

Recent Developments and Recommendations for Housing *Xenopus laevis* (Xl) frogs commonly used for Laboratory Testing

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Introduction

For many decades, the *Xenopus laevis* (Xl) frog has been the amphibian most widely used in laboratories around the world (Archard, 2012). In recent years it has been a test subject for embryological, biomedical and genetic experiments, chiefly because the outcome of genetic alterations made to the embryo emerge within days as they transform into tadpoles (Pearl *et al.*, 2012). Despite the heavy reliance on this species, there have been few studies researching the optimal housing requirements and the benefits of enrichment for captive Xl. This paper surveys some recent studies conducted in these areas, as well as briefly discussing the effect of vibrations on Xl in the laboratory. Optimising the conditions and welfare for captive Xl is prudent, as more researchers opt to use frogs rather than higher vertebrates in experiments (Pearl *et al.*, 2012).

Discussion

Xl tadpoles are an aquatic species that require air to breath, either via aquatic respiration or air-breathing from the surface when there is less dissolved oxygen available (Calich & Wassersug, 2012). In the wild they live in medium to deep pools of water, such as ponds or rivers (Chum *et al.*, 2013) and maintain a head-down position in the water column (Calich & Wassersug, 2012). A study conducted by Calich and Wassersug (2012) investigated the effect of water depth on the growth and development of Xl tadpoles. The experiment investigated three different container depths, each with equal water volume. Observation and testing showed that as the depth of the tank increased, dissolved oxygen decreased, and the amount of air-breathing undertaken by tadpoles increased. The experiment was repeated three times, with 10 tadpoles used in each 14-day trial (Calich & Wassersug, 2012).

Interestingly, the results showed that the tadpoles kept in the deeper tanks had greater length and development, despite having less dissolved oxygen available than in shallower tanks. This was due to the inability of the tadpoles in the shallower tanks to build up enough momentum to break the surface tension of the water. To compensate, they spent more time floating close to the water's surface where there was a higher oxygen concentration, an unnatural behaviour in this species (Calich & Wassersug, 2012). This finding is significant, as many captive Xl are housed in shallow tanks and are therefore susceptible to reduced growth and development, secondary to respiratory disease caused by their inability to air-breathe properly (Calich & Wassersug, 2012).

While tank parameters can influence the health of Xl, recent studies also demonstrate the benefits of environmental enrichment that provides shelter, reduces stress and decreases in-tank aggression (Chum *et al.*, 2013). Archard (2012) wanted to assess enrichment to determine the effects it had on Xl behaviour, size and growth. The study looked at two different forms of enrichment provided to juvenile Xl (n=9) over 30 weeks: a large plastic plant and a 15cm piece of plastic tubing, compared to no shelter in a control tank. Size-matched Xl (n=9) were used in each of the environments (Archard, 2012). The results showed that enrichment did not affect how much the Xl ate compared to the controls, nor were there any significant effects on weight, growth or reproductive potential of Xl over the 30 weeks (Archard, 2012). However, enrichment did alter the areas of the tank where Xl spent their time. As shelter seeking is a normal behaviour in Xl, the tadpoles in tanks with no enrichment often used the tank edges or each other for concealment (Archard, 2012). Comparing the two enrichment types, Xl were found to use the plant more than the tube. It was hypothesised that this was because the plant provided more opportunities to hide both under it, next to it, and within the fronds (Archard, 2012). Further, enrichment in the tanks reduced the tadpoles' startle response, which was interpreted as a reduction in stress resulting from use of the shelter (Archard, 2012). Stress was defined as an over-taxing of the animals' control systems and a reduction in their fitness. This can be compared to wild Xl, who will choose water with surrounding vegetable matter in order to lay their eggs and take refuge under cover when threatened (Chum *et al.*, 2013). Research also showed that

aggression and cannibalism in tanks decreased when there was refuge available (Chum *et al.*, 2013). Currently, 46% of laboratories provide enrichment for *XI* (Chum *et al.*, 2013). Archard's findings confirm that this should become standard practice.

Not only are conditions within the tank important, *XI* are also susceptible to external forces such as vibrations. The effects of vibrations not only pose health risks for *XI*, they can also lead to misinterpretation of research data. In 2008 a case was documented in the USA in which construction work in a room adjacent to the laboratory caused severe stress responses in 168 captive *XI*, including skin sloughing, air gulping, and stomach eversion. A further 7 adult *XI* died (Felt *et al.*, 2012). Following this, a study conducted by Vandenberg *et al.* (2012) investigated the effect vibrations had on developing *XI* tadpoles. Eighteen different frequency, waveform and directional combinations were applied to groups of 50-200 embryos from their one-cell stage until they had undergone neurulation (1 night). The study found that frequencies of 7, 15 and 100Hz all induced significant levels of heterotaxia and neural tube defects in 6-20% of animals (controls 1%), as well as deformed tails (Vandenberg *et al.*, 2012). To minimise the inadvertent effects of construction work occurring near a laboratory, *XI* should be relocated elsewhere, placed in a noise-dampening room or on anti-vibration shelving (Felt *et al.*, 2012).

Conclusion

Despite being known as hardy animals, the research presented here demonstrates the fragility of *XI* in captivity. Their optimal housing requirements in the laboratory are not necessarily intuitive. Although there are many successful breeding programs for *XI* around the world, there are still advances to be made to enhance their welfare and health in captivity. In 2012-13 alone, discoveries were made about optimal tank depth, environmental enrichment and the susceptibility of *XI* to low-frequency vibrations.

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