 1988]. Careful female choice would explain the observed differences in reproductive success in the calling males, but this is not consistent with the results of this study where only weak preferences were found (cf. female choice). As females did not choose

bigger males and showed only slight size-assortative pairing, the success of a male was mainly influenced by chance. Male persistency could be another possible feature for female choice, but although there is some evidence from other frog species that females choose males by recognising their duration of stay [Dyson et al. 1998, Jacobson 1985], no pattern was found predicting higher amplexus probability for males after calling several nights, or a higher probability of successive amplexus after the first one.

Males calling longer at the same place seem to have a greater probability of obtaining a female because they had a better chance, not because they were actively chosen by a female.

As the females come from the land the best places for males to call can be predicted to be the pond edges. At *Hyperolius* pond, the calling sites of males were recorded and compared to the calling sites of another species (*Hyperolius nasutus*), where females do not move around but are very often found sitting alone, not approaching a male for amplexus. It was found that most *H. nitidulus* males sit calling on the edges of the pond away from the actual spawning water, and only very few of them call in the water. Most couples were also found at the pond edges. Males with calling sites at the edges of the pond seem to stay for several nights while males with “bad” positions in the middle of the pond wander around and change their calling site from night to night.

Male reproductive success depends mainly on their access to a female. As male frogs in this species do not contribute anything to their offspring other than sperm, the main effort for the males should be in getting a mating partner, i.e. their behaviour should focus on attracting females. However, as a male cannot increase its own reproductive success by displaying special traits, females do not seem to choose very carefully. The male strategy of calling (as females would otherwise not recognise them, and as there could be some call criteria that females prefer possibly because they are linked with physiological fitness rather than with size) preferably at the pond edges (because females come from the land) and of staying at the pond as long as possible are clearly adaptations to increase the probability of an encounter with a female.

Male-male competition is reported for several anuran species [Wells 1978, Arak 1983, Robertson 1986a, Crump 1988] and was observed in *Hyperolius marmoratus* [Dyson & Telford 1992 a,b] and in the males of *Hyperolius nitidulus* defending small calling territories at the pond site. Inter-male spacing of minimum 30 cm resembled the minimum distances between two calling *Hyperolius marmoratus* males of 50 cm found by Telford (1985). Silent

males were observed, but there was no evidence for satellite behaviour [Lucas et al. 1996] as silent males were never observed mating.

Male choice for larger females as documented by Verrell (1985, 1986) for some newt species seems rather unlikely in the lek-breeding system. There was no observation in this direction during the investigated three years, but a male preference for larger, more fecund females cannot be excluded.

Clutch size

The in this study found negative clutch size - egg size correlation is typical for all ovipositing animals. There seems to be a trade-off because energy supplies are not infinite and have to be allocated: one can either produce large or many eggs [Begon et al. 1996, Duellman 1985, Duellman & Trueb 1986]. The correlation of clutch size with female size found is widely known in anurans: larger females have larger clutches [Begon et al. 1996, Duellman & Trueb 1986].

The correlation between male size and clutch size that was found is not very strong, it may be an effect of covariation of male and female size. Possibly, females could hold eggs back if they consider their male suboptimal, but so far there is no evidence for this theory. The possibility seems rather unlikely because females that were taken from the pond, started spawning even without a male and/or water when kept in the sample glass for too long.

Tadpoles

Due to the pond environment, tadpole observation in the field was difficult. The water was very turbid (visibility around 5cm), and the dense vegetation made it impossible to see tadpoles. Electro-fishing revealed that there were tadpoles, but the data were merely qualitative and not quantitative, thus no further statistical analysis was performed. The results, however, supported our observations. They showed that many tadpoles were present at Hyperolius pond despite a large number of mainly invertebrate predators whereas at the Gansé Bridge pond many fishes but sometimes no tadpoles could be found. The same pattern was observed in the number of juvenile frogs leaving the ponds. Ponds seem to differ greatly in their amount and kind of predators, which greatly affects the tadpoles in the pond. The patterns may fluctuate from year to year, which would explain the total failure of Hyperolius pond to produce any *Hyperolius* juveniles in 1998.

Juveniles

Size differences of juveniles may be an indication of different living conditions for the tadpoles in the ponds. Differences in food supply seem likely to play an important role for the observed differences in juvenile size [Anholt & Werner 1995, Skelly and Werner 1990, Leips & Travis 1994, Beck 1997].

It has been shown for many other species that, under unfavourable conditions, tadpoles can develop faster and leave the ponds earlier but smaller, e.g. to escape predators [Brönmark & Miner 1992, Skelly and Werner 1990, Smith and Buskirk 1995] or pond desiccation [Tejedo & Reques 1994, Denver et al. 1998, Brady & Griffith 2000]. Predator impact probably differed between the ponds (cf. 3.1.3 tadpoles).

Water chemistry can inhibit or slow down larval growth [Buchholz & Linsenmair 1991, Schmuck et al. 1994, Glos 1998] and water temperature has a strong effect on larval development and metamorphic size [Blaustein et al. 1999, Atkinson 1994]. Water quality and temperature were similar among the ponds, hence these factors are unlikely to have caused the observed size differences.

Tadpole density may also be an important factor influencing tadpole growth [Brockelman 1969, Wilbur 1976, 1977a+b] as competition and interference inhibit larval growth. Considering the number of freshly metamorphosed juveniles leaving the ponds, Hyperolius pond had the higher tadpole density, but considering the number of couples spawning in the different ponds, Gansé Bridge pond might have had a higher tadpole density (Table 19), which could explain the smaller juveniles. High predator pressure in Gansé Bridge pond, indicated by the presence of many fish and by the total failure to release any tadpoles during September, may be responsible for the small output of metamorphosing juveniles and it could also reduce metamorph sizes [Laurila et al 1997, 1998, Laurila & Kujasalo 1999].

Table 19: Indicators of tadpole density calculated for the Gansé and the Hyperolius pond in 1999.

	Freshly metamorphosed frogs		Couples found at the ponds	
	Hyperolius pond	Gansé Bridge pond	Hyperolius pond	Gansé Bridge pond
Number of juveniles/couples	1353	346	78	47
Catching days	80	26	80	26
Ratio animals/catching day	17	13	0.98	1.8

Pond attractiveness

There was no clear pond characteristic that made a prediction of its attractiveness possible. Nevertheless, the capability of water storage seemed to be an important factor. The negative effects of already present tadpoles are known for several anuran species, for example *Hoplobatrachus occipitalis* (due to cannibalistic tadpoles also a predation problem) [Spieler & Linsenmair 1997] and *Hyla chrysoscellis* [Resetarits & Wilbur 1989]. There are also laboratory observations indicating that the presence of tadpoles inhibits further spawning [Linsenmair personal communication].

No negative correlation between spawning willingness and tadpole density could be found in this study. Maybe this factor plays a role in small ponds, which may reach their capacity limits earlier and where competition may play a greater role, but not in the large observed ones where predation by invertebrates, fish or turtles may have higher impact on larval survival than density effects. Predation rate should be taken into account before spawning in a particular pond and high predation pressure should be avoided if possible. Olfactory cues seem to be used to identify conspecifics [Hews 1988], fish [Petranka et al. 1987] and invertebrate larvae and female frogs react by avoiding crowded and dangerous ponds [Hopey & Petranka 1994]. However, several authors found no evidence for the avoidance of breeding sites with a high predation rate [Laurila & Aho 1997] and argued that other effects are possibly more critical for the reproductive success in these cases and likewise no evidence of predation avoidance behaviour was found for *Hyperolius nitidulus* in this study.

Females did not change the ponds when laying several clutches. Of all females found more than once only one changed from a very small to a larger pond, all the others chose the same pond they had already put the first clutch in. Using a known pond therefore seems to be more advantageous than changing, either because all ponds are equal in their adult and tadpole survival rate, or because the costs of changing are higher than the benefits of it. However, the number of females found more than once is very low (N = 30). It cannot be excluded that a considerable proportion of females changed ponds maybe on a much larger scale than was detected. Eventually two strategies coexist within the female population, with some individuals dispersing and others residing (see below).

Dispersal

Dispersal is often explained as a mechanism avoiding inbreeding and sibling competition [Dobson & Jones 1985, Gandon 1999]. For different species, the primary reason to leave can vary [Pusey & Wolf 1996, Kasuya 2000] and several models that predict different

evolutionary stable dispersal rates for different environmental situations were created and tested with field data [Felsenstein 1976, Motro 1991]. These models have in common that dispersal is predicted to be high when the costs are low and the possible benefits are high, while dispersal is low, when the costs (e.g. mortality rate) are high [Comins et al. 1980]. Sex-biased dispersal strategies have been observed in mammals and birds [Liberg & Von Schantz 1985, Juillard 2000, Perrin & Mazalov 2000] and have often been explained by differing mating strategies [Liberg & Von Schantz 1985, Perrin & Mazalov 2000].

Little dispersal between the ponds was observed in this study. Only 19% of all males found more than once at the Gansé Plaine were recaptured at a pond other than the first one. It is interesting to note that dispersal behaviour changed towards the end of the rainy season. During investigation weeks 25-32, marked individuals were recaptured more frequently, but changed ponds less often. This behaviour could be an adaptation to already desiccating small ponds. Possibly the larger ponds investigated in this study could provide enough water for tadpole development, while small ones had already become risky. Changes between ponds would, therefore, be no longer advantageous.

Females could not be observed changing the ponds; only one female out of 30 found more than once changed from a very small to a larger pond for her second clutch.

Males changed between ponds more than females and thus sex-biased dispersal behaviour with males leaving their birth area more regularly is predicted, but due to the small number of females found more than once no definite statement can be given.

Generally, the costs of dispersal seem to be high in *Hyperolius nitidulus*, while the benefits at least to change between the large ponds observed seem low.

Dispersal can nevertheless play a role. The high number of unknown individuals coming to the ponds late during the rainy season may indicate dispersal and not only a methodological problem (see above). If two strategies exist where part of the population stays relatively close to its birth place while another part disperses over a wider range than covered in this study, they could not be detected by our sampling design.

3.2 Existence of a second generation per year

3.2.1 The problem

Physiological adaptations of *Hyperolius nitidulus* to harsh climate conditions have been well studied. Juvenile frogs show two very different behavioural patterns correlating with the time during rainy season at which they metamorphose. Juveniles metamorphosing during the first two thirds of the rainy season hide near the ground when leaving the water and are very sensitive to low air humidity and high air temperatures, which cause high mortality. On the contrary, juveniles metamorphosing during the last third of the rainy season can be seen sitting uncovered in the grass up to 1 m above ground. Sunlight hits them directly, but they can cope very well with high air temperatures and low humidity.

It is known from laboratory experiments that juvenile frogs do not need to go through a dry season before reproduction can take place [Schmuck et al. 1994, Linsenmair 1998]. They can mature in only two months. As tadpoles need about 6-8 weeks to metamorphose, the total time for a *Hyperolius nitidulus* to reach adulthood is about 4 months. Therefore, the reproductive period from May to October (6 months) appears very long.

The two behavioural strategies and the relatively short developmental times led to the hypothesis that *Hyperolius nitidulus* does have more than one reproductive strategy, with either one or two generations per year. Such a mixed strategy may be considered adaptive in an unpredictable environment, if the length of rainy season varies between years. If the rainy season is short, at least one generation will be finished and ready to aestivate, but a second generation may be successful if the ponds persist longer.

The frogs that leave the pond and come back during the same rainy season to reproduce are defined as the second generation, because they are placed in between the reproduction of the “normal” animals (one reproductive cycle = first generation).

To directly prove the existence of a second generation, it would be easiest to mark juvenile frogs when they leave the water, release them and try to recapture them when they come back to the pond in the same season to reproduce. It was, however, difficult to find a proper marking technique that lasts at least several months and does not affect juvenile behaviour or growth. Juvenile *Hyperolius* are difficult to mark for three reasons: they are very small (12 mm body length, 0.15 g), the skin is shed daily, and their toes regenerate [Richards et al. 1975]. These properties render all classical marking methods impossible: colour marks on the skin will last exactly one day, rings are impossible to fix because of the smooth skin,

transponders are too big and toe-clipping, an otherwise very common method for marking frogs, is risky as the toes possibly regenerate before the frogs return to the pond. The problem could only be solved by the development of a new marking technique, but until such a technique was available (cf. 3.2.3), this study concentrated on indirect evidence for the existence of a second generation. Body size variation in time revealing multiple peaks can indicate cohorts or different generations [Begon et al. 1996]. Considering that the clutch size of an individual female frog decreased with time, a similar bimodal time curve for clutch size would indicate the arrival of a second generation of females. The beginning of a new generation could be indicated by a sudden increase in mean clutch size during one breeding season (Figure 26).

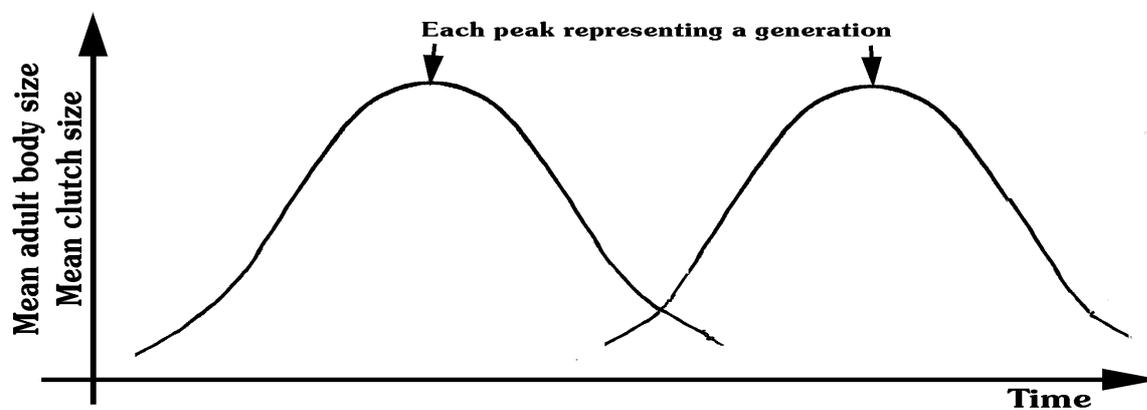


Figure 26: Schematic representation of multiple generations per season indicated by peaks in the time course of mean adult body size or clutch size.

More indirect evidence may be drawn from a careful documentation of changes in juveniles behaviour and skin morphology to distinguish between dry-season adapted and rainy-season adapted juveniles.

Finally, in 1998, a technique originally invented for marking fish was modified and adapted to permanently label juvenile frogs. This technique then enabled long-term marking and the search for direct evidence of a second generation.

3.2.2 Indirect evidence for the existence of a second generation

3.2.2.1 Methods

Adult size of calling males and solitary females at the ponds was measured frequently during the rainy seasons of all three years. Frogs were toe-clipped individually and released after the last frog had been treated. Coupled males and females were measured, marked and released after spawning in captivity the following day. Only newly arrived (previously unknown = without toe clipping number) adults were considered for the calculation of mean body size. Measurements were grouped in eight week intervals and differences in group means were tested by One way ANOVA with size as main effect. The Scheffé test was used for the post-hoc tests.

Clutch sizes were determined with couples held in aquaria as described in 2.2.3. As for the adult frog measurements, data were grouped in eight week intervals to test whether the observed changes in clutch size over time were significant. A Kruskal-Wallis ANOVA and subsequent Nemenyi test was used for statistical analysis as clutch size was not normally distributed.

Freshly metamorphosed frogs were brought into the camp to test juvenile reaction to high air temperatures and low air humidity throughout the rainy season 1999. They were kept in groups of 7 to 25 in cages 40x40x80 cm in size which provided dry grass for sitting above ground and a dense layer of leaves on the ground for hiding, and were exposed to the savannah sun light. To protect the froglets from ground predators (mainly army ants), the cages were placed on a bench with posts immersed in bowls filled with a water and soap mixture, which prevented insects from climbing up and into the cages.

On the following two days, the number of juveniles sitting in the sun was recorded five times a day (8, 11, 14, 17 and 20:00 h). After two days, young that had survived were returned to the ponds.

Juvenile skin morphology was studied in the laboratory in Würzburg preparing skins of 5 dry season adapted (freely sitting) and five rainy season adapted (hiding) juveniles' (Pictures 13 - 16). Froglets were collected in Africa directly when leaving the pond after metamorphosis. The juveniles were kept in the laboratory (28° C/ 20°C, 50 %/ 70 % R. H. (day/night)) and

prepared two weeks later in Würzburg. Small pieces of dorsal and ventral skin (~ 1 mm²) were fixed in 2.5 % glutaraldehyde, postfixed in 2 % osmium tetroxide and contrasted with uranyl acetate. The tissue was dehydrated and flat-embedded in a propylenoxid/epon 1:1 mixture. Photographs were taken with a Zeiss EM 900 electron microscope (details see appendix).

In particular, differences in the thickness of the purine crystal layer were subject to investigation. To measure crystal number and orientation, the Stratum spongiosum of the ventral and dorsal skin of each individual was divided into 10 µm layers. Each layer was subdivided into distances of 10 µm, this resulted in 10 µm x 10 µm squares. For each individual 3 squares were randomly chosen by throwing a dice first to determine the layer and a second time to determine the square inside the layer. For each square the number of purine crystals was counted and their orientation towards the surface of the skin was measured (0-90°). Mann-Whitney U-tests were used to obtain differences between the behavioural types. Bonferroni correction was used to compensate multiple test usage.

3.2.2.2 Results

Adult size

Despite significant differences in average male size between years and ponds (see 3.1.2.1 male behaviour), male size in all years and at all ponds showed the same pattern towards the end of the rainy season as it declined strongly and during a short period of time. The decrease in male size occurred in each year at about the same time at the end of September/beginning of October (investigation week 24) and was significant for each year and each pond (Figure 27).

Eight-week group means of male SVL showed a significant size reduction for weeks 25-32 compared with all other groups (Table 20, Figure 28)

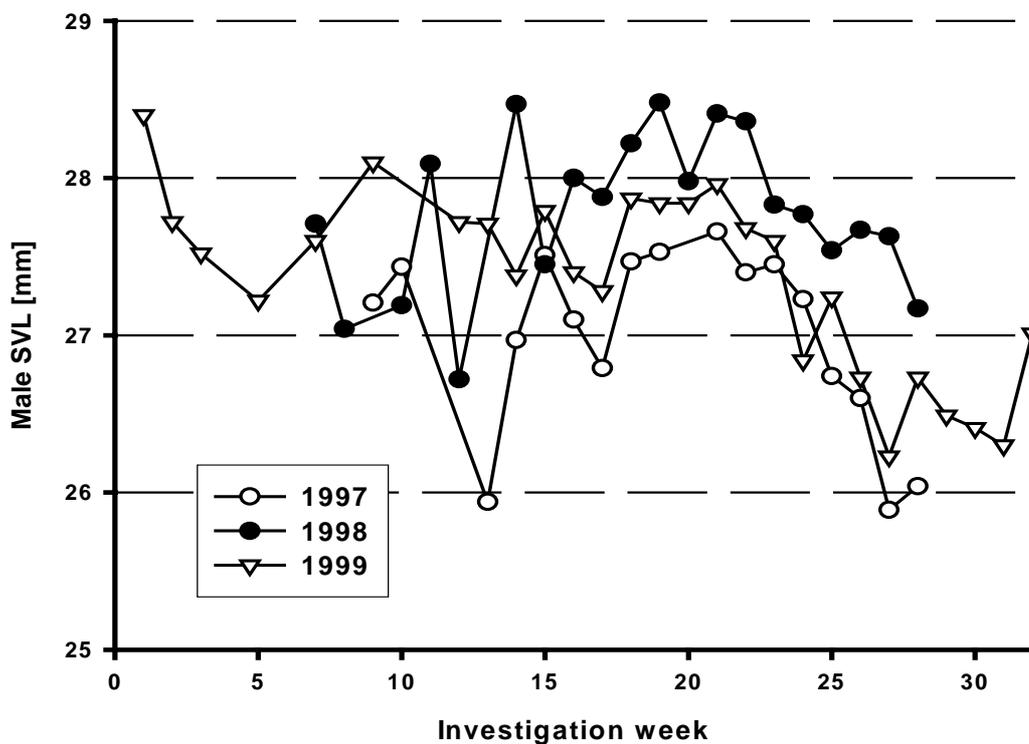


Figure 27: Time course of mean male size at all observation sites during the three study years.

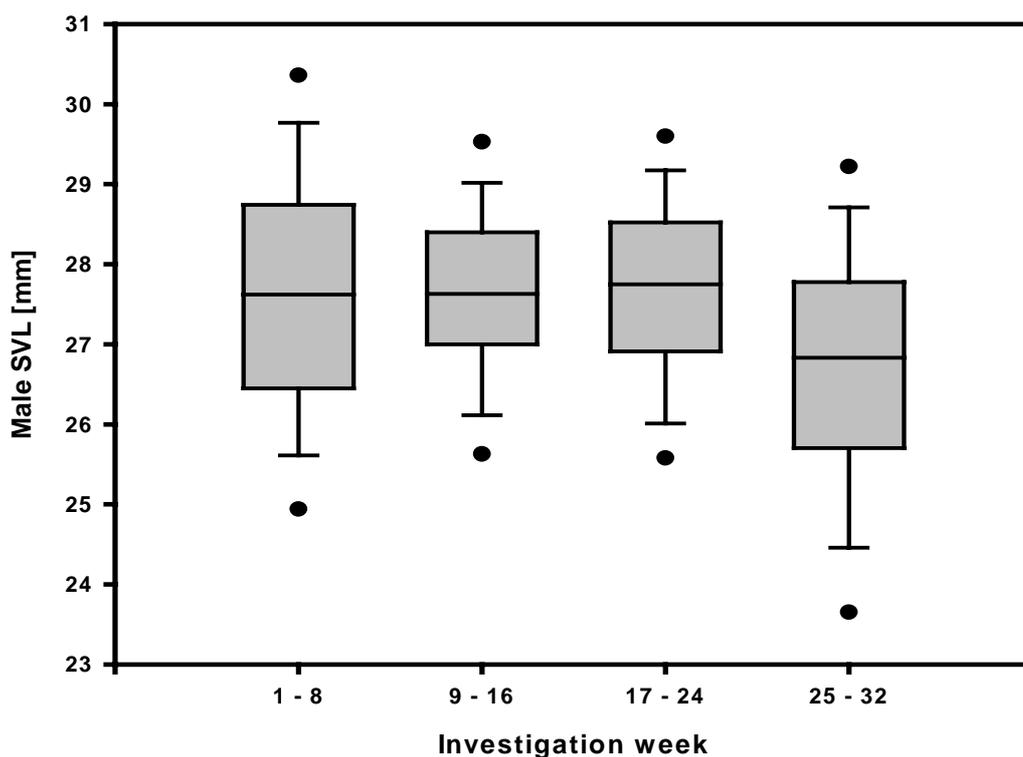


Figure 28: Box plot (median (line), 10th, 25th, 75th and 90th percentiles (box with error bars), 5th and 95th percentiles (circle)) of male sizes pooled for all three investigation years and grouped in eight week intervals.

Table 20: ANOVA table for male size measurements grouped in eight week intervals and results of subsequent Scheffé test for SVL.

<u>ANOVA for all measurements</u>	Number	F	p
SVL	2431	83.07	< 0.001
TF	2431	60.94	< 0.001
Weight	2428	217.81	< 0.001
<u>Scheffé test (SVL)</u>			
	weeks 9-16	weeks 17-24	weeks 25-32
Weeks 1-8	0.999	0.972	0.00000*
Weeks 9-16	----	0.991	0.00000*
Weeks 17-24	----	----	0.00000*

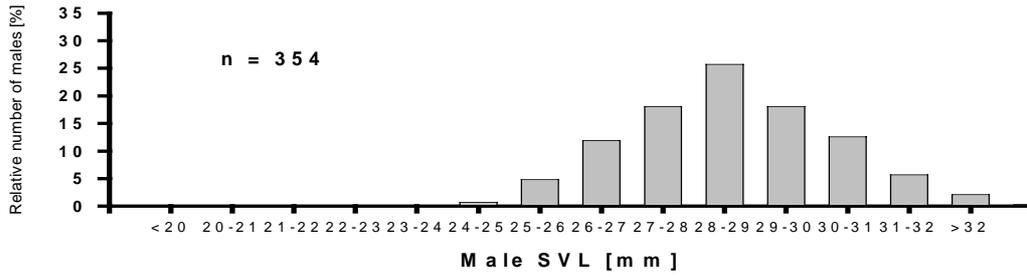


Figure 29a: Male size class distribution in investigation weeks 1-8.

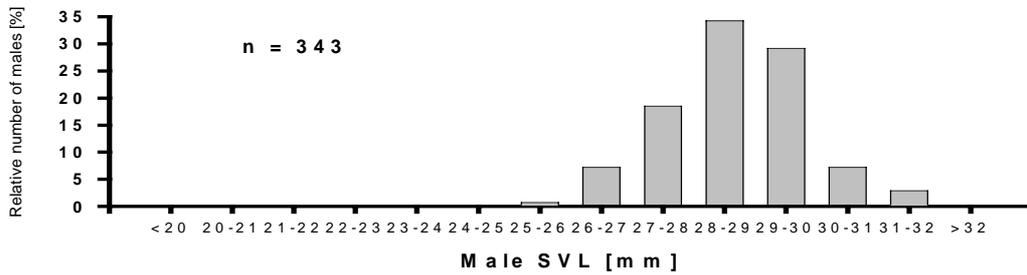


Figure 29b: Male size class distribution in investigation weeks 9-16.

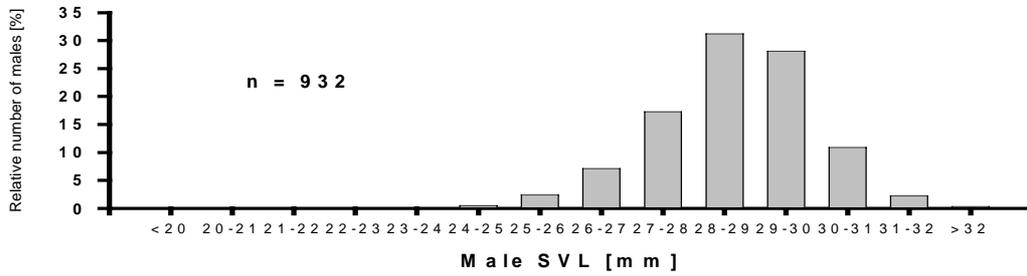


Figure 29c: Male size class distribution in investigation weeks 17-24.

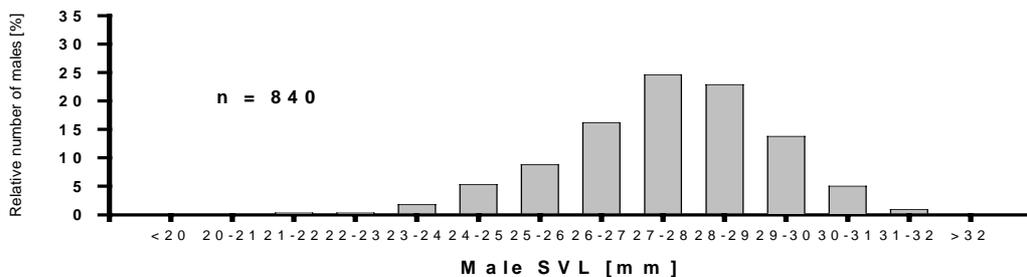


Figure 29d: Male size class distribution in investigation weeks 25-32.

A closer investigation of size classes revealed that very small males (<24 mm) that did not occur earlier were present later in the rainy season. This is demonstrated with detailed size frequency diagrams in Figure 29 a-d. Very few small males (<24 mm) were present at the beginning of the rainy season (Figure 29a), but in the ongoing rainy season (Figure 29b) this size class disappeared (grew?), and could only be found again in increasing numbers towards the end of the rainy season, in particular in weeks 25-32 (Figure 29c+d). A similar occurrence of small males at the ponds was detected for all three years.

By plotting mean females SVL versus time, no obvious changes could be observed (Figure 30), but a one-way ANOVA for pooled data of all ponds and years for eight-week groups revealed significant differences in SVL, TF and weight similar to the male results (for all size measurements $df = 1036.2$, $F > 22.9$, $p < 0.0001$; Table 21, Figure 31). A subsequent Scheffé test indicated that females were significantly larger during investigation weeks 1-8 compared with investigation weeks 9-16 and 17-24 and significantly smaller in investigation weeks 25-32.

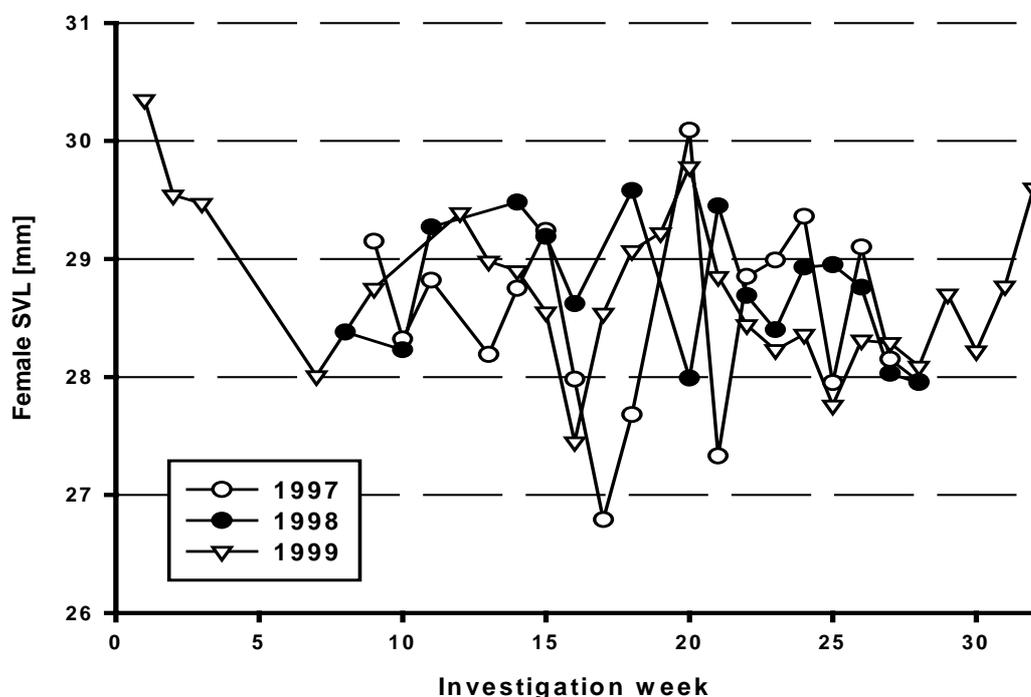


Figure 30: Time course of female size (SVL [mm]) in all years. Weekly mean values of newly arriving females pooled for all investigated ponds are given.

For both mean male and female sizes there were significant differences between the years and the ponds that can be explained by the different sampling times.

Table 21: ANOVA table for female size grouped in eight-week intervals (cf. Figure 31).

One-way Anova	df	F	p
SVL [mm]	1036.2	27.8	<0.001
TF [mm]	1036.2	22.9	<0.001
Weight [g]	1017.2	85.5	<0.001

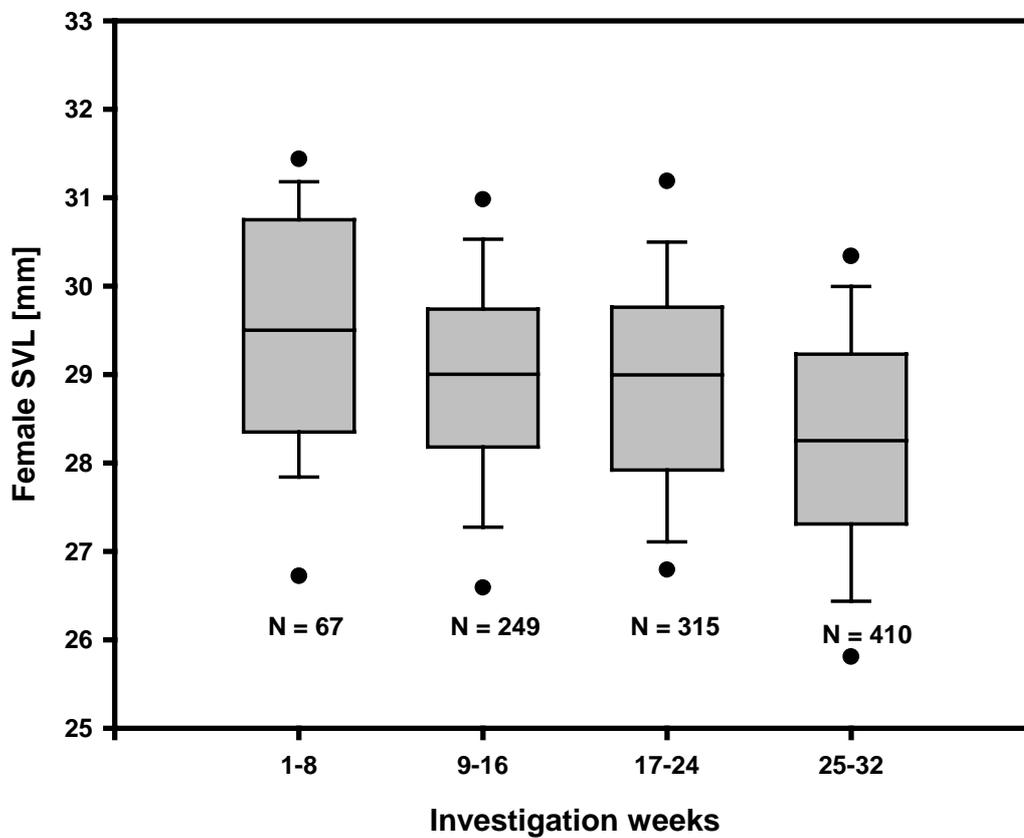


Figure 31: Box plot (median (line), 10th, 25th, 75th and 90th percentiles (box with error bars), 5th and 95th percentiles (circle)) of female sizes pooled for all three investigation years and grouped in eight week intervals.

Clutch size

Clutch size showed a consistent pattern during the three years of observation (Figure 32). It started with very large average clutches of around 380 eggs per clutch with maximum numbers of up to 730 eggs for individual clutches and stayed around this level for 2/3rds of the breeding season. Towards the end of the breeding season, mean clutch sizes dropped very rapidly to about 200 eggs. Individual clutches with only 100 or even fewer eggs occurred quite regularly. The marked and sudden decline in mean clutch size occurred at the same time (investigation week 24) in all investigation years (Figure 32), i.e., simultaneously with the decline in male size (cf. Figure 27).

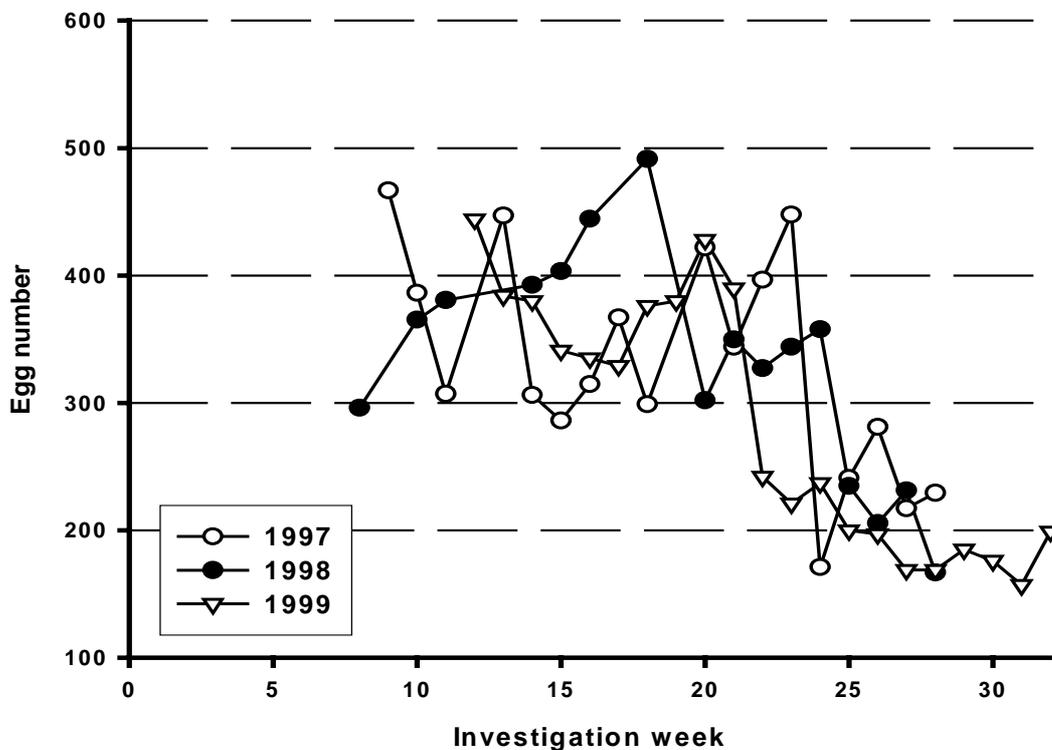


Figure 32: Time course of clutch size in the three years of investigation. Mean values per week pooled for all investigated ponds are given.

The decline of clutch size at the end of the rainy season was significant when data were grouped in eight week intervals (Kruskal-Wallis ANOVA and Nemenyi test $p < 0.01$ – Table 22, Figure 33). There are only three time intervals as clutches were only measured after investigation week 8.

Table 22: Kruskal-Wallis ANOVA and Nemenyi test for clutch size grouped in eight week intervals in 1999.

K-W ANOVA	Number	F	p
Clutch size	475	258.02	0.00
Nemenyi test	weeks 17-24	weeks 25-32	
Weeks 9-16	0.0000*	0.0000*	
Weeks 17-24	----	0.0000*	

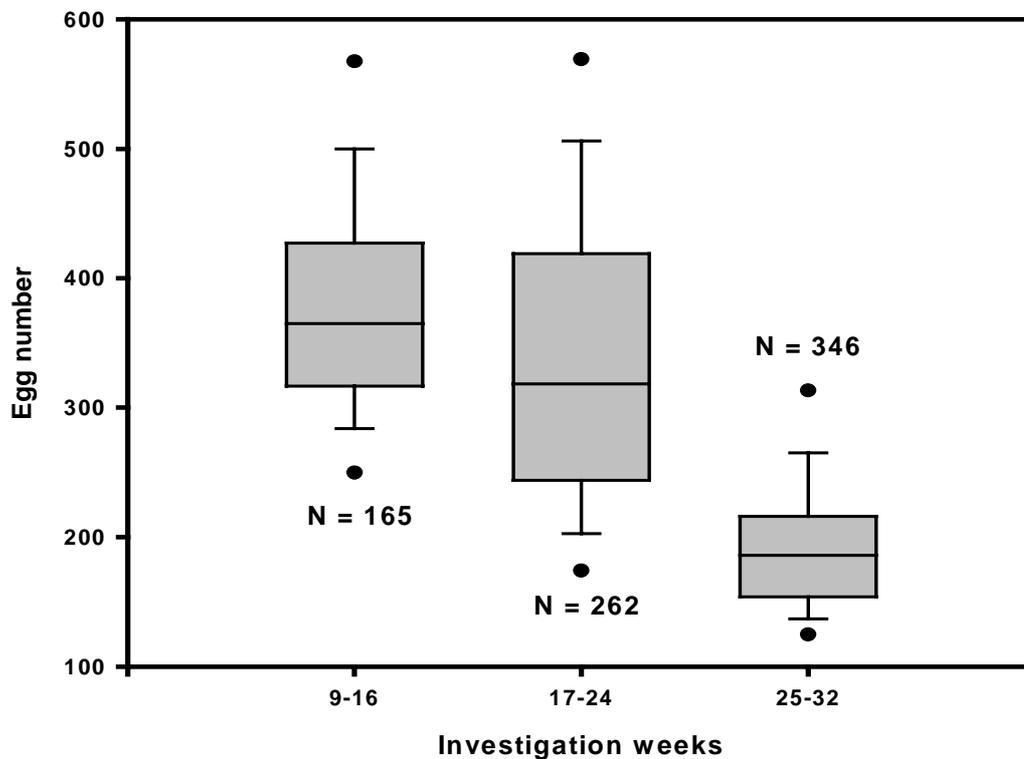


Figure 33: Box plots: median (line), 10th, 25th, 75th and 90th percentiles (box with error bars), 5th and 95th percentiles (circle)) of clutch sizes pooled for all three investigation years and grouped in four week intervals.

Egg diameter differed between years, but generally stayed fairly constant after an initial increase (Figure 34).

One-way ANOVA and subsequent Scheffé test for four-week groups revealed significantly lower egg diameters at the beginning of the rainy season (investigation week 9-12) than in all other weeks, but no other differences.

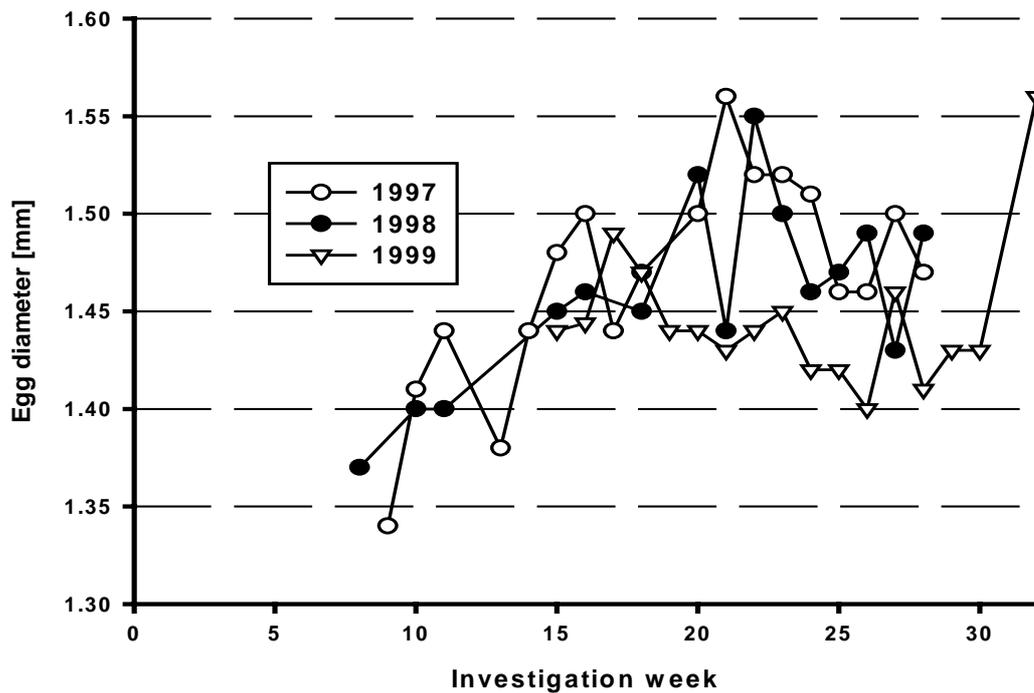


Figure 34: Time course of egg diameters during three investigation periods. Weekly mean values of all ponds are given.

Juvenile behaviour

Two juvenile behaviour types were found in the field: either juveniles hid near the ground obviously avoiding direct sunlight or they sat freely at the grass between 10 cm and 90 cm above ground exposing themselves directly to the sunlight. Juveniles sitting freely were assumed to be dry season adapted, juveniles hiding were thought to be rainy season adapted. To document the changes in juveniles behaviour and therefrom get more indirect evidence for the existence of a second generation, juvenile behaviour was tested in the camp.

Juvenile mortality in the behaviour test was low as long as a thick layer of leaves was present where the young could hide and find higher air humidity and lower temperatures. There were no differences in behaviour between the first and the second day of each test, but nevertheless only data of day two were used to make sure that all animals had been in the cages almost equally long and had settled down to show normal behaviour. The reactions of juveniles were very clearly separable; either an animal hid and was not visible or it sat directly in the sun clearly visible for the observer. As there was no transition between the behavioural patterns

(for example juveniles sitting halfway visible near the ground), dry season or wet season adapted froglets were clearly distinguishable. In July, August and September all animals hid on the ground under the leaves. They died very easily when the leaf layer was not thick enough (less than 5 cm). However, in October (12%) and November (30%) some juveniles preferred to sit on the dry grass, directly exposed to the sunlight (Figure 35), hence they were dry season adapted.

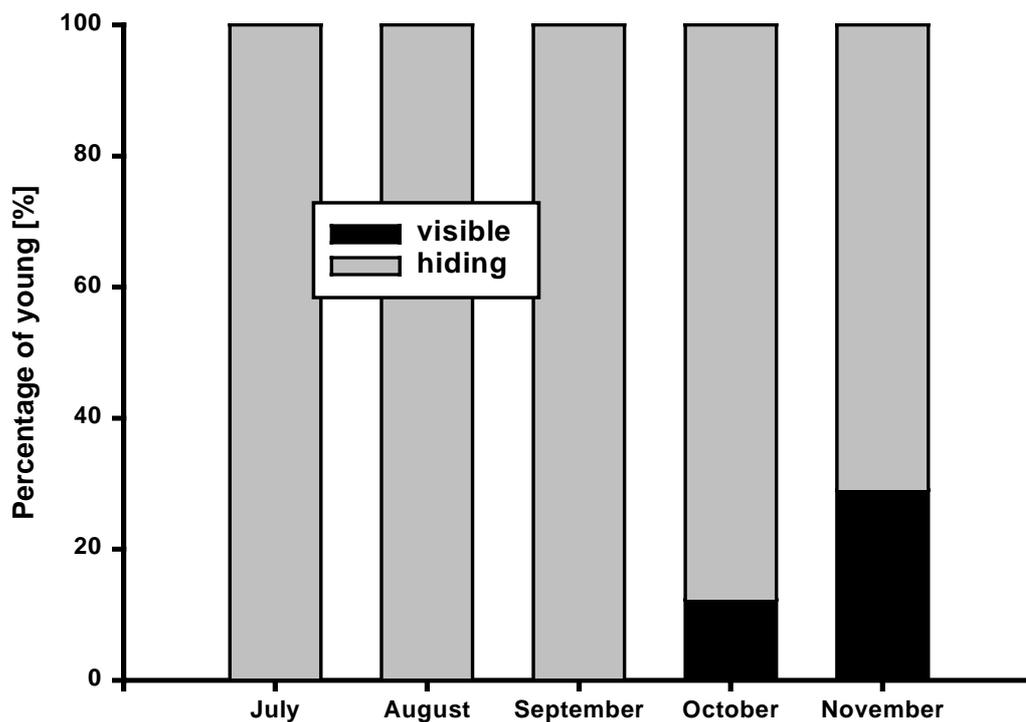


Figure 35: Result of the juvenile behaviour experiment in different months, 1999. Hiding individuals were considered rainy season adapted, visible individuals were sitting freely in the sun and therefore considered dry season adapted.

A nonparametric multiple comparison test based on Zar revealed significant differences between the percentage of young visible in November and July to September (Table 23).

(This result does not mean that the 70 % percent of the juveniles that showed no dry season adaptation will inevitably die during the dry season, in captivity slow post metamorphic adaptation to dry season conditions has already been observed.)

Table 23: Summary of the behaviour tests during July to November 1999. Exposed juveniles sat in the sun, i.e. they were adapted to dry season conditions.

	Number of individuals tested	Number of test	% visible (median values)	Nonparametric multiple comparison test					
				Mean rank sum	Q-values				
					July	August	September	October	November
July	162	10	0	16.6	----	0.002	0.05	1.53	3.36*
August	79	9	0	16.6	0.002	----	0.05	1.49	3.28*
September	45	7	0	16.3	0.05	0.05	----	1.45	3.12*
October	51	8	12.24	25.25	1.53	1.49	1.45	----	1.68
November	65	9	29	35	3.36*	3.28*	3.12*	1.68	----

* significant for $p < 0.02$

Juvenile skin morphology

Freshly metamorphosed juveniles' skin was investigated to possibly obtain morphological differences between the differing behaviour types of the juveniles. Juvenile dorsal and ventral skin was about 80 μm thick. No differences could be found between dry season and wet season adapted juveniles concerning total skin thickness or the thickness of the skin layers: Epidermis $\sim 15 \mu\text{m}$, Stratum spongiosum $\sim 40 \mu\text{m}$, Stratum compactum $\sim 15 \mu\text{m}$, Tela subcutanea $\sim 10 \mu\text{m}$. Differences were, however, very clearly visible in the number of iridophores in the dorsal skin and in the number of purine platelets in these iridophores (Table 24). The number of iridophores was nearly twice as high in the dry season adapted frogs than in the rainy season adapted ones (dry season adapted: 212 \pm 13 iridophores/ mm^2 ; wet season adapted: 118 \pm 15 iridophores/ mm^2). Individuals of both behavioural types had significantly more purine crystals in their dorsal than in their ventral skin (Table 24). The dry season adapted juveniles' dorsal Stratum spongiosum contained significantly more purine crystal platelets (Table 25).

Dry season adapted juvenile crystals also showed a higher degree of order (Table 26). The variance of the platelets' angles towards the epidermal surface was about twice as high in the wet season adapted juveniles.

Table 24: Numbers of purine crystals in the dorsal and ventral skin of dry season (DS) and wet season (WS) adapted freshly metamorphosed froglets.

	Number of platelets/100 μm^2	Platelet angles towards the epidermal surface (0-90°)			Number of frogs
		median*	quartile distance*	variance	
DS dorsal	102.4 +/- 6.6	42	27	391.6	5
DS ventral	33.8 +/- 5.4	42	38	615.4	5
WS dorsal	56.8 +/- 16.2	45	34	663.8	5
WS ventral	29.8 +/- 8.1	43	43	669.4	5

* mean values for the medians, quartile distances and variances are given

Table 25: Results of Mann-Whitney U-tests on the number of purine crystals per 100 μm^2 in ventral and dorsal skin of dry season (DS) and wet season (WS) adapted juvenile *Hyperolius nitidulus*. (Significant results are marked with bold letters. Bonferroni correction was used to compensate multiple test usage)

	U	Z	p	N ₁	N ₂
DS ventral versus dorsal skin	0.00	2.61	0.009	5	5
WS ventral versus dorsal skin	0.00	2.61	0.009	5	5
DS versus WS dorsal	0.00	2.61	0.009	5	5
DS versus WS ventral	10.0	0.52	0.60	5	5

Table 26: Results of Mann-Whitney U-tests on the variance of the angles of the crystal platelets towards the epidermal surface. The ventral and dorsal skin of dry season (DS) and wet season (WS) adapted juvenile *Hyperolius nitidulus* was compared. (Significant results are marked with bold letters. Bonferroni correction was use to compensate multiple test usage)

	U	Z	p	N ₁	N ₂
DS versus WS dorsal	0.00	2.61	0.009	5	5
DS versus WS ventral	11	-0.31	0.75	5	5

Summary

Adult size decreased suddenly and significantly toward the end of the rainy season. Mean clutch size showed a similar seasonal pattern as adult size while egg size increased at the beginning of the rainy season and remained constant. Juvenile frogs showed different behavioural patterns towards the beginning and the end of the rainy season that were clearly correlated to skin morphology.

3.2.3 Direct evidence for a second generation

3.2.3.1 Methods

The earliest juveniles had the greatest probability of returning to the ponds in the second part of the rainy season and reproducing successfully during the same season. Therefore, an intense daily sampling programme was carried out from July to September to collect as many individuals as possible. Freshly metamorphosed froglets were collected every day from 6.7.99 to the 30.9.99. Around mid September the main effort shifted towards catching adult frogs as it was supposed that the first marked juveniles could return to the ponds to reproduce at that time.

Hyperolius pond, the main investigation pond for this problem, was visited every night in order to find at least some juveniles. All other ponds were visited at regular intervals, at least once a week. All juveniles collected, were brought to the camp and measured and weighed the following day.

The freshly metamorphosed frogs were marked with Visible Implant Fluorescent Elastomer (VIE) Tags, a special injectable dye originally invented to mark fish. VIE is produced by NMT (North-Western Marine Technologies). It contains two components: the dye and a special gluing component. These have to be mixed at a 10:1 ratio in order for proper curing to occur after a workable time of about two hours. The time needed for solidifying depends on temperature (the higher the temperature, the shorter the injectable time). The liquid state makes it possible to inject the marks under the skin in exactly the amount needed and the fact that the dye solidifies guarantees a solid mark with clear mark edges.

Four colours which fluoresce under light blue light are available. They can be identified with a special filter even under pigmented skin. The dye is injected with a small hypodermic needle. A special injection device makes it easier to press the syringe and helps to apportion the material for an optimal mark size. As *Hyperolius nitidulus* juveniles are very small (12 mm body length) some practice is needed before large numbers of juveniles can be marked during the time before the dye solidifies. The easiest way is to take the juvenile's legs between thumb and index finger, holding the frog's head facing away from the marking person who then injects the dye carefully under the dorsal skin right and left of the spine. In juveniles, the marks can be seen without using special light and filter (Picture 10 shows the

marking procedure – note the juveniles’ size! Picture 11 a-c shows the marked juveniles under different light conditions).

Frogllets were collected until at least 50 individuals were available or until the first had been kept in captivity for 5 days before they were marked with the special dye. They were marked in the morning when it was cooler and were released in the evening after the search for adults and other juveniles. The first marked young frogs were caged and observed for several days. No mortality in connection with the marking was detected. Freshly marked juveniles were active but settled down after a short while. They showed no sign of injuries and behaved naturally, i.e., were for instance aware of potential predators and preyed on small insect. Therefore they were released as soon as possible to reduce the risk of death due to caging under unfavourable conditions. The most serious problem with caged juveniles was to obtain adequate amounts of food.

The four fluorescent colours did not allow individual marking, but marking cohorts of frogs was sufficient for our task. Because the dye marks float inside the frogs’ lymphatic system, moving not only up and down but also from left to right positional information could not be used for a possible code number.

The use of the dye started at Gansé pond in 1998. The code consisted of two marks: one for the place, where the animal was found (for example: Gansé pond = orange); the other for the month when it was found (August = orange; September = green; October = yellow).

Juvenile colour-codes: 1998

<u>Location/Month</u>	August	September	October
Gansé Plaine	orange/orange	orange/green	orange/yellow
Hyperolius pond	no juveniles		

Two marks were used at Hyperolius pond and three marks at the Gansé ponds in 1999 in order to differentiate between marking locations and times (month and year).

Juvenile colour-codes: 1999

<u>Location/Month</u>	July	August	September
Gansé Central pond	yellow/orange/yellow	red/yellow/red	yellow/orange/red
Hyperolius pond	orange/orange	orange/yellow	orange/red

3.2.3.2 Results

Freshly metamorphosed frogs

The first attempt to obtain direct evidence of a second generation started in the breeding season of 1998 (cf. 2.3.3 Juvenile behaviour). The first couples were found in mid June; the first freshly metamorphosed juvenile occurred about 8 weeks later at Gansé Plaine. In August, 41 juveniles were found and 40 of them were marked and released. Numbers increased to 196 marked (of 203 found) froglets in September and 472 of 479 in October. The sizes of the freshly metamorphosed frogs did not change during this time. They ranged between 11.5 mm to 15.5 mm snout-vent-length (mean +/- SD 13.16 +/- 0.67 mm) (Table 24).

Table 27: Size and weight of juvenile frogs in 1998.

	SVL [mm]	TF [mm]	weight [g]	tail length [mm]
Mean	13.2	6.1	0.17	2
Standard deviation	0.7	0.4	0.03	3

Not a single one of the 708 marked frogs was found returning to the pond for reproduction until the rainy season ended at the end of October (no more rain falls, no more calling and female activity). No dye-marked frog was found anywhere in the area.

In 1999, however, the breeding season started very early at the end of April, so that the first juveniles could be marked at the beginning of July. Also, many more juveniles left the water than in 1998. Marking efforts were concentrated on Hyperolius pond as a large number of juveniles emerged in the beginning and as no juveniles had been marked before, hence there was no possibility of confusing second generation young with last year's frogs. In total, 1154 froglets were marked at the pond, 472 in July, 575 in August and 107 in September. In addition, a small number was marked at Gansé Plaine (78 in July and 122 in August) where separate codes were used for different ponds to investigate dispersal.

The first marked male was found calling at Hyperolius pond on 26th September 1999. It was marked with two orange spots indicating its first sighting in July 1999. A total of 46 marked individuals was found reproducing at all ponds (Figure 36). Of the 46 frogs recaptured, 3 were found at the Gansé Plaine (Picture 12a+b) (total number of marked: 200 -> recapture rate: 1.5%) and 43 at Hyperolius pond (total number of marked juveniles: 1154 -> 3.7%). At

Hyperolius pond, 11 juveniles marked in July (2.3% recapture rate), 30 marked in August (recapture rate: 5.4%) and one marked in September (recapture rate 0.9%) were found. One juvenile was detected with one missing mark, which rendered it impossible to determine the month of marking (Figure 36).

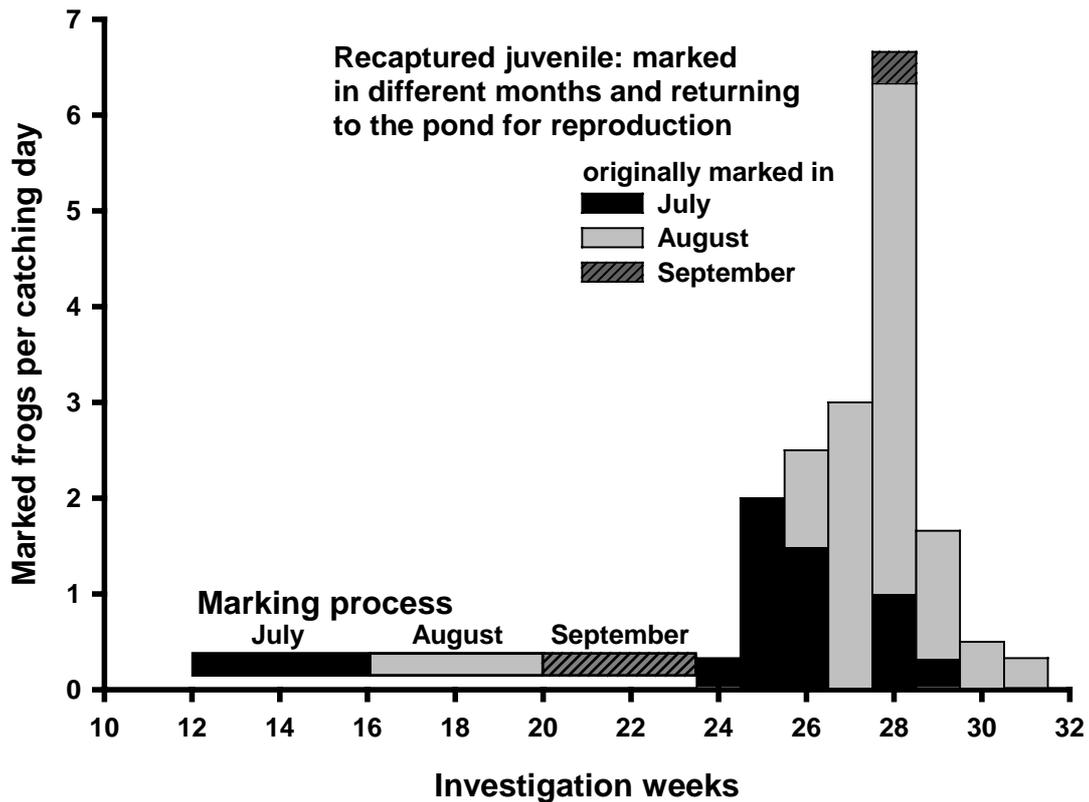


Figure 36: Recapture rates of marked juveniles that returned during the same season to Hyperolius pond for reproduction. Patterns code for the marking months: July to September. The horizontal bar shows the marking period, vertical bars give the number of recaptured individuals per catching day.

On average it took froglets 83 days to reach maturity after being marked. The fastest individual needed only 45 days (6.5 weeks). Although second generation females were significantly larger than males (male SVL: 25.3 +/- 1.25 mm, female SVL: 26.9 +/- 1.74 mm; T-test: $t = -2.99$, $p = 0.005$) they did not need more time to mature (Mann-Whitney-U-test: $U = 118$, $Z = 0.263$, $p = 0.79$) (Figure 37).

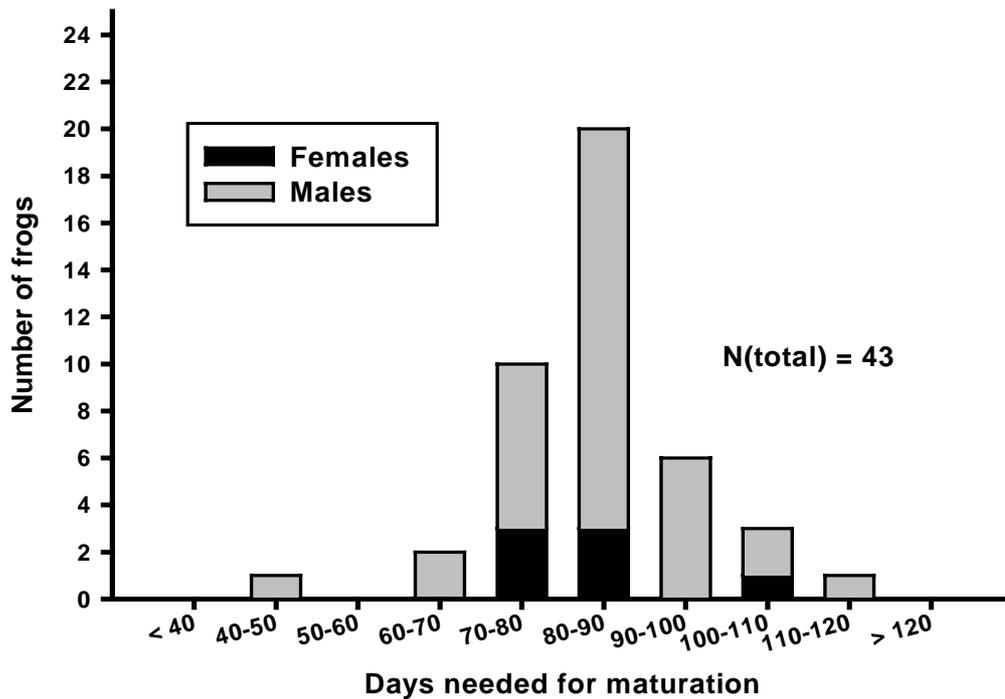


Figure 37: Time needed for maturation by male and female second generation frogs found at Hyperolius pond.

Direct and indirect evidence for a second generation matched well in the data. The appearance of the second generation surely influenced mean adult and clutch size towards the end of the reproductive season. In 1999, the changes in adult and clutch size seemed to start before the first marked juveniles came for reproduction (Figure 38), this is due to a sampling problem. Since reproduction started already on the 21st of April (investigation week 1) in 1999 first juveniles leaving the ponds could have been expected for the 31st of May (investigation week 7) already (indirect data on tadpole developmental times in the field were given by first amplexus sighting and first juveniles found in 1997 and 1998). Juvenile marking, however, did not start before 7th of July (investigation week 12). Calculating with a median developmental time of 83 days for maturation first second generation frogs could have been at the ponds from 22nd of August (investigation week 19). First second generation frogs were found in investigation week 24. This 5 week delay matches exactly the 5 weeks delay in marking juvenile frogs. The calculated onset of second generation appearance in investigation

week 19 resembles the onset of adult and clutch size changes in investigation week 20 very well.

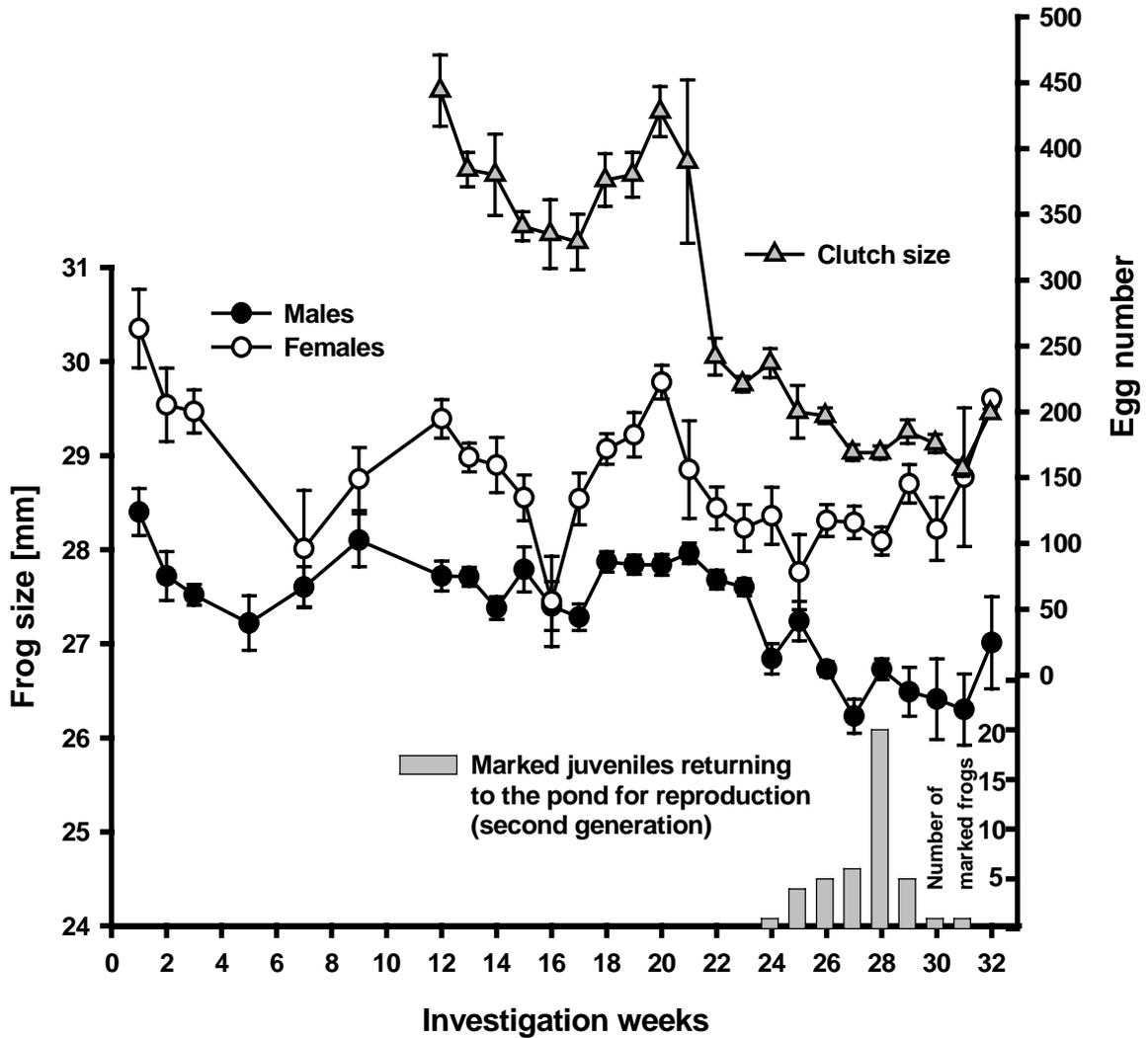


Figure 38: Indirect (adult SVL, clutch size) and direct (number of marked and recaptured juveniles) evidence for the second generation in 1999. (For adult and clutch sizes mean values and standard errors are given. Grey bars give the number of juveniles returning to Hyperolius pond for reproduction.)

Characteristics of the second generation

Among the 46 juveniles belonging to the second generation were 39 males and 7 females, which is a slightly more male-biased sex ratio than the totally observed operational sex ratio (total sex ratio: 3.6 males per female; second generation sex ratio: 5.4 males per female).

Marked and unmarked frogs were present simultaneously during investigation weeks 25-32, hence their morphometric characteristics could be compared. While the marked frogs definitely belonged to the second generation, unmarked frogs could not be assigned to a particular generation with certainty. It is possible that some of the unmarked frogs belonged to the second generation, too. Nevertheless, the group of unmarked frogs are furthermore called “first” generation and the marked frogs “second”. This implies that estimates of the differences between the two groups are conservative.

Both male and female second-generation frogs were significantly smaller than those from the “first” (Figure 39). Not only SVL but also TF and weight were significantly reduced for second generation frogs (Table 28) (T-Test: for males $p < 0.001$, for females $p < 0.01$). Differences became even more clear when the second generation frogs were compared to adults found during investigation weeks 1-8 when it was impossible that second generation frogs were already present. (Table 28 – first generation investigation weeks 1-8)

Five of the seven second generation females found were in amplexus and spawned in captivity.

Clutch size of second-generation females did not differ from clutch size of unmarked females if all clutches during investigation weeks 25-32 were taken. (Mann-Whitney U-test $p = 0.57$) (Figure 39). If second generation females clutches (that were all found at Hyperolius pond) were compared the clutches of the other clutches of females present at Hyperolius pond, second generation clutches were larger than the “first” generation ones (Mann-Whitney U-test: $U = 134$, $p = 0.013$). Calculating expected clutch sizes for the females weight using the correlation for female weight and clutch size found for females that most likely spawned for the first time (females of 1997 and 1998 found during the first two weeks of reproductive activity; $N = 47$: $\text{egg number} = 79.848 + 227.04 * \text{female weight}$) showed that clutches of the second generation were smaller than expected from the correlation. The five clutches found were in the median 58 eggs smaller than expected from female weight.

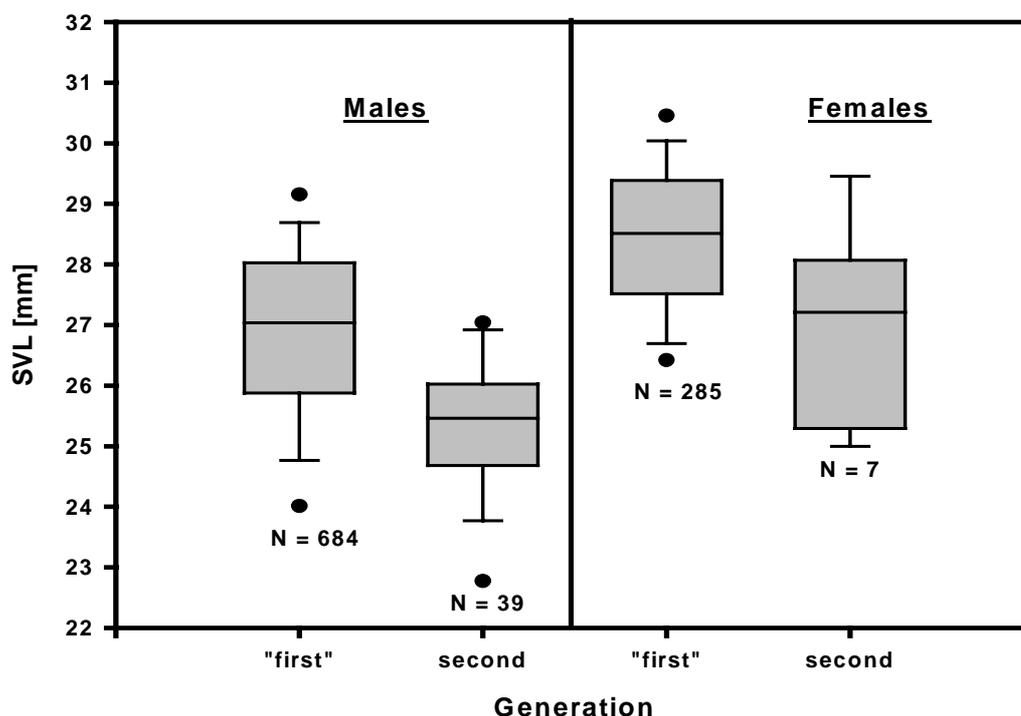


Figure 39: Sizes (SVL) of marked (second generation) and unmarked (first and unmarked part of second generation) males and females during investigation weeks 25-32 in 1999. (Box plots: median (line), 10th, 25th, 75th and 90th percentiles (box with error bars), 5th and 95th percentiles (circle))

Table 28: Summary of the sizes of second-generation frogs and comparison to the mean of the “first”-generation frogs (mean values +/- standard deviation). Differences between generations are significant (Mann-Whitney U-test: for males $p < 0.01$, for females $p = 0.039$).

Males	SVL [mm]	TF [mm]	Weight [g]
First generation (investigation weeks 1-8)	27.6 +/- 1.6	13.0 +/- 0.9	1.23 +/- 0.22
“First” generation (investigation weeks 25-32)	27.3 +/- 1.5	12.8 +/- 0.8	1.14 +/- 0.20
Second generation	25.1 +/- 1.4	11.7 +/- 0.8	0.88 +/- 0.15
Females	SVL [mm]	TF [mm]	Weight [g]
First generation (investigation weeks 1-8)	29.5 +/- 1.3	13.8 +/- 0.9	1.72 +/- 0.29
“First” generation (investigation weeks 25-32)	28.7 +/- 1.4	13.5 +/- 0.8	1.39 +/- 0.29
Second generation	26.9 +/- 1.7	12.5 +/- 0.8	1.09 +/- 0.16

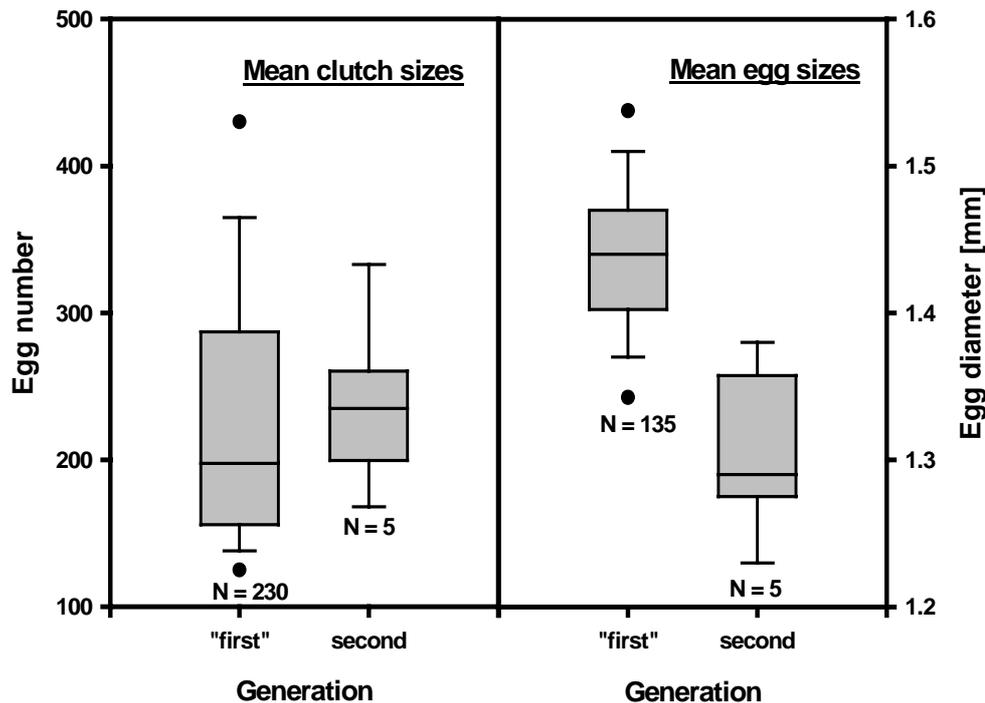


Figure 40: Comparison of mean egg number and egg diameter between "first" and second generation during investigation weeks 25-32, 1999. (Box plots: median (line), 10th, 25th, 75th and 90th percentiles (box with error bars), 5th and 95th percentiles (circle))

Egg size differed between "first" and second generation: eggs laid by females belonging to the second generation were significantly smaller than the mean egg size of the "first" generation. (Mann-Whitney U-test $p=0.0007$) (Figure 40)

8.5 % of the newly arriving males during investigation weeks 25-32 at Hyperolius pond were from the second generation. Less second generation females were found. Only 7 (3.3%) of the 213 newly arriving females during investigation weeks 25-32 at Hyperolius pond belonged to the second generation.

Reproductive success was similar for the second generation males than for the first generation ones. 6.6 % of the males found in amplexus were from the second generation. Although this percentage is bit lower than the percentage second generation males have in the population (8.5 %), this difference was not significant (Chi² test: Chi²: 0.64, $p = 0.42$). Although second-generation males were significantly smaller than the rest of the males, females chose them for mating in the same proportion as they appeared in the population.

One of the 7 second-generation females spawned twice, but no second-generation male was observed in a second pairing.

To estimate the mistake made by missing freshly metamorphosed froglets, adult size (SVL) was used as a criterion to differentiate between first and second generation. Very small frogs (males: < 25 mm; females: < 26.5 mm) found reproducing at the Hyperolius pond were classified as second generation frogs. This resulted in 82 second generation males and 21 second generation females present at the pond during investigation weeks 25-32. 16.4 % of the newly arriving frogs belonged to the group of very small reproducing frogs. Given that size is a reliable classification criterion about 20 % of the newly arriving males and about 10 % of the females would belong to the second generation.

An overlap of generations towards the end of the rainy season became clear, when SVL was used to differentiate between first and second generation. Figure 41 shows that during investigation weeks 25-32 although the number very small reproducing males (SVL < 25 mm) increases there were still many large males (SVL > 27 mm) present, which most possibly belonged to the first generation.

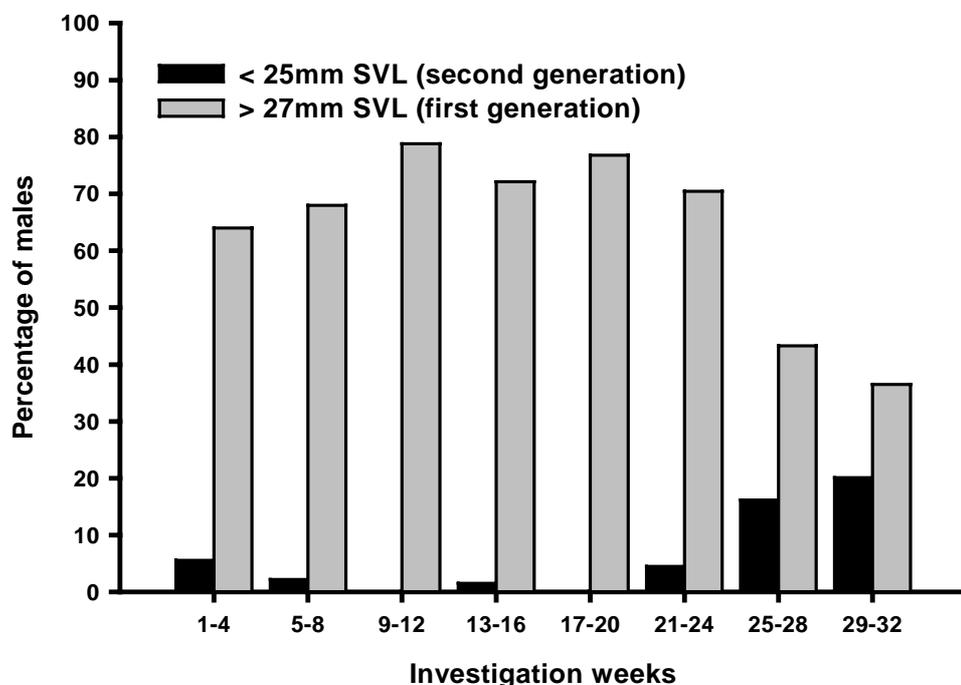


Figure 41: Percentage of very small (second generation) and very large (first generation) males present at the ponds during the investigated period in 1999.

Because most second generation males were found at Hyperolius pond where dispersal behaviour could not be investigated, the size classification for second generation males (> 25 mm) was used in combination with the pond changing data (see chapter 3.1.2.1) to estimate the numbers of wandering second generation males. It was found that of all males found during investigation weeks 25-32 more often than once at the ponds, possibly second generation males did not change ponds more often than the rest of the males ($\text{Chi}^2 : 0.20$; $p = 0.65$).

Another sampling mistake was made by not sampling every night. To estimate the number of marked frogs that could possibly have been found, when all nights had been sampled, the mean recapture rate of 2.38 marked individuals per catching night at Hyperolius pond for the investigation weeks 25-32 was multiplied with the maximum number of 56 catching nights. 133 second generation frogs was the outcome of this calculation resulting in a juvenile recapture rate of 11.5 %. 21 % of all newly arriving frogs at Hyperolius pond would have belonged to the second generation.

Summary

There was direct evidence for the existence of a second generation. Some juveniles marked with a special dye when leaving the water, came back to the pond to reproduce during the same season. Both male and female individuals from the second generation were smaller than the rest of the reproducing adults. Eggs laid by females originating from the second generation were smaller although clutch sizes did not differ significantly.

3.2.4 Discussion

Indirect evidence of a second generation was derived from relative abrupt changes in male and female size. Adult size decreased in 1997 and 1998 very suddenly during week 24, in 1999, the decrease in size was even clearer but happened a few weeks earlier (investigation week 20). The earlier decrease in adult size can easily be explained by the earlier start of reproduction in this year. The changes in adult size were in part due to disappearing larger size classes, but mainly to the arrival of very small individuals (< 24 mm) that were not present at the ponds earlier during the rainy season (Figure 29 and Figure 41). It seems very unlikely that these frogs had been aestivating as they would have had about 5 months to grow and should not have been so small. Rather, these frogs could belong to a second generation which only had about two months to mature, hence the small size could be due to time constraints. The small size of the late males would not lead to a reproductive disadvantage, as females show no size preference. Blanckenhorn (1997, 2000 a+b) for example also interprets distinct size differences in the course of time as indicators of different life histories.

Further indirect evidence is yielded by clutch sizes. Clutches were very small towards the end of the rainy season. There are two possible explanations for this phenomenon: clutches originated either from aestivated (first generation) females that already laid several bigger clutches before, or from females that belong to the second generation. If these females decided under time constraints to reproduce early, they had to reduce energy supplies for egg production, hence smaller clutch sizes were to be expected. This hypothesis was supported by the finding that second generation clutches were in the median 58 eggs smaller than expected from the females' weight.

Changes in size and female fecundity were also found and interpreted as indirect evidence for life-cycles including two generations per year by Barbault (1984). He found that at least two savannah frog species (*Arthroleptis poecilonotus* [Barbault & Trefault Rodriques 1979] and *Phrynobatrachus calcaratus* [Barbault & Pilorge 1980]) were likely to reproduce in two, but contrasting to *Hyperolius nitidulus*, rather distinct generations.

Production of smaller eggs was assumed to indicate the energy limitation of second generation females. Larger eggs result in larger tadpoles [Crump 1984] and are, therefore, thought to be advantageous, due to, e.g. predation survival rates [Warkentin 1999 but see Crump & Vaira 1991]. Berven & Chadra (1988) found in addition that tadpoles from smaller eggs were more sensible to tadpole density. It is interesting to note with this respect that

females spawning for a second or following time, which should be energy limited, typically reduced the number of eggs per clutch (cf. 3.1.2.3) not the egg size.

Behavioural differences in accordance with different life histories are reported for example by Blanckenhorn & Perner 1996. In *Hyperolius nitidulus* juvenile behaviour changed at about the time when male and clutch size decreased. Juvenile *Hyperolius nitidulus* were able to sit freely in the sun, directly after metamorphosis. The sudden appearance of juveniles able to resist dry season conditions as low humidity and high air temperatures can be interpreted as the appearance of the first juveniles adapted to aestivation, delaying their reproduction to the next year. The two clearly separable behavioural types can also be interpreted as indirect evidence of different reproductive strategies. The results of the histological investigations of the juveniles' skin (dry season adapted juveniles have more purine crystals in a higher order than rainy season adapted) support this interpretation by showing that the different behavioural types also differ in their skin morphology. This could possibly indicate alternative developmental processes. The skin morphology of juvenile *Hyperolius nitidulus* was very different according to its behaviour shown directly after metamorphosis. Juveniles hiding at the ground had fewer iridophores and fewer purine crystals than juveniles already sitting in the sun, and the crystals showed a lower degree of order. Dry-season adapted frogs had a thick iridophore layer containing highly ordered crystals. Kobelt & Linsenmair studied the skin morphology of *Hyperolius nitidulus* already in 1986. They used juveniles that were kept for six months under wet season and dry season conditions in the laboratory. Comparing their results to the results of this study reveals many similarities: Kobelt & Linsenmair (1986) also found higher numbers of purine crystals in a higher degree of order in the dry season adapted juveniles. The iridophore density in dry season adapted juveniles was a bit lower in this study than in the study by Kobelt & Linsenmair (this study: 212/mm²; Kobelt & Linsenmair: ~230/mm²). This may be due to the early age of the juveniles that although they were already able to sit freely in the sun, still were not fully adapted to the dry season conditions. The iridophore densities found for the wet season adapted juveniles was higher in this study than in the study by Kobelt & Linsenmair (this study: 118/mm²; Kobelt & Linsenmair: 40/mm²). It seems possible that the freshly metamorphosed juveniles although they still showed wet season behaviour were already on their way to aestivation adaptation. Another possibility to explain this observation could be that the juveniles kept by Kobelt & Linsenmair grew 6 months under wet season conditions. If no new crystals were produced during this period of time, the existing ones should be more widely distributed in the now larger juveniles.

The dorsal skin of the dry season adapted juveniles in this study was thinner (80 μm) than the dorsal skin of the juveniles investigated by Kobelt & Linsenmair (125 μm). This is due to the younger age of the juveniles used in this study.

However, it could clearly be seen that hiding juveniles had less iridophores and less purine crystals than juveniles sitting in the sun. As the radiation reflective qualities of the iridophores containing highly ordered crystal layers are known [Kobelt & Linsenmair 1992], hiding juveniles should not be very radiation tolerant and, therefore, be poorly adapted to dry season conditions. Juveniles sitting in the sun seem to be able to do so as their dorsal skin shows already a higher degree of adaptation to dry season conditions.

The clear differences found among the investigated individuals that were of the same age, came from the same pond and were after metamorphosis kept under the same environmental conditions, show that the decision whether to reproduce during this same season or to aestivate is made already in the tadpole stage of these frogs' development.

The high percentage of juveniles (70%) in the behavioural test still unable to resist dry season conditions may, however, be due to night-time sampling: all juveniles for the behaviour tests were collected at night as no juveniles were found coming out of the water during day time. On the contrary, Linsenmair (personal communication) observed juveniles leaving the ponds during the day with a high proportion exposing themselves fully to the sunlight directly after leaving the water. In 1999, the rainy season may not have been advanced enough to detect the peak emerging of juveniles ready to aestivate. Environmental conditions were eventually not yet harsh enough to force the juveniles to aestivate. Consequently, not all individuals that were theoretically able to aestivate, actually displayed aestivation behaviour. There is also the possibility that results were biased by fleeing behaviour of froglets. Juveniles sitting freely in the sun dive when disturbed into the dense vegetation below. Checking the cages at regular intervals may have been a disturbance answered by some of the juveniles with diving, although this behaviour was never observed in the caged frogs. Finally, there is a possibility of later adaptation to dry season conditions. It is known from laboratory experiments [Schmuck et al. 1994] that rainy-season adapted juveniles can slowly become dry-season adapted when the conditions are favourable. However, the advantage of being dry-season adapted should increase with the increasing probability of the last rains occurring. Drying-out vegetation and soil will be followed by a lack of micro-climatically suitable shelters, which rainy-season adapted juveniles depend on, at least during the day.

The final proof for the existence of a second generation came with the recapture of individuals which had been marked as juveniles when they left the pond. The 46 recaptured frogs definitely came back to the pond to reproduce during the same season in 1999. As site fidelity of the juveniles was assumed [cf. Sinsch 1990], and considering the short period of time the frogs had to survive (several weeks) the recapture rate was low (~3.7%). This can be a consequence of high juvenile mortality or of dispersal [Berven and Gruzien 1990; Samollow 1980]. Only 8.5% of all newly arriving (=unmarked by toe-clipping) males found at Hyperolius pond during investigation weeks 25-32 could clearly be identified as belonging to the second generation. This can either be due to a very small fraction of all juveniles coming back to reproduce during the same year or, more likely, to the fact that only a small fraction of all juveniles leaving the ponds had been marked. Both would result in many undetected second generation frogs that would automatically be assigned to the “first” generation. If characteristics of the “first” and the second generation are compared, i.e. SVL, differences become clearer when the second-generation frogs (found only in investigation weeks 25-32) are contrasted with the frogs of investigation weeks 1-8 only, a time when it was definitely impossible that the second generation was already present. A “dilution” effect (comparing the second-generation animals to a mixture of first- and second-generation animals) may have reduced the apparent differences in investigation weeks 25-32.

Apart from the large number of undetected second generation frogs, low recapture rates possibly also had methodological reasons. Most ponds at Gansé Plaine were probed only once a week and even Hyperolius pond could not be sampled every night. Recapture rates surely would have been higher if more ponds had been sampled more regularly. This may also explain the differences in the recapture rates found between the investigated areas: the most thoroughly investigated Hyperolius pond had a recapture rate of 3.7%, Gansé Plaine a recapture rate of 1.5%. An additional reason for the low recapture rate at the Gansé Plaine is that the landscape offers a huge number of reproduction possibilities, such as small and large ponds with dense vegetation which makes dispersal more likely. In fact, one juvenile marked at Gansé Bridge pond was found reproducing at Gansé pond (distance: 400m).

Moreover, first Hyperolius pond juveniles may have remained undetected as early investigations concentrated on the Gansé Plaine ponds.

A negative effect of the dye marks on juvenile survival rates cannot be ruled out as no comparison was possible. The behaviour of the juveniles directly after marking (3.2.3.1) suggests no direct problems concerning the marks. Other authors also found no negative

effects of internal marks on short-term survival [Sinsch 1997b, Anholt & Negovetic 1998]. However, the long-term effects and a possibly higher predation pressure can not be judged.

Indirect and direct evidence for a second generation matched very well. The sudden size decrease in adults (indirect evidence) can be explained by the small size of newly arriving second-generation adults and the sudden decrease in adult size occurred just at the time when the first marked males returned (direct evidence). Parallel to the adult size decreases and the first occurrence of marked frogs clutch size decreased significantly and juveniles changed their behaviour towards dry season adaptation. Due to these data there is no doubt that at least some individuals of *Hyperolius nitidulus* follow the suggested alternative life-cycle including two generations per year (Figure 2).

3.3 Other reproductive strategies

3.3.1 Introduction

A second generation per year seems to be highly advantageous at least during years with good environmental conditions. It should, therefore, always be profitable to reproduce as fast as possible in order to ensure the survival of the second generation juveniles. However, in all investigation years previously unknown (unmarked = no toe clipping number) frogs arrived at the ponds very late during rainy season. The question arose whether these animals had migrated and had already reproduced at some other pond or whether they started reproduction very late and if so, why.

It is possible that not all individuals are physiologically able to start reproduction as soon as the rainfalls start. The individual frog's aestivating condition may differ, depending on its physiological condition at the start of and foraging success during the transient season.

To investigate whether all animals try to reproduce as fast as possible, aestivating juveniles were marked towards the end of the dry season with the special dye. The aim of this study was to discover dispersal events and to see whether aestivating condition (size) has an influence on the starting point of reproduction.

3.3.2 Methods

Aestivating *Hyperolius nitidulus* were marked in March and April 1999. They were spotted walking into the savannah, leaving the road at an angle of 90°. The best success in finding frogs is between 10:00 and 16:00 h when it is hottest and the sun is high. The whitest frogs are seen best on sunny, cloudless days, but with some practice it is possible to find even darker beige to brown individuals. The frogs were marked in the field with the special dye injected under the skin as described in 3.2.3.1. The colours coded for the area where a frog was found (e.g. northern Gansé Plaine: yellow, central Gansé Plaine: red, southern Gansé Plaine: green, Gansé Swamp: red/green (Map 3)). The number of marks were coded for the size class (one mark: small, two marks: medium, three marks: large). Frogs were divided into three size classes: small (<16 mm SVL), medium (16-18 mm SVL) and large (>18 mm SVL). Because of the rapid growth of the animals there were no frogs left in size class 1 by week three. Therefore, all size classes were shifted up one millimetre (e.g. medium size class: 17-19 mm SVL). All animals were marked between 25th March and 15th April 1999. Recapturing

started with the beginning of the calling behaviour of the males on the 21st April 1999, one week after the last juvenile had been marked.

3.3.3 Results

Aestivating juveniles

Aestivating juveniles of *Hyperolius nitidulus* were found at Gansé Plaine, the Bretelle, near the View point pond and at Iringou. In total, 680 juveniles were marked, and 60 individuals from Iringou were additionally measured but not marked.

Aestivating juveniles varied widely in size. A maximum SVL difference of 11.9 mm was registered in the same patch (Table 29). There were also size differences in aestivating juveniles from various locations: those found at Iringou and Bretelle were significantly smaller than juveniles from View Point and Gansé Plaine (Kruskal Wallis ANOVA $p < 0.01$) (Table 29).

Table 29: Summary of aestivating juveniles' morphometrics.

All (N=742)	Mean +/- sd	Minimum	Maximum
SVL [mm]	17.7 +/- 1.65	12.7	24.3
TF [mm]	8.4 +/- 0.91	6.0	11.7
weight [g]	0.39 +/- 0.12	0.17	1.07
View point (N=34)	Mean +/- sd	Minimum	Maximum
SVL [mm]	18.6 +/- 1.63	15.64	22.4
TF [mm]	8.7 +/- 0.92	7.5	10.98
weight [g]	0.44 +/- 0.11	0.25	0.75
Bretelle (N=226)	Mean +/- sd	Minimum	Maximum
SVL [mm]	17.4 +/- 1.43	14.5	22.8
TF [mm]	8.2 +/- 0.79	6.3	11.2
weight [g]	0.38 +/- 0.09	0.2	0.73
Gansé Plaine (N=422)	Mean +/- sd	Minimum	Maximum
SVL [mm]	17.9 +/- 1.74	12.7	24.3
TF [mm]	8.5 +/- 0.97	6.0	11.7
weight [g]	0.40 +/- 0.12	0.17	1.07
Iringou (N=60)	Mean +/- sd	Minimum	Maximum
SVL [mm]	17.1 +/- 1.45	14.4	21.1
TF [mm]	8.3 +/- 0.81	6.8	10.8
weight [g]	0.32 +/- 0.09	0.19	0.56

The main investigation effort was concentrated on Gansé Plaine as the ponds located there were known to be breeding sites of *Hyperolius*. No breeding ponds could be found at Bretelle.

There were three separate main patches of aestivating *Hyperolius nitidulus* (Map 3): the northern Gansé Plaine stretched 55-500 m east of the Gansé bridge and was about 500 m wide, the central Gansé Plaine surrounded the Gansé Main pond and reached up to 500 m east; the southern part of Gansé Plaine was adjacent to the central part, starting about 200 m further south than the central Gansé pond and reaching up to 800 m east. Few aestivating *Hyperolius nitidulus* were found in a fourth patch at Gansé Swamp, south of the others.

A total number of 422 aestivating juveniles was marked at Gansé Plaine. The sampled areas differed in the number of juveniles found and juveniles coming from the various sites differed in size. (Table 30)

Table 30: Summary of marked juveniles abundance and measurements.

	Marking colour	Number of individuals found	Mean size +/- standard deviation (SVL [mm])	Minimum SVL [mm]	Maximum SVL [mm]
Northern G-PL	yellow	155	17.45 +/- 1.85	12.7	24.3
Middle G-PL	red	144	18.16 +/- 1.80	14.4	23.5
Southern G-PL	green	88	18.23 +/- 1.40	14.9	22.8
Gansé swamp	red + green	35	17.91 +/- 1.31	15.4	21.5
All	----	422	17.90 +/- 1.74	12.7	24.3

Size differences occurred between juveniles marked in different areas (Kruskal-Wallis ANOVA $p < 0.05$) and in different investigation weeks. Juvenile size increased rapidly from marking week 1 to 2 (Kruskal-Wallis ANOVA $p < 0.05$) (Figure 42).

The ponds varied also in the proportion of different size classes found. More frogs were classified as small in the northern parts of Gansé Plaine than in all other parts.

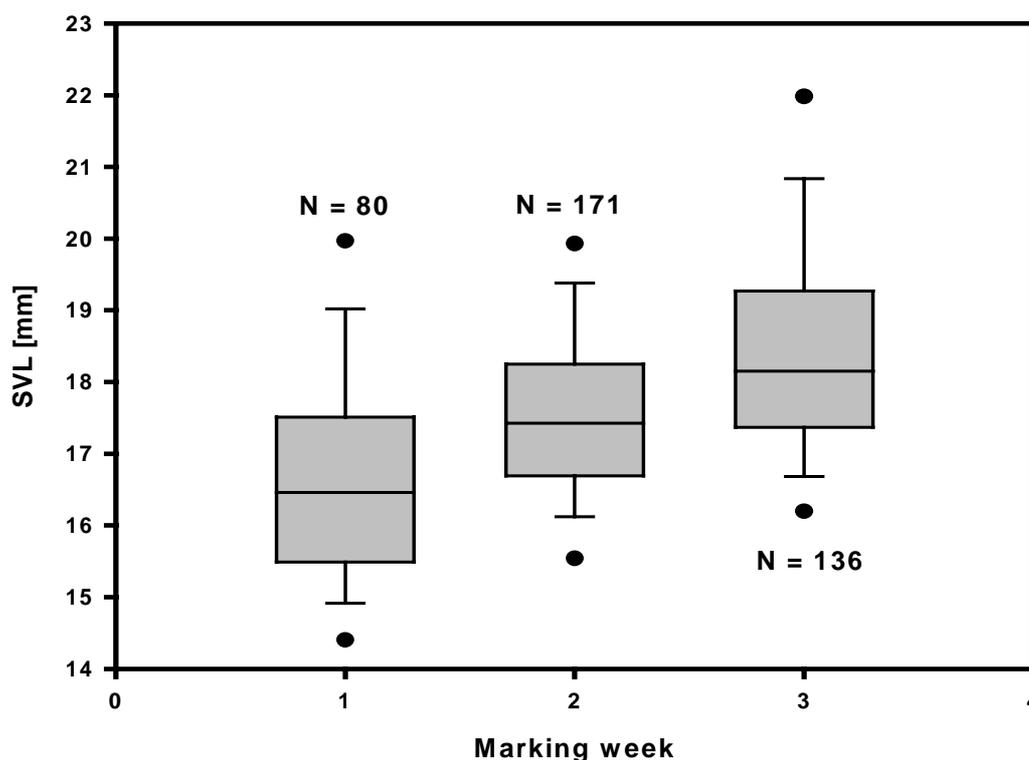


Figure 42: Size differences of aestivating juveniles marked in successive weeks. (Box plots: median (line), 10th, 25th, 75th and 90th percentiles (box with error bars), 5th and 95th percentiles (circle))

Recapture

Of all the 422 marked juveniles at the Gansé Plaine, 25 (6%) came to the investigated ponds to reproduce. Most individuals detected (12) were originally marked at Central Gansé Plaine (Table 31) and the highest number of frogs found was at the central Gansé pond (Table 31).

Table 31: Numbers of recaptured, marked aestivating *Hyperolius nitidulus*. (Numbers in parentheses indicate percentage of marked aestivating juvenile detected at the ponds)

		Found in the			
		North	Central	South	Total found
Marked in the	North	4	6	0	10 (6.5%)
	Central	3	8	1	13 (9.0%)
	South	1	2	0	2 (2.3%)
	Total	8	10	1	

Table 32: Percentage of young recaptured at the Gansé Plaine sites with respect to their marking site and dispersal of young coming from different Gansé Plaine sites.

Recapture			Dispersal		
		Recaptured in the			Coming from the
		North (n = 8)			North (n=10)
Marked in the	North	50	Going to the	North	40
	Central	37.5		Central	60
	South	12.5		South	0
		Recaptured in the			Coming from the
		Central (n = 16)			Central (n = 12)
Marked in the	North	37.5	Going to the	North	25
	Central	50		Central	67
	South	12.5		South	8
		Recaptured in the			Coming from the
		South (n = 1)			South (n = 3)
Marked in the	North	0	Going to the	North	33
	Central	100		Central	67
	South	0		South	0

Dispersal away from the aestivation areas was rarely found (Table 32). In general a high percentage of marked individuals reproducing in one region had been marked in the same region (Table 31). Nevertheless, there was a general trend of movements towards the central Gansé Plaine (Table 32), although these results must be viewed with caution because of the low number of frogs (20) involved.

Of the 25 recaptured aestivating frogs, 4 were females (16%), which results in a sex ratio of 5.25 males per female, a slightly larger male bias than in the overall sex ratio of 3.6 males per female. It resembles to a high degree the sex ratio found in the second-generation animals (5.4 males per female).

The activity pattern of the marked frogs matched the activity pattern of the rest of the frogs (Figure 43) The graph demonstrates the large discrepancy between the total number of calling males and the marked calling males. Although the search for aestivating frogs was intense in order to mark as many frogs as possible, evidently only a small fraction of the aestivating

population had been marked. It is unclear, where the rest of the calling male population came from.

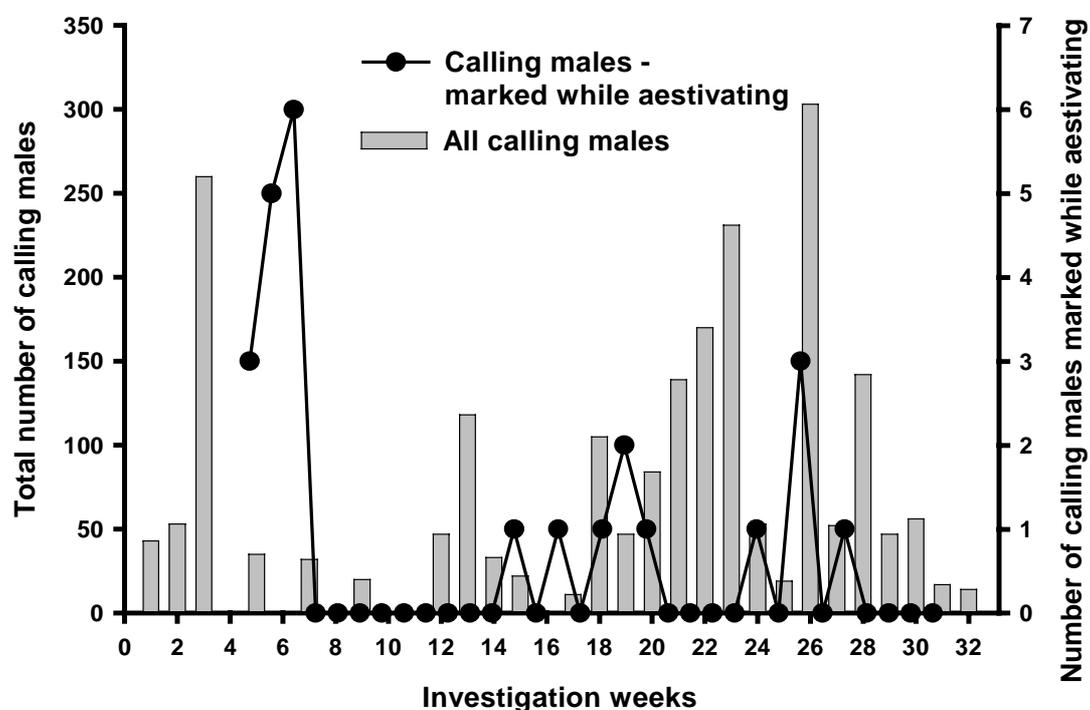


Figure 43: Activity of marked and unmarked individuals of *Hyperolius nitidulus* at Gansé Plaine (note the different scales).

Although sizes of marked frogs differed in different areas (cf. Table 30) these differences were no longer significant after the frogs had come to the ponds, probably because the numbers of marked frogs found were too low (25). Moreover, the mean size of frogs marked in different size classes no longer differed after they turned up for reproduction (Table 33), i.e. all frogs grew to similar size and frogs marked at a large size did not remain bigger than the other frogs.

Table 33: Median sizes (SVL [mm]) of returning aestivated *Hyperolius nitidulus* at Gansé Plaine.

		Snout-vent-length [mm]					
<u>Site of marking</u>	Number	Median	Minimum	Maximum	Percentiles		Mean
					25%	75%	
North	10	27.7	25.8	30.8	27.3	28.1	27.9
Central	13	29.0	26.0	30.8	27.0	29.3	28.3
South	2	26.4	25.9	26.9	25.9	26.9	26.4
		Snout-vent-length [mm]					
<u>Size-class at marking</u>	Number	Median	Minimum	Maximum	25%	75%	Mean
Small	5	27.7	25.8	30.8	27.6	27.7	27.9
Medium	15	28.1	26.0	30.8	26.9	29.1	28.1
Large	5	27.3	25.9	29.8	27.0	29.0	27.8

There was no significant difference (Kruskal-Wallis ANOVA $p=0.42$) in the timing of reproduction between frogs marked in different size classes, although, contrary to expectations, there seems to be a trend that originally large frogs reproduce later (Table 32).

Table 34: Time of reproduction of frogs belonging to different size classes at the time they were marked as aestivating juveniles.

		Time of reproduction (week)					
<u>Size-class at marking</u>	number	median	minimum	maximum	25%	75%	mean
Small	5	3	2	26	2	3	7.2
Medium	15	3	1	26	2	28	10.0
Large	5	17	1	28	3	26	15.0

Marked frogs occurred at the northern Gansé Plaine (Bridge ponds) significantly later than at the central Plaine pond (week 19 and 2, respectively) (Figure 44) (Mann Whitney U-test $p=0.023$; $Z=-2.27$, $U=27$).

There was also a significant difference between the times of occurrence of the sexes at the ponds. Females came significantly later than males (Mann Whitney U-test $p=0.013$) (Figure 45).

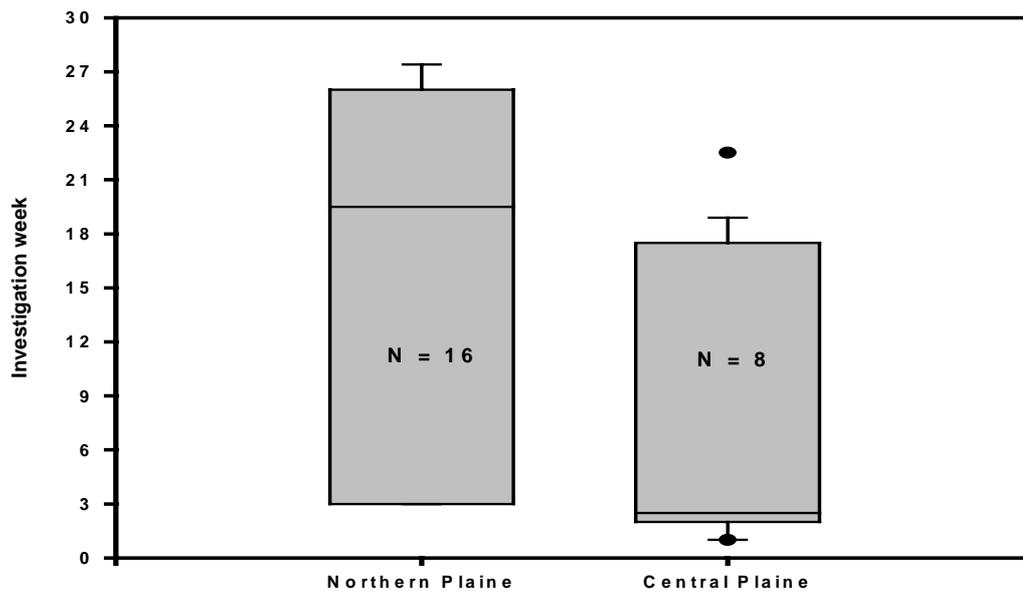


Figure 44: Time of reproduction of marked aestivating *Hyperolius nitidulus* in ponds of the northern and central Gansé Plaine. (Box plots: median (line), 10th, 25th, 75th and 90th percentiles (box with error bars), 5th and 95th percentiles (circle))

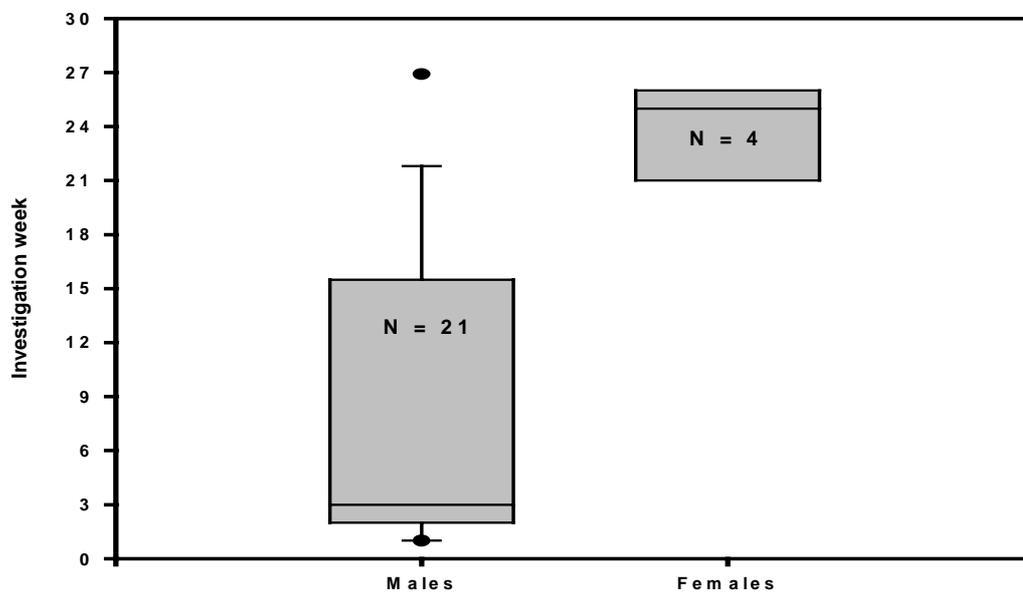


Figure 45: Time of reproduction of male and female *Hyperolius* marked as aestivating juveniles in the savannah. (Box plots: median (line), 10th, 25th, 75th and 90th percentiles (box with error bars), 5th and 95th percentiles (circle))

The main problem in interpreting the observed delay in reproduction is the possibility that these individuals had reproduced earlier at another pond that had not been investigated. Three of the four females that were found had rather small clutches (228, 216, 166 eggs/female – arrival weeks 26 and 28). This suggests that they had already spawned when coming to the investigated ponds, especially because the fourth female with the largest clutch (392 eggs) arrived first (investigation week 18). To estimate whether the aestivated females oviposited for the first or a following time, female weight and clutch size was correlated using females that most likely spawned for the first time (females of 1997 and 1998 found during the first two weeks of reproductive activity; N = 47). The correlation (egg number = $79.848 + 227.04 \cdot \text{female weight}$) was used to calculate expected clutch sizes based on female weight. Figure 46 shows the differences between expected and found clutch size in different months calculated for all years and all ponds.

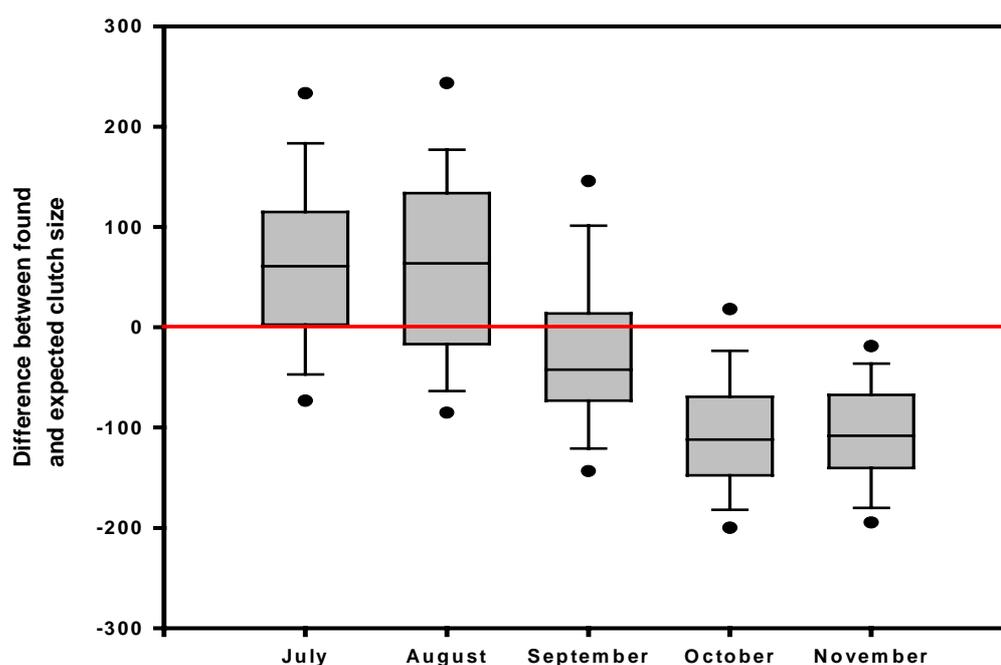


Figure 46: Difference between expected and found clutch sizes based on the correlations for first spawning females for all ponds and years in the different months. (Box plots: median (line), 10th, 25th, 75th and 90th percentiles (box with error bars), 5th and 95th percentiles (circle))

The ANOVA showed that clutch size in July and August were quite similar to each other but very different from September, October and November values. November and October also showed no significant difference (Table 35).

Table 35: ANOVA results for monthly differences between expected and found clutch size, with year as covariable and subsequent Scheffé test.

ANOVA		df = 4			F = 143		P < 0.001	
Scheffé	August	September	October	November	Mean difference	Co-variable		
July	0.99	0.000*	0.000*	0.000*	63.7	1998.67		
August	----	0.000*	0.000*	0.000*	62.8	1998.54		
September	----	----	0.000*	0.000*	-21.98	1998.43		
October	----	----	----	1.0	-104.99	1998.39		
November	----	----	----	----	-105.1	1999.0		

Table 36: Aestivated females' clutch sizes.

Number	Females		Egg number		
	Arrival week	Weight [g]	Found	Expected	Difference
3011	18	1.08	392	324	68
3234	24	1.02	166	311	-145
3255	26	1.20	216	354	-138
3341	26	1.32	228	380	-152

Table 36 demonstrates, that the early female with the larger clutch had a higher egg number than expected for her weight, i.e. the clutch could be classified as first clutch. The other three females had lower clutch sizes than expected, thus these clutches can be considered at least as second ones. This would mean they had already reproduced. They may have been missed during their first spawning either because they reproduced at Gansé Plaine without being noticed, or they spawned somewhere else.

Summary

Six percent of the marked aestivating juveniles were found reproducing at the different Gansé ponds. There was no evidence for physical constraints influencing the onset of reproduction of aestivating frogs. Frogs classified as “large” during marking were not larger than the rest of the population when they came to the pond and they did not start reproducing earlier.

3.3.4 Discussion

Size differences

There were distinct size differences between aestivating juveniles from different areas during the marking process.

Size differences among frogs from different areas possibly reflect the environmental conditions. Rainfall is very local and juveniles depend on rain for foraging. For example, at Gansé Plaine, rainfall usually occurs earlier and more often than at Bretelle, which could explain why frogs from Bretelle were smaller than those from Gansé Plaine. Due to the fact that the Gansé Plaine is large, a similar correlation of rainfall with frog size during aestivation might be responsible for size differences between its northern and southern parts. Local size differences can vary from year to year. Juveniles found at Iringou were very small in 1999, but larger than all other juveniles found in other years [Linsenmair unpublished].

Individuals from the same aestivation patch also varied greatly in size. This may reflect the different physiological conditions of individuals at the start of the dry season. Another possible explanation is the existence of individual differences in hunting success during humid periods, despite identical weather conditions in one patch.

Size differences between frogs found in marking week one and two show that juveniles grew rapidly towards the end of the dry season although they still show partly aestivating behaviour. There was probably already enough moisture at night to allow them to hunt.

Size differences vanished in the recaptured frogs coming to the ponds to reproduce. Individuals that were large during aestivation did not seem to have any advantage from this size in starting reproduction earlier or keeping their size for reproduction. Maybe being large during aestivation is not a sign of good condition, but a handicap that has to be compensated for. Large frogs may have been juveniles that started to grow for reproduction but had to switch afterwards towards aestivation. Due to the large size they used more energy during the dry season [Kobelt & Linsenmair 1995] and, therefore, required more time to grow afterwards. Different behavioural strategies may be another reason. If juveniles that managed to grow very fast towards the end of the dry season reduce activity to minimise predation risk, they will not arrive earlier and/or larger than the rest.

In addition, 1999 seems to have been an extraordinary year with regard to the reproductive period, which was very long and started early. The last juveniles measured aestivating were on average 10 mm smaller than the males calling just one week later. This seems to be an indication that part of the population had already left the aestivation areas and started reproducing, while other frogs were still aestivating.

Recapture

Only 6% of the marked juveniles could be found reproducing at the investigated ponds. The same reasons as mentioned in chapter 3.2.4 may be responsible for the low number of juveniles found after reproduction started. High mortality rates may be one reason, but it seems far more likely that only a small fraction of the active males was caught and/or that not enough ponds were monitored.

Only 1.2% of all reproducing frogs found at Gansé Plaine, had been marked aestivating. The percentage of recaptured females was even lower (0.96%) than the proportion of aestivated males (1.3%). This very low number raises some unresolved questions concerning dispersal that are still under investigation :

- 1) Where did all the other marked juveniles go to? Towards the end of the dry season, mortality caused by unfavourable and harsh climate conditions should be fairly low, because the up-coming rain provides water and makes hunting possible.
- 2) Where did all the unmarked juveniles come from? Other aestivation sites than the exploited ones could not be found.

Juveniles very likely suffer a high predation pressure, when moving around searching for prey. Juveniles possibly have to wander towards the ponds from long distances. Around the *Hyperolius* pond, for example, it seems rather unlikely to find a safe aestivation place within a distance of several hundred meters. Eventually, some juvenile *Hyperolius* aestivate in trees like in Lamto [Vuattoux 1968] and were not found.

In anurans, dispersal events at the beginning of the reproductive season are a very common phenomenon in savannah regions [Spieler & Linsenmair 1998, Kaminski et al. 1999, Marsh et al. 1999]. However, dispersal on the scale investigated in this study seems to be of rather low importance, as in general a high percentage of marked individuals reproducing in one region had been marked in the same region (Table 29). Nevertheless, there was a general trend of

movements towards the central Gansé Plaine (Table 30), although these results must be viewed with caution because of the low number of frogs (25) involved.

It also is plausible that two strategies exist: some of the frogs could return after aestivation to the area where they emerged and others could disperse after aestivation into areas far apart from the investigated ones.

Another interesting result of the marking of aestivating frogs was that 44% of the 25 frogs marked during aestivation and found reproducing at the Gansé Plaine ponds arrived at the investigated ponds in week 13 and later. This high percentage led to the hypothesis of an additional reproductive strategy, where individuals do not reproduce as fast as possible to produce a second generation, but reproduce rather late, which may guarantee high reproductive success in years with unfavourable conditions for the second generation.

The results of the clutch size investigations including the expected clutch size of females to determine whether the females spawned already, showed that most of the females especially when arriving very late were likely to have spawned at least once before captured. However, one aestivated female arriving in investigation week 18 probably spawned for the first time, thus supporting the hypothesis of another late-starting reproductive strategy. This clutch size hypothesis is based on the assumption that no differences between the early and late first-spawning females exist. Females delaying reproduction may follow different strategies than females spawning very early during the reproductive period. If later clutches had for example generally better survival rates, smaller clutches could even be advantageous.

3.4 Environmental factors influencing the decision between aestivation and reproduction

3.4.1 Introduction

The observed polymorphism in life-history strategies of *Hyperolius nitidulus* could possibly follow bet-hedging theory, where the strategy decision either to aestivate or to reproduce in the same season is made by chance. However, it seems instead to be a conditional strategy where the decision is made due to environmental factors or physiological condition [McNamara & Houston 1996]. Since part of the freshly metamorphosed frogs is already dry-season adapted when leaving the water (cf. 3.2.2.2), a mechanism was predicted allowing the tadpoles to differentiate whether the rainy season has just started or whether the rainy season has already been going on for a while. Maybe they can distinguish between an early and a late rainy season phase.

The duration of the larval phase of amphibians and the size at metamorphosis is in most amphibians very plastic and responds to a number of abiotic and biotic factors. Abiotic factors are, for example, water temperature [Smith-Gill & Berven 1979, Newman 1989, Hayes et al 1993, Blaustein et al. 1999], photoperiod [Wright et al. 1990] and pond duration [Denver et al. 1998, Newman 1992, Reques & Tejedo 1997]. Biotic factors could be food availability [D'Angelo et al. 1941, Wilbur 1977b, Travis 1984, Alford & Harris 1988, Berven & Chadra 1988, Crump 1989, Skelly & Werner 1990, Reznick & Yang 1993, Leips & Travis 1994, Anholt & Werner 1995], predation [Skelly & Werner 1990, Wilbur & Fauth 1990, Skelly 1992, 1995, Jackson & Semlitsch 1993, Smith & Van Buskirk 1995, Horat & Semlitsch 1994, McCollum & VanBuskirk 1996, Babbitt & Tanner 1998, Laurila et al. 1998, Laurila & Kujasalo 1999, Chivers et al. 1999, Lardner 2000], density effects [Bragg 1940, Richards 1958, Licht 1967, Brockelman 1969, Wilbur 1972, 1976, 1977 a,b, Gromko et al. 1973, Wilbur & Collins 1973, John & Fenster 1975, Smith-Gill & Gill 1978, Crump 1981, Semlitsch & Caldwell 1982, Parris & Semlitsch 1998, Taylor & Scott 1997], inter- and intraspecific chemical signalling [Rose 1960, Smith-Gill & Berven 1979, Werner 1986, Kupferberg 1997] and water quality, for example pH-levels and ion contents [Glos 1998, Banks & Beebee 1988]. All these factors are known to shape the life-history of amphibians. The effects are well documented and currently under investigation (see literature cited).

For *Hyperolius nitidulus* Schmuck et al. (1994) in their laboratory study found that freshly metamorphosed froglets showed higher tolerance to dry season conditions when raised under high density conditions in water that was changed rarely, than tadpoles raised under low density conditions in water that was changed daily. The latter tried to hide on the ground and suffered high mortality rates when kept under high air temperatures and low air humidity.

Therefore, this study focussed on water quality in the 1997 experiments, but light was afterwards included as it is probably an important environmental factor controlling tadpole development. The investigation followed two lines: (1) changes of environmental factors during the rainy season were monitored in order to find a correlation that might be used by tadpoles to predict the duration of the rainy season; (2) *Hyperolius nitidulus* tadpoles were raised in the laboratory under controlled conditions to test whether water quality or light affect the occurrence of a certain adaptation type (rainy season or dry season).

3.4.2 Methods

Environmental factors in the field

Water quality parameters were measured once a week. Conductivity, pH-value, temperature and water level were recorded in the field when ponds were visited. A hound net was used to check if tadpoles were present, which species they belonged to and if predators could be found. In 1998, water samples were collected in addition every third week. They were analysed photometrically in the laboratory for their nutrient contents (nitrogen and phosphate) (cf. 2.2.4).

In order to detect significant differences in light conditions between rainy and dry season, light intensity (radiation) was measured automatically every quarter of an hour (Campbell weather station – Li-Cor Radiation sensor 200 SZ). Additionally exemplary light intensity was measured during clouded and sunny days using a luxmeter (Gossen: Mavolux digital) The number of sunny hours per day was recorded during one rainy season as an integrative parameter.

Tadpole experiments

The effect of water quality on tadpole development was studied in the laboratory. Tadpoles in Gosner stadium 20 (free swimming and already feeding) with a total length of 12 mm and a body length of 6 mm were selected for the experiments. They were measured (body length, total length) every 4th day and the developmental stage was determined using a Gosner table

[Gosner 1960]. Water quality was checked by measuring pH-value, conductivity level, temperature and sometimes oxygen content. The water was changed regularly every 5th day except for some particular treatments. In 1997 and 1998 rain water was used for all treatments. All aquaria for the water quality experiments were placed in the shade of the forest. Aquaria for the light effect tests were placed in the savannah. Light intensity was measured with a luxmeter (Gossen: Mavolux digital). In the experiments concerning water quality (fresh versus contaminated water) the water already used by the tadpoles was left in the aquaria as long as conductivity did not exceed 300 μ siemens/cm and only partial changes of water (one half of the used water volume was replaced by fresh water) were made (contaminated water) while the fresh water treatments received a total water change even more often than the rest of the groups. In 1998, cloudy water was produced by dissolving one table spoon of sieved savannah soil in 2 l of rain water.

Each treatment consisted of two different aquaria to minimise the risk of total failure due to pathogens dispersing in the water. Experiments started with 120 and 190 tadpoles in 1997 and 1998, respectively. All experimental conditions are summarised in Table 37.

Table 37: Summary of tadpole experiments' conditions.

Year	Group	Conditions	Volume of water	Tadpoles/aquarium	Special conditions
1997	A	large volume	2.5l	5	
	B	small volume	0.5l	5	
	C	high density	0.75l	25	
	D	low density	0.75l	5	
	E	sun light	0.75l	5	placed in the savannah
	F	shade	0.75l	5	placed in the forest
	G	fresh water	0.75l	5	water exchange every day
	H	contaminated water	0.75l	5	no water exchange as long as possible
Year	Group	Conditions	Volume of water	Tadpoles/aquarium	Special conditions
1998	A	sunlight, cloudy water	2l	20	placed in the savannah
	B	shade, cloudy water	2l	20	placed in the forest
	C	sunlight, clear water	2l	20	placed in the savannah
	D	shade, clear water	2l	20	placed in the forest
	E	low density	2l	20	
	F	high density	2l	50	
	G	fresh water	2l	20	water change every day
	H	contaminated water	2l	20	no water change as long as possible

At the end of 1999, four clutches of *Hyperolius nitidulus* were brought to Würzburg, to raise tadpoles under completely controllable conditions. All hatching tadpoles died within several days possibly due to unfavourable water conditions.

In 2000, eleven clutches came to Würzburg in July. This time a special water addition binding heavy metal and calcium-carbonate (VivAqua Wasseraufbereitung, Astra-Products) was used and most tadpoles survived. They were raised under several different experimental conditions to test whether water quality (conductivity) or light influenced the adaptation of juvenile frogs. Water qualities were: 1) low conductivity water, which means tap water (conductivity ~ 1200 $\mu\text{siemens/cm}$) prepared with the water addition and mixed with distilled water (conductivity 0 $\mu\text{siemens/cm}$) until a conductivity of 30 – 50 $\mu\text{siemens/cm}$ was reached; 2) high conductivity water: tap water prepared with the water addition and mixed with distilled water until a conductivity of 250-300 $\mu\text{siemens/cm}$ was reached. For the contaminated water only 0.5 l of the water already used by the tadpoles were replaced by fresh high conductivity water every fourth day. For the fresh water treatment low conductivity water was changed every second day. Low conductivity water was used to raise the tadpoles under differing light conditions. The experimental conditions are summarised in Table 38. A small pocket conductivity metre (neoLab 4-1091 range: 10/1990 μS) was used to control for conductivity of the experimental groups. Light and dark treatments were generated by using uncovered aquaria (light intensity in the climatic chambers: ~ 4400 lx) and aquaria covered with a double layer of paper (light intensity ~430 lx). The aquaria had the normal night day rhythm but the covered aquaria were about ten times darker. Water temperature did not differ between the treatments.

Table 38: Experimental conditions for tadpole raising in 2000

Year	Group	Conditions	Volume of water	Tadpoles/aquarium	Water conditions
<u>2000</u>	F	fresh water	1l	10	water change every second day 30-50 $\mu\text{siemens/cm}$
	C	contaminated water	1l	10	partial water change every fourth day 250-300 $\mu\text{siemens/cm}$
	LC	low conductivity	1l	10	water change every fourth day 30-50 $\mu\text{siemens/cm}$
	HC	high conductivity	1l	10	water change every fourth day 250 – 300 $\mu\text{siemens/cm}$
	Light	day/night rhythm uncovered aquaria	1l	10	water change every fourth day 30-50 $\mu\text{siemens/cm}$
	Dark	day/night rhythm covered aquaria	1l	10	water change every fourth day 30-50 $\mu\text{siemens/cm}$

Each experimental design was started in two aquaria separating small (total length < 10 mm) from large tadpoles (total length >12 mm) of the same clutches, to investigate possible size effects. The aquaria were placed in a climatic chamber where day temperature was 28°C and night temperature was 20°C (water temperature resembled programmed air temperature) until the first juveniles metamorphosed. Environmental conditions for the behaviour tests are summarised in Table 39.

Day/night rhythm was 11h light to 13h dark. Day rhythm was shifted lasting from 4:00h until 15:00h to make observation of night activity easier.

Table 39: Climatic chamber programming for the behaviour tests of freshly metamorphosed juveniles.

	Day	Night
Temperature	32°C	20°C
Air humidity	30%	70%

An exact copy of the tadpole treatments in the climatic chamber was placed in a bureau to have nearly natural light conditions (only filtered through a window). Light varied between 2500 lux at cloudy days to 20.000 lux at sunny days. Covering the “dark” treatment also reduced the light intensity about factor 10. Water temperature was around 26°C because the aquaria were placed by the heater. Behaviour experiments were carried out in the climatic chamber to have dry-season-like conditions.

Altogether 240 tadpoles were used in this experiment.

Another experiment investigating the importance of duration of light instead of light intensity was started shortly after the main experiment. 120 tadpoles from 6 clutches were placed into aquaria (10 tadpoles/1l aquarium) and put in a climatic chamber. The tadpoles belonging to one clutch were kept separate to investigate probable kinship effects. 10 tadpoles of each clutch were kept under normal climatic chamber light conditions including an 11/13h change of light/dark cycle simulating dry-season conditions. Another 10 tadpoles were kept under a non-transparent plastic box that was opened between one and four hours a day to have only short periods of light simulating rainy-season (compare 3.4.3 b) light conditions in the field, Figure 4). Although it was clear that the light intensities in the field were much higher

(compare to p. 115) than what could be given in the laboratory, it was thought that the difference between total darkness under the plastic box and the given light intensity (4400 lux) would be enough to stimulate a reaction.

Juvenile behaviour

The resulting freshly metamorphosed juveniles were tested for their reaction to dry-season conditions (bright light, high temperatures and low air humidity). In 1997 and 1998 in Africa, similar cages were used as for the behavioural tests with juveniles coming from the field in 1999 (see chapter 3.2.2.1). In 2000, when tadpoles and juveniles were kept in climatic chambers, the tests were performed in plexiglas tubes 50cm in height with a diameter of 12 cm. The tubes had lids prepared with a gauze and small openings in the lower part to guarantee air circulation. The tubes were prepared with dry grass to sit on and a leaf layer to provide cover if wanted. Juveniles were fed with drosophila and water was sprayed in the tubes shortly after dusk. Juveniles stayed in the tubes for two days and sitting position (free or covered) was noted during the day and at night.

3.4.3 Results

3.4.3.1 Environmental conditions

Water quality in the field

Although in all investigation years the rainy season had already started, water depth was low and even decreased during the first few weeks. All ponds filled simultaneously between weeks 14 and 18 (August) when the first productive rains fell after the short dry period. The maximum filling of the ponds occurred in all years rather late (August, week 23). Water loss due to evaporation and seeping was highly variable between ponds, Hyperolius pond, e.g., held the water for quite a long time while View Point pond dried up very fast (Figure 47).

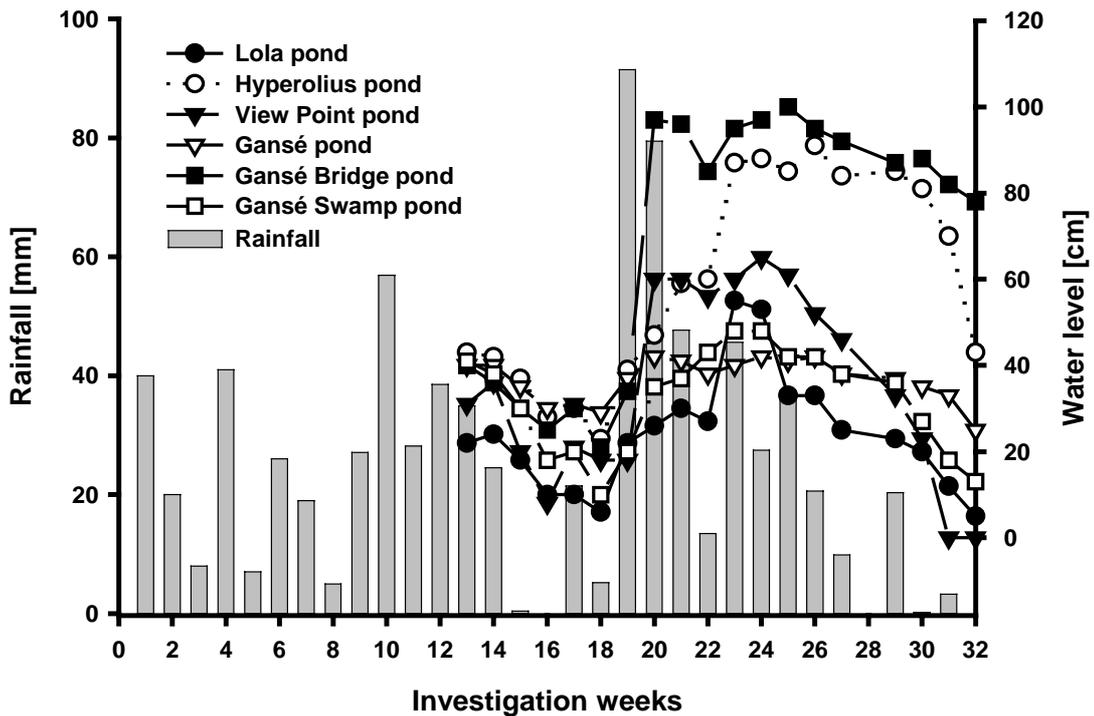


Figure 47: Water level (lines) and rainfall (bars) during 1999. (Symbols denote different ponds.)

Throughout the year, pH-values varied between 6 and 7 and stayed mostly slightly below 7, indicating rain as the major source. There were no marked changes during the rainy season of 1998, but in 1999 the pH-value varied according to heavy rainfalls (Figure 48). Correlations of pH-values with water depth or conductivity level were not detectable.

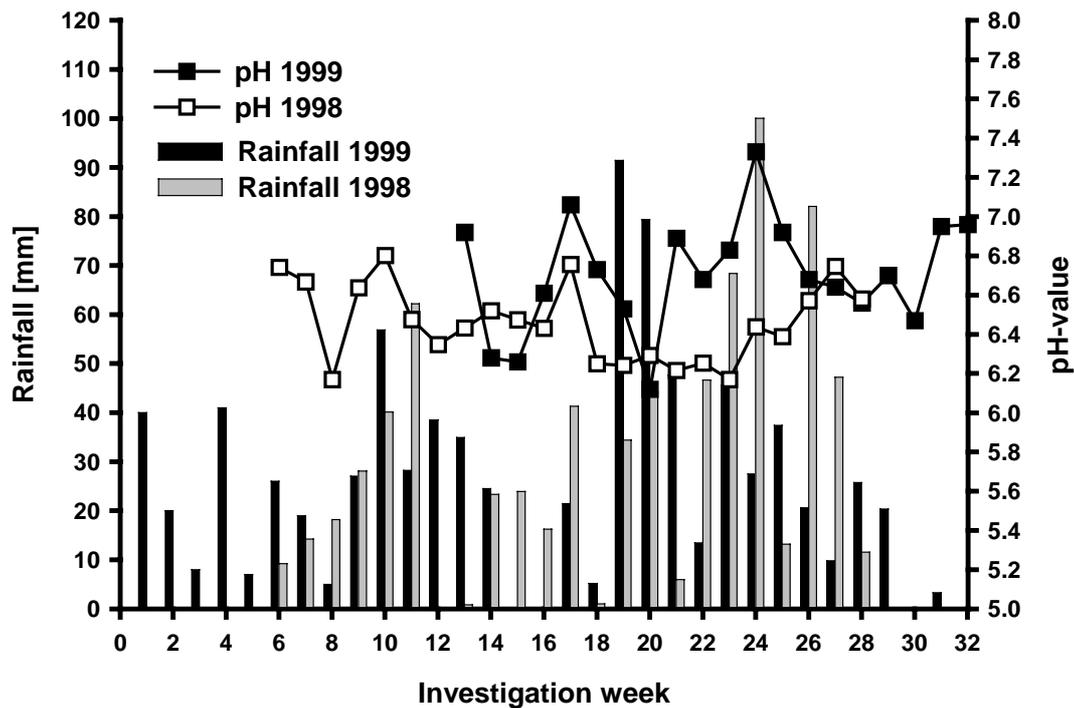


Figure 48: Mean pH-values (lines) of all ponds and precipitation (bars) in 1998 and 1999.

Conductivity levels rose towards the end of the rainy season but the ponds showed very different patterns of conductivity changes (Figure 49). For example, conductivity remained very low, in View Point pond (mean around $10 \mu\text{S}/\text{cm}$) until the pond was nearly dry, but it increased suddenly to a very high value ($342 \mu\text{S}/\text{cm}$) within one week at the end of the rainy season. Lola pond water fluctuated widely and decreased during the last phase while Hyperolius pond showed a slow but steady increase in conductivity.

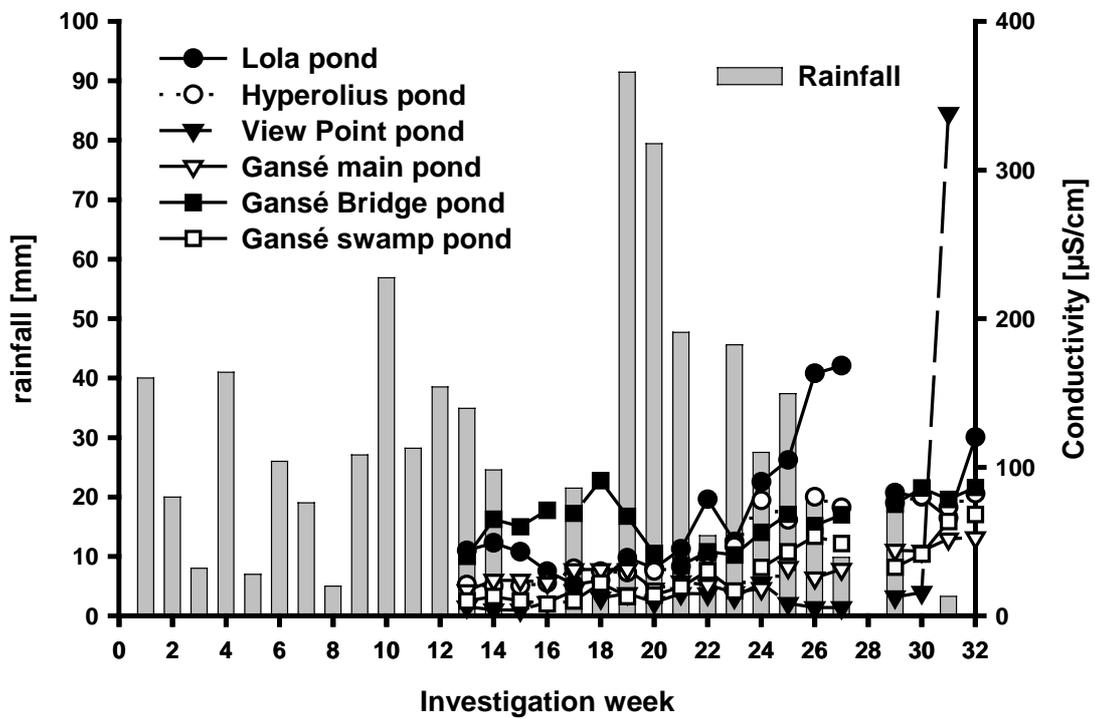


Figure 49: Conductivity levels (lines) and rainfall (bars) in 1999. (Symbols denote different ponds)

Changes in conductivity could be a criterion to estimate changes in water levels and the probability of drying. However, only half of the investigated ponds showed the expected correlation between water level and conductivity (high water levels resulted in low conductivity). Table 40 summarises the results of Spearman rank correlations of conductivity and water level for all ponds in 1998 and 1999.

Table 40: Summary of all results of the Spearman rank correlations for conductivity versus water level at different ponds in 1998 and 1999, starting in investigation week 23 after the ponds were filled to a maximum. Significant correlations ($p < 0.05$) are marked in bold letters.

Pond (year)	Spearman rank correlation		Conclusion
Hyperolius pond (1998)	p=0.38	R= -0.5	n. s.
Hyperolius pond (1999)	p=0.39	R= -0.3	n. s.
Lola pond (1998)	p=1.00	R= 0.0	n. s.
Lola pond (1999)	p= -0.57	R= -0.2	n. s.
View point pond (1998)	p=0.80	R= -0.1	n. s.
View point pond (1999)	p=0.023	R= -0.7	the higher the water level the lower the conductivity
Gansé main pond (1998)	p=0.32	R= 0.4	n. s.
Gansé main pond (1999)	p= 0.005	R= -0.8	the higher the water level the lower the conductivity
Afrixalus pond (1998)	p= 0.49	R= 0.3	n. s.
Gansé bridge pond (1999)	p= 0.021	R= -0.7	the higher the water level the lower the conductivity
Gansé swamp pond (1999)	p= 0.007	R= -0.8	the higher the water level the lower the conductivity

The nutrient contents measured in 1998 in the investigated ponds were unexpectedly high (N: 0.525 mg/l, P: 0.072 mg/l) (Figure 50). Especially due to the high phosphate levels much more algae growth would have been expected (details and values – for comparison see discussion page 125). They were not correlated to any other parameter, e.g. water depth. No temporal pattern was detected.

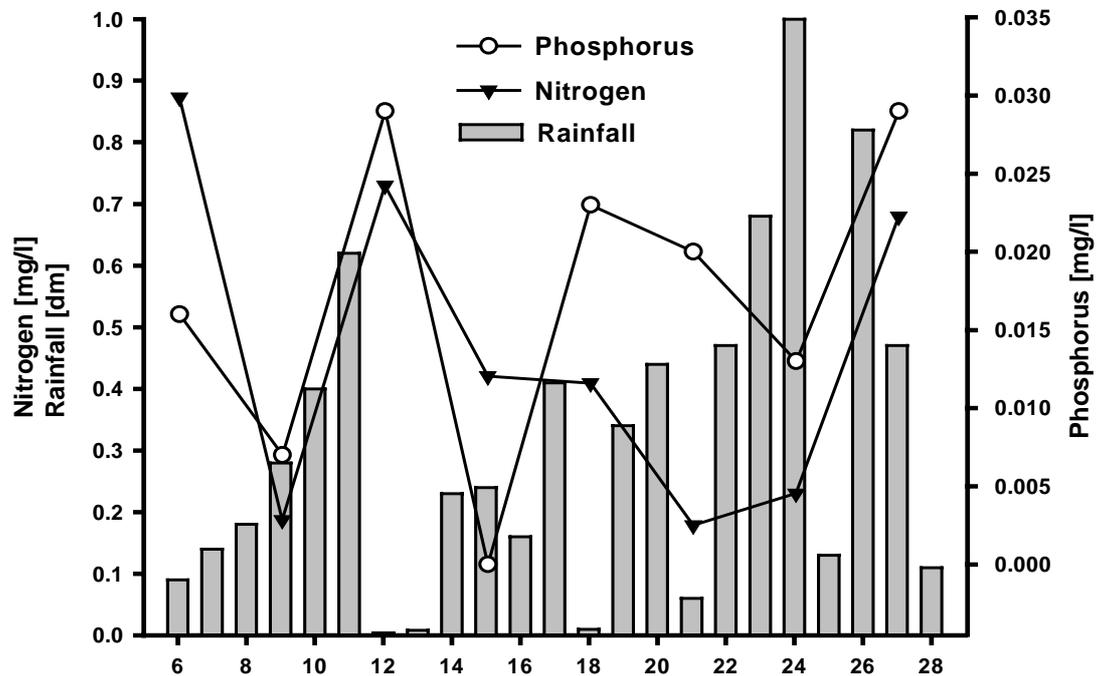


Figure 50: Nutrient contents of Gansé pond and rainfall in 1998. (Rainfall unit is dm to fit on the nitrogen axis.)

Light conditions in the field

Light intensity and duration changed significantly at the end of the rainy season. While it was very cloudy from July to September, sunny hours became more frequent in October and mainly in November (Figure 51). A nonparametric multiple comparison test following Zar (1984) revealed significantly more sunny hours per day in November than in July, August, or September. Although there was a trend towards more sunny hours in October, the differences were not significant.

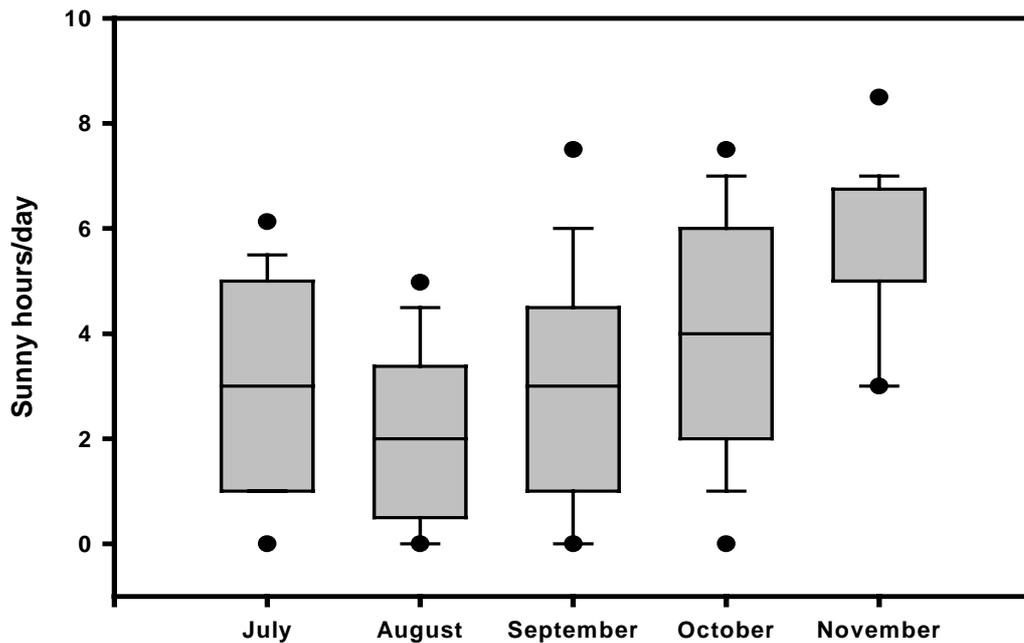


Figure 51: Box-plot of median numbers of sunny hours per day for the rainy season 1999 (July to November). (Box plots: median (line), 10th, 25th, 75th and 90th percentiles (box with error bars), 5th and 95th percentiles (circle))

Light intensity differed considerably between cloudy and sunny days. On sunny days light intensity (up to 100.000 lux) was up to five times as high as on cloudy days (20.000 lux). Also the stability of light intensities varied, while during dry season light intensity was constantly high (raising steadily until 13:00 h and going down slowly afterwards), during rainy season light intensity could change rapidly due to variable amounts of clouds varying from 20.000 lux up to 100.000 lux within minutes.

Summary

The ephemeral ponds in Comoé National park that were used by *Hyperolius nitidulus* for reproduction showed some similarities: conductivity levels were very low varying normally between 4 and 90 $\mu\text{S}/\text{cm}$ but increasing up to 338 $\mu\text{S}/\text{cm}$ towards the end of the rainy season. Throughout the year, pH-values varied between 5.5 and 7.5 and stayed slightly below 7 most of the time, indicating rain as the major source. Correlations of pH-values with water depth or conductivity level were not detectable. The number of sunny hours/day increased significantly towards the end of the rainy season.

3.4.3.2 Tadpole experiments

Tadpole development

Tadpole developmental stage and size was very closely correlated (Pearson Product Moment correlation $p < 0.001$; Table 41), therefore developmental stage in accordance with Gosner (1960) was taken as a reliable measurement to describe tadpole growth plus development.

Table 41 : Results of a Pearson-Product-Moment correlation among development and growth in *Hyperolius nitidulus* tadpoles. R^2 values are given. (Data from week 14 in 2000.)

	developmental stage	body length [mm]
body length [mm]	0.81	-----
total length [mm]	0.69	0.76

Differences between the years investigated

All years investigated differed significantly in tadpole mortality rates (Table 42) and developmental times (Kruskal-Wallis ANOVA $H=75.75$ $p < 0.001$). In 1997 less than 50% of all tadpoles used during the experiment died before reaching metamorphosis, in 1998 76% died before metamorphosing and in 2000, when tadpoles were brought to Würzburg to be raised under laboratory conditions, almost 93% of them died before metamorphosis (Table II - Appendix). χ^2 tests with Bonferroni correction to compensate for multiple test usage revealed the significance of the observed differences between the years (Table 43).

Table 42: Results of χ^2 tests on differences in mortality rates between the investigated years. (Results significant after Bonferroni correction are marked in bold letters.)

	Chi²	p
1997 versus 1998	16.31	0.0001
1998 versus 2000	73.64	0.00001
2000 versus 1997	20.25	0.00001

Developmental times considerably extended in 2000 when first metamorphs left the water 105 days after hatching. In 1997 the first metamorphosing tadpole was found after 48, in 1998 after 56 days. Indirect data from the field (first amplexant couple found compared to the first

juveniles seen) indicate that field tadpole developmental time is around 40 days. Figure 52 shows differences in tadpole development and occurrence of first metamorphs for all years.

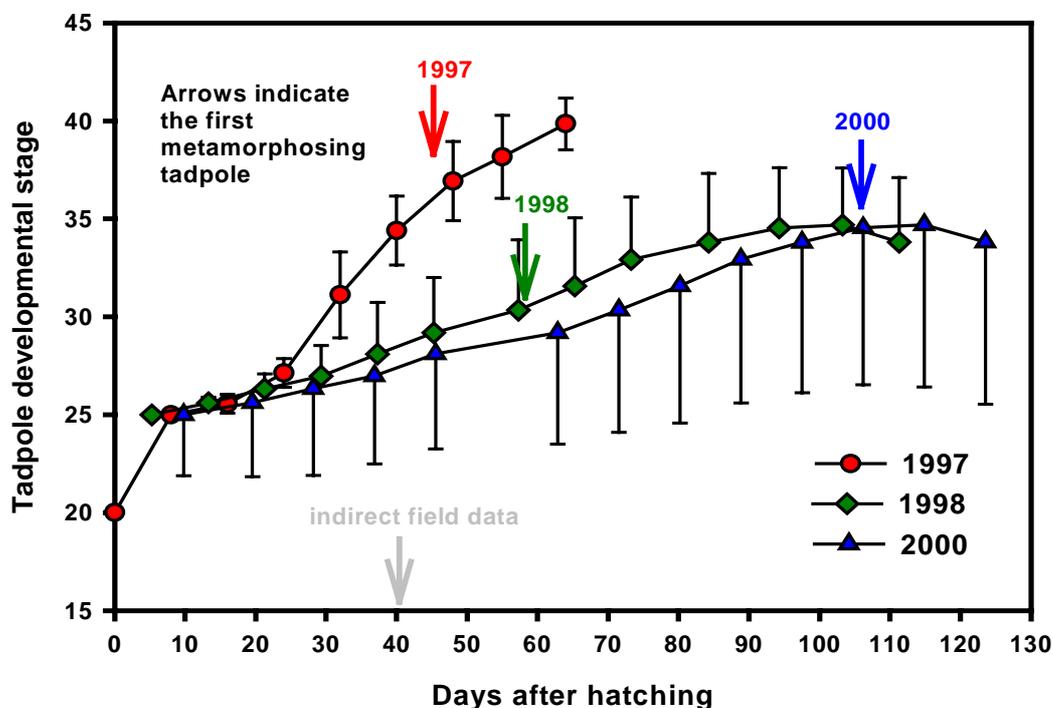


Figure 52: Tadpole growth curves for the different years. Plotted are mean values of all experimental groups with standard deviation. Arrows indicate the first metamorphosing tadpole (colour code for the investigated year: red = 1997, green = 1998, blue = 2000, grey = indirect field data from 1998)

Table 43: Days until metamorphosis and results of Mann Whitney U-tests for differences between the years. (Bold letters indicate significant differences, Bonferroni correction was used to compensate multiple test usage)

Days until metamorphosis						
	Median	25% Percentile	75% Percentile	Minimum	Maximum	N
1997	62.5	55	68	48	99	59
1998	89	77	106	56	108	33
2000	126	117	130	105	133	19
Mann-Whitney U-tests						
	U	Z	p			
1997 versus 1998	169	-6.549	< 0.00001			
1998 versus 2000	0	5.615	< 0.00001			
2000 versus 1997	18	6.525	< 0.00001			

Differences between experimental groups

Mortality rates between different experimental groups were found to be significantly higher in 1997 for tadpoles raised in much versus little water (Chi² test $p = 0.0098$) and in high versus low densities (Chi² test $p = 0.0006$). For all other groups and years no significant differences in mortality could be found (Table III - Appendix).

Differences in development and growth between experimental groups were found but a high intragroup variability and high mortalities which sometimes shrunk group size from 10 to 2 during the experiment, made proper statistical analysis difficult. However, growth curves (Figure 53) are given to illustrate tadpole growth (mean values per group in the time course) and the high variability among individuals of the same group (standard deviation). To give an example two experimental groups present in all three years were chosen: fresh water and contaminated water was thought may influence life-history decisions of tadpoles [cf. Schmuck et al.1994]. Tadpoles raised in contaminated water seemed to have higher mortality rates than tadpoles raised in fresh water (Table IV - Appendix), but these results were not statistically significant. In 1998 tadpoles raised in contaminated water seemed to grow faster than tadpoles in fresh water, but the opposite result was obtained in 1997 and 2000.

Significant differences in growth and development between different treatments were found only in 1997 between tadpoles raised in low and high density (Mann-Whitney U-test $p < 0.004$) and in 1998 between tadpoles raised in low and high density (Mann-Whitney-U-test $p < 0.004$). Mann-Whitney-U-tests were followed by Bonferroni correction, to correct for multiple test usage [Sachs 1999] but the differences remained significant (Table V - Appendix). Figure 54 illustrates the differences showing that tadpoles raised under low density conditions developed faster than tadpoles raised under high density conditions. Even though the comparison between the groups was made after 7 weeks in 1997 and after 10 weeks in 1998, tadpoles raised in 1998 did not reach the developmental levels of 1997 tadpoles.

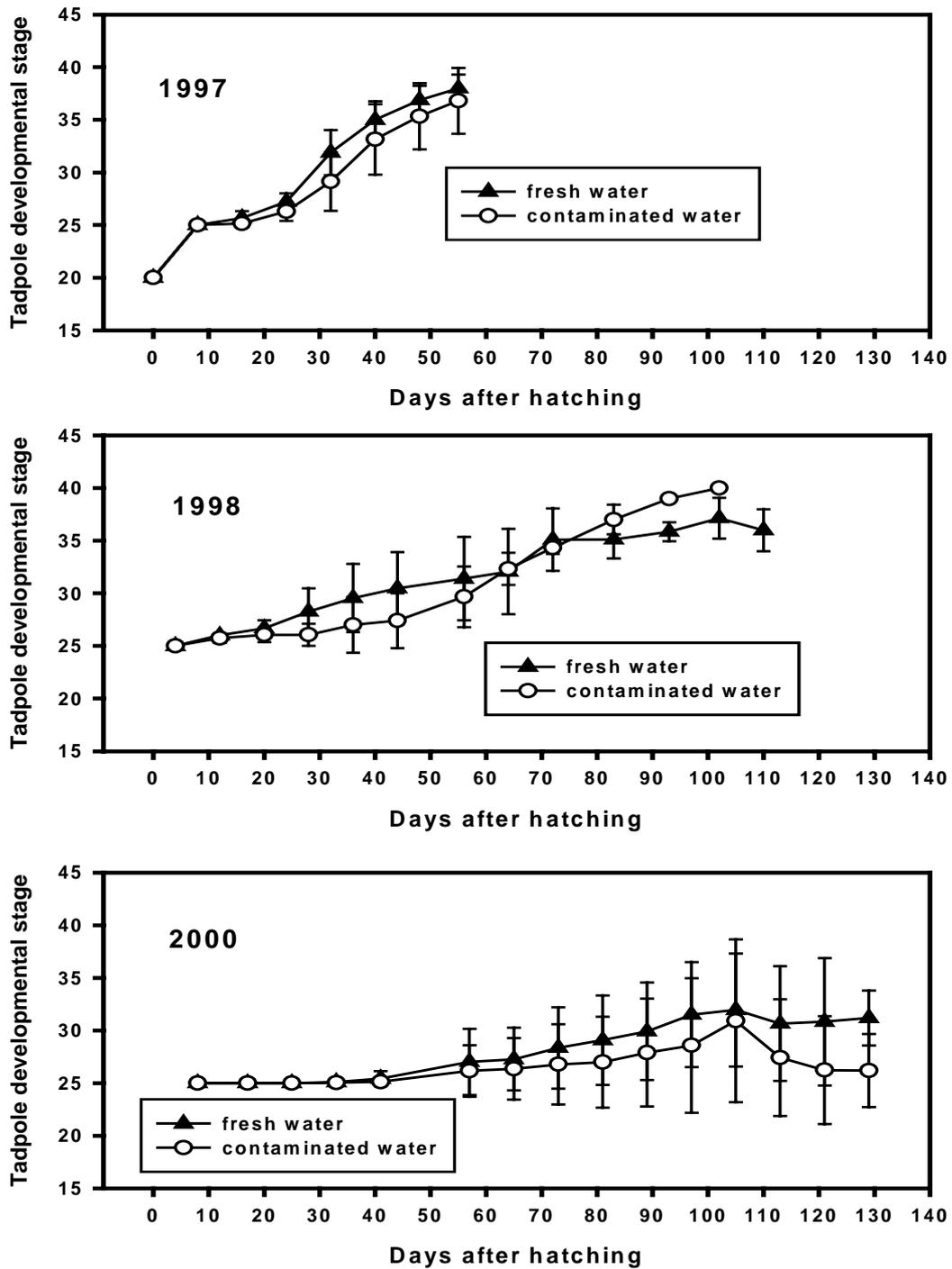


Figure 53: Exemplary growth curves of tadpoles in two experimental groups present in all three years: fresh versus contaminated water. Treatment mean values plus standard deviations are given.

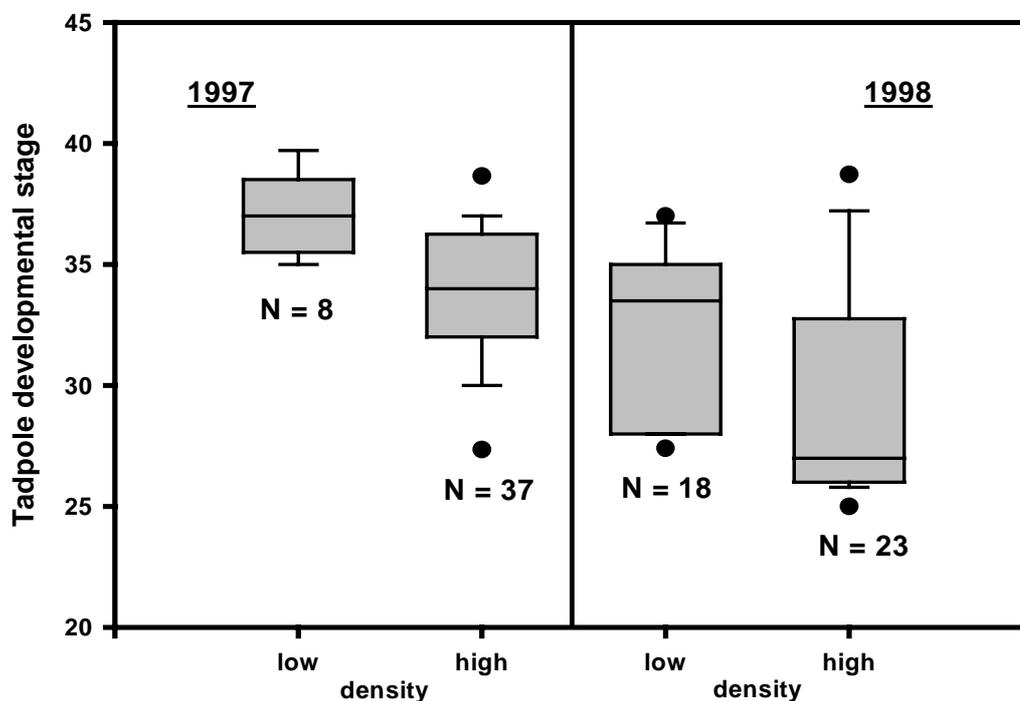


Figure 54: Differences in development between tadpoles raised in low versus tadpoles raised in high densities. Tadpoles developed faster under low density conditions in both years 1997 and 1998. (Box plot (median (line), 10th, 25th, 75th and 90th percentiles (box with error bars) and 5th and 95th percentiles (circle))

In 2000 significant differences between the two treatments climatic chamber and bureau were found. Mortality was higher in the climatic chamber (Chi²-test: Chi² = 36.94, $\alpha < 0.0001$) but development was significantly faster and tadpoles were larger (Table 44).

Table 44: Differences between the climatic chamber and bureau treatment in 2000. (Mann-Whitney U-tests comparing growth and development 10 weeks after hatching. Significant results are marked with bold letters)

Mann-Whitney-U-test					
	U	Z	p	N ₁	N ₂
Developmental stage	800	5.15	<0.00001	38	98
Body length [mm]	1327	2.59	0.0081	38	98
Total length [mm]	1523.5	1.64	0.0998	38	98

For this reason these groups had to be tested separately. In the climatic chamber and bureau treatments in 2000 no significant differences in development or growth between the experimental groups was found (Appendix - Table V).

Size differences in the treatments vanished very early. After about 6 weeks of development the intra-group variability in size and developmental stages was higher than the differences between the originally large and small tadpoles.

Juvenile behaviour

Although juvenile behaviour was clearly separable in the dry season adapted and the rainy season adapted type (cf. 3.2.2.2) and juveniles tested for it showed either one or the other behaviour, no factor could be identified triggering the decision between aestivation and immediate reproduction. In 1997, 1998 and 2000 most juveniles responded to the dry season adaptation test by hiding, showing low tolerance for high temperatures and low humidity and high mortality rates when directly exposed to the sunlight. No differences between treatments could be found when the years were separated. Due to high mortality the responding individual numbers in 1998 and 2000 were too low for statistical analysis. The only trend that could be found was a slight tendency for juveniles raised in contaminated water to be dry-season adapted or at least more tolerable towards dry season conditions. To get sufficient numbers for this test, all years were pooled. Additionally all groups with water conditions other than contaminated were counted as fresh water, and tadpoles raised in contaminated water but not for this experiment also had to be taken into account.

Summary

Tadpole raising in the laboratory was characterised by high mortality rates and slow development especially in 2000, when tadpoles were raised in Würzburg. Density was the only factor influencing tadpole development and growth. Juvenile behaviour was not influenced by the water conditions the tadpoles were raised in, contaminated water showed a trend towards biasing tadpole development towards dry-season adaptation.

3.4.4 Discussion

Due to the fact that freshly metamorphosed juveniles of *Hyperolius nitidulus* were already dry or rainy season adapted (see chapter 3.2.2.2), it was assumed that the decision for aestivation or immediate reproduction was already made during the tadpole stage. Many tadpoles are very variable in their developmental response to different environmental factors (Newman 1998, Beachy et al. 1999, Niecieza 2000) and this adaptive plasticity is generally thought to be advantageous when unpredictable environments are inhabited (Van Buskirk et al. 1997). Pond desiccation is a phenomenon that often gets drastic developmental responses from tadpoles [Tejedo & Reques 1994, Brady & Griffith 2000]. It is also a reliable sign of the end of the rainy season. Therefore it was investigated if any environmental factor measurable in the field correlated with pond desiccation and was possibly able to trigger tadpole development and juvenile behaviour.

Possible indicators of rainy season duration

pH

pH-values did not change much, but showed a certain dependence on rainfall. They increased during phases with low rainfall up to a maximum of 7.8, possibly because of photosynthetic activity and dropped after rainfalls due to the slightly acidic inflow of water (rain water pH: 6). As a clear trend for pH-values could not be found, e.g. increasing toward the end of the rainy season, it does not seem very likely that tadpoles use pH to estimate the duration of the rainy season.

Conductivity

Conductivity was measured because of the expected negative correlation with water level. Detecting changes of ion content in the pond water by tadpoles could be a possibility to estimate water level and predict rates of water loss. The conductivity was very low compared to African or European lakes; rain collected in the camp had a conductivity of 5-10 $\mu\text{S}/\text{cm}$. The expected correlation with water level (the lower the water level, the higher the conductivity) was found in some of the investigated ponds for the period after maximum filling, but it was not very clear. The ponds showed a rather individual conductivity increase towards the end of the rainy season. View Point pond, e.g., had very low conductivity levels until it almost dried out, when conductivity rose to more than 10 times the former value in one week (20 $\mu\text{S}/\text{cm}$ -> 338 $\mu\text{S}/\text{cm}$). This sudden increase seems far too late as a signal for a

tadpole since the pond dried out in the same week. In all other ponds conductivity increased slower and could possibly be used as a criterion for estimating the length of time the pond would still contain water. A combination of this factor with other factors, e.g. light (see below), is also possible.

Nutrient content

Nutrient content was high but stayed in a normal range (Golterman 1975). The water colour (brown) indicates a high level of humic acids and a low Ca level and conductivity.

Table 45 compares results of Golterman's investigations at Lake George (East Africa) to the phosphate and nitrogen content of the Comoé ponds. Ammonium levels were higher in the Comoé ponds than in Lake George. This may be due to the smaller size of the Comoé ponds and the frequent input from animal sources.

Table 45: Comparison of nutrient contents of Lake George and the Comoé ponds.

	<u>Lake George</u>		<u>Comoé ponds</u>	
	Range	Mean	Range	Mean +/- SD
NH₄-N [µg/l]	3-135	24.4	10-1240	120 +/- 270
NO₃-N [µg/l]	0.0 – 2390	533	70-1650	390 +/- 270
PO₄-P [µg/l]	2.5 – 215	78.8	10-80	70 +/- 20

The nutrient content seems to fluctuate on a much shorter scale than would be needed to predict how long the rainy season will last. Because of the small pond size, the water is frequently loaded with nutrients coming from buffalo and hippopotami that use the ponds for drinking and bathing and thereby defecate and urinate into the water. These short term changes in nutrient content may overrule long term changes in nutrient content, which may explain the lack of a temporal pattern in nutrient content for the ponds and make it impossible for the tadpoles to use this factor as a zeitgeber.

Light

Full light duration changed significantly with a clear temporal pattern during the rainy season and could therefore be used as a criterion to estimate the duration of the rainy season. Light intensity is very different between sunny and cloudy days, hence it is possible that tadpoles use the increase of sunny hours as indication of the end of the rainy season.

Water volume

Although it was not investigated in this study tadpoles might possibly estimate pond desiccation directly by realising swimming volume changes [Denver et al. 1998] rather than indirectly by high tadpole densities or changing water quality.

Tadpole raising

Tadpoles were raised mainly to investigate possible effects of water quality or light conditions on life-history decisions of juvenile frogs.

Tadpole development is influenced by many biotic and abiotic factors and tadpoles show a high adaptive plasticity in reacting to these traits (see Introduction).

Tadpole development seemed fairly natural in 1997, while in 1998 mortality rates were higher and developmental times elongated. In 2000, when the tadpoles were raised under laboratory conditions, mortality rates were unexpectedly high and growth rates were unexpectedly low. Differences between the years can possibly be explained by density effects. In 1997 5 tadpoles were raised in 0.75l of water, in 1998 7.5 tadpoles/0.75l of water (20 tadpoles per 2 l of water) and in 2000 10 tadpoles were raised in 0.75l of water. Reduced tadpole success showing in lower developmental rates, slower growth and small size at metamorphosis due to high tadpole density are found in many anuran species and are well documented [Brockelman 1969, Crump 1981, Wilbur 1976, 1997a,b]. Higher variability in growth (cf. intragroup variability in tadpole size) and developmental times are also often observed among tadpoles raised in high densities [Crump 1981]. The experimental results of 1997 and 1998 showing that tadpoles raised under higher densities suffered significantly higher mortality rates and developed significantly slower than tadpoles raised under lower density conditions point in the same direction. These density effects are explained either by competition for food [Brockelman 1969], infection with growth inhibiting cells (“colourless algae like cells 5-19µm in diameter, possibly bacteria” [Richards 1958]) [Licht 1967] or inhibiting agents released from larger tadpoles [Rose 1960]. Except for a possible competition for food all these explanations seem rather irrelevant for *Hyperolius nitidulus*. In the field, density dependent mortality is possibly mainly mediated by predation [Anholt and Werner 1995, Calef 1973].

Although density effects may have contributed to differences in the development of tadpoles and survival between the years in the laboratory, tadpole densities in the field can be much higher than the ones used in the experiment. Bragg (1940) found *Bufo* densities between 3.5 and 27 tadpoles per litre and Rödel (1998) reported tadpole densities up to 30.7 individuals per litre in the Comoé National Park. Thus, density effects are surely not sufficient to explain the poor performance of tadpoles in the laboratory in 2000. Tadpoles in the climatic chamber and bureau suffered significantly differently in mortality and developmental traits but tadpole density, at least at the beginning, was the same for both treatments. Tadpoles were fed ad libitum, but competition for food, at least for some limiting ingredients, cannot totally be excluded although in all years the same food was used. Skelly and Werner (1990) showed that food limitation results in lower metamorphic size and in longer time needed to reach metamorphosis. Temperature can have very strong effects on tadpole development [Blaustein et al. 1999], thus the lower office temperature may be responsible for slower metamorphosis of tadpoles.

A possible explanation for high mortality rates may be stress caused by the regular measurements. Crump (1981) reported survival rates between 73% and 90% in undisturbed bowls with tadpoles. But as experimental stress was the same in all years this does not explain the differences between the years. However, undisturbed tadpoles not used for the experiments seemed to grow faster (first freshly metamorphosed juvenile about two weeks earlier than the experiment ones), but these effects were not systematically observed and other factors like water change frequency differed additionally between these tadpoles and the experimentally used ones.

Juvenile behaviour

The freshly metamorphosed juveniles coming from the different experimental treatments were tested for their behavioural response to dry season conditions (bright light, high temperatures, low air humidity).

In 1997, towards the end of the rainy season 24% of the 59 metamorphs were found sitting directly in the sun. Differences between the treatments could not be found. In all groups, most individuals preferred covering. In 1998, 33% of the investigated 33 juveniles preferred sitting in the sun, but due to high mortality rates during tadpole stages, no differences between the groups could be found. In 2000, when mortality rates were even higher, no differences in the behavioural outcome could be found between the investigated groups. However, when all

tadpoles raised in contaminated water and fresh water were pooled there was a trend indicating that juveniles raised in contaminated water were more often dry-season adapted. This is in accordance with the results of Schmuck et al. (1994) who found that contaminated water increased the percentage of freshly metamorphosed juveniles able to tolerate dry season conditions.

The trend found in this study has to be interpreted very carefully. Considering high mortality rates, extraordinarily slow development and developmental malformations in 2000, tadpole raising conditions were far from the natural situation. The observed results may be a laboratory artefact although it seems far more likely that the possible effect of water quality on the developmental decision of tadpoles was masked due to the unfavourable conditions. It is possible that in the field tadpole density plays a role not only for the development of tadpoles but also for the behaviour shown afterwards, strongly pointing to a decision for either aestivation or immediate reproduction. Light conditions had no influence, but this may also be due to the experimental conditions not simulating the field situation in enough detail (light intensities constantly around 4400 lux, instead of over day changing intensities up to 100.000 lux) or stressing the wrong light characteristic.

The tadpole-raising experiments showed that water quality may play a role in the decision process but further work has to be done testing light intensity and duration (e.g. sunny hours per day) under more natural conditions. It also has to be tested whether tadpoles of *Hyperolius nitidulus* are able to recognise pond desiccation by decreasing water volume rather than changes in water quality or encounter rates with other tadpoles [Denver et al. 1998].

Maternal effects could also influence the tadpoles decisions to aestivate or to immediately reproduce. Long term maternal effects that effected offspring life-history were found for example by Groeters & Dingle (1987) and by Sibly et al. (1997) and although seem rather unlikely can not be excluded in this study.

4 Discussion

Different reproductive strategies

Distinct juvenile behaviour differences, changes in adult sizes and reproductive capacity and a long reproductive period triggered the working hypothesis of two alternative life-cycle strategies favouring aestivation or immediate reproduction. Our hypothesis for the life-cycles of *Hyperolius nitidulus* that differed from the commonly assumed reproductive pattern was confirmed by the results of this study.

Aestivated juveniles start to mature at the beginning of the rainy season and reproduce subsequently. Their tadpoles grow until metamorphosis and either reproduce in this same season in which case their offspring aestivates (one year – two generations) or they delay reproduction to the following year and aestivate themselves (one year – one generation). Juveniles trying to reproduce as fast as possible should invest in growth and differentiation and show no costly adaptations to aestivation, while juveniles delaying reproduction to the following rainy season should be well adapted to dry season conditions. The chances of a particular strategy being successful must change with time: for example, the second generation strategy including fast maturation and reproduction during the same rainy season seems to be far more successful when started as early as possible. Juveniles that metamorphose early during the rainy season can expect some more months of rain and enough time for their offspring to leave the water and prepare for aestivation. For juveniles metamorphosing late during the rainy season, the risk of total reproductive failure due to drying out ponds may be higher than the risk of their own death during the dry season.

Different life-history strategies can be highly adaptive in a rather unpredictable environment, where the duration of the reproductive phase varies strongly between years [Menu and Roebuck 2000; Cooper and Kaplan 1982; Semlitsch and Gibbons 1985]. On the one hand, individuals reproducing with a second summer generation should have strong advantages: juveniles do not have to survive a hard dry season with high mortality rates, but instead can be sure to reproduce in one season and, if the dry season is favourable, even reproduce a second season. On the other hand, the costs may be high if the conditions are unfavourable and the second generation fails because of a lack of developmental time. A hint on the possibly high costs of the strategy is the result of the reproductive season in 1998, when reproduction started late and of 700 marked juveniles not one could be found reproducing.

Because time seemed to be a very important factor, it was assumed that juveniles start to reproduce as fast as possible and that differences in the starting point of reproduction were due to physiological constraints resulting from differing aestivation outcome. However, it was found that at least some individuals arrived very late at the ponds and that larger size at the end of the aestivation period did not predict earlier arrival at the reproduction ponds. Possibly larger size does not indicate a better survival during the aestivation period and better foraging success but is a handicap indicating that, due to its non-optimal size, this frog had used more energy during aestivation [Kobelt & Linsenmair 1994] and now needs more time to prepare for reproduction. Juveniles that are able to grow very fast at the end of the aestivation period could also reduce activity after reaching a size limit to reduce predation risk and, therefore, follow another foraging strategy. This behaviour would also lead to uniform body sizes at the start of reproduction. Another possibility is the existence of a reproductive strategy characterised by the aestivated adults waiting and reproducing late rather than early. This strategy could be advantageous when the environmental conditions are unfavourable and the second generation fails.

The role of female choice

Leks are generally thought to be ideal systems to study female choice [Bradbury & Gibson 1983, Ryan 1997]. Female preference for larger males or size assortative mating is documented for many lek breeding frog species [Licht 1976, Marquez –M. De Orense & Tejedo-Madueno 1990, Tejedo 1992, Howard & Young 1998, Howard et al. 1998, Castellano & Giacoma 1998, Robertson 1986b, 1990]. Therefore, the absence of female discrimination between second and first year males despite their significant differences in size and age is one of the most interesting results of the investigation into the reproductive behaviour of *Hyperolius nitidulus*. The second-generation strategy would have been expected to fail if female *H. nitidulus* showed a significant preference for large or older males, because the reproductive success of second-generation males, which are smaller and have no proof of their capability to aestivate, would be reduced. Eventually the reduction of fitness for the second-generation males would be too high to be compensated for by the advantage of having more offspring in case of a long rainy season. Female mating behaviour seems to be the key trait for the coexistence of two life-history strategies in the population of *Hyperolius nitidulus*.

However, the possibility cannot be ruled out that females choose their mates anyway. Females possibly do not select for older or larger males but it is possible that they

prefer male traits that were not investigated. As calling is a very costly behaviour [MacNally 1981, Grafe et al. 1992] it is assumed that females use male calls to choose among males. They possibly prefer high call rates indicating physiological fitness rather than call frequency indicating size [Passmore et al. 1992]. Female *Hyperolius nitidulus* possibly minimise predatory costs by choosing between a very small fraction of all present males (by minimising the time spent moving around in the lek). However, of the few males in her direct surrounding the female could choose the fittest using his call rate as a reliable indicator. This would explain males calling and males higher calling effort when male densities are high (cf. 3.1.2.1). As it is very likely that the call rate indicates individual fitness rather than individual size or age the female lack of discrimination between first and second generation males could be explained.

Male calls could also give information about different genotypes of males independent of the males' size or age [Abt & Reyer 1993, Ryan et al. 1996].

Kin competition

At the beginning of the rainy season, several males were found reproducing that were undetectable for several weeks but found again at the ponds later during the rainy season. These long-reproducing males probably face an interesting problem: they possibly compete with their own sons for mates. This aspect seems important as it may have great influence on the individual's fitness.

It was found that in *Hyperolius nitidulus* both sexes could find themselves in the situation of competing directly with their offspring. The maximum number of days between the first and last capturing were 180 for males and 181 for females, while 85 days was the assumed the lower limit to possibly encounter own offspring.

Most studies about competition between adults and offspring are conducted on mammals and birds [Greenwood 1980, Dobson 1982, Moore & Ali 1984, Packer 1984] and the evolution of dispersal is often discussed in this context [Gandon 1999]. Sex-biased dispersal is widespread in these animals and mainly explained by competition avoidance [Clark 1978, Harvey et al. 1984, Gowaty 1993] and inbreeding avoidance [Packer 1984, Liberg & Von Schantz 1985, Motro 1991, Perrin & Mazalov 2000].

The lek mating system is a very special reproductive mode and inbreeding avoidance and sibling competition are discussed rarely in this context. In the evolution of leks, high and low

ranked males often have different optimal lek sizes [Widemo & Owens 1995]. High ranked males have a certain optimal lek size because larger leks are more attractive for females, but over a certain lek size, high ranked males lose fitness by losing too many copulations to lower ranked individuals. Low ranked males are supposed to always try to enlarge a lek because they benefit from larger lek sizes [Alatalo et al. 1992]. Another aspect is the possible indirect benefit a low ranked male could derive from joining a lek including many high ranked relatives. Increasing the lek size will attract more females and, therefore, increase the reproductive success of the higher ranked males that it is related to [Kokko & Lindström 1996]. In most lek systems, older males are higher in rank and young males that could be their sons are in a low rank position.

The lek system of *Hyperolius nitidulus* is somewhat different from the one investigated by Kokko & Lindström (1996). No higher or lower ranked males were found in the sense that females preferred a special male type. Reproductive success of males varied mainly by chance not by careful female choice. As no differentiation of female frogs between the first and the second generation males could be detected, sons are equivalent competitors for their fathers. Therefore, to maximise their direct (own mating success) and indirect (sons' mating success) fitness, males should avoid competing directly with their sons, which means they should avoid ponds where the probability of encountering their offspring is high. In fact, the number of migrating males (found at another pond than where they were initially marked) was significantly higher in the long reproducing males than in all males found more than once. An alternative explanation for the higher dispersal rates of longer absent males could be that males that are absent from the ponds for longer times possibly go farther away and when are again ready to reproduce are by chance closer to another pond for calling. Although this hypothesis can not be excluded, it seems as if at least part of the long reproducing males actively choose different reproduction ponds. Actually one of them was found regularly over several weeks at the same pond and suddenly within one week changed its reproduction pond over a distance of 1 km.

Second generation adult dispersal rates were estimated with the help of the size classification. The second generation adult dispersal rate did not differ from all other males present at that time of the rainy season. Juvenile dispersal could not be estimated because a reliable number of marked juveniles was found only at *Hyperolius* pond. At Gansé Plaine only three juveniles were found of which one changed the pond (distance 400 m). The low number of recaptures make interpretations whether this event was dispersal, or whether it was a by chance product unreasonable.

Therefore, it is just hypothesised that dispersal of the adult males rather than dispersal of the young seems to be used to avoid interacting with relatives.

In the lek breeding system of *Hyperolius nitidulus*, no other kin relationship should have a similar degree of competition as the father/son interaction [Liberg & Von Schantz 1985].

Siblings possibly compete for resources [Pusey & Wolf 1996, Kasuya 2000, Dobson & Jones 1985, Comins et al. 1980], what might be a reason for dispersal. In fact the mortality rate of juvenile *Hyperolius nitidulus* has to be considered very high, therefore, resource competition is likely to be rather unimportant. Changes between ponds possibly occur by chance and to minimise distances. A juvenile leaving its natal pond after metamorphosis could when reaching maturity be nearer to another reproductive pond and, therefore, possibly attracted by the calls of other males choose to reproduce there.

As females do not compete for resources (sex ratio is highly male biased and no limit in spawning sites was observed) females are unlikely to have any direct fitness loss when reproducing at the same ponds as their daughters. Dispersal in females occurred only once and never in the five individuals that were observed again after 12 weeks or more and who could, therefore, be in the situation to compete with their own daughters. The only female wandering from one pond to another moved from a very small to a rather large pond, indicating that possibly other reasons than kin competition were the reason for this movement.

The costs of interactions between fathers and daughters, or mothers and sons vary with the costs of inbreeding in a species [Gandon 1999, Dobson & Jones 1985]. They cannot be estimated in this study, but seem to be rather low in comparison with a possible direct mate competition as seen in the father/son interaction. Despite the fact that females possibly do not prefer larger leks, because a larger offer of males to choose from could be advantageous, larger leks could have the advantage of more females spawning at the same time, therefore, generating a surplus of eggs and tadpoles that can not adequately be preyed on by predators. The predator satiation theory is widely accepted for seed production in plants (mainly trees) [Silvertown 1980, Ayal 1994], but also verified for many animals species for example cicadas [Williams et al. 1993], moths [Harrison & Wilcox 1995], reptiles [Eckrich & Owens 1995] and ungulates [Sinclair et al. 2000].

The costs of kin competition were probably highest for males because due to the mating system and female behaviour, fathers lose direct fitness by possibly losing a mating opportunity to their son and indirect fitness, if they make their sons lose a mating opportunity.

Adult males seen over a very long period of reproductive time seem to avoid the encounter with their sons by changing their breeding pond. At very large ponds (e.g. *Hyperolius* pond) it could be sufficient to change the calling site from one edge of the pond to another if tadpoles stay where the eggs were laid and if juveniles returned mainly to the pond site they came from.

Conclusions

In the Comoé National Park, *Hyperolius nitidulus* reproduces with at least two different life history strategies: either one or two generations per year are produced. Individuals reproducing early may later face the problem of competing with their own offspring. Competition would be most serious for male frogs due to the reproductive system of *Hyperolius nitidulus* where female choice for male size or age is lacking and sons are equal competitors for access to females. Evidence that males avoid competing with their sons by changing reproductive ponds later in the season, was found in this study.

The factors influencing the tadpole's decision whether to aestivate or to reproduce immediately are still under investigation. Water quality seems to play a role but light and possibly reduced water volume towards the end of the rainy season could also trigger the decision.

There were also some hints that despite the obvious advantage of early reproduction there may be another strategy to delay reproduction, which may be highly advantageous during years with unfavourable conditions when the second generation fails.

Tadpoles, therefore, have to solve the dilemma whether to aestivate or to reproduce. Bet-hedging strategies are a possible solution for this dilemma [Rowe & Ludwig 1991]. A very well known model for life-history evolution in a variable environment is Cohen's model for the evolution of seed dormancy [Cohen 1970]. Seger and Brockmann (1987) extended and slightly altered the model to explain the insect diapause problem. Their theory predicts two characteristic outcomes: 1) No sudden switch between the production of diapausing and reproducing offspring would occur, but a transition shifting proportions of nondiapausing towards diapausing individuals; 2) The first diapausing offspring would be found as soon as the probability of developing directly in order to reproduce falls below unity. Although *Hyperolius nitidulus* does not exactly perform a diapause it is faced with a similar problem: adult death at an uncertain date.

Whether the life history polymorphism observed in *Hyperolius nitidulus* is due to phenotypic plasticity or genetic polymorphism is not yet known. Juveniles' response to environmental factors and the partial reversibility of the decision to aestivate or to reproduce seems to favour phenotypic plasticity as a possible explanation [Semlitsch et al. 1990, Gotthart & Nylin 1995, Via et al. 1995, Schlichting & Pigliucci 1998, Laurila & Kujasalo 1999]. However, Levins (1963) suggested that a genetic polymorphism would be favoured when the reliability of the environmental cue is low [see also Lively 1986a and Daan & Tinbergen 1997]. This is surely the case for the water quality in the ponds used by *Hyperolius nitidulus*. Laboratory breeding experiments [Linsenmair unpubl. results] revealed that juveniles coming from a line of frogs (more than 5 generations) selected for their ability to reproduce as fast as possible were no longer able to aestivate properly.

Despite this uncertainty, there is no doubt that the optimal combination of different life histories is profitable and may be a reason for the wide range and high local abundance of *Hyperolius nitidulus*.

Prospects

The detailed study of the life history strategies of *Hyperolius nitidulus* in Comoé National Park revealed a reproductive system that is very well adapted to the local environmental conditions. Every aspect of the reproductive behaviour seemed to fit in the picture.

Due to its wide distribution range (West African savannah belt) *Hyperolius nitidulus* is confronted with a wide range of environmental conditions containing very short rainy seasons of only several weeks in the north to nearly all year rain climates in the south. It would be very interesting to investigate the reproductive behaviour of *Hyperolius nitidulus* over the range of these extremely different habitats. The results of these investigations could contribute much knowledge to the general discussion on the evolution of life histories.

5 Summary

Distinct juvenile behaviour differences, changes in adult sizes and reproductive capacity and a long reproductive period triggered the working hypothesis of two alternative life-cycle strategies favouring aestivation or immediate reproduction. The hypothesis for the life-cycles of *Hyperolius nitidulus* that differed from the commonly assumed reproductive strategy for this species was confirmed by the results of this study.

Aestivated juveniles start to mature at the beginning of the rainy season and reproduce subsequently. Their tadpoles grow until metamorphosis and either reproduce in this same season, in which case their offspring aestivates (one year – two generations), or they delay reproduction to the following year and aestivate themselves (one year – one generation). Juveniles trying to reproduce as fast as possible will invest in growth and differentiation and show no costly adaptations to aestivation, while juveniles delaying reproduction to the following rainy season will be well adapted to dry season conditions.

Indirect evidence for the existence of a second generation was found in all three investigation years: adult size decreased abruptly towards the end of the rainy season, mainly due to the arrival of very small individuals, and clutch size decreased abruptly. Also at the end of the rainy season juveniles had two behavioural types: one hiding on the ground and clearly avoiding direct sunlight and another sitting freely above ground showing higher tolerance towards dry season conditions (high air temperatures and low humidity). Skin morphology differed between the types showing many more purine crystals in a higher order in the dry-season adapted juveniles.

The final proof for the existence of a second generation came with the recapture of individuals marked as juveniles when they left the pond. The 45 recaptured frogs definitely came back to the pond to reproduce during the same season in 1999.

Second generation frogs (males and females) were significantly smaller than the rest of all adults and egg diameter was reduced. Clutch size did not differ significantly. It was found that females did not discriminate against second generation males when coming to the ponds to reproduce. Second generation males had a similar chance to be found in amplexus as first generation males.

Indirect and direct evidence for a second generation matched very well. The sudden size decrease in adults occurred just at the time when the first marked frogs returned.

The observation that freshly metamorphosed froglets were able to sit in the sun directly after leaving the water led to the assumption that the decision whether to aestivate or to reproduce already happens during the frogs' larval period. Water chemistry and the influence of light was investigated to look for the factors triggering the decision, but only contaminated water increased the number of juveniles ready for aestivation.

Despite the second generation strategy that would enforce early reproduction, this study revealed the possibility of another strategy of starting very late with reproduction. This strategy could be advantageous when the rainy season is short and the second generation fails.

Whether the life history polymorphism observed in *Hyperolius nitidulus* is due to phenotypic plasticity or genetic polymorphism is still not known. Despite this uncertainty, there is no doubt that the optimal combination of different life histories is profitable and may be a reason for the wide range and high local abundance of *Hyperolius nitidulus*.

6 Zusammenfassung

Deutliche Verhaltensunterschiede bei Juvenilen, Veränderungen in der Größe von Adultfröschen, reduzierte Gelegegrößen und eine lange Reproduktionsphase führten zu der Arbeitshypothese von möglicherweise zwei verschiedenen life-history Strategien für *Hyperolius nitidulus*: Ästivation oder unmittelbare Reproduktion. Die Hypothese der alternativen life-cycles wich vom allgemein angenommenen Lebensverlauf der Frösche ab, wurde aber in dieser Arbeit bestätigt.

Ästivierte Jungfrösche entwickeln sich zu Beginn der Regenzeit zu Adulten und reproduzieren sich dann (= erste Generation). Ihre Kaulquappen wachsen entweder bis zur Metamorphose und reproduzieren sich dann in dieser Saison (Sommergeneration), in welchem Fall erst ihre Nachkommen wieder ästivieren (ein Jahr – zwei Generationen) oder sie verschieben ihre Reproduktion auf das nächste Jahr und ästivieren selbst (ein Jahr - eine Generation). Jungfrösche, die sofort versuchen, sich zu reproduzieren, sollten in schnelles Wachstum und Differenzierung investieren und keine teuren Anpassungen an die Ästivation aufweisen, während Jungtiere, die die Reproduktion auf das nächste Jahr verschieben, gut an Trockenzeitbedingungen angepasst sein sollten.

Indirekte Hinweise auf eine kurzlebige Sommergeneration (= zweite Generation) gab es in allen Untersuchungsjahren: Die mittlere Adultgröße nahm gegen Ende der Regenzeit abrupt ab, hauptsächlich aufgrund der Ankunft extrem kleiner Tiere, und auch die Gelegegröße ging rapide zurück. Gegen Ende der Regenzeit gab es bei den Jungfröschen außerdem zwei verschiedene Verhaltenstypen: einen, der sich am Boden versteckte und deutlich direkte Sonnenbestrahlung mied und ein anderer, der frei an Grashalmen über dem Boden saß und höhere Toleranz gegenüber Trockenzeitbedingungen (hohe Temperaturen und niedrige Luftfeuchtigkeit) aufwies. Die Hautmorphologie dieser Verhaltenstypen war ebenfalls unterschiedlich. Die trockenadaptierten Tiere hatten mehr Purinkristalle, die außerdem stärker geordnet waren.

Der Wiederfang von Tieren, die sich in derselben Regenzeit in der sie als Jungfrösche markiert worden waren, fortpflanzten, war der direkte Beweis für die Existenz Sommergeneration. 45 Frösche kamen 1999 in derselben Saison zurück, um sich zu reproduzieren.

Frösche aus der Sommergeneration (=Fortpflanzung in der selben Saison) waren signifikant kleiner als der Rest der Frösche am Tümpel, und der Eidurchmesser von Gelegen von

Zweitgenerationsweibchen war reduziert. Gelegegrößen zwischen erster und zweiter Generation waren nicht unterschiedlich. Reproduktionsbereite Weibchen unterschieden nicht zwischen Männchen der ersten und zweiten Generation. Männchen der zweiten Generation hatten daher dieselben Amplexuschancen.

Direkte und indirekte Hinweise auf eine zweite Generation passten zeitlich sehr gut zusammen. Die plötzliche Größenreduktion in den Adulten trat genau zu dem Zeitpunkt auf, zu dem die ersten markierten Frösche zurückkamen, um sich zu reproduzieren.

Die Beobachtung, dass frischmetamorphosierte Jungtiere in der Lage waren, direkt nach dem Verlassen des Wassers in der Sonne zu sitzen, führten zu der Annahme, dass die Entscheidung für Reproduktion oder Ästivation bereits während der larvalen Phase gefällt werden muss. Wasserchemie und der Einfluss von Licht wurden untersucht, allerdings erhöhte nur stark verschmutztes Wasser die Anzahl ästivationsbereiter Juveniler.

Neben der nachgewiesenen zweiten Generationsstrategie, die eine möglichst schnelle Reproduktion fördern sollte, gab es Hinweise auf eine weitere mögliche Reproduktionsstrategie, bei der sehr spät mit der Reproduktion begonnen wird. Diese Strategie könnte vorteilhaft sein, wenn die Regenzeit kurz ist und die zweite Generation scheitert.

Ob der in *Hyperolius nitidulus* gefundenen life-history Polymorphismus genetisch ist, oder ob es sich um phänotypische Plastizität handelt, ist noch nicht bekannt.

Trotz dieser Unsicherheit gibt es keinen Zweifel, dass die optimale Kombination von verschiedenen life-history Strategien profitabel und vielleicht ein Grund für die weite Verbreitung und hohe lokale Abundanz von *Hyperolius nitidulus* ist.

7 Literature

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8 Appendix

Table I: Male size differences between the years investigated. Mean values +/- standard deviation and ANOVA and Scheffé post hoc test results are shown. Only weeks when in all years reproduction took place were taken into account.

Descriptive statistics				
	SVL [mm]	TF [mm]	Weight [g]	Number
1997	27 +/- 1.33	12.7 +/- 0.79	1.13 +/- 0.17	557
1998	27.8 +/- 1.33	13.1 +/- 0.87	1.20 +/- 0.17	697
1999	27.3 +/- 1.50	12.8 +/- 0.80	1.27 +/- 0.18	2012
ANOVA results				
	F	p	df	
SVL [mm]	51.25	< 0.00001	2	
TF [mm]	37.27	< 0.00001	2	
Weight [g]	53.07	< 0.00001	2	
Scheffé test results				
SVL	1998	1999		
1997	< 0.00001	0.0009		
1998	-----	< 0.00001		
TF	1998	1999		
1997	< 0.00001	0.07		
1998	-----	< 0.00001		
Weight	1998	1999		
1997	< 0.00001	0.99		
1998		< 0.00001		

Table II: Mortality and metamorphosis of *Hyperolius nitidulus* tadpoles raised in the laboratory to investigate factors influencing the decision: aestivation or reproduction.

1997: Finished developed 64 days after hatching:						
Group	total number of tadpoles finished	percentage of all tadpoles in this group	dead		metamorphosis	
			number	percentage	number	percentage of individuals
much water	10	100	5	50	5	50
little water	10	100	0	0	10	100
high density	43	86	33	77	10	23
low density	10	100	2	20	8	80
light	10	100	2	20	8	80
shadow	10	100	2	20	8	80
fresh water	10	100	3	30	7	70
contaminated water	10	100	7	70	3	30
total	113	94	54	48	59	52
1998: Finished development 110 days after hatching						
Group	total number of tadpoles finished	percentage of all tadpoles in this group	dead		metamorphosis	
			number	percentage	number	percentage of individuals
light (cloudy water)	20	100	13	65	7	35
shadow (cloudy water)	12	60	6	50	6	50
light (clear water)	10	50	6	60	4	40
shadow (clear water)	9	45	6	67	3	33
low density	7	35	4	57	3	43
high density	35	70	29	83	6	17
fresh water	14	70	11	79	3	21
contaminated water	20	100	19	95	1	5
total	127	67	94	76	33	26
2000: Finished development 129 days after hatching						
Group	total number of tadpoles finished	percentage of all tadpoles in this group	dead		metamorphosis	
			number	percentage	number	percentage of individuals
low conductivity	29	72.5	27	93	2	7
high conductivity	29	72.5	28	97	1	3
fresh water	28	70	25	89	3	11
contaminated water	30	75	27	90	3	10
light	31	77.5	28	90	3	10
shadow	23	57.5	23	100	0	0
total	170	71	158	93	12	7

**Table III: Summary of Chi²-test results comparing mortality rates in different groups.
(Significant results are marked with bold letters.)**

Chi²- tests: Mortality 1997		
	Chi²	p
much versus little water	6.67	0.0098
high versus low density	11.65	0.0006
light versus shadow	0	1
fresh versus contaminated water	3.2	0.0736
Chi²- tests: Mortality 1998		
	Chi²	p
light versus shadow (cloudy water)	0.7	0.403
light versus shadow (clear water)	0.09	0.763
low versus high density	2.29	0.130
fresh versus contaminated water	2.14	0.143
Chi²- tests: Mortality 2000		
	Chi²	p
low versus high conductivity	0.35	0.553
fresh versus contaminated water	0.01	0.93
light versus shadow	2.36	0.125

Table IV: Differences in developmental stage and growth among tadpoles raised under differing water conditions. 1997: comparison 7 weeks after hatching, 1998 and 2000 comparison 10 weeks after hatching. Bonferroni correction was used to compensate for multiple test usage. Significant differences after the correction are marked with bold numbers.

Mann-Whitney U tests 1997					
much versus little water					
	U	Z	p	N₁	N₂
developmental stage	22.5	0	1	5	9
body length [mm]	16	0.867	0.386	5	9
total length [mm]	14	1.13	0.257	5	9
high versus low density					
	U	Z	p	N₁	N₂
developmental stage	50.5	-2.89	0.004	37	8
body length [mm]	50	-2.91	0.003	37	8
total length [mm]	48.5	-2.95	0.003	37	8
light versus shadow					
	U	Z	p	N₁	N₂
developmental stage	39.5	0.04	0.96	10	8
body length [mm]	30	-0.89	0.37	10	8
total length [mm]	20	-1.78	0.08	10	8
fresh versus contaminated water					
	U	Z	p	N₁	N₂
developmental stage	19.5	0.581	0.561	8	6
body length [mm]	11	1.678	0.093	8	6
total length [mm]	16	1.033	0.302	8	6
Mann-Whitney U-tests 1998					
light versus shadow (cloudy water)					
	U	Z	p	N₁	N₂
developmental stage	65.5	2.062	0.039	13	18
body length [mm]	84	1.321	0.186	13	18
total length [mm]	81	1.441	0.15	13	18
light versus shadow (clear water)					
	U	Z	p	N₁	N₂
developmental stage	116.5	1.205	0.228	17	18
body length [mm]	123	0.99	0.298	17	18
total length [mm]	106.5	106.5	0.124	17	18
high versus low density					
	U	Z	p	N₁	N₂
developmental stage	105	2.68	0.007	18	23
body length [mm]	97.5	2.877	0.004	18	23
total length [mm]	102	2.758	0.006	18	23

Mann-Whitney U-tests 1998					
fresh versus contaminated water					
	U	Z	p	N₁	N₂
developmental stage	16	-0.08	0.937	11	3
body length [mm]	6	-1.77	0.077	11	3
total length [mm]	3	-2.11	0.035	11	3
Mann-Whitney U-tests 2000 (climatic chamber)					
low versus high conductivity					
	U	Z	p	N₁	N₂
developmental stage	29	0.5855	0.558	10	7
body length [mm]	27.5	0.7319	0.464	10	7
total length [mm]	29	0.5855	0.558	10	7
fresh versus contaminated water					
	U	Z	p	N₁	N₂
developmental stage	11	0.567	0.571	7	4
body length [mm]	13	0.189	0.850	7	4
total length [mm]	12	0.378	0.706	7	4
light versus shadow					
	U	Z	p	N₁	N₂
developmental stage	42	0.570	0.569	9	11
body length [mm]	44	0.418	0.676	9	11
total length [mm]	35.5	1.064	0.288	9	11
Mann-Whitney U-tests 2000 (bureau)					
low versus high conductivity					
	U	Z	p	N₁	N₂
developmental stage	105.5	0.460	0.645	13	18
body length [mm]	79	1.521	0.128	13	18
total length [mm]	81	1.441	0.149	13	18
fresh versus contaminated water					
	U	Z	p	N₁	N₂
developmental stage	132.5	0.347	0.729	19	15
body length [mm]	125	0.607	0.544	19	15
total length [mm]	89.5	1.838	0.066	19	15
light versus shadow					
	U	Z	p	N₁	N₂
developmental stage	126	0.360	0.719	16	17
body length [mm]	130	-0.216	0.829	16	17
total length [mm]	129.5	0.234	0.815	16	17

Table V: Summary of sitting positions of freshly metamorphosed juveniles.

1997				Fisher test p-values
Group	visible	hiding	total number	
much water	1	4	5	1.000
little water	3	7	10	
high density	4	6	10	0.3137
low density	1	7	8	
light	2	6	8	0.4667
shadow	0	8	8	
fresh water	2	5	7	1.000
contaminated water	1	2	3	
1998				Fisher test p-values
Group	visible	hiding	total number	
light (cloudy water)	3	4	7	---
shadow (cloudy water)	1	5	6	
light (clear water)	2	2	4	---
shadow (clear water)	1	2	3	
low density	1	2	3	---
high density	3	3	6	
fresh water	0	3	3	---
contaminated water	0	1	1	
2000				Fisher test p-values
Group	visible	hiding	total number	
low conductivity	0	3	3	---
high conductivity	1	1	2	
fresh water	1	1	2	---
contaminated water	1	2	3	
light	0	2	2	---
shadow	0	2	2	
pooled data for water quality (all years)				Chi² test p-value
highly contaminated	9	10	19	0.063
fresh water	27	75	102	

Detailed description of REM preparation of juvenile skin (cf. 3.2.2.1)

Froglets were collected in Africa at night (22/11/1999), brought to Würzburg (25/11/1999) and kept in an aquarium (30 cm x 25 cm x 30 cm) to restore natural behaviour. On 7/12/1999 5 individuals sitting freely and 5 individuals hiding were chosen and killed with an overdose of ethyl m-aminobenzoate (MS-222) a frequently used anaesthetic for aquatic vertebrates [Yuwiler & Samuel 1974, Hensel et al. 1975, Spieler 1997]. Small pieces (~1mm²) of dorsal and ventral skin were prepared and treated as follows:

- Fixation in 2.5 % buffered glutaraldehyde (30-45 minutes)
- Washing: 5 times (3 minutes each time) with 50 mM monophosphate buffer pH 7.2
- Fixation in 2 % buffered OsO₄ (90-120 minutes)
- Washing (H₂O): 5 times 3minutes each
- Contrasting over night: 0.5% or 2% watery uranyl acetate
- Washing (H₂O): 5 times 3minutes each
- Dehydration

30%	ethanol	30 minutes	4°C
50%	ethanol	30 minutes	4°C
70%	ethanol	30 minutes	4°C
90%	ethanol	30 minutes	4°C
96%	ethanol	30 minutes	4°C
100%	ethanol	30 minutes	4°C
100%	ethanol	30 minutes	4°C
100%	ethanol	30 minutes	room temperature
	propylenoxide	30 minutes	room temperature
	propylenoxide	30 minutes	room temperature
	propylenoxide	30 minutes	room temperature
- Embedding

propylenoxide : epon	= 1:1	2-4h
propylenoxide : epon	= 1:1	over night

The tissue pieces were embedded as flat as possible to achieve comparable cross-sections.



Picture 1: Aestivating juvenile



Picture 2: Reproductive adult



Picture 7: Fighting males



Picture 6: Calling male



Picture 8: Amplexus



Picture 9a+b: Freshly metamorphosed juveniles



Picture 3: Gansé Plaine



Picture 5: View Point pond

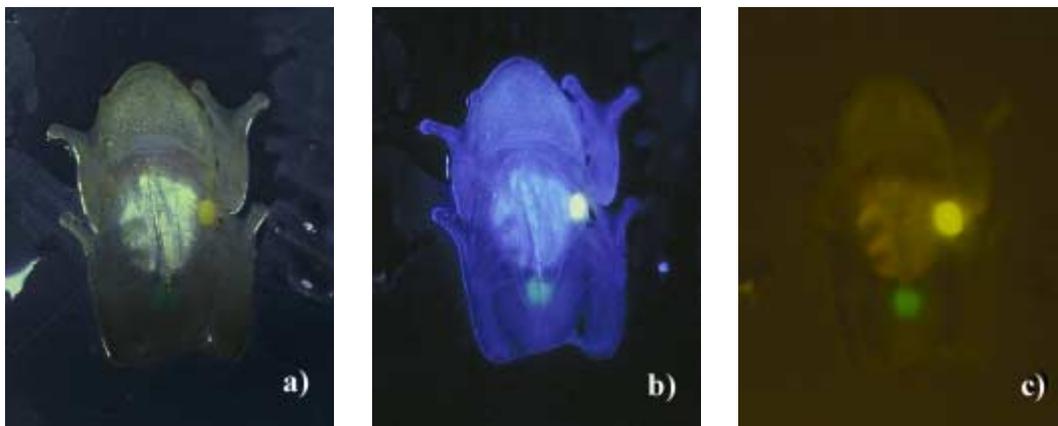


Picture 4: Hyperolius pond

Marking Method



Picture 10: Juvenile marking procedure



Picture 11: Marked juvenile a) plain b) special light c) special light and filter glass

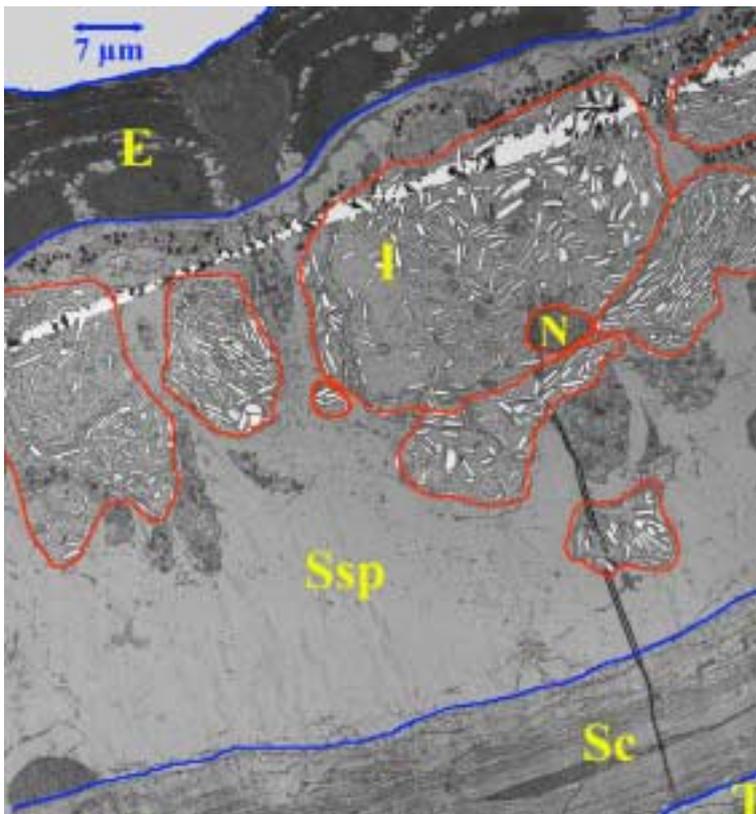


Picture 12a: Recaptured adult

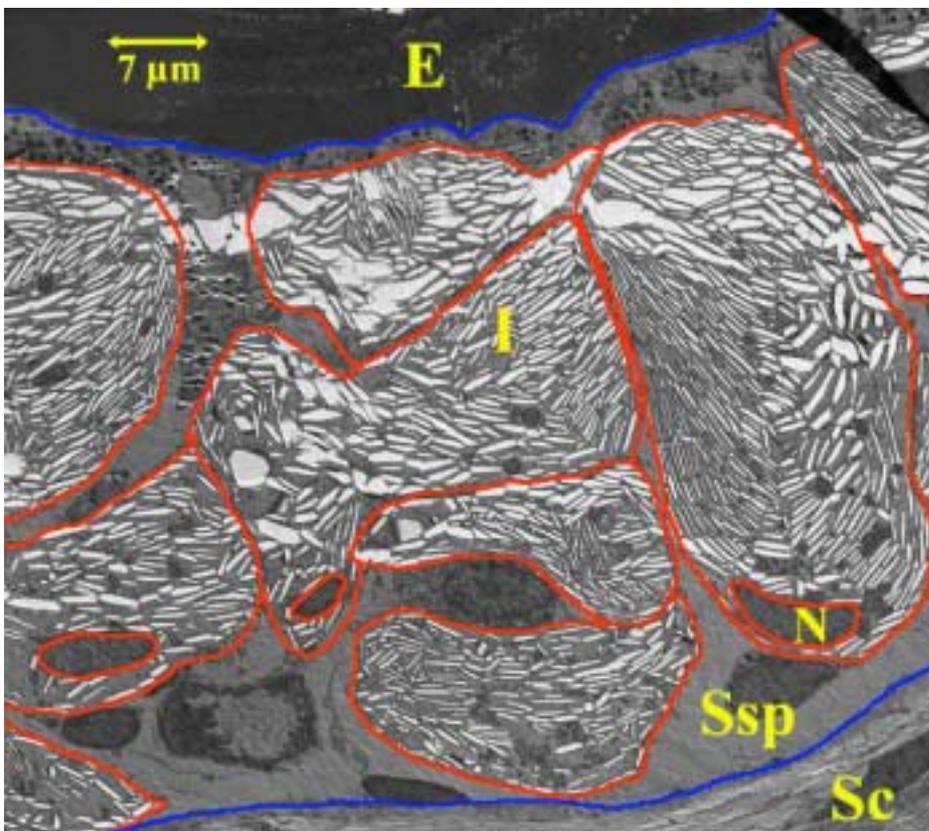


Picture 12b: The same adult looked at with special light and filter

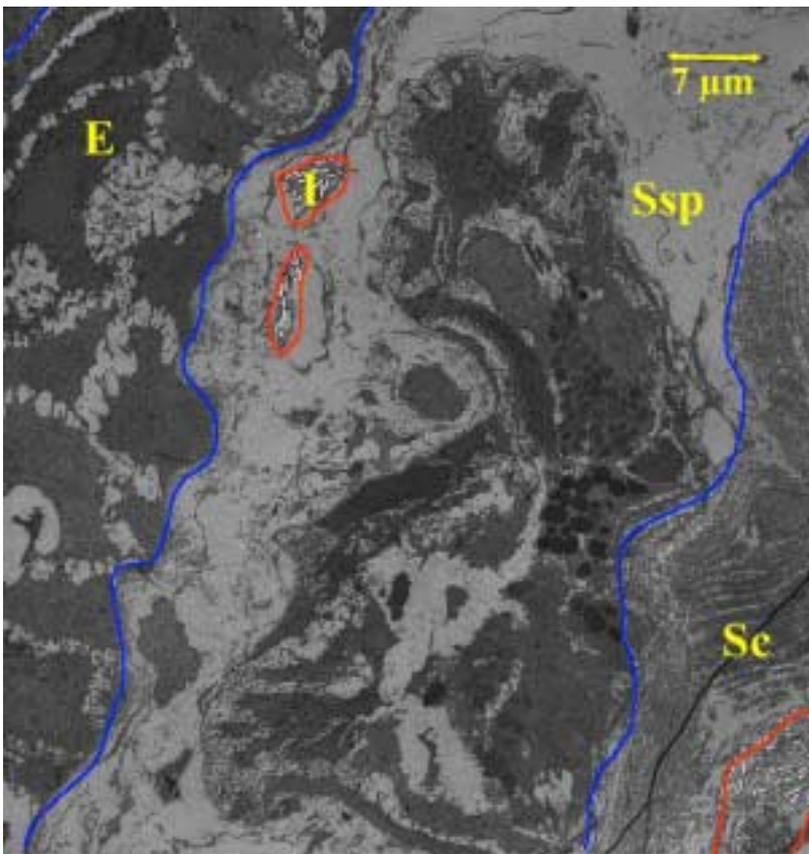
Juvenile skin morphology



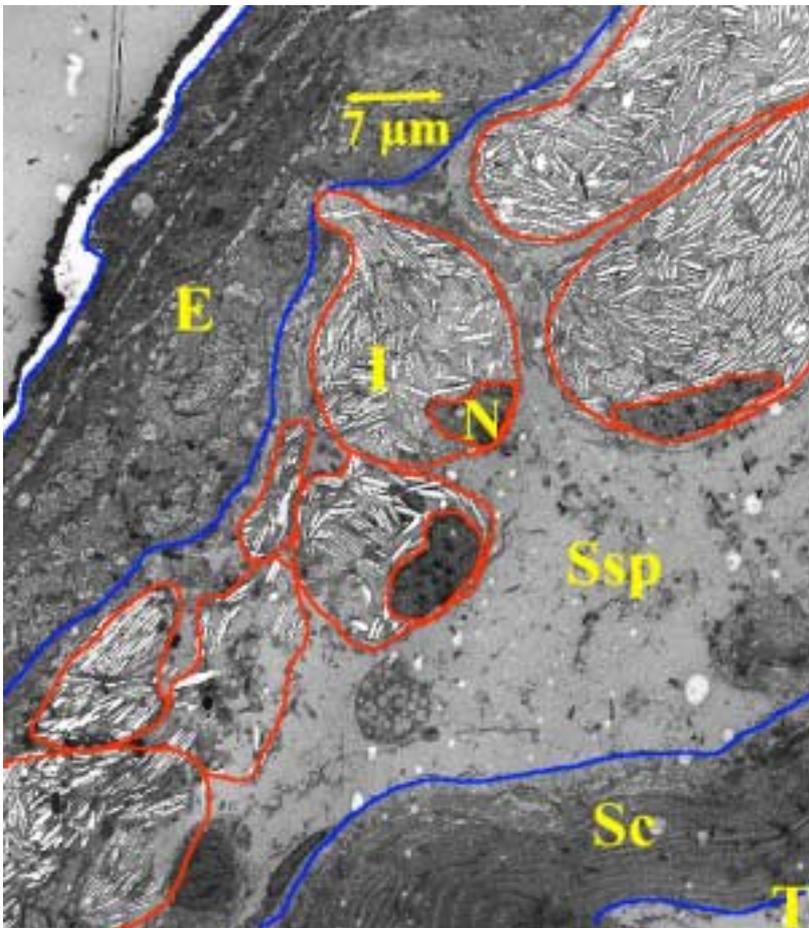
Picture 13: Wet season adapted juvenile: dorsal skin. (E Epidermis, Sc Stratum compactum, Ssp Stratum spongiosum, T Tela subcutanae, I Iridophore, N Nucleus)



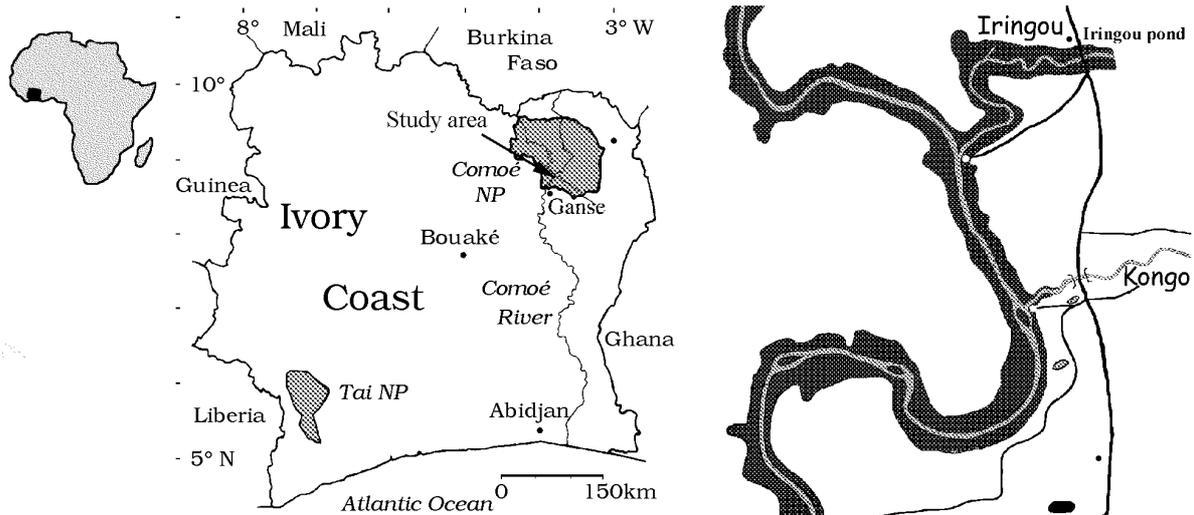
Picture 14: Dry season adapted juvenile: dorsal skin. (Abbr. see Picture 13)



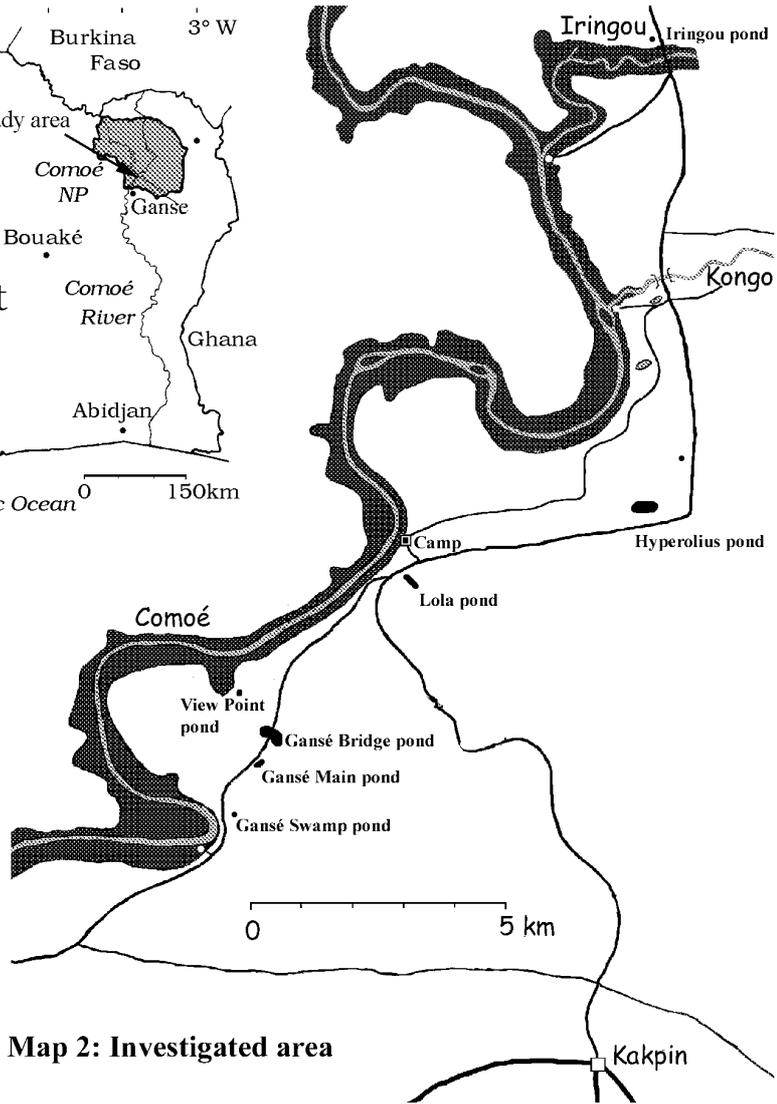
Picture 15: Wet season adapted juvenile: ventral skin. (Abbr. see Picture 13)



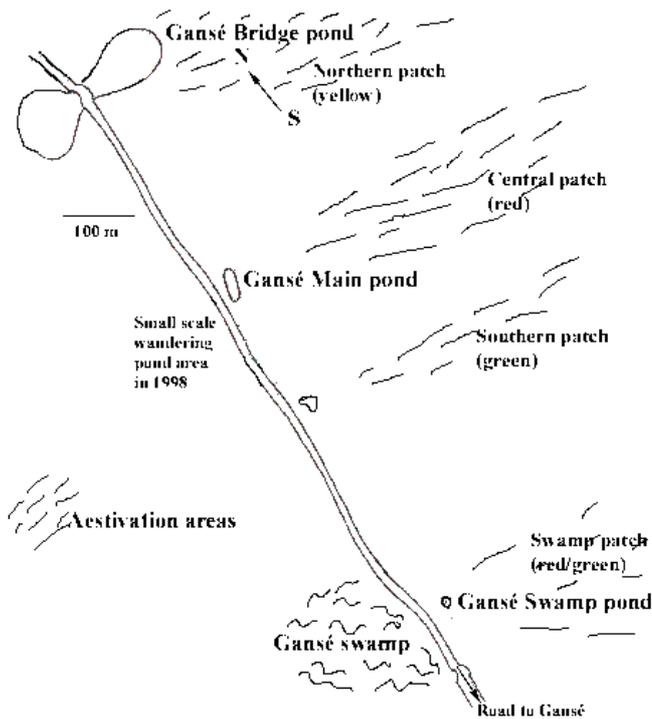
Picture 16: Dry season adapted juvenile: ventral skin. (Abbr. see Picture 13)



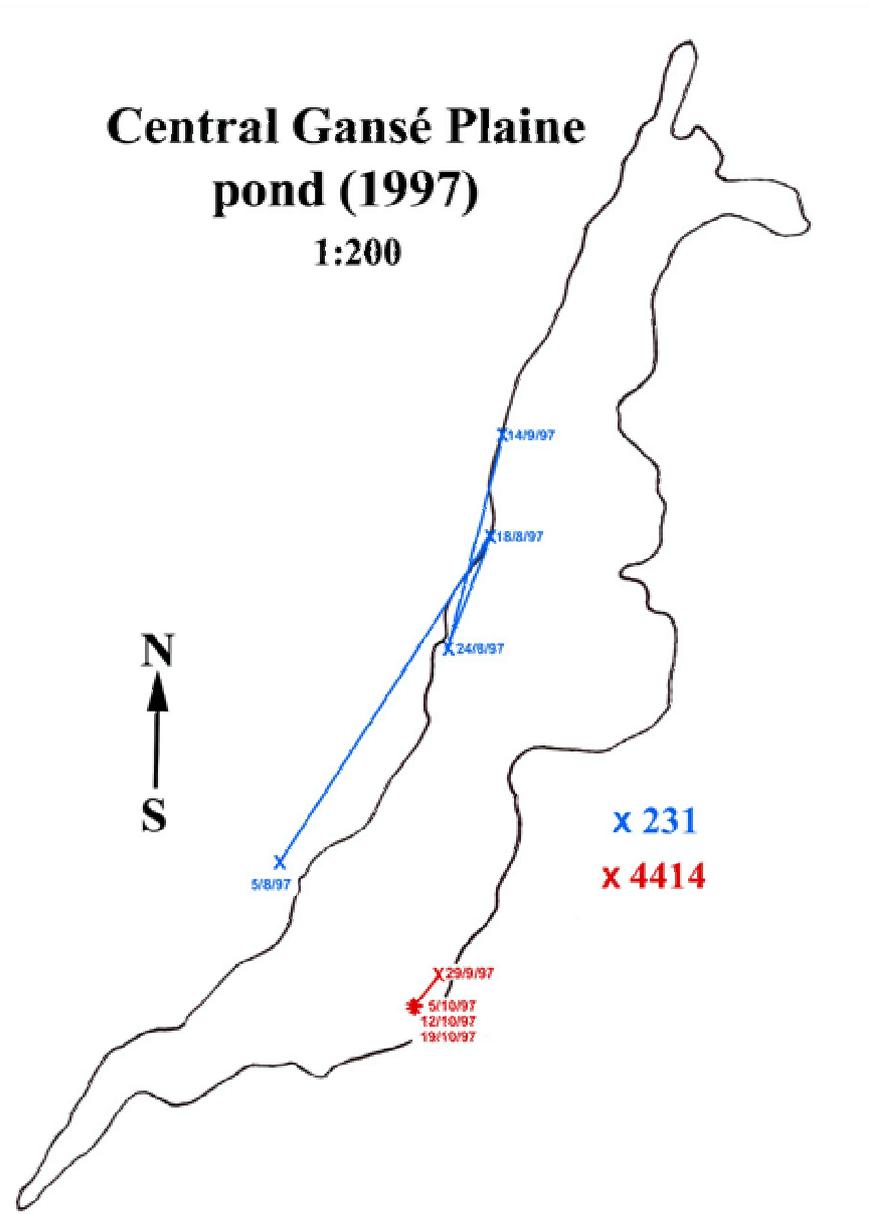
**Map 1: Ivory Coast
(West Africa)**



Map 2: Investigated area



Map 3: Gansé Plaine



Map 4: Different site preferences of two male *Hyperolius nitidulus*.