Canadian Journal of Zoology
Revue canadienne de zoologie

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Journal:	<i>Canadian Journal of Zoology</i>
Manuscript ID	cjz-2016-0200.R1
Manuscript Type:	Note
Date Submitted by the Author:	27-Oct-2016
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Keyword:	Anolis [Norops] aquaticus, aquatic anole, cryptic, metachrosis, physiological color change, STRESS < Organ System

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October 27, 2016

Revised manuscript for consideration as a Note in the *Canadian Journal of Zoology*

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Rapid body color brightening is associated with exposure to a stressor in an *Anolis* lizard

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Abstract

Many species use color change to optimize body coloration to changing environmental conditions, and drivers of rapid color change in natural populations are numerous and poorly understood. We examined factors influencing body coloration in *Anolis aquaticus* (Taylor, 1956), a lizard possessing color-changing stripes along the length of its body. We quantified the color of three body regions (the eye stripe, lateral stripe, and dorsum) before and after exposure to a mild stressor (handling and restraint). Based on current understanding of the *Anolis* genus, we hypothesized that exposure to a stressor would generate genus-typical skin darkening (i.e., increased melanism). Contrary to expectations, stress consistently brightened body coloration: eye and lateral stripes transitioned from brown to pale blue and green, and the dorsum became lighter brown. Sex, size, and body temperature did not correlate with any aspect of body coloration, and a laboratory experiment confirmed that light exposure did not drive brightening. We propose that color change may serve to reduce conspicuousness through disruptive camouflage; lizards tended to display brighter stripes on mottled green-brown substrates. Together, these results improve our understanding of *Anolis* color change diversity and emphasize the need for a broader interpretation of the mechanism and functions of color change across taxa.

Keywords: *Anolis* [*Norops*] *aquaticus*, *aquatic anole*, *cryptic*, *metachrosis*, *physiological color change*, *stress*

Introduction

Body color plasticity plays a vital role in predator avoidance, signaling, and thermoregulation across animal taxa (see reviews by Caro 2005; Stuart-Fox and Moussalli 2009; Sköld et al. 2013; Stevens 2016). In particular, to rapidly respond to quickly changing conditions, individuals of many species (including fish, reptiles, amphibians, and arthropods) reversibly alter their body coloration in seconds to minutes (physiological color change; Thurman 1988). These rapid changes in body coloration permit individuals to dynamically respond to predators (e.g., Stuart-Fox and Moussalli 2008; Langridge 2009; Kronstadt et al. 2013), abiotic challenges (e.g., Fulgione et al. 2014; Smith et al. 2016), prey availability (e.g., Anderson and Dodson 2015), and conspecifics (e.g., Umbers et al. 2013; Ligon 2014; Maruska 2015). Together, these color changes offer insight into, and have sometimes been used as proxies of, physiological or cognitive state (e.g., Yang et al. 2001; Greenberg 2002; Plavicki et al. 2004; Adamo et al. 2006).

Lizards within the *Anolis* genus have historically attracted attention for their ability to change from a bright green to dark brown within only a few minutes (e.g., Parker and Starratt 1904; Kleinholz 1936). Rapid color change in *Anolis* continues to be a topic of interest because of its broad relevance to physiological ecology and the evolution of animal signals (Plavicki et al. 2004; Yabuta and Suzuki-Watanabe 2011; Wilczynski et al. 2015). The green-to-brown transition is induced by the stimulation of melanophores, cells that contain melanin pigments (Hadley and Goldman 1969; Vaughan 1987). Melanophores are found below xanthophores (containing yellow pigmentary organelles, pterinosomes) and iridophores (containing reflecting platelets responsible for blue-green coloration) in *Anolis* skin. When melanophores are stimulated, melanin migrates upward and surrounds xanthophores and iridophores, effectively blocking light to these cells and resulting in a visible skin coloration of brown or black; re-

aggregation of melanin within the melanophores returns skin to a green coloration (Taylor and Hadley 1970).

The environmental factors inducing color change in *Anolis* body coloration are numerous and synergistic: experiments on excised portions of *A. carolinensis* skin demonstrate that green-to-brown color change occurs in response to increased light exposure and brown-to-green color change occurs in response to onset of total darkness (Hadley and Goldman 1969; Vaughan 1987). Relatedly, diel timing strongly correlates with *Anolis* color: most anoles are green at night, but their color is more variable and somewhat substrate-matching during the day (Gordon and Fox 1960). Temperature likewise plays a role, with warmer lizards generally greener and cooler lizard browner (Claussen and Art 1981; but see Yabuta and Suzuki-Watanabe 2011); early work on *Anolis* also suggests that light exposure moderates coloration at intermediate temperatures (Parker and Starratt 1904).

Anolis body coloration is directly influenced by stressors and serves as a conspecific signal (Greenberg et al. 1984; Greenberg and Crews 1990; Summers and Greenberg 1994; Wilczynski et al. 2015). Current understanding of *Anolis* physiology suggests that color changes are exclusively due to circulating hormones and lack direct neural control. The physiological stress response, i.e., the activation of hormonal secretion pathways that allow individuals to cope with acute stressors, induces a rapid shift from green to brown in *A. carolinensis* (reviewed in Greenberg 2003). In staged laboratory contests, subordinate male anoles turned brown within 30 to 40 minutes of being paired with new rivals (Wilczynski et al. 2015), maintained brown coloration for the duration of their interaction (Plavicki et al. 2004), and have higher circulating corticosterone (Greenberg et al. 1984) and lower androgen (Greenberg and Crews 1990) concentrations. For these reasons, it has generally been assumed that brown coloration

corresponds to stress and subordination, whereas green coloration is a sign of social dominance.

We examined the effect of a mild stressor (handling and temporary restraint in a well-lit environment) on rapid physiological color change in *Anolis aquaticus* (Taylor, 1956), a semi-aquatic anole with color-changing eye stripes and lateral stripes, with the expectation that stressors would lead to genus-typical skin darkening. To isolate the potential effect of one component of the stressor, we additionally tested how light exposure influences color change in a controlled laboratory experiment.

Methods

This study was conducted from 30 June to 22 July 2015 at the Las Cruces Biological Station (LCBS), Coto Brus County, Costa Rica. *Anolis aquaticus* is found along small streams on lowland and pre-montane slopes in southwestern Costa Rica and northwestern Panama (Savage 2005). This medium-sized lizard (adult snout-vent length: 52 to 77 mm; Márquez and Márquez 2009) bears darker and lighter brown stripes with small yellow spots on its dorsum. Two stripes, an eye stripe and a lateral stripe, each 2 to 4 mm wide, run the length of the body (Fig. 1). An eye stripe is found below each eye, beginning behind the tip of the snout, bordering the upper mouth, and diminishing past the ear. The lateral stripe is irregularly shaped, beginning behind the lower jaw and running, more or less continuously, to the hind limbs on both sides of the body. *A. aquaticus* adults and juveniles of both sexes can be found with either dark (brown) (Fig. 1a) or bright (blue and green) (Fig. 1b) eye and lateral stripes.

Field data collection

Upon sighting *A. aquaticus* in the field, we obtained body temperatures using an infrared thermometer (IRT) (eT650D, EnnoLogic, Eugene, Oregon, USA) following the methods of Hare et al. (2007); in brief, the IRT was held in-line with the lizard's body axis and the laser pointer was directed at the center of the lizard's dorsal posterior abdomen, at an approximate IRT-to-lizard distance of 20 to 30 cm as per the manufacturer's recommendations. The lizard was then captured by hand or noose, and was immediately photographed using a Sony NEX-3 digital camera (Sony Corp., Thailand) with the camera flash on under canopy cover to standardize field lighting conditions to the extent possible (as per Calisi and Hews 2007). The camera was set to manual with an ISO of 800, shutter speed of 30, aperture of F22, and 14.20-megapixel resolution. Each lizard was positioned against a white background exactly 20 cm from the camera lens, which faced directly down. Two photographs were taken in succession: 1) a dorsal photograph, in which the lizard was held at the base of its tail with its ventral surface flat against the background and 2) a lateral photograph, in which the lizard was held on its right side so that the left eye stripe and lateral stripe were visible and parallel with the background.

To subject all lizards to a standardized mild stressor, after capture each lizard (N = 31; 12 males and 19 females) was placed into a mesh cotton bag and relocated to a well-lit, warm surface (~ 25 °C) in the immediate vicinity of its capture location for 240 seconds. Immediately thereafter, we returned the lizard to the original (shaded) photography location and took a second set of photographs (dorsal and lateral, as described). All photographs were saved as RAW files to prevent the loss of information (Stevens et al. 2007). We recorded sex and measured snout-vent length (SVL) with a clear plastic ruler. Each lizard was then released at its location of capture after being given individual identification markings at the base of its tail with nail polish.

Manipulation of lighting environment

A separate set of 30 lizards was collected from the field and transported to the LCBS laboratory to examine the effect of lighting environment on coloration. Following transport, lizards were placed into individual experimental arenas: plastic aquaria (30 x 19 x 20.5 cm, $l \times w \times h$) that were secured at the top with window screening and furnished with a petri dish of water. Arena walls were lined with black paper, and white paper was glued to the arena floors. The experiment was conducted in a windowless, temperature-controlled room (21° C) that was lit by two full spectrum daylight fluorescent bulbs (F32T8 TL865 ALTO Plus, Philips Lighting, Somerset, NJ, USA) on a 12 h light cycle (6:00 to 18:00 h). All lizards experienced identical lighting conditions (total darkness) overnight. At 08:00 h, before manipulating the lighting environment, each lizard was photographed dorsally and laterally in the experimental room using handling procedures and photographic backgrounds described previously. Half of the lizards ($n = 15$) were then randomly selected for the dark treatment, and the other half ($n = 15$) was selected for the light exposure treatment. To simulate dark conditions (such as those experienced when lizards are in rock crevices in their natural environments), the enclosures holding lizards in the dark treatment were covered with heavy cardboard, resulting in almost complete darkness within the enclosure. The enclosures containing lizards in the light treatment remained uncovered. Lizards were left undisturbed in these conditions for 6 hours. Lizards' lighting environments were maintained until immediately before each lizard was removed for its second round of photographs. Each lizard was photographed within less than 20 seconds of being removed from its respective treatment, and no detectable color change was noted during this time. Lizards were then given nail polish identification marks at the base of the tail and released at their sites of capture.

Our work adhered to the *Guide to the Care and Use of Experimental Animals* (Canadian Council on Animal Care, 1993). Use of animals was reviewed and approved by the Organization for Tropical Studies, and research permits were obtained from the Ministry of the Environment and Energy, Republic of Costa Rica (SINAC-SE-GASP-PI-R-092-2015).

Image analysis

Digital photography as a method of studying animal coloration provides several advantages but requires careful interpretation (for recent reviews, see Hutton et al. 2015; Kemp et al. 2015; Troscianko and Stevens 2015; Johnsen 2016). The aims of our study (namely, quantifying differences in color brightness) could be achieved using digital photography within the human-visible color range, similar to its use in a variety of other recent studies that quantify basic differences in color (e.g., Sowersby et al. 2015; Escudero et al. 2016; Keren-Rotem et al. 2016). However, we do not extrapolate these results to *Anolis* color vision or that of its predators, as anoles are known to use and perceive ultraviolet colors (Loew et al. 2002). Therefore, our results solely quantify color variation within the human-visible color range.

We ensured that all photographs were standardized during photo capture (as described previously) and during post-processing according to recent recommendations (Johnsen 2016). Prior to analysis, all images were converted to uncompressed TIFF files and image brightness was calibrated in ImageJ (Schneider et al. 2012) using the background standard (Calisi and Hews 2007). To quantify dorsal coloration, an ellipse was drawn on the dorsum using the “oval” tool. The eye stripe and lateral stripe were traced with the “freehand” tool; we then obtained the % luminance (i.e., brightness), % saturation (i.e., chroma, or deviation from a neutral grey tone) and hue (in degrees) of each of these three locations.

Statistical analyses

All continuous variables were log-10 transformed prior to analyses to better fit the assumptions of parametric tests. We examined whether sex, SVL, and body temperature affected the luminance, saturation, or hue of the dorsum, eye stripe, and lateral stripe using separate linear models; these measurements were obtained from the first set of photographs taken immediately after field capture. We then used repeated measures analyses of variance (ANOVA) to 1) quantify differences in the luminance, saturation, and hue of each body region (dorsum, eye stripe, and lateral stripe) following stressor-induced rapid color change and 2) determine if the degree of this color change was sex dependent. Luminance, saturation, or hue were dependent variables in each model, sex was a categorical predictor variable, and time (pre- or post-stressor) was the within-subject factor.

Using repeated-measures ANOVA, we examined the effect of light exposure on body coloration in our laboratory experiment. Our repeated dependent variables were the luminance, saturation, or hue of each body region, and predictors included a between-subject factor (treatment: light vs. dark), a within-subject factor (time: before and after), and their interaction.

Data were analyzed using R (R Foundation for Statistical Computing, Vienna, Austria). All tests were two-tailed, and alpha was set at 0.05.

Results

Linear models indicate that the luminance, saturation, and hue of the dorsum, eye stripes and lateral stripes were not influenced by sex, SVL, or body temperature (all $P > 0.135$) in our field survey. Following exposure to a mild stressor, each of the three body regions that we examined

brightened substantially (Table 1). Without exception, the % luminance of the eye stripe and lateral stripe of every lizard in our survey increased after exposure to the mild stressor (Fig. 2). Differences in dorsal % luminance varied somewhat in direction among individuals, though on average also increased after stressor exposure (Fig. 2). In addition, the eye stripe and lateral stripe, but not the dorsum, experienced a post-stressor increase in hue towards green-blue colors (Table 1). Changes in coloration were not affected by sex ($P > 0.136$ for all sex * time interactions; Table 1).

The % luminance of the dorsum ($F_{1,28} = 20.859$, $P < 0.001$) and lateral stripe ($F_{1,28} = 6.015$, $P = 0.021$), but not the eye stripe ($F_{1,28} = 2.128$, $P = 0.156$), increased with time during our laboratory experiment. There was no effect of lighting environment on the % luminance of the dorsum ($F_{1,28} = 0.006$, $P = 0.936$; Fig. 3a) or eye stripe ($F_{1,28} = 0.571$, $P = 0.456$; Fig. 3b), though a non-significant trend suggests an influence of treatment on lateral stripe % luminance ($F_{1,28} = 3.700$, $P = 0.064$): lateral stripes were somewhat brighter in the dark treatment than in the light exposure treatment (Fig. 3c). Saturation (%) and hue were not affected by time (saturation: all $P > 0.253$; hue: all $P > 0.171$) or treatment (saturation: all $P > 0.428$; hue: all $P > 0.288$).

Discussion

We examined correlates of the coloration of three body regions (dorsum, eye stripe, and lateral stripe) of a species within the well-studied *Anolis* genus to address hypotheses regarding the causes of rapid changes in body coloration. We demonstrate that the brightness (luminance) of all three body regions increases rapidly following exposure to a mild stressor (i.e., handling and restraint) in *A. aquaticus*. Hue also changed rapidly: from more uniform brown or dark colors,

eye stripes became white-blue and lateral stripes became green. These atypical directional changes even occurred in individuals that originally exhibited brighter colors (e.g., Fig. 1b) upon initial capture. Sex, SVL, and body temperature did not correlate with any measure of color in our field study. Contrary to expectations based on our field observations, in which body color brightening correlated with well-lit environments, a controlled laboratory experiment in which lighting environment was manipulated suggested that darkness, not light, produces somewhat brighter lateral stripe colors.

To consistently elicit a physiological stress response, our standardized stressor included handling, restraint, and temporary relocation into a warmer and more well-lit environment. Our results unexpectedly suggest that the physiological stress response correlates with brighter coloration in this species. Every *A. aquaticus* individual exposed to the stressor increased the % luminance and hue of the eye stripe and lateral stripe between their initial state at capture (i.e., prior to an anticipated increase in plasma corticosterone and therefore a pre-stress measurement; Moore 1991) and their final state (i.e., following field manipulation, which correlates with an increased plasma stress hormone concentrations in other species; Matt et al. 1997). The relationship of stress and brightness exhibited in *A. aquaticus* is therefore at odds with the accepted paradigm in which stress induces dark coloration (reviewed by Greenberg 2002, 2003 for *A. carolinensis*). Glucocorticoids (corticosterone) and stress-relevant catecholamines are elevated following exposure to a stressor, and both are implicated in the darkening of body coloration (Greenberg et al. 1984; Greenberg and Crews 1990; Summers and Greenberg 1994) through co-production of melanocyte-stimulating hormone (MSH) and corticotropin (ACTH) (Hadley and Goldman 1969). Elevated corticosterone concentrations can also increase rates of synthesis of two stress-relevant catecholamines, epinephrine and norepinephrine (Greenberg

2002). Using *in vitro* experiments, Hadley and Goldman (1969) demonstrated that these catecholamines cause melanin to aggregate (i.e., cause color brightening) or disperse (become darker) from melanophores depending on the initial state of the skin: green skin turns brown, and “somewhat dark” skin may brighten to mottled green. However, *A. aquaticus* with bright eye and lateral stripes never adopted dark coloration following capture in our study, but consistently became brighter green-blue regardless of their initial coloration.

In *A. aquaticus*, MSH, epinephrine, and norepinephrine may interact with receptors along the eye stripe and lateral stripe to cause skin brightening. Although MSH produces melanin dispersion, two types of receptors (alpha- and beta-adrenergic receptors) modulate its effects on skin coloration (Hadley and Goldman 1969; Goldman and Hadley 1970); stimulation of alpha-receptors can override MSH-induced skin darkening. *A. carolinensis*, for example, lack alpha-receptors in a patch of post-orbital skin that serves as a signal and darkens during male-male contests (Vaughan and Greenberg 1987). It is possible that uneven distribution of melanophore receptor type (e.g., a lack of beta-receptors) along eye stripes and lateral stripes in *A. aquaticus* explains the rapid brown-to-green color change associated with acute stress. In addition, distinctions between acute and chronic stressors will be a critical step forward when identifying the proximate causes of color changes in animals, as the severity and duration of stressors are broadly known to non-linearly relate to color changes in anoles (Hadley 1928; Greenberg 2002) and other species (e.g., Kindermann et al. 2013), though their mechanistic bases and potential functions are generally unknown.

When lizards were subjected to our standardized stressor of handling and restraint in a cloth bag, two abiotic factors also increased simultaneously: light and temperature. We therefore examined whether either of these factors could be in part responsible for *A. aquaticus*'s rapid

color change. Because our field survey indicated no correlation between temperature and color, we subsequently tested the effect of lighting environment on coloration in the laboratory. Our results suggest that *A. aquaticus* again diverges from patterns found in other *Anolis* species, in which green-to-brown transitions occur when exposed to light, including lighting lacking a UV-component (e.g., Vaughan 1987) such as ours. In our study, most *A. aquaticus* body regions did not exhibit color change with respect to the lighting environment, with a weak exception of a slight increase in lateral stripe brightness in dark environments. The failure to respond to changes in lighting environment suggests that light exposure is not a likely cause of rapid color change in this species.

To our knowledge, there is no precedent for stress-related rapid color brightening in anoline lizards (see Table S1). The only documented examples of rapid brown-to-green transitions were induced by simultaneous interruption of the nervous and circulatory systems (Carlton 1903), exposure to artificial green light (Wilson 1939), or as a component of multiple rapid color changes that was always preceded by a green-to-brown shift (Hadley 1928). Because of the atypical directionality of *A. aquaticus*'s rapid color change, identifying the functional significance of stress-related stripe brightening may help improve understanding of the functions of rapid color change in this genus as a whole. In our survey, we noted that *A. aquaticus* are primarily found on two substrate types: a) moss-speckled riverbanks and boulders or b) dark surfaces including crevices or recessed riverbank walls. We observed that *A. aquaticus* on moss-speckled surfaces often bore more brightly colored stripes, and those on darker substrates bore inconspicuous stripe coloration (dark brown) (L. Swierk, pers. obs.). Adopting brown stripes may enhance substrate-matching on the dark substrates, whereas displaying bright, irregular stripes could be a form of disruptive camouflage against brightly-mottled surfaces (Cuthill et al.

2005; Resetarits and Raxworthy 2015); an examination of predator vision models and the light environments of these habitats might confirm this prediction. Such a link between coloration and camouflage would also complement our finding that stress is associated with brighter body coloration: green speckled surfaces tend to be much more exposed than uniformly dark crevices or walls, and thus green perches could be more stressful for *A. aquaticus*. Alternatively, rapid color change may play a role in social signaling; rapid color brightening relates to contest success in other (non-anoline) taxa (e.g., Ligon and McGraw 2013), though sex and size did not influence body color in *A. aquaticus*.

Knowledge of body color change physiology and function in anoline lizards is primarily founded on classic (but dated) studies that are biased toward a single species, *A. carolinensis* (see Table S1). A broad foundational understanding of the evolution and function of color change in this model taxon therefore has yet to be formalized. Although studies on dewlap evolution and function are abundant, whole-body dynamic color changes in anoles have been recently overlooked despite their suspected significance to some species' inter- and intraspecific communication (e.g., Jenssen et al. 1995; Korzan et al. 2002). *A. aquaticus*'s atypical rapid color change broadens our understanding of color change diversity within this genus and highlights the need for future studies on the mechanistic and functional significance of physiological color change across taxa.

Acknowledgements

We thank J. Losos and D. Skelly for valuable suggestions, M. Petelo, J. Montemarano, and J. Moreira for field assistance, B. Dugelby, R. Quiros Flores, and Z. Zahawi for logistical support and research supplies, and two anonymous reviewers for their insightful comments. J.F.F.B. was

funded by the National Science Foundation (HRD-1249135) through the Native American and Pacific Island Research Experience program for undergraduates and was supported by the Organization for Tropical Studies.

Draft

Literature Cited

- Adamo, S.A., Ehgoetz, K., Sangster, C., and Whitehorne, I. 2006. Signaling to the enemy? Body pattern expression and its response to external cues during hunting in the cuttlefish *Sepia officinalis* (Cephalopoda). *Biol. Bull. (Woods Hole)*, **210**(3): 192–200. doi:10.2307/4134557.
- Anderson, A.G., and Dodson, G.N. 2015. Colour change ability and its effect on prey capture success in female *Misumenoides formosipes* crab spiders. *Ecol. Entomol.* **40**(2): 106–113. doi:10.1111/een.12167.
- Calisi, R.M., and Hews, D.K. 2007. Steroid correlates of multiple color traits in the spiny lizard, *Sceloporus pyrocephalus*. *J. Comp. Physiol. B* **177**(6): 641–654. doi:10.1007/s00360-007-0162-7.
- Carlton, F.C. 1903. The color changes in the skin of the so-called Florida chameleon, *Anolis carolinensis* Cuv. *Proc. Am. Acad. Arts Sci.* **39**(10): 259–276.
- Caro, T. 2005. The adaptive significance of coloration in mammals. *Bioscience*, **55**(2): 125. doi:10.1641/0006-3568(2005)055[0125:TASOCI]2.0.CO;2.
- Claussen, D.L., and Art, G.R. 1981. Heating and cooling rates in *Anolis carolinensis* and comparisons with other lizards. *Comp. Biochem. Physiol.* **69A**: 23–29. doi:10.1016/0300-9629(81)90634-4.
- Cuthill, I.C., Stevens, M., Sheppard, J., Maddocks, T., Párraga, C.A., and Troscianko, T.S. 2005. Disruptive coloration and background pattern matching. *Nature*, **434**(7029): 72–74. doi:10.1038/nature03312.
- Escudero, P.C., Minoli, I., Marín, M.A.G., Morando, M., and Avila, L.J. 2016. Melanism and ontogeny: a case study in lizards of the *Liolaemus fitzingerii* group (Squamata: Liolaemini). *Can. J. Zool.* **206**: 199–206. doi:10.1139/cjz-2015-0112.
- Fulgione, D., Trapanese, M., Maselli, V., Rippa, D., Itri, F., Avallone, B., Van Damme, R., Monti, D.M., and Raia, P. 2014. Seeing through the skin: dermal light sensitivity provides cryptism in moorish gecko. *J. Zool. (Lond.)* **294**(2): 122–128. doi:10.1111/jzo.12159.
- Goldman, J.M., and Hadley, M.E. 1970. Evidence for separate receptors for melanophore stimulating hormone and catecholamine regulation of cyclic AMP in the control of melanophore responses. *Br. J. Pharmacol.* **39**(1): 160–6.
- Gordon, C., and Fox, W. 1960. The normal daily rhythm of color changes in the lizard, *Anolis carolinensis*, when exposed to the contrasting background color. *Herpetologica*, **16**(4): 233–235.

- Greenberg, N., Chen, T., and Crews, D. 1984. Social status, gonadal state, and the adrenal stress response in the lizard, *Anolis carolinensis*. *Horm. Behav.* **18**(1): 1–11. doi:10.1016/0018-506X(84)90045-X.
- Greenberg, N. 2002. Ethological aspects of stress in a model lizard, *Anolis carolinensis*. *Integr. Comp. Biol.* **42**(3): 526–40. doi:10.1093/icb/42.3.526.
- Greenberg, N. 2003. Sociality, stress, and the corpus striatum of the green anolis lizard. *Physiol. Behav.* **79**(3): 429–440. doi:10.1016/S0031-9384(03)00162-8.
- Greenberg, N., and Crews, D. 1990. Endocrine and behavioral responses to aggression and social dominance in the green anole lizard, *Anolis carolinensis*. *Gen. Comp. Endocrinol.* **77**(2): 246–255. doi:10.1016/0016-6480(90)90309-A.
- Hadley, C.E. 1928. Color changes in excised pieces of integument of *Anolis equestris* under the influence of light. *Proc. Natl. Acad. Sci. U.S.A.* **14**: 822–824. doi:10.1073/pnas.14.10.822.
- Hadley, M.E., and Goldman, J.M. 1969. Physiological color changes in reptiles. *Am. Zool.* **9**(2): 489–504.
- Hutton, P., Seymoure, B.M., McGraw, K.J., Ligon, R.A., and Simpson, R.K. 2015. Dynamic color communication. *Curr. Opin. Behav. Sci.* **6**: 41–49. doi:10.1016/j.cobeha.2015.08.007.
- Jenssen, T. A., Greenberg, N., and Hovde, K. A. 1995. Behavioral profile of free-ranging male lizards, *Anolis carolinensis*, across breeding and post-breeding seasons. *Herpetol. Monogr.* **9**: 41–62. doi: 10.2307/1466995.
- Johnsen, S. 2016. How to measure color using spectrometers and calibrated photographs. *J. Exp. Biol.* **219**(6): 772–778. doi:10.1242/jeb.124008.
- Kemp, D.J., Herberstein, M.E., Fleishman, L.J., Endler, J.A., Bennett, A.T.D., Dyer, A.G., Hart, N.S., Marshall, J., and Whiting, M.J. 2015. An integrative framework for the appraisal of coloration in nature. *Am. Nat.* **185**(6): 705–724. doi:10.1086/681021.
- Keren-Rotem, T., Levy, N., Wolf, L., Bouskila, A., and Geffen, E. 2016. Male preference for sexual signalling over crypsis is associated with alternative mating tactics. *Anim. Behav.* **117**: 43–49. doi:10.1016/j.anbehav.2016.04.021.
- Kindermann, C., Narayan, E.J., Wild, F., Wild, C.H., and Hero, J.M. 2013. The effect of stress and stress hormones on dynamic colour-change in a sexually dichromatic Australian frog. *Comp. Biochem. Physiol. A* **165**(2): 223–227. doi:10.1016/j.cbpa.2013.03.011.
- Kleinholz, L.H. 1936. Studies in reptilian color changes. I. A preliminary report. *Proc. Natl. Acad. Sci. U.S.A.* **22**: 454–456. doi:10.1073/pnas.22.7.454.
- Korzan, W. J., Summers, T. R., and Summers, C. H. 2002. Manipulation of visual sympathetic

- sign stimulus modifies social status and plasma catecholamines. *Gen. Comp. Endocrinol.* **128**: 153–161. doi: 10.1016/S0016-6480(02)00077-1.
- Kronstadt, S.M., Darnell, M.Z., and Munguia, P. 2013. Background and temperature effects on *Uca panacea* color change. *Mar. Biol.* **160**(6): 1373–1381. doi:10.1007/s00227-013-2189-5.
- Langridge, K. V. 2009. Cuttlefish use startle displays, but not against large predators. *Anim. Behav.* **77**(4): 847–856. doi:10.1016/j.anbehav.2008.11.023.
- Ligon, R.A. 2014. Defeated chameleons darken dynamically during dyadic disputes to decrease danger from dominants. *Behav. Ecol. Sociobiol.* **68**(6): 1007–1017. doi:10.1007/s00265-014-1713-z.
- Ligon, R.A., and McGraw, K.J. 2013. Chameleons communicate with complex colour changes during contests: different body regions convey different information. *Biol. Lett.* **9**(6): 20130892. doi:10.1098/rsbl.2013.0892.
- Loew, E.R., Fleishman, L.J., Foster, R.G., and Provencio, I. 2002. Visual pigments and oil droplets in diurnal lizards: a comparative study of Caribbean anoles. *J. Exp. Biol.* **205**(7): 927–938.
- Márquez, C.M.M., and Márquez, L.D. 2009. Reproductive biology in the wild and in captivity of *Anolis aquaticus* (Sauria: Polychrotidae) in Costa Rica. *Boletín Técnico Ser. Zoológica*, **8**(4-5): 50–73.
- Maruska, K.P. 2015. Social transitions cause rapid behavioral and neuroendocrine changes. *Integr. Comp. Biol.* **55**(2): 1–13. doi:10.1093/icb/icv057.
- Matt, K.S., Moore, M.C., Knapp, R., and Moore, I.T. 1997. Sympathetic mediation of stress and aggressive competition: plasma catecholamines in free-living male tree lizards. *Physiol. Behav.* **61**(5): 639–47. doi:10.1016/S0031-9384(96)00500-8.
- Moore, M.C. 1991. Application of organization-activation theory to alternative male reproductive strategies: A review. *Horm. Behav.* **25**(2): 154–179. doi:10.1016/0018-506X(91)90048-M.
- Ng, J., Kelly, A.L., MacGuigan, D.J., and Glor, R.E. 2013. The role of heritable and dietary factors in the sexual signal of a Hispaniolan *Anolis* lizard, *Anolis distichus*. *J. Hered.* **104**(6): 862–873. doi:10.1093/jhered/est060.
- Parker, G.H., and Starratt, S.A. 1904. The effect of heat on the color changes in the skin of *Anolis carolinensis* Cuv. *Proc. Am. Acad. Arts Sci.* **40**(10): 457–466.
- Plavicki, J., Yang, E.J., and Wilczynski, W. 2004. Dominance status predicts response to nonsocial forced movement stress in the green anole lizard (*Anolis carolinensis*). *Physiol. Behav.* **80**(4): 547–555. doi:10.1016/j.physbeh.2003.10.009.

- Resetarits, E.J., and Raxworthy, C.J. 2015. Hidden in plain sight: How ventral line markings in chameleons may enhance camouflage. *Am. Nat.* **187**(2): 262–273. doi:10.1086/684412.
- Savage, J.M. 2005. *The Amphibians and Reptiles of Costa Rica: A Herpetofauna between Two Continents, between Two Seas*. University of Chicago Press, Chicago.
- Schneider, C.A., Rasband, W.S., and Eliceiri, K.W. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods*, **9**(7): 671–675. doi:10.1038/nmeth.2089.
- Sköld, H.N., Aspögren, S., and Wallin, M. 2013. Rapid color change in fish and amphibians - function, regulation, and emerging applications. *Pigment Cell Melanoma Res.* **26**(1): 29–38. doi:10.1111/pcmr.12040.
- Smith, K.R., Cadena, V., Endler, J.A., Porter, W.P., Kearney, M.R., and Stuart-Fox, D. 2016. Colour change on different body regions provides thermal and signalling advantages in bearded dragon lizards. *Proc. R. Soc. B Biol. Sci.* **283**: 20160626. doi:10.1098/rspb.2016.0626.
- Sowersby, W., Lehtonen, T.K., and Wong, B.B.M. 2015. Background matching ability and the maintenance of a colour polymorphism in the red devil cichlid. *J. Evol. Biol.* **28**(2): 395–402. doi:10.1111/jeb.12572.
- Stevens, M., Párraga, C.A., Cuthill, I.C., Partridge, J.C., and Troscianko, T.S. 2007. Using digital photography to study animal coloration. *Biol. J. Linn. Soc.* **90**(2): 211–237. doi:10.1111/j.1095-8312.2007.00725.x.
- Stevens, M. 2016. Color change, phenotypic plasticity, and camouflage. *Front. Ecol. Evol.* **4**(51): 1–10. doi:10.3389/fevo.2016.00051.
- Stuart-Fox, D., and Moussalli, A. 2008. Selection for social signalling drives the evolution of chameleon colour change. *PLoS Biol.* **6**(1): e25. doi:10.1371/journal.pbio.0060025.
- Stuart-Fox, D., and Moussalli, A. 2009. Camouflage, communication and thermoregulation: lessons from colour changing organisms. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* No. **364**(1516): 463–70. doi:10.1098/rstb.2008.0254.
- Summers, C.H., and Greenberg, N. 1994. Somatic correlates of adrenergic activity during aggression in the lizard, *Anolis carolinensis*. *Horm. Behav.* **28**: 29–40. doi:10.1006/hbeh.1994.1003.
- Taylor, J.D., and Hadley, M.E. 1970. Chromatophores and color change in the lizard, *Anolis carolinensis*. *Z. Zellforsch. Mikrosk. Anat.* **104**(2): 282–94. doi:10.1007/BF00309737.
- Thurman, C.L., 1988. Rhythmic physiological colour change in Crustacea—a review. *Comp. Biochem. Physiol. C* **91**(1): 171–185. doi:10.1016/0742-8413(88)90184-3.

- Troscianko, J., and Stevens, M. 2015. Image calibration and analysis toolbox - a free software suite for objectively measuring reflectance, colour and pattern. *Methods Ecol. Evol.* **6**: 1320–1331. doi:10.1111/2041-210X.12439.
- Umbers, K.D.L., Tatarnic, N.J., Holwell, G.I., and Herberstein, M.E. 2013. Bright turquoise as an intraspecific signal in the chameleon grasshopper (*Kosciuscola tristis*). *Behav. Ecol. Sociobiol.* **67**(3): 439–447. doi:10.1007/s00265-012-1464-7.
- Vaughan, G.L. 1987. Photosensitivity in the skin of the lizard, *Anolis carolinensis*. *Photochem. Photobiol.* **46**(1): 109–114. doi:10.1111/j.1751-1097.1987.tb04743.x.
- Vaughan, G.L., and Greenberg, N. 1987. Propranolol, a beta-adrenergic antagonist, retards response to MSH in skin of *Anolis carolinensis*. *Physiol. Behav.* **40**(5): 555–558. doi:10.1016/0031-9384(87)90095-3.
- Wilczynski, W., Black, M.P., Salem, S.J., and Ezeoke, C.B. 2015. Behavioural persistence during an agonistic encounter differentiates winners from losers in green anole lizards. *Behaviour*, **152**(5): 563–591. doi:10.1163/1568539X-00003243.
- Wilson, F.H. 1939. Preliminary experiments on the color changes of *Anolis carolinensis* (Cuvier). *Am. Nat.* **73**(745): 190–192.
- Yabuta, S., and Suzuki-Watanabe, A. 2011. Function of body coloration in green anoles (*Anolis carolinensis*) at the beginning of the breeding season: Advertisement signaling and thermoregulation. *Curr. Herpetol.* **30**(2): 155–158. doi:10.5358/hsj.30.155.
- Yang, E., Phelps, S.M., Crews, D., and Wilczynski, W. 2001. The effects of social experience on aggressive behavior in the green anole lizard (*Anolis carolinensis*). *Ethology*, **793**: 777–793. doi:10.1046/j.1439-0310.2001.00704.x.

Table 1. Test for differences in luminance, saturation, and hue of the eye stripe, lateral stripe, and dorsum, before and after exposure to a stressor and between the sexes in *Anolis aquaticus*.

		df	F	P
Eye stripe	<i>Luminance</i>			
	Time	1, 27	47.944	< 0.001 **
	Sex	1, 27	0.579	0.453
	Time x Sex	1, 27	2.359	0.136
	<i>Saturation</i>			
	Time	1, 27	1.098	0.304
	Sex	1, 27	0.456	0.505
	Time x Sex	1, 27	1.179	0.287
	<i>Hue</i>			
	Time	1, 27	40.477	< 0.001 **
Lateral stripe				
	<i>Luminance</i>			
	Time	1, 27	47.260	< 0.001 **
	Sex	1, 27	0.085	0.773
	Time x Sex	1, 27	0.380	0.543
	<i>Saturation</i>			
	Time	1, 27	6.713	0.015 *
	Sex	1, 27	2.524	0.124
	Time x Sex	1, 27	1.529	0.227
	<i>Hue</i>			
Dorsum				
	<i>Luminance</i>			
	Time	1, 27	7.870	0.009 *
	Sex	1, 27	3.802	0.062
	Time x Sex	1, 27	0.009	0.926
	<i>Saturation</i>			
	Time	1, 27	0.531	0.472
	Sex	1, 27	5.009	0.034 *
	Time x Sex	1, 27	1.449	0.239
	<i>Hue</i>			
	Time	1, 27	0.398	0.534
	Sex	1, 27	2.112	0.158
	Time x Sex	1, 27	0.178	0.677

Note: Output of repeated measures analyses of variance (ANOVA). Luminance (%), saturation (%), and hue (degrees) were dependent variables. Time was a within-subject factor (pre- or post-stressor), and Time x Sex indicates the interaction of time and sex. Luminance, saturation, and hue were log-10 transformed prior to analysis. A single asterisk (*) denotes significance ($P < 0.05$), double asterisks (**) indicates significance at $P < 0.001$, and df is degrees of freedom.

Draft

Figure Legends

Fig. 1. Two adult male aquatic anoles, *Anolis aquaticus*, representing the range of eye stripe and lateral stripe colors: a) dark and b) bright. Eye stripe and lateral stripe locations are noted in the lower photo. Note that *A. aquaticus* also display eye and lateral stripes that are more intense blue and green, respectively. Photo credit: a) J. Montemarano, b) L. Swierk.

Fig. 2. Average % luminance (brightness) of the dorsum, eye stripe, and lateral stripe of the aquatic anole, *Anolis aquaticus*, before (gray bars) and after (white bars) exposure to a standardized mild stressor of human handling, restraint, and relocation. Error bars represent ± 1 standard error. Asterisks (*) indicate significant differences.

Fig. 3. Average % luminance (brightness) of the a) dorsum, b) eye stripe, and c) lateral stripe in the aquatic anole, *Anolis aquaticus*, before and after experimental manipulation of the lighting environment. The light exposure treatment is represented by open white squares and dotted lines, and the dark treatment is represented by black circles and solid lines. Error bars represent ± 1 standard error.

(a)

(b)

Luminance (%)

35
30
25
20
15
10
5
0<https://mc.manuscriptcentral.com/cjz-pubs>

Dorsum

Eye Stripe

Lateral Stripe

*

*

*