

Colour methods.

Reflectance of the skin was measured using spectrophotometry (Avantes, AvaSoft spectrophotometer, AvaSpec-2048-USB2-UA-50, range 250-1000 nm) with a deuterium-halogen light source and fitted with a fibre-optic probe (probe diameter 1.3 mm). The probe, which was mounted within a metal holder to ensure reading at a constant distance from the surface, was always placed perpendicular to the skin of the animals. All measurements were expressed in relation to a white reference tile (WS2; Avantes). Four points on the body of each animal were sampled: One mid-dorsally on the head directly posterior to the eyes, one on the center of the dewlap and a central point on both flanks. These points were chosen to reflect the overall coloration of the animals' body. Reflectance spectra were calculated automatically by the software (AvaSpec75USB2).

Each of our reflectance spectra originally comprised 690 data points (0.59 nm reflectance intervals from 300 to 700 nm). Reflectance data were grouped into 10 nm bins resulting in mean values for 40 bins ranging from 300 to 700 nm. Principal component analysis (PCA) was used to reduce the number of variables for the reflectance data. Additionally, six commonly used reflectance indices were calculated from the reflectance spectra and these were then correlated with the principal component scores received from the principal component analyses of the four points measured (Griggio et al., 2009): mean brightness (the mean percentage of reflectance from 300 to 700 nm), UV chroma ($R_{300-400}/R_{300-700}$), blue chroma ($R_{400-475}/R_{300-700}$), green chroma ($R_{475-550}/R_{300-700}$), yellow chroma ($R_{550-625}/R_{300-700}$) and red chroma ($R_{625-700}/R_{300-700}$) (Endler, 1990; Montgomerie, 2006). In analyses of spectral data one of the PC's inevitably represents brightness variation and other PCs represent color variation (Cuthill et al., 1999). A PCA was run for the three points on the body (head, flanks and dewlap). The reflectance data obtained from the left and right flank were averaged. Components with eigenvalues > 1.5 were retained and one-way repeated measures ANOVAs were carried out on the component scores to test for statistical differences between treatments.

Colour results

In all three body parts measured, the second principal component (Eigenvalue = 26.63, 22.19% of variance explained) correlated positively with reflectance at almost all wavelengths (table 3, appendix) and the scores were positively correlated with the mean brightness colour chroma (Pearson correlation: heads 0.712, flanks 0.623 and dewlap 0.437). The second principal axis can therefore be considered a 'brightness' axis. The brightness of the head, flank and dewlap colours exhibited significant variation among treatments (fig. 1 appendix, $F_{2,58} = 22.02$, $P < 0.001$), the change in brightness was similar between sexes (sex*treatment-effect: $F_{2,58} = 0.46$, $P = 0.64$), but males did have an overall higher brightness than females (sex-effect: $F_{1,29} = 7.14$, $P < 0.05$). Brightness was lower in the "acclimatisation" period compared to the "small cage" (post-hoc, $P < 0.001$) and the "large cage" treatments ($P < 0.001$). Brightness did not differ between the "small cage" and "large cage" treatment ($P = 1$).

The first principal component (Eigenvalue = 35.03, 29.19% of variance explained) correlated positively with reflectances at almost all wavelengths for the flanks and the dewlap (table 3, appendix), indicating that the second component represents colour variation in the flanks and dewlap. The colouration of the flanks and dewlap exhibited significant variation among treatments (fig. 1B, C, appendix $F_{1,49,43,12} = 7.12$, $P < 0.01$). The scores for PC1 were found to be lower in the "acclimatisation" period compared to the "small cage" ($P < 0.001$) and "large cage" ($P < 0.05$)

treatments. The scores did not differ between the “small cage” and “large cage” treatments ($P = 0.83$). the changes in colouration were similar between sexes (sex*treatment-effect: $F_{1,49,43.12} = 3.04$, $P = 0.07$), but males did have lower scores than females (sex-effect: $F_{1,29} = 7.14$, $P < 0.05$). When these scores are correlated to the previously mentioned colour chromas, we find that they are negatively correlated with the green (-0.243) and yellow (-0.279) chromas for the flanks and with the UV-chroma (-0.274) for the dewlap. This indicates that the flanks reflected more green and yellow in the “acclimatisation” period, while the dewlap reflected more UV.

The third principal component (Eigenvalue = 19.6, 16.3% of variance explained) correlated positively with reflectances between 500 and 650 nm for the head, indicating that the third component represents colour variation in the heads. Also here did the colouration show significant variation among the treatments (fig. 1A appendix, $F_{2,58} = 5.24$, $P < 0.01$). The scores for PC3 were found to be higher in the “acclimatisation” period compared to the “small cage” treatment ($P < 0.01$) and higher but not significant compared to the “large cage” ($P = 0.06$) treatment. The scores did not differ between the “small cage” and “large cage” treatments ($P=1$). the changes in colouration differed between sexes (sex*treatment-effect: $F_{2,58} = 4.45$, $P < 0.05$), and males had lower scores than females (sex-effect: $F_{1,29} = 21.91$, $P < 0.001$). When these scores are correlated to the previously mentioned colour chromas, we find that they are positively correlated with the green (0.342) and yellow (0.354) chromas. This indicates that the heads also reflected more green and yellow in the “acclimation” period.

Table 3. Component matrix colour analysis.

Head	PC1	PC2	PC3	Flanks	PC1	PC2	PC3	Dewlap	PC1	PC2	PC3
H300-310	-0.113	0.703	0.023	F300-310	0.557	0.433	-0.346	D300-310	-0.111	0.68	0.071
H310-320	-0.082	0.718	-0.042	F310-320	0.632	0.4	-0.35	D310-320	-0.123	0.683	0.035
H320-330	-0.108	0.8	-0.077	F320-330	0.804	0.182	-0.381	D320-330	-0.204	0.687	-0.128
H330-340	-0.129	0.865	-0.113	F330-340	0.847	0.081	-0.357	D330-340	0.037	0.755	-0.024
H340-350	-0.135	0.866	-0.113	F340-350	0.861	0.065	-0.34	D340-350	0.214	0.752	0.086
H350-360	-0.132	0.866	-0.107	F350-360	0.862	0.067	-0.338	D350-360	0.25	0.749	0.124
H360-370	-0.158	0.867	-0.086	F360-370	0.864	0.068	-0.337	D360-370	0.266	0.739	0.17
H370-380	-0.165	0.874	-0.081	F370-380	0.864	0.072	-0.338	D370-380	0.297	0.725	0.222
H380-390	-0.177	0.877	-0.066	F380-390	0.867	0.079	-0.339	D380-390	0.333	0.695	0.31
H390-400	-0.194	0.888	-0.049	F390-400	0.873	0.089	-0.339	D390-400	0.389	0.644	0.409
H400-410	-0.192	0.91	-0.05	F400-410	0.881	0.107	-0.343	D400-410	0.451	0.565	0.502
H410-420	-0.199	0.931	-0.049	F410-420	0.887	0.128	-0.354	D410-420	0.501	0.465	0.588
H420-430	-0.215	0.931	-0.054	F420-430	0.892	0.147	-0.362	D420-430	0.537	0.372	0.638
H430-440	-0.252	0.915	-0.039	F430-440	0.896	0.158	-0.366	D430-440	0.539	0.303	0.684
H440-450	-0.257	0.896	-0.046	F440-450	0.897	0.164	-0.363	D440-450	0.566	0.24	0.694
H450-460	-0.281	0.875	-0.045	F450-460	0.896	0.168	-0.362	D450-460	0.577	0.193	0.703
H460-470	-0.281	0.88	-0.022	F460-470	0.896	0.178	-0.358	D460-470	0.576	0.164	0.712
H470-480	-0.282	0.879	-0.005	F470-480	0.895	0.183	-0.355	D470-480	0.583	0.128	0.715
H480-490	-0.29	0.868	0.008	F480-490	0.891	0.186	-0.353	D480-490	0.583	0.099	0.718
H490-500	-0.293	0.88	0.049	F490-500	0.889	0.201	-0.343	D490-500	0.573	0.077	0.73
H500-510	-0.244	0.862	0.121	F500-510	0.875	0.214	-0.331	D500-510	0.582	0.05	0.725
H510-520	-0.233	0.725	0.234	F510-520	0.817	0.224	-0.291	D510-520	0.568	0.018	0.736
H520-530	-0.246	0.575	0.296	F520-530	0.714	0.209	-0.224	D520-530	0.552	-0.003	0.744
H530-540	-0.235	0.478	0.31	F530-540	0.634	0.176	-0.177	D530-540	0.561	-0.023	0.737
H540-550	-0.252	0.428	0.349	F540-550	0.57	0.162	-0.14	D540-550	0.538	-0.024	0.756
H550-560	-0.232	0.404	0.343	F550-560	0.593	0.144	-0.133	D550-560	0.561	-0.037	0.752
H560-570	-0.233	0.389	0.346	F560-570	0.622	0.126	-0.143	D560-570	0.566	-0.054	0.767
H570-580	-0.238	0.389	0.351	F570-580	0.665	0.123	-0.165	D570-580	0.563	-0.077	0.788
H580-590	-0.252	0.389	0.359	F580-590	0.705	0.117	-0.181	D580-590	0.529	-0.114	0.806
H590-600	-0.272	0.39	0.354	F590-600	0.754	0.107	-0.209	D590-600	0.485	-0.158	0.803
H600-610	-0.314	0.411	0.334	F600-610	0.805	0.107	-0.249	D600-610	0.458	-0.188	0.776
H610-620	-0.36	0.439	0.284	F610-620	0.829	0.108	-0.282	D610-620	0.42	-0.208	0.755
H620-630	-0.379	0.461	0.187	F620-630	0.823	0.106	-0.317	D620-630	0.402	-0.223	0.717
H630-640	-0.377	0.448	0.073	F630-640	0.802	0.1	-0.331	D630-640	0.374	-0.225	0.675
H640-650	-0.345	0.408	-0.029	F640-650	0.771	0.093	-0.333	D640-650	0.342	-0.222	0.628
H650-660	-0.302	0.355	-0.106	F650-660	0.737	0.085	-0.331	D650-660	0.318	-0.22	0.567
H660-670	-0.26	0.306	-0.157	F660-670	0.696	0.077	-0.319	D660-670	0.28	-0.209	0.508
H670-680	-0.221	0.266	-0.193	F670-680	0.658	0.069	-0.308	D670-680	0.25	-0.197	0.445
H680-690	-0.186	0.23	-0.217	F680-690	0.621	0.061	-0.293	D680-690	0.218	-0.19	0.381
H690-700	-0.154	0.206	-0.231	F690-700	0.589	0.055	-0.279	D690-700	0.192	-0.172	0.321

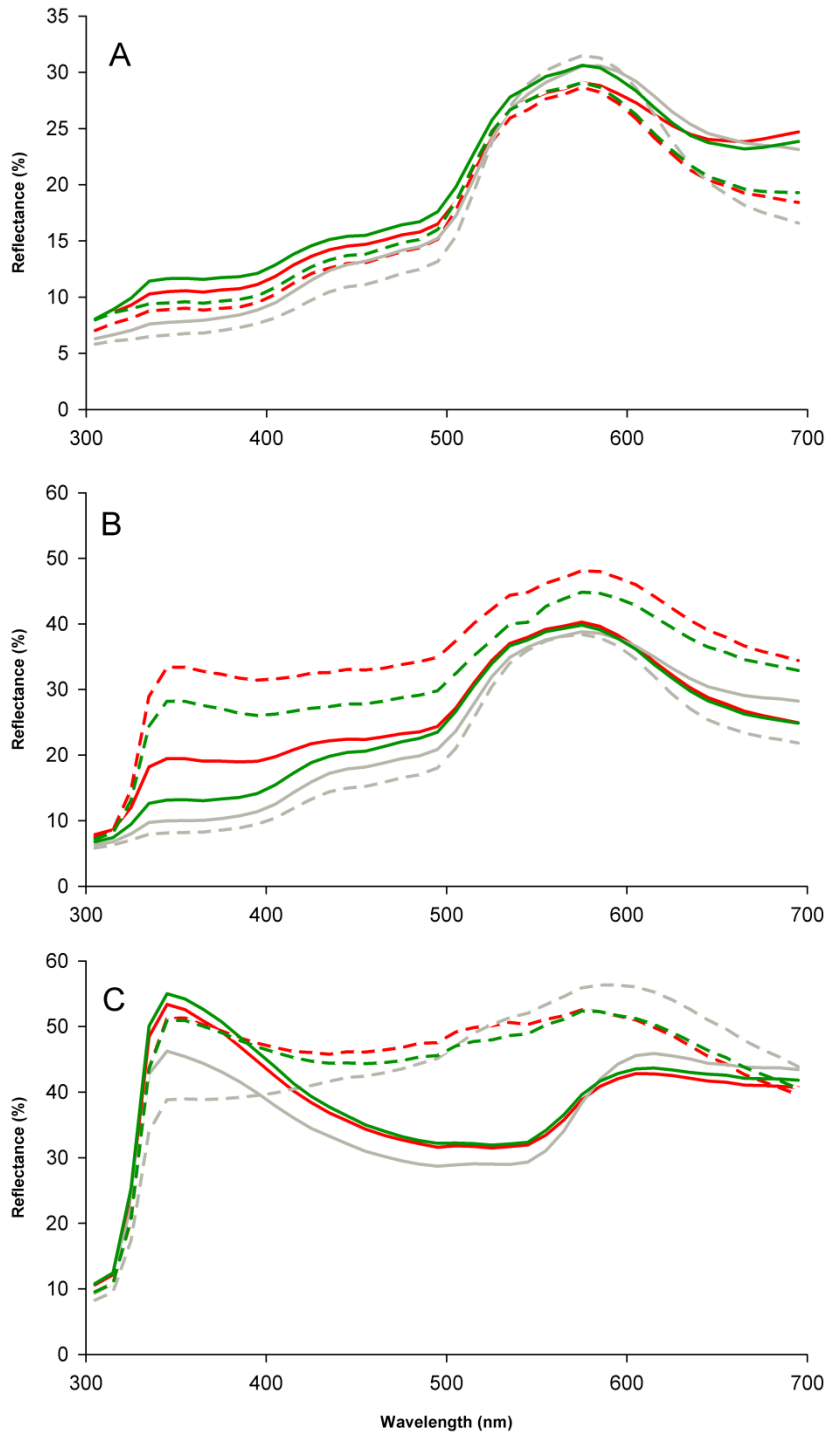


Figure 1.
reflection
the head

(B) and the dewlap (C) of *Anolis carolinensis* lizards in the “acclimatisation” period (grey lines), the small cage (red lines) and the large cage treatments (green lines). Values for males (full lines) and females (dashed lines) are shown separately

Average
of the skin of
(A), the flanks