JZAR JOURNAL OF ZOO AND AQUARIUM RESEARCH
www.jzar.org

Research article

Keeping the golden mantella golden: The effect of dietary carotenoid supplementation and UV provision on the colouration and growth of *Mantella aurantiaca*

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Keywords: amphibian, carotenoid, colour, ex situ, husbandry, mantella, UVB

Article history:
Received: 13 Jan 2021
Accepted: 09 Mar 2022
Published online: 30 Apr 2022

Abstract

One of the limitations affecting the success of captive breeding programmes for amphibians is a lack of knowledge relating to the diet of wild animals. Carotenoids are known to be an important dietary component for the health and proper development of colouration in many vertebrates, but information relating to amphibians is limited. We investigated the influence of carotenoid supplementation in the Critically Endangered golden mantella *Mantella aurantiaca*. In a fully factorial design, 60 juvenile M. aurantiaca were provided with two dietary carotenoid treatments, standard and enhanced, and two ultraviolet B (UVB) treatments, no UVB and low-level UVB. There was a significant interaction of the treatments on the skin colouration of the study animals. The provision of both an enhanced carotenoid diet and UVB light resulted in the frogs being more red coloured, however when provided in combination the increase in redness was lower than when the treatments were provided alone. In the second part of the study, 64 juvenile M. aurantiaca were reared on one of two diets containing the same concentration of carotenoids, but different profiles: a 'red' diet and a 'yellow' diet. These treatments also had a significant effect on colouration, with those receiving the red diet becoming a more intense red colour. Our results demonstrate that the bright colouration of *M. aurantiaca* is influenced by both the concentration and profile of carotenoids in the diet, in addition to the presence of low-level UVB exposure. None of the treatments had any reported effect on the growth of the study animals, however further work should investigate other physiological responses not investigated here.

Introduction

Amphibians are globally threatened (IUCN 2020) and captive breeding programs (CBPs) are integral to their conservation (Wren et al. 2015). Amphibians are underrepresented in CBPs (Conde et al. 2011; Dawson et al. 2016) and their complex husbandry requirements have limited success in some cases (Pessier et al. 2014). Diet provision in captivity is one important limitation (Ferrie et al. 2014), due to a combination of reduced prey diversity and limited prey nutritional value compared with that available in the wild (Augustine et al. 2016; Clugston and Blaner 2014; Finke 2002; Jayson et al. 2018; Livingston et al. 2014; Nicholson et al. 2017).

Carotenoids are a large group of organic pigments consisting of xanthophylls and carotenes, which confer the red, orange and yellow colouration of a broad range of plants and animals (Fraser and Bramley 2004). Carotenoids have numerous roles in biological processes and may protect against disease in model mammal species (Chew and Park 2004; Stahl and Sies 2003, 2005). With the exception of a few invertebrate species, only plants and fungi are able to produce carotenoids de novo, with the majority of animals being completely dependent on dietary sources (Fraser and Bramley 2004). Captive amphibian diets are often deficient in carotenoids (Finke 2002; Ogilvy et al. 2012a), leading to relatively dull colouration and reduced carotenoid diversity in the skin compared with wild animals

(Matsui et al. 2002), which may have direct fitness consequences (Ogilvy et al. 2012b). Natural colouration may be recovered in captive animals through supplementation with a diverse range of carotenoids (Brenes-Soto and Dierenfeld 2014; Ogilvy et al. 2012b; Umbers et al. 2016).

Studies investigating health benefits of carotenoids in amphibians have so far been limited. A small but growing number of studies have demonstrated positive benefits of carotenoid supplementation, including increased or improved growth rates, reproductive success, vitamin A levels and immune response (Brenes-Soto and Dierenfeld 2014; Dugas et al. 2013; Ogilvy and Preziosi 2012; Ogilvy et al. 2012b; Szuroczki et al. 2016, 2019). However, others have shown carotenoids to have no effect on any measures recorded (Byrne and Silla 2017; McInerney et al. 2019), and in some cases have even shown possible detrimental effects on growth and survivorship (Cothran et al. 2015; Keogh et al. 2018). Currently only a small number of carotenoids have been investigated in a limited number of amphibian species, and in many cases model taxa have not been those with the most striking colouration (e.g. *Hyla versicolor, Lithobates sylvatica, Ranoidea booroolongensis, Xenopus tropicalis*). Given the broad diversity of amphibians it is likely that species will have different requirements for carotenoids, both in concentration and profile (Byrne and Silla 2017; McInerney et al. 2019).

In addition to carotenoid provision, ultraviolet B (UVB) radiation is an important component of amphibian husbandry. Although understudied compared to other vertebrate taxa, exposure to UVB has been shown to have numerous health benefits in a range of amphibian species, through the synthesis of vitamin D_3 and associated calcium metabolism (Antwis and Browne 2009). Observed benefits include increased growth rates, improved skeletal development and reductions in the incidence of metabolic bone disease, a common disorder seen in captive amphibian populations (Michaels et al. 2015; Tapley et al. 2015; Verschooren et al. 2011). Despite these benefits, UVB exposure can also have deleterious effects, including increased embryo or larvae mortality rates, decreased growth rates and deformities (Blaustein et al. 2003). It is well documented in mammals that some carotenoids when deposited in the dermis can prevent or limit the damage caused by UVB radiation through the mitigation of free radicals (Heinrich et al. 2003; Lee et al. 2000; Stahl and Sies 2002, 2005; Sies and Stahl 2004). The only study to date investigating this in amphibians found no effect, despite other benefits, although positive effects of UVB exposure were only found in larvae (Ogilvy and Preziosi 2012).

Here we investigate the roles that dietary carotenoids have on the golden mantella *Mantella aurantiaca*, a Critically Endangered species (Vences and Raxworthy 2008) found relatively commonly in captivity. Golden mantella exhibit yellow-orange through to red colouration in the wild, but can exhibit a comparatively dull appearance in captivity (Passos et al. 2020). Our aim was to investigate the species' requirements for carotenoid supplementation, focusing on the roles that different concentrations and profiles of carotenoids have on the colouration and growth of *M. aurantiaca*, in addition to interactions with UVB radiation.

Methods

To determine the influence of dietary carotenoids on the growth and colouration of *Mantella aurantiaca*, two studies were conducted. The first combined the provision of different concentrations of dietary carotenoids with UVB exposure (from now on referred to as the UV-carotenoid study). The second looked at the profile of carotenoids in the diet (carotenoid profile study).

Ethics statement

These studies were approved by the University of Manchester Ethics Committee prior to commencement. A Home Office License under the Animals (Scientific Procedures) Act 1986 was not required as all variables fell within normal husbandry practices for captive amphibians. All animals were regularly monitored for any negative signs, of which none were observed.

Study animals and basic husbandry for both studies

Studies were conducted at the University of Manchester, UK, during 2014–2015. All individuals were captive-bred from existing individuals at the university. Experimental animals were housed in 30 × 30 × 45 cm vivaria (Exo Terra, UK), with naturalistic set ups consisting of a soil and coir substrate and furnished with moss, oak leaves, small branches and ferns. All tanks were set up with identical furnishings and were maintained over the course of the studies to ensure the layouts remained consistent. Vivaria were located in a climate-controlled unit maintained at 24°C during the day and 18°C at night on a 12:12 photo- and thermo-period. All vivaria were lit with standard 'daylight' lamps (30 W T8 'warm white' 36" fluorescent tubes, GE Lighting, Hungary). Relative humidity was maintained at around 80% through regular misting of enclosures.

UV-carotenoid study

In the first study, two dietary and two UVB treatments were provided to 60 *M. aurantiaca* in a fully factorial design. Animals originated from a single clutch, which prior to the start of the study has been reared on a spirulina and fish flake mixture as tadpoles, and hatchling black crickets *Gryllus bimaculatus* gut-loaded with fresh vegetables as new metamorphs. During this time all animals were group-housed within a single enclosure. At four weeks post metamorphosis, froglets were randomly allocated to one of four treatment groups. Study animals were housed in groups of five for the duration of the study, with three tanks per treatment. The study ran for a duration of 12 months.

The two diets consisted of a 'standard' carotenoid diet and an 'enhanced' carotenoid diet, which were fed to hatchling *G. bimaculatus* that were then consumed by froglets. The standard diet contained red bell pepper, carrot and savoy cabbage in a ratio of 1:1:1 by weight (Ogilvy et al. 2012a). The enhanced diet had the same ingredients as the standard diet plus an extra part of a commercially available broad-spectrum carotenoid supplement aimed at the herpetological market (Superpig, Repashy Ventures, USA). This equated to an additional ~1.7 mg/g of carotenoids in the enhanced diet. The diets were provided to hatchling *G. bimaculatus* (Ogilvy et al. 2012a) for a minimum of 24 hours before they were fed to the study animals. To prevent preferential feeding, the ingredients for both diets were blended to produce more uniform mixtures and were proportioned into approximate 5 g blocks to allow consistent presentation to crickets. Two batches of each diet were made throughout the study and stored at −20°C until required. Prior to feeding to the froglets, the crickets were lightly dusted with a calcium and vitamin supplement (Nutrobal, Vetark Professional, UK; see Supplementary Information for composition). Feeding occurred three times a week.

The two UVB treatments consisted of no UVB (-UV) and low-level UVB provision (+UV). In addition to daylight lamps (see ' Study animals and basic husbandry for both studies'), all experimental tanks were lit with UVB-emitting bulbs (Reptisun 10.0, ZooMed, USA). To create the -UV treatment, the bulbs above half of the groups were covered with a transparent UV blocking filter (Mylar, DuPont Teijin Films, USA). Bulbs were changed halfway through the study (month 6) due to the reduction in UVB strength over time. The UV index in the tanks was measured every three months over the course of the study (Digital UV Index Radiometer, ZooMed,

USA). For each tank, three recordings were taken at different locations (front, middle and rear) at a height of 6–10 cm above the substrate surface, corresponding to the heights of the low to mid-level furnishings used by the frogs. Over the duration of the study the UV index in the +UV tanks was between 0.5 and 1.0, and 0.0 and 0.1 for the -UV tanks (see Supplementary Table S1 for full UVI readings). Due to the shelters provided within the enclosures all animals in the +UV treatment had access to areas of 0.0.

Carotenoid profile study

For the second study, two diets containing different profiles of carotenoids were provided to crickets fed to 64 *M. aurantiaca*. Individuals originated from two clutches laid on the same day. When froglets were approximately 10 days post-metamorphosis they were randomly allocated to one of the two treatment groups. Each treatment group contained equal numbers of animals from both clutches. Froglets were housed in groups of four, with eight tanks per treatment. The study ran for a duration of five months. The two cricket diets consisted of a 'yellow' carotenoid diet and a 'red' carotenoid diet. For both diets the total concentration of carotenoids was 5 mg/g. The red diet contained lycopene, astaxanthin and beta-carotene in a ratio of 6.5:2.5:1. The yellow diet contained lutein, zeaxanthin and beta-carotene in a ratio of 6.5:2.5:1. The small amount of beta-carotene (corresponding to 0.5 mg/g) was included in both diets due to the potential health benefits of carotenoids acting as a source of vitamin A; none of the main carotenoids of focus had provitamin A activity. All carotenoids were manufactured by Swanson Health Products (Canada). The carotenoids were incorporated into a basal diet (Table 1) naturally low in carotenoids. Batches of each diet were made at the start of the study and frozen at −20°C until required. Frogs were fed hatchling black crickets, *G. bimaculatus* (Ogilvy et al. 2012a), which had been gut-loaded on one of the diets for at least 24 hours prior to feeding. Crickets were lightly dusted with a calcium and vitamin supplement (Nutrobal, Vetark Professional, UK) before feeding. Feeding occurred three times a week. No UVB was provided to animals during the experiment, ensuring any changes in colour were solely due to the diets.

Data collection

Over the course of both studies the colouration of the frogs was measured, along with the mass and snout-to-vent length (SVL). For the five months of the carotenoid profile study, measurements were taken every c. 30 days, and for the 12 months of the UV-carotenoid study every c. 60 days. At each time point frogs were weighed and photographed three times on grid-lined paper next to an orange colour standard (ColorChecker Classic, X-Rite, USA). Photographs were taken under consistent parameters (the same camera, lighting and colour standard), so standardisation of measurements was not necessary (Ogilvy et al. 2012b). Using ImageJ (Schneider et al. 2012), photographs were analysed to measure SVL and dorsum colouration; the values for which were averaged across the three images. The colour of the frogs was measured in terms of 'redness', as described in Ogilvy et al. (2012b). We have previously demonstrated that these methods have high repeatability (Michaels et al. 2015; Ogilvy et al. 2012b).

Table 1. Composition of the basal diet used in the carotenoid profile study. [1]The Co-operative Group Ltd, UK, [2]NBTY Europe Ltd, UK, [3]Vetark Professional, UK

Ingredient	Content (% by volume)
Wheat flour[1]	30
Soy flour[2]	30
White potato[1]	20
Sunflower oil[1]	15
Nutrobal[3]	5

Data analysis

All statistical analysis was conducted in RStudio (Version 1.1.463). The data from both studies were analysed with linear mixed models (LMM) and Wald chi squared tests, using the lme4 and car R packages, respectively (Bates et al. 2015; Fox and Weisberg 2019). Normality of residuals was examined using the plot command; all residuals appeared to be normally distributed.

For the UV-carotenoid study, separate LMMs were used for colouration, mass and SVL. Each model included diet and UV treatment as fixed effects, with tank and time as random effects. The interaction between diet and UV treatment was also included. As individual frogs could not be identified within each tank of five, it was not possible to track specific individuals over the course of the study. Therefore, each individual was tracked by its tank number, which was included in the model.

Similar analyses were used for the carotenoid profile study. Linear mixed models were run for colouration, mass and SVL, with diet as a fixed effect, and time and tank nested within clutch as random effects.

Results

UV-carotenoid study

The treatments had a significant effect on the colouration of the frogs in the UV-carotenoid study, with a significant interaction between diet and UV treatment (Wald χ^2=4.04, P=0.044). Frogs receiving the enhanced diet were redder in colour than those on the standard diet, as were frogs in the +UV groups compared with those in the -UV group (Figure 1). Both diet and UV treatment had a similar impact on the increase in redness; when compared to the palest group, standard/-UV (redness value of 1.008 at month 12 for example), the provision of enhanced diet alone or +UV alone led to a similar increase in redness (1.049 and 1.058 respectively). However, the effects were not additive; the provision of both the enhanced diet and +UV together led to only slightly redder individuals (1.069 at month 12) than those receiving an enhanced diet or +UV alone.

At the start of the study, colouration changed rapidly for all groups until month 4 when it began to stabilise, reflecting the change from the brown colouration of metamorphosis, to the orange colouration seen in adults. From this point onwards the standard diet/-UV treatment group was consistently the palest (Figure 1A and 1B), with the other three treatment groups being of a similar redness. There was some fluctuation in the colouration of these three groups, but after month 6, those on the enhanced diet/+UV were the reddest (Figure 1A and 1C).

Neither diet nor UV treatment had an effect on the mass or SVL of the frogs (mass: diet Wald χ^2=2.19, P=0.14; UV Wald χ^2=0.35, P=0.55; SVL: diet Wald χ^2=0.68, P=0.41; UV Wald χ^2=0.04, P=0.84; Supplementary Figures).

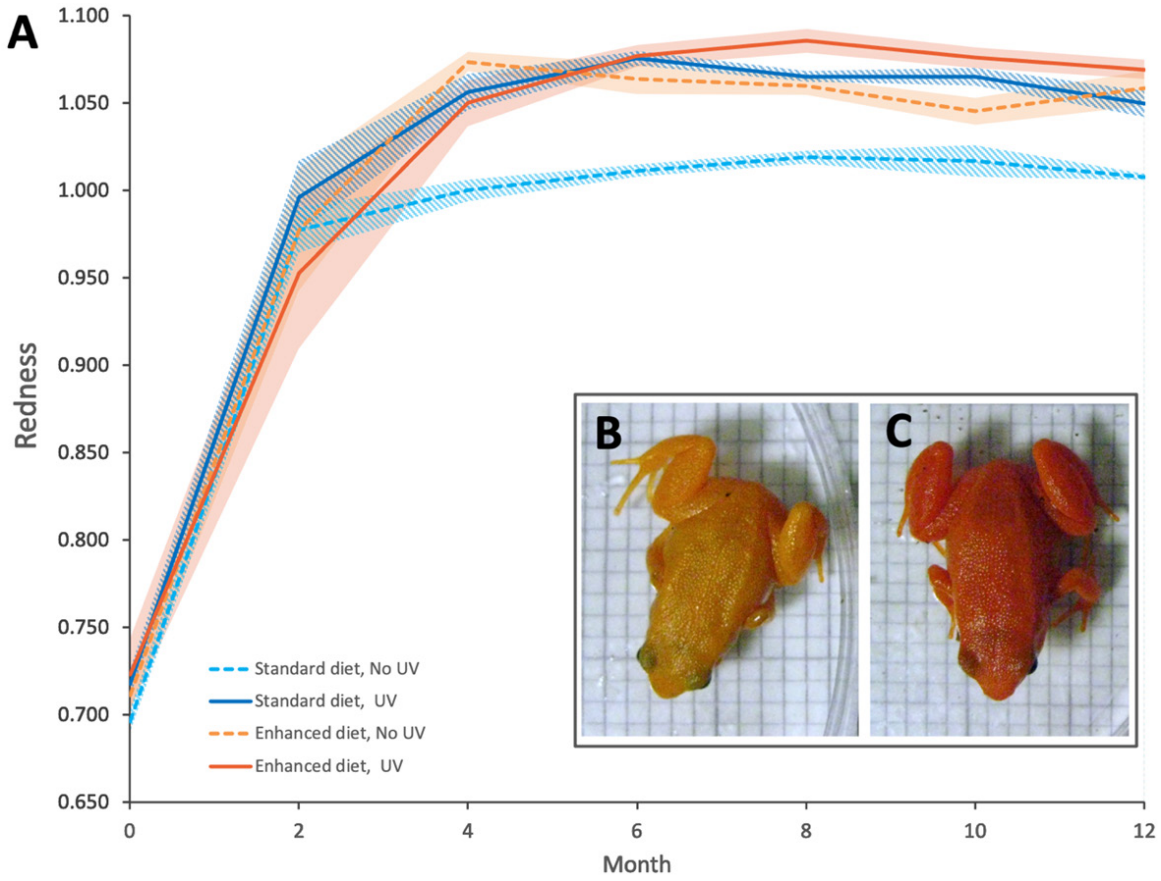

Figure 1. UV-carotenoid study. A) Colouration of frogs during the course of the study. Lines represent the mean redness of each treatment group, with shaded areas representing the standard error of the mean. Standard diet in blue (hashed shading) and enhanced diet in orange (solid shading). UV+ represented with darker solid lines, and -UV with dashed lines. B) Photograph of a frog from the standard diet/-UV group (redness value of 0.992). C) Photograph of a frog from the enhanced diet/+UV group (redness value of 1.090). Both photographs were taken at month 12 of the study.

Carotenoid profile study

The profile of carotenoids in the diets had a significant effect on colouration, with individuals receiving the crickets fed on the 'red' carotenoid diet being more red in colour than those receiving crickets fed the 'yellow' diet (Wald χ^2=6.36, P=0.012; Figure 2). In the UV-carotenoid study, there was a rapid increase in colouration for both groups at the start of the study, which stabilised around month 4. This is also when the colouration of the groups became evident, with those in the red diet group being distinctly redder from month 3 (Figure 2).

Over the course of the study there was no effect of diet on the SVL or mass of the study animals (Wald χ^2=0.73, P=0.39 and Wald χ^2=0.15, P=0.70 respectively; Supplementary Figures).

Discussion

Effect of carotenoids and UVB on colouration

The results from both studies demonstrate that dietary carotenoids can influence the colouration of *Mantella aurantiaca*. Supplementation of a vegetable diet for prey insects with a broad-spectrum carotenoid supplement resulted in a significant increase in redness of frogs in the UV-carotenoid study. This is in keeping with other supplementation studies involving different amphibian species, which have found the addition of supplementary carotenoids to the diet of prey insects fed to captive amphibians can lead to the development of more intense colouration (Brenes-Soto and Dierenfeld 2014; Ogilvy et al. 2012b; Umbers et al. 2016). Previous studies have predominantly compared the effects of a supplemented diet to a zero-carotenoid diet. The current experimental design addresses the full influence of dietary carotenoids; a zero-carotenoid diet may not be typical of a 'standard' diet provided in CBPs. One of the goals of the UV-carotenoid study was to determine if such a standard diet may be sufficient in producing bright colouration. These results show that a vegetable mixture can be successful in producing an orange colouration similar to that of an enhanced diet so long as UVB is provided simultaneously; in the absence of UVB, supplementary dietary carotenoids were necessary for more intensely coloured animals.

Given the broad range of carotenoids in the supplement used during the UV-carotenoid study, it is not possible to say with certainty whether it was the increase in total carotenoid content that led to greater red colouration in individuals fed the enhanced diet, or if it was due to differences in the types of carotenoids being provided. The results from the carotenoid profile study help to show that even when concentration is controlled, the profile of carotenoids can have a significant impact on colouration. Similar results were found by Brenes-Soto and Dierenfeld (2014)

Journal of Zoo and Aquarium Research 10(2) 2022
https://doi.org/10.19227/jzar.v10i2.598

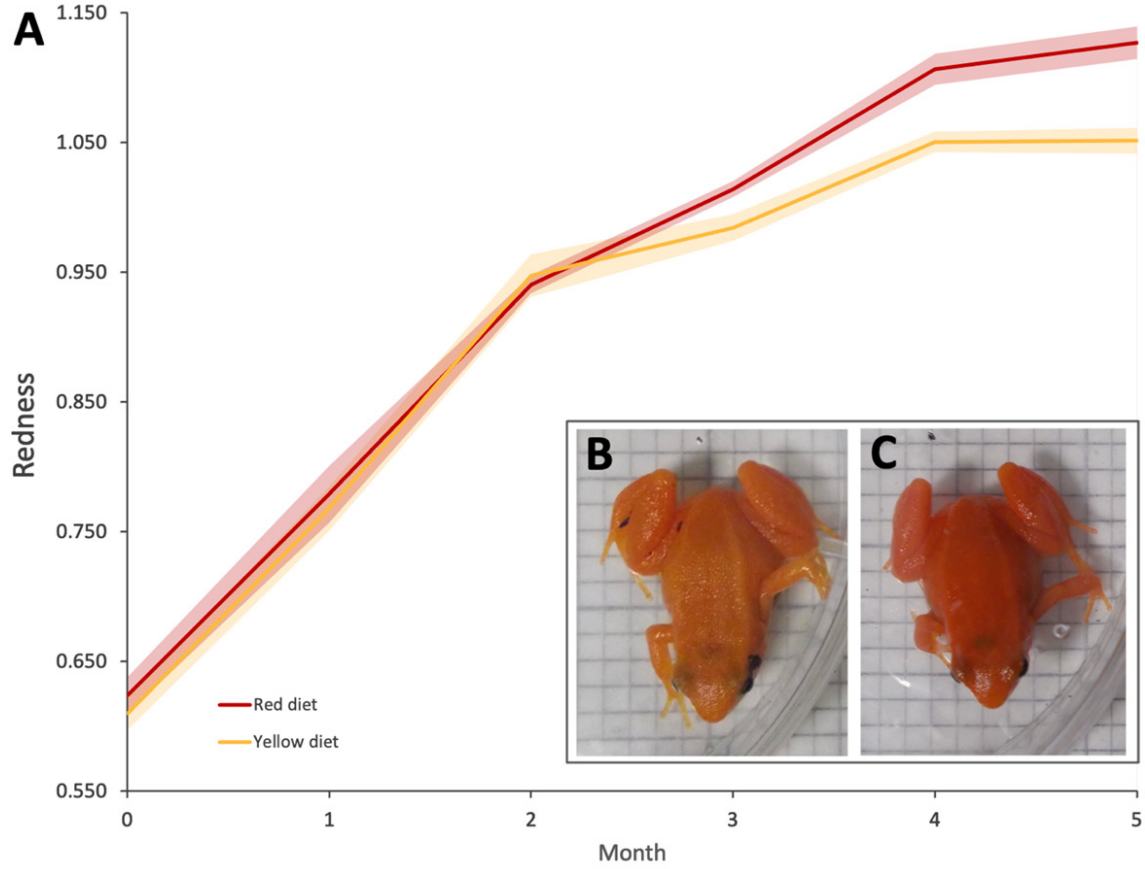

Figure 2. Carotenoid profile study. A) Colouration of frogs during the course of the study. Lines represent the mean redness for each treatment group, with shaded areas representing the standard error of the mean. B) Photograph of a frog from the yellow diet treatment group (redness value of 1.031) C) Photograph of a frog from the red diet group (redness value of 1.104). Both photographs were taken at month 5 of the study.

in the false tomato frog *Dyscophus guineti*: frogs fed a beta-carotene only supplement were less colourful than animals fed a mix of carotenoids. However, in that case the concentration of carotenoids differed between diets, with the mixed diet having a higher overall concentration. Our results demonstrate that when providing carotenoids to captive amphibians it is important to consider not just overall concentration, but also the types being selected. Although there are some data available on which carotenoids are found in amphibian skin, information is limited to a relatively small number of species (Brenes-Soto et al. 2017). Even in the absence of this information, it may still be possible to tailor supplements for different species in captivity based on their known colouration in the wild. In addition, there may be further impacts of carotenoid profile on health, which were not measured in this study, as different carotenoid compounds can have different specific roles in other physiological processes (McInerney et al. 2019).

In addition to dietary carotenoids, the presence of UVB radiation also had an impact on colouration. The colouration of amphibian skin in part comes from carotenoids stored within xanthophores but is also due to melanin found in melanosomes (and structural pigments in iridophores). It has been demonstrated in other amphibian species that exposure to UVB light can lead to increases in melanin levels in the dermis (Blaustein and Belden 2003). It is possible that the increased redness observed in *M. aurantiaca* in the +UV treatment groups may be due to elevated levels of dermal melanin. Michaels et al. (2015) found that *Bombina orientalis* exposed to UVB radiation achieved their adult colouration quicker than those with no UVB, and detected differences in colour within one month following metamorphosis. In the UV-carotenoid study presented here, the treatments had no apparent influence on colouration until month 4 post-metamorphosis. Golden mantella metamorphose brown in colouration, with their distinctive orange not appearing until several weeks to months later, possibly due to the time taken to accumulate sufficient levels of carotenoids to achieve this colour. This transition is accompanied by a behavioural change from cryptic behaviour reliant on camouflage in leaf litter, to bold behaviour presumably linked to aposematism once the colour change has occurred; carotenoid accumulation may therefore be linked with accumulation of skin toxins also derived from the diet.

Given the protective effects of carotenoids against UVB damage in other vertebrate species (Sies and Stahl 2004), it may be possible that the increased redness was due to additional carotenoids deposited within the dermis. This idea may be supported by the comparatively small increase in redness in the enhanced/+UV group compared to the others; provision of either the enhanced diet alone or UV alone resulted in a similar increase in redness (Figure 1A), however when both were provided, although there was an increase, it was not additive. It is possible

that *M. aurantiaca* have a 'maximum redness' due to limits on the number of carotenoids being stored in the skin, and that either the enhanced diet or UV were enough to reach this potential maximum and the combination of both in tandem had little additional effect. However, without additional analysis of the skin, it is not possible to determine the mechanisms behind the change in colouration. It is also important to consider that although more intense colouration developed in response to UVB radiation may be desirable in some ways, it is possible that this mechanism may be diverting carotenoids away from other functions (Baeta et al. 2008; McGraw and Hill 2000).

Different populations of wild golden mantella exhibit variations in colour, with some populations being more yellow-orange, while others are an intense red (Daly et al. 1996; Passos et al. 2020). Recent genetic work investigating the relationships between *M. aurantiaca* and other closely related species (*M. crocea* and *M. milotympanum*) found that genetically similar populations can have large variations in phenotype, and in contrast phenotypically similar populations can be genetically distinct (Klonoski et al. 2019). Although the reasons for this are likely to be multifaceted, the results from both studies here suggest that some phenotypic variation may be caused by variation in the diets of different populations. Given the variation in the availability of certain invertebrates, both temporally and spatially, some amphibian species have shown differences in diet between seasons as well as between populations (Kovács et al. 2007; Moskowitz et al. 2018; Quiroga et al. 2011). *Mantella aurantiaca* are thought to be generalist feeders, with diets consisting of a broad range of invertebrate prey (Woodhead et al. 2007). It is therefore probable that the diet composition of different populations of *M. aurantiaca* may vary in the wild, potentially leading to differences in concentration and profile of carotenoids being consumed. Differences in the environment may also potentially lead to some of this variation. Reductions in canopy cover or foliage density may lead to increased ambient UV levels, again leading to potential differences in colouration.

As well as differences in colour between wild populations of *M. aurantiaca*, marked differences in colour can be seen between wild and captive-bred individuals, with some captive animals suffering from a much duller appearance than their wild counterparts (Passos et al. 2020). The bright colour of golden mantella is due to aposematic signalling, warning potential predators of their toxicity (Daly et al. 1996). As part of the action plan for this species there are plans for reintroductions of captive-bred animals to the wild (Edmonds et al. 2015). The impact on potential predation rates of releasing dull individuals is unknown, but important to consider when selecting animals for release (Passos et al. 2020). Given the extensive knowledge of use of carotenoids by other vertebrate groups as an intraspecific signal of health, e.g. in birds (Alonso-Alvarez et al. 2004) and fish (Pike et al. 2010), it is possible that *M. aurantiaca* may also use colouration as an indicator of health in mate choice. There is limited information on the roles of colour in sexual selection in amphibians, but for some species male colouration is thought to influence female mate choice (Gomez et al. 2009; Vasquez and Pfennig 2007), however this has not been found for all species (Ogilvy 2011).

Effect of carotenoids and UVB on growth

Neither study found any impact of the treatments on the growth of the animals. A small but growing number of studies have investigated the influence of dietary carotenoids on amphibian growth, but the results are mixed depending on the species and their life stage, the carotenoids investigated and the dosage (Byrne and Silla 2017; Cothran et al. 2015; Keogh et al. 2018; McInerney et al. 2019; Ogilvy and Preziosi 2012; Ogilvy et al. 2012b). In general, studies that have reported negative consequences of carotenoid supplementation have investigated high concentrations (up to 10 mg/g being fed to invertebrate prey). The levels given during the studies presented here (5 mg/g for the carotenoid profile study and an additional 1.7 mg/g for the enhanced diet in the UV-carotenoid study) represent an 'intermediate' dose in relation to other works. It may be that certain concentrations of carotenoids are tolerable or even required by amphibian species, whereas over this concentration negative impacts become apparent (Keogh et al. 2018). Given the lack of data available on carotenoid concentrations consumed by wild amphibians, it is not possible to know what actually constitutes a 'high' or 'low' carotenoid dose, and it is also likely to be highly species-specific.

Given the broad diversity of the properties of different carotenoids, concentration is only one factor that may influence growth or development. The carotenoids used in the carotenoid profile study (lutein and zeaxanthin in the yellow diet, and lycopene and astaxanthin in the red diet) were chosen due to their use in prior studies, as well as their known presence in the skin of other amphibian species (Baruah and Goswami 2012; Bonansea et al. 2017; Czeczuga 1980). Despite these carotenoids influencing growth in other studies, they appeared to have no influence here. It is important to note that the growth of animals in both red and yellow treatments were in keeping with normal *M. aurantiaca* development, with similar sizes being seen between both studies (Supplementary Figures) and in general rearing of the species (personal observation, J Newton-Youens, C. Michaels). The dietary choices made for both studies were primarily to investigate the influence on colouration. Therefore, to fully explore the influence of carotenoids on growth and development in *M. aurantiaca*, future work should focus on single carotenoid supplements.

The provision of UVB can have varying impacts on amphibians depending on the light intensity and the species. As for many animals, high levels of UVB exposure can have very damaging effects on amphibians (Antwis and Browne 2009). It has been demonstrated in a number of species, however, that a low level of UVB exposure in combination with appropriate heat provision is necessary for adequate production of vitamin D_3 and proper bone morphology and development (Michaels et al. 2015; Tapley et al. 2015; Verschooren et al. 2011). Here we found no evidence of UVB having an influence on the growth (or health) of the study animals, either positive or negative. Given the impact that UVB exposure had on the colouration of the animals, it could be assumed that this provision was sufficient to trigger other physiological responses. Although no impact was detected on growth, it is possible other effects, such as vitamin D_3 levels, may have been affected (Michaels et al. 2015). However, this was not possible to test as given the small body size of the species, it would not have been possible to collect adequate blood samples. It may also be that the UV levels were insufficient to stimulate additional physiological responses. The levels provided during the study were within the natural range recorded within the species' habitat in the wild, however higher levels up to 5.4 UVI have been observed (R. Griffiths 2014, unpublished data).

It is possible that the vitamin and mineral supplement dusted onto the food items may have masked the impact of either UVB provision or carotenoid supplementation such that any self-synthesised (D_3 via UVB irradiation) or absorbed from experimental supplement (carotenoids) had no discernible effect. However, this was not the case for *Bombina orientalis*, for which the effect of UVB on vitamin D_3 levels was not masked despite frequent dusting with the same supplement as that used in this study (Michaels et al. 2015). As for carotenoids and vitamin A, there it still much unknown as to how widespread the ability for conversion from the former to the latter is in amphibians (Clugston and Blaner 2014), and it has not been investigated in *Mantella*. This therefore remains an untested possible contributor to our results.

Conclusion

Captive bred *Mantella aurantiaca*, like many other amphibian species, are often less intensely coloured than their wild counterparts (Passos et al. 2020). Our results demonstrate that it is possible to improve the colouration of *M. aurantiaca* in captivity through the relative ease of incorporating supplements into the diet of feeder invertebrates, and that this can be enhanced through the simultaneous provision of UVB. UVB-emitting lamps should also be considered in captive breeding institutions, however due to difficulties in sourcing and resource constraints in some areas of the world, this may not be possible. Based on the apparent lack of influence the treatments had on the growth of the animals in these studies, it not possible to make recommendations for their use in this species. However, given the marked influence carotenoids can have in other amphibian species (both positive and negative) more information on the health requirements of amphibians for carotenoids should be gained. Moving forward, more information is required as to which carotenoids are being consumed by wild amphibians, so that appropriate diets can be formulated for captive populations.

Acknowledgments

The authors would like to thank Rachael Antwis for early discussions about the project and comments on the manuscript.

References

Alonso-Alvarez C., Bertrand S., Devevey G., Gaillard M., Prost J., Faivre B., Sorci G. (2004) An experimental test of the dose-dependent effect of carotenoids and immune activation on sexual signals and antioxidant activity. *American Naturalist* 164(5): 651–659. doi:10.1086/424971

Antwis R.E., Browne R.K. (2009) Ultraviolet radiation and Vitamin D_3 in amphibian health, behaviour, diet and conservation. *Comparative Biochemistry and Physiology Part A* 154(2): 184–190. doi:10.1016/j.cbpa.2009.06.008

Augustine L., Terrell K.A., Petzinger C., Nissen B., Maslanka M. (2016) Nutritional analysis of diet items available to captive and free-ranging Hellbenders (*Cryptobranchus alleganiensis*). *Herpetological Review* 47(1): 63–69.

Baeta R., Faivre B., Motreuil S., Gaillard M., Moreau J. (2008) Carotenoid trade-off between parasitic resistance and sexual display: An experimental study in the blackbird (*Turdus merula*). *Proceedings of the Royal Society B* 275(1633): 427–434. doi:10.1098/rspb.2007.1383

Baruah P., Goswami U.C. (2012) Characterization of carotenoid pigments in amphibian, *Rhacophorous bipunctatus. Journal of Research in Biology* 2(2): 114–118.

Bates D., Mächler M., Bolker B.M., Walker S.C. (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67(1): 1–48. doi:10.18637/jss.v067.i01

Blaustein A.R., Romansic J.M., Kiesecker J.M., Hatch A.C. (2003) Ultraviolet radiation, toxic chemicals and amphibian population declines. *Diversity and Distributions* 9(2): 123–140. doi:10.1046/j.1472-4642.2003.00015.x

Blaustein A.R., Belden L.K. (2003) Amphibian defenses against ultraviolet-B radiation. Evolution & Development 5(1): 89–97. doi:10.1046/j.1525-142X.2003.03014.x

Bonansea M.I., Heit C., Vaira M. (2017) Pigment composition of the bright skin in the poison toad *Melanophryniscus rubriventris* (Anura: Bufonidae) from Argentina. *Salamandra* 53(1): 142–147.

Brenes-Soto A., Dierenfeld E.S. (2014) Effect of dietary carotenoids on vitamin A status and skin pigmentation in false tomato frogs (*Dyscophus guineti*). *Zoo Biology* 33(6): 544–552. doi:10.1002/zoo.21175

Brenes-Soto A., Dierenfeld E.S., Janssens G.P.J. (2017) Colouration in amphibians as a reflection of nutritional status: The case of tree frogs in Costa Rica. *PLoS ONE* 12(8): e0182020. doi:10.1371/journal.pone.0182020

Byrne P.G., Silla A.J. (2017) Testing the effect of dietary carotenoids on larval survival, growth and development in the critically endangered southern corroboree frog. *Zoo Biology* 36(2): 161–169. doi:10.1002/zoo.21352

Chew B.P., Park J.S. (2004) Carotenoid action on the immune response. *The Journal of Nutrition* 134(1): 257S–261S. doi:10.1093/jn/134.1.257S

Clugston R.D., Blaner W.S. (2014) Vitamin A (retinoid) metabolism and actions: What we know and what we need to know about amphibians. *Zoo Biology* 33(6): 527–535. doi:10.1002/zoo.21140

Conde D.A., Flesness N., Colchero F., Jones O.R., Scheuerlein A. (2011) An emerging role of zoos to conserve biodiversity. *Science* 331(6023): 1390–1391. doi:10.1126/science.1200674

Cothran R.D., Gervasi S.S., Murray C., French B.J., Bradley P.W., Urbina J., Blaustein A.R., Relyea R.A. (2015) Carotenoids and amphibians: Effects on life history and susceptibility to the infectious pathogen, *Batrachochytrium dendrobatidis. Conservation Physiology* 3(1): cov005. doi:10.1093/conphys/cov005

Czeczuga B. (1980) Investigations on carotenoids in Amphibia-II. Carotenoids occuring in various parts of the body of certain species. *Comparative Biochemistry and Physiology Part B* 65(4): 623–630. doi:10.1016/0305-0491(80)90170-4

Daly J.W., Andriamaharavo N.R., Andriantsiferana M., Myers C.W. (1996) Madagascan poison frogs (*Mantella*) and their skin alkaloids. *American Museum Novitates* 3177: 1–34.

Dawson J., Patel F., Griffiths R.A., Young R.P. (2016) Assessing the global zoo response to the amphibian crisis through 20-year trends in captive collections. *Conservation Biology* 30(1): 82–91. doi:10.1111/cobi.12563

Dugas M.B., Yeager J., Richards-Zawacki C.L. (2013) Carotenoid supplementation enhances reproductive success in captive strawberry poison frogs (*Oophaga pumilio*). *Zoo Biology* 32(6): 655–658. doi:10.1002/zoo.21102

Edmonds D., Rakotoarisoa J.C., Rasoanantenaina S., Sam S.S., Soamiarimampionona J., Tsimialomanana E., Youssouf, Dolch R., Rabemananjara F., Rabibisoa N., Robsomanitrandrasana E. (2015) Captive husbandry, reproduction, and fecundity of the golden mantella (*Mantella aurantiaca*) at the Mitsinjo breeding facility in Madagascar. *Salamandra* 51(4): 315–325.

Ferrie G.M., Alford V.C., Atkinson J., Baitchman E., Barber D., Blaner W.S., Crawshaw G., Daneault A., Dierenfeld E., Finke M., Fleming G., Gagliardo R., Hoffman E.A., Karasov W., Klasing K., Koutsos E., Lankton J., Lavin S.R., Lentini A., Livingston S., Lock B., Mason T., McComb A., Morris C., Pessier A.P., Olea-Popelka F., Probst T., Rodriguez C., Schad K., Semmen K., Sincage J., Stamper M.A., Steinmetz J., Sullivan K., Terrell S., Wertan N., Wheaton C.J., Wilson B., Valdes E.V. (2014) Nutrition and health in amphibian husbandry. *Zoo Biology* 33(6): 485–501. doi:10.1002/zoo.21180

Finke M.D. (2002) Complete nutrient composition of commercially raised invertebrates used as food for insectivores. *Zoo Biology* 21(3): 269–285. doi:10.1002/zoo.10031

Fox J., Weisberg S. (2019) *An R Companion to Applied Regression Third edition.* Thousand Oaks, CA: Sage Publications.

Fraser P.D., Bramley P.M. (2004) The biosynthesis and nutritional uses of carotenoids. *Progress in Lipid Research* 43(3): 228–265. doi:10.1016/j.plipres.2003.10.002

Gomez D., Richardson C., Lengagne T., Plenet S., Joly P., Léna J.P., Théry M. (2009) The role of nocturnal vision in mate choice: Females prefer conspicuous males in the European tree frog (*Hyla arborea*). *Proceedings of the Royal Society B* 276(1666): 2351–2358. doi:10.1098/rspb.2009.0168

Heinrich U., Gärtner C., Wiebusch M., Eichler O., Sies H., Tronnier H., Stahl W. (2003) Supplementation with beta-carotene or a similar amount of mixed carotenoids protects humans from UV-induced erythema. *The Journal of Nutrition* 133(1): 98–101. doi:10.1093/jn/133.1.98

IUCN (2020) The IUCN Red List of Threatened Species. Version 2020-2. Available at: https://www.iucnredlist.org.

Jayson S., Ferguson A., Goetz M., Routh A., Tapley B., Harding L., Michaels C.J., Dawson J. (2018) Comparison of the nutritional content of the captive and wild diets of the critically endangered mountain chicken frog (*Leptodactylus fallax*) to improve its captive husbandry. *Zoo Biology* 37(5): 332–346. doi:10.1002/zoo.21442

Keogh L.M., Silla A.J., McFadden M.S., Byrne P.G. (2018) Dose and life stage-dependent effects of dietary beta-carotene supplementation on the growth and development of the Booroolong frog. *Conservation Physiology* 6(1): coy052. doi:10.1093/conphys/coy052

Klonoski K., Bi K., Rosenblum E.B. (2019) Phenotypic and genetic diversity in aposematic Malagasy poison frogs (genus *Mantella*). *Ecology and Evolution* 9(5): 2725–2742. doi:10.1002/ece3.4943

Kovács É.H., Sas I., Covaciu-Marcov S.D., Hartel T., Cupsa D., Groza M. (2007) Seasonal variation in the diet of a population of Hyla arborea from Romania. *Amphibia-Reptilia* 28(4): 485–491. doi:10.1163/156853807782152534

Lee J., Jiang S., Levine N., Watson R.R. (2000) Carotenoid supplementation reduces erythema in human skin after simulated solar radiation exposure. *Experimental Biology and Medicine* 223(2): 170–174. doi:10.1177/153537020022300209

Livingston S., Lavin S.R., Sullivan K., Attard L., Valdes E.V. (2014) Challenges with effective nutrient supplementation for amphibians: A review of cricket studies. *Zoo Biology* 33(6): 565–576. doi:10.1002/zoo.21177

Matsui K., Marunouchi J., Nakamura M. (2002) An ultrastructural and carotenoid analysis of the red ventrum of the Japanese newt, *Cynops pyrrhogaster. Pigment Cell Research* 15(4): 265–272. doi:10.1034/j.1600-0749.2002.01085.x

McGraw K.J., Hill G.E. (2000) Differential effects of endoparasitism on the expression of carotenoid- and melanin-based ornamental coloration. *Proceedings of the Royal Society B* 267(1452): 1525–1531. doi:10.1098/rspb.2000.1174

McInerney E.P., Silla A.J., Byrne P.G. (2019) Effect of carotenoid class and dose on the larval growth and development of the critically endangered southern corroboree frog. *Conservation Physiology* 7(1): coz009. doi:10.1093/conphys/coz009

Michaels C.J., Antwis R.E., Preziosi R.F. (2015) Impacts of UVB provision and dietary calcium content on serum vitamin D_3, growth rates, skeletal structure and coloration in captive oriental fire-bellied toads (*Bombina orientalis*). *Journal of Animal Physiology and Animal Nutrition* 99(2): 391–403. doi:10.1111/jpn.12203

Moskowitz N.A., Roland A.B., Fischer E.K., Ranaivorazo N., Vidoudez C., Aguilar M.T., Caldera S.M., Chea J., Cristus M.G., Crowdis J.P., DeMessie B., desJardins-Park C.R., Effenberger A.H., Flores F., Giles M., He E.Y., Izmaylov N.S., Lee C.W.C., Pagel N.A., Phu K.K., Rosen L.U., Seda D.A., Shen Y., Vargas S., Murray A.W., Abebe E., Trauger S.A., Donoso D.A., Vences M., O'Connell L.A. (2018) Seasonal changes in diet and chemical defense in the Climbing Mantella frog (*Mantella laevigata*). *PLoS ONE* 13(12): e0207940. doi:10.1371/journal.pone.0207940

Nicholson D.J., Tapley B., Jayson S., Dale J., Harding L., Spencer J., Sulton M., Durand S., Cunningham A. (2017) Development of in-country live food production for amphibian conservation: The mountain chicken frog (*Leptodactylus fallax*) on Dominica, West Indies. *Amphibian and Reptile Conservation* 11(2): 59–68.

Ogilvy V. (2011) The colourful sex life of *Agalychnis moreletii* - The influence of carotenoid-based colouration on mate choice in a nocturnal tree frog. In: The Influence of Carotenoids on Fitness Related Traits in Anurans: Implications for Ex Situ Conservation. PhD Thesis, Univeristy of Manchester, UK, 75–96.

Ogilvy V., Preziosi R.F. (2012) Can carotenoids mediate the potentially harmful effects of ultraviolet light in *Silurana (Xenopus) tropicalis* larvae? *Journal of Animal Physiology and Animal Nutrition* 96(4): 693–699. doi:10.1111/j.1439-0396.2011.01197.x

Ogilvy V, Fidgett A.L., Preziosi R.F. (2012a) Differences in carotenoid accumulation among three feeder-cricket species: Implications for carotenoid delivery to captive insectivores. *Zoo Biology* 31(4): 470–478. doi:10.1002/zoo.20416

Ogilvy V., Preziosi R.F., Fidgett A.L. (2012b) A brighter future for frogs? The influence of carotenoids on the health, development and reproductive success of the red-eye tree frog. *Animal Conservation* 15(5): 480–488. doi:10.1111/j.1469-1795.2012.00536.x

Passos L.F., Garcia G., Young R. (2020) How does captivity affect skin colour reflectance of golden mantella frogs? *The Herpetological Journal* 30(1): 13–19. doi:10.33256/hj30.1.1319

Pessier A.P., Baitchman E.J., Crump P., Wilson B., Griffith E., Ross H. (2014) Causes of mortality in anuran amphibians from an ex situ survival assurance colony in Panama. *Zoo Biology* 33(6): 516–526. doi:10.1002/zoo.21166

Pike T.W., Blount J.D., Lindström J., Metcalfe N.B. (2010) Dietary carotenoid availability, sexual signalling and functional fertility in sticklebacks. *Biology Letters* 6(2): 191–193. doi:10.1098/rsbl.2009.0815

Quiroga M.F., Bonansea M.I., Vaira M. (2011) Population diet variation and individual specialization in the poison toad, *Melanophryniscus rubriventris* (Vellard, 1947). *Amphibia-Reptilia* 32(2): 261–265. doi:10.1163/017353710X546530

Schneider C.A., Rasband W.S., Eliceiri K.W. (2012) NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* 9: 671–675. doi: 10.1038/nmeth.2089

Sies H., Stahl W. (2004) Carotenoids and UV protection. *Photochemical and Photobiological Sciences* 3(8): 749–752. doi:10.1039/b316082c

Stahl W., Sies H. (2002) Carotenoids and Protection against Solar UV Radiation. *Skin Pharmacology and Applied Skin Physiology* 15:291-296. doi: 10.1159/000064532

Stahl W., Sies H. (2003) Antioxidant activity of carotenoids. *Molecular Aspects of Medicine* 24(6): 345–351. doi:10.1016/S0098-2997(03)00030-X

Stahl W., Sies H. (2005) Bioactivity and protective effects of natural carotenoids. *Biochimica et Biophysica Acta - Molecular Basis of Disease* 1740(2): 101–107. doi:10.1016/j.bbadis.2004.12.006

Szuroczki D., Koprivnikar J., Baker R.L. (2016) Dietary antioxidants enhance immunocompetence in larval amphibians. *Comparative Biochemistry and Physiology Part A* 201: 182–188. doi:10.1016/j.cbpa.2016.07.014

Szuroczki D., Koprivnikar J., Baker R.L. (2019) Effects of dietary antioxidants and environmental stressors on immune function and condition in *Lithobates (Rana) sylvaticus. Comparative Biochemistry and Physiology Part A* 229: 25–32. doi:10.1016/j.cbpa.2018.11.017

Tapley B., Rendle M., Baines F.M., Goetz M., Bradfield K.S., Rood D., Lopez J., Garcia G., Routh A. (2015) Meeting ultraviolet B radiation requirements of amphibians in captivity: A case study with mountain chicken frogs (*Leptodactylus fallax*) and general recommendations for pre-release health screening. *Zoo Biology* 34(1): 46–52. doi:10.1002/zoo.21170

Umbers K.D.L., Silla A.J., Bailey J.A., Shaw A.K., Byrne P.G. (2016) Dietary carotenoids change the colour of Southern corroboree frogs. *Biological Journal of the Linnean Society* 119(2): 436–444. doi:10.1111/bij.12818

Vásquez T., Pfennig K.S. (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. doi:10.1007/s00265-007-0446-7

Vences M., Raxworthy C.J. (2008) *Mantella aurantiaca.* The IUCN Red List of Threatened Species 2008: e.T12776A3381123. Available at: https://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T12776A3381123.en [Accessed 30 November 2020].

Verschooren E., Brown R.K., Vercammen F., Pereboom J. (2011) Ultraviolet B radiation (UV-B) and the growth and skeletal development of the Amazonian milk frog (*Trachycephalus resinifictrix*) from metamorphosis. *Journal of Physiology and Pathophysiology* 2(3): 34–42. doi:10.5897/JPAP.9000026

Woodhead C., Vences M., Vieites D.R., Gamboni I., Fisher B.L., Griffiths R.A. (2007) Specialist or generalist? Feeding ecology of the Malagasy poison frog *Mantella aurantiaca. The Herpetological Journal* 17(4): 225–236.

Wren S., Angulo A., Meredith H., Kielgast J., Dos Santos M., Bishop P. (eds.) (2015) Amphibian Conservation Action Plan. IUCN SSC Amphibian Specialist Group. Available at: https://www.iucn-amphibians.org/resources/acap/.