Preference of *Xenopus laevis* for different housing conditions

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1. *Introduction*

With the use of European *Rana* frogs being more restrictively legislated, there is a pressing need for an alternative animal model. The South African Clawed Frog has been considered a suitable laboratory animal for a long time already (*Sharpio & Zwarenstein* 1934, *Grimm* 1951). Attempts to optimize housing conditions have been made repeatedly (*Gasche* 1943, *Parker et al.* 1947, *Ochse* 1948). Breeding procedures reported in the literature are still, largely empirically based. Difficulties are even today encountered in the breeding of South African Clawed Frogs. The significance of the more important housing factors for the development of body weight and body length of *Xenopus laevis* were therefore recently studied by *Hilken et al.* (submitted). The following factors were found to obviously influence the development of these animals: feeding, water care, stocking density and availability of hiding places. Since development of growth should not be used as sole measure in determining optimal husbandry conditions for amphibian species, experiments determining preference values for the important housing factors were performed subsequently as described below.

2. *Materials and methods*

All experiments were repeated with the basins rotated 180° to rule out preferences for one part of the basin due to influences other than the factors studied.

2.1 Animals

All Clawed Frogs, used for the preference tests were bred in the laboratory according to established methods (*Gasche* 1943, *Ochse* 1948, *Hilken et al.* submitted). For the evaluation of preference behaviour only siblings of the same breeding cycle were used for each factor. Within such groups a large variability in body weight and length is found (*Hilken et al.* submitted). A large number of animals (2.3; hundred frogs) were used to evaluate the water depth preference behaviour. This group therefore exhibited the greatest variability in body size. Tab. 1 describes about the conditions at the beginning of the investigation.

2.2 Temperature

20 frogs were placed into an aquarium (150 cm x 50 cm, water level: 15 cm) divided into eight equal compartments by the insertion of plastic partitions (Fig. 1a). Only small openings (4 cm x 2 cm) connected the compartments, in order to minimize heat exchange between them. Water temperature was regulated using one (t $\leq$ 24°C) or two (t > 24°C) heating rods on one side of the aquarium, and an air pump adding cold air (2–6°C) on the other side. Temperature differences of up to 10°C were possible between the two ends of the aquarium. Towards the middle of the basin, temperature differences decreased. The mean value of the temperatures of 4 compartments of one side was recorded. Temperatures given in Fig. 1b are therefore mean values of the temperatures found in the two halves of the aquarium. Frogs were observed for their preferences. Distribution of frogs were counted 10 times. Counts were performed without animals noticing the observer hidden behind a curtain.

2.3 Water depth

For this experiment 100 five months old frogs (body weight: 12.5–50 g) were kept in a basin measuring 230 cm x 90 cm. Water temperature was maintained at a constant 20°C (±

Table 1. Frogs.

Environmental factors	Number of frogs (Number of observations)	Age of frogs after metamorphosis (month)	Weight [g] × ± SD	Length [cm] × ± SD
Water temperature	20 (n = 10)	12	34.0 ± 8.7	6.5 ± 0.7
Water depth	100 (n = 28)	5	18.5 ± 6.5	5.3 ± 0.9
Background	15/10 (n = 10/4)	12	32.2 ± 5.1	6.2 ± 0.4
Hiding place	8 (n = 10)	8	25.7 ± 8.1	6.8 ± 0.8
Light	15 (n = 10)	12	35.2 ± 8.1	6.8 + 0.8

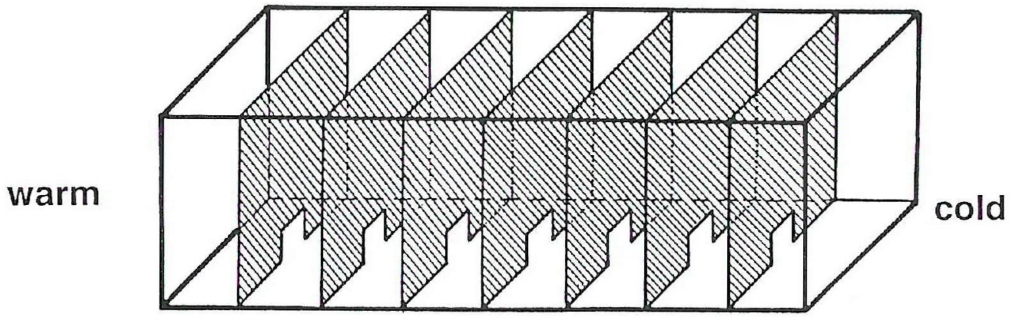


Fig. 1a. Experimental design: water temperature.

1°C). The floor had a gradient of 4° (Fig. 2a). This angle was chosen so frogs could sit relaxed near the top, without sliding down. Thin colour markings divided the floor into 5 segments. The water level ranged from 14.5 cm (most shallow segment) to 25.0 cm (deepest segment). Animals present in each segment were counted with the observer remaining behind a curtain in order to prevent disturbance of the animals. Distribution of frogs were determined 28 times within 14 days.

2.4 Background colour

Two experimental procedures were used to study preferences of *X. laevis* for different background colours. The water temperature was maintained at 20°C (± 1°C). Counts were performed without animals noticing the observer.

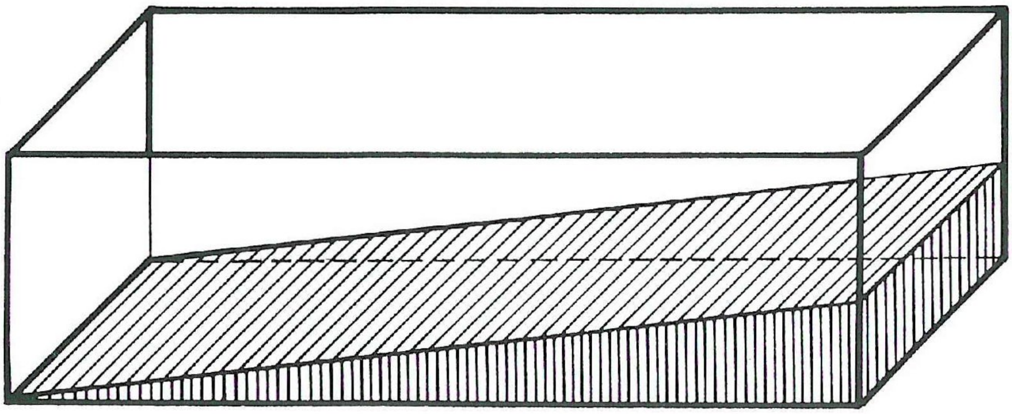


Fig. 2a. Experimental design: water depth.

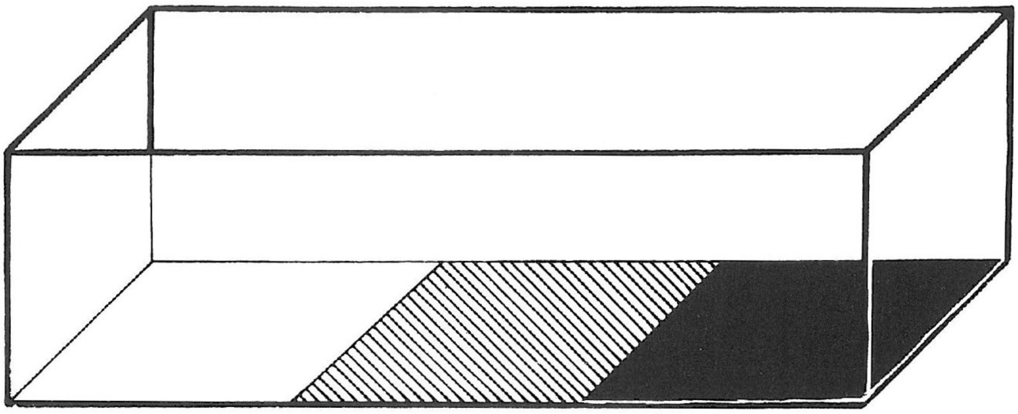


Fig. 3a. Experimental design: background colouring.

2.4.1 Frogs adapted to a three-coloured test basin

For this procedure 15 animals were kept for 3 weeks in an aquarium (150 cm × 50 cm; water level 15 cm) whose floor was covered to equal parts with white, grey and black glass plate (Fig. 3a). Animals present in each segment were counted 10 times within 5 days. Following this, the three glass plates were shifted and the animals again observed over a period of five days. All five colour combinations were in turn tried on the basin floor.

2.4.2 Frogs adapted to one background colour

Prior to beginning this procedure 10 frogs were kept in either white, grey or black coloured polyethylene basins until colour adaptation was complete. After no further colour change could be noticed (max. 45 minutes) animals were placed into an aquarium with

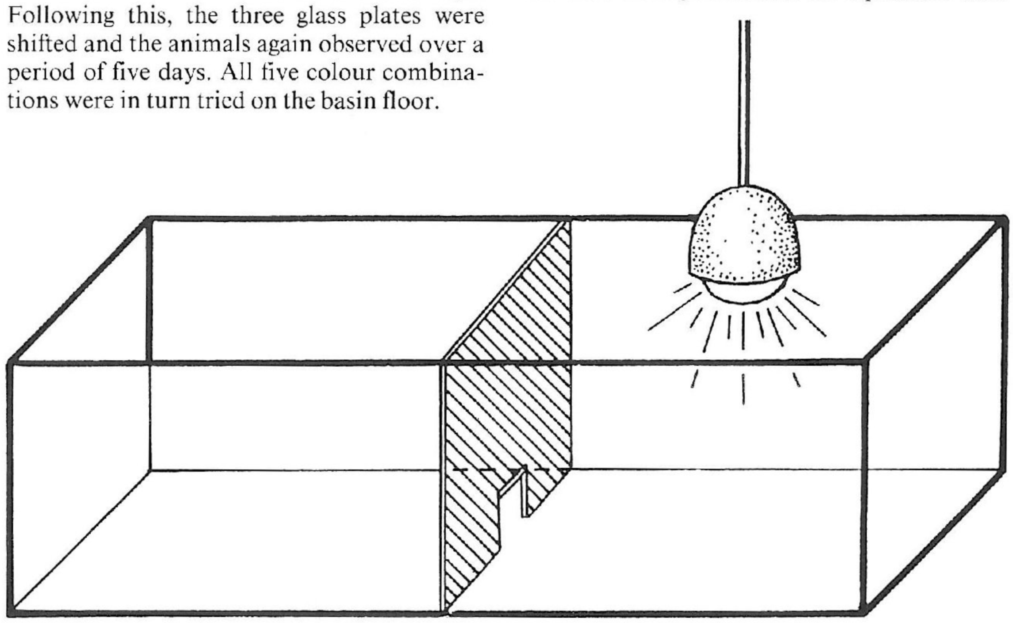


Fig. 6a. Experimental design: light intensity.

three differently coloured floor segments (see 2.4.1). The extent to which frog colour determined a preference for a certain background colouring in the new environment was observed. After one hour animals hardly left their preferred glass plate and the experiment was terminated. Animal counts (n = 4) were performed 1, 15, 30, 45 and 60 minutes after placement into the new aquarium.

2.5 Availability of hiding places

2.5.1 Preferences without disturbance

Eight frogs were placed in each of 3 polyethylene basins (60,5 × 41 cm; water level 15 cm) fitted with different hiding places. Water temperature was maintained at 20°C (± 1°C). The first basin contained 6 pieces of red earthenware pipe, and an uncovered part of equal surface area. A second basin was fitted with 6 pieces of transparent pipe (plexiglass). The third basin contained 3 red opaque and 3 transparent pieces of pipe as shelter, as well as an uncovered area. All pipes were of the same size. Distribution of the animals within a basin, whether under or outside the provided shelter, was determined ten times at one hour intervals. Counts (n = 10) were performed without animals noticing the observer.

2.5.2 Preferences during disturbance

Experimental procedure was as described above under 2.5.1. With one exception, counts were performed by an observer whose approach to the basin obviously startled the frogs. Counts were taken approximately 30 seconds later, when the animals had settled again.

2.6 Light

An aquarium (150 cm × 50 cm; water level 15 cm) was divided into two halves with an opaque plastic partition (Fig. 6a). Water temperature was maintained at 20°C (± 1°C). A 4 cm × 2 cm opening was provided for frog passage. Distribution of frogs in equally illuminated compartments was measured 10 times. Following, the room was completely darkened. One half of the basin was illuminated by an artificial light beam with an intensity of 400 lux (experiment 1) or with an intensity of 200 lux (experiment 2), the other half remained dark. The illumination caused no measurable temperature differences. For this experiment the distribution of frogs was determined 10 times.

3. *Results*

3.1 Temperature

Temperatures between 20–22°C were preferred (Fig. 1b). Given a choice of water ranging in temperature from 20.2 to 23.1°C, the frogs were found more frequently in the colder region (55.9 %: 41.3 %). However, in a basin with water ranging in temperature from 16°C to 20.2°C, only 10 % of the animals were found in the colder region. Although temperatures even below 16°C and above 25°C were tolerated. Animals found at these extreme temperatures tended to soon move on to regions with temperatures closer to the preferred range. Usually temperatures higher than 25°C were avoided completely.

3.2 Water depth

Rarely more than 10 of the 100 animals were found in the shallow half of the basin (Fig. 2b). The larger ones always aggregated in

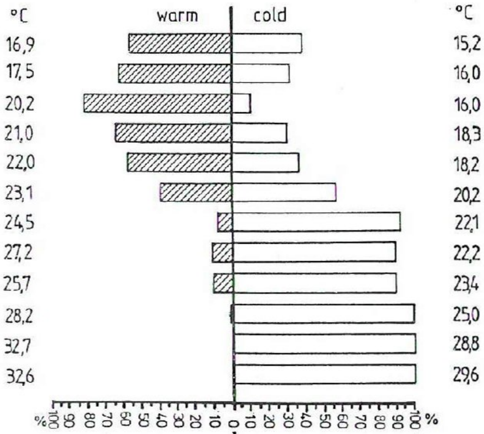


Fig. 1b. Temperature preference of *Xenopus laevis.*

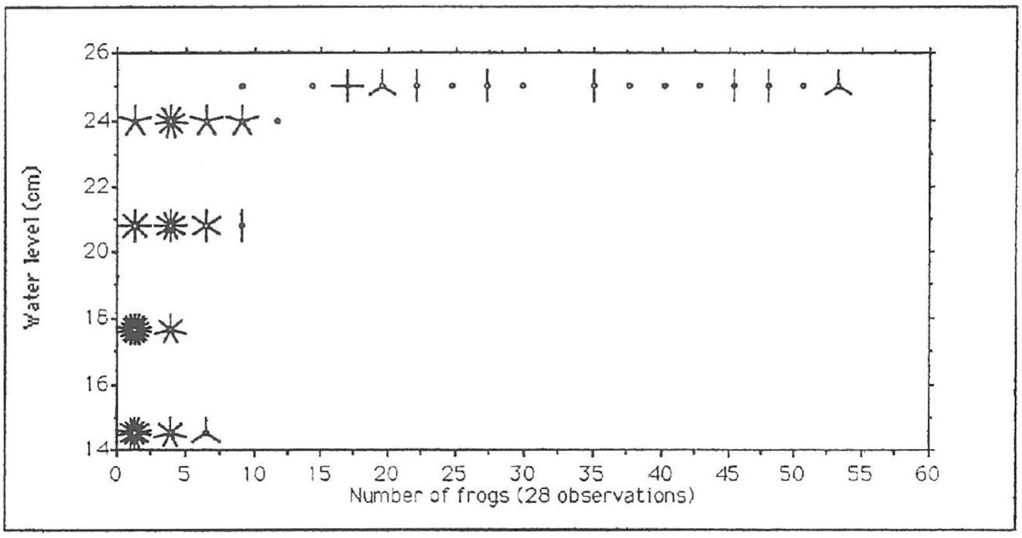


Fig. 2b. Water depth preference of *Xenopus laevis.*

the deeper half of the basin, the smaller ones in the more shallow half.

3.3 Basin floor colour

3.3.1 Frogs adapted to a three-coloured test basin

Regardless of its position, frogs were found more often over the black floor plate (Fig. 4). With the black plate positioned at one end of the basin, nearly 90 % (92.1 %, respectively 91.3 % of the frogs appeared on the black plate, arranged at the right hand side; nearly 81.2, when it was positioned on the left hand side; see Fig. 4) of the animals were found over it, resulting in no evident differences between grey and white. Differences between preferences for grey and white could only be evaluated, when the black plate was positioned in the middle. With both colour combinations around it, a markedly preference for grey was found.

3.3.2 Frogs adapted to one background colour

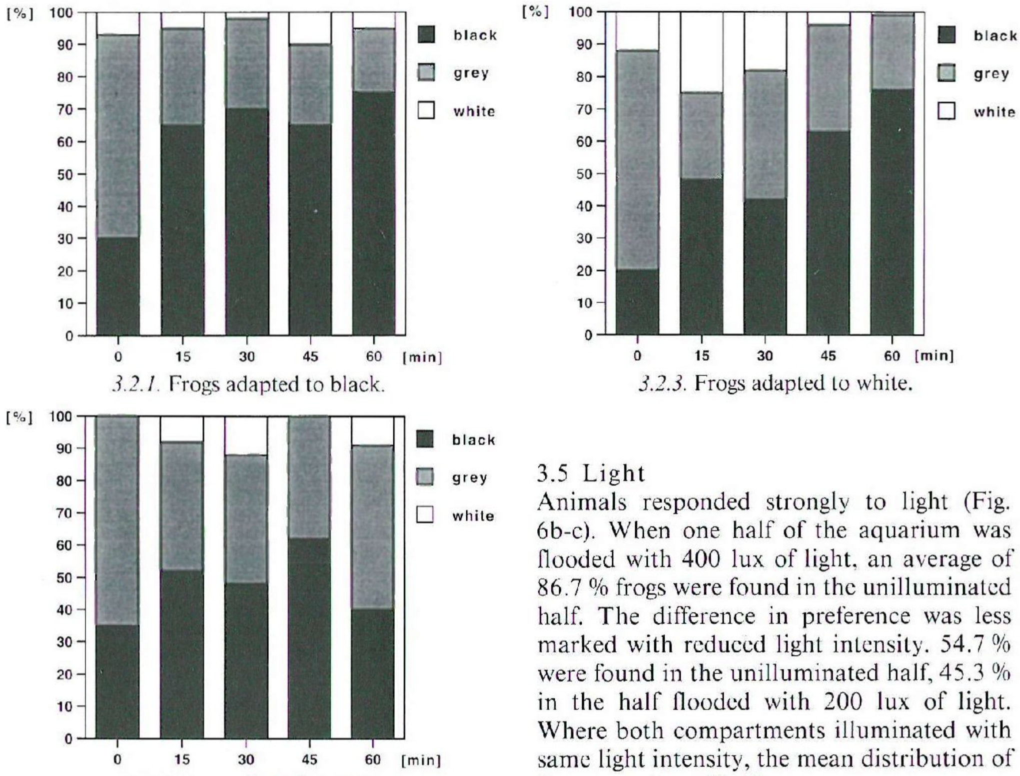
Regardless of body colour, frogs always aggregated first over the grey floor plate (white adapted: 68 %; grey adapted: 65 %; black adapted: 63 %). After a short time period, however, animals which had previously adapted to black or white backgrounds showed a definite preference for black background colour (Fig. 3.2.1, 3.2.3). In contrast, animals which had previously adapted to a grey (Fig. 3.2.2) background showed about equal preferences for the black (40 %) and grey floor plate (51 %) after 60 minutes. Black and grey backgrounds were always preferred over a white one.

3.4 Availability of hiding places

3.4.1 Preferences without disturbance

With transparent pipe available as shelter, frogs showed a marked preference for uncovered areas of the basin (Fig. 5). Red opaque pipe, on the other hand, was immediately accepted as shelter by the animals. Here, animals avoided the uncovered parts of the basin and were found predominantly under the pipes. Frogs living in the basin with both transparent and opaque pipe available as shelter were found most frequently under the opaque (51.3 %). Here, 38.7 % remained in uncovered areas and only 10 % were found under

Fig. 3.2. Mean frequency with previously colour adapted frogs settled over differently coloured backgrounds during the first hour of placement.



3.2.1. Frogs adapted to black.

3.2.2. Frogs adapted to grey.

3.2.3. Frogs adapted to white.

transparent pipe. Animals preferred uncovered areas to areas under transparent pipe.

3.4.2 Preferences during disturbance

Even after environmental disturbance, frogs preferred remaining in uncovered areas (65.9 %) rather than taking cover under the transparent pipe (34.1 %). In contrast, most frogs immediately fled under the opaque pipes, when available (94.3 %). With both, transparent and opaque red pipe available as shelter, an average of 75 % of animals were found under opaque pipe, 11.4 % under transparent pipe and 13.6 % in uncovered areas, following environmental disturbance.

3.5 Light

Animals responded strongly to light (Fig. 6b-c). When one half of the aquarium was flooded with 400 lux of light, an average of 86.7 % frogs were found in the unilluminated half. The difference in preference was less marked with reduced light intensity. 54.7 % were found in the unilluminated half, 45.3 % in the half flooded with 200 lux of light. Where both compartments illuminated with same light intensity, the mean distribution of frogs was about 50:50.

4. *Discussion*

Many laboratories still use exclusively *Xenopus laevis* captured in the wild. Those laboratories with breeding colonies often use frogs captured in the wild as starting stock. Breeding the animals to be used for laboratory purposes is desirable for two reasons:

a) Destruction of their natural habitats during capture is avoided.

b) Hygienic status and genetic quality of the animals are improved.

Xenopus laevis is not domesticated as yet. In other words, animals bred in the laboratory still require husbandry conditions similar to captured ones. Results of the preference experiments described here should be interpreted with this in mind.

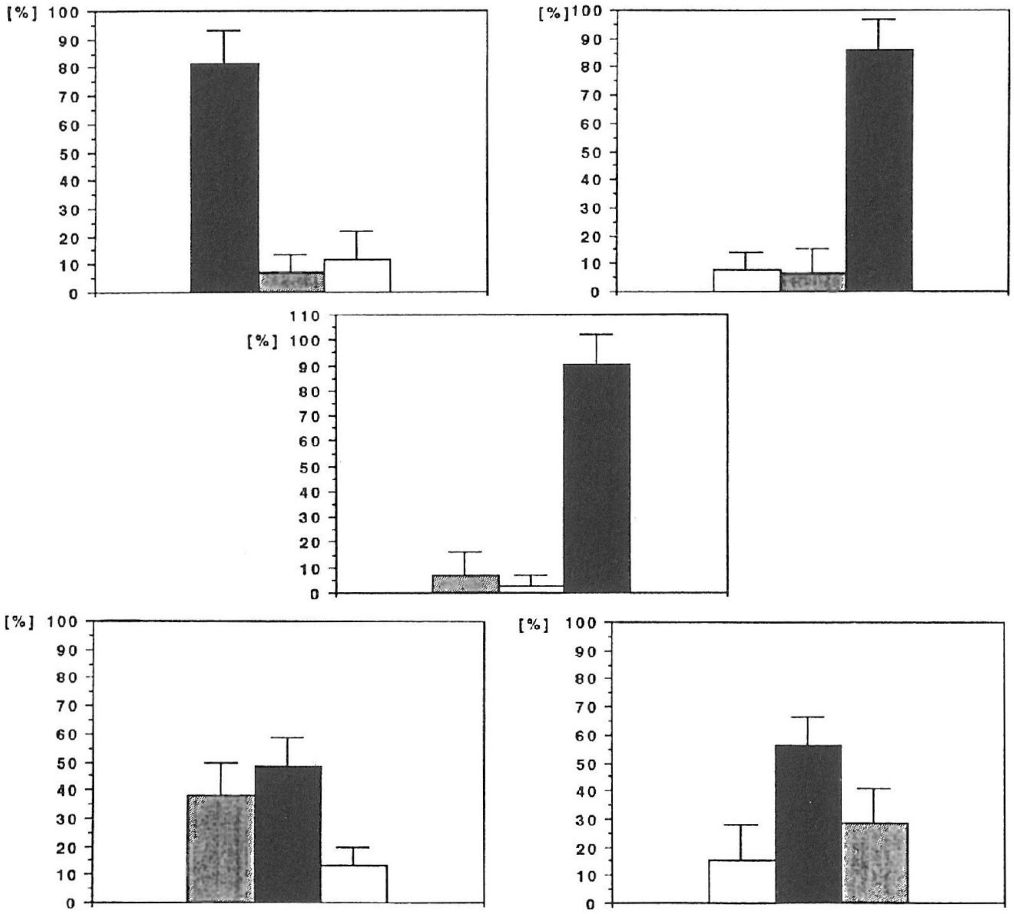


Fig. 4. Mean frequency with which frogs accustomed to a basin settled over differently coloured backgrounds (five arrangements).

4.1 Water temperature and water depth

Xenopus laevis can tolerate large differences in environmental temperature. It can therefore be considered eurythermic. Nevertheless, animals preferred colder and deeper water in our experiments. *Lerch* (1948) kept his breeding animals for months at a temperature of 2–3°C without losing any. *Wu & Gerhart* (1991) however report temperatures below 14°C and above 26°C to be stress factors. Adult animals survive at these temperatures, but the quality of the oocytes is markedly reduced. These observations are confirmed by *Goldin* (1992). A greater sensitivity of Clawed Frogs to warm water than to cold water was also noted by him. Similar observations were made during the course of this experiment. For example, rigidity was observed in frogs adapted to a cold environment following placement into water at 30°C (unpublished observations).

It is assumed that searching out the upper and warmer water is advantageous only for the filtering tadpoles. The density of plankton is highest there. Already *Ochse* (1948) showed the development of *Xenopus* tad-

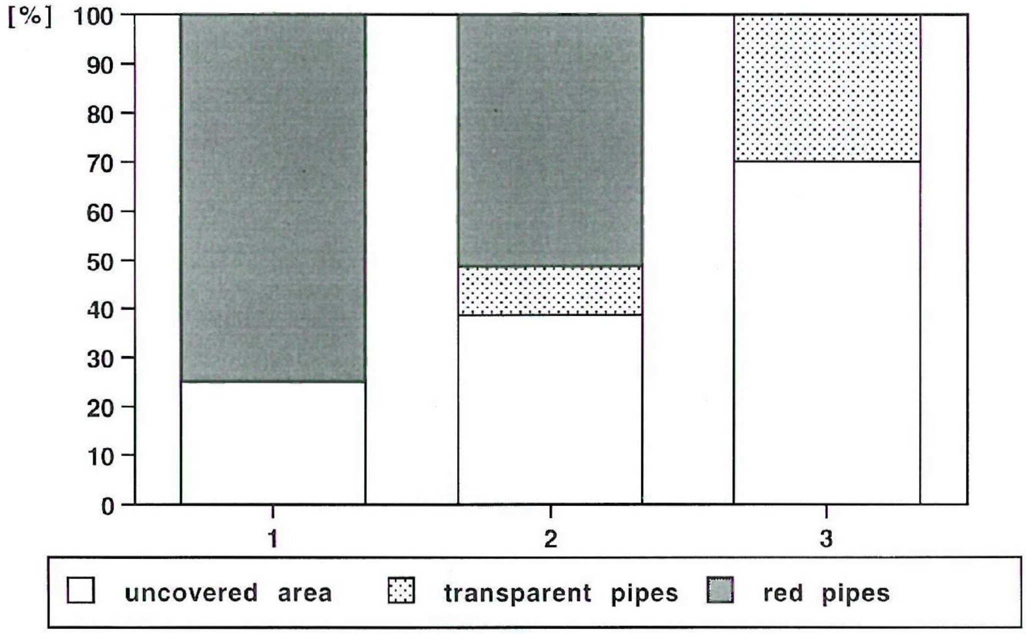


Fig. 5. Choice of different areas of the basin without environmental disturbance.

poles to be fastest in cultures kept in warm water. Similar observations were made by *Nieuwkopp & Faber* (1975) for the development of *Xenopus* embryos.

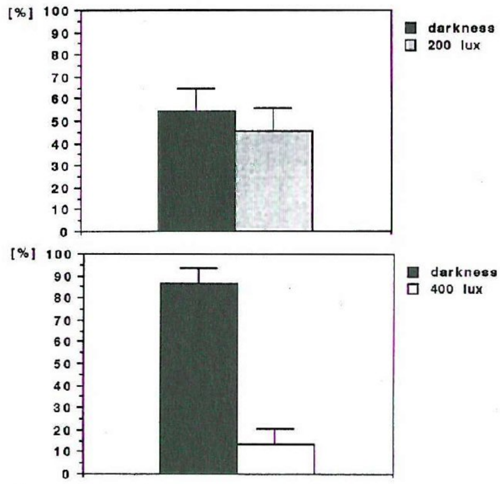


Fig. 6b-c: Light intensity preferences of *Xenopus laevis. b)* 400 lux vs darkness. *c)* 200 lux vs darkness.

In the wild, frogs are most in danger of predators in the warmer, more shallow water regions. This may explain the avoidance of these areas in the experimental basin by the larger animals, even though this results in a greater ground to water surface distance. Animals kept at a water depth of 20 cm did not grow any slower than ones kept at a depth of 5 cm (*Hilken et al.* submitted). Smaller frogs were forced into the more shallow areas of the basin. As well, smaller individuals were displaced from the feeding areas. Indeed, when differences in size were more evident, the smaller ones are in constant danger of being devoured by the larger animals. In the wild, water temperature is lower in the preferred deeper water regions. The preferred temperature range between 20–22°C documented in this study, agrees largely with the empirical observations made by other authors (*Andres et al.* 1948; *Lerch* 1948).

4.2 Background colour

The ability of *X. laevis* to adapt its colour to the background has been known for a long time. *Slome & Hogben* (1929, 1934) already described this to be under hormonal, rather than nervous, control.

A definite preference was shown in these experiments for dark ground colouring. This is in keeping with the preference shown for deeper water regions, which are usually also darker (see also section 4.1). Black background was preferred over grey, which in turn was preferred over white. Animals adapted to a lighter coloured background moved on into a darker area as soon as possible, despite being very noticable until adaptation to the new background was complete. As well, preferences for areas near the basin edge were noted. Preference for black ground colour was greatest when this was found at the edge of the basin. Even when arranged in the middle of the basin, however, marked preference was displayed for the black plate. Further, development of body weight and body length are positively influenced by black background colour (*Hilken et al.* submitted). On the other hand, basin colour is of no significance for the raising of tadpoles (*Gasche* 1944).

4.3 Availability of hiding places and light

As described previously, Clawed Frogs kept without hiding places grow faster than ones with hiding places available to them (*Hilken et al.* submitted). Frogs without shelter become tame quickly and move and feed without fear throughout the basin. *Grimm* (1952) already described that Clawed Frogs can become completely tame. On the other hand frogs with shelter available, rarely leave it.

In the preference tests, transparent pipe was made available as shelter for two reasons:

a) It was hoped the continued visibility would aid the taming process.

b) By allowing contact between the pipe and the backs of the animals, it was hoped the pipes would make the frogs to feel protected.

Contacting shelter with their backs is thought to be important for Clawed Frogs. Coverage just high enough to allow back contact is preferred. We had found previously that pipes of larger diameter were avoided by single frogs; they were only used by groups of frogs. A pile of frogs then again allowed contact of a back with the pipe ceiling. In most cases, frogs hid in the narrow spaces found between pieces when segments of large pipe were arranged next to one another. *Grimm* (1952) also describes frogs as preferring to have contact with the environment.

Nevertheless, this study made clear that the frogs do not regard transparent pipe as suitable shelter. Animals preferred remaining in uncovered areas of the basin. When given a choice, a strong preference for opaque pipe was evident. Disturbances in the environment reinforced this preference. Opaque pipe was accepted as real shelter by the frogs, while the transparent was not. The main reason for this was probably that no visual or light protection was provided by the transparent pipes. Light was seen to be an obvious factor influencing the distribution of frogs. In this study, only very few frogs were counted in the basin half flooded with 400 lux of light. With the illuminated half of the basin receiving only 200 lux, the distribution became more equal. It appears that 200 lux approaches the light intensity no longer avoided by *Xenopus laevis*. This aversion to light should be made allowance for when keeping *Xenopus laevis* in captivity. The usual lighting systems for aquariums are considerably brighter. *Parker et al.* (1947) and *Grimm* (1952) as well *Wu & Gerhart* (1991) already mentioned Clawed Frogs to have an 'aversion to light'. The animals observed here exhibited a strong aversion to light and a strong preference for dark background colour. Clawed frogs should therefore not be kept in aquariums or other lightly coloured containers, but rather in dark, dimly illuminated basins.

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Summary
Since the European frogs (*Rana* spp.) have been included in the German endangered species regulations, *Xenopus laevis* (South African Clawed Frog) is being used increasingly in animal research and in teaching. In this study, the preference of *X. laevis* for different housing conditions were examined. *X. laevis* preferred dark backgrounds, a water temperature between 20°C–22°C, and the deeper parts of the test basin. Red earthenware pipe was accepted as cover, transparent pipe was not. The frogs avoided areas illuminated with more than 200 lux.

Zusammenfassung
Seitdem Europäische Froscharten (*Rana* spp.) unter deutsche Artenschutzbestimmungen fallen, wird zunehmend der permanent aquatisch lebende Südafrikanische Krallenfrosch (*Xenopus laevis*) in der Forschung und Lehre eingesetzt. Die Präferenzen von *X. laevis* für unterschiedliche Umgebungsbedingungen wurden überprüft. Die Untersuchung zeigt, daß *X. laevis* Wassertemperaturen zwischen 20°C und 22°C, einen dunkelen Untergrund und die tieferen Teile des Wasserbeckens bevorzugt. Rote Tonröhren wurden im Gegensatz zu durchsichtigen Röhren als Unterschlupf akzeptiert. Es wird angenommen, daß dies auf der starken Lichtscheue der Tiere beruht, da sie Beleuchtungsintensitäten über 200 Lux meiden.

Yhteenveto / K. Pelkonen
Etelä-Afrikkalaista kynsisammakkoa (*Xenopus laevis*) käytään enenevässä määrin tutkimuksessa ja opetuksessa, koska eurooppalaiset sammakot (*Rana* spp.) on otettu mukaan Saksan uhanalaisia lajeja koskeviin säännöksiin. Tässä tutkimuksessa selvitettiin kynsisammakon erilaisia ympäristövalintoja. Kynsisammakko suosil tummia taustoja, 20–22°C veden lämpötilaa ja koealtaiden syvempää päätä. Eläimet valitsivat punaisen keramiikkaputken suojapaikaksi, mutta eivät läpinäkyvää. Sammakot eivät viihtyneet yli 200 luxin valaistuksen voimakkuudessa.

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