Colour methods.

Reflectance of the skin was measured using spectrophotometry (Avantes, Avasoft spectrophotometer, AvaSpec-2048-USB2-UA-50, range 250-1,000 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 ($R_{300\text{-}400}/R_{300\text{-}700}$), blue chroma ($R_{400\text{-}475}/R_{300\text{-}700}$), green chroma ($R_{475\text{-}550}/R_{300\text{-}700}$), yellow chroma ($R_{550\text{-}625}$/$R_{300\text{-}700}$) and red chroma ($R_{625\text{-}700}/R_{300\text{-}700}$) (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 first principal component (Eigenvalue = 32.003, % of variance = 26.67) correlated positively with reflectance at all wavelengths (Table 3, appendix) and the scores were positively correlated with the mean reflectance colour chroma (Pearson correlation: heads 0.486, flanks 0.512 and dewlap 0.342). The first principal axis can therefore be considered a 'brightness' axis. The brightness of the head, flank and dewlap colours exhibited significant variation among treatments (Figure 1 Appendix, $F_{2,62} = 43.77$, $P < 0.001$), but not between sexes (sex-effect: $F_{1,31} = 0.110$, $P = 0.742$; sex × treatment-effect: $F_{2,62} = 1.985$, $P = 0.146$). Brightness was lower in the 'acclimatisation' period than in the 'manipulation' (*post hoc*, $P < 0.001$) and the 'no manipulation' treatment ($P < 0.001$). Brightness was slightly higher in the 'manipulation' treatment but the difference was not significant ($P = 0.086$).

The second principal component (Eigenvalue = 22.3, % of variance = 18.58) correlated positively with reflectances at almost all wavelengths for the dewlap, indicating that the second component represents colour variation in the dewlap. There were no significant differences between the treatments for the colouration of the dewlaps (Figure 1C Appendix, $F_{2,62} = 0.962$, $P = 0.388$). We did find a significant difference for the colouration of the dewlap between both sexes ($F_{1,31} = 51.486$, $P < 0.001$).

The third principal component (Eigenvalue = 19.32, % of variance = 16.1) correlated positively with reflectances at almost all wavelengths for the head and negatively with reflectances for the flanks, indicating that the third component represents colour variation in the heads and flanks. Also, here there were no significant differences between the treatments for the colouration of the heads and flanks (Figure 1A and 1B Appendix, $F_{2,62} = 1.985$, $P = 0.954$).

Table 3. component matrix colour analysis.

Head	PC1	PC2	PC3	Flanks	PC1	PC2	PC3	Dewlap	PC1	PC2	PC3
H300-310	0,547	-0,344	0,315	F300-310	0,619	0,106	-0,131	D300-310	0,264	-0,125	0,007
H310-320	0,562	-0,370	0,301	F310-320	0,651	0,113	-0,135	D310-320	0,266	-0,160	-0,053
H320-330	0,617	-0,416	0,294	F320-330	0,759	0,088	-0,273	D320-330	0,271	-0,360	-0,228
H330-340	0,651	-0,468	0,292	F330-340	0,769	0,059	-0,338	D330-340	0,419	-0,237	-0,090
H340-350	0,653	-0,470	0,290	F340-350	0,765	0,061	-0,342	D340-350	0,486	-0,117	0,031
H350-360	0,653	-0,468	0,288	F350-360	0,768	0,066	-0,335	D350-360	0,504	-0,078	0,068
H360-370	0,656	-0,475	0,281	F360-370	0,774	0,061	-0,336	D360-370	0,512	-0,046	0,097
H370-380	0,663	-0,475	0,275	F370-380	0,781	0,067	-0,335	D370-380	0,529	0,009	0,139
H380-390	0,674	-0,476	0,268	F380-390	0,791	0,071	-0,336	D380-390	0,545	0,099	0,205
H390-400	0,685	-0,472	0,271	F390-400	0,804	0,079	-0,344	D390-400	0,554	0,219	0,293
H400-410	0,699	-0,475	0,287	F400-410	0,825	0,096	-0,367	D400-410	0,542	0,352	0,387
H410-420	0,715	-0,483	0,319	F410-420	0,844	0,13	-0,392	D410-420	0,505	0,481	0,470
H420-430	0,713	-0,494	0,355	F420-430	0,847	0,159	-0,407	D420-430	0,449	0,571	0,520
H430-440	0,692	-0,510	0,374	F430-440	0,837	0,172	-0,412	D430-440	0,403	0,618	0,541
H440-450	0,674	-0,508	0,375	F440-450	0,827	0,184	-0,402	D440-450	0,366	0,657	0,544
H450-460	0,662	-0,501	0,368	F450-460	0,82	0,188	-0,396	D450-460	0,338	0,683	0,540
H460-470	0,661	-0,502	0,381	F460-470	0,823	0,187	-0,403	D460-470	0,318	0,696	0,541
H470-480	0,647	-0,506	0,402	F470-480	0,818	0,195	-0,417	D470-480	0,291	0,711	0,540
H480-490	0,633	-0,503	0,413	F480-490	0,812	0,199	-0,421	D480-490	0,270	0,722	0,538
H490-500	0,638	-0,500	0,449	F490-500	0,817	0,199	-0,441	D490-500	0,255	0,729	0,537
H500-510	0,600	-0,489	0,545	F500-510	0,797	0,192	-0,483	D500-510	0,236	0,734	0,541
H510-520	0,450	-0,421	0,621	F510-520	0,716	0,187	-0,504	D510-520	0,218	0,737	0,543
H520-530	0,284	-0,350	0,626	F520-530	0,608	0,193	-0,506	D520-530	0,201	0,737	0,544
H530-540	0,184	-0,300	0,617	F530-540	0,531	0,202	-0,498	D530-540	0,189	0,738	0,545
H540-550	0,136	-0,275	0,617	F540-550	0,503	0,214	-0,495	D540-550	0,183	0,742	0,546
H550-560	0,121	-0,259	0,614	F550-560	0,496	0,229	-0,49	D550-560	0,178	0,758	0,539
H560-570	0,116	-0,254	0,616	F560-570	0,501	0,238	-0,492	D560-570	0,176	0,783	0,528
H570-580	0,117	-0,262	0,620	F570-580	0,513	0,242	-0,499	D570-580	0,170	0,812	0,507
H580-590	0,124	-0,269	0,625	F580-590	0,53	0,248	-0,504	D580-590	0,151	0,829	0,471
H590-600	0,141	-0,283	0,634	F590-600	0,552	0,257	-0,505	D590-600	0,123	0,820	0,422
H600-610	0,183	-0,310	0,643	F600-610	0,579	0,261	-0,495	D600-610	0,097	0,801	0,367
H610-620	0,244	-0,348	0,623	F610-620	0,595	0,254	-0,461	D610-620	0,081	0,779	0,318
H620-630	0,300	-0,380	0,548	F620-630	0,584	0,232	-0,397	D620-630	0,074	0,755	0,266
H630-640	0,323	-0,391	0,429	F630-640	0,545	0,206	-0,326	D630-640	0,071	0,725	0,216
H640-650	0,317	-0,379	0,293	F640-650	0,493	0,174	-0,255	D640-650	0,073	0,689	0,164
H650-660	0,288	-0,355	0,172	F650-660	0,435	0,143	-0,186	D650-660	0,070	0,644	0,109
H660-670	0,261	-0,322	0,070	F660-670	0,379	0,111	-0,122	D660-670	0,070	0,593	0,056
H670-680	0,235	-0,293	-0,009	F670-680	0,33	0,075	-0,064	D670-680	0,070	0,537	0,009
H680-690	0,219	-0,262	-0,074	F680-690	0,286	0,042	-0,011	D680-690	0,069	0,478	-0,036
H690-700	0,209	-0,233	-0,125	F690-700	0,252	0,014	0,032	D690-700	0,072	0,424	-0,075

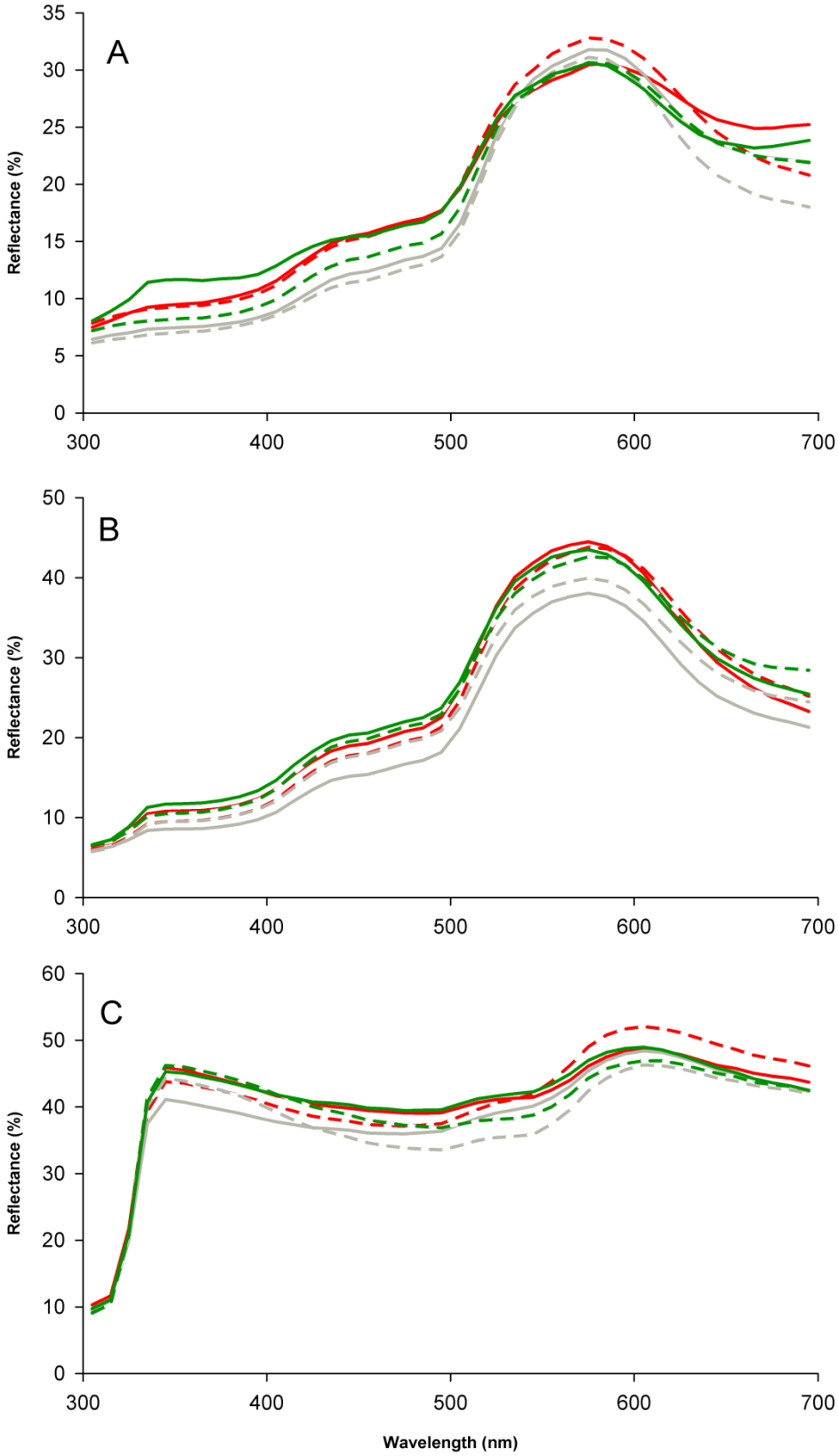

Figure 1 Average reflection of the skin of the head (A), the flanks (B) and the dewlap (C) of *Anolis carolinensis* lizards in the 'acclimatisation' period (grey lines), the 'handled' treatment (red lines) and the 'unhandled' treatment (green lines). Values for males (full lines) and females (dashed lines) are shown separately