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1 **Full title: How does captivity affect skin colour reflectance of golden mantella frogs?**

2 **Running Title: Golden mantellas' skin colouration**

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20 Abstract

21 Colouration is an important trait for social communication in amphibians, being used in intra-
22 and intersexual signaling to express information about individual body condition, health state,
23 amongst other things. The striking colour pattern exhibited by some anuran species are also
24 used in “aposematic” signals to advertise unpalatability to predators. The aim of this study was
25 to investigate how the captive environment affects the colour of golden mantella frogs by
26 comparing captive reared frogs with wild conspecifics. A USB-2000 portable diode-array
27 spectrometer and a xenon strobe light source were used to perform spectrophotometric
28 measurements on captive and wild populations. Hue, chroma and brightness of skin colour
29 were analyzed as well as body condition using the scaled mass index. Analyses showed
30 variation among populations, but significant differences were only found between captive and
31 wild populations. Generalized linear mixed models were used to evaluate the effects of body
32 condition on colour variation and showed that animals with lower body condition from one
33 captive population had significantly different colouration than their wild counterparts.
34 Importantly, one captive population was not greatly different in colouration from their wild
35 counterparts – demonstrating that this problem is not inevitable in captivity. These results can
36 have important implications for reintroduction programmes.

37

38

39 Introduction

58 The current global amphibian crisis has resulted in an unprecedented rate of amphibian-
59 biodiversity loss, resulting in a growth of captive breeding as a conservation tool for
60 amphibians (Griffiths & Pavajeau, 2008). Maintaining captive populations is important in
61 terms of species conservation for potential reintroduction into the wild (Harding, Griffiths &
62 Pavajeau, 2016).

63 One of the biggest challenges faced while keeping amphibians in captivity is mimicking
64 their diet and nutritional needs (Livingston et al.,2014) this can directly impact many aspects
65 of the individual's ecology, including skin pigmentation (Brenes-Soto & Dierenfeld, 2014). It
66 is common to observe amphibians kept in captivity displaying a faded colouration in
67 comparison to their wild counterparts (Brenes-Soto & Dierenfeld, 2014). In amphibians, skin
68 colouration influences courtship and mate preference, recognition of breeding partners and
69 perception of fitness, consequently affecting breeding success, resulting in advantages for
70 selective females and for strikingly coloured males (Brenes-Soto et al. 2017).

71 Skin colour may also inform about physiological conditions, such as oxidative and
72 immune status (McGraw 2005;), and parasite load (Molnár et al. 2013). Carotenoid based
73 colourations can be used to assess physiological and health status of an individual while
74 colouration associated with melanin informs about hormonal levels and social dominance
75 (Candolin 2003).

76 The remarkable colour patterns displayed by many anuran species (Hoffman & Blouin,
77 2000) are also used as conspicuous “aposematic” indicators, warning signals to advertise
78 unpalatability to potential predators (Hegna et al., 2013; Mann & Cumming, 2012; Ruxton et
79 al., 2004). By reducing the frequency of costly encounters with predators, the protection that

80 aposematism confers can lower the costs of otherwise risky behaviours, including foraging and
81 sexual displays (Dugas et al., 2015).

82 Divergent antipredator strategies such as aposematism not only require integration of
83 physiology, morphology and behaviour; they also alter the way selection acts on other suites
84 of traits (Stankowich & Blumstein, 2005). It is expected that, in some scenarios, an increase in
85 the toxicity should also coincide with a greater visual contrast of the warning signal, with
86 “nastier” animals “shouting loudest” (Mann & Cumming, 2012; Speed & Ruxton, 2007). This
87 is because the greater risk of detection and attack on highly conspicuous prey can be
88 compensated for by the stronger predator deterrence induced by high toxicity (Darst et al.,
89 2006). A positive relationship may also emerge from physiological or energetic trade-offs
90 between the two traits (Blount et al., 2012).

91 Colour refers to a sensory experience, not an objective quantity, and how animals
92 perceive this information can vary quite considerably according to their visual system and how
93 they process colour (Maia et al., 2013). The vertebrate retina contains two types of
94 photoreceptor: rods that function at low light levels, and cones that function in daylight and
95 provide the basis for colour vision. Colour vision requires at least two spectrally distinct classes
96 of cone cells combined with a nervous system that can compare the quantum catch of one class
97 of cone with the quantum catch of another. (Bowmaker and Hunt, 2006)

98 Animals have evolved their visual sensitivity to match aspects of their photic
99 environment (Bowmaker and Hunt, 2006), with modern teleosts, reptiles and birds possessing
100 rods and four spectral classes of cones each representing one of the five visual pigment families
101 giving these species the potential for tetra chromatic colour vision. In contrast, mammals, due
102 to their nocturnal ancestry, have rod-dominated retinas with colour vision reduced to a basic
103 dichromatic system (Bowmaker, 2015).

114 The role of colouration and colour vision in animal communication has been a
115 fundamental question in evolutionary biology for many decades (Maia et al., 2013). Colour is
116 involved in a wide range of biological phenomena such as thermoregulation, crypsis, mimicry,
117 communication as well as indicating health status of an individual (Endler,1993; Forsman et
118 al., 2002; Robertson & Rosenblum, 2009). Therefore, the quantification of animal colour
119 variation is a crucial component of conservation and ecological studies. If captive animals are
120 bred for conservation purposes and a reintroduction is a future goal, these issues are of major
121 concern. The aim of this study was to investigate if the captive environment is affecting the
122 colour of golden mantella frogs and, if so, to quantify this difference.

123 Methodology

124 Ethical Approval

125 All the research reported in this study was approved by the Ethics Commission of
126 Chester Zoo, UK, and from the Research Ethics Committee from the University of Salford and
127 it conforms to all regulations and laws in all relevant countries in relation to care of
128 experimental animal subjects. To collect data from wild individuals, permission (permit) was
129 obtained from the government of Madagascar. Furthermore, we can confirm, from our post-
130 experimental monitoring, that no animals suffered any injuries, became ill or had their
131 survivorship negatively affected because of this study.

132 Study subject:

133 The golden mantella frog (*Mantella aurantiaca*) is a critically endangered species
134 (Vence & Raxworthy, 2009), found only in Madagascar with a distribution restricted to a
135 fragment of forest that is under severe threat from mining, agriculture, timber extraction and
136 over-collecting for the pet trade (Randrianelona et al., 2010). According to the Amphibian
137 Ark, *ex situ* assistance is vital for the long-term survival of the golden mantella frog (Johnson,

138 2008). This is an ideal species to test the effects of captivity on colouration because the species
139 is naturally only one consistent orange colour.

140 Study sites:

141 Mangabe area (Madagascar wild): Most breeding ponds for the golden mantella frogs are found
142 in this area according to recent studies concerning conservation priority sites for mantella frogs.
143 Also known as, the “blue forest”, Mangabe is a site of international biodiversity importance,
144 divided between two administrative districts, Moramanga in the north and Anosibe An'ala to
145 the south. Data sampling (15 males and 15 females) for this study was done in a protected area
146 of the Moramanga region.

147 Ambatovy Mining Site (Madagascar wild): Ambatovy’s Mine is located within a species-rich
148 region of Madagascar at the southern end of the remaining Eastern Forest Corridor at
149 Moramanga region. As part of the Environmental Management Plan, there is a Conservation
150 zone of native forest kept under pristine conditions by the mining company. During this study
151 animals from the Conservation zone (15 males and 15 females) were sampled.

152 Mitsinjo Association Captive Breeding Centre (Madagascar captive): Mitsinjo Association is
153 a community-run conservation organization. This is Madagascar’s first biosecure facility to
154 safeguard amphibians from extinction, currently maintaining a genetically viable population of
155 the golden mantella frog taken from the Ambatovy mining site (i.e., genetic founders), their
156 offspring (F1) that are intended for reintroductions at artificially created breeding and natural
157 ponds. Animals are kept in tanks with aquarium gravel as substrate, a potted plant, coconut
158 shells for hiding. Animals were fed a variety of live invertebrates (fruit flies- *Drosophila sp.*,
159 isopods – *Trichorhina sp.*, springtails - Collembolas). We sampled 8 males and 8 females
160 founder frogs (i.e. wild caught) and the same number from their F1 frogs.

161 Chester Zoo (UK): Chester Zoo is actively involved in the conservation of the golden mantella
162 frogs in Madagascar. The zoo currently maintains two *ex situ* groups of *M. aurantiaca*, one is

163 on public display at the Zoo's Tropical Realm exhibit and a second group is kept off show in a
164 biosecurity container specifically for conservation-related research. Animals have been in
165 captivity for more than 5 generations. Animals are kept in naturalistic tanks with different live
166 species of plants, moss for substrate, water, hiding places under rocks and UV light. Animals
167 are fed different live invertebrates (fruit flies- *Drosophila sp.*, isopods – *Trichorhina sp.*,
168 springtails - Collembolas). We sampled 8 males and 8 females from the Chester Zoo off-show
169 colony.

170

171 Spectrophotometric measurements

172 We used a USB-2000 portable diode-array spectrometer and a PX-2 xenon strobe light
173 source (both from Ocean Optics, Dunedin, USA), probe positioned at an angle of 90°, to
174 perform spectrophotometric measurements. To exclude ambient light and standardize
175 measuring distance a cylindrical plastic tube was mounted on the fibre optic probe. The
176 equipment permitted that the spectral analyses were conducted in the 300 and 700 nm range.
177 Spectral reflectance measurements were always taken of each individual from the dorsum,
178 three consecutive measurements per frog, only adult frogs during breeding season were used
179 during this study. Colour measurements should sample the most visible surfaces to obtain a
180 representative sample (within an individual) of the spectral shape of the entire body. Golden
181 mantellas do not show any sexual dichromatism, allowing the use of both male and females.
182 Summary variables for the colour measurements were calculated. Spectralon white standard
183 measurements were taken between each individual to account for lamp drift. This methodology
184 was based on previous studies measuring colour variation in different species (Crothers, et al.,
185 2011; Maan & Cummings, 2008; Siddiqi et al., 2005).

186 Colour analyses

187 Colour may be described by three essential parameters: Hue, Chroma, and Brightness
188 and all three variables were analysed as they are customarily used in studies of animal
189 coloration, thereby facilitating comparisons between studies. Brightness (Q_t) may be defined
190 as the total intensity of light (Endler, 1990). Q_t was calculated by summing the percentage
191 reflectance (R) across the entire spectrum (R_{300} and R_{700}).

192 Hue represents the common meaning of colour, for example, violet, blue, orange, green
193 (Endler, 1990); In general, the hue of a spectrum is a function of its shape. Hue is correlated
194 with the wavelength of the maximum slope, as well as the sign of the slope (Endler, 1990). It
195 is the wavelength within the visible-light spectrum at which the energy output from a source is
196 greatest Hue (nm) was measured as the wavelength of maximum reflectance.

197 Chroma is a measure of the ‘purity’ or ‘saturation’ of a colour and is a function of how
198 rapidly intensity changes with wavelength (Endler, 1990). Chroma was calculated as relative
199 medium wavelength chroma (MC, calculated as $(R_{max} - R_{min})/Q_t$).

200 Brightness, Hue and Chroma differences between populations were analysed with a
201 mixed model with origin (wild or captive) as fixed factors and populations as random factors.
202 Data were analysed using the Pavo (Maia et al., 2013) package from R studio (R Studio Team
203 2015). The data from each population were plotted on the same graph to confirm
204 standardization of sampling, no error from the sampling design was found. Data from different
205 populations were compared based on colour distance and colorimetric variables. Four different
206 visual systems: human, a snake (Boidae, Bowmaker, 2015), a Scincidae lizard (New et al.,
207 2012), representing potential predators, and a diurnal poison frog (*D. pumilio*, Siddiqi et al.,
208 2004) under an ideal illumination condition, were used to calculate colour distance. The colour
209 distance analyses in Just Noticeable Distance units (JND) (Wen, 2012) - were used to infer if
210 different visual systems would be able to notice differences between the different populations.

211 In general, when $JND < 1$, the spectral pair is barely distinguishable under ideal conditions,
212 and as JND becomes greater, discrimination can be made more rapidly and under increasingly
213 unfavourable viewing conditions (Siddiqi et al., 2004). Due to the lack of data on golden
214 mantella photoreceptor sensitivity, sensitivity data used in the analysis were those from a
215 species with similar activity pattern (i.e. aposematic diurnal).

216 Body Condition

217 Body condition is a valuable index that can be assessed using reliable, non-invasive
218 techniques, and it can identify the health condition of a population before any deleterious
219 effects can be observed (MacCracken & Stebbing, 2012). Body condition (BC) was assessed
220 using the scaled mass index proposed by Peig & Green (2009). This method is independent of
221 size and can be used for comparison between different populations; those characteristics
222 potentially make it superior to the traditional residual indices and, reportedly have worked well
223 in amphibian studies (MacCracken & Stebbing, 2012, Michaels et al., 2014). The scaled mass
224 index of condition (SMI) was calculated as follows:

$$225 \quad \text{SMI} = M * [\text{SVL}_0 / \text{SVL}]^{\text{bSMA}}$$

226 Where M and SVL are the mass and snout-ventral length of the individual, SVL_0 is the
227 arithmetic mean SVL of the population, and bSMA is the standardized major axis slope from
228 the regression of ln mass on ln SVL for the population (Peig & Green, 2009). Each individual
229 SVL was measured ($\pm 0.01\text{mm}$) using a digital caliper (Lujii 150mm, Omiky) mass was
230 measured using a precision scale (accurate to 0.01g, Smart Weigh ACC200 AccuStar). Body
231 condition was calculated for each individual and groups were compared using an ANOVA test
232 followed up by a *post-hoc* test.

233 Results

234 The colorimetric variables analysis showed no differences between wild and captive
235 animals for brightness, but significant differences ($p < 0.0001$) for hue and chroma were found.

236 The colour distance analyses (Table 1) showed that all visual system tested would be
237 able to detect a noticeable difference when comparing wild individuals from Mangabe and with
238 individuals from Mitsinjo, both founder and F1, on all visual systems used. The individuals
239 from Chester Zoo and the animals from Mangabe had a low or non-detectable difference in the
240 colour distance analyses.

241 Body condition analyses showed a significant difference between the groups ($F = 7.109$, $df =$
242 7 , $p < 0.001$). The *post-hoc* analyses confirmed a significant difference ($p < 0.0001$) between the
243 Mitsinjo group and all other groups. Frogs kept at Mitsinjo had a significantly lower body
244 condition, both founder and F1.

245 Generalized linear mixed models were used to evaluate the effects of body condition
246 on the chroma, and hue variation. Location was included as a random factor (chroma: variance
247 0.38 , St. Dev. ± 0.62 , hue: variance 118.13 , St. Dev. ± 10.86). The selected model with an
248 Akaike information criterion (AIC) of 1195.1 for chroma and AIC of 332.80 for hue, showed
249 that body condition had a strong impact on both chroma ($F = 7.17$, $df = 1$, $p < 0.001$) and hue
250 ($F = 25.83$, $df = 1$, $p < 0.001$).

251

252 Discussion

253 In this study, we showed that different populations of golden mantella frogs vary in
254 colour, most notably between captive and wild conditions. In general, wild frogs were brighter,
255 more colourful and were a different shade of orange/red in comparison to captive frogs,
256 especially those from the captive populations in Madagascar (Figure 1). A relationship
257 between lower body condition and duller colouration was also observed. The implication of

258 the differences observed could be negative survival or lower reproductive success if captive
259 frogs were to be released to the wild (Rojas, 2016).

260 The hue comparison results showed that the golden mantella frogs' skin coloration has
261 been affected by captivity with a significant difference when compared to wild conspecifics.
262 However, during the colour distance analysis, the visual systems used would only have the
263 ability to distinguish between animals kept at Mitsinjo and the other populations. The results
264 showed that, even though, there were significant differences between all populations, most of
265 them would be low or non-detectable by the visual systems tested, with the exception of the
266 Mitsinjo colony. Both founders and F1 at Mitsinjo presented a colouration that differed
267 significantly from their wild counterparts. This shows that the change in the skin coloration is
268 not a generalized effect of captivity since frogs kept at Chester Zoo did not display such a
269 dramatic change.

270 Results showed that the individuals kept at the Mitsinjo breeding centre had a much
271 lower body condition than any other group. Body condition is a result of many variables
272 including nutritional status, stress levels and abiotic variables (MacCracken & Stebbings,
273 2012). Replicating diverse diets in captivity creates a range of challenges including issues of
274 environment, economics and practicality of insect husbandry (the main food item - Livingston
275 et al., 2014). Animals tend to have nutritional imbalances, due to deficiencies of one or several
276 nutrients; for example, low quantities of carotenoids (which are known to affect frog
277 colouration) are common in commercially-reared insects (Finke, 2015).

278 A relationship between body condition and loss of skin coloration was also detected;
279 animals with lower body condition also had a greater difference in skin colouration according
280 to the colour distance analyses. Animal coloration is a product of different variables, including
281 pigments obtained from the diet, such as carotenoids. Dietary carotenoids are associated with
282 yellow, orange, and red coloration and increased levels can lead to brighter coloration and

283 changes in hue (Umbers et al., 2016). The consequences of limited carotenoid availability on
284 ornamental coloration have been shown in both field and captive conditions (Hill, 2009;
285 Brenes-Soto & Dierenfeld, 2014). Healthier animals in good body condition, after meeting
286 their physiological needs of pigments for immune and antioxidant response, can accumulate
287 enough pigments to increase colouration, maximizing sexual display (Hill, 2009). The
288 preference of females for males with brighter colouration shows the choice for phenotypic
289 quality connected with direct or indirect genetic benefits (Brenes-Soto et al., 2017). Striking
290 colouration reveals an individual animal's ability to provide material advantages, such as
291 fertility, high quality territory, nutrition and the maintenance of the genetic variation
292 (Andersson & Simmons, 2006, Zamora-Camacho & Comas, 2019).

293 Previous studies have shown a clear link between skin colouration, body condition and
294 health status in amphibians (Brenes-Soto et al., 2017), with animals with higher levels of
295 glucose and protein concentration in the blood showing a darker yellow and orange colouration
296 (Brenes-Soto et al., 2017). The faded colouration and lower body condition observed on
297 animals kept at Mitsinjo breeding centre could be interpreted as a warning sign of animals'
298 health conditions.

299 Alteration of pigmentation could hypothetically affect potential recognition of breeding
300 partners, perception of fitness, and could thus have an indirect effect on health and reproductive
301 output (Crothers et al., 2011, Brenes-Soto & Dierenfeld, 2014, Ogilvy et al., 2012). All these
302 factors contribute to the complexity of maintaining the frogs' wellbeing in captivity (Speed &
303 Ruxton, 2007).

304 Species recognition is a fundamental problem for animals in social contexts
305 (Kraaijeveld-Smit et al., 2006); skin coloration is also involved in the sexual behaviour of many
306 species. The colour distance analyses using the spectral sensitivity of a diurnal poison frog have

307 shown that frogs would be able to detect colouration differences. Diurnal species of
308 amphibians, such as the golden mantella frogs, use visual signals as an important part of their
309 courtship and mate selection (Mann et al., 2004), and bright colouration is an important one
310 (Bowmaker, 2015). For example, females of different taxa prefer to mate with more colourful
311 or brighter individuals (Maan & Cummings, 2008; Ogilvy et al., 2012). Releasing animals with
312 different skin coloration could, potentially, compromise their breeding opportunities and, for a
313 reintroduction to be successful, individuals released for conservation purposes must not only
314 survive but also must breed (Giligan & Frankham, 2003; Mathews et al., 2005). If reintroduced
315 animals survived, there is a chance that due to this phenotypic difference, captive animals may
316 be more likely to mate with other captive-born animals, which could lead to producing two
317 morphologically separate populations of animals (Slade et al., 2014). A low frequency of
318 breeding between captive-bred and wild animals also means that no improvement of the wild
319 population's genetic diversity and any possible negative genetic changes acquired in captivity
320 would continue to be expressed in their offspring, limiting their fitness in the wild (Slade et al.,
321 2014).

322 Aposematism is an important anti-predator strategy, which signals unprofitability of
323 prey to a predator via conspicuous traits associated with unpalatability (Dreher et al., 2015;),
324 Captive-raised frogs have no alkaloids detectable in skin extracts, these mantelline frogs are
325 dependent on dietary sources for their skin alkaloids (Daly et al., 1997) making them
326 unpalatable. Potential predators for the golden mantella frogs would be reptile species such as
327 *Zonosaurus madagascariensis* and *Tamnosophis lateralis* (Jovanic et al., 2009). The colour
328 distance analysis using a model of snake and a lizard visual system showed significant and
329 highly detectable differences in the skin coloration of frogs from Mistinjo breeding centre in
330 comparison to wild populations, suggesting that predators would be able to perceive these
331 different colourations.

332 The colour distance analyses done using the human visual system (Bowmaker, 2015)
333 demonstrated that keepers would be able to detect the different colouration in the animals they
334 manage from wild conspecifics. This could be used as a measurement to select animals with
335 greater colour similarities to the wild populations for reintroduction purposes. Colour charts
336 are commonly used to evaluate colour scores of animals in zoos (Brenes-Soto & Dierenfeld,
337 2014); although this is a qualitative measurement, a species-specific colouration chart, could
338 be produced and used as a health/management parameter. Frogs kept under optimal condition
339 would have a more similar colouration to their wild counterparts; that is, a health check with
340 less need to handle animals.

341 The *M. aurantiaca* is a critically endangered frog with reintroduction as part of its Species'
342 Action Plan to help mitigate the environmental impacts on the species' natural distribution
343 (Edmonds et al., 2015). It is important to consider the present results when thinking about
344 releasing *M. aurantiaca* back to the wild. The aposematic coloration plays an important role
345 on the behaviour and ecology of many species, making the results presented here important to
346 consider when planning reintroductions. Pre-release assessment should also take into account
347 the physical condition, and include a colour assessment as part of the process.

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494 Figure 1. Examples of skin colouration from the three groups of golden mantellas. A) Wild
495 individuals from Mangabe; B) Chester Zoo off-show individuals; C) Mitisinjo F1
496 individuals.

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