

Correspondence

Eye malformation and body coloration in *Litoria aurea* tadpoles

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In amphibians, colour, patterns, and their changes are determined by pigmented chromatophores (melanophores and xanthophores) and reflective iridophores. The synthesis or destruction of pigments due to a persistent or continuous lack of stimulation of the chromatophores result in a slow and long-lasting (days to months) morphological colour change (DULLMAN & TRUEB 1994). On the other hand, a rapid, hormone-stimulated rearrangement of pigment organelles within the chromatophores results in a physiological colour change (reviewed by THIBAudeau & ALTIG 2012). Physiological colour change can be a direct response of chromatophores to light (primary response), or a secondary response, mediated by the eyes and controlled by the neural and/or endocrine systems (OSHIMA 2001, SKÖLD et al. 2013). In addition to light (HOGBEN & SLOME 1931, BAGNARA 1960, WITHERS 1995), colour change in amphibians can be affected by temperature (KING et al. 1994), humidity (WITHERS 1995), background colour (HOGBEN & SLOME 1931, NIELSEN & DYCK 1978, NIELSEN 1979, KING et al. 1994), circadian cycle (FILADELFI et al. 2005), social interactions, and sexual selection (SHELDON et al. 2003, RIES et al. 2008, DOUCET & MENNILL 2010, KINDERMANN et al. 2013, SKÖLD et al. 2013). In tadpoles, predation pressure can also affect colour development (MCCOLLUM & LEIMBERGER 1997, TOUCHON & WARKETIN 2008), but colour change to match background seems less common than in post-metamorphic stages (WELLS 2007).

Tadpoles of the endangered green and golden bell frog, *Litoria aurea* (LESSON, 1827), from southeastern Australia are often light yellowish in colour, but individuals living in shaded or dark waters, or against a dark pond background, become blackish. Individual tadpoles change between light and dark colours (L. PIZZATTO pers. obs., S. CLULOW pers. comm.), but timeframe and exact conditions required for them to do so are as yet unknown.

In 2013, several captive-bred tadpoles, from couples originally collected on Koorangang Island (32°51'47" S,

151°43'39" E) in 2010 and their offspring, were missing either one eye or both (orbits present but eyeballs missing). Individuals from the same clutch were kept jointly and thus exposed to the same environmental conditions in outdoor tanks. In samples from five clutches, the percentage of individuals without both eyes varied from 0 to 8.85%, and that of one-eyed individuals from 7.48 to 22.0% (Table 1). Both conditions had been observed in previous years but more rarely. Eyeless tadpoles appeared conspicuously darker than normal and one-eyed conspecifics in their clutches. To test for a relationship between eye malformation and body colour, we randomly selected from each clutch tadpoles with two eyes, one eye, or no eyes (N = 20 per group) and placed them individually in plastic containers (14 × 11 × 7 cm) filled with 300 ml of rainwater. Each container was placed arbitrarily on a bench with a white background, and tadpoles were left undisturbed for one hour. The experiment was run in two batches (December and April 2013), depending on clutch availability. A group of four volunteers (unaware of the hypothesis being tested or the conditions of the tadpoles), one at time, scored the colour (light/yellowish or dark/blackish) of each tadpole. When a tadpole had three or more scores for a specific colour this judgement was regarded as final. In the second batch, we used one extra volunteer, as two tadpoles had records for both colours. Colour scoring was consistent between all but one volunteer; 12 tadpoles had one score differing from the others, and four tadpoles had two scores differing from the others. Inconsistencies were evenly distributed among the groups of tadpoles.

After the volunteers had scored the first batch, the white background was replaced with a black background, and the containers were covered with black fabric. We inspected tadpole colours within 1 and 24 h, but no change had occurred in any of them. After the observations were completed, all eyeless tadpoles were euthanised by immersion in buffered tricaine methane sulfonate (4 g/l).

Table 1. Percentage of *Litoria aurea* tadpoles, from five different captive-bred clutches, that had both eyes, one eye only, and no eyes.

Clutch ID	% individuals with no eyes	% individuals with one eye	% individuals with both eyes	Sample size
K1	8.85	19.5	71.65	113
S3-3	5.03	22.01	72.96	159
S4-3	0	7.48	92.52	107
J1-2	0.44	18.22	81.3	225
S1-3	0.82	21.15	78.02	364

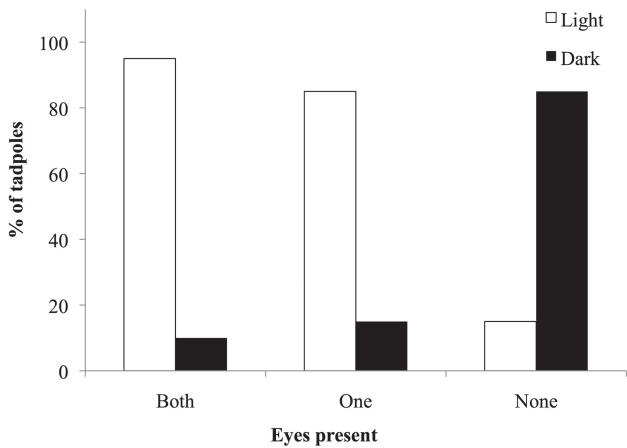


Figure 1. Percentage of dark- and light-coloured *Litoria aurea* tadpoles with and without eye malformations.

Tadpole colour depended on the presence of at least one eye ($\chi^2 = 35.9$, $df = 2$, $p < 0.0001$); after having being kept against a white background, tadpoles with both or one eye were light in colour, whereas tadpoles without any eyes were dark (Figs 1, 2).

The frequency of eyeless individuals was low, but individuals with only one eye reached up to 22%. While the cause of this malformation is unknown and deserves further investigation, it may have a genetic basis, as frequency varied among clutches, and all tadpoles were exposed to very similar environmental conditions. One-eyed tadpoles are able to feed, complete metamorphosis, and can survive to adulthood in both captivity and field conditions (L. PIZZATTO pers. obs). In contrast, while eyeless tadpoles feed and behave normally (e.g., form schools with conspecifics), they are unable to feed efficiently enough to improve their body condition after metamorphosis (L. PIZZATTO, pers. obs. based on 3 post-metamorphs found in the tanks; Fig. 3), and are likely doomed in natural conditions.

Secondary response to colour background similar to what we recorded occurs in post-metamorphic *Xenopus laevis*: individuals turn dark when positioned against black backgrounds or in darkness, and pale against a white background or in bright conditions (HOGBEN & SLOME 1931). Like in blind *L. aurea* tadpoles, the same lack of response to background colour occurs for surgically blinded *X. laevis* and possibly in fish (HOGBEN & SLOME 1931), reinforcing the notion that vision impairment affects background perception, and background-matching is mostly mediated by the eyes (WELLS 2007).

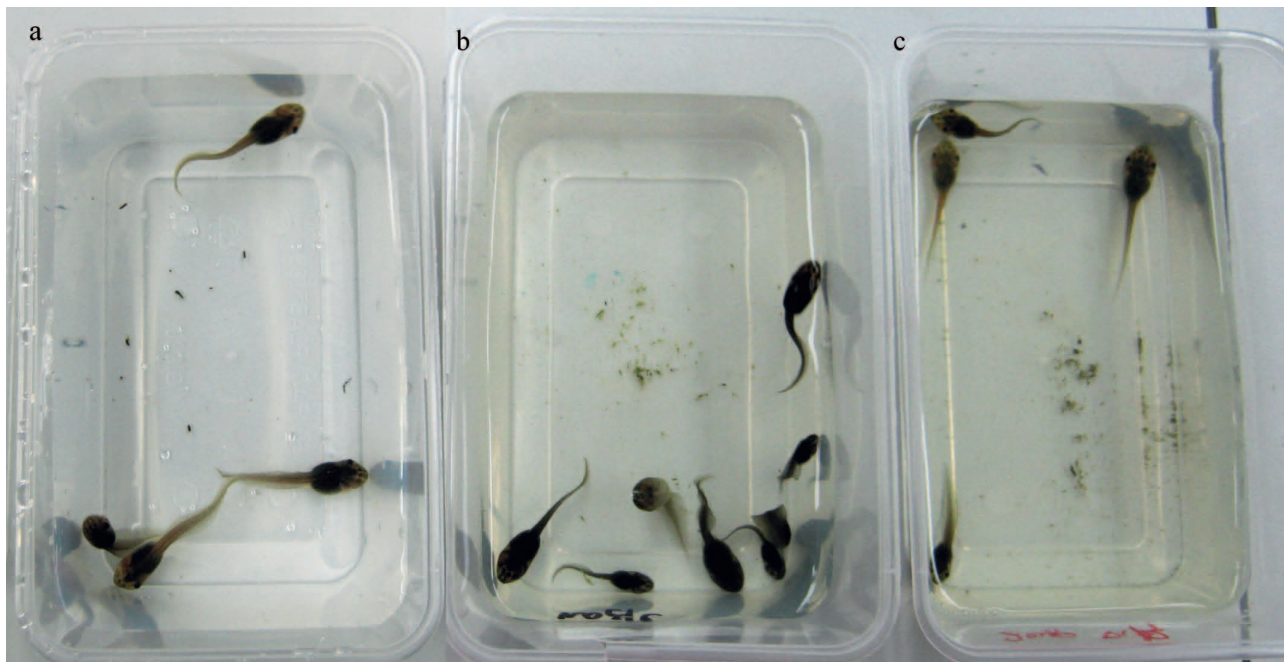


Figure 2. Coloration of a) one-eye, b) eyeless, and c) two-eyed *Litoria aurea* tadpoles against a white background.

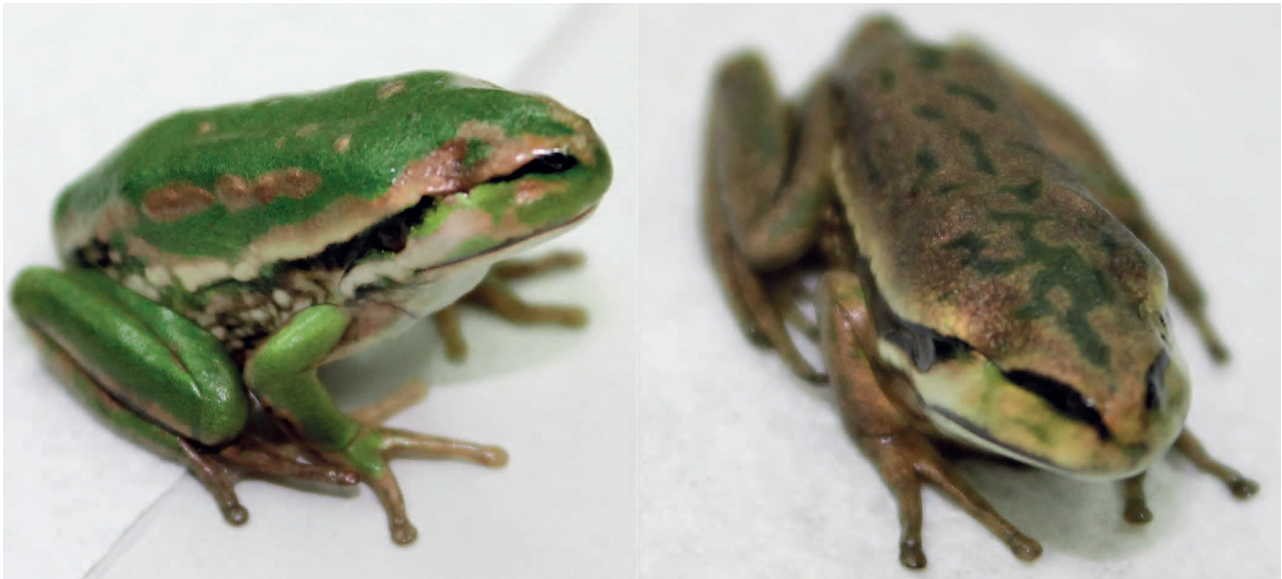


Figure 3. Eyeless *Litoria aurea*, a few days post metamorphosis.

Background-matching is an essentially anti-predator strategy (STEVENS & MERILAITA 2011). However, in natural conditions, a dark coloration of tadpoles living in shaded areas (dark background) may also improve thermoregulation. Accordingly, some amphibians change their colour in response to temperature (KING et al. 1994, WITHERS 1995, WELLS 2007). While the colour change observed in *L. aurea* tadpoles was affected by background brightness in laboratory conditions, continuously shaded conditions also induced dark coloration in outdoor tanks. In this later case, an effect of temperature on colour change cannot be ruled out. Similarly, juvenile and adult *L. aurea* have also been observed to turn dark in shaded habitats (L. PIZZATTO pers. obs.).

Physiological colour change in *Xenopus* can also be controlled by the pineal and concentration of melatonin (BAGNARA 1960, BINKLEY et al. 1988), a response induced by illumination (primary), not background colour (BAGNARA 1960, BINKLEY et al. 1988). In our white-background experiment, lighting conditions were equal and constant for all test containers, and only blind tadpoles remained dark. Thus, unless the absence of eyes is accompanied by other structural damage that compromises pineal functioning, colour control by the gland is unlikely to explain our results. Additionally, bell frog tadpoles kept in white opaque containers do not become dark at night (L. PIZZATTO, pers. obs.). It is not impossible, however, that there is individual variation in light sensitivity or melatonin production, and a primary response determines the colour of those few light-coloured blind individuals. Another plausible hypothesis would be that those tadpoles were exhibiting a morphological response to long-term exposure to sunlight in their raising tanks.

Very rapid physiological changes have been reported in invertebrates, fish and reptiles (within seconds or minutes:

THURMAN 1988, STUART-FOX & MOUSSALLI 2008), and also occur in some amphibians (within minutes: WELLS 2007, KINDERMANN et al. 2013). However, our opportunistic observations show that background-induced colours change in *L. aurea* tadpoles progress at a much slower pace as in *X. laevis* (several hours to days: BAGNARA 1960). The longer response times in colour change of amphibians likely result from a higher degree of reliance on hormonal control as opposed to the neurohumoral regulation of chromatophores in fishes (ASPENGRÉN et al. 2009). Our observations also demonstrate that the response is faster for lightening than darkening, which may be due to the process of chromatophores dispersion being more complex than their aggregation (ASPENGRÉN et al. 2009).

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