**Use of translucent refuge use in *Xenopus tropicalis* with an aim for improving welfare**

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**Abstract**

*Xenopus tropicalis* is an increasingly important animal model in a variety of biological research fields. In many countries legislation exists to promote and increase welfare wherever possible, including the ability to view animals during daily husbandry with minimal stress to the animal. *X. tropicalis* (n = 16) refuge use was investigated, it was found that they significantly preferred black opaque overhead cover to open-ended pipes or closed off ceramic plants pots in refuge choice experiments. This experiment was repeated by replacing the opaque black overhead cover with red filters. A significant preference for overhead cover was seen for the red translucent cover, compared to other available refuges suggesting that *X. tropicalis* may adopt translucent refuges due to their visual inabilities. The incapability of frogs to see certain wavelengths of light may allow staff to view them whilst simultaneously providing the refuge of choice.

Keywords: Xenopus; refuge use; husbandry; welfare;

*Xenopus* *laevis* and *Xenopus tropicalis* are a significant model organism in biomedical research and related subject areas [1,2] but there are few scientific studies on captive amphibian enrichment [1,3]. Major and Wassersug [4] found that 46% of *Xenopus* laboratories provided a refuge; 26% offered cover or shading; 20% offered sinkable rocks, ceramic plant pots and other refuges capable of covering part or the entire animal. Hilken et al. [5] have reported that refuge/cover reduced activity and increased growth and [6] showed that they prefer tanks with covers with a trend towards a greater quantity and quality of eggs produced. Refuges and cover also reduce aggression and cannibalism [7].

 *X. laevis* vision is believed to have a peak absorbance sensitivity between 519nm [8]; see figure 1 in [9]) and 522nm (figure 1in [10]), possessing only one visual pigment [9], indicating they may not be able to perceive colour at longer wavelengths e.g. reds and shorter wavelengths in blue range. Close phylogenetic relatedness means it is plausible that *X. tropicalis* has similar visual inabilities. Translucent plastic hides with a red tint called ‘mouse houses’ have been designed for laboratory mice; they are thought to perceive translucent red as darkness [11].

16 laboratory reared *X. tropicalis* were sexed (10 females, six males), weighed/measured (mean mass = 20.3g ± 1.7; mean length = 107mm ± 10.1) and isolated before the experiments began. They were approximately three years old, all from the same batch of eggs (strain unknown). They were, kept according to standard EU/UK housing regulations (ASPA 1986) fed a mixture of sinking pellets and blood worm throughout their live). The experiments took place over four weeks in early 2014.

The first experiment tested preference of refuges typically given to laboratory amphibians [2]. A grey opaque tank measuring (lxwxh) 86.5cm x 40cm x 55cm, and was filled with water to 38cm. Three types of typical refuges (terra cotta plant pot measuring 8cm aperture, 5cm base, and 8cm length; a black open-ended PVC pipe measuring 7.5cm diameter, 9.5cm length; a black overhead cover covering one quarter of the surface area of tank). One refuge was placed in one of the quadrants in the tank. The other two refuges were placed in two of the remaining three quadrants, the fourth was left free as an open area. Each frog was added to the tank and allowed to acclimatise for 5 minutes. A ‘blind’ observer then timed how long the frog used each type of refuge using a stop watch. Time started when a frog stopped within a refuge but not if it simply passed through. Times were collected for each refuge use over 5 minutes, and were also compared these times to the time spent not using any refuge at all (300 seconds minus total time of all refuge use). The position of the refuges in each quadrant were randomized daily.

The second experiment repeated the first experiment with the acceptation of replacing the black opaque cover with a laminated red filter (Lee Filters™ 106 ‘primary red’ <http://www.leefilters.com/lighting/colour-details.html#106&filter=cf&sort=number> ).

All experiments were tested for equality of variances and normality and statistics were carried out in SPSS v 20. All experiments were conducted after an ethical review carried out by the institutions NACWO and conform to ARRIVE guidelines.

Significant difference in the amount of time spent in different refuges or non-refuge use was found (Friedman test Chi Square = 10.563 d.f = 3, p = 0.001) in experiment one. Post hoc testing (Wilcoxon matched pairs) found significant difference between the black opaque cover and plant pot use (p = 0.023); not using any refuge and plant pot (p = 0.02); not using any refuge and pipe (p = 0.012); a strong tendency towards black cover use more than the pipe (p = 0.055).

Significant difference between the time used in the refuges (translucent red cover, pipe, plant pot) or non-refuge use was found (ANOVA, F6.2883, 15, p = 0.001) in experiment 2. Post hoc testing (Tukey) revealed that there was significantly more time spent using the red translucent cover than the pipe (p = 0.033), than the plant pot (p = 0.005) but not compared to using no refuge (p = 0.995). There was also significantly more time spent using no refuge than the plant pot (p = 0.01). There were no significant differences between sexes in either experiment.

The results from the first experiment (Figure 2A) suggest that overhead cover is preferred as a refuge for *X. tropicalis*. The surface area of cover offered by the overhead cover far exceeds that of the ceramic plant pot and PVC pipework. The cover gives more protected space for behaviours such as foraging.

*X. tropicalis* utilise translucent red covers in the same way that they utilise black, opaque covers. A non-opaque cover, such as that used in experiment two, has many advantages; it allows staff to observe the frogs without disturbing them and causing stress, monitor waste, uneaten food or reproduction. Furthermore, it may allow experimentation or observation of natural behaviours without interference. Given the knowledge of *Xenopus* vision [8], [9],10]) it is perhaps plausible that the frogs are unable to see the remaining light after it has passed through the red filter; therefore, providing adequate cover for the frogs.

Given that ‘no cover’ did not differ significantly from using the preferred refuge is possibly down to exploration of the environment, looking for food perhaps: these times include movement between areas which may have inflated the recorded time for ‘no cover’ use.

Using laboratory animal colour vision to improve welfare has been approached before in laboratory mice (e.g. [11]). It is to be noted that not all common laboratory mice strains accepted the translucent red refuges in [11] and there are numerous laboratory *Xenopus* strains, some may not react in the same way as the animals studied here. Nevertheless, we find this pilot study offers future directions in research regarding *Xenopu*s spp. welfare, by taking advantage of an animal’s deficit in colour vision.

**References**

[1] Chum H, Felt S, Garner J and Green S 2013 Biology, behavior, and environmental enrichment for the captive African clawed frog (*Xenopus* spp). Applied Animal Behaviour Science 143(2-4): 150-156

[2] Green SL 2009 The Laboratory *Xenopus* sp. CRC Press, Boca Raton

[3] Archard GA 2013 Refuge use affects daily activity patterns in female *Xenopus laevis*. Applied Animal Behaviour Science 145(3): 123-128

[4] Major N and Wassersug RJ 1998 Survey of current techniques in the care and maintenance of the African clawed frog (*Xenopus laevis*). Journal of the American Association for Laboratory Animal Science 37(5): 57-60

[5] Hilken G, Dimigen J and Iglauer F 1995 Growth of Xenopus laevis under different laboratory rearing conditions. Laboratory Animals 29(2): 152-162

[6] Brown MJ and Nixon RM 2004 Enrichment for a captive environment-the *Xenopus laevis*. Animal Technology and Welfare 3: 87-95

[7] Torreilles SL and Green SL 2007 Refuge cover decreases the incidence of bite wounds in laboratory South African clawed frogs (*Xenopus laevis*). Journal of the American Association for Laboratory Animal Science 46(5): 33-36

[8] Dartnall HJA 1954 A study of the visual pigments of the clawed toad. The Journal of Physiology 125(1): 25-42

[9] Bridges CDB, Hollyfield JG, Witkovsky P and Gallin E 1977 The visual pigment and vitamin A of *Xenopus laevis* embryos, larvae and adults. Experimental Eye Research 24(1): 7-13

[10] Crescitelli F 1973 The visual pigment system of *Xenopus laevis*: tadpoles and adults. Vision Research 13(4): 855-865

[11] Key D and Hewett A 2002 Developing and testing a novel cage insert, the" Mouse House", designed to enrich the lives of laboratory mice without adversely affecting the science. Animal Technology and Welfare 1: 55-64