Kindermann, C., **Narayan, E.**, Wild, C., Wild, F., Hero, J-M. (2013). The effect of stress and stress hormones on dynamic colour-change in a sexually dichromatic Australian frog. Comp Biochem Physiol-A. 165: 223-227. ***Publication arising from supervised Honours student research project.***

The effect of stress and stress hormones on dynamic colour-change in a sexually dichromatic Australian frog

Christina Kindermann[a]*, Edward J. Narayan[a], Francis Wild[b], Clyde H.Wild[a] and Jean-Marc Hero[a]

[a] Environmental Futures Centre, School of Environment, Griffith University, Gold Coast campus, QLD 4222, Australia.

[b] School of Information and Communication Technology, Griffith University, Gold Coast campus, QLD 4222, Australia

Running headline: Colour change in *Litoria wilcoxii*

* Corresponding author. Tel.: +61 403689539; fax: +61 755528067

E-mail address: christina.kindermann@griffithuni.edu.au

Postal address: School of Environment, Griffith University, Parklands Dr, Southport, QLD 4222, Australia

Abstract

Rapid colour changes in vertebrates have fascinated biologists for centuries, herein we demonstrate dynamic colour change in an anuran amphibian, the stony creek frog (*Litoria wilcoxii*), which turns from brown to bright (lemon) yellow during amplexus. We show this by comparing the colour of baseline (unpaired males) and amplecting (paired) males. We also investigate the possible role of stress and stress hormones on this colour change. Frogs were subjected to four different levels of stressors (handling, toe-clipping, saline injection and adrenocorticotropic hormone [ACTH] injection) and the colour change was measured using digital photography. A comparison of baseline colour and stress hormone (corticosterone) levels was also conducted to give further insight to this topic. From the images, the Red Blue Green (RGB) colour values were calculated, and a principal components analysis (PCA) was used to create a single colour metric (the major axis) as an index of colour in the visible spectrum. A moderate stressor (toe-clipping) led to a significant change in colour (within 10 minutes) similar to that of amplecting males. Surprisingly, neither a mild stressor (handling and saline injection) nor the maximum stressor (handling and ACTH injection) led to a lightening response. This study confirms the dynamic male colour change in this species in response to medium stressors add adds new knowledge to the understanding of the functional mechanisms of dynamic colour change in amphibians.

Key Words: dynamic colour change; *Litoria wilcoxii*; breeding; hormones, stress

Introduction

Colour change is an ecologically important morphological trait in many vertebrate species, which is controlled by complex neuro-endocrine processes involving the pars intermedia of the brain (Aspengren et al., 2009; Bagnara et al., 1968; Nery and Castrucci, 1997). Darkening or lightening of skin colouration is attained via hormonal control of pigment aggregation (lightening) or dispersion (darkening) in cells called chromatophores. Chromatophores are made up of three cell types that contain different pigments, melanophores (contain melanin) , iridophores (light reflecting structures) and xanthophores [contain carotenoids] (Bagnara et al., 1968). Several hormones and other external stimulants (such as light and temperature) have been shown to trigger colour change in anuran amphibians (see review by Sköld et al., 2012); however responses vary between species (Nielsen, 1978a; Salim and Ali, 2011; Sköld et al., 2012).

The phenomenon of dynamic colour change in animals could be essential for many reasons, such as camouflage to avoid predators (Camargo et al., 1999; Stevens and Merilaita, 2009; Stuart-Fox and Moussalli, 2009; Stuart-Fox et al., 2008), thermoregulation (Norris, 1967 ; Tattersall et al., 2006) and sexual selection - to stand out and attract mates (Chen et al., 2012; Stuart-Fox and Moussalli, 2008, 2009). Although there have been numerous studies on the function of colour change in amphibians (Edgren, 1954; Kats and Vandragt, 1986; King et al., 1994; King and King, 1991; Nielsen, 1978a; Nielsen, 1978b; Nielsen, 1979, 1980; Stegen et al., 2004; Tattersall et al., 2006; Wente and Phillips, 2003), only a few of these have focussed on this in the context of rapid changes (a change within minutes) for social signalling and breeding. Dynamic sexual dichromatism (reversible colour change during breeding) has been observed in approximately 31 anuran species and due to the ephemeral nature of colour change this number may increase as species are studied in more detail (Bell and Zamudio, 2012).

The Stony Creek Frog (*Litoria wilcoxii*) is a common species of Hylidae found on rocky creek sections across eastern Australia. They are a nocturnal species, sexually dimorphic and dichromatic, males are approximately half the size of females and have yellow colouring on their bellies and sides compared females who are brown in colouration (Donnellan and Mahony, 2004; Vanderduys, 2013). They are prolonged breeders and during the breeding season males aggregate in groups and call from stream banks close to the water. Males are bright lemon yellow whilst in amplexus (mating) with a female (Vanderduys, 2013), yet the

timing and physiological mechanisms behind this dynamic change in colour are unknown (Fig 1. c). During a recent field exploration, we observed rapid colour change in adult male frogs in response to toe-clipping (an established amphibian marking technique) which is also considered as a moderate physiological stressor in amphibians (Narayan et al., 2011c). This suggested possible role of the stress endocrine system (the precursor hormone Adrenocorticotropic hormone [ACTH] and the stress hormone corticosterone) in influencing rapid colour change in this species.

Previous works on the role of stress (handling or capture stress) in colour change in amphibians conclude that it leads to a darkening of skin colouration or no change at all (Doucet and Mennill, 2010; Nielsen, 1978a). However, the interplay between stress hormones like ACTH and corticosterone is more complex and less well studied. Early works by Ide (1973) and Butman (1979) identified that ACTH injection induces melanosome dispersion in cultured frog skin cells (Butman et al., 1979; Ide, 1973). Studies in other colour changing vertebrates (fish and reptiles) show differing responses to stress hormones for example cortisol treatment led to pigment dispersal in xanthophores but pigment contraction it in melanophores hence resulting in lightening of the teleost fish [*Gillichthys mirabilis*](Sage, 1970). However, in the lizard (*Anolis carolinensis*) males with elevated corticosterone levels were darker (Greenberg, 2002; Greenberg, 2003). More research is therefore warranted to determine the role of stress and stress hormones in amphibian color change.

In this study, we identify the existence of dynamic male colour change, by comparing colouration of paired and unpaired males and provide novel data demonstrating the role stressors (handling plus toe-clipping and handling only) and stress hormones (ACTH and saline as control) have on rapid colour change in *L. wilcoxii*. We also compare baseline colour with corticosterone (a product of ACTH stimulation of the interrenal glands) to further investigate the stress-colour relationship in this species.

Materials and Methods

Field methods

Adult male *L. wilcoxii* studied in January 2011 (during the breeding season) in Numinbah Valley, South East Queensland (28.219°S, 153.232°E, 196 m altitude). Field sampling went from dusk until approximately 2300 or until all visible frogs had been captured. Immediately following capture and urine sampling (based on the protocols of Kindermann et al. 2012) frogs were placed into 50cm×30cm×20cm sterilized plastic boxes (dark so no light could get through) and photographed for a baseline (time 0) photo (Fig.1 a and b). Frogs were then subjected to four levels of experimental stressors Experiments were undertaken in the field and frogs were released back into their natural habitats immediately after the fieldwork was completed (with 5 hours of initial capture). All work was undertaken using Queensland Government Scientific Purpose Permit (WISP07106310) and Griffith University Animal Ethics Committee (AEC) permit (ENV/08/10/AEC). Amplecting pairs were photographed in the same way when they were found opportunistically.

Experimental stressors

After the first photograph was taken (time 0 min) frogs were either handled manually (n = 12 male frogs), handled and toe-clipped (n =12 male frogs), injected with ACTH (n=6) or injected with saline (n=5). Handling protocol included measurements of snout-urostyle-length (SUL, mm) and weighing frogs. Toe-clipping methods followed those in (Hero, 1989) and the number of toes clipped from each frog was one (n-2), two (n=6) and three (n=4). ACTH and saline solution was injected in the coelomic cavity (away from any vital organs), using a sterile 25-gauge needle. ACTH dose for each frog was 0.446 mg ACTH g–1 bodyweight (Sigma Chemical Co., A-0298) in a 100 µL saline vehicle (0.9% NaCl) and the dose for the saline injection was 100 µl of 0.9 % saline solution (Kindermann et al., 2012; Narayan et al., 2010a). Photographs were taken at around 5 to 10 minutes and then at 20-30 minute intervals over 100 minutes (Fig 1.a and b). Due to time constrains not all frogs were photographed at equal time periods or an equal number and analysis therefore looked at changes in each individual frog.

Photographing methodology and colour analysis

A digital camera (Canon PowerShot S5, Japan) was used to acquire RAW format images of adult male frogs over time to observe baseline colour and colour change in response. To

ensure consistency among all images the camera was held at the same distance from all frogs (30cm) with setting at sync macro and flash at full. A Munsell 24 Colour Checker Chart was placed next to each frog while it was photographed to demonstrate the similarity of exposure levels between photos and allow photographs to be calibrated if differences occurred (de Velasco and Tattersall, 2008; Tattersall et al., 2006). Photographing time (as well as the time frogs were exposed to light) was less than 30 seconds to ensure that light did not affect colour change (Stegen et al., 2004). Digital image analysis rather than spectrometry was used as it is less invasive and therefore less likely to course stress-induced skin colour changes (Greenberg, 2002). Frog photographs were at first corrected to account for differences in lighting using Adobe Photoshop C35 Extended (AdobeSystems, 2010). A rectangle was then cut from the frog's eyes to the back legs using the crop tool, such that only the dorsal body surface of the frog was analysed. Any pure white pixels (that were a result of flash reflection) were removed using the magic wand tool. The average RGB value was then calculated for each photograph (Ohta et al., 2008). Although RGB colour is designed for the human visual system and may not explain the colour amphibians (or predators) see (Endler, 1990) it allows us to measure differences in colour which is all that is required for this purpose (Touchon and Warkentin, 2008).

Because RGB values are in a sense 3-dimensional and not strictly independent of each other we used principal components analysis (PCA) to reduce the variables to one dimension that could explain the colour change (Vásquez and Pfennig, 2007; Wang and Shaffer, 2008). The eigenvalues for each axis were 2.4, 0.5 and 0.04, accounting for 80%, 18% and 1.4% of the variance respectively. Factors with eigenvalues less than 1 have less explanatory power than the original variables (on average) and there is no statistical reason to retain them (Kaiser, 1960)This analysis provides a single factor (or axis), which describes the changes in colour (from dark brown to bright yellow), and the variance not accounted by that factor is considerably less than that the average variance in each of the three original variables. To convert the RGB values for each data point to scores on the major axis, the standardised R, G and B values are multiplied by the factor loadings. The loadings for factor (or axis 1) are: for R 0.392472, for G 0.399892 and for B 0.318050. These steps are all calculated automatically in R with the psych package (Revelle, 2011). Factor scores are rescaled to recover the differences in variance between the factors; these values (which range from -3 to 3) become the best single (and sufficient) measure of colour (Kaiser, 1960).

Corticosterone enzyme immunoassays

Urinary corticosterone enzyme-immunoassay was performed as described in Narayan et al. (2010ab, 2011abc, 2012) and recently established for adult male *L. wilcoxii* (Kindermann et al., 2012). The same assay was used to measure the concentration of urinary corticosterone metabolites (hereafter referred to as urinary corticosterone) in adult male *L. wilcoxii* in this study. Urine steroid conjugates were standardized to creatinine (Cr) levels to control for water content based on the on previous studies(Kindermann et al., 2012; Narayan et al., 2010a; Narayan et al., 2011a; Narayan et al., 2010b; Narayan et al., 2011b, 2012; Narayan et al., 2011c)

(Narayan et al., 2010a; Narayan et al., 2011a; Narayan et al., 2010b; Narayan et al., 2011b, 2012; Narayan et al., 2011c) and inter-assay coefficients of variation (CV) were determined from high-(~70%) and low-(~30%) binding internal controls run on all assays. Intra-assay CV were 6.3 % and 2.5 % for low-and high-percentage-bound controls, and inter-assay CV were 15.0 % and 13.2 % for low-and high-percentage-bound controls respectively. The assay sensitivity was calculated as the value 2 standard deviations from the mean response of the blank samples (zero binding) and was 2.68 + 0.70 pg/well (n = 8).

Statistical analysis

A t-test was used to compare the difference between colour between baseline frogs and frogs found in amplexus (p-values were considered significant at = 0.05). Linear regression was used to test the relationship between colour values and baseline corticosterone levels, (p-values were considered significant at α=0.05). To compare the effect of experimental stressors (ACTH, saline, handling and toe-clipping) on colour change, linear mixed effect models (to account for unequal variances, non-independence through time, and unequal repetitions at each time point) were used. Time (levels ranged from 4 to 203 min) was classed as a fixed affect and the individual frog as a random effect. All data were analysed using R (team, 2011) and the R packages lme4 (Bates et al., 2010) and languageR (Baayen, 2009). P-values were based on Markov-chain Monte Carlo sampling (MCMC) using the languageR package as the lme4 package does not give degrees of freedom or p values (Bates et al., 2010). MCMC-estimated p-values were considered significant at α=0.05. Results are presented as mean (± SE).

Results

The mean baseline colour of male frogs (unpaired) was -0.45 (± 0.09) and the mean colour of male frogs in amplexus were + 2.45 (± 0.16). There was a significant difference in colour between unpaired and amplexing males (Fig. 2; $n = 51$, $t = 17.89$, $p<0.001$).

The relationship between baseline colour and baseline urinary corticosterone Baseline colour values for individual male frogs were highly variable ranging from -2.2 to +3.13 and baseline urinary corticosterone ranged from 11.0 pg/ µg Cr to 36.0 pg/ µg Cr. There was no significant relationship between baseline colour and urinary corticosterone (Fig. 3; $n= 39$, $R= 0.01$, $p=0.52$).

There was a variation of baseline colours in all frogs colour values ranged from -1.53 to 1.08 (Fig. 4). For toe-clipped frogs colour score values increased considerably within 10 minutes up until 20 minutes and there were significant differences in colour scores between time 0 and all time periods up to 23 minutes (Fig. 4 top right, $p < 0.05$ for each comparison between times) however no significant differences between time 0 and 50-90 min ($p > 0.05$ for all time comparisons). There was no significant change in colour between time 0 and all time periods after ACTH and saline injection and handling only (Fig. 4, $p >0.05$), however some individual increases and decreases in colour scores can be observed graphically.

Discussion

We have provided novel data demonstrating rapid colour change in an amphibian and given more detailed analysis on the nature of sexual dichromatism in *L.wilcoxii*. Amplexing males were significantly more yellow in comparison to unpaired males; this indicates that sexual selection may regulate colour change. Most recently studied in the chameleon (Stuart-Fox and Moussalli, 2008)and amphibians *Bufo luetkenii* (Doucet and Mennill, 2010)and *Rana arvalis* (Ries et al., 2008; Sheldon et al., 2003)the influence of sexual selection on colour change is a fundamental evolutionary trait. The colour change observed *L. wilcoxii* provides an additional example examine the proximate and ultimate basis of colour change.

We have given evidence that this colour change is very rapid and to our knowledge *L. wilcoxii* is one of few (if any) known amphibian species in the world that can undergo such a fast change for breeding. Other species which change colour for display do so at a slower rate, over several hours and months rather than minutes (Doucet and Mennill, 2010; Greenberg, 1942; Ries et al., 2008; Sheldon et al., 2003). In a recent review by Sköld 2012, it was stated amphibians (and reptiles) tend to change colour over an hour or more as colour change is regulated by hormones rather than neuro-hormones as in fish [which can change colour within seconds] (Fujii, 2000; Sköld et al., 2012), our study contradicts this idea which leads to the suggestion of the possible role of neuro-hormones. Toe-clipping is a short term stressor (Narayan et al., 2011c) and could potentially cause activation of neuro-endocrine hormones such as catecholamines (epinephrine and nor-epinephrine).

A study by Doucet and Mennill (2010) showed opposing results to our study as male toads *B. luetkenii* faded from yellow to brown once in amplexus. The authors speculated that rapid darkening indicates the cost of expressing bright colour during breeding. This was supported by results showing colour also faded rapidly after captivity (Doucet and Mennill, 2010). *L. wilcoxii* stay bright yellow during amplexus and turn yellow after medium stressors, this result is surprising as one would expect yellow to be a costly colour, allowing animals to be detected more easily by predators. In an earlier study by Nielson (1978) between species differences were found in response to stressors. When exposed to a stressor (being pressed into a box) *Hyla cinerea* darkened in colour whereas *Hyla arborea* showed no change. Both species however went lighter in colour when injected with epinephrine (Nielsen, 1978a). These differing displays support the theory that hormonal responses as well as evolutionary reasons for colour change vary among species. It can be hypothesised that the darkening

response in *H. cinerea* and *B. luetkenii* may be due to the release of ACTH as ACTH injection induced melanosome dispersion in cultured frog skin cells (Butman et al., 1979; Ide, 1973) however more work is needed to confirm this. The injection dose of ACTH caused a stress response in the frogs in this study (see Kindermann et al. 2012) and therefore it would be expected that if ACTH was the trigger for colour change a colour response would have been observed, however more detailed analysis is needed to confirm this.

The complex relationship between different levels of stress and colour change has been studied extensively in the lizard (*Anolis carolinensis*). This species has is a reliable indicator of the stress response, whereby dominant green (lighter) males had lower stress levels then subordinate brown(dark) males (Greenberg, 2002; Greenberg, 2003; Greenberg and Crews, 1990). They also found out that short-term stressor (physical restraint) resulted in a reduced aggregation of MSH and increased release of epinephrine hence resulting an increase in brightness (in this case green colouration), whereas a chronic stressor (social subordination) increased MSH and therefore pigment expansion (darkening) leading to a colour change from green to brown (Greenberg, 2002). Therefore the role of stressors and stress hormones in *L. wilcoxii* cannot be disregarded completely, as different levels of stress result in the release of different hormones. The fact that toe-clipping a frog induced the same colouration as a frog in amplexus poses several questions. Are both responses under the same hormonal control or is this colour change under control of multiple hormones? Although the hormones released by toe-clipping stress may turn the frog yellow, the hormones released for amplexus may be completely different.

Further research is needed to examine this in more detail and determine whether this striking colour change is the product of sexual selection or just an accidental response to hormonal stimulation in response to mating activity (e.g. increased sperm activity during amplexus, induced by increases in testosterone levels). Along with behavioural and chemical cues (signalling, male competition, and female choice), more information is needed the influence of reproductive hormones to obtain a better picture of this colour system. Female and non-breeding male *Acris gryllus* that were treated with testosterone pellets developed breeding male colouration within one week of treatment (Greenberg, 1942)and juvenile *Hyperolius argus* administered with testosterone and estradiol assumed male or female colour patterns within 5-34 days (Richards, 1982). Although these colour changes occurred at a much slower rate than the rapid responses observed in *L.wilcoxii*, they may still be part of a multiple hormone system.

Conclusion

In summary, this study has provided new evidence of rapid and reversible colour change in an anuran amphibian. Our results expand on current knowledge of dynamic sexual dichromatism in amphibians, and add information to the complex interaction between stressors, stress hormones and colour change.

Acknowledgements

We acknowledge financial support from the Environmental Futures Centre and the School of Environment at Griffith University, Australia. Queensland Government Scientific Purpose Permit (WISP07106310) and Griffith University Animal Ethics Committee (AEC) permit (ENV/08/10/AEC) were obtained for this study. We thank the volunteers who assisted with the field work.

Figures:

Figure 1: Photographs of brown unpaired male (a), a yellow male 7 min after toe-clipping (b) and a pair in amplexus (c).

Figure 2: Colour values of adult male *Litoria wilcoxii* found in amplexus compared to unpaired frogs. There was a significant difference between groups ($n=51$, $t = 17.89$, $p<0.001$). Each dot represents an individual frog at first capture.

Figure 3: Scatter plot showing baseline corticosterone (pg/µg Cr) versus baseline colour in *Litoria wilcoxii*. The association between them was not significant ($n= 39$, $R= 0.01$, $p=0.52$). Each point on the graph represents an individual frog at first capture (baseline photo).

Figure 4: Colour change response to saline injections (n=6), ACTH injection (n=6), toe-clipping (n=12) and handling only (n=12) showing individual changes in colour scores through time (min), each line represents in individual frog and each point represents the time it was photographed.

References

AdobeSystems, 2010. Adobe Photoshop Extended. Adobe Systems Incorporated, San Jose, California, United States.

Aspengren, S., Skold, H.N., Wallin, M., 2009. Different strategies for color change. Cellular and Molecular Life Sciences 66, 187-191.

Baayen, R.H., 2009. LanguageR: Data sets and functions with "Analyzing Linguistic Data: A practical introduction to statistics". R package version 0.955.

Bagnara, J.T., Taylor, J.D., Hadley, M.E., 1968. Dermal chromatophore unit. Journal of Cell Biology 38, 67-69.

Bates, D., Maechler, M., Bolker, B., 2010. lme4: Linear mixed-effects models using S4 classes. R package version 0.999375-33.

Bell, R.C., Zamudio, K.R., 2012. Sexual dichromatism in frogs: natural selection, sexual selection and unexpected diversity. Proceedings of the Royal Society B: Biological Sciences.

Butman, B.T., Obika, M., Tchen, T.T., Taylor, J.D., 1979. Hormone-induced pigment translocations in amphibian dermal iridophores, in vitro: Changes in cell shape. Journal of Experimental Zoology 208, 17-33.

Camargo, C.R., Visconti, M.A., Castrucci, A.M.L., 1999. Physiological color change in the bullfrog, *Rana catesbeiana*. Journal of Experimental Zoology 283, 160-169.

Chen, I., Stuart-Fox, D., Hugall, A.F., Symonds, M.R.E., 2012. Sexual selection and the evolution of complex colour patterns in dragon lizards. Evolution.

de Velasco, J.B., Tattersall, G.J., 2008. The influence of hypoxia on the thermal sensitivity of skin colouration in the bearded dragon, *Pogona vitticeps*. Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology 178, 867-875.

Donnellan, S.C., Mahony, M.J., 2004. Allozyme, chromosomal and morphological variability in the *Litoria lesueuri* species group (Anura:Hylidae), including a description of a new species. Australian Journal of Zoology 52.

Doucet, S.M., Mennill, D.J., 2010. Dynamic sexual dichromatism in an explosively breeding Neotropical toad. Biology Letters 6, 63-66.

Edgren, R.A., 1954. Factors Controlling Color Change in the Tree Frog, *Hyla versicolor* Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine (New York, NY). Royal Society of Medicine, 20-23.

Endler, J.A., 1990. On the measurement and classification of color in studies of animal colour patterns Biological Journal of the Linnean Society 41, 315-352.

Fujii, R., 2000. The regulation of motile activity in fish chromatophores. Pigment Cell Research 13, 300-319.

Greenberg, B., 1942. Some effects of testosterone on the sexual pigmentation and other sex characters of the cricket frog (*Acris gryllus*). Journal of Experimental Zoology 91, 435-451.

Greenberg, N., 2002. Ethological aspects of stress in a model lizard, *Anolis carolinensis*. Integrative and Comparative Biology 42, 526-540.

Greenberg, N., 2003. Sociality, stress, and the corpus striatum of the green anolis lizard. Physiology & Behavior 79, 429-440.

Greenberg, N., Crews, D., 1990. Endocrine and behavioral responses to aggression and social dominance in the green anole lizard, *Anolis carolinensis*. General and Comparative Endocrinology 77, 246-255.

Hero, J.M., 1989. A simple code for toe clipping anurans. Herpetological Review 20, 66–67

Ide, H., 1973. Effects of ACTH on melanophores and iridophores isolated from bullfrog tadpoles. General and Comparative Endocrinology 21, 390-397.

Kaiser, H.F., 1960. The application of electronic computers to factor analysis. Educational and Psychological Measurement 20, 141–151.

Kats, L.B., Vandragt, R.G., 1986. Background colour matching in the spring peeper, *Hyla crucifer*. Copeia, 109-115.
Kindermann, C., Narayan, E.J., Hero, J.M., 2012. Urinary corticosterone metabolites and chytridiomycosis disease prevalence in a free-living population of male Stony Creek frogs (*Litoria wilcoxii*). Comparative Biochemistry and Physiology-Part A: Molecular & Integrative Physiology.
King, R.B., Hauff, S., Phillips, J.B., 1994. Physiological colour change on the green treefrog-responses to background brightness and temperiture. Copeia, 422-432.
King, R.B., King, B., 1991. Sexual differences in color and color change in wood frogs. Canadian Journal of Zoology 69, 1963-1968.
Narayan, E., Molinia, F., Christi, K., Morley, C., Cockrem, J., 2010a. Urinary corticosterone metabolite responses to capture, and annual patterns of urinary corticosterone in wild and captive endangered Fijian ground frogs (*Platymantis vitiana*). Australian Journal of Zoology 58, 189-197.
Narayan, E.J., Cockrem, J.F., Hero, J.M., 2011a. Urinary corticosterone metabolite responses to capture and captivity in the cane toad (*Rhinella marina*). General and Comparative Endocrinology 173, 371-377.
Narayan, E.J., Molinia, F.C., Christi, K.S., Morley, C.G., Cockrem, J.F., 2010b. Annual cycles of urinary reproductive steroid concentrations in wild and captive endangered Fijian ground frogs (*Platymantis vitiana*). General and Comparative Endocrinology 166, 172-179.
Narayan, E.J., Molinia, F.C., Cockrem, J.F., Hero, J.M., 2011b. Individual variation and repeatability in urinary corticosterone metabolite responses to capture in the cane toad (*Rhinella marina*). General and Comparative Endocrinology.
Narayan, E.J., Molinia, F.C., Cockrem, J.F., Hero, J.M., 2012. Changes in urinary testosterone and corticosterone metabolites during short-term confinement with repeated handling in wild male cane toads (*Rhinella marina*). Australian Journal of Zoology 59, 264-269.
Narayan, E.J., Molinia, F.C., Kindermann, C., Cockrem, J.F., Hero, J.M., 2011c. Urinary corticosterone responses to capture and toe-clipping in the cane toad (*Rhinella marina*) indicate that toe-clipping is a stressor for amphibians. General and Comparative Endocrinology 174, 238-245.
Nery, L.E.M., Castrucci, A.M.D., 1997. Pigment cell signalling for physiological color change. Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology 118, 1135-1144.
Nielsen, H.I., 1978a. The effect of stress and adrenaline on the color of *Hyla cinerea* and *Hyla arborea*. General and Comparative Endocrinology 36, 543-552.
Nielsen, H.I., 1978b. Ultrastructural changes in the dermal chromatophore unit of *Hyla arborea* during color change. Cell and Tissue Research 194, 405-418.
Nielsen, H.I., 1979. Chromatophores and adaptation to coloured backgrounds in two colour types of the edible frog, *Rana esculenta*. Journal of Zoology 189, 349-358.
Nielsen, H.I., 1980. Color and color adaptation of the European tree frog, *Hyla arborea*. Journal of Experimental Zoology 211, 143-151.
Norris, K.S., 1967 Color Adaptation in Desert Reptiles and its Thermal Relationships, in: W.W. Milstead (Ed.), Lizard Ecology - a Symposium. University of Missouri Press, Columbia, Missouri,USA, 162-229.
Ohta, K., Hirano, M., Mine, T., Mizutani, H., Yamaguchi, A., Matsuyama, M., 2008. Body color change and serum steroid hormone levels throughout the process of sex change in the adult wrasse, *Pseudolabrus sieboldi*. Marine Biology 153, 843-852.
Revelle, W., 2011. Procedures for Psychological, Psychometric, and Personality Research, R package version 1.2.8. North-western University, Evanston. .
Richards, C.M., 1982. The alteration of chromatophore expression by sex hormones in the kenyan reed frog, *Hyperolius viridiflavus*. General and Comparative Endocrinology 46, 59-67.
Ries, C., Spaethe, J., Sztatecsny, M., Strondl, C., Hödl, W., 2008. Turning blue and ultraviolet: sex-specific colour change during the mating season in the Balkan moor frog. Journal of Zoology 276, 229-236.

Sage, M., 1970. Control of prolactin release and its role in color change in the teleost *Gillichthys mirabilis*. Journal of Experimental Zoology 173, 121-127.
Salim, S., Ali, S.A., 2011. Vertebrate melanophores as potential model for drug discovery and development: A review. Cellular & Molecular Biology Letters 16, 162-200.
Sheldon, B.C., Arponen, H., Laurila, A., Crochet, P.A., Merilä, J., 2003. Sire coloration influences offspring survival under predation risk in the moorfrog. Evolutionary biology 16, 1288-1295.
Sköld, H.N., Aspengren, S., Wallin, M., 2012. Rapid color change in fish and amphibians–function, regulation, and emerging applications. Pigment Cell & Melanoma Research.
Stegen, J.C., Gienger, C.M., Sun, L.X., 2004. The control of color change in the Pacific tree frog, *Hyla regilla*. Canadian Journal of Zoology-Revue Canadienne De Zoologie 82, 889-896.
Stevens, M., Merilaita, S., 2009. Animal camouflage: current issues and new perspectives. Philosophical Transactions of the Royal Society B: Biological Sciences 364, 423-427.
Stuart-Fox, D., Moussalli, A., 2008. Selection for Social Signalling Drives the Evolution of Chameleon Colour Change. PLoS Biol 6, e25.
Stuart-Fox, D., Moussalli, A., 2009. Camouflage, communication and thermoregulation: lessons from colour changing organisms. Philosophical Transactions of the Royal Society B: Biological Sciences 364, 463-470.
Stuart-Fox, D., Moussalli, A., Whiting, M.J., 2008. Predator-specific camouflage in chameleons. Biology Letters 4, 326-329.
Tattersall, G.J., Eterovick, P.C., de Andrade, D.V., 2006. Tribute to R. G. Boutilier: Skin colour and body temperature changes in basking *Bokermannohyla alvarengai* (Bokermann 1956). The Journal of Experimental Biology 209, 1185-1196.
team, R.d.c., 2011. R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria.
Touchon, J.C., Warkentin, K.M., 2008. Fish and dragonfly nymph predators induce opposite shifts in color and morphology of tadpoles. Oikos 117, 634-640.
Vanderduys, E., 2013. Field Guide to the Frogs of Queensland. CSIRO Publishing.
Vásquez, T., Pfennig, K., 2007. Looking on the bright side: females prefer coloration indicative of male size and condition in the sexually dichromatic spadefoot toad,*Scaphiopus couchii*. Behavioral Ecology and Sociobiology 62, 127-135.
Wang, I.J., Shaffer, H.B., 2008. Rapid color evolution in an aposematic species: a phylogenetic analysis of color variation in the strikingly polymorphic strewberry poison-dart frog. Evolution 62, 2742-2759.
Wente, W.H., Phillips, J.B., 2003. Fixed Green and Brown Color Morphs and a Novel Color Changing Morph of the Pacific Tree Frog *Hyla regilla*. The American Naturalist 162, 461-473.

FIGURE 1

FIGURE 2

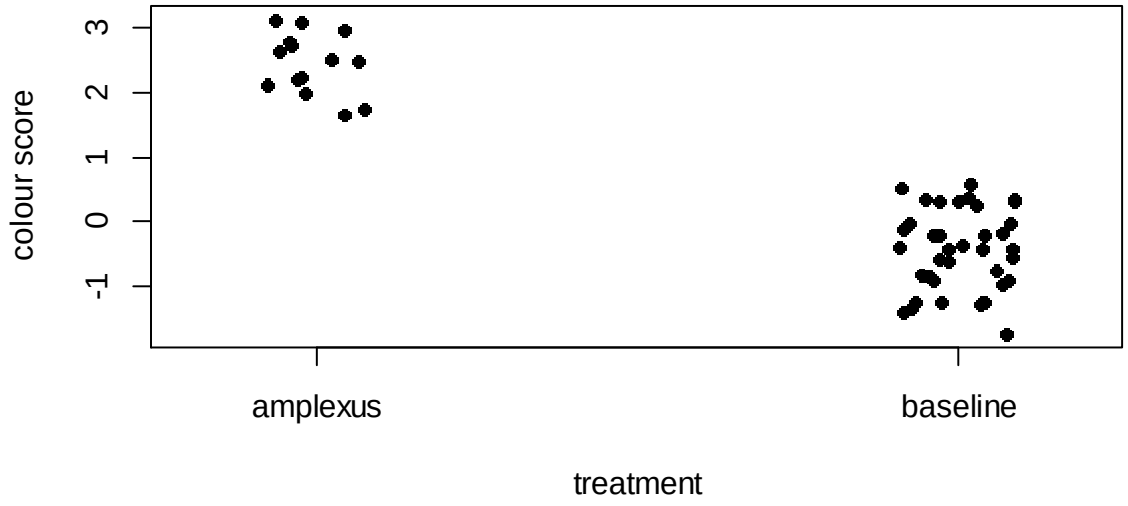

FIGURE 3

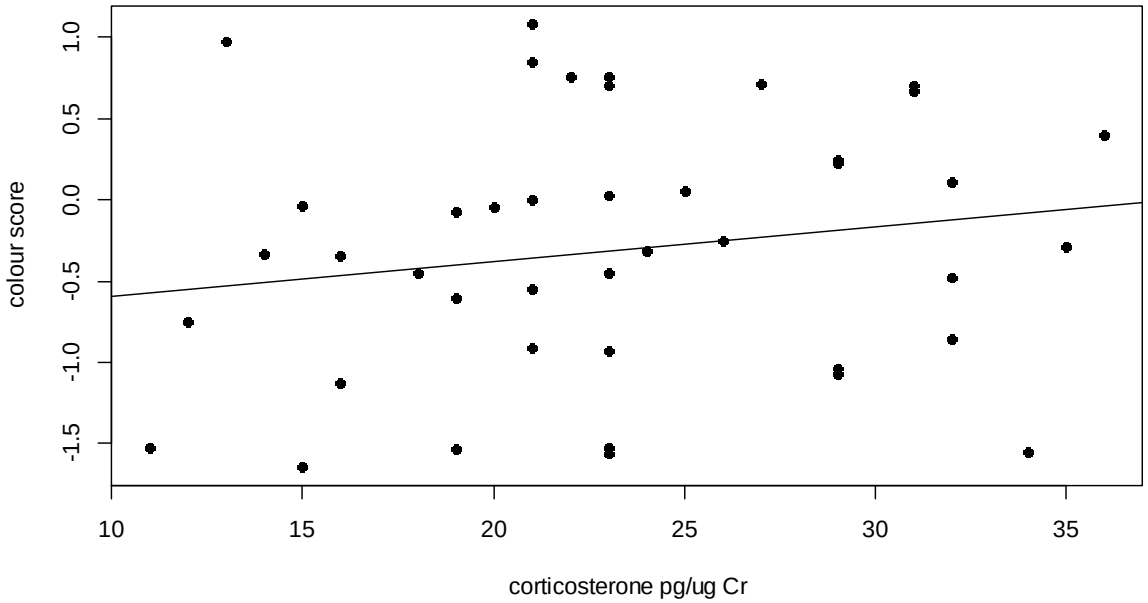

FIGURE 4