Bonn zoological Bulletin 67 (2): 117–128
2018 · Galunder K. & Rödder D.
https://doi.org/10.20363/BZB-2018.67.2.117

ISSN 2190–7307
http://www.zoologicalbulletin.de

Research article

urn:lsid:zoobank.org:pub:3646985F-4125-49CF-BBF2-8EDAACA3AA58

Developmental Ecology and Larval Staging in *Polypedates otilophus* (Boulenger, 1893) (Anura: Rhacophoridae)

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Abstract. Tadpoles of *Polypedates otilophus* originating from two different foam nests were raised at water temperatures between 19°C and 27°C. A larval staging table according to Gosner is provided for the first time for the genus. The clutch sizes of the foam nests differed (19 and 49 eggs, respectively), but only 14 and 17 tadpoles hatched at stage 25. The first tadpole of nest one completed metamorphosis after 120 days and the first tadpole of nest two after 131 days. Before metamorphosis is completed, the metamorphs developed significant brown striation which is first visible on the hind legs and subsequently also on the dorsal side of the body. Detailed characteristics of each larval stage are provided. We herein provide the first detailed report on the larval development of *P. otilophus*, which can be used as surrogate species for captive management of other *Polypedates* taxa. Most interestingly, the temporal development of larvae in this species appears to be extremely plastic and strongly depending on ambient temperature.

Key words. Developmental ecology, tadpole morphology, environmental plasticity.

INTRODUCTION

The genus *Polypedates*, which is distributed in Eastern India, southeastern Asia, the Philippines, and Borneo (Frost 2017), was first described by J. J. Tschudi in 1838. Currently 24 species of the genus are recognized (Frost 2017) and 15 are listed at the IUCN Red List of Threatened Species (IUCN 2017). One species is listed as "Endangered" (*Polypedates insularis* Das, 1995) and four species are listed as "Data Deficient" by IUCN (*Polypedates chlorophthalmus* Das, 2005; *P. hecticus* Peters, 1868; *P. occidentalis* Das & Dutta, 2006; and *P. zed* [Dubois, 1986]) and ten species are listed as Least Concern (*Polypedates colletti* [Boulenger, 1890]; *P. cruciger* Blyth, 1852; *P. leucomystax* [Gravenhorst, 1829]; *P. macrotis* [Boulenger, 1891]; *P. maculatus* [Gray, 1830]; *P. megacephalus* Hallowell, 1860; *P. mutus* [Smith, 1940]; *P. otilophus* [Boulenger, 1893]; *P. pseudocruciger* Das & Ravichandran, 1998; and *P. taeniatus* [Boulenger, 1906]; IUCN 2017).

Polypedates otilophus was first described as *Rhacophorus otilophus* by Boulenger in 1893, who characterized *Rhacophorus otilophus* by its much depressed head which is large and a little broader than long. Furthermore, according to the original description, it possesses a pointed snout which is a little longer than the diameter of the orbit and a nostril which is close to the tip of the snout. The forehead is concave and the fingers long with rudimentary webs. The tips are dilated into rather large disks and the toes are two-thirds webbed, but the disks are smaller than those of the fingers. The skin texture of the dorsum is finely granular, whereas the skin of the belly and the lower surface of the thighs are coarsely granular. The dorsal coloration is pale olive with dark grey spots and longitudinal streaks (Fig. 1). Further, the hind limbs are dark cross-banded which become thinner and denser on the concealed surfaces of the hind limbs. Males have internal vocal sacs. A male specimen measured in Bongon, North Borneo, had a snout to vent length (SVL) of 80 mm (Boulenger 1893) and Iskandar (2004) reported that females can reach up to 100 mm SVL.

Polypedates otilophus is listed as Least Concern by IUCN (2017) because of its wide distribution, its presumed large population and because of the tolerance of a degree of habitat modification. According to the most recent assessment the species is unlikely to be declining fast enough to qualify for being listed in a higher threat category. Matsui et al. (2014) noted that the species occurs at many sites in Borneo and on Sumatra at elevations below 1,000 m a.s.l. The species is arboreal prefering lowland forests in flat and hilly terrain. Under natural conditons breeding takes place in temporary rain pools; specimens are also frequently found in disturbed

Received: 06.04.2018
Accepted: 25.10.2018

Corresponding editor: W. Böhme
Published: 01.11.2018

Fig. 1. Adult specimen of *Polypedates otilophus*. Photo: M. Flecks.

habitats, such as logged areas at the forest edge, which apparently do not possess a threat (Inger et al. 2004).

There are few studies available reporting on the reproduction and tadpole development of species of the genus *Polypedates*. Tapley and Girgin (2015) raised 14 clutches of *P. otilophus* and reported that tadpoles need 74 to 84 days to reach metamorphosis at 22°C to 26°C. In this study foam nests were five times produced in the early morning about 6 a.m., wherein the entire process of the nest construction took about 45 minutes. Three of the nests, which were dissected within 24 hours, contained 42 to 119 eggs. The authors reported that the tadpoles hatched after approximately ten days and that the first tadpole metamorphosed after 74 days. Metamorphosis within the cohort took place within ten days (Tapley & Girgin 2015).

Chakravarty et al. (2011) reported for *Polypedates teraiensis* the entire development from ovum fertilization up to emergence of the froglet with 58 days at 26°C to 32°C. They examined five different foam nests of which clutch sizes varied between 67 and 127 white-coloured eggs. Some eggs located on the outermost surface of the foam nest sometimes did not develop and turned pale yellow due to desiccation. Embryos of *P. teraiensis* hatched at stage 20 and stayed within the foam nest until stage 22 (*sensu* Gosner 1960). In another study, Tamuly & Dey (2014) reported on the larval morphology and development of *Polypedates teraiensis* within 42 days after hatching at temperatures between 26°C and 33°C. Under these conditions the keratodent jaws developed at Gosner stage 25 and were assimilated at stage 42.

Yorke (1983) presented data on the survival of embryos and larvae for *Polypedates leucomystax*. The average embryonic mortality was 34% in field-collected egg masses, wherein fertilization rates were approximately 100%. The pooled mortility data showed that 98% occurred prior to tail-bud stage. In this study the highest frequency and proportion of mortality occurred in the early neural stages, whereas no embryonic mortality was found beyond tail-bud stages. The mortality increased in stages 31 to 35 (Yorke 1983).

Hsu et al. (2012) stated that breeding in *Polypedates braueri* on the Bagua Terrace takes place from March to August though tadpoles can be found during the entire year, hibernating in man-made water containers in lowland orchands. Laboratory experiments showed that the overwintering is facultative and can be initialized by low temperatures and limited food, wherein the role of food availability was confirmed in the wild (Hsu et al. 2012).

Information on captive breeding of *P. otilophus* is scarce. Iskandar (2004) reported that *P. otilophus* does not do well in captivity. Janzen (2014) and Tapley and Girgin (2015) reported on husbandry and breeding events in captivity. Detailed information on larval staging and development are currently lacking.

Fig. 2. Tanks used for captive breeding of *Polypedates otilophus*: the terrarium of the initial breeding group (A); one of the aquaria used for tadpole raising (B); and one of the terraria for raising the froglets after metamorphosis (C).

MATERIAL AND METHODS

Captive Management and Breeding

In 2014, 27 tadpoles of *Polypedates otilophus* were donated to the Zoologisches Forschungsmuseum Alexander Koenig (ZFMK) by a private German breeder who previously reported on captive management of the species (Janzen 2014). At the time the present study was conducted our breeding group consisted of 20 specimens, which were kept in a terrarium in the animal keeping unit of the ZFMK measuring 60 x 80 x 140 cm lxbxh (Fig. 2A). They were fed ad libitum with adult crickets (*Acheta domesticus* or *Gryllus assimilis*) two or three times per week and irregularly with flies (*Musca domestica*), which were fed with fresh fruit or fruit puree to absorb more vitamins.

The terrarium was divided into two different parts: a land part and a water part (25 x 80 x 18 cm lxbxh) containing a water pump (Eheim Universalpumpe, Typ 1260 210) connected to a sprinkler system and some *Cryptocoryne* sp. The land part consisted of several layers of filter padding reaching a total height of 20 cm and was equipped with different plants (*Monstera deliciosa* and *Syngonium* sp.) and some branches, partly extending from the water part to the land part. The back side as well as the right side of the terrarium were covered with Hygrolon® to keep the humidity high.

Next to natural daylight, LED light strips (Solar Stinger 1100 mm Sunstrip Dimmable Diver) served as light source between 8 a.m. and 8 p.m. In order to stimulate reproduction, the sprinkler system was activated daily for about three hours, resulting in a relative humidity between 65% and 90%. Air temperature varied between 20°C and 30°C and the water temperature varied between 20°C and 28°C.

Under these conditions two foam nests were produced, wherein the first one was attached on a leaf above the water part on February 26, 2017 and the second nest was attached to the glass wall above the water part on March 15, 2017.

Raising of tadpoles and froglets

To provide suitable water conditions fresh osmosis water was remineralized in an aquarium (50 x 40 x 30 cm lxbxh), which was equipped with aquatic plants (*Cryptocoryne* sp.), dried leaves (*Fagus sylvatica*) and circulated with a water pump (Eheim Powerhead 650). After extracting water for exchanges on a weekly basis the aquarium was refilled with fresh osmosis water.

The leaf which contained the first foam nest (group 1) was cut off the plant and placed in a second terrarium (50 x 40 x 40 cm lxbxh) to avoid disturbances of the adults. It was similarly equipped as the terrarium of the adults and automatically sprayed with water three times per day for each 30 seconds. A box (25 x 18 x 7.5 cm lxbxh) providing remineralized osmosis water was placed under the foam nest to allow hatching of group 1 in a monitored environment. The second foam nest could not be placed in a separate terrarium without damage as it was attached to a glass wall. In order to collect the hatching tadpoles of group 2 a plastic box (10 x 10 x 10 cm lxbxh) was placed underneath it. Foam nests were checked daily and hatched tadpoles were moved into aquaria (see below). Both foam nests were torn apart when no tadpole hatched after at least one week and the remaining eggs were counted and photographed. Air and water temperature ranged between 21°C and 22°C during the developmental phase of both groups.

Tadpoles of both groups were separated in two similar aquaria (30 x 30 x 30 cm lxbxh, water level 25 cm) providing equivalent environmental conditions (Fig. 2B). Each aquarium contained aquatic plants (*Cryptocoryne* sp.), algae-covered stones, dried leaves of *Fagus sylvatica*, which served as additional food, and snails (*Physella* sp. and *Planorbella* sp.) to remove food remains. Tadpoles were fed three times per week ad libitum. Due to availability the food composition changed during the larval phase, starting with a mixture of three different minced fish foods (Sera Vipan Großflocke XL-Hauptfutter für alle Zierfische, O.S.I. Spirulina Flakes and Tetra Tablets TabiMin). Tadpoles of group 1 were fed with this mixture until day 108, whereas the tadpoles of group 2 were fed with it until day 91. Later on the tadpoles were

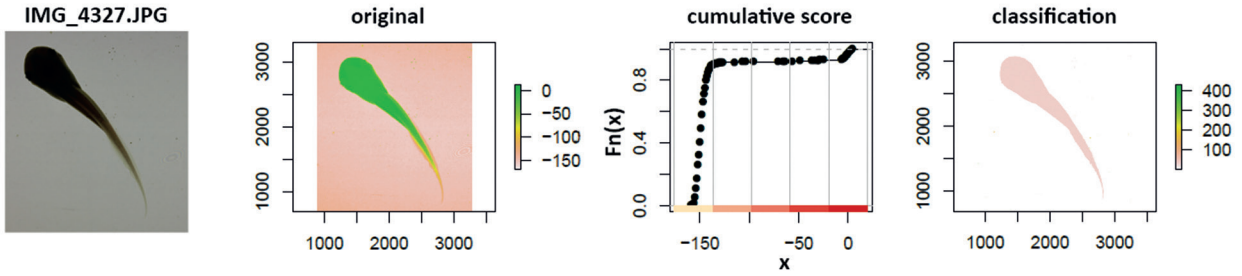

Fig. 3. Example of a tadpole picture which was processed with SAISAQ to calculate the body size. The original photo is transferred into a monochromatic Figure and a threshold is computed to select the most appropriate color intensity to delimit the largest dark object, which in turn is measured in terms of the total number of pixels. Based on this pixel score the surface is computed using a standard.

fed with fish food tablets (Tetra Tablets TabiMin, 1–2 tablets/aquarium).

Two-thirds of the water of each aquarium was exchanged weekly with remineralized osmosis water and the algae-covered stones were exchanged. No artificial light source was provided as the aquaria were placed in a room providing daylight. The water temperature varied between 19°C and 27°C (group 1) and 25°C (group 2). During an unexpected cold period in Germany, the water temperature of both aquariums sank to 19°C which was counterbalanced by a heater (Sera Automatic Heater 50 W), thus reestablishing a minimum of 21°C afterwards. A piece of floating cork was placed in each aquarium to enable the metamorphosed froglets to leave the water.

Froglets were moved to terraria (50 x 40 x 40 cm lxbxh; Fig. 2C) which were equipped similarly to those of the adults, with a filter pad as substrate layer and a small water part (1.5 cm depth) and Hygrolon® to keep humidity high. The terraria were automatically misted with water three times per day for 30 seconds each resulting in a relative humidity between 70 and 90 %. The land parts of both terraria were equipped with *Monstera deliciosa*, *Pilea* sp., bromeliads, different mosses and ferns, and as light source in both terraria LED light strips were used (Solar Stinger 1100 mm Sunstrip Dimmable Diver). The photoperiod was set to daylight hours between 8 a.m. and 8 p.m.. Air temperature varied between 19.0°C and 25.0°C and water temperature between 20.0°C and 25.5°C.

Froglets were fed with flightless fruit flies (*Drosophila melanogaster* and *D. hydei*) and crickets (*Acheta domesticus*) two to three times per week, which were fed with fresh vegetables, fruit or fruit puree to absorb vitamins. Additionally, prey items were dusted with mineral or vitamin powder (herpetal Amphib, herpetal Mineral + Vitamin D3 and herpetal Complete Terrarium).

Data Collection and Evaluation

The development of the nests, tadpoles and froglets was monitored between March 2nd and August 1st, 2017. After the tadpoles hatched, standardized photos were taken of every tadpole every day for a week (Canon Eos 550D) as described in Kurth et al. (2014). Afterwards the interval was extended and photos were taken two to four times per week until day 114 depending on the developmental progress of the tadpoles. When the tadpoles metamorphosed to froglets, photos were taken of every specimen before they were placed into the terrarium belonging to their groups. Photos of the frogs were taken weekly.

The growth of the tadpoles was determined using the SAISAQ pipeline (Kurth et al. 2014), programmed in the open source statistics software R (R Developmental Core Team 2017). SAISAQ analyses photograph files and computes the surface of a tadpole (Fig. 3), which is highly correlated with tadpole mass and can be used to describe body size in tadpoles (Kurth et al. 2014).

RESULTS

Development and Staging

During the night from February 25 to February 26 and the night from March 14 to March 15, the foam nests were produced, while nest 2 was larger and not as compact as nest 1. The part of the foam nest containing the eggs sank in a fluid transition. Both foam nests were beige to yellow coloured becoming darker with drying. On day 9 the foam nest of group 1 showed the first holes when the first tadpoles hatched (Fig. 4). On day 10 and 11, the foam nest sank further. From day 14 onwards, the foam started to disorganize, holes became larger and remaining eggs became visible (Fig. 4). The foam nest of group 2 started disorganization on day 6 and most tadpoles had hatched on day 10 (Fig. 4). The clutch of group 1 contained 49

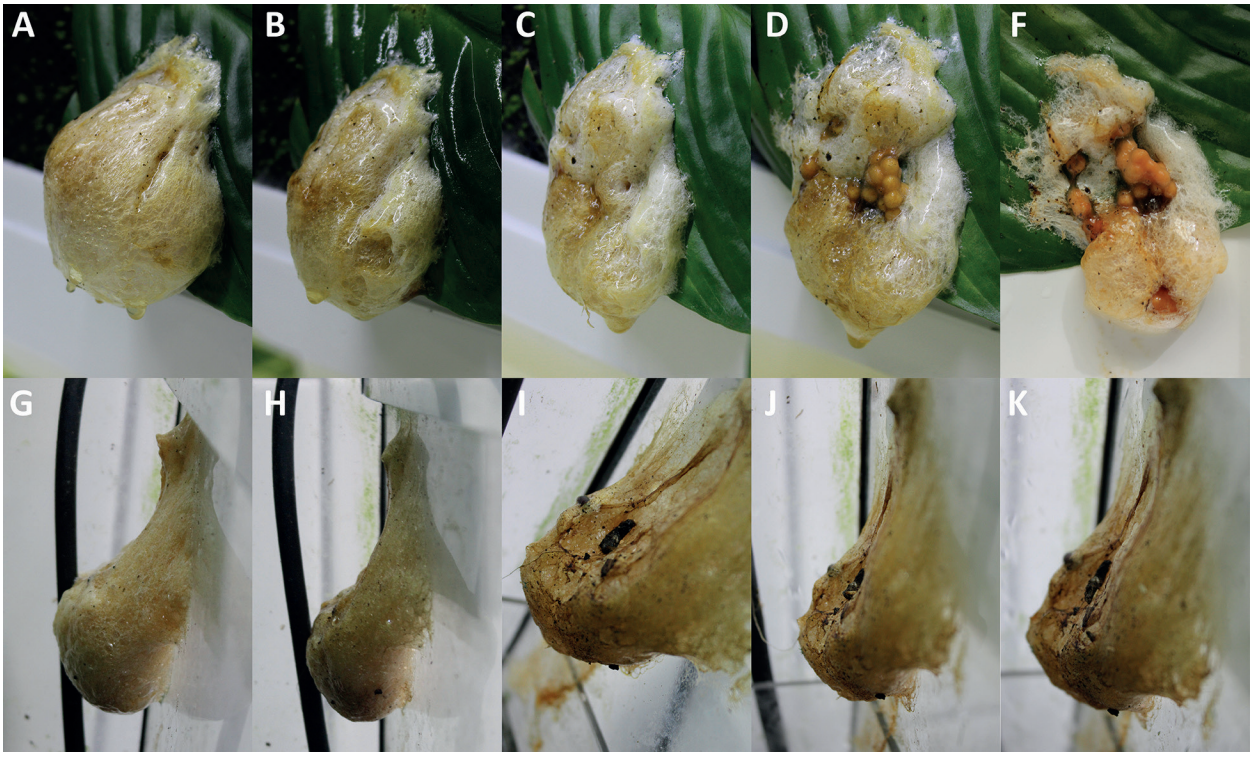

Fig. 4. Foam nests of *Polypedates otilophus*. Upper row shows the development of the nest of group 1 (5, 10, 13, 16, and 19 days), bottom row group 2 (1, 6, 10, 13, and 16 days).

eggs including 35 unhatched eggs, whereas the clutch of group 2 contained 19 eggs including 2 unhatched.

On day 11 the first two tadpoles of group 1 hatched. Afterwards, five tadpoles hatched on day 12 and another five tadpoles the next day. The last two tadpoles hatched on day 14. On day 8 the first tadpole of group 2 hatched. On day 9, thirteen tadpoles hatched and on day 10 the last three did.

A detailed staging table according to Gosner (1960) is provided in Appendix 1 and corresponding photos are provided in Figs 4–5, and 7–9. The tadpoles hatched when they were in Gosner stage 25 (Fig. 6). Between day 21 and day 50 the hind limb buds grew (Fig. 8). On day 41 the first tadpoles of group 1 reached Gosner stage 30. The first tadpoles of group 1 reached stage 31 on day 51. Stages 32 to 36 were hard to identify because the musculature of the tail overlapped the foot bud partly. On day 84 only the first tadpole of group 1 reached stage 37. At this time two tadpoles of group 1 were in stage 30, seven tadpoles of group 1 were in stages 31 to 33 and the remaining three tadpoles of group 1 were in stages 34 to 36. The first tadpole completed metamorphosis after 120 days (group 1). The other tadpoles were slower in their development with the last specimen completing metamorphosis on day 169.

Some tadpoles of both groups showed a spinal curvature in the area between the body and the tail fin (Fig. 9) starting on day 55 when a weak spinal curvature became visible in some tadpoles of group 1. On day 84, most tadpoles of group 1 showed the spinal curvature which became more distinctive in the course of time. The spinal curvature vanished after completing metamorphosis having apparently no effects on the frogs.

Comparing the development in the two groups it became evident that the first froglet of group 2 completed metamorphosis later than the first froglet of group 1, although hatching earlier. In group 1 one specimen developed faster than the remaining cohort while in group 2, the tadpoles metamorphosed shortly after one another (Gosner stages 37 to 40).

Mortality

During the study, five tadpoles died in group 1, and none in group 2. The tadpoles died on days 18, 109, 117, 128 and 137. Noteworthy, other tadpoles fed on dead specimens proving for the first time adelphophagy in *P. otilophus*.

Growth Rate

The tadpoles of group 2, which hatched earlier than the tadpoles of group 1, hatched also with a smaller body size (Fig. 6; group 1: 0.176 cm^2–0.306 cm^2; group2:

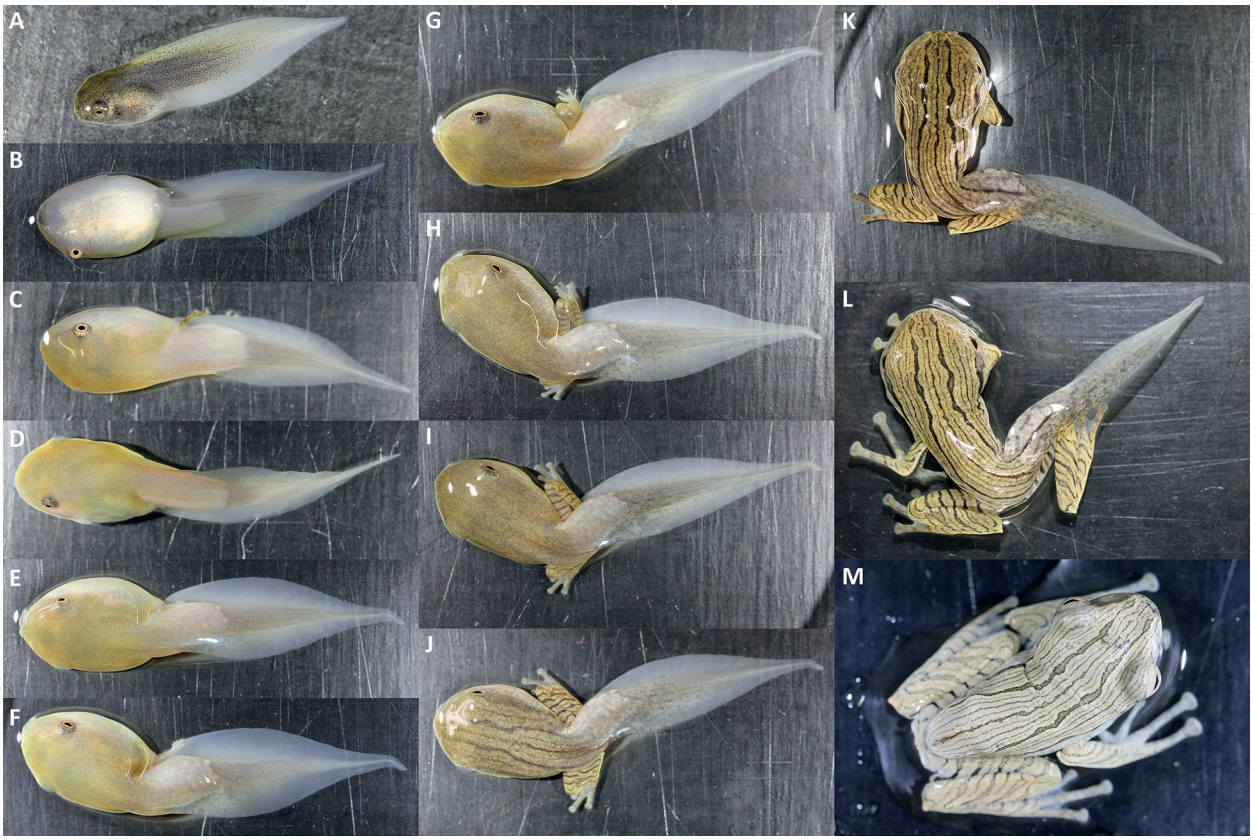

Fig. 5. Larval development of *Polypedates otilophus*. Gosner stage 25, 14 days old (A); stage 25, 39 days, ventral view (B); stage 29–30, 48 days (C); stage 31–32, 59 days (D); stage 36, 81 days (E); stage 37–38, 87 days (F); stage 39, 96 days (G); stage 39–40, 99 days (H); stage 39–40, 102 days (I); stage 39–40, 105 days (J); stage 42, 109 days (K); stage 43–44, 111 days (L); stage 46, 120 days (M).

0.184–0.247 cm^2). Larger body sizes were reached by tadpoles of both groups which hatched last. Body sizes were similar in both groups between day 15 and day 26 (0.7 cm^2). Afterwards, the body sizes differed between the two groups between day 27 and day 67. During this period, the body sizes of group 1 were larger than the body sizes of group 2. The body sizes of group 1 developed from the range of 0.687 cm^2–0.868 cm^2 to the range of 1.287 cm^2–2.618 cm^2. At their peaks, the body sizes of the largest tadpoles of both groups had a difference of 0.697 cm^2. The body sizes of group 2 varied between 0.525 cm^2 and 0.823 cm^2 at the beginning of this period on day 27 and between 1.187 cm^2 and 2.744 cm^2 at the end of this period on day 67. From day 67 to day 79, the body sizes of the two groups were similar. Between day 67 and 79, there was only one day at which a tadpole of group 1 clearly had a larger body size which was than the other tadpoles (3.246 cm^2 on day 75). Later on, there was a period between day 80 and day 102 when the body sizes of group 1 were again larger than the body sizes of group 2. The body sizes of group 1 varied between the range of 1.386 cm^2 and 3.589 cm^2 at the beginning of this period and the range of 2.094 cm^2 and 4.545 cm^2 at the end of this period; whereas the body sizes of group 2 varied between the range of 1.147 cm^2 and 2.912 cm^2 at the beginning and the range of 1.726 cm^2 and 3.877 cm^2 at the end of this period.

Between day 103 and day 129 the body sizes of the two groups of tadpoles were similar and the body sizes of some tadpoles of group 2 were larger than those of group 1. The body size span of group 1 varied between 2.017 cm^2 and 4.232 cm^2 at the beginning and between 4.456 cm^2 and 7.339 cm^2 at the end of this period. On day 103 the range of the body sizes of group 2 was between 1.528 cm^2 and 4.271 cm^2. At the end of this time period, the range of the body sizes was between 3.256 cm^2 and 6.178 cm^2. The maximum body size on day 115 had a tadpole of group 2 with 6.181 cm^2. After day 130 the remaining tadpoles of group 1 had larger body sizes than the remaining ones of group 2. At this time, some tadpoles went ashore. The body sizes of group 2 were only collected until day 140, but around this time the body sizes of the tadpoles of group 1 were noticeably larger than the body sizes of group 2, for example, on day 135 the maximum body size of a tadpole of group 1 was 8.094 cm^2, whereas the maximum body size of a

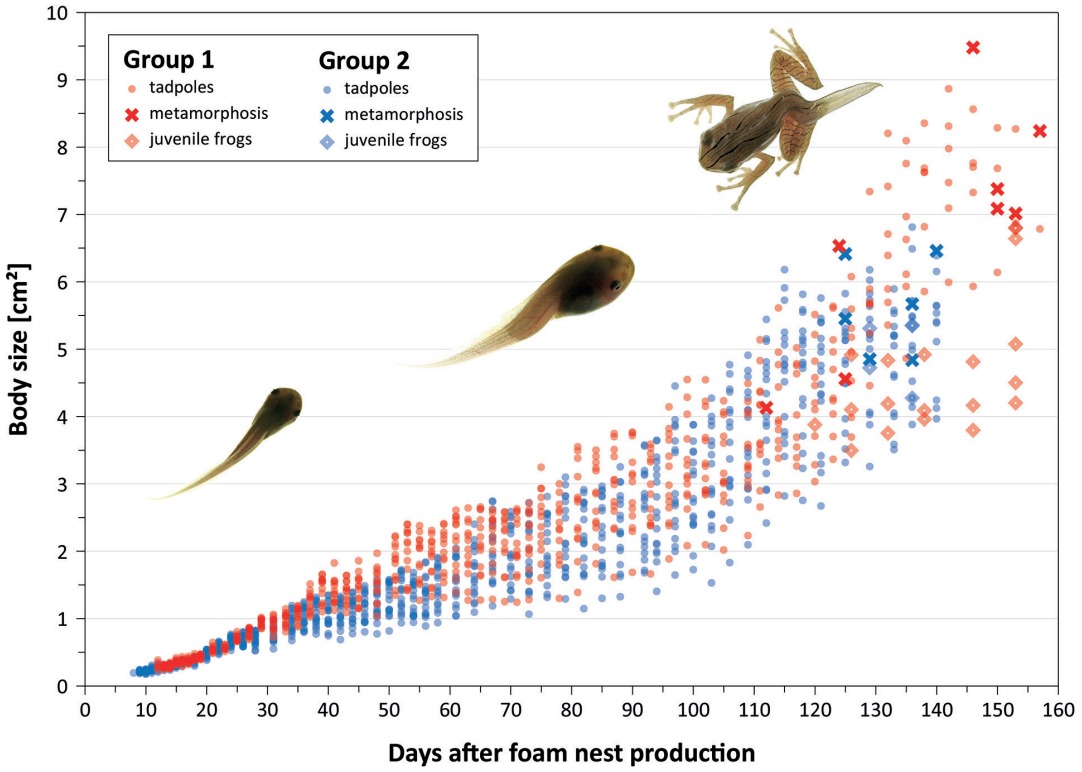

Fig. 6. Comparison of the growth rates of both groups of tadpoles, metamorphs and juvenile frogs.

tadpole of group 2 was 6.811 cm² on day 136. Moreover, the maximum body size of all recorded body sizes had a tadpole of group 1 with 8.864 cm² on day 142. After this day, the body sizes of the tadpoles of group 1 decreased.

Metamorphosis

Froglets entered the landpart between day 112 and day 157. Body sizes ranged from 4.130 to 9.477 cm². The first tadpoles went ashore with a body size of 4.130 cm² and 6.534 cm² on day 112 and 124 (group 1) highlighting a huge difference of the body sizes of the first two tadpoles (Fig. 6). On day 125, three tadpoles went ashore (Fig. 6; two specimens belonging to group 2; 5.450 cm² and 6.413 cm²). Tadpoles of group 1 left the water earlier starting on day 112 than those of group 2 (day 125), while in each cohort the first tadpoles going ashore had smaller body sizes than those which went ashore later.

Mortality and Growth Rate of the Froglets

During the study, no metamorphosed frog died. The first frog which went ashore on day 112 had a body size of 4.130 cm² (Fig. 6), which was reduced to 3.876 cm² on day 120. Two frogs went ashore with body sizes of 5.450 cm² and 6.413 cm² on day 125 and later on day 129, with their body sizes decreasing to 5.307 cm² and 4.723 cm². At this time, their body size span was only at 0.584 cm². The smallest body size had a frog of group 1 with 3.495 cm² on day 126. The three frogs of group 1 had a body size span of 1.419 cm² at the beginning. Moreover, it became evident that all body sizes of these three frogs developed similarly. From day 126 to day 132 the body sizes decreased, whereas the body sizes increased from day 132 to day 138.

DISCUSSION

In comparison to the clutch size of 44 to 119 eggs of *P. otilophus* reported of Tapley and Girgin (2015), the clutch size of 19 and 49 eggs in our study seemed very small. The frogs of our study were sexually mature, but they were apparently not full-grown. Tapley and Girgin (2015) report that three foam nests which were observed by the authors were deposited on leaves and the other eleven clutches were deposited on the glass walls of a vivarium. A similar behaviour of the frogs was evident in our breeding group.

Chakravarty et al. (2011) reported for *Polypedates teraiensis* that this species begins to breed sporadically after the first few rains of the rainy season. Reproduction in our group of *P. otilophus* was also induced by an artificial rainy season. Chakravarty et al. (2011) observed

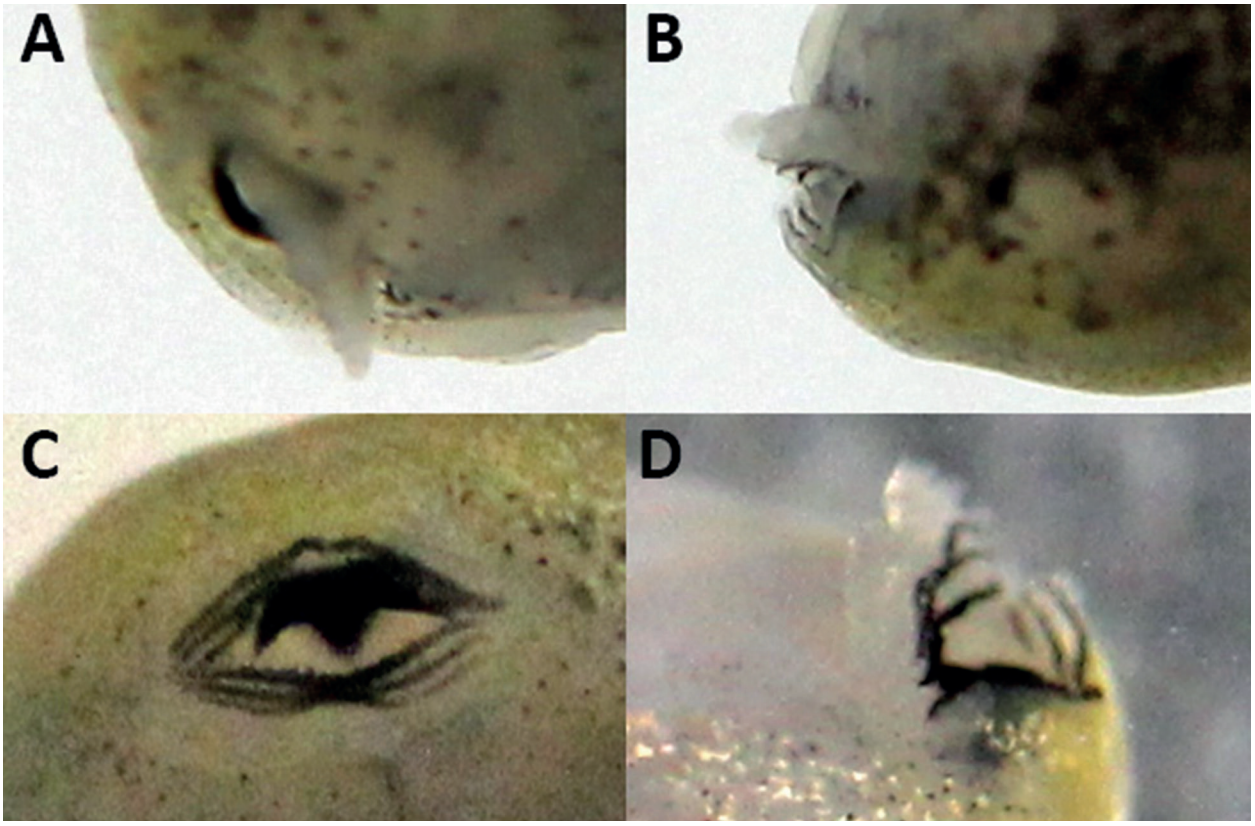

Fig. 7. Enlarged views of the mouths of *Polypedates otilophus* tadpoles from three different perspectives: a tadpole of group 1 on day 15 (A); a tadpole of group 1 on day 16 (B); a tadpole of group 2 on day 20 (C); and a tadpole of group 2 on day 22 (D).

that commonly foam nests were deposited on vegetation above shallow temporary water, but also that some individuals of *P. teraiensis* deposited some foam nests on logs or walls of human habitations far from water, which desiccated and decayed. This behaviour is similar to the just described behaviour of *P. otilophus* depositing eggs on glass walls in captivity.

The large number of eggs found in the foam nest of group 1 was conspicuous, but it is not clear, whether the eggs were unfertilized or whether the tadpoles died during the early development before they could hatch. Yorke (1983) reported for *Polypedates leucomystax* that the embryonic mortality was 34% and that all eggs in all observed foam nests were fertilized. Only two eggs of group 2 did not hatch. One of these two eggs was visible through the glass side from day 8 onwards. Chakravarty et al. (2011) reported for *P. teraiensis* that some eggs which were found on the outermost surface of the foam nest, which may not develop and turned pale yellow due to desiccation. The two eggs of group 2 which were found in the foam nest after the tadpoles hatched were intensively yellow. Moreover, this foam nest was the one which was exposed to drier environmental conditions resulting in a dry and hard consistency. There was a huge difference to the consistency of the foam of group 1 of which the foam nest was regularly sprayed with water. Probably the reason why the tadpoles of group 2 hatched earlier from day 8 to day 10 than those of group 1 which hatched from day 10 to day 14.

Tapley and Girgin (2015) reported that *P. otilophus* tadpoles hatched on about day 10 at a temperature between 22°C and 26°C, but it was not clear whether the ambient temperature was the same where the foam nests were deposited (Tapley & Girgin, 2015). The ambient temperature where the foam nest of group 1 was deposited was about 21.5°C and the temperature where the foam nest of group 2 was placed was about 22°C suggesting constancy in developmental time despite different ambient temperatures.

Gosner (1960) reported that the embryos of most species hatch between the stages 17 to 20. In our study tadpoles of *Polypedates otilophus* dropped into the water at stage 25. Chakravarty et al. (2011) reported for *Polypedates teraiensis* that tadpoles of this species hatched in stage 20 and they dropped into the water in stage 22 suggesting some delay between hatching and leaving the nest. Such a delay may also be present in *P. otilophus*, wherein the trigger of leaving the nest is currently unknown but may be related to rain fall or emerging predators.

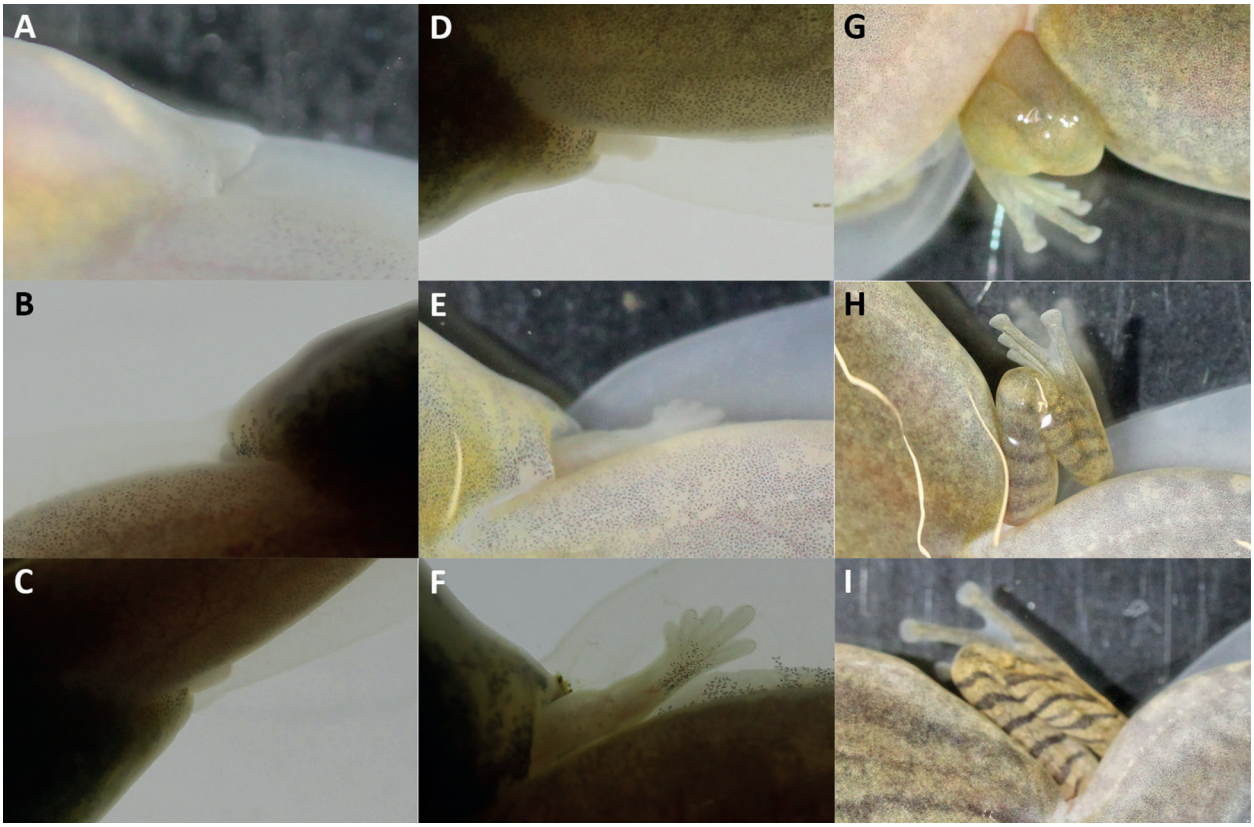

Fig. 8. Development of the hind extremities in *Polypedates otilophus*. Hind limb bud of a tadpole of group 1 in stage 26 on day 21 (A), in stage 28 on day 29 (B), in stage 30 on day 48 (C), in stage 31 on day 51 (D), in stage 36 on day 81 (E), in stage 37 on day 84 with five separated toes (F), in stage 39 on day 96 (G), in stage 39–40 on day 99 (H) and in stage 39–40 on day 105 (I).

In our study tadpoles of *P. otilophus* developed the lower and upper jaw sheaths in stage 25. In contrast to this, the upper and lower jaw sheaths of *P. teraiensis* developed already in stage 22 and the jaw sheaths had completely disappeared in stage 42. The reduction of the jaw sheaths happened in both species at the same time, but the development of the jaw sheaths is earlier in *P. teraiensis* than in other species in which the formations of keratodonts and jaw sheaths are slightly delayed (Chakravarty et al. 2011).

The first tadpoles of group 1 needed 120 days for the entire metamorphosis, whereas the first tadpoles of group 2 completed their metamorphosis after 131 days. This time which the tadpoles needed differs hugely from the period of time which the *P. otilophus* of Tapley and Girgin (2015) required, completing metamorphosis between 74 days and 84 days. They reported only ten days for the complete metamorphosis of the first tadpole and the last metamorphosed tadpole. In our group 1, there was a difference of 49 days between the complete metamorphosis of the first and the last tadpole indicating huge plasticity. One factor could be lower water temperatures, but conditions were largely similar in both studies: Tapley and Girgin (2015) raised their tadpoles at 22°C to 26°C while ours were raised between 19°C and 27°C. Moreover, Tapley and Girgin (2015) reported that their tadpoles were fed daily. In contrast, our tadpoles were fed only three times per week but aditionally fed with dried leaves and algae-covered stones available ad libitum.

The factors food availability and temperature are important ones and strongly affect developmental times. Hsu et al. (2012) reported for *Polypedates braueri* that these tadpoles hybernate if food ressources are limited and, when the ambient temperatures are too low for completing metamorphosis in one year. Furthermore, the authors reported on laboratory experiments in which some tadpoles raised at 15°C never reached metamorphosis (Hsu et al. 2012). These results highlight two points: On the one hand, the development of the tadpoles is influenced by low temperatures so that their development decelerates or is disrupted. On the other hand, the quantity of food is pivotal. These factors could be the reason of the different lengths of the larval phase in our study compared to previous publications. Our two groups were exposed to lower temperatures at a different point of time in their development. That might be the reason why group 2 was behind the development of group 1 at the beginning on day 36, before both groups got a heater. On day 36, it

Fig. 9. Specimens of *Polypedates otilophus* with spinal curvatures: a tadpole of group 1 on day 55 (left) and a tadpole of group 1 on day 124 (right).

was visible that the tadpoles of group 1 had larger body sizes than the tadpoles of group 2. At this point of time, the water temperature of group 1 was only about 19°C. After the heater was in use, the water temperature was not lower than 21°C. Furthermore, group 2 comprised more tadpoles. Maybe, the larger group size has caused a slower development.

In contrast to this hypothesis, Chang et al. (2014) reported for *Rhacophorus moltrechti* that the tadpoles reared under water temperatures of 17°C and 22°C and increased tadpole density, enhanced their larval growth, translating into greater metamorphic mass without changing time to metamorphosis or decreasing survival rates. This process was only reported on the tadpoles which were raised under the just described temperatures. The tadpoles which were raised under a water temperature of 27°C did not show this kind of development (Chang et al. 2014).

Some tadpoles died in group 1, whereas none died in group 2. The first tadpole died on day 18. At this time, tadpoles of group 1 were in stage 25. The next tadpole died on day 109. There is a huge time span during which no tadpole died. Yorke (1983) reported for *Polypedates leucomystax* that the mortality increased between the 31th and 35th day. Moreover, the pooled mortality data showed that 98% of the mortality occurred prior to the tail-bud stage, but in contrast to this, most tadpoles of group 1 died after the tail-bud stages. Some of the tadpoles of group 1 which died had an extremely distinct spinal curvature. Although the species appears to tolerate a broad range of temperature regimes, more studies are necessary to determine the optimal temperature for larval development.

Acknowledgements. We would like to thank Morris Flecks who helped with the camera equipment and arrangements of plates. Furthermore, thanks to Timo Hartmann, David Hörnes and Nils Behr who assisted with the animal care. Ursula Bott helped with her excellent proofreading skills. Very helpful comments by Thomas Ziegler significantly improved the manuscript.

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APPENDIX I

Appendix 1. Larval development of *Polypedates otilophus* according to the Gosner (1960) stages. Gr. refers to group 1 and 2 respectively, Day refers to the number of days after nest deposition.

Stages	Date [dd.mm.yy]	Day	Characteristic Traits
	Gr. 1: 11.03.2017	14	The eggs are round and brown coloured with a different intensity;
	Gr. 2: 22.03.2017	8	
25	Gr. 1: 08.03.2017	11	The tadpoles dropped into the water; the body of the tadpole was silver coloured underneath the eyes in the lateral view, whereas the body of the tadpole was yellow coloured in dorsal view; the part between the eyes to the tail fin was yellow coloured; the tadpoles were flesh-coloured on the ventral side; the tail fin was transparent with black and silver dots and with a significant visible musculature; the black dots were mainly visible from the transition of the body to the tail fin to two-thirds of the tail fin; variations: the black pigmentation on the whole tail fin; some show a stronger pigmentation on the whole tail fin; mostly the lower as well as the rear tail fin area were free of dots; the number, the order and the size of the dots were individually different; most tadpoles had also black dots around the eyes, on eye level and on the yellow coloured area as well; these dots were also individually variable in size, number and order; the silver dots on the body were on the same area like the black dots, but in comparison to the black dots, the silver dots were visible only above the musculature on the tail fin; body shape was more or less oval in the dorsal view; the body became wider behind the eyes and narrower near the tail like a paunch;
	22.03.2017	8	
25	Gr. 1: 12.03.2017	15	Oral disc with black lower and upper jaw sheaths, black tooth rows developed (Fig. 7A/B);
	Gr. 2: 27.03.2017	13	
25	Gr. 1: 16.03.2017	19	One third of the musculature of the tail fin became thicker (Fig. 5A);
	Gr. 2: 30.03.2017	16	
26	Gr. 1: 18.03.2017	21	Tadpoles developed the hind limb buds (Fig. 8);
	Gr. 2: 03.04.2017	20	
26	Gr. 1: 22.03.2017	25	Tadpoles increased their body weight; the body shape was oval; the body did not become wider beyond the eyes;
	Gr. 2: 05.04.2017	22	
27	Gr. 1: 24.03.2017	27	The hind limb buds increased their growth;
	Gr. 2: 09.04.2017	26	
28	Gr. 1: 26.03.2017	29	The growth of the hind limb buds still went on; the colouring of the tadpoles was still the same;
	Gr. 2: 11.04.2017	28	
29	Gr. 1: 01.04.2017	35	The hind limb buds had approximately 1.5 % size of their diameter;
	Gr. 2: 17.04.2017	34	

Appendix 1. *(continued)*

Stages	Date [dd.mm.yy]	Day	Characteristic Traits
30	Gr. 1: 07.04.2017	41	The hind limb buds grew to the double size of their diameter; the body was silver coloured with black dots around the mouth from the ventral view (Fig. 5B);
	Gr. 2: 23.04.2017	40	
31	Gr. 1: 17.04.2017	51	At this time the first tadpoles reached this stage; the other tadpoles were still in stage 30;
	Gr. 2: 20.05.2017	67	
31	Gr. 1: 21.04.2017	55	Some tadpoles had a light spinal curvature in the transition between the body and the tail fin (Fig. 9A);
31–35	Gr. 2: 26.05.2017	73	
31–35	Gr. 1: 25.04.2017	59	The spinal curvature became more distinctive by the concerned tadpoles of group 1; the tadpoles grew;
	Gr. 2: see stage 37 on 10.06.2017	–	
31–35	Gr. 1: 01.05.2017	65	The spinal curvature became thicker in the concerned tadpoles of group 1;
	–	–	
36	Gr. 1: 17.05.2017	81	All toes with the exception of the first and the second ones were separated; no pigmentation on the hind legs visible; the body shape was rounder;
	Gr. 2: 04.06.2017	82	
37	Gr. 1: 20.05.2017	84	All five toes were separated in one tadpole of group 1 (Fig. 8D); the foot was flesh-coloured with black dots; most tadpoles of group 1 developed a spinal curvature; the spinal curvature became more distinctive in the concerned tadpoles of group 2;
	Gr. 2: 10.06.2017	88	
37–38	Gr. 1: 23.05.2017	87	The hind legs became thicker; it seemed like the hind legs were ruffled; during this time the skin of the hind legs looked transparent so that the underlying skeleton was visible;
	Gr. 2: 22.06.207	100	
37–38	Gr. 1: 26.05.2017	90	The pigmentation on the hind legs increased (Fig. 8F);
	Gr. 2: 25.06.2017	103	
38	Gr. 1: 29.05.2017	93	The inner metatarsal tubercle was formed; the skin on the hind legs still seemed ruffled; moreover, the skin on the hind legs became slowly non-transparent and yellow coloured;
	Gr. 2: 25.06.2017	103	
39	Gr. 1: 01.06.2017	96	The hind legs were non-transparent and yellow coloured;
	Gr. 2: 28.06.2017	106	
39–40	Gr. 1: 04.06.2017	99	The hind legs had a light striation with brownish stripes;
	Gr. 2: 01.07.2017	109	
39–40	Gr. 1: 07.06.2017	102	The hind legs show a significant brown striation; the body has a very light brown striation; the forelimbs are poorly visible under the skin; there was only a light thickening visible where the forelimbs were; the hind legs became larger;
	Gr. 2: 07.07.2017	115	
39–40	Gr. 1: 10.06.2017	105	The brown striation becomes more pronounced on the body;
	Gr. 2: 10.07.2017	118	
41	Gr. 1: 12.06.2017	107	The forelimbs are visible under the skin; the brown striation of the body was significant and the basis of the tail fin also had the brown striation (group 2);
	Gr. 2: 13.07.2017	121	
42	Gr. 1: 14.06.2017	109	Brown striation of the body is significant and the basis of the tail fin shows also a brown striation (group 1); forelimbs protruded;
	Gr. 2: 16.07.2017	124	
43–44	Gr. 1: 16.06.2017	111	The tail fin started to regress; first frogs went ashore; they often held onto the glass walls of the aquarium instead of sitting on the cork;
	Gr. 2: 17.07.2017	125	
45	Gr. 1: 19.06.2017	114	There was only a stub of the original tail fin;
	Gr. 2: 19.07.2017	127	
46	Gr. 1: 25.06.2017	120	The metamorphosis of the first tadpoles was completed;
	Gr. 2: 23.07.2017	131	