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Effect of enrichment on the behaviour and growth of juvenile *Xenopus laevis*

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ABSTRACT

Xenopus laevis is the most widely used model amphibian species in laboratories, yet there is almost no experimental evidence to guide best practice for captive housing. Enrichment is an important component of maintenance and welfare. A split-sibship experimental design was used to rear juvenile *X. laevis* under one of three treatments for 30 weeks: a control with no enrichment, enrichment from plastic drainpipe, or enrichment from a plastic plant. Location and clustering behaviour were quantified, along with the amount of food eaten and the time to start eating. Many *Xenopus* users work with eggs or embryos, and are concerned with the growth and size of animals because female size determines reproductive output. Snout-vent length (SVL) and mass were therefore measured every 3 weeks. After 30 weeks, final measurements included fat body mass in both males and females, and ovary mass and stage of development in females. Enrichment did not effect the time to start eating, or the amount of food eaten, and there were no differences in growth (SVL or mass) between treatments. There were also no differences in final mean per tank body mass, SVL, head width or fat body mass, or the coefficients of variation of these per tank measures. Ovary mass and developmental stage were both correlated with final body mass, but there was no effect of enrichment on these measures of female reproductive potential. However, there were clear differences in behaviour between treatments. Animals used enrichment in a variety of ways. Animals in tanks with drainpipes lay inside or alongside the pipe. Animals in tanks with plants lay under, against the base of, or among the fronds of the plant. Plants were used more than drainpipes, but enrichment in either form resulted in animals spending less time against the edge of tanks, and less time clustered together. Animals from enriched tanks also had smaller startle responses, suggesting they may have been less stressed. They were harder to catch in tanks than controls, but this was the result of the enrichment itself: once the enrichment was removed there was no difference in capture times. Overall, there is no evidence that enrichment limited the growth or reproductive potential of *X. laevis*, and clear evidence that they used it when it was available. Enrichment should therefore be provided to captive *X. laevis* as standard unless there is a well-defined reason not to do so.

1. Introduction

Environmental enrichment includes any process that manipulates the surroundings of captive animals to increase their welfare. Physical manipulations can include providing shelter, environmental complexity and stimulation, and are an important component of captive animal housing (Young, 2003). As well as improving welfare,

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enrichment may increase research validity if it allows animals to show a normal range of behavioural and physiological responses to stimuli (Brydges and Braithwaite, 2009). It may also decrease the number of animals required for research, for example if animal tissues produced for experimental use are of higher quality.

In ectotherms there is very little experimental evidence available with which to determine best practice for maintenance in captivity in general (Reed, 2005; Tinsley, 2010), and environmental enrichment in particular (Young, 2003). However, the use of ectotherms in scientific research, such as zebrafish, *Danio rerio*, and the African clawed frog, *Xenopus laevis*, is significant. For example, more than 10,000 wild *X. laevis* were exported annually from South Africa to supply laboratories between 1993 and 2004 (Weldon et al., 2007). These numbers are increasing, as scientists seek to replace the use of higher vertebrates such as mammals and birds with species with a perceived lower capacity to experience pain and distress, and that are less emotive to the public (Reed, 2005).

X. laevis is the model amphibian organism in laboratories. In particular, tadpoles are used in studies of vertebrate development, and oocytes are used in molecular studies of cell membrane function and protein expression (Gurdon and Hopwood, 2000; Green, 2002). Poor maintenance is an acknowledged problem in the literature, for example it is often blamed for low egg production, or low egg quality (Green, 2002; Schultz and Dawson, 2003). Studies on the growth and behaviour of *X. laevis* reared under experimentally manipulated housing regimes therefore have the potential to improve welfare by determining (1) the preferences of these animals, and (2) the most favourable conditions for growth and reproduction, thus reducing the numbers of animal required for tissue production.

X. laevis in laboratories are housed in a wide range of conditions (Major and Wassersug, 1998; Reed, 2005; Tinsley, 2010). Traditionally, they are kept in tanks with standing water that is completely replaced at intervals, but increasingly they are housed in recirculation tanks, which require less maintenance. However, both types of tank frequently contain no enrichment of any kind, despite the fact that *X. laevis* uses enrichment when it is available (Hilken et al., 1994; Brown and Nixon, 2004). *X. laevis* in bare tanks spend a lot of time clustered together, often piled on top of one another, and may experience higher rates of physical aggression and cannibalism (Torreilles and Green, 2007).

Here, the effect of enrichment on the behaviour and growth of juvenile *X. laevis* was investigated. Animals received either no enrichment, a readily available form of enrichment, in use in some laboratories (plastic drainpipe; Hilken et al., 1994; Brown and Nixon, 2004), or a more naturalistic enrichment item (plastic plant). Our first aim was to determine how *X. laevis* utilize enrichment when it is available, and whether feeding behaviours and behavioural responses to standard maintenance procedures vary between enrichment treatments and controls with no enrichment.

It is unclear whether enrichment will affect growth. Perhaps counterintuitively, a study comparing *X. laevis* housed in a tank with no enrichment to one with cover (halves of earthenware pipe, with radius approximately 40 mm) found animals were heavier, but not longer, when enrichment was absent (Hilken et al., 1995). This suggests that enrichment may negatively affect condition and/or growth rates, and agrees with results found in fish (e.g. in cod, *Gadus morhua*, Braithwaite and Salvanes, 2005).

The potential negative effect of enrichment on *X. laevis* growth is important to *Xenopus* users because size is an important determinant of age at sexual maturity and reproductive success (Tinsley et al., 1996). Given that *Xenopus* users are primarily concerned with the efficient production of oocytes and tadpoles, our second aim was therefore to determine whether enrichment causes juvenile *X. laevis* to grow more slowly, or be maintained in lower condition. Growth of juveniles was measured over a 30 week period, at the end of which terminal measures were used to quantify the size of fat bodies in all individuals, and the size and development of ovaries in females.

2. Methods

2.1. Source of animals used

Nine pairs of adult, wild-caught South Africa *X. laevis* were induced to breed using human chorionic gonadotropin (see Warren, 2008 for details). The resulting nine sibships of tadpoles were split between a number of experiments. For this experiment, 20 tadpoles per sibship were moved to new tanks for rearing to metamorphosis. During rearing, tadpoles were maintained at $20 \pm 1\,^{\circ}$C, with a natural light cycle. Each sibship was housed in an opaque white 40 L plastic tank with 30 L aerated dechlorinated water, which was changed weekly. Tadpoles were fed crushed Blades toad pellets (Blades Biological Ltd., UK) ad lib. twice daily. As animals started to metamorphose (at 7 weeks post hatch), the amount of crushed food was reduced accordingly, and metamorphs were fed with uncrushed #1 Blades pellets three times weekly.

2.2. Experimental manipulations and maintenance

During the experiment, animals were maintained in one of three treatments, which varied only in environmental enrichment. The three treatments consisted of two different types of enrichment, and a control where no enrichment was provided. One enrichment treatment was a 150 mm length of grey plastic drainpipe (internal diameter 105 mm). The other was a 380 mm long, multi-fronded green plastic *Ambulia* plant with a weighted base.

At the start of the experiment (11 weeks post hatch, approximately 2 weeks post metamorphosis), all 20 animals per sibship were weighed and their SVL[1] recorded. Each sibship was then divided into three size-matched groups of three animals. The three groups had the same mean size (within 1 mm), and as constant a variance in size as was possible. One group per sibship was then randomly assigned to each of the three treatments. These initial measurements were used as baseline ('week 0') measurements. The remaining eleven animals per sibship were culled in

[1] SVL, snout-vent length.